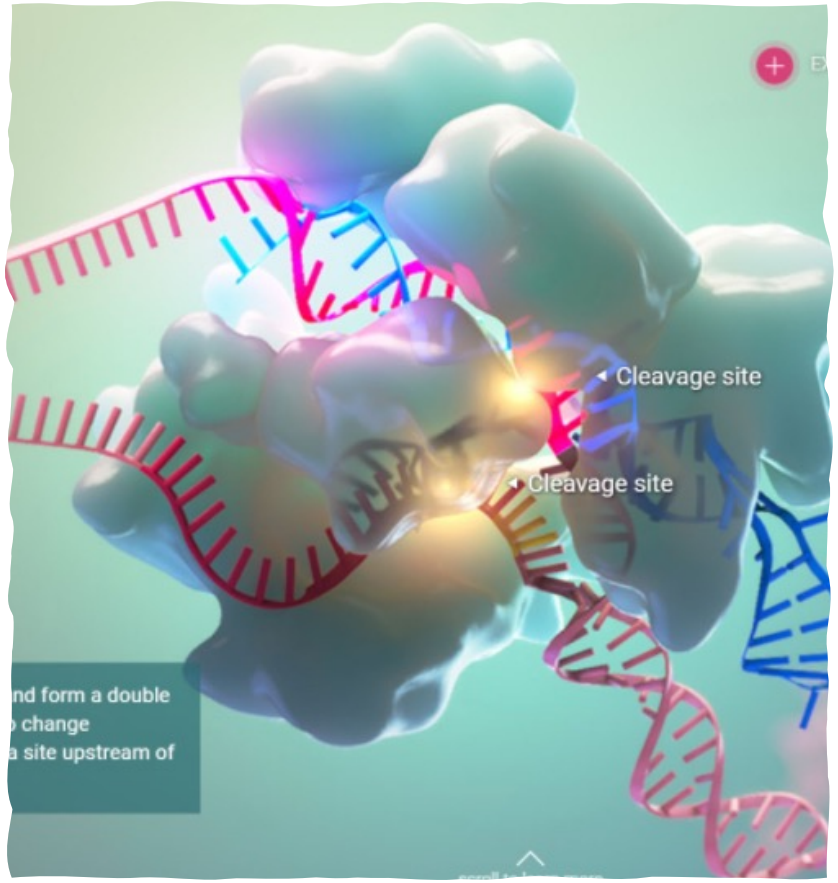
Boosting Plant Genome Editing With a Versatile CRISPR-Combo System



- Yiping Qi, PhD
- University of Maryland, College Park

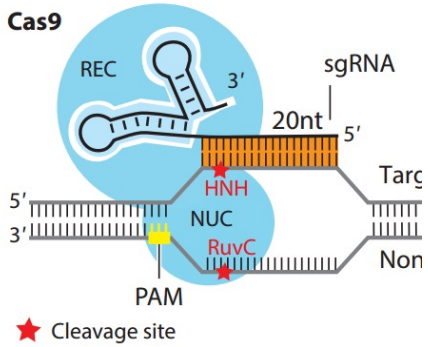
- 6/29/2022

PlantEd

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

CRISPR/Cas9 in Genome Editing and Beyond

Cas9 nuclease



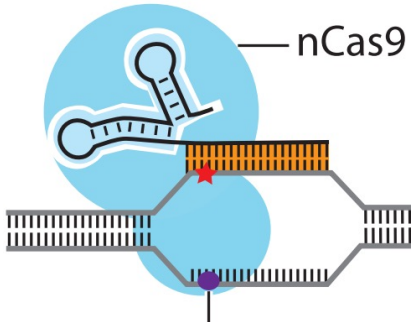
Double-stranded break (DSB)

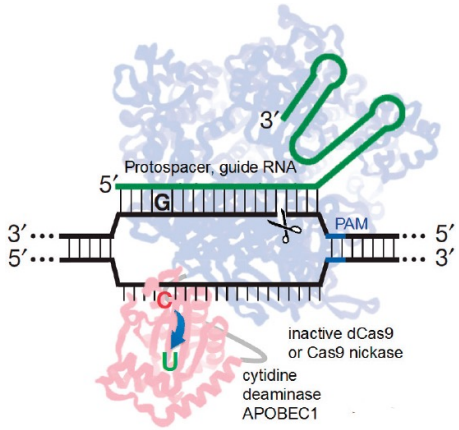
NHEJ
(non-homologous end joining)

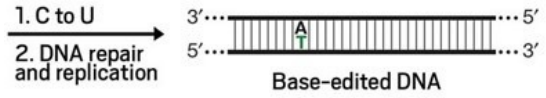
HDR
(homology-directed repair)

Gene Editing

nCas9
(Cas9 nickase)

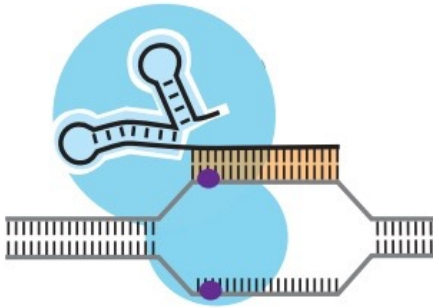


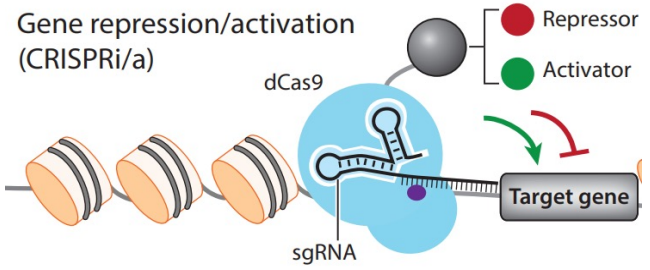




Base Editing

dCas9
(nuclease-dead Cas9)

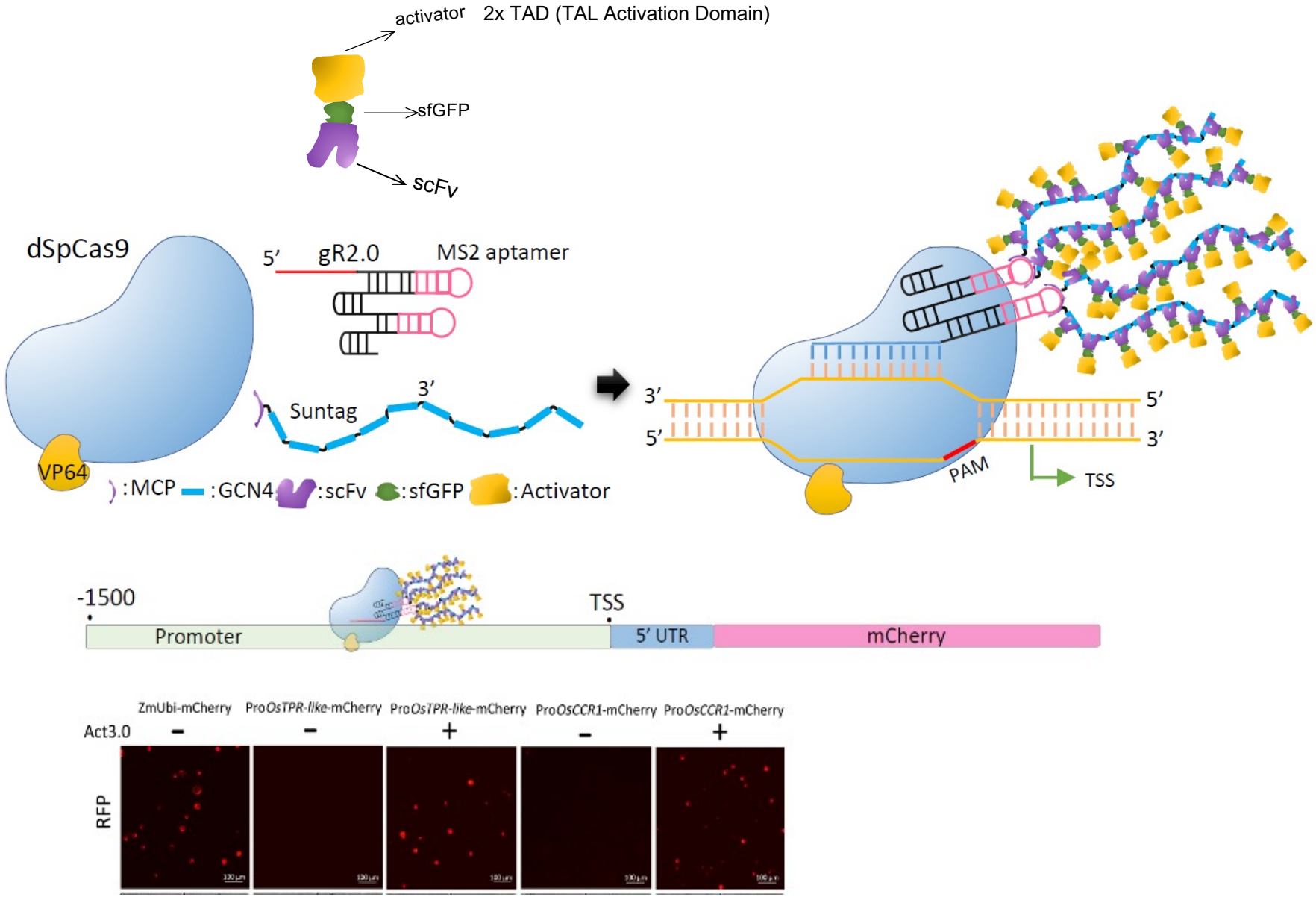




CRISPRa and CRISPRi

(Lei S. Qi et al., *Annual Review of Biochemistry* 2016 ; Gaudelli et al., *Nature* 2017)

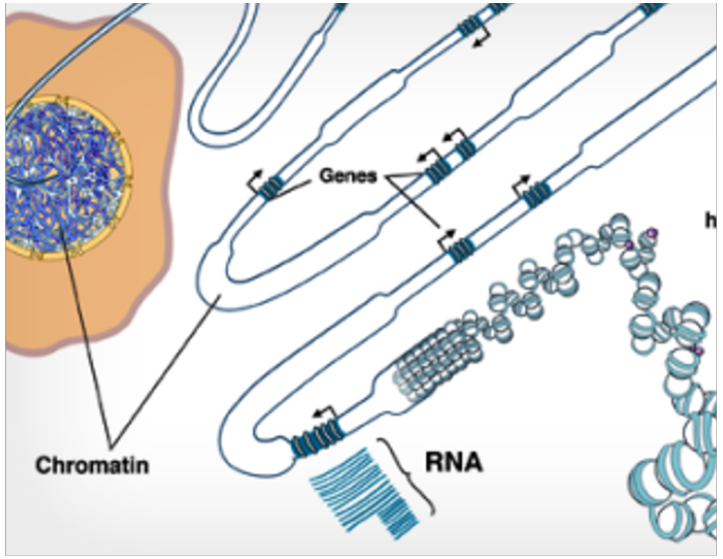
CRISPR-Act3.0 Gene Activation System



(Pan et al., *Nature Plants*, 2021)

From CRISPR-Act3.0 to CRISPR-Combo

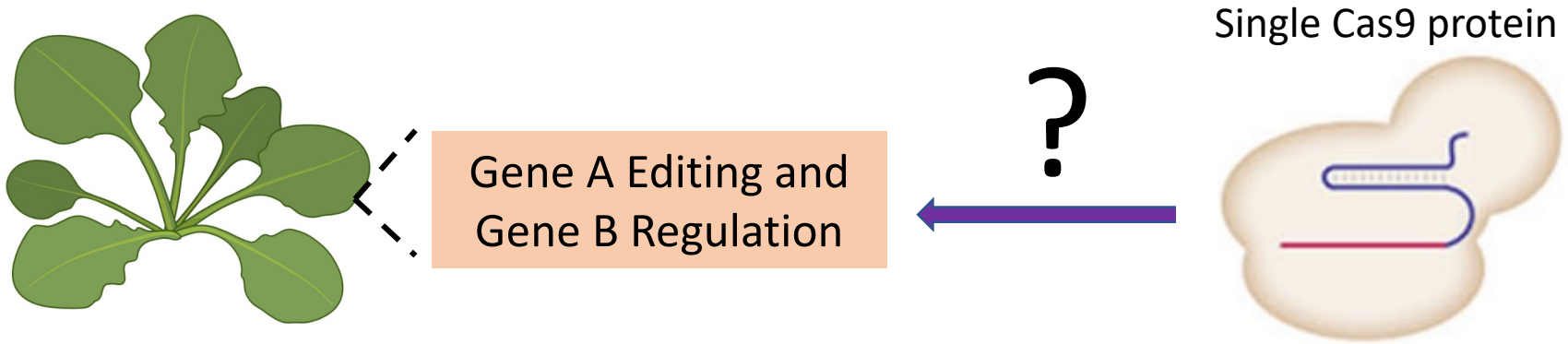
CRISPR-Combo



Genome Transcriptome

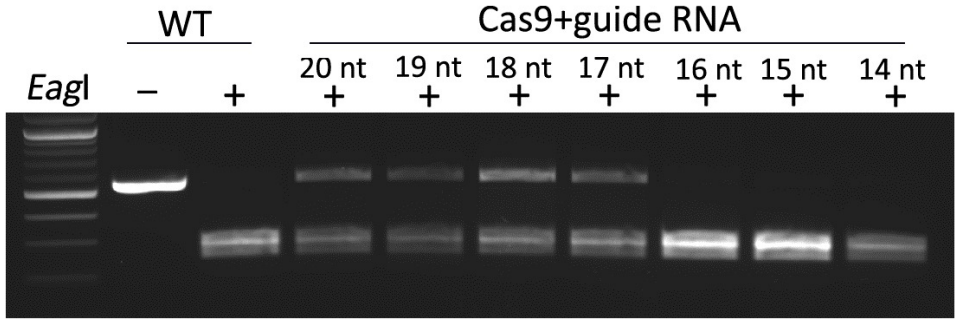
Conventional CRISPR toolbox

From CRISPR-Act3.0 to CRISPR-Combo

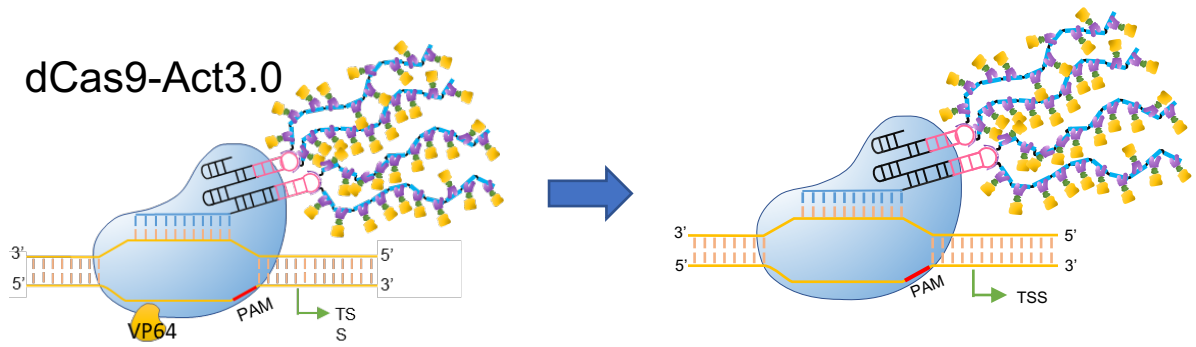


Development of CRISPR-Combo System

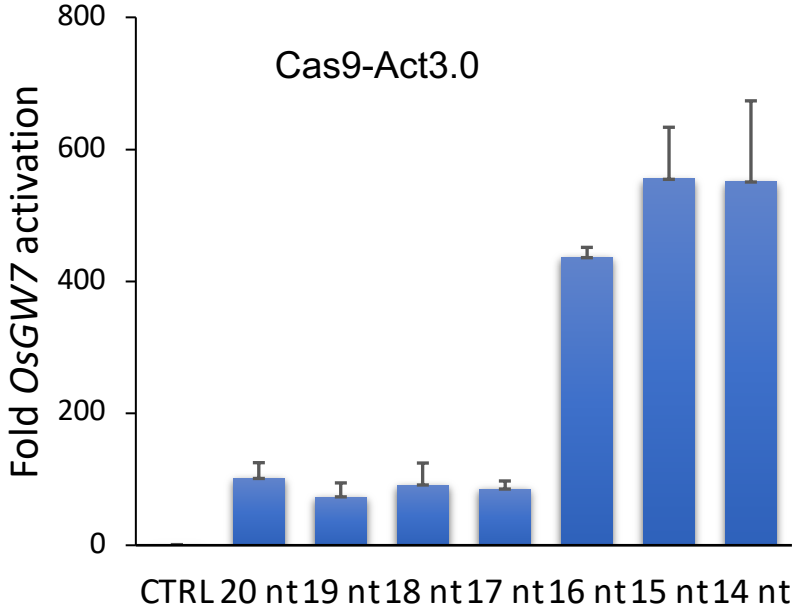
Modulation of **Cas9 nuclease activity** by **altering the protospacer length of the sgRNA**



Cas9 nuclease = dCas9, protospacer length ≤ 16 nt



Cas9-Act3.0
(CRISPR-Combo)



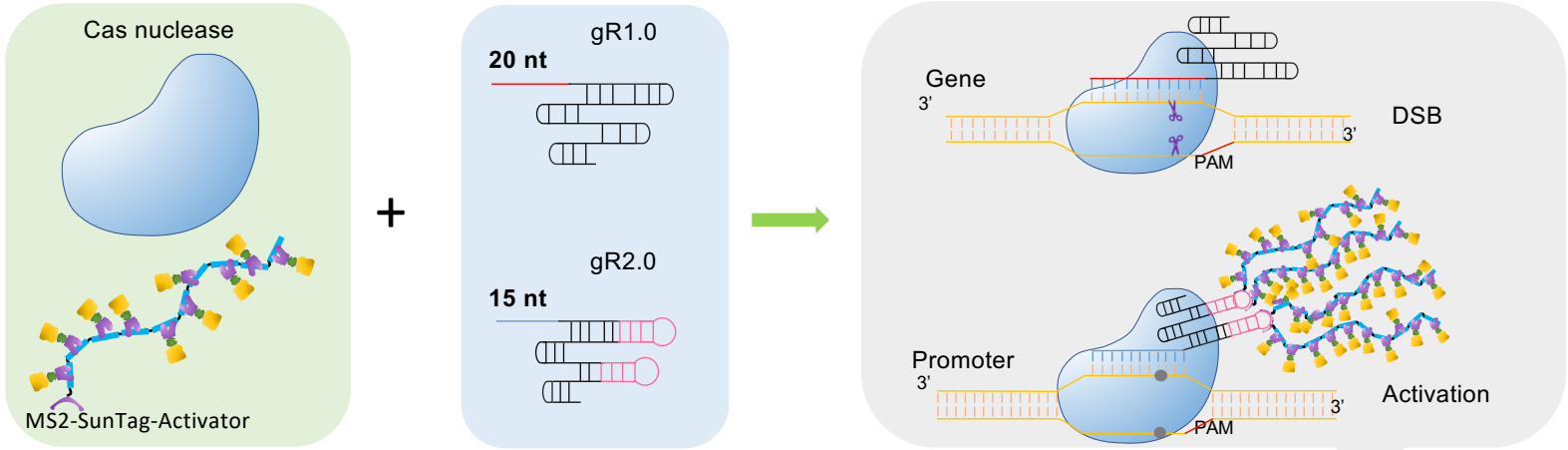
Protospacer ≥ 17 nt, gene editing

Protospacer ≤ 16 nt, gene activation

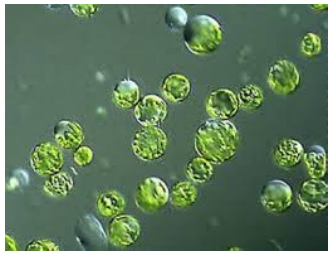
(Pan et al., *Nature Plants* 2022)

CRISPR-Combo system for simultaneous gene editing and activation.

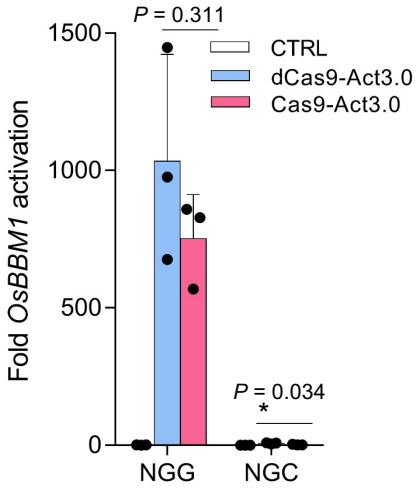
Cas9-Act3.0: orthogonal gene knockout and activation

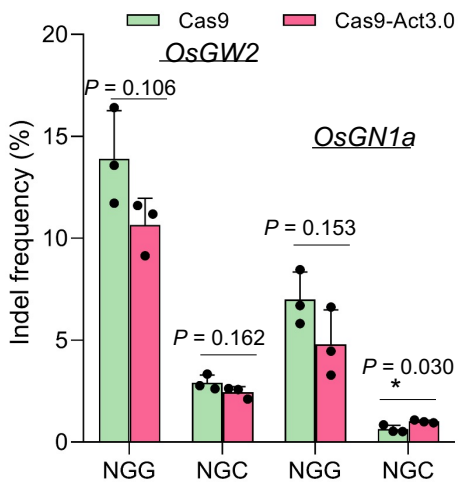


Protoplasts

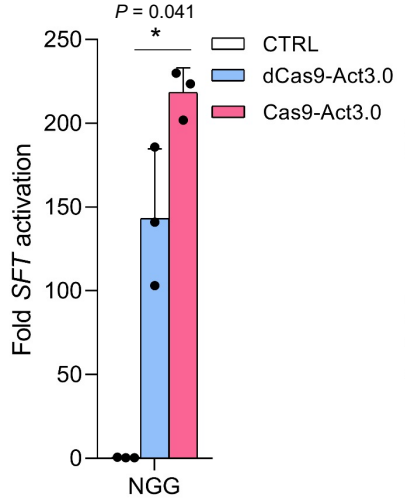


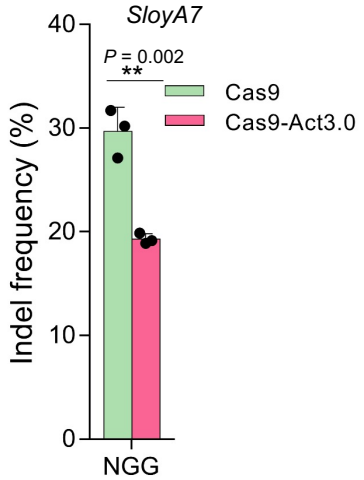
Rice





Tomato

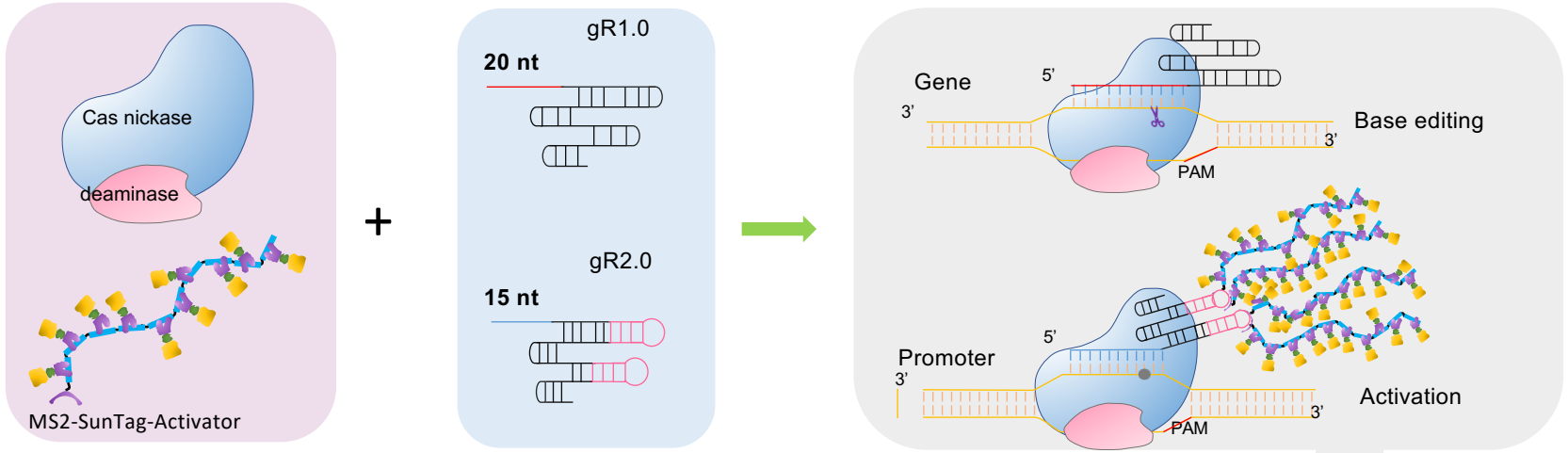




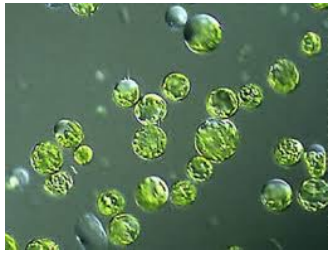
(Pan et al., *Nature Plants* 2022)

CRISPR-Combo system for simultaneous gene editing and activation.

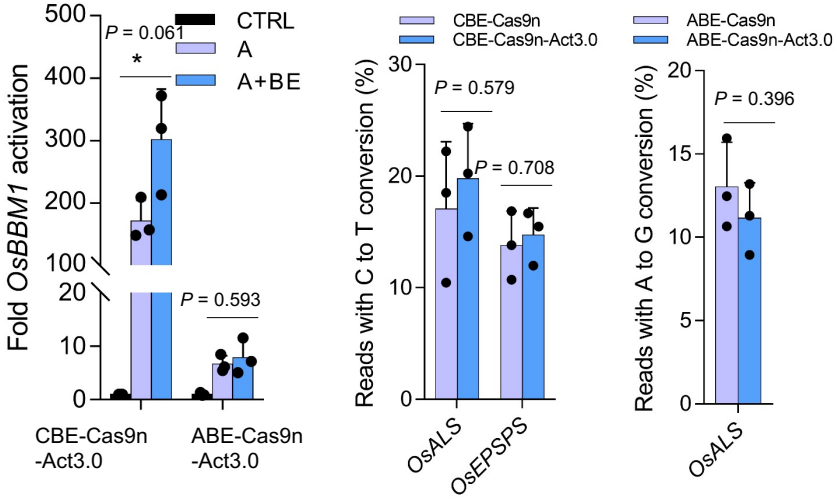
CBE-Cas9n-Act3.0: orthogonal base editing and activation



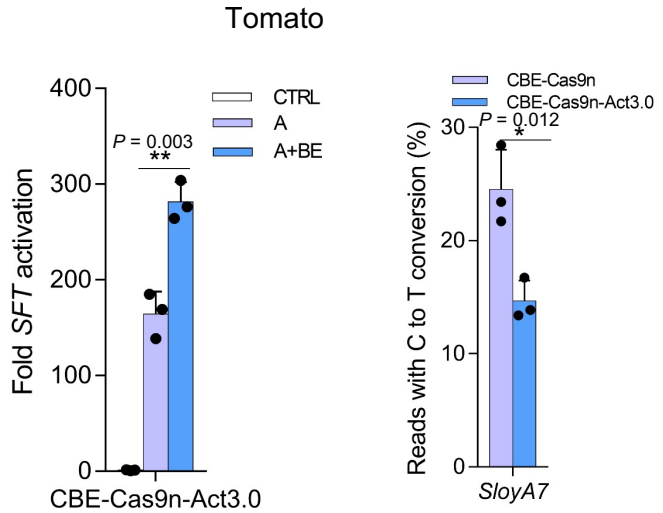
Protoplasts



Rice

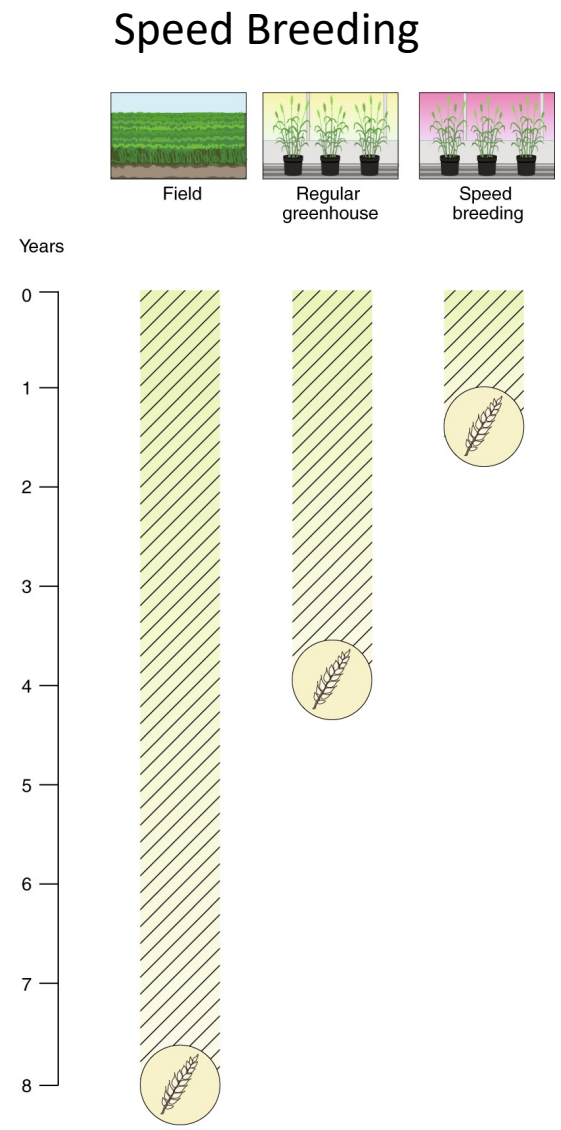


Tomato

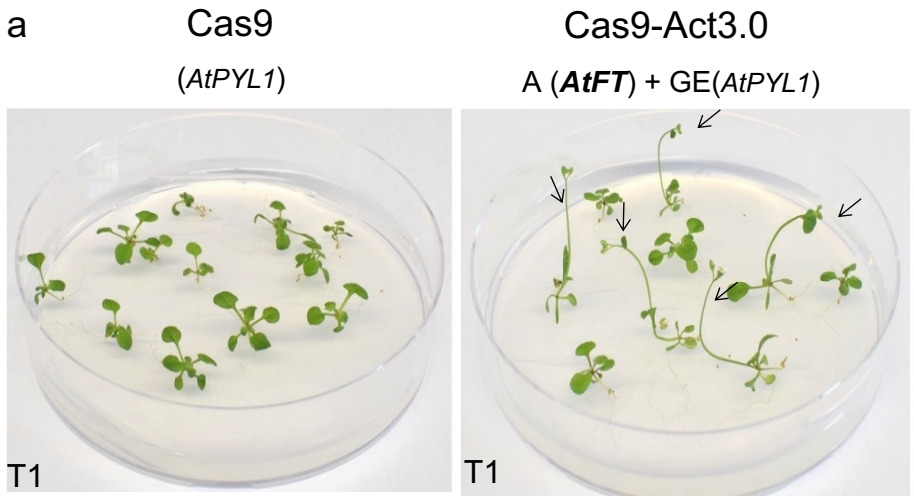


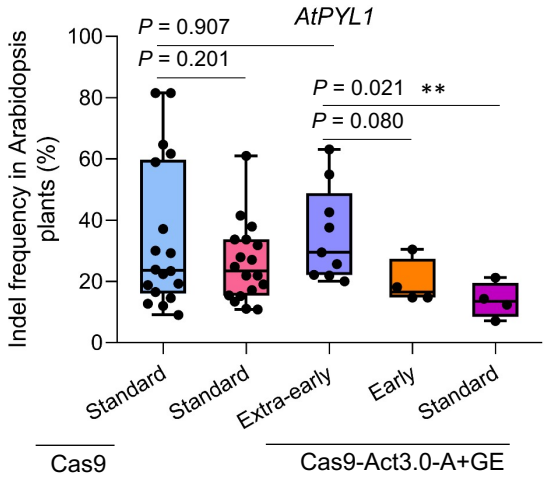
(Pan et al., *Nature Plants* 2022)

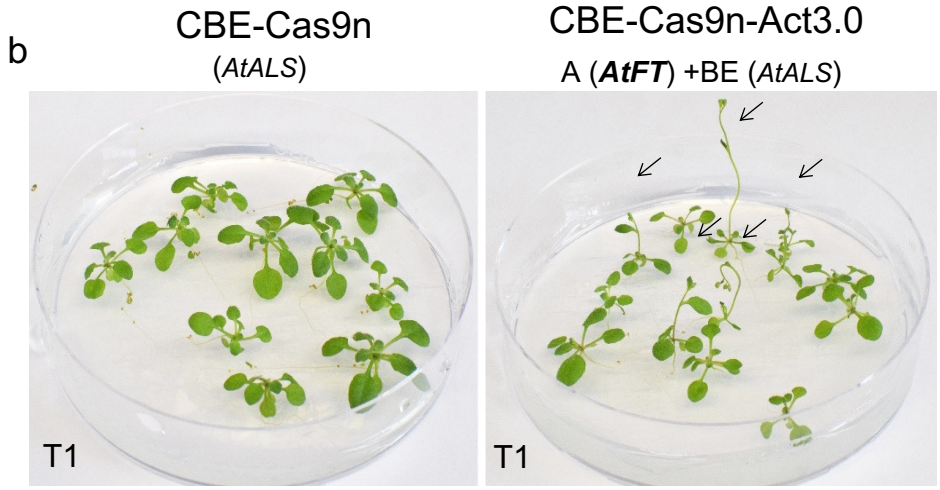
CRISPR-Combo: Rapid breeding of transgene-free edited plants by promoting flowering.

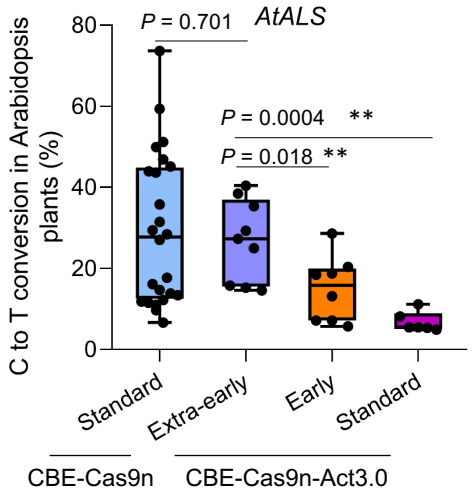


(Hickey et al., *Nature Biotechnology* 2019)









(Pan et al., *Nature Plants* 2022)

CRISPR-Combo: Rapid breeding of transgene-free edited plants by promoting flowering.

T2 progeny of T1 extra-early flowering plants

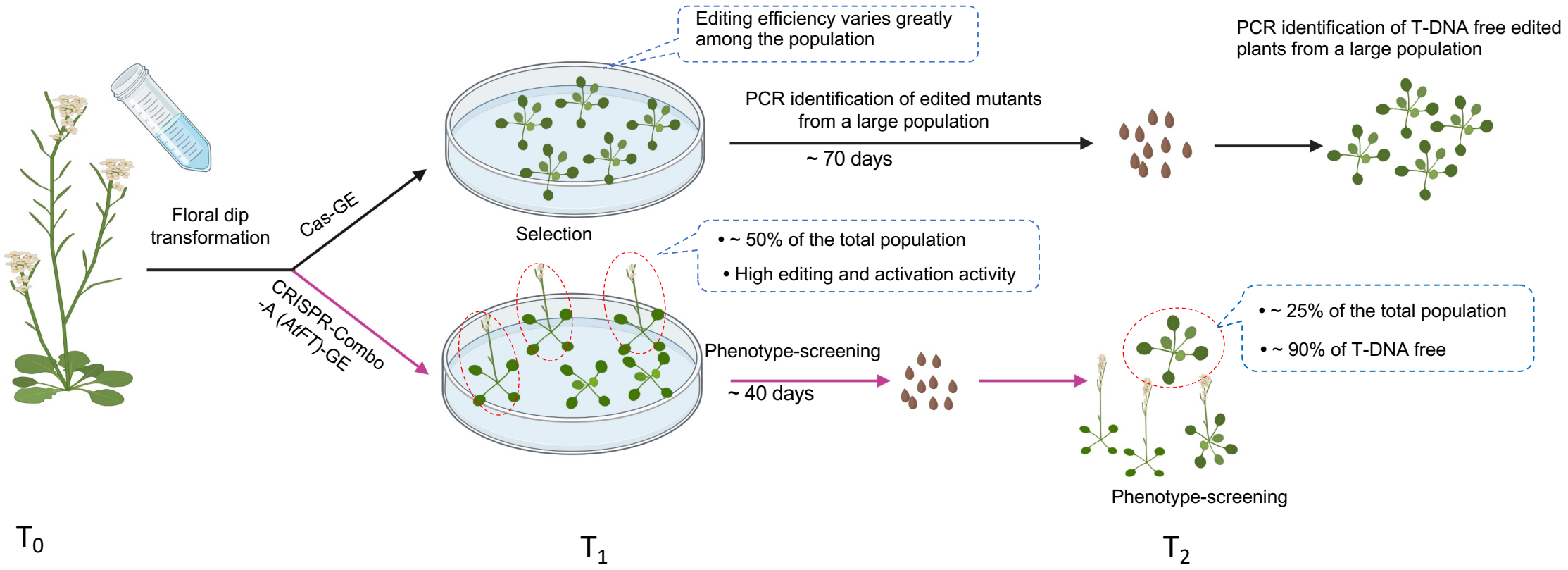
T2

Cas9-Act3.0-A+GE							
T1 transgenic lines	#1	#2	#3	#4	#11	#14	Total
Total examined	94	137	94	110	111	114	660
Extra-Early (EE)	47	47	47	79	24	16	260
Early (E)	23	44	19	6	57	77	226
Standard (S)	24	46	28	25	30	21	174
(EE+E) vs S	2.9 vs 1	2.0 vs 1	2.4 vs 1	3.4 vs 1	2.7 vs 1	4.4 vs 1	2.8 vs 1
T-DNA free S	23 (96%)	42 (91%)	24 (86%)	25 (100%)	29 (97%)	17 (81%)	160 (92%)

CBE-Cas9n-Act3.0-A+BE							
T1 transgenic lines	#5	#14	#15	#16	#17	#19	Total
Total examined	104	120	139	120	81	163	727
Extra-Early (EE)	43	38	41	73	39	30	264
Early (E)	31	57	57	6	10	85	246
Standard (S)	30	25	41	41	32	48	217
(EE+E) vs S	2.5 vs 1	3.8 vs 1	2.4 vs 1	1.9 vs 1	1.5 vs 1	2.4 vs 1	2.4 vs 1
T-DNA free S	29 (97%)	24 (96%)	37 (90%)	38 (93%)	32 (100%)	42 (88%)	202 (93%)

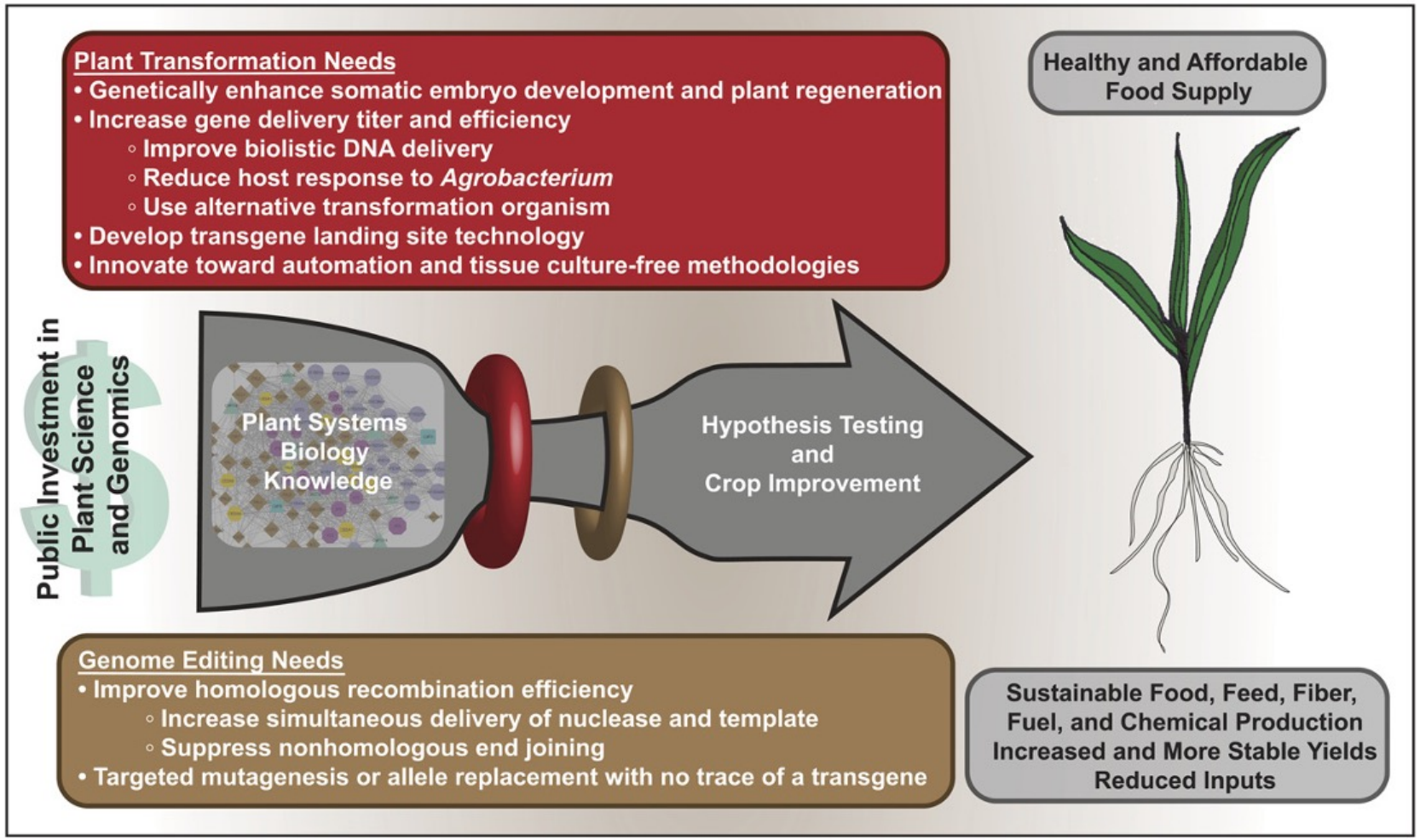
(Pan et al., *Nature Plants* 2022)

CRISPR-Combo: Rapid breeding of transgene-free edited plants by promoting flowering.



(Pan et al., *Nature Plants* 2022)

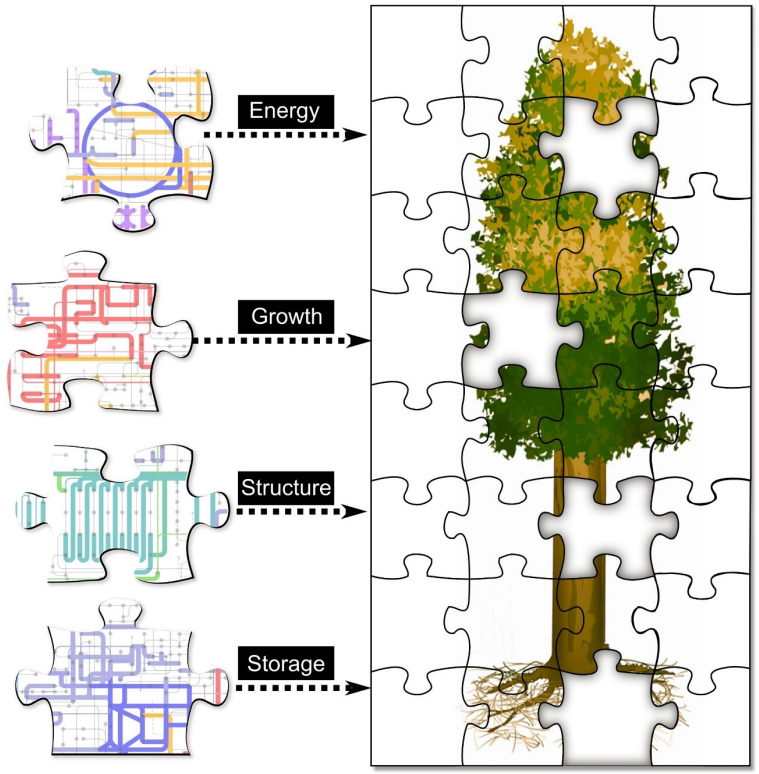
Plant Transformation and Regeneration is a Major Bottleneck of Genome Editing Applications in Crops



Altpeter et. al, *Plant Cell* 2016

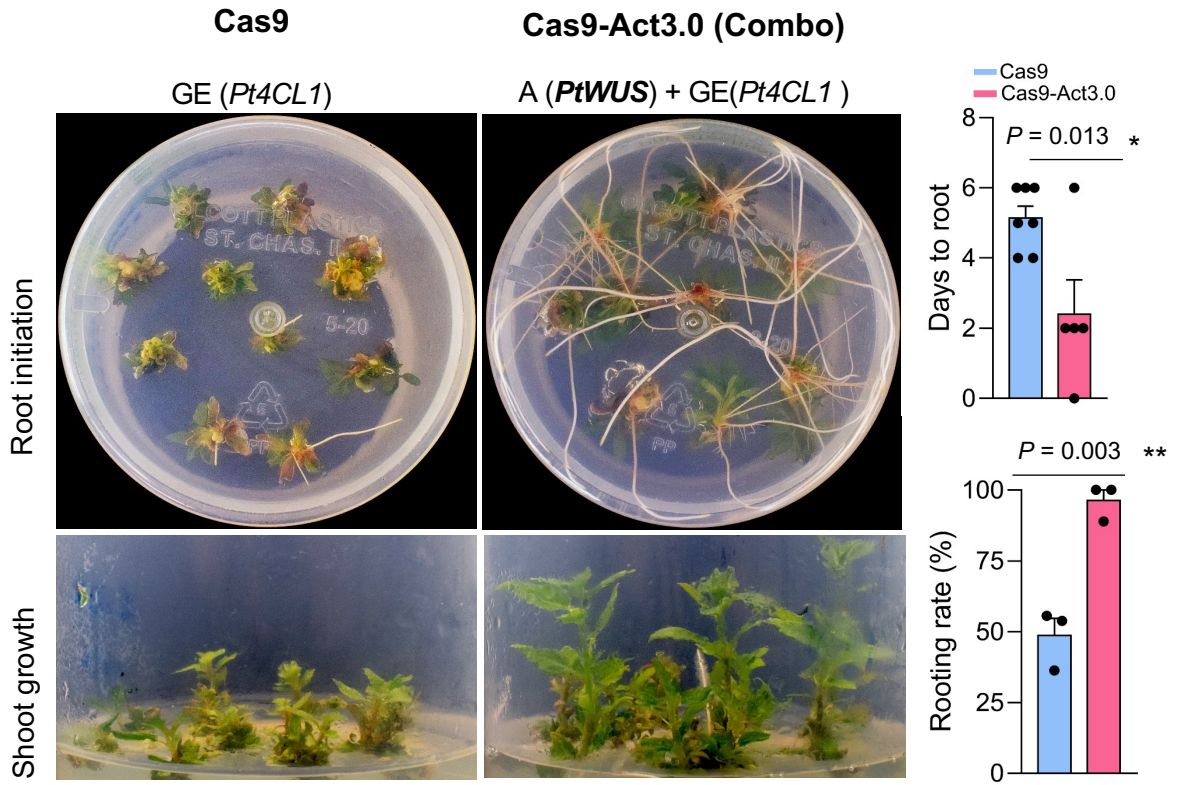
CRISPR-Combo: Rapid breeding of genome-edited plants by promoting regeneration in **poplar**.

Poplar—a Bioenergy and Biomaterial Source



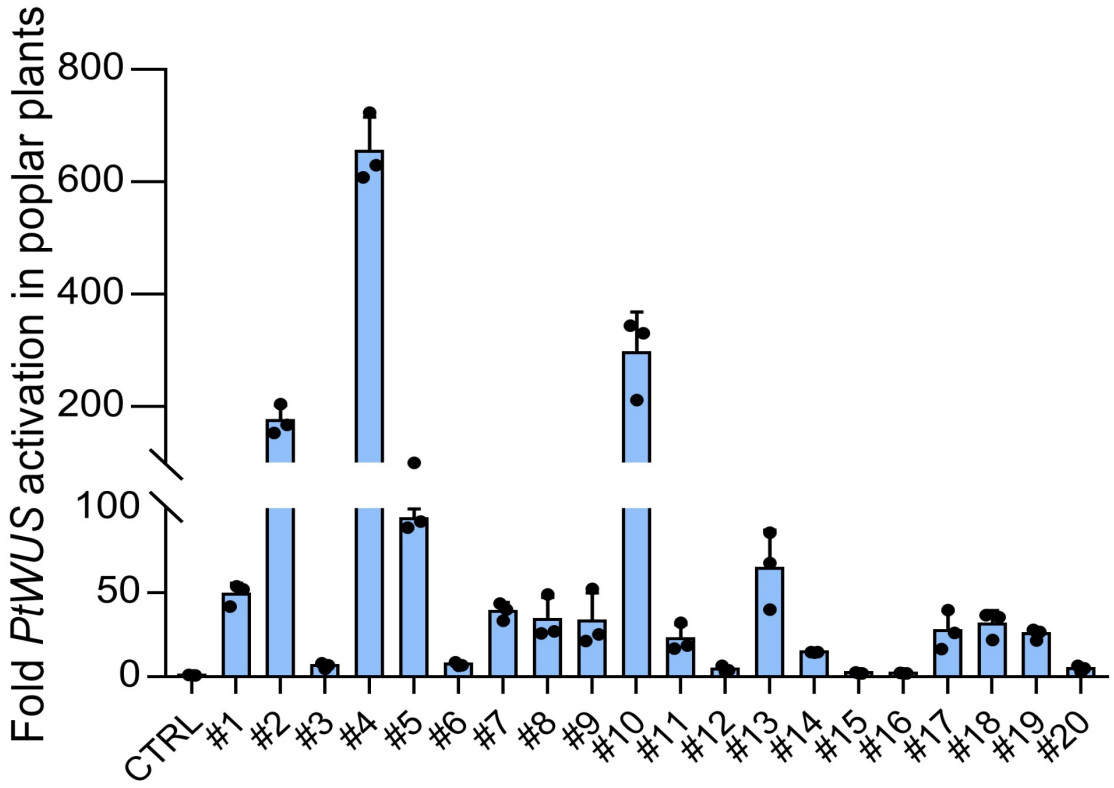
[Oak Ridge National Laboratory]

Cas9-Act3.0/CRISPR-Combo promotes root initiation and shoot growth by activation of *PtWUS*.

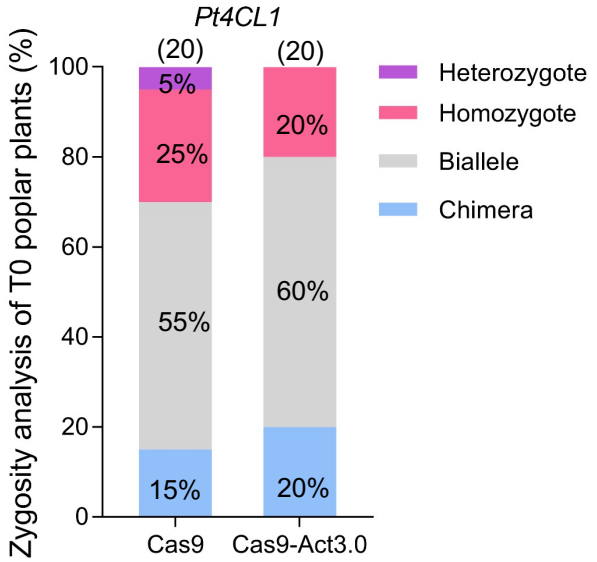


(Pan et al., *Nature Plants* 2022)

CRISPR-Combo: Rapid breeding of genome-edited plants by promoting regeneration in **poplar**.



Gene activation

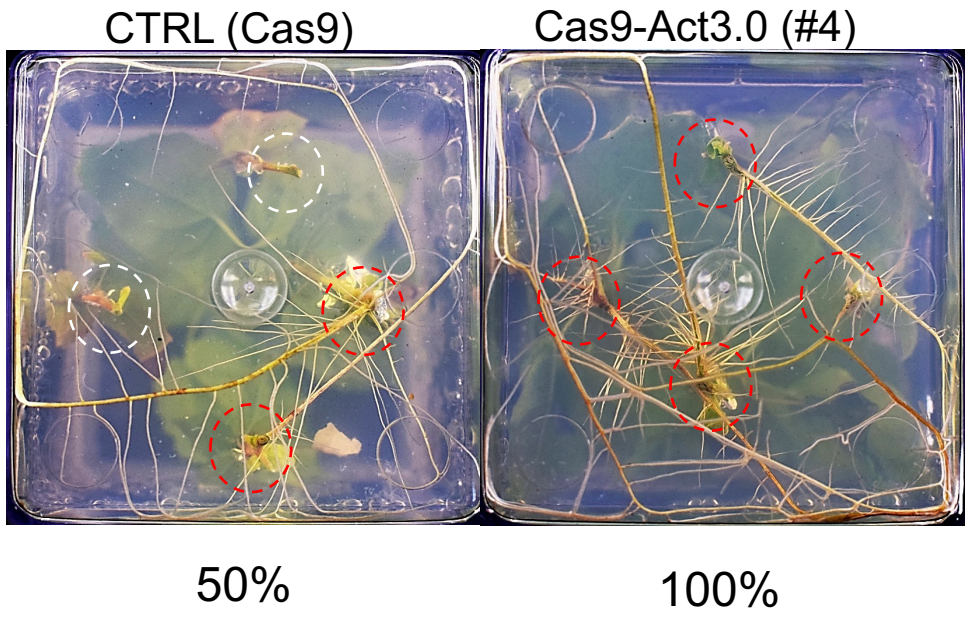


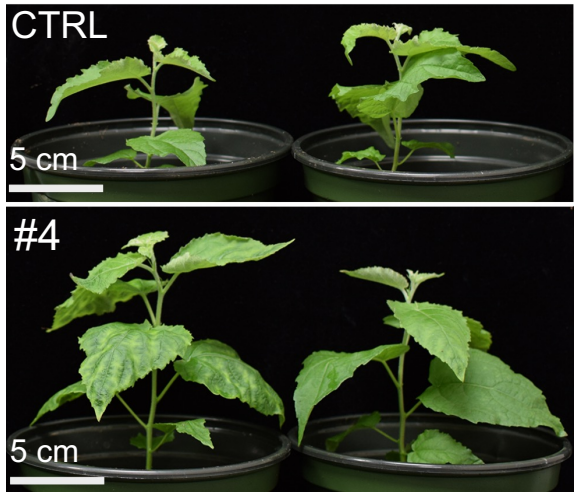
Gene editing

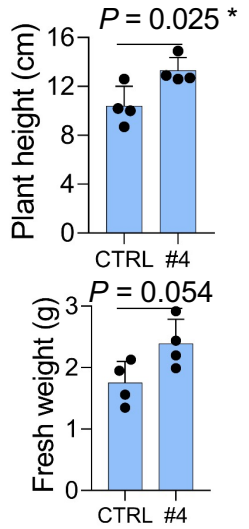
(Pan et al., *Nature Plants* 2022)

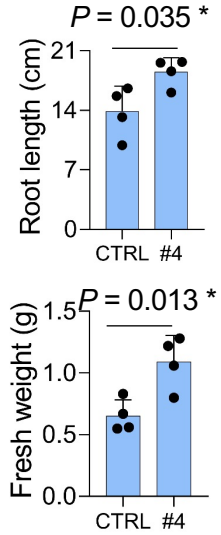
CRISPR-Combo: Rapid breeding of genome-edited plants by promoting regeneration in **poplar**.

- Cas9-Act3.0 (CRISPR-Combo) promotes *de novo* root initiation of stem cuttings.
- The *PtWUS* high-activation line showed significantly **enhanced shoot biomass** and **increased root biomass**.



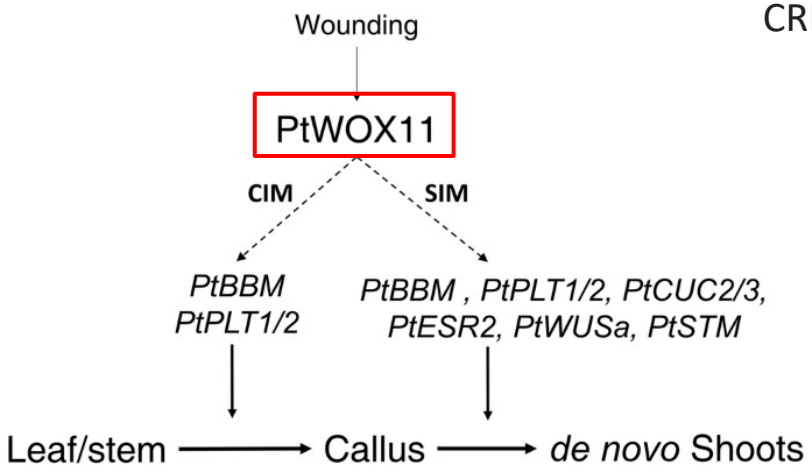






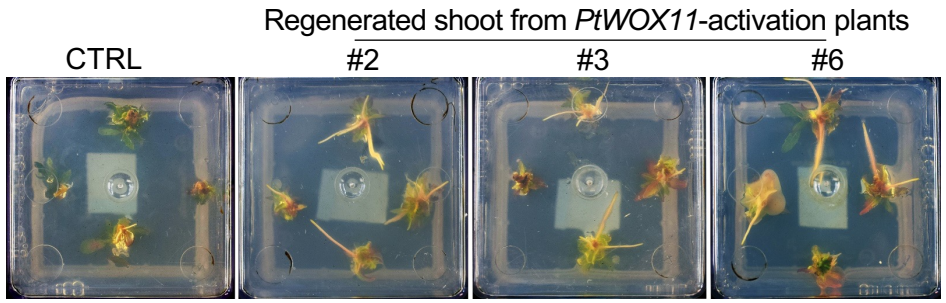
(Pan et al., *Nature Plants* 2022)

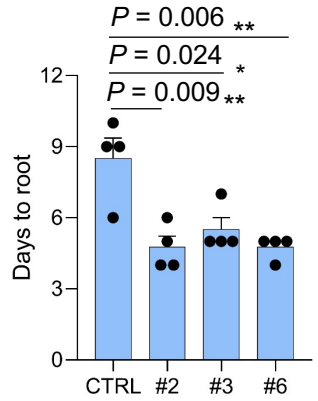
CRISPR-Combo: Rapid breeding of genome-edited plants by promoting regeneration in **poplar**.

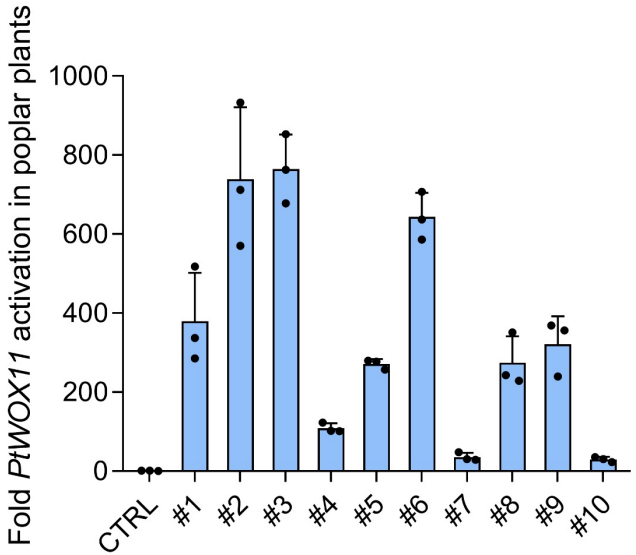


(Liu et al., 2018)

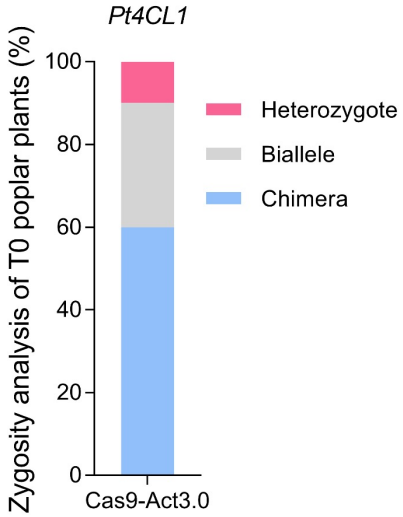
CRISPR-Combo T_0 lines showed high level of ***PtWOX11*** **activation and** ***Pt4CL1*** **editing.**







Gene activation

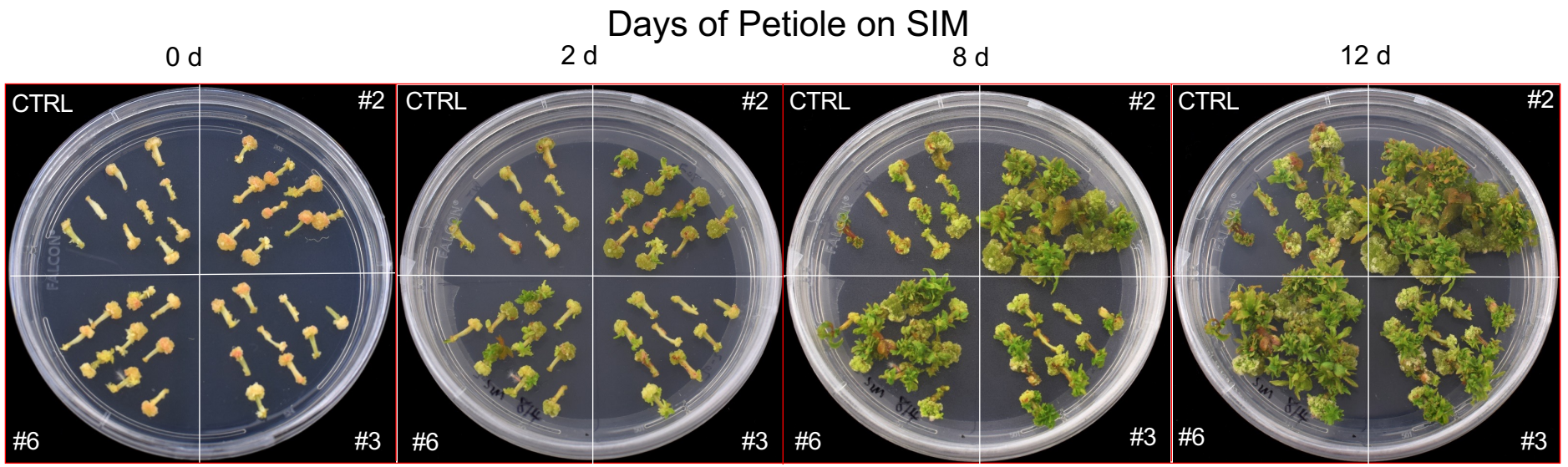


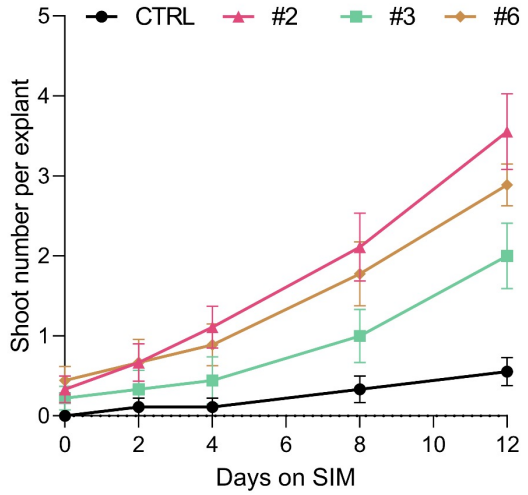
Gene editing

(Pan et al., *Nature Plants* 2022)

CRISPR-Combo: Rapid breeding of genome-edited plants by promoting regeneration in **poplar**.

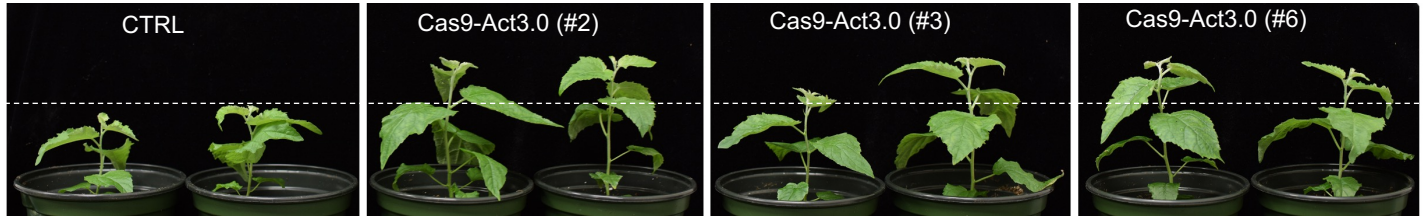
Higher *PtWOX11* activation lines showed **rapid *de novo* callus regeneration** from **petiole cuttings** and resulting in **more adventitious shoots** per explant.



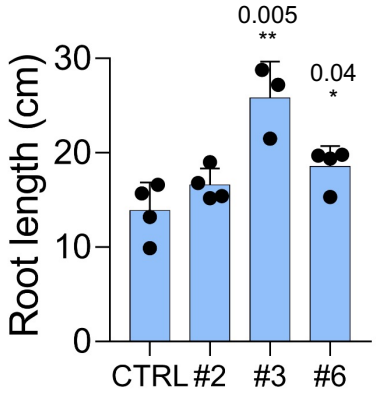


(Pan et al., *Nature Plants* 2022)

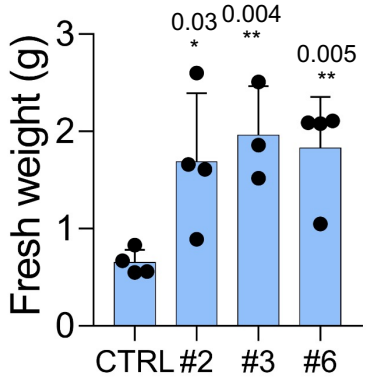
CRISPR-Combo: Rapid breeding of genome-edited plants by promoting regeneration in **poplar**.



The plant **height, shoot biomass and leaf area** were **increased** in some *PtWOX11* activation lines.



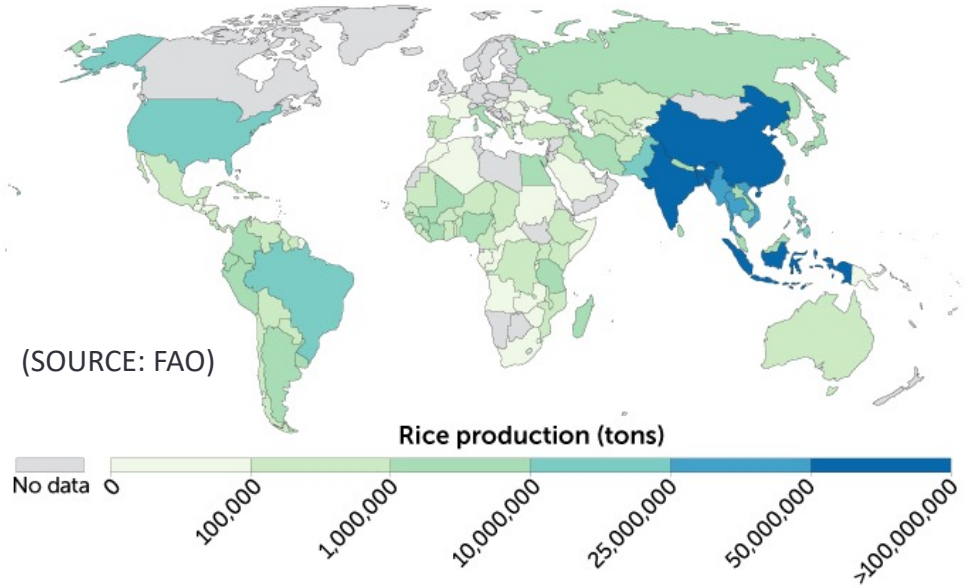
Higher *PtWOX11* activation lines showed **increased root length** and **root biomass** production



(Pan et al., *Nature Plants* 2022)

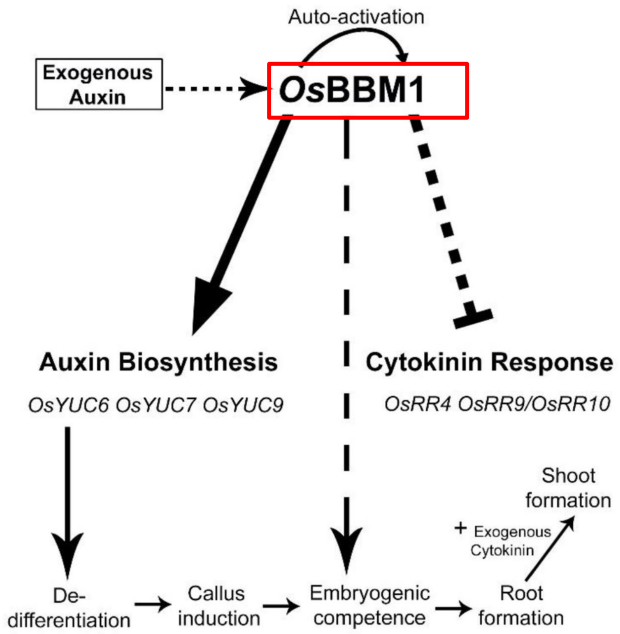
CRISPR-Combo: Rapid breeding of genome-edited plants by promoting regeneration in **rice**.

Rice feeds half the world.



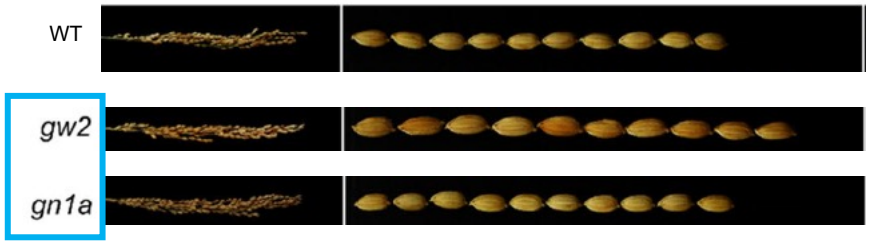
(SOURCE: FAO)

Gene activation



(Khanday et al., bioRxiv 2020)

Gene editing



(Zhou et al., Plant Cell Rep 2018)

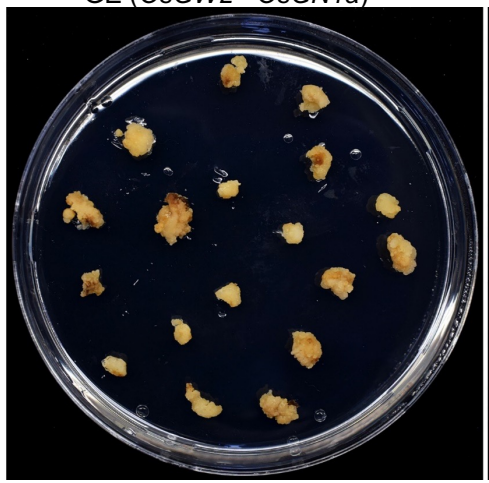
(Pan et al., *Nature Plants* 2022)

CRISPR-Combo: Rapid breeding of genome-edited plants by promoting regeneration in **rice**.

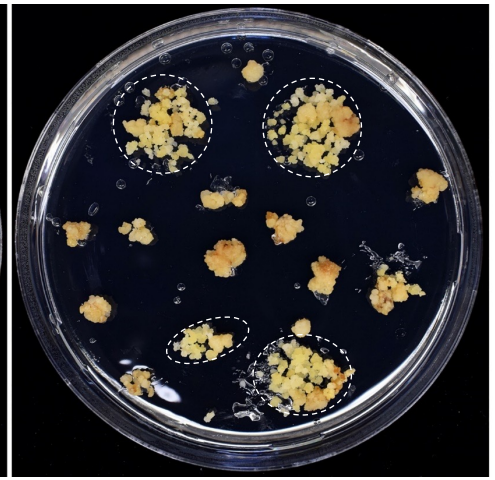
Regeneration of genome-edited rice plants in a **hormone-free manner** by activation of *OsBBM1.*

Cas9-Act3.0 RSM-hormone-free (HF)

GE (*OsGW2 +OsGN1a*)

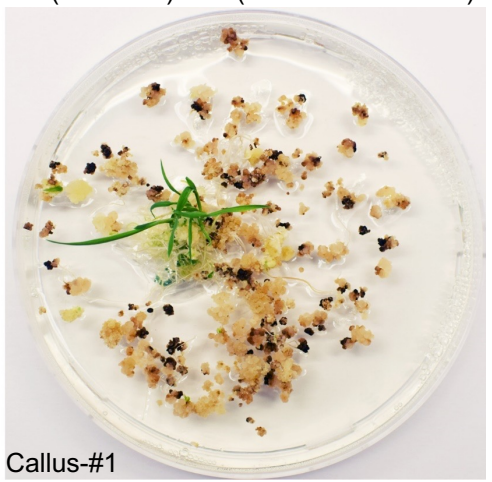


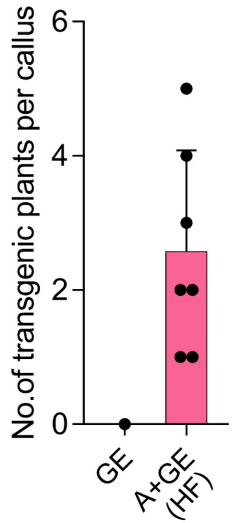
A (***OsBBM1***)+GE (*OsGW2 +OsGN1a*)



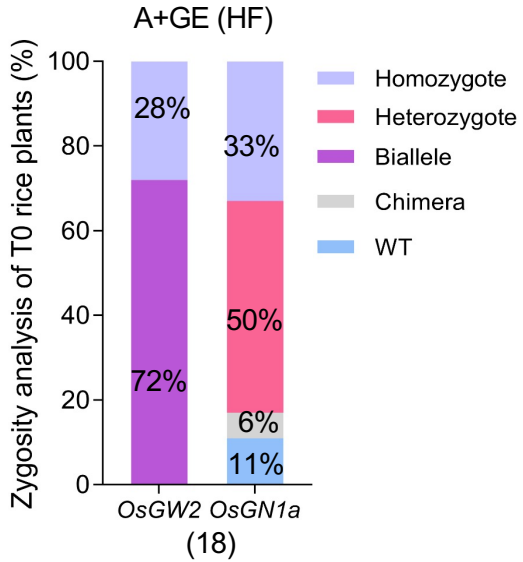
Cas9-Act3.0 Reg I-HF

A (*OsBBM1*)+GE (*OsGW2 +OsGN1a*)



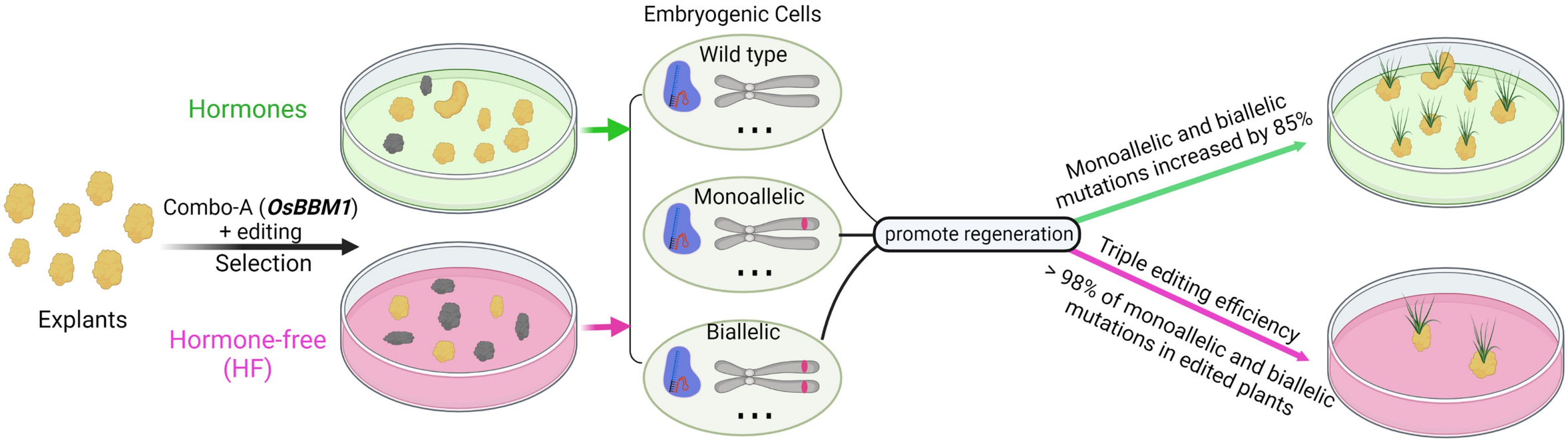


Almost all *OsGW2*- and *OsGN1a*-edited plants contained **heritable targeted mutations** including homozygous, biallelic or heterozygous mutations.



(Pan et al., *Nature Plants* 2022)

CRISPR-Combo: Rapid breeding of genome-edited plants by promoting regeneration in **rice**.

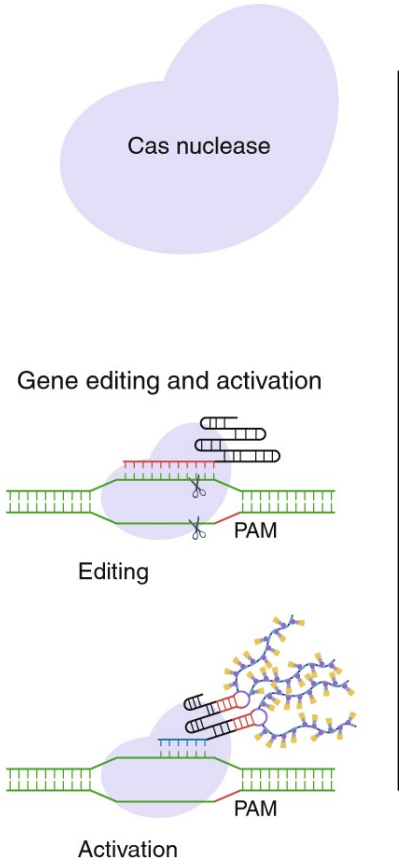


(Pan et al., *Nature Plants* 2022)

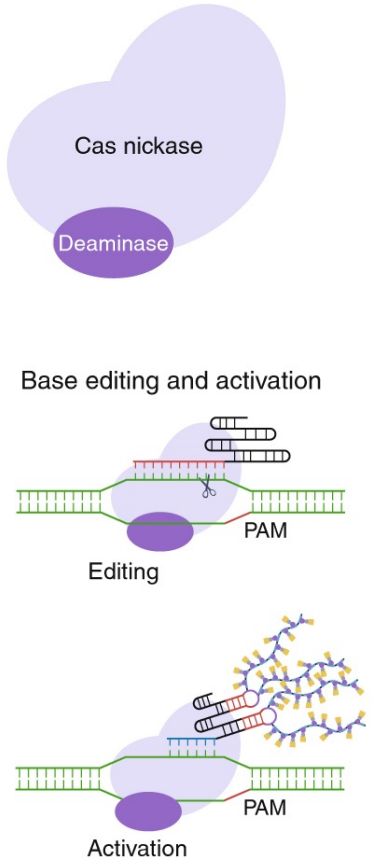
Summary

Two CRISPR-Combo systems:

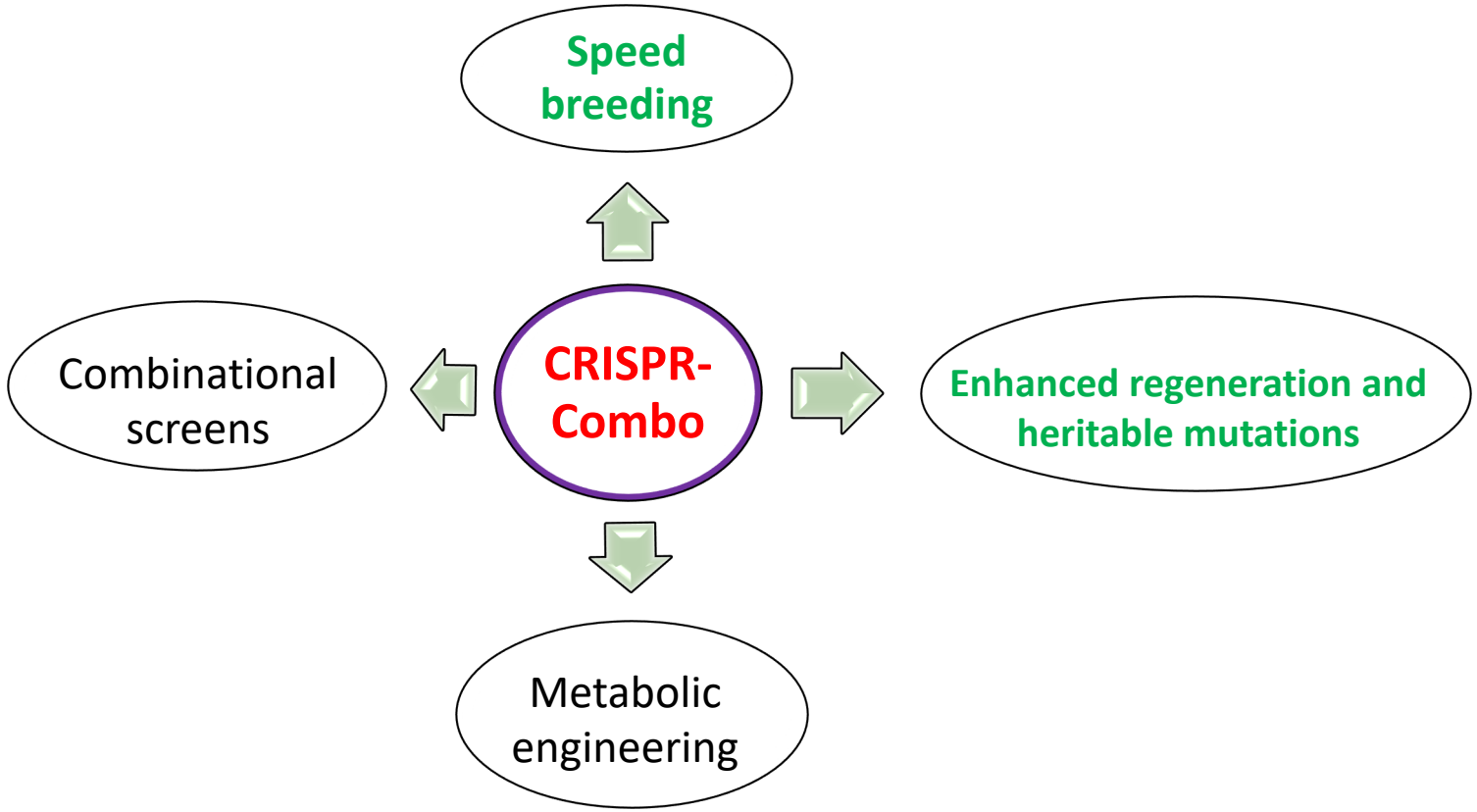
1. Cas9-Act3.0



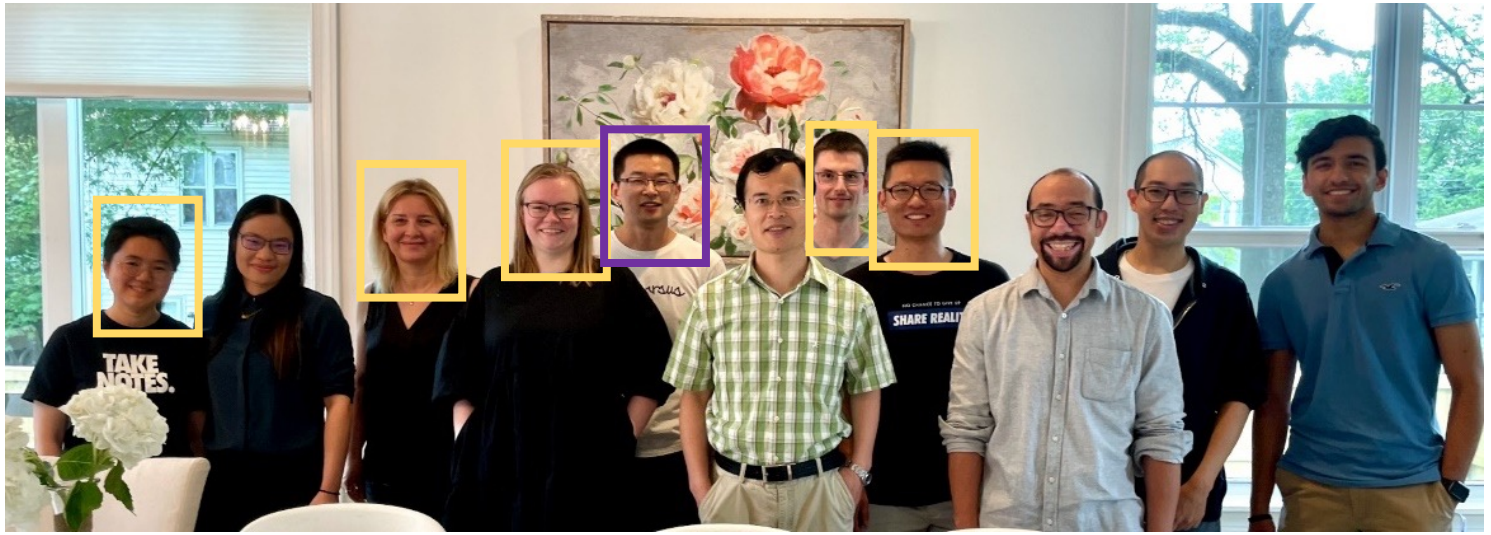
2. CBE-Cas9n-Act3.0



(Debernardi and Rowan, *Nature Plants* 2022)



ACKNOWLEDGEMENTS



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