# 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

KWS

# Breeding & Research at KWS

| | |
|---|---|
| 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 |

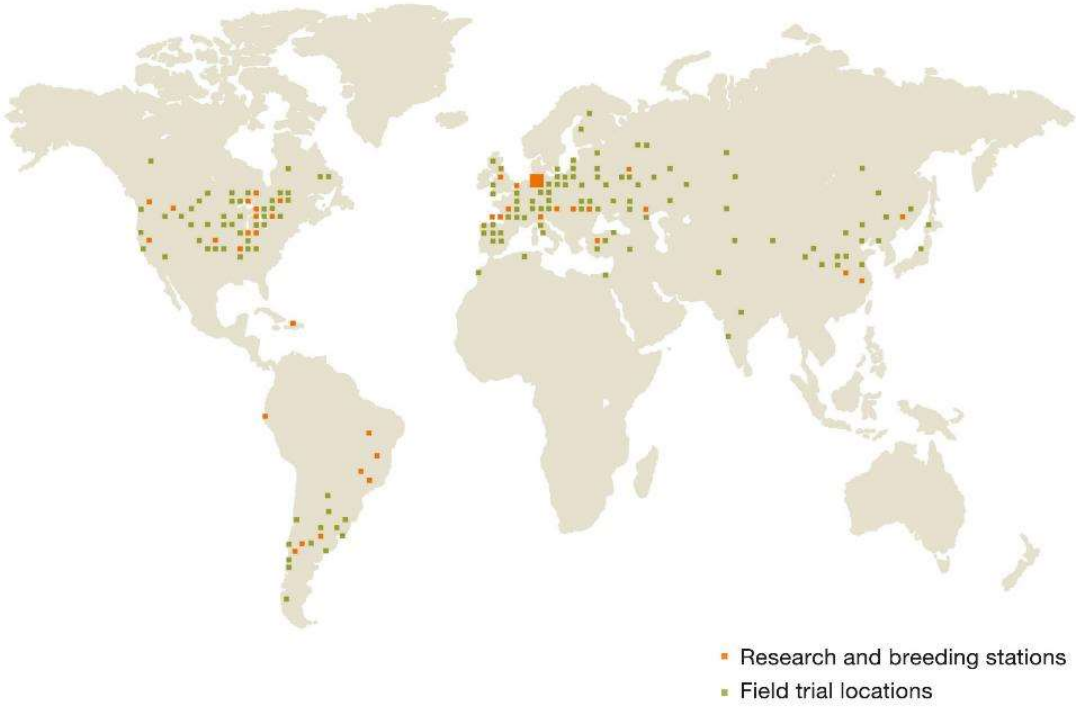
Core markets:

- 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



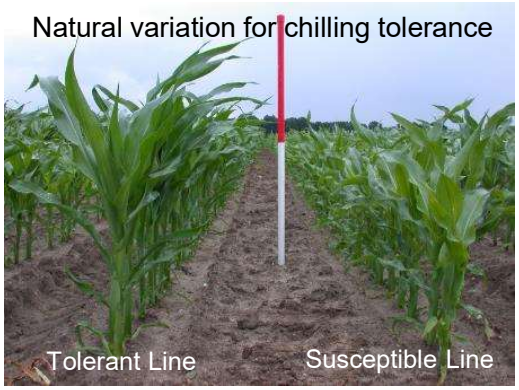
# Breeding & Research at KWS

# Breeding & Research at KWS - Candidate genes for trait production

Candidate Genes → Bioinformatics → Transformation & experimental tests → Field tests → Product development

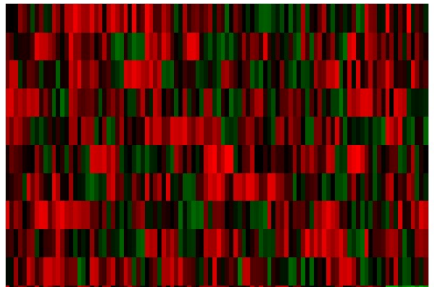
- Molecular breeding
- Own research projects

Natural variation for chilling tolerance




**Gene expression**

**Various stress conditions** (drought, chilling, ...)




Growth phenotyping reveals yield effect

Validated gene → Breeding product; GM product; Gene editing product

A gene effect is considered validated only after field experiments

# 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% |
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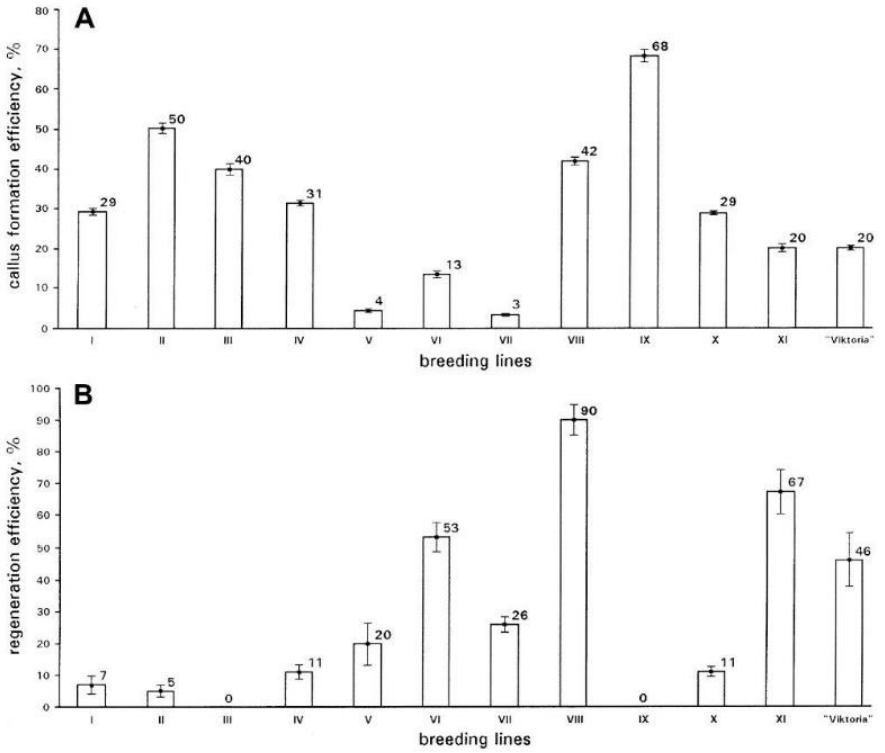
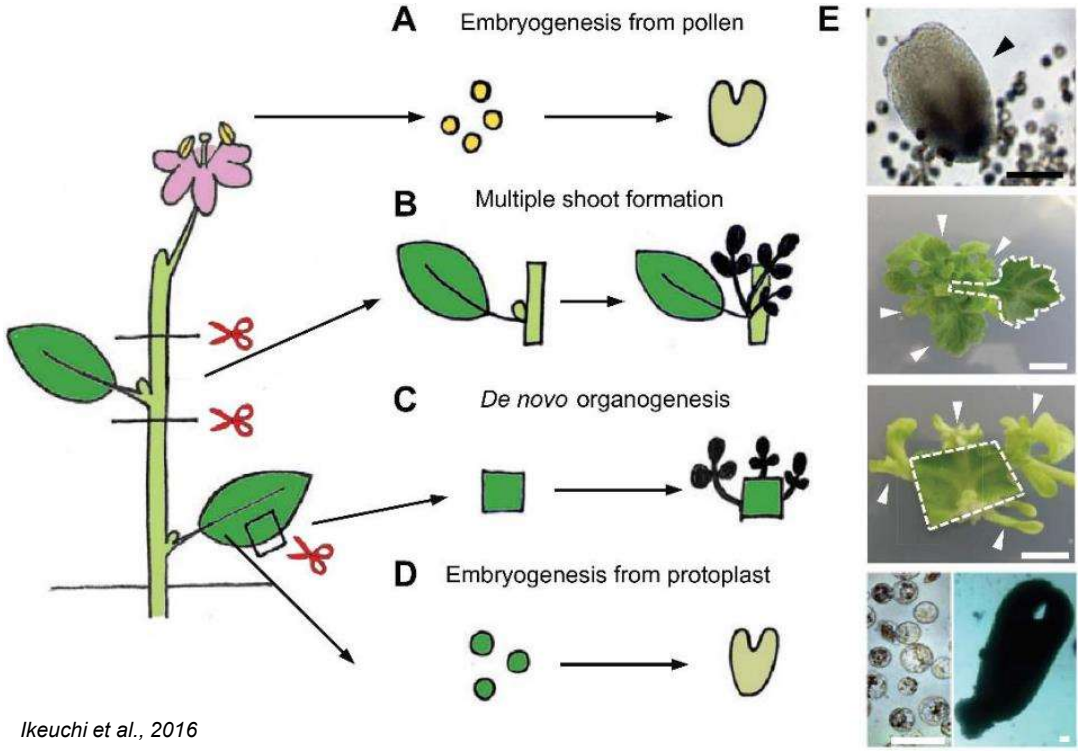
| Zhang et al. 2001 | 0.1 to 1.0% |
| Kuykendall et al. 2003 | 0.1 to 1.0% |
| Hisano et al. 2004 | Av. 11.5 % |
| Norouzi et al. 2005 | Av. 6.2 % |
| Yang at el. 2005 | 8 to 30% |
| Kishchenko et al. 2005 | 37 to 45% |
| Kagami at el. 2015 | 10% |

## Genotype dependency of plant regeneration in sugar beet

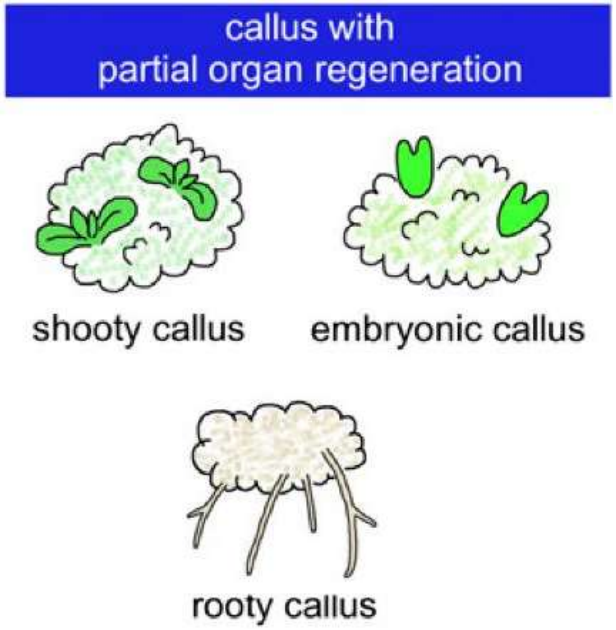



*Dovzhenko & Koop (2003) Planta*

# Morphogenic plasticity in plants

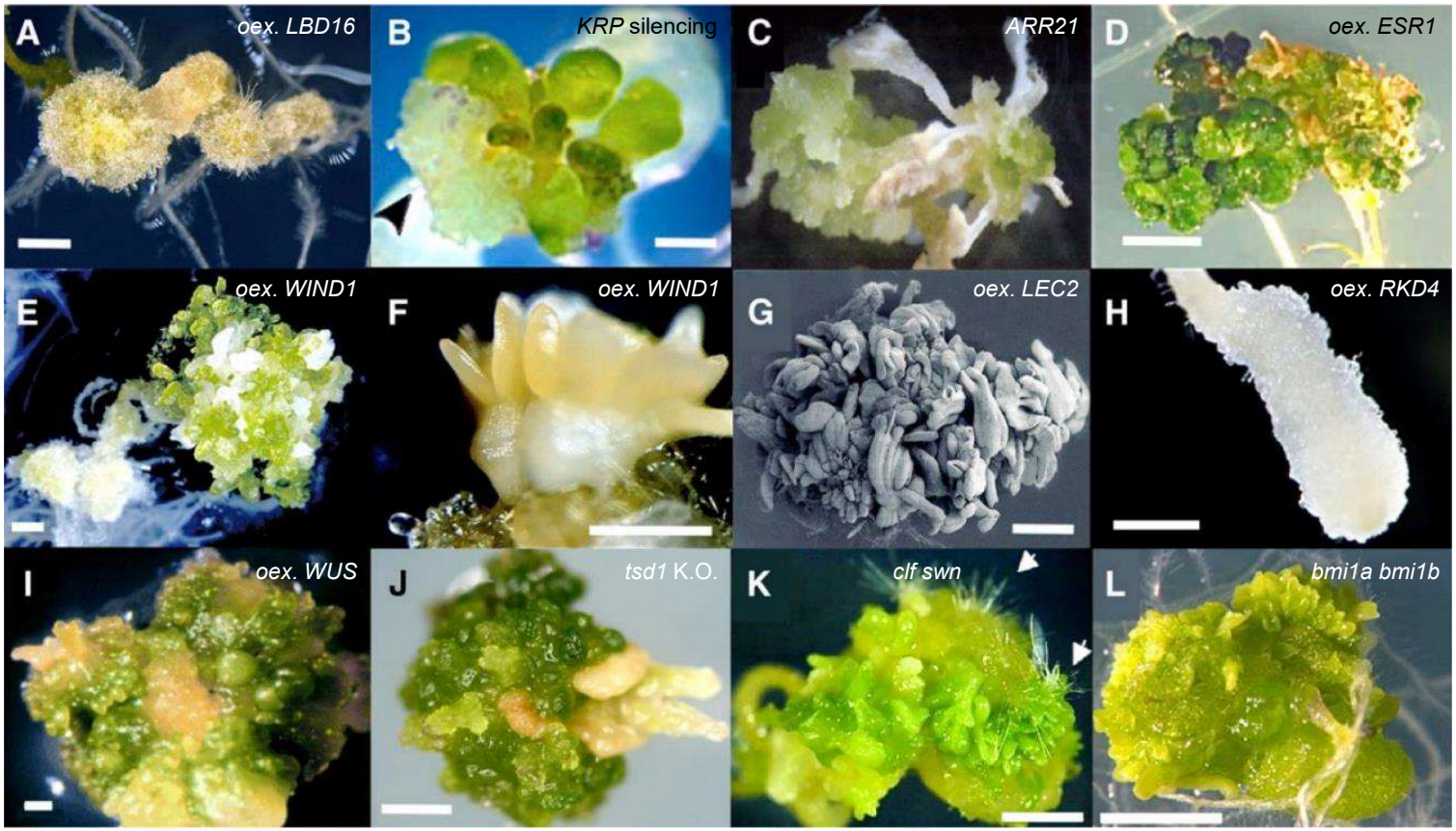



*Ikeuchi et al., 2016*




*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


Leu-rich repeat (LRR) transmembrane receptor-like kinase (RLK)

MADS-box transcription factors

Three-amino-acid-loop-extension (TALE) class of homeoproteins, e.g. STM

WUSCHEL; homeobox (HB) transcription factors

AP2/ERF superfamily, e.g. BBM

Nin-like Family; e.g. RKD4

NF-YB transcription factors; e.g. LEC1

B3 domain family; e.g. FUS3

NAM, ATAF, and CUC (NAC) transcription factors

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

MYB Family

OTHER GENE FAMILIES?

YES!

THE GROWTH-REGULATING FACTOR (GRF) FAMILY

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

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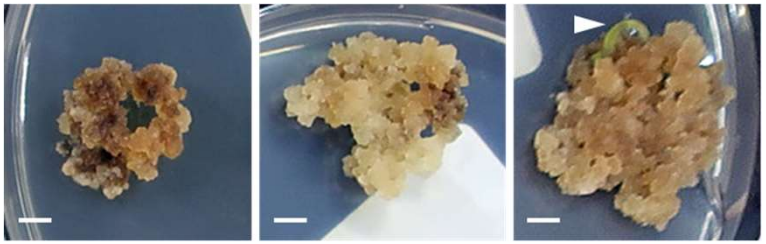
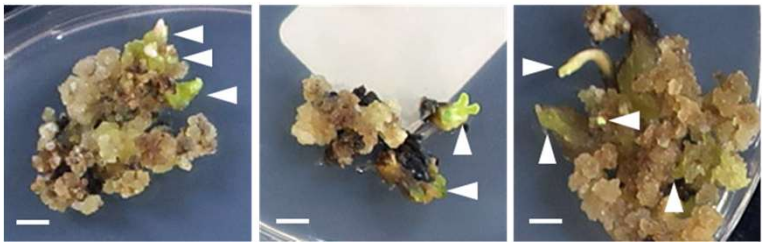
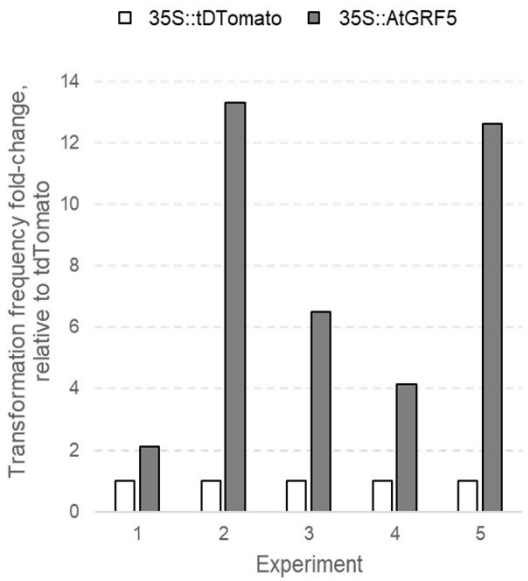
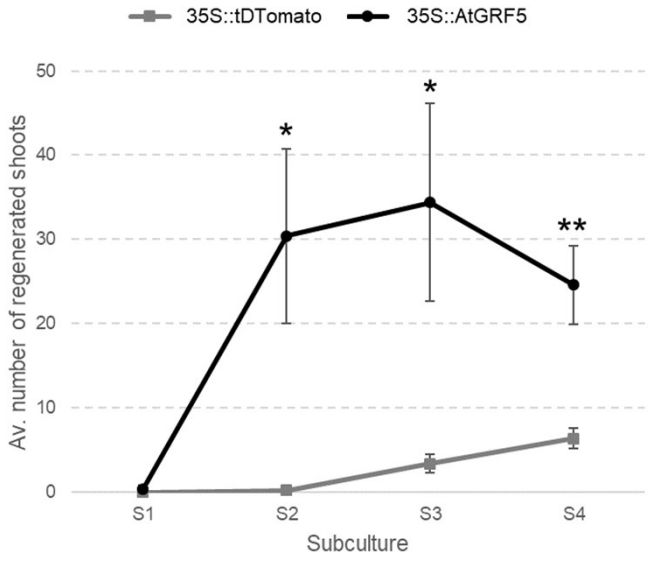
*35S::tDTomato*



*35S::AtGRF5*





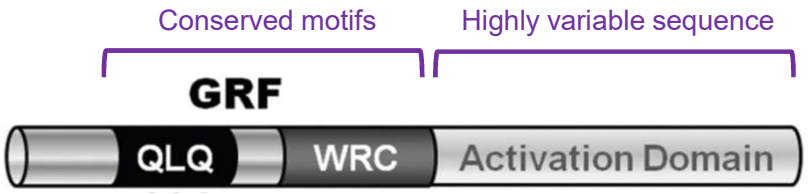



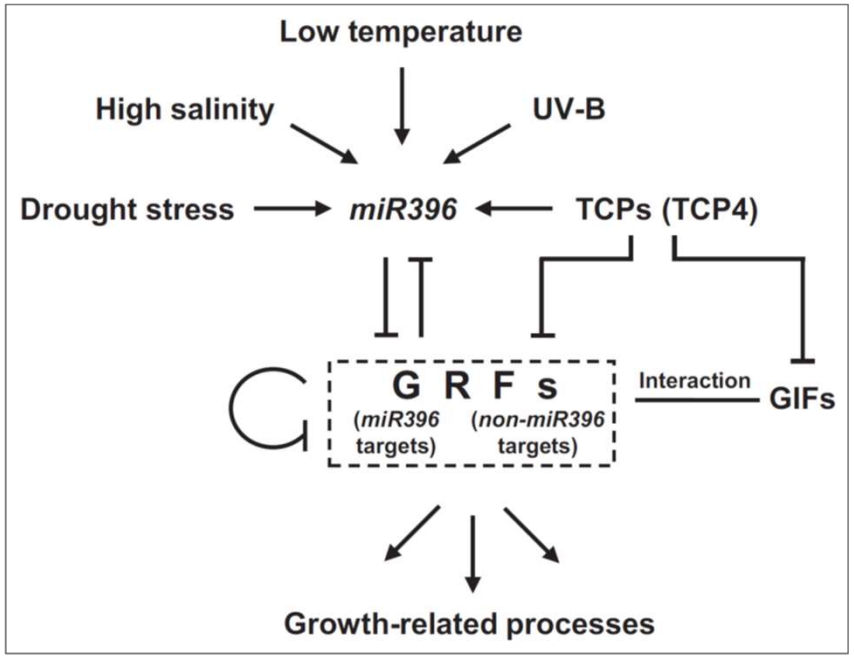

Significant differences as compared to the control (35S::tdTomato): * = p<0.05; ** = p<0.01

# The *GRF* gene family

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

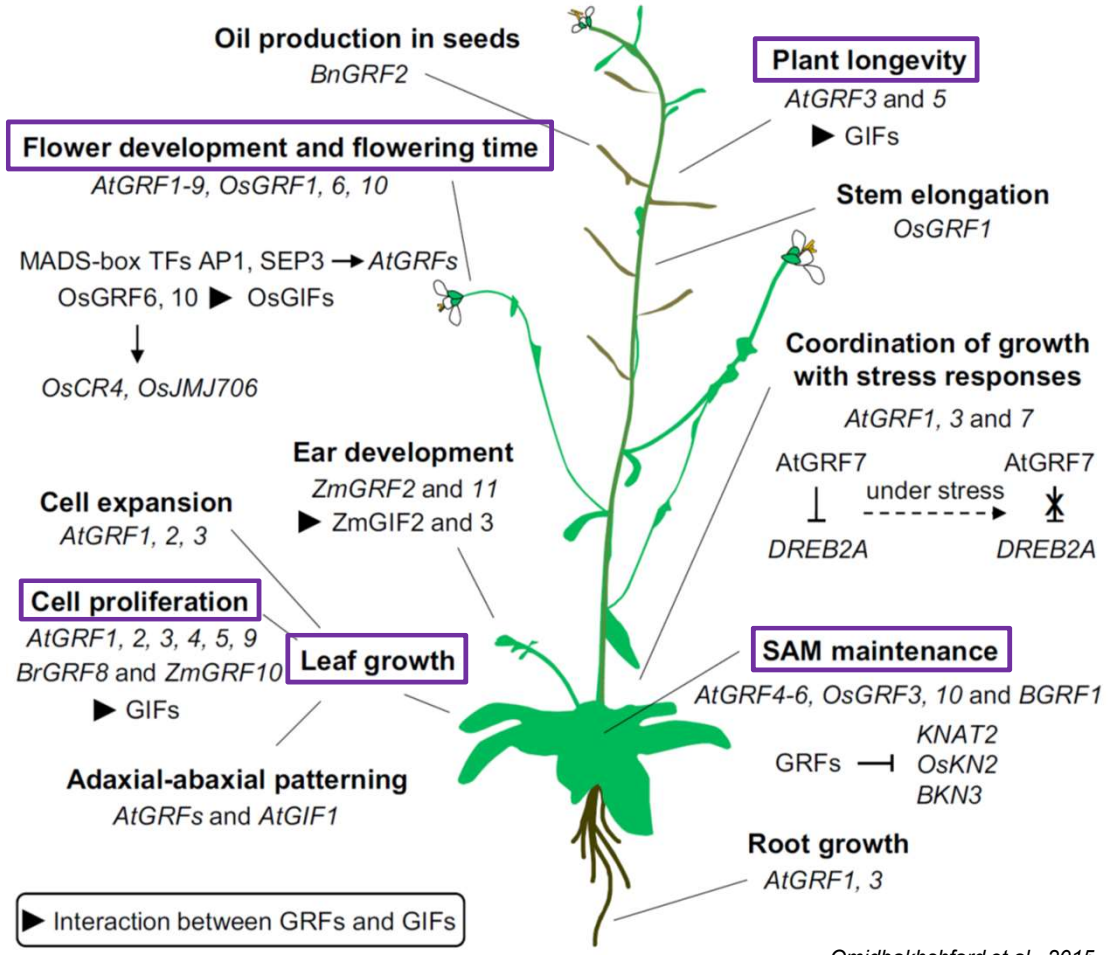
*Omidbakhshfard et al., 2015*




*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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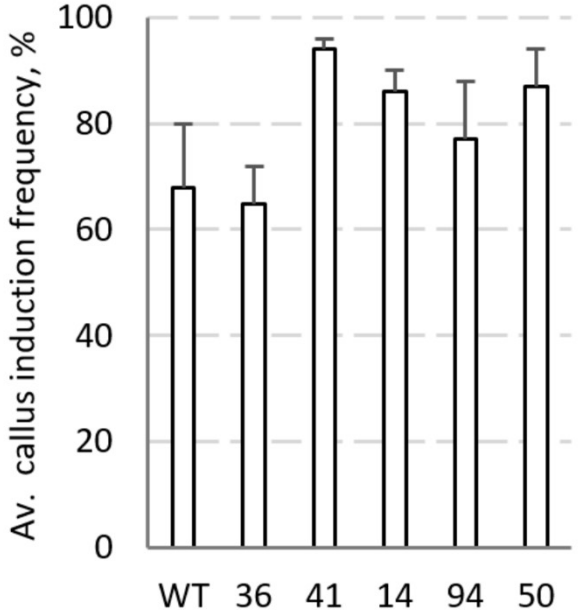


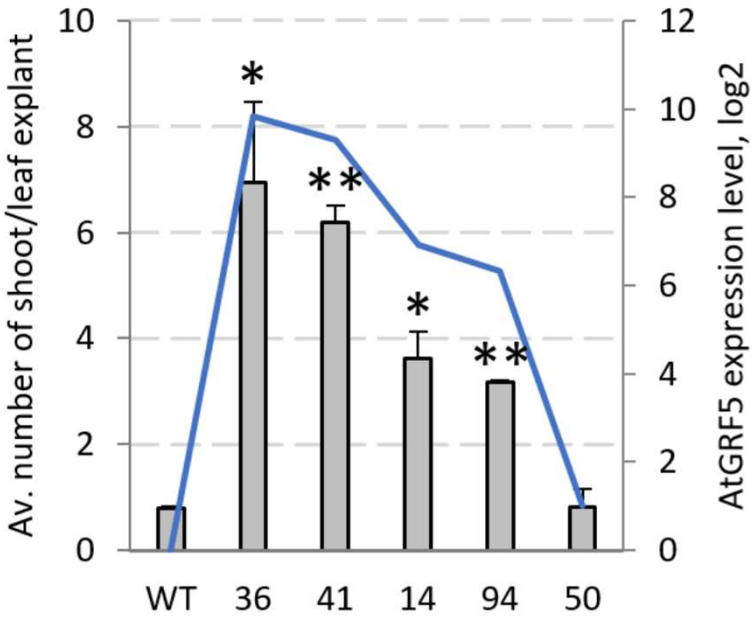

# *AtGRF5* allows efficient recovery of co-transformed events

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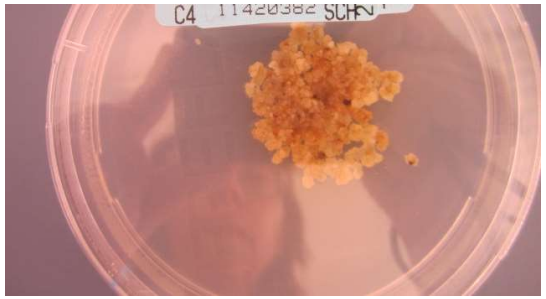
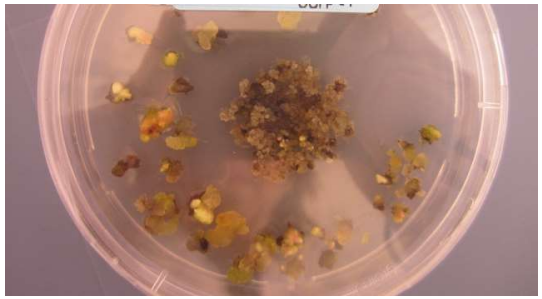
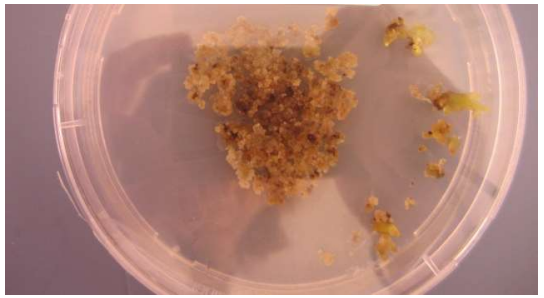
35S::tdTomato

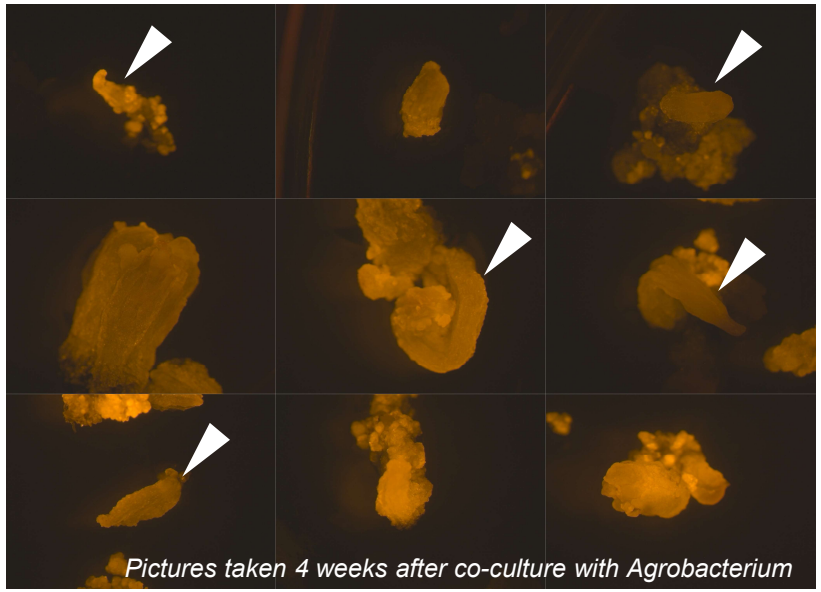


35S::AtGRF5

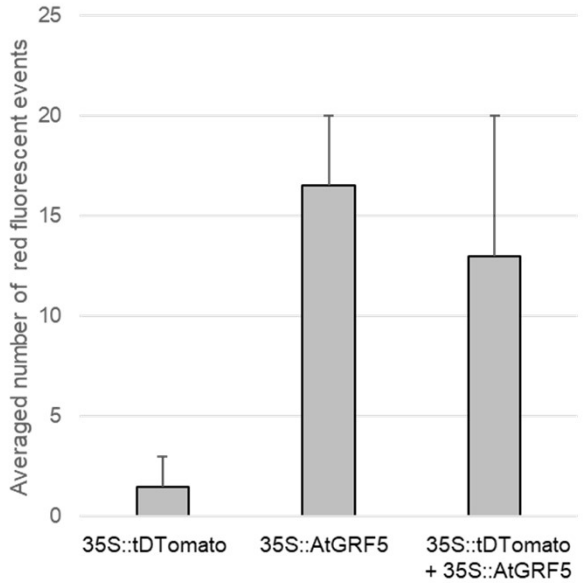


Co-transformation 35S::tdTomato + 35S::AtGRF5





*Pictures taken 4 weeks after co-culture with Agrobacterium*



# *AtGRF5* promotes transformation of recalcitrant sugar beet genotypes

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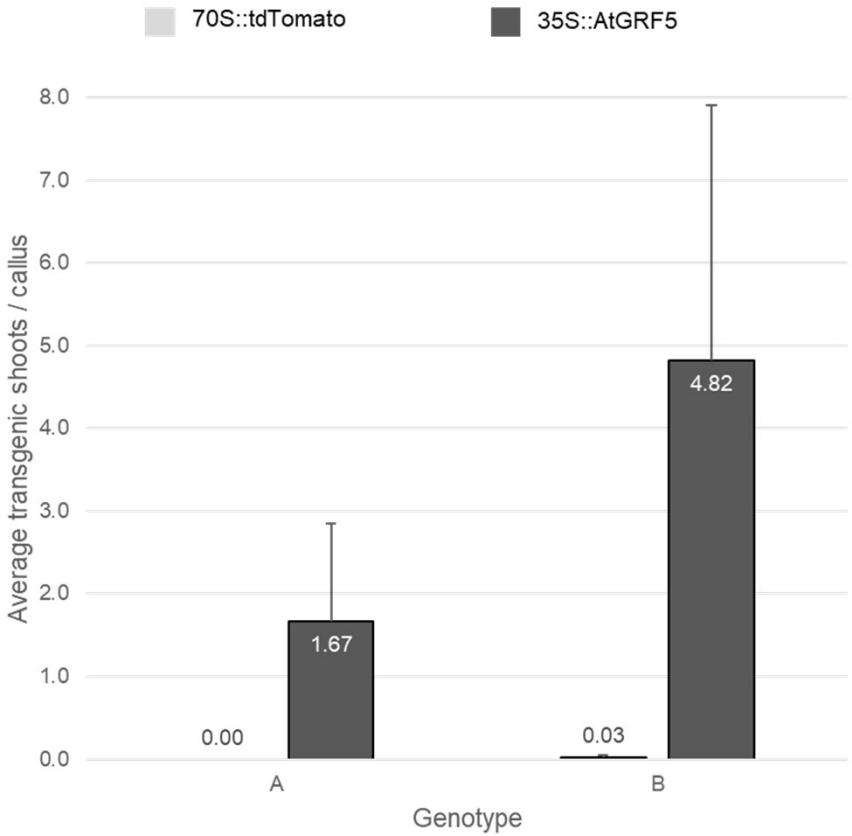

# Overexpression of *GRF5* orthologs increases the transformation frequency in Corn

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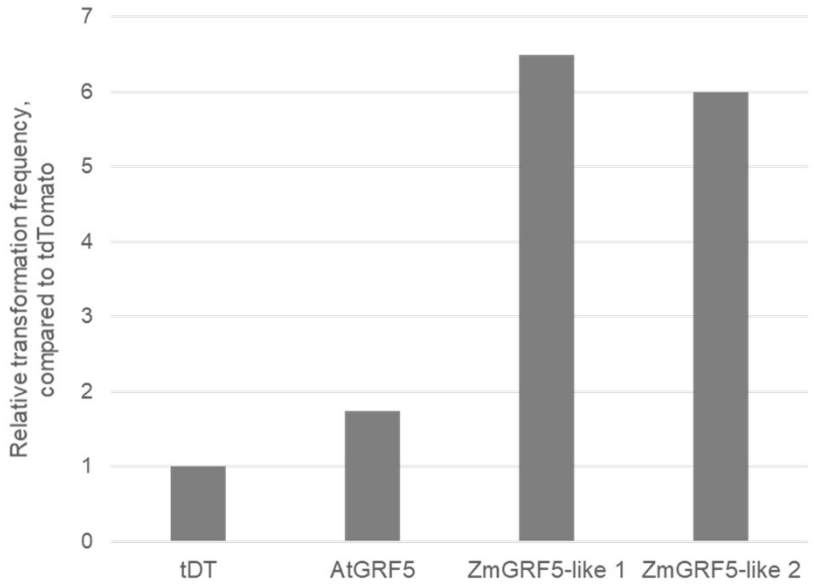
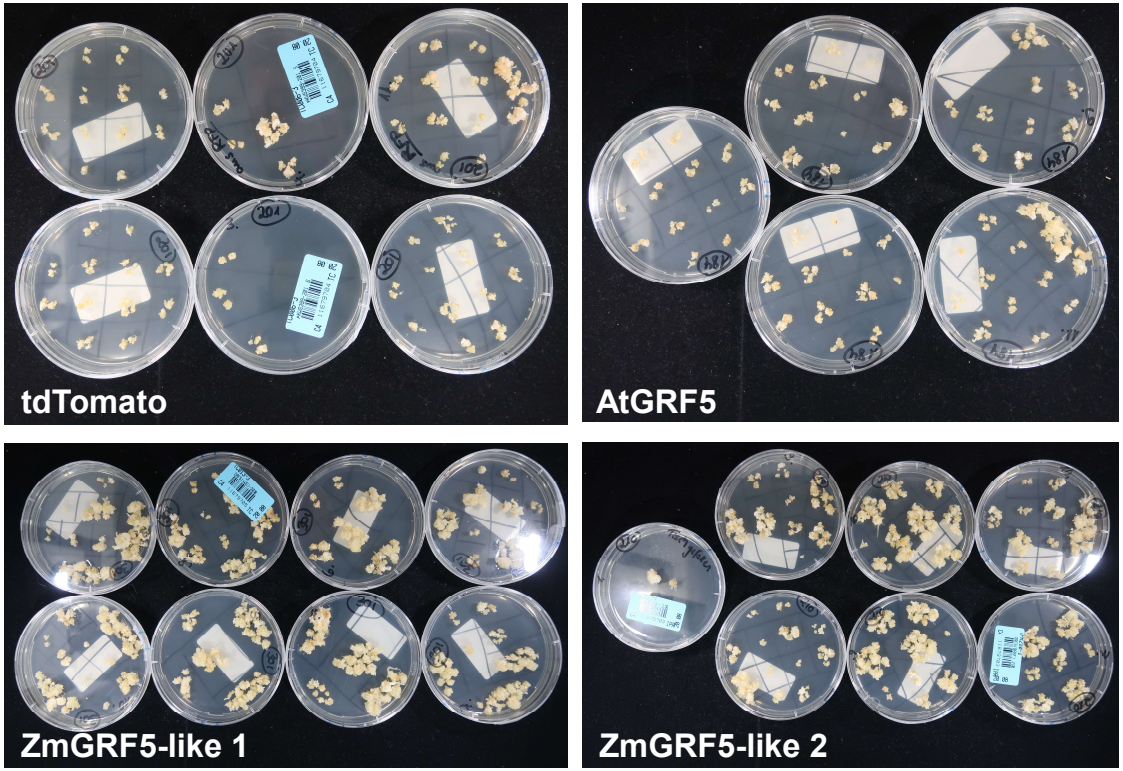
Two putative GRF5 orthologs in maize:

*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 !