Coordination between GROWTH-REGULATING FACTOR1 and GRF-INTERACTING FACTOR1 plays a key role in regulating leaf growth in rice

Yuzhu Lu1,2,3*, Yunlong Meng3, Jia Zeng3, Ying Luo3, Zhen Feng3, Liying Bian3 and Suyun Gao1,2

Abstract

Background: The interactions between Growth-regulating factors (GRFs) and GRF-Interacting Factors (GIFs) have been well demonstrated but it remains unclear whether different combinations of GRF and GIF play distinctive roles in the pathway downstream of the complex.

Results: Here we showed that OsGRF1 and OsGIF1 synergistically regulate leaf growth in rice. The expression of OsGIF1 emerged in all tissues with much higher level while that of OsGRF1 appeared preferentially only in the stem tips containing shoot apical meristem (SAM) and younger leaves containing leaf primordium. Overexpression of an OsmiR396-resistant version of mOsGRF1 resulted in expanded leaves due to increased cell proliferation while knockdown of OsGRF1 displayed an opposite phenotype. Overexpression of OsGIF1 did not exhibit new phenotype while knockdown lines displayed pleiotropic growth defects including shrunken leaves. The crossed lines of mOsGRF1 overexpression and OsGIF1 knockdown still exhibited shrunken leaves, indicating that OsGIF1 is indispensable in leaf growth regulated by OsGRF1. The expression of OsGRF1 could be upregulated by gibberellins (GAs) and downregulated by various stresses while that of OsGIF1 could not.

Conclusion: Our results suggest that OsGIF1 is in an excessive expression in various tissues and play roles in various aspects of growth while OsGRF1 may specifically involve in leaf growth through titrating OsGIF1. Both internal and external conditions impacting leaf growth are likely via way of regulating the expression of OsGRF1.

Keywords: OsGRF1, OsGIF1, miR396, Leaf growth, Stress response

Background

Interactions between transcription factors (TFs) and their coactivators are usually essential in regulating downstream genes expression and thus in properly modulating individual growth. Despite a wide range of genes’ interactions existed in vivo, only limited numbers have been identified due to the lack of assuredly reliable methods. In plants, GROWTH-REGULATING FACTOR (GRF) and GRF-INTERACTING FACTOR (GIF) were well known to interact with each other and this complex duo has been proved to participate in many aspects of the development and growth in plants [15, 17, 21, 22, 31].

OsGRF1 is the first member found to be induced by gibberellic acid (GA) in rice [43, 44]. Based on the features of OsGRF1’s amino acid sequence, a family of 12
members has been found in rice [6]. This plant-specific family was defined by two conserved domains, QLQ (Gln, Leu, Gln) and WRC (Trp, Arg, Cys), in the N-terminal region of GRF proteins. The QLQ domain is essential for protein-protein interaction [17] and WRC domain comprising a C_xH motif is believed to bind DNA with its nuclear localization signal (NLS) [6]. The roles of GRFs were initially thought to regulate the growth of leaf and stem [13, 16, 18, 44]. Thereafter growing number of studies reported other functions of GRFs, such as seed and root development, stress response, flowing, and plant longevity [3, 7, 11, 19, 24, 26, 33]. As a highly conserved family, GRFs have been found in all land plants including Arabidopsis thaliana, Brassica napus, Glycine max, Solanum tuberosum, Zea mays, the moss Physcomitrella patens [2, 8, 19, 20, 27, 32, 46, 51, 53]. Most members of GRFs are negatively regulated by miR396, which cleave their targets at the transcript level [14]. In rice, 11 of 12 members of OsGRFs are targets of OsmiR396, except OsGIF11 [40]. MiR396 was firstly identified in Arabidopsis and rice by computational and experimental means [14, 40, 49, 50]. Like GRFs, miR396 family is also a highly conserved plant microRNA family found in all land plants [1]. MiR396 has been demonstrated to be involved in various aspects of plant growth and development [4, 5, 9, 11, 25, 26, 29, 35–37, 47]. As a regulatory molecule, the roles of miR396 depend on the functions of its targets as well as the ways how it regulates its targets.

Compared with GRF family which usually comprises 8–20 numbers, GIF family is much smaller with only few members, usually below 5 copies, in different plants [31]. However, the phenomenon that GIF genes exist in most eukaryotic species including embryophytes, green algae, and metazoan shows this family is more conserved than GRF family [17]. The amino acid sequences of GIF are featured by having two domains, SNH (SYT N-terminal homology) and QG, which is rich of glutamine (Q) and glycine (G). Binding and Y2H assays demonstrated that the GRF QLQ domain and GIF SNH domain mediate the interaction between the two families [13, 17, 26]. In Arabidopsis, the interactions between different AtGRFs and AtGIFs have been well identified [7, 24, 45]. So far, it still remains unclear whether different combinations of GRF and GIF play their unique roles in the downstream of the complex. Interestingly, overexpression of ZmGRF10, which has no transactivation activity due to the lack of almost entire C-terminal domain, was found to fine-tune the homeostasis of the GRF-GIF complex via way of competitive combination [48]. Also, different combinations of GRFs and GIFs have been observed in the different regions of maize leaf [30]. These results showed that the combinations between different members of GRFs and GIFs are widely existed and in a competitive way.

Here we probed into the precise titration relationship between OsGRF1 and OsGIF1. By analyzing their expression and the phenotypes of the transgenic lines, we proposed a coordinated relationship between them.

Results

Different expression patterns of OsGIF1 and OsGRF1

Although the interactions between different GRFs and GIFs have been well tested ([13, 17, 21]; Lee et al., 2014 [26]), the precise functions of different combinations still remain unclear. There are 12 members of OsGRF and 2 members of OsGIF in rice (Oryza sativa ssp. japonica, [6, 34]). Earlier studies revealed that OsGRF1 is a GA induced gene and can affect the stem elongation in Arabidopsis [6, 43, 44]. The functions of OsGIF1 have been reported to be involved in regulating growth of multiple organs such as leaves, stems and grains [10, 23]. Investigating genes expression patterns is necessary for probing into their functions because genes expression patterns are usually consistent with their roles. Here, we chose OsGRF1 and OsGIF1 as objectives to fully investigate their expression profiles. We selected flowers from the adult plants and different older and younger tissues such as leaves, stems, and roots from 4-week-old seedlings as objects for analyzing genes expression. Quantitative Reverse Transcription-PCR (qRT-PCR) showed that the expression levels of OsGRF1 were relatively higher in the younger tissues including younger leaves, shoots, and roots, especially in shoot apical meristem (SAM) and leaf primordium (Fig. 1a). By contrast, the expression of OsGIF1 seemed to be constitutive with similar levels in almost all tested tissues (Fig. 1a).

qRT-PCR may show the expressional tendencies of OsGRF1 and OsGIF1 in different tissues, but cannot reflect the intensity differences of the expressions. To further compare their expression level, especially for the intensities between OsGRF1 and OsGIF1, we measured the two genes’ expression by northern blot. We elaborately employed two probes containing same content of radioactively labeled α-32P-dCTP which was inserted into probes by PCR for hybridizing the two genes respectively. As Fig. 1b shown, the RNA abundance of OsGIF1 was much higher than that of OsGRF1 in all tested tissues, even in younger leaves and shoots where the expression levels OsGRF1 were also relatively higher. Overall, the expression levels detected by northern blot and qRT-PCR were consistent with each other (Fig. 1a and b). These results indicated that the expression of OsGIF1 is in a constitutive manner with much higher level, however, the expression of OsGRF1 displays a tissue-specific preference with relatively lower level. To further investigate the two genes expression on protein level, total protein was extracted from 2-week-old seedlings and was immunoblotted by anti-OsGIF1 and anti-OsGRF1 respectively.
The molecular weight of the OsGIF1 was about 25 kDa while that of the OsGRF1 is about 43.5 kDa. As shown in Fig. 1c, the blot intensity of OsGIF1 was much stronger than that of OsGRF1, further indicating the protein abundance OsGIF1 was more abundant than OsGRF1.

The expression of OsGRF1 can be regulated by phytohormones and stresses while that of OsGIF1 cannot. It is well known that the OsGRF1 and most other OsGRFs are GA-inducible [6, 43, 44]. As a kind of basic plant hormone, gibberellins (GAs) are often in a pivotal hub of different pathways. Usually the concentration of endogenous gibberellins is likely affected by other factors, such as biotic and abiotic stresses [42]. Additionally we did not know whether or how OsGRF1 and OsGIF1 respond to these factors. We chose 2-week-old seedlings exposed to different treatments including GA, salt, drought, UV, pathogen, and ABA for designated time. Then the total RNA from these seedlings was extracted and the two genes’ expressions were measured by qRT-PCR respectively. As expected, the expression of OsGRF1 was gradually increased with the extension of GA3 treatment, while that of OsGIF1 was not affected during the identical period (Fig. 2a). By contrast, the expressions of OsGRF1 were gradually reduced under the treatments of ABA and various stresses (Fig. 2b to f). Similar as GA treatment, the expressions of OsGIF1 were also unaffected in the other treatments (Fig. 2). Interestingly, the variation of OsGRF1 expression under ABA treatment was more remarkable than other stresses, and the expression of OsGIF1 was also slightly fallen under ABA treatment (Fig. 2b to f), indicating that ABA, one of stresses-associated hormones, may have a rapid effect upon the genes’ expression than other
stresses. These results showed that the expression of OsGRF1 could be regulated by various factors, but that of OsGIF1 could not.

Overexpression of miR396-resistant version of OsGRF1 results in expanded leaves
OsGRF1 has already been identified as the target of miR396 in plants [14, 15, 40]. A gain-of-function mutant overexpressing a microRNA-resistant-version of target has been used for elucidating the roles of a given microRNA (Axtell and [1]). OsGRF1 was found to be highly expressed in shoot tips and young leaves (Fig. 1), indicating it likely plays role in regulating the growth of leaf and shoot. To avoid being targeted by OsmiR396, we got a miR396-resistant-version of OsGRF1 by mutating five bases of OsGRF1 mRNA in miR396-acted region without alteration of amino acid sequence according to the degeneracy of codons (Fig. 3a). Then we introduced miR396-resistant-version of OsGRF1 (named as mOsGRF1) and wild-type OsGRF1 into rice respectively, both were driven by the native promoter of OsGRF1. The transgenic plants were propagated and the homozygous lines were selected on hygromycin in T2 generation. Northern blot and qRT-PCR showed that the RNA abundance of miR396 was nearly at same level in 4-week-old seedlings of the non-transformed plants, OsGRF1OE and mOsGRF1OE, but the OsGRF1 mRNA levels in mOsGRF1OE lines were significantly higher than that of the other two lines (Fig. 3b). The overall growth rates of the three genetic backgrounds are close to each other but an overgrowth of leaves was observed in mOsGRF1OE lines (Table 1). The first leaf of seedlings in both wild type and OsGRF1OE was an incomplete leaf of which shape likes a needle, whereas that of mOsGRF1OE lines had a tongue-like shape with a leaf-stalk (Fig. 3d). The sizes of other leaves of mOsGRF1OE lines were also bigger than that of the non-transformed plants and OsGRF1OE in the 3-week-old seedlings (Fig. 3c). Besides these, no other obvious difference of characteristics was observed in the three backgrounds. These results showed that OsGRF1 plays roles in promoting the leaf growth. It is intriguing that mOsGRF1OE lines had no apparent difference in stem growth even though mRNA of OsGRF1 also highly accumulated in the stem (Fig. 1). We speculated that overexpression of only one member of this family in shoot tips where all 12 members are highly expressed [6] might not be sufficient to produce an apparent stem elongation.
Knockdown of OsGRF1 by RNAi displays a phenotype of shrunken leaves

To further investigate the roles of OsGRF1 in rice development and growth, we knocked down OsGRF1 by RNAi technology. We chose a specific sequence corresponding to 3′ region of OsGRF1 as object to construct RNAi vector and brought it into rice by Agrobacterium-mediated transformation. As shown in Fig. 4a, the expression of OsGRF1 measured by qRT-PCR was significantly lower in the OsGRF1RNAi lines. The most prominent phenotype of knockdown of OsGRF1 was that the transgenic lines exhibited smaller leaves (Fig. 4b). The differences between leaf sizes of the three genetic backgrounds (NT, mOsGRF1OE, OsGRF1RNAi) were more remarkable in the position closer to the base (Fig. 4b, Table 1). To investigate

**Table 1** Comparisons of the leaf phenotypes of the non-transformed plants, OsGRF1OE and mOsGRF1OE as well as OsGRF1RNAi lines

|                              | Length and width of the first leaf (cm) | Length and width of the second leaf (cm) | Length and width of the third leaf (cm) | Length and width of the fourth leaf (cm) |
|------------------------------|----------------------------------------|-----------------------------------------|----------------------------------------|----------------------------------------|
| Non-transformed plants       | 0.54 ± 0.06 (L)                         | 1.51 ± 0.19 (L)                         | 5.03 ± 0.40 (L)                        | 15.00 ± 0.88 (L)                       |
| OsGRF1OE                     | 0.6 ± 0.05 (L)                          | 1.60 ± 0.15 (L)                         | 5.50 ± 0.5 (L)                         | 15.5 ± 0.77 (L)                        |
| mOsGRF1OE                    | 0.91 ± 0.07 (L) *                       | 3.11 ± 0.22 (L) *                       | 8.41 ± 0.71 (L) *                      | 19.02 ± 1.12 (L) *                     |
| OsGRF1RNAi                   | 0.33 ± 0.04 (L) *                       | 0.85 ± 0.15 (L) *                       | 3.8 ± 0.35 (L) *                       | 13.20 ± 0.65 (L) *                     |
| mOsGRF1RNAi                  | 0.07 ± 0.01 (W) *                       | 0.16 ± 0.02 (W) *                       | 0.41 ± 0.04 (W) *                      | 0.72 ± 0.05 (W) *                      |
| x                            | 0.37 ± 0.05 (L) *                       | 0.91 ± 0.12 (L) *                       | 4.1 ± 0.41 (L) *                       | 13.90 ± 0.81 (L) *                     |
| OsGRF1RNAi                   | 0.08 ± 0.01 (W) *                       | 0.17 ± 0.03 (W) *                       | 0.48 ± 0.04 (W) *                      | 0.81 ± 0.04 (W) *                      |

* Means Significant difference at P < 0.01 compared with the leaf data of non-transformed plants by Student's t-test (n = 7; means ± SDs)

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whether the difference in leaf growth was caused by cell proliferation or cell elongation, suspension-cultured cells stemmed from leaf calli of the three genetic backgrounds of OsGRF1 were made. Plants suspension systems usually are made up of numerous lumps in which dozens cells clump together, and very few dissociated cells can be observed in suspension-cultured system. After 6 days of being cultured, the biomass increment of suspension-cultured cells of OsGRF1RNAi lines was significantly lower than that of the non-transformed plants, while that of mOsGRF1OE lines was remarkably higher (Fig. 4c). Additionally, there were no significant differences in the size of dissociated cells between the three genetic backgrounds (Fig. 4d). These results showed that the bigger differences of the leaf size between the three genetic lines are like caused by the activities of cell diversion rather than cell elongation. Some cell-cycle-related genes such as cyclin Oryza sativa1 (cycOs1), cyclin Oryza sativa2 (cycOs2) have been believed to be GA-induced [38, 39] even though it remained unknown whether these cell-cycle-related genes were related with OsGRF1. We measured the expressions of cycOs1 and cycOs2 in the leaves of 3-week-old seedlings of the three backgrounds of OsGRF1. As shown in Fig. 4e, the expressions of cycOs1 and cycOs2 are upregulated in lines of mOsGRF1OE and downregulated in lines of OsGRF1RNAi. These results fully demonstrated the activities of cell division could be affected by OsGRF1 in rice leaf.

Knockdown of OsGIF1 exhibits pleiotropic growth defects including shrunken leaves while overexpression shows no variation

To investigate the functions of OsGIF1 in rice, we made transgenic lines with overexpression or knockdown.
(RNAi by specific sequence) of OsGIF1. Then OsGIF1 expression levels were measured by qRT-PCR in the three backgrounds (NT, OsGIF1OE, OsGIF1RNAi) (Fig. 5b). Expectedly, the expression of OsGIF1 was significantly higher in OsGIF1OE lines while much lower in OsGIF1RNAi lines (Fig. 5b). Then the growth traits were carefully investigated during the whole lifetime. The plants overexpressing OsGIF1 did not display any new phenotype all the time in their life cycle (Fig. 5a). Their stem length, tiller number, leaf size, and thousand-grain weight, are identical to the non-transformed plants (Fig. 5a). However, the knockdown lines of OsGIF1 displayed multiple defects in their lifecycle, such as shorter stems, withered seeds, slender roots with reduced number, and shrunk leaves (Fig. 5c to e; Table 1). We had previously shown that the expressions of cycOs1 and cycOs2 could be affected by OsGRF1 in the leaves (Fig. 4e), but we did not know whether this impact needs the partner of OsGIF1. To determine this uncertainty, the expressions of cycOs1 and cycOs2 were also measured by qRT-PCR in the leaves of the three genetic backgrounds of OsGIF1. As shown in Fig. 5g, the expression levels of cycOs1 and cycOs2 were significantly lower in OsGIF1RNAi lines while did not change apparently in the OsGIF1OE lines (Fig. 5g). These results indicated the activity of cell division was inhibited in the leaves of the knockdown lines. For the reason why the expressions of cycOs1 and cycOs2 were not changed in the lines with overexpression of OsGIF1, we speculated that the expressions of cycOs1 and cycOs2 are under control of OsGIF1-OsGRF1 duo in which the expression of OsGIF1 is already in an excessive state in the non-transformed plants (Fig. 1b and c). This assumption was further

![Image](image_url)
supported by the observation that the size of leaves still exhibited as shrunk in the crossed lines of mOsGRF1OE and OsGIF1RNAi (Fig. 5c), because only overexpression of mOsGRF1 but lack the partner of OsGIF1 is not enough to promote leaf growth. This observation was also supported by molecular evidence that the expression of cycOs1 and cycOs2 were still significantly lower in the leaves of hybrid lines of mOsGRF1OE and OsGIF1RNAi (Fig. 5g), in which the expression of OsGRF1 is higher while that of OsGIF1 is lower (Fig. 5f).

Discussion

The roles of GRF-GIF duo have been revealed to be involved in many aspects of plant development and growth [15, 31]. However, compared with GIF family which usually comprises very few members, the GRF family is much bigger. So, the diverse functions of GRFs involving many aspects of plants development may reflect the combinations of the specific individual role of different family members. The roles of single member of GIFs seemed to be more versatile due to very fewer members in this family. GIF family found in most eukaryotic species is more conserved than GRF family [17], indicating they may have other roles beyond combination with GRFs. The observation that Arabidopsis gif1/2/3 triplet mutant displayed severe defects in the growth and development [22] further supports this assumption. So far, most studies focused on revealing the roles of individual member of the two families but not on the distinct roles of the different combinations [7, 24, 26, 30, 45, 48, 52]. Currently, the precise correlation between the individual members of the two families remains largely unclear.

Here we elaborately compared the expression patterns of OsGRF1 and OsGIF1 and profoundly analyzed the overlap of the phenotypes of transgenic plants with ectopic expression of OsGRF1 and OsGIF1. From our results we can draw the key points as the following:

(1) The expression of OsGIF1 is in a constitutive manner with much higher levels while the expression levels of OsGRF1 are in a tissue-specific preference with relative lower levels overall (Fig. 1). The reasons for higher expression level of OsGIF1 are likely caused by two aspects: the lower copies of OsGIFs (only two in rice) in this family and the assumptions that GIFs also probably interact with other transcription factor, in addition to GRFs, based on some ChIP assays [45, 52];

(2) The specific roles of OsGRF1 may only be involved in regulating leaf growth while the roles of OsGIF may be involved in various aspects of plants growth. To explore the roles of OsGRF1, which prefers to express in tips of both stem and leaf, we use its native promoter rather than a constitutive promoter because we did not intend to sabotage its inherent expression way. The specific role of OsGRF1 in regulating leaf growth was manifested due to avoid being targeted by miR396 (Fig. 3c and d). Additionally the observation that shrunk size of leaves also emerged in the lines of knockdown of OsGRF1 by RNAi, further suggesting its role in regulating leaf growth (Fig. 4b). However the phenotype of knockdown of OsGIF1 displayed multiple defects including shrunk leaves, indicating it may have multiple roles in plant growth (Fig. 5c to e). For reasons why overexpression of OsGIF1 had no new phenotype, we speculated this would be caused by the fact that the expression of OsGIF1 is already in an excessive manner in various tissues (Fig. 1b and c);

(3) The expression of OsGRF1 can be affected by various stresses and some kinds of hormones while that of OsGIF1 is unaffected. The expressions of cell-cycle-related genes such as cycOs1 and cycOs2 in rice leaf are under control of OsGIF1-OsGRF1 duo. Even OsGRF1 and cell-cycle-related genes such as cycOs1 and cyclin were believed to be induced by GA [6, 38, 39, 43, 44], but it remained unknown whether there is a link between OsGIF1 and cell-cycle-related genes in GA response. Here we suggest that cycOs1 and cycOs2 are in the downstream of OsGRF1 in response to GA because higher level of OsGRF1 promoted the expression of cycOs1 and cycOs2 while lower level of OsGRF1 inhibited them (Fig. 4e). Even OsGIF1 did not respond to GA (Fig. 2a), the fact that OsGRF1 can interact with OsGRF1 and OsGIF1RNAi also presented a phenotype of shrunk leaves (Fig. 5c), indicating that the expression of cycOs1 and cycOs2 may need OsGIF1-OsGRF1 duo.

In summary, here we probed into the distinct role of a combination between the given members of OsGRFs and OsGIFs, and found their specific function in regulating leaf growth. The future studies may probably focus more on revealing the distinct roles of different combinations of OsGRFs and OsGIFs.

Conclusions

Based on the above results, we propose a working model here to interpret how OsGRF1 and OsGIF1 work together in regulating growth (Fig. 6). We suggest that the expression of OsGRF1 is suppressed by various stresses, ABA, and mir396 while promoted by GA. The interaction between OsGRF1 and OsGIF1 is necessary in specifically promoting leaf growth by promoting the expression of cell-cycle-related genes. However, OsGIF1,
which expresses in a higher level, may also work with other factor(s) in regulating other aspects of growth.

Methods
Plant materials and growth conditions
Rice cultivar (Oryza sativa ssp. japonica) was used as the control plants and serves the genetic background for all transgenic plants. All seeds involved in this study were taken from Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, China. Y.L. undertook the formal identification of the plant materials used in his study. The voucher specimen of this material has not been deposited in a publicly available herbarium yet. The conditions for normal growth of the control plants and transgenic plants were performed as described by Lu et al. [28].

Stress and hormone treatments
Salt stress, UV light stress, pathogen (Magnaporthe grisea) stress, drought stress, and abscisic acid (ABA) treatment, were performed as described previously [28]. For gibberellin (GA) treatment, the whole 2-week-old seedlings were incubated into N6 liquid solution containing 50 μM GA3 and 0.02% Tween 20. Then total RNA was extracted from the above seedlings at the point of designated time for genes analysis.

Observation of cells and creation of suspension-cultured cells
For creation of suspension cells, the rice calli derived from sterilized leaves of the non-transformed plants, OsGRF1OE, and mOsGRF1OE lines were grown on N6 culture medium (solid). Four weeks later, 1 g of the fresh calli from different lines was incubated into 500 mL AA
medium [41] and biomass was measured at given intervals. The suspension cells were observed and recorded under microscope.

**Quantitative RT-PCR**

For quantitative RT-PCR analysis of OsGRF1 (Gene ID: Os02g0776900), OsGIF1 (Gene ID: Os03g52320), cycOs1 (Gene ID: Os04g0563700) and cycOs2 (Gene ID: Os06g0726800), 2 μg of total RNA was reversely transcribed in a total volume of 20 μL with 0.5 mg oligo (dT)15, 0.75 mM dNTPs, 10 mM DTT, and 100 U SuperScript II RNase H2 reverse transcriptase (Invitrogen). The reaction volume for PCR was 20 μL with 1 μL of the RT reactions [28]. The primers for quantitative RT-PCR are listed as the following: OsGRF1, FW: 5′-TGATCTTTCTAAAGAGGGCAGG-3′, RV: 5′-TGTTGGTGATCGGGAGGTCGTT-3′; OsGIF1, FW: 5′-GCAGCAGCAGCAGCCGCCGC-3′, RV: 5′-TGCCCTTGAGGTACTCCCCGT-3′; cycOs1: FW: 5′-GTGTCTTCTAGGATGGTAGA-3′, RV: 5′-GTATCTTTCTAAAGAGGGCAGG-3′; cycOs2: FW: 5′-CATGAGAAGGTTCTCTCAAGGCT-3′, RV: 5′-TGTTGCACCTGACGATGTTAGA-3′. The two probes were designated to contain same content of radioactive 32P by designed primers (OsGRF1, FW: 5′-TGATCTTTCTAAAGAGGGCAGG-3′, RV: 5′-TGTTGGTGATCGGGAGGTCGTT-3′; OsGIF1, FW: 5′-GCAGCAGCAGCAGCCGCCGC-3′, RV: 5′-TGCCCTTGAGGTACTCCCCGT-3′). The OsGIF1 was then constructed into pCAMBIA1301 under Ubi1 promoter for overexpression. For RNAi (RNA interference) of both OsGRF1 and OsGIF1, the specific regions (probe region for northern blot) of the two genes were used for silencing the targets and were brought into pCAMBIA1301 forward and backward respectively, separated by an intron. All the constructed expression vectors were introduced into rice calli through Agrobacterium tumefaciens (EHA105) mediated methods [12].

**Western blot**

For western blot of OsGRF1 and OsGIF1, total protein from the 2-week-old seedlings of the non-transformed plants was extracted by SDS sample buffer and boiled for 10 min. Then the extracted proteins were separated by SDS-PAGE and immunoblotted with antibody of anti-OsGRF1 and anti-OsGIF1 at 1:1000 dilution. To prepare the antibodies of OsGRF1 and OsGIF1, 6× His-OsGRF1 and 6× His-OsGIF1 constructed into pET28 vector were expressed and used as antigens to produce monoclonal antibodies in rabbits (Purchased from Junhui Biotech, Co, China). During the process of making monoclonal antibodies, the rabbits were immunized four times at least and the purities of the extracted antibodies should be kept greater than 90%. Finally, the values of enzyme linked immunosorbent assay (ELISA) should be greater than 1:128000.

**Abbreviations**

ABA: Abscisic acid; GRFs: Growth-regulating factors; GIFs: GRF-Interacting Factors; NLS: Nuclear localization signal; qRT-PCR: Quantitative Reverse-Transcription Polymerase-Chain-Reaction; SAM: Shoot Apical Meristem; RNAi: RNA interference

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The datasets generated and analyzed during the current study are available. Availability of data and materials

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The plant materials (Oryza sativa L. Nipponbare) used as control and background for all transgenic lines come from Yangzhou University. The experimental research on plants carried out in his work complies with institutional, national, and international guidelines.

Competing interests

The authors declare that they have no competing interests.

Author details

1Jiangsu Key Laboratory of Crop Genetics and Physiology/ Key Laboratory of Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou 225009, China. 2Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University, Yangzhou 225009, Jiangsu, China. 3College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China.

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