



GRF5, a Novel Regeneration Booster Gene that Improves Transformation of Monocot and Dicot Species

Molecular Breeding Workshop 2019
(University of Geisenheim)
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SEEDING
THE FUTURE
SINCE 1856

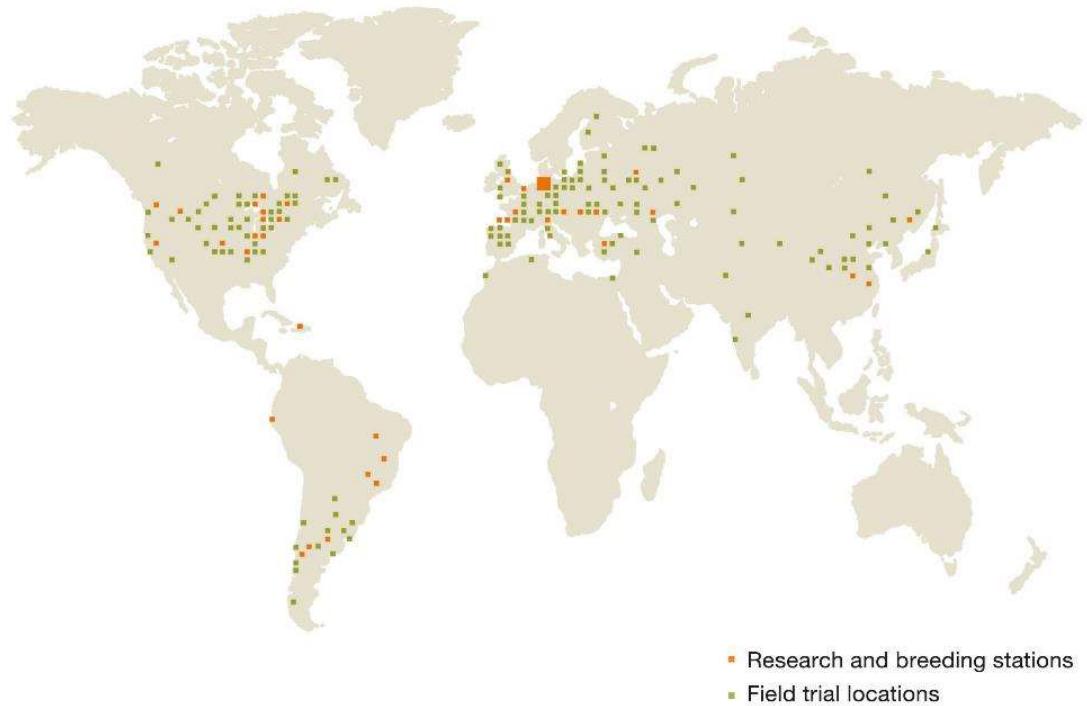


Founded: 1856 in Klein Wanzleben, Saxony-Anhalt
Headquarters: Einbeck, Lower Saxony
RD activities in US: GRC – St. Louis
Employees: 4950, including 1900 in R&D

Core markets: ▪ Europe, North and South America and China

Seed for: ▪ Sugar beet, fodder beet
▪ Corn
▪ Cereals: wheat, rye, barley, oats, triticale
▪ Oil and protein plants: rapeseed, sunflower, soybean, grain peas.
▪ Sorghum, sweet sorghum

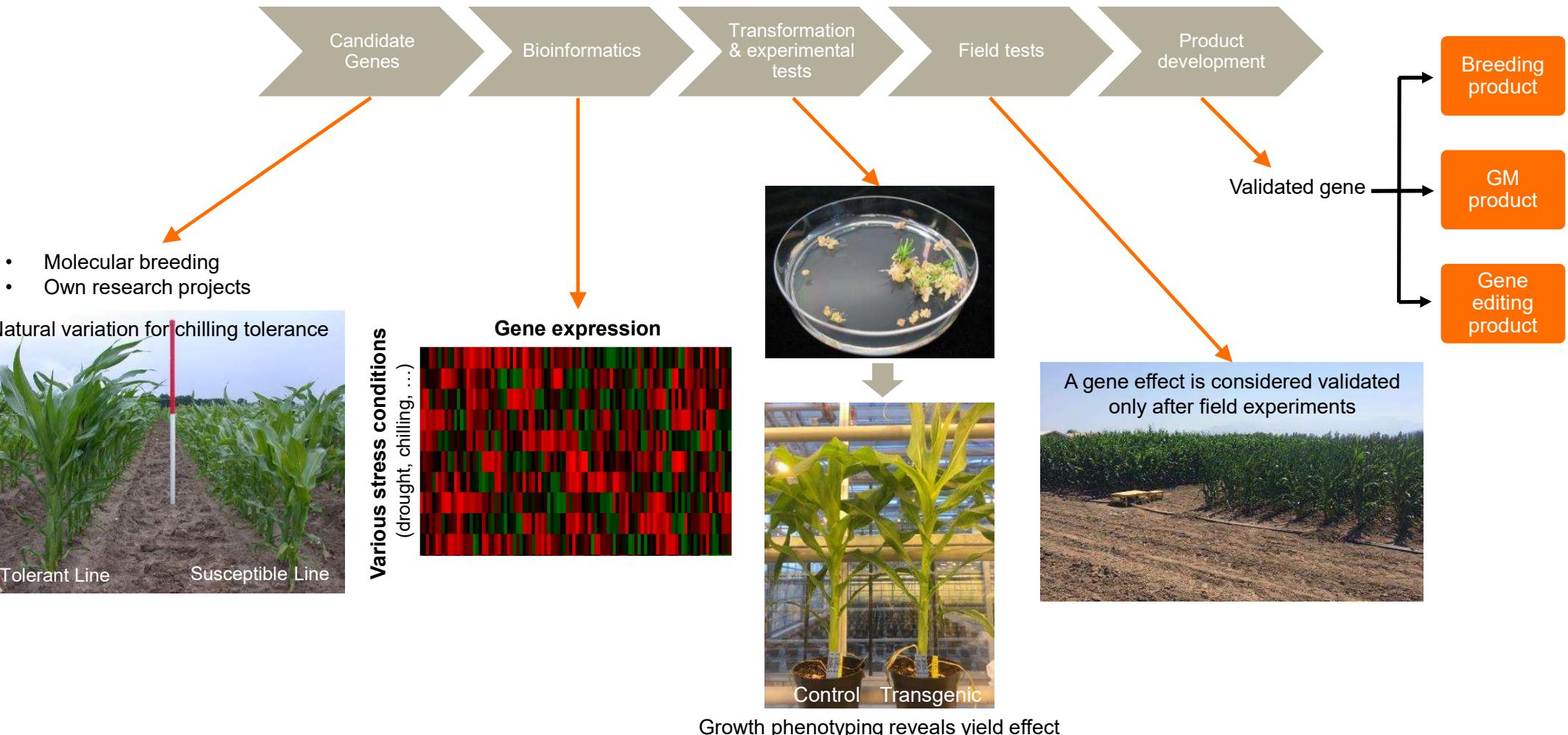
Distribution activities in over 70 countries



Breeding & Research at KWS



Breeding & Research at KWS - Candidate genes for trait production



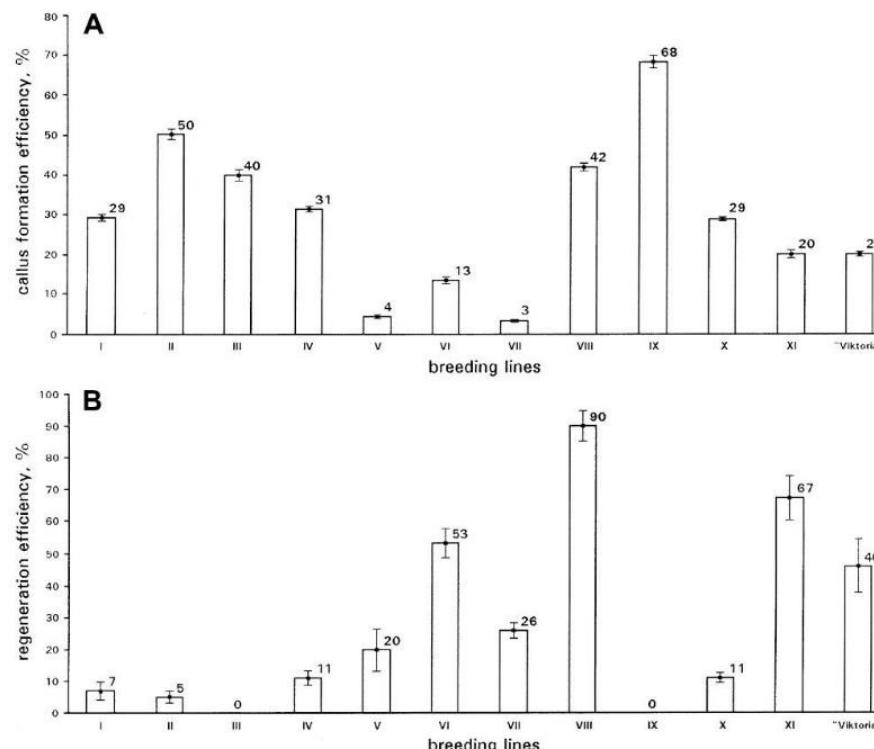
Genotype-dependency of plant regeneration – Sugar beet transformation



Examples from the literature

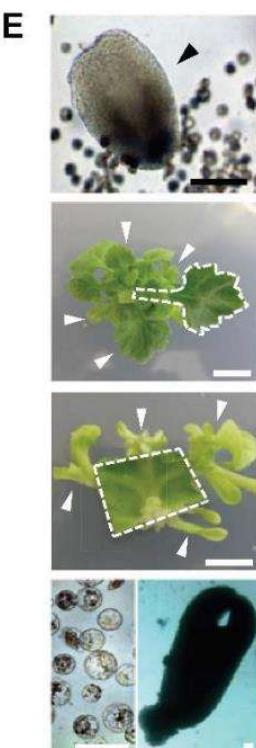
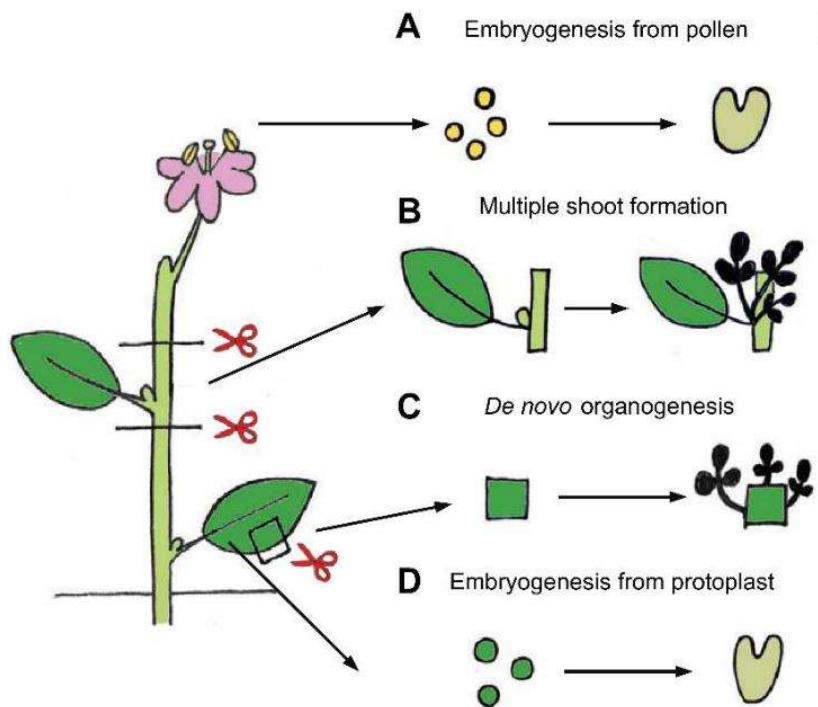
Reference	Transformation efficiency
Lindsey & Gallois 1990	0.1 to 1.0%
D'Halluin et al. 1992	0.1 to 1.0%
Krens et al. 1996	0.1 to 1.0%
Mannerlof et al. 1997	0.1 to 1.0%
Joersbo et al. 1998	0.1 to 1.0%
Snyder et al. 1999	0.1 to 1.0%
Joersbo et al. 2000	0.1 to 1.0%
Zhang et al. 2001	0.1 to 1.0%
Kuykendall et al. 2003	0.1 to 1.0%
Hisano et al. 2004	Av. 11.5 %
Norouzi et al. 2005	Av. 6.2 %
Yang et al. 2005	8 to 30%
Kishchenko et al. 2005	37 to 45%
Kagami et al. 2015	10%

Genotype dependency of plant regeneration in sugar beet

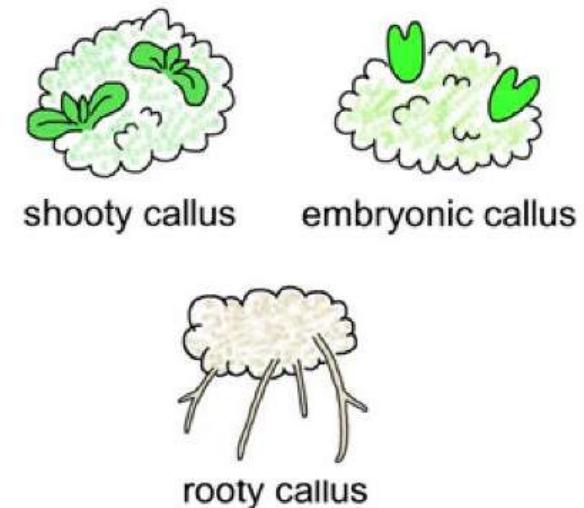


Dovzhenko & Koop (2003) *Planta*

Morphogenic plasticity in plants

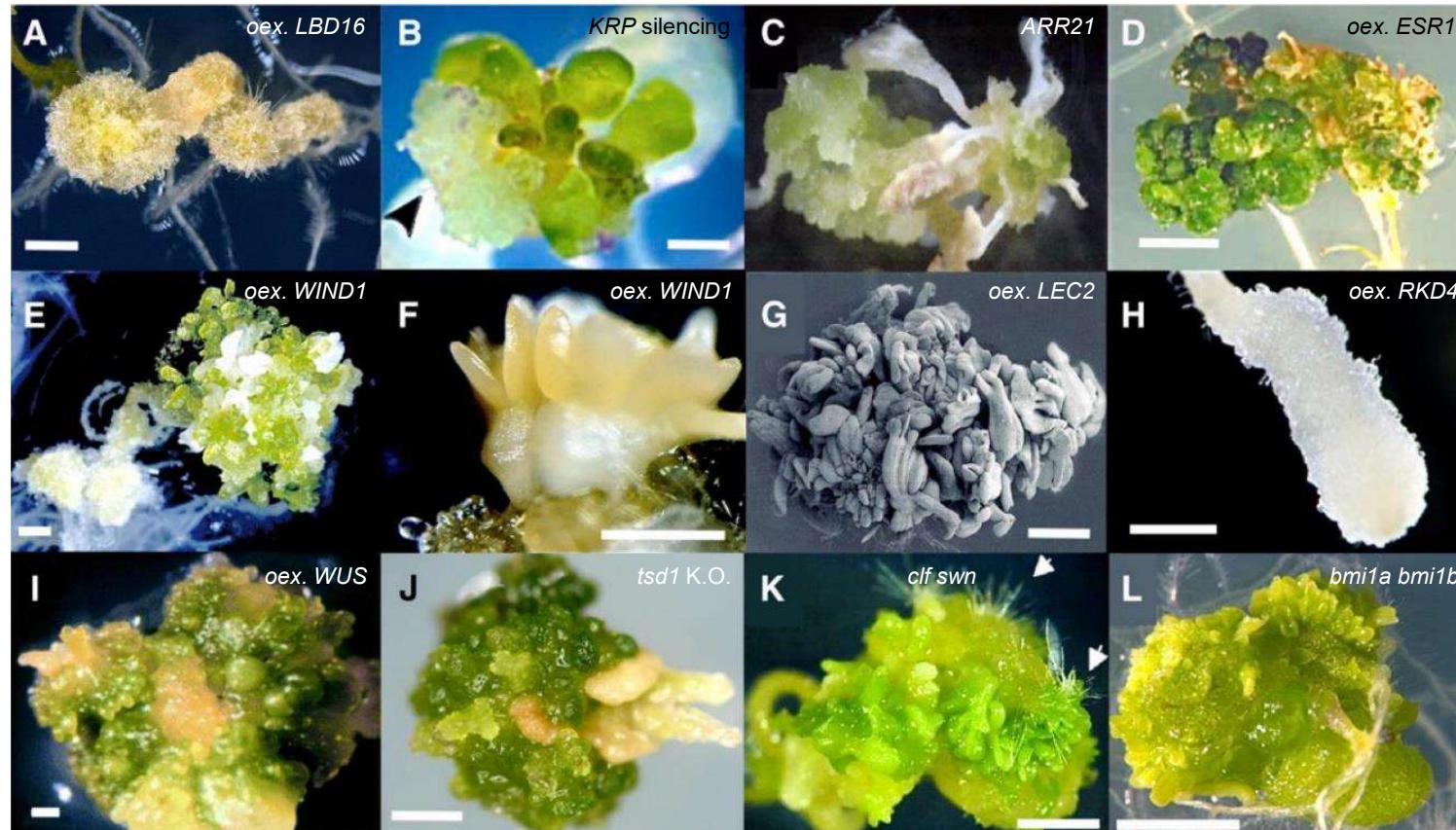


callus with
partial organ regeneration



Ikeuchi et al., 2013

The potential of morphogenic genes to improve plant regeneration



Ikeuchi et al., (2013); Anzola et al., 2010; Tajima et al., 2004; Banno et al., 2001; Iwase et al., 2011; Stone et al., 2001; Waki et al., 2011; Zuo et al., 2002; Krupková and Schmülling, 2009; Chanvivattana et al., 2004; Bratzel et al., 2010

Morphogenic gene families reported to improve regeneration or transformation



Examples					
Strategy	CDS	Promoter for Transgene	Transformed Species	Variety *	Ref.
(A) Enhance pre-existing somatic embryogenic culture response	<i>AiSERK1</i>	35S	<i>Arabidopsis thaliana</i>	Ws	[18]
	<i>CsSERK1 for RNAi</i>	35S and Inducible	<i>Coffea canephora</i>	cv. Robusta	[19]
	<i>AtAGL15</i>	35S	<i>A. thaliana</i>	Ws	[20]
	<i>GmAGL15</i>	35S	<i>Glycine max</i>	"Jack"	[21]
	<i>GhAGL15</i>	35S	<i>Sorghum bicolor</i>	cv. CRI24	[22]
	<i>AtWUS</i>	Inducible	<i>C. canephora</i>	cv. Robusta	[23]
	<i>BnSTM, BeSTM</i>	35S	<i>A. thaliana</i>	Col	[24]
	<i>BnSTM, BeSTM</i>	35S	<i>Broussaisia arguta</i>	cv. Topas	[24]
	<i>BrSTM</i>	35S	<i>A. thaliana</i>	Col	[24]
	<i>BrSTM</i>	35S	<i>B. napus</i>	cv. Topas	[24]
(B) Ectopic formation of somatic embryos or meristems	<i>AtWUS</i>	35S	<i>G. hirsutum</i>	var. Coker 310	[25]
	<i>BnBBM</i>	35S	<i>A. thaliana</i>	Col and C24	[26]
	<i>ABBM-GR</i>	Inducible	<i>Nicotiana tabacum</i>	Wisconsin 38	[27]
	<i>ABBM-GR</i>	Inducible	<i>N. tabacum</i>	Petit Havana SR1	[27]
	<i>GmBBM</i>	35S	<i>Thlaspi arvense</i>	cv. "White wine"	[28]
	<i>TcBBM</i>	35S	<i>A. thaliana</i>	Col-0	[29]
	<i>EgBBM</i>	35S	<i>A. thaliana</i>	"Sogo Vivian"	[29]
	<i>AtEMK</i>	35S	<i>Psp (Orchid)</i>	Ws-0	[30]
	<i>AtRKD4</i>	Inducible	<i>A. thaliana</i>	Col	[31]
	<i>AtLCC1</i>	35S	<i>Cornus officinalis</i>	cv. "Olimex"	[32]
(C) Restrict morphogenic response to enable recovery of normal plants	<i>CaLH</i>	35S	<i>Picea abies</i>	cv. "Blue Spruce"	[33]
	<i>PaHAP7A</i>	Inducible	<i>A. thaliana</i>	Col	[34]
	<i>AtFUS3</i>	AtML1	<i>A. thaliana</i>	Col	[35]
	<i>AtLCC2</i>	35S	<i>Artemesia annua</i>	cv. "Blue Spruce"	[36]
	<i>AtWUS, AtEMK</i>	Activated	<i>A. thaliana</i>	Ler	[30]
	<i>AtWUS</i>	Inducible	<i>N. tabacum</i>	cv. Samsun	[31]
	<i>AtWOX5</i>	Inducible	<i>N. tabacum</i>	cv. Samsun	[32]
	<i>ZmKN1</i>	35S	<i>N. tabacum</i>	cv. Kintu	[33]
	<i>NIKNI</i>	35S	<i>N. tabacum</i>	cv. Samsun	[34]
	<i>AICU1, AICU2</i>	35S	<i>A. thaliana</i>	Ler	[35]
	<i>AtLEC1</i>	Inducible	<i>A. thaliana</i>	Col-0	[36]
	<i>AtESR1</i>	Inducible	<i>A. thaliana</i>	Ws	[37]
	<i>AtESR2</i>	Inducible	<i>A. thaliana</i>	Ler and Ws	[38]
	<i>AtMPA</i>	MP Promoter	<i>A. thaliana</i>	Col-0	[39]
	<i>BnBBM</i>	Inducible	<i>Capitium annuum</i>	Three hybrids ^b	[40]
	<i>ABBM</i>	Inducible	<i>A. thaliana</i>	RDL and Ler	[51]
	<i>AtPGA37</i>	Inducible	<i>A. thaliana</i>	Col-0, Ws, Ler	[52]
	<i>AtLEC2</i>	Inducible	<i>T. cacao</i>	var. SCA6	[53]
	<i>AtWOX2 WOX8 WOX9</i>	Inducible	<i>N. tabacum</i>	cv. Samsun	[54]
	<i>BnBBM^a</i>	35S	<i>Populus tremuloides</i>	not specified	[55]
	<i>ZmBBM/ZmWUS2^a</i>	Ubi + NOS	<i>Zea mays</i>	4 Pioneer hybrids ^c	[17]
	<i>ZmBBM/ZmWUS2^a</i>	Ubi + NOS	<i>Oryza sativa</i>	(indica) cv. IRV95	[17]
	<i>ZmBBM/ZmWUS2^a</i>	Ubi + NOS	<i>Sorghum bicolor</i>	var. Ts430	[17]
	<i>ZmBBM/ZmWUS2^a</i>	Ubi + NOS	<i>Solanum lycopersicum</i>	var. CP01-1372	[17]
	<i>ZmBBM/ZmWUS2^a</i>	Ubi + NOS	<i>Z. mays</i>	public inbred B73	[56]
	<i>ZmBBM/ZmWUS2^a</i>	Ubi + NOS	<i>S. lycopersicum</i>	var. P898012	[56]
	<i>ZmBBM/ZmWUS2</i>	PLTP + AXIG1	<i>Z. mays</i>	maize inbreds ^d	[57]

OTHER GENE FAMILIES?

YES!

THE GROWTH-REGULATING FACTOR (GRF) FAMILY

NF-YB transcription factors; e.g. LEC1

B3 domain family; e.g. FUS3

Auxin response factors (ARF); e.g. MP

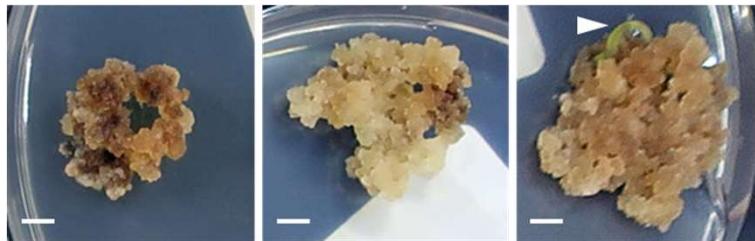
MYB Family

Ectopic expression of *AtGRF5* enhances shoot regeneration in sugar beet

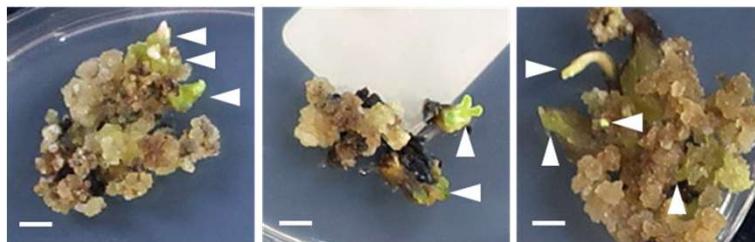


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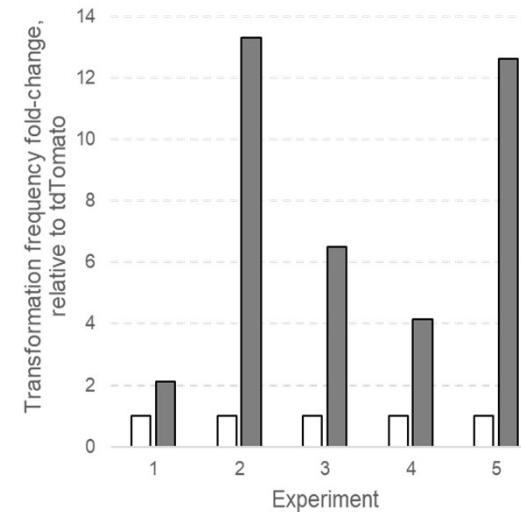
35S::*tDTomato*



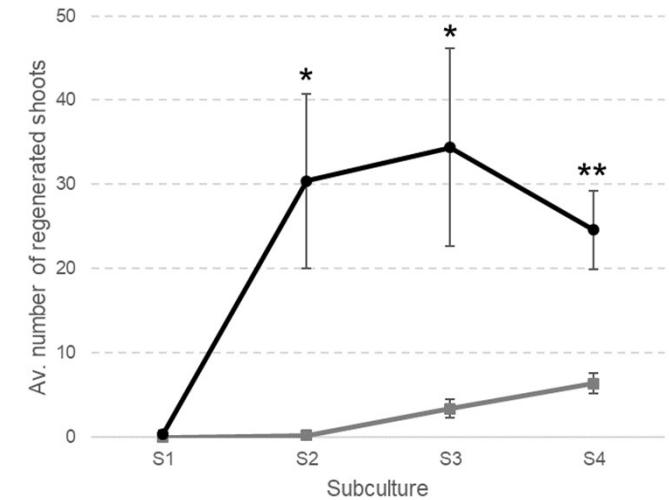
35S::*AtGRF5*



□ 35S::*tDTomato* ■ 35S::*AtGRF5*



■ 35S::*tDTomato* ● 35S::*AtGRF5*



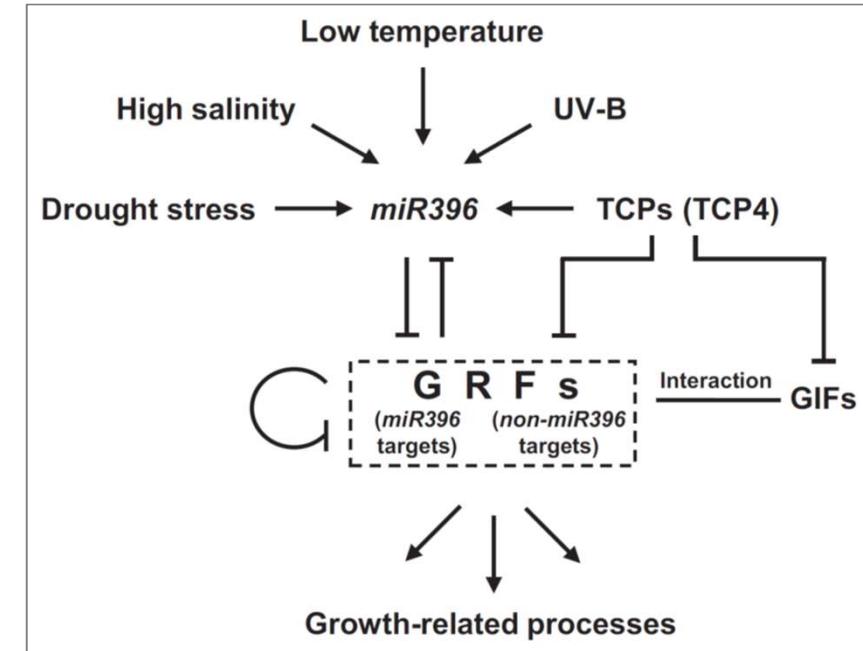
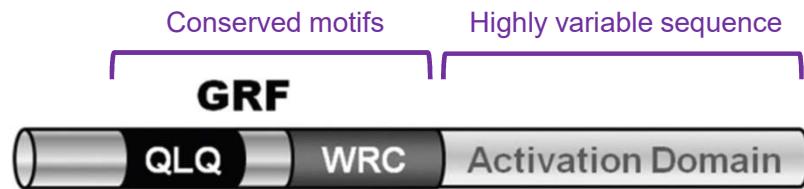
Significant differences as compared to the control
(35S::*tDTomato*): * = $p < 0.05$; ** = $p < 0.01$

The *GRF* gene family



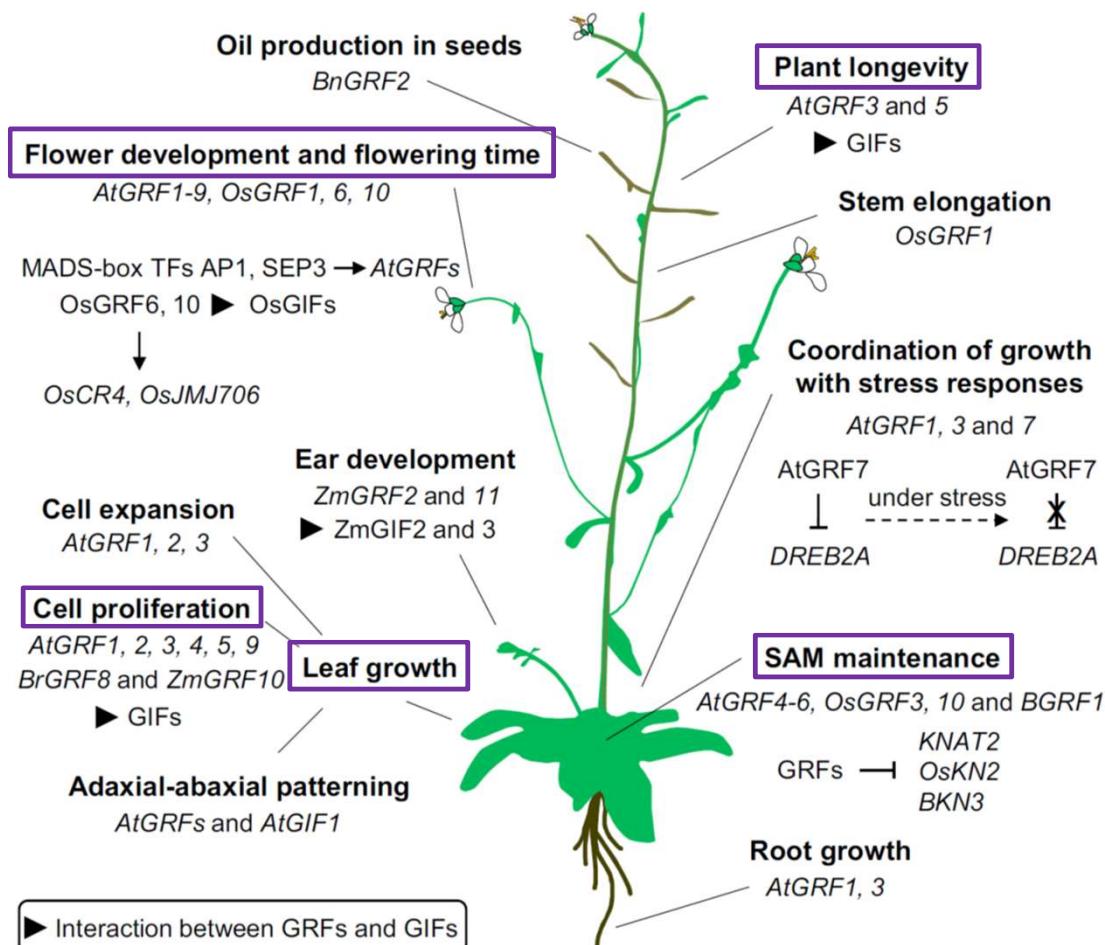
Plant species	GRFs
Arabidopsis thaliana	9
Beta vulgaris	7
Hordeum vulgare	8
Zea mays	17

Omidbakhshfard et al., 2015



Omidbakhshfard et al., 2015

GRFs control diverse processes of plant development

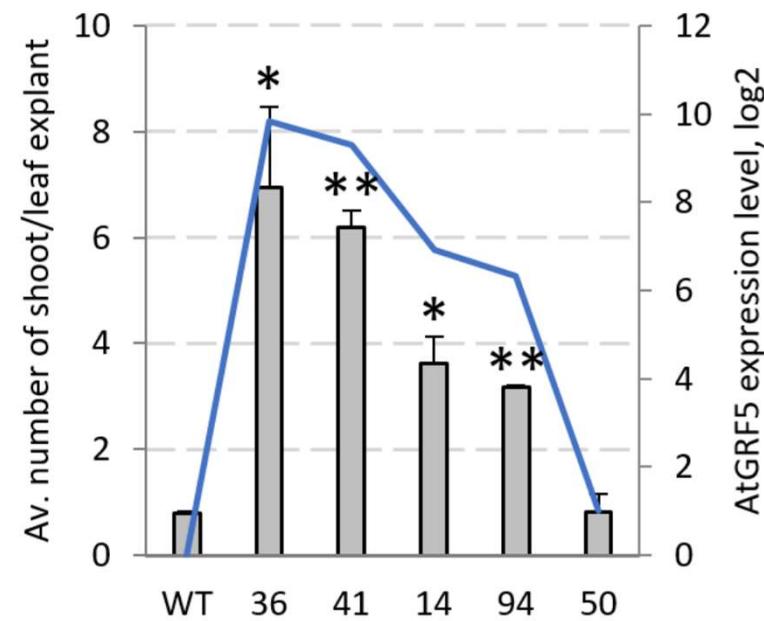
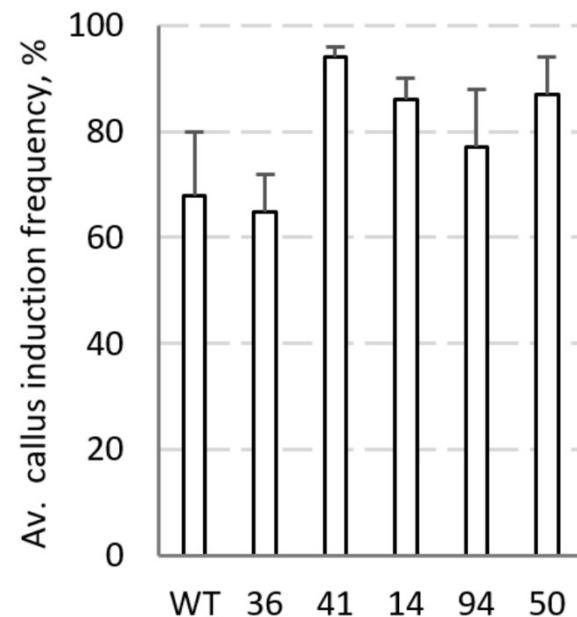


Omidbakhshfard et al., 2015

AtGRF5 promotes shoot formation, but not callus formation in sugar beet



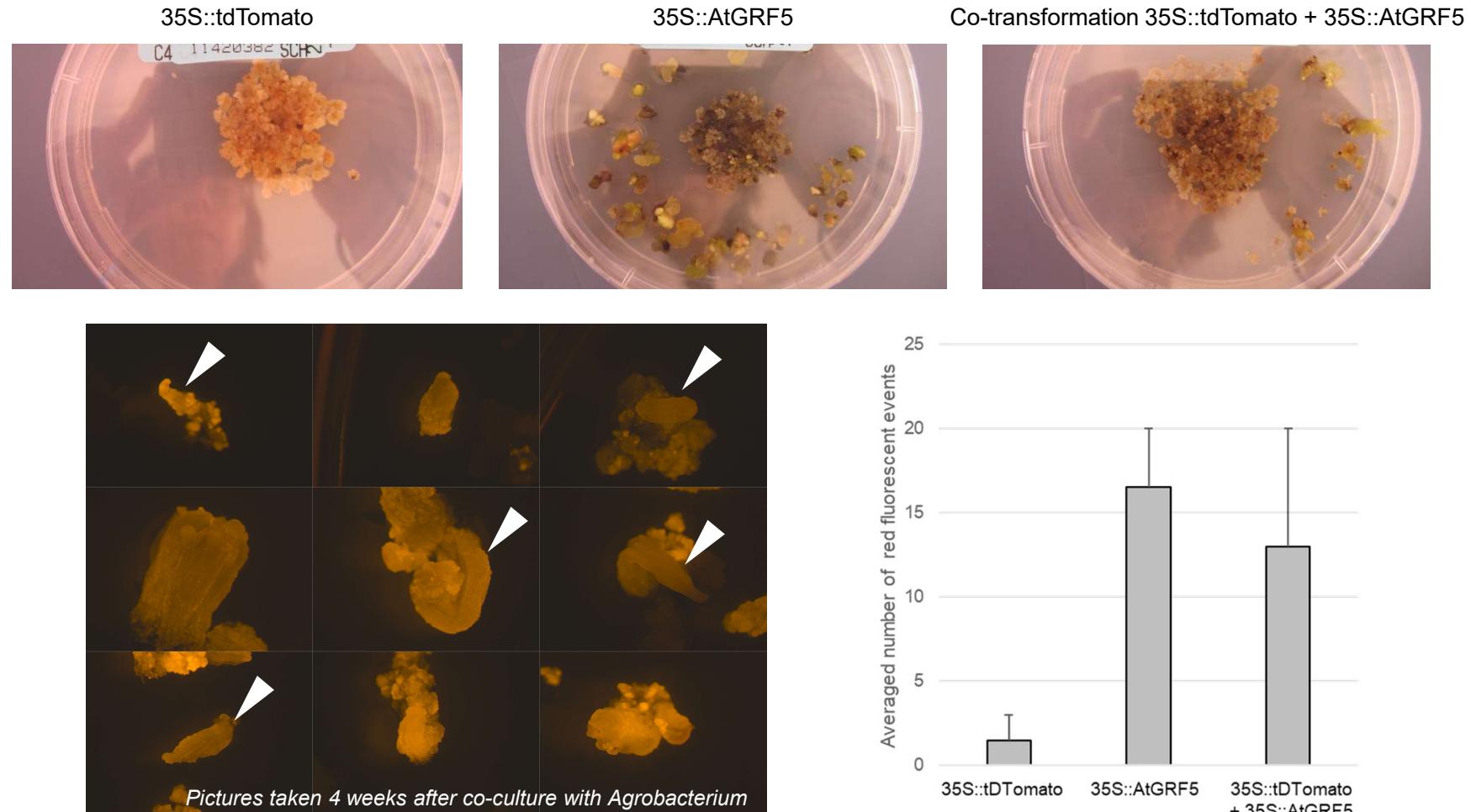
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AtGRF5 allows efficient recovery of co-transformed events



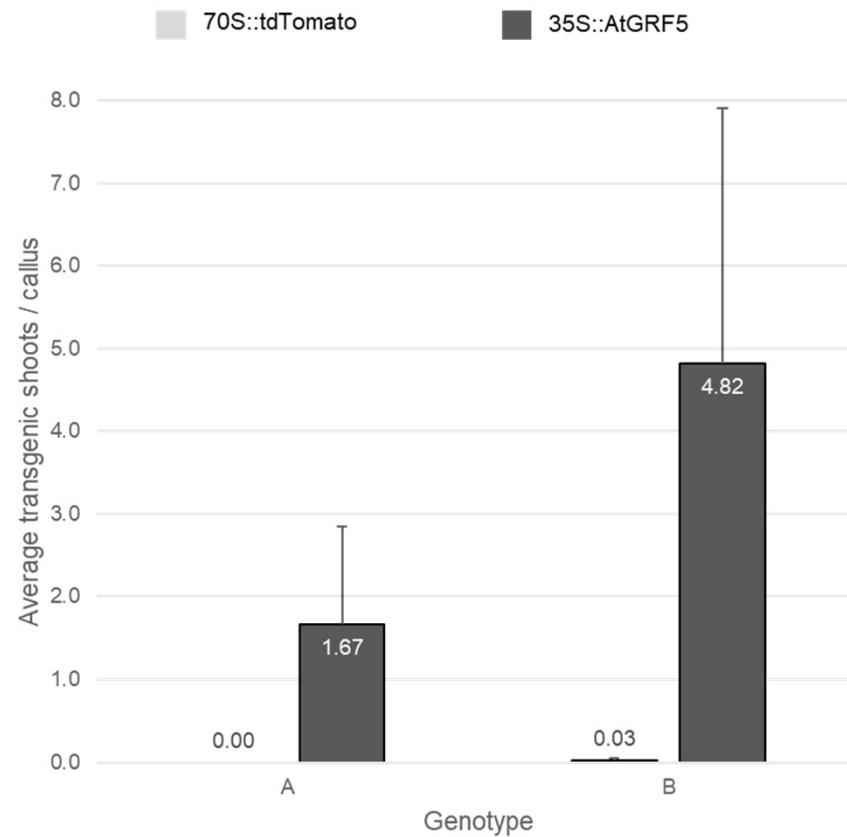
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AtGRF5 promotes transformation of recalcitrant sugar beet genotypes



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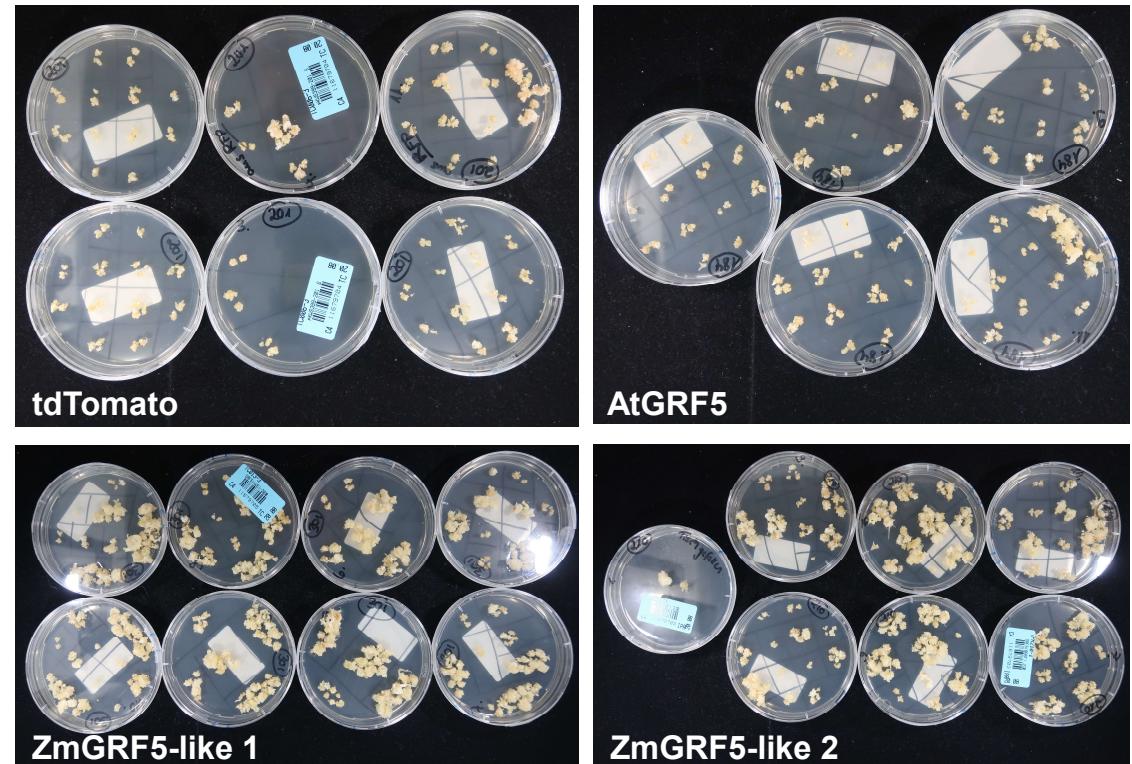
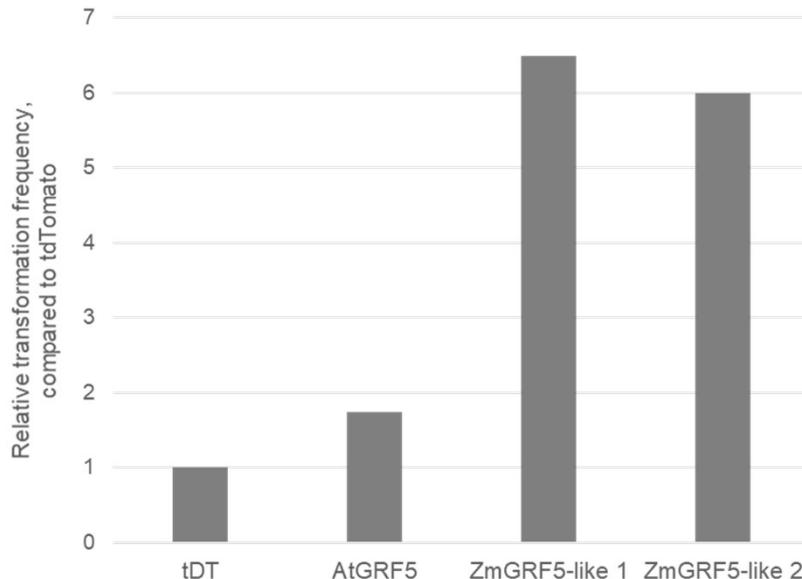
Overexpression of *GRF5* orthologs increases the transformation frequency in Corn



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Two putative *GRF5* orthologs in maize:

ZmGRF5-like1 and *ZmGRF5-like2*



Summary



- Discover the potential of GRF gene family to improve transformation methods
- *GRFs* seems to be powerful regeneration booster genes in monocot and dicot species
- *AtGRF5* promotes regeneration of sugar beet recalcitrant genotypes
- Maize putative *GRF5* orthologs are required for promoting regeneration
- *GRF5* is promoting transformation in both organogenesis and somatic embryogenesis-based methods
- Continuing collaboration with BASF on other crops



Acknowledgements:

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Thank you for your attention !

