Agrobacterium tumefaciens vs *rhizogenes*

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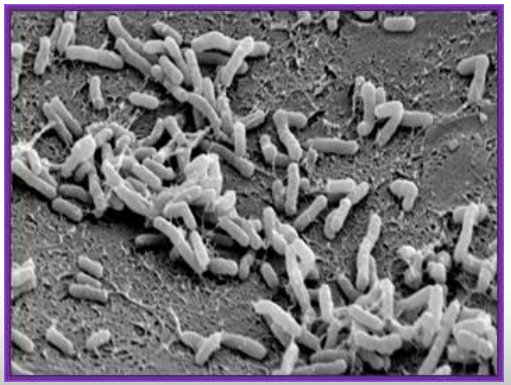
Ext. 245



Mérida Yucatán 14 de julio 2017

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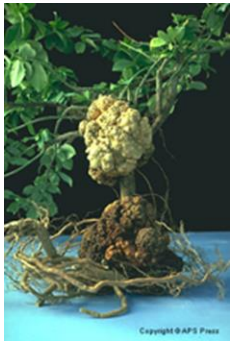

Crown gall on Euonymus caused by *Agrobacterium tumefaciens*. (Courtesy R.L. Forster)


S. Vann

Tobacco Plant Showing Symptoms of Hairy Root Disease
Photo Credit: Adriana M. Alippi, Facultad de Ciencias Agrarias y Forestales, Argentina

Potato Plant Showing Symptoms of Hairy Root Disease
Photo Credit: Potato Gene Engineering Network

Foliar Wilt Caused by *Rhizobium rhizogenes* infection on Mulberry
Photo Credit: William M. Brown Jr.

Roots of a Young Mulberry Tree Showing Symptoms of Hairy Root Disease
Photo Credit: William M. Brown Jr.

Common Bean Genetically Transformed with *Rhizobium rhizogenes*

Photo Courtesy of Federico Sanchez, Universidad Nacional Autónoma de México

Soybean Plant With *Rhizobium rhizogenes* Infection

Photo Courtesy of Peter M. Gresshoff, The University of Queensland

International Journal of Systematic and Evolutionary Microbiology (2001), 51, 89–103 Printed in Great Britain

A revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie *et al.* 1998 as new combinations: *Rhizobium radiobacter*, *R. rhizogenes*, *R. rubi*, *R. undicola* and *R. vitis*

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Former name	Current Name	Symptoms
Agrobacterium larrymoorei	*Rhizobium larrymoorei* (Young et al., 2001)	aerial tumours
Agrobacterium radiobacter = *A. tumefaciens*	*Rhizobium radiobacter* (Young et al., 2001)	crown gall
Agrobacterium rhizogenes	*Rhizobium rhizogenes* (Young et al., 2001)	hairy-root disease
Agrobacterium rubi	*Rhizobium rubi* (Young et al., 2001)	cane gall
Agrobacterium vitis	*Rhizobium vitis* (Young et al., 2001)	crown gall

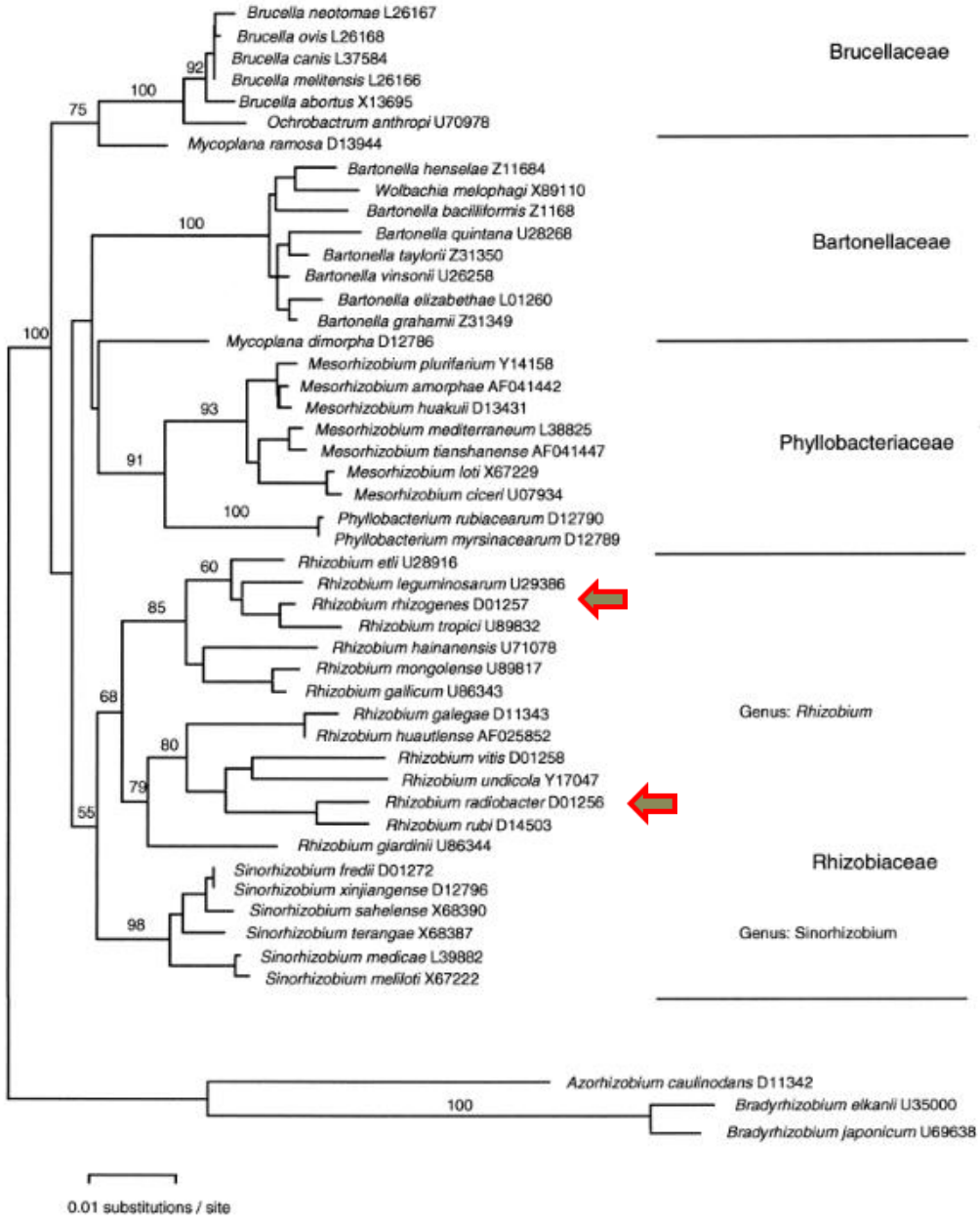

Fig. 2. Neighbour-joining tree expressing the relationships among the *Rhizobiaceae* and their relatives, based on 16S rDNA sequences. Sites that include gaps in more than one sequence were excluded. Horizontal branch lengths are proportional to the estimated number of nucleotide substitutions and bootstrap probabilities (as percentages) are determined from 1000 resamplings.

International Journal of Systematic and Evolutionary Microbiology (2003), **53**, 1681–1687 DOI 10.1099/ijs.0.02445-0

Taxonomic Note

Agrobacterium is a definable genus of the family *Rhizobiaceae*

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This paper is co-signed by the following individuals, all of whom have communicated to the Editor of IJSEM their agreement with the positions of the authors concerning the taxonomic validity of the genus *Agrobacterium*. Some of the co-signatories have contributed to the Editor additional information in support of the position of the authors.

Drs Fredrick M. Ausubel, Department of Genetics, Harvard Medical School, Boston, MA, USA; Jacques Balandreau, Université Claude Bernard, Lyon, France; Rene Bally, Université Claude Bernard, Lyon, France; Lois Banta, Department of Biological Sciences, Williams College, Williamstown, MA, USA; Andrew Binns, Department of Biology, University of Pennsylvania, Philadelphia, PA, USA; Peter Bottomley, Department of Microbiology, Oregon State University, Corvallis, OR, USA; Hacène Bouzar, Sakata Seed America, Inc., Salinas, CA, USA; Susana Brom, Centro de Investigación sobre Fijación Nitrógeno, Cuernevaca, Mexico; William J. Broughton, Laboratoire de Biologie Moléculaire des Plantes Superieures, Université Genéve, Switzerland; Thomas J. Burr, Department of Plant Pathology, Cornell University, Geneva, NY, USA; Miguel A. Cevallos, Centro de Investigación sobre Fijación Nitrógeno, Cuernavaca Mexico; Jai-Soo Cha, Chung Buk National University, Chongju, Korea; Mary-Dell Chilton, Syngenta, Research Triangle Park, NC, USA; William Scott Chilton, Department of Botany, North Carolina State University, Raleigh, NC, USA; Peter Christie, Department of Microbiology and Molecular Genetics, University of Texas, Houston Medical School, Houston, TX, USA;

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Netherlands; Todd R. Steck, Department of Biology, University of North Carolina – Charlotte, Charlotte, NC, USA; Gary Stacey, Department of Plant Microbiology and Pathology, University of Missouri, Columbia, MO, USA; Sandor Süle, Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary; Laurent Sutra, UMR de Pathologie Végétale, INRA, Beaucouzé, France; Nobukazu Tanaka, Center for Gene Science, Hiroshima University, Hiroshima, Japan; David Tepfer, Laboratoire de Biologie de la Rhizosphére, INRA, Versailles, France; Encarna Velázquez Perez, Departmento Microbíologia y Genética Campus M. Unamuno, Salamanca, Spain; Graham C. Walker, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA; Stephen C. Winans, Department of Microbiology, Cornell University, Ithaca, NY, USA; Derek Wood, Department of Microbiology, University of Washington, Seattle, WA, USA; Shaw-Jyre Wu, Central National University, Chung Li, Taiwan,

Note Added in Proof

Two recent publications (Weller *et al.*, 2002; Van Berkum *et al.*, 2003) present results from the phylogenetic analysis of nucleotide sequences that are consistent with our position that there is no sound scientific evidence that warrants combining the members of the genus *Agrobacterium* into the genus *Rhizobium*.

Weller, S. A., Simpkins, S. A., Stead, D. E., Kurdziel, A., Hird, H. & Weeks, R. J. (2002). Identification of *Agrobacterium* spp. present within *Brassica napus* seed by TaqMan PCR - implications for GM screening procedures. Arch Microbiol 178, 338–343.

Van Berkum, P., Terefework, Z., Paulin, L., Soumalainen, S.,Lindstrom, K. & Eardly, B. D. (2003). Discordant phylogenies within the *rrn* loci of Rhizobia. J Bacteriol 185, 2988–2998.

International Journal of Systematic and Evolutionary Microbiology (2011), **61**, 3089–3093 DOI 10.1099/ijs.0.036913-0

Minutes

International Committee on Systematics of Prokaryotes

Subcommittee on the taxonomy of *Agrobacterium* and *Rhizobium*

Minutes of the meeting, 7 September 2010, Geneva, Switzerland

Kristina Lindström, *Secretary*
J. P. W. Young, *Acting Chairperson*

Chapter 12
Agrobacterium tumefaciens and its Use in Plant Biotechnology

İbrahim İlker Özyiğit

M. Ashraf et al. (eds.), *Crop Production for Agricultural Improvement*,
DOI 10.1007/978-94-007-4116-4_12, © Springer Science+Business Media B.V. 2012

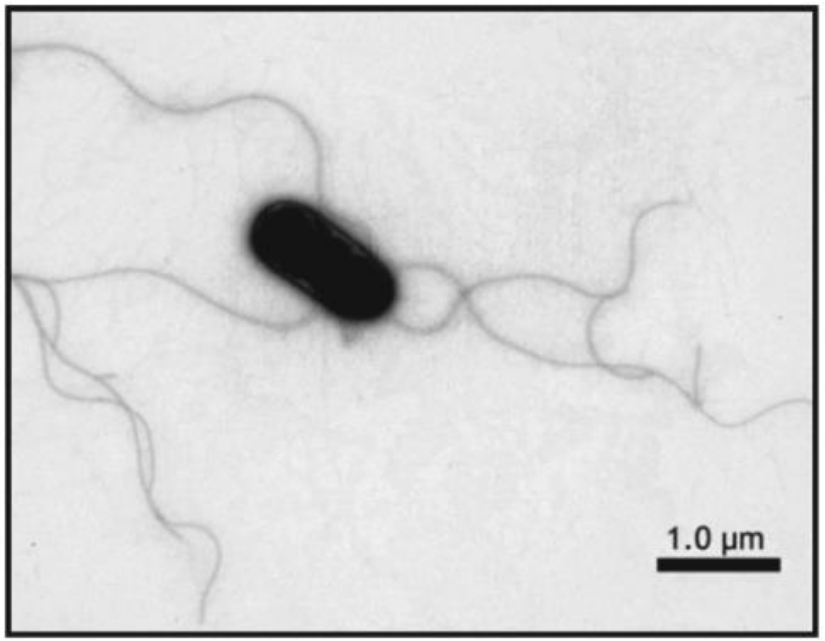

Fig. 12.1 Electron microscopic view of *Agrobacterium tumefaciens* with peritrichous flagella (Modified from Jeon et al. 2008)

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rhizobiales
Family	Rhizobiaceae
Genus	*Agrobacterium*
Species	*Agrobacterium tumefaciens*

Table 12.1 Systematics of *Agrobacterium tumefaciens* (Bergey's Manual of Systematic Bacteriology 2005)

Table 12.4 The brief history of plant biotechnology related to *Agrobacterium* sp

1853	The first written report of crown gall disease (Fabre and Dunal 1853)
1897	*Agrobacterium vitis* identified as causal agent of crown gall in grape (Cavara 1897)
1902	First attempt of plant tissue culture (Haberlandt 1902)
1907	*A. tumefaciens* identified as causal agent of crown gall in Paris daisy (*Argyranthemum frutescens*) (Smith and Townsend 1907)
1941	*In vitro* culture of crown gall tissues (Braun 1941)
1947	Sterile plant tumor tissue can proliferate indefinitely on hormone-free medium in culture. Tumor cells are proposed to be 'transformed' by an *Agrobacterium*-derived tumor-inducing principle (TIP) (Braun 1947)
1952	First application of micrografting (Morel and Martin 1952)
1954	First plant from single cell (Muir et al. 1954)
1956	Unusual low-molecular weight nitrogenous compounds (opines) are identified exclusively in tumor tissue (Lioret 1956)
1959	Publication of first handbook on plant tissue culture (Gautheret 1959)
1962	Development of Murashige and Skoog nutrient medium (Murashige and Skoog 1962)
1970	Discovery of first restriction endonuclease from *Haemophilus influenzae* Rd. It was later purified and named *Hind*I (Smith and Wilcox 1970)
1971	*A. tumefaciens* loses virulence when grown at 37°C. The TIP can be transferred between virulent and avirulent *A. tumefaciens* strains (Hamilton and Fall 1971; Kerr 1971)
	Preparation of first restriction map using *Hind*I enzyme to cut circular DNA of SV 40 into 11 specific fragments was prepared (Danna and Nathans 1971)
1974	*A. tumefaciens* virulence depends on the presence of a large 'tumor-inducing' (Ti) plasmid. The TIP is probably a component of the Ti plasmid (Zaenen 1974)
	Biotransformation in plant tissue cultures (Reinhard 1974)
1976	Octopine and nopaline synthesis and breakdown found to be genetically controlled by the Ti plasmid of *A. tumefaciens* (Bomhoff et al. 1976)

1977	The T-DNA region of the Ti plasmid is present in the genome of crown gall tumor cells: the T-DNA is the TIP (Chilton et al. 1977)
1980	The opine concept: the synthesis of opines by transformed cells creates an ecological niche for the infecting strain of *Agrobacterium* (Guyon et al. 1980)
1983	The first plant transformed with a recombinant gene using *A. tumefaciens* as a vector (Zambryski et al. 1983)
1984	T-DNA oncogenes are identified that mediate overproduction of auxin and cytokinin (Klee et al. 1984; Lichtenstein et al. 1984)
	Development of the genetic fingerprinting technique for identifying individuals by analyzing polymorphism at DNA sequence level (Jeffreys et al. 1984)
1985	The *vir*A/*vir*G two-component regulatory system is identified as a central component of signal perception and transduction in *Agrobacterium* transformation (Stachel and Zambryski 1986)
1987	Isolation of *Bt* gene from bacterium (*Bacillus thuringiensis*) (Barton et al. 1987)
1990	Development of the random amplified polymorphic DNA (RAPD) technique (Williams et al. 1990; Welsh and McClelland 1990)
2001	Publication of the complete genome sequence of two *A. tumefaciens* strains (Goodner et al. 2001; Wood et al. 2001)
2005	220 million acres of biotechnologic crops with herbicide tolerance and/or insect resistance traits were cultivated in 21 countries worldwide (James 2010)
2007	The global area of biotech crops reached 114.3 million hectares (282.4 million acres) worldwide (James 2010)
2010	The global area of biotech crops continued to soar for the fifteenth consecutive year at a sustained growth rate of 10% or 14 million hectares (35 million acres), reaching 148 million hectares or 365 million acres (James 2010)

Title: Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector

Authors: Herrera-Estrella, Luis; Depicker, Ann; van Montagu, Marc; Schell, Jeff

Publication: Nature, Volume 303, Issue 5914, pp. 209-213 (1983). (Nature Homepage)

Publication Date: 05/1983

Origin: NATURE

DOI: 10.1038/303209a0

Bibliographic Code:1983Natur.303..209H

The EMBO Journal Vol.2 No.12 pp.2143–2150, 1983

Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity

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Communicated by M. Van Montagu
Received on 22 July 1983

Genes presentes en el ADN-T del Plásmido Ti

Physiological and Molecular Plant Pathology 76 (2011) 76–81

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Mini Review

Agrobacterium tumefaciens: From crown gall tumors to genetic transformation

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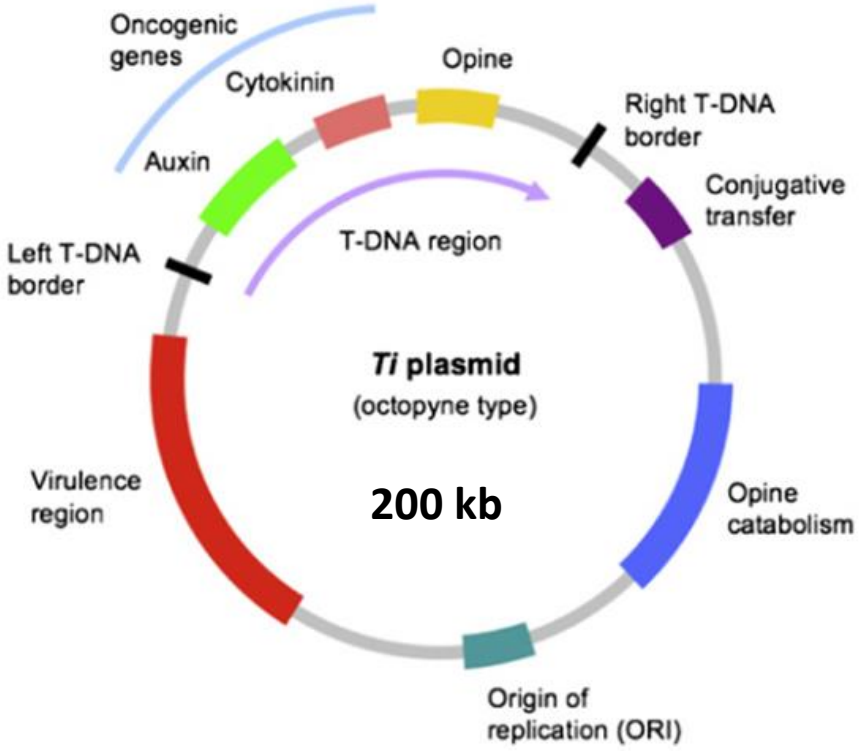

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JOURNAL OF BACTERIOLOGY, July 2000, p. 3885–3895
0021-9193/00/$04.00+0

Vol. 182, No. 14 ESTIGACIÓN CONACYT

MINIREVIEW

The Bases of Crown Gall Tumorigenesis

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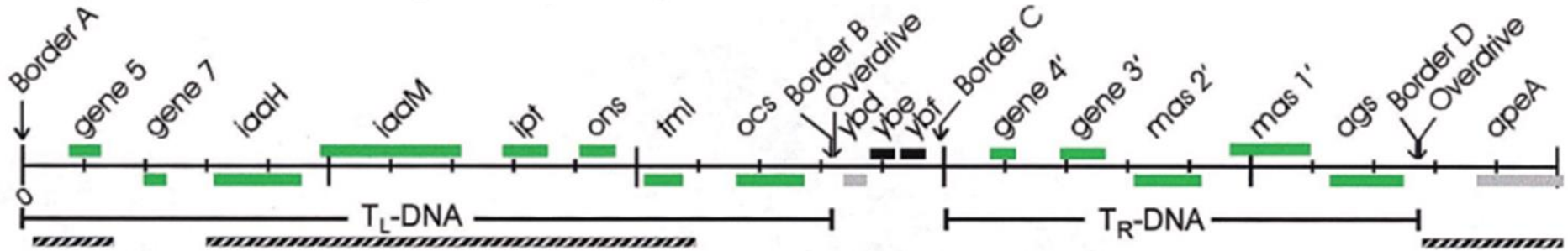
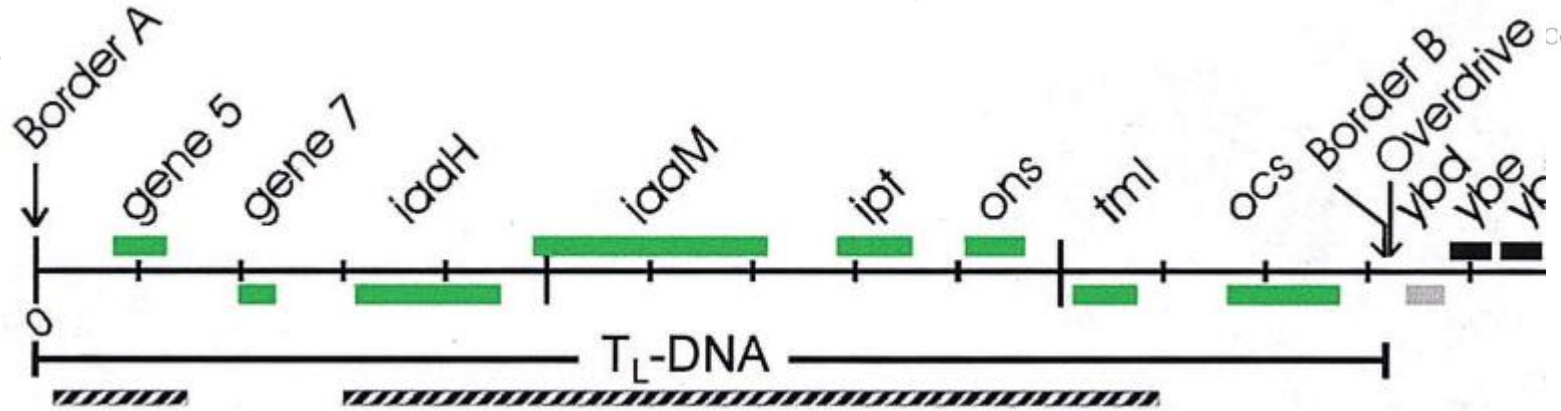


***tms* = tumour morphology shoot**

TABLE 1. Genes encoded by the octopine-type Ti plasmid[a]

Genetic locus	Description	Reference(s)
T-DNA genes		
ags	Agropine synthase, lactonization of mannopine	24, 40
Gene 5	Synthesis of indole-3-lactate, an auxin antagonist	57
iaaH and *iaaM*	Conversion of tryptophan to indole acetic acid (auxin)	55
ipt	Condensation of AMP and isopentenylpyrophosphate to form isopentenyl-AMP, a cytokinin	66
mas1' and *mas2'*	Mannopine synthase; condensation of glucose with glutamine or glutamate followed by reduction	24
ocs	Octopine synthase, reductive condensation of pyruvate with four basic amino acids	21
ons	Opine export from plant cells	75
tml (gene *6b*)	Auxin sensitivity	108
Borders A, B, C, D	*cis*-acting sites required for T-DNA processing, functionally equivalent to conjugal origins of transfer	125
Overdrive	*cis*-acting site for optimal T-DNA transfer; VirC1 binding site	110, 113



13 kb

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 1728–1732, March 1984
Cell Biology

tms **= tumour morphology shoot**

Nucleotide sequence of the *tms* genes of the pTiA6NC octopine Ti plasmid: Two gene products involved in plant tumorigenesis

(*Agrobacterium tumefaciens*)

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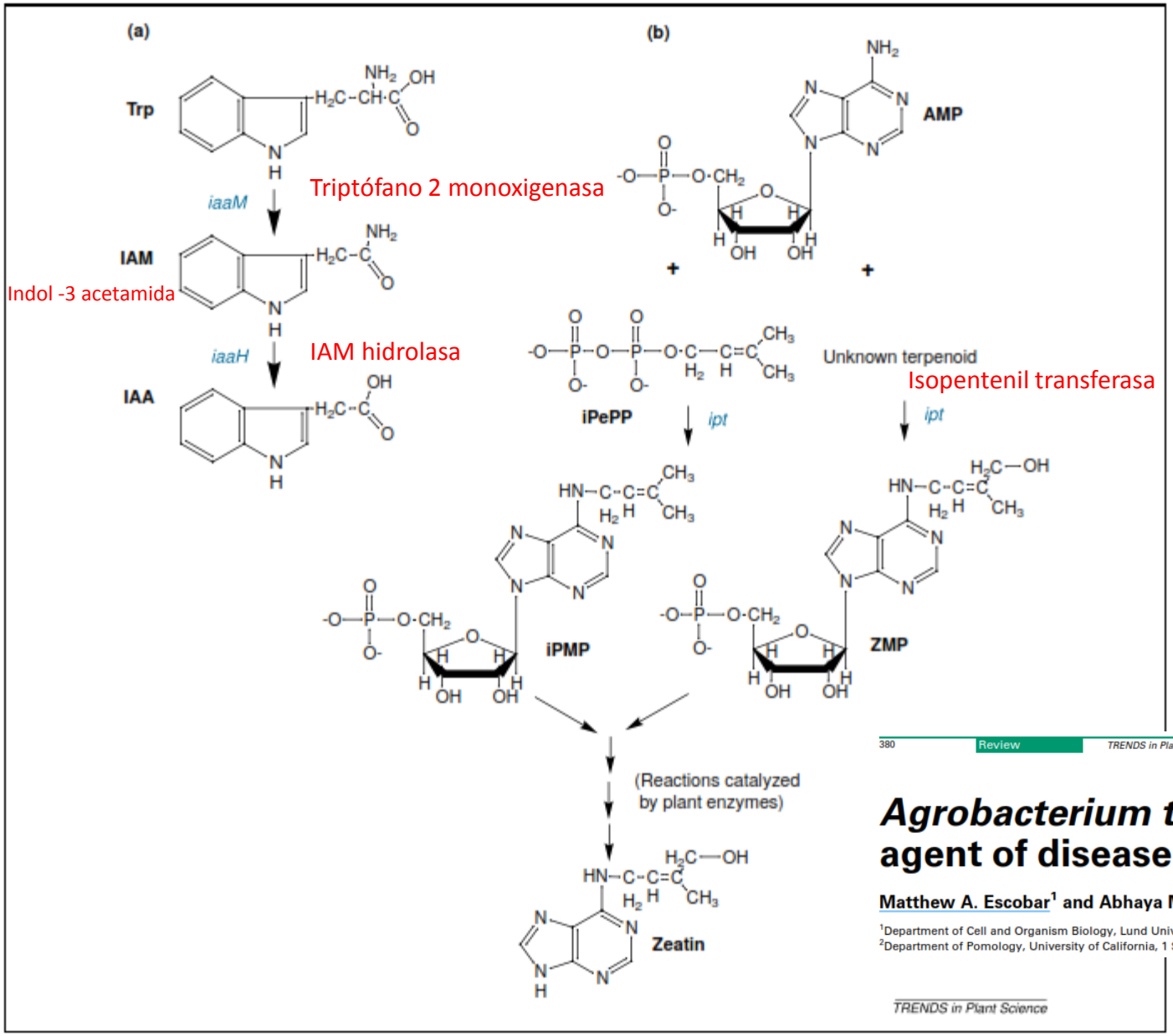
Communicated by Earl W. Davie, December 12, 1983

tms1 **= Transcrito 1 = proteína 83,769 daltons = ¿?**

tms2 **= Transcrito 2 = Proteína 49,588 daltons = convierte el indol-3-acetamide al ácido indolacético (AIA)**



380 Review TRENDS in Plant Science Vol.8 No.8 August 2003

Agrobacterium tumefaciens as an agent of disease

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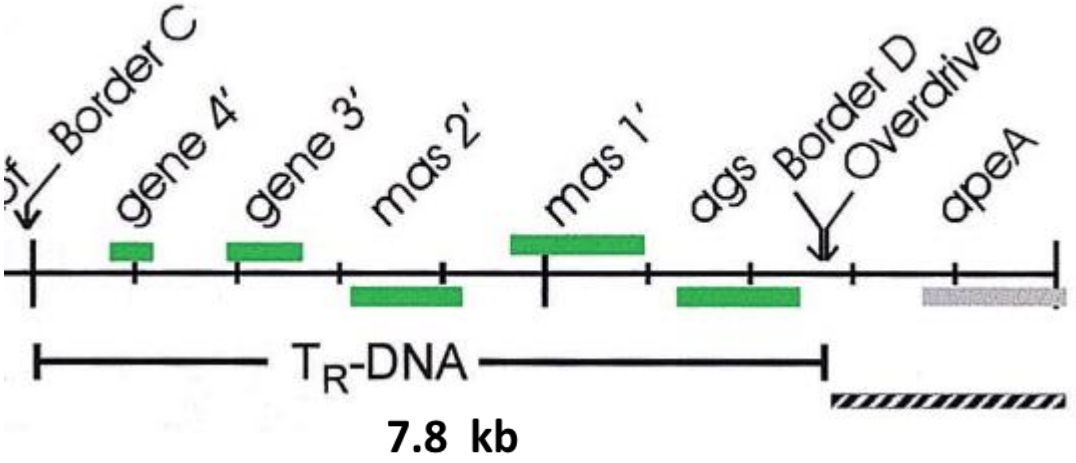
TRENDS in Plant Science

Fig. 1. *Agrobacterium tumefaciens* derived phytohormone biosynthesis pathways. (a) Auxin biosynthesis catalyzed by the *iaaM* and *iaaH* oncogenes. (b) Cytokinin biosynthesis catalyzed by the *ipt* oncogene. Adapted from [28,80].



mas1' y **mas2'**: Manopina sintasa; condensación de glucosa con glutamine o glutamate seguido de una redución

Ags: Agropine sintasa, lactonización of manopina

Phytochemistry, Vol. 34, No. 1, pp. 31–38, 1993
Printed in Great Britain.

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REVIEW ARTICLE NUMBER 82

CHEMISTRY AND BIOCHEMISTRY OF OPINES, CHEMICAL MEDIATORS OF PARASITISM

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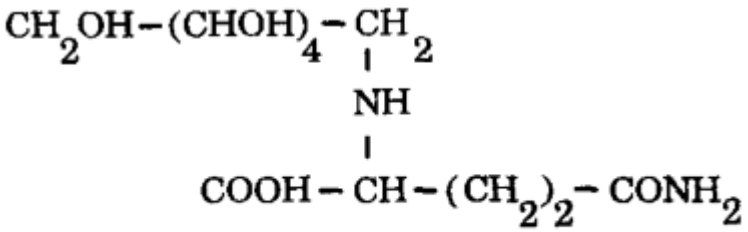
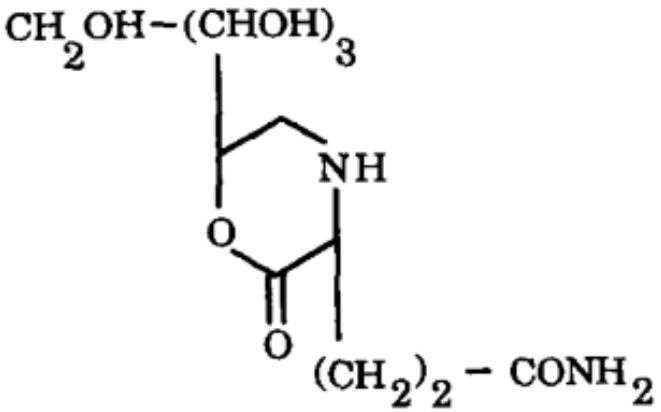


MANNOPINE



AGROPINE

Proceso de infección de *Agrobacterium*

Chapter 12
Agrobacterium tumefaciens and its Use in Plant Biotechnology

İbrahim İlker Özyiğit

M. Ashraf et al. (eds.), *Crop Production for Agricultural Improvement*,
DOI 10.1007/978-94-007-4116-4_12, © Springer Science+Business Media B.V. 2012

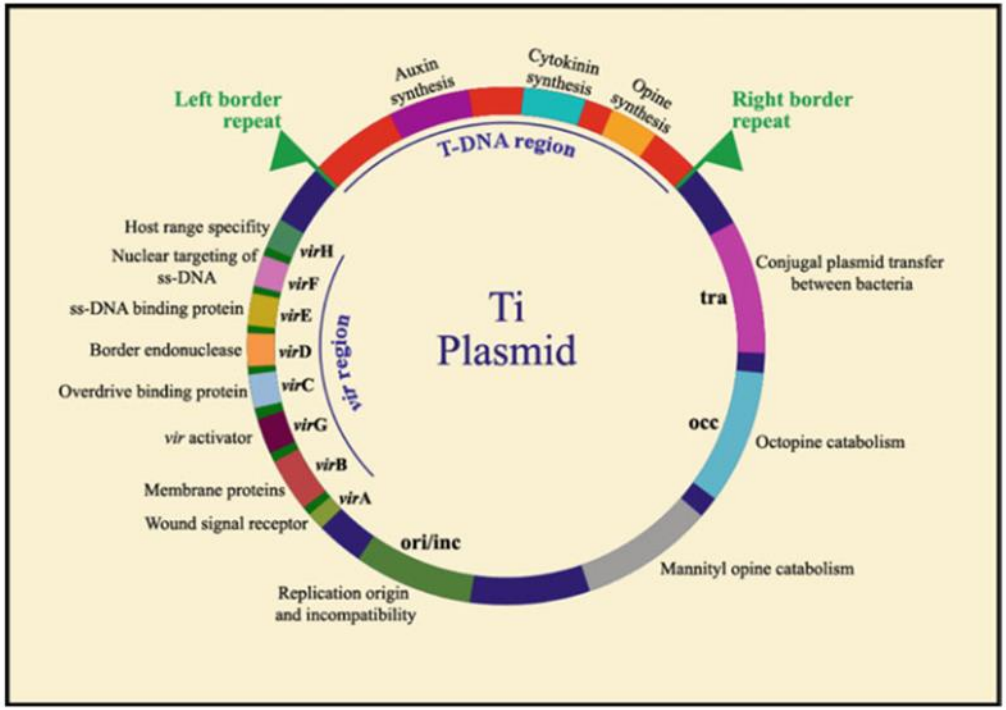
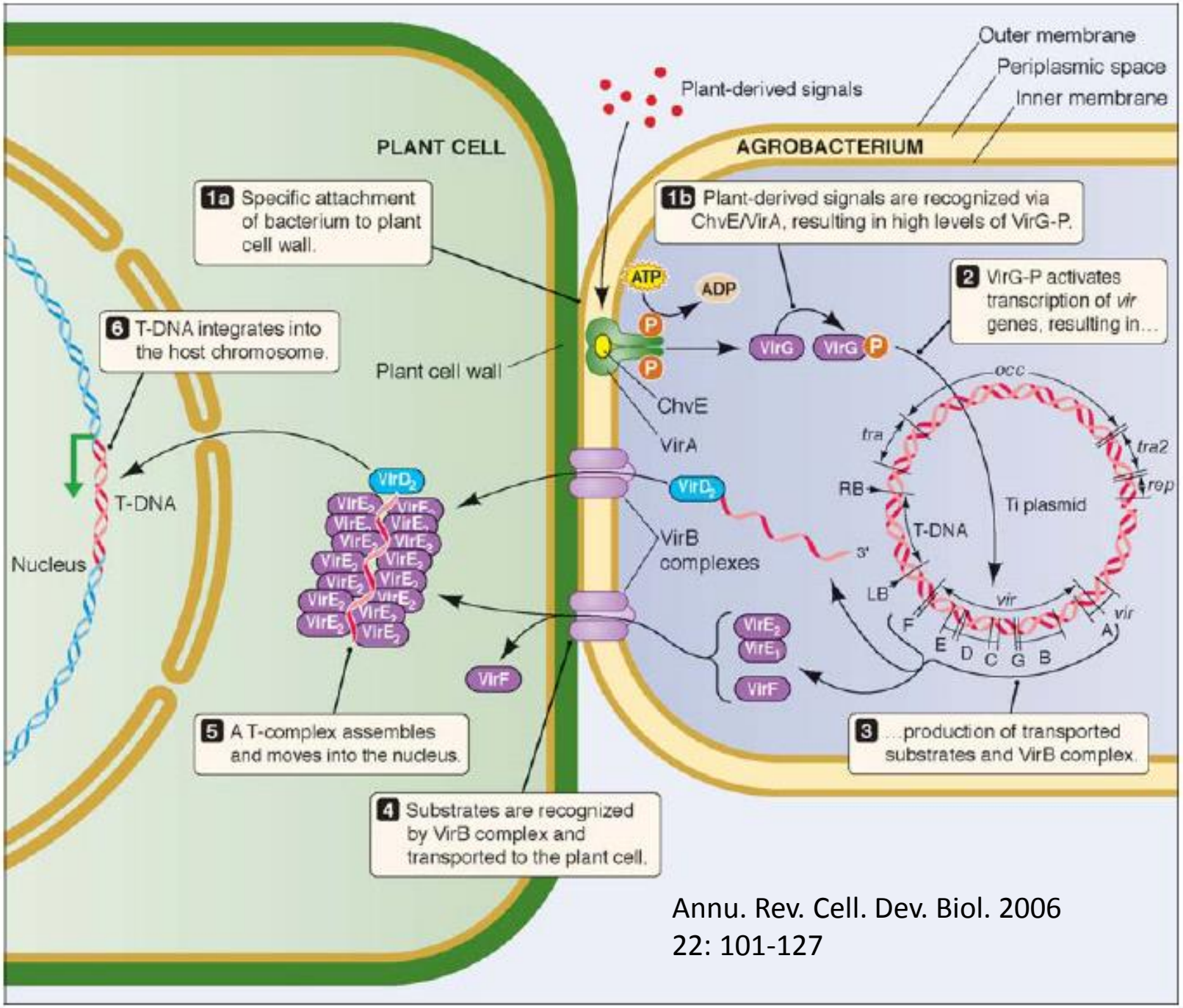


Fig. 12.5 Genetic map of octopine-type Ti plasmid (Modified from Ream 2002 and Özcan et al. 2004)

Table 12.2 The *vir* regions of Ti plasmids and their functions

vir Locus	Function
***vir*H**	Encodes VirH1 and VirH2 proteins (they could enhance the transfer efficiency, detoxifying certain plant compounds that can affect bacterial growth)
***vir*F**	Encodes 23 kDa protein (functions once the T-DNA complex is inside the plant cells via the conjugal channel or independently, as it was assumed for VirE2 export)
***vir*E**	Encodes ss-T-DNA binding protein (stabilizes T-DNA during or after transfer)
***vir*D**	Nicks Ti plasmid at T-DNA borders, covalently attaches to T-strand
***vir*C**	Binds to the 'overdrive' region to promote high efficiency T-strand synthesis
***vir*G**	Regulatory (transcriptional activator of other *vir* loci)
***vir*B**	Transfer apparatus (required for export of the T-complex and VirE2 into the plant cell)
***vir*A**	Regulatory (recognizes plant metabolites, activates *vir*G)



Annu. Rev. Cell. Dev. Biol. 2006
22: 101-127

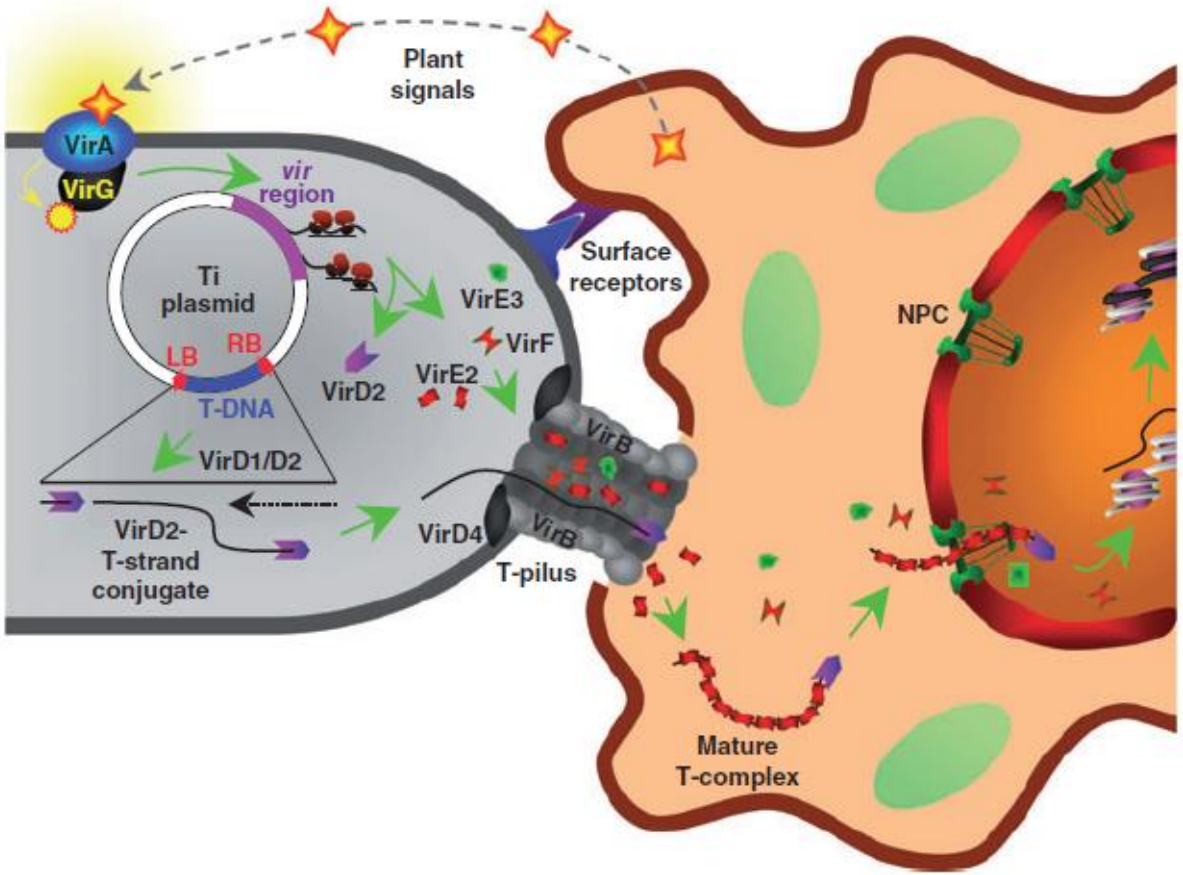
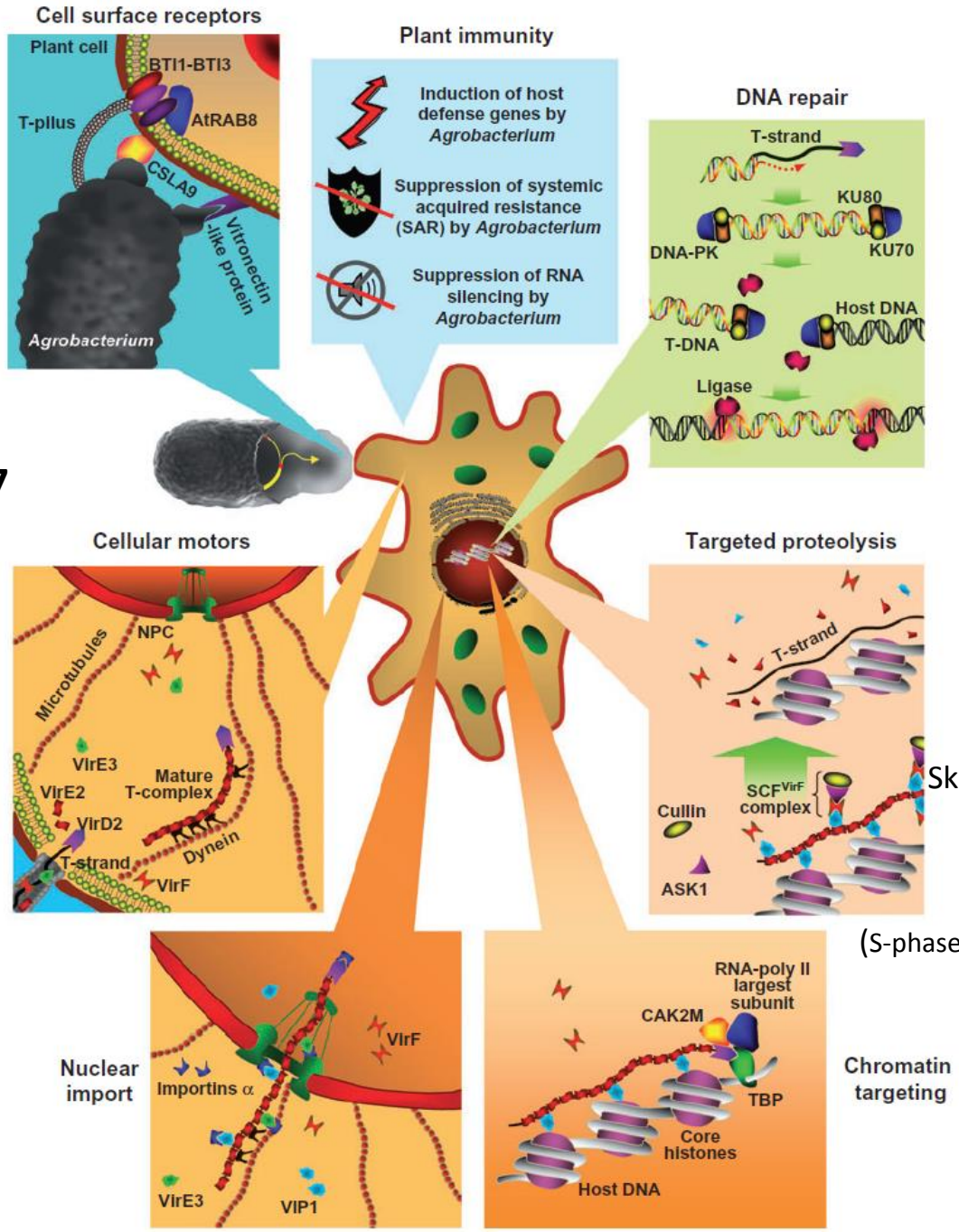


Fig. 1. Summary of major molecular events and structures within the *Agrobacterium* cell that generate the Vir protein machinery and T-strands which then are transported into the plant cell, enter its nucleus and integrate into the genome. The transformation process begins with recognition of plant signals by the bacterial VirA/VirG sensory system, followed by activation of the *vir* loci and attachment of the bacterium to the host cell. The T-strand is excised from the T-DNA region by VirD2/VirD1 and exported, *in cis* with a covalently attached VirD2 molecule and *in trans* with several other Vir proteins, into the plant cell cytoplasm via a VirB/D4 type IV secretion system. Inside the host cell, the VirD2–T-strand conjugate is packaged by numerous molecules of VirE2 to form a mature T-complex. For in-depth discussion on the T-complex transport and nuclear import, and T-DNA integration, see text.

Citovsky V. et al. 2007. Biological systems of the host cell involved in *Agrobacterium infection.* **Cellular Microbiology**. **9(1): 9–20**

Citovsky V. et al. 2007
Cellular Microbiology.
9(1): 9–20



Skp1-Cullin-F-box protein

(S-phase kinase-associated protein 1)

Fig. 2. Summary of major biological systems of the host cell that are involved in the *Agrobacterium*-mediate genetic transformation. Main molecular events associated with each biological system are depicted. For further details, see text.

Physiological and Molecular Plant Pathology 76 (2011) 76–81

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

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Mini Review

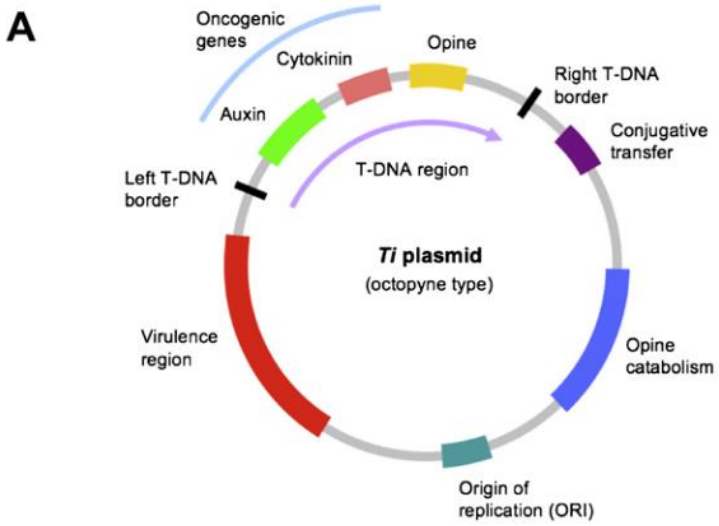
Agrobacterium tumefaciens: From crown gall tumors to genetic transformation

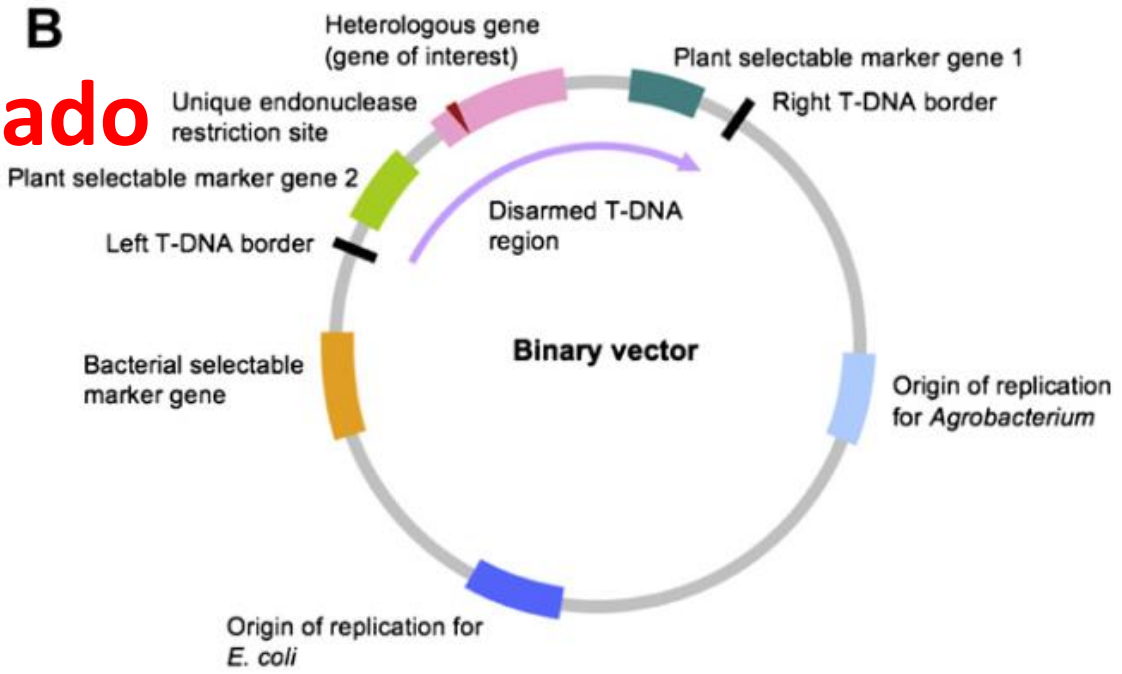
Daniel I. Păcurar [a,c,*], Hans Thordal-Christensen [d], Monica L. Păcurar [b,c], Doru Pamfil [c], Constantin Botez [c], Catherine Bellini [a,e]

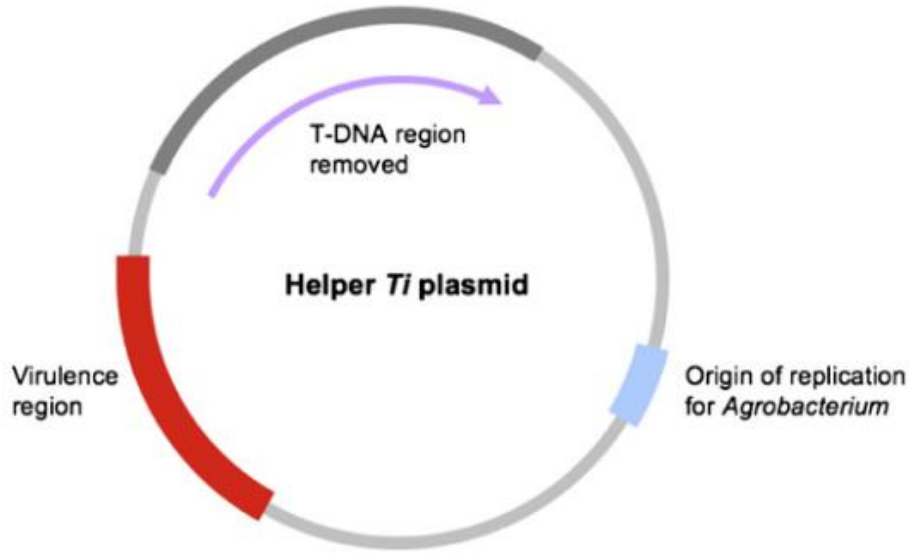
[a] Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, SE-90187 Umeå, Sweden
[b] Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-90183 Umeå, Sweden
[c] University of Agricultural Sciences and Veterinary Medicine, 400372 Cluj Napoca, Romania
[d] Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, DK-1871 Frederiksberg C, Denmark
[e] Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech, 78026 Versailles Cedex, France

Fig. 1. Crown gall tumor on an oak tree.

Plásmido Ti desarmado







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[e] Institut Jean-Pierre Bourgin, UMR1318 INRA-AgroParisTech, 78026 Versailles Cedex, France

Chapter 12
Agrobacterium tumefaciens and its Use in Plant Biotechnology

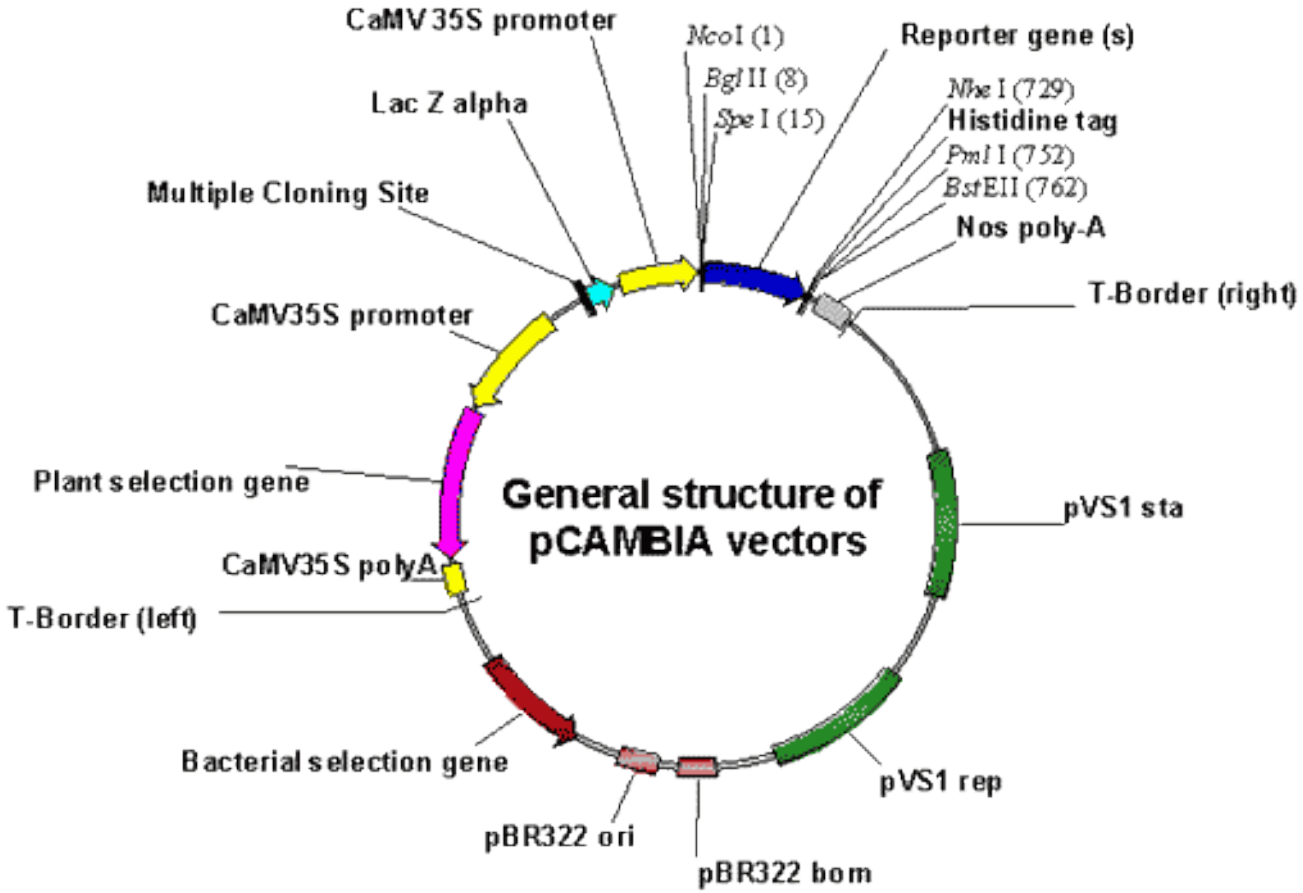
İbrahim İlker Özyiğit

M. Ashraf et al. (eds.), *Crop Production for Agricultural Improvement*,
DOI 10.1007/978-94-007-4116-4_12, © Springer Science+Business Media B.V. 2012

Table 12.5 The mostly used marker and reporter genes, their encoded enzymes and conferred resistances

Marker Gene	Enzyme Encoded	Resistance Conferred
Antibiotics		
npt II	Neomycin phosphotransferase	Kanamycin, neomycin, G418, paromycine
hpt or *aph* IV	Hygromycin -phosphotransferase	Hygromycin
dhfr bacterial or mouse	Dihydrofolate reductase	Methotrexate
bla	TEM-1 β-lactamase	Ampicillin
aadA	Association with several transposons (Tn7, Tn21, …)	Streptomycin and spectinomycin
Herbicides		
bar	Phosphinothricin acetyltransferase	Phosphinothricin
aro A	5-enolpyruvylshikimate-3-phosphate synthase	Glyphosate
Modified *als* genes	Acetohydroxyacid synthase (or acetolactate synthase)	Chlorsulfuron, imidazolanones
Reporter Genes		
CAT	Chloramphenicol acetyltransferase	
GUS	β-glucuronidase	
npt II	Neomycin phosphotransferase	
Luc	Luciferase	
bar	Phosphinothricin acetyltransferase	
β-gal	β-galactosidase	

Vectores binarios



http://www.cambia.org/daisy/cambia/585

pCambia Vectors

The transformation of plants is common place in hundreds of laboratories worldwide, tranformation is achieved using bacterially-mediated or direct DNA transfer methods. A sizeable technical limitations faced by many labs is that several vectors still used are historical relics with substandard features that make DNA constructions awkward or cumbersome such as: low-copy origin of replication resulting in low yield DNA preps, large size and lack of convenient restriction sites for manipulation.

pCambia1380 Plant Expression Vector

$134.18

Add to Cart

Product size is: 20ug
Vector contains a kanamycin resistance gene for bacterial selection, a hygromycin B resistance gene for plant selection, and no reporter gene to allow construction of user's own system. Learn More
Product ID: M1598
Availability: In Stock

https://www.markergene.com/pcambia-vectors

Agrobacterium tumefaciens

1. En un gran porcentaje una copia del ADN-T se integra al genoma vegetal

2. Están disponibles un gran número de vectores conteniendo los bordes del ADN-T con varios genes reporteros y marcadores de selección, lo que permite a los investigadores seleccionar la combinación más apropiada para insertar los genes heterólogos

3. Es posible transferir grandes fragmentos de ADN.

Al sistema de transformación vía *Agrobacterium tumefaciens*, se le considera mas precisa, mas controlable y por lo tanto mas "limpia" que el bombardeo de micropartículas.

Herrera-Estrella L. et al. 2005. Transgenic plants. In Methods in Molecular Biology: Transgenic plants: Methodos and Protocols. Edited by L. Peña. Humana Press. Inc. Totowa NJ Vol. 286. pag 3-31

Raíces vegetales

Daucos carota
Manihot esculenta
Solanum tuberosum
Ipomea batatas

Pastos

Cynodon spp
Panicum virgatum
Festuca arundinacea
Lolium perenne

Especies leñosas

Ulmus americana
Quercus suber
Eucaliptus
Pinus radiata
Populus spp
Hevea brasiliensis

Plantas tropicales

Musa sp
Citrus
Coffea sp
Carica papaya
Ananas comusus
Saccharum spp

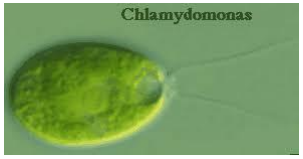
Nueces y frutas

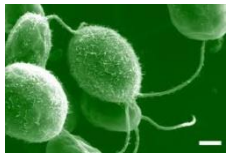
Castanea dentata
Malus x domestica
Vaccinium corymbosum
Vitis vinifera
Fragaria x ananassa
Juglans

Plantas Ornamentales

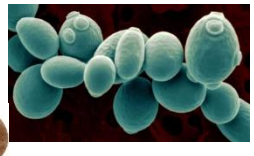
Dianthus caryophylus
Dendranthema x grandiflora
Phaelenopsis, Oncidium, Cymbidium
Petunia hybrida
Rosa hybrida

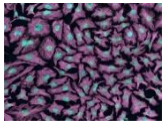
Plantas medicinales: *Panax ginseng, Cannabis sativa, Papaver somniferum*



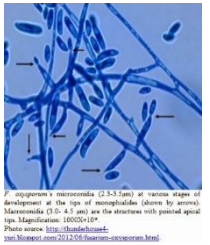


Algas verdes (*Chlamydomonas reinhardtii*)





Levaduras (*Saccharomyces cerevisiae*)
Actinomicetos (*Streptomyces lividans***)**
Hongos filamentosos (*Magnaporthe grisea* y *Fusarium oxysporum***)**
Champiñones (Agaricus bisporus)
Células de mamíferos (HeLa**) He**nrietta **La**cks



Wang K. 2006**.** *Agrobacterium* Protocols. Second edition. Vol 2. **Methods in Molecular Biology 344**

Chapter 2

New Approaches to *Agrobacterium tumefaciens*-Mediated Gene Transfer to Plants

Mustafa Yildiz, Murat Aycan and Sunjung Park

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66465

Para lograr el éxito de la tabla anterior

Se ha manipulado algunas variables para aumentar la Eficiencia de Transformación:

Densidad bacteriana
pH del medio
Concentración de acetosiringona
Infiltración a vacío
Tiempo de cocultivo
Temperatura de cocultivo

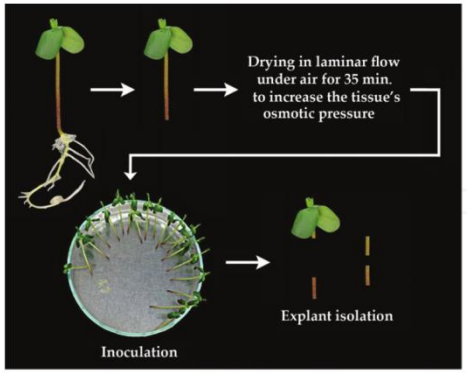
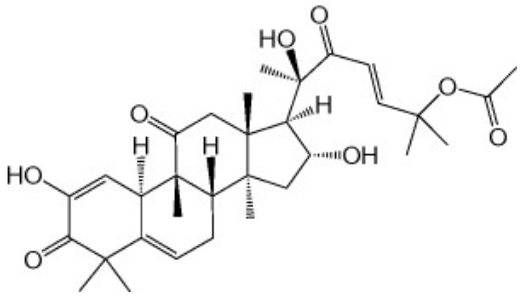


Figure 2. Explant isolation from inoculated 7-old-day flax seedlings having cotyledon leaves without root system.



1. Utilizing explant's negative atmospheric pressure for increased gene transformation

2. The effect of squirting cucumber (*Ecballium elaterium (L.) A. Rich)* fruit juice *on A. tumefaciens*-mediated transformation

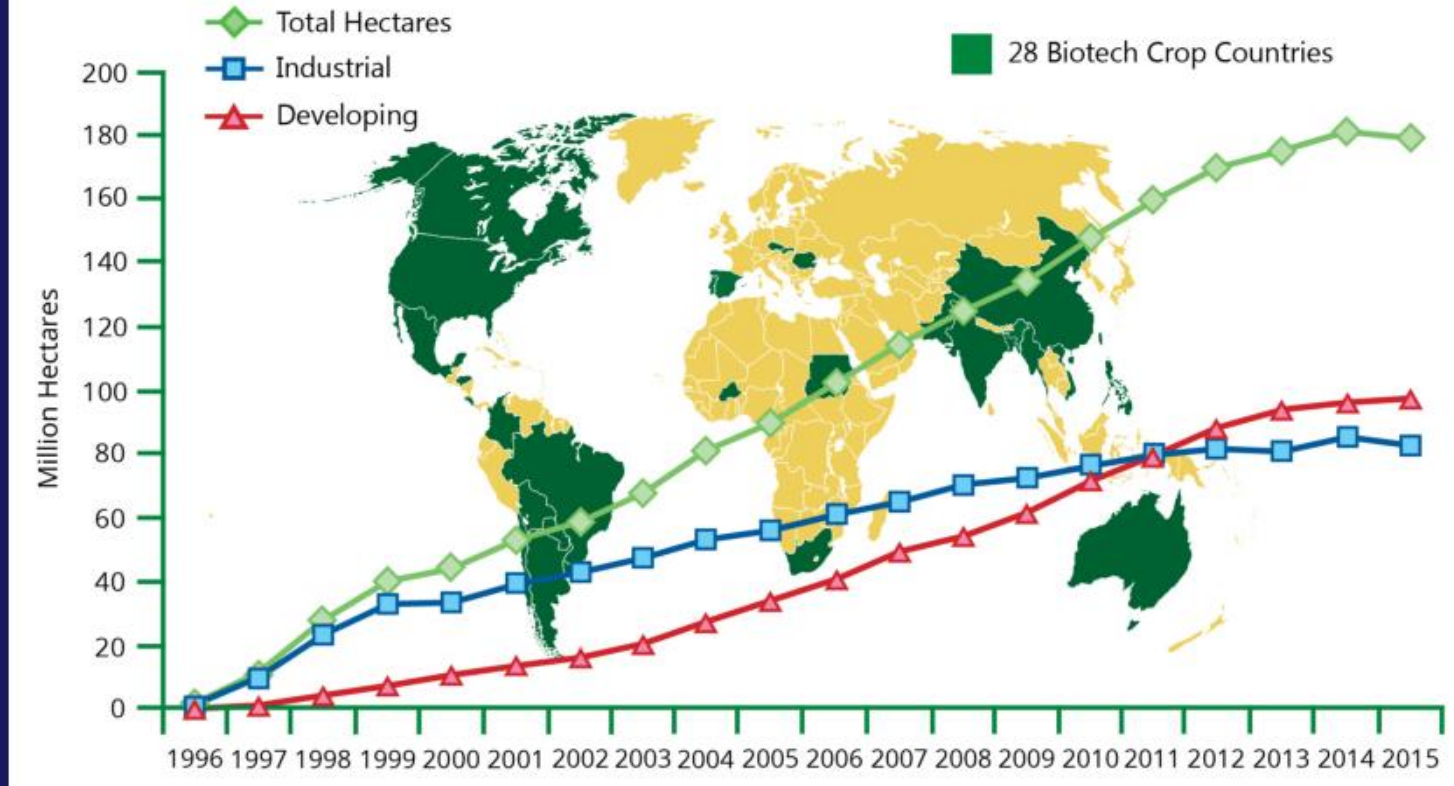
Pepinillo del diablo (purgante)

α-elaterin (cucurbitacin E), ß-elaterin (cucurbitacin B), elatericine A (cucurbitacin D), and elatericine B (cucurbitacin I) that are poisonous and showed antibacterial activities

3. Use of magnetic field strength (0-control, 75, 150, and 300 mT) **for high-transformation frequency via *A. tumefaciens***

4. The effect of gamma radiation (0-control, 40, 80, and 120 Gy) **on A. tumefaciens-mediated transformation**

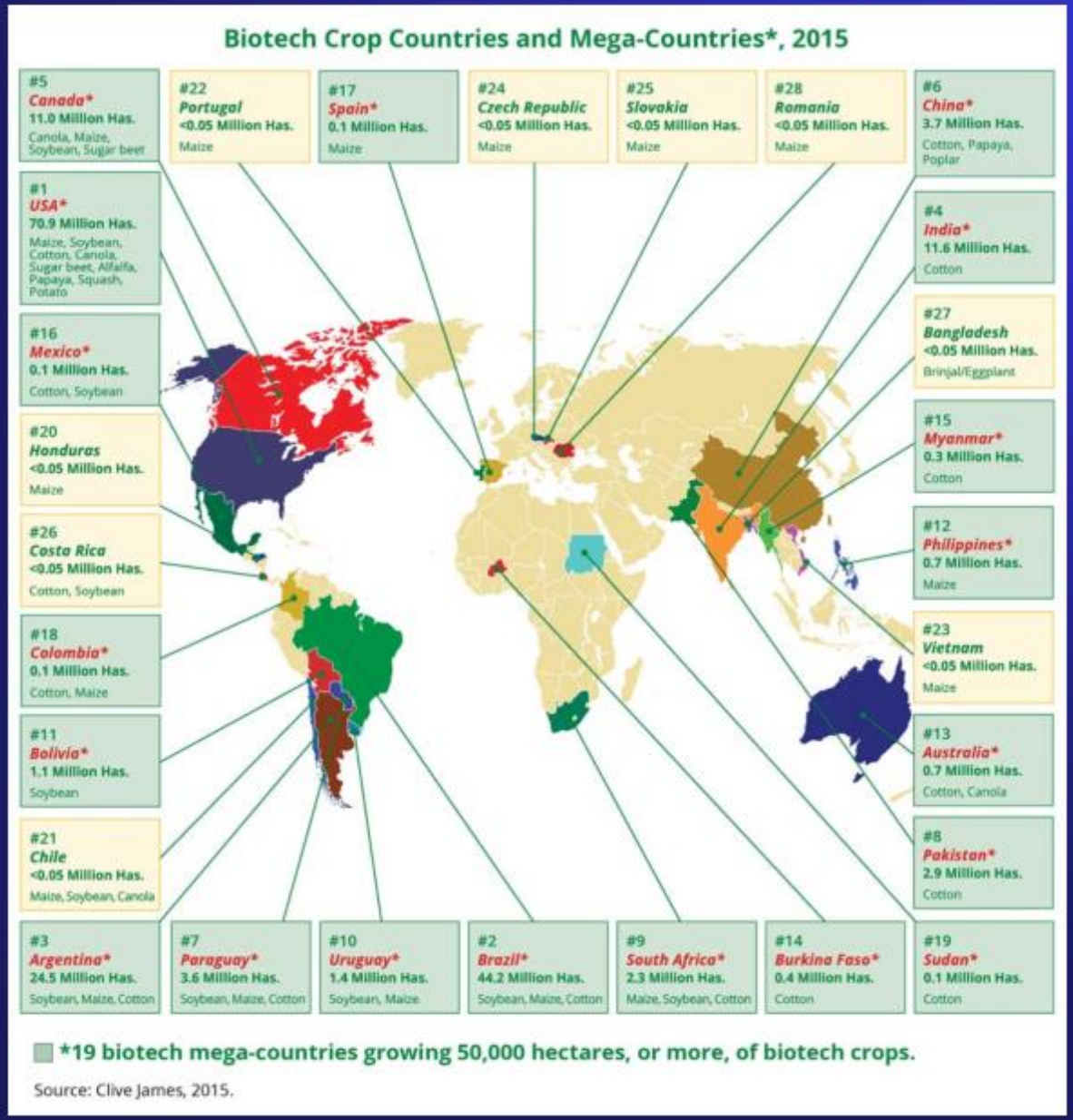
Producción Mundial de Cultivos Genéticamente Modificados



Up to ~18 million farmers, in 28 countries planted 179.7 million hectares (444 million acres) in 2015, a marginal decrease of 1% or 1.8 million hectares (4.4 million acres) from 2014.

Source: Clive James, 2015.

Biotech Crop Countries and Mega-Countries*, 2015



ISAAA
INTERNATIONAL SERVICE FOR THE ACQUISITION OF AGRI-BIOTECH APPLICATIONS

ISAAA Briefs

BRIEF 52

Global Status of Commercialized Biotech/GM Crops: 2016

Up to ~18 million farmers in 26 countries planted 185.1 million hectares (457.4 million acres) in 2016, an increase of 3% or 5.4 million hectares (13.1 million acres) from 2015.

Table 1. Global Area of Biotech Crops, the First 21 Years, 1996 to 2016

Year	Hectares (million)	Acres (million)
1996	1.7	4.2
1997	11.0	27.2
1998	27.8	68.7
1999	39.9	98.6
2000	44.2	109.2
2001	52.6	130.0
2002	58.7	145.0
2003	67.7	167.3
2004	81.0	200.2
2005	90.0	222.4
2006	102.0	252.0
2007	114.3	282.4
2008	125.0	308.9
2009	134.0	331.1
2010	148.0	365.7
2011	160.0	395.4
2012	170.3	420.8
2013	175.2	432.9
2014	181.5	448.5
2015	179.7	444.0
2016	**185.1**	**457.4**
Total	**2,149.7**	**5,312.0**

Global hectarage of biotech crops in 2016 increased to 185.1 million hectares compared with 179.7 million hectares in 2016, equivalent to 3% or 5.4 million hectares.

Agrobacterium rhizogenes

Genes presentes en el ADN-T del Plásmido Ri
Genes *rolA, rolB, rolC* y *rolD*
Plantas transgénicas a los genes *rol*

Genes presentes en el ADN-T del Plásmido Ri

In Vitro Cell.Dev.Biol.—Plant (2007) 43:383–403
DOI 10.1007/s11627-007-9096-8

REVIEW

Agrobacterium rhizogenes: recent developments and promising applications

Veena Veena · Christopher G. Taylor

Received: 21 August 2007 / Accepted: 24 September 2007 / Published online: 16 November 2007 / Editor: Christian Walter

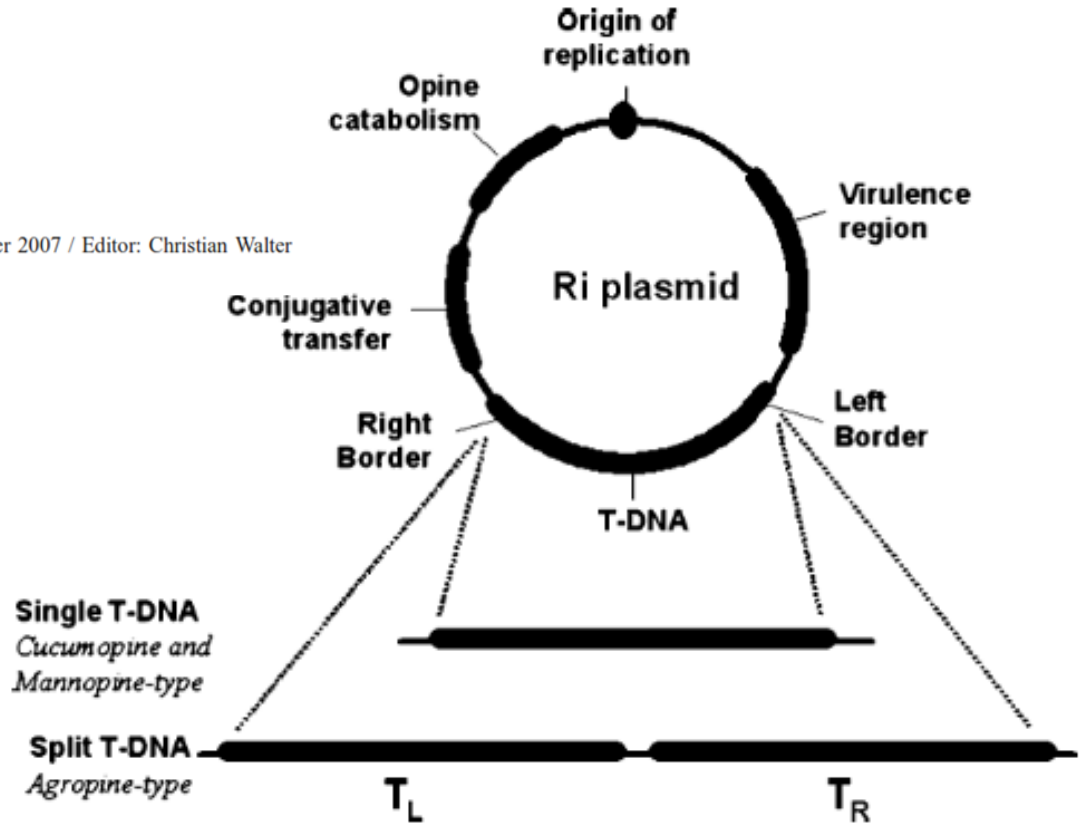
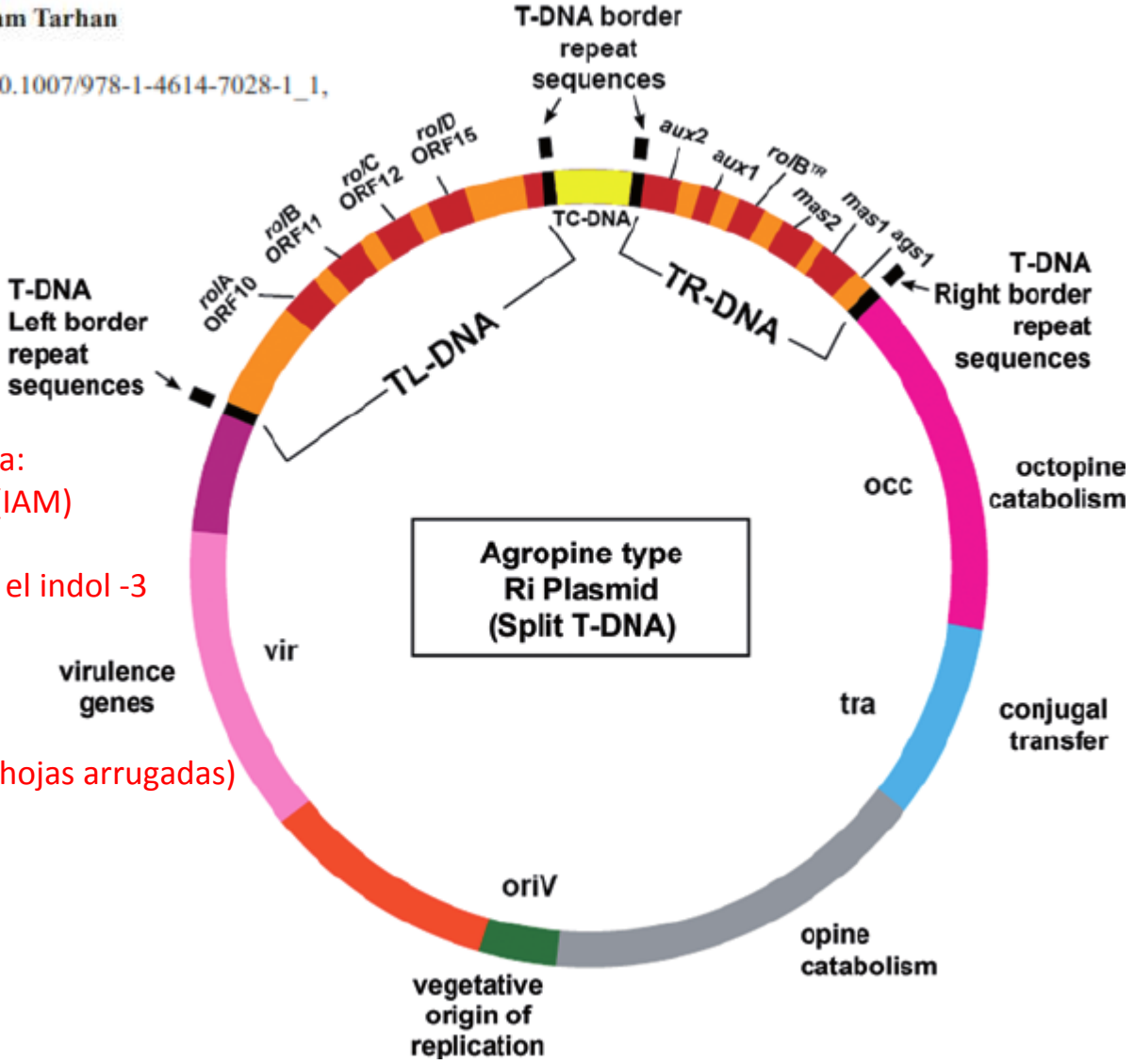


Figure 2. Structure of the Ri-plasmids of *A. rhizogenes*.

Chapter 1
Agrobacterium rhizogenes-Mediated Transformation and Its Biotechnological Applications in Crops

Ibrahim Ilker Ozyigit, Ilhan Dogan and Ebru Artam Tarhan

K. R. Hakeem et al. (eds.), *Crop Improvement*, DOI 10.1007/978-1-4614-7028-1_1,



***tmr* = tumour morphology root**

***aux*1** = 749 aa = Triptófano 2 monoxigenasa: Convierte triptófano a indol -3 acetamida (IAM)

***aux*2** = 466 aa = IAM hidrolasa: Convierte el indol -3 acetamida (IAM) a AIA

***rolB*TR** = gen homólogo del *rolB* (produce hojas arrugadas)

***mas*1 *y mas*2** = Manopina sintasa
***ags*1** = Agropina sintasa

Chapter 1
Agrobacterium rhizogenes-Mediated Transformation and Its Biotechnological Applications in Crops

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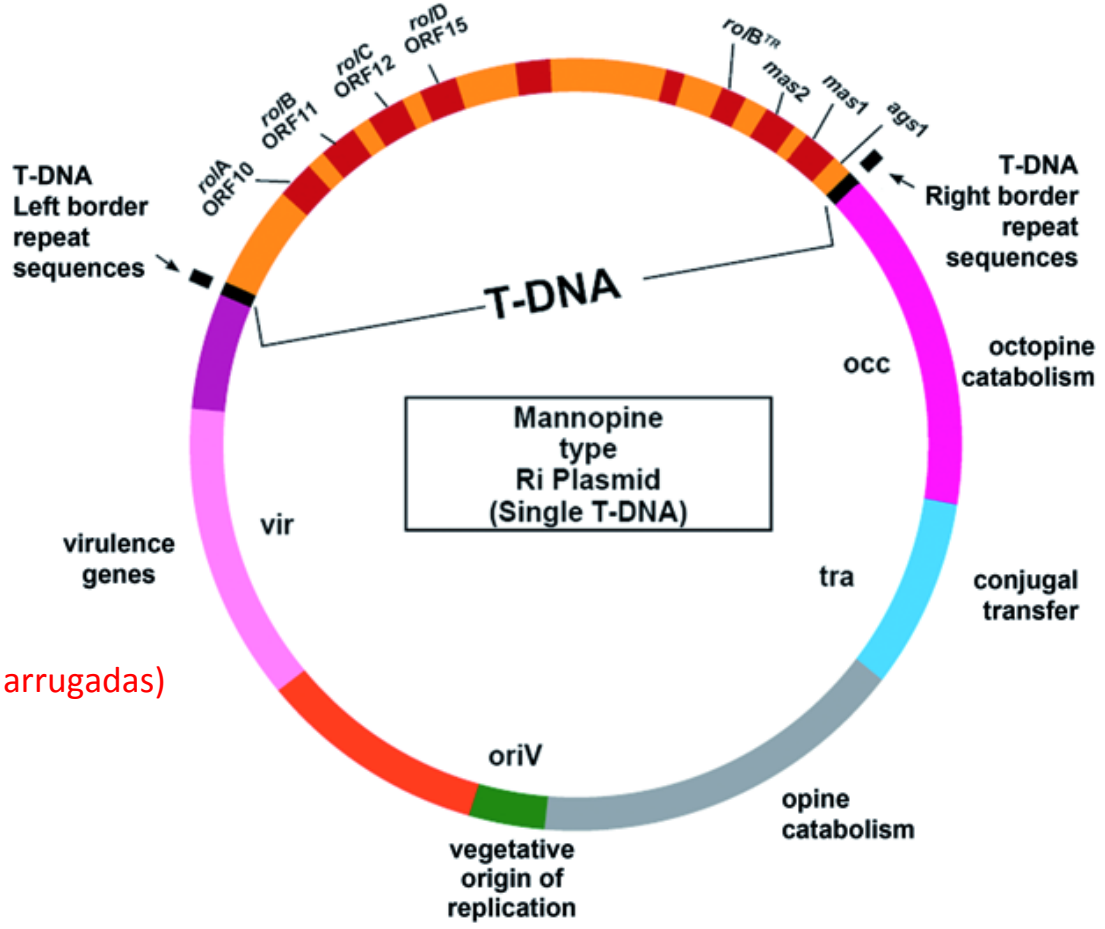
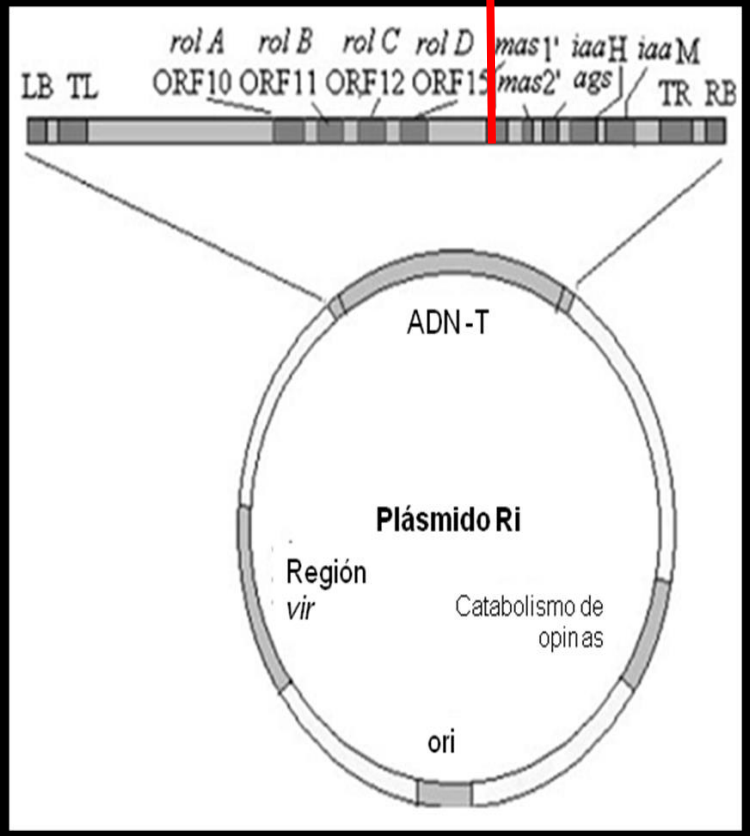


***rolB*TR** = gen homólogo del *rolB* (produce hojas arrugadas)

***mas*1 *y mas*2** = Manopina sintasa
***ags*1** = Agropina sintasa

ADN -T– BORDE IZQUIERDO

ADN- T – BORDE DERECHO



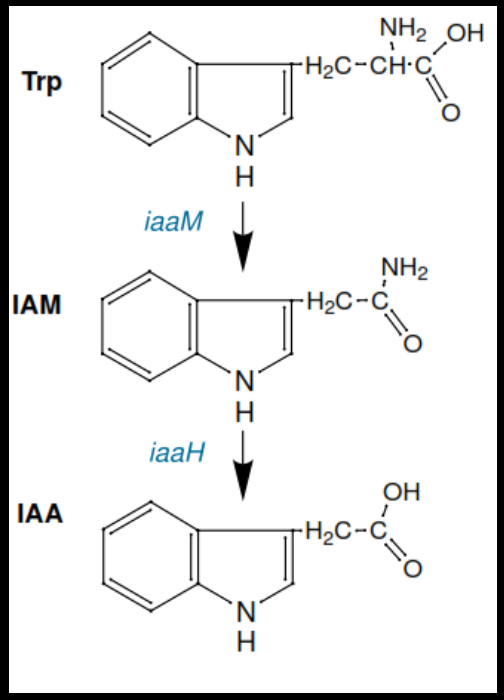
Biotechnol Lett (2012) 34:407–415
DOI 10.1007/s10529-011-0785-3

REVIEW

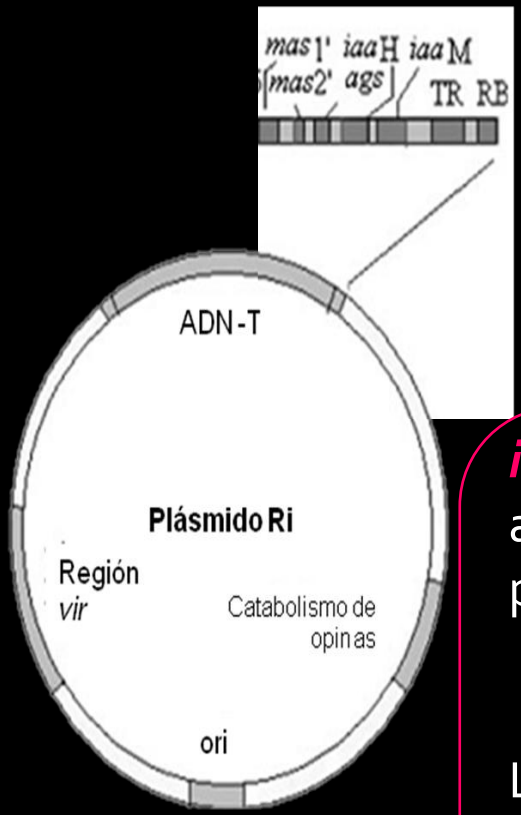
Natural plant genetic engineer *Agrobacterium rhizogenes*: role of T-DNA in plant secondary metabolism

Sheela Chandra

iaaM = Triptofano monooxigenasa



El ADN T – BORDE DERECHO



iaaH = Indol-3-acetamida-hidrolasa = produce AIA biosíntesis de auxinas.

La síntesis de las opinas, manopina (*mas1 'y mas2'*) y agropina (ags).

Genes *rolA, rolB, rolC* y *rolD*

Biotechnology Advances 26 (2008) 318–324

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Functions of *rol* genes in plant secondary metabolism

Victor P. Bulgakov *

Bioengineering Group, Institute of Biology and Soil Science, Far East Branch of Russian Academy of Sciences, Vladivostok, 690022, Russia

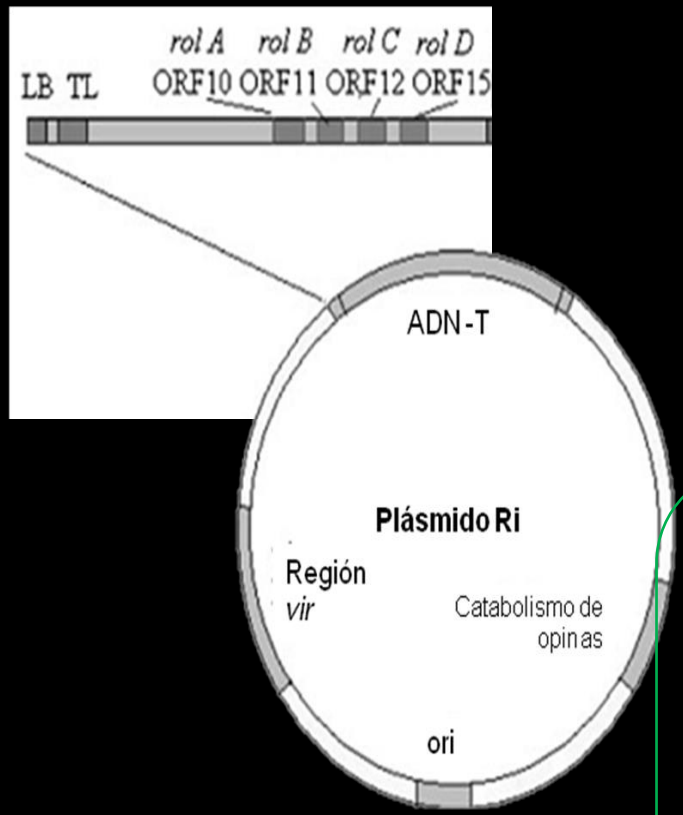
Contents

rol = root locus

***rol* A (ORF10)** - *proteína de unión de ácidos nucléicos .*

Papillomavirus E2 DNA-binding domain

ADN T– BORDE IZQUIERDO



***rol* C (ORF12)** - *activa procesos del metabolismo secundario, libera citocininas y auxinas.*

***rol* B (ORF11)** – *Fosfatasa de tirosina, que inhibe el crecimiento del tejido vegetal y aumenta la producción de metabolitos.*

Moriuchi et al. (2004) identified the nuclear localization of the RolB protein and, based on this finding and by analogy with the *A. tumefaciens* **6b protein,** hypothesized that RolB might function as a transcriptional coactivator/mediator.

***rol* D (ORF15)** - ornitina ciclodesaminasa que convierte la ornitina en prolina

(estabilidad en el crecimiento de las raíces y abundante floración en las plantas transformadas).

Plant Cell Tiss Organ Cult (2014) 117:477–481
DOI 10.1007/s11240-014-0450-3

RESEARCH NOTE

Enhanced production of isoflavones by elicitation in hairy root cultures of Soybean

Jeevaraj Theboral · Ganeshan Sivanandhan · Kondeti Subramanyam · Muthukrishnan Arun · Natesan Selvaraj · Markandan Manickavasagam · Andy Ganapathi

Supplementary Fig. 1

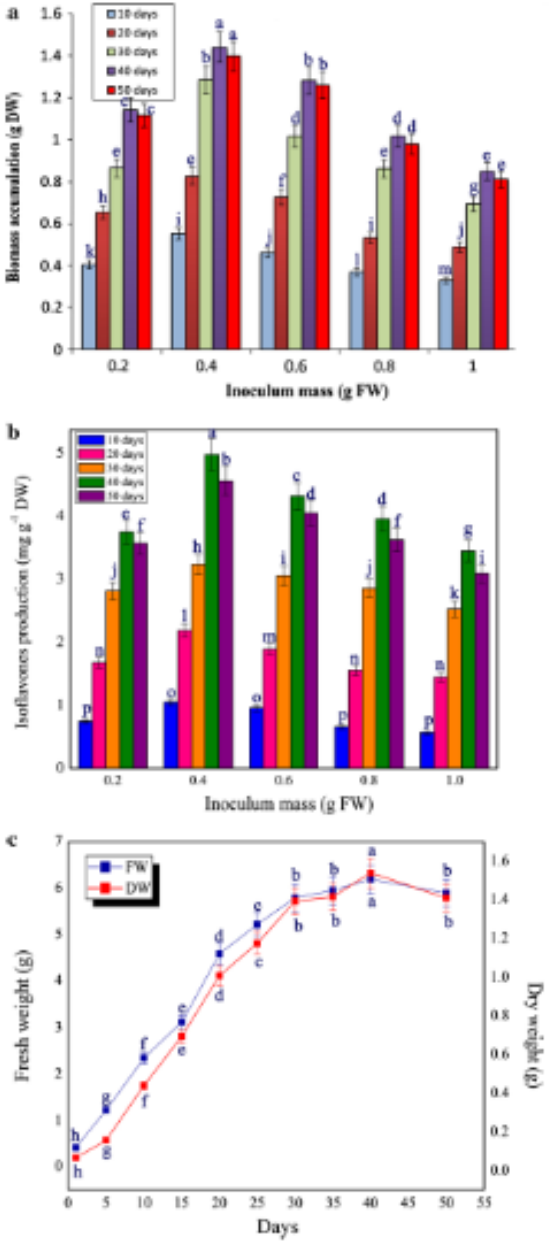


Fig. 1 Effect of inoculum mass on biomass accumulation (**a**) and isoflavones production (**b**) in half-strength MS liquid media. Data were recorded for 50 days at every 10 days interval. (**c**) Time-course study of soybean hairy roots. Fresh and dry weight of the biomass is taken at every 5 days interval. Data are represented as the mean ± standard error from three replicates

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The growth and saponin production of *Platycodon grandiflorum* (Jacq.) A. DC. (Chinese bellflower) hairy roots cultures maintained in shake flasks and mist bioreactor

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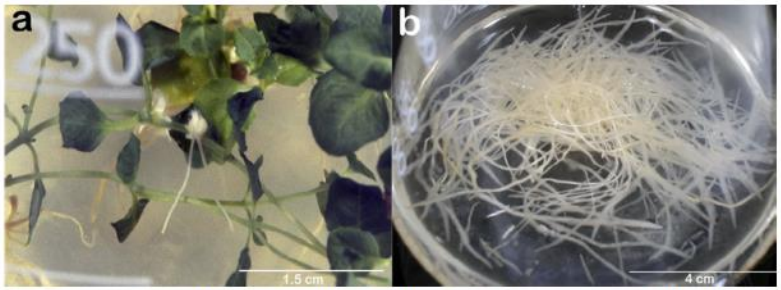

Fig. 1 **a** *Platycodon grandiflorum* hairy roots growing from inoculated site of plantlet, week 2 after inoculation. **b** Hairy roots on hormone-free WPM medium with sucrose (40 g/l). Culture week 2.

Fig. 6 *P. grandiflorum* hairy root biomass of transgenic line Pl 17 after the culture period of 12 weeks in 5-l bioreactor with liquid WPM medium supplemented with 40 g/l sucrose.

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RESEARCH ARTICLE
CENTRO PÚBLICO DE INVESTIGACIÓN CONACYT

Genetic transformation studies and scale up of hairy root culture of *Glycyrrhiza glabra* in bioreactor

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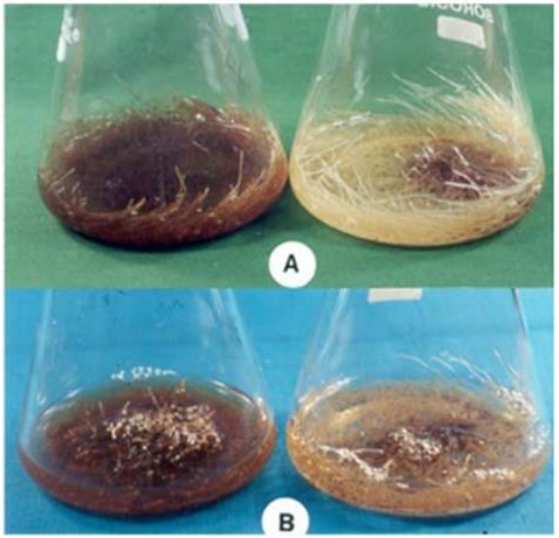

Figure 3. *Agrobacterium rhizogenes* mediated transformed roots of *Glycyrrhiza glabra* cultured in different basal medium. (From left to right) B5 and NB (A); WP and MS (B).

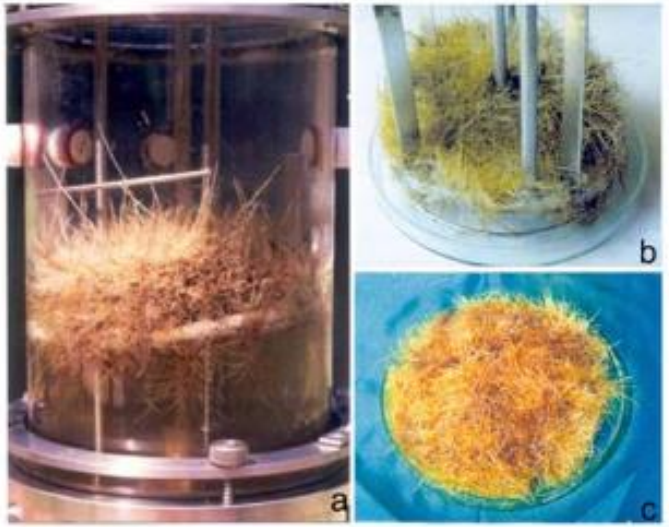

Figure 4. *G. glabra* hairy roots in bioreactor (a) Biomass harvested after 30 day of inoculation (b and c).

Bubble column with
Hairy roots of *B. vulgaris*

Stirred tank reactor with
suspension culture of
Helianthus annuus

3/30/2011

ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Individual and Combined Effects of the *rolA, B,* and *C* Genes on Anthraquinone Production in *Rubia cordifolia* Transformed Calli

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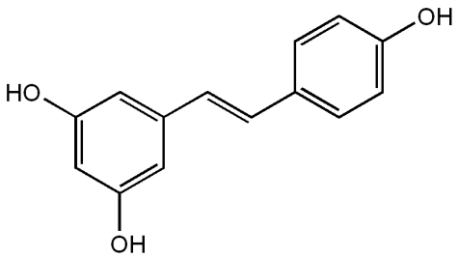


Callos de *Vitis amurensis* transformados con el gen *rolB,* resultan en un aumento de más de 100 veces en la producción de resveratrol (Kiselev et al., 2007). (3,5,4´-trihidroxiestilbeno) es un potente antioxidante (antiedad).

Nc = Testigo negativo (PCR sin ADN)
Pc = Testigo positivo (pPCV002-*rol*ABC)
R = Callos sin transformar (silvestres)
RA = Callos transformados con el gen *rolA*
RABC = Callos transformados con los 3 genes
RA4 = Callos transformados con cepa silvestre de *Agrobacterium rhizogenes*

L, M y H = Bajo, medio y altos niveles de expresión del gen respectivo

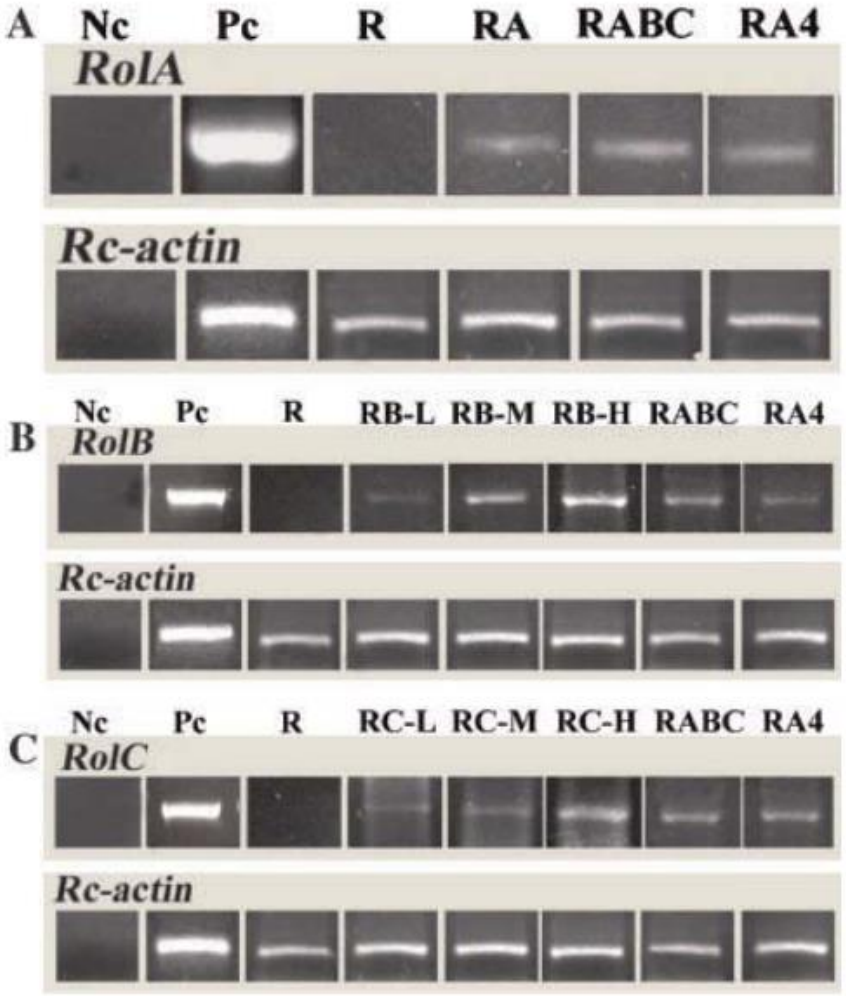


Figure 1. Semiquantitative RT-PCR analysis of *rolA* (**A**), *rolB* (**B**), and *rolC* (**C**) transcripts in comparison with actin gene (*Rc-act*) transcripts. R (untransformed callus line), RA (*rolA*-transformed callus line), RB [*rolB*-transformed calli lines with low (L), medium (M), and high (H) level of *rolB* gene expression], RC [*rolC*-transformed calli lines with low (L), medium (M), and high (H) level of *rolC* gene expression], RABC (callus line transformed with three genes *rolA*, *rolB*, and *rolC*), RA4 (callus line transformed with wild-type strain of *A. rhizogenes* A_4). Total RNA was isolated from the 30-day callus cultures. Nc, negative control (PCR mixture without plant cDNA); Pc, positive control (pPCV002-*rolABC* for the *rol* genes and PCR fragment of the actin cDNA for *Rc-act*).

ICS = Isocorismato sintasa

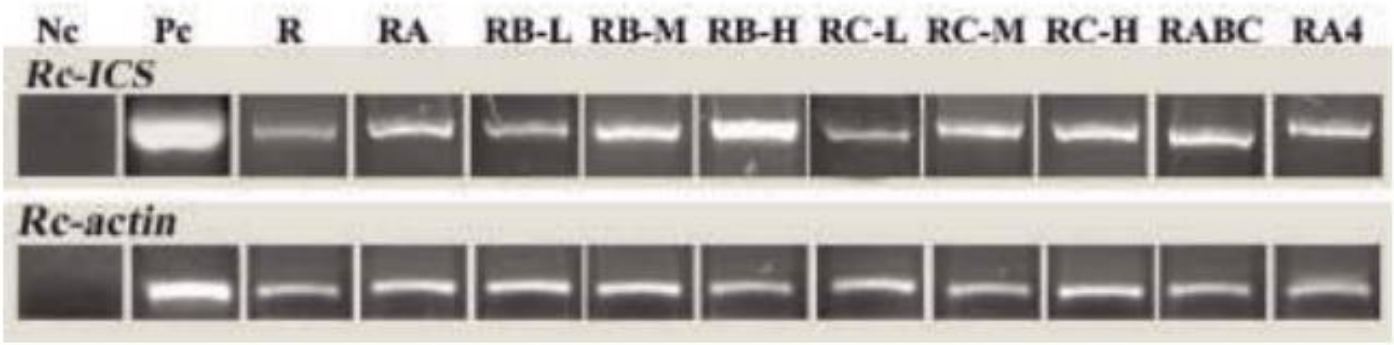


Figure 3. Semiquantitative RT-PCR analysis of *R. cordifolia* isochorismate synthase (*Rc-ICS*) transcripts in comparison with actin (*Rc-act*) transcripts. R (untransformed callus line), RA (*rolA*-transformed callus line), RB [*rolB*-transformed calli lines with low (L), medium (M), and high (H) level of *rolB* gene expression], RC [*rolC*-transformed calli lines with low (L), medium (M), and high (H) level of *rolC* gene expression], RABC (callus line transformed with *rolA*, *rolB*, and *rolC*), RA4 (callus line transformed with wild-type strain of *A. rhizogenes* A_4). Total RNA was isolated from the 30-day callus cultures. Nc, negative control (PCR mixture without plant cDNA); Pc, positive control (PCR fragment of the *ICS* cDNA for *Rc-ICS* and PCR fragment of actin cDNA for *Rc-act*).

ICS = Isocorismato sintasa

Table I. Expression of the *rolA*, *rolB*, *rolC*, *Rc-ICS* genes[a], biomass accumulation[b], and anthraquinone content[c] in non-transgenic and different variants of transgenic callus cultures of *R. cordifolia*.

	Gene expression, RFU					
Cell lines	*rolA*	*rolB*	*rolC*	*Rc-ICS*	Fresh biomass (g)	Total AQ content (% dry wt)
R	—	—	—	0.80 ± 0.12	5.40 ± 0.27	0.41 ± 0.02
RA	1.54 ± 0.11	—	—	4.00 ± 0.30	8.28 ± 0.60	1.10 ± 0.08
RB-L	—	2.10 ± 0.14	—	3.60 ± 0.16	4.53 ± 0.27	1.13 ± 0.01
RB-M	—	8.20 ± 0.30	—	17.00 ± 0.50	3.21 ± 0.21	4.70 ± 0.01
RB-H	—	14.00 ± 0.50	—	26.00 ± 0.90	1.89 ± 0.21	5.85 ± 0.08
RC-L	—	—	1.50 ± 0.10	3.10 ± 0.30	6.21 ± 0.21	0.81 ± 0.02
RC-M	—	—	2.58 ± 0.15	5.00 ± 0.45	4.98 ± 0.39	1.14 ± 0.02
RC-H	—	—	5.60 ± 0.35	9.00 ± 0.60	5.43 ± 0.21	1.78 ± 0.01
RABC	3.15 ± 0.15	7.80 ± 0.40	3.10 ± 0.13	14.00 ± 0.41	3.93 ± 0.24	2.54 ± 0.02
RA_4	2.96 ± 0.17	5.10 ± 0.20	2.90 ± 0.16	10.00 ± 0.60	5.61 ± 0.21	1.80 ± 0.02

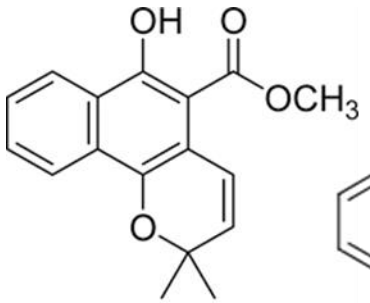
R (untransformed callus line), RA (*rolA*-transformed callus line), RB [*rolB*-transformed calli lines with low (L), medium (M), and high (H) level of *rolB* gene expression], RC [*rolC*-transformed calli lines with low (L), medium (M), and high (H) level of *rolC* gene expression], RABC (callus line transformed with three genes *rolA*, *rolB*, and *rolC*), RA4 (callus line transformed with wild-type strain of *A. rhizogenes* A_4).

[a]Semiquantitative analysis was performed by RT-PCR followed by microchip-based DNA measurement. Data (mean ± SEM) represent measurements of three independent replicates from two different RNA isolations and are presented as relative fluorescent units (RFU) normalized to the expression of the *R. cordifolia* actin gene, as described in the Materials and Methods Section.

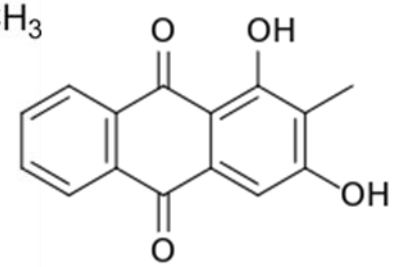
[b]Data are expressed as mean ± SEM from five separate experiments with three replicates each.

[c]Total AQ content representing sum of munjistin and purpurin (% of dry callus weight). Data are expressed as mean ± SEM from five separate experiments with three replicates each.

Las antraquinones (AQs) posen actvidades antimicrobianas y hepatoprotectoras y son consideradas útiles en la desintegración y eliminación de piedras urinarias (Singh et al., 2004). Ejemplos, mollugina, rubiadina,



Mollugin



JOURNAL OF Plant Physiology

Relation Between the Amount of *rol*C Gene Product and Indole Alkaloid Accumulation in *Catharanthus roseus* Transformed Root Cultures

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Received October 30, 1997 · Accepted January 28, 1998

Table 1: Indole alkaloid contents of *C. roseus* transformed root lines at week 6 of culture on B5/2 solid medium. Values are expressed as mg g^{-1} DW ± SD or % of total alkaloid content. Each value is the average of two replicates during 3 successive subcultures.

Line	Vindoline		Ajmalicine		Catharanthine	
	mg g^{-1} DW	% of total	mg g^{-1} DW	% of total	mg g^{-1} DW	% of total
13*	0.197±0.039	14.8	0.490±0.038	36.8	0.644±0.087	48.2
19*	0.321±0.056	23.3	0.789±0.014	42.0	0.768±0.048	40.9
101*	0.342±0.078	17.0	0.808±0.075	38.9	0.925±0.177	44.6
104	0.426±0.076	9.4	1.495±0.298	32.8	2.630±0.713	57.8
106	0.277±0.033	7.1	1.741±0.337	45.0	1.851±0.258	47.8
107	0.244±0.046	6.9	1.621±0.318	44.9	1.743±0.343	48.3
108*	0.192±0.038	14.4	0.423±0.103	31.7	0.721±0.145	53.9
109	0.339±0.059	9.9	1.840±0.335	54.1	1.221±0.231	35.9
110	0.354±0.069	6.6	2.106±0.385	39.6	2.864±0.558	53.8
114	0.321±0.023	7.3	2.308±0.457	52.8	1.740±0.336	39.8
115	0.533±0.098	14.3	1.243±0.239	33.2	1.940±0.357	52.2
116	0.261±0.052	7.4	1.828±0.359	51.9	1.436±0.139	40.7
117	0.652±0.123	13.7	1.306±0.306	27.4	2.810±0.548	58.9
118	0.609±0.119	14.9	1.337±0.257	32.7	2.142±0.386	52.4
126	0.612±0.099	11.1	1.935±0.375	35.2	2.956±0.572	53.7
127	0.253±0.051	4.7	2.660±0.518	49.1	2.504±0.498	46.2
128	0.399±0.076	10.3	1.430±0.275	37.1	2.028±0.386	52.6
137	0.338±0.025	6.9	1.869±0.359	38.6	2.630±0.585	54.4
140*	0.113±0.022	11.7	0.513±0.106	53.3	0.336±0.015	34.9
141	0.275±0.052	6.3	2.166±0.415	49.4	1.939±0.376	44.3
146	0.275±0.053	10.3	1.032±0.180	38.6	1.370±0.265	51.2
152	0.417±0.079	10.6	1.141±0.218	28.9	2.436±0.493	61.8
154	0.783±0.158	15.2	1.679±0.335	32.5	2.689±0.710	52.3

* Roots with thick morphology.

園学雑. (J. Japan. Soc. Hort. Sci.) 75 (4): 312–317. 2006.

Morphological and Physiological Changes in Transgenic *Chrysanthemum morifolium* Ramat. 'Ogura-nishiki' with *rolC*

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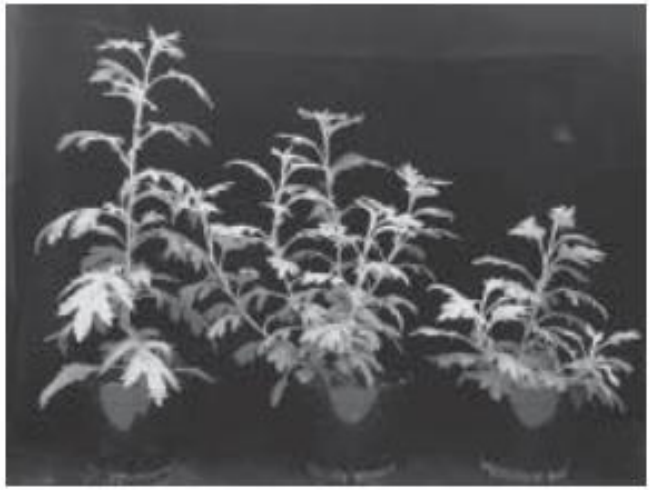

Wild-type oprolC-17 oprolC-18

Fig. 3. Morphological differences between a wild-type and *rolC*-transformed plants grown in a greenhouse kept at 25°C for 8 weeks.

Table 1. Number of shoots, and shoot and internode lengths of wild-type and transformants 12 weeks after being transferred to a greenhouse.

	Number of shoots[z]	Shoot length[y] (cm)	Internode length[x] (cm)
Wild-type	1.0 ± 0[w]	39.9 ± 0.6	2.4 ± 0.1
oprolC-17	4.2 ± 0.2*	25.3 ± 2.1*	1.3 ± 0.1*
oprolC-18	3.8 ± 0.2*	15.9 ± 2.2*	0.8 ± 0.2*

* indicates significant differences between *rolC*-transgenic and wild-type lines according to the *t*-test ($P < 0.05$).
[z] Numbers of shoots over 5 cm were counted.
[y] Shoot lengths of the longest shoots were measured.
[x] Average values of the fifth to tenth leaves.
[w] Means ± SE (n = 5).

Table 2. Florets number and length of wild-type and transformants.

	Floret number per inflorescence[z]		Floret length (cm)[y]	
	Tubular florets	Ray florets	Tubular florets	Ray florets
Wild-type	68.5 ± 6.9[x]	49.8 ± 6.1	0.73 ± 0.08	3.0 ± 0.2
oprolC-17	75.8 ± 9.6	43.2 ± 8.2	0.71 ± 0.13	2.0 ± 0.3*
oprolC-18	59.0 ± 9.6	43.3 ± 6.6	0.79 ± 0.12	1.7 ± 0.2*

* indicates significant differences between *rolC*-transgenic and wild-type lines according to the *t*-test ($P < 0.05$).
[z] The numbers of ray and tubular florets were counted from a random sample of 10 inflorescences.
[y] The length of each five of ray and tubular florets per inflorescence were measured.
[x] Means ± SE (n = 10).

Wild-type *rolC*-transformants

Fig. 4. Morphological differences between inflorescences of a wild-type and *rolC*-transformant.

The plant oncogene *rolD* encodes a functional ornithine cyclodeaminase

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PNAS | November 6, 2001 | vol. 98 | no. 23 | 13449–13453

The plant oncogene *rolD* stimulates the reproductive phase transition in plants. We define here the function of its gene product. We show that the RolD protein bears sequence homology with ornithine cyclodeaminase, an uncommon enzyme of specialized-niche eubacteria and archaea that catalyzes the unusual NAD-dependent conversion of ornithine to proline. To confirm the prediction of the bioinformatic analysis, the RolD protein was expressed in *Escherichia coli* and purifed. An ornithine-dependent *NAD* Reduction that can be ascribed only to ornithine cyclodeaminase (OCD) activity was detected both in bacterial extracts containing RolD and in assays on the purified RolD protein. Furthermore, OCD activity was observed in soluble extracts from plants overexpressing *rolD*. The role of rolD in plant pathogenesis and its effect on plant reproductive development are discussed in light of the newly demonstrated enzymatic activity of its gene product.

DEVELOPMENTAL BIOLOGY 180, 693–700 (1996)
ARTICLE NO. 0338

The Plant Oncogene *rolD* Stimulates Flowering in Transgenic Tobacco Plants

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FIG. 1. A vegetative untransformed SR1 plant (left) and a flowering *rolD* plant (right) at the stage of 10–11 internodes.
FIG. 3. A flowering untransformed SR1 plant (left) and a flowering *rolD* plant after the development of sylleptic axillary inflorescences (right).

Theor Appl Genet (2003) 107:831–836
DOI 10.1007/s00122-003-1322-0

P. Bettini · S. Michelotti · D. Bindi · R. Giannini ·
M. Capuana · M. Buiatti

Pleiotropic effect of the insertion of the *Agrobacterium rhizogenes rolD* gene in tomato (*Lycopersicon esculentum* Mill.)

Table 1 Student's *t*-test analysis for characters observed in the *rolD*-transgenic plants and the corresponding untransformed regenerated controls

Character	Groups	Mean ± SE	*df*	*t*	*P*-value
No. inflorescences/plant	rolD3	15.1 ± 1.8	21	0.6	n.s.
	Controls	14 ± 1			
	rolD4	17 ± 0.8	20	1.5	n.s.
	Controls	14 ± 1			
	rolD23	22.6 ± 2.5	20	3.7	< 0.01
	controls	14 ± 1			
No. fruits/plant	rolD3	40.5 ± 1.8	21	4.3	< 0.01
	Controls	23.6 ± 2.2			
	rolD4	37.6 ± 2.2	20	3.3	< 0.01
	Controls	23.6 ± 2.2			
	rolD23	37.6 ± 1.3	20	3.4	< 0.01
	Controls	23.6 ± 2.2			
Mean weight fruits/plant	rolD3	8.3 ± 0.3	21	2	n.s.
	Controls	9.2 ± 0.2			
	rolD4	10 ± 0.35	20	1.8	n.s.
	Controls	9.2 ± 0.2			
	rolD23	9.2 ± 0.7	20	0.012	n.s.
	Controls	9.2 ± 0.2			
Plant height (first inflorescence)	rolD3	16.5 ± 1.4	22	1.067	n.s.
	Controls	18 ± 0.6			
	rolD4	19.6 ± 2	21	0.9	n.s.
	Controls	18 ± 0.6			
	rolD23	21 ± 1.8	21	1.82	n.s.
	Controls	18 ± 0.6			
Plant height (first fruit)	rolD3	68 ± 4.4	21	1.6	n.s.
	Controls	59.5 ± 2.6			
	rolD4	66.2 ± 3.1	20	1.28	n.s.
	Controls	59.5 ± 2.6			
	rolD23	64.2 ± 0.6	20	0.9	n.s.
	Controls	59.5 ± 2.6			
No. days before flowering	rolD3	15.6 ± 0.8	22	3.5	< 0.01
	Controls	18.5 ± 0.4			
	rolD4	15.2 ± 0.9	21	3.8	< 0.01
	Controls	18.5 ± 0.4			
	rolD23	15.8 ± 0.7	21	3.3	< 0.01
	Controls	18.5 ± 0.4			

Plantas transgénicas a los genes *rol*

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Biotechnology Advances 23 (2005) 3–39

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Research review paper

Influence of *rol* genes in floriculture

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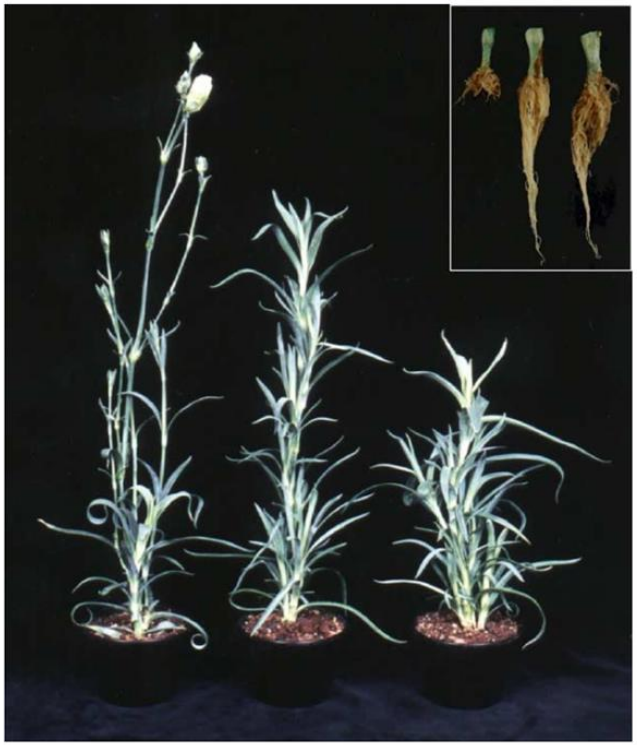


Fig. 2. Morphological alterations and root formation in *rolC*-transgenic carnation plants. Left, control (*uidA*-transgenic) carnations; middle and right, *rolC*-transgenic carnations.

In Vitro Cell.Dev.Biol.—Plant (2007) 43:383–403
DOI 10.1007/s11627-007-9096-8

REVIEW

Agrobacterium rhizogenes: recent developments and promising applications

Veena Veena · Christopher G. Taylor

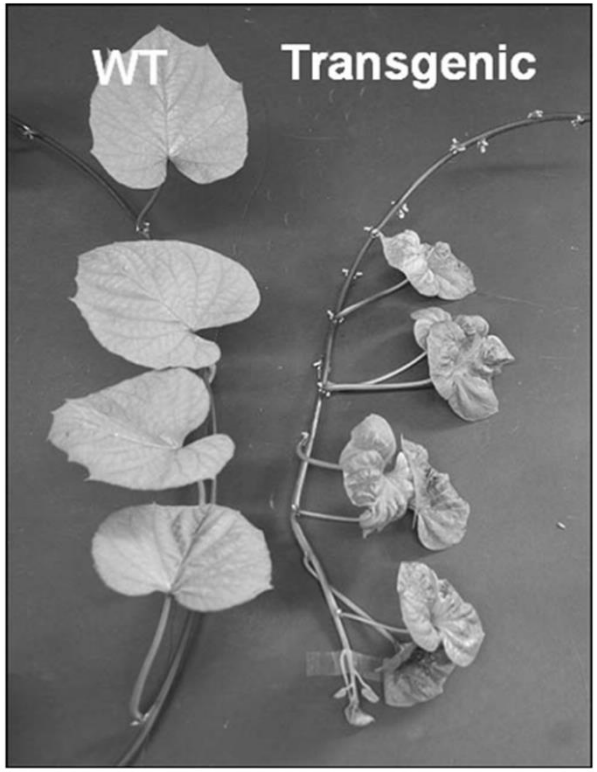


Figure 3. Hairy root phenotype of sweet potato stably transformed by *A. rhizogenes* and regenerated from transgenic roots. Note the short internode distances, curled leaves, and adventitious root buds at the nodes.

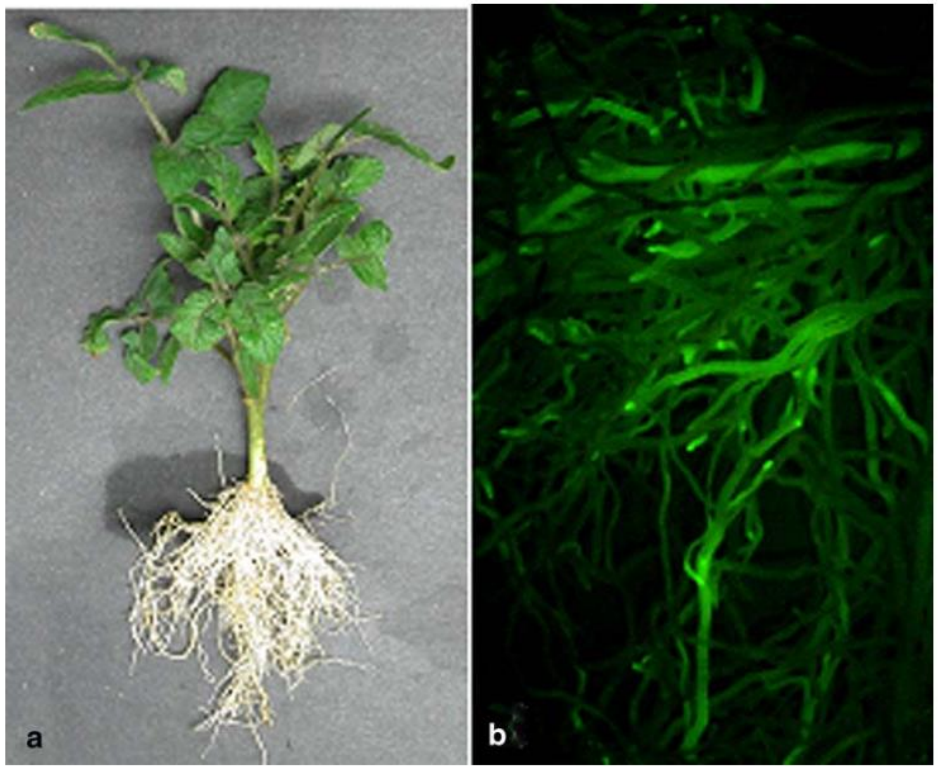


Figure 4. Composite tomato plants. (*a*) Transgenic roots produced on wild-type shoots of tomato after infection with *A. rhizogenes*. (*b*) Expression of GFP scorable marker in transgenic roots.

Europ.J.Hort.Sci., 74 (6). S. 275–287, 2009, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

The Use of *Agrobacterium rhizogenes* and its *rol*-Genes for Quality Improvement in Ornamentals

B. Christensen[1] and R. Müller[2]
([1]AgroTech A/S, Taastrup, Denmark and [2]University of Copenhagen, Faculty of Life Sciences, Taastrup, Denmark)

Table 3. Examples of phenotypical changes induced by *rol*A-gene in different plant species.

Plant species	Promoter	Phenotype	Reference
Daucus carota (Carrot)	Native	Increased number of shoots. Some plants converted from biennial to annual flowering	(Limami et al. 1998)
Lycopersicon esculentum (Tomato)	Native	Small, dark-green, strongly wrinkled leaves, high ratio between dry weight and leaf area, long internodes, reduced flower bud length, hyperstyly, reduced pollen production. Male sterility	(Van Altvorst et al. 1992)
Malus x domestica M26 (Apple rootstock)	Native	Reduced stem growth, leaf area and internode length, some transformants showed reduced leaf area, shoot dry weight and plant dry weight	(Holefors et al. 1998)
Nicotiana tabacum (Tobacco)	Native	Wrinkled leaves, condensed flower inflorescence, increased stigma size, larger flowers	(Schmulling et al. 1988)
	Native	Severely stunted, extremely reduced internode length, altered leaf morphology, severe leaf wrinkling, low length-to-width ratio	(Sinkar et al. 1988a)
	Native	Wrinkled leaves, reduced internode length, deficient root growth	(Carneiro and Vilaine 1993)
	Native	Male sterility, abnormal flower morphology, wrinkled leaves and shortened internodes	(Sun et al. 1991a)
	35S	Wrinkled leaves, reduced leaf size, reduced number of flowers, altered flower morphology, short internodes, inhibited or delayed flowering, compact inflorescence, shorter flowers, female and male sterility	(Martin-Tanguy et al. 1993, 1996)
	35S	Stunted growth, dark green, wrinkled leaves, changed leaf length to width ratio, delayed flowering, reduced number of flowers, condenses inflorescence, reduced length of styles	(Dehio et al. 1993)
Oryza sativa (Rice)	35S	Dark, severely, wrinkled leaves, reduced number of branches	(Lee et al. 2001)

Table 4. Examples of phenotypical changes induced by *rol*B-gene in different plant species.

Plant species	Promoter	Phenotype	Reference
Actinidia deliciosa (Kiwi)	Native	Normal phenotype, increased rooting ability	(Rugini et al. 1997)
Lycopersicon esculentum (Tomato)	Native	Wider and shorter leaves without leaf wrinkling, strong reduction of apical dominance, reduced number of flowers	(Van Altvorst et al. 1992)
Malus x domestica 'Florina' (Apple)	Native	Increased rooting ability and root fresh weight. No other change in phenotype	(Radchuk and Korkhovoy 2005)
Malus x domestica M.9/29 (Apple rootstock)	Native	Increased *in vitro* rooting, reduced number of nodes, reduced stem length, same relative growth rate, root length and morphology as control plants	(Zhu et al. 2001b)
Medicago sativa (Alfalfa, lucerne)	Native	Delayed flowering, increased stem number, increased root and shoot dry weight	(Frugis et al. 1995)
Nicotiana tabacum (Tobacco)	Native	Altered leaf morphology, increased stigma, increased flower size, abnormal flowers, adventitious roots on stems, slightly reduced pollen production	(Schmulling et al. 1988)
	Native	Wrinkled, dark-green leaves, excessive, partially non-geotropic roots	(Cardarelli et al. 1987)
	35S	Necrotic leaves, rounded leaf edge, heterostyly	(Schmulling et al. 1988)

Table 5. Examples of phenotypical changes induced by *rol*C-gene in different plant species.

Plant species	Promoter	Phenotype	Reference
Atropa belladonna (Deadly nightshade)	35S	Reduced apical dominance, pale and lanceolated leaves, smaller flowers, increased flowering	(Kurioka et al. 1992)
	Native	Normal phenotype	(Kurioka et al. 1992)
Cichorium intybus (Belgian Endive)	Native	Conversion from biennial to annual flowering	(Kamada et al. 1992)
Chrysanthemum morifolium (Chrysanthemum)	Native	Reduced plant height and internode length, increased number of shoots	(Kubo et al. 2006)
Dianthus caryophyllus (Carnation)	35S	Reduced apical dominance, increased number of lateral shoots, flowering and rooting ability	(Zuker et al. 2001)
Diospyros kaki (Japanese persimmon)	35S	Reduced plant height and internode length and leaf size, increased number of lateral shoots	(Koshita et al. 2002)
Nicotiana tabacum (Tobacco)	Native	Altered leaf morphology, increased branching, reduced flower size, pollen production and size of seed capsules	(Schmulling et al. 1988)
	Native	Reduced plant height, internode length and leaf size, earlier flowering, reduced flower size, pollen viability, size of seed capsules and number of seeds	(Scorza et al. 1994)
	Native	Reduced plant height, apical dominance, and flower size	(Oono et al. 1987)
	35S	Dwarf and bushy due to decreased internode length, increased number of shoots and leaves, small lanceolated leaves, reduced female fertility, male sterility	(Schmulling et al. 1988)
	35S	Reduced apical dominance and internode length, increased number of nodes, light green, lanceolate leaves, reduced flower size, male sterility	(Nilsson et al. 1993)
	35S	Reduced height and flower size, altered leaf shape, slightly increased petiole length, male sterile	(Martin-Tanguy et al. 1993)
	35S	Reduced plant height, lanceolated leaves, earlier flowering, reduced fertility	(Faiss et al. 1996)
Osteospermum ecklonis (African daisy)	35S	Pale green leaves, erect plant habit, earlier flowering, increased number of flowers	(Giovannini et al. 1999)
Pelargonium x *domestica* (Regal pelargonium)	35S	Reduced plant height, leaf area and flower diameter, earlier flowering	(Boase et al. 2004)

Table 6. Examples of phenotypical changes induced by *rol*D-gene in different plant species.

Plant species	Promoter	Phenotype	Reference
Daucus carota (Carrot)	Native	Severely dwarfed, wrinkled leaves, curved petioles	(Limami et al. 1998)
Lycopersicon esculentum (Tomato)	Native	Early flowering, increased number of inflorescences, higher fruit yield, perfect normal fruits and fertile seeds.	(Bettini et al. 2003)
Nicotiana tabacum (Tobacco)	Native	Early flowering, increased number of inflorescences	(Mauro et al. 1996)
	Native/35S	Normal phenotype	(Lemcke and Schmulling 1998)

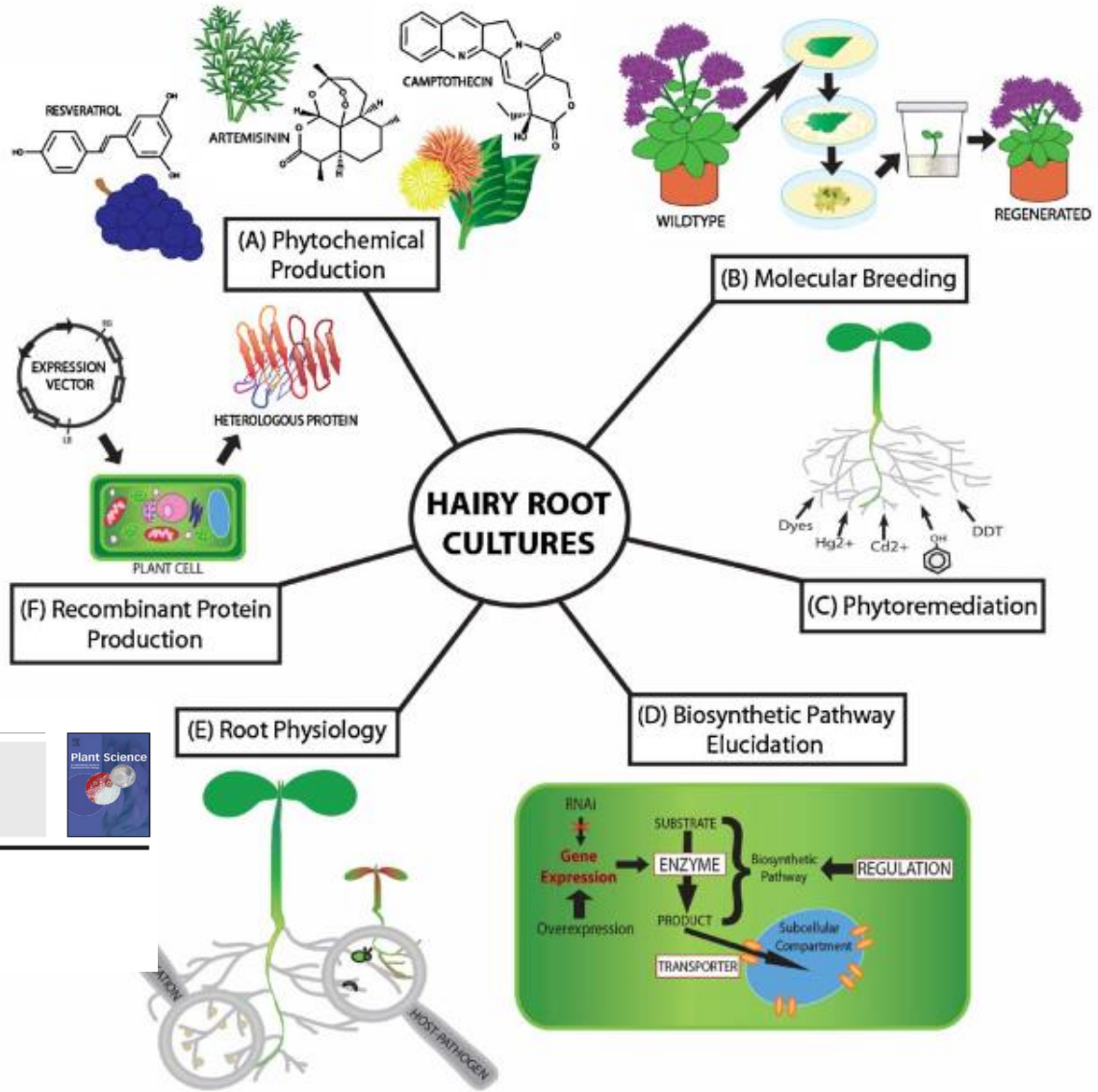
Chapter 1
Agrobacterium rhizogenes-Mediated Transformation and Its Biotechnological Applications in Crops

Ibrahim Ilker Ozyigit, Ilhan Dogan and Ebru Artam Tarhan

K. R. Hakeem et al. (eds.), *Crop Improvement*, DOI 10.1007/978-1-4614-7028-1_1,

Table 1.1 Oncogenes of *A. rhizogenes*, their encoded proteins, functions and phenotypic changes in host plants

Gene	Protein	Function	Phenotype
*rol*A	Sequence motif common in DNA-binding proteins Regulatory transcription factor	Inhibits cell elongation via diffusible factor Decreases hormone concentrations Increase sensitivity to auxin Modulating hormone physiology of GA Interfere polyamine metabolism Correlate with plasma membrane H^+ ATPase activity	Stunted growth, dark green wrinkled leaves with an altered length to width ratio, condensed inflorescences, retarded onset of flowering, compact reduced number of flowers
*rol*B	Localizes to plasma membrane	Alterations in the reception/transduction of the auxin signal Stimulates new meristem formation Induce secondary metabolism	Fast growth, root meristem neoformation, high branching and plagiotropism
*rol*C	Phloem-specific expression in the root, low expression in the leaf, and no expression in the shoot tip	Reduces cell size Reduces abscisic acid (ABA), polyamine, and ethylene levels Formation of shoot meristems Regulate sugar metabolism and transport Stimulate the production of high levels of secondary metabolites	Increased branching, dwarfed plants with short internodes, reduced epidermal cell size in internodes, lanceolate leaves, early flowering, reduced flower size and reduced pollen production
*rol*D	Only expresses in Agropine type strains Cytosolic protein Exhibits poor tissue- or organ-specific expression	Incapable of inducing root formation on its own Provide defense response as a result of environmental stress	Increased flowering, reduced rooting, elongating and expanding tissues of each organ but not on apical meristem, callus growth giving rise to initiation of tumor resemble formation



Plant Science 180 (2011) 439–446

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journal homepage: www.elsevier.com/locate/plantsci

Review

The multiplicity of hairy root cultures: Prolific possibilities

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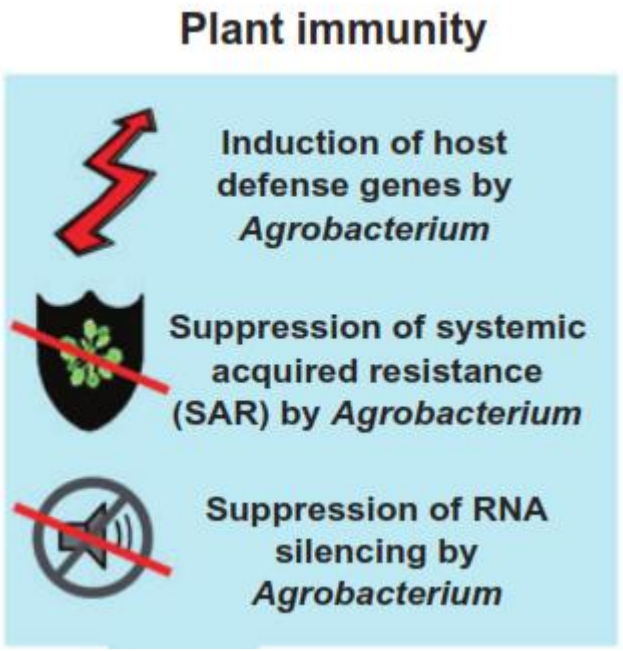
Fig. 1. The diverse and abundant uses of hairy root cultures. (A) *Phytochemical production* in hairy roots is a major topic of study that spans several classes of phytochemicals, including alkaloids, terpenoids, and phenolics. (B) *Molecular breeding* by infection of ornamental plants with *Agrobacterium rhizogenes* and regeneration of whole plants from hairy roots yields plants with desirable phenotypes, such as compact size, for horticultural purposes. (C) Hairy root culture has been used as a model system for studying *Phytoremediation* of toxic substances and reactive dyes. (D) Molecular, biochemical and genetic studies in hairy roots have accelerated *Biosynthetic pathway elucidation* for phytochemicals, which, in turn, facilitates metabolic engineering in hairy root cultures. (E) *Root physiology* studies ranging from nitrogen fixation, iron-deficiency, aluminum toxicity, to host–pathogen interactions have been conducted in hairy root cultures. (F) *Recombinant protein production* in this system has been explored as a rapid, contained, low-cost, genetically stable means of producing human antibodies, cytokines, and other protein therapeutics.

RECALCITRANCIA A LA TRANSFORMACIÓN GENÉTICA VÍA *AGROBACTERIUM*?

Genotipos y sus metabolitos
AIA
Acido salicilico
Etileno
Mutantes rats



Citovsky V. et al. 2007. Biological systems of the host cell involved in *Agrobacterium infection.* **Cellular Microbiology** . **9(1): 9–20**

PAMPS = **P**athogen **A**ssociates **M**olecular **P**atterns

PTI = **P**AMP **T**riggered **I**mmunity

ETS = **E**ffector-**T**riggered **S**usceptibility

ETI = **E**ffector-**T**riggered **I**mmunity)

PRRs = **P**attern **R**ecognition **R**eceptor**s**

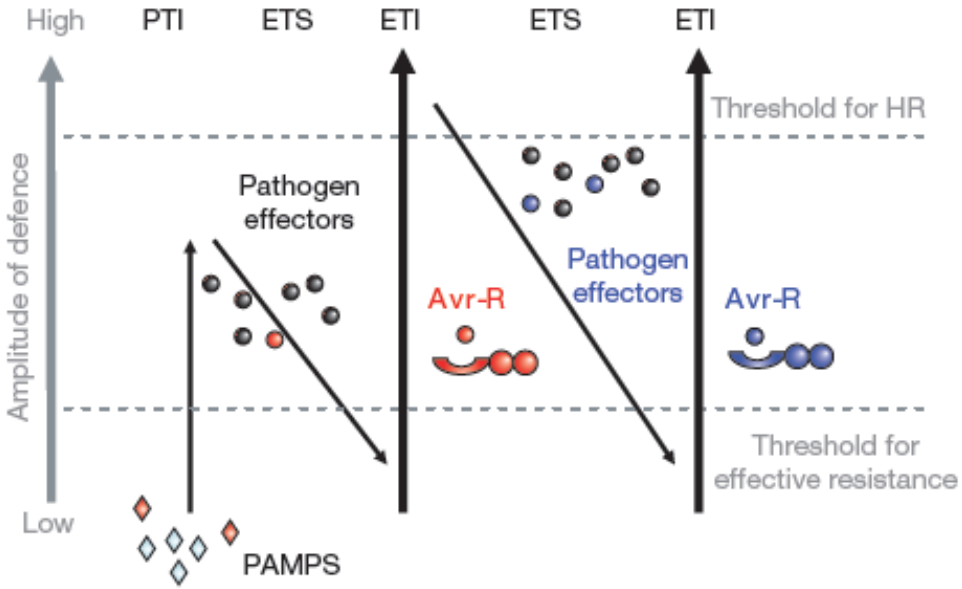


Figure 1 | A zigzag model illustrates the quantitative output of the plant immune system. In this scheme, the ultimate amplitude of disease resistance or susceptibility is proportional to [PTI – ETS + ETI]. In phase 1, plants detect microbial/pathogen-associated molecular patterns (MAMPs/PAMPs, red diamonds) via PRRs to trigger PAMP-triggered immunity (PTI). In phase 2, successful pathogens deliver effectors that interfere with PTI, or otherwise enable pathogen nutrition and dispersal, resulting in effector-triggered susceptibility (ETS). In phase 3, one effector (indicated in red) is recognized by an NB-LRR protein, activating effector-triggered immunity (ETI), an amplified version of PTI that often passes a threshold for induction of hypersensitive cell death (HR). In phase 4, pathogen isolates are selected that have lost the red effector, and perhaps gained new effectors through horizontal gene flow (in blue)—these can help pathogens to suppress ETI. Selection favours new plant NB-LRR alleles that can recognize one of the newly acquired effectors, resulting again in ETI.

Jones J. D. G. 1 & Dangl J.L. 2006. The plant inmmune sytems. Nature 444.323-329

TRANSFORMACIONES GENÉTICAS DE MONOCOTILEDÓNEAS

Arroz	**Hiei et al. 1994; 1997** **Kant et al. 2007** **Toki et al. 1997**
Cebada	**Tingay et al. 1997** **Shrawat et al 2007**
Trigo	**Cheng et al. 1997**
Sorgo	**Zhao et al. 2000** **Carlos et al. 2004** **Carvalho et al. 2004**
Maíz	**Ishida et al. 1996**

DEPENDIENTE DEL GENOTIPO

Arroz (40 genotipos de Japonica, Indica y Javonica)

Cebada (cvs . Golden Promise y Ingri)

Trigo (cv. Bobwhite)

Maíz (cv. A188 o sus híbridos)

Caña de azúcar (cv . Ja 60-5)

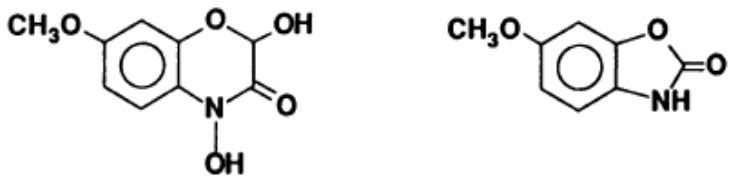
¿A QUE SE DEBE?

LA DIFERENCIA EN LA SUSCEPTIBILIDAD DE LOS GENOTIPOS A LA TRANSFORMACIÓN

1. Presencia de un sistema de inhibición de la maquinaria de *Agrobacterium*

DIMBOA

Homogenados de plántulas de maíz. Inhibían el crecimiento y virulencia *Agrobacterium tumefaciens.* Y era causado por el 2,4-dihidroxi-7-metoxi-2H-1,4-benzoxazinona



1: DIMBOA 2: MBOA

FIG. 1. Structure of DIMBOA and MBOA.

DIMBOA a 0.5 mM bloquea completamente el crecimiento de *Agrobacterium*. El maíz contienen entre 1-20 mM de DIMBOA, dependiendo de la variedad, localización y estado de desarrollo.

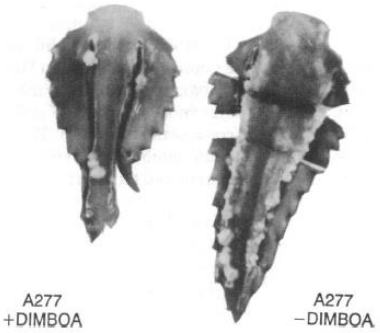


FIG. 7. *Kalanchoe* leaves inoculated with *A. tumefaciens* A277 in the presence of 25 μl of 0.5 mM DIMBOA.

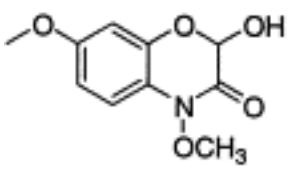
Sahi S.V., Chilton M.D. and W.S. 1990. Corn metabolites affect growth and virulence of *Agrobacterium tumefaciens*. PNAS. 87: 3879-3883,

¿A QUE SE DEBE?

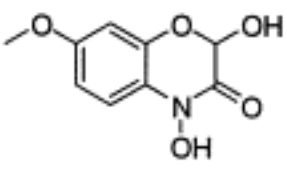
LA DIFERENCIA EN LA SUSCEPTIBILIDAD DE LOS GENOTIPOS A LA TRANSFORMACIÓN

MDIBOA (2-hidroxi-4,7-dimetoxibenzoxazinona)

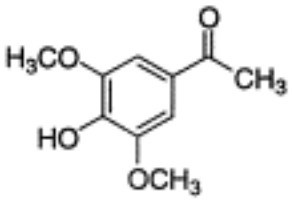
Es una molécula similar a DIMBOA y se encuentra presente a alta concentraciones (98%) en los exudados de plántulas de maíz. Es un inhibidor mas potente de la virulencia de *Agrobacterium* y un efecto más limitado en su crecimiento.



MDIBOA



DIMBOA



AS

Chemistry & Biology

Inhibidor de la cinasa de histidina (histidine kinase)

Zhang et al. 2000. At the maize/*Agrobacterium* interface: natural factors limiting host transformation. Chemistry & Biology. 7: 611-621

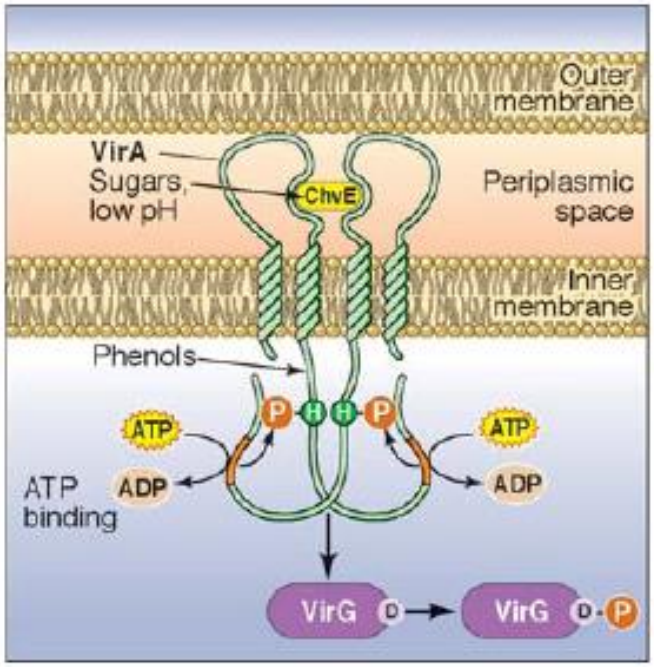
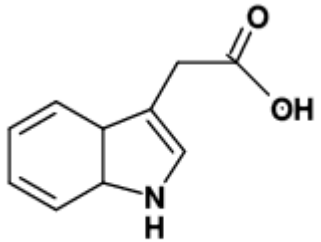


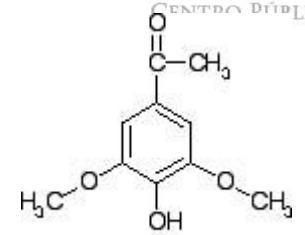
Figure 2

The ChvE/VirA/VirG signal transducing system (note that stoichiometry of ChvE:VirA is not known). See text for details.

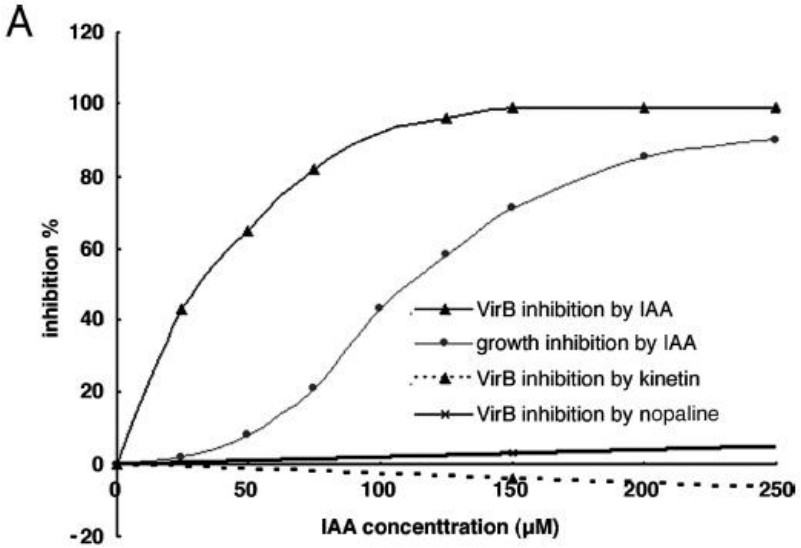
McCullen C.A. and Binns A.N. *2006. Agrobacterium tumefaciens* and Plant Cell Interactions and Activities Required for Interkingdom Macromolecular Transfer. **Annu. Rev. Cell. Dev. Biol. 22: 101-127**



Indole-3-Acetic Acid



Acetosyringone



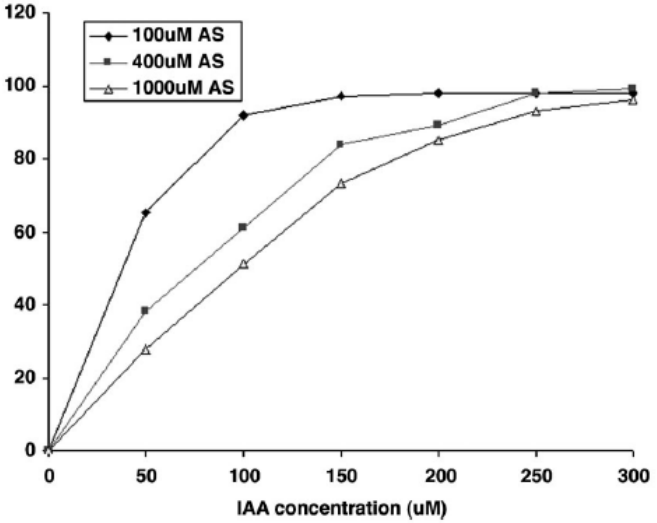
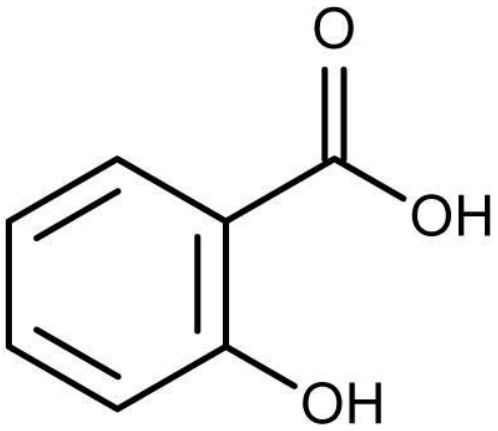


Fig. 3. Competition between IAA and AS.

Fig. 1. The effect of IAA on *vir gene expression and growth of A. tumefaciens.*
(A) Effect of IAA, kinetin, and nopaline on virB gene expression.
(B) Only one concentration of kinetin and nopaline was used.

AIA reprime la expresión de los genes *vir* por competir con la acetosiringona en su interacción con VirA

Liu P. and Nester E.W. 2006. Indoleacetic acid, a product of transferred DNA, inhibits vir gene expression and growth of *Agrobacterium tumefaciens* C58. PNAS.103(12): 4658–4662



ACIDO SALICÍLICO (SA)

La evidencia sugiere que SA atenúa la función del dominio cinasa de VirA

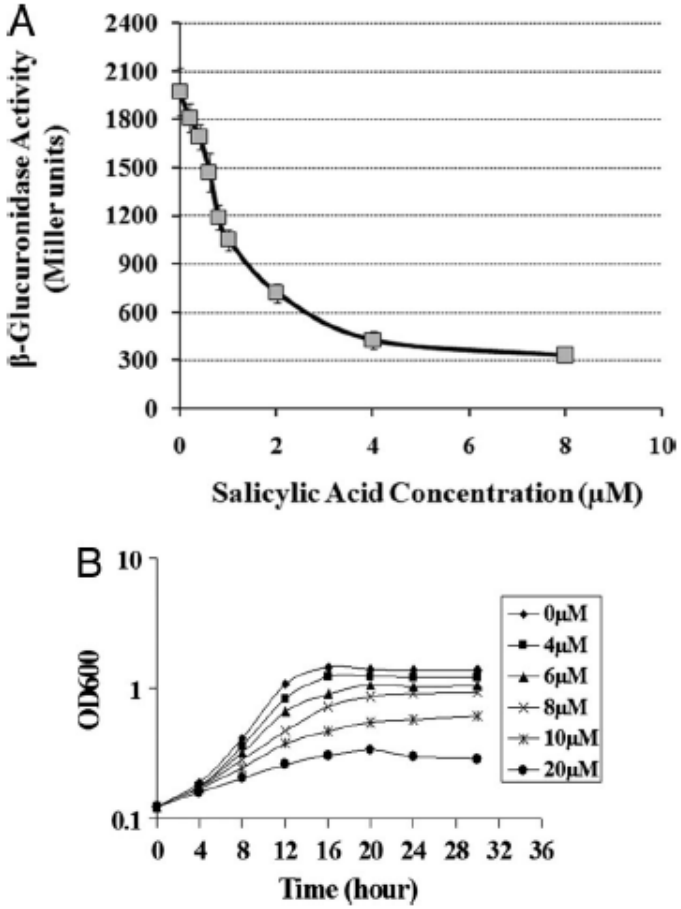
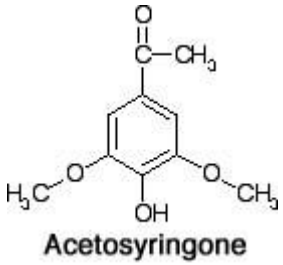
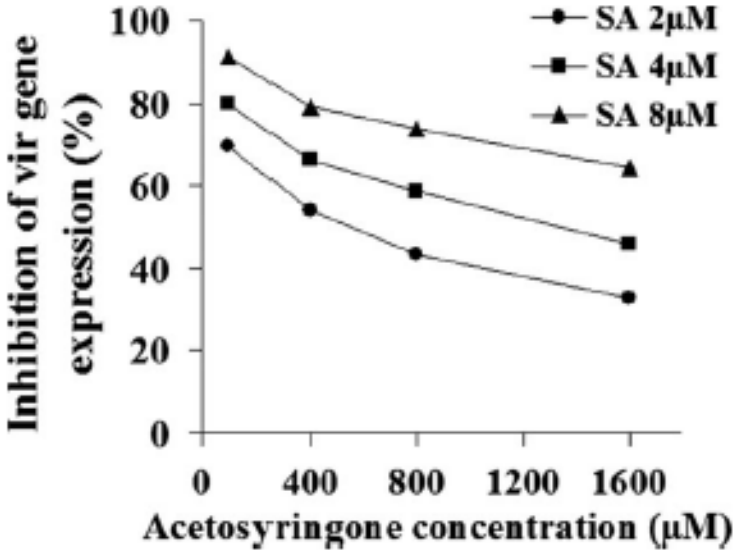


Fig. 1. SA inhibits *vir* gene induction and, at higher concentrations, bacterial growth. (*A*) Effect of SA on *Agrobacterium vir* gene expression. Expression of a plasmid-borne *virB1*::*gusA* transcriptional gene fusion in *Agrobacterium* C58 grown in induction medium with acetosyringone (100 μM), carbenicillin (100 μg/ml), and various concentrations of SA (0–8 μM) is shown. After 16 h, β-glucuronidase activity was measured as described in *Materials and Methods*. (*B*) Effect of SA on *Agrobacterium* growth. *Agrobacterium* growth under various concentrations of SA (0–20 μM) is shown. Cells were grown in acidified AB minimal medium (pH 5.5) supplied with various concentrations of SA. Readings at A_{600} (OD600) are the mean of three independent experiments.

Yuan et al. 2007. The plant signal salicylic acid shuts down expression of the vir regulon and activates quormone-quenching genes in *Agrobacterium.* PNAS 104 (28): 11790–11795



Acetosyringone



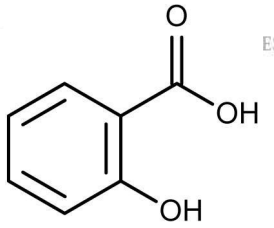
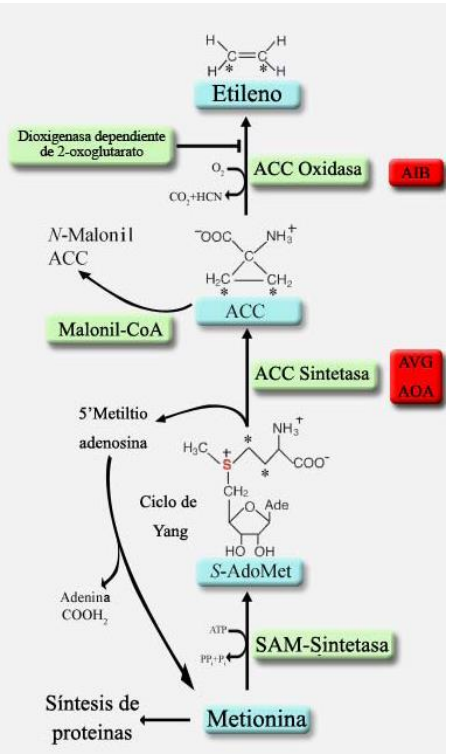


Fig. 4. Increasing acetosyringone attenuates the inhibition of *vir* gene expression by SA. Acetosyringone dose–response curves were obtained for cells grown in the absence or presence of the indicated concentrations of SA for 24 h, and the percentage inhibition in *virB1*::*gusA* expression was calculated. The data represent the averages of three independent determinations.

Mutantes de *Arabidopsis* defectuosas en la acumulación de SA son mas susceptibles a la infección por *Agrobacterium* , mientras que las sobreproductoras de SA son mas resistentes

Lo mismo ocurre con mutantes de *Nicotiana benthamiana* (Anand et al. 2008)

Yuan et al. 2007. The plant signal salicylic acid shuts down expression of the vir regulon and activates quormone-quenching genes in *Agrobacterium.* PNAS 104 (28): 11790–11795



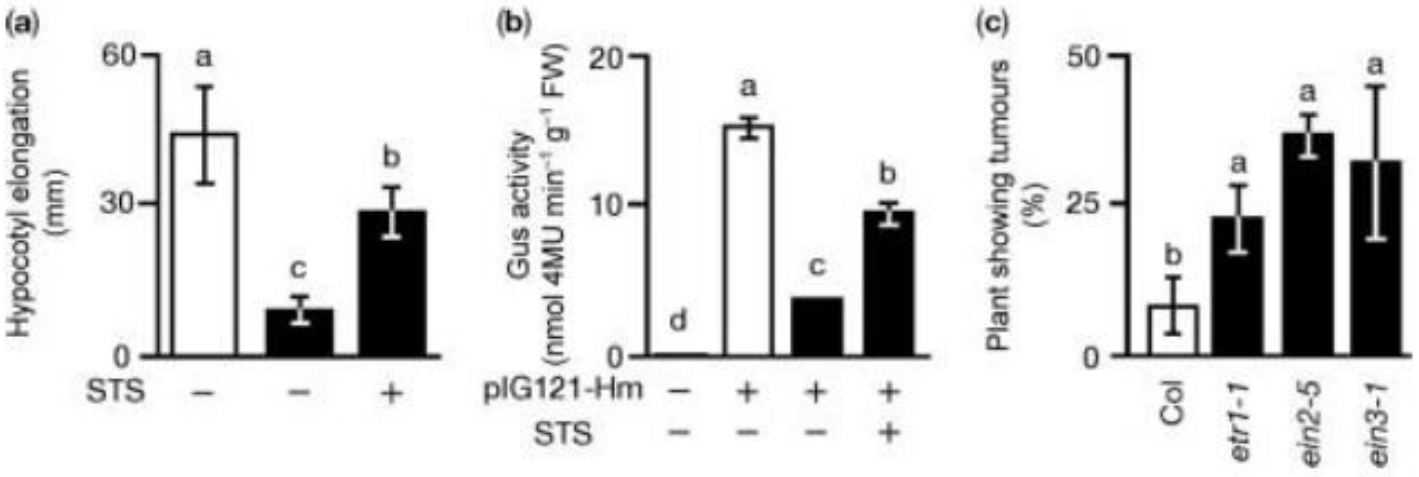


Fig. 1 Effect of the ethylene response on T-DNA transfer in melon (*Cucumis melo)* cotyledon segments and the ethylene *response in Arabidopsis* mutants. (a) Hypocotyl lengths of the melon seedlings. Seedlings grown under light on medium with (+) or without (–) 100 μ M silver thiosulfate (STS) were measured. The open and closed columns represent the absence and presence of 1-aminocyclopropane-1-carboxylic acid (ACC) in the medium, respectively. Bars indicate SD (*n = 30*). *(b)* Occurrence of T-DNA transfer in segments of seedling cotyledons. The occurrence of T-DNA transfer was indicated by ß-glucuronidase (GUS) activity in the segments. Bars indicate SD (*n = 3*). The open and closed columns represent the absence and presence of ACC, respectively. ACC was added to the germination and co-cultivation media. STS was applied only to the germination medium. *Agrobacterium* cells without (–) or carrying (+) the plasmid pIG121-Hm. Bacterial cell suspensions were prepared at 10^8 cells ml–1for inoculation. Bars indicate SD (n = 3). The letters indicate statistical significance at the 5% confidence level based on Student's t-test. *(c)* Frequency of tumour formation in ethylene-insensitive *Arabidopsis mutants*. Each value is the average of three independent experiments. The letters represent statistically significant differences based on chi-square testing (*P < 0.05).*

Nonaka S. et al. 2008. Ethylene production in plants during transformation suppresses *vir* gene expression in *Agrobacterium tumefaciens*. New Phytologist.***178: 647–656***

Resistant to Agrobacterium Transformation

Identification of Arabidopsis *rat* Mutants

Yanmin Zhu, Jaesung Nam, Jaime M. Humara, Kirankumar S. Mysore, Lan-Ying Lee, Hongbin Cao, Lisa Valentine, Jingling Li, Anthony D. Kaiser, Andrea L. Kopecky, Hau-Hsuan Hwang, Saikat Bhattacharjee, Praveen K. Rao, Tzvi Tzfira, Jyothi Rajagopal, HoChul Yi, Veena, Badam S. Yadav, Yan M. Crane, Kui Lin, Yves Larcher, Matthew J.K. Gelvin, Marnie Knue, Cynthia Ramos, Xiaowen Zhao, Susan J. Davis, Sang-Ic Kim, C.T. Ranjith-Kumar, Yoo-Jin Choi, Vipin K. Hallan, Sudip Chattopadhyay, Xiangzhen Sui, Alicja Ziemienowicz, Ann G. Matthysse, Vitaly Citovsky, Barbara Hohn, and Stanton B. Gelvin*

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Plant Physiology Vol. 132, Issue 2 (494-505 Jun 2003)

Table I. *rat mutants*

*, Mutant complemented with wild-type gene; **, attempted complementation failed; ***, kanamycin (kan) resistance does not cosegregate with rat phenotype; +, mutant scores less than 25% of wild-type; ++, mutant scores less than 33% of wild-type; +++, mutant scores less than 50% of wild-type; ++++, mutant scores more than 50% of wild-type but still a rat mutant; +++++, mutant scores at the level of wild-type for transient GUS activity; N/A, not applicable.

Mutant	Identification[a]	Collection	Tumorigenesis	Phosphinothricin (ppt) Resistance	Transient GUS	Zygosity	Resistance Marker	Gene Affected	Insertion Site
*rat1**	F	Feldmann	+	+	+	homo	kan	Arabinogalactan protein	5′-Untranslated region (UTR)
*rat3**	F	Feldmann	+	+	+++	homo	kan	Likely cell wall protein	Intergenic
*rat4**	F	Feldmann	+	+	+	homo	kan	Cellulose synthase-like protein (CslA-09)	3′-UTR
*rat5**	F	Feldmann	+	+	+++++	homo	kan	Histone H2A-1	3′-UTR
rat6	F	Feldmann	+	+	+		kan		
rat7	F	Feldmann	+	+	+		kan	Unknown protein	
rat8	F	Feldmann	+++	+++	++		kan		
rat9	F	Feldmann	+	+	+		kan	Unknown protein	
rat10	F	Feldmann	+	+	++		kan		
rat11	F	Feldmann	+	+	++		kan		
rat12	F	Feldmann	+	+	+		kan		
rat13	F	Feldmann	+	+	+		kan		
rat14	F	Feldmann	+	+	++		kan	Unknown protein	3′-UTR
rat15	F	Feldmann	+	+	+		kan		
rat16	F	Feldmann	+	+++	++		kan		
*rat17***	F	Feldmann	+	+	+++++		kan	Myb transcription factor (*cpc*)	3′-UTR
rat18	F	Feldmann	+	++	+++++		kan		
rat19	F	Feldmann	+	+	+		kan		Intergenic
rat20	F	Feldmann	+	+++	+++++		kan		
rat21	F	Feldmann	+	+	+++		kan		
rat22	F	Feldmann	+	++	++++		kan	Unknown protein	Intergenic
rat A1	F	Feldmann	+	+		homo	kan		
*rat A2**	F, R	Feldmann	+	+	+	homo	kan	phosphatase 2A (*rcn1*)	Sixth exon
rat A3	F	Feldmann	+	+		homo	kan		
rat A4	F	Feldmann	+	++++		homo	kan	Kinesin protein	First intron
rat A5	F	Feldmann	+	++++		homo	kan	Unknown protein	
rat A6	F	Feldmann	+	+		homo	kan		
*ratJ1**	F	Feldmann	+	++	+	homo	kan	Importin β-3	18th intron
ratJ2	F	Feldmann	+	+			kan	MADS box protein	Fifth intron
ratJ3	F	Feldmann	+				kan		
ratJ4	F	Feldmann	+	++			kan		
ratJ5	F	Feldmann	+	+			kan		
ratJ6	F	Feldmann	+	+			kan	3- Isopropylmalate dehydrogenase	Sixth exon
ratJ7	F	Feldmann	+	+		homo	kan	DEAD box RNA helicase	Third intron

Table II. *Steps of the transformation process putatively disrupted in selected Arabidopsis rat mutants*

+, Mutant scores less than 25% of the wild-type; ++, mutant scores less than 33% of the wild-type; +++, mutant scores less than 50% of the wild-type; ++++, mutant scores more than 50% of the wild-type but still a *rat* mutant; +++++, mutant scores at the level of wild-type for transient GUS activity; *, mutant has been complemented with the wild-type gene; ND, not determined.

Mutant	Tumorigenesis	Transient GUS	Gene Affected
Bacterial attachment/T-DNA transfer			
*rat1**	+	+	Arabinogalactan protein
*rat3**	+	+++	Likely cell wall protein
*rat4**	+	+	AtCslA-09
ratT18	+	ND	β-Expansin
Antisense *rat4*	+++	ND	AtCslA-09
Antisense F9	++	+	Unknown protein
Antisense F8	+	+	Unknown protein
Antisense RAB8	+	+++	AtRAB8
RNAi BTI1	+	+	Unknown protein
RNAi BTI2	+	+	Unknown protein
RNAi BTI3	+	+	Unknown protein
RNAi AtRAB8	+	+	AtRAB8
Cytoplasmic trafficking/cytoskeleton			
act2-1	+	++	Actin-2
act7-4*	+	+++	Actin-7
act7-1*	+	+++	Actin-7
rat A4	+	ND	Kinesin protein
Nuclear targeting			
*ratJ1**	+	+	Importin β-3
Importin α-7*	+	+++	Importin α-7
Antisense importin α-1	++/+++	++/+++	Importin α-1
T-DNA integration/chromatin structure and remodeling			
*rat5**	+	++++	Histone H2A-1
HTA2	++	ND	Histone H2A-2
HTA3	+	ND	Histone H2A-3
HTA10	++++	+++++	Histone H2A-10
HTA11	+	ND	Histone H2A-11
HTA13	++++	+++++	Histone H2A-13
HTB5	++	ND	Histone H2B-5
HTB6	++	+++++	Histone H2B-6
HTR4/5	+++	ND	Histone H3-4/5
HFO3	+	ND	Histone H4-3
HFO4	+	+++++	Histone H4-4
HDA1	+	+++++	Histone deacetylase-1
HDA2	++++	+++++	Histone deacetylase-2
HDA6	+++	ND	Histone deacetylase-6
HDA9	++++	ND	Histone deacetylase-9
HAT6	+	+++	Histone acetyl transferase-6
HAC11	++	+	Histone acetyl transferase-11
HXA1	+++	+	Histone acetylase complex HXA1
HXA2	+	++++	Histone acetylase complex HXA2
RNAi CHA6	++++	ND	Chromatin-remodeling complex subunit 6
RNAi HAC 8-1	+	ND	Histone acetyl transferase-8
RNAi NFA2-1	+++	ND	Nucleosome assembly factor A
RNAi SGA1	+	ND	Chromatin-silencing group 1

Zhu Y. et al. 2003. Identification of Arabidopsis *rat Mutants.* Plant Physiology. 132: 494–505

Table 1 Plant proteins and subcellular structures important for *Agrobacterium*-mediated transformation

Transformation process	Plant protein/structure involved	Reference
Bacterial attachment/biofilm formation	Arabinogalactan protein AtAGP17	44, 129
	Cellulose synthase-like CslA-09	128, 129
	Cellulose synthase-like CslB-05	N Sardesai & SB Gelvin, unpublished data
	Plant defense reaction proteins	5, 44, Veena & SB Gelvin, unpublished data
T-DNA and virulence protein transfer	Reticulon domain proteins BTI1 (AtRTNLB1), BTI2 (AtRTNLB2), and BTI3 (AtRTNLB4)	54, 129
	Rab8 GTPase	54, 129
Cytoplasmic trafficking	Microtubules[b]/kinesin	91, 129
	Actin and Myosin	129, P Rao, Y Yu, L-Y Lee, SB Gelvin, unpublished data
	Cyclophilin[a]	28
Nuclear targeting	Importin α	10, 11, 12, 65, 129
	Importin β/Transportin	129
	CAK2Ms kinase	10
	Protein phosphatase 2C (PP2C)	101
	VIP1	32, 50, 62, 66, 108
	Caspase[a]	16, 88
	GALLS interacting protein (GIP[a])	Y Wang, L-Y Lee, L Hodges, W Ream, SB Gelvin, unpublished data
Targeting T-DNA to chromatin	CAK2Ms[a]	10
	VIP1	61, 66, 69
Vir protein removal/T-DNA and protein stability	Ask/Skp proteins	95, 109, 125, 129
	Caspase	16, 88
	Histones	102
	pCsn5-1[a]	43
T-DNA integration	DNA ligase IV[a]	40, 112, 113, 114, 129, 130
	Ku70, Rad50, Mre11, Xrs2, Sir4[b]	112
	Ku80[a]	40, 42, 67
	VIP1	66
	VIP2	4
	Histones	6, 78, 80, 122, 123, 129
	Nucleosome assembly CAF-1	37
	Histone H3 chaperone SGA1	22, 129, G Tenea & SB Gelvin, unpublished data
	Histone deacetylases	22, 129
Transgene expression	Histones H2A, H3-11, and H4	102
Susceptibility to transformation	Myb transcription factor	N Sardesai, H Chen, J Spantzel, B Yadav, SB Gelvin, unpublished data

[a]Role in this transformation step is likely but not yet proven, or the literature indicates conflicting results.
[b]Shown in a *Xenopus* in vitro system only.
[c]Important for *Agrobacterium*-mediated transformation of yeast, but not yet shown in plants.

Gelvin S.B. 2010. Plant proteins involved in *Agrobacterium*-mediated genetic transformation. Annu Rev. Phytopathol 48:45-68

Table 2 Plant proteins whose overexpression increases *Agrobacterium*-mediated plant transformation

Protein	Likely role in transformation	Reference
BTI1 (AtRTNLB1)	T-DNA and virulence protein transfer	54
VIP1	Nuclear targeting	32, 109
Ku80	T-DNA integration	67
Histones H2A, H3-11, and H4	T-DNA stability, transgene expression	78, 102, 123, 127
SGA1 (ASF1)	T-DNA integration	G Tenea & SB Gelvin, unpublished data
UDP-glucosyltransferase	Defense response	N Sardesai & SB Gelvin, unpublished data
GALLS interacting protein (GIP)	Nuclear targeting[a]	Y Wang, L-Y Lee, L Hodges, W Ream, SB Gelvin, unpublished data

[a]Role in this transformation step is likely but not yet proven.

Gelvin S.B. 2010. Plant proteins involved in *Agrobacterium*-mediated genetic transformation. Annu Rev. Phytopathol 48:45-68

Funciones nuevas de los genes de *Agrobacterium*

Adv Biochem Eng Biotechnol (2013) 134: 1–22
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Recent Advances in the Understanding of *Agrobacterium rhizogenes*-Derived Genes and Their Effects on Stress Resistance and Plant Metabolism

Victor P. Bulgakov, Yuri N. Shkryl, Galina N. Veremeichik, Tatiana Y. Gorpenchenko and Yuliya V. Vereshchagina

1. Funciones nuevas de los genes rolB y rolC en células vegetales

1.1. Homeostasis de ROS y expresión de genes antioxidantes

Table 1 Individual and combined expression of the *rol* genes in suspension-cultivated *R. cordifolia* cells decreases ROS levels

Intracellular ROS level	Nontransformed control	*rolA*	*rolB*	*rolC*	*rolABC*	*pRiA4*
Steady-state conditions	100 ± 3	93 ± 3[a]	78 ± 4 [14]	56 ± 6 [11]	90 ± 3 [55]	80 ± 4 [55]
ROS-inducing treatment	160 ± 4 [11, 14]	109 ± 5[a]	96 ± 5 [14]	74 ± 5 [11]	101 ± 4[a]	113 ± 4[a]

The data are presented as percentage concentrations of intracellular ROS. The basic level of ROS in control nonstressed cells has been accepted for 100 %. The 5-day-old cell suspension cultures were loaded with H_2DCF-DA and analyzed by confocal microscopy as described previously [11]. In the ROS-inducing treatment, *R. cordifolia* cells were grown for 4 days in the dark and treated with paraquat (Aldrich, 10 μM, final conc.) for 1 h under continuous light exposure (200 μmol m^{-2} s^{-1} radiation). The data were obtained in three independent experiments and presented as mean ± SE

[a] Our unpublished results

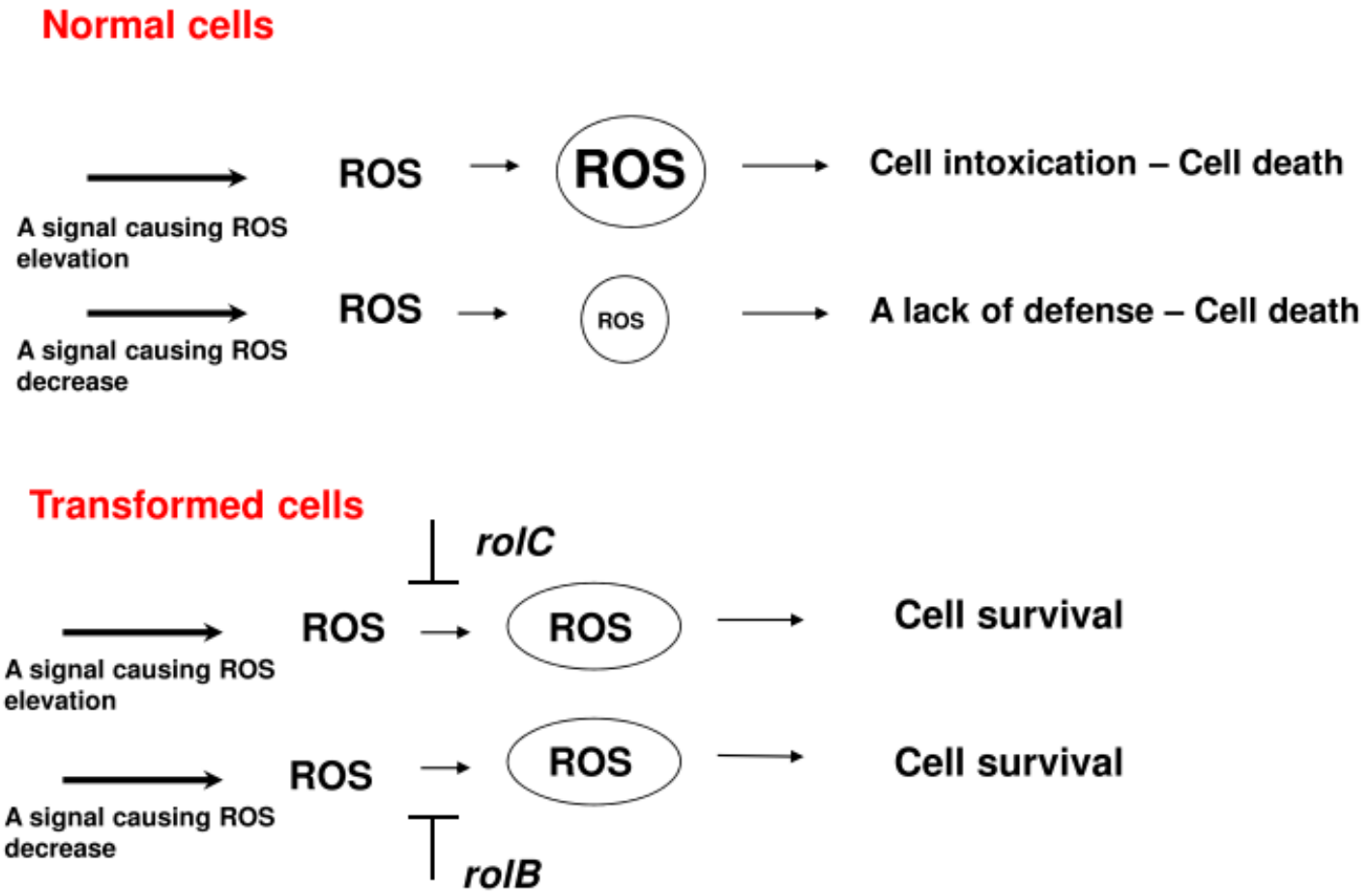
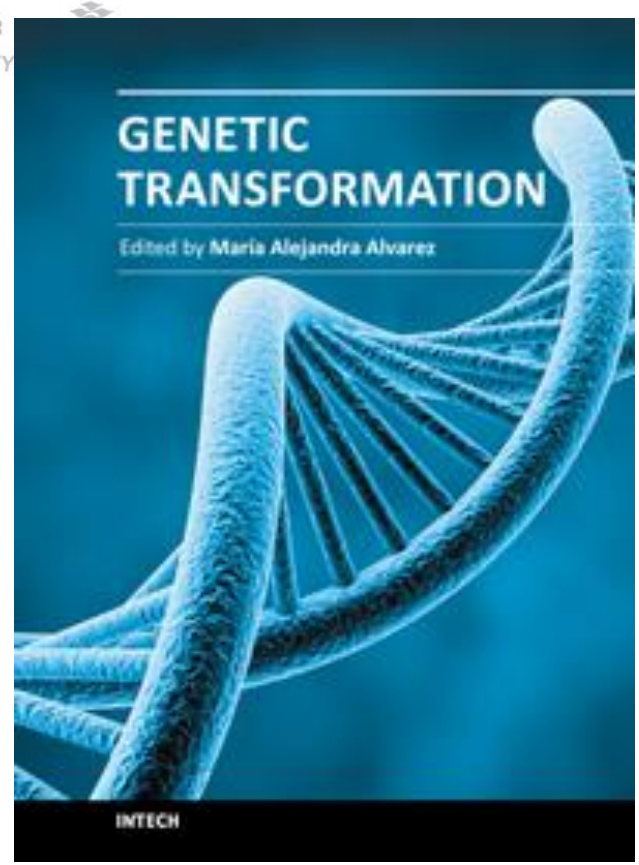


Fig. 2. A scheme illustrating the possible role of *rol* genes in cell survival. The *rolC* and *rolB* genes mitigate ROS changes caused by environmental stimuli. The signals causing acute ROS elevations are high temperature, cold, high salt conditions, excessive light and others. Signals causing decreased intracellular ROS levels are provoked by many pathogens.



Application of Agrobacterium *Rol* Genes in Plant Biotechnology: A Natural Phenomenon of Secondary Metabolism Regulation

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Biochemistry, Genetics and Molecular Biology
Genetic Transformation

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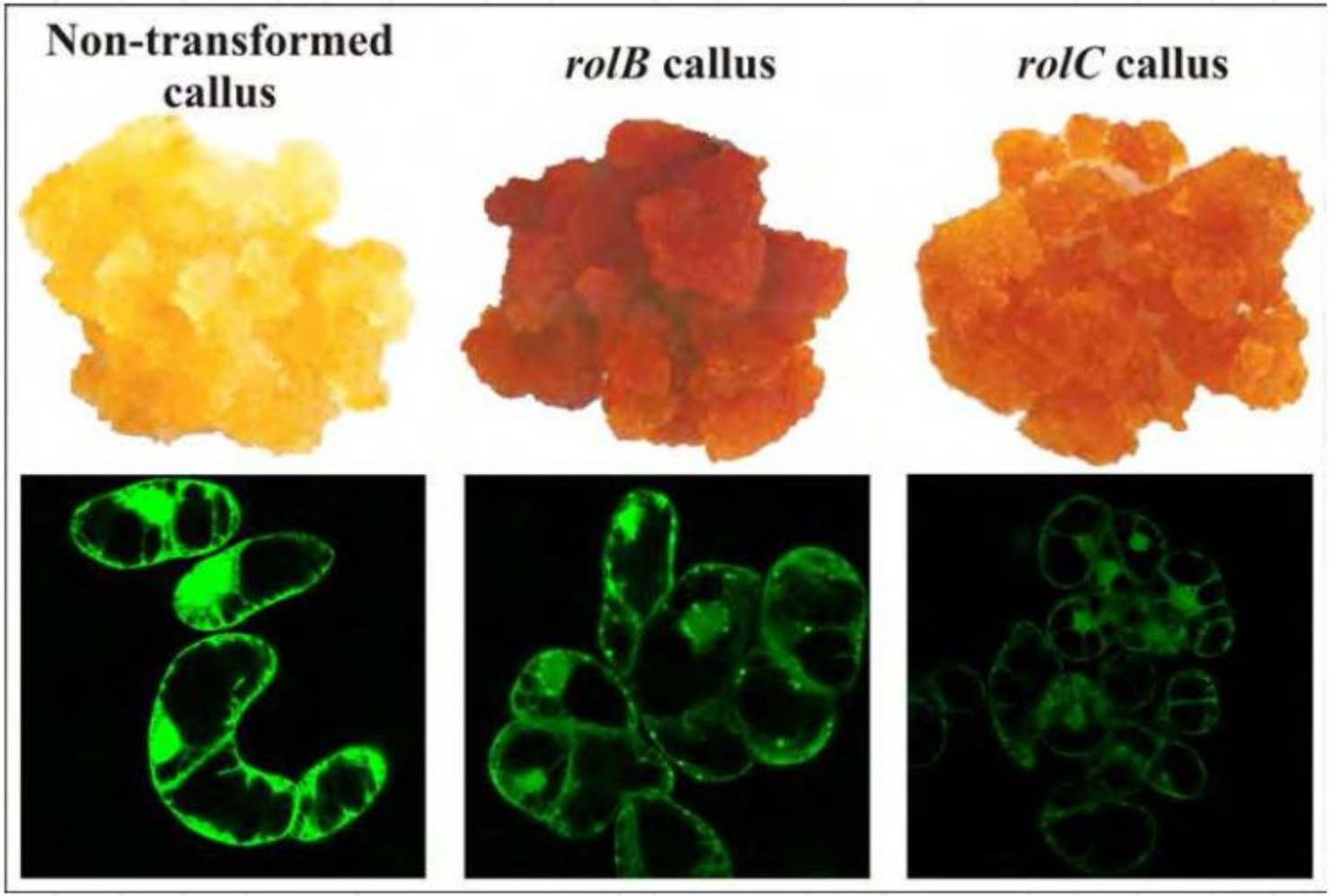

Fig. 1. Relation between secondary metabolism and intracellular ROS level in *R. cordifolia* cells. The upper panel presents phenotypes of *R. cordifolia* calli transformed with the *rol* genes. The *rolB*-calli and *rolC*-calli contained ten times and six times more anthraquinones, respectively, compared to the non-transformed calli. At the same time, ROS levels in cells of these transformed calli were low (see the bottom panel). Green fluorescence inside cells reflects summarized ROS (such as hydrogen peroxide, peroxyl radicals and peroxynitrite) levels measured by laser-scanning confocal microscopy and visualized by dichlorofluorescein diacetate.

1.2. Apoptosis

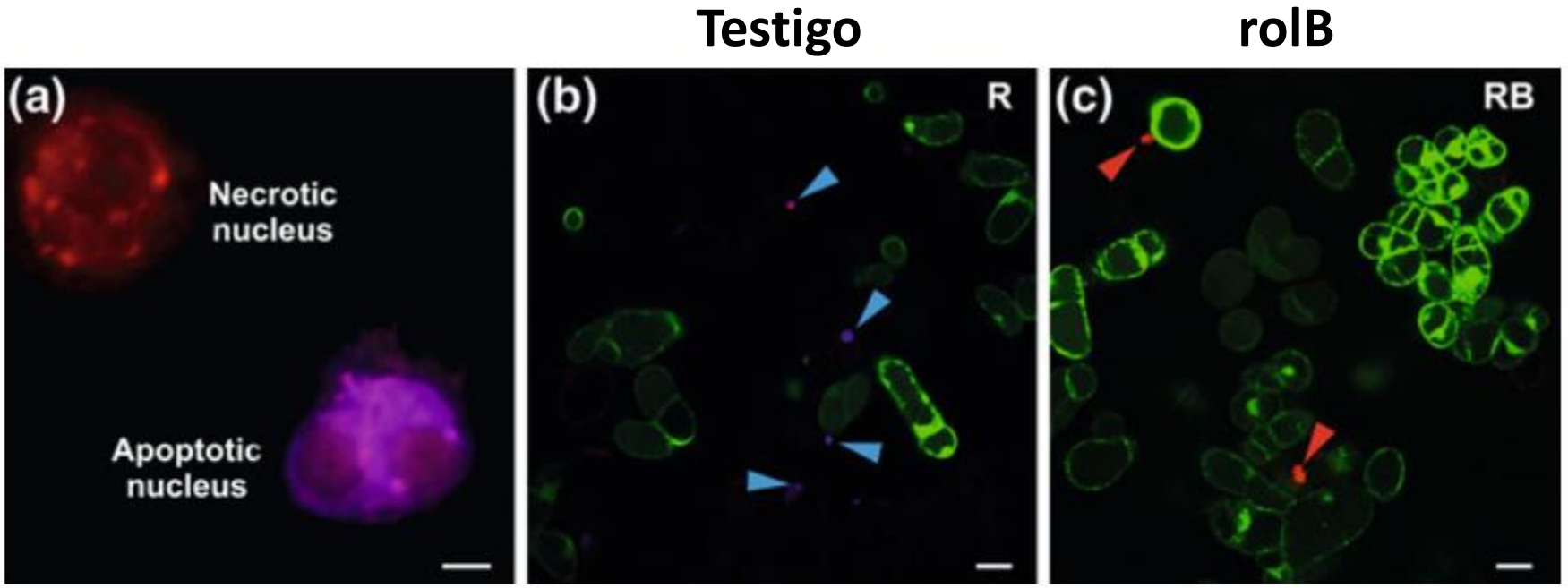

Fig. 2 Expression of *rolB* in *R. cordifolia* cells diminishes apoptosislike symptoms [30]. Cells of the control (R) and *rolB*-transformed (RB) cultures were analyzed by laser confocal microscopy. Propidium iodide and Hoechst 33342 were used to detect collapsed nuclei of necrotic cells and nuclei of apoptotic cells, respectively. **a** A view of nuclei of *R. cordifolia* cells dyed by necrosis (*red*) and apoptosis (*blue* + *red*). In control nontransformed cells (**b**), blue staining of the nuclei (*cyan arrows*) indicates an early phase of apoptosis and violet staining of the nucleus reflects a later phase of apoptosis. Necrotic cells with red-stained nuclei are indicated by *red arrows* (**c**). Scale bars, 2 μm (**a**) and 50 μm (**b**, **c**)

1.3. Respuesta al estrés de la células transformadas

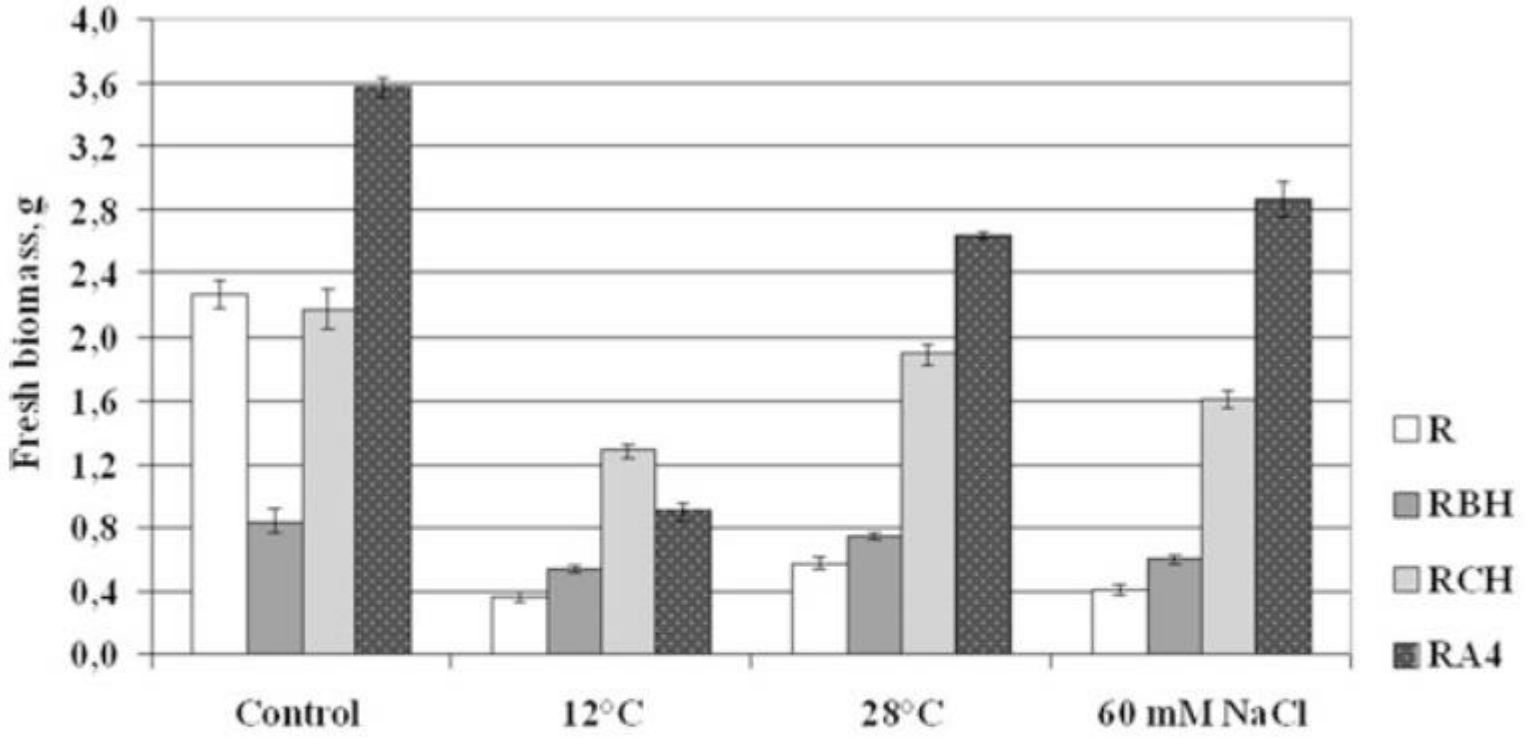

Fig. 3 Resistance of *R. cordifolia* cells to low and high temperatures and salt stress. The data are summarized from earlier publications [11, 14, 55]. Control: normal conditions of cultivation (24 °C). *R* nontransformed control, *RBH* high-*rolB*-expressing cells, *RCH* high-*rolC*-expressing cells, *RA4 A. rhizogenes* A4 transformed cells. The inoculum biomass was 0.2 g fresh weight. The R, RBH, and RCH cultures were grown in the presence of 6-benzylaminopurine (0.5 mg l^{-1}) and α-naphthaleneacetic acid (2.0 mg l^{-1}). The A4 cells were grown in hormone-free medium

1.4. Oncogenes del ADN-T y el Metabolismo Secundario

Los genes rolB y rolC, son activadores del metabolismo secundario en las familias:

Solanaceae, Araliaceae, Rubiaceae, Vitaceae, and Rosaceae.

Among the activated metabolites were
Antraquinones (AQs),
Alcaloids (tropano, piridin e indólicos, and indole groups),
Estilbenes (resveratrol),
Isoflavonoides (isoflavones and pterocarpans),
Glucósidos tupo dammarano (ginsenósidos).

Dependiendo del grupo de metabolitos secundarios, estos pueden elevarse de 2-300 veces.

En células transformadas de *R. cordifolia* el efecto estimulador ha sido estable durante un período de cultivo de 12 años.

2. Fosforilación de tirosina y patogénesis microbiana

El gen RolB codifica a una proteína con actividad de fosfatasa de tirosina (tyrosine phosphatase = TyP). La cual reprime la inducción de ROS en plantas y la muerte celular programada

En *Arabidopsis* el gen HopAO1 codifica para una proteína con la misma actividad de TyP y reprime las respuesta de defensa asociadas con la inmunidad innata disparada por los Patrones Moleculares Asociadas a Patógenos = PAMP)

Entre los genes desregulados por HopAO1, se encuentran los relacionados por proteínas PR-1, PR-2 y PR-5 y el factor transcripcional MYB122 involucrado en la regulación el metabolismo Secundario y la proteína XET que participa en el endurecimiento de la pared celular.

A plant oncogene as a phosphatase

SIR — The plant oncogene *rolB*, from *Agrobacterium rhizogenes*, induces differentiation and growth of neoplastic roots (hairy-roots[1]) in dicotyledonous plants. *rolB*-transformed plant cells show an increased membrane sensitivity to[2,3], and binding capacity of[4], auxin, the most extensively studied plant hormone. The oncogene *rolB* may thus provide a tool for elucidating the still elusive mechanism of auxin signal perception/transduction and for shedding light on the role of this plant hormone in the control of plant growth and differentiation. So far, all attempts to clarify the biochemical activity and subcellular localization of the *rolB* gene product have been inconclusive. Here we show that the RolB protein overproduced in *Escherichia coli* has tyrosine phosphatase activity, and that in transformed plant cells it is localized in the plasma membrane.

The full-length *rolB* gene was cloned in vector ptrc97B (provided by E. Amman) and the resulting construct (pMTB4) transferred in *E. coli* W3110*lac* I^{q}L8. On induction by isopropyl-β-D-thiogalactoside, the production of the RolB protein was confirmed by immunoblotting. The lysates of bacteria producing RolB (MTB4) have a fivefold higher phos-

Filippini F, Rossi R, Marin O, Trovato M, Costantino P, Downey PM, Lo Schiavo F, Terzi M (1996) A plant oncogene as a phosphatase. Nature 379:499–500

The Plant Journal (2004) **38**, 260–275

doi: 10.1111/j.1365-313X.2004.02041.x

Nuclear localization and interaction of RolB with plant 14-3-3 proteins correlates with induction of adventitious roots by the oncogene *rolB*

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The rooting-locus gene B (*rolB*) on the T-DNA of the root-inducing (Ri) plasmid in *Agrobacterium rhizogenes* is responsible for the induction of transformed adventitious roots, although the root induction mechanism is unknown. We report here that the RolB protein of pRi1724 (1724RolB) is associated with *Nicotiana tabacum* 14-3-3-like protein ωII (Nt14-3-3 ωII) in tobacco bright yellow (BY)-2 cells. Nt14-3-3 ωII directly interacts with 1724RolB protein. Green fluorescent protein (GFP)-fused 1724RolB is localized to the nucleus. GFP-fused mutant 1724RolB proteins having a deletion or amino acid substitution are unable to interact with Nt14-3-3 ωII and also show impaired nuclear localization. Moreover, these 1724RolB mutants show decreased capacity for adventitious root induction. These results suggest that adventitious root induction by 1724RolB protein correlates with its interaction with Nt14-3-3 ωII and the nuclear localization of 1724RolB protein.

La localización nuclear de la proteína rolB mediada por proteínas 14-3-3 favorecen la Hipótesis que su blanco primario son las proteínas nucleares y más específicamente la desfosforilación de los residuos de tirosina de las MAP CINASAS

3. Supresores de la inmunidad innata o reguladores de la adaptabilidad celular

3.1 Mecanismo de acción de 6b

Actividad dual: Activador transcripcional

Modulador y mediador de la maquinaria de Silenciamiento de ARN

JOURNAL OF BACTERIOLOGY, July 2000, p. 3885–3895
0021-9193/00/$04.00+0

Vol. 182, No. 14

MINIREVIEW

The Bases of Crown Gall Tumorigenesis

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13 kb **7.8 kb**

TABLE 1. Genes encoded by the octopine-type Ti plasmid[a]

Genetic locus	Description	Reference(s)
T-DNA genes		
ags	Agropine synthase, lactonization of mannopine	24, 40
Gene *5*	Synthesis of indole-3-lactate, an auxin antagonist	57
iaaH and *iaaM*	Conversion of tryptophan to indole acetic acid (auxin)	55
ipt	Condensation of AMP and isopentenylpyrophosphate to form isopentenyl-AMP, a cytokinin	66
mas1' and *mas2'*	Mannopine synthase; condensation of glucose with glutamine or glutamate followed by reduction	24
ocs	Octopine synthase, reductive condensation of pyruvate with four basic amino acids	21
ons	Opine export from plant cells	75
tml (gene *6b*)	Auxin sensitivity	108
Borders A, B, C, D	*cis*-acting sites required for T-DNA processing, functionally equivalent to conjugal origins of transfer	125
Overdrive	*cis*-acting site for optimal T-DNA transfer; VirC1 binding site	110, 113

Plant Cell Physiol. 47(5): 664–672 (2006)
doi:10.1093/pcp/pcj036, available online at www.pcp.oupjournals.org

Oncogene *6b* from *Agrobacterium tumefaciens* Induces Abaxial Cell Division at Late Stages of Leaf Development and Modifies Vascular Development in Petioles

Shinji Terakura [1], Saeko Kitakura [1], Masaki Ishikawa [1,4], Yoshihisa Ueno [1], Tomomichi Fujita [1,5], Chiyoko Machida [2], Hiroetsu Wabiko [3] and Yasunori Machida [1,*]

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The *6b* gene in the T-DNA region of the Ti plasmids of *Agrobacterium tumefaciens* and *A. vitis* is able to generate shooty calli in phytohormone-free culture of leaf sections of tobacco transformed with *6b*. In the present study, we report characteristic morphological abnormalities of the leaves of transgenic tobacco and Arabidopsis that express *6b* from pTiAKE10 (AK-6b), and altered expression of genes related to cell division and meristem formation in the transgenic plants. Cotyledons and leaves of both transgenic tobacco and Arabidopsis exhibited various abnormalities including upward curling of leaf blades, and transgenic tobacco leaves produced leaf-like outgrowths from the abaxial side. Transcripts of some class 1 *KNOX* homeobox genes, which are thought to be related to meristem functions, and cell cycle regulating genes were ectopically accumulated in mature leaves. M phase-specific genes were also ectopically expressed at the abaxial sides of mature leaves. These results suggest that the AK-*6b* gene stimulates the cellular potential for division and meristematic functions preferentially in the abaxial side of leaves and that the leaf phenotypes generated by AK-*6b* are at least in part due to such biased cell division during polar development of leaves. The results of the present experiments with a fusion gene between the AK-*6b* gene and the glucocorticoid receptor gene showed that nuclear import of the AK-6b protein was essential for upward curling of leaves and hormone-free callus formation, suggesting a role for AK-6b in nuclear events.

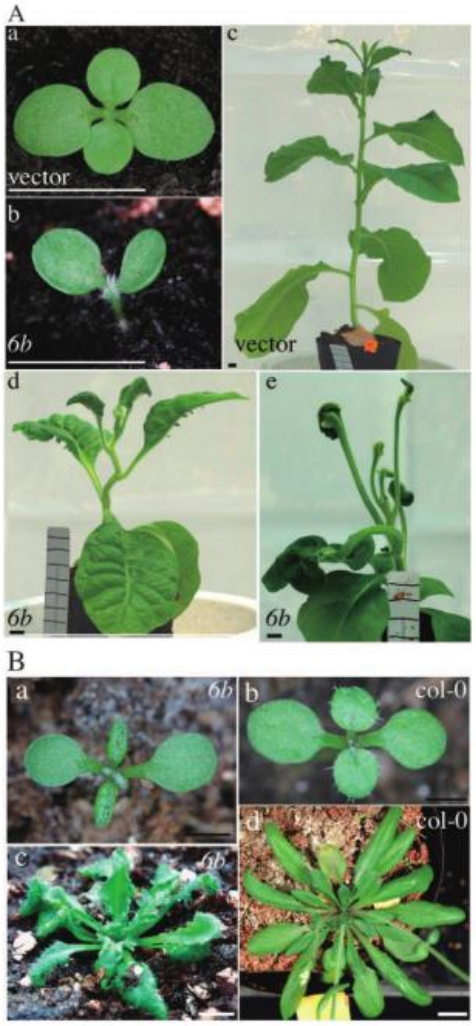

Fig. 1 The typical phenotype of AK-*6b* transgenic tobacco. (A) Gross morphology of aerial parts of tobacco plants. Plants were soil-grown in a greenhouse. (a) A tobacco plant transformed with empty vector pBI121, 14 days old. (b) A transgenic tobacco plant that had been transformed with the P35S-AK-*6b* gene, 14 days old. (c) A tobacco plant transformed with empty vector pBI121, 2 months old. (d) An AK-*6b* transgenic tobacco plant that exhibited a mild phenotype, 2 months old. (e) An AK-*6b* transgenic tobacco plant that exhibited a severe phenotype, 2 months old. Scale bars = 1 cm. (B) Gross morphology of aerial parts of Arabidopsis plants. Plants were soil-grown in a covered container and the cover was removed 3 d after vernalization (DAV). (a) An AK-*6b* transgenic Arabidopsis plant, 10 DAV. (b) A non-transgenic Arabidopsis plant, 10 DAV. (c) An AK-*6b* transgenic Arabidopsis plant, 41 DAV. (d) A non-transgenic Arabidopsis plant, 41 DAV. Scale bars = 1 mm (a, b) and 1 cm (c, d).

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An Oncoprotein from the Plant Pathogen *Agrobacterium* Has Histone Chaperone–Like Activity

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Protein 6b, encoded by T-DNA from the pathogen *Agrobacterium tumefaciens*, stimulates the plant hormone–independent division of cells in culture in vitro and induces aberrant cell growth and the ectopic expression of various genes, including genes related to cell division and meristem-related class 1 KNOX homeobox genes, in 6b-expressing transgenic *Arabidopsis thaliana* and *Nicotiana tabacum* plants. Protein 6b is found in nuclei and binds to several plant nuclear proteins. Here, we report that 6b binds specifically to histone H3 in vitro but not to other core histones. Analysis by bimolecular fluorescence complementation revealed an interaction in vivo between 6b and histone H3. We recovered 6b from a chromatin fraction from 6b-expressing plant cells. A supercoiling assay and digestion with micrococcal nuclease indicated that 6b acts as a histone chaperone with the ability to mediate formation of nucleosomes *in vitro*. Mutant 6b, lacking the C-terminal region that is required for cell division–stimulating activity and interaction with histone H3, was deficient in histone chaperone activity. Our results suggest a relationship between alterations in nucleosome structure and the expression of growth-regulating genes on the one hand and the induction of aberrant cell proliferation on the other.

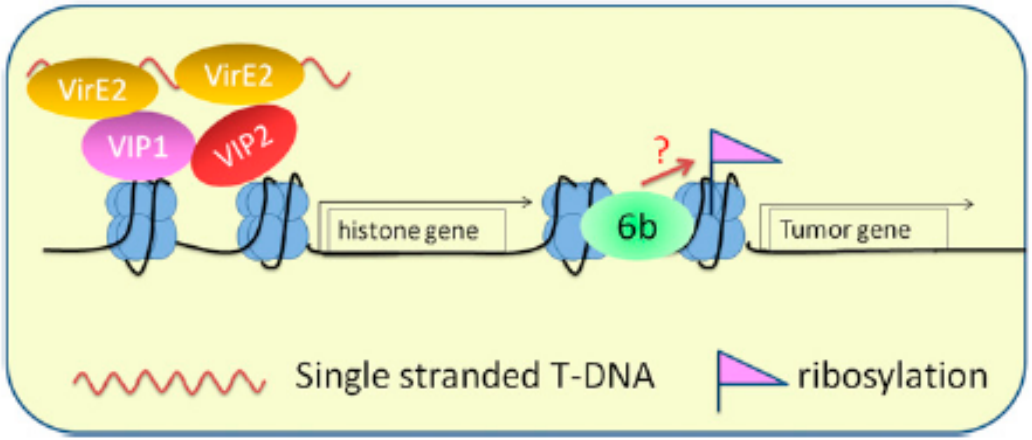

Figure 2. A model for the *Agrobacterium* effectors VirE2 and 6b in modulating chromatin configurations and gene expression in the plant nucleus. VirE2 directly interacts with VIP1 and VIP2, which also interact with each other. VIP1 facilitates the association of T-DNA complexes with histones and thereby promotes T-DNA integration. VIP2 may activate histone gene expression. 6b directly interacts with histone H3 and possesses an ADP-ribosyltransferase activity. 6b could potentially modulate histone modification and induce genes involved in abnormal cell growth.

Ma K-W. Flores C. and Ma W. 2011Chromatin Configuration as a Battlefield in Plant-Bacteria Interactions. Plant Physiology.157: 535–543

3.2. T-DNA Versus Virus: Suppression de las defensas del huésped

Molecular insights into plant cell proliferation disturbance by *Agrobacterium* protein 6b

GENES & DEVELOPMENT 25:64–76 © 2011

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The *Agrobacterium* Ti plasmid (T-DNA) 6b proteins interact with many different host proteins implicated in plant cell proliferation. Here, we show that *Arabidopsis* plants overexpressing 6b display microRNA (miRNA) deficiency by directly targeting SERRATE and AGO1 via a specific loop fragment (residues 40–55). In addition, we report the crystal structures of *Agrobacterium tumefaciens* AK6b at 2.1 A°, *Agrobacterium vitis* AB6b at 1.65 A and *Arabidopsis* ADP ribosylation factor (ARF) at 1.8 A°. The 6b structure adopts an ADP-ribosylating toxin fold closely related to cholera toxin. In vitro ADP ribosylation analysis demonstrates that 6b represents a new toxin family, with Tyr 66, Thr 93, and Tyr 153 as the ADP ribosylation catalytic residues in the presence of Arabidopsis ARF and GTP. Our work provides molecular insights, suggesting that 6b regulates plant cell growth by the disturbance of the miRNA pathway through its ADP ribosylation activity.

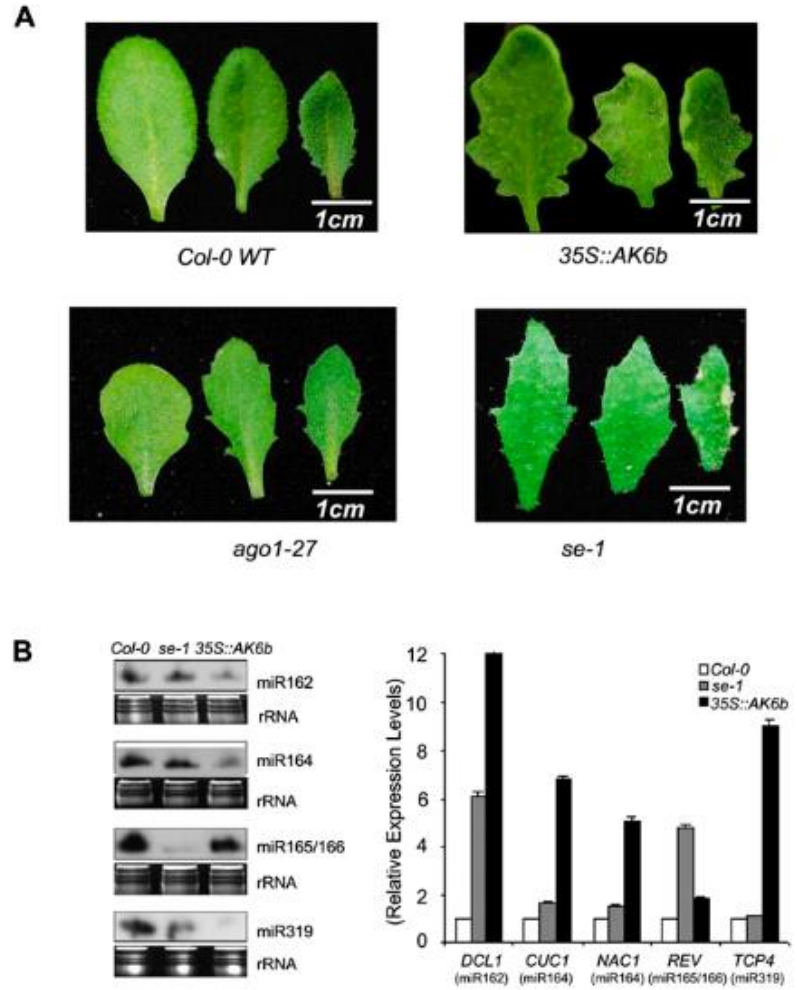

Figure 1. Expression of miRNA in *AK6b* transgenic plants. (*A*) Comparison of the morphological phenotype between *35S::AK6b*, *ago1-27*, and *se-1* plants. Photographs were taken for the second, third, and fourth true leaves of 4-wk-old seedlings. Bar, 1 cm. (*B*) Accumulation of small RNAs and target mRNAs in wild-type (*Col-0*), *se-1*, and *35S::AK6b* plants. Each lane contained 12 μg of RNA. rRNAs were used as a loading control. For analysis of target mRNAs, quantifications of each cDNA sample were made in triplicate, and consistent results from at least two independent RNA samples were used.

MicroRNAs (miRNAs): Spm RNAs regulatorios de 21-24 nucleotidos. Funcionan:

(1) Respuesta al estrés biótico y abiótico
(2) Metabolismo
(3) Señalización a reguladores de crecimiento
(4) Transcription
(5) Desarollo
(6) Regulation de maquinaria de miRNA

DCL1 = RNAse III DICER-LIKE
CUC1 = CUP-SHAPED COTYLEDON1 (meristems)
NAC = (NAM, ATAF1/2 y CUC2). Familia de factores transcripcionales relacionadas con estrés abiótico
REV = REVOLUTA (desarrollo meristemo apical)
TCP4 = Familia de factores transcripcionales (desarrollo floral)

The results show that 6b executes its suppressor function by directly targeting SE and AGO1, two key components of the miRNA machinery in *Arabidopsis*.

The EMBO Journal Vol.17 No.1 pp.170–180, 1998

AGO1 defines a novel locus of *Arabidopsis* controlling leaf development

Karen Bohmert, Isabelle Camus[1], Catherine Bellini[1], David Bouchez[1], Michel Caboche[1] and Christoph Benning[2]

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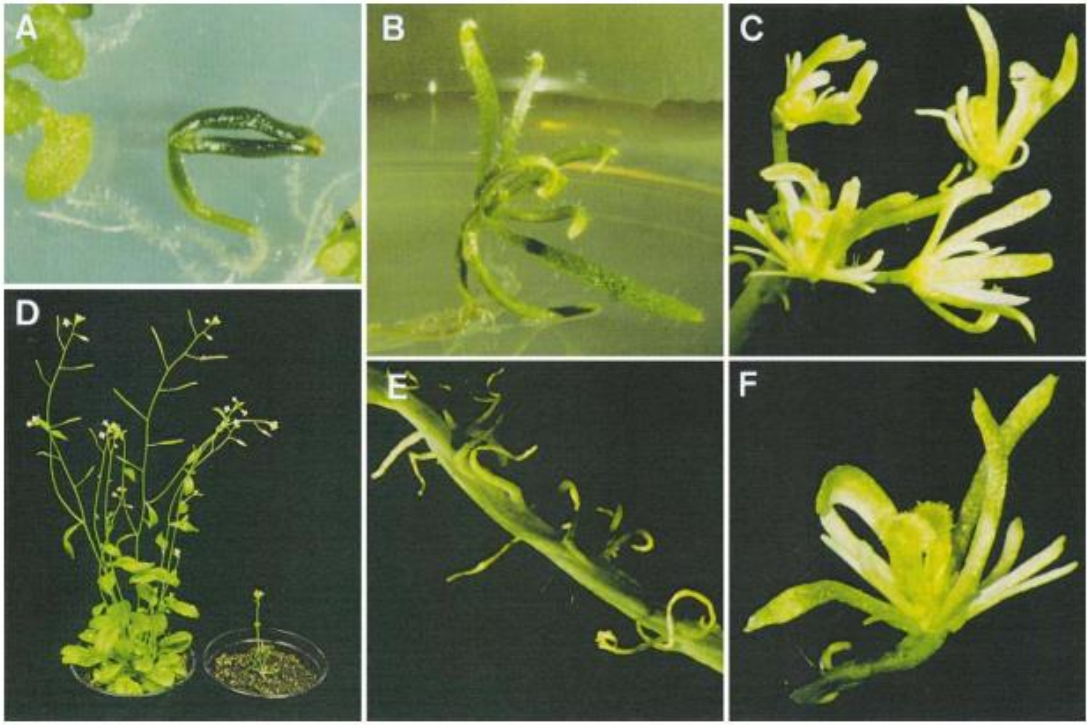


Fig. 1. Phenotype of the *ago1-1* mutant. (**A**) 11-day-old *ago1-1* seedling (center) and hygromycin B resistant heterozygous plant (upper left corne (**B**) 35-day-old *ago1-1* seedling; (**C**) inflorescence of a 6-week-old *ago1-1* mutant; (**D**) 6-week-old wild-type (left) and *ago1-1* mutant (right); (**E**) cauline leaf-like structures on an *ago1-1* inflorescence stem (6-weeks-old); (**F**) *ago1-1* flower (6-weeks-old).

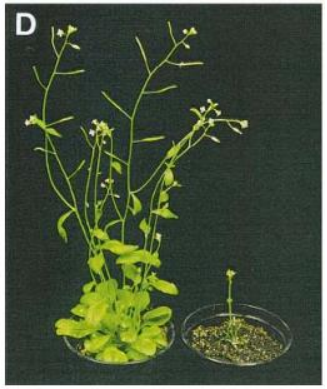
Fig. 8. Phenotype of transgenic wild-type and heterozygous *AGO1-1/ago1-1* lines transformed with sense- or antisense-35S-CMV::*AGO1*-cDNA constructs. (**A**) untransformed wild-type (4 weeks old); (**B**) antisense plant (5 weeks old); (**C**) sense plant (4 weeks old); (**D**) and (**E**) leaves of antisense plants; (**F**) leaves of a sense plant (4 weeks old); (**G**) petal of a sense plant.

Plant Physiology. June 2008. 147: 456–468,

RNA Silencing in Plants: Yesterday, Today, and Tomorrow

Andrew Eamens*, Ming-Bo Wang, Neil A. Smith, and Peter M. Waterhouse

Commonwealth Scientific and Industrial Research Organization Plant Industry, Canberra, Australian Capital Territory 2601, Australia (A.E., M.-B.W., N.A.S., P.M.W.); and University of Sydney, Sydney, New South Wales 2006, Australia (P.M.W.)



ARGONAUTE1 (AGO1): Cataliza el corte del ARNm

RNA-dependent ARN polymerase (RDR)
Methyltransferase HUA ENHANCER1(HEN1)
RNAse III DICER-LIKE 1(DCL1)

RISC, RNA-induced silencing complex

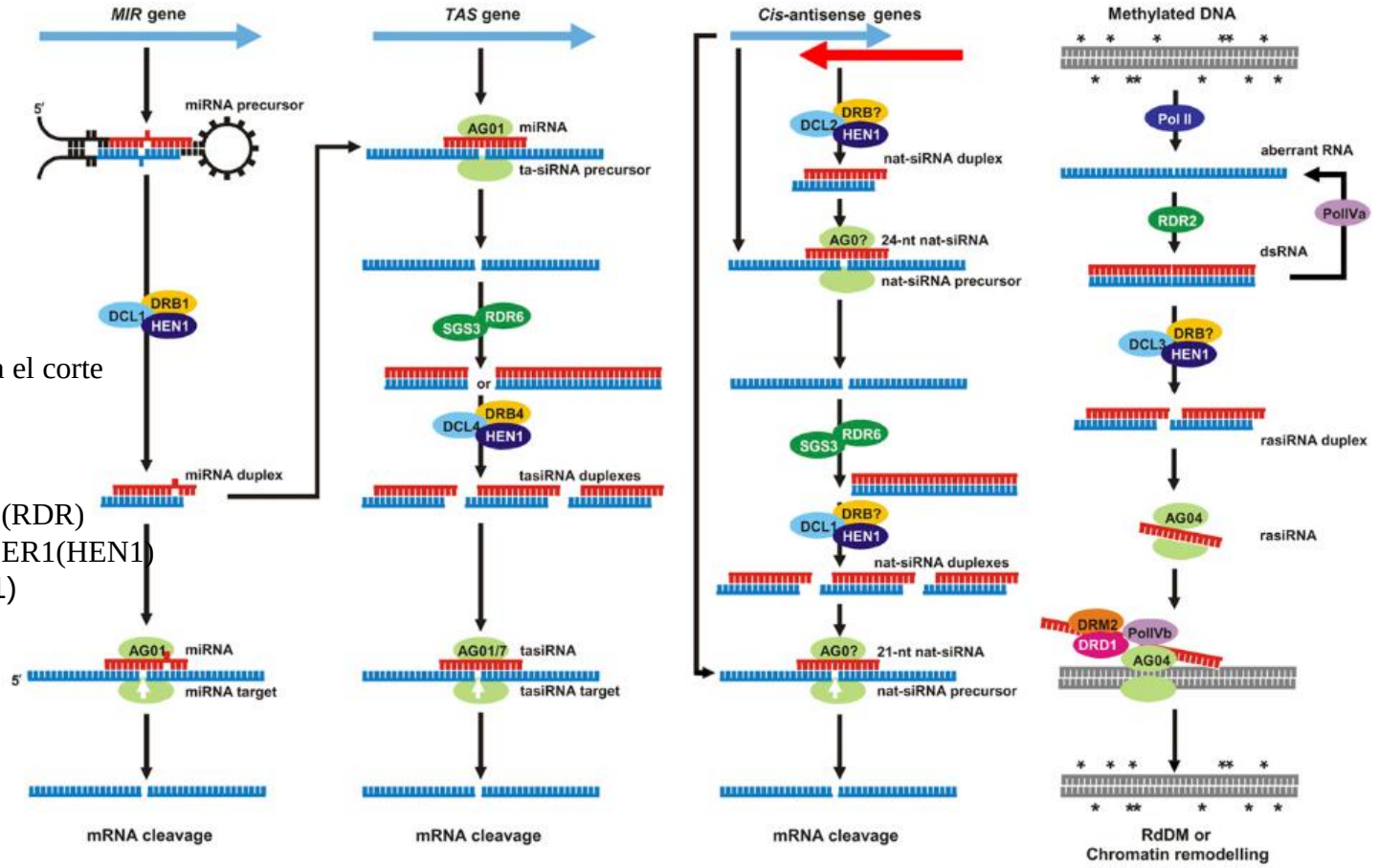


Figure 3. The parallel RNA silencing pathways of Arabidopsis. Schematic representation of the parallel DCL/sRNA-directed RNA silencing pathways in the model dicotyledonous species Arabidopsis, outlining the specific step or steps in each pathway for the individual RNA silencing-associated proteins mentioned in the text of this review.

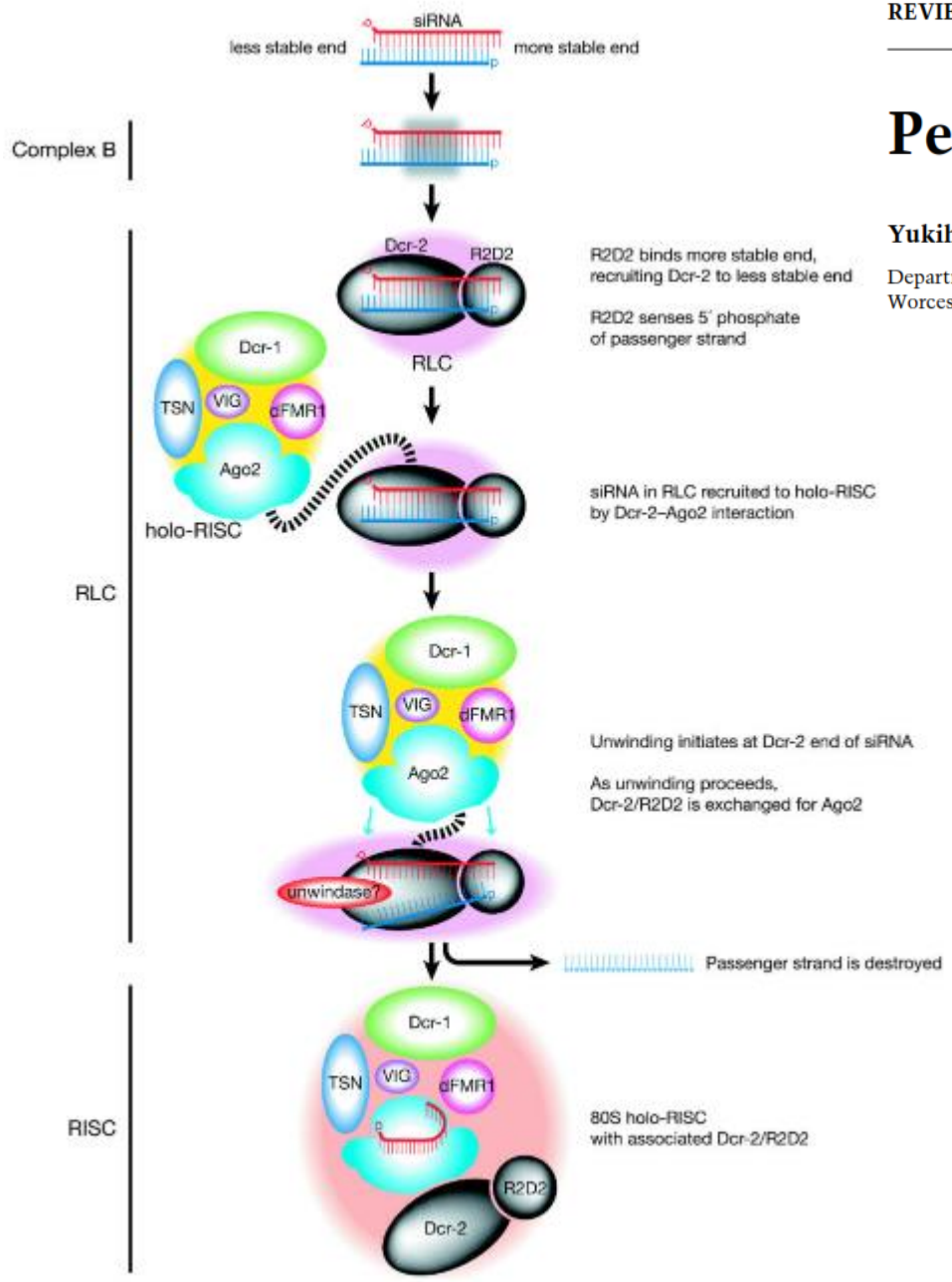


Figure 2. The RISC assembly pathway for siRNA in flies.

Perspective: machines for RNAi

Yukihide Tomari and Phillip D. Zamore[1]

Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605, USA

GENES & DEVELOPMENT 19:517–529 (2005)

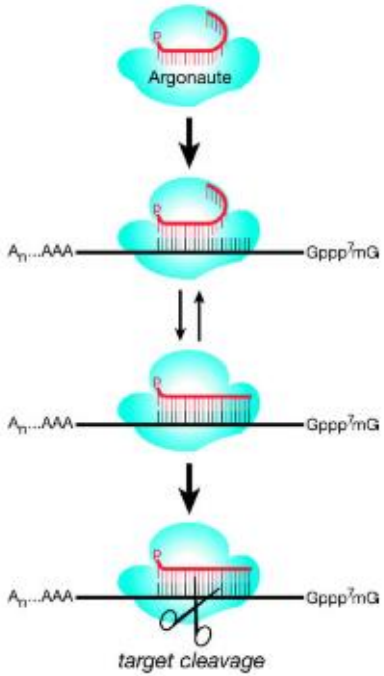


Figure 3. A two-state model for Argonaute function in RISC.

nature genetics

Induction, suppression and requirement of RNA silencing pathways in virulent *Agrobacterium tumefaciens* infections

Patrice Dunoyer, Christophe Himber & Olivier Voinnet

VOLUME 38 | NUMBER 2 | FEBRUARY 2006 NATURE GENETICS

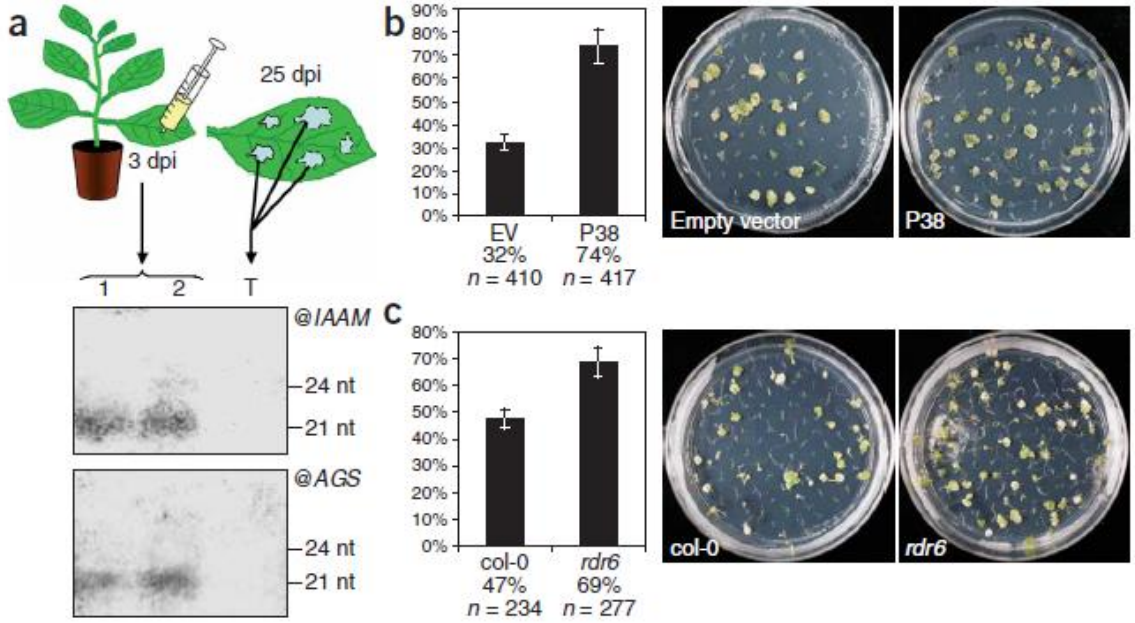


Figure 1 Induction and suppression of RNA silencing in *A. tumefaciens*-infected tissues. (**a**) *N. benthamiana* leaves were syringe infiltrated with a low inoculum of *A. tumefaciens*. Whole leaves were collected at 3 dpi or left to allow the development of tumors and individually dissected at 25 dpi. Accumulation of *iaaM* and *ags* siRNA was assessed by RNA blot analysis. The RNAs in tracks 1 and 2 were extracted from separate leaf samples. T, tumor. (**b**) Dissected roots from P38 transgenic *A. thaliana* (right) show enhanced susceptibility to virulent *A. tumefaciens* compared with root segments from empty vector transformants (left). (**c**) Roots of the *rdr6* loss-of-function mutant of *A. thaliana* also show enhanced susceptibility to *A. tumefaciens* compared with roots from wild-type plants. *n*, total number of individually inoculated root segments in three (P38) or two (*rdr6*) independent experiments involving 130–150 segments each. The total number of tumors eventually developing on the inoculated roots is expressed as a percentage. The P38, empty vector transgenes and *rdr6* mutations are all in the *A. thaliana* ecotype Col-0, used here as a reference.

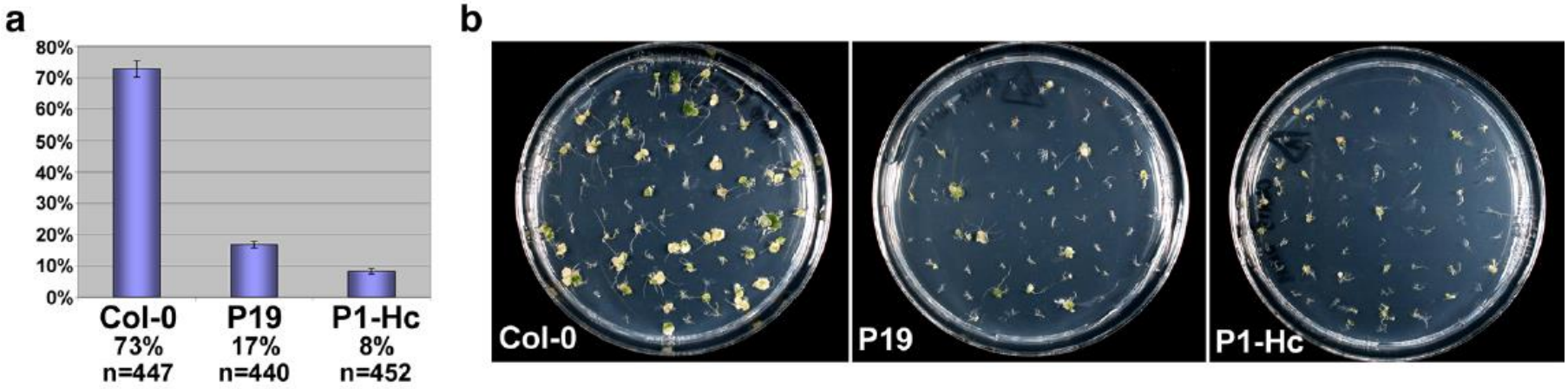
tryptophan 2-monooxygenase (iaaM)

agropine synthase (ags)

RDR6 is required for sense-transgene silencing

Dicer-like (DCL) enzymes define separate endogenous silencing pathways

Dunoyer P. et al. 2006. Induction suppression and requirement of RNA silencing pathways in virulent *Agrobacterium tumefaciens* infections. Nature Genetics. 38 (2): 258-263



Supplementary Fig. 1. ***Arabidopsis plants expressing the P19 and P1-HcPro viral silencing*** **suppressors show decrease susceptibility to** ***Agrobacterium.*** *Transgenic lines expressing the Tomato bushy stunt virus P19 protein and the Turnip mosaic virus P1-HcPro were described* previously. Unlike P38, P19 and P1-HcPro were shown to inhibit both RNAi from IR transgene and miRNA-guided function in *Arabidopsis. The experimental procedure is the* same as in Fig.1; the total values are from three independent inoculation experiments involving 140-150 root segments each. The P19 and P1-HcPro transgenes are all in the *Arabidopsis* ecotype Col-0 used here as a reference.

Dunoyer P. et al. 2006. Induction suppression and requirement of RNA silencing pathways in virulent *Agrobacterium tumefaciens* infections. **Nature Genetics.** 38 (2): 258-263

Chapter 1
Agrobacterium rhizogenes-Mediated Transformation and Its Biotechnological Applications in Crops

Ibrahim Ilker Ozyigit, Ilhan Dogan and Ebru Artam Tarhan

K. R. Hakeem et al. (eds.), *Crop Improvement*, DOI 10.1007/978-1-4614-7028-1_1,

Table 1.1 Oncogenes of *A. rhizogenes*, their encoded proteins, functions and phenotypic changes in host plants

Gene	Protein	Function	Phenotype
*rol*A	Sequence motif common in DNA-binding proteins Regulatory transcription factor	Inhibits cell elongation via diffusible factor Decreases hormone concentrations Increase sensitivity to auxin Modulating hormone physiology of GA Interfere polyamine metabolism Correlate with plasma membrane H^+ ATPase activity	Stunted growth, dark green wrinkled leaves with an altered length to width ratio, condensed inflorescences, retarded onset of flowering, compact reduced number of flowers
*rol*B	Localizes to plasma membrane	Alterations in the reception/transduction of the auxin signal Stimulates new meristem formation Induce secondary metabolism	Fast growth, root meristem neoformation, high branching and plagiotropism
*rol*C	Phloem-specific expression in the root, low expression in the leaf, and no expression in the shoot tip	Reduces cell size Reduces abscisic acid (ABA), polyamine, and ethylene levels Formation of shoot meristems Regulate sugar metabolism and transport Stimulate the production of high levels of secondary metabolites	Increased branching, dwarfed plants with short internodes, reduced epidermal cell size in internodes, lanceolate leaves, early flowering, reduced flower size and reduced pollen production
*rol*D	Only expresses in Agropine type strains Cytosolic protein Exhibits poor tissue- or organ-specific expression	Incapable of inducing root formation on its own Provide defense response as a result of environmental stress	Increased flowering, reduced rooting, elongating and expanding tissues of each organ but not on apical meristem, callus growth giving rise to initiation of tumor resemble formation

*rol*B^{TR}	CX5R motif is absent N-terminal part contain 14 amino acids	*rol*B homolog on TR-DNA in the agropine type Ri plasmid	Wrinkled leaves bent strongly downward, formed shoots at the base of the stem and retarded growth
ORF3n	Modification of phenolic enzymes and involve secondary metabolism and/or the transport of hormones	Negative regulator to the dedifferentiation of tissues	Retarded flowering, less dense inflorescences, altered internode elongation and leaf morphology and necrotic tips of upper leaves, sepals and bracts no sign of necrosis on the basal leaves

Table 1.1 (continued)

Gene	Protein	Function	Phenotype
ORF8	Fusion protein consisting of N-terminal domain (NORF8) and C-terminal part (CORF8) Tryptophan monooxygenase activity	Modifies sucrose transport N-terminal domain causes sugar/starch accumulation C-terminal domain reduces sugar/starch accumulation	Growth retardation, chlorotic and necrotic leaves and accumulation of high levels of sugars (glucose, fructose and sucrose) and starch
ORF13	Contains a conservative retinoblastoma (RB)-binding motif LxCxE	Hormone homeostasis and regulation of the cell cycle Increases number of mitoses in shoot apical meristem Induces dedifferentiation (prerequisite to competence) Graft transmissible	Induce cell proliferation such as dense green and rapidly proliferating callus, including irregular formation of leaves, severe leaf nervure, shortened and variable internode length, abnormal and asymmetric flowers, agravitropic root growth and a reduced cell number and cell size in the root
ORF13a	Tissue specific manner in plants, primarily in leaf vascular tissues May interact directly with DNA SPXX repeat motif	Necessary for root induction Regulatory function of itself	Not yield a visible phenotype
ORF14	Auxin like effect	Act together with ORF13 to induce root induction	No morphological change

3.3. Causan los oncogenes efectos adversos a las células vegetales?

Los oncogenes del ADN-Ti o ADN-Ri, alteran los procesos fisiológicos normales y causan daños en el desarrollo de las plantas, lo que representa pérdidas agrícolas.

Sin embargo la transformación con el gen rolC, tiene un efecto positivo en la viabilidad de las células cultivadas por largos períodos.

Raíces de Panax ginseng transformadas con el gen rolC , continuan creciendo a pesar de tener 20 años de cultivo, mientras que el cultivo de raíces normales presentan menos viabilidad.

Cultivos de callos transformados con el gen rolC de *R. cordifolia* han sido estables y con crecimiento vigoroso durante 12 años de cultivo

FIN

GRACIAS POR SU ATENCIÓN