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# Salicylate Activity. 1. Protection of Plants from Paraquat Injury

F. Paul Silverman,* Peter D. Petracek, Christina M. Fledderman, Zhiguo Ju,† Daniel F. Heiman, and Prem Warrior

Valent BioSciences Corporation, 6131 Oakwood Road, Long Grove, Illinois 60047

Paraquat (1,1′-dimethyl-4,4′-bipyridinium; methylviologen) is a widely used, nonselective contact herbicide that rapidly stimulates free radical generation. It has been found that the addition of sodium salicylate (sodium 2-hydroxybenzoate; NaSA) to paraquat spray solutions significantly decreased herbicidal activity. This protection was observed in tobacco (*Nicotiana tabacum*) regardless of whether NaSA was foliar-applied along with or prior to paraquat application or NaSA was soil-applied prior to paraquat application. Because salicylic acid (SA) is an inducer of systemic acquired resistance (SAR) to plant disease, paraquat protection by three SAR inducers (acibenzolar-*S*-methyl, harpin, and probenazole) and selected salicylate derivatives was assessed. Twenty-two of 24 compounds tested decreased herbicidal activity when foliar-applied with paraquat. Protection from paraquat was greatest with 5-chlorosalicylate, and no protection was observed with benzoic acid. NaSA decreased paraquat activity on *npr1-2*, an *Arabidopsis* mutant that is compromised in NaSA-induced SAR, and on *ein2-1*, an ethylene-insensitive *Arabidopsis* mutant. Thus, salicylate protection from paraquat is independent of disease resistance and ethylene perception. This suggests the existence of an NaSA-mediated pathway capable of protecting plants from reactive oxygen stress.

**KEYWORDS: Photosystem I inhibitor; PSI; alternative respiration; salicylhydroxamic acid; SHAM; aminoethoxyvinylglycine; AVG; benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester; BTH; 3-(2-propenyloxy)-1,2-benzisothiazole-1,1-dioxide**

## INTRODUCTION

Salicylic acid (SA) is a simple plant phenolic. The role of SA as an endogenous signal was first shown by the induction of thermogensis in *Arum* lilies (*1*). SA is an important signal molecule in the defense response of many plants and is integral in the establishment of resistance to pathogen attack known as systemic acquired resistance (SAR) (*2*). SA is also involved in plant stress tolerance. Exogenous applications of SA protect mustard plants from heat stress (*3*) and maize from chilling stress (*4*). SA application also induces antioxidant defenses, including superoxide dismutase (*5*). Moreover, SA levels have been shown to increase in response to the free radical generators ozone and UV illumination (*6*).

Paraquat is a free radical-generating herbicide that inhibits photosysnthesis by accepting electrons from photosystem I, which in turn generates reactive oxygen species (ROS) in light (*7*). The ROS generated, which include superoxide anion, hydrogen peroxide, and the hydroxyl radical, cause lipid peroxidation and membrane damage (*8*).

In the present study, we examined the relationship between salicylates and paraquat damage. Strobel and Kuc (*9*) found that SA pretreatment protects tobacco plants from paraquat injury. Ananieva et al. (*10*) and Kim et al. (*11*) also determined that pretreatment of plants with SA provided protection from subsequent paraquat treatment. In these papers, resistance to paraquat was significant, but all assumed a time element necessary for the induction of ROS-associated enzymes to quench paraquat activity. It is well-known that synergies and antagonisms of crop protection agents may significantly affect the performance of pesticides in the field. In our studies, we have determined that salicylate protection from paraquat injury does not require any pretreatment interval and that resistance to paraquat is independent of SAR and ethylene perception.

## MATERIALS AND METHODS

**Chemicals.** Paraquat (1,1′-dimethyl-4,4′-bipyridinium; methylviologen), sodium salicylate (sodium 2-hydroxybenzoate; NaSA), and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. 3-Fluorosalicylate, 6-methylsalicylate, and 3,5-difluorosalicylate were produced by synthesis as described elsewhere (*12*). Aminoethoxyvinylglycine (AVG) was obtained from Valent BioSciences Corp. (Libertyville, IL). Actigard 50WG with the active ingredient acibenzolar-*S*-methyl [benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester] was obtained from Syngenta Crop Protection (Greensboro, NC). Messenger, with the active ingredient harpin, was obtained from Eden Bioscience (Bothell, WA). Oryzemate, with the active ingredient probenazole [3-(2-propenyloxy)-1,2-benzisothiazole-1,1-dioxide], was obtained from Meiji Seika Kaisha Ltd. (Tokyo, Japan). The crop oil concentrate (COC) used in all foliar sprays comprised

* Author to whom correspondence should be addressee (e-mail paul.silverman@valent.com).
† Present address: Shandong University of Technology, Zibo, Shandong, People's Republic of China.

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83% Orchex 796 (Exxon Co., Houston, TX) and 17% AT Plus 300F (Uniqema, New Castle, DE).

**Plant Material.** Tobacco (*Nicotiana tabacum* cv. Xanthi-nc) seed was obtained from Dr. Ilya Raskin (Rutgers University, New Brunswick, NJ). Tobacco plants were grown as previously described (*13*). Briefly, tobacco was sown onto Pro-Mix PGX and grown under cool white fluorescent lamps at 250 $\mu$mol·m$^{-2}$·s$^{-1}$ (16:8 h light/dark cycle; 25 °C). Three weeks after sowing, individual plants were transplanted into pots (7.6 cm diameter) containing Pro-Mix PGX, grown in the greenhouse for 3 weeks, and treated at the 4−5 leaf stage.

Seed for the *Arabidopsis thaliana* (L.) Heyn. *npr1-2* and *ein2-1* mutants was obtained from the Arabidopsis Biological Resource Center (The Ohio State University, Columbus, OH). The corresponding wild type (Columbia) seed was obtained from Lehle Seed (Round Rock, TX). *Arabidopsis* plants were grown in Pro-Mix PGX under cool white fluorescent lamps at 150 $\mu$mol·m$^{-2}$·s$^{-1}$ (16:8 h light/dark cycle; 25 °C) and treated at maturity.

**Herbicide and Combination Treatments.** In all herbicide applications, plants were sprayed with a hand sprayer with a volume sufficient to ensure complete coverage. The pH of spray solutions was unaffected by the addition of NaSA. COC blend was added to all spray solutions at rates of 0.1% (v/v) for *Arabidopsis* and 0.25% (v/v) for tobacco. All foliar treatments containing both herbicide and salicylate or SAR inducer were mixed and applied in a single spray solution as soon as possible after mixing. After spraying, plants were returned to their previous growth conditions, light banks (*Arabidopsis*) or greenhouse (tobacco), and arranged in a randomized complete block. Herbicidal activity was determined by visual inspection and expressed as percent leaf area damaged. Protection was defined as a decrease in percent leaf area damaged (herbicidal activity) as compared to the herbicide alone. Protection data were normalized for the damage observed on paraquat-treated plants in the same trial.

**Statistical Analysis.** Data were subjected to analysis of variance, and means were separated by Duncan's new multiple-range test using PlotIT software (Scientific Programming Enterprises, Haslett, MI).

## RESULTS

Leaves of tobacco plants sprayed with paraquat (780 $\mu$M) desiccated within 3 h after application and quickly became necrotic. The addition of NaSA (10 mM) to the foliar spray solution (simultaneous application) significantly decreased paraquat damage (**Figure 1A**). Paraquat damage increased with concentration (29, 145, 290, or 780 $\mu$M) and was inhibited by NaSA (10 mM) at all paraquat concentrations (**Figure 1B**).

Simultaneous foliar application of NaSA with paraquat provided greater protection than application of NaSA either 1, 2, or 4 days prior to paraquat application (**Figure 1C**). The effectiveness of simultaneous NaSA treatment with paraquat suggests that changes in gene expression are not necessary for protection of tobacco from paraquat. NaSA applied to the soil 1 day prior to foliar application of paraquat reduced herbicidal activity (percent herbicide-induced leaf damage) by 15, 45, and 70% for 1, 10, and 50 mM NaSA, respectively, on tobacco (**Figure 1D**). Moreover, NaSA itself caused only minor phytotoxicity under the conditions used in these studies and only at 50 mM, thus suggesting that paraquat protection is not due to salicylate-induced cell death. Because protection from paraquat does not require either NaSA pretreatment (**Figure 1C**) or application to the same part of the plant as NaSA (**Figure 1D**), the effect of NaSA is assumed to be on the plant rather than on the chemical stability or cuticular penetration of paraquat.

Protection from paraquat damage was conferred by other compounds, including commercially available SAR inducers and salicylate derivatives (**Table 1**). The SAR inducers acibenzolar-*S*-methyl, harpin, and probenazole protected against paraquat. Most of the chloro-, fluoro-, methyl-, and methoxysalicylates tested provided protection from paraquat comparable to that of NaSA. The most effective salicylates were 5-chlorosalicylate, 3-chlorosalicylate, 5-methoxysalicylate, and 3-fluorosalicylate (70, 26, 23, and 13% greater protection than NaSA, respectively), and the least effective was 3-methylsalicylate (~80% less protection than NaSA; **Table 1**). The halogenated salicylates that were the most effective at protection from paraquat are also as active as salicylate at induction of the defense-related protein PR-1a (*12*). Benzoic acid, which did not protect plants from paraquat (**Table 1**), was also not active as an inducer of PR-1a

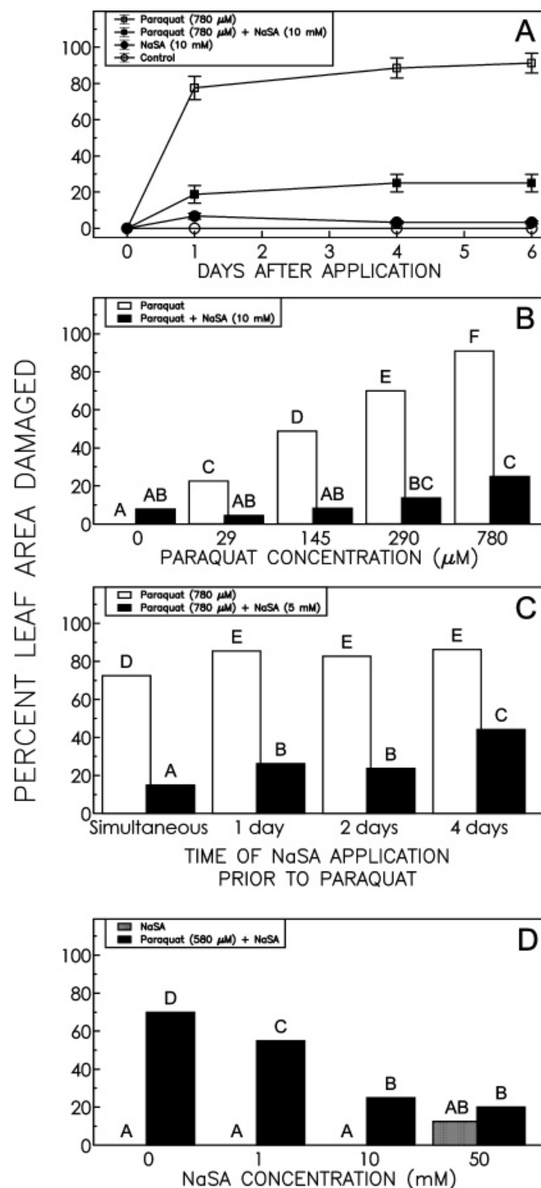


**Figure 1.** Sodium salicylate (NaSA) protection of tobacco from paraquat damage: (A) effect of NaSA (10 mM) on the time course of paraquat (780 $\mu$M) activity (bar represents ± SE); (**B**) effect of paraquat concentration (29, 145, 290, or 780 $\mu$M) on NaSA (10 mM) reduction of herbicidal activity; (**C**) effect of time of NaSA (5 mM) application on paraquat (780 $\mu$M) activity (NaSA was applied with or 1, 2, or 4 days prior to application of paraquat); (**D**) effect of soil application of NaSA (1, 10, and 50 mM) on paraquat (580 $\mu$M) activity. NaSA was foliar-applied in **A**–**C**. Paraquat was foliar-applied in all studies. Crop oil concentrate (0.25% v/v) was used in all foliar treatments. Leaf area damage was assessed 6 days after treatment except in the time course. Means were separated by Duncan's new multiple-range test ($p = 0.05$; $n = 6$ plants per treatment). Means with the same letter are not statistically different.

**Table 1.** Protection of Xanthi-nc Tobacco from Paraquat Damage with Selected Salicylates, Systemic Acquired Resistance Inducers, and Other Compounds

| compound[a] | protection from paraquat (% reduction in damage compared to paraquat alone)[b] | relative protection from paraquat (compared with salicylate)[c] |
|---|---|---|
| control (paraquat alone) | 0 | 0.00 |
| sodium salicylate (NaSA) | 46 | 1.00 |
| 3-chlorosalicylate | 58 | 1.26 |
| 3-fluorosalicylate | 52 | 1.13 |
| 3-methylsalicylate | 9 | 0.20 |
| 4-chlorosalicylate | 47 | 1.02 |
| 4-fluorosalicylate | 45 | 0.98 |
| 4-methoxysalicylate | 15 | 0.33 |
| 4-methylsalicylate | 30 | 0.65 |
| 5-chlorosalicylate | 78 | 1.70 |
| 5-fluorosalicylate | 45 | 0.98 |
| 5-methoxysalicylate | 57 | 1.23 |
| 5-methylsalicylate | 42 | 0.91 |
| 6-fluorosalicylate | 47 | 1.02 |
| 6-methoxysalicylate | 43 | 0.93 |
| 6-methylsalicylate | 31 | 0.67 |
| 3,5-dichorosalicylate | 31 | 0.67 |
| 3,5-difluorosalicylate | 28 | 0.61 |
| benzoic acid | 0 | 0.00 |
| thiosalicylic acid | 10 | 0.22 |
| benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester (acibenzolar-*S*-methyl) | 40 | 0.87 |
| harpin | 31 | 0.67 |
| 3-(2-propenyloxy)-1,2-benz-isothiazole-1,1-dioxide (probenazole) | 30 | 0.65 |
| aminoethoxyvinylglycine (AVG) | 1 | 0.02 |
| salicylhydroxamic acid (SHAM) | 32 | 0.70 |

[a] Paraquat (580 $\mu$M) and test compounds (2 mM) were simultaneously foliarly applied to tobacco. Probenazole and SHAM were applied at 5 mM, harpin was foliarly applied at 11.2 g of Messenger/L, and AVG was applied at 0.5 mM. [b] Protection is the percent reduction in the percent leaf area damaged compared to paraquat alone. Value is the mean of protection of at least two trials with $n = 6$ replicate plants per trial. [c] Relative activity is leaf area damage for compound plus paraquat divided by damage for NaSA plus paraquat.

(*12*). These results suggest a link between SAR and protection from paraquat.

To determine the role of SAR in reducing paraquat activity, we tested whether NaSA protected the *Arabidopsis* mutant *npr1-2* from paraquat. Plants with mutations in NPR1 are more susceptible to pathogen attack and are less able to express defense genes in response to SA (*14*). Simultaneous application of NaSA with paraquat protected *npr1-2* plants from herbicidal damage to an extent similar to the protection observed on NPR1 (Columbia wild type) plants (**Figure 2A**). These results argue against SAR involvement in the NaSA protection of plants from paraquat injury.

The role of the plant hormone ethylene in NaSA protection from paraquat was examined using either the *ein2-1* mutant or AVG, an inhibitor of ethylene biosynthesis. Plants with mutations in EIN2 are unable to perceive ethylene due to a lesion in the signal transducer (*15*). Simultaneous NaSA application protected the *ein2-1* mutant from paraquat to the same extent as for plants with wild-type EIN2 (**Figure 2B**). Furthermore, AVG did not protect tobacco from paraquat (**Table 1**). These two experiments indicate that neither ethylene perception nor production is involved in NaSA protection of plants from paraquat.

Salicylhydroxamic acid (SHAM), an inhibitor of alternative respiration and salicylate structural analogue, protected tobacco from paraquat (**Table 1**). However, the protection observed from the combination of SHAM (2 mM), NaSA (2 mM), and paraquat (580 $\mu$M) was the same as for the combination of NaSA and paraquat. This experiment demonstrates that SHAM does not affect NaSA protection from paraquat and indicates that alternative respiration is not involved in NaSA protection of plants from paraquat.

## DISCUSSION

SA is an important inducer of the plant defense response. Since 1970, when SA application was shown to induce the synthesis of resistance-associated proteins (cited in ref *16*), SA has been used in the analysis of plant response to pathogens. The subsequent discovery of its role as a signal in the induction of SAR further detailed the complexity of SA action. The absolute requirement for SA in the induction of SAR was demonstrated by the use of the *nahG* transgene, which rapidly metabolizes SA to catechol, thus preventing SA accumulation and blocking the establishment of SAR (*17*). Although the SAR pathway is not the only plant disease resistance pathway, all commercial SAR inducers to date either mimic SA (e.g., acibenzolar-S-methyl) or induce the SA-dependent SAR pathway (*18*).

The role of SA in relation to oxidative stress is less clear. In many cases, SA has been shown to increase oxidative stress tolerance. Pretreatment with SA decreased the oxidative damage and increased the survival of *Arabidopsis* following heat stress, whereas *nahG* transgenics showed increased susceptibility to heat (*19*). SA has been shown to increase stress tolerance in bean and tomato (*20*), chilling tolerance in maize (*21*), and

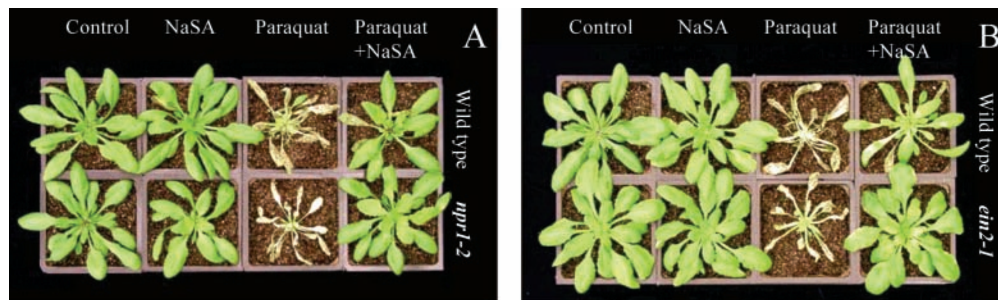


**Figure 2.** Simultaneous application of sodium salicylate (NaSA; 2.5 mM) protects *Arabidopsis* from paraquat (80 $\mu$M) damage: (**A**) protection from paraquat by NaSA of Columbia (wild type) and *npr1-2*, mutant compromised in systemic acquired resistance (SAR); (**B**) protection from paraquat by NaSA of Columbia (wild type) and *ein2-1*, mutant insensitive to ethylene. Controls were sprayed with water plus adjuvant. Plants were photographed 4 days after foliar application.

thermal tolerance in mustard (*22*). In contrast, some studies with *nahG* transgenics show that SA increases the sensitivity of plants to free radical generators including paraquat, possibly through a feedback mechanism increasing the response to ROS (*23*, *24*).

We examined three processes (SAR, ethylene, and alternative respiration) that are regulated by SA in an attempt to determine the mode of action by which salicylate protects plants from paraquat. (1) SA induces SAR. Nevertheless, the *Arabidopsis* mutant *npr1-2*, which is compromised in SAR, was protected from paraquat by NaSA (**Figure 2A**). Thus, NaSA protection of plants from paraquat is independent of NPR1-dependent SAR. The *Arabidopsis* mutant *ein2-1*, which is ethylene-insensitive and compromised in the induction of the induced systemic resistance (ISR) pathway (*25*), was also protected from paraquat by NaSA (**Figure 2B**). Thus, the NaSA protection of SAR- and ISR-compromised mutants from paraquat damage argues against a disease resistance mechanism. (2) SA inhibits ethylene synthesis (*26*). NaSA protected the ethylene-insensitive *ein2-1* mutant from paraquat (**Figure 2B**). Furthermore, the ethylene biosynthesis inhibitor, AVG, neither protected plants from paraquat (**Table 1**) nor inhibited NaSA protection (data not shown). Interestingly, ethylene itself provided weak protection from paraquat that is independent of NaSA protection (*27*). These data illustrate the independence of NaSA protection from paraquat from ethylene inhibition. (3) SA stimulates alternative respiration (*28*). Although the alternative respiration inhibitor SHAM reduced paraquat activity (**Table 1**), it did not block or synergize NaSA protection from paraquat. Thus, NaSA protection from paraquat is independent of SAR induction, ethylene inhibition, and alternative respiration stimulation.

We have shown that simultaneous application of NaSA with paraquat greatly reduced free radical herbicide damage. In all previous studies on protection from paraquat damage by SA, plants were pretreated prior to paraquat application. Although our work demonstrates that NaSA was effective in a pretreatment (**Figure 1C**), it was more effective as a simultaneous treatment with paraquat in foliar applications. Strobel and Kuc (*9*), Ananieva (*5*, *10*), and Kim (*11*) used significant time intervals ($\geq$24 h) between SA treatment and challenge with paraquat. All of these SA pretreatments increased antioxidative defenses and may function through SA regulation of the cellular redox state (*23*). In fact, it is possible that there may be two independent mechanisms of protection from paraquat: one that protects with simultaneous treatment and the other that protects after pretreatment with NaSA.

Although SAR, ethylene, and alternative respiration are not apparently involved with NaSA protection from paraquat, there are several mechanisms that may be operative. First, SA may interfere with paraquat uptake or translocation in the plant. In comparisons of paraquat-resistant and -susceptible biotypes of weeds, only translocation in the leaf is more limited in resistant weeds than in susceptible weeds (*29*), although uptake of paraquat was similar between biotypes. SA may inhibit uptake of xenobiotics, either through an effect on pH or by inhibiting uptake directly (*30*). Although the pH of the spray solutions was unchanged by the addition of NaSA, acids such as SA may interfere with transport processes by depolarizing the plasma membrane (*31*). Second, SA may act directly as a scavenger of ROS generated by PQ. This mode of action has been suggested for chlorogenic acid, which may protect rats from paraquat by acting as a superoxide acceptor (*32*). Third, exogenous SA may lead to greater maintenance of cellular redox state in response to paraquat. Studies have shown that SA is necessary to maintain and increase glutathione reduction in response to ROS (*23*). Therefore, exogenous salicylate may induce cellular redox changes, resulting in better paraquat protection. Fourth, SA may be inducing other pathways that lead to the protection of plants from paraquat. For example, SA protection of paraquat may function in SAR in an NPR1-independent manner (*33*).

In summary, protection of plants from paraquat through simultaneous application of NaSA, other salicylates, or inducers of plant disease resistance is a novel means of limiting the herbicidal activity of paraquat. The protection does not require induction of SAR defenses, inhibition of ethylene, or stimulation of alternative respiratory pathway. This suggests that salicylate protection from paraquat is likely through a mode of action not yet determined.

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## LITERATURE CITED

(1) Raskin, I.; Turner, I.; Melander, W. Regulation of heat production in the influorescences of an *Arum* lily by endogenous salicylic acid. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2214−2218.

(2) Heil, M.; Bostock, R. Induced Systemic Resistance (ISR) Against Pathogens in the Context of Induced Plant Defences. *Ann. Bot.* **2002**, *89*, 503−512.

(3) Dat, J.; Lopez-Delgado, H.; Foyer, C.; Scott, I. Parallel changes in $H_2O_2$ and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. *Plant Physiol.* **1998**, *116*, 1351−1357.

(4) Janda, T.; Szalai, G.; Tari, I.; Paldi, E. Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays*) plants. *Planta* **1999**, *208*, 175−180.

(5) Ananieva, E.; Christov, K.; Popova, L. Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. *J. Plant Physiol*. **2004**, *161*, 319−328.

(6) Yalpani, N.; Enyedi, A.; Leon, J.; Raskin, I. Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance in tobacco. *Planta* **1994**, *193*, 372−376.

(7) Anonomyous. Paraquat. In *Herbicide Handbook*, 7th ed.; Ahrens, W. H., Ed.; Weed Science Society of America: Champaign, IL, 1994; pp 226−228.

(8) Fujibe, T.; Saji, H.; Arakawa, K.; Yabe, N.; Takeuchi, Y.; Yamamoto, K. A methyl viologen-resistant mutant of Arabidopsis, which is allelic to ozone-sensitive *rcd1*, is tolerant to supplemental ultraviolet-B irradiation. *Plant Physiol.* **2004**, *134,* 275−285.

(9) Strobel, N.; Kuc, J. Chemical and biological inducers of systemic resistance to pathogens protect cucumber and tobacco plants from damage caused by paraquat and cupric chloride. *Phytopathology* **1995**, *85,* 1306−1310.

(10) Ananieva, E.; Alexieva, V.; Popova, L. Treatment with salicylic acid decreases the effects of paraquat on photosynthesis. *J. Plant Physiol.* **2002**, *159*, 685−693.

(11) Kim, H.-S.; Lim, C.-J.; Han, T.-J.; Kim, J.-C.; Jin, C.-D. Effects of salicylic acid on paraquat tolerance in *Arabidopsis thaliana* plants. *J. Plant Biol.* **2003**, *46*, 31−37.

(12) Silverman, F. P.; Petracek, P. D.; Heiman, D. F.; Fledderman, C. M.; Warrior, P. Salicylate activity. 3. Structure relationship to systemic acquired resistance. *J. Agric. Food Chem.* **2005**, *53*, 9775−9780.

(13) Silverman, P.; Nuckles, E.; Ye, X.; Kuc, J.; Raskin, I. Salicylic acid, ethylene, and pathogen resistance in tobacco. *Mol. Plant−Microbe Interact.* **1993**, *6*, 775−781.

(14) Cao, H.; Glazebrook, J.; Clarke, J.; Volko, S.; Dong, X. The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell* **1997**, *88*, 57−63.

(15) Alonso, J. M.; Hirayama, T.; Roman, G.; Nourizadeh, S.; Ecker, J. R. EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. *Science* **1999**, *284*, 2148−2152.
(16) Raskin, I. Role of salicylic acid in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1992**, *43*, 439−463.
(17) Gaffney, T.; Friedrich, L.; Vernooij, B.; Negrotto, D.; Nye, G.; Uknes, S.; Ward, E.; Kessmann, H.; Ryals, J. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* **1993**, *261*, 754−756.
(18) Gozzo, F. Systemic acquired resistance in crop protection: From nature to a chemical approach. *J. Agric. Food Chem.* **2003**, *51*, 4487−4503.
(19) Larkindale, J.; Knight, M. Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid. *Plant Physiol.* **2002**, *128*, 682−695.
(20) Senaratna, T.; Touchell, D.; Bunn, E.; Dixon, K. Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regul.* **2000**, *30*, 157−161.
(21) Kang, H.-M.; Saltveit, M. Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. *Physiol. Plant.* **2002**, *115*, 571−576.
(22) Dat, J.; Foyer, C.; Scott, I. Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. *Plant Physiol.* **1998**, *118*, 1455−1461.
(23) Rao, M.; Davis, R. Ozone-induced cell death occurs via two distinct mechanisms in Arabidopsis: the role of salicylic acid. *Plant J.* **1999**, *17*, 603−614.
(24) Borsani, O.; Valpuesta, V.; Botella, M. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. *Plant Physiol.* **2001**, *126*, 1024−1030.
(25) Thomma, B. P. H. J.; Eggermont, K.; Tierens, K. F. M.-J.; Broekaert, W. F. Requirement of functional *Ethylene-Insensitive 2* gene for efficient resistance of Arabidopsis to infection by *Botrytis cinerea*. *Plant Physiol.* **1999**, *121*, 1093−1101.
(26) Leslie, C.; Romani, R. Inhibition of ethylene biosynthesis by salicylic acid. *Plant Physiol.* **1988**, *88*, 833−837.
(27) Silverman, F. P.; Petracek, P. D.; Ju, Z.; Heiman, D. F.; Warrior, P. Enhanced herbicide composition. U.S. Patent 6,919,298, 2005.
(28) Rhoads, D. M.; McIntosh, L. Cytochrome and alternative pathway respiration in tobacco. Effects of salicylic acid. *Plant Physiol.* **1993**, *103*, 877−883.
(29) Yu, Q.; Cairns, A.; Powles, S. Paraquat resistance in a population of *Lolium rigidum*. *Funct. Plant Biol.* **2004**, *31*, 247−254.
(30) Harper, J. R.; Balke, N. E. Characterization of the inhibition of $K^+$ absorption in oat roots by salicylic acid. *Plant Physiol.* **1981**, *68*, 1349−1353.
(31) Sterling, T. M. Mechanisms of herbicide absorption across plant membranes and accumulation in plant cells. *Weed Sci.* **1994**, *42*, 263−276.
(32) Tsuchiya, T.; Suzuki, O.; Igarashi, K. Protective effects of chlorogenic acid on paraquat-induced oxidative stress in rats. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 765−768.
(33) Shah, J.; Kachroo, P.; Nandi, A.; Klessig, D. A recessive mutation in the *Arabidopsis SSI2* gene confers SA- and *NPR1*-independent expression of *PR* genes and resistance against bacterial and oomycete pathogens. *Plant J.* **2001**, *25*, 563−574.

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