NOVEDADES SOBRE CRISPR–Cas9

(Hoy con autores santafesinos)

A GRF–GIF chimeric protein improves the regeneration efficiency of transgenic plants

David M. Tricoli, Maria F. Ercoli, Sadiye Hayta, Pamela Ronald, **Javier F. Palatnik** & Jorge Dubcovsky

Nature Biotechnology (2020)Cite this article

- Published: 12 October 2020

Abstract

The potential of genome editing to improve the agronomic performance of crops is often limited by low plant regeneration efficiencies and few transformable genotypes. Here, we show that expression of a fusion protein combining wheat GROWTH-REGULATING FACTOR 4 (GRF4) and its cofactor GRF-INTERACTING FACTOR 1 (GIF1) substantially increases the efficiency and speed of regeneration in wheat, triticale and rice and increases the number of transformable wheat genotypes. *GRF4–GIF1* transgenic plants were fertile and without obvious developmental defects. Moreover, *GRF4–GIF1* induced efficient wheat regeneration in the absence of exogenous cytokinins, which facilitates selection of transgenic plants without selectable markers. We also combined *GRF4–GIF1* with CRISPR–Cas9 genome editing and generated 30 edited wheat plants with disruptions in the gene *Q* (*AP2L-A5*). Finally, we show that a dicot *GRF–GIF* chimera improves regeneration efficiency in citrus, suggesting that this strategy can be applied to dicot crops.

Data availability

Accession numbers and gene names are available in the phylogenetic tree in Supplementary Fig. 1. All wheat gene names are based on genome release RefSeq v1.0. The raw data for the different experiments are available in Supplementary Tables 3, 4, 6 and 7. The steps for the generation of the different vectors and the transformation protocols are described in the Methods. The following vectors will be available through Addgene (http://www.addgene.org/): JD553-wheat *GRF4–GIF1* in pDONR, JD633-wheat *GRF4–GIF1* in the CRISPR vector, JD630-*Vitis GRF4–GIF1* in pDONR, JD638-*Vitis* miR396-resistant *GRF4–GIF1* in pDONR, JD689-*Citrus GRF4–GIF1* in pDONR, JD690-*Citrus GRF4–GIF1* in pGWB14, JD631-*Vitis GRF4–GIF1* in pGWB14 and JD639-*Vitis* miR396-resistant *GRF4–GIF1* in pGWB14.

References

1. 1.

   Lotan, T. et al. *Arabidopsis LEAFY COTYLEDON1* is sufficient to induce embryo development in vegetative cells. *Cell* **93**, 1195–1205 (1998).

   CAS Article Google Scholar

2. 2.

   Lowe, K. et al. In *Plant Biotechnology 2002 and Beyond: Proceedings of the 10th International Association for Plant Tissue Culture and Biotechnology Congress* (ed. Vasil, I. K.) 283–284 (Springer, 2003).

3. 3.

   Stone, S. L. et al. *LEAFY COTYLEDON2* encodes a B3 domain transcription factor that induces embryo development. *Proc. Natl Acad. Sci. USA* **98**, 11806–11811 (2001).

CAS Article Google Scholar

4. 4.

Zuo, J. R., Niu, Q. W., Frugis, G. & Chua, N. H. The *WUSCHEL* gene promotes vegetative-to-embryonic transition in *Arabidopsis*. *Plant J.* **30**, 349–359 (2002).

CAS Article Google Scholar

5. 5.

Boutilier, K. et al. Ectopic expression of *BABY BOOM* triggers a conversion from vegetative to embryonic growth. *Plant Cell* **14**, 1737–1749 (2002).

CAS Article Google Scholar

6. 6.

Gordon-Kamm, B. et al. Using morphogenic genes to improve recovery and regeneration of transgenic plants. *Plants* **8**, 38 (2019).

7. 7.

Lowe, K. et al. Rapid genotype "independent" *Zea mays* L. (maize) transformation via direct somatic embryogenesis. *In Vitro Cell. Dev. Biol. Plant* **54**, 240–252 (2018).

CAS Article Google Scholar

8. 8.

Lowe, K. et al. Morphogenic regulators *Baby boom* and *Wuschel* improve monocot transformation. *Plant Cell* **28**, 1998–2015 (2016).

CAS Article Google Scholar

9. 9.

Maher, M. F. et al. Plant gene editing through de novo induction of meristems. *Nat. Biotechnol.* **38**, 84–89 (2020).

CAS Article Google Scholar

10. 10.

Omidbakhshfard, M. A., Proost, S., Fujikura, U. & Mueller-Roeber, B. Growth-regulating factors (GRFs): a small transcription factor family with important functions in plant biology. *Mol. Plant* **8**, 998–1010 (2015).

CAS Article Google Scholar

11. 11.

Kim, J. H., Choi, D. S. & Kende, H. The *AtGRF* family of putative transcription factors is involved in leaf and cotyledon growth in *Arabidopsis*. *Plant J.* **36**, 94–104 (2003).

CAS Article Google Scholar

12. 12.

Kim, J. H. & Kende, H. A transcriptional coactivator, *AtGIF1*, is involved in regulating leaf growth and morphology in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **101**, 13374–13379 (2004).

CAS Article Google Scholar

13.13.

Horiguchi, G., Kim, G. T. & Tsukaya, H. The transcription factor *AtGRF5* and the transcription coactivator *AN3* regulate cell proliferation in leaf primordia of *Arabidopsis thaliana*. *Plant J.* **43**, 68–78 (2005).

CAS Article Google Scholar

14.14.

Debernardi, J. M., Rodriguez, R. E., Mecchia, M. A. & Palatnik, J. F. Functional specialization of the plant miR396 regulatory network through distinct microRNA–target interactions. *PLoS Genet.* **8**, e1002419 (2012).

15.15.

Debernardi, J. M. et al. Post-transcriptional control of GRF transcription factors by microRNA miR396 and GIF co-activator affects leaf size and longevity. *Plant J.* **79**, 413–426 (2014).

CAS Article Google Scholar

16.16.

Vercruyssen, L. et al. ANGUSTIFOLIA3 binds to SWI/SNF chromatin remodeling complexes to regulate transcription during *Arabidopsis* leaf development. *Plant Cell* **26**, 210–229 (2014).

CAS Article Google Scholar

17.17.

Liebsch, D. & Palatnik, J. F. MicroRNA miR396, GRF transcription factors and GIF co-regulators: a conserved plant growth regulatory module with potential for breeding and biotechnology. *Curr. Opin. Plant Biol.* 53, 31–42 (2020).

CAS Article Google Scholar

18.18.

Li, S. C. et al. The *OsmiR396c–OsGRF4–OsGIF1* regulatory module determines grain size and yield in rice. *Plant Biotechnol. J.* **14**, 2134–2146 (2016).

CAS Article Google Scholar

19.19.

Rodriguez, R. E. et al. Control of cell proliferation in *Arabidopsis thaliana* by microRNA miR396. *Development* **137**, 103–112 (2010).

CAS Article Google Scholar

20.20.

He, Z. S. et al. *OsGIF1* positively regulates the sizes of stems, leaves, and grains in rice. *Front. Plant Sci.* **8**, 1730 (2017).

21.21.

Shimano, S. et al. Conserved functional control, but distinct regulation, of cell proliferation in rice and *Arabidopsis* leaves revealed by comparative analysis of *GRF-INTERACTING FACTOR 1* orthologs. *Development* **145**, dev159624 (2018).

22.22.

Zhang, D. et al. *GRF-Interacting Factor1* regulates shoot architecture and meristem determinacy in maize. *Plant Cell* **30**, 360–374 (2018).

CAS Article Google Scholar

23.23.

Duan, P. et al. Regulation of OsGRF4 by OsmiR396 controls grain size and yield in rice. *Nat. Plants* **2**, 15203 (2015).

Article Google Scholar

24.24.

Hu, J. et al. A rare allele of *GS2* enhances grain size and grain yield in rice. *Mol. Plant* **8**, 1455–1465 (2015).

CAS Article Google Scholar

25.25.

Che, R. H. et al. Control of grain size and rice yield by GL2-mediated brassinosteroid responses. *Nat. Plants* **2**, 15195 (2016).

CAS Article Google Scholar

26.26.

Sun, P. Y. et al. OsGRF4 controls grain shape, panicle length and seed shattering in rice. *J. Integ. Plant Biol.* **58**, 836–847 (2016).

CAS Article Google Scholar

27.27.

Li, S. et al. Modulating plant growth—metabolism coordination for sustainable agriculture. *Nature* **560**, 595–600 (2018).

CAS Article Google Scholar

28.28.

Rodriguez, R. E. et al. MicroRNA miR396 regulates the switch between stem cells and transit-amplifying cells in *Arabidopsis* roots. *Plant Cell* **27**, 3354–3366 (2015).

CAS Article Google Scholar

29.29.

Ishida, Y., Hiei, Y. & Komari, T. In *Proceedings of the 12th International Wheat Genetics Symposium* (eds. Ogihara, Y. et al.) 167–173 (Springer, 2015).

30.30.

Richardson, T., Thistleton, J., Higgins, T. J., Howitt, C. & Ayliffe, M. Efficient *Agrobacterium* transformation of elite wheat germplasm without selection. *Plant Cell Tiss. Organ Cult.* **119**, 647–659 (2014).

CAS Article Google Scholar

31.31.

Wang, K., Liu, H. Y., Du, L. P. & Ye, X. G. Generation of marker-free transgenic hexaploid wheat via an *Agrobacterium*-mediated co-transformation strategy in commercial Chinese wheat varieties. *Plant Biotechnol. J.* **15**, 614–623 (2017).

CAS Article Google Scholar

32.32.

Hayta, S. et al. An efficient and reproducible *Agrobacterium*-mediated transformation method for hexaploid wheat (*Triticum aestivum* L.). *Plant Methods* **15**, 121 (2019).

CAS Article Google Scholar

33.33.

Debernardi, J. M., Lin, H., Chuck, G., Faris, J. D. & Dubcovsky, J. microRNA172 plays a crucial role in wheat spike morphogenesis and grain threshability. *Development* **144**, 1966–1975 (2017).

CAS Article Google Scholar

34.34.

Kong, J. et al. Overexpression of the transcription factor *GROWTH-REGULATING FACTOR5* improves transformation of dicot and monocot species. *Front. Plant Sci*. https://doi.org/10.3389/fpls.2020.572319 (2020).

35.35.

Wang, W., Akhunova, A., Chao, S. & Akhunov, E. Optimizing multiplex CRISPR/Cas9-based genome editing for wheat. Preprint at *bioRxiv* https://doi.org/10.1101/051342 (2016).

36.36.

Chern, M. S. et al. Evidence for a disease-resistance pathway in rice similar to the NPR1-mediated signaling pathway in *Arabidopsis*. *Plant J.* **27**, 101–113 (2001).

CAS Article Google Scholar

Download references

Acknowledgements

This project was supported by the Howard Hughes Medical Institute, NRI Competitive Grant 2017-67007-25939 from the USDA National Institute of Food and Agriculture (NIFA) and the International Wheat Partnership Initiative (IWYP). **J.F.P. acknowledges support from the Argentinean Research Council (CONICET); Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación; and International Centre for Genetic Engineering and Biotechnology, grant ICGEB/ARG17-01.** P.R. was supported by NIH grant GM122968. S.H. acknowledges support from the Biotechnology and Biological Sciences Research Council Genes in the Environment Institute Strategic Programme BB/P013511/1. J.M.D. was supported by a fellowship (LT000590/2014-L) from the Human Frontier Science Program. M.F.E. is a Latin American Fellow in the Biomedical Sciences, supported by the Pew Charitable Trusts. We thank Y. Wang (Chinese Academy of Sciences, Beijing) for the pYP25F binary vector, M. Padilla, R. Rasia, G. Rabasa, B. Van Bockern and M. Smedley for excellent technical support and C. Uauy for coordinating the testing of the *GRF4–GIF1* chimera at the John Innes Centre.

Author information

Affiliations

1. **Department of Plant Sciences, University of California, Davis, CA, USA**
   Juan M. Debernardi & Jorge Dubcovsky

2. **Howard Hughes Medical Institute, Chevy Chase, MD, USA**
   Juan M. Debernardi & Jorge Dubcovsky

3. **Plant Transformation Facility, University of California, Davis, CA, USA**
   David M. Tricoli

4. **Department of Plant Pathology, University of California, Davis, CA, USA**
   Maria F. Ercoli & Pamela Ronald

5. **Genome Center, University of California, Davis, CA, USA**
   Maria F. Ercoli & Pamela Ronald

6. **Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich, UK**
   Sadiye Hayta

7. **Instituto de Biología Molecular y Celular de Rosario, CONICET and Universidad Nacional de Rosario, Santa Fe, Argentina**
   Javier F. Palatnik

8. **Centro de Estudios Interdisciplinarios, Universidad Nacional de Rosario, Santa Fe, Argentina**
   Javier F. Palatnik

Corresponding author

Correspondence to Jorge Dubcovsky.