Diuretics Prime Plant Immunity in *Arabidopsis thaliana*

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Abstract

Plant activators are agrochemicals that activate the plant immune system, thereby enhancing disease resistance. Due to their prophylactic and durable effects on a wide spectrum of diseases, plant activators can provide synergistic crop protection when used in combination with traditional pest controls. Although plant activators have achieved great success in wet-rice farming practices in Asia, their use is still limited. To isolate novel plant activators applicable to other crops, we screened a chemical library using a method that can selectively identify immune-priming compounds. Here, we report the isolation and characterization of three diuretics, bumetanide, bendroflumethiazide and clopamide, as immune-priming compounds. These drugs upregulate the immunity-related cell death of *Arabidopsis* suspension-cultured cells induced with an avirulent strain of *Pseudomonas syringae pv. tomato* in a concentration-dependent manner. The application of these compounds to *Arabidopsis* plants confers disease resistance to not only the avirulent but also a virulent strain of the pathogen. Unlike salicylic acid, an endogenous phytohormone that governs disease resistance in response to biotrophic pathogens, the three diuretic compounds analyzed here do not induce PR1 or inhibit plant growth, showing potential as lead compounds in a practical application.

Introduction

Pesticides such as microbicides, fungicides and insecticides are widely used to cover the demand for the expanding world population and biomass [1]. Even with pesticides, the total crop loss from disease is estimated to be approximately 14% of the total potential production ability of the planet. Importantly, the percentage of crop loss due to disease and insects has not changed over the last 30 years [2]. In addition, the effects of pesticides are often overcome by spontaneous mutations in the pathogenic organisms. Many pesticides are associated with risks to both the environment and food production. Furthermore, pesticides often affect not only the targeted organisms but also useful predators or antagonistic microorganisms.

In contrast to the agrochemicals that target pests, plant activators work in plants and increase their ability to resist pests [3]. For example, the most successful plant activator, probenazole, was originally developed 35 years ago [4]. Probenazole has been widely used in the cultivation of irrigated rice in East Asia to protect against blast fungus and bacterial leaf blight. The remarkable advantage of probenazole is its durability, and indeed, no drug-resistant pathogens have emerged, most likely because it acts on host crops but not pathogens. Interestingly, probenazole potentiates but does not directly induce the defense responses in plants. Probenazole stimulates the accumulation of salicylic acid (SA), a defense hormone in plants, but its target and its precise mode of action remain unclear [5]. In contrast, benzothiadiazole (BTH), another practical plant activator, functions as a synthetic chemical analog of SA [6,7]. BTH and SA effectively mount a disease resistance response in plants. However, the application of BTH has been confined to specific crops, mainly due to accompanying drug injuries at high dosages. These injuries are most likely due to the strong induction of the defense response, which is often associated with severe growth suppression [8,9,10,11].

To obtain novel lead compounds, we established a method for selectively identifying agents that enhance but do not induce the disease resistance response in plants [12]. The method was miniaturized for high-throughput screening to explore chemical libraries that contain a large number of compounds. From a comprehensive survey of a commercially available library of diversity-oriented small molecules, we have successfully identified several compounds that prime the immune response in *Arabidopsis* plants and have investigated five of these compounds, which was designated as imprimatinA1, A2, A3, B1 and B2 [12]. In this study, we screened an additional chemical library containing pharmaceutical drugs and natural products, and identified three diuretics as plant immune-system-priming compounds. These compounds potentiate but do not directly induce the defense responses and show potential as lead structures for novel plant activators.

Results

Isolation of three diuretics as plant immune-priming compounds

Using a previously established high-throughput chemical screening method for plant immune-priming agents [12], we searched for chemical compounds that enhance cell death in cultured *Arabidopsis* cells after infection with an avirulent patho-
genic bacteria, *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* avrRpm1). We used a commercially available chemical library comprised of 2,000 small organic molecules, including known pharmaceutical drugs, experimental bioactive compounds and natural products (MicroSource Discovery Systems Inc.). After performing an initial screen made up of three replicates, the concentration dependency of the candidate chemicals was analyzed. The candidate compounds that reproducibly enhanced cell death were classified into different groups based on both their pharmaceutical properties and their molecular structures. For this study, we focused on a group containing three different diuretics, bumetanide, bendroflumethiazide and clopamide (Fig. 1).

Arabidopsis suspension cells were treated with these diuretics at different concentrations, and the rates of cell death were quantitatively measured after inoculation with *Pst-avrRpm1*. These compounds upregulated the cell death induced by the pathogen in a concentration-dependent manner (Fig. 2). Their effects on cell death ranged from half to one-third of SA, which can function as an endogenous potentiator for pathogen-triggered cell death [13]. Bumetanide appeared to have a stronger effect than the other diuretics, however, it would be due to its concentration-dependent toxic effects on the cells in the absence of the pathogens (Fig. 2). This chemical activity is similar to those of ImprimatinA1, A3, B1 and B2 which were the plant activators isolated from our previous screening and the commercial plant activator tiadinil [12]. Therefore, we further analyzed these compounds including BMT.

The diuretics increased disease resistance in *Arabidopsis* plants

Next, we examined if these diuretics functioned in plants. *Arabidopsis* seedlings were grown hydroponically on rockwool, and their roots were immersed in water solutions containing each diuretic at 100 μM. SA and DMSO were applied at 50 μM as a positive and negative control, respectively. Three days after incubation, a virulent and an avirulent *Pst* strains were inoculated into the leaves, and the numbers of bacteria inside the leaves were counted at the indicated days post inoculation (DPI). All chemicals significantly decreased the growth of avirulent bacteria compared with the mock control experiment (Fig. 3a). Interestingly, the diuretic treatments also suppressed the growth of the virulent *Pst* (Fig. 3b). The levels of enhanced resistance to both the avirulent and virulent *Pst* were almost the same as those of 50 μM SA (Figs. 3a and 3b). The effects of these diuretics on disease resistance in planta were consistent with those on the pathogen-induced cell death of the suspension cells (Fig. 2). According to the upregulation of the disease resistance in the diuretics-treated *Arabidopsis* plants, the greater levels of *PR1* gene expression were detected at 24 hours after infection of *Pst* (Fig. 4). We also tested if the diuretics had inhibitory effects on bacterial growth and found that these compounds did not prevent bacterial growth at a concentration of 200 μM (Fig. 5).

The diuretics did not directly induce PR1 unlike SA

Next, we examined if these diuretics possess activity analogous to that of SA. Young *Arabidopsis* seedlings were treated with the diuretic compounds for 24 or 48 hours at 100 μM, and the...
expression of defense genes was analyzed. The transcription levels of the pathogenesis-related 1 (PR1) gene, a marker of SA-dependent defense responses, and A3g57260, an SA-inducible gene [14], were examined by real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Although SA effectively induced the expression of these genes at both time points, none of the examined diuretics induced these genes (Fig. 6). These results suggest that the tested diuretics and SA function differently.

The diuretics did not inhibit SA glucosyltransferase
In the previous study, we revealed that the perturbation of SA glucosylation, a major metabolic pathway used during defense responses, is one of the modes of action for plant immune-priming agents [12]. Therefore, we examined whether the isolated diuretics inhibit the enzymatic activity of SA glucosyltransferase (SAGT).

Each diuretic was added to an in vitro reaction mixture containing UGT74F1, one of the Arabidopsis SAGTs, and their effects were evaluated. ImprimatinA2, a positive control, clearly inhibited the SAGT activity, whereas clopamide had no inhibitory effects on the SAGT enzymatic activity at 100 μM (Fig. 7). Considering that Imprimatins had more than 83% inhibitory activities of the Arabidopsis SAGTs at 100 μM [12], 10–30% inhibitions of the SAGT activity of BMT and BFM were likely to be insufficient to account for their priming effects on plant immunity.

The diuretics did not inhibit plant growth
To evaluate the potential of these diuretics for practical applications as plant activators, the effects of the compounds on plant growth were examined. After sterilization and stratification, Arabidopsis seeds were incubated with liquid media containing each of the diuretics at varied concentrations. In contrast to SA, which inhibited the growth of Arabidopsis seedlings in a concentration-dependent manner, the tested compounds did not severely prevent germination or growth at an effective concentration range for the enhancement of cell death and disease resistance (Fig. 8). Consistent with the toxicity effect of BMT on the suspension cells (Fig. 2), it seems to have some negative effect on germination/growth of Arabidopsis seedlings (Fig. 8).

Figure 3. The increased disease resistance induced by the diuretics in Arabidopsis plants. Arabidopsis seedlings were grown on rockwool for three weeks under short-day conditions, and their roots were drenched with water supplemented with 100 μM of the compounds for three days. As a positive control, 50 μM of sodium salicylate (SA) was used. Then, the avirulent Pst-avrRpm1 (A) and the virulent Pst (B) were inoculated into the leaves by syringe infiltration, and the bacterial numbers were counted at the indicated days (n = 4). **P<0.01; Student’s t-test.

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Figure 4. The expression of PR1 gene 24 hours after pathogen inoculation with or without the application of the diuretics. The PR1 transcript level was determined by qRT-PCR. cDNA were prepared from seedlings 24 hours after inoculation of Pst-avrRpm1 with or without 100 μM of the diuretics. DMSO was used as mock treatment. The expression values were normalized to Actin2 as an internal standard. The bar represents the SE of three independent replicates. **P<0.01, *P<0.05; Student’s t-test.

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Figure 5. Effect of the diuretics on the growth of pathogenic bacteria. Pst was cultured in liquid minimal medium supplemented with 200 μM of the indicated chemicals or 100 μg/mL hygromycin, and bacterial growth was monitored as the optical density of the bacteria at 600 nm at the indicated times after inoculation. The bar represents the SE of three independent replicates.

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Discussion

In this study, a high-throughput chemical screening method was used to isolate three diuretics, bumetanide, bendroflumethiazide and clopamide, that act as plant immune-priming compounds. These compounds enhanced the pathogen-induced cell death of suspension-cultured Arabidopsis cells in a concentration-dependent manner (Fig. 2). The application of the diuretics to Arabidopsis seedlings enhanced disease resistance (Fig. 3), likely due to the immune-priming effects of the compounds, which is supported by the absence of any toxic effects on pathogenic bacteria (Fig. 5). Thus, the screen using the suspension-cultured cells is suitable for identifying lead compounds for priming plant immunity.

Consistent with our screening strategy, the isolated compounds did not induce the two tested defense genes in the absence of pathogens (Fig. 6). Likewise, these compounds did not prevent germination and plant growth during the early seedling stage (Fig. 8). Compared with other screening methods, which use activation potency of defense gene expression as an indicator, our screening method can provide unique lead compounds with less phytotoxic effects and is thus suitable for practical use.

In addition to being lead compounds for crop protectants, the diuretics can also be useful probes for understanding plant immunity at the molecular level. We demonstrated that these diuretics do not function as analogs of SA, unlike BTH and INA (Fig. 6). Furthermore, they did not inhibit the SA glucosylation enzyme that was previously identified as a target of other immune-priming compounds [12] (Fig. 7). These results imply that these diuretics potentiate plant immunity by an unknown mechanism.

Diuretics are pharmaceutical drugs used in clinical practice to treat hypertension or edema [15]. Diuretics target the sodium-chloride symporters, a type of electroneutral cation-coupled chloride cotransporter known as SLC12 (solute carrier family 12), which are broadly conserved in the animal kingdom [16]. Diuretics act on the SLC12A family members, which are sodium (Na$^+$)-coupled chloride (Cl$^-$) cotransporters located along the renal tubule of the kidney nephron. By binding to the Cl$^-$ binding site of the SLC12A proteins and competing with Cl$^-$ [16], diuretics inhibit Na$^+$ and Cl$^-$ reabsorption from the tubular fluid into the cells. This interferes with urine concentration and dilution [15]. In the Arabidopsis genome, only a single protein, AtCCC1/HAP5 (At1g30450), has a high similarity to animal cation-Cl$^-$
cotransporters (CCC) such as SLC12A [17]. AtCCC1 was shown to have Cl− transport activity that depended on both Na+ and K+ using a cRNA expression system with Xenopus laevis oocytes. Interestingly, the Cl− uptake activity of AtCCC1 was also demonstrated to be reduced in the presence of 100 µM bumetanide in vitro [17]. Thus, the inhibition of AtCCC1 might be a potential target of the isolated diuretics for cell death and disease resistance in Arabidopsis. However, the target of each of the diuretics is different in animals, although their inhibitory mechanism is shared. For instance, bumetanide, a loop diuretic, targets the Na+-K+-2Cl− cotransporters SLC12A1 and SLC12A2 [15,16], whereas bendroflumethiazide, a thiazide diuretic, and clopamide, a thiazide-like diuretic, act on the Na+-Cl− cotransporter SLC12A3 [15,16]. In our experiments, the activities of these diuretics are nearly the same in priming plant immunity (Figs. 2 and 3). This situation suggests the possibility that these diuretics might act on other proteins to potentiate plant immunity.

Some diuretics are known to have the potential to cause a hypertensive allergic reaction when used as medication, and this effect is thought to be derived from the sulfonamide groups of the diuretics [18]. Accordingly, the isolated diuretics in this study have molecular structures containing the sulfonamide functional group (Fig. 1). Plants do not have allergic reaction, however, the immune-priming effect of the diuretics might be dependent on this sulfonamide moiety with unknown mechanism. The treatment of Arabidopsis seedlings with the sulfonamide compounds, sulfaethoxazole, sulfadiazine and sulfapyridine, decreased the growth of pathogenic bacteria inside of the plants [19]. Sulfaethoxazole had the strongest effect among the tested sulfonamides, and it can confer disease resistance in soil-grown Arabidopsis seedlings at 100 µM. The effective concentration range of these sulfonamides is very similar to those of the diuretics. The chemical library used in this study contains ethacrynic acid, spironolactone and triamterene, which are diuretics without a sulfonamide group. None of these non-sulfonamide diuretic compounds were identified as positives in our screen, although ethacrynic acid targets Na+-K+-2Cl− cotransporters and is a loop diuretic [20]. Further analysis of the mode of action of the diuretics or sulfonamides will reveal new insights into plant immunity and provide a novel tool for conferring disease tolerance in plants.

Materials and Methods

Chemicals

A commercial chemical library of the Spectrum Collection containing 1,000 medical drugs, 500 natural products with unknown biological properties and 420 non-drug bioactive compounds was purchased (The Spectrum Collection, 10 mM in DMSO, MicroSource Discovery System Inc., Gaylordsville, CT, USA). The prohibited imports in the library were excluded. Compounds were further obtained from the chemical companies indicated below. Bumetanide, bendroflumethiazide and sodium salicylate were purchased from Sigma-Aldrich (St Louis, MO, USA). Clopamide was purchased from BIOMOL International (Plymouth meeting, PA, USA).

Plant materials and growth conditions

Arabidopsis suspension-cultured cells were grown in liquid media containing MS with 3% sucrose supplemented with 0.5 µM/L MES (pH 5.7), 0.5 µg/L naphthaleneacetic acid and 0.05 µg/L 6-benzylaminopurine under long-day conditions (16-h light/8-h dark cycles) [21,22]. For the gene expression analysis, Arabidopsis thaliana ecotype Columbia was grown on half-MS agar media (1% sucrose) at 22°C under long-day conditions. To assay for disease resistance, the plants were grown in soil at 22°C under short-day conditions (8-h light/16-h dark cycles). For the growth assay, Arabidopsis seeds were sterilized and stored at 4°C for 4 days to break dormancy. Then, the seeds were dispensed into 96-well plates, and half-MS liquid media supplemented with 1% sucrose containing each chemical was applied at the indicated concentrations. The plates were then incubated at 22°C under long-day conditions.

Quantitative measurement assay for the chemical’s effect on cell death

The method was performed essentially as described previously [12]. Arabidopsis suspension cells were dispensed into each well of 96-well plates, and the chemicals were added at the indicated concentrations. After a one hour incubation with occasional tapping, Pseudomonas syringae pv. tomato DC3000 (Pst avrRpm1 (final concentration: OD=0.2 in MS medium) and MgCl2 as mock were applied to each duplicated well, respectively. After cocultivation on a shaker for 21 hours under long-day conditions at 22°C, the cells were stained with 1% Evans blue dye. Then, the cells were washed with 1 mL of water four times, and the incorporated dye was eluted with 400 µL of elution solution (50% methanol, 1% SDS). The absorbance at 593 nm was measured with a microplate reader and was taken as the degree of cell death. The cell death rates were calculated as the value relative to the mock control at 100%. The data are represented as the mean ± SD (n = 5).

Chemical treatment of plants and RNA experiments

Arabidopsis seedlings grown on agar media for two weeks were soaked in liquid half-MS media (1% sucrose) supplemented with 100 µM of the chemicals. The plants were incubated for 24 or 48 hours at 22°C. The seedlings were collected in a 2-mL tube and frozen in liquid nitrogen. Then, the samples were crushed with four zirconia balls (ø 2 mm) using the Shake Master Neo (BMS, Tokyo, Japan). Total RNA was extracted with the PureLink Micro-to-Midi Total RNA Purification System with on-column DNase treatment (Invitrogen, Carlsbad, CA, USA). The RNA concentration and purity were validated by a spectrometer (BioPhotometer plus; Eppendorf, Germany). cDNA was synthesized from each sample with the PrimeScript RT reagent kit with gDNA Eraser (Perfect Real Time) (Takara, Shiga, Japan). The qRT-PCRs were performed using the KAPA SYBR Fast qPCR Kit (KAPA BIOSYSTEMS, Woburn, MA, USA) with a LightCycler® 480 real-time PCR instrument (Roche Diagnostics, Basel, Switzerland). The quantitation of the target transcript was conducted using the LightCycler 480 internal software “Absolute Quantification 2nd Derivative Max” and normalized to Actin2. The primers used in this study were 5′-CCGGTTCTTTTCTTTCCAAAGG-3′ and 5′-CCGGTACTGTCACACACG-3′ for Actin2, 5′-TGATCTCTGTTGG-GAATTATGT-3′ and 5′-TGATCTCTGTTGG-GAATTATGT-3′ for 5′-TCGGTAGCATCACACTGATTTTG-3′. The cell death rates were calculated as the value relative to the mock control at 100%. The data are represented as the mean ± SD (n = 5).
with 100 μM of the chemicals was supplied. The plants were then grown for 3 days prior to the inoculation with the pathogenic bacteria. As a positive control, 50 μM of SA was used. Freshly grown bacteria of both the Pst and Pst avrRpm1 strains resuspended in 10 mM MgCl2 to an OD600 of 0.002 were inoculated into leaves with a needleless syringe. After 3 days, leaf disks were punched out with a sterile cork-borer and placed in a 2-mL tube. Then, the disks were crushed with zirconia balls in 500 μL of 10 mM MgCl2. A serial dilution of each sample was spread onto agar media containing kanamycin and rifampicin, and the number of bacteria inside the leaves was calculated.

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Author Contributions
Conceived and designed the experiments: YN KS. Performed the experiments: YN MI. Analyzed the data: YN MI. Wrote the paper: YN KS.

References
1. Editorial (2010) How to feed a hungry world. Nature 466: 531–532.
2. Agrios GN (2004) Plant pathology 5th edn. (Academic Press, Harcourt Brace Jonannovich, Publishers, New York).
3. Oostendorp M, Kunz W, Dietrich B, Staub T (2001) Induced Disease Resistance in Plants by Chemicals. Eur J Plant Pathol 107: 19–28.
4. Watanabe T, Igarashi H, Matsumoto K, Seki S, Mase S, et al. (1977) The Characteristics of Probenazole (Oryzemate®) for the Control of Rice Blast. J Pestic Sci 2: 291–296.
5. Nakashita H, Yoshikawa K, Yasuda M, Nitta T, Araoi Y, et al. (2002) Probenazole induces systemic acquired resistance in tobacco through salicylic acid accumulation. Physiol Mol Plant Pathol 61: 197–203.
6. Gorlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, et al. (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8: 629–643.
7. Vlot AC, Dempey DA, Klessig DF (2009) Salicylic Acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol 47: 177–206.
8. Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb C (1997) Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. Plant Cell 9: 261–270.
9. Knoch C, Saha MS, Girke T, Klessig T (2009) The synthetic elicitor 3,5-dichloroanthranilic acid induces NPR1-dependent and NPR1-independent mechanisms of disease resistance in Arabidopsis. Plant Physiol 150: 333–347.
10. Zhou J, Volz C, Pfeiffer K, Klessig DF (2007) Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. Plant Physiol 145: 883–894.
11. Yang DI, Yao J, Mei CS, Tong XH, Zeng LJ, et al. (2012) Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc Natl Acad Sci U S A 109: E1192–E1200.
12. Noutoshi Y, Okazaki M, Kida T, Nishina Y, Morishita Y, et al. (2012) Novel plant immune-priming compounds identified via high-throughput chemical screening target salicylic acid glucosyltransferases in Arabidopsis. Plant Cell doi:10.1105/tpc.112.098343.
13. Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb C (1997) Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. Plant Cell 9: 261–270.
14. Knoch C, Saha MS, Girke T, Klessig T (2009) The synthetic elicitor 3,5-dichloroanthranilic acid induces NPR1-dependent and NPR1-independent mechanisms of disease resistance in Arabidopsis. Plant Physiol 150: 333–347.
15. Plant L (2003) Clinical use of diuretics. Clin Med 3: 517–520.
16. Gamba G (2005) Molecular physiology and pathophysiology of electroneutral cation-chloride cotransporters. Physiol Rev 85: 423–493.
17. Colmenero-Flores JM, Martinez G, Gamba G, Vazquez N, Iglesias DJ, et al. (2007) Identification and functional characterization of cation-chloride cotransporters in plants. Plant J 50: 278–292.
18. Healy R, Jankowski TA, Crowenover B (2007) Clinical inquiries. Which diuretics are safe and effective for patients with a sulfa allergy? J Fam Pract 56: 488–490.
19. Schreiber K, Bijlsma R, Boryczka J, Desveaux D (2008) A high-throughput chemical screen for resistance to Pseudomonas syringae in Arabidopsis. Plant Cell 54: 522–531.
20. Owen NE, Prastein ML (1985) Na/K/Cl cotransport in cultured human fibroblasts. J Biol Chem 260: 1443–1451.
21. Menges M, Murray JA (2002) Synchronous Arabidopsis suspension cultures for analysis of cell cycle gene activity. Plant J 30: 522–531.
22. Wang Y, Li D, You H, Li B, Zou J, et al. (2012) Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc Natl Acad Sci U S A 109: E1192–E1200.

References
1. Editorial (2010) How to feed a hungry world. Nature 466: 531–532.
2. Agrios GN (2004) Plant pathology 5th edn. (Academic Press, Harcourt Brace Jonannovich, Publishers, New York).
3. Oostendorp M, Kunz W, Dietrich B, Staub T (2001) Induced Disease Resistance in Plants by Chemicals. Eur J Plant Pathol 107: 19–28.
4. Watanabe T, Igarashi H, Matsumoto K, Seki S, Mase S, et al. (1977) The Characteristics of Probenazole (Oryzemate®) for the Control of Rice Blast. J Pestic Sci 2: 291–296.
5. Nakashita H, Yoshikawa K, Yasuda M, Nitta T, Araoi Y, et al. (2002) Probenazole induces systemic acquired resistance in tobacco through salicylic acid accumulation. Physiol Mol Plant Pathol 61: 197–203.
6. Gorlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, et al. (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8: 629–643.
7. Vlot AC, Dempey DA, Klessig DF (2009) Salicylic Acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol 47: 177–206.
8. Shirasu K, Yachiro P, Shah J, Klessig DF (2002) A gain-of-function mutation in an Arabidopsis Toll Interleukin1 receptor-nucleotide binding site-leucine-rich repeat type R gene triggers defense responses and results in enhanced disease resistance. Plant Cell 14: 3149–3162.
9. Zhang Y, Gerincshag D, Dong X, Li X (2003) A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of npi-l-1, constitutive 1. Plant Cell 15: 2636–2646.
10. Noutoshi Y, Ito T, Seki M, Nakashita H, Yoshioka K, et al. (2005) A single amino acid insertion in the WRKY domain of the Arabidopsis 3-cis-3,5-dichloroanthranilic acid induces NPR1-dependent and NPR1-independent mechanisms of disease resistance in Arabidopsis. Plant J 30: 522–531.
11. Yang DI, Yao J, Mei CS, Tong XH, Zeng LJ, et al. (2012) Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc Natl Acad Sci U S A 109: E1192–E1200.