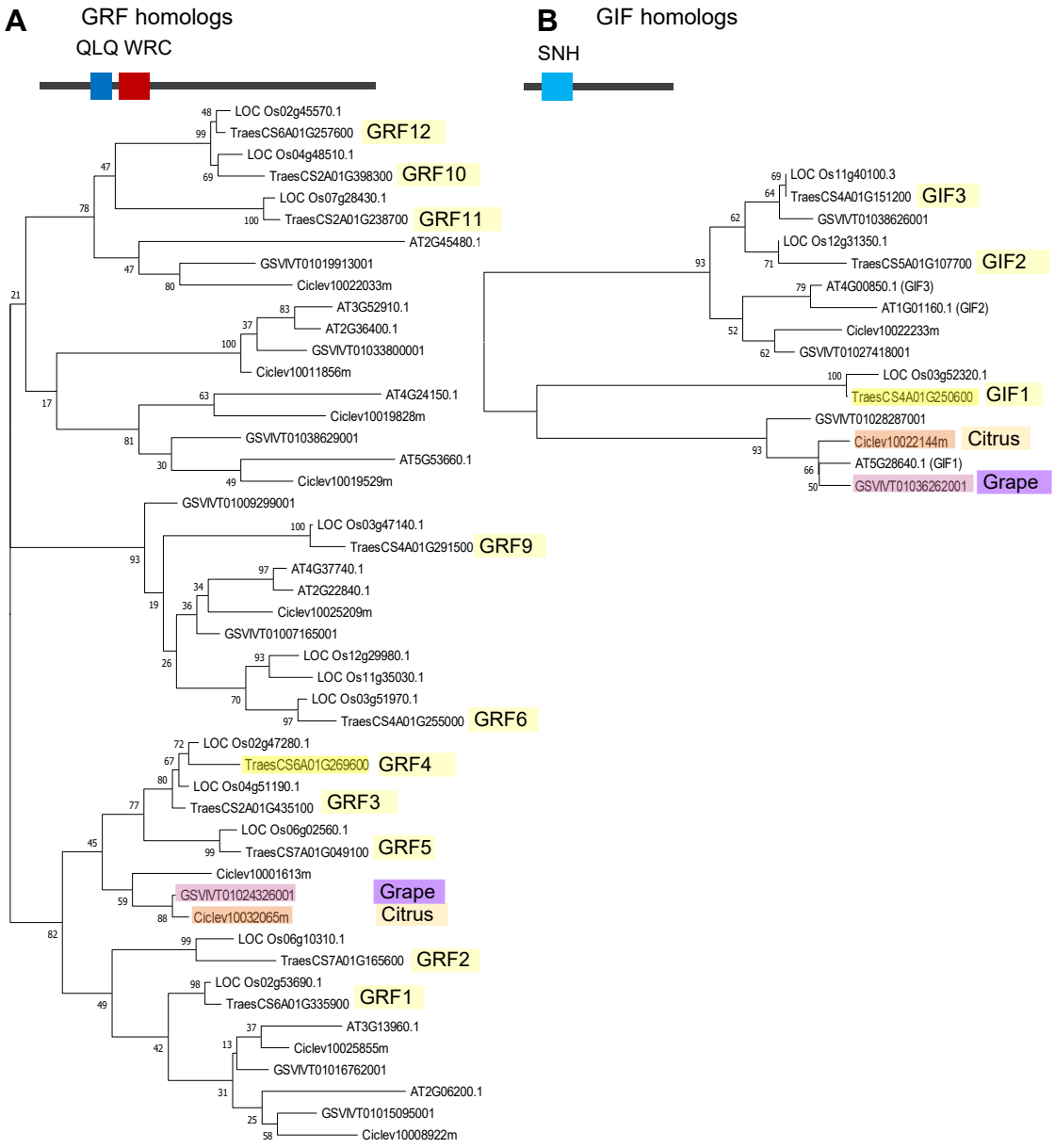
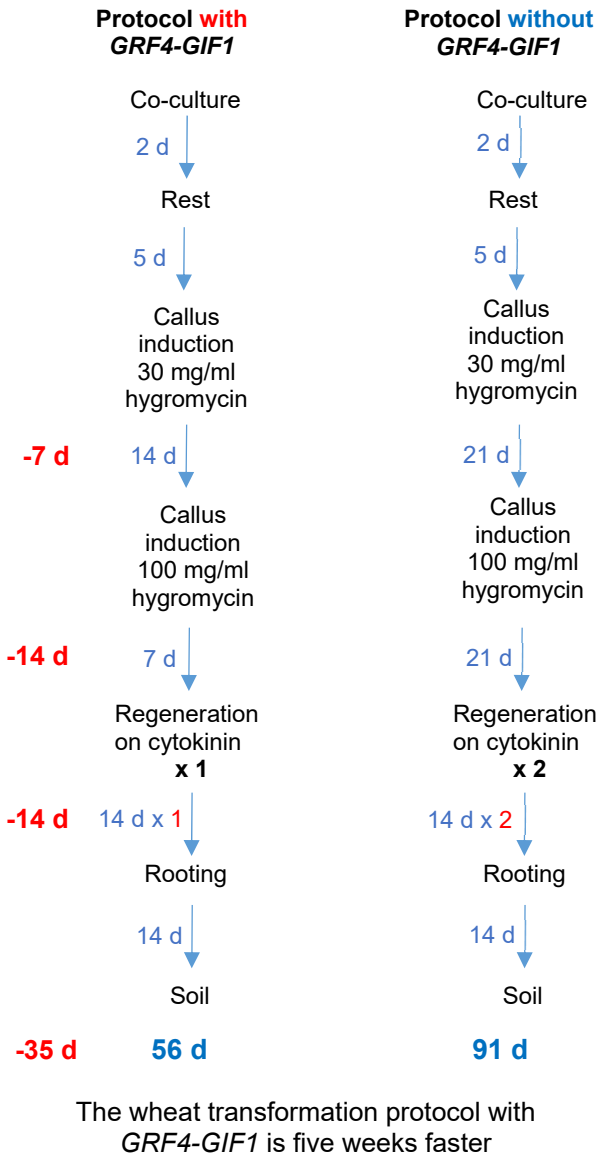
Supplementary Figures

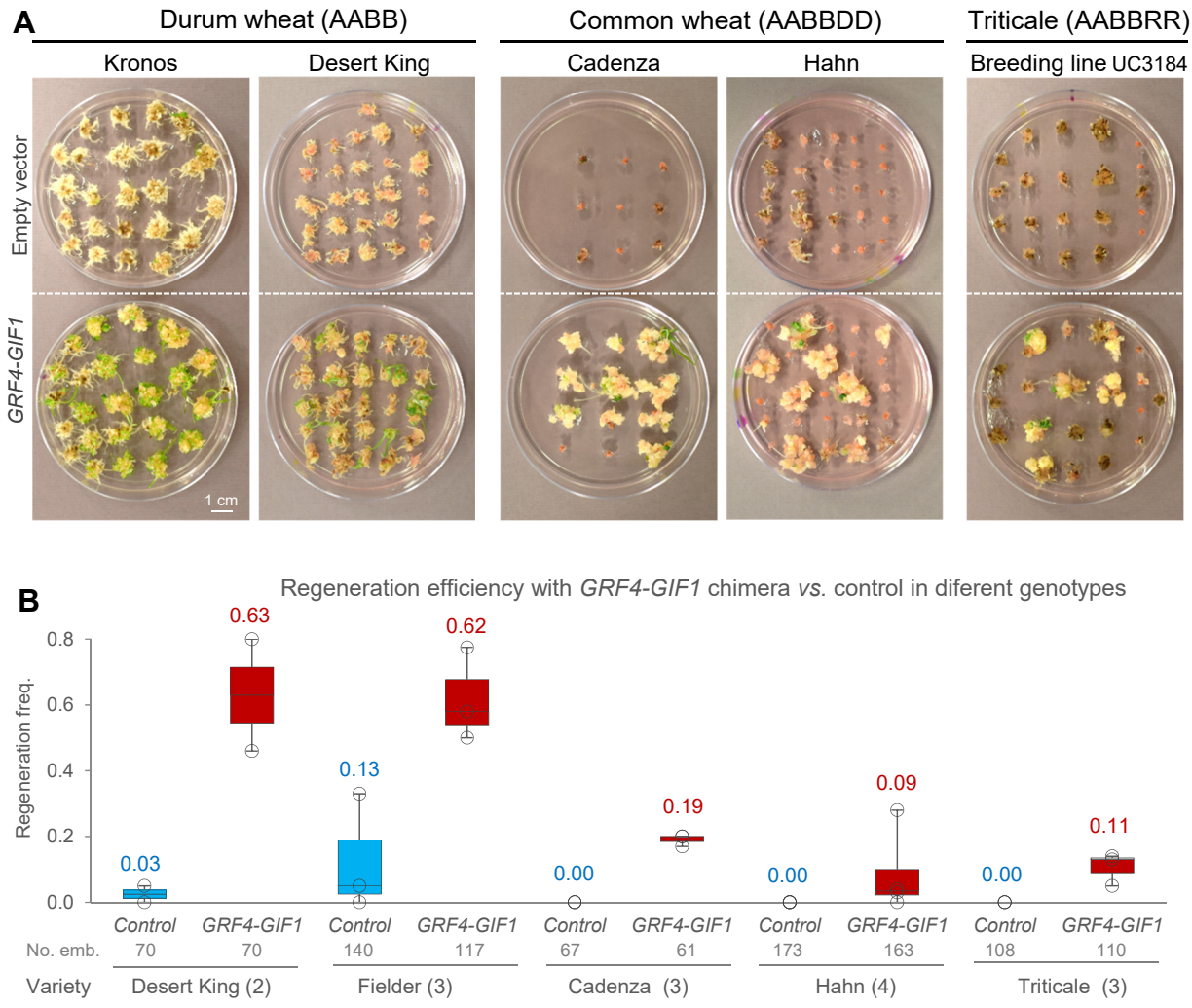
Supplementary Figure 1. Phylogenetic trees of GRF and GIF families for wheat (yellow highlight and corresponding RefSeq v1.0 names), rice, Arabidopsis, citrus and grape. The closest homologs to wheat GRF4 and GIF1 are highlighted in orange for citrus and in violet for grape. **A**) We used the QLQ and WRC domains for the analysis of the GRF proteins and **B**) the SNH domain for the analysis of the GIF proteins. The evolutionary history was inferred by using the Maximum Likelihood method. We show the tree with the highest log-likelihood. The percentage of trees in which the associated taxa clustered together is shown next to the branches. We conducted the evolutionary analysis in MEGA X [1]. Yellow highlight: wheat. Orange highlight: selected *Citrus* homolog. Violet highlight: selected *Vitis* homolog. Note that the cluster including wheat and rice GRF3, GRF4, and GRF5 proteins does not include any Arabidopsis protein.



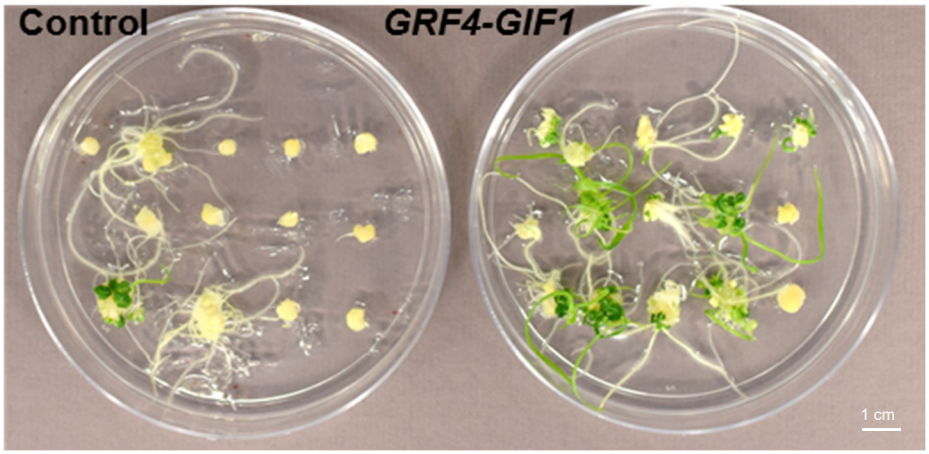
Supplementary Figure 2. Accelerated wheat transformation protocol using the *GRF4-GIF1* chimera relative to normal protocol of wheat transformation at the UC Davis transformation facility. The protocol with the *GRF4-GIF1* chimera is faster, reducing the overall process by five weeks.



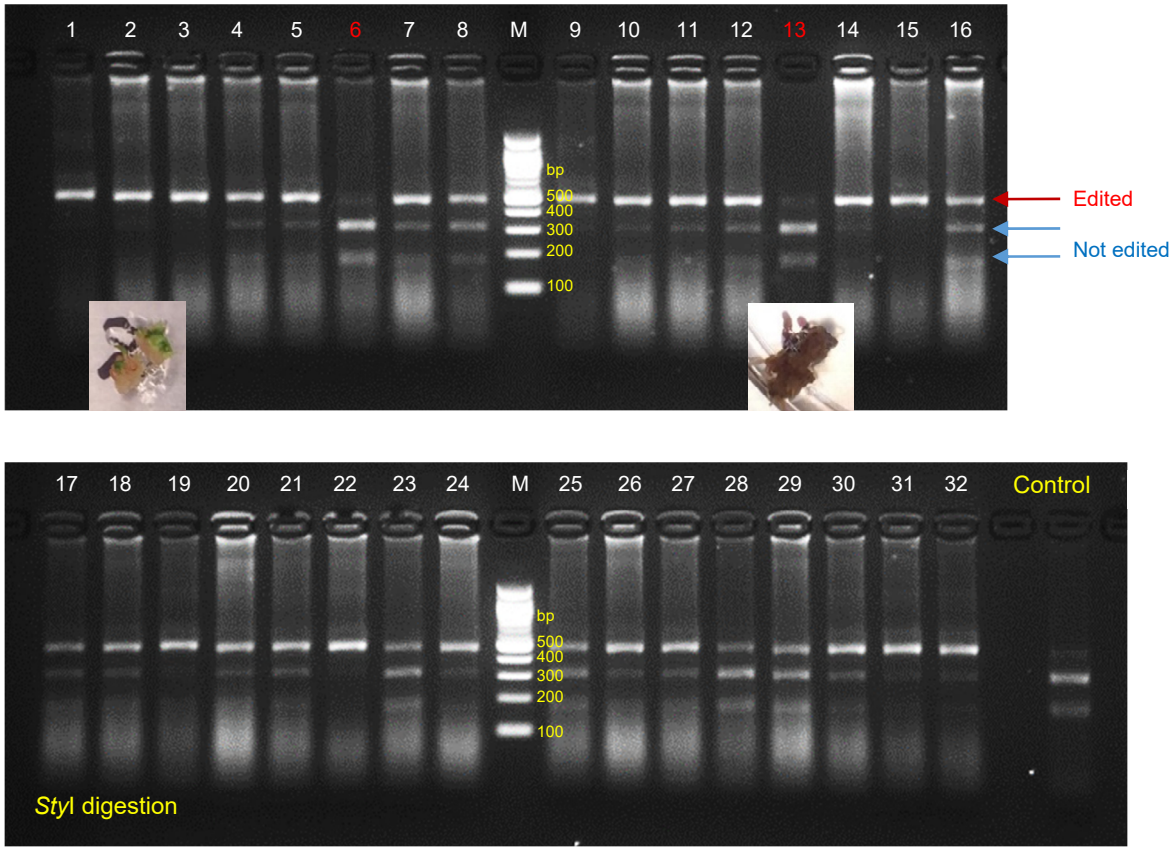
Supplementary Figure 3. Effect of the *GRF4-GIF1* chimera in regeneration efficiency in different genotypes. **A**) Representative transformations showing higher frequency of regenerated shoots in the presence of the *GRF4-GIF1* chimera than in the control (empty vector) in different wheat and Triticale genotypes. **B**) Box-plots showing regeneration efficiencies of *GRF4-GIF1 vs.* control in the same cultivars as in A. The raw data is available in Supplementary Table 4A and B. The number of independent experiments is indicated in parenthesis after the genotype name and the total number of inoculated embryos is indicated below. The box shows the range from first to third quartiles, and is divided by the median. The whiskers span down to the minimum, and up to the maximum observation. Empty black circles are regeneration results from individual experiments. No statistical analysis is presented for these experiments because transformations of many of these cultivars without the *GRF4-GIF1* chimera showed 0 or close to 0 regeneration frequencies. Averages are presented above the box-plots.



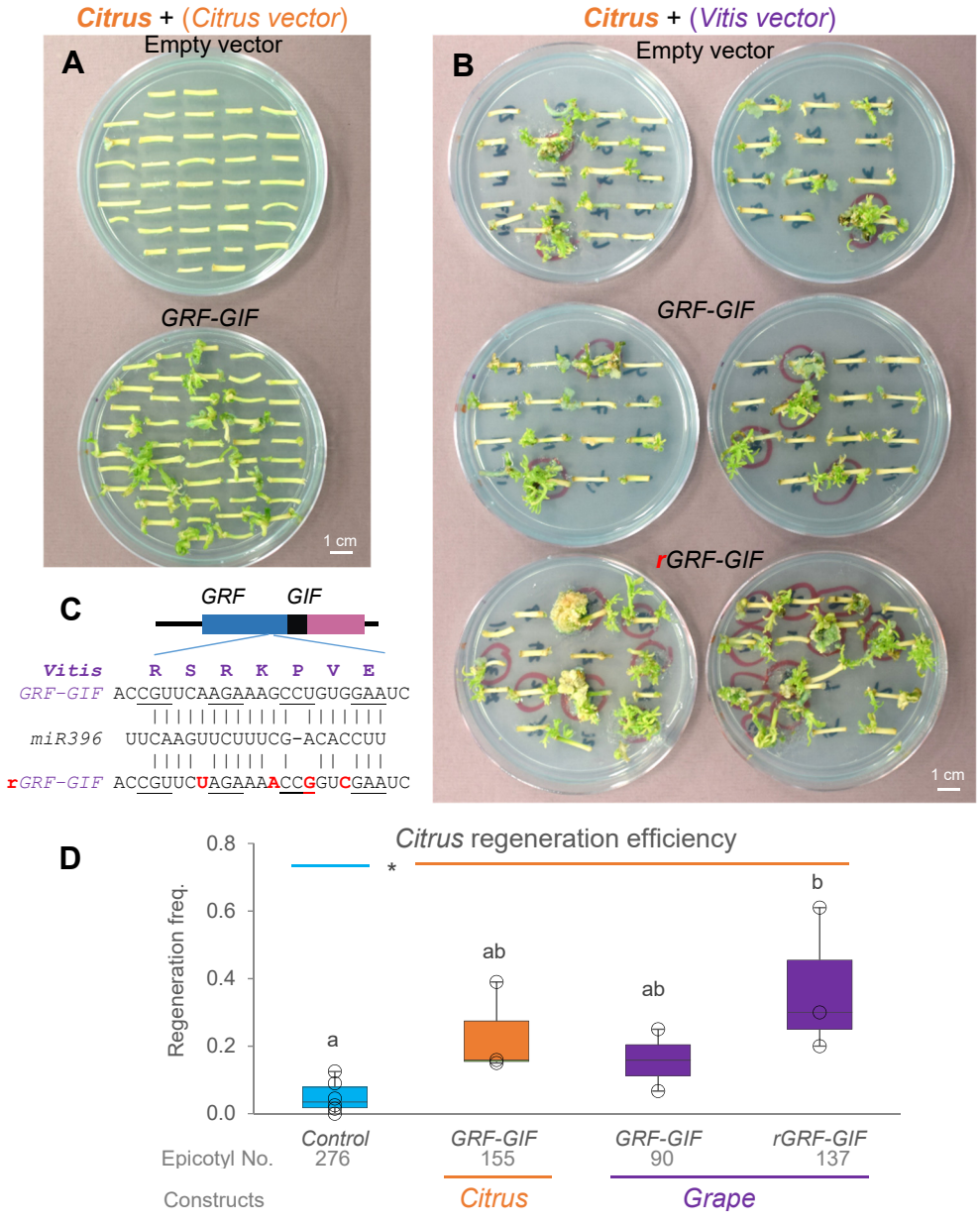
Supplementary Figure 4. Effect of the *GRF4-GIF1* chimera in regeneration efficiency in the absence of exogenous cytokinin. Immature wheat embryos from a *GRF4-GIF1* transgenic Kronos T_1 plant and a segregating non-transgenic T_1 sister line where treated following the standard transformation protocol, excluding the *Agrobacterium* inoculation and the addition of hygromycin to the plates. In the last step, the calli where transferred to regeneration media in the absence of cytokinin. The number of calli regenerating green shoots was significantly higher in the *GRF4-GIF1* transgenic plant (21 out of 27) than in the non-transgenic sister control (3 out of 26). The picture shows representative plates with calli in regeneration media without cytokinin.



Supplementary Figure 5. Genome edited wheat plants using combined *GRF4-GIF1* – CRISPR-Cas9 technology. We recovered 30 independent transgenic events (white numbers) out of 32 infected callus. Calli 6 and 13 (red numbers) were not transgenic. Figures are examples of a transgenic (2) and non-transgenic (13) callus. Editing disrupts a *Sty*I restriction site in the target region resulting in an undigested band (red arrow). Not edited sequences are digested (blue arrows). Ten of the edited events were sequenced and the detected mutations are presented in Figure 3D. M = 100 bp ladder. Control = non-transgenic Kronos.



Supplementary Figure 6. Transformation of dicot species with GRF-GIF chimers. A) *Citrus* epicotyls transformed with an empty vector and the *Citrus GRF-GIF* chimera (60 d after *Agrobacterium* inoculation). **B**) *Citrus* epicotyls transformed with an empty vector and the *Vitis GRF-GIF* and miR396-resistant *Vitis GRF-GIF* (*rGRF-GIF*) (120 d after inoculation). **C**) Scheme of a *Vitis GRF-GIF* chimera showing the miR396 target site and its interaction with miR396 below. In the miR396-resistant *rGRF-GIF* version, we introduced silent mutations (in red) to reduce interactions with miR396. **D**) Box-plots comparing three *Citrus* experiments. Regeneration results from individual experiments are indicated by empty black circles. Box-plot definition is the same as in Supplementary Figure 3. Different letters above the box-plots indicate significant Tukey test ($P < 0.05$). Horizontal lines on top indicate a significant contrast between the control and combined *GRF-GIF* constructs ($P = 0.0136$). Normality of residuals was confirmed by Shapiro-Wilk's test and homogeneity of variances by Levene's test.



SUPPLEMEN

SUPPLEMENTARY TABLES

Supplementary Table 1. Primers used in this study.

Name	Sequence	Gene
Fw-GRF4a	GGGGACAAGTTTGTACAAAAAAGCTGCCACCATGGCGATGCCGTATGCCTCT	GRF4
Rev-GRF4a	GGGGACCACTTTGTACAAGAAAGCTGAACGGTACATYTCGCCGGCGAACAG	
Fw-GIF1a	GGGGACAAGTTTGTACAAAAAAGCTGCCACCATGCAGCAGCAACACCTGATG	GIF1
Rev-GIF1a	GGGGACCACTTTGTACAAGAAAGCTGAACGGCTTCCTTCCTCCTCGGT	
Fw-GRF4a	GGGGACAAGTTTGTACAAAAAAGCTGCCACCATGGCGATGCCGTATGCCTCT	GRF4-GIF1
Rev-GRF4b	GGCAGCGGCCGCGTACATYTCGCCGGCGAACAG	
Fw-GIF1b	GCGGCCGCTGCCATGCAGCAGCAACACCTGATG	
Rev-GIF1b	GGGGACCACTTTGTACAAGAAAGCTGAACGCTAGCTTCCTTCCTCCTCGGT	
Fw_HindIII	GCCACTCAGCAAGCTTTGCAGCGT	Ubi::GRF4-term
Rev-HindIII	TCACGCTGCAAAGCTCTAATTCCCGATCTAGTAAC	
Fw_ZmUbi-AscI	GGATCTGCAGGCGCGTGCAGCGTGACCCGGTCGTG	Ubi::GRF4-GIF1-term
Rev_NosTerm-AscI	TGCACTGCAGGCGCGCTAATTCCCGATCTAGTAAC	
QT1-F-GG	ACTTGATGAGGAACTGGACCAAGG	Q gene gRNA
QT1-R-GG	AAACCCTTGGTCCAGTTCCTCATC	
QT1check-F	TGAGCGACTACGAGGAGGAT	Q gene genotyping
QT1check-R	CAGCTGCCCTGTCACATCTA	
Fw-rGRF-Vvi	TCTAGAAAACCGGTCGAATCACAAACTA	rGRF-GIF
Rev-rGRF-Vvi	TCGACCGGTTTTCTAGAACGGTTGCGG	
Fw-GRF-Vvi	GGGGACAAGTTTGTACAAAAAAGCTGCCACCATGAAGCAAAGCTTTGTGG	
Rev-GIF-Vvi	GGGGACCACTTTGTACAAGAAAGCTGAACGTCAATTCCCATCTTCAGCA	
pLC41_1064	TCGCTTATTTAAAGGGCGAAT	Transgenic plants genotyping
pLC41_1061	AGCGCGCAAACTAGGATAAA	

Supplementary Table 2. Grain measurements in *GRF4-GIF1* T1 transgenic plants and their sister negative controls in a growth chamber (16 h light at 22 °C and 8 h darkness at 18 °C, light intensity 260 μM m^{-2} s^{-1}). Two statistical analyses are presented: 1) A more conservative test using the averages of the families from each event as experimental units (5 negatives *vs*. 3 positives). 2) A more liberal test using the individual plants as experimental units (38 negatives *vs*. 16 positives). Parameters from individual plants were obtained from an average of 23 grains estimated from a Marvin Grain Analyzer. The JD561 numbers indicate independent transformation events with the same *GRF4-GIF1* construct.

GRF4-GIF1 (JD561)	Plants	Spikelets / spike	Grains / spike	TGW(g)	Area (mm^2)	Width (mm)	Length (mm)
Negative (JD561#2-1)	8	13.25	26	41.48	17.75	3.31	7.43
Negative (JD561#12-1)	9	12.78	23	57.04	20.85	3.69	7.80
Negative (JD561#20-6)	4	11.50	23	55.92	20.07	3.66	7.63
Negative (JD561#21-1)	12	11.67	27	55.50	19.74	3.60	7.63
Negative (JD561#23-8)	5	12.80	26	51.81	19.27	3.55	7.43
Positive (JD561#13-1)	9	9.67	18	59.68	22.18	3.67	8.31
Positive (JD561#20-11)	2	10.00	13	57.09	21.60	3.69	8.08
Positive (JD561#23-6)	5	11.00	21	60.66	21.38	3.68	8.00
Weighted Avg. negatives	38	12.39	25.10	52.47	19.56	3.56	7.60
Weighted Avg. transgenic	16	10.13	19.10	59.66	21.86	3.68	8.18
% increase		-18.3%	-23.9%	13.7%	11.7%	3.2%	7.7%
Two side *t*-test (family as e.u)		0.007	0.007	0.131	0.022	0.239	0.003
Two side *t*-test (plant as e.u.)		3.3E-04	8.9E-05	2.9E-03	5.7E-06	1.5E-02	1.5E-08

Supplementary Table 3. Regeneration frequencies for different *GRF-GIF* combinations compared with empty vector in tetraploid wheat Kronos. The number of embryos used is indicated below each frequency. Regeneration frequencies were estimated as the number of calluses showing at least one regenerating shoot / total number of inoculated embryos. The blue "x" indicate the experiments included in the statistical analyses presented in the different figures and supplementary figures. All experiments in this Table used the regular 91 d protocol and *Agrobacterium* strain EHA105.

		GRF4-	Fig.	GRF4 &	Fig.			Fig.	GRF4-		Fig.	GRF5	GRF1	GRF9	Fig.
Exp.	pLC41	GIF1	1D	GIF1	1E	GIF1	GRF4	1F	GIF2	GIF3	1G		-GIF1		S1
1-a	0.04 25	0.90 48	x												
1-b	0.08 25	0.96 25	x												
2		0.27 60		0.06 32	x										
3		0.91 79		0.77 83	x										
3b[a]		0.60 47		0.14 41	x										
4	0.13 53	0.70 50	x	0.46 48	x	0.57 47									
6	0.20 20	0.65 20	x	0.50 20	x	0.35 20									
22	0.16 24	0.82 28	x			0.16 24	0.64 25	x							
25	0.00 15	0.70 20	x			0.40 15	0.06 15	x							
25b[a]	0.00 15	0.35 17	x			0.00 15	0.00 15	x							
26	0.08 24	0.55 20	x			0.20 20	0.20 20	x							
26b[b]	0.06 16	0.31 16	x			0.10 10	0.12 16	x							
12	0.00 10	0.50 10	x						0.20 10	0.20 10	x				
13	0.17 24	0.72 25	x						0.50 24	0.46 24	x				
24	0.10 10	0.50 10	x						0.50 10	0.30 10	x	.	0.10 10	0.30 10	x
17	0.00 21	0.67 21	x									0.57 21	0.16 19	0.19 21	x
18	0.20 24	0.88 24	x									0.70 23	0.79 24	0.76 21	x
28	0.02 44	0.56 43	x									0.21 42	0.16 45	0.06 18	x

"-" indicates a fused protein or chimera, "&" indicates individual genes induced by separate promoters.

[a] No embryo dissection.

[b] No cytokinin.

Supplementary Table 4. Regeneration frequencies in plants transformed with the *Ubi::GRF4-GIF1* chimera or the empty vector pLC41. **A**) Tetraploid and hexaploid wheat commercial cultivars. **B**) Triticale breeding line UC3190. EHA105 and AGL1 are two different *Agrobacterium* strains (no differences were observed between the two strains). The number of embryos used for each genotype is indicated below the regeneration frequency.

A. Wheat

Desert King (4x)	Exp1 EHA105	Exp2 EHA105	**Average**	**SE**
pLC41	0.05 20	0 50	**0.025**	0.025
Ubi::GRF4-GIF1	0.80 20	0.46 50	**0.630**	0.170

Fielder (6x)	Exp1 UCD EHA105	Exp2 UCD EHA105	UCD **Average**	UCD **SE**
pLC41	0.05 49	0 10	**0.025**	0.025
Ubi::GRF4-GIF1	0.58 67	0.5 10	**0.540**	0.040

Fielder (6x)	Exp1 JIC AGL1	Three Fielder experiments **Average**	 **SE**
pAGM8031	0.33 81	0.127	0.103
Ubi::GRF4-GIF1	0.775 40	0.618	0.082

Cadenza (6x)	Exp1 AGL1	Exp2 AGL1	Exp3 EHA105	**Average**	**SE**
pLC41	0 19	0 23	0 25	**0.000**	0.000
Ubi::GRF4-GIF1	0.20 12	0.17 24	0.20 25	**0.190**	0.010

Hahn (6x)	Exp1 EHA105	Exp2 EHA105	Exp3 EHA105	Exp4 AGL1	**Average**	**SE**
pLC41	0 31	0 48	0 69	0 25	**0.000**	0.000
Ubi::GRF4-GIF1	0.03 37	0.04 50	0 51	0.28 25	**0.088**	0.087

Supplementary Table 4B. Triticale

Triticale UC3190 (6x)	Exp9 EHA105	Exp11 EHA105	Exp15 EHA105	**Average**	**SE**
pLC41	0 45	0 21	0 42	**0.000**	0.000
Ubi::GRF4-GIF1	0.05 45	0.13 22	0.14 43	**0.107**	0.028

Supplementary Table 5. Regeneration frequencies in wheat transformation with *Agrobacterium*

Wheat methods	Explant	Average efficiency	Marker	Agro strain	Cultivars
This study without *GRF4-GIF1*	immature embryos	8.3 / 2.5 % 12.7 % / 0.0 %	HPT	EHA105	Kronos (4x) / 1 other (4x) Fielder (6x) / 2 other (6x)
GRF4-GIF1	immature embryos	65.1 / 63.0 % 61.8 / 13.9 %	HPT	EHA105	Kronos (4x) / 1 other (4x) Fielder (6x) / 2 other (6x)
Cheng et al., 1997 [2]	immature embryos	2.2 %	NPT	C58 (ABI)	Bobwhite (6x)
Khanna HK, Daggard GE 2003 [3]	immature embryos	3.9 %	PPT	LBA4404	Veery5 (6x)
Wu et al., 2003 [4]	immature embryos	9.5 / 4.5 %	PPT	AGL1	Bobwhite / 3 other (6x)
Cheng et al., 2003 [5]	immature embryos	1.1%	NPT II	C58 (ABI)	Bobwhite (6x)
Hu et al., 2003 [6]	immature embryos	4.4 %	Glyphosate	C58 (ABI)	Bobwhite (6x)
Przetakiewicz et al., 2004 [7]	immature embryos	12.6 / 2.3 %	NPT II	EHA101 / LBA4404	Kontesa, Torka & Eta (6x)
Mitic et al., 2004 [8]	immature embryos	0.6 %	PPT / HPT	AGL1 / LBA4404	Vesna (6x)
Wu et al., 2008 [9]	immature embryos	3.0 %	PPT	AGL1	Ofanto (4x)
Risacher et al., 2009 [10]	immature seeds *in planta*	5.0 %	NPT II	EHA105	NB1 (6x)
He et al., 2010 [11]	immature embryos	6.3 %	PPT	AGL1	Stewart (4x)
Bińka et al., 2012 [12]	immature embryos	3.4 %	NPT / PPT	EHA101/AGL1	Kontesa, Torka (6x)
Hensel et al., 2017 [13]	immature embryos	5 to 15 % [a]	HPT	AGL1	Bobwhite (6x)
Hayta et al., 2019 [14]	immature embryos	19 %	HPT	AGL1	Fielder (6x)
Proprietary Japan Tobacco [b]					
Ishida et al., 2015 [c] [15]	immature embryos	76.2 / 60.8 %	PPT / HPT	EHA101/EHA105	Fielder (6x, PPT *vs* HPT)
Richardson et al., 2014 [16]	immature embryos	40.9 / 12.1 % 50.8 / 26.0 %	PPT	AGL1	Fielder / 9 other (6x) Kronos / 1 other (4x)
Wang et al., 2017 [17]	immature embryos	45.3 / 10.8 %	PPT	C58C1	Fielder / 17 other (6x)

[a] Only range provided

[b] At UCD, we purchased the JT license and received training at their company. However, without the *GRF4-GIF1*, we have not been able to obtain the high regeneration efficiencies reported in Ishida et al. 2015 [15] (likely because we use a wider range of embryo sizes collected from plant grown under different conditions)

[c] Report by the Japan Tobacco company in a non-peer reviewed journal

Supplementary Table 6. Regeneration frequencies in rice (*Oryza sativa*) cultivar Kitaake. Experiments 1 and 6 utilized the wheat-optimized vector pLC41 with or without the *Ubi::GRF4-GIF1* chimera. Experiments 2-5 used pCAMBIA1300, a vector frequently utilized in rice transformation, with or without the *Ubi::GRF4-GIF1* chimera. In each of these three experiments, calli generated from the same seed stock were inoculated with *Agrobacterium* containing the designated vector construct. In experiment 2, pCAMBIA1300-sgRNA refers to the pCAMBIA1300 vector carrying *Ubi::GRF4-GIF1* chimera plus a sgRNA targeted to gene *OsKitaake06g041700* encoding a TYROSYLPROTEIN SULFOTRANSFERASE (TPST). In experiments 3 and 4, pCAMBIA1300-gus refers to the control pCAMBIA1300-gus without the chimera. All experiments employed *Agrobacterium* strain EHA105. The number of calli used for each genotype is indicated below the regeneration frequency

Rice Kitaake	Exp1	Exp2-4	Exp5	Exp6		
No. calli inoc.	n=85	n=100 x 3	n=50	n=50	**Average**	**SE**
No *GRF4-GIF1*	0.118	0.235	0.22	0.24	**0.2033**	0.028
	pLC41	pCAMBIA1300-gus	pCambia1300	pLC41		
Ubi::GRF4-GIF1	0.353	0.460	0.44	0.46	**0.4283**	0.025
	pLC41	pCAMBIA1300-sgRNA	pCambia1300	pLC41		

Two sided paired *t*-test *GRF4-GIF1 vs.* control: $P < 0.0001$ (n = 4 experiments)

Supplementary Table 7. Regeneration frequencies in *Citrus*. Experiments 1 to 3 used a *GRF-GIF* chimera based on *Citrus* sequences whereas experiments 4 to 6 used a *GRF-GIF* chimera based on *Vitis* sequences. In addition, the last three experiments included a second *Vitis* construct with mutations in the miR396 binding site (*rGRF4-GIF1*) that precludes its cleavage. The number of epicotyls used for each genotype is indicated below the regeneration frequency.

Carrizo	Exp1	Exp2	Exp3	Exp4	Exp5	Exp6	**Average**	**SE**
Empty vector	0.04 45	0.00 38	0.12 56	0.02 65	0.09 32	0.02 40	**0.05**	0.02
Citrus GRF-GIF	0.15 45	0.39 41	0.16 69	-	-	-	**0.21**	0.09
Vitis GRF-GIF	-	-	-	0.07 59	0.25 31	-	**0.16**	0.09
Vitis rGRF4-GIF1	-	-	-	0.20 66	0.61 31	0.30 40	**0.37**	0.12

Supplementary Table 8. Comparisons of *GRF4-GIF1* with transformation technologies using different morphogenic genes.

Technology	Ref.	Advantages	Disadvantages / limitations
GRF4-GIF1	This one	1. Publicly available for research 2. No developmental defects 3. Expands the range of genotypes that can be transformed 4. Rapid transformation protocol (60 days in wheat) 5. Robust regeneration efficiencies under broader set of protocols, including embryogenic and organogenic methods 6. Simple to implement and combine with gene editing 7. Efficient selection without selectable markers (wheat) 8. Tested in monocot and dicot species	1. No tested yet in mature tissues in monocots 2. Transgene incorporated together with the *GRF4-GIF1* chimera [a]. 3. Only tested in protocols that require in vitro tissue culture
Bbm-Wus2 (CORTEVA)	[18-20]	1. High regeneration efficiencies in maize 2. Rapid transformation protocol (35 days maize) 3. Expanded range of maize germplasm that can be transformed 4. Works in mature tissues 5. Advanced vectors worked well in sorghum, Indica rice, and sugar cane	1. Proprietary (but available for research) 2. Protocol optimized for maize. Use of specific maize promoters required to avoid regeneration problems and developmental defects 3. Tested only in monocots 4. If the BBM-WUS2 is not excised it induces developmental defects. Vectors with a CRE-LOX system are available 5. Only tested in methods that require in vitro tissue culture
De novo meristem induction Fast-TrACC *Wus2*/*ipt*	[21]	1. Sidesteps the need for tissue culture 2. Co-delivery of developmental regulators and guide RNAs can generate edited shoots in plants constitutively expressing Cas9 3. It worked in *Benthamiana* soil grown plants	1. Tested only in dicot plants (*Benthamiana*, tomato, potato, grape) 2. Fertile plants showed only in *Benthamiana* 3. Many edited plants show developmental defects and failed to produce seeds 4. Specific developmental regulators need to be defined in each new species 5. Needs transgenic plants previously transformed with Cas9

[a] This is not a problem for gene editing because both the CRISPR-Cas9 and the GRF4-GIF1 constructs are segregated out after editing. Although the presence of the *GRF4-GIF1* is not associated with developmental defects, the user can separate the transgene from the *GRF4-GIF1* chimera by using a line previously transformed with the *GRF4-GIF1* without a selectable marker, and then retransforming the same line with the desired transgene. Since the transgene and the *GRF4-GIF1* construct are incorporated in different loci, they can be segregated apart.

Supplementary References

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