Biotechnology Tools For The Future Capsicum

http://mindbodysoulpatio.files.wordpress.com/2008/05/chile.jpg

Suman Bagga

Champa Sengupta-Gopalan

(Depart. Plant & Environmental Sciences)

Importance of chile

- High value cash crop in the world and in New Mexico
- Has been cultivated in the NM Rio Grande valley for four centuries
- It is considered state's signature crop and it contributes to the NM's economy

Importance of chile

- It is an indispensible spice used in cuisines all over the world
- High nutritive value, excellent source of vitamin C, A, B-complex and E along with minerals Mo, Mn, Folate, K & thiamine.
- Powerful antioxident
- Therapeutic properties by Capsaicinoids
- Hotness due to Capsaicins

Factors Affecting Chile Production

- Chile production is negatively affected by:

- Biotic factors – phytopathogenic fungi, bacteria, viruses, weeds and other pests like root knot nematodes

- Abiotic factors – temperature, moisture, light, pesticides and herbicides

Factors affecting chile


Phytophthora affected field


Curly top virus


Bacterial leaf spot


Drought


Russian thistle and Kochia

Strategies for Combating Disease

- Application/use of chemicals like fungicide sprays, soil fumigants, pesticides and herbicides
- Conventional Plant Breeding combined with improved agricultural practices
- Use of molecular markers for rapid selection of desired traits

Traditional Breeding

Resistant

X

Susceptible

Elite Traits

Progeny- Screen for resistance

Linkage drag

Resistant Progeny

X

Elite Traits

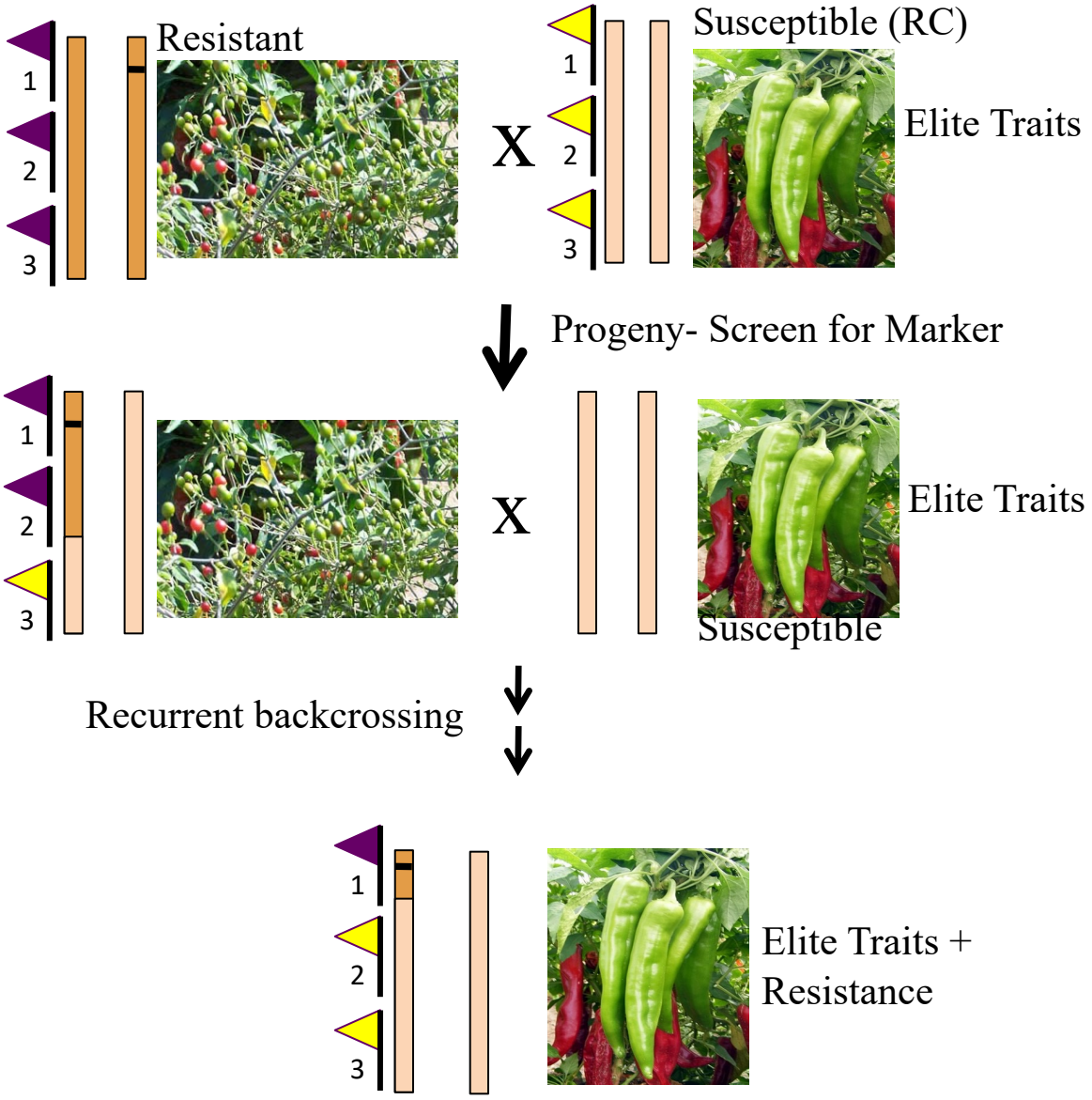
Susceptible

Recurrent backcrossing

Elite Traits + Resistance

www.daylife.com/ photo/02IK67t0Qo58p

Marker assisted breeding



-Track traits of interest using markers

-Uses markers to compare backcross progeny to the recurrent parent (RC)

- Identifies rare progeny with very high similarity to RC

-Accelerates selection process

- Allows selection of traits that are difficult to evaluate phenotypically

Crop Improvement involves changing the plant's genetic makeup

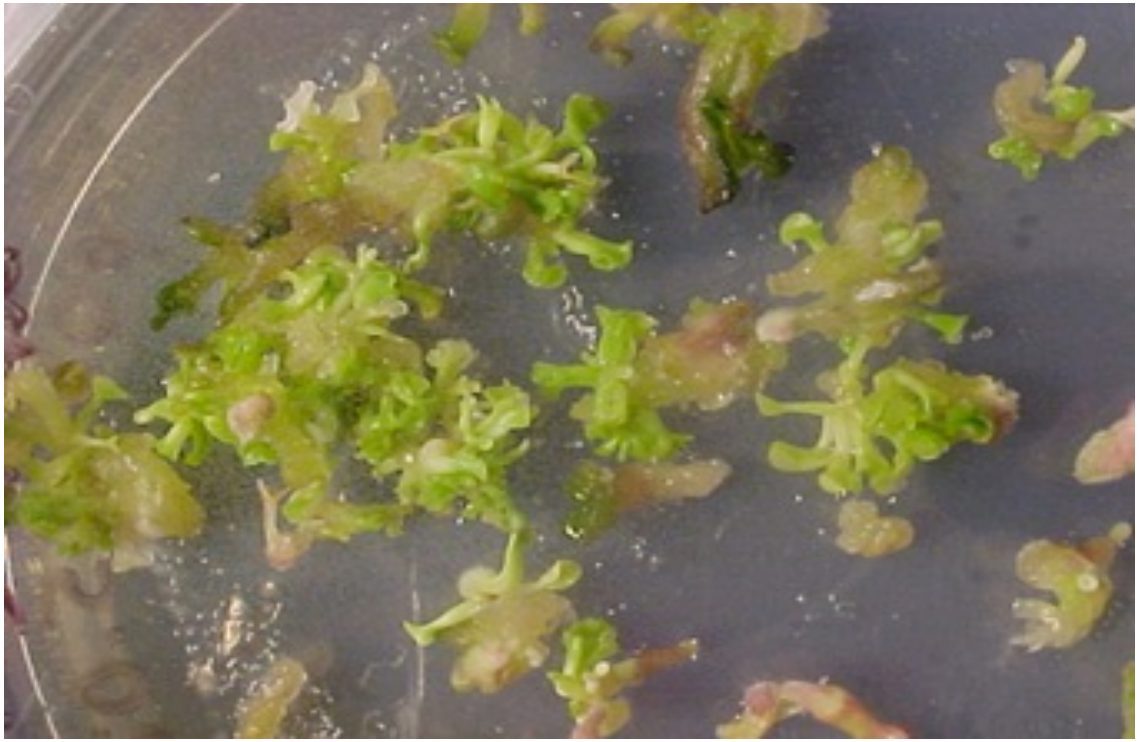
Conventional and Marker assisted Breeding: Making deliberate crosses between two parents.

Plant Genetic Engineering: Introducing genes of desired traits into recipient plant by methods other than sexual crosses.

Introduction of Resistance by Transgenic Technology

- Introduction of a cloned resistance gene into a plant by transgenic technology
- Can overcome the limitations of interspecies sterility
- Allows insertion of multiple genes simultaneously

Plant Regeneration Technology - A key step in Genetic Engineering

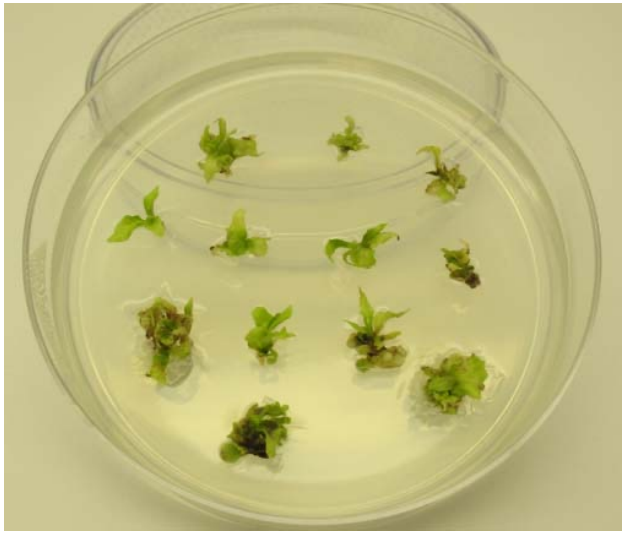


Regenerating whole plants from single cells following introduction of a gene into the cells

Plant Regeneration in Tissue Culture

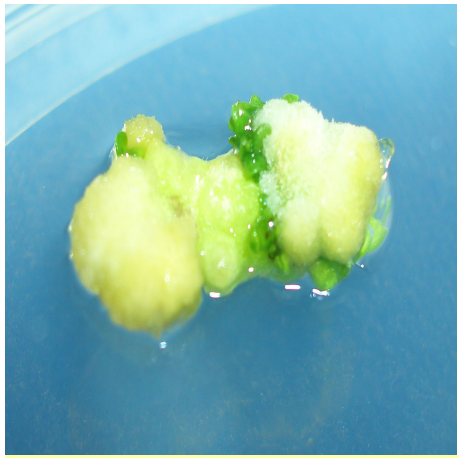
Steps:

1. Survey of cultivars for high regeneration potential
2. Selection of explant type and growth phase
3. Plant growth media
4. Growth regulators and other culture conditions (temperature, light and dark regime, etc.)

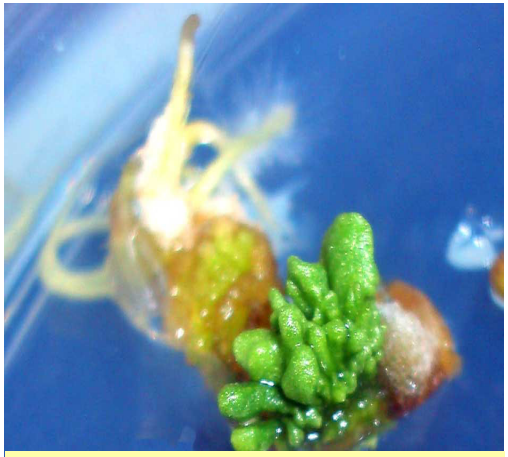


Chile Regeneration

Callusing



Multiple Embryo Formation



Embryo Development

Plantlet with multiple shoots

Plant Genetic engineering

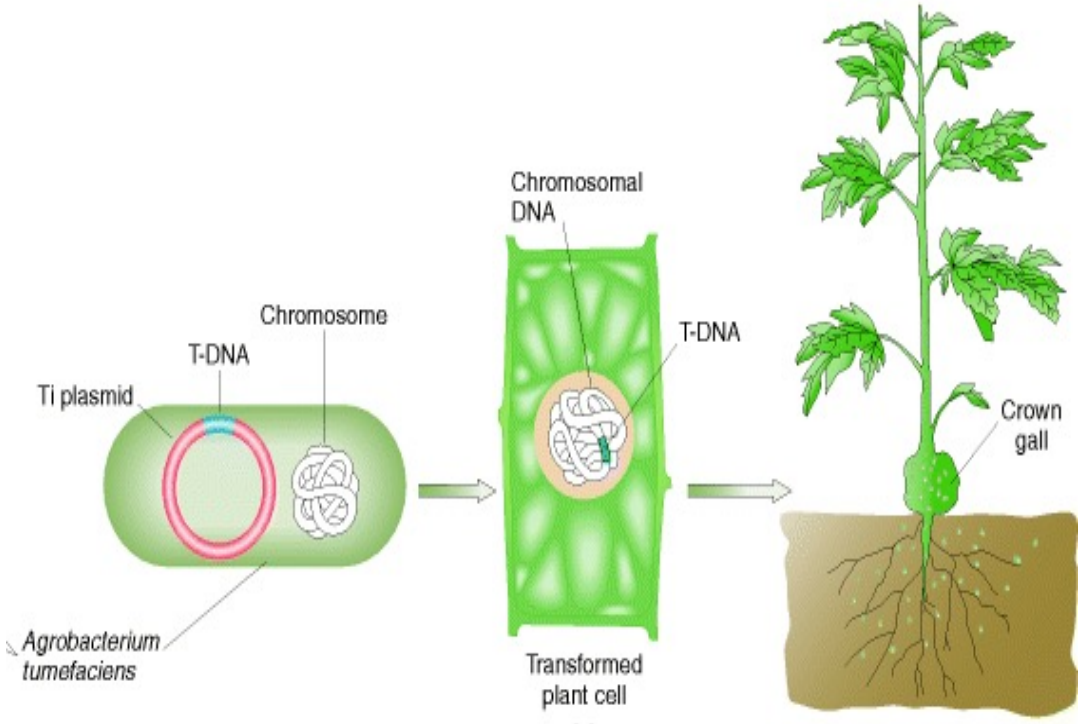
- Introduction of genes using methods other than sexual crosses.

- The genes can originate from a crossable sexually compatible plant - **cisgenics**.

- The genes can originate from any organism or be synthetic - **transgenics**

Plant Transformation - Introduction of cloned DNA into plant cells

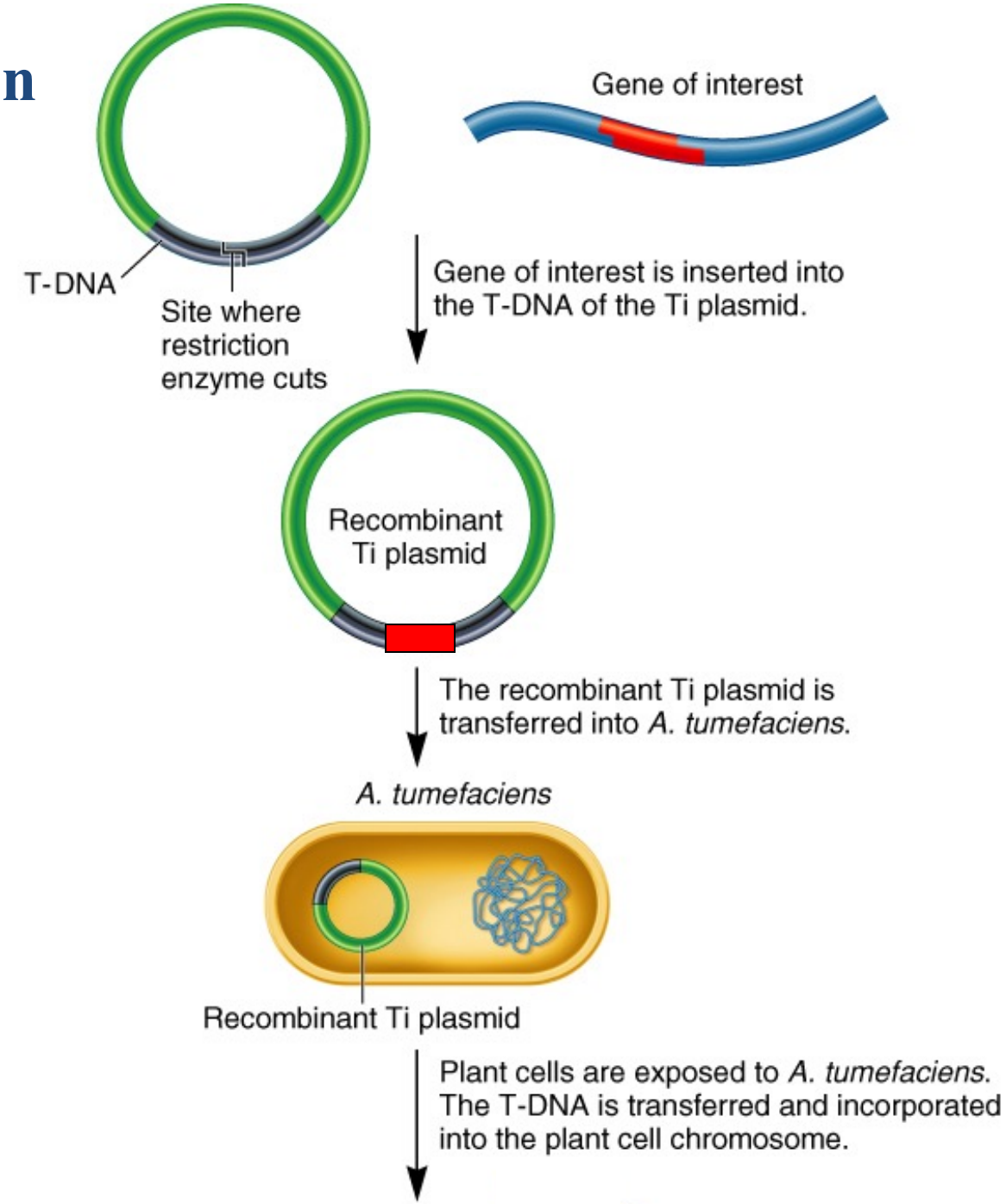
Agrobacterium tumefaciens
(Nature's own genetic engineer)



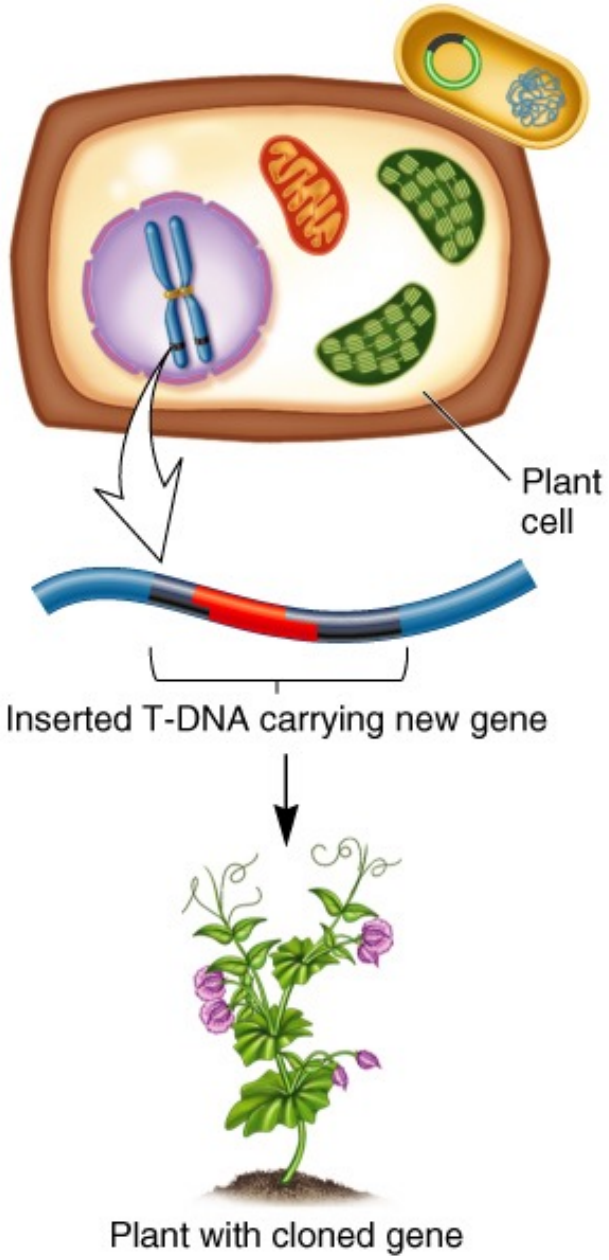
(http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Talbert/crowngall.gif)

(http://arabidopsis.info/students/agrobacterium/gall1.jpg)

Plant Transformation



Plant Transformation
(contd)



What are some of our objectives towards Chile improvement?

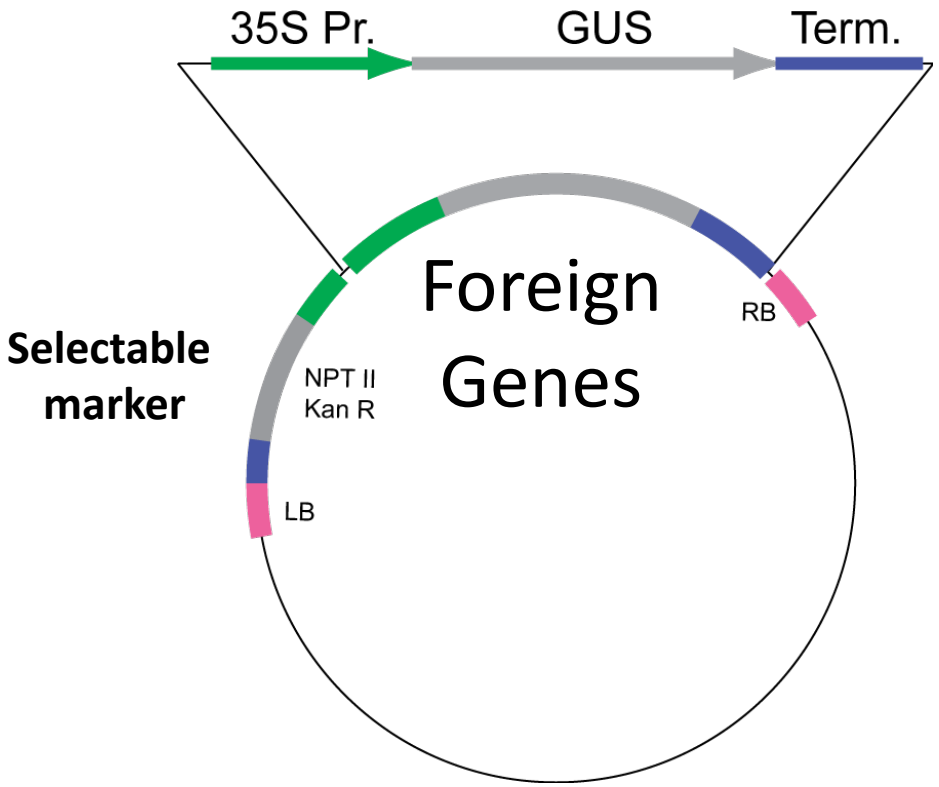
Chile production is negatively affected by:

- Biotic factors – phytopathogenic fungi, bacteria, viruses and other pests like root knot nematodes

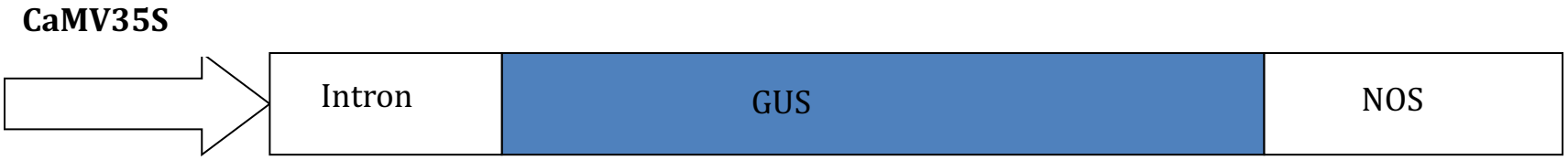
-Abiotic factors – temperature, moisture, light, pesticides and herbicides

To develop resistance in Chile to both biotic and abiotic stresses

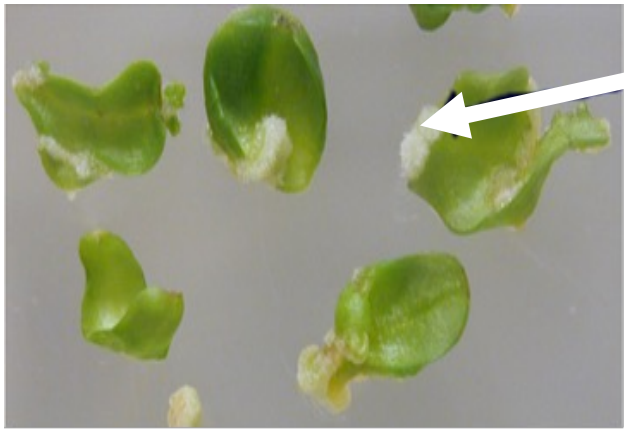
Transformation Vectors



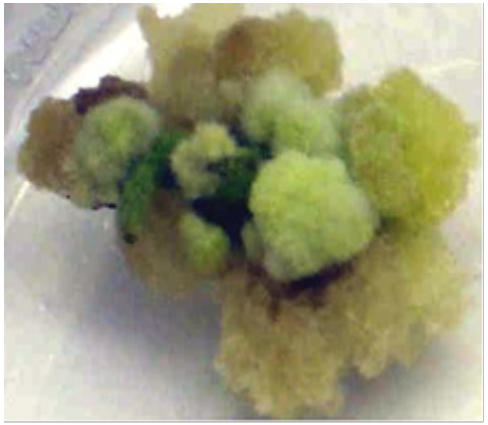
Transgenic Vector



Selection stages of transgenic plantlets



Explants



Callus

Plantlet

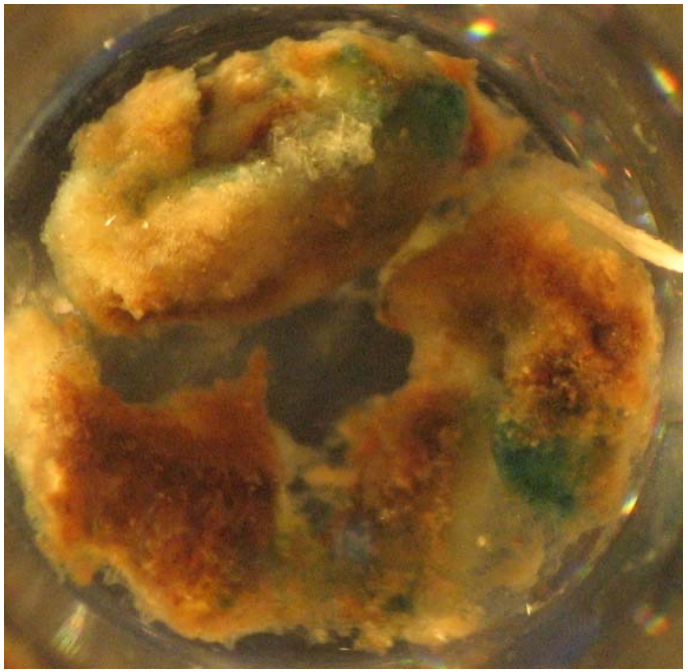
Bleached plantlet

β-glucuronidase

-Reporter gene

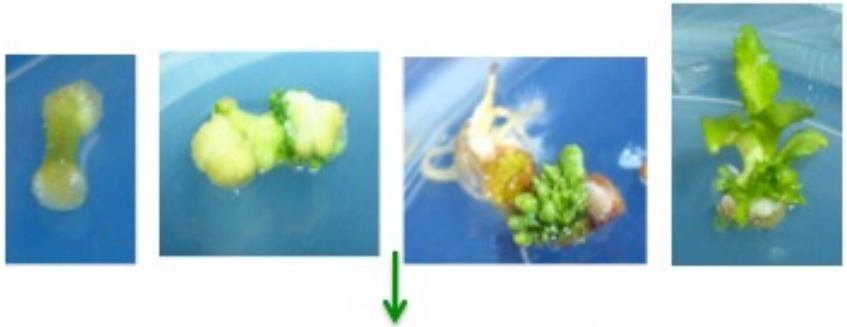
-Transformed plant cells expressing gene appear blue when stained with substrate

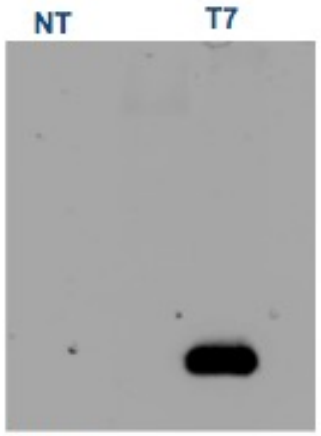
-GUS with intron



Putative Transformants:

Stages in Chile transformation and regeneration with the GUS (β-glucuronidase) reporter gene





Native gel

Polyacrylamide gel stained with fluorogenic substrate, ELF-97-D glucuronide, for β-glucuronidase.

ELF-97 β-D glucuronide allows the detection of enzymatic activity in situ. Yields a hydrolytic product, minimizes interference from autofluorescence in plant tissue.

Chile Biotechnology

Developing a regeneration and transformation protocols for NM chile

Use biotechnology for gene identification and discovery

A cisgenic and transgenic approach to develop herbicide and disease resistance in Chile

Making designer genes for transgenics

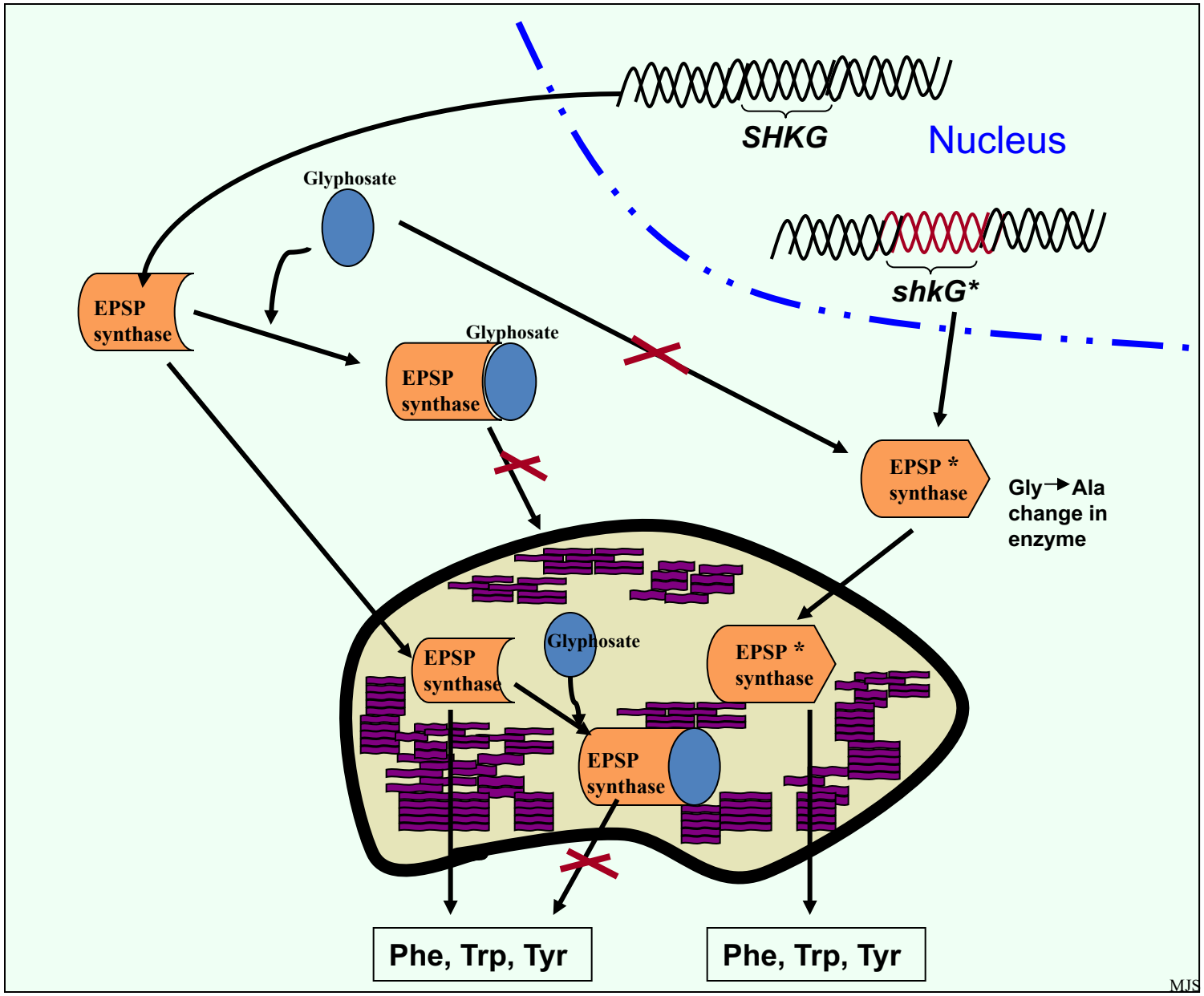
- Herbicide resistant gene (Chile gene) - protein coding sequence engineered behind plant regulatory sequence

Regulatory Region

Herbicide resistance gene

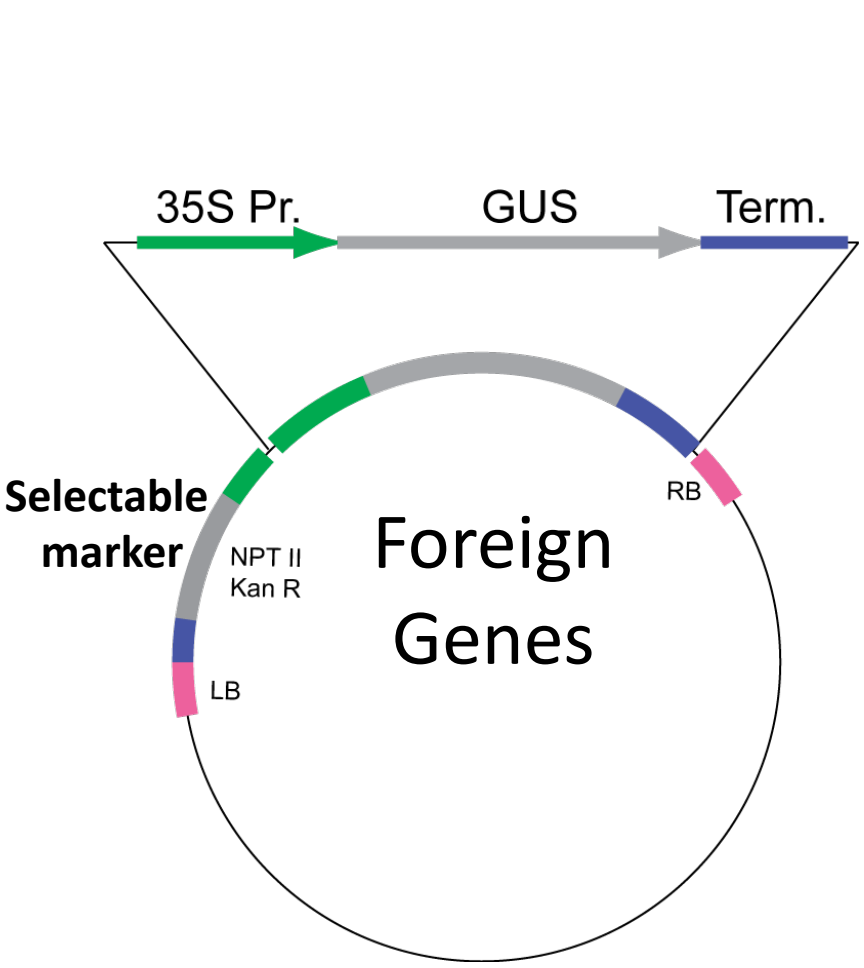
Constitutive promoter OR
Leaf Specific Promoter

Herbicide Tolerance

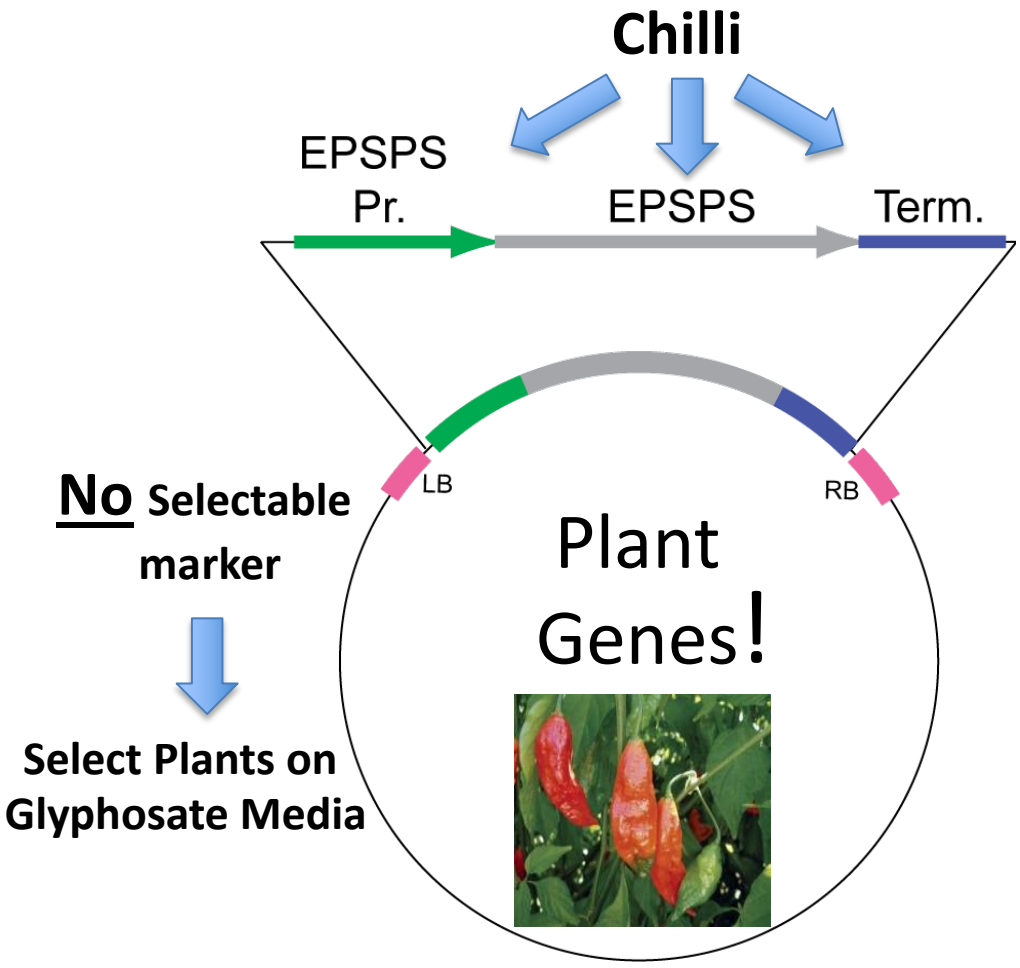


Modified from: *Biochemistry and Molecular Biology of Plants*. Edited by Buchanan B., Gruissem W., Jones R. (2000) 2000 Courier Companies, Inc., Rockville , MD.

Transformation Vectors

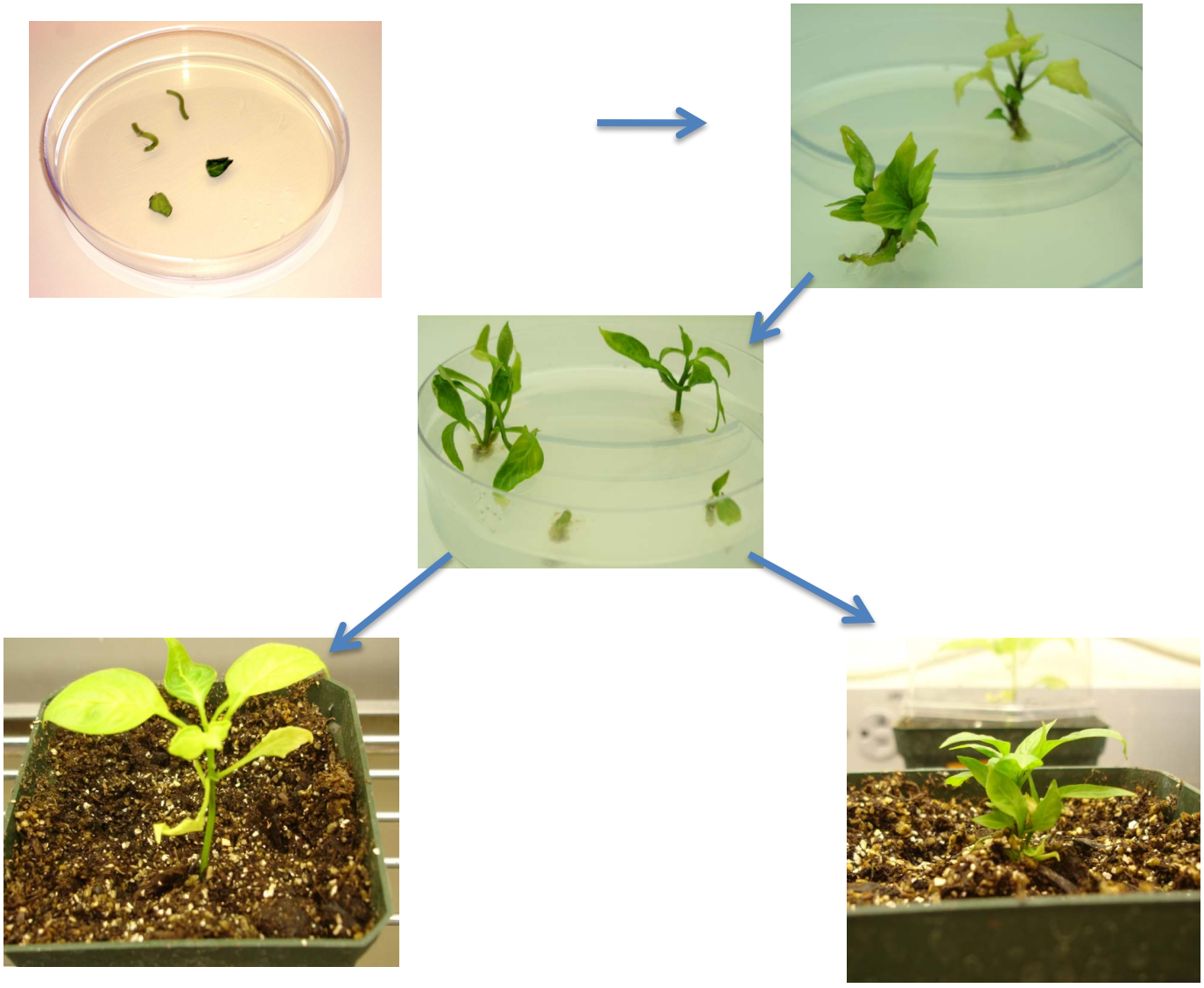


Transgenic Vector

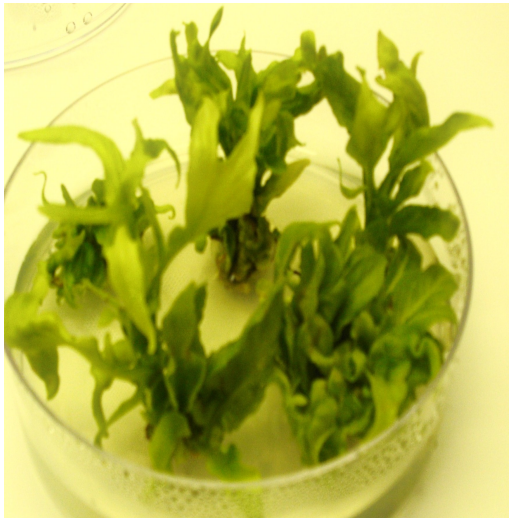


Intragenic Vector

Development of Transgenic Plants in Tissue Culture



- **Regeneration Of Transgenic Chile Plants**



Intragenic Vector

Gene of interest from the same or related plants.

Gene driven by its own regulatory sequence to target gene expression at a specific time, location or in response to a specific signal.

No selectable markers

No foreign DNA is inserted to produce non transgenic GM plants.

- Isolation and engineering of a broad spectrum resistance gene (***RB)*** gene from *Solanum bulbocastanum* into cultivated potato and for resistance to *P. infestans.*

Phytophthora affected field

- The *RB* gene from the *Solanum bulbocastanum* (wild relative of potato) confers broad spectrum resistance to many strains of *Phytophthora infestans* (potato late blight) when engineered into commercial potatos lines.
- When tested in the wild-type and transgenic potatoes it was found that 1) *P. capsici* causes disease on potatoes and 2) that potatoes containing the *RB* gene had reduced disease.
- Engineered the *RB* gene into tobacco and tomato to ask if it would confer resistance to *P. capsici* when moved into a different Solanaceous species.

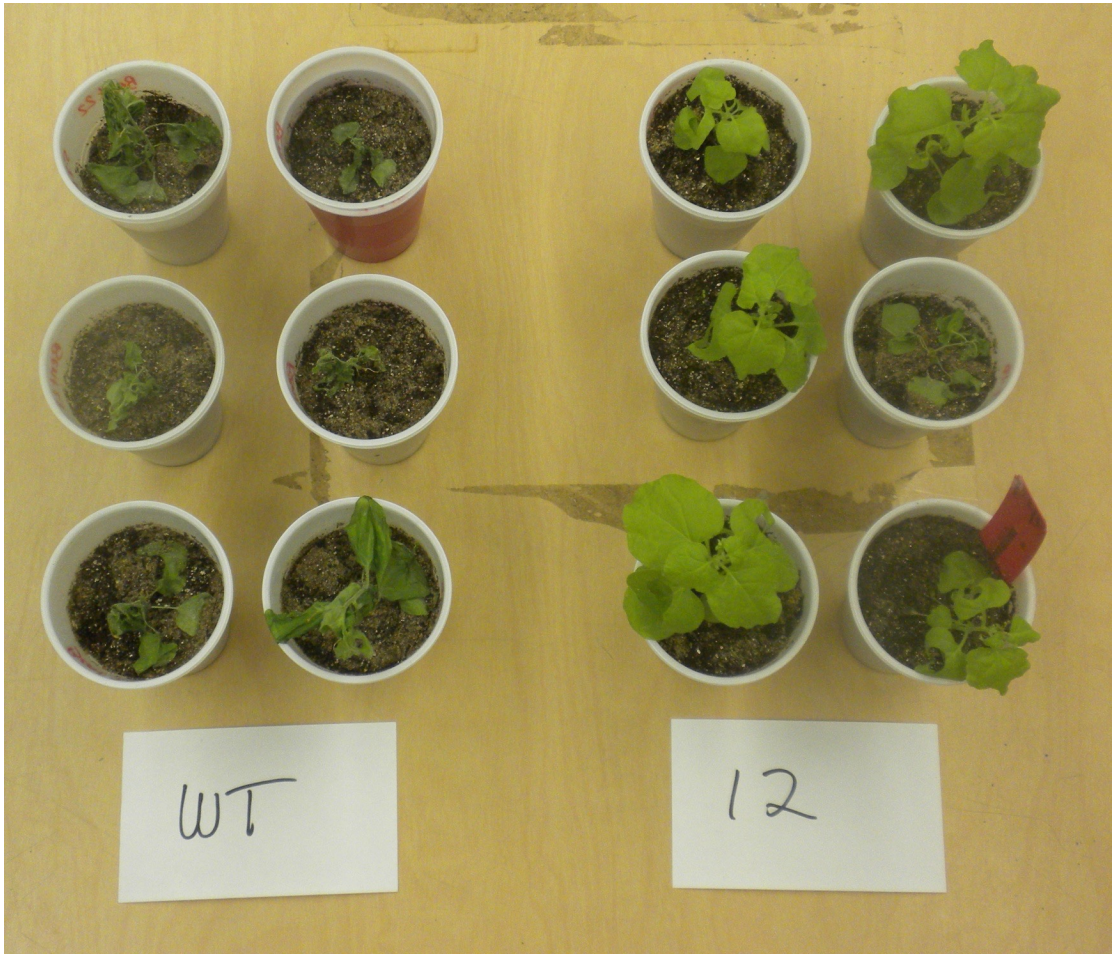
- Current work

- Twelve transgenic tobacco lines containing the *RB* gene were created and self pollinated to create segregating populations for each line.

- Seed from each line was planted and young plants were challenged with *P. capsici*.

- Plants were rated over a 14 day window for mild symptoms, severe wilt, or death. Disease progress curves were plotted for each line.

Resistance to *Phytophthora capsici* in transgenic tobacco plants containing the *RB* resistance gene from potato.



Chile Biotechnology Group

(Prof. Champa Gopalan's Lab.)

Melina Sedano M.S.

Hanna Jesko
Carolina Burgos-Vega
Charleen Carr

Dr Jose Louis Ortega

Collaborators:
Dr Steve Hanson

Dr Paul Bosland

Funding and support for this research from NMCA is appreciated & acknowledged

Nobel Prize laureate, Dr Norman E. Borlaug

- "There is no evidence to indicate that biotechnology is dangerous. After all, mother nature has been doing this kind of thing for God knows how long."

Factors affecting chile


Phytophthora affected field


Curly top virus


Bacterial leaf spot


Drought


Russian thistle and Kochia

Other Strategies/Approaches:

- Protein-mediated resistance-expression of transgenic **coat protein** genes to block the progression of virus infection process.
- RNA based resistance by degrading the viral RNA by using the plants post-transcriptional gene silencing (**PTGS)** mechanism
- mRNA pathway by designing **artificial microRNA** against viruses whose expression in transgenic plants can confer resistance against these viruses.

Comparison of conventional breeding with cisgenics

- Cisgenics is better than traditional introgression and translocation breeding because of the lack of linkage drag and the reduced number of steps.
- Cisgenics is now also referred to as **Precision breeding**
- Cisgenics also allows for gene stacking

Plant Genetic Engineering - *Transgenics*

Increasing the gene pool for crop improvement.

Transgenics is the introduction of a gene obtained from any source into a recipient plant

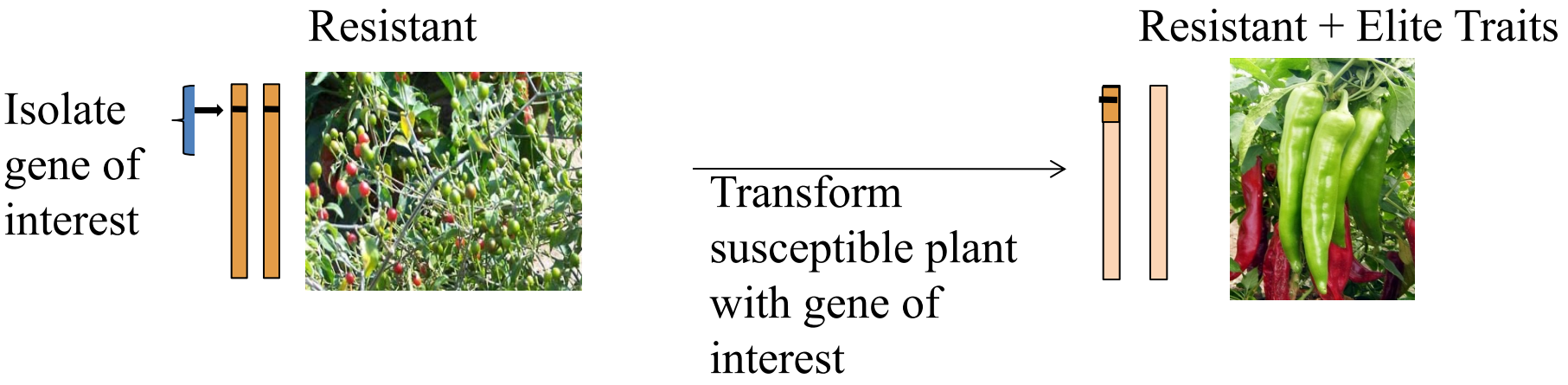
(***Genetic code is universal***)

Bt gene from bacteria encoding for protein with insecticidal activity can be expressed in plants.

Involves manipulation of the bacterial gene for expression in plants

Plant Genetic Engineering –Cisgenics

- Cisgenesis is the genetic modification of the recipient plant with a natural gene from a crossable sexually compatible plant



PRECISION BREEDING:

- Intragenic vectors for developing non-transgenic genetically modified (GM) plants.
- Production of plants with no DNA from outside the pool as is already available to the plant breeders.
- Resulting GM plants are not “TRANSGENIC”, although they are derived using the molecular biology and plant transformation tools.
- Socially acceptable/responsible way for developing GM crops.