Transformation of *Zea mays* seedlings with *Agrobacterium tumefaciens*

Resesarch topics and seminar 510

Presented by

Mohamed Abdellatif

11th of May 2021

Outline

- The presented Paper
- History of *Zea mays*
- History of *Agrobacterium tumefaciens*
- Aim
- Methods
- Results
- Discussion

The Paper to be presented

Plant Molecular Biology 7: 43–50, 1986

The transformation of *Zea mays* seedlings with *Agrobacterium tumefaciens*

Detection of T-DNA specific enzyme activities

Anne C. F. Graves & S. L. Goldman*

Department of Biology, University of Toledo, Toledo, OH 43606, U.S.A.

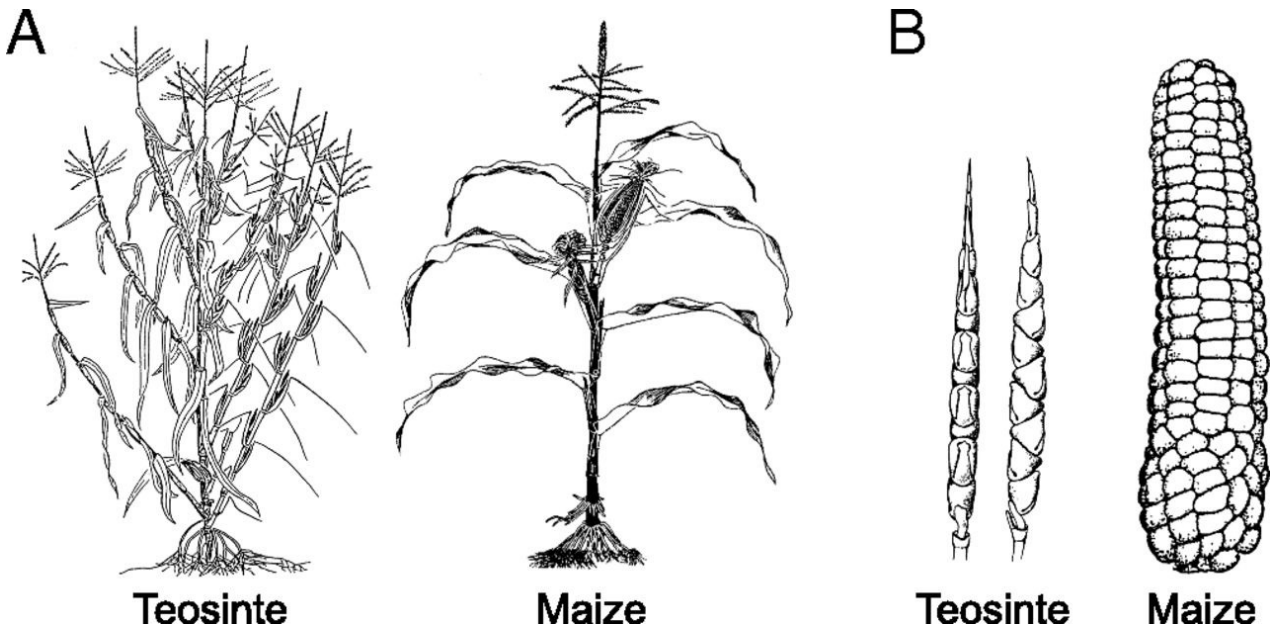
Keywords: transformation, *Agrobacterium tumefaciens, Zea mays*

History

Mexico was the center of domestication of Zea mays 9-10000 years ago

History

The original Maize have been cross bred with a native grain called teosinte



History

The process of heterosis helped in producing superior hybrids than the parents specially in *Zea mays.*

History of *Agrobacterium tumefaciens*

- **Rod shaped**
- **Gram negative**
- **Carries a Ti-plasmid**
- **The causing agent of crown gall disease.**

History of *Agrobacterium tumefaciens*

- **Crown gall disease is a tumor that is formed in dicots.**
- **Caused by the insertion of the T-DNA in the plant genome.**

Can It affect monocots?

Aim

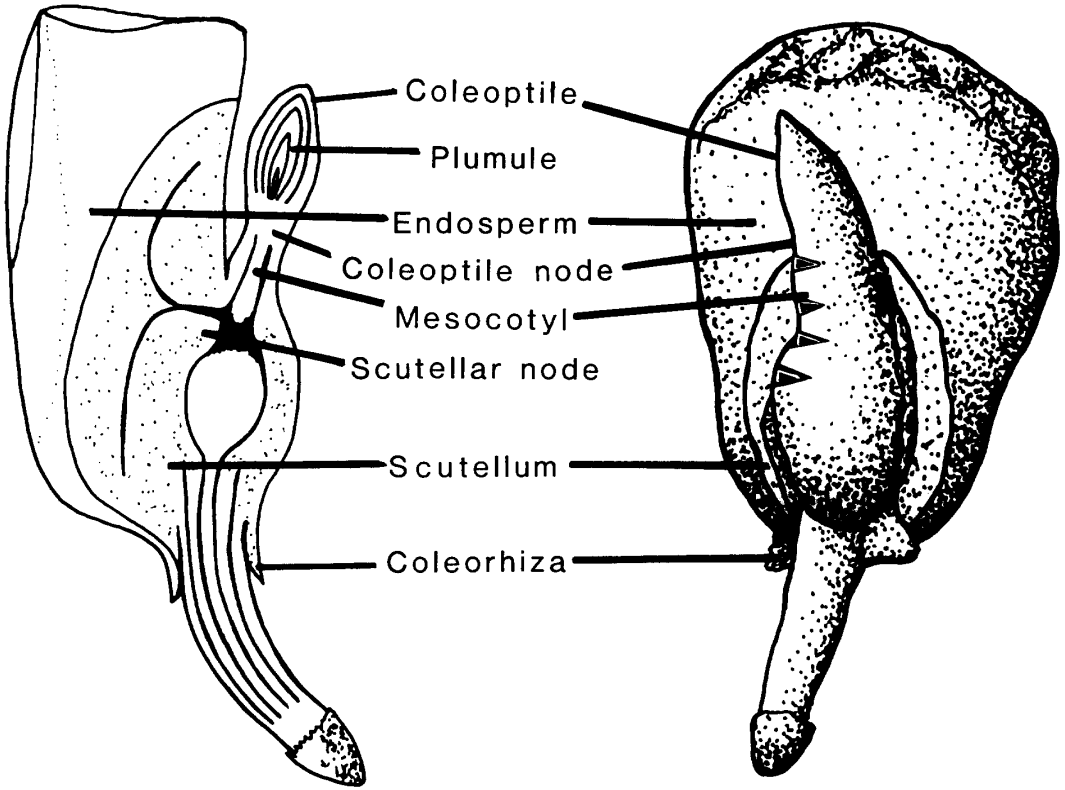
- **To prove that members of commercially important *Gramineae* are also susciptable for *A. tumefaciens* infection.**

Method

- **Five *A. tumefaciens* strains were used**
- **Two of them (B6 and, C58N) produce lysopine and nopaline dehydrogenase activities, respectively.**
- **The other 3 work as controls where A348 is a positive control while 238MX, and JK195 are negative controls**

Method

- **Four wound sites were introduced to sterilized germinating seedlings of hybrid yellow lochief.**
- **10^8 cells were dripped into the wounds.**



Method

- The seedlings are incubated.
- After incubation they are ground in Tris.HCl Sucrose extraction buffer.
- Reaction medium was used for the detection of lysopine and nopaline dehydrogenase activities.
- Electrophoretic separation was used to separate products of the enzyme activity.
- The presence of the opines was tested using staining with phenanthrenequinone.

Results

A. Detection of products of octopine synthesizing enzymes.

Lane 1, B6 inoculated seedling extract at time zero

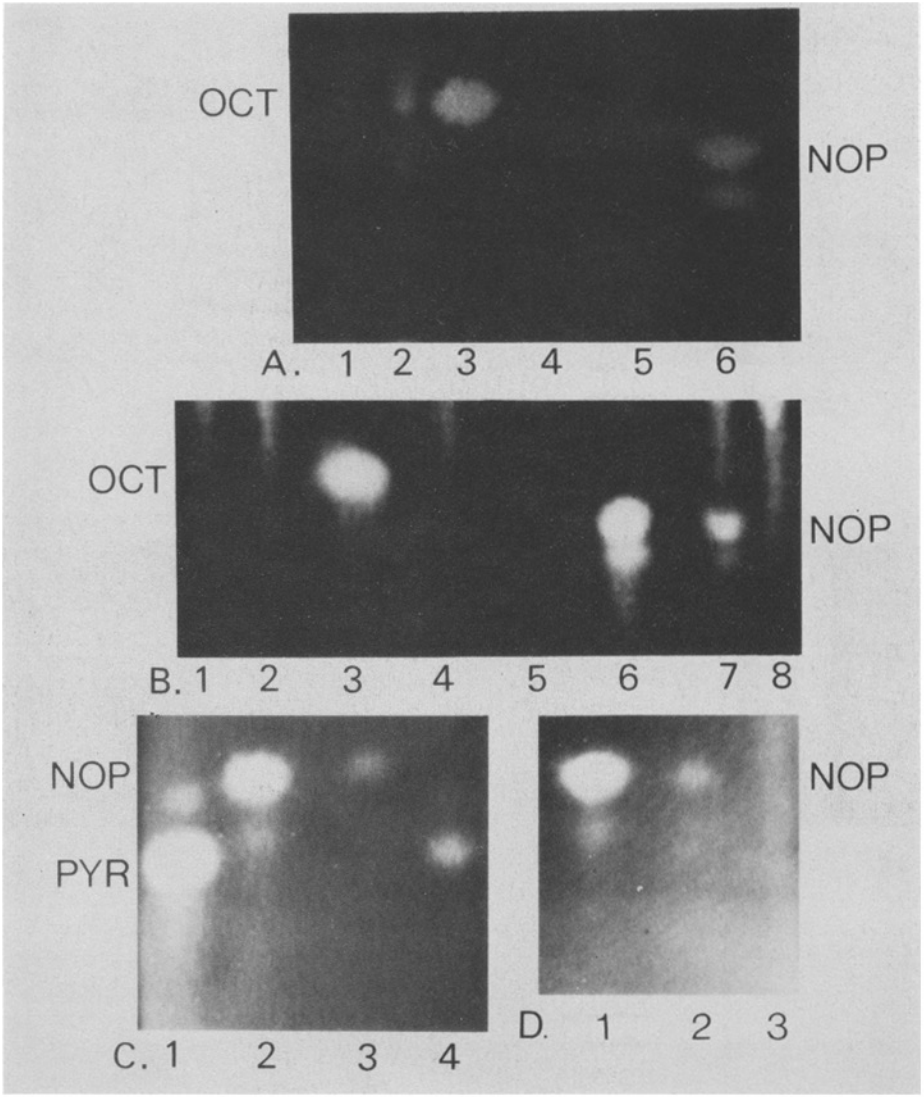
Lane 2, the same after 15 h.

Lane 3, synthetic octopine (Calbiochem)

Lane 4, 0.9% NaCl inoculated seedlings extract, at time zero

Lane 5, the same after 15 hr

Lane 6, Synthetic nopaline.



Results

B. Detection of products of nopaline synthesizing enzymes.

Lane 1, B6 inoculated seedlings extract at time zero

Lane 2, the same after 15 hr

Lane 3, synthetic octopine

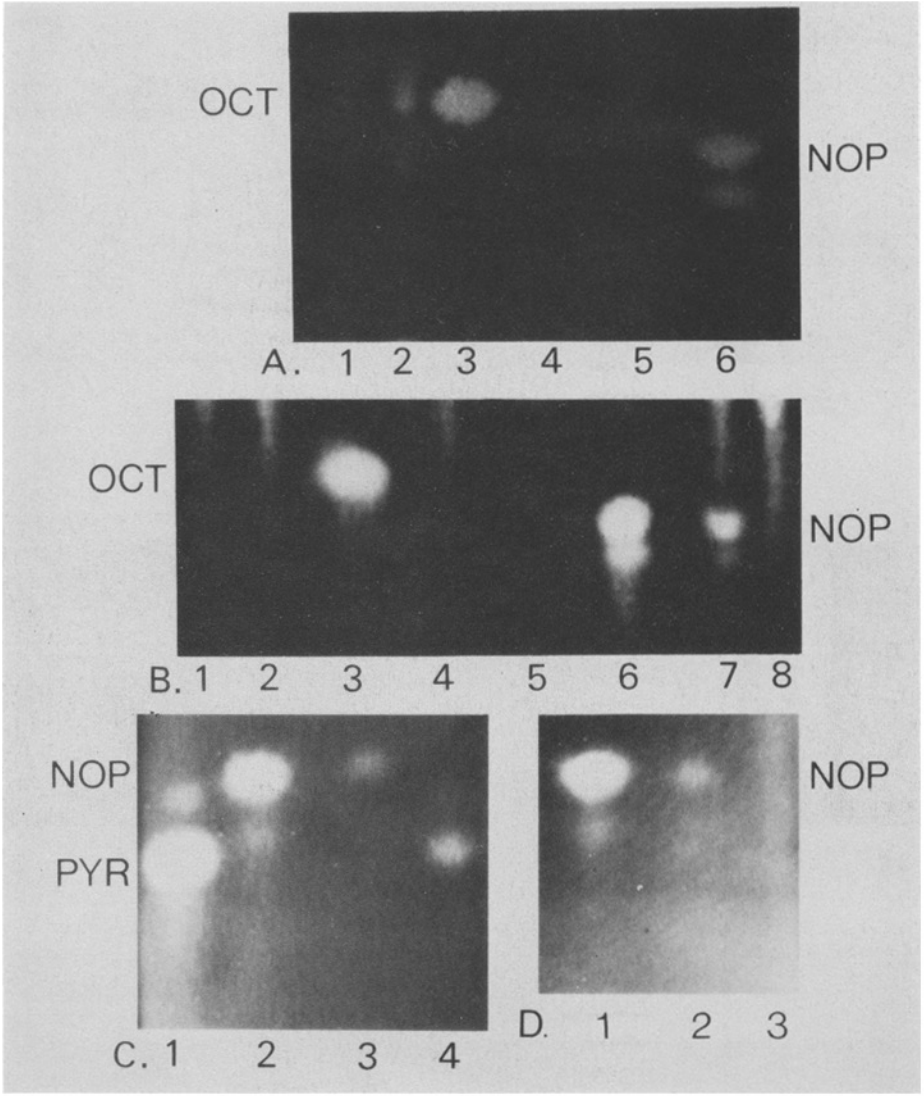
Lane 4, extract of 0.9% NaCl inoculated seedlings at time zero

Lane 5, the same, after 15 hr

Lane 6, synthetic nopaline (Sigma)

Lane 7, strain C58N inoculated seedling extract after 15 hr

Lane 8, the same at time zero.



Results

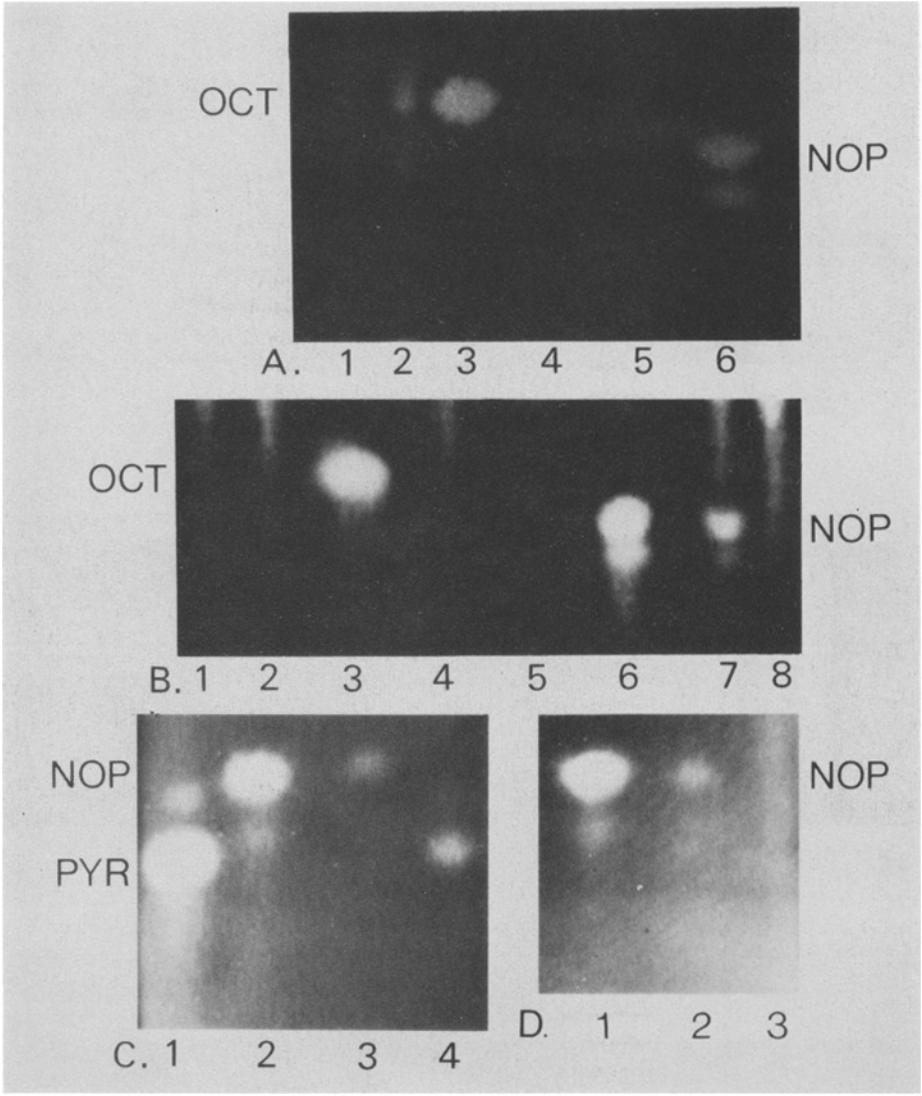
C. Confirmation of nopaline by conversion to pyro-nopaline

Lane 1, pyronopaline (produced from synthetic nopaline by treating with hot 2M acetic acid for 1 hour

Lane 2, synthetic nopaline (some of which converts spontaneously to pyronopaline)

Lane 3, product of enzymatic activity of C58N inoculated seedlings

Lane 4, the same treated with hot 2M acetic acid for 1 hr



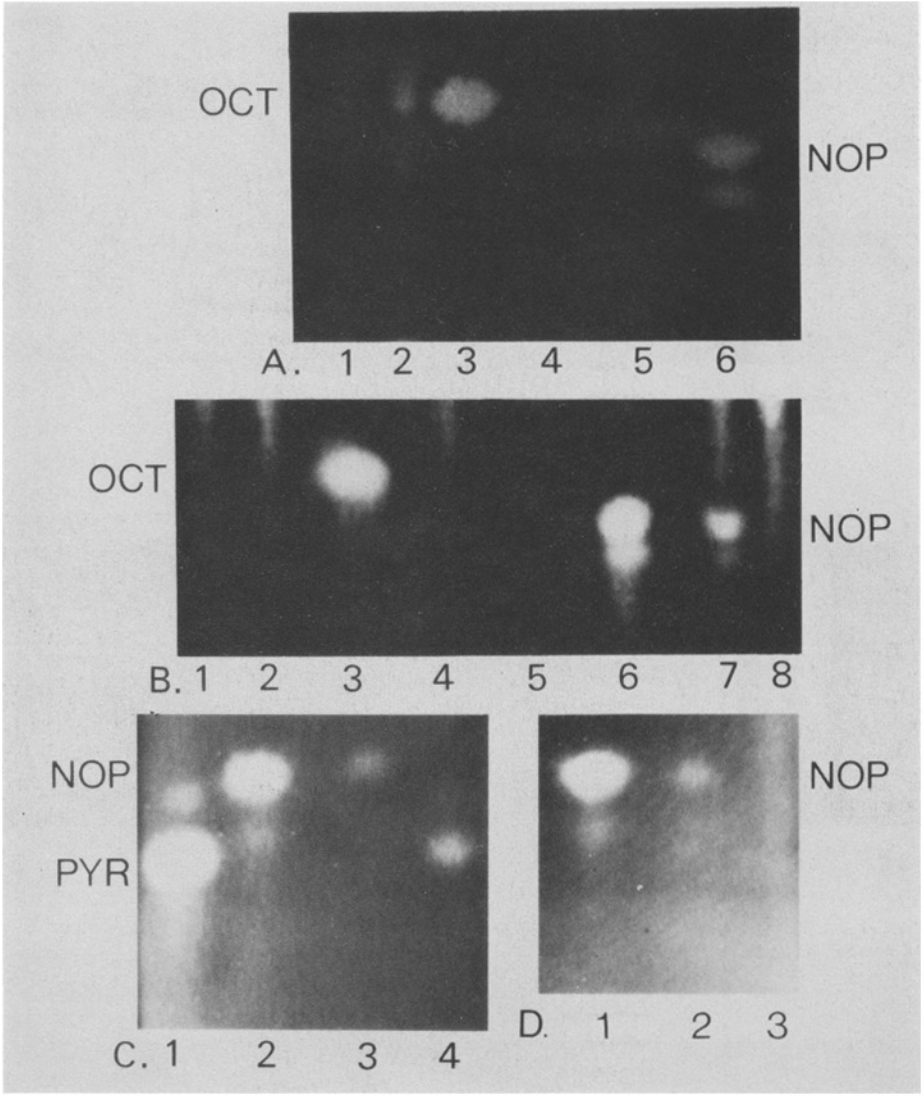
Results

D. Confirmation of nopaline identity by its Ti-plasmid mediated catabolism.

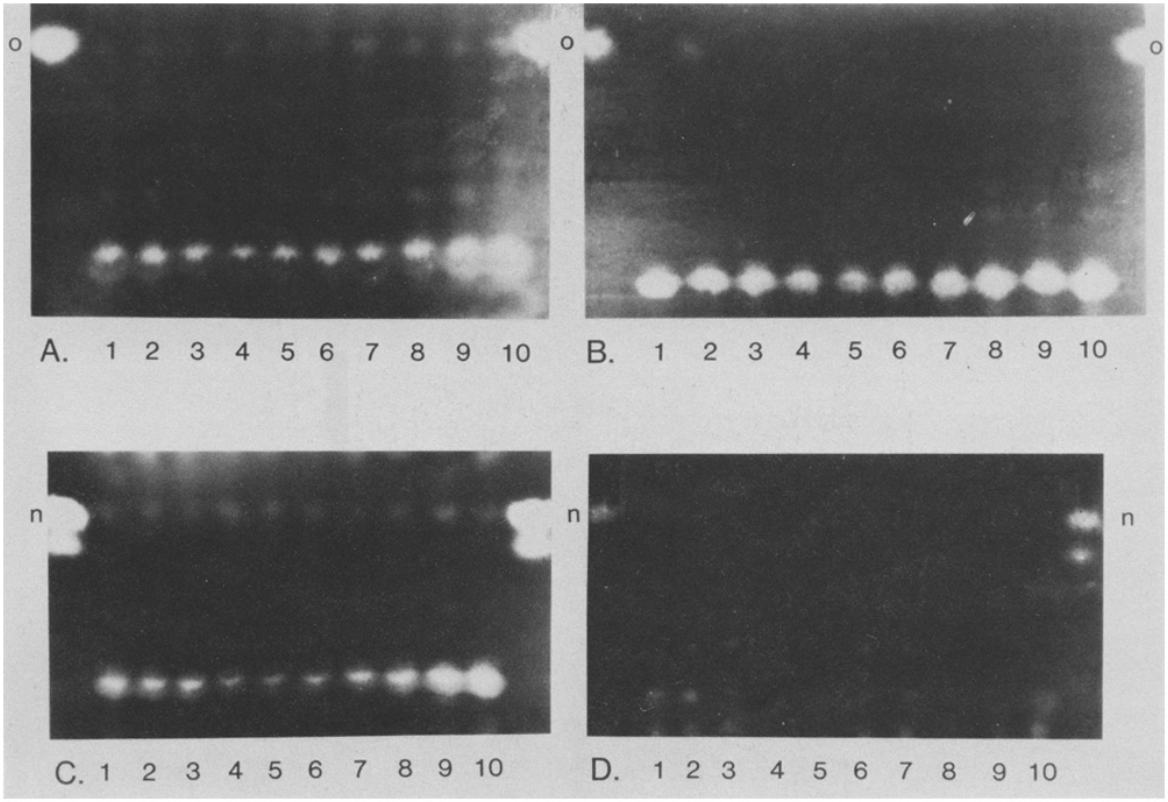
Lane 1, synthetic nopaline

Lane 2, nopaline produced by A. tumefaciens strain C58N inoculated seedlings (this material was electrophoresed and eluted) incubated with strain B6 for 24 hours

Lane 3, the same after incubation with strain C58N (a nopaline-catabolizing strain) for 24 hr.



Results



Opine synthesizing enzyme activities in single Zea mays seedlings inoculated with A. tumefaciens strains: B6 in panel A. lanes 1- 10; A348 in panel B. lanes 1-5; 238 MX in panel B. lanes 6-10; C58 in panel C. lanes 1- 10 and JK195 in panel D. lanes 1- 10. O = octopine standard and n = nopaline standard.

Discussion

- Only bacterial strains capable of transferring T-DNA managed to transform the seedlings.
- Strains that carry mutations in the *vir* region didn't trigger opine synthase activity.
- Neither lysopine nor nopaline dehydrogenase activity was detected in extracts prepared only from sonicated bacterial suspensions.

Conclusion

A. tumefaciens had delivered the T-DNA successfully to meristematic regions of intact seedlings including the scutellar node and mesocotyl.

The enzymes produced were the result from a plant/bacterial interaction and depend on functional vir genes to intoduce the T-DNA, permitting that segment to enter its eukaryotic host.

Thanks for listening

Teosinte, the wild ancestor of maize, which was domesticated in tropical southern Mexico around 7,000 B.C.

Representation of an early Agricultural "small cob" maize grown in the Tucson basin around 2000 B.C. Although there is not enough data to accurately illustrate its form, archeologists speculate that it was small and not a reliable food source until much later.

Popcorn, the hard-kernelled early corn grown in the Southwest.

Chapalote maize has both popcorn kernel varieties, and the less-hard flint kernels.

Tohono O'odham 60-day flour corn, probably similar to a type of Hohokam maize that appeared around A.D. 300 and may be a type found in Dempsey's pit house site.

Modern Tohono O'odham June dent corn appearing post-Spanish contact, probably the late 1700s.

Sweet corn from a local supermarket.