Plant Physiol. (1995) 108: 633–639

Salicylic Acid in Rice[1]

Biosynthesis, Conjugation, and Possible Role

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Salicylic acid (SA) is a natural inducer of disease resistance in some dicotyledonous plants. Rice seedlings (*Oryza sativa* L.) had the highest levels of SA among all plants tested for SA content (between 0.01 and 37.19 μg/g fresh weight). The second leaf of rice seedlings had slightly lower SA levels than any younger leaves. To investigate the role of SA in rice disease resistance, we examined the levels of SA in rice (cv M-201) after inoculation with bacterial and fungal pathogens. SA levels did not increase after inoculation with either the avirulent pathogen *Pseudomonas syringae* D20 or with the rice pathogens *Magnaporthe grisea*, the causal agent of rice blast, and *Rhizoctonia solani*, the causal agent of sheath blight. However, leaf SA levels in 28 rice varieties showed a correlation with generalized blast resistance, indicating that SA may play a role as a constitutive defense compound. Biosynthesis and metabolism of SA in rice was studied and compared to that of tobacco. Rice shoots converted [^{14}C]cinnamic acid to SA and the lignin precursors *p*-coumaric and ferulic acids, whereas [^{14}C]benzoic acid was readily converted to SA. The data suggest that in rice, as in tobacco, SA is synthesized from cinnamic acid via benzoic acid. In rice shoots, SA is largely present as a free acid; however, exogenously supplied SA was converted to β-*O*-D-glucosylSA by an SA-inducible glucosyltransferase (SA-GTase). A 7-fold induction of SA-GTase activity was observed after 6 h of feeding 1 mM SA. Both rice roots and shoots showed similar patterns of SA-GTase induction by SA, with maximal induction after feeding with 1 mM SA.

The first indication that SA, a common plant phenolic (Malamy and Klessig, 1992; Métraux and Raskin, 1993; Ryals et al., 1994), plays an important regulatory role in plants came from the study on thermogenesis in the inflorescences of *Arum* lilies (Raskin et al., 1987). Subsequently, SA was shown to be a signal in acquired resistance to pathogens in tobacco (Malamy et al., 1990; Gaffney et al., 1993; Vernooij et al., 1994) and cucumber (Métraux et al., 1990). The development of acquired resistance often follows a localized tissue death at the site of pathogen penetration called the HR (Klement, 1982). After the inoculation with the inducing pathogen, SAR to subsequent pathogen attack develops in the pathogen-free tissues (Ross, 1961). SA may be a signal responsible for the induction and maintenance of SAR (Yalpani and Raskin, 1993; Ryals et al., 1994). Increases in SA are required for the induction of tobacco SAR (Gaffney et al., 1993), and SA likely acts via the local and systemic induction of PR proteins, which possess antipathogen activity (Ward et al., 1991).

Systemic increases in the endogenous levels of SA parallel the induction of PR proteins and SAR. Although the levels of total SA in healthy tobacco (*Nicotiana tabacum* L. cv Xanthi-nc) leaves rarely exceed 100 to 200 ng/g fresh weight, they increase to as much as 75 μg/g fresh weight locally and up to 1.5 μg/g fresh weight systemically after the HR induced by TMV (Enyedi et al., 1992).

The pathway of SA biosynthesis was recently elucidated (Yalpani et al., 1993a) in tobacco, where SA is synthesized from CA via BA. The latter reaction is catalyzed by a BA-inducible BA2H, which functions as a Cyt P450 monooxygenase (León et al., 1993). GSA is the major metabolite of exogenous and endogenous SA in tobacco (Enyedi et al., 1992; Malamy et al., 1992), although production of a Glc ester has also been reported (Edwards, 1994). Large quantities of GSA (50 μg/g fresh weight) accumulate in and around the TMV-induced HR lesions in tobacco, whereas little GSA is present in phloem and in systemically protected leaves (Enyedi et al., 1992). The enzyme that catalyzes SA conjugation was characterized in oat roots (Yalpani et al., 1992) and tobacco leaves (Enyedi and Raskin, 1993). This enzyme, SA-GTase, is strongly and specifically induced by SA, which accumulates in the vicinity of the HR lesions.

The phenomenon of acquired resistance is not limited to dicotyledonous plants. Both wheat and barley developed resistance to powdery mildew (*Erysiphe graminis* f.sp.

[1] This study was supported by the Rockefeller Foundation and the New Jersey Commission for Science and Technology. Additional support was provided by the U.S. Department of Agriculture (Competitive Research Grants Office) and by the Division of Energy Biosciences of the U.S. Department of Energy.

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Abbreviations: BA, benzoic acid; BA2H, benzoic acid 2-hydroxylase; CA, cinnamic acid; cfu, colony-forming units; FA, ferulic acid; GSA, β-*O*-D-glucosylSA; HR, hypersensitive response; *o*-CO, *o*-coumaric acid; *p*-CO, *p*-coumaric acid; PR, pathogenesis-related; RT, retention time; SA, salicylic acid; SA-GTase, SA-glucosyltransferase; SAR, systemic acquired resistance; TMV, tobacco mosaic virus.

tritici) after inoculation with incompatible strains of powdery mildew (Ouchi et al., 1976; Schweizer et al., 1989). One of the best demonstrations of SAR in cereals was obtained in rice (*Oryza sativa* L.). Upper leaves of rice showed greater resistance to *Magnaporthe grisea*, the causal agent of rice blast, after inoculation of lower leaves with a wheat-infecting *Pseudomonas syringae* (Smith and Métraux, 1991). The importance of the PR proteins in rice SAR is not as clear as in tobacco and cucumber. Increases in both chitinase and glucanase activities were detected only in *P. syringae*-inoculated leaves and not systemically. However, the transcription of a chitinase (Nishizawa and Hibi, 1991) and a glucanase (Simmons et al., 1992) was induced in rice by pathogen-derived elicitors and SA. Additionally, application of 2,6-dichloro-isonicotinic acid, a possible SA analog that activates natural plant defenses, induced resistance in rice to *M. grisea* and *Xanthamonas oryzae* (Métraux et al., 1991).

Rice has the highest endogenous SA levels among all plants surveyed (Raskin et al., 1990). SA levels reported for healthy rice leaves were 37.19 μg/g fresh weight, which is 2 orders of magnitude higher than SA levels in healthy tobacco (cv Xanthi-nc). This observation, along with the demonstration of SAR in rice and the induction of rice PR proteins by SA, led us to investigate the biosynthesis and metabolism of SA in rice and its possible involvement in SAR.

MATERIALS AND METHODS

Plant Culture

All experiments were carried out with the rice (*Oryza sativa* L.) cultivar M-201, with the exception of the variety study. Seeds were allowed to imbibe on water-saturated germination paper (Anchor Paper, Saint Paul, MN) for 2 d at 25°C before sowing. Seeds were sown in a 1:1 (v/v) peat moss:washed fritted clay mixture and were fertilized twice a week with 0.25 g/L $CaHPO_4$, 0.375 g/L KNO_3, and 1.05 g/L Miracid (Stern's Miracle-Gro Products, Port Washington, NY). Plants were grown in an environmentally controlled growth chamber at 25°C, 75% RH, with a 16-h photoperiod (500 μmol m^{-2} s^{-1}) that was provided by a combination of incandescent and cool-white fluorescent lights. Except for the developmental and root studies, all experiments were performed on rice seedlings at the three-leaf stage, 12 to 14 d postplanting.

Rice roots were grown in an aeroponic system adapted from Yalpani et al. (1992). Rice seeds were pre-sterilized in 5% household bleach and germinated in the dark through cheesecloth suspended over an aerated beaker containing 10 mM $CaSO_4$. The roots of 12-d-old seedlings were used for SA-GTase induction studies.

SA Biosynthesis and Conjugation

For feeding experiments, rice seedlings excised 5 mm above the soil surface were recut under 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA. Seedlings were fed with 50 μL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 μCi of either [7-^{14}C]BA (40 mCi $mmol^{-1}$, ICN, Costa Mesa, CA) or [3-^{14}C]CA (53.8 mCi $mmol^{-1}$, Isotopchim, Ganagobie-Peyruis, France). The label was supplied for 30 min and chased with buffer. Harvested tissue was frozen in liquid N_2 and stored at −20°C until analyzed.

For SA turnover studies, the second leaf of the seedling was syringe-infiltrated with buffer (5 mM KPO_4, pH 5.8) containing 500 μM [7-^{14}C]SA (56.0 mCi $mmol^{-1}$, NEN) and leaves were excised at the times indicated. Turnover of SA was determined by measuring the decrease in labeled SA after β-glucosidase (EC 3.2.1.21; almond, Sigma) digestion (Enyedi et al., 1992). β-Glucosidase digestion was used to determine the levels of total tissue SA, a sum of free SA and GSA. For SA-GTase studies, excised rice shoots (see above) were fed different concentrations of SA for the times indicated as previously described (Yalpani et al., 1992). Roots (approximately 10 cm in length) used in SA-GTase induction experiments were excised, rinsed with water, and submerged in a continuously aerated medium (30 mL/g fresh weight of tissue) containing 0.5 mM KCl, 0.25 mM $CaSO_4$, 25 mM Mes/Tris, pH 6.5, that was supplemented with different concentrations of SA. The roots were harvested after 12 h of incubation in darkness.

Extraction and Quantitation of SA, GSA, and Biosynthetic Precursors

Tissue samples were analyzed for SA as previously described (Yalpani et al., 1991; Enyedi et al., 1992). Conjugates were quantified after either chemical (base followed by acid) hydrolysis or enzymatic hydrolysis with β-glucosidase as previously described (Enyedi et al., 1992). For the experiments in Figure 5, dried methanolic extracts were rehydrated in distilled water and filtered (0.2 μm) prior to HPLC analysis.

SA and other plant phenolics were separated and quantified using an HPLC protocol adapted from Yalpani et al. (1993a). The RTs for plant phenolics were: GSA, 2.30 min; SA, 4.5 min; *p*-CO, 6.5 min; BA, 6.8 min; FA, 7.5 min, *o*-CO, 11.8 min; and CA, 15.2 min. SA was verified by mass spectral analysis, and other phenolics were confirmed by co-elution with authentic standards. All data are corrected for recovery using internally spiked samples.

SA-GTase and BA2H Measurements

SA-GTase activity was determined as previously described (Yalpani et al., 1992) with the exception that Dowex-1 was omitted. A unit of SA-GTase activity was defined as the amount of enzyme activity required to convert 1 μmol of SA to GSA min^{-1} under the assay conditions. BA2H activity was determined according to León et al. (1993).

Pathogens and Plant Inoculations

Pseudomonas syringae pv *syringae* D20, a gift from R. Hammerschmidt (Michigan State University, East Lansing, MI) was maintained on *Pseudomonas* F agar (Difco, Detroit, MI) and was prepared for plant inoculations as previously described for *P. syringae* pv *tomato* (Silverman et al.,

1993). The concentration of inoculum averaged 1×10^9 cfu/mL. The second leaf of the rice seedling was inoculated at five separate sites with the bacterial suspension as previously described (Smith and Métraux, 1991).

Magnaporthe grisea (Hebert) Barr race IG-1, a gift from V. Cotter (American Cyanamid Co., Princeton, NJ), was maintained on fresh oatmeal agar at 25°C. Spores were harvested 7 to 9 d after plating. The spore-harvesting solution (Smith and Métraux, 1991) was modified to contain 0.05% (v/v) Tween 20 instead of sodium oleate. Spore concentration was adjusted to 2.5×10^5 spores/mL. Plants were sprayed to run off with inoculum and were maintained at 100% RH for the duration of the experiment.

Generalized resistance was determined in field nurseries and/or greenhouses after inoculation with three defined races of *M. grisea*. Resistance to each race was scored individually for each cultivar and averaged to obtain a generalized resistance rating. Field nurseries were also in contact with naturally occurring inocula of *M. grisea*.

Experiments using *Rhizoctonia solani* AG-1 IA, the causal agent of sheath blight, were conducted at American Cyanamid Co. For inoculum preparation, plugs were cut from colonies grown on V-8 juice agar and incubated with gentle agitation (100 rpm) in potato dextrose broth at 24°C for 5 d, and the resulting mycelium was fragmented at low speed in a Waring blender for 20 s. Thereafter, 16 mL of inoculum were applied to the soil surface of 4-inch pots containing 20 rice seedlings. After inoculation, rice plants were maintained at 100% RH for the times indicated. In control inoculations, an equal volume of water was applied to the soil surface. In mock-inoculations with *M. grisea* the plants were sprayed with the spore-harvesting solution only. Controls for *P. syringae* inoculation were infiltrated with sterile water.

RESULTS

Developmental Changes in SA Levels of Rice Seedlings

The levels of free SA determined in the leaves of M-201 rice seedlings varied from 7.4 to 15.3 μg/g fresh weight of free SA (Table I). The first leaf was not analyzed because of its small size. The second leaf always contained lower levels of SA than the leaves above. Acid-base hydrolysis of leaf extracts did not produce significant changes in SA content (data not shown). Therefore, all data reflect the levels of free SA, unless specifically indicated.

SA Levels in Pathogen-Inoculated Rice

The interaction between rice and the avirulent pathogen *P. syringae* D20 resulted in an HR and the induction of PR proteins (Smith and Métraux, 1991). However, inoculation with *P. syringae* D20 did not cause significant local or systemic changes in the SA levels in the rice leaves for the duration of the experiment (Fig. 1A). SA levels in the areas of *P. syringae*-induced lesions also did not change significantly (data not shown). An HR response developed within 48 h at the sites of pathogen inoculation. Moreover, the levels of SA in pathogen-free leaves (leaf 3) were almost unchanged throughout the experiments, except for the 168-h point. The small increase in SA detected at this time was not duplicated in the repeat experiment and was attributed to variability associated with development of the third leaf, which was expanding throughout the experiment. *M. grisea* inoculation, like inoculation with *P. syringae*, did not alter SA levels in rice (Fig. 1B). *M. grisea*-induced lesions became visible 48 h after inoculation. The spindle-shaped lesions continued to expand throughout the course of the experiment, averaging 1.1 mm in length 7 d postinoculation.

Table II illustrates the levels of SA in the different tissues of M-201 rice after infection with the virulent fungal pathogen *R. solani*, which produced extensive water-soaked lesions of the leaf sheath. Within 48 h after inoculation, the *R. solani* lesions extended up the leaf sheath to the first leaf blade and up to the second leaf blade by 120 h. SA levels decreased in the leaf sheath of *R. solani*-inoculated plants. This decrease was attributed to a massive tissue disorganization and death associated with infection.

Although the above results do not support a signaling role for SA in acquired disease resistance in rice, the possibility remains that unusually high levels of SA in rice may function as a constitutive defense against *M. grisea*. To test this possibility, we determined the correlation between generalized blast resistance and SA content of 28 modern rice varieties (Fig. 2). The rice varieties tested included American japonicas (e.g. M-201 and A-301) and indicas (e.g. Katy and Texmont) as well as varieties from various international breeding programs. Of all the tested varieties, Jasmine 85 had the highest SA level (30.4 μg/g fresh weight), whereas A-301 had only 7.6 μg/g fresh weight of SA, a 3.9-fold difference. Resistance was determined in field nurseries and/or greenhouses after inoculation with three defined races of *M. grisea*. Field nurseries were also in

Table I. *Levels of free SA (μg/g fresh weight) in M-201 rice seedlings*

Rice seedlings were grown for 35 d, and leaves were sampled weekly for SA. The experiment was repeated twice with similar results. The results are the mean of triplicates ± SE.

Days after Sowing	SA at Leaf Position				
	2	3	4	5	6
	μg/g fresh weight				
7	9.28 ± 1.23				
14	9.40 ± 0.69	11.05 ± 0.33			
21	7.37 ± 1.11	12.66 ± 1.13	14.94 ± 1.95		
28	8.75 ± 0.59	13.05 ± 1.65	14.16 ± 2.02	12.86 ± 0.52	
35	8.32 ± 1.29	15.29 ± 2.19	12.66 ± 0.82	11.88 ± 1.41	11.79 ± 0.24

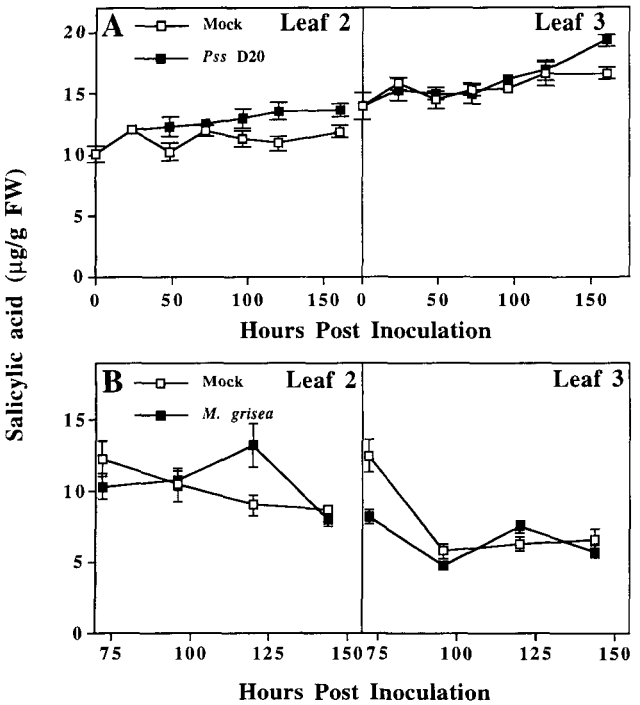

Figure 1. Levels of free SA in rice seedlings after inoculation with virulent or avirulent pathogens. A, SA levels in rice infiltrated in the second leaf with *P. syringae* D20 (1×10^9 cfu/mL). B, SA levels in rice inoculated with *M. grisea* (2.5×10^5 spores/mL). Each experiment was repeated twice with similar results. Each SA value is the mean ± SE of triplicate samples.

contact with naturally occurring races of *M. grisea*. The relation between generalized resistance to blast and seedling SA was significant ($r^2 = 0.477$, P = 0.00005) using Pearson's correlation (Steel and Torrie, 1980).

SA Biosynthesis in Rice

To elucidate the pathway of SA biosynthesis in rice, seedlings were fed likely SA precursors. Pulse feeding with [^{14}C]CA (53.8 mCi mmol^{-1}) gave rise to labeled *p*-CO, FA, and SA (Fig. 3A). The highest incorporation of label into these compounds occurred 1 h after CA feeding. Between 1 and 6 h after pulse feeding, the specific activity of *p*-CO, FA, and CA declined 6.6-fold, 3.6-fold, and 15.8-fold, respectively. No radioactivity was eluted at the RT of *o*-CO, indicating that it is not a major metabolite of CA in rice.

[^{14}C]BA (40 mCi mmol^{-1}) fed to excised rice seedlings was rapidly incorporated into SA (Fig. 3B). The relatively low specific activity of the SA after [^{14}C]BA feeding is consistent with the large size of the endogenous SA pool in rice leaves. Feeding with 500 µM BA resulted in 32% increases in SA, whereas feeding with *o*-CO acid resulted in no changes in SA levels (data not shown). In addition, the turnover rate of total SA in rice leaves was only 4% per day, as determined by [^{14}C]SA feeding (data not shown). BA2H activity in healthy 2-week-old rice seedlings was 5-fold higher than that observed in healthy tobacco leaves (8.2 ± 1.2 compared to 1.7 ± 0.3 nmol h^{-1} g^{-1}) (León et al., 1993).

Table II. *Levels of salicylic acid (µg/g fresh weight) in M-201 rice after inoculation with R. solani*

Two-week-old rice seedlings were inoculated with *R. solani*, and the levels of SA were determined at 48 and 120 h postinoculation. Lower sheath designates the portion between the soil surface and the first leaf blade. Upper sheath designates the portion between the first and second leaf blades. Leaf blade designates the blades of the second and third leaves. The experiment was repeated twice with similar results. Each value is the mean ± SE of triplicate samples.

Time after Inoculation	SA		
	Lower sheath	Upper sheath	Leaf blade
	µg/g fresh weight		
R. solani infected			
48 h	5.06 ± 0.51[a]	9.87 ± 0.41	9.69 ± 0.98
120 h	2.17 ± 0.02[a]	3.88 ± 0.55[a]	8.69 ± 0.28
Mock inoculated			
48 h	9.21 ± 0.66	8.79 ± 0.31	9.11 ± 0.33
120 h	9.06 ± 0.44	11.69 ± 0.59	8.71 ± 1.45

[a] Denotes collapsed tissue.

Conjugation of SA by Rice

As already mentioned, rice leaves, in contrast to tobacco (Enyedi et al., 1992), contain little if any of the acid-base hydrolyzable conjugates of SA, such as GSA. To test whether rice still possesses SA-inducible SA-GTase activ-

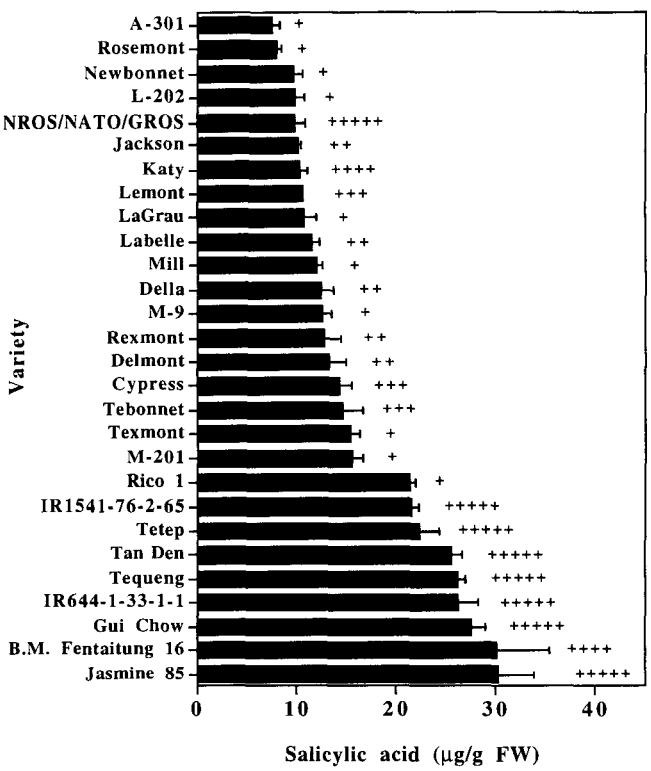

Figure 2. Levels of free SA in 2-week-old rice seedlings of varieties differing in resistance to rice blast. Blast resistance ratings: +, highly susceptible; ++, susceptible; +++, moderately resistant; ++++, resistant; +++++, highly resistant. The experiment was repeated twice with similar results. Each SA value is the mean ± SE of triplicate samples.

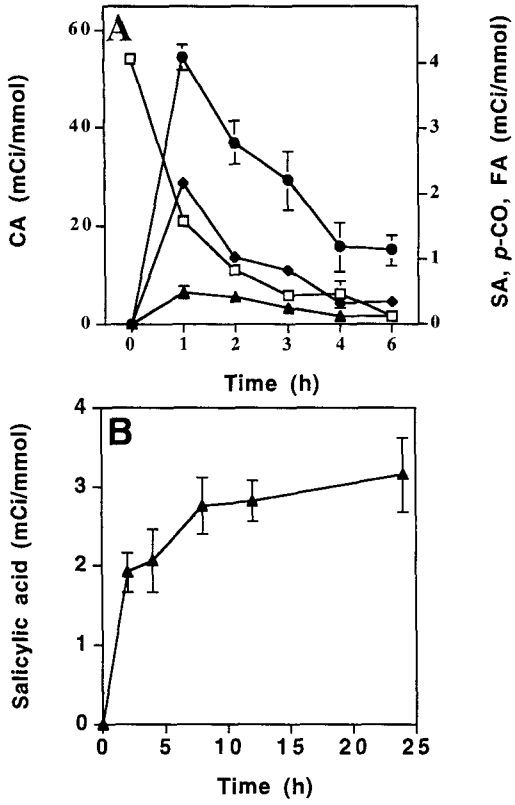

Figure 3. Tracer studies of SA biosynthesis in M-201 rice. A, Specific activities of free CA (□), *p*-CO (♦), FA (●), and SA (▲) after pulse labeling with [^{14}C]CA. B, Specific activity of free SA produced after pulse labeling with [^{14}C]BA. Each experiment was repeated twice with similar results. Each point is the mean ± SE of triplicate samples.

ity, excised seedlings were fed with 1 mM SA. Prior to SA treatment, rice leaves contained significant levels of SA-GTase activity (Fig. 4A). After 6 h of SA feeding, SA-GTase activity was induced 9.4-fold and 13-fold on a per g fresh weight basis or a per mg protein basis, respectively. Upon feeding with 1 mM SA, the levels of free SA increased from 7.2 to 116.2 μg/g fresh weight, whereas the levels of total SA (free SA plus β-glucosidase-digestible conjugate) increased to 187.0 μg/g fresh weight (Fig. 4B). This means that 70.8 μg/g fresh weight of GSA was formed by the end of the experiment in parallel with the increase in SA-GTase activity. The total amount of SA recovered after digestion with β-glucosidase accounted for the SA removed from the feeding solution, suggesting that GSA was the major metabolite of SA in rice (data not shown). The formation of GSA was confirmed by feeding [^{14}C]SA to excised rice shoots (Fig. 5). Two major peaks of radioactivity were observed in rice extracts after SA feeding (Fig. 5C): one with an RT of 2.3 min, which co-eluted with a GSA standard (Fig. 5A), and a second with an RT of 4.5 min, which co-eluted with an SA standard (Fig. 5B). After digestion with β-glucosidase, greater than 90% of the radioactivity in the RT peak at 2.3 min was liberated and appeared in the SA peak (Fig. 5D).

To establish a dose dependence of SA-GTase induction by SA, excised rice seedlings and aeroponically grown roots were fed different SA concentrations for 6 and 12 h, respectively (Fig. 6). The basal levels of SA-GTase activity were doubled in both the shoots and roots by incubations with 50 μM SA. Maximal induction of the SA-GTase was by 1 mM SA, with increases in the shoots and roots of 7.5- and 7.7-fold, respectively. Incubation with SA concentrations exceeding 1 mM led to a decreased induction of SA-GTase activity. No visible symptoms of plant toxicity were observed at the SA concentrations used in these experiments.

DISCUSSION

Our results suggest that the role of SA in rice disease resistance may differ from that observed in tobacco. First, the basal levels of SA in M-201 rice are 50-fold higher than those observed in tobacco (Enyedi et al., 1992). Second, no changes were detected in the SA levels in rice after interactions with either avirulent or virulent pathogens (Fig. 1; Table II). This phenomenon is similar to that documented for the amphidiploid *Nicotiana glutinosa* × *Nicotiana debneyi* hybrid in which constitutively high SA levels were not affected by inoculation with TMV (Yalpani et al., 1993b). Third, in rice endogenously produced SA remains as a free acid for a long time, with little if any GSA or other metabolites formed in either pathogen-free or infected tissues. In contrast, in TMV-inoculated *N. tabacum* cv Xanthi-nc, de novo-synthesized SA is rapidly converted to GSA.

This observation suggests that in rice, unlike tobacco, SA may not play a signaling role in disease resistance. However, it is possible that in rice SA is part of a constitutively expressed pathogen defense system. The correlation be-

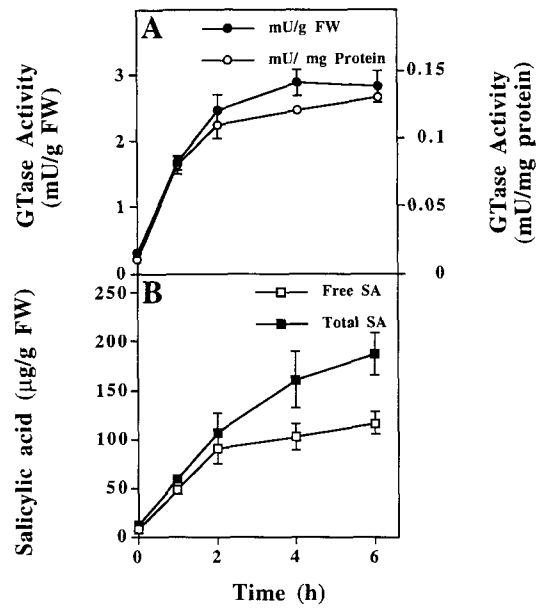

Figure 4. Time course of SA-GTase induction and SA accumulation in rice seedlings. A, Induction of SA-GTase activity in rice seedlings during continuous feeding with 1 mM SA. B, Accumulation of free SA and total SA (free SA plus GSA) during feeding with SA. Each experiment was repeated twice with similar results. Each point is the mean ± SE of triplicate samples. One milliunit (mU) of SA-GTase activity denotes the conversion of 1 nmol of SA to GSA per min at 25°C.

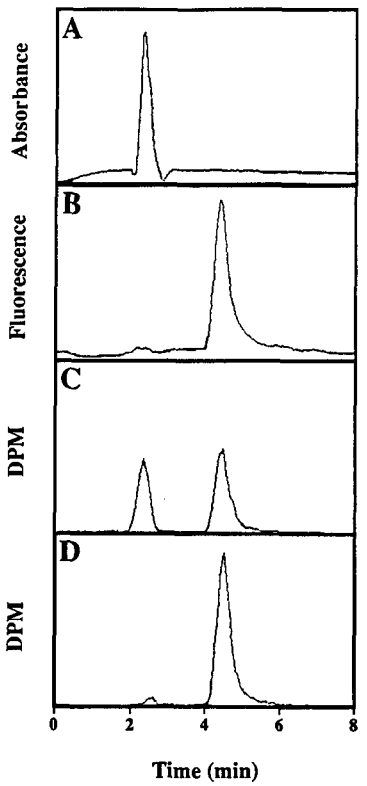

Figure 5. HPLC profiles of GSA standard (UV, A_{274}) (A); SA standard (fluorescence, excitation at 313 nm, emission at 405 nm) (B); extract of rice seedlings fed with 1 mM [^{14}C]SA for 6 h (radioactivity detector) (C); and tissue extract from C digested with β-glucosidase (D).

tween SA levels and generalized blast resistance supports this hypothesis (Fig. 2). However, a thorough genetic analysis of rice populations segregating for blast resistance and SA levels is required to draw more definitive conclusions from these data. In addition, it is not known whether the correlation holds for other races of *M. grisea* and other rice cultivars. Therefore, caution should be used to interpret the observed correlation between SA levels and resistance to blast. Nevertheless, it is tempting to speculate that breeding for pest and pathogen resistance over the last millennium produced rice varieties with constitutively high SA levels. As a result, increased levels of SA in cultivated rice are no longer induced by pathogen infection. However, the levels of SA present in rice do not directly inhibit *M. grisea* germination and growth. Even at concentrations approximately 10-fold higher than endogenous SA levels in rice (1 mM), SA did not inhibit *M. grisea* growth, respiration, or spore germination (data not shown). Higher SA levels (7.8 mM) were shown to inhibit spore germination of both *M. grisea* and *Cochliobolus miyabeanus*, the causal agent of brown spot (Ishii et al., 1962). Therefore, in rice as in tobacco, SA may not act on the pathogen directly. Rather, it may keep endogenous defense systems at a higher state of alert. Because of the correlation of increased SA with blast resistance in rice, it may be possible to use SA levels as a biochemical marker for blast resistance in rice breeding programs.

To understand how SA levels are regulated in rice, we have studied SA biosynthesis and conjugation. Tracer studies indicated that in rice, SA is synthesized from CA, which also serves as a precursor for *p*-CO and FA (Fig. 3A). Formation of ^{14}C-labeled *p*-CO and FA, which are lignin precursors, was earlier reported in rice after feeding of [^{14}C]Phe (El-Basyouni et al., 1964). ^{14}C-labeled *p*-CO and FA were also formed after [^{14}C]CA feeding to elicitor-treated bean cell suspensions (Edwards et al., 1990). The accumulation of large amounts of labeled lignin precursors interfered with the detection of labeled BA. However, feeding [^{14}C]BA to rice shoots caused significant accumulation of labeled SA (Fig. 3B). The results indicate that in rice, as in tobacco, SA is synthesized from CA via BA. In addition, rice leaves divert large amounts of CA to the production of lignin precursors such as *o*-CO and FA.

Studies of SA metabolism in rice were conducted to understand the biochemical mechanisms that allow rice to maintain exceptionally high levels of free SA, without converting it to GSA. Surprisingly, the basal level of SA-GTase activity in rice shoots (0.3 milliunits/g fresh weight) was similar to that in TMV-inoculated tobacco leaves that formed large amounts of GSA (cf. Enyedi and Raskin, 1993, and Fig. 4). Furthermore, exogenously supplied SA induced SA-GTase activity in rice roots and shoots in a dose-dependent manner (Fig. 6). Similar increases of SA-GTase activity after SA feeding were observed in oat roots (Yalpani et al., 1992), tobacco leaves (Enyedi and Raskin, 1993), and cell cultures of *Malotus japonicus* (Tanaka et al.,

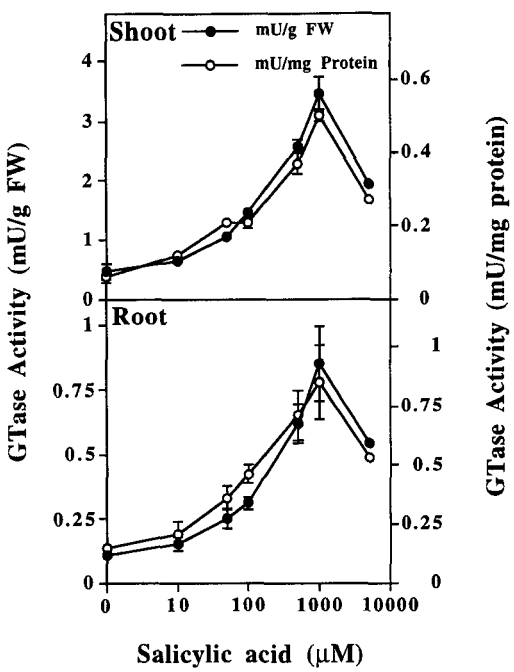

Figure 6. Effect of different levels of SA on SA-GTase activity in M-201 rice. Shoot, SA-GTase activity after 6 h of continuous feeding with SA. Root, SA-GTase activity in 12-d-old rice roots treated with SA. Rice roots were grown aeroponically and treated with SA for 12 h. Each experiment was repeated twice with similar results. Each point is the mean ± SE of triplicate samples.

1990). There are several possible explanations for the ability of rice to accumulate high levels of free SA in spite of the high basal levels of SA-GTase activity. First, free SA in rice may be spatially separated from the SA-GTase, whereas exogenously fed SA comes in contact with SA-GTase. Second, UDP-Glc or other required cofactors may limit the reaction in spite of the overabundance of SA. Finally, several proteins may exhibit SA-GTase activity in vitro. However, the enzyme responsible for the glucosylation of SA in vivo may only be induced by higher than basal levels of SA. Only further research can distinguish between these possibilities. Meanwhile, our results suggest that the role of SA in rice disease resistance differs significantly from that observed in tobacco, whereas SA biosynthesis in both species was similar. The much greater levels of free SA present in rice may be at least partially explained by the slow metabolism of de novo-synthesized SA.

ACKNOWLEDGMENTS

The authors are grateful to Van Cotter, Jose León, and Peter Day for helpful discussions and to Thomas Meagher for assistance with the statistical analysis.

Received October 26, 1994; accepted February 17, 1995.

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