Two New Tools for Genetic Improvement of Cellulosic Energy Crop:

‘Growth Promoting’ and ‘Gene-Deletor’ Technologies

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Technology 1:

A ‘Growth Promoting’ Gene Technology for Cellulosic Bioenergy Crops

Why do we need to improve yield of cellulosic bioenergy crops?

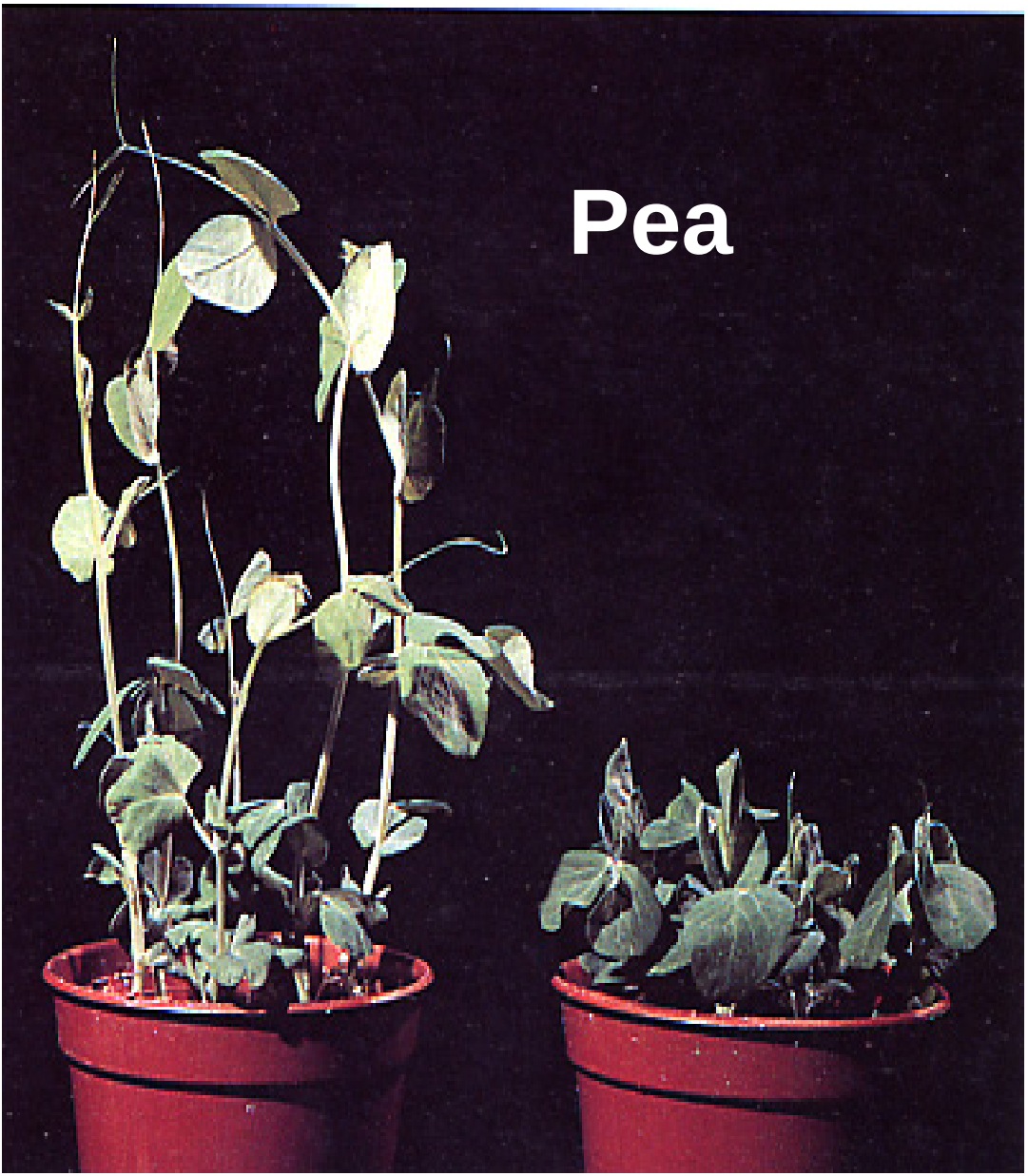
-- Use less land to grow biofuel crops.

-- Reduce cost of feedstock and therefore reduce biofuel price.

	Today ($/gal) (@$60/dt, 60 gal/dt)	2010-12 ($/gal) (@$30/dt, 90 gal/dt)
Feedstock	$1.00 (40%)	$0.33
Total	$2.65 (100%) (Not competitive with gasoline)	$1.10 (Competitive with gasoline)

Our strategy to improve the yield of cellulosic bioenergy crops is through manupulation of plant homones

The plant hormone gibberellin (GA) plays a key role in plant growth

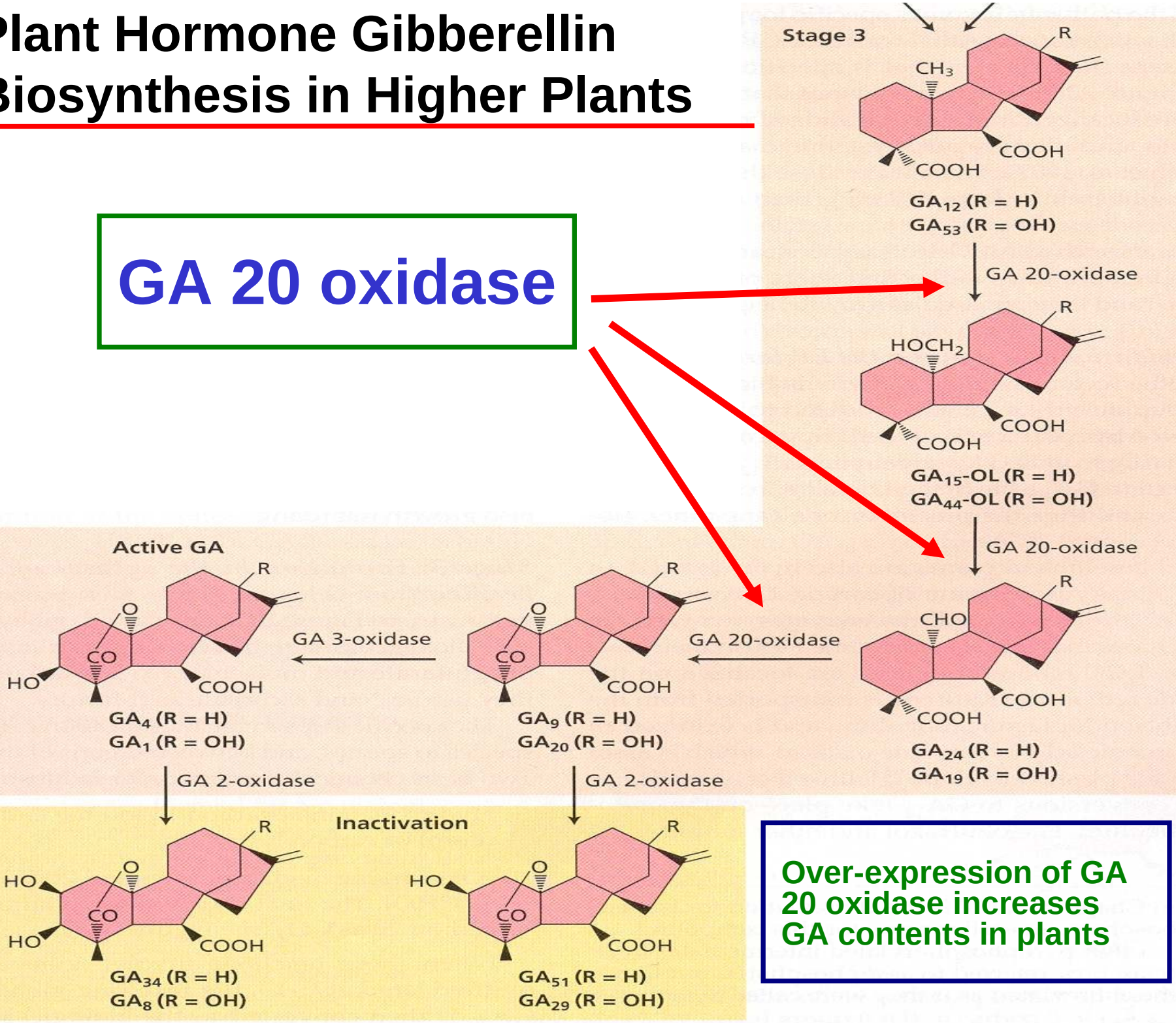


Wild-type GA deficient

Wild-type Sprayed w/ GA

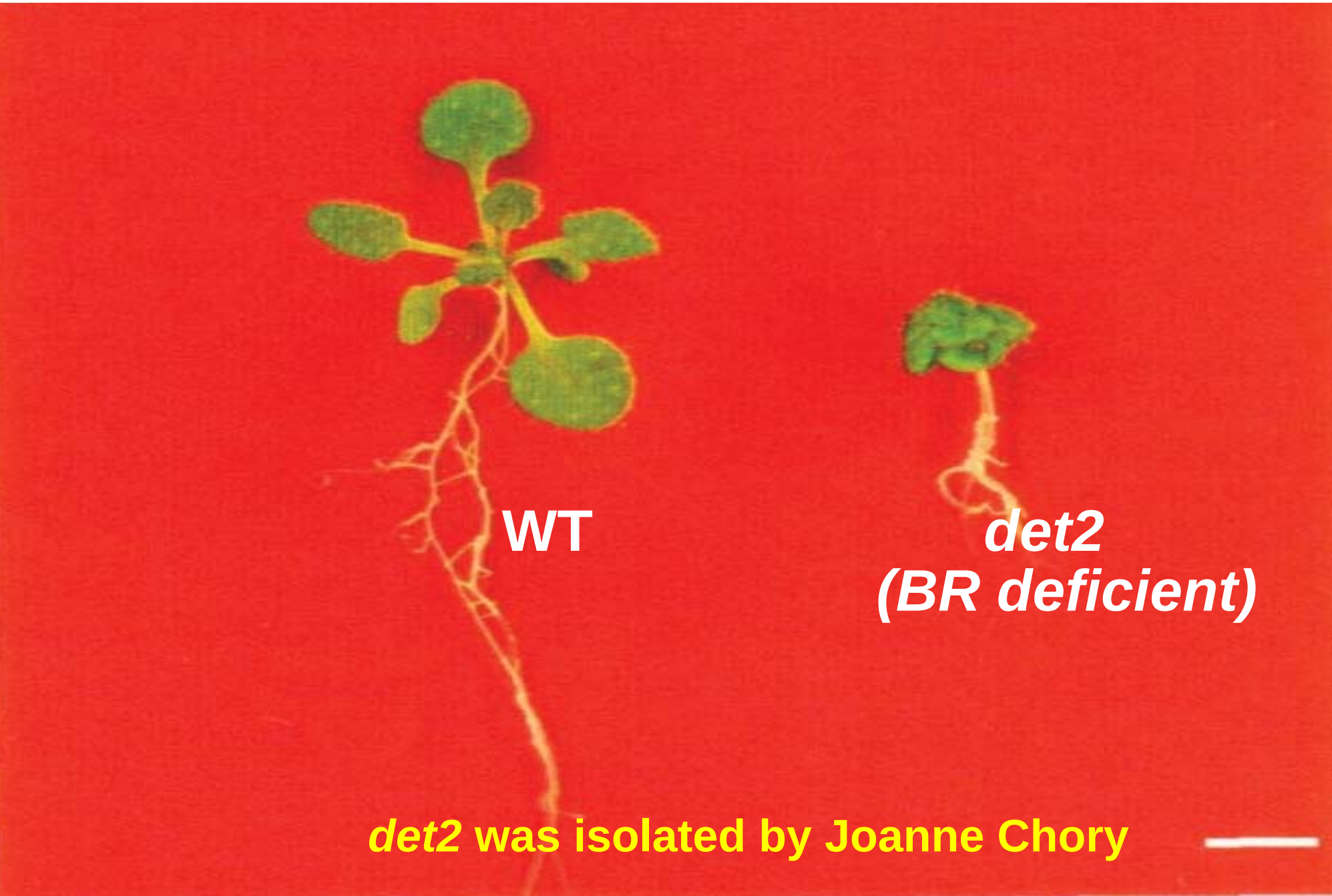
Plant Hormone Gibberellin Biosynthesis in Higher Plants

GA 20 oxidase

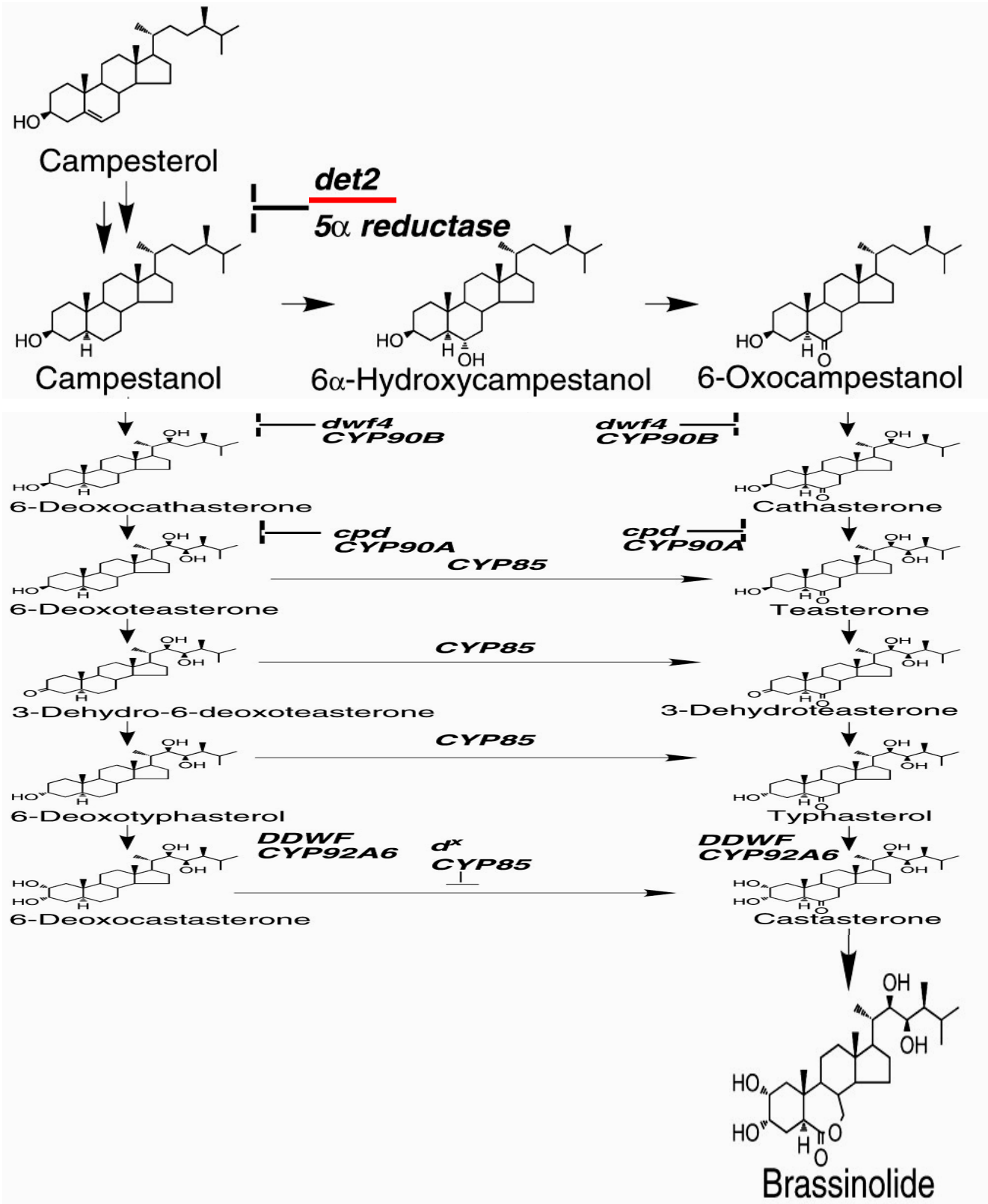


Over-expression of GA 20 oxidase increases GA contents in plants

The plant hormone brassinosteroid (BR) plays a key role in plant growth



Brassinosteroid Biosynthesis and Det2 (5α reductase)

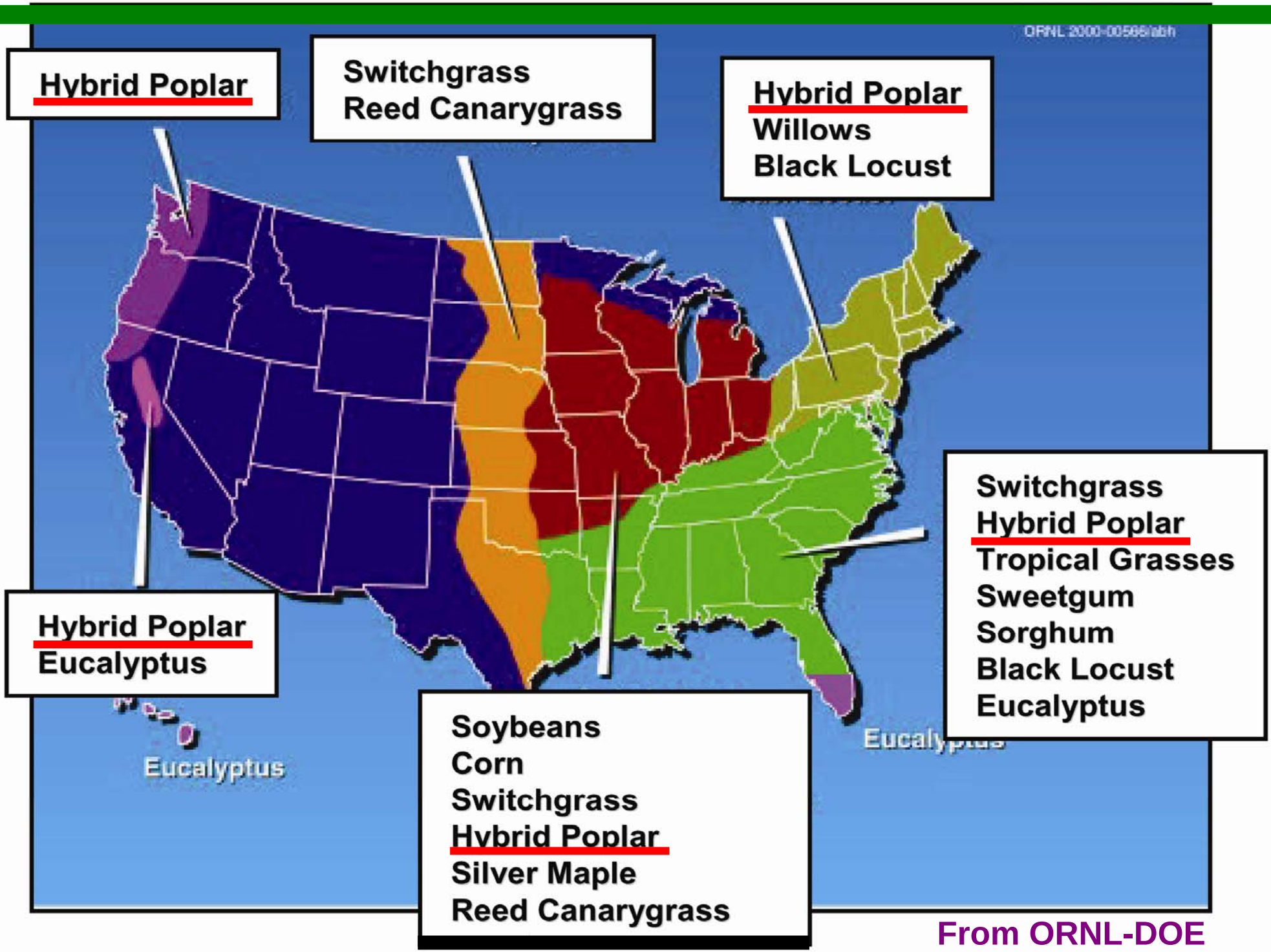


GA & BR over-production:

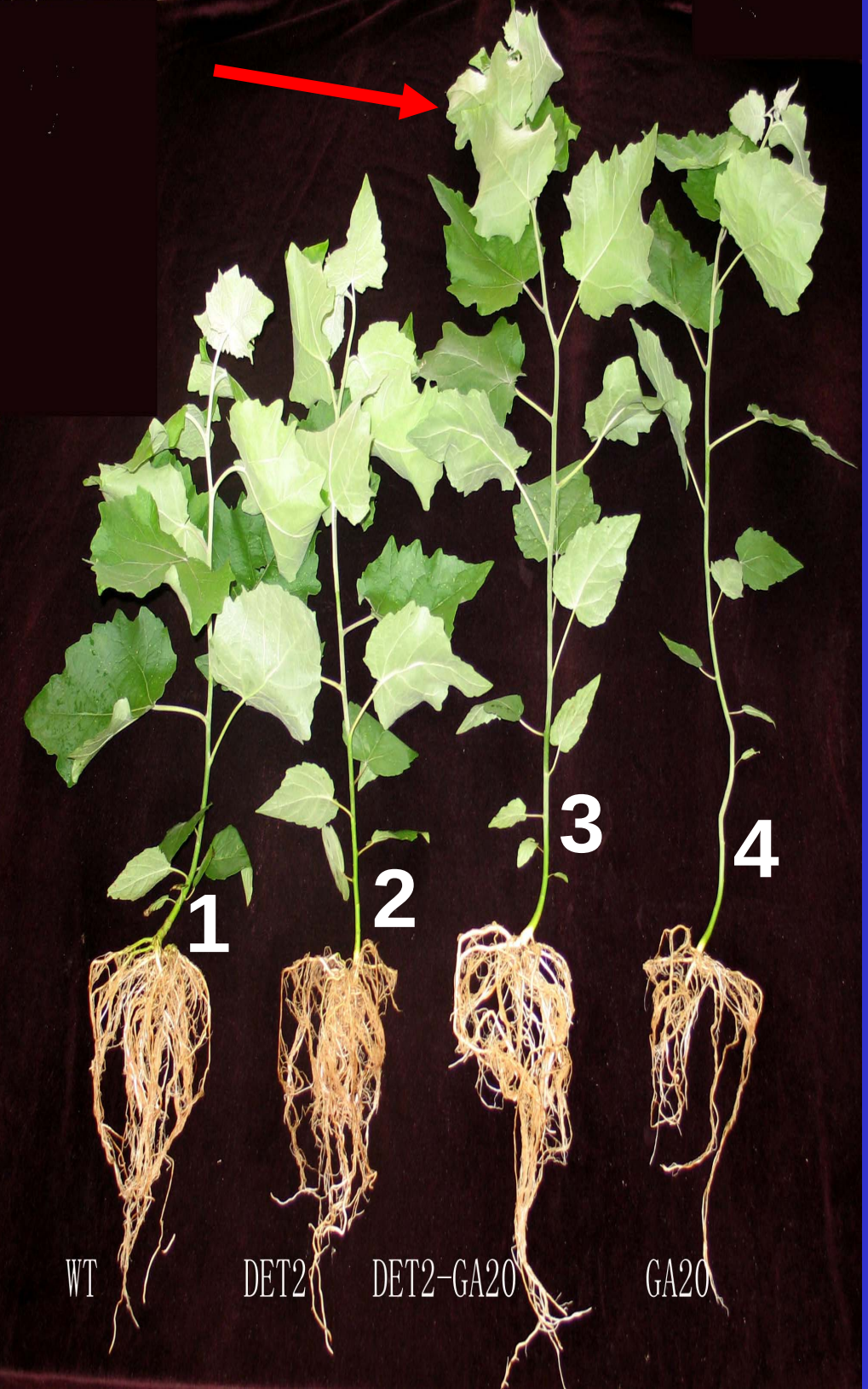
GA20: Gibberellin biosynthetic gene

Det2: Brassinosteroid biosynthetic gene

Poplar has been identified as an energy crop in Northeast U.S.



From ORNL-DOE



1: Wild type poplar

2: Det2 poplar

3: GA20-Det2 poplar

4: GA20 poplar


Stem cuttings:
GA20 + Det2 over-expressors: roots similar to wt but better shoot growth
WT
DET2-GA20

Overexpression of GA20 + Det2 genes enhances biomass production of poplar plants*

	WT	GA + Det2 - #5	GA + Det2 - #12
Stem	1.3±0.1	6.0±0.6	5.7±0.5
Total	8.0	15.4	15.6

*Dry weight was obtained from two months aspen plants grown in greenhouse

Conclusions:

-- The GA20-oxidase and Det2 genes may be used to improve growth of cellulosic feedstock crops and therefore to reduce the total cost of ethanol.

Science (2006) 313:1742.

ECOLOGY

Adding Biofuels to the Invasive Species Fire?

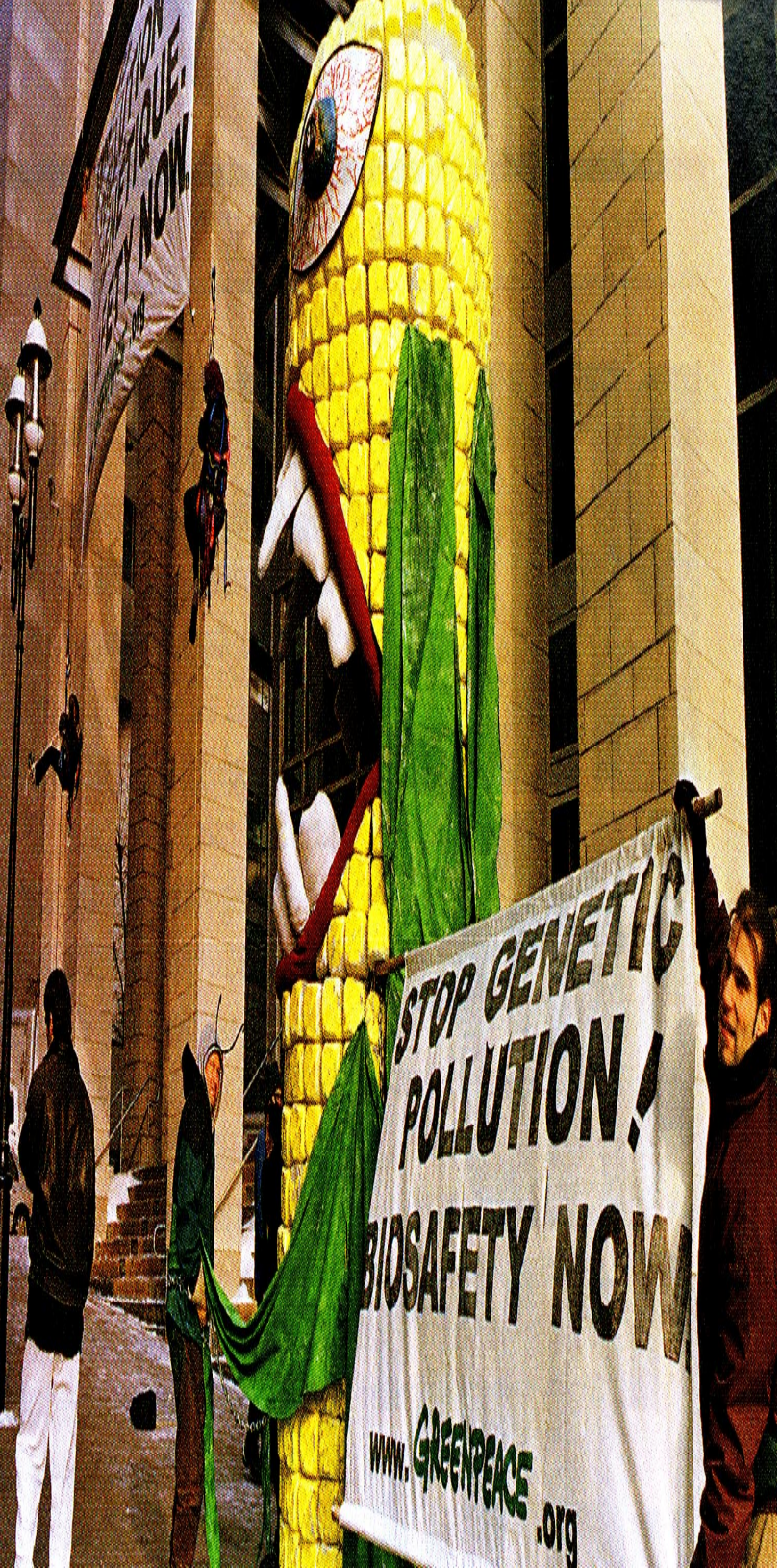
S. Raghu,[1]* R. C. Anderson,[2] C. C. Daehler,[3] A. S. Davis,[4] R. N. Wiedenmann,[5] D. Simberloff, [6] R. N. Mack[7]

"Most of the traits that are touted as great for biofuel crops - no known pests or diseases, rapid growth, high water-use efficiency - are red flags for invasion biologists."

Damages of invasive plants to the US per year:

-- $35 billion in costs for damage and control

-- 3 million acres of new land taken over by invasive plants



Also, although transgenic plant technology provides a powerful tool to improve growth and quality of bioenergy crops, transgenes may escape to the environment via seed- and pollen-mediated gene flow.

This is particularly relevant for cellulosic energy crops because these plants are less domesticated.

Technology 2:

The ‘Gene-Deletor’ Technology

Bio-Based Technologies for Transgene Containment

-- **Male sterility** (non-effective for seed-mediated gene flow)

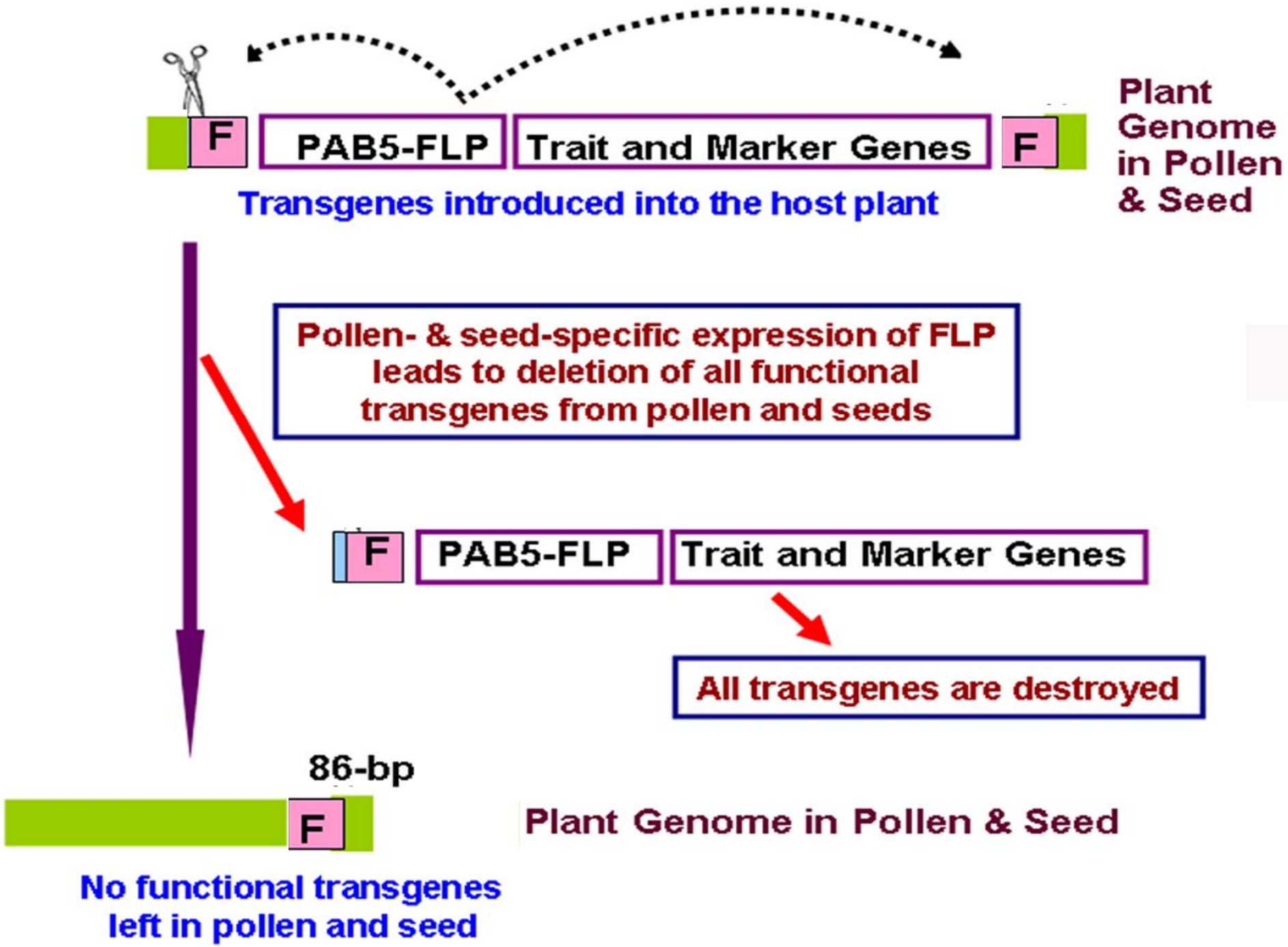
-- **Chloroplast transformation**
(non-effective for seed-mediated gene flow)

-- **Female sterility** (non-effective for pollen-mediated gene flow)

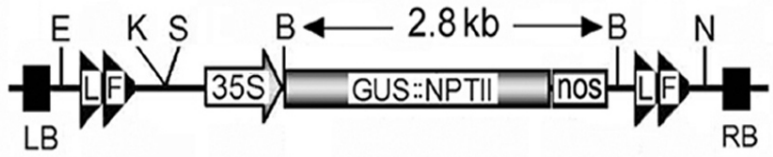
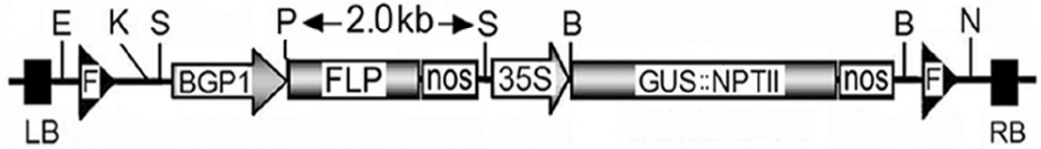
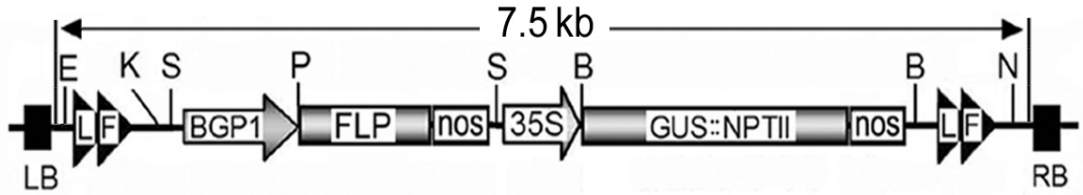
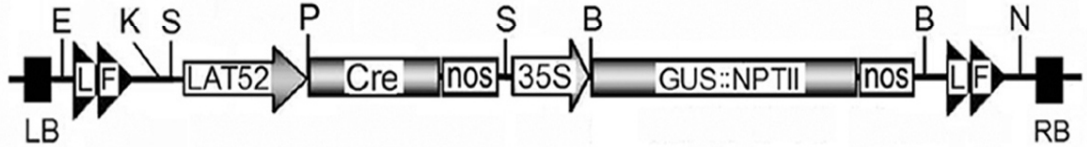
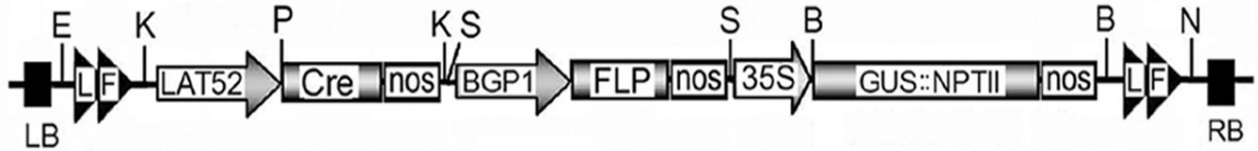
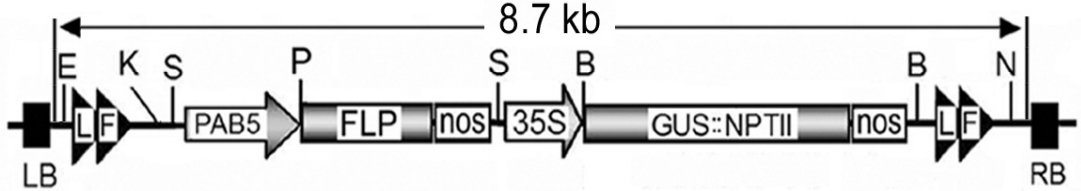
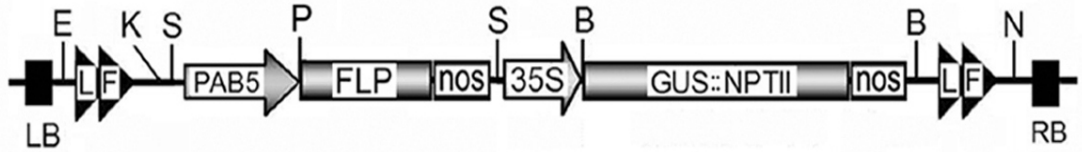
-- **Terminator seed** (cannot be used due to social reasons)

-- **Seedless fruit** (not for grain or seed crops)

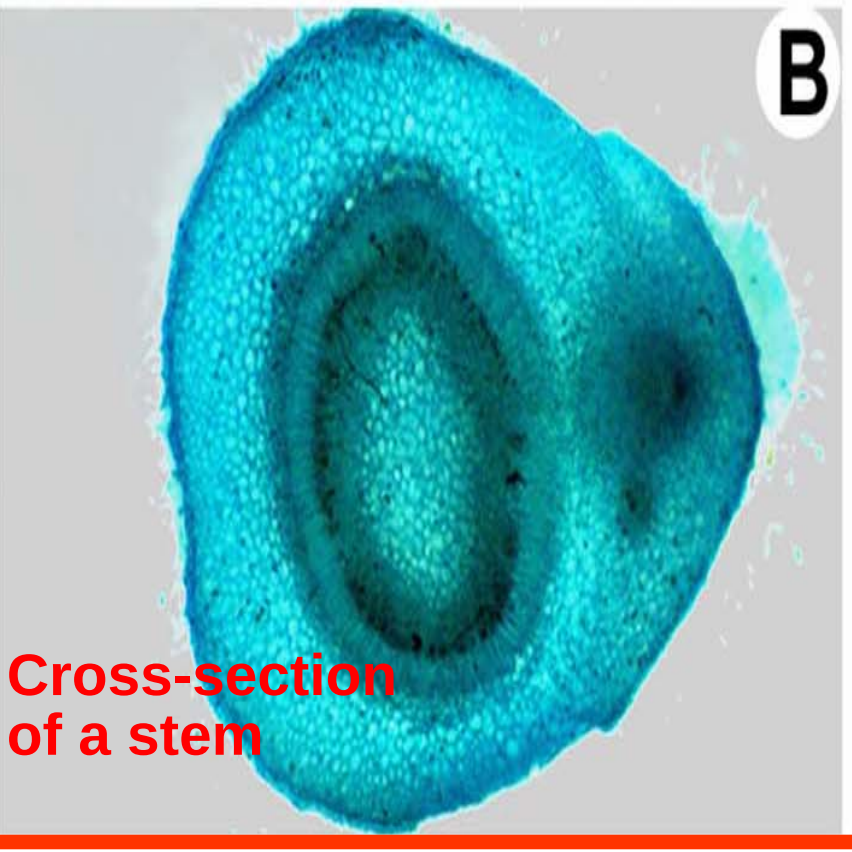
The Principle of the Gene-Deletor Technology

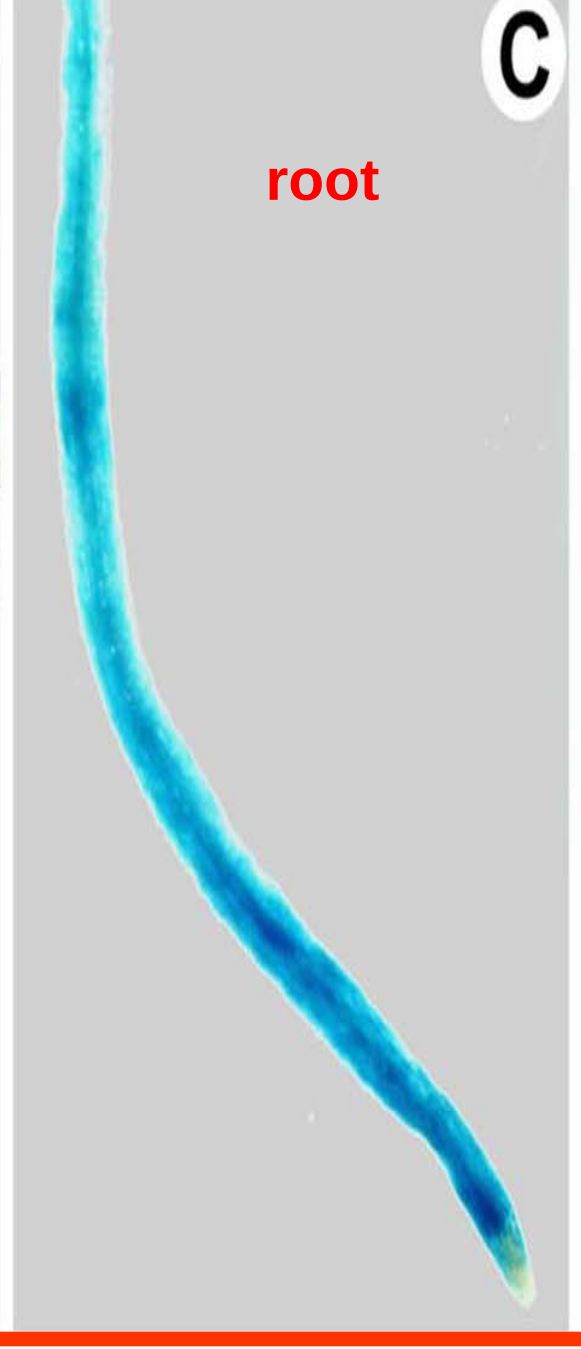


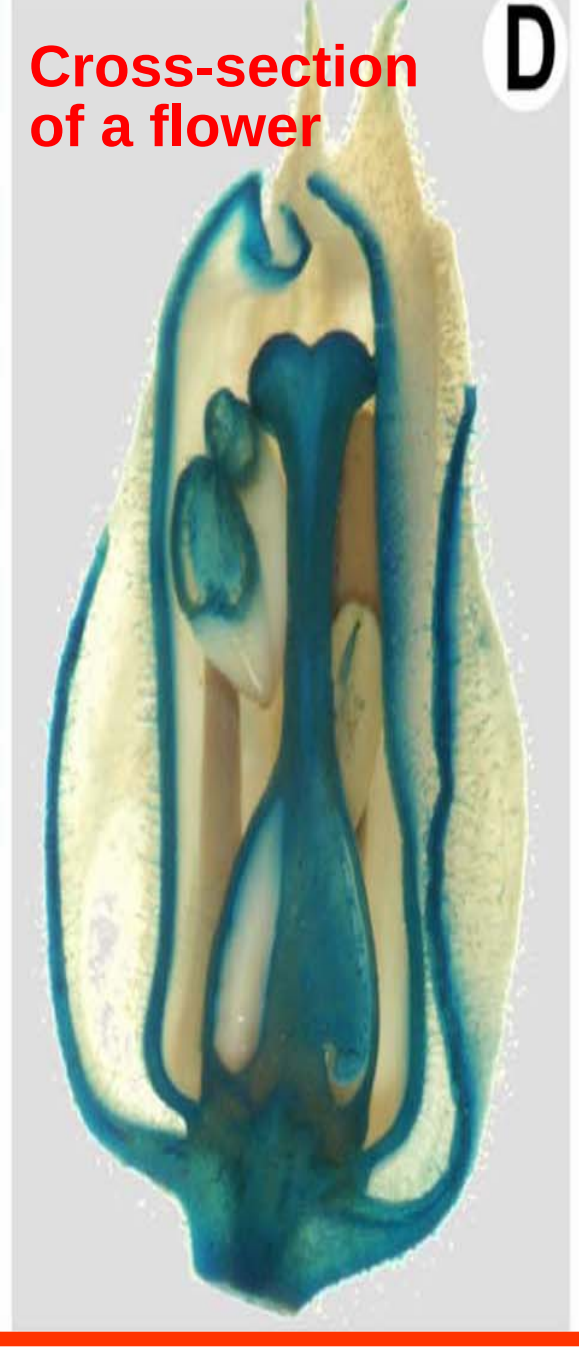
The major challenge is that such a deleting system must be highly efficient so that it can be used under field conditions.

Gene Cassette Names	Diagrams of Gene Cassettes
pLF	
pF_polB-FLP	
pLF_polB-FLP	
pLF_polL-Cre	
pLF_pol-Cre+FLP	
pF_polseed-FLP	
pLF_polseed-FLP	


No “Gene-Deletor’
With “Gene-Deletor”

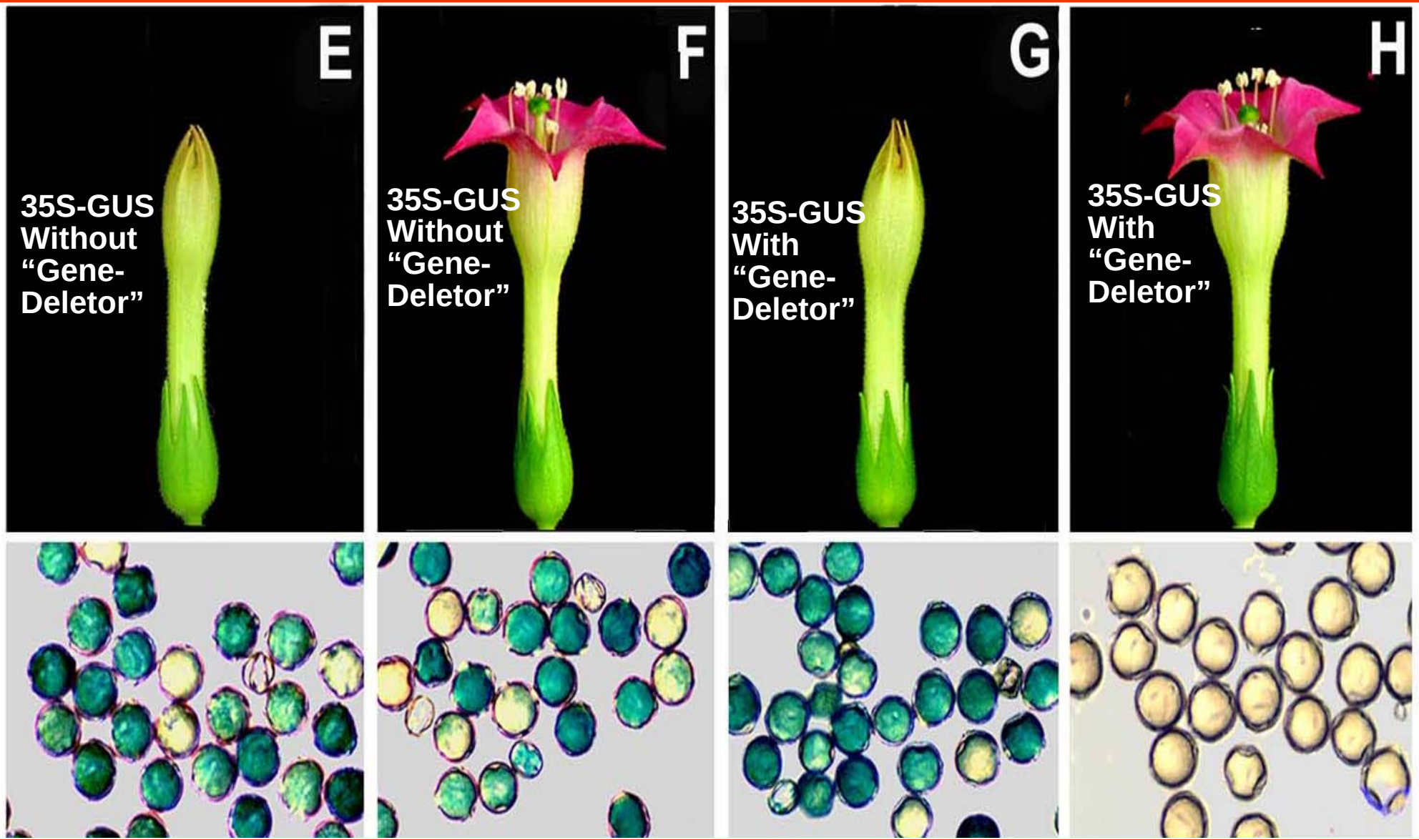






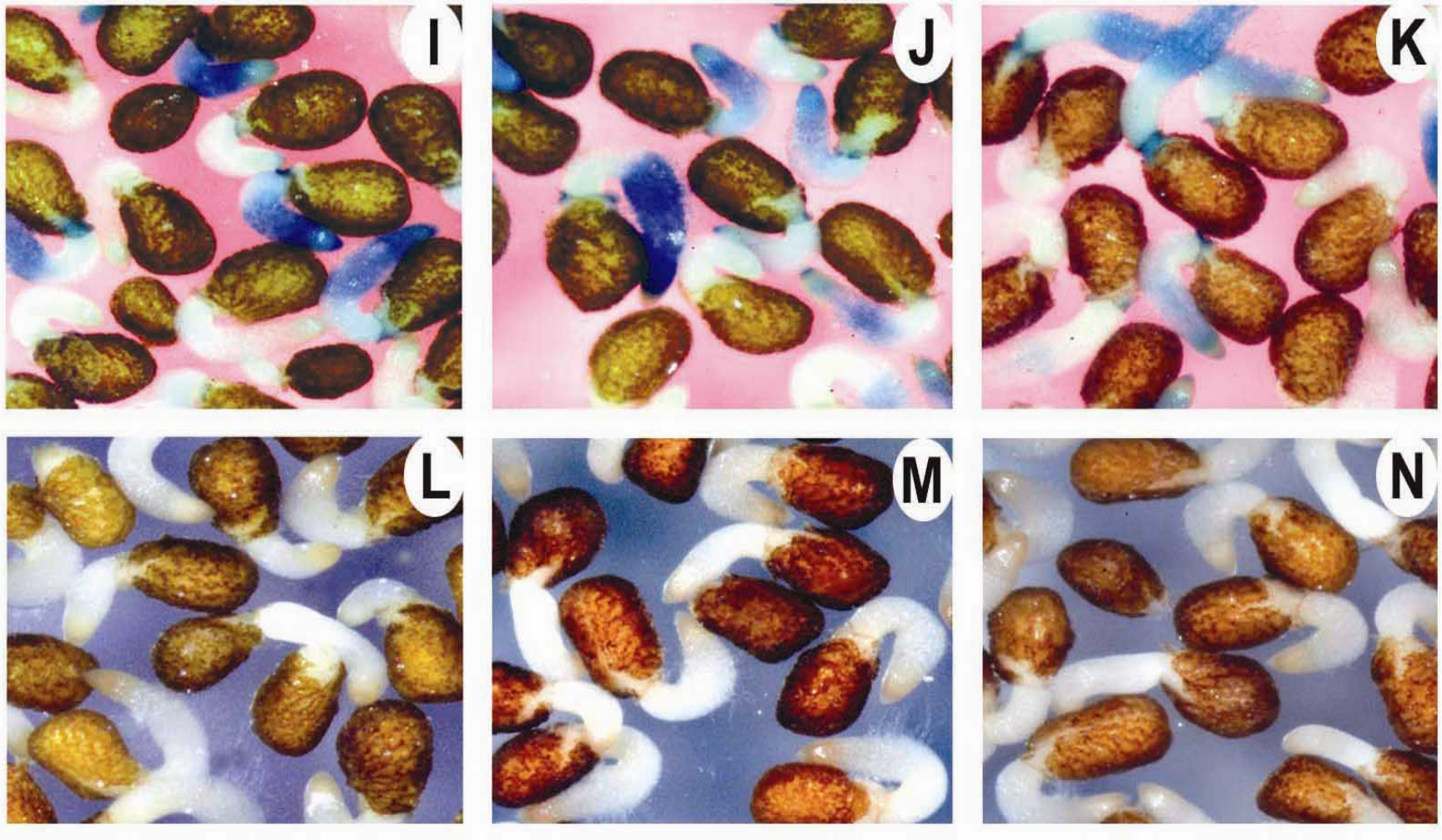
The presence of blue color (an indicator for the presence of transgenes) shows that transgenes are present in leaf, stem, root & flower.

Deletion of Transgenes from Pollen



The absence of blue color in mature pollen indicates that the transgenes have been deleted in pollen.

Deletion of Transgenes from Pollen and Seeds



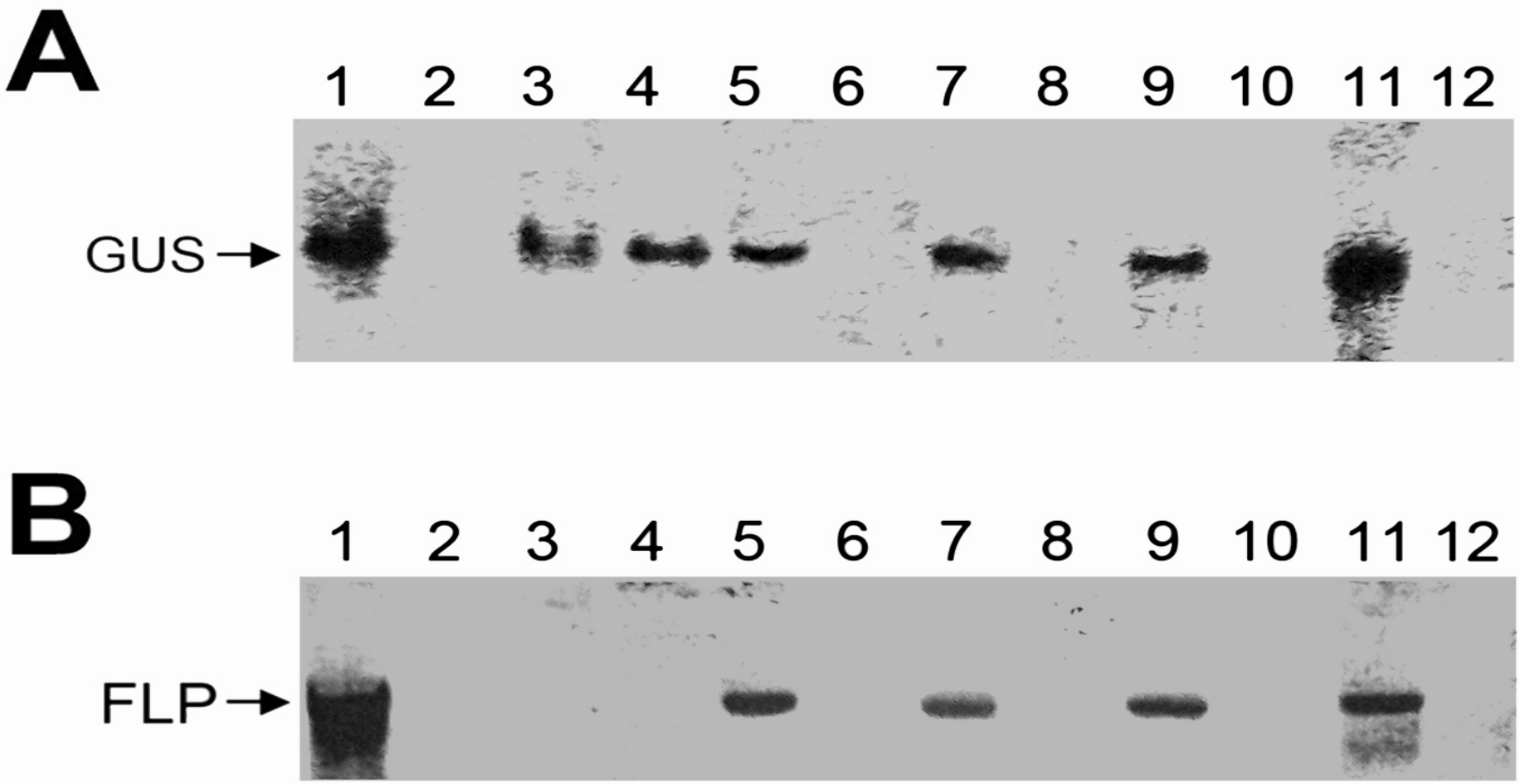
No ‘gene-deletor’: **I (self-pollination) , J (cross, wt-pollen) & K (cross, transgenic-pollen)**

With ‘gene-deletor’: **L (self-pollination) , M (cross, wt-pollen) & N (cross, transgenic-pollen)**

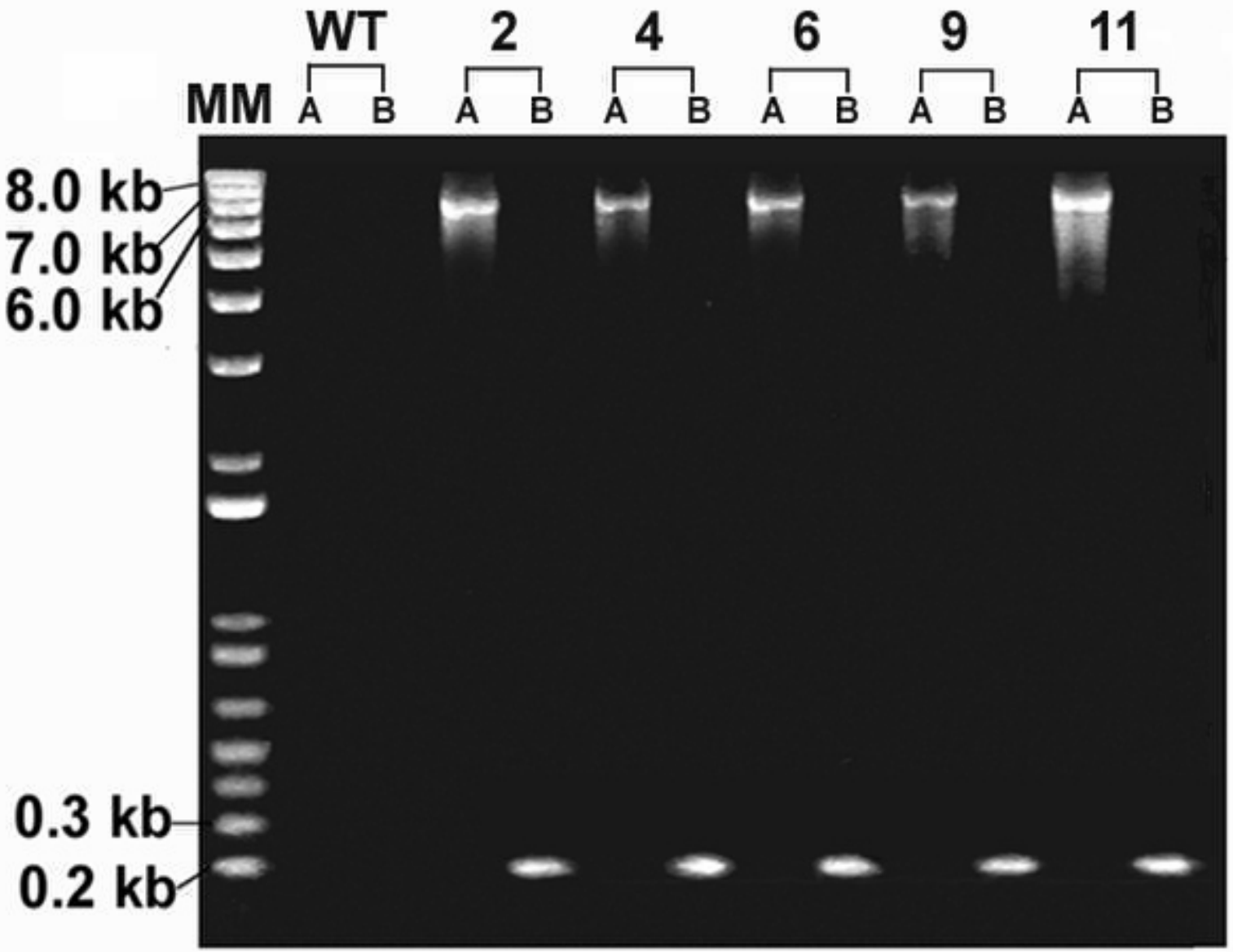
Efficiency of the ‘Gene-Deletor’ Technology

Plant	GUS^- : GUS^+ ratio in progeny from various genetic crosses			Observed effect
	Self-pollinated	WT as pollen recipient	WT as pollen donor	
pLF-5	776 : 2,127	987 : 1,028	1,009 : 985	No excision in pollen or seed
pLF_polseed-FLP-2	32,990 : 0	26,343 : 0	32,120 : 0	100% excision in both pollen seeds

Molecular Evidence for Deletion of Transgenes from Pollen & Seed
(Southern Blot Hybridizations)



Before deletion: #4(Lane 5), #6(Lane 7), #9(Lane 9), #11(Lane 11)
After deletion: #4(Lane 6), #6(Lane 8), #9(Lane 10), #11(Lane 12)



Transgenes

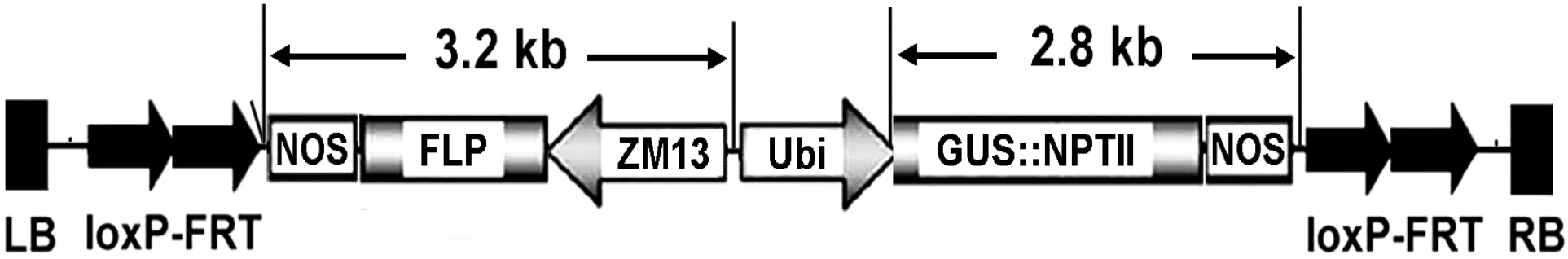
Post-excisional signal

A: before deletion B: after deletion

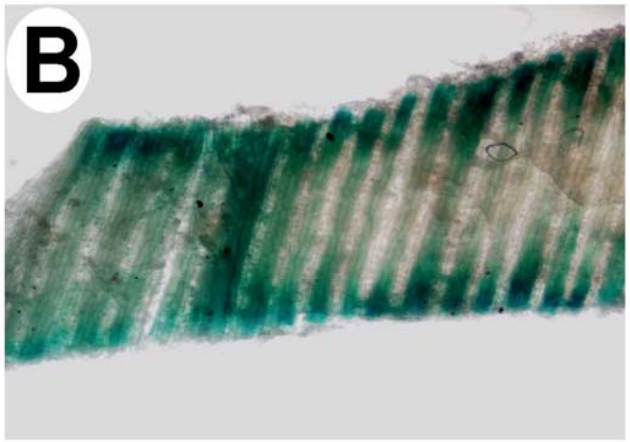
PCR analysis confirms a complete deletion of transgenes in pollen and seeds of GM plants

Also, our preliminary results have also demonstrated that the ‘gene-deletor’ technology may work well in monocot plants.

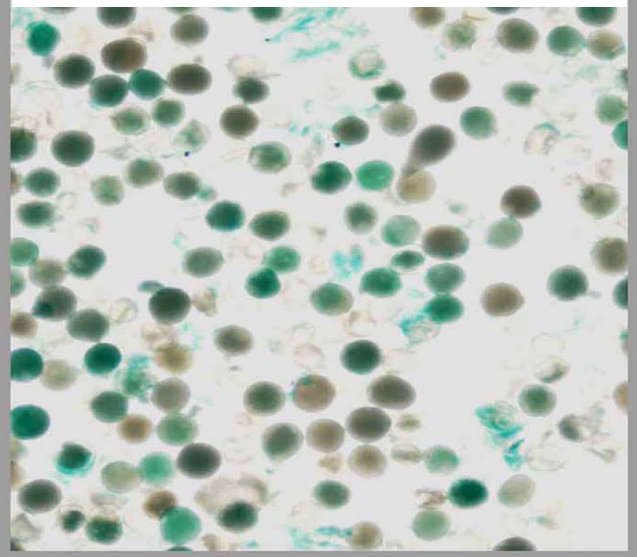
Monocot Version of the ‘Gene-Deletor’ Technology



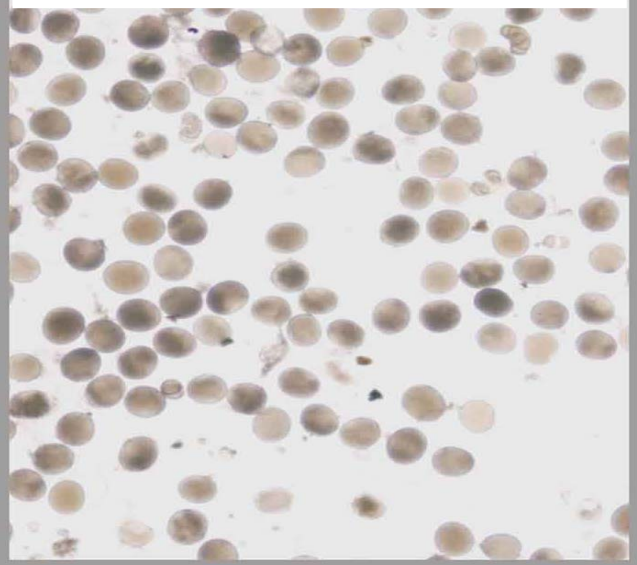
Transgenes were deleted from corn pollen



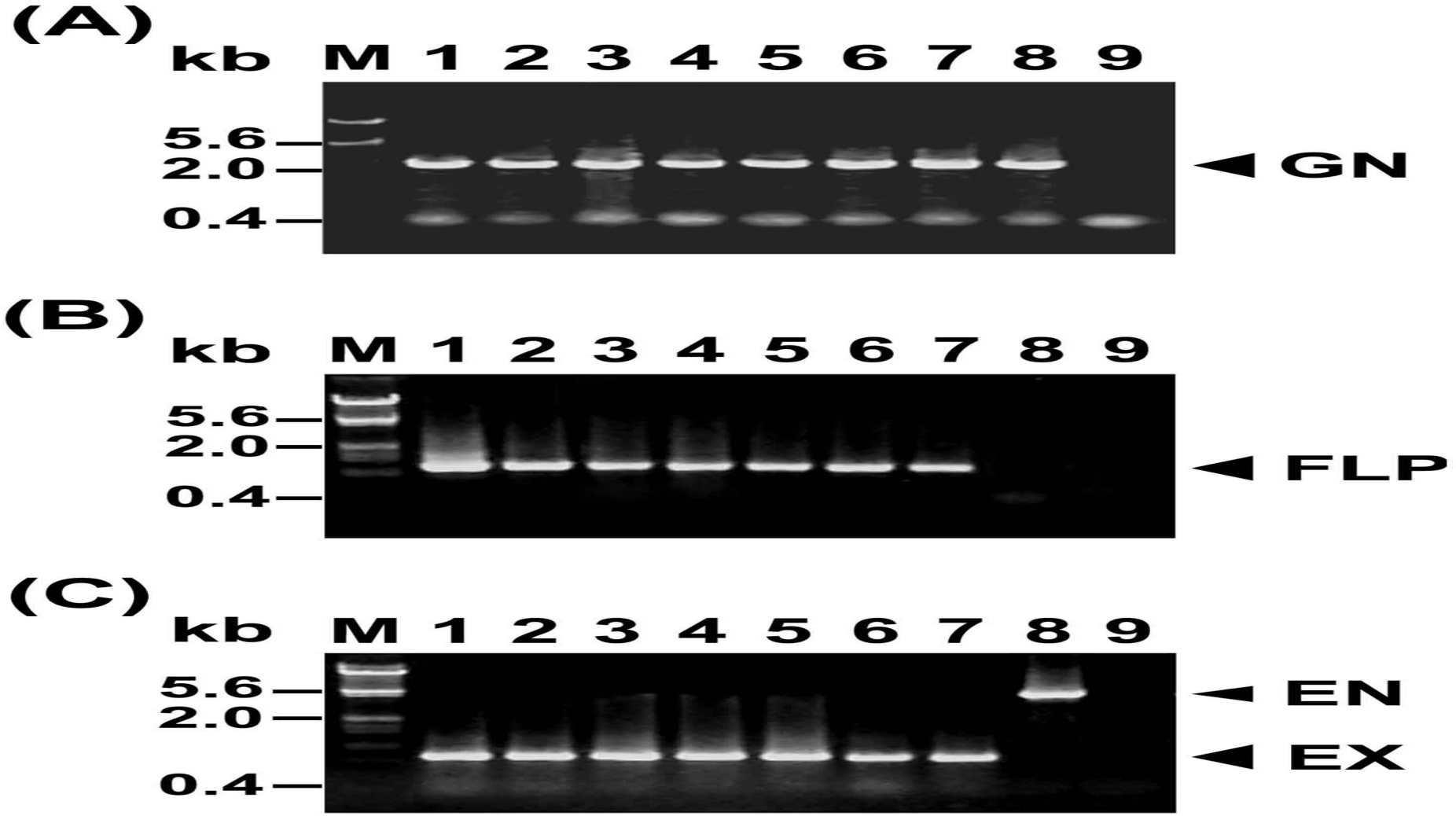
Pollen from conventional transgenic corn



Pollen from transgenic corn with ‘gene-deletor’



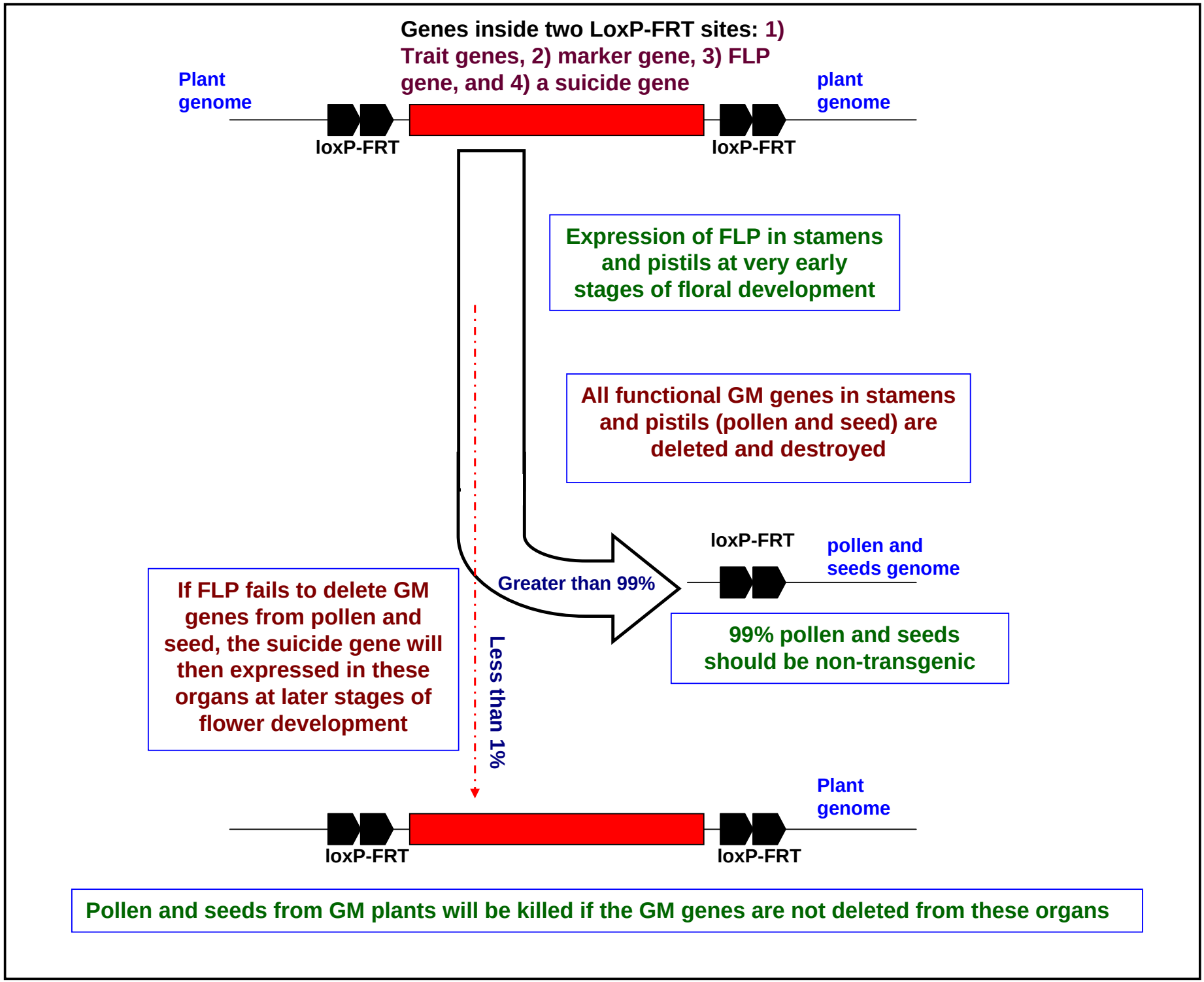
Molecular evidence confirms that all transgenes were deleted from pollen



Can we further improve the deletion efficiency of the ‘gene-deletor’ technology?

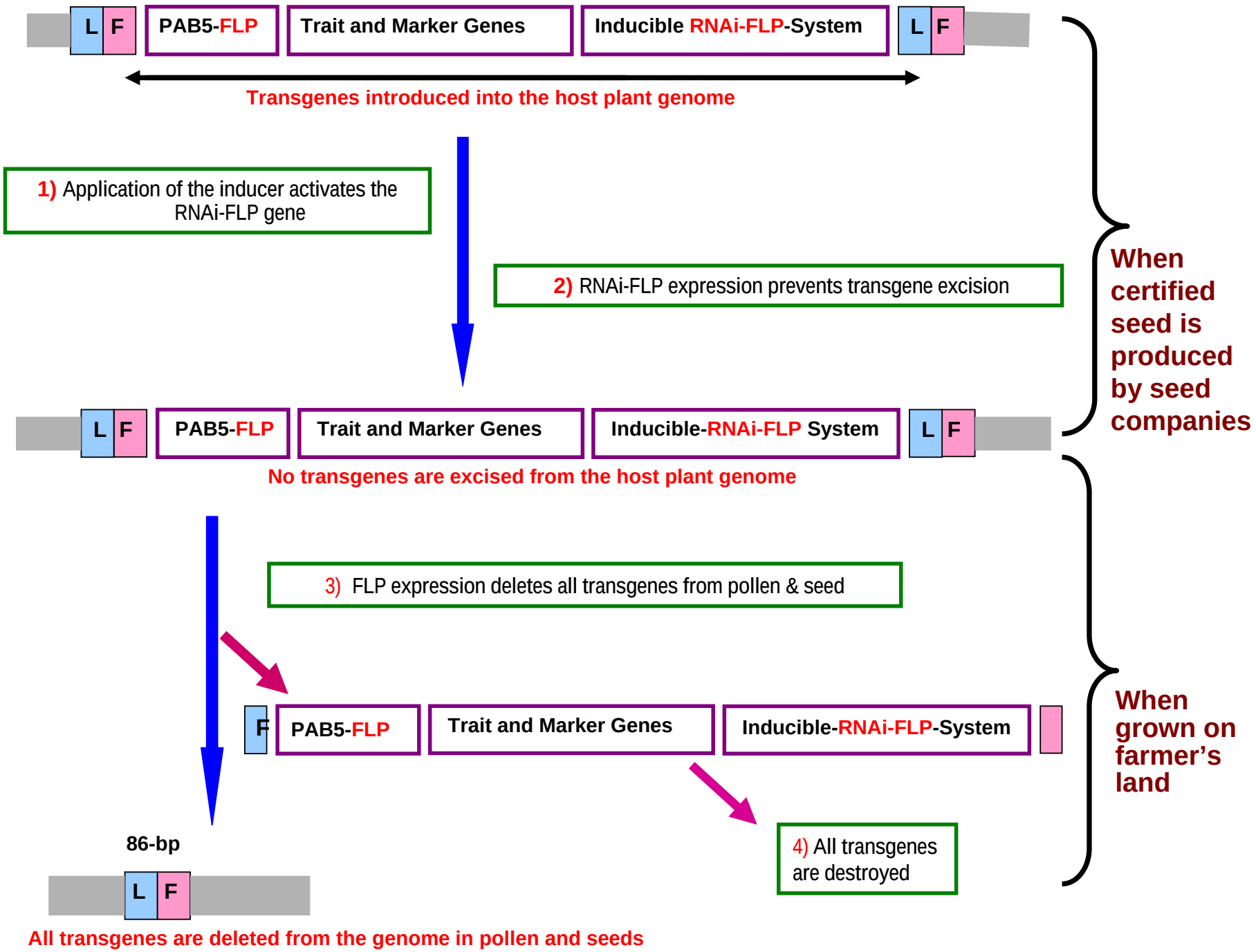
The answer is Yes.

If transgenes are not deleted in pollen and seeds, these organs will be killed

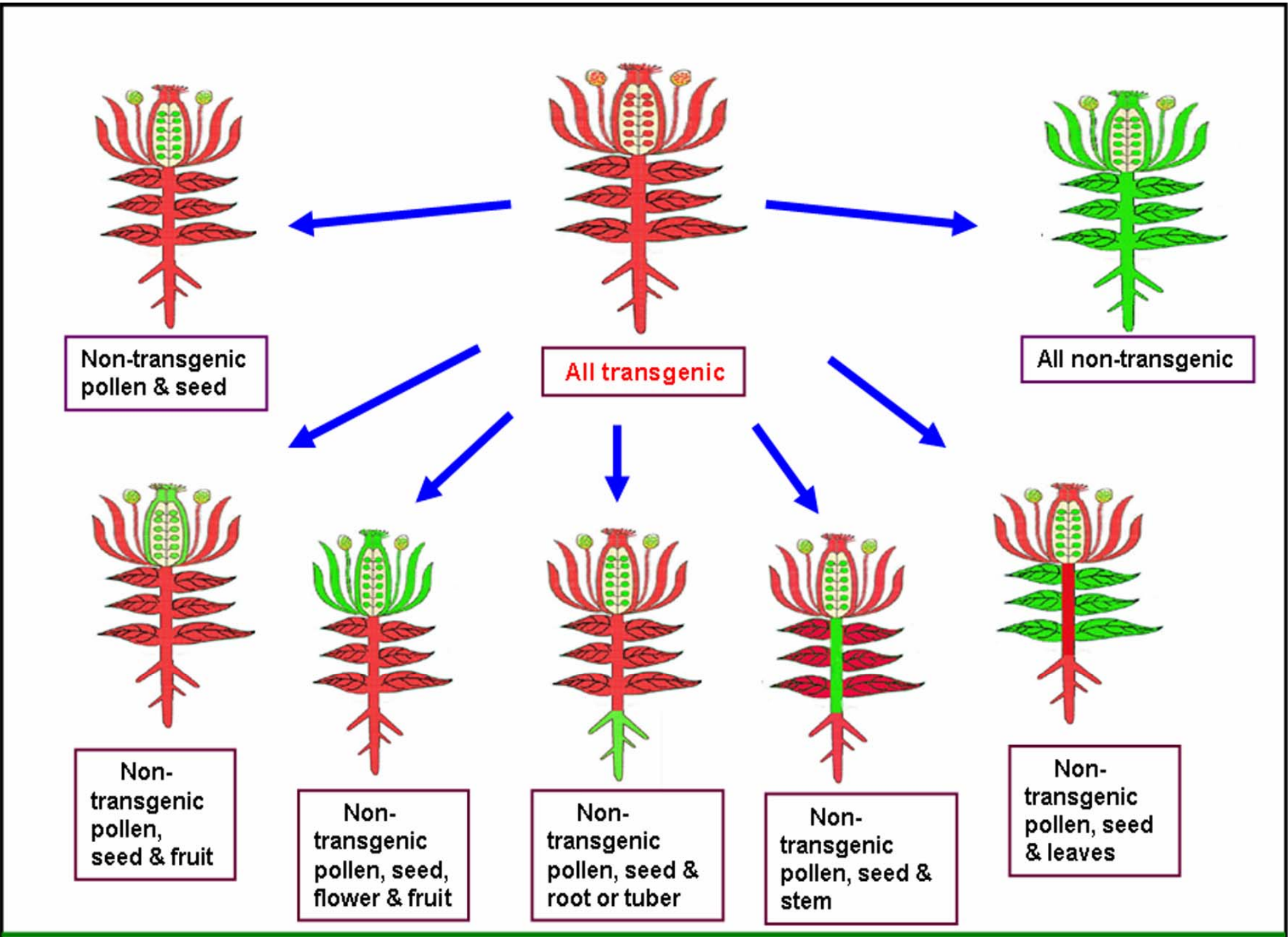


Can we use the ‘gene-deletor’ technology in sexually propagated crops such as switchgrass, corn, soybean and canola?

The answer is Yes!



The Gene-Deletor Technology Can Be Used in Sexually Propagated Crops



The ‘gene-deletor’ technology could be used to produce non-transgenic products from transgenic crops

The ‘gene-deletor’ technology can be readily used in vegetatively propagated crops

-- to reduce pollen- and seed-mediated transgene flow problem.

-- to alleviate food safety concerns over transgenic crops.

-- to reduce transgene-mediated invasiveness of bioenergy crops.

One of our goals is to have the both the "gene-deletor" and "growth promoting" gene technologies used bioenergy crops such as poplar, willow and swicthgrass.

For more information about the gene-deletor technology, please:

Please Google “gene-deletor”

Acknowledgments

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