14-3-3 Gene of *Zostera Japonica* ZjGRF1 Participates in Gibberellin Signaling Pathway

Siting Chen (✉ c105043041@126.com)

Guangxi Mangrove Research Center <https://orcid.org/0000-0003-3468-9320>

Guanglong Qiu

Guangxi Mangrove Research Center

Research Article

Keywords: 14-3-3, *Zostera japonica*, ZjGRF1, gibberellin, *Arabidopsis thaliana*

Posted Date: November 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1063446/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Molecular Biology Reports on April 15th, 2022. See the published version at <https://doi.org/10.1007/s11033-022-07330-3>.

Abstract

Background *Zostera japonica* (*Z. japonica*) is a unique seagrass species in Asia, which has important ecological value. Gibberellin is an important plant hormone, which plays an important role in the regulation of plant growth and development, including seed longevity, seed germination, anti-aging, hypocotyl elongation, leaf development, reproductive organ development and abiotic stress. However, the role of *14-3-3* gene of *Z. japonica* in GA signaling pathway is still unclear.

Methods and results Here, we found that the gibberellin content of *Arabidopsis* overexpressing *ZjGRF1*, *14-3-3* gene of *Z. japonica*, is lower than that of wild type (WT), and the expression of gibberellin synthesis genes is lower than that of WT. The expression level of gibberellin receptors in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT. The expression level of GA response genes *EXP8* in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT, and the expression levels of *PRE1*, *PRE5* and *SCL3* were higher than that in WT. Transgenic *ZjGRF1 Arabidopsis* is insensitive to paclobutrazol, an inhibitor of gibberellin synthesis. The expression level of flowering regulatory genes in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT.

Conclusion This is the first report on the function of *14-3-3* gene family in seagrass. Our findings can be further utilized in future studies on regulation of *14-3-3* gene on plant development.

Introduction

Gibberellins (GAs) are a kind of growth factor widely existing in plants. Their chemical structures belong to diterpenes and consist of four isoprene units. 136 kinds of GAs with different structures have been isolated and identified from plants, fungi and bacteria, but only a few have biological activities. GA₁ and GA₄ are the main endogenous active molecules in most plants. Most GAs have no biological activity, but are precursors or inactive metabolites of active GAs. The concentration of active GAs in plant tissue is determined by the rate of GAs synthesis and inactivation. Gibberellin with biological activity regulates many aspects of plant growth and development by promoting cell proliferation and expansion, including flower and fruit development, seed germination, stem extension, leaf expansion and so on.

14-3-3 proteins were first found in bovine brain tissue cells [1], and was named according to the number of fragments separated by DEAE cellulose and the mobility in starch gel electrophoresis. Brain tissue is rich in 14-3-3 protein, accounting for about 1% of all soluble proteins. For a long time, 14-3-3 protein is considered to be a brain specific protein. Later, it was found that it is a highly conserved regulatory protein widely existing in eukaryotes. Since 1992, a variety of 14-3-3 proteins have been found in plants. *Arabidopsis*, for example, has 13 genes encoding 14-3-3 protein. 14-3-3 proteins interact with target proteins by recognizing specific phosphorylation sequences. More than 300 target proteins have been found to interact with 14-3-3 proteins in plants. Different 14-3-3 isoforms are expressed in different tissues of plants, and they are very different. They specifically regulate target proteins, participate in a

series of signal transduction and metabolic processes in plant cells, and affect the growth and development of plants.

REPRESSION OF SHOOT GROWTH (RSG) is a kind of bZIP transcription factor, which regulates the GA level in plants by regulating the transcription of related genes in the GA synthesis pathway. In tobacco, 14-3-3 is an RSG interaction protein, and the site RSG interacting with 14-3-3 protein was RSG-Ser114. The interaction between 14-3-3 protein and RSG depends on Ser-114 phosphorylation of RSG. Exogenous application of GA leads to the temporary increase of intracellular Ca^{2+} level. The transient change of Ca^{2+} level makes Ca^{2+} dependent CDPK kinase phosphorylate Ser-114 of RSG and promote the interaction between 14-3-3 protein and phosphorylated RSG. In this process, 14-3-3 protein, as a negative regulator, binds RSG, changes the localization of RSG protein in cells, so that RSG remains in the cytoplasm and cannot enter the nucleus to regulate the genes encoding GA biosynthesis related proteins, inhibits the transcription of GA biosynthesis related genes in the nucleus, and thus controls the GA level in vivo [2, 3].

Seagrass meadow ecosystem is a highly productive ecosystem in coastal waters [4]. Although it accounts for only 0.15% of the global ocean area, it contributes 1% of the net primary productivity of the global ocean. In recent years, the ecological function and economic value of seagrass meadows have been gradually recognized by people [5].

Zostera japonica (*Z. japonica*) is an endemic *Subgenus Zosterella* species in Asia. Its plant is short, but it has important ecological value. *Z. japonica* can live in the shallow intertidal zone where the water temperature and light fluctuation are strong, which is difficult for *Zostera marina* (*Z. marina*) to adapt to, showing stronger adaptability and vitality [6]. It is the most widely distributed seagrass species in China, and it is distributed in the Yellow Sea, Bohai Sea, South China Sea and other coastal areas in China. In the Yellow Sea and Bohai Sea area, *Z. japonica* have appeared in Weifang, Qingdao, Yantai, Weihai, Rizhao and other places in Shandong Province [7]. In recent years, the seagrass habitat in the Yellow Sea and Bohai Sea has been seriously damaged, and it is difficult to find a large area of continuous distribution of *Z. japonica* seagrass meadows.

However, to our knowledge, no studies have been conducted on the physiological function of seagrass 14-3-3 gene. In our previous study, we cloned a 14-3-3 gene *ZjGRF1* of *Z. japonica* and overexpressed it in *Arabidopsis* with 35S promoter and obtained homozygous plants (Unpublished results). the gibberellin content of transgenic *ZjGRF1 Arabidopsis* is lower than that of wild type (WT), and the expression of gibberellin synthesis genes is lower than that of WT. The expression level of gibberellin receptors in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT. The expression level of GA response genes *EXP8* in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT, and the expression levels of *PRE1*, *PRE5* and *SCL3* were higher than that in WT. Transgenic *ZjGRF1 Arabidopsis* is insensitive to paclobutrazol, an inhibitor of gibberellin synthesis. The expression level of flowering regulatory genes in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT. In conclusion, these results suggest that *ZjGRF1* is involved in gibberellin signaling pathway.

Materials And Methods

Plant materials and growth conditions

Arabidopsis Columbia ecotype was used as WT. 17-d-old Seedlings were grown on 1/2 Murashige and Skoog (1/2 MS) solid medium (0.8% agar) in a growth chamber (ZMD-260, Yanghui, Ningbo, China) under long-day conditions, with 16 h of white light ($80 \mu\text{mol m}^{-2} \text{sec}^{-1}$) and 8 h of dark, and with 60% relative air humidity at 21°C.

qRT-PCR

Quantitative real-time PCR was performed with TB Green Premix Ex Taq II (Takara) using QuantStudio 3 real-time PCR detection system (Applied Biosystems by Thermo Fisher Scientific). According to the manufacturer's protocol, RNeasy pure plant Kit (Qiagen) was used to isolate total RNA from frozen samples in liquid nitrogen. cDNA was synthesized with 1 μg total RNA and PrimeScript RT reagent kit with gDNA eraser (Takara). *Actin2* was used as internal reference, and the relative transcription level was determined by comparative threshold cycle (Ct). The primer name and sequence are shown in Table S1. Three independent biological replicates of each genotype were statistically analyzed.

GA content measurement

17-d-old seedlings were used to measure the GA content. The GA content was determined using a Plant GA ELISA Kit (Senbeijia, Nanjing, China).

Statistical analysis

Values presented are the means $\pm$ SD. An asterisk indicates that there was a significant difference between the WT using a Student's *t*-test (* $P < 0.05$, ** $P < 0.01$). All statistical analyses were performed using Microsoft Excel for Windows (Microsoft office 365, Redmond, WA, USA). For qRT-PCR, three independent biological replicates of each genotype were statistically analyzed. For measurement of the root length, 18 seedlings were statistically analyzed. For measurement of the hypocotyl length, 16 seedlings were statistically analyzed.

Results

ZjGRF1 inhibits gibberellin synthesis

Gibberellin is one of the essential plant hormones in the process of plant growth and development. It regulates seed germination, stem elongation, flower transformation, flower and fruit development. The results showed that the GA content of transgenic *ZjGRF1 Arabidopsis* plants was lower than that of WT (Fig. 1a). We measured the expression level of GA synthesis genes in 17-day-old seedlings of transgenic *ZjGRF1 Arabidopsis* plants. The results showed that the expression level of *GA3ox1* and *GA3ox2* in transgenic *ZjGRF1 Arabidopsis* plants was significantly lower than that of WT (Fig. 1b).

The expression level of gibberellin receptors in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT

In rice [8] and *Arabidopsis* [9], the research on gibberellin signaling pathway has been relatively perfect, in which DELLA protein is the key negative regulator of plant growth and development [10, 11], *GIBBERELLIN-INSENSITIVE DWARF 1* (*GID1*) are gibberellin receptors [12]. *GID1* change their conformation after recognizing and binding GA, and bind with DELLA protein to form GA-*GID1*-DELLA trimer, It leads to the degradation of DELLA protein through SCF^{*GID2*/SLY1} complex, relieves its inhibition on plant growth, and promotes plants to show gibberellin effect [13-15]. We measured the expression level of GA receptors in 17-day-old seedlings of transgenic *ZjGRF1 Arabidopsis* plants. The results showed that the expression level of *GID1a*, *GID1b* and *GID1c* in transgenic *ZjGRF1 Arabidopsis* plants was significantly lower than that of WT (Fig. 2).

***ZjGRF1* affects the expression level of gibberellin response related genes**

We measured the expression level of GA response related genes in 17-day-old seedlings of transgenic *ZjGRF1 Arabidopsis* plants. The results showed that the expression level of *EXP8* in transgenic *ZjGRF1 Arabidopsis* plants was significantly lower than that of WT, while the expression level of *PRE1*, *PRE5* and *SCL3* in transgenic *ZjGRF1 Arabidopsis* plants was significantly higher than that of WT (Fig. 3).

The sensitivity of transgenic *ZjGRF1 Arabidopsis* to Paclobutrazol (PAC) decreased

We analyzed the sensitivity of transgenic *ZjGRF1 Arabidopsis* to Gibberellin synthesis inhibitor PAC and found that transgenic *ZjGRF1 Arabidopsis* was less sensitive to PAC (Fig. 4). When plants were grown in the presence of 1 μ M PAC, the root length of WT plants decreased by 65%, and that of transgenic *ZjGRF1 Arabidopsis* decreased by 53%, which was significantly longer than that of WT (Fig. 4b). We then studied the seed germination rate of transgenic *ZjGRF1 Arabidopsis* in the presence of PAC. We studied the germination rate under white light at 40 μ M PAC. WT seeds could not germinate in the presence of 40 μ M PAC, while 18% of transgenic *ZjGRF1 Arabidopsis* seeds germinated (Fig. 4c). To further investigate this effect, we measured the length of hypocotyl in the presence of 1 μ M PAC. Seedlings were grown in the dark on 1/2 MS medium containing 1 μ M PAC for 12 days. In WT plants, when PAC concentration increased from 0 to 1 μ M, hypocotyl length decreased from 2.8 cm to 0.7 cm. In transgenic *ZjGRF1 Arabidopsis*, when PAC concentration increased from 0 to 1 μ M, hypocotyl length decreased from 2.9 cm to 1.0 cm, which was significantly longer than that of WT (Fig. 4d).

The expression level of flowering regulatory genes in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT

It is well known that GA promotes flowering by activating the expression of *LEAFY* (*LFY*) and *SUPPRESSOR OF CONSTANS1* (*SOC1*) [16, 17]. Therefore, we detected the mRNA expression levels of *LFY* and *SOC1* in samples collected 12 hours after illumination under LD conditions. Compared with the WT, transgenic *ZjGRF1 Arabidopsis* expressed lower levels of *SOC1* and *LFY* mRNA (Fig. 5). These results suggest that transgenic *ZjGRF1 Arabidopsis* inhibits flowering by inhibiting the expression of *LFY* mRNA.

Discussion

In this article, we show that the gibberellin content of transgenic *ZjGRF1 Arabidopsis* is lower than that of WT, and the expression of gibberellin synthesis genes is lower than that of WT (Fig. 1). The expression level of gibberellin receptors in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT (Fig. 2). The expression level of GA response genes *EXP8* in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT, and the expression levels of *PRE1*, *PRE5* and *SCL3* were higher than that in WT (Fig. 3). Transgenic *ZjGRF1 Arabidopsis* is insensitive to paclobutrazol, an inhibitor of gibberellin synthesis (Fig. 4). The expression level of flowering regulatory genes in transgenic *ZjGRF1 Arabidopsis* was lower than that in WT (Fig. 5). Taken together, our results indicate that *ZjGRF1* is involved in gibberellin signaling pathway.

The content of gibberellin in plants is determined by the relative strength of synthesis and decomposition, and its rate is regulated by a series of key enzymes. There are many enzymes involved in the synthesis and decomposition of gibberellin in higher plants. GA20-oxidase and GA3-oxidase, as the key enzymes in the last step of gibberellin biosynthesis pathway, can catalyze GA₁₂ and GA₅₃ to synthesize active GA₁ and GA₄ [18, 19]. Among them, GA20ox converts GA₁₂ and GA₅₃ into GA₉ and GA₂₀, and GA3ox further converts GA₉ and GA₂₀ into GA₁ and GA₄ [20]. In addition, GA20ox and GA3ox are regulated by the negative feedback of active GA [21, 22]. GA2-oxidase is a key enzyme in the process of GA degradation. It can decompose and inactivate bioactive GAs and its precursors and other intermediates in plants, so as to maintain the balance between bioactive GAs and intermediates in plants [23]. These three types of gibberellin oxidase genes belong to the 2OG-Fe(II) oxygenase subfamily and are encoded by the polygenic family [18]. When GA20ox and GA3ox are absent, the amount of bioactive GA synthesized is reduced. Similarly, overexpression of GA2ox class degrades bioactive GA in plants, resulting in dwarf phenotype [19, 24]. In addition, many studies have found that GA2ox, GA3ox and GA20ox family members have certain effects on flowering through overexpression or mutation [25–28]. So far, *GA2ox*, *GA3ox* and *GA20ox* family members have been reported in many species, such as rice [29], *Arabidopsis*, soybean [30], cucumber, jatropha, etc. The gibberellin content of transgenic *ZjGRF1 Arabidopsis* is lower than that of WT, which may be due to the lower expression levels of *GA3ox1* and *GA3ox2* (Fig. 1).

GID1 proteins are soluble GA receptors. GID1 genes encode a kind of proteins similar to Hormone sensitive lipases (HSL). Although they contain conserved HSL motifs: HGG and GX SXG, it was found that GID1s do not have hydrolase activity. GID1s can directly interact with DELLA of rice, and this interaction depends on the presence of active GA [12]. The expression level of *GID1a*, *GID1b* and *GID1c* in transgenic *ZjGRF1 Arabidopsis* plants was significantly lower than that of WT (Fig. 2).

PREs are GA-responsive genes. *PRE* gene family consists of six members (*PRE1–PRE6*) in *Arabidopsis*. The HLH motif of *PRE* members is a highly conserved motif that mediates protein-protein interactions among basic HLH proteins [31]. In contrast to the true basic HLH proteins composed of at least 147 members in *Arabidopsis* [32], *PREs* lack the basic region near the motif which is involved in DNA binding activity. The expression level of *PRE1* and *PRE5* in transgenic *ZjGRF1 Arabidopsis* plants was significantly higher than that of WT (Fig. 3).

In *Arabidopsis*, SCARECROW-LIKE 3 (SCL3) is a positive regulator of GA signal. The expression of SCL3 is induced by DELLA but inhibited by GA. SCL3 interacts with DELLA to regulate root growth and seed germination by antagonizing the activity of DELLA protein [33, 34]. The expression level of *SCL3* in transgenic *ZjGRF1 Arabidopsis* plants was significantly higher than that of WT (Fig. 3), which may be due to the lower GA content in transgenic *ZjGRF1 Arabidopsis* (Fig. 1a).

Paclobutrazol is an inhibitor of gibberellin synthesis and inhibits plant growth. In this study, we found that the sensitivity of transgenic *ZjGRF1 Arabidopsis* to PAC decreased (Fig. 4).

The molecular mechanism by which GA regulates *SOC1* expression is unclear. GA promotes the expression of *LFY* by binding to the cis-acting element on the *LFY* promoter [16, 35], while *SOC1* regulates the expression of *LFY* by directly binding to the promoter of *LFY* gene. Therefore, GA regulates the transcription of *LFY* through *SOC1* dependent and independent pathways. Gibberellin may affect the flowering development of plants by regulating the expression of *SOC1* and *LFY* in apical meristem. Compared with the WT, transgenic *ZjGRF1 Arabidopsis* expressed lower levels of *LFY* mRNA (Fig. 5), which may be due to the lower GA content in transgenic *ZjGRF1 Arabidopsis* (Fig. 1a).

Conclusion

In this study, we first studied the role of *ZjGRF1*, *14-3-3* gene of *Z. japonica*, in GA signaling pathway. These results are helpful to further study the function of *14-3-3* genes of seagrass.

Declarations

Acknowledgments

This study was funded by the Natural Science Foundation of Guangxi Province (2020GXNSFAA297067, 2018GXNSFBA281062), the Research Fund Program of Guangxi Key Lab of Mangrove Conservation and Utilization (grant numbers GKLMC-20A04, GKLMC-20A01), the National Science & Technology Basic Work Program (2015FY110600) and the National Key Research and Development Program of China (2017YFC0506100). These funding bodies had no role in the design of the study, collection, analysis, and interpretation of data, or in the writing of the manuscript.

Authors' contributions

SC designed the study and conducted the experiments. GQ conducted field sampling and identification. SC and GQ wrote the manuscript. All the authors reviewed the manuscript.

References

1. Moore, B.W. and V.J. Perez, *Specific acidic proteins of the nervous system*. Physiological Biochemical Aspects of Nervous Integration, 1967: p. 343-359.

2. Ishida, S., et al., *Involvement of 14-3-3 signaling protein binding in the functional regulation of the transcriptional activator REPRESSION OF SHOOT GROWTH by gibberellins*. Plant Cell, 2004. **16**(10): p. 2641-2651.
3. Ishida, S., et al., *A tobacco calcium-dependent protein kinase, CDPK1, regulates the transcription factor REPRESSION OF SHOOT GROWTH in response to gibberellins*. Plant Cell, 2008. **20**(12): p. 3273-3288.
4. Duarte, C.M. and C.L. Chiscano, *Seagrass biomass and production: A reassessment*. Aquatic Botany, 1999. **65**(1-4): p. 159-174.
5. Costanza, R., et al., *The value of the world's ecosystem services and natural capital*. Nature, 1997. **387**(15): p. 253-260.
6. Shin, H. and H.K. Choi, *Taxonomy and distribution of Zostera (Zosteraceae) in eastern Asia, with special reference to Korea*. Aquatic Botany, 1998. **60**: p. 47-66.
7. Hartog, C.D. and Z. Yang, *A catalogue of the seagrasses of China*. Chinese Journal of Oceanology and Limnology, 1990. **8**(1): p. 74-91.
8. Ueguchi-Tanaka, M., et al., *Gibberellin receptor and its role in gibberellin signaling in plants*. Annu Rev Plant Biol, 2007. **58**: p. 183-198.
9. Sun, T.P., *Gibberellin metabolism, perception and signaling pathways in Arabidopsis*. Arabidopsis Book, 2008. **6**: p. e0103.
10. Peng, J., et al., *The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses*. Genes Dev, 1997. **11**(23): p. 3194-3205.
11. Silverstone, A.L., C.N. Ciampaglio, and T. Sun, *The Arabidopsis RGA gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway*. Plant Cell, 1998. **10**(2): p. 155-169.
12. Ueguchi-Tanaka, M., et al., *GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin*. Nature, 2005. **437**(7059): p. 693-698.
13. Hirano, K., et al., *The GID1-mediated gibberellin perception mechanism is conserved in the Lycophyte Selaginella moellendorffii but not in the Bryophyte Physcomitrella patens*. Plant Cell, 2007. **19**(10): p. 3058-3079.
14. Ueguchi-Tanaka, M., et al., *Molecular interactions of a soluble gibberellin receptor, GID1, with a rice DELLA protein, SLR1, and gibberellin*. Plant Cell, 2007. **19**(7): p. 2140-2155.
15. Itoh, H., M. Ueguchi-Tanaka, and M. Matsuoka, *Molecular biology of gibberellins signaling in higher plants*. Int Rev Cell Mol Biol, 2008. **268**: p. 191-221.
16. Blazquez, M.A., et al., *Gibberellins promote flowering of arabidopsis by activating the LEAFY promoter*. Plant Cell, 1998. **10**(5): p. 791-800.
17. Moon, J., et al., *The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis*. Plant J, 2003. **35**(5): p. 613-623.
18. Hedden, P. and A.L. Phillips, *Gibberellin metabolism: new insights revealed by the genes*. Trends Plant Sci, 2000. **5**(12): p. 523-530.

19. Giacomelli, L., et al., *Gibberellin metabolism in Vitis vinifera L. during bloom and fruit-set: functional characterization and evolution of grapevine gibberellin oxidases*. J Exp Bot, 2013. **64**(14): p. 4403-4419.
20. Zhao, H., J. Dong, and T. Wang, *Function and Expression Analysis of Gibberellin Oxidases in Apple*. Plant Molecular Biology Reporter, 2010. **26**(2): p. 231-238.
21. Fleet, C.M. and T.P. Sun, *A DELLAcate balance: the role of gibberellin in plant morphogenesis*. Curr Opin Plant Biol, 2005. **8**(1): p. 77-85.
22. Mitchum, M.G., et al., *Distinct and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development*. Plant J, 2006. **45**(5): p. 804-818.
23. Wuddineh, W.A., et al., *Identification and overexpression of gibberellin 2-oxidase (GA2ox) in switchgrass (Panicum virgatum L.) for improved plant architecture and reduced biomass recalcitrance*. Plant Biotechnol J, 2015. **13**(5): p. 636-647.
24. Schomburg, F.M., et al., *Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants*. Plant Cell, 2003. **15**(1): p. 151-163.
25. Rieu, I., et al., *The gibberellin biosynthetic genes AtGA20ox1 and AtGA20ox2 act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle*. Plant J, 2008. **53**(3): p. 488-504.
26. Reinecke, D.M., et al., *Gibberellin 3-oxidase gene expression patterns influence gibberellin biosynthesis, growth, and development in pea*. Plant Physiol, 2013. **163**(2): p. 929-945.
27. Xiao, et al., *Overexpression of the Gibberellin 2-Oxidase Gene from Camellia lipoensis Induces Dwarfism and Smaller Flowers in Nicotiana tabacum*. PLANT MOL BIOL REP, 2016. **34**(1): p. 182-191.
28. Yan, J., et al., *Ectopic expression of GA 2-oxidase 6 from rapeseed (Brassica napus L.) causes dwarfism, late flowering and enhanced chlorophyll accumulation in Arabidopsis thaliana*. Plant Physiol Biochem, 2017. **111**: p. 10-19.
29. Kaneko, M., et al., *Where do gibberellin biosynthesis and gibberellin signaling occur in rice plants?* Plant J, 2003. **35**(1): p. 104-115.
30. Han, F. and B. Zhu, *Evolutionary analysis of three gibberellin oxidase genes in rice, Arabidopsis, and soybean*. Gene, 2011. **473**(1): p. 23-35.
31. Jones, N., *Transcriptional regulation by dimerization: two sides to an incestuous relationship*. Cell, 1990. **61**(1): p. 9-11.
32. Toledo-Ortiz, G., E. Huq, and P.H. Quail, *The Arabidopsis basic/helix-loop-helix transcription factor family*. Plant Cell, 2003. **15**(8): p. 1749-1770.
33. Heo, J.O., et al., *Funneling of gibberellin signaling by the GRAS transcription regulator scarecrow-like 3 in the Arabidopsis root*. Proc Natl Acad Sci U S A, 2011. **108**(5): p. 2166-2171.
34. Zhang, Z.L., et al., *Scarecrow-like 3 promotes gibberellin signaling by antagonizing master growth repressor DELLA in Arabidopsis*. Proc Natl Acad Sci U S A, 2011. **108**(5): p. 2160-2165.

35. Gocal, G.F., et al., *Long-day up-regulation of a GAMYB gene during Lolium temulentum inflorescence formation*. Plant Physiol, 1999. **119**(4): p. 1271-1278.

Figures

Figure 1

ZjGRF1 inhibits GA synthesis. (a) GA content in 17-d-old seedlings of WT and transgenic ZjGRF1 Arabidopsis (SD, n=3). (b) GA synthesis genes expression level in 17-d-old seedlings of WT and transgenic ZjGRF1 Arabidopsis (SD, n=3). Actin2 was used as internal reference, and the relative transcription level was determined by comparative threshold cycle (Ct). Three independent biological replicates of each genotype were statistically analyzed. * P<0.05

Figure 2

GA receptors expression level in 17-d-old seedlings of WT and transgenic ZjGRF1 Arabidopsis (SD, n=3). Actin2 was used as internal reference, and the relative transcription level was determined by comparative threshold cycle (Ct). Three independent biological replicates of each genotype were statistically analyzed. * P<0.05, ** P<0.01

Figure 3

GA response genes expression level in 17-d-old seedlings of WT and transgenic ZjGRF1 Arabidopsis (SD, n=3). Actin2 was used as internal reference, and the relative transcription level was determined by comparative threshold cycle (Ct). Three independent biological replicates of each genotype were statistically analyzed. * P<0.05, ** P<0.01

Figure 4

Transgenic ZjGRF1 Arabidopsis is insensitive to GA synthesis inhibitor PAC. (a) Graphical representation of the seedlings treated with PAC (2 μ M). Seedlings were transferred at day 4 and photographed 10 d later. (b) Root elongation of transgenic ZjGRF1 Arabidopsis grown under the LD condition in the absence or presence of 1 μ M PAC for 14 d (SD, n=18). (c) Increased seed germination in the transgenic ZjGRF1 Arabidopsis in the presence of 40 μ M PAC. Imbibed seeds were germinated under white light with or without 40 μ M PAC (SD, n=3). (d) Hypocotyl elongation of transgenic ZjGRF1 Arabidopsis grown in the dark in the presence of 1 μ M PAC for 12 d. (SD, n=3). ** P<0.01

Figure 5

Expression of *SOC1* and *LFY* mRNAs in transgenic ZjGRF1 Arabidopsis grown under the LD condition. Seventeen-day-old LD-grown plants were sampled for expression analysis at the 12th hour of light. Actin2 was used as internal reference, and the relative transcription level was determined by comparative threshold cycle (Ct). Three independent biological replicates of each genotype were statistically analyzed. Error bars indicate SD. * $P < 0.05$

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryfile1.pdf](#)