# Overexpression of *GbERF* confers alteration of ethylene-responsive gene expression and enhanced resistance to *Pseudomonas syringae* in transgenic tobacco

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GbERF belongs to the ERF (ethylene responsive factor) family of transcription factors and regulates the GCC-box containing pathogen-related (PR) genes in the ethylene signal transduction pathway. To study the function of GbERF in the process of biotic stress, transgenic tobacco plants expressing *GbERF* were generated. Overexpression of *GbERF* did not change transgenic plant's phenotype and endogenous ethylene level. However, the expression profile of some ethylene-inducible GCC-box and non-GCC-box containing genes was altered, such as *PR1b*, *PR2*, *PR3*, *PR4*, *Osmotin*, *CHN50*, ACC oxidase and ACC synthase genes. These data indicate that the cotton GbERF could act as a transcriptional activator or repressor to regulate the differential expression of ethylene-inducible genes via GCC and non-GCC *cis*-elements. Moreover, the constitutive expression of *GbERF* in transgenic tobacco enhanced the plant's resistance to *Pseudomonas syringae* pv *tabaci* infection. In conclusion, *GbERF* mediates the expression of a wide array of *PR* and ethylene-responsive genes and plays an important role in the plant's response to biotic stress.

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## 1. Introduction

The ethylene responsive factor (ERF) transcription factor family has been found to participate in plant response to biotic and abiotic stresses (Reichmann and Meyerowitz 1998; Kizis *et al* 2001). The ERF proteins were first isolated as GCC box binding proteins from tobacco (Ohme-Takagi and Shinshi 1995). Until now many ERF proteins have been identified from various plant species, such as *ERF*s and *AtERF*s from *Arabidopsis thaliana* (Fujimoto *et al* 2000), *Pti* 4/5/6 (Gu *et al* 2002), *Tsi1* (Park *et al* 2001) and *TERF1* (Huang *et al* 2004) from tomato, *NtERF1* to *NtERF5* from tobacco (Ohme-Takagi and Shinshi 1995; Ohta *et al* 2000; Fischer and Dröge-Laser 2004). Accumulated evidences have shown that the ERF is a subfamily of the large AP2 transcription factor family (Reichmann *et al* 2000). The ERF proteins contain a highly conserved DNA binding domain, consisting of 58-59 amino acids (Ohme-Takagi and Shinshi 1995) and the determined 3D solution structure shows that it is comprised of a three

**Keywords.** Constitutive expression; *GbERF*; *Nicotiana tabacum*; *Pseudomonas syringae*; transgenic plant

Abbreviations used: ABA, Abscisic acid; ACC, 1-aminocyclopropane carboxylic aicd; ACO, ACC oxidase; ACS, ACC synthase; ERF, ethylene responsive factor; PCR, polymerase chain reaction; RT, reverse transcription.

stranded antiparellel $\beta$-sheet and an $\alpha$-helix packed approximately parallel to the $\beta$-sheet. The $\beta$-sheet interacts with specific motifs located inside the major groove of the DNA helix (Allen *et al* 1998).

The ERF transcription factors are shown to be involved in regulating plant pathogen-related (*PR*) genes expression via the GCC-box and non-GCC *cis*-elements (Reichmann and Meyerowitz 1998; Kizis *et al* 2001; Chakravarthy *et al* 2003). As an example, the expression of tomato Pti4 and Pti5 can be induced by *Pseudomonas*, and Pti4/5/6 regulate *PR* gene expression (Zhou *et al* 1997; Wu *et al* 2002). After bacterial infection, Pti4 was phosphorylated by the Pto kinase, which was thought to mediate transcriptional activation of *PR* gene (Gu *et al* 2000). The overexpression of *ERF* genes resulted in enhanced tolerance to pathogen attack in transgenic plants. As an example, overexpression of the *Arabidopsis ERF1* enhanced resistance of transgenic plants to *Botrytis cinerea*, but the resistance to *Pseudomonas syringae* infection was decreased (Berrocal-Lobo *et al* 2002). Moreover, the overexpression of tobacco *Tsi1* gene led to constitutive expression of *PR* gene and the enhanced resistance against pathogen attack and osmotic stress (Park *et al* 2001), while overexpression of *Pti5* did not lead to constitutive expression of *PR* gene but accelerated pathogen induced transcription (He *et al* 2001). In summary, defined members of the ERF family seem to play specific roles in pathogen defense and plant response to environmental stress.

The *GbERF* was cloned from *Gossypium barbadense* through SSH approach in our previous studies (Qin *et al* 2004; Zuo *et al* 2005). The transcripts of *GbERF* accumulated highly and rapidly after treated with exogenous ethylene and *Verticillium dahliae* infection, slightly in response to salt, cold, drought stress and exogenous abscisic acid (ABA) treatment (Qin *et al* 2004). However, the transcription of *GbERF* declined in response to water stress. Here we report that constitutive expression of *GbERF* under the control of cauliflower mosaic virus (CaMV) 35S promoter in tobacco *(Nicotiana tabacum* cv *bairihong*) leads to the constitutive expression changes of some ethylene responsive genes in normal conditions and the enhanced resistance to the infection of virulence *Pseudomonas syringae* pv *tabaci*.

## 2. Materials and methods

### 2.1 *Plant material and growth conditions*

All plants were grown at 25°C with a 16:8 h light/dark cycle. The tobacco used for transformation is *N. tabacum* cv *bairihong*. Leaves from transgenic tobacco plants were used for analysis of the expression of *GbERF* and the downstream genes.

### 2.2 *Generation of transgenic GbERF tobacco plants*

The plasmid (pBI121-*GbERF* ) used in the transformation of tobacco was constructed by cloning the whole coding sequence of *GbERF* cDNA into pBI121 in the sense orientation under the control of CaMV 35S promoter (figure 1). The pBI121-*GbERF* was introduced into *Agrobacterium tumefaciens* strain EHA105 by triparental mating and the resulting strain was used to transform tobacco leaf discs by *Agrobacterium*-mediated transformation (Hoekema *et al* 1983).

### 2.3 *Polymerase chain reaction screening and Northern blot analysis of transgenic tobacco plants*

Genomic DNA and total RNA of transgenic and wild-type tobacco plants were extracted using the cetyltrimethylammonium bromide (CTAB) method (Sambrook *et al* 1989). RNase (Takara, Japan) was used to digest RNA from genomic DNA and RNase-free DNaseI (Takara, Japan) was used to digest the genomic DNA from the total RNA.

All the putative transgenic plants (kanamycin resistant) were analyzed by polymerase chain reaction (PCR) with the specific primers FT1 (5´-GACAAATTCTCAGAAACCACTTTCC-3´) and RT1 (5´-CTCAAGAAACTCTTCACCCACTACA-3´) for the presence of the *GbERF* gene in the genome. Primer positions and PCR product size are indicated in figure 1. The PCR reactions were carried out in

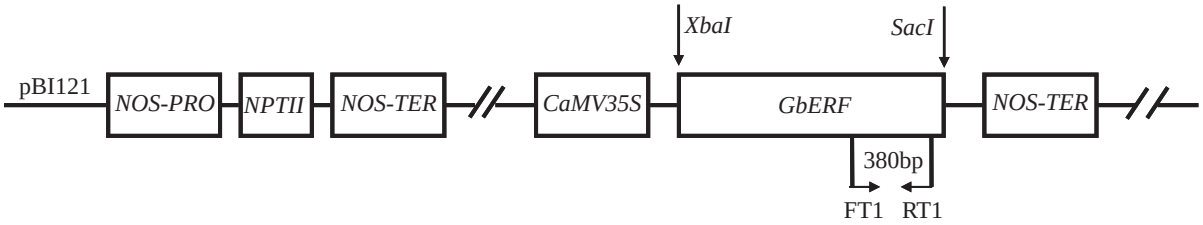


**Figure 1.** Schematic representation of the plasmid pBI121-GbERF used in tobacco transformation. The relevant restriction sites (*Xba*I and *Sac*I) and the positions of PCR primers (FT1 and RT1) as well as the PCR product size were indicated.

a total volume of 30 $\mu$l comprising 50 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 M each of dNTPs, 1.25 units of *Taq* DNA polymerase and 125 pmol of each primer by denaturing DNA at 94°C for 3 min followed by 30 cycles of amplification (94°C for 50 s, 60°C for 50 s, 72°C for 50 s) and elongation by 10 min at 72°C.

PCR-positive plants were subjected to Northern blot analysis for the expression of *GbERF* in plants. An aliquot of 20 $\mu$g total RNA was denatured and electrophoresed on a 1.2% agarose gel containing 6% formaldehyde in 1×MOPS buffer, transferred onto a Hybond-$N^+$ nylon membrane (Amersham Pharmacia, USA) and hybridized with a fragment of the *GbERF* 3´UTR as the probe. Equal loading of samples was confirmed by hybridization of rRNA. The Gene Images random priming labelling module and Gene Images CDP-Star detection module (Amersham Pharmacia, USA) were used for probe labelling, hybridization and detection procedures. The blotting was also performed following manufacturer's protocol. Six independent transgenic lines (G1, G2, G3, G4, G5 and G6) constitutively expressing *GbERF* and wild-type (WT) tobacco plants were used in the later analysis.

### 2.4 *RNA transcripts analysis*

Semi-quantitative reverse trascription (RT)-PCR was used to analyse the expression profiles of ethylene-inducible genes in transgenic and wild-type plants using one-step RT-PCR kit (Takara, Japan). The RT-PCR was performed using an aliquot of 1 $\mu$g total RNA as the template by 95°C for 5 min followed by 20-28 cycles of amplification (95°C for 1 min, 55°C for 1 min, 72°C for 1 min). The PCR products were analysed by Gene Tools software from Gene Company (CA, USA). The RT-PCR reaction for the house-keeping actin gene was performed at the same condition as described above to estimate if equal amounts of RNA among samples were used in the RT-PCR reaction. The ethylene-inducible genes and their respective primers used in the analysis are listed in table 1.

**Table 1.** PCR primers used in the analysis of expression of ethylene-inducible genes in transgenic *GbERF* tobacco lines.

| | Gene name | Primer (5´-3´) | Reference |
|---|---|---|---|
| GCC box containing genes | *PR1b* | Forward: ACTGCAACCTCGTACATTCT<br>Reverse: CACTTAACCCTAGCACATCC | Matsuoka *et al* 1987 |
| | *PR2* | Forward: CACAACAAGAAGCAAATCCT<br>Reverse: TATCAAAGTGAAACCAGAGT | Ward *et al* 1991 |
| | *PR3* | Forward: AACGACGGTAGATGTCCTGC<br>Reverse: AGATGGCTTGTTGTCCTGTG | Linthorst *et al* 1990 |
| | *PR4* | Forward: GCTACTTGGGATGCCGATAA<br>Reverse: TAACAGCGCACGTCTGAAAA | Linthorst *et al* 1991 |
| | *CHN50* | Forward: TCAGTTAATCATTTCGCCAG<br>Reverse: CTCCATGTTGATAGCGGTGC | Melchers *et al* 1994 |
| | *Osmotin* | Forward: GTTTTCTTCCTCCTTGCCTT<br>Reverse: ACATTCGCCGTATATTAGCC | Kumar and Spencer 1992 |
| | *ACO* | Forward: GAGAAAGAGGCAGCGGAGAG<br>Reverse: ACCAAAACAAGCAAATAATG | GenBank Acc. No. X98493 |
| Non- GCC-box containing genes | *ACS* | Forward: AATCGCATAGTTATGAGTGG<br>Reverse: TCAAGAAGAGTGCCTAGTGG | Liu *et al* 1998 |
| | *Actin* | Forward: CTTGACGGAAAGAGGTTATT<br>Reverse: GATCCTCCAATCCAGACACT | GenBank Acc. No. AB158612 |

### 2.5 *Disease resistance assay*

Wild-type and transgenic tobacco plants were grown in soil in a growth chamber at 25°C with a 16:8 h light/dark photoperiod. Healthy and fully expanded young leaves from wild-type and transgenic tobacco plants were washed briefly in distilled water. *Pseudomonas syringae* pv *tabaci* was grown in King's medium B (Martin *et al* 1993). The bacterial culture was washed and resuspended in 10 mM $MgCl_2$ to $10^7$ cfu/ml. Bacterial suspensions were infiltrated into fully expanded tobacco leaves using a 1 ml plastic syringe with a needle. An aliquot of 20 $\mu$l suspension was infiltrated in the left side of the leaf, and the right side was infiltrated with the same amount of distilled water. At 7 days after inoculation, the infected leaves were collected and the bacterial populations were determined (Park *et al* 2001). All the experiments were repeated three times.

### 2.6 *Analysis of seed germination*

For the ethylene triple-response assay, surface sterilized seeds were plated onto MS media plates containing 10 $\mu$M 1-aminocyclopropane-carboxylic acid (ACC, Sigma), or 15 $\mu$M $AgNO_3$ and incubated at 23°C in darkness for 6 days (Huang *et al* 2004).

## 3. Results

### 3.1 *Transformation and regeneration of transgenic plants expressing GbERF*

In order to analyse the biological function of *GbERF* as transcription factor in plants, *GbERF*-overexpressing transgenic tobacco plants were generated by transforming tobacco leaf discs with EHA105 (pBI121-*GbERF*) and subsequent kanamycin selection. The transgenic status of the putative transgenic plants was confirmed by PCR analysis for the presence of the *GbERF* gene in the genome (figure 2A), and the expression of *GbERF* in transgenic plants was demonstrated by Northern blot analysis (figure 2B). In total, 57 independent transgenic tobacco plants were obtained.

### 3.2 *Heterotrophic expression of GbERF in tobacco shows no ethylene triple response and phenotypic changes*

Northern blot analysis showed that the expression levels of *GbERF* in independent transgenic plants were different (figure 2B). *GbERF* transcripts in plants G4 and G5 were far less than those in other plants.

Ethylene promotes the so-called "triple response": an exaggeration of the transient developmental structure

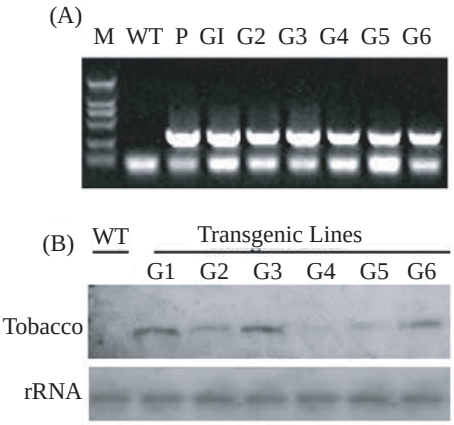


**Figure 2.** PCR (**A**) and Northern blot (**B**) analyses for the presence and expression of *GbERF* in transgenic tobacco plants. M, molecular size marker DL2000, the respective bands were 2000, 1000, 750, 500, 250 and 100 bp; P, pBI121-*GbERF*; WT, wild-type untransformed control plant; G1-G6, independent transgenic *GbERF* tobacco plants.

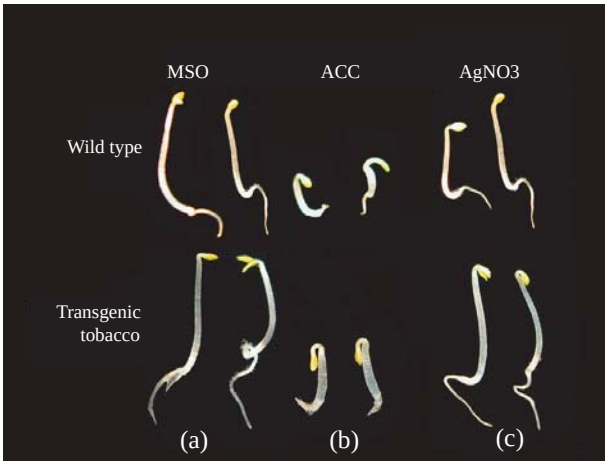


**Figure 3.** Germination assay of wild-type and transgenic tobacco. Tobacco seeds were germinated in dark on MS plates, (**a**) without any additional addendum (MSO); (**b**) with 10 $\mu$M 1-aminocyclopropane-carboxylic acid (ACC); (**c**) with 15 $\mu$M $AgNO_3$. Six days later, the germinated transgenic and wild-type plants displayed ethylene triple-response in ACC containing medium, but grew normally in $AgNO_3$ containing and MS0 media.

known as the apical hook, together with an inhibition of hypocotyl and root extension growth. The triple response is thought to protect the shoot and root apical meristems from damage during growth through the soil. It was reported that the overexpression of *Pti4* in transgenic *Arabidopsis* (Gu *et al* 2002) and *TERF* in tobacco (Huang *et al* 2004) could lead to phenotypic alteration and cause the ethylene triple response. In our study, *GbERF*-expressing transgenic

tobacco plants did not show any phenotypic changes in seed germination and vegetative stage. As shown in figure 3, compared with the wild-type seed germination, the transgenic T1 seedlings showed no ethylene triple-response in MS medium. The germination of both wild-type and transgenic seeds on ACC containing medium displayed the typical ethylene triple response: the inhibition of root and hypocotyl elongation and curvature of the apical hook. Moreover, the endogenous ethylene level had little difference in matured wild-type and transgenic tobacco (data not shown). These results indicated that overexpression of *GbERF* in tobacco plant did not involve in ethylene biosynthesis and evoke phenotypic changes in all growth stages.

### 3.3 GbERF *overexpression alters GCC-box containing* PR *gene expression in transgenic plants*

The GCC box, a conserved DNA sequence element (AGCCGCC) in the promoter sequence of ethylene inducible genes especially in many *PR* genes, such as pathogenesis related-1 protein gene (*PR1*), *β-1, 3-glucanase* (*PR2*), *chitinase* (*PR3*) and *Osmotin* (*PR5*) (Ohme-Takagi and Shinshi 1995). It has been suggested that the GCC-box is a target in the ethylene signal transduction pathway and deletion of this sequence eliminates ethylene responsiveness (Shinshi *et al* 1990). Furthermore, the GCC-box was found to be presented in many basic *PR* genes from bean, tobacco, tomato, *Arabidopsis* and potato (Zhou *et al* 1997; Jia and Martin 1999). In this study, the expression of *GbERF* and its possible target genes were analysed in transgenic plants and compared with that of wild-type plants (figure 4A). The transcript of *PR3* was increased in all transgenic lines, and the transcript of *PR1b* (pathogenesis related-1b protein gene) was also increased except in line G2. There was an obvious expression increase of *PR2* in lines G1 and G5, and of *PR4* in lines G3, G5 and G6. However, the transcripts of *Osmotin* were decreased significantly in all transgenic lines and the transcripts of *CHN50* were decreased slightly in lines G1, G3, G4 and G6.

It has been well demonstrated that *PR1b*, *PR2*, *PR3*, *PR4*, *Osmotin* and *CHN50* are GCC-box containing genes and their expression could be activated or repressed by different ERF transcription factors (Park *et al* 2001; Gu *et al* 2002; Fischer and Dröge-Laser 2004), and the expression of these genes were related to the onset of systemic acquired resistance (SAR) in tobacco (Ward *et al* 1991). The expression of *PR1b*, *PR3* and *PR4* was regulated by the ET/JA-dependent signalling pathway (Zhou *et al* 1997; Gu *et al* 2002). In our previous studies, the expression of *GbERF* could be induced by ethylene and ABA, but could not be

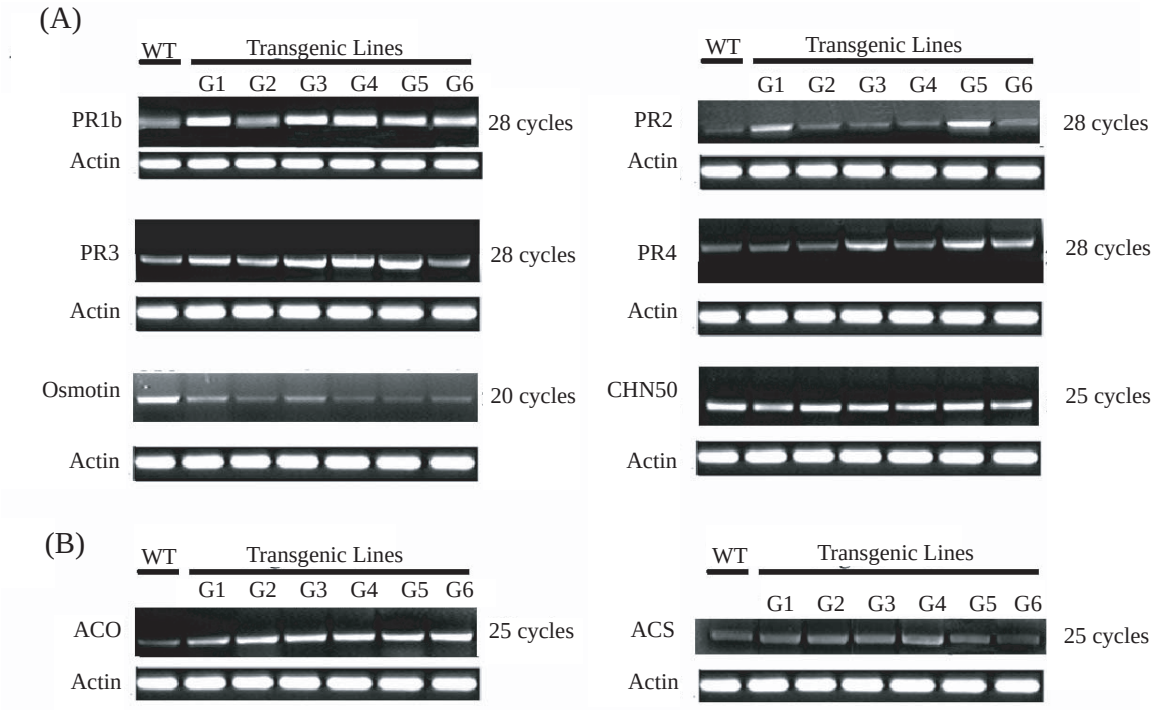


**Figure 4.** The expression profiles of (**A**) GCC-box containing PR genes [*Osmotin*, *PR2*, *PR1b*, *PR4*, *CHN50* (Chitinase V) and *PR3*], and (**B**) ethylene responsive genes (*ACO*, *ACS*) in wild-type and transgenic tobacco lines revealed by RT-PCR analysis. The entire experiment was repeated three times. WT, wild-type untransformed tobacco line. G1-G6, independent transgenic tobacco lines. *Actin* was used to normalize the amount of templates used in PCR reactions (lower panel). Cycling of RT-PCR reaction was listed on the right side.

induced by salicylic acid (Qin *et al* 2004; Zuo *et al* 2005). The current result of expression profile analysis suggests that *GbERF* may play an important role in plant's defense response by regulating the expression of various *PR* genes.

### 3.4 *Expression profile of some ethylene-inducible genes*

Ethylene treatment can induce the expression of many genes without GCC-box in their promoter regions (Chakravarthy *et al* 2003). ACC oxidase (*ACO*) is highly repressed by ethylene treatment in microarray analysis (Schenk *et al* 2000). In the present study, the transcript of *ACO* was increased in all transgenic lines compared with wild-type tobacco (figure 4B). ACC synthase (*ACS*) is the key enzyme in ethylene biosynthesis in plant, and the transcripts of *ACS* had little changes between transgenic and wild-type tobacco (figure 4B). Ethylene regulates its own biosynthesis and receptor genes (Wang *et al* 2002) and microarray analysis revealed that pathogen attack and ethylene treatment could induce the transcription of *ACO* gene (Wang *et al* 2002; Guang and Jacqueline 2003). ACO catalyses ACC and generates ethylene. In the present study, although the transcript of *ACO* was increased, the endogenous ethylene synthesis was not enhanced (data not shown), implying that the overexpression of *ACO* alone may be not enough to promote the ethylene synthesis. The germination of T1 transgenic seeds showed no ethylene triple response and the endogenous ethylene in matured plants was not increased compared with wild-type tobacco. The fact that the expression of ACS, the key enzyme in ethylene synthesis (Kende 1993), in transgenic tobacco was not changed might be responsible for why endogenous ethylene was not increased in transgenic tobacco.

### 3.5 *Overexpression of* GbERF *enhances tobacco's defense response against bacterial pathogen* P. syringae *pv* tabaci

To determine whether overexpression of *GbERF* enhances resistance to pathogens, we inoculate wild-type and

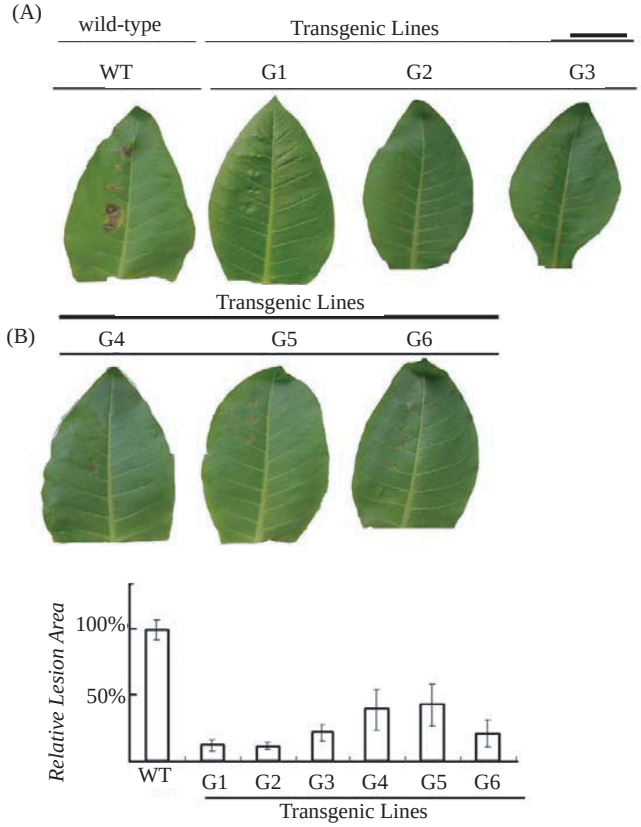


**Figure 5.** Disease resistance assay. (**A**) The first full-expanded leaf from independent tobacco lines was inoculated with *P. syringae* at the left side, and the right side of each leaf was dipped with distilled water. The disease symptoms were scored 7 days later. (**B**) The relative lesion area. The average lesion area of each independent transgenic line was calculated ($n$=3) and their relative lesion areas were shown in columns after comparison with the average lesion area on wild type tobacco. The data represented the means ± SE.

transgenic tobacco plants with *P. syringae* pv *tabaci*, a virulent bacterial pathogen. As shown in figure 5A**,** inhibition of pathogen growth was observed in all transgenic plants. Seven days after inoculation, growth of *P. syringae* in transgenic tobacco was significantly inhibited compared with wild-type tobacco. There were lesions developed on the leaves of transgenic tobacco, but the sizes of the lesions were significantly smaller than those on wild-type tobacco. The lesions on wild-type tobacco leaves were 4-5-fold larger than those on leaves of lines G1 and G2 (figure 5B). The enhanced resistance to *P. syringae* in all these six transgenic tobacco demonstrates that disease resistance conferred by overexpression of *GbERF* is effective and sufficient against bacterial pathogens even at low expression level.

## 4. Discussion

Increasing evidence indicates that the ERF proteins in plants belong to the huge AP2 transcription factor family (Fujimoto *et al* 2000; Reichmann *et al* 2000), suggesting that the ERF proteins might have crucial roles in regulating development and the responses to environmental stresses. The overexpression of tomato *TERF1* in tobacco (Huang *et al* 2004) and Pti4 in *Arabidopsis* (Gu *et al* 2002) causes phenotypic changes associated with the ethylene response. Our results show that overexpression of *GbERF*, which is a downstream component in ethylene signal pathway, could increase the expression of ACO, an enzyme involved in ethylene biosynthesis, but could not mediate the biosynthesis of endogenous ethylene and change the phenotype in transgenic plants. Thus it is clear that GbERF is not involved in ethylene synthesis. At present, little is known about the relationship of endogenous ethylene changes and the overexpression of ERFs. Understanding the role of ERFs in ethylene biosynthesis might give a fresh view of ERFs in ethylene signal pathway.

*GbERF* shows no tissue-specific expression (Qin *et al* 2004) and the overexpression of *GbERF* does not lead to changes in vegetative or generative plant growth, indicating a possible global function in cotton response to biotic stress. *PR* genes containing GCC-box in their promoters have been shown to be ERF targets in ethylene responsive signal pathway (Ohme-Takagi and Shinshi 1995; Ohta *et al* 2000; Chakravarthy *et al* 2003). However, numerous reports have revealed that different ERF proteins induce only a subset of *PR* genes. Overexpression of *Arabidopsis* ERF1 results in constitutive activation of *β-Glu, b-CHI* or *PDF1.2*, whereas overexpression of tomato *Pti4* in *Arabidopsis* leads to activation of *PR1*, *Chitinase* (*PR3*), *PR4* and *PDF1.2* (Gu *et al* 2002; Chakravarthy *et al* 2003). Our results showed that the constitutive expression of *GbERF* in tobacco could induce the expression of some PR genes containing GCC box. The function of *GbERF* as transcription factor was thus clear. Moreover, *Pti4* could regulate defense-related gene expression via GCC box and non-GCC box *cis*-elements (Chakravarthy *et al* 2003). In our study, we also observed that *GbERF* could induce non-GCC box containing *ACO* gene expression. This might indicate that the GCC box containing *PR* genes is only a subset of ERF targets, and the ERF could regulate its downstream gene expression via additional *cis*-elements. We deduce that the structure complexity in the promoter of defense-related genes and the various ERFs existed in plant genome might be an explanation to the differential exhibition of different defense-related ethylene inducible genes. Further studies on the function of *GbERF* in mediating expression of *PR* genes might throw a fresh light on the role of *ERF* in plant response to environmental stress.

It has been well documented that the overexpression of some *ERF* genes changes the expression of some PR genes and results in the increased resistance to bacterial, fungal and virus pathogens and abiotic stress (He *et al* 2001; Berrocal-Lobo *et al* 2002; Gu *et al* 2002; Chakravarthy *et al* 2003; Fischer and Dröge-Laser 2004). Overexpression of the pepper transcription factor *CaPF1* in transgenic Virginia pine (*Pinus virginiana* Mill.) confers multiple stress tolerance and enhances organ growth (Tang *et al* 2005) while ectopic overexpression of tomato *JERF3* in tobacco activates downstream gene expression and enhances salt tolerance (Wang *et al* 2004). The overexpression of *Tsi1* increases plant's resistance to *P. syringae* and also increases plant's tolerance to salt (Park *et al* 2001). More recently, four rice genes encoding ethylene-responsive transcriptional factors were cloned and their expressions were found to be responsive to biotic and abiotic stress (Cao *et al* 2006). In our study, the overexpression of *GbERF* in transgenic tobacco increased plant resistance to *P. syringae* infection. Furthermore, the plant resistance to pathogen was not related to the expression level of *GbERF*. Although the expression levels of *GbERF* among the tested six transgenic lines were different, the resistance of each line to *P. syringae* was all enhanced significantly. This phenomenon, to some extent, suggests a threshold value in the expression of *GbERF* to enhance plant's resistance to pathogens. Taken together with the alteration in *PR* genes' expression, it indicates that *GbERF* may play important roles in plant's response to biotic stress rather than to abiotic stress. These data also support the view that huge number of *ERF* genes in plant genome reflects not only diversities but distinct functions of the plant to deal with specific environmental stress conditions (Fischer and Dröge-Laser 2004).

In summary, our results show that GbERF could mediate GCC-box and non-GCC box containing genes' transcription. Expression of *GbERF* in transgenic tobacco confers an enhanced resistance to *P. syringae* infection. This study provides evidence that *GbERF* gene products are involved

in the regulation of a subset of ethylene responsive genes related to response to biotic stress.

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