The Plant Cell, Vol. 15, 2647–2653, November 2003, www.plantcell.org © 2003 American Society of Plant Biologists

Knockout Analysis of Arabidopsis Transcription Factors *TGA2*, *TGA5*, and *TGA6* Reveals Their Redundant and Essential Roles in Systemic Acquired Resistance

Yuelin Zhang,[a,1] Mark J. Tessaro,[a] Michael Lassner,[b] and Xin Li[a]

[a] Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada
[b] Maxygen, Inc., Redwood City, California 94063

Arabidopsis nonexpresser of pathogenesis-related (PR) genes (NPR1) is the sole positive regulator that has been shown to be essential for the induction of systemic acquired resistance. In *npr1* mutant plants, salicylic acid (SA)–mediated PR gene expression and pathogen resistance are abolished completely. NPR1 has been shown to interact with three closely related TGA transcription factors—TGA2, TGA5, and TGA6—in yeast two-hybrid assays. To elucidate the biological functions of these three TGA transcription factors, we analyzed single and combined deletion knockout mutants of *TGA2*, *TGA5*, and *TGA6* for SA-induced *PR* gene expression and pathogen resistance. Induction of *PR* gene expression and pathogen resistance by the SA analog 2,6-dichloroisonicotinic acid (INA) was blocked in *tga6-1 tga2-1 tga5-1* but not in *tga6-1* or *tga2-1 tga5-1* plants. Loss of INA-induced resistance to *Peronospora parasitica* Noco2 cosegregated with the *tga6-1* mutation in progeny of multiple lines that were heterozygous for *tga6-1* and homozygous for *tga2-1 tga5-1* and could be complemented by genomic clones of wild-type *TGA2* or *TGA5*, indicating that *TGA2*, *TGA5*, and *TGA6* encode redundant and essential functions in the positive regulation of systemic acquired resistance. In addition, *tga6-1 tga2-1 tga5-1* plants had reduced tolerance to high levels of SA and accumulated higher basal levels of *PR-1* under noninducing conditions, suggesting that these TGA factors also are important for SA tolerance and the negative regulation of the basal expression of *PR-1*.

INTRODUCTION

Systemic acquired resistance (SAR) is a general defense response that develops in the distal, uninfected parts of plants after local infection by an avirulent pathogen (Ryals et al., 1996). SAR is effective against a broad spectrum of microbial pathogens. One important signal molecule in SAR is salicylic acid (SA), which is required and sufficient for the induction of *pathogenesis-related* (*PR*) genes and pathogen resistance during SAR. When attacked by pathogens, plants synthesize and accumulate higher levels of SA in both infected and systemic tissues (Malamy et al., 1990; Métraux et al., 1990; Rasmussen et al., 1991). Application of SA or SA analogs, such as 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole, also induces both *PR* gene expression and pathogen resistance in plants (White, 1979; Métraux et al., 1991; Görlach et al., 1996). In addition, blocking SA accumulation by expressing the bacterial SA-degrading enzyme salicylate hydroxylase prevents the induction of *PR* genes and SAR in transgenic plants (Gaffney et al., 1993). The importance of SA in plant defense also is confirmed by analyzing mutants that are deficient in SA synthesis. In Arabidopsis, mutations in *enhanced disease susceptibility5* (*EDS5*) and *SA induction-deficient2* (*SID2*) block pathogen-induced SA synthesis and render the plants more susceptible to pathogen infection (Rogers and Ausubel, 1997; Nawrath and Métraux, 1999). *EDS5* encodes a member of the MATE transporter family and is likely to be involved in transporting one of the precursors for the biosynthesis of SA (Nawrath et al., 2002). *SID2* encodes an isochorismate synthase, suggesting that SA accumulated during pathogen infection is derived from chorismate (Wildermuth et al., 2001).

Several different genetic screens were conducted to identify regulatory genes downstream of SA. All 12 SA-nonresponsive mutants identified contain mutations in nonexpresser of *PR* genes (*NPR1*) (also known as *NIM1* and *SAI1*) (Cao et al., 1994; Delaney et al., 1995; Glazebrook et al., 1996; Shah et al., 1997). In *npr1* plants, induction of *PR* genes and pathogen resistance by SA are abolished. *NPR1* encodes a protein with no obvious biochemical functions except the presence of two protein–protein interaction domains, a BTB/POZ domain at the N-terminal end and an ankyrin-repeat domain in the central region (Cao et al., 1997; Ryals et al., 1997; Aravind and Koonin, 1999). The presence of protein–protein interaction domains in NPR1 suggests that NPR1 may regulate SA signaling through an association with other proteins.

Several groups have performed yeast two-hybrid screens using NPR1 as bait and found multiple TGA transcription factors that can interact with NPR1 (Zhang et al., 1999; Després et al., 2000; Niggeweg et al., 2000a; Zhou et al., 2000; Chern et al., 2001, Kim and Delaney, 2002). We showed previously that TGA2 (also known as AHBP-1b), TGA5 (also known as OBF5), and TGA6 interact with NPR1, with TGA2 and TGA6 exhibiting strong affinity and TGA5 showing weaker affinity to NPR1 in the yeast two-hybrid assay (Zhang et al., 1999). The interaction between

[1] To whom correspondence should be addressed. E-mail yuelin@interchange.ubc.ca; fax 1-604-822-6089.
Article, publication date, and citation information can be found at www.plantcell.org/cgi/doi/10.1105/tpc.014894.

NPR1 and TGA2 was demonstrated in vivo (Subramaniam et al., 2001; Fan and Dong, 2002), and the involvement of TGA transcription factors in SA signaling is supported further by the presence of a TGA binding site in the *PR-1* promoter that is essential for SA-induced *PR-1* expression (Lebel et al., 1998). However, genetic evidence for the roles of these TGA transcription factors in SAR is lacking. Here, we report that *TGA2*, *TGA5*, and *TGA6* encode redundant functions and are essential for the induction of SAR.

RESULTS

Isolation of *tga6-1* and Construction of the *tga6-1 tga2-1 tga5-1* Triple Mutant

Using primers flanking *TGA6* (*At3g12250*), we screened an Arabidopsis deletion mutant population by PCR and identified a deletion mutant for *TGA6* named *tga6-1*. Sequence analysis of the deletion mutation revealed that a fragment of ~2.7 kb between nucleotides 30,861 and 33,564 on BAC clone F28J15 was deleted (Figure 1). Sequence comparison between *TGA6* cDNA and the genomic sequence revealed that the cDNA of *TGA6* is transcribed from the region between nucleotides 30,249 and 33,651 of F28J15 and that the coding sequence is located between nucleotides 31,032 and 33,264. Thus, the deletion in *tga6-1* occurred within *TGA6* and removed the entire coding region. *TGA2* (*At5g06950*) and *TGA5* (*At5g06960*) are linked directly, and the distance between these two genes is <2 kb. We reported previously the identification of a mutant with both *TGA2* and *TGA5* deleted (Li et al., 2001). This mutant is named *tga2-1 tga5-1*. To obtain a triple mutant for *TGA2*, *TGA5*, and *TGA6*, we crossed *tga6-1* with *tga2-1 tga5-1* and screened the resulting F2 population for homozygous mutants at both loci. Two independent F2 lines that are homozygous at both loci were obtained, and the progeny of these two lines were used for subsequent phenotypic analysis. F2 lines heterozygous at the *tga6-1* locus and homozygous at the *tga2-1 tga5-1* locus also were obtained, and they were used later for cosegregation analysis.

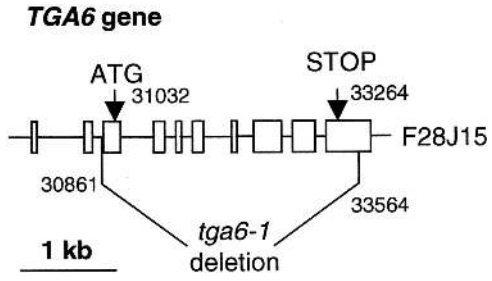

Figure 1. Structure of the Deletion in *tga6-1*.

The fast neutron–induced deletion in *TGA6* spans the region between position 30,861 in the second intron and position 33,564 in the last exon. The numbering is based on the Arabidopsis BAC clone F28J15 that contains *TGA6*.

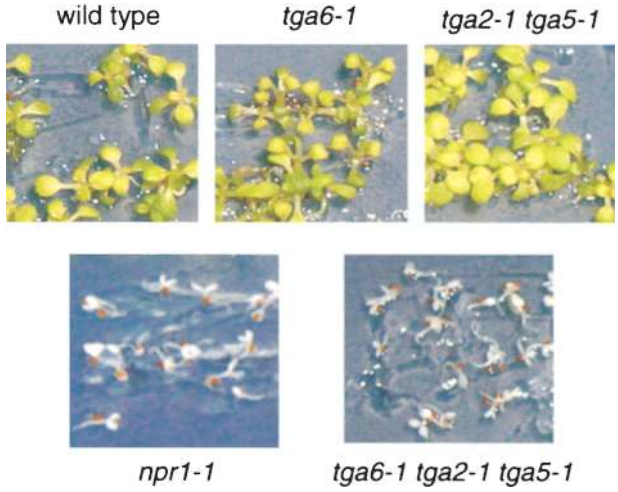

Figure 2. Tolerance of Wild-Type, *npr1-1*, *tga6-1*, *tga2-1 tga5-1*, and *tga6-1 tga2-1 tga5-1* Plants to 0.2 mM SA.

Seeds were plated on MS medium containing 0.2 mM SA, and the photographs were taken 14 days after germination. This experiment was repeated twice with similar results.

The *TGA* Triple Knockout Mutant Is More Sensitive to the Toxicity of SA

Previously, *npr1-1* plants were shown to be more sensitive to high concentrations of SA (Cao et al., 1997). To determine whether the *tga* mutants also have altered responses to SA, the mutant seeds were plated on MS medium (Murashige and Skoog, 1962) containing 0.2 mM SA. Similar to *npr1-1*, *tga6-1 tga2-1 tga5-1* plants were highly sensitive to SA. As shown in Figure 2, the growth of *tga6-1 tga2-1 tga5-1* plants was arrested at the cotyledon stage and the seedlings were bleached, whereas *tga6-1* and *tga2-1 tga5-1* grew like the wild type under the same conditions. Thus, these TGA transcription factors play roles similar to that of NPR1 in the regulation of tolerance to SA.

Induction of *PR-1* by INA Is Blocked in the *TGA* Triple Knockout Mutant

In Arabidopsis, *PR-1* is highly induced during SAR (Uknes et al., 1992). The expression of *PR-1* also can be induced by exogenous application of SA or the SA analog INA. Mutations in *NPR1* completely block the induction of *PR-1* by SA or INA. To determine whether *TGA2*, *TGA5*, and *TGA6* encode functions similar to those of *NPR1*, we analyzed the expression levels of *PR-1* in the *TGA* knockout mutants under inducing and noninducing conditions. Because SA is highly toxic to the *TGA* triple mutant, we used INA as the inducing agent.

In wild-type plants, *PR-1* was induced strongly by INA treatment. This induction was not affected by either *tga6-1* or the *tga2-1 tga5-1*, because both *tga6-1* and *tga2-1 tga5-1* plants accumulated levels of *PR-1* similar to that in wild-type plants after INA induction (Figure 3). By contrast, *PR-1* was no longer induced by INA in the *tga6-1 tga2-1 tga5-1* triple knockout mutant, sug-

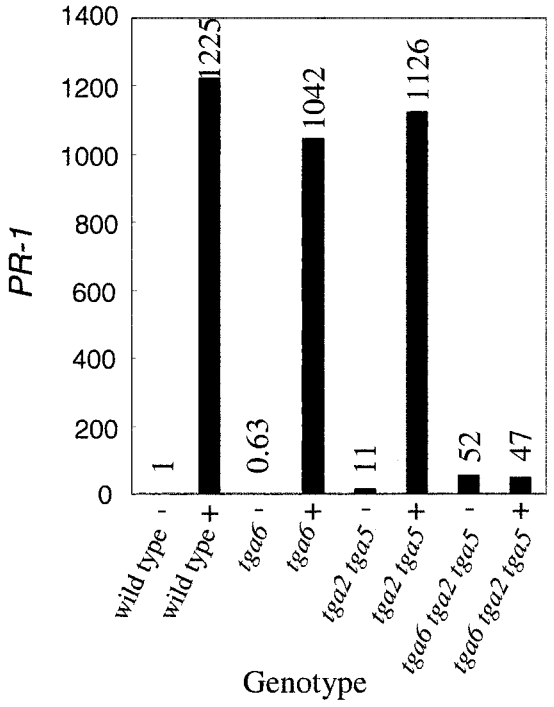

Figure 3. *PR-1* Expression in Wild-Type, *tga6-1*, *tga2-1 tga5-1*, and *tga6-1 tga2-1 tga5-1* Plants in Response to Treatment with INA.

Total RNA was extracted from 20-day-old seedlings grown on MS medium in the presence (+) or absence (−) of 50 μM INA. Relative levels of *PR-1* were determined by real-time PCR using SYBR Green I chemistry. Values were normalized to the expression of *ACTIN1* and are expressed relative to the level in wild-type plants. This experiment was repeated twice with similar results.

gesting that SAR is compromised when all three TGA factors are mutated.

Interestingly, under noninducing conditions, the basal level of *PR-1* was affected in the *TGA* knockout mutants. In *tga6-1 tga2-1 tga5-1* and *tga2-1 tga5-1* plants, the expression level of *PR-1* was ~50-fold and 10-fold higher than that of wild-type plants, respectively (Figure 3). No significant change in the basal level of *PR-1* was observed in *tga6-1* plants.

SAR Is Abolished in *TGA* Triple Knockout Mutants

To determine whether INA-induced pathogen resistance was affected in the *TGA* knockout mutants, 2-week-old wild-type and mutant plants were treated with 0.33 mM INA and inoculated with the virulent oomycete pathogen *Peronospora parasitica* Noco2 after 3 days. The INA-treated wild-type plants were immune to *P. parasitica* Noco2 infection, because no conidiophores were observed on the plants 7 days after inoculation. This induced immunity was not affected by the *tga6-1* or *tga2-1 tga5-1* mutations, because both mutants were as resistant as the wild type (Figures 4A and 4B). By contrast, the INA-induced resistance was abolished completely in *tga6-1 tga2-1 tga5-1*, indicating that SAR is compromised in the *TGA* triple mutant.

We further tested whether systemic resistance can be induced by an avirulent pathogen in *tga6-1 tga2-1 tga5-1* plants. As shown in Figure 5, *Pseudomonas syringae* pv *tomato* (*P.s.t.*) DC3000 carrying *avrRpt2* induced systemic resistance to *Pseudomonas syringae* pv *maculicola* (*P.s.m.*) ES4326 in wild-type plants but not in *tga6-1 tga2-1 tga5-1* plants, further suggesting that SAR is compromised in the *TGA* triple mutant.

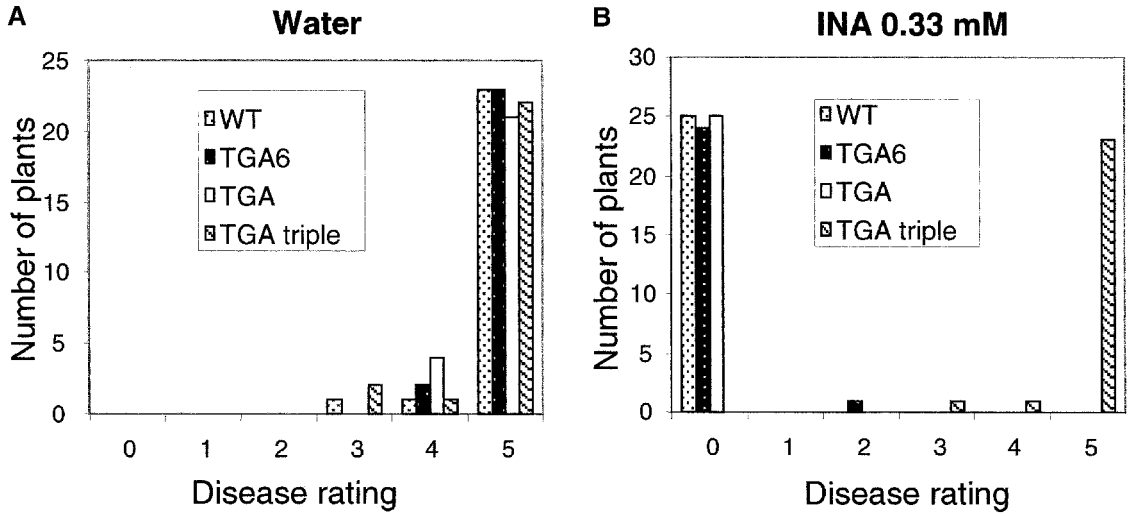

Figure 4. Growth of *P. parasitica* Noco2 on Wild-Type, *tga6-1*, *tga2-1 tga5-1*, and *tga6-1 tga2-1 tga5-1* Plants.

Two-week-old seedlings were pretreated with water **(A)** or 0.33 mM INA **(B)** and sprayed with *P. parasitica* Noco2 spores (5×10^3 spores/mL) 3 days later. Infection was scored at 7 days after inoculation by counting the number of conidiophores per infected leaf. A total of 25 plants were scored for each treatment. Disease rating scores are as follows: 0, no conidiophores on the plants; 1, no more than 5 conidiophores per infected leaf; 2, 6 to 20 conidiophores on a few of the infected leaves; 3, 6 to 20 conidiophores on most of the infected leaves; 4, 5 or more conidiophores on all infected leaves; 5, 20 or more conidiophores on all infected leaves. This experiment was repeated once with similar results. WT, wild type.

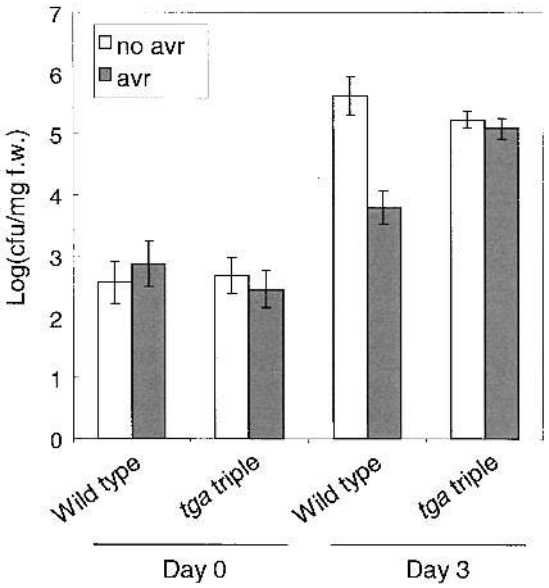

Figure 5. Growth of *P.s.m.* ES4326 in Wild-Type and *tga6-1 tga2-1 tga5-1* Plants Preinoculated with *P.s.t.* DC3000 *avrRpt2*.

Two leaves from each plant were infiltrated with *P.s.t.* DC3000 *avrRpt2* (OD_{600} = 0.02) in 10 mM $MgCl_2$ solution or with the buffer alone 3 days before *P.s.m.* ES4326 infection (OD_{600} = 0.001). Leaf discs within the inoculated area were taken at 0 and 3 days after *P.s.m.* ES4326 infection, and the bacterial titers were measured. Error bars represent 95% confidence limits of log-transformed data. Four samples were taken for each time point. This experiment was repeated once with similar results. avr, *P.s.t.* DC3000 *avrRpt2*; cfu, colony-forming units; f.w., fresh weight; *tga* triple, *tga6-1 tga2-1 tga5-1*.

Compromised SAR in *tga6-1 tga2-1 tga5-1* Cosegregates with *tga6-1*

As shown in Figure 1, the deletion in *tga6-1* affected only *TGA6*. To determine whether the loss of INA-induced resistance in the *TGA* triple mutant cosegregated with the *tga6-1* deletion, we analyzed four independent F2 lines that were heterozygous for the *tga6-1* deletion but homozygous for the *tga2-1 tga5-1* deletion. The F3 plants of these lines were analyzed for susceptibility to *P. parasitica* Noco2 after INA treatment. As shown in Table 1, in all four lines tested, approximately one-fourth of the progeny lost INA-induced resistance to *P. parasitica* Noco2. To determine whether the susceptible plants were homozygous at the *tga6-1* locus, DNA from each individual susceptible plant was analyzed by PCR using primers within the *TGA6* deletion. All susceptible plants were found to be homozygous for the *tga6-1* deletion, indicating that the loss of INA-induced resistance cosegregated with the *tga6-1* deletion.

Both *TGA2* and *TGA5* Can Restore INA-Induced *PR* Gene Expression and Pathogen Resistance in *tga6-1 tga2-1 tga5-1* Plants

To determine whether *TGA2* and *TGA5* can complement the mutant phenotypes of *tga6-1 tga2-1 tga5-1*, we transformed *tga6-1 tga2-1 tga5-1* plants with genomic clones containing either *TGA2* or *TGA5*. Three independent lines for each construct were used for phenotypic analysis. Because similar results were obtained from all lines, only results from one line for each construct is shown in Figure 6. INA-induced *PR-1* expression was restored in the *TGA* triple mutant plants transformed with genomic DNA clones of *TGA2* or *TGA5* (Figure 6A). Treatment with INA also resulted in strong resistance to *P. parasitica* Noco2 in these transgenic plants (Figures 6C and 6D), indicating that SAR was restored in the triple mutant expressing either of the two *TGA* transcription factors. Furthermore, tolerance to SA was restored in *tga6-1 tga2-1 tga5-1* plants transformed with the genomic clone of *TGA2* or *TGA5* (Figure 6B).

DISCUSSION

Although TGA transcription factors have been suggested to be important regulators of SA signaling, it was unclear whether they are essential for the establishment of SAR. In previous studies, transgenic plants overexpressing dominant-negative forms of *TGA2* or a tobacco *TGA2* homolog were used to probe the functions of TGA transcription factors in Arabidopsis and tobacco (Niggeweg et al., 2000b; Pontier et al., 2001; Fan and Dong, 2002). Phenotypes of the transgenic plants differed dramatically depending on the specific dominant-negative mutant used. In one study, tobacco plants overexpressing a dominant-negative form of TGA2 exhibited higher levels of *PR* gene induction by pathogen challenge and an enhanced SAR, leading to the conclusion that TGA factors are not essential for *PR* gene activation or SAR (Pontier et al., 2001). In another study, INA-induced *PR-1* expression was reduced in plants accumulating high levels of a truncated form of TGA2, although it is unclear whether INA-induced pathogen resistance was affected in these plants (Fan and Dong, 2002). Similarly, a reduction of SA-induced *PR* gene expression also was observed in transgenic plants overexpressing a dominant-negative mutant of tobacco TGA2.2 (Niggeweg et al., 2000b).

Because dominant-negative mutants most likely affect multiple independent TGA factors to various degrees, it is difficult to determine the functions of individual TGA factors using this approach. Instead, we created knockout plants of *TGA2*, *TGA5*, and *TGA6* and assayed the single and combined mutants for altered regulation of SA signaling. We found that both *PR* gene expression and pathogen resistance cannot be induced by INA or avirulent pathogens in the *tga6-1 tga2-1 tga5-1* triple knockout mutant, suggesting that these TGA transcription factors serve as essential positive regulators of SAR. We also showed

Table 1. Segregation of the Progeny of *TGA6/tga6-1 tga2-1/tga2-1 tga5-1/tga5-1* Lines

Plant Line	No. of Resistant Plants	No. of Susceptible Plants
Line 1	36	11
Line 2	30	9
Line 3	31	12
Line 4	40	12

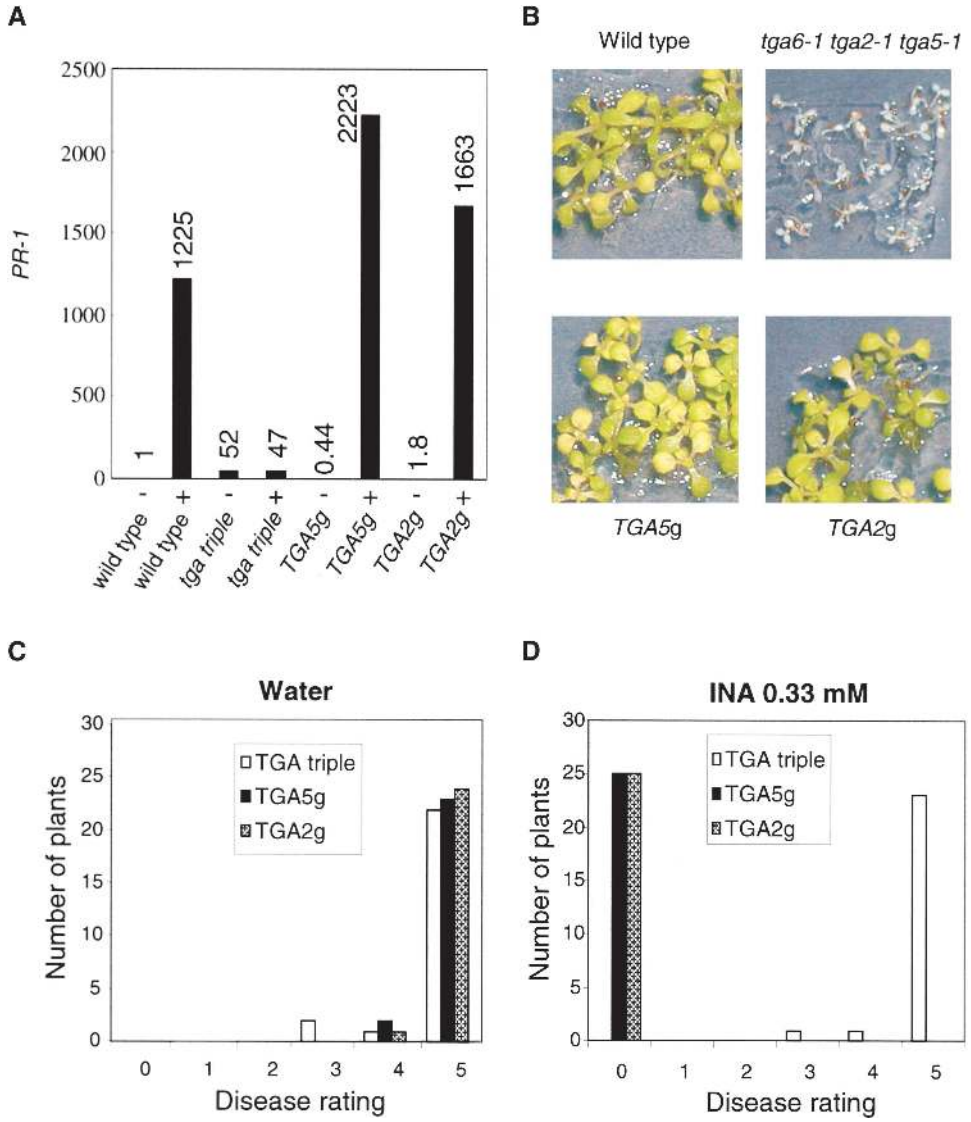

Figure 6. Complementation of *tga6-1 tga2-1 tga5-1* by Genomic Clones of *TGA2* or *TGA5*.

tga triple, *tga6-1 tga2-1 tga5-1*; *TGA2*g, a representative *tga6-1 tga2-1 tga5-1* line transformed with the genomic clone of *TGA2*; *TGA5*g, a representative *tga6-1 tga2-1 tga5-1* line transformed with the genomic clone of *TGA5*.

(A) Complementation of *tga6-1 tga2-1 tga5-1* in *PR-1* expression. The relative expression levels of *PR-1* in *tga6-1 tga2-1 tga5-1*, *TGA2*g, and *TGA5*g were determined as described for Figure 3. +, grown on MS medium + 50 mM INA; −, MS medium alone.

(B) Complementation of *tga6-1 tga2-1 tga5-1* in SA tolerance. T3 seeds of *tga6-1 tga2-1 tga5-1* homozygous for the *TGA2* or *TGA5* transgene were plated on MS medium with 0.2 mM SA along with seeds of *tga6-1 tga2-1 tga5-1*. The photographs were taken 14 days after gemination.

(C) and **(D)** Complementation of *tga6-1 tga2-1 tga5-1* in response to pathogen infection. Using protocols described in Figure 4, water-treated **(C)** or INA-treated **(D)** plants were inoculated with *P. parasitica* Noco2. Infection was scored at 7 days after inoculation by counting the number of conidiophores per infected leaf. A total of 25 plants were scored for each treatment. Each experiment was repeated at least once with similar results.

that genomic clones containing either TGA2 or TGA5 can complement the loss of the SAR phenotype in the *TGA* triple mutant. Thus, either TGA2 or TGA5 is sufficient for INA-induced *PR* gene expression and pathogen resistance. Because the loss of the SAR phenotype cosegregated with the *tga6-1* mutation and was observed only in the triple mutant but not in *tga2-1 tga5-1*, TGA6 also is sufficient for INA-induced *PR* gene expression and pathogen resistance. These data demonstrate that *TGA2*, *TGA5*, and *TGA6* encode redundant functions in the induction of SAR.

Furthermore, we found that *tga2-1 tga5-1* and *tga6-1 tga2-1 tga5-1* accumulated increased levels of *PR-1* under noninducing conditions, suggesting that these TGA transcription factors repress the basal expression of *PR-1*. The higher basal level of

PR-1 in the triple mutant, compared with the double mutant, suggests that *TGA6* is partially responsible for the negative regulation of basal levels of *PR-1*. On the other hand, either *TGA2* or *TGA5* appeared to be sufficient to suppress the basal expression of *PR-1*, because transforming the genomic clone of either *TGA2* or *TGA5* into the triple mutant reverted *PR-1* expression to the wild-type level. The increased *PR-1* expression probably is the result of the loss of binding of TGA factors to a negative element on the *PR-1* promoter. This hypothesis is supported by the presence of an *as-1*–related TGACG element (LS5) that functions as a weak silencer in the *PR-1* promoter (Lebel et al., 1998).

In addition to the loss of SAR phenotypes, *npr1* plants also exhibited reduced tolerance to high concentrations of SA and enhanced susceptibility to the bacterial pathogen *P.s.m.* ES4326. We found that *tga6-1 tga2-1 tga5-1* seedlings were very sensitive to SA toxicity, suggesting that these TGA transcription factors may work together with NPR1 to regulate tolerance to high levels of SA. Unlike *npr1* plants, *TGA* triple knockout plants did not exhibit enhanced susceptibility to *P.s.m.* ES4326 (Figure 5). It is possible that the basal resistance to *P.s.m.* ES4326 is regulated by other mechanisms. In Arabidopsis, there are at least two other TGA transcription factors that can bind to NPR1 in the yeast two-hybrid system (Després et al., 2000; Zhou et al., 2000). Our data suggest that the other TGA transcription factors do not have the same function as TGA2, TGA5, and TGA6 in the positive regulation of SAR. Whether they serve other biological functions regulated by NPR1 remains to be determined.

METHODS

Generation of Triple Knockout Mutants for *TGA2*, *TGA5*, and *TGA6*

TGA2 and *TGA5* are located next to each other, and the identification of *tga2-1 tga5-1* was described previously (Li et al., 2001). To identify a deletion mutant for *TGA6*, an *Arabidopsis thaliana* deletion mutant population of 51,840 lines was screened by PCR using primers flanking the gene. The distance between the primers is ~9 kb. The PCR extension time was set at 1.5 min to avoid amplification of the wild-type DNA fragment. A single deletion mutant, *tga6-1*, was detected initially in one of the megapools containing 2592 lines. Individual mutant plants were isolated subsequently by deconvolution as described previously (Li et al., 2001). *tga6-1 tga2-1 tga5-1* was generated using pollen from *tga6-1* plants to fertilize *tga2-1 tga5-1* plants. The F1 plants were selfed, and *tga6-1 tga2-1 tga5-1* was identified in the F2 generation by PCR using primers within the deletions to confirm homozygosity at both loci.

Analysis of *PR* Gene Expression in the Mutant Plants

To analyze gene expression levels by real-time reverse transcription–PCR, total RNA samples were prepared from 20-day-old plants grown on MS medium (Murashige and Skoog, 1962), with or without INA, using the Totally RNA kit from Ambion (Austin, TX). Reverse transcription of the cDNA was performed using the RT-for-PCR kit from Clontech (Palo Alto, CA). Real-time PCR was performed using the QuantiTect SYBR Green PCR kit from Qiagen (Valencia, CA). The primers used to amplify *PR-1* were 5′-GTAGGTGCTCTTGTTCTTCCC-3′ and 5′-CACATAATTCCCACGAGGATC-3′. The primers used to amplify *ACTIN1* were 5′-CGATGAAGCTCAATCCAAACGA-3′ and 5′-CAGAGTCGAGCACAATACCG-3′.

Pathogen Infections

Both *tga6-1* and *tga2-1 tga5-1* are in the Columbia ecotype background, and *Peronospora parasitica* Noco2 is virulent on this ecotype. Infection of wild-type and *tga* plants with *P. parasitica* Noco2 was performed by spraying a suspension of conidia (~5×10^3 spores/mL water) on 2-week-old soil-grown plants. Inoculated plants were maintained subsequently in a Conviron TC16 growth chamber (Winnipeg, Canada) at 18°C with a 12-h photoperiod and ~80% RH. A disease rating was determined for each plant according to Cao et al. (1998) at 7 days after inoculation. For each genotype and treatment, 25 plants were scored.

Infection with the virulent bacterial pathogen *Pseudomonas syringae* pv *maculicola* (*P.s.m.*) ES4326 was performed by infiltrating leaves of 4-week-old soil-grown wild-type and mutant plants with a bacterial suspension at $OD_{600} = 0.001$, which is the dose that normally causes disease in wild-type plants. Symptoms were examined 3 days after inoculation. The bacterial titer in the leaves was measured according to a previously described procedure (Cao et al., 1994).

Complementation of *tga6-1 tga2-1 tga5-1* Plants by Wild-Type Genes

A 5.9-kb KpnI-BamHI fragment (MOJ9, nucleotides 28,249 to 34,162) containing *TGA2* was subcloned from P1 clone MOJ9 to pGreen229 (Hellens et al., 2000) to create pG229-TGA2. A 4.6-kb EcoRI-SacI fragment (MOJ9, nucleotides 33,110 to 37,773) containing *TGA5* was subcloned from MOJ9 to pGreen229 to create pG229-TGA5. pG229-TGA2 and pG229-TGA5 were transformed into *tga6-1 tga2-1 tga5-1* using the floral-dip method (Clough and Bent, 1998), and transformants were selected on soil by spraying the T1 plants with the herbicide glufosinate. At least 10 transformants were obtained for each construct. All assays on the complementing lines were performed on glufosinate-resistant T2 plants.

Upon request, materials integral to the findings presented in this publication will be made available in a timely manner to all investigators on similar terms for noncommercial research purposes. To obtain materials, please contact Yuelin Zhang, yuelin@interchange.ubc.ca

Accession Number

The accession number for BAC clone F28J15 is AC069472.

ACKNOWLEDGMENTS

We thank Brian Staskawicz for *P.s.t.* DC3000 *avrRpt2* strain, Jian-min Zhou and Jane Parker for sending materials used in this study, and Sandra Goritschnig and Kristoffer Palma for careful reading of the manuscript. We are grateful for financial support to Y.Z. from the Natural Sciences and Engineering Council of Canada and to X.L. from the Canadian Foundation for Innovation and the Natural Sciences and Engineering Council of Canada.

Received June 18, 2003; accepted September 6, 2003.

REFERENCES

Aravind, L., and Koonin, E.V. (1999). Fold prediction and evolutionary analysis of the POZ domain: Structural and evolutionary relationship with the potassium channel tetramerization domain. J. Mol. Biol. **285,** 1353–1361.

Cao, H., Bowling, S.A., Gordon, A.S., and Dong, X. (1994). Character-

ization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell **6,** 1583–1592.

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

Cao, H., Li, X., and Dong, X. (1998). Generation of broad-spectrum disease resistance by overexpression of an essential regulatory gene in systemic acquired resistance. Proc. Natl. Acad. Sci. USA **95,** 6531–6536.

Chern, M.S., Fitzgerald, H.A., Yadav, R.C., Canlas, P.E., Dong, X., and Ronald, P.C. (2001). Evidence for a disease-resistance pathway in rice similar to the NPR1-mediated signaling pathway in Arabidopsis. Plant J. **27,** 101–113.

Clough, S.J., and Bent, A.F. (1998). Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J. **16,** 735–743.

Delaney, T.P., Friedrich, L., and Ryals, J.A. (1995). Arabidopsis signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Natl. Acad. Sci. USA **92,** 6602–6606.

Després, C., DeLong, C., Glaze, S., Liu, E., and Fobert, P.R. (2000). The Arabidopsis NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors. Plant Cell **12,** 279–290.

Fan, W., and Dong, X. (2002). In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid–mediated gene activation in Arabidopsis. Plant Cell **14,** 1377–1389.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. (1993). Requirement of salicylic acid for the induction of systemic acquired resistance. Science **261,** 754–756.

Glazebrook, J., Rogers, E.E., and Ausubel, F.M. (1996). Isolation of Arabidopsis mutants with enhanced disease susceptibility by direct screening. Genetics **143,** 973–982.

Görlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.-H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., and Ryals, J. (1996). Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell **8,** 629–643.

Hellens, R.P., Edwards, E.A., Leyland, N.R., Bean, S., and Mullineaux, P.M. (2000). pGreen: A versatile and flexible binary Ti vector for *Agrobacterium*-mediated plant transformation. Plant Mol. Biol. **42,** 819–832.

Kim, H.S., and Delaney, T.P. (2002). Over-expression of TGA5, which encodes a bZIP transcription factor that interacts with NIM1/NPR1, confers SAR-independent resistance in *Arabidopsis thaliana* to *Peronospora parasitica*. Plant J. **32,** 151–163.

Lebel, E., Heifetz, P., Thorne, L., Uknes, S., Ryals, J., and Ward, E. (1998). Functional analysis of regulatory sequences controlling PR-1 gene expression in Arabidopsis. Plant J. **16,** 223–233.

Li, X., Song, Y., Century, K., Straight, S., Ronald, P., Dong, X., Lassner, M., and Zhang, Y. (2001). A fast neutron deletion mutagenesis-based reverse genetics system for plants. Plant J. **27,** 235–242.

Malamy, J., Carr, J.P., Klessig, D.F., and Raskin, I. (1990). Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. Science **250,** 1002–1004.

Métraux, J.P., Ahl-Goy, P., Staub, T., Speich, J., Steinemann, A., Ryals, J., and Ward, E. (1991). Induced resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens. In Advances in Molecular Genetics of Plant-Microbe Interactions, Vol. 1, H. Hennecke and D.P.S. Verma, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 432–439.

Métraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W., and Inverardi, B. (1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science **250,** 1004–1006.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. **15,** 473–497.

Nawrath, C., Heck, S., Parinthawong, N., and Metraux, J.P. (2002). EDS5, an essential component of salicylic acid–dependent signaling for disease resistance in Arabidopsis, is a member of the MATE transporter family. Plant Cell **14,** 275–286.

Nawrath, C., and Métraux, J.-P. (1999). Salicylic acid induction–deficient mutants of Arabidopsis express *PR-2* and *PR-5* and accumulate high levels of camalexin after pathogen inoculation. Plant Cell **11,** 1393–1404.

Niggeweg, R., Thurow, C., Kegler, C., and Gatz, C. (2000a). Tobacco transcription factor TGA2.2 is the main component of as-1-binding factor ASF-1 and is involved in salicylic acid- and auxin-inducible expression of as-1-containing target promoters. J. Biol. Chem. **275,** 19897–19905.

Niggeweg, R., Thurow, C., Weigel, R., Pfitzner, U., and Gatz, C. (2000b). Tobacco TGA factors differ with respect to interaction with NPR1, activation potential and DNA-binding properties. Plant Mol. Biol. **42,** 775–788.

Pontier, D., Miao, Z.H., and Lam, E. (2001). Trans-dominant suppression of plant TGA factors reveals their negative and positive roles in plant defense responses. Plant J. **27,** 529–538.

Rasmussen, J.B., Hammerschmidt, R., and Zook, M.N. (1991). Systemic induction of salicylic acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*. Plant Physiol. **97,** 1342–1347.

Rogers, E.E., and Ausubel, F.M. (1997). Arabidopsis enhanced disease susceptibility mutants exhibit enhanced susceptibility to several bacterial pathogens and alterations in *PR-1* gene expression. Plant Cell **9,** 305–316.

Ryals, J., Weymann, K., Lawton, K., Friedrich, L., Ellis, D., Steiner, H.-Y., Johnson, J., Delaney, T.P., Jesse, T., Vos, P., and Uknes, S. (1997). The Arabidopsis NIM1 protein shows homology to the mammalian transcription factor inhibitor IκB. Plant Cell **9,** 425–439.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y., and Hunt, M.D. (1996). Systemic acquired resistance. Plant Cell **8,** 1809–1819.

Shah, J., Tsui, F., and Klessig, D.F. (1997). Characterization of a salicylic acid-insensitive mutant (*sai1*) of *Arabidopsis thaliana*, identified in a selective screen utilizing the SA-inducible expression of the *tms2* gene. Mol. Plant-Microbe Interact. **10,** 69–78.

Subramaniam, R., Desveaux, D., Spickler, C., Michnick, S.W., and Brisson, N. (2001). Direct visualization of protein interactions in plant cells. Nat. Biotechnol. **19,** 769–772.

Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E., and Ryals, J. (1992). Acquired resistance in *Arabidopsis*. Plant Cell **4,** 645–656.

White, R.F. (1979). Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology **99,** 410–412.

Wildermuth, M.C., Dewdney, J., Wu, G., and Ausubel, F.M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature **414,** 562–565.

Zhang, Y.L., Fan, W.H., Kinkema, M., Li, X., and Dong, X. (1999). Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the PR-1 gene. Proc. Natl. Acad. Sci. USA **96,** 6523–6528.

Zhou, J.M., Trifa, Y., Silva, H., Pontier, D., Lam, E., Shah, J., and Klessig, D.F. (2000). NPR1 differentially interacts with members of the TGA/OBF family of transcription factors that bind an element of the PR-1 gene required for induction by salicylic acid. Mol. Plant-Microbe Interact. **13,** 191–202.