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Do ethylene response factors-9 and -14 repress *PR* gene expression in the interaction between *Piriformospora indica* and Arabidopsis?

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The plant hormone ethylene (ET) plays a crucial role in the signalling network when plants have to respond to biotic stresses. We investigate the beneficial interaction between the model plant *Arabidopsis thaliana* and the endophytic fungus *Piriformospora indica*. Recently, we showed that ET signalling and ETHYLENE RESPONSE FACTOR (ERF)1 are important to balance beneficial and nonbeneficial traits in this symbiosis. 147 *ERF* genes in Arabidopsis encode transcriptional regulators with a variety of functions involved in development, physiological processes as well as plant/microbe interactions. In the beneficial symbiosis between Arabidopsis and *P. indica*, overexpression of *ERF1* activates defence responses, strongly reduces root colonization and thus abolishes the benefits for the plants. Here we show that additional transcription factors of the ERF family, the ERF DOMAIN PROTEIN9 (ERF9) and the ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR14 (ERF14) are involved in the interaction between the two symbionts and are required for growth promotion of the host plant. Expression of these genes is upregulated in colonized wild-type roots. Insertional inactivation of *ERF9* and *ERF14* diminishes the *P. indica*-induced growth promotion and activates the expression of the *PATHOGENESIS-RELATED* (*PR*)*-1* and *PR-2* genes. We propose that ERF9 and ERF14 repress *PR* gene expression in colonized Arabidopsis roots and thus contribute to the establishment of a beneficial interaction.

Introduction

Recently we have shown that ethylene (ET) signalling and ET-targeted transcription factors are required to balance beneficial and nonbeneficial traits in the symbiosis between the endophytic fungus *Piriformospora indica* and the model plant *Arabidopsis thaliana*.[1] *P. indica* belongs to the Sebacinales and colonizes roots of many plant species inter- and intracellularly including Arabidopsis. The fungus forms pear-shaped spores which accumulate in the roots as well as on the root surface, stimulates growth and seed production, confers resistance against abiotic (water and salt) stress and protects the plant against pathogen infections.[2-4] Mutants impaired in ET perception, signal transduction or ET-targeted transcription factors were examined in Camehl et al.[1] Growth of *ETR1*, *EIN2* and *EIN3/EIL1* deletion mutants was not promoted or even inhibited by *P. indica*. Overexpression of *ERF1* promoted defence responses in the presence of the fungus and abolished the benefits for the plants. Besides ET, ERF1 is a target of jasmonic acid (JA) signalling in Arabidopsis.[5] Inactivation of *ERF1* is not feasible because of the redundant function of the ERF family members. Therefore we (like others) investigated seedlings overexpressing *ERF1* under the control of the *35S* promoter. *P. indica*-induced promotion of shoot growth was reduced and of root growth was completely abrogated compared to uncolonized *35S::ERF1* control seedlings. Expression of the defence genes *PR-1, PR-2, PR-5* and *PDF1.2,* but not of *PR-3, PR-4* and *LOX1* was stimulated by the fungus in *35S::ERF1*, but not in wild-type roots. We concluded that defense responses become more efficiently activated against *P. indica* in *35S::ERF1* plants.

Here, we present data for two other transcription factors belonging to the ERF family within the superfamily AP2/ERF, which contains 147 members in Arabidopsis.[6] The AP2/ERF superfamily is defined by the ERF domain, which consists of 60 to 70 conserved amino acids involved in DNA binding. The ERF domain was first identified in the four DNA-binding proteins NtERF1-4 from *Nicotiana tabacum*,[7] which recognize a conserved GCC box present in several ET-inducible genes, e.g., in those for pathogenesis-related (PR) proteins.[8,9] Flanking sequences of the GCC box affect the binding of ERFs, thus it is likely that different ERFs regulate different target genes with a conserved GCC sequence in their promters.[10]

The AP2/ERF superfamily is divided into the RAV, AP2 and ERF families. Among them, the ERF family is the largest with 122 members and is further divided into two subfamilies, the CBF/DREB subfamily also called subgroup A and the ERF subfamily, called subgroup B.[11] The roles of the transcription factors of subgroup A are mainly involved in the regulation of abiotic

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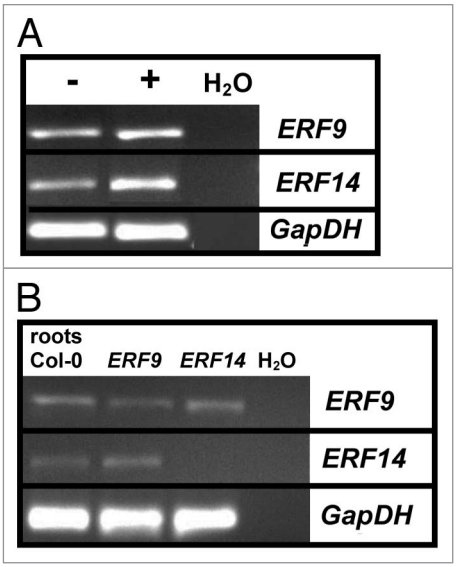

Figure 1. (A) RNA was isolated from Arabidopsis wild type roots seven days after inoculation with *P. indica* (+) or without (-) prior to RT-PCR analysis. (B) RNA was isolated from the knock-out mutant and Col-0 wild type roots prior to RT-PCR analysis.

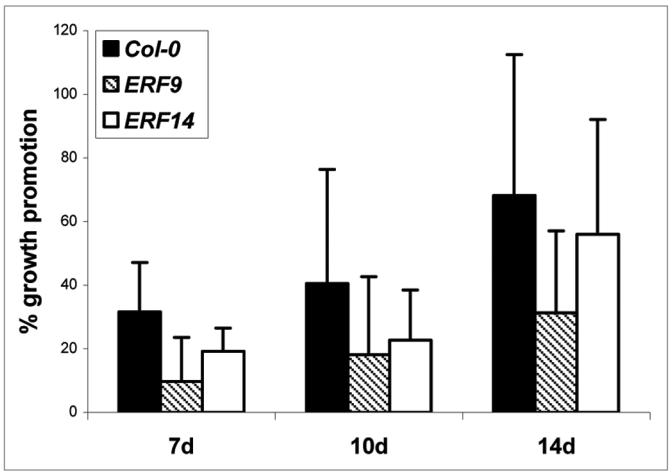

Figure 2. Root fresh weights of wild-type (Col-0) and mutant seedlings (*ERF9/ERF14*) 7, 10 and 14 days after inoculation with *P. indica*. The graph shows percent growth promotion by the fungus. Mean of four independent experiments with SE.

stress responses. For example, *DREB1A* is induced by low-temperature stress, and *DREB2A* by dehydration[12] and salt stress[13] in Arabidopsis. In contrast all transcription factors involved in disease resistance are found in the subgroup B which includes 65 members.[6,11] Since many of them are regulated by similar stimuli, a high degree of functional redundancy is expected, therefore, isolation of knock-out mutants for single transcription factor genes encoding members of the B family to discover specific phenotypes is not common. The best studied transcription factor is ERF1, and various overexpressor lines were generated and investigated.[5,14] In 2007, Oñate-Sánchez et al.[15] showed that ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR14 (ERF14) plays a key role in defense against several pathogens including *Fusarium oxysporum*. Here, we demonstrate that *ERF14* and another member of the B-family, *ERF9*, are also involved in the beneficial interaction between Arabidopsis and *P. indica*.

Tsutsui et al.[16] showed in 2009 that DEAR1 (a member of the A family) plays a regulatory role in both freezing tolerance and response to pathogen infection. The authors concluded that DEAR1 mediates the crosstalk between abiotic and biotic stress signalling pathways in plants. *ERF9* has a motif in its promoter region which can act as a target of DEAR1.[16] *ERF9* expression is strongly reduced in *DEAR1* overexpressor lines whereas *PR-1*, *-2*, *-3* and *-5* are constitutively upregulated.[16] Here, we present evidence that ERF9 might be a negative regulator of *PR* gene expression. Since the *ERF14* and *ERF9* mRNA levels are upregulated in *P. indica*-colonized Arabidopsis roots, both members of the subgroup B were further investigated in this study.

Results

***ERF9* and *ERF14* seedlings are affected in their growth response to *P. indica*.** The transcript levels of *ERF9* and *ERF14* are upregulated in the roots of wild type plants exposed to *P. indica* for 7 days (**Fig. 1A**). Therefore, we generated homozygote knock-out lines for *ERF9* and *ERF14*. Since the insertion in *ERF14* is in the exon, no transcripts can be detected in a semi-quantitative RT-PCR analysis, whereas *ERF9* contains a T-DNA insertion in the 5'-untranslated region and, thus, transcripts are still present in the mutant, but the amount is less than in the wild type (**Fig. 1B**). Databank analysis reveals that the *ERF9* mRNA might be longer than the annotated full length cDNA, therefore, besides a reduction in the mRNA level in the insertion line, also the stability and/or translatability of the *ERF9* transcript might be affected.

Under our co-cultivation conditions of the two symbionts, growth of wild-type Arabidopsis seedlings is stimulated by *P. indica* and the seedlings are taller than the uncolonized control seedlings.[3] Also growth of the two *ERF* insertion lines is stimulated by the beneficial fungus, however the stimulatory effect is less compared to wild type seedlings (**Fig. 2**). The same trend was observed for adult insertion plants transferred to soil (**Fig. 3**). Although these differences are not significant according to the students T-test, the tendency of the response to the fungus in all individual experiments is similar and comparable to the behaviour of *35S::ERF1*,[1] for which we could detect significant differences in the response to *P. indica*. Upregulation of the mRNA levels and the response of the insertion lines to the fungus suggest that ERF9 and ERF14 participate in the beneficial interaction between the two symbionts.

***PR-1* is upregulated in colonized *ERF9* and *PR-2* in colonized *ERF14* seedlings.** Since it is known that ERF14 plays a role in plant defense,[15] we tested several marker genes for different defense pathways in Arabidopsis roots. PR-1, PR-2, PR-3 and PR-4 are believed to be involved in systemic acquired resistance (SAR).[20] Interestingly, under our growth conditions *PR-1* is downregulated in *ERF14*, irrespective of whether the mutant is

grown in the presence or absence of *P. indica* for 7 days (**Fig. 4**). *PR-1* expression in *ERF9* is comparable to wild type seedlings, which may be caused by the residual amount of the *ERF9* mRNA in the insertion line (**Fig. 1B**).

However, we observe an upregulation of the *PR-1* mRNA level in colonized *ERF9* roots relative to the uncolonized *ERF9* control, similar to the results obtained for *35S::ERF1*.[1] In contrast, *PR-2* is clearly upregulated in colonized *ERF14* seedlings relative to the uncolonized *ERF14* control, again similar to the results obtained for *35S::ERF1*. No regulation of *PR-1* is observed in colonized *ERF14* and no regulation of *PR-2* in colonized *ERF9* roots, and both *PR* genes are also not upregulated in colonized wild-type roots (**Fig. 4**). These results suggest ERF9 represses *P. indica*-induced *PR-1* and ERF14 repress *P. indica*-induced *PR-2* expression in wild-type roots (cf. Discussion). Although the individual responses are difficult to compare due to different manipulations of the ET signalling pathway in the individual *ERF* mutants tested, it is reasonable to assume that repression of *PR* expression in the *ERF* mutants may be involved in the establishment of a beneficial interaction. The reduced growth response of the *ERF* mutants to *P. indica* correlates with the upregulation of *PR* expression in these mutants.

The *PR-3* and *PR-4* mRNA level do not respond to *P. indica* in the two insertion lines (**Fig. 4**), similar to their regulation in *35S::ERF1*.[1] Furthermore, *PDF1.2* expression is also not regulated in the two insertion lines, although this gene responded strongly to the fungus in the *35S::ERF1* overexpressor. Finally, in none of the ET signalling and ET-related transcription factor mutants analysed, the *ERF1* mRNA level responded to *P. indica* in the roots (**Fig. 4**).[1]

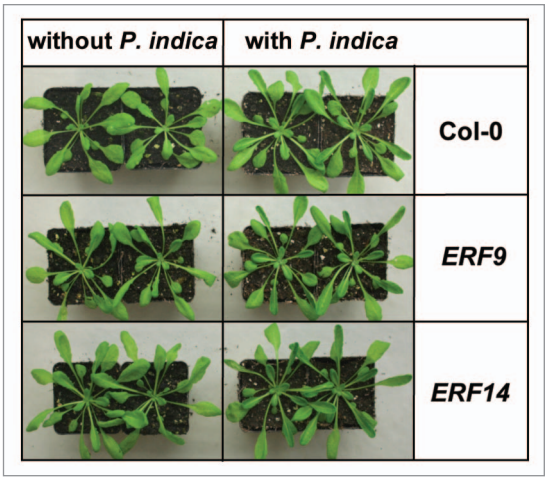

Figure 3. Plants grown 10 days on MS media were transferred to soil which was either inoculated with or without 1% *P. indica* mycel. Plants were grown for four weeks under short day conditions.

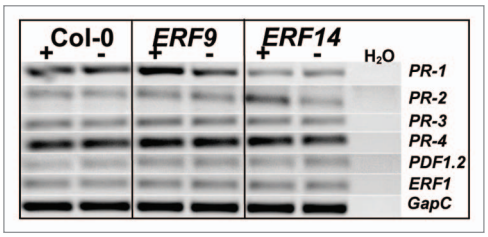

Figure 4. mRNA levels for different defence genes in the roots of wild type (Col-0) and mutant roots (*ERF9/ERF14*) cocultivated with (+) or without (-) the fungus for seven days.

Discussion

In the article by Camehl et al.[1] we showed that upregulation of *ERF1* causes an imbalance of beneficial and nonbeneficial traits in the *P. indica*/Arabidopsis symbiosis. Here we extend these studies for two other ET-responsive transcription factors, ERF9 and ERF14, by studying the response of corresponding insertion lines to the fungus. Growth of *ERF14* and *ERF9* is still promoted by *P. indica*, but less than in wild type (**Figs. 2 and 3**). Thus, complete (*ERF14*) or partial (*ERF9*) inactivation of a single ERF transcription factor results already in an altered response to *P. indica*. The weaker effects observed in the studies here compared to those reported for the *35S::ERF1* line are probably caused by different experimental manipulations of the ERF protein levels (overexpression vs. knock-out or knock-down), by redundant functions of the ERF proteins which prevents a complete loss of function in insertion lines, or by the fact that *ERF9* is only partially inactivated. Nevertheless, all results support the idea that ERFs participate in the interaction between Arabidopsis and *P. indica*. Furthermore, there are unique features of the investigated transcription factors which have not yet been considered so far.

ERF9 is unique within the ERF family because it contains an "ET response factor-associated amphiphilic repression" (EAR) motif, which might function as a transcriptional repressor.[21] Furthermore, a "dehydration-responsive element binding" motif (5'-TACCGACAT-3')—first identified in the promoter of the drought-responsive gene *RD29A* from Arabidopsis[22]—is also present in the *ERF9* promoter. This motif binds DREB transcriptional regulators,[23] one of them is DEAR1. Tsutsui et al.[16] have shown that overexpression of DEAR1 leads to a repression of *ERF9* expression. On the other hand *PR* genes and *PDF1.2* are constitutive upregulated in the *DEAR1* overexpressor line, which also accumulates endogenous salicylic acid (SA). The authors proposed that DEAR1 functions as a negative transcriptional regulator of the SA- and the ET/JA-induced signalling pathways. The higher *ERF9* mRNA level in the *P. indica*-colonized roots is consistent with conclusions from Tsutsui et al.[16] that ERF9 might be involved in *PR* gene expression. Since relatively little is known about the function of ERF9, a complete knock-out line or an overexpression line for *ERF9* would help to understand the role of this putative repressor in both beneficial and pathogenic plant/microbe interactions.

PR-1 and *PR-2* expression is differentially regulated in the roots of colonized *ERF9* and *ERF14* seedlings. *PR-1* is upregulated in *ERF9* roots after treatment with *P. indica*, but not in *ERF14* and wild-type roots (**Fig. 4**). Likewise, *PR-2* is upregulated in *ERF14* roots after treatment with *P. indica*, but not in *ERF9* and wild-type roots. This is consistent with the idea that *P. indica* stimulates *ERF9* (*ERF14*) gene expression (**Fig. 1**) in

order to repress *PR-1* (*PR-2*) gene expression for establishing a mutualistic interaction (**Fig. 4**). It is tempting to speculate that such regulatory circuits may be crucial for restricting defense gene activation in beneficial plant/microbe symbioses, in particular, since *ERF9* and *ERF14* are upregulated in colonized wild-type roots (**Fig. 1**). ERFs may play a crucial role in beneficial plant/microbe interactions, since this transcription factor family contain both transcriptional activators and repressors.[24] It remains to be determined whether selected activation of specific members of this gene family may participate in the decision whether a microbe is accepted as friend or foe. Mutualism or parasitism is directly connected to defense gene expression, which affects microbial growth and root colonization.[1,4,25]

In addition, *PR-1* expression is constitutively downregulated in *ERF14*, but not in *ERF9* roots when compared to the wild-type, independent of fungal colonization (**Fig. 4**). This clearly defines a stimulatory role for ERF14 in *PR-1* expression in the roots. Oñate-Sánchez[15] found no difference in *PR-1* expression in the leaves of knock-out and the wild type plants and under their conditions *PR-1* expression was at the detection limit. In *ERF14* overexpressor lines, *PR-1* and *PR-3* were upregulated in the leaves compared to the wild type. Thus, *ERF14* acts directly or indirectly as an activator of *PR-1* in both leaves and roots.

PR-1 and *PR-2* are specific marker genes for the SA pathway, whereas *PR-3* and *PDF1.2* are marker genes for ET/JA pathway.[5,26] *PR-1* and *PR-2* are regulated differently in the two ERF mutants whereas they are not regulated in the wild-type. *PR-3* and *PDF1.2* are not regulated by the fungus in neither the wild type nor the mutants (**Fig. 4**). Thus, defense gene activation by *P. indica* cannot be attributed unambiguously to any of the two proposed pathways. Most strikingly, *PR-1*, but not *PR-2* is upregulated in colonized *ERF9* and vice versa in colonized *ERF14* seedlings, although both genes are believed to be regulated by the SA pathway. PR-1 has antifungal properties but the microbial targets are unkonwn.[27] The proposed antimicrobial target of PR-2 is the fungal cell wall component β-1,3 glucan.[28] PR-3 and PR-4 have chitinase activity.[29] The exact role of the PR proteins and their regulation during the establishment of the beneficial interaction between *P. indica* and Arabidopsis remains to be determined.

We could lately show that elevation of the *ERF1* mRNA level triggers defense gene activation in colonized Arabidopsis roots and both, SA- and ET/JA-regulated defence genes respond to the fungus. We concluded that the activation is not pathway specific.[1] The data shown here support this idea. Many responses depend on a cross-talk between the two signalling pathways in pathogenic plant/microbe interactions.[30]

While ERF1 may function as a putative activator of *PR-1*, *PR-2*, *PR-5* and *PDF1.2* in the beneficial interaction between the two symbionts,[1] ERF9/ERF14 may repress *PR-1*/*PR-2* expression. Considering that ERF transcription factors can function as transcriptional activators and repressors of defense genes, it appears that we are only at the beginning to understand the signalling events that occur in both beneficial and pathogenic interactions.

Material and Methods

Growth conditions of plant and fungus. Wild type (ecotype *Columbia*) and homozygote T-DNA insertion Arabidopsis seeds (*ERF9*: SALK_091532O and *ERF14*: SALK_118494C) were surface-sterilized and placed on Petri dishes containing MS nutrient medium.[17] After cold treatment at 4°C for 48 h, plates were incubated for 7 days at 22°C under continuous illumination (100 μmol m^{-2} sec^{-1}). *P. indica* was cultured as described previously[2,18] on Kaefer medium.[19] For solid medium 1% (w/v) agar was included.

Co-cultivation experiments and estimation of plant growth. Nine days after plating Arabidopsis seeds on MS medium, the seedlings were transferred to nylon disks (mesh size 70 μm) and placed on top of a modified plant nutrient culture medium (5 mM KNO_3, 2 mM $MgSO_4$, 2 mM $Ca(NO_3)_2$, 0.01 μM $FeSO_4$, 70 μM H_3BO_3, 14 μM $MnCl_2$, 0.5 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.2 μM Na_2MoO_4, 0.01 μM $CoCl_2$, 10.5 g l^{-1} agar, pH 5.6), in Petri dishes. One seedling was used per Petri dish and one fungal plug of 5 mm in diameter was placed at a distance of 1 cm from the roots. The plates were incubated at 22°C under continuous illumination from the side (80 μmol m^{-2} sec^{-1}). Fresh weights were determined directly after seedlings were removed from the plates.

Experiments on soil. Arabidopsis plants were cultivated on MS medium as described above for 10 days. The soil was mixed carefully with the mycelium (1%, w/v), which was obtained from liquid cultures after the medium was removed. Cultivation occurred in small plastic pots with Aracon tubes in a temperature-controlled growth chamber at 22°C under long-day conditions (light intensity: 80 μmol m^{-2} sec^{-1}). The sizes of the plants were daily monitored.

Semiquantitative RT-PCR. Total RNA was isolated from 10 pooled replicates of Arabidopsis roots with the RNeasy kit from Qiagen according to the protocol provided by the manufacturer. cDNA synthesis was performed with the Omniscript kit from Qiagen. All reactions were repeated with two independent biological replicates.

The transcript levels of *ERF9* and *ERF14* were tested with the following primer pairs: *ERF14* (At1g04370), GGA TCA AGG AGG TCG TAG CAG TGG and TTA TTG CCT CTT GCC CAT GTT G; *ERF9* (At5g44210), GCT CCA AGA CAG GCG AAC GGT AGA and CTA AAC GTC CAC CAC CGG TGG A.

Expression of selected defense genes was analysed after 7 days of co-cultivation of *P. indica* with Arabidopsis roots with the following primer pairs: *PR-1* (At2g14610), TGT ATG AGT CTG CAG TTG CC and CAA CTG CAG ACT CAT ACA; *PR-2* (At3g57260), ACC ACA CAG CTG GAC AAA TCG and ATG AGC TCG ATG TCA GAG CCA; *PR-3* (At3g12500), TCA TGG GGC TAC TGT TTC AAG and TAT TGC TCT ACC GCA TAG ACC; *PR-4* (At3g04720), GAC CTC GTG GTC AAG CTT CTT and TTG CTA CAT CCA AAT CCA AGC; *PDF1.2* (At5g44420), CTT GTG TGC TGG GAA GAC ATA and AGC ACA GAA GTT GTG CGA GAA and *ERF1* (At3g23240), CCT TCC GAT CAA ATC CGT AAG

and TCC CGA GCC AAA CCC TAA TAC. For the housekeeping gene *GAPC2* (At3g04120) GAG CTG ACT ACG TTG TTG AG and GGA GAC AAT GTC AAG GTC GG were used.

Acknowledgements

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References

1. Camehl I, Sherameti I, Venus Y, Bethke G, Varma A, Lee J, et al. Ethylene signalling and ethylene-targeted transcription factors are required to balance beneficial and nonbeneficial traits in the symbiosis between the endophytic fungus *Piriformospora indica* and *Arabidopsis thaliana*. New Phytol 2010; 4:1062-73.
2. Peškan-Berghöfer T, Shahollari B, Giang PH, Hehl S, Markert C, Blanke V, et al. Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant-microbe interactions and involves early plant protein modifications in the endoplasmatic reticulum and at the plasma membrane. Physiol Plant 2004; 122:465-77.
3. Oelmüller R, Sherameti I, Tripathi S, Varma A. *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis 2009; 49:1-17.
4. Johnson JM, Oelmüller R. Mutualism or parasitism: life in an unstable continuum. What can we lean from the mutualistic interaction between *Piriformospora indica* and *Arabidopsis thaliana*? Endocyt Cell Res 2009; 19:81-110.
5. Lorenzo O, Piqueras R, Sanchez-Serrano JJ, Solano R. ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 2003; 15:165-78.
6. Nakano T, Suzuki K, Fujimura T, Shinshi H. Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant Physiol 2006; 140:411-32.
7. Ohme-Takagi M, Shinshi H. Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell 1995; 7:173-82.
8. Eyal Y, Meller Y, Lev-Yadun S, Fluhr R. A basic-type PR-1 promoter directs ethylene responsiveness, vascular and abscission zone-specific expression. Plant J 1993; 4:225-34.
9. Meller Y, Sessa G, Eyal Y, Fluhr R. DNA-protein interactions on a cis-DNA element essential for ethylene regulation. Plant Mol Biol 1993; 23:453-63.
10. Tournier B, Sanchez-Ballesta MT, Jones B, Pesquet E, Regad F, Latche A, et al. New members of the tomato ERF family show specific expression pattern and diverse DNA-binding capacity to the GCC box element. FEBS Lett 2003; 550:149-54.
11. Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, Yamaguchi-Shinozaki K. DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem Biophys Res Commun 2002; 290:998-1009.
12. Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, et al. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 1998; 20:1391-406.
13. Nakashima K, Shinwari ZK, Sakuma Y, Seki M, Miura S, Shinozaki K, et al. Organization and expression of two Arabidopsis *DREB2* genes encoding DRE-binding proteins involved in dehydration- and high-salinity-responsive gene expression. Plant Mol Biol 2000; 42:657-65.
14. Solano R, Stepanova A, Chao Q, Ecker JR. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev 1998; 12:3703-14.
15. Oñate-Sánchez L, Anderson J, Young J, Singh K. AtERF14, a member of the ERF family of transcription factors, plays a nonredundant role in plant defense. Plant Physiol 2007; 143:400-9.
16. Tsutsui T, Kato W, Asada Y, Sako K, Sato T, Sonoda Y, et al. DEAR1, a transcriptional repressor of DREB protein that mediates plant defense and freezing stress responses in Arabidopsis. J Plant Res 2009; 122:633-43.
17. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol 1962; 15:473-97.
18. Verma SA, Varma A, Rexer K-H, Hassel A, Kost G, Sarbhoy A, et al. *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. Mycologia 1998; 90:898-905.
19. Hill TW, Kaefer E. Improved protocols for Aspergillus medium: trace elements and minimum medium salt stock solutions. Fungal Genet Newsl 2001; 48:20-1.
20 Van Loon LC, Van Strien EA. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol Mol Plant Pathol 1999; 55:85-97.
21. Otha M, Matsui K, Hiratsu K, Shinshi H, Ohme-Takagi M. Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. Plant Cell 2001; 8:1959-68.
22. Yamaguchi-Shinozaki K, Shinozaki K. A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature or high-salt stress. Plant Cell 1994; 6:251-64.
23. Huang B, Liu JY. A cotton dehydration responsive element binding protein functions as a transcriptional repressor of DRE-mediated gene expression. Biochem Biophys Res Commun 2006; 343:1023-2031.
24. Broekaert WF, Delauré SL, De Bolle MF, Cammue BP. The role of ethylene in host-pathogen interactions. Annu Rev Phytopathol 2006; 44:393-416.
25. Sherameti I, Venus Y, Drzewiecki C, Tripathi S, Dan VM, Nitz I, et al. PYK10, a beta-glucosidase located in the endoplasmatic reticulum, is crucial for the beneficial interaction between *Arabidopsis thaliana* and the endophytic fungus *Piriformospora indica*. Plant J 2008; 54:428-39.
26. Penninckx IA, Thomma BP, Buchala A, Métraux JP, Broekaert WF. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. Plant Cell 1998; 12:2103-13.
27. Antoniw JF, Ritter CE, Pierpoint WS, Van Loon LC. Comparison of three pathogenesis-related proteins from plants of two cultivars of tobacco infected with TMV. J Gen Virol 1980; 47:79-87.
28. Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, et al. Acquired resistance in Arabidopsis. Plant Cell 1992; 4:645-56.
29. Van Loon LC. Regulation of changes in proteins and enzymes associated with active defense against virus infection, in: Wood RKS, (Ed.), Active Defense Mechanisms in Plants. Plenum Press, New York 1982; 247-73.
30. Leon-Reyes A, Spoel S, De Lange E, Abe H, Kobayashi M, Tsuda S, et al. Ethylene modulates the role of NON EXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. Plant Physiol 2009; 149:1797-809.