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The Rice 14-3-3 Gene Family and its Involvement in Responses to Biotic and Abiotic Stress

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Abstract

14-3-3 proteins function as major regulators of primary metabolism and cellular signal transduction in plants. However, their involvement in plant defense and stress responses is largely unknown. In order to better address functions of the rice 14-3-3/GF14 proteins in defense and abiotic stress responses, we examined the rice GF14 family that comprises eight numbers. The phylogenetic comparison with the *Arabidopsis* 14-3-3 family revealed that the majority of rice GF14s might have evolved as an independent branch. At least four rice *GF14* genes, *GF14b*, *GF14c*, *GF14e* and *Gf14f* were differentially regulated in the interactions of rice-*Magnaporthe grisea* and rice-*Xanthomonas oryzae* pv. *oryzae*, and the incompatible interactions stronger induced the genes than the compatible interactions. These *GF14* genes were also induced by the defense compounds, benzothiadiazole, methyl jasmonate, ethephon and hydrogen peroxide. Similarly, they were differentially regulated by salinity, drought, wounding and abscisic acid. Tissue-specific analysis and expression of GF14-YFP fusions revealed that the four GF14 isoforms were expressed with tissue specificity and accumulated differentially in the cytoplasm and nucleus. Our current study provides fundamental information for the further investigation of the rice GF14 proteins.

Key words: rice; 14-3-3 family; biotic and abiotic stress; expression regulation; subcellular localization

1. Introduction

All plant biological processes are controlled by signal transduction and metabolism regulation that have been known to occur via phosphorylation-mediated transition of protein states. In many cases, to complete their regulatory actions, these phosphorylated proteins must physically associate with the specialized adapter proteins, which are known as 14-3-3 phosphoserine/threonine binding proteins.[1–4] It is one hallmark of signal transduction and metabolism events to identify 14-3-3 participation in cellular regulatory pathways in which 14-3-3s, generally acting as adapters, chaperones, activators or repressors, interact physically with target (client) proteins phosphorylated to execute an important step in signal transduction and metabolism.

There are several lines of evidence to show that 14-3-3s are involved in many metabolic and signaling pathways for plant growth regulation and responses to environmental stress. The most prominent paradigms involving 14-3-3s in plant regulatory events include the regulation of plasma membrane H^+-ATPase, nitrate reductase (NR) and sucrose phosphate synthase (SPS).[1,4] 14-3-3s have been known as positive regulators of H^+-ATPase activity, with 14-3-3s and Mg^{2+} bound to the C-terminal region of the H^+-ATPase, pump activity is stimulated. Interestingly, a wilt-inducing phytotoxin fusicoccin (FC) binds to the complex of 14-3-3s and H^+-ATPase, resulting in continuous high-activity state of H^+-ATPase, guard cell solute uptake and excessive transpiration that leads to leaf wilt.[5–7] Whereas, 14-3-3s function as inhibitors of NR and SPS. Mg^{2+} binding causes a structural change in 14-3-3s that allows binding to the phosphorylated target enzymes, a process known to inactivate NR and SPS.[1] 14-3-3 proteins also bind a range of

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transcription factors for their activation and translocation.[8] For example, the tobacco transcription factor RSG (repression of shoot growth) is bound to 14-3-3s and involved in GA biosynthesis.[9,10]

Interestingly, 14-3-3 proteins function as regulators of a wide range of target proteins that are involved in responses to abiotic and biotic stress, by regulating target proteins with functions of either signaling or transcription activation or defense.[11] For example, the tobacco *T14-3-3* is induced in the perception of the salt stress[12]; two *Arabidopsis* 14-3-3 genes, *RCI1/RCI1A* and *RCI2/RCI1B*, are regulated during cold acclimation[13]; *Arabidopsis* 14-3-3 isoforms could bind to and probably activate a stress-responsive calcium-dependent protein kinase, CPK-1.[14,15] The activity of mitochondrial and chloroplast F0F1 ATP synthases, regulated by 14-3-3s, is involved in responses to light/dark transitions, anoxia changes in nutrient supply.[16] Recently, Yan et al.[17] reported that the transgenic cotton plants with constitutive expression of *GF14*λ displayed phenotypes of 'stay-green' and enhanced tolerance to drought stress, probably through the regulation of the stomatal aperture by the complex of 14-3-3s and H^+-ATPase. 14-3-3s have been found involved in plant defense responses.[11,18] For instance, a 14-3-3 gene was identified to be regulated in the non-host hypersensitive response (HR) between barley and *Blumeria graminis* f.sp. *tritici*.[19] Furthermore, it is well characterized that 14-3-3s bind to and activate H^+-ATPase, creating a binding site for the phytotoxin FC, and FC-binding activity of an epidermal microsomal fraction increases upon the pathogen attack, suggesting that 14-3-3s are involved in an epidermis-specific response to the fungus probably through activating the proton pump (H^+-ATPase) to stimulate the HR.[20] Caffeic acid/5-hydroxyferulic acid *O*-methyltransferase (OMT1) and ascorbate peroxidase implicated in plant defense or oxidative stress are identified to interact with 14-3-3s by yeast two-hybrid screening.[21,22] It is also shown that the white spruce and hybrid poplar 14-3-3s are up-regulated by wounding or wounding elicitors, or by chitosan and jasmonic acid, two defensive elicitors, and H^+-ATPase is a potential target for the 14-3-3-mediated regulation during stress.[23] Similar to FC, other two inducers of programmed cell death, tunicamycin and brefeldin A, also induce the accumulation of 14-3-3 proteins.[24] Interestingly, a 14-3-3-interactor, AKR2, is an ankyrin-repeat containing protein and negatively regulates transcription factors that mediate defense responses.[25,26] The *AKR2*-antisense plants developed HR-like lesions with increased H_2O_2 generation and exhibited increased resistance to a bacterial pathogen.[26]

Plants have large 14-3-3 gene families, and various 14-3-3 isoforms have differential affinities for certain target proteins. The dicotyledonous model plant *Arabidopsis* has hitherto the most complete and largest 14-3-3 family which consists of 15 members with at least 12 expressed members classified into two evolutionary branches, the ε group and the non-ε group.[3,4] These 14-3-3 isoforms exhibit a high cell and tissue-type specificity and are localized within the cytoplasm and organelles such as nuclei, plastids and mitochondria.[3] The expression specificity and subcellular compartment of isoforms contribute to their diverse interactions with partners and differential functions in cellular activities. However, our understanding of the significance of 14-3-3 proteins in the regulation of plant development and environmental adaptation is still in its infancy.

Rice, with its relative small genome and important staple food crop, has been adopted as a monocotyledonous model plant for dissecting genetic networks of biotic and abiotic stress responses in cereal crops. The completion of the rice genome project allows genome-wide searching for the 14-3-3 family in the genome,[27] which has been found involved in rice stress responses as well as development. For example, the rice 14-3-3 proteins, GF14b, GF14c, GF14e and GF14f (previously named SR14-3-3), interact with target proteins that are involved in stress responses.[28] Interestingly, GF14b and GF14f may interact with mitogen-activated protein (MAP) kinase BIMK1, a component induced by rice blast fungus and known to participate in systemic acquired resistance.[28] In a large-scale microarry analysis for rice genes involved in defense responses, we identified some 14-3-3 genes were regulated by different interactions between rice and the pathogens or by defense signaling compounds. In this report, we analyzed the phylogenetics of the rice 14-3-3 family comprising eight members that are all expressed. We systemically analyzed expression patterns of four 14-3-3 genes that were differentially regulated by the rice blast fungus *Magnaporthe grisea* (*M. grisea*) and bacterial blight *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), defense signaling compounds and diverse abiotic stress stimuli. We also observed subcellular locations of these isoforms.

2. Materials and methods

2.1. Mining of rice14-3-3s and phylogenetic analysis

Rice 14-3-3s were searched from the whole rice genome (*japonica* Nipponbare) with the conversed 14-3-3 protein sequence via the public GenBank database (http://www.ncbi.nlm.nih.gov/). The retrieved cDNAs or coding regions of 14-3-3s then were searched for their full-length cDNAs in the KOME database (http://cdna01.dna.affrc.go.jp/cDNA/). Genomic structures of the rice 14-3-3 genes were analyzed by comparing the cDNA sequences and the corresponding genomic DNA sequences. ClustalX 1.81 and Genedoc were used to multi-align nucleotide acid and amino acid sequences of

the 14-3-3s. ClustalX 1.81 and TreeView 1.6.1 were used to perform phylogenetic analysis.

2.2. Plant growth and inoculation

A pair of rice (*Oryza sativa* L. ssp. *indica*) near isogenic lines, H7R (resistant to rice blast, with the resistance gene *Pi-k/Pi-r1(t)* from the donor Tetep) and H7S (susceptible) were grown in a growth chamber under 28°C/22°C and 14 h/10 h (day/night) for 2 weeks, then were spray-inoculated with *M. grisea* race ZB1 as described.[29] Rice suspension cells were made from the transgenic rice line 106 expressing the resistance gene *Xa21* against *Xoo*[30] and were co-incubated with 10^7 cells per ml of the incompatible *Xoo* race P6 (99A) and the compatible race K1 (J18) as described.[31] Inoculated leaves/cells were harvested at a time course of 0–24 h postinoculation. All tissues were washed to remove surface microbes and frozen in liquid nitrogen immediately then stored at −80°C for RNA preparation.

2.3. Treatments with defense signaling compounds

Two-week-old seedlings of H7R were spray-treated with 300 μM benzothiadiazole (BTH, a salicylic acid analog), 100 μM methyl jasmonate (MeJA), and 100 μM ethephon (ETH, a precursor of ethylene), in the closed pots at a growth chamber. Water-spray was used as mock controls. Leaves were collected at 0, 4, 8, 12 and 24 h after treatments. The rice suspension cells were incubated for 0, 2, 6 and 12 h with various concentrations of H_2O_2.

2.4. Treatments with abiotic stress

Two-week-old seedlings of H7R were cultured with 150 mM NaCl, 20% PEG 6000 for salt and drought stress treatments, and with 100 μM abscisic acid (ABA) solution for 0–48 h. For wounding treatment, leaves were cut into 2–3 cm pieces and kept in 50 mM potassium phosphate buffer (pH 7.0) for 0–48 h.

2.5. RNA preparation and northern blot

Total RNAs were isolated from cells by using TRIzol tragent according to the manufacturer's protocol (Waston Biot). Each 30 μg of total RNAs was separated on a 1% formaldehyde-agarose gel for capillary blotting onto Hybond nylon membrane (Amersham Pharmacia), and hybridized with probes derived from a 320 bp *Sac*II/*Sfi*I fragment from *GF14b*, a 420 bp *Sfi*I fragment from *GF14c*, a 400 bp *Bcl*I/*Sfi*I fragment from *GF14e* and a 350 bp *Cfr13I/Sfi*I fragment from *GF14f*. Similarities between each probe and the other *GF14* genes are <55% to ensure probe specificity.

2.6. Tissue-specific expression of GF14 genes

Total RNA was prepared from seedling leaves, leaves, roots, internodes and panicles followed by the first cDNA strand synthesis for RT–PCR using SuperScript™ RT–PCR kit (Invitrogen). Primers were designed for *GF14b* (5′-AGGACATTGCTTTGGCTGAG-3′ and 5′-TTACTGCCCCTCGCTGGAG-3′), *GF14c* (5′-CGTTTGACGAAGCCATCTCC-3′ and 5′-CTAGTAGAACAGGAGAAGAATC-3′), *GF14e* (5′-GATATTGCCCTGGCAGAGTTG-3′ and 5′-GAGATATCGGAAGTCCACAGC-3′) and *GF14f* (5′-GATATTGCCCTGGCAGAGTTG-3′ and 5′-TTAGTGGCCCTCTCCTTCAG-3′), to amplify the fragments of 300 bp (*GF14b*), 280 bp (*GF14c*), 240 bp (*GF14e*) and 300 bp (*GF14f*), respectively. The rice *ubi-1* was used as the control.[32]

2.7. Subcellular localization of GF14 protein

GF14-YFP fusions were made by in-frame fusion of the full-length GF14 cDNAs with YFP (accession number AA048591). The fusion genes were inserted into the vector pCAMBIAC1301 driven by the 35S promoter and were bombard-transformed into the wild-type cultivar TP309 calli by using a helium biolistic device (Bio-Rad PDS-1000/He). Calli were grown and selected on medium supplemented with hygromycin (20 mg/L) to generate GF14-YFP transgenic calli. Transient expression of the GF14-GFP fusions in onion epidermal cells was performed as previously described.[33] The transgenic rice and onion cells were observed with a confocal laser scanning microscope (Zeiss LSM510). 4′-6-diamidino-2-phenylindole (DAPI) fluorescence staining was performed to detect the nuclei. The cells were dipped in 30% sucrose solution for 30 min to perform plasmolysis.

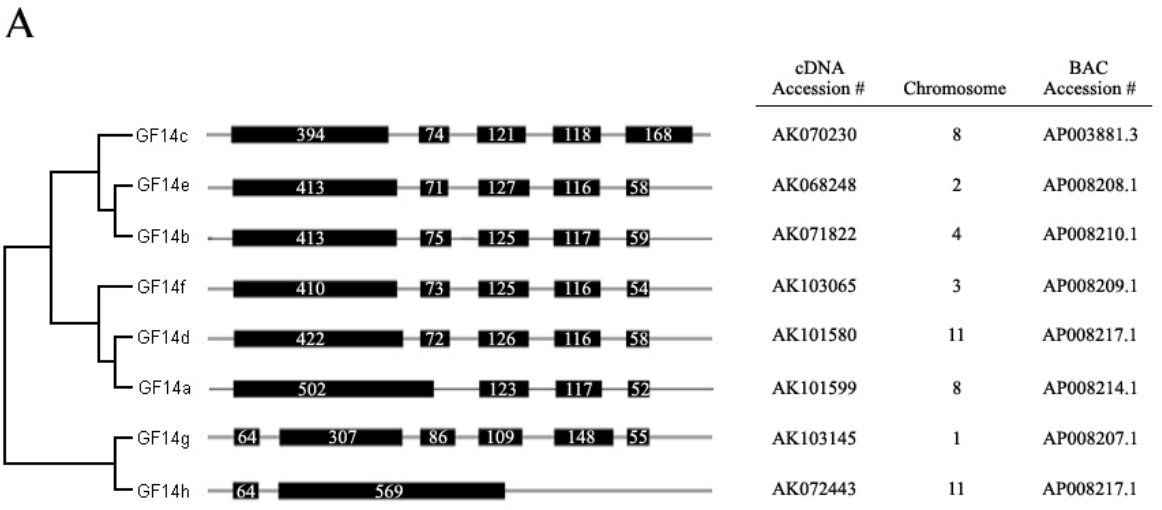
2.8. Analysis of cis-acting elements in promoter regions of GF14 genes

According to the full-length cDNA sequences, the putative promoter regions were retrieved by searching the matched genomic sequences of the genes in GenBank (http://www.ncbi.nlm.nih.gov/BLAST/), with 2000 bp upstream of the 5′ termini of the full-length cDNAs. The *cis*-acting elements, such as W, GCC and GCC-like, ABRE and DRE/CRT boxes in the promoter regions were analyzed.

3. Results

3.1. The rice GF14 family and comparison with the Arabidopsis family

By taking advantage of the completion of the rice genome project, we found that this model crop has a total of eight 14-3-3 genes located to six chromosomes that all have matched full-length cDNAs, suggesting that these genes are all expressed (Fig. 1A). Following the previous report,[28] the members of the rice family are named GF14a through GF14h (G-box factor 14-3-3 homologs), with SR14-3-3 named as GF14f. These GF14 proteins share



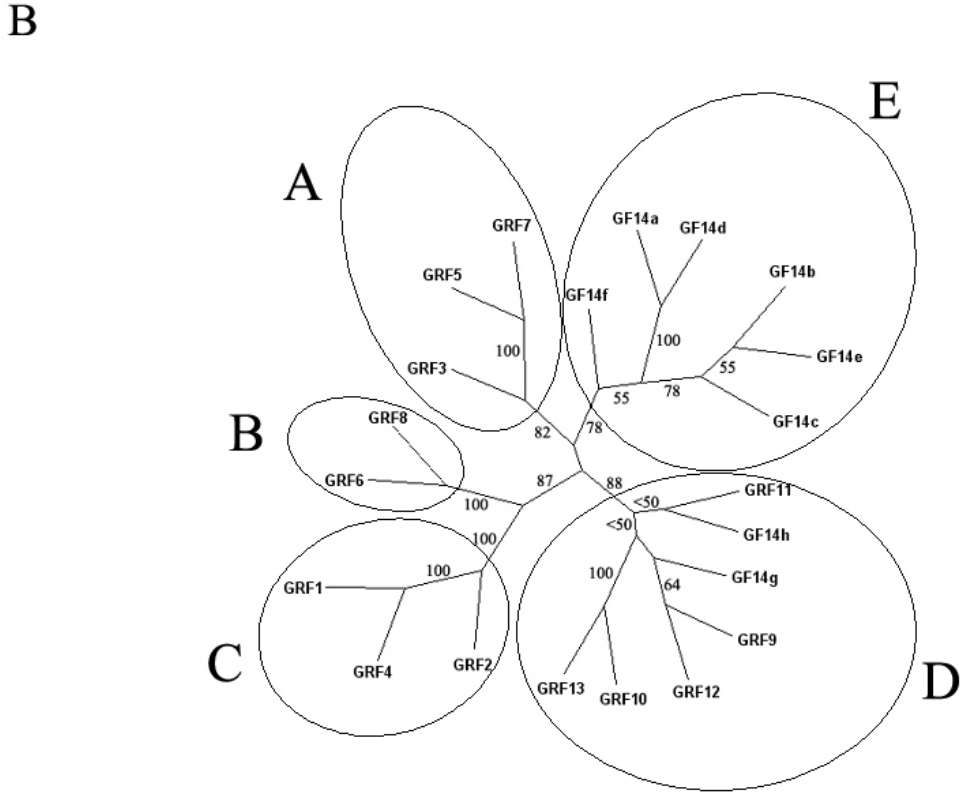


Figure 1. Rice 14-3-3 gene structure and phylogenetic tree of rice and *Arabidopsis* 14-3-3 families. (**A**) Genomic structure of eight rice 14-3-3 genes. Individual exons are delineated by segmentation. Numbers show nucleotide lengths of exons. The accession numbers for full-length cDNA and BAC clones are indicated. (**B**) A phylogenetic tree shows the topology of the rice (GF14) and *Arabidopsis* (GRF) 14-3-3 families within five major groups, based on cDNA sequences. A, B, C group are *Arabidopsis* non-ε groups. Group D belongs to ε group. Note that the majority of rice GF14s are classified into group E (non-ε group). Branch numbers represent as percentage of bootstrap values in 1000 sampling replicates.

highly conserved structures (Fig. 1A). According to the 14-3-3 classification,[4] GF14g and GF14h belong to the ε group, while the rest belong to the non-ε group. The phylogenetic topology of the rice family compared with the *Arabidopsis* family reveals five groups (Fig. 1B), of which groups A–D are similar to those for the *Arabidopsis* family and other plant 14-3-3s.[4] GF14g and GF14h fall into group D with five *Arabidopsis* 14-3-3s. However,

the majority of the rice GF14s (six out of eight) constitute an additional group E. This result suggests that separate gene duplication of the non-ε group could occur before the differentiation of the two species. The richness of diversity in these 14-3-3s indicates divergent roles of the members in biological processes in rice as well as in *Arabidopsis*.

3.2. *GF14 genes induced during the early rice-M. grisea and rice-Xoo interactions*

In a large-scale microarray analysis of rice genes involved in defense responses, we found >1000 genes regulated by pathogen infection and other stress stimuli (Li et al., unpublished). The microarray data are available in our rice genomics database (http://www.nlpmg.labs.gov.cn/Signal/Ricearray/Supplemental1.xls). Among these regulated genes, we found four of the *GF14* genes, *GF14b*, *GF14c*, *GF14e* and *GF14f*. The four GF14s share high similarity (overall 85.5–95.4% identities) on their amino acid sequences (Fig. 2A), but comparatively low similarity (overall 34.8–51.7% identities) on cDNA sequences. We analyzed the induction of these genes in the early incompatible and compatible rice-*M. grisea* interactions. Northern blot analysis showed that all four *GF14* genes were induced by the fungal pathogen in a time course of 0–24 h postinoculation (Fig. 2B). Similarly, these genes except *GF14f* were also induced in the rice-*Xoo* interactions (Fig. 2C). Furthermore, it was shown that these genes appeared to be earlier or stronger induced in the incompatible interactions than in the compatible interactions between rice and the pathogens (Fig. 2B and C), consistent with the well-recognized expression pattern of defense-related genes during the host–pathogen interactions.[34]

3.3. *GF14 genes regulated by abiotic stress*

It has been known that plant 14-3-3 proteins are involved in abiotic stress responses.[11] We further analyzed the expression patterns of these *GF14* genes in responses to abiotic stress including salinity, drought and wounding. Northern hybridization indicated that these *GF14* genes were differentially regulated by the stress stimuli (Fig. 3). The transcription of *GF14b* was rapidly induced at 2–4 h by the salt and PEG6000 (drought-mimic) treatments, then declined to basal levels. Similarly, *GF14c* and *GF14e* were also induced at the early stage of 2–8 h by salt and drought stresses, consistent with the observation that the transcription of *GF14b*, *GF14e* was induced by a variety of stress stimuli.[28] *GF14f* appeared to be induced by salinity and drought through the whole time course of 2–48 h. Wounding also induced the *GF14* genes except *GF14b* with peak levels at 24 h after treatment. Interestingly, the transcription of *GF14e* was decreased at 48 h in wounding treatment (Fig. 3). These differential expression patterns of the *GF14* genes tested in face of abiotic stresses implicate that they may play regulatory roles in environmental responses.

3.4. *GF14 genes regulated by defense and stress compounds*

It has been known that the phytohormones, salicylic acid, jasmonic acid and ethylene are signaling compounds involved in plant defense,[34] and hydrogen peroxide (H_2O_2), one of reactive oxygen intermediates resulting from an oxidative burst during the plant HR, acts as an activator of plant defense.[35] We systemically analyzed the induction of the four *GF14* genes by these molecules. As shown in Fig. 4A, *GF14f* was induced by BTH, ETH and MeJA, similar was *GF14e* except it was first slightly repressed at 4 and 8 h by BTH. *GF14b* was slightly induced by BTH and ETH but not by MeJA. Similarly *GF14c* was significantly induced at 24 h by ETH and slightly induced by BTH and MeJA. Because of no consistent information for H_2O_2 treatment of rice cells, we tested the effect of different H_2O_2 concentrations on the induction of the 14-3-3 genes. All of the four *GF14* genes were up-regulated by H_2O_2 even with low concentration (Fig. 4B). Low H_2O_2 concentration (0.01 mM) seemed to be more efficient to activate *GF14c*.

ABA acts as a stress-responsive hormone and involves in modulating tolerance to drought and salinity.[36] Our analysis showed that ABA induced *GF14e* and *GF14f* during the time course (Fig. 4C). Similar to the observation in the salt and drought treatments (Fig. 3), *GF14b* and *GF14c* were obviously induced by ABA only within early 2–8 h of treatments (Fig. 4C). In addition, *GF14c* appeared to be more subject to environmental regulation, because even its basal expression level was somewhat different among independent treatments (Figs 2B and C, 3 and 4).

3.5. *Cis-elements in promoter regions of GF14 genes*

It has been known that the *cis*-acting elements, W, GCC and GCC-like boxes, play important roles in expression regulation of defense-related genes,[37] and that the major *cis*-elements involved in abiotic stress response are ABRE and DRE/CRT.[36,38] We systematically analyzed these elements in the predicted 2.0 kb promoter regions of the four *GF14* genes (Table 1). The analysis shows that the ABRE, W and GCC/GCC-like sequences are predominant elements in the predicted promoters of the *GF14* genes analyzed, indicating their features of biotic and abiotic stress-mediated induction. We also found that the same *cis*-elements are present in the other four *GF14* genes (Table 1). The biological significance of these elements in the regulation of gene expression is yet to be investigated.

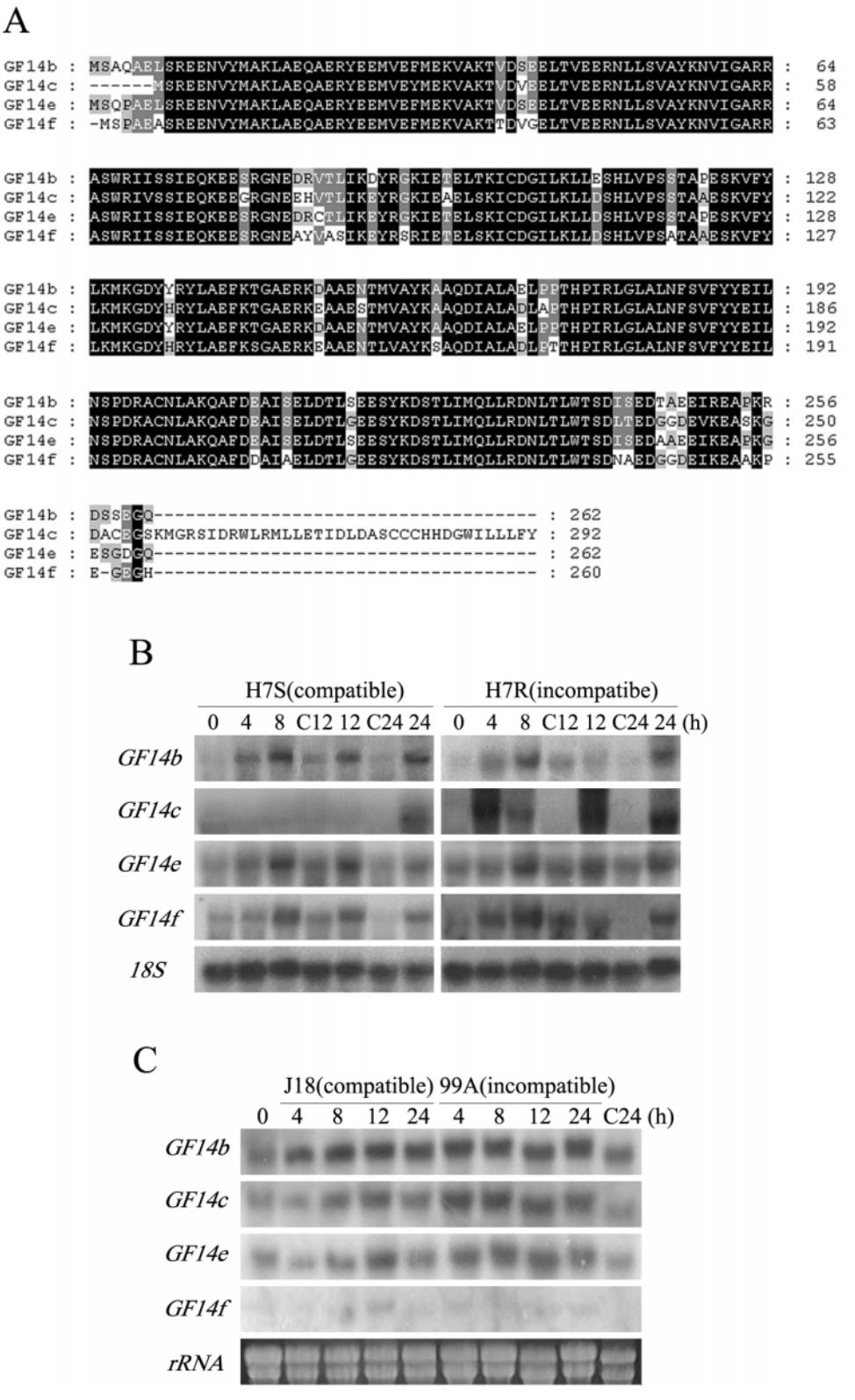


Figure 2. Alignments of four rice GF14 isoforms and their induction during rice defense responses. (**A**) Protein sequence alignment of the four GF14 isoforms with overall 85.5–95.4% identity. (**B**) Expression patterns of the *GF14* genes in the incompatible and compatible rice-*M. grisea* interactions in a time course of 0–24 h postinoculation. C12, control for 12 h; C24, control for 24 h. (**C**) Expression patterns of the *GF14* genes in the incompatible and compatible rice-*Xoo* interactions in a time course of 0–24 h postinoculation. Independent experiments were carried out with similar results. C24, control for 24 h.

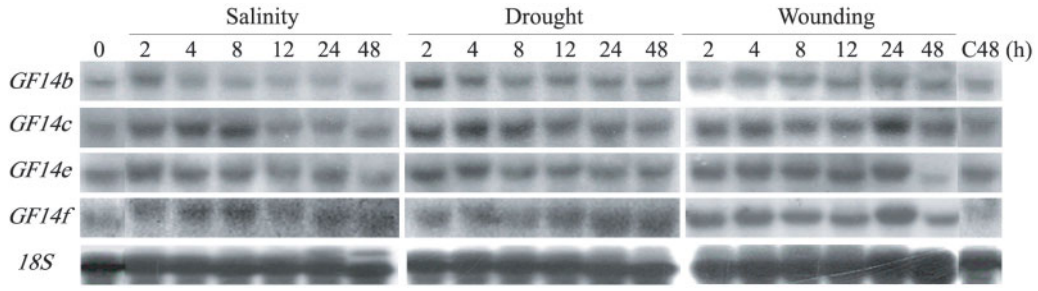


Figure 3. Differential regulation of *GF14* gene expression by abiotic stress. The transcripts of *GF14b, GF14c, GF14e* and *GF14f* were detected after salt (150 mM NaCl), drought (20% PEG6000) and wounding treatments as described. C48, control for 48 h.

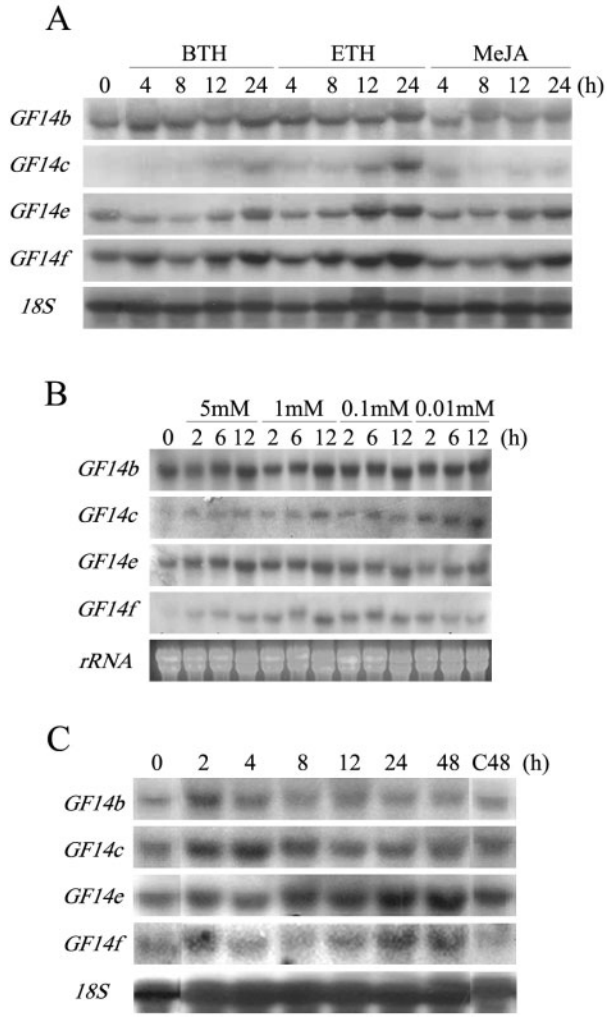


Figure 4. Differential induction of *GF14* genes by defense and stress molecules. (**A**) BTH (300 μM), MeJA (100 μM) and ETH (100 μM) differentially induced *GF14b, GF14c, GF14e* and *GF14f*. (**B**) H_2O_2 induced the *GF14* genes. Note that low H_2O_2 concentration (0.01 mM) seemed to stronger induce *GF14c*. (**C**) Induction of the *GF14* genes by ABA (100 μM). C48, control for 48 h.

3.6. Tissue-specific expression and subcellular localization of GF14 proteins

We further analyzed the tissue-specific expression patterns of the *GF14* genes, and found that these *GF14s* exhibited a degree of tissue specificity (Fig. 5). *GF14e* was expressed in all tissues tested, with the highest expression level in the panicle, similar was the expression pattern of *GF14f* except its low expression level in the flag leaf and internode of the heading plant. Whereas the transcript of *GF14b* was hardly detected in the flag leaf, internode and panicle of the heading plant, with higher level detected in the root. Similarly, the transcript of *GF14c* was hardly detected in the leaf and root of the heading plant, with the highest level detected in the seedling leaves. These tissue-specific patterns of *GF14s*' expression are probably associated with their differential functions.

14-3-3 proteins are widely localized in diverse subcellular compartments, implicating their multifunctional roles in cellular processes.[3] We made GF14-YFP fusion constructs and expressed them in rice cells. Because localization of YFP/GFP fusion proteins is usually obscure in rice callus, the cytoplasmic location of the fusion proteins was difficult to be imaged in the rice cells. We could observe obvious YFP fluorescence only in the compartments where the fusion proteins accumulated (Fig. 6A). With DAPI fluorescence probing to localize the nuclei, we observed that GF14b-YFP, GF14e-YFP and GF14f-YFP accumulated in the nuclei. For a clear vision of the localization of the YFP fusion proteins, transient expression was performed in onion epidermic cells. This additional experiment clearly showed that GF14-YFP fusion proteins were all targeted to the cytoplasm (Fig. 6B). Same to the observation with the transformed rice calli, GF14b-YFP, GF14e-YFP and GF14f also accumulated in the nuclei. Similar subcellular localization was also observed for the *Arabidopsis* 14-3-3 proteins.[3,4] Hence, diversity in the expression and subcellular localization of the rice 14-3-3 proteins is present to ensure their functions in certain cells or tissues.

4. Discussions

14-3-3 proteins, acting as ubiquitous regulators, have been known to play important roles in many plant biological processes. However, their involvement in abiotic

Table 1. Stress-responsive *cis*-elements in promoter regions of *GF14* genes[a]

	W box [(T)TGAC(Y)]	GCC/GCC-like box [(A)GCCG/ACC]	ABRE [(RY)ACGTS(BSC)]	DRE/CRT [RCCGA(SA)]
GF14a	15	3	10	4
GF14b	6	5	18	5
GF14c	9	4	22	4
GF14d	6	3	7	3
GF14e	4	10	4	1
GF14f	12	2	9	3
GF14g	1	5	2	0
GF14h	11	1	2	0

Y = C or T; R = G or A; S = C or G; B = C, G or T.
[a]2000 bp putative promoter regions were analyzed for *cis*-elements.

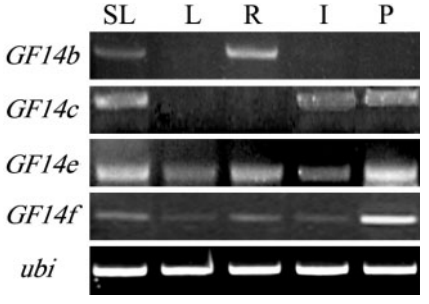


Figure 5. Tissue-specific expression and subcellular localizations of the GF14 isoforms. Expression of the *GF14* genes in different tissues, detected by RT–PCR with the rice *ubi-1* as a control. SL, seedling leaf; L, R, I, P for flag leaf, root, internode, panicle of heading plants, respectively.

and abiotic stress responses, two biological activities with agricultural significance, has not been well studied. In this paper, we have shown that the rice 14-3-3 genes, *GF14b, GF14c, GF14e* and *GF14f*, were induced differentially by the rice fungal and bacterial pathogens, defense signaling compounds and abiotic stress stimuli (Figs 2–4). Our current study adds to the previous findings of the rice 14-3-3 family[28,39,40] and would serve as a start point to explore their functions in disease resistance and stress tolerance in the model cereal crop.

It is intriguing that the rice 14-3-3 family has most likely only eight isoforms classified into 2 groups in comparison with 12 isoforms expressed in the *Arabidopsis* family (Fig. 1), given the much larger genome of rice than that of *Arabidopsis*. It has been known that, in spite of the conservation of their core structure, 14-3-3 isoforms have a degree of specificity with regard to the target proteins with which they interact.[11] It is also interesting that the rice 14-3-3 proteins except two constitute a separate group (group E) (Fig. 1B). A systemic and comparative analysis of the target proteins of the rice and *Arabidopsis* 14-3-3 isoforms would contribute to fundamental understanding of the conservation and divergence of biological processes between the two model plants.

We found that all of four *GF14* genes studied were differentially regulated by the rice pathogen challenge and abiotic stress, indicating they may play important roles in rice defense and abiotic stress tolerance. Similar involvement of 14-3-3s in defense and stress responses has also been frequently observed in other plants. In addition to the evidence described above, a 14-3-3 gene is induced during the HR of soybean inoculated with *P. syringae*,[41] and in cotton inoculated with the wilt pathogen *V. dahliae*.[42] In tomato, ten 14-3-3 genes were found to be differentially regulated in resistant or susceptible plants challenged with the avirulence elicitor Avr9.[18] In ABA-mediated stress responses, 14-3-3 proteins are constituents of transcription factor complexes for ABA-induced gene expression.[2] Direct evidence for 14-3-3s involved in disease resistance or stress tolerance also comes from genetic studies. A quantitative resistance marker (QTL) against fungal pathogens is mapped onto a region of chromosome 4AL of wheat containing a 14-3-3 gene.[43] *GF14c* is localized within the interval of drought tolerance QTL *oa8.1*, and SR14-3-3/GF14f interacts with an ATPase known to locate to the region of drought tolerance QTL *oa3.1*.[28,39] More recently, *GF14e* (previously named as 14-3-3 protein-A) is localized in the QTL region for blast resistance on chromosome 2.[40] It has been extensively documented that transmembrane ion flux is involved in early defense signaling, therefore, a role for some 14-3-3 proteins in plant defense can be speculated, which probably interact with some membrane-bound clients such as H^+-ATPase. Interestingly, *GF14b* and *GF14c* exhibited similar induction patterns during the salinity, drought and ABA treatments (Figs 3 and 4C), whether they play an important role in face of these stress conditions remains to be evaluated. We also found the *cis*-elements responsive to pathogen attack and abiotic stress in the rice *GF14* genes (Table 1), appropriately explaining the features of the induction of the genes by biotic and abiotic stresses. Therefore, 14-3-3s are also subject to the regulation by certain transcript factors.

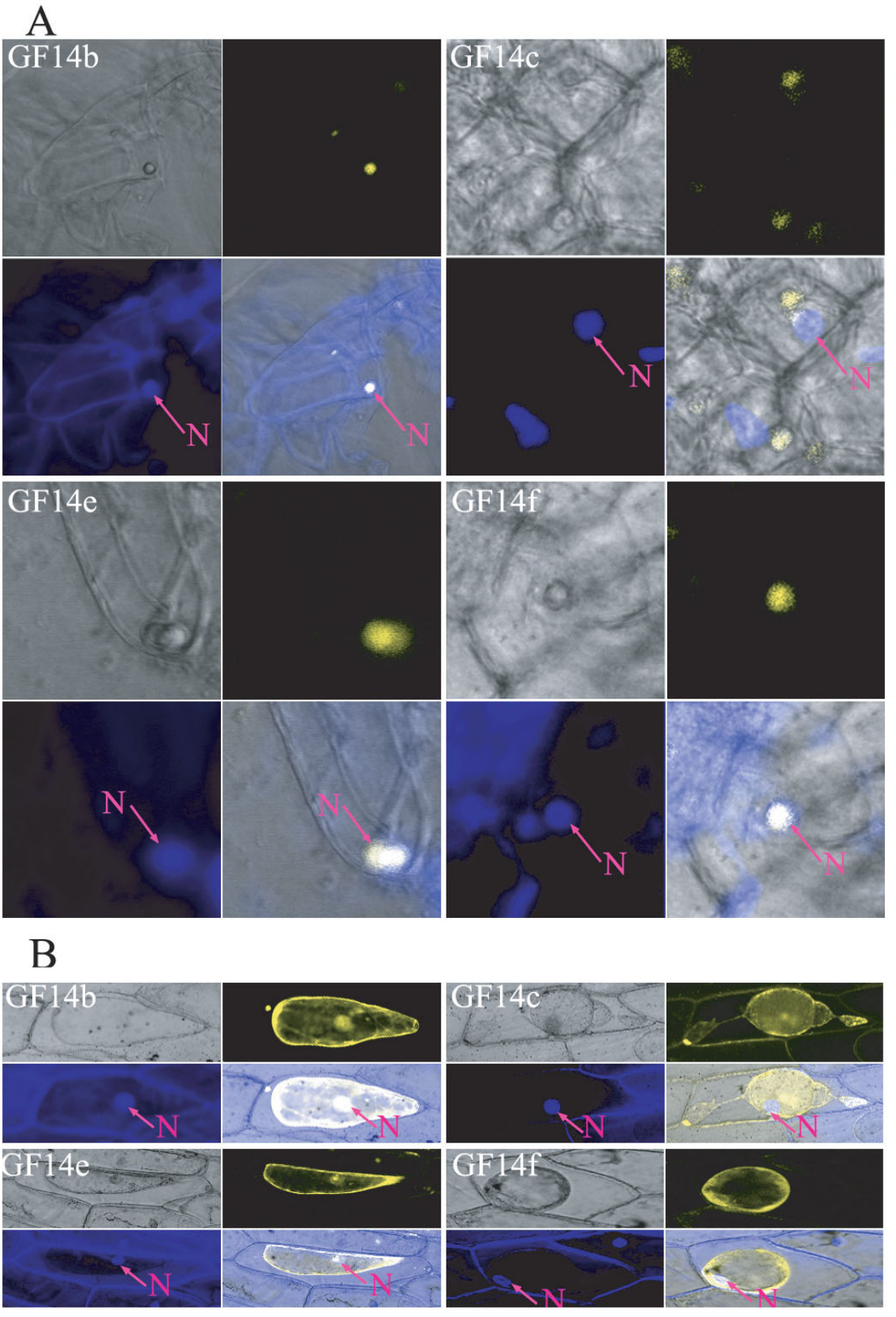


Figure 6. Subcellular localizations of the GF14 isoforms in rice callus and onion epidermal cells. (**A**) Subcellular localizations of the GF14 isoforms in rice callus cells. (**B**) Subcellular localizations of the GF14 isoforms in onion epidermal cells. Plasmolysis was performed to confirm subcellular localization of the GF14s. YFP fluorescence (top right) was detected together with bright field images of cells (top left); DAPI fluorescence images (bottom left) and all images merged (bottom right). N, nucleus.

Given the size of 14-3-3 gene families and the wide range of pathways in which they have been involved, it would be difficult or trivial to identify the phenotypes of individual 14-3-3 mutations.[4,8] 14-3-3s act as scaffolds for the assembly of large signaling complexes, and various 14-3-3 isoforms have different affinities for at least certain target proteins that are most likely with the same cellular localization for functioning. Complete understanding of 14-3-3 roles will require the determination of cellular and subcellular localizations and targets of specific isoforms. For example, GF14c is associated with at least 10 proteins known to accumulate in the thylakoid.[28] Therefore, searching for and identifying functions of the target proteins of a given 14-3-3 through the forward or reverse genetic approach would provide a clue for the 14-3-3-mediated cellular activities.

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