The genome of cultivated sweetpotato contains functional Agrobacterium T-DNAs: an example of a naturally transgenic food crop

PNAS

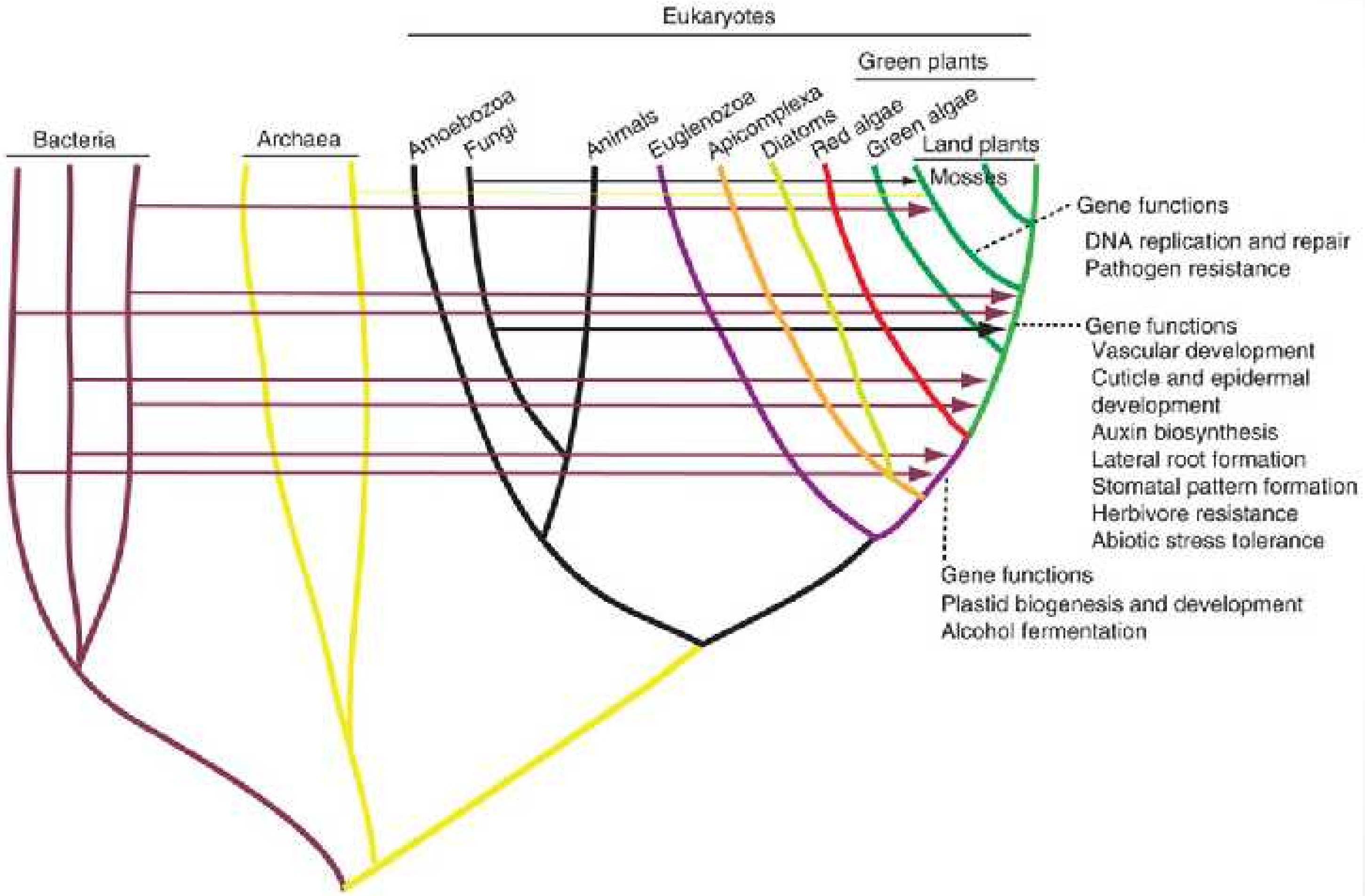
Natural transgenes in sweet potato

Proceedings of the National Academy of Sciences of the United States of America
www.pnas.org/cgi/doi/10.1073/pnas.1419685112

Sexual vs. horizontal gene transfer

- Sexual or vertical gene transfer happens between members of the same species, and thus the genes are closely related and have the same evolutionary history
- Horizontal or lateral gene transfer is when a gene gets transferred from one species to another with which they are not normally sexually compatible
- One can detect such occurrences when a gene in a species is clearly different or related to other organisms as compared to all other genes from that same or related species

horizontal gene transfer is detected more and more in the genomic era



Widespread impact of horizontal gene transfer on plant colonization of land

Jipei Yue, Xiangyang Hu, Hang Sun, Yongping Yang & Jinling Huang

Nature Communications 3, Article number: 1152 doi:10.1038/ncomms2148

horizontal gene transfer is detected more and more in the genomic era

FISH Labeling Reveals a Horizontally Transferred Algal (*Vaucheria litorea*) Nuclear Gene on a Sea Slug (*Elysia chlorotica*) Chromosome

Julie A. Schwartz, Nicholas E. Curtis and Sidney K. Pierce

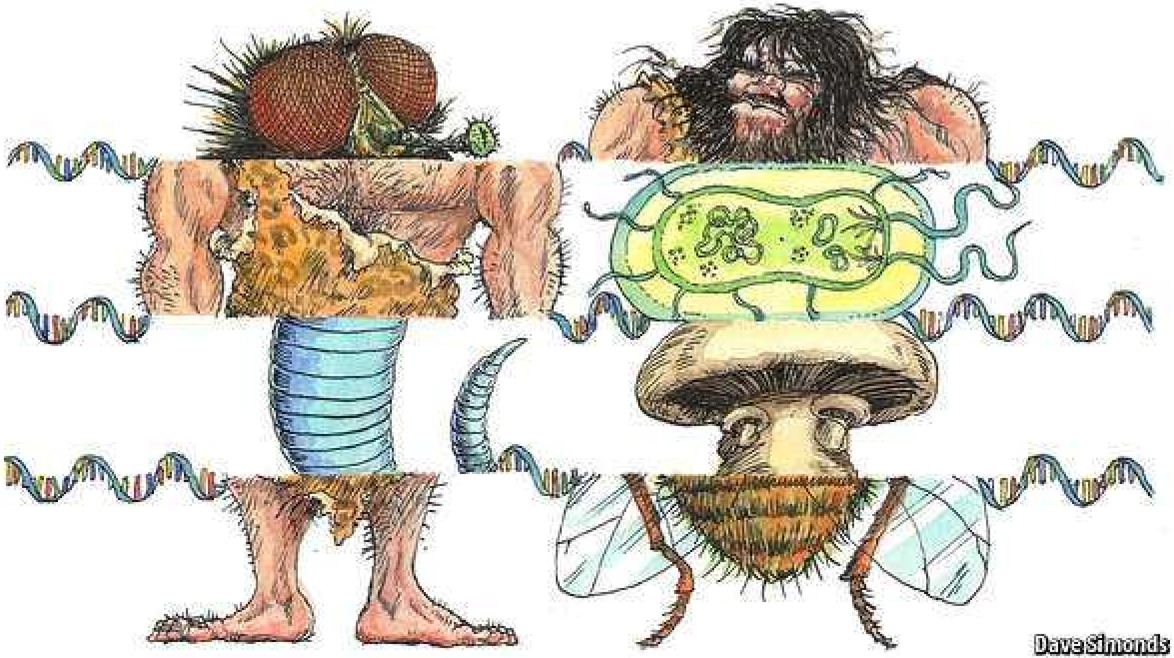
Biol. Bull. December 1, 2014 vol. 227 no. 3 300-312

Even in humans!

Horizontal gene transfer

Genetically modified people

Human beings' ancestors have routinely stolen genes from other species



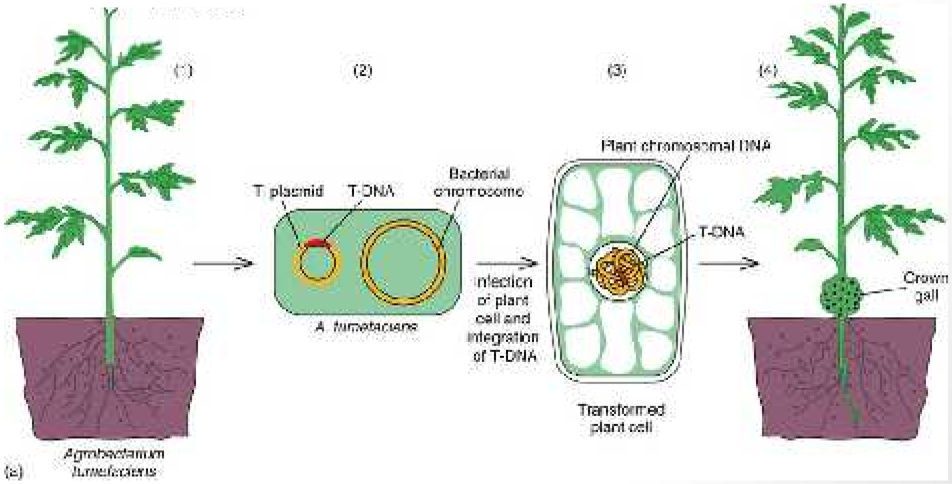
Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes

Alastair Crisp, Chiara Boschetti, Malcolm Perry, Alan Tunnacliffe and Gos Micklem

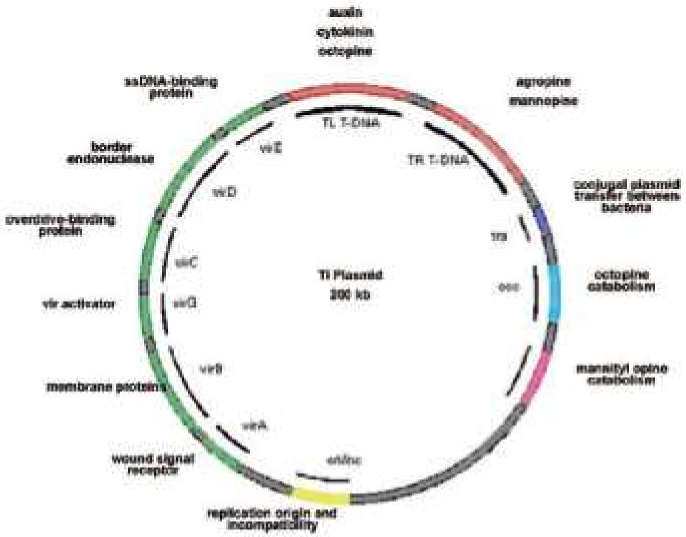
Genome Biology 2015, **16**:50 doi:10.1186/s13059-015-0607-3

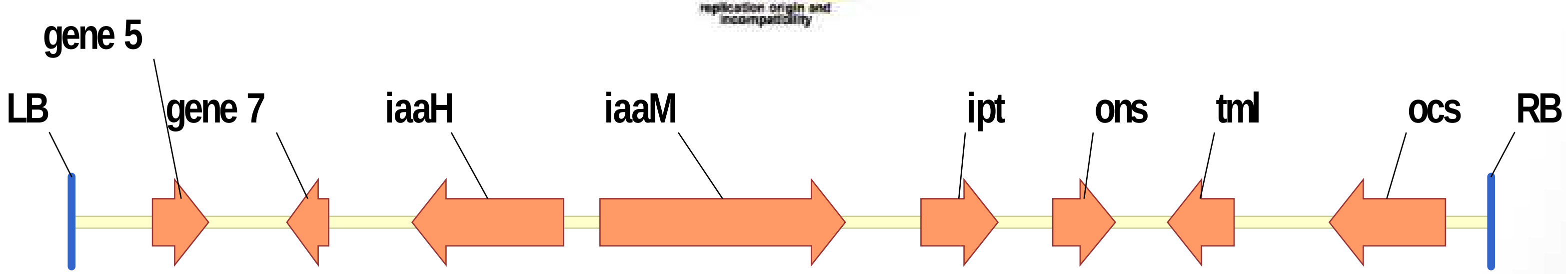
Crown gall disease (affects 140 species)

DNA of the Ti plasmid of *Agrobacterium tumefaciens/rhizogenes* is transferred to the plants and integrated into the plant genome to produce plant hormones that cause the plant cells to proliferate and form a source of nutrition for the bacteria. We can replace the bacterial DNA with our favorite DNA and let the bacteria transfer it.



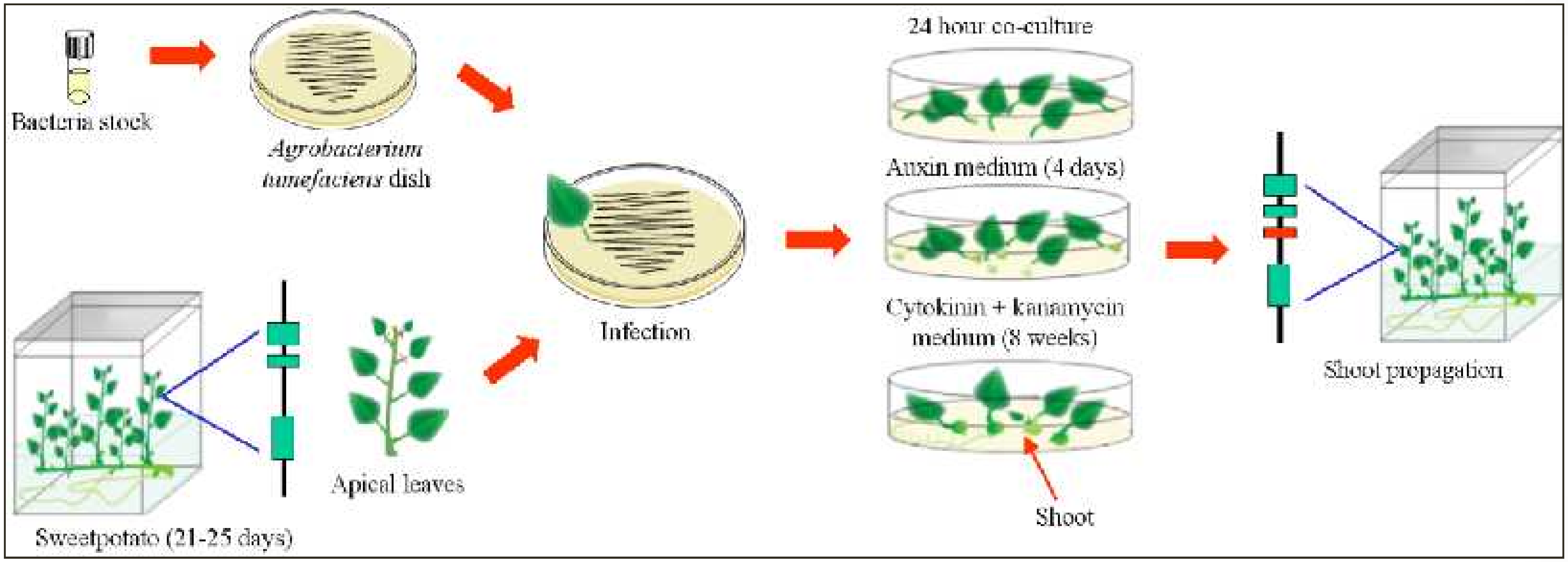
T-DNAs encode genes involved in auxin biosynthesis and sensitivity and opine synthesis located between T-DNA border regions





- Scientists can replace these genes by other genes and use Agrobacterium to introduce them into transgenic plants.

Agrobacterium: a tool for producing transgenic plants



- Prefered method of producing transgenic plants

Natural transgenic plants exist (1)

- *rol* genes from Agrobacterium into *Nicotiana glauca* - 30 years ago

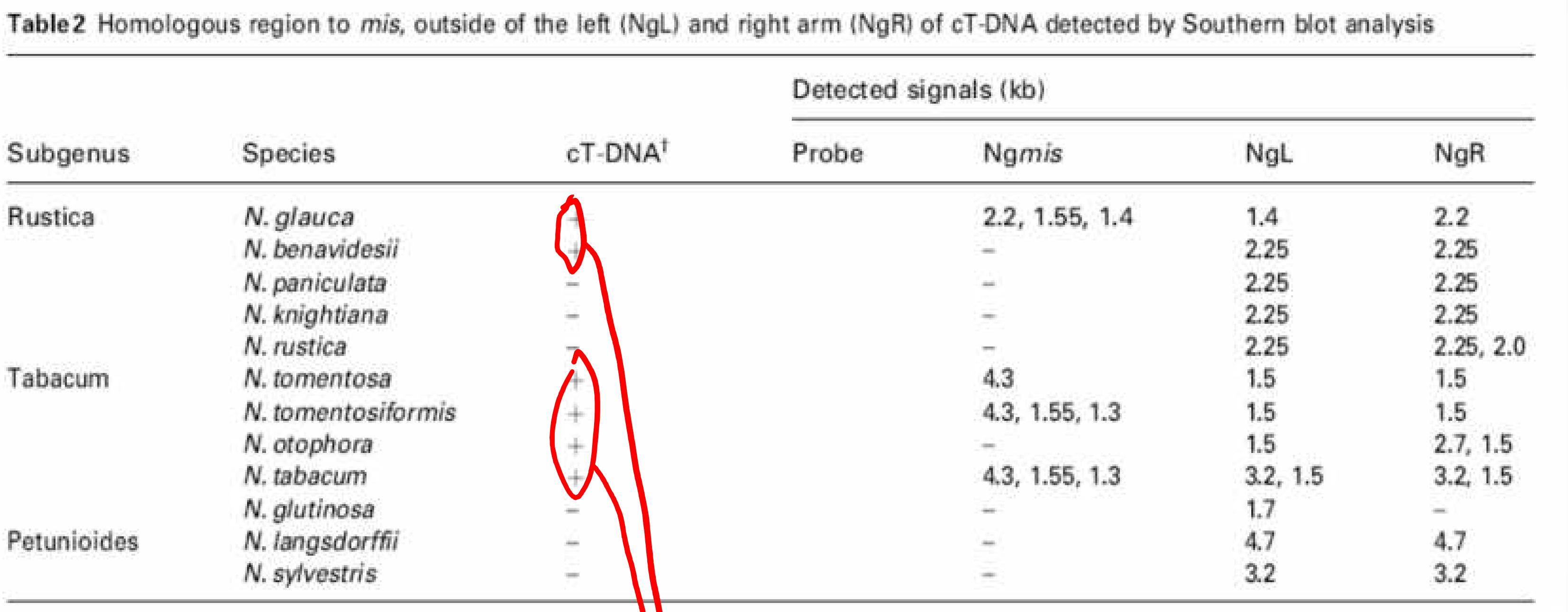
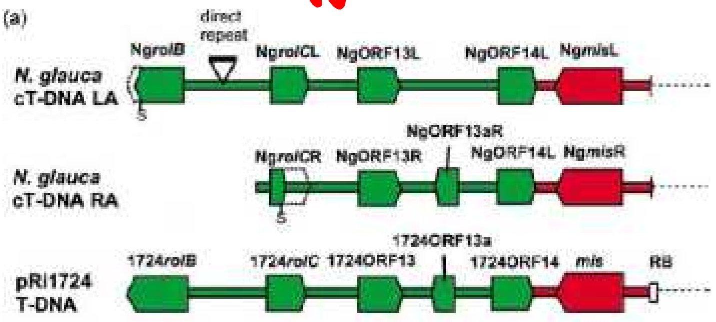


Table 2 Homologous region to *mis*, outside of the left (NgL) and right arm (NgR) of cT-DNA detected by Southern blot analysis

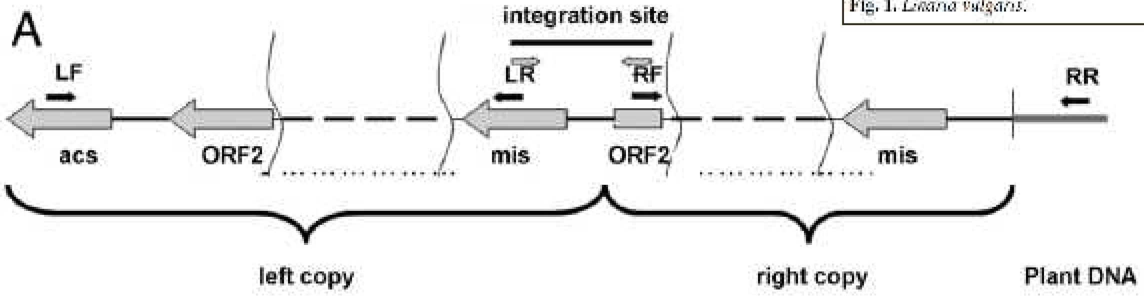
			Detected signals (kb)			
Subgenus	Species	cT-DNA†	Probe	Ng*mis*	NgL	NgR
Rustica	*N. glauca*	+		2.2, 1.55, 1.4	1.4	2.2
	N. benavidesii	+		–	2.25	2.25
	N. paniculata	–		–	2.25	2.25
	N. knightiana	–		–	2.25	2.25
	N. rustica	–		–	2.25	2.25, 2.0
Tabacum	*N. tomentosa*	+		4.3	1.5	1.5
	N. tomentosiformis	+		4.3, 1.55, 1.3	1.5	1.5
	N. otophora	+		–	1.5	2.7, 1.5
	N. tabacum	+		4.3, 1.55, 1.3	3.2, 1.5	3.2, 1.5
	N. glutinosa	–		–	1.7	–
Petunioides	*N. langsdorffii*	–		–	4.7	4.7
	N. sylvestris	–		–	3.2	3.2



Natural transgenic plants exist (2)

- Matveeva et al. (2014). **Horizontal Gene Transfer from Genus Agrobacterium to the Plant Linaria in Nature** [MPMI Vol. 25, No. 12, 2012, pp. 1542–1551]
- *Linaria vulgaris* is a species of **toadflax** (Linaria), native to most of Europe and northern Asia (also been introduced in North America).
- Most commonly found as a weed, toadflax is sometimes cultivated for cut flowers

Fig. 1. *Linaria vulgaris.*



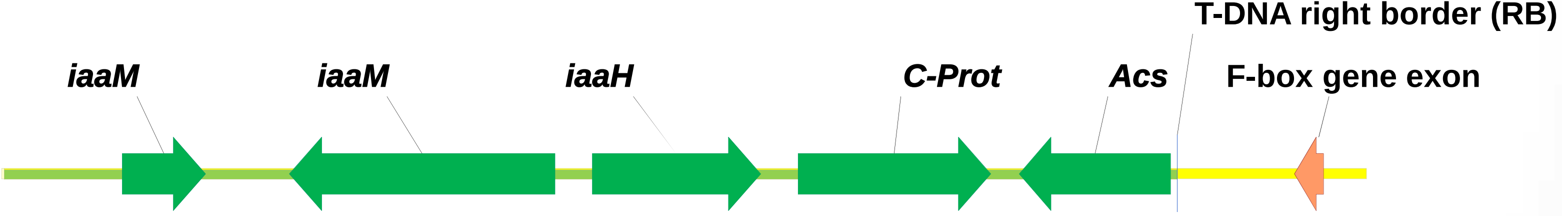
2008: a fortuitous discovery

- Analyzing results of small RNA sequencing and assembly of cultivar ‘Huachano’
- New viruses discovered leading to proof of concept of new generic diagnostic method
- Also other sequences identified:
  - Transposons/retrotransposons [control mechanism]
  - NBS-LRR (=resistance) genes [control/evolution?]
  - Bacterial genes (iaaH, iaaM, Acs, C-prot, Orf8..) [contamination?]

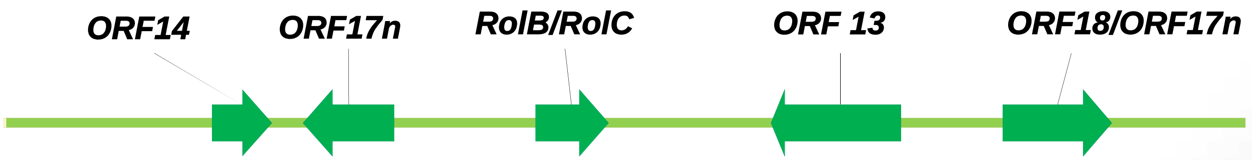
Confirmation by PCR

- PCR confirmation of presence in Huachano, and amplification of intervening & flanking sequences:

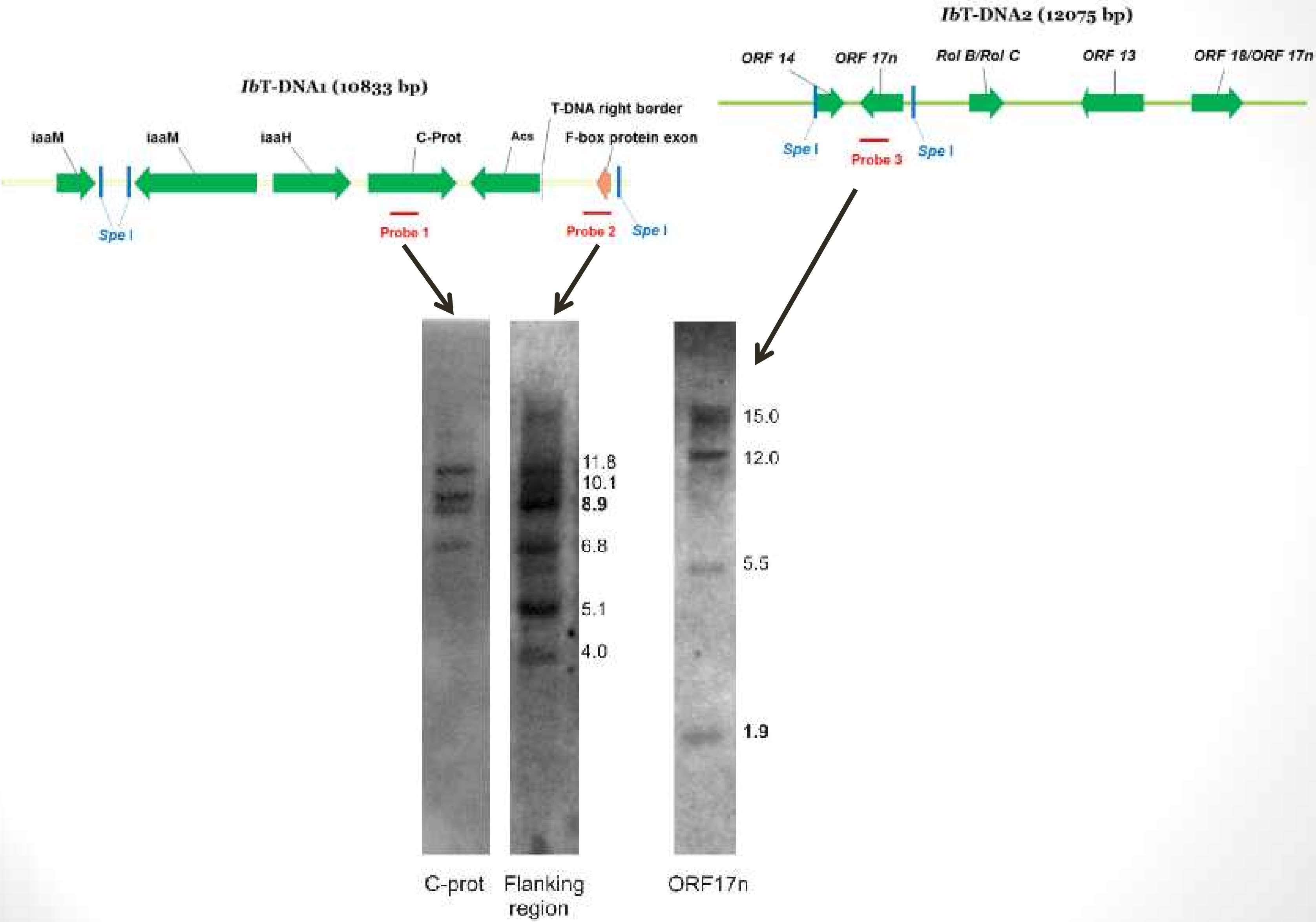
‘Huachano’ *Ib*T-DNA1 (KM052616)
10,833 bp



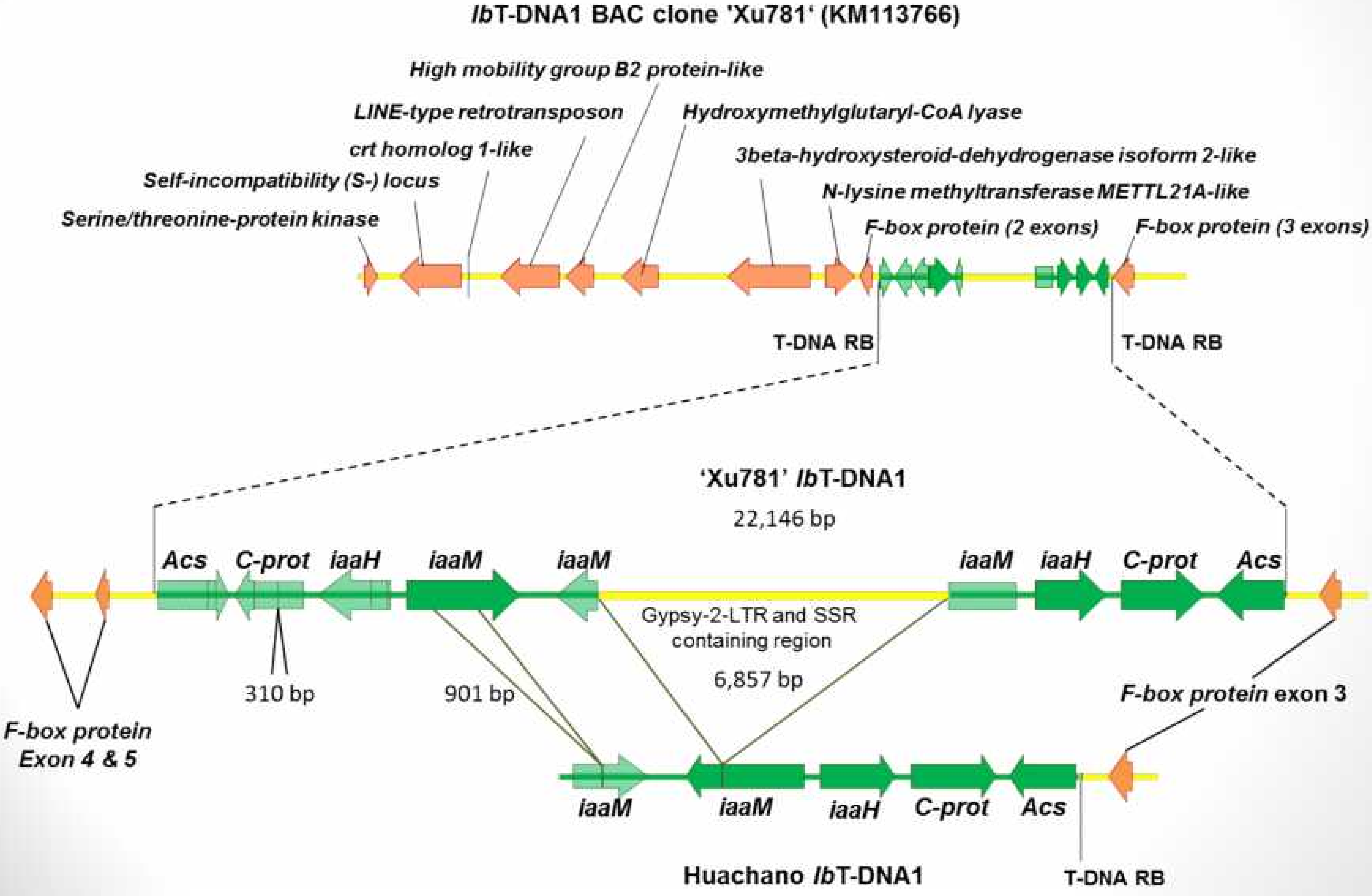
‘Huachano’ *Ib*T-DNA2 (KM052617)
12,075 bp



Confirmation by Southern blot

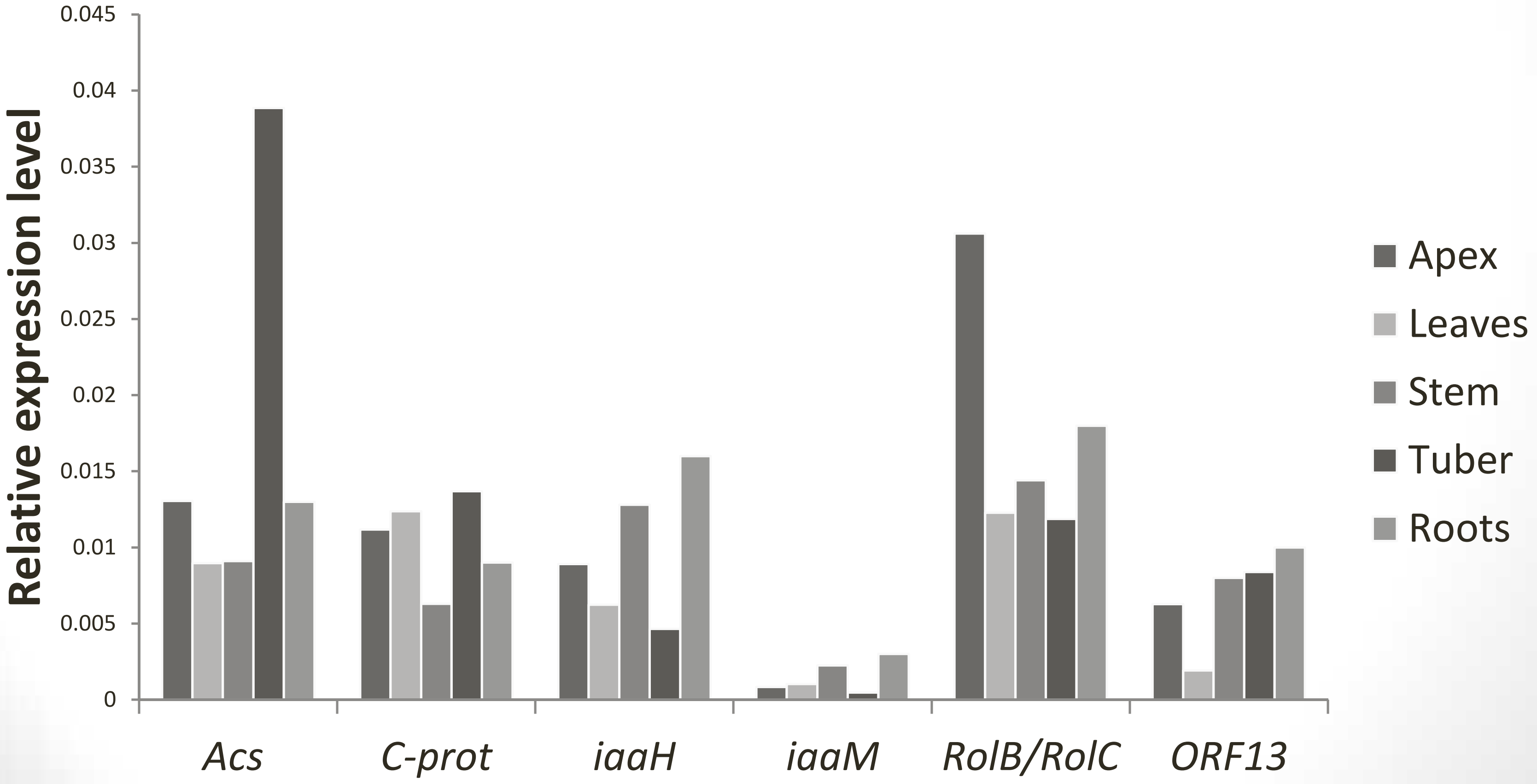


Confirmation by screening BAC library and sequencing



Are the genes encoded by the T-DNAs expressed through mRNA?

- Tested by real-time PCR



Is it present in all sweetpotatoes?

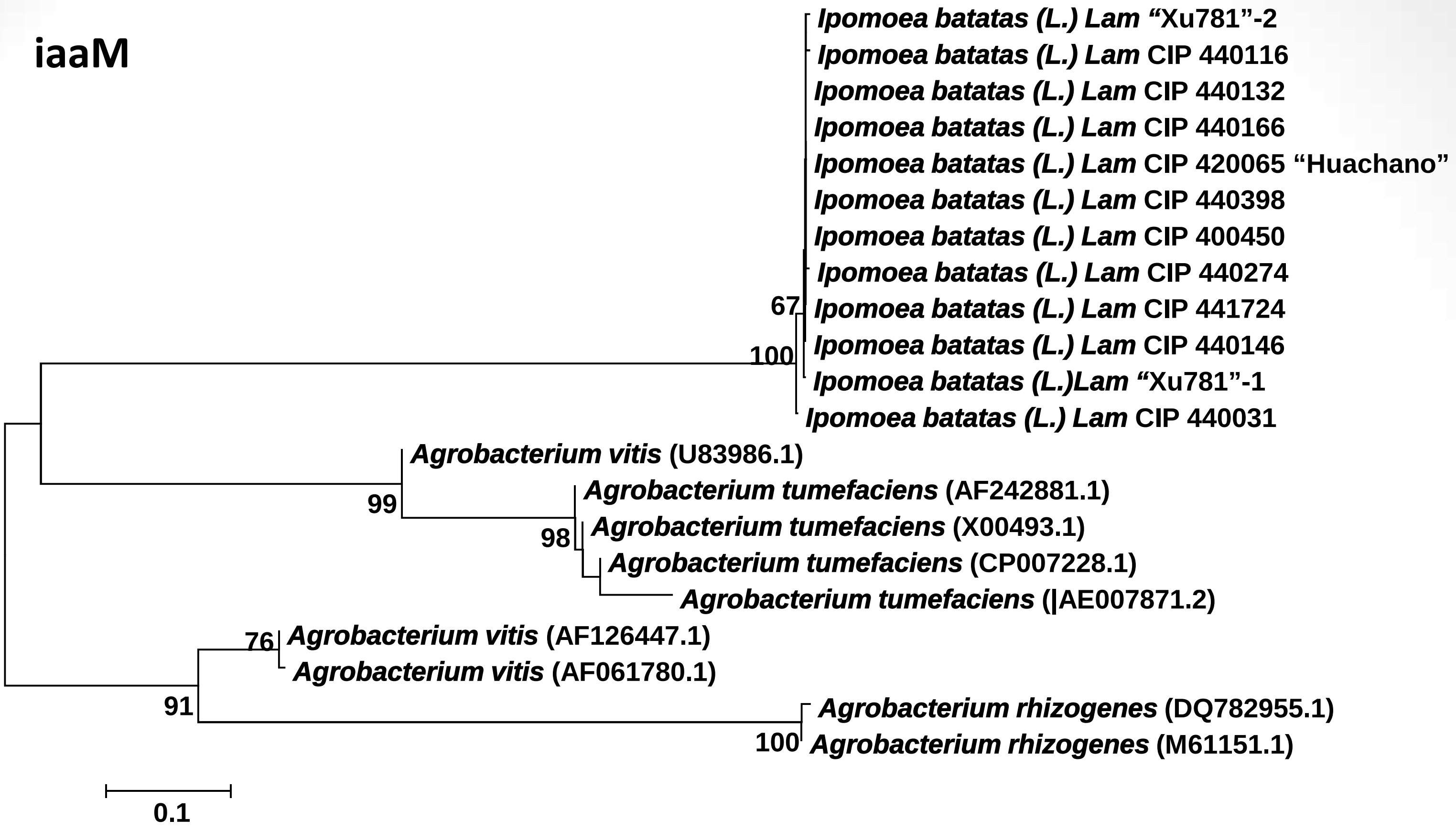
- PCR testing of CIP and USDA collections:

	IbT-DNA 1	IbT-DNA 2
CIP genebank (80)	100%	31%
USDA genebank (211)	100%	15% (137 tested)

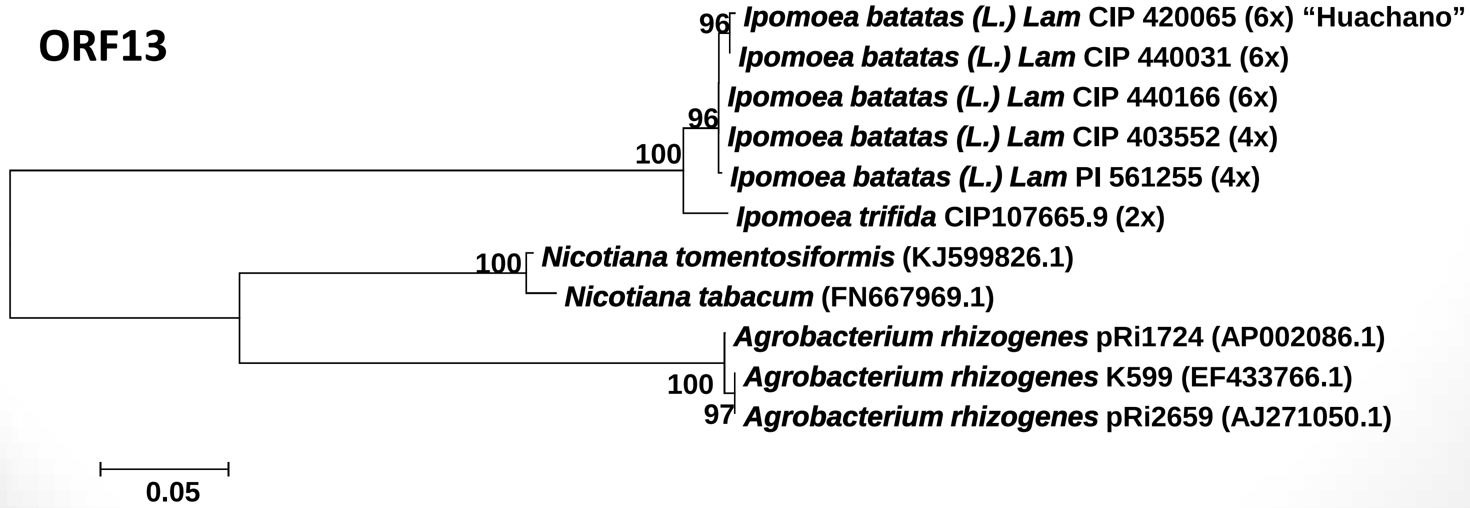
- And wild relatives?

	IbT-DNA 1	IbT-DNA 2
Ipomoea batatas/tabascana 4x (10)	0%	20%
Ipomoea trifida/triloba 2x (3)	0%	30%

iaaM

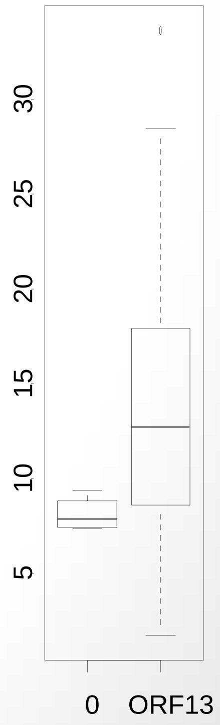

Ipomoea batatas (L.) Lam “Xu781”-2
Ipomoea batatas (L.) Lam CIP 440116
Ipomoea batatas (L.) Lam CIP 440132
Ipomoea batatas (L.) Lam CIP 440166
Ipomoea batatas (L.) Lam CIP 420065 “Huachano”
Ipomoea batatas (L.) Lam CIP 440398
Ipomoea batatas (L.) Lam CIP 400450
Ipomoea batatas (L.) Lam CIP 440274
67
Ipomoea batatas (L.) Lam CIP 441724
100
Ipomoea batatas (L.) Lam CIP 440146
Ipomoea batatas (L.)Lam “Xu781”-1
Ipomoea batatas (L.) Lam CIP 440031
Agrobacterium vitis (U83986.1)
99
Agrobacterium tumefaciens (AF242881.1)
98
Agrobacterium tumefaciens (X00493.1)
Agrobacterium tumefaciens (CP007228.1)
Agrobacterium tumefaciens (|AE007871.2)
76
Agrobacterium vitis (AF126447.1)
Agrobacterium vitis (AF061780.1)
91
Agrobacterium rhizogenes (DQ782955.1)
100
Agrobacterium rhizogenes (M61151.1)
0.1

ORF13


96
Ipomoea batatas (L.) Lam CIP 420065 (6x) “Huachano”
Ipomoea batatas (L.) Lam CIP 440031 (6x)
Ipomoea batatas (L.) Lam CIP 440166 (6x)
96
Ipomoea batatas (L.) Lam CIP 403552 (4x)
100
Ipomoea batatas (L.) Lam PI 561255 (4x)
Ipomoea trifida CIP107665.9 (2x)
100
Nicotiana tomentosiformis (KJ599826.1)
Nicotiana tabacum (FN667969.1)
Agrobacterium rhizogenes pRi1724 (AP002086.1)
100
Agrobacterium rhizogenes K599 (EF433766.1)
97
Agrobacterium rhizogenes pRi2659 (AJ271050.1)
0.05

Do these genes have any role in sweetpotato root development?

- Available: mapping population Beauregard x Tanzania including phenotypic data from two locations (La Molina & San Ramon)
- Both are positive for IbT-DNA1, but IbT-DNA2 is only present in Tanzania
- Segregation of IbT-DNA2 was observed in the population: 13 genotypes lacking RolB/RolC, and of these 4 were also negative for ORF13
- Presence of ORF13 was significantly associated with total root yield under San Ramon conditions (Wilcoxon rank sum test with continuity correction).



Conclusions

- One (or two) ancestor(s) of sweetpotato were transformed through infection by Agrobacterium, integrating two T-DNA regions into the *Ipomoea* genome.
- One of the T-DNAs appears to be fixed (it does not segregate) in cultivated sweetpotato as opposed to wild relatives.
- Because some of the genes are active, this provides the possibility that they may have conferred a trait that has been selected for during domestication.
- **Sweetpotato is naturally transgenic**

Remaining questions and research opportunities

- What are the roles of the T-DNA encoded genes, if any, in sweetpotato physiology/phenotype:
  - Storage root formation?
  - Rapid rooting of cuttings?
- Are closely linked genes involved in domestication traits or the interruption of F-box gene?
- Study of IbT-DNAs in related Ipomoea provide opportunity to better understand progenitors of cultivated sweetpotato & fate of exogenous transgenes after horizontal gene-transfer (speed of evolution, control of expression)
- None of the 5 Ipomoea sequenced to date contain either of the T-DNAs ☹

What does it not mean?

- These genes were required for the domestication of sweetpotato (we have not shown this yet, correlation is not causation)
- These plants were purposely transformed by ancient farmers to make sweetpotato (the T-DNAs can be found in wild relatives and were thus likely introduced long before domestication)
- Transgenics or GMOs are good or bad (the worse weeds and toxic plants are all natural)
- **Sweetpotato is a GMO in the general sense (a GMO is a regulated crop, not a naturally-found crop by legal definitions)**