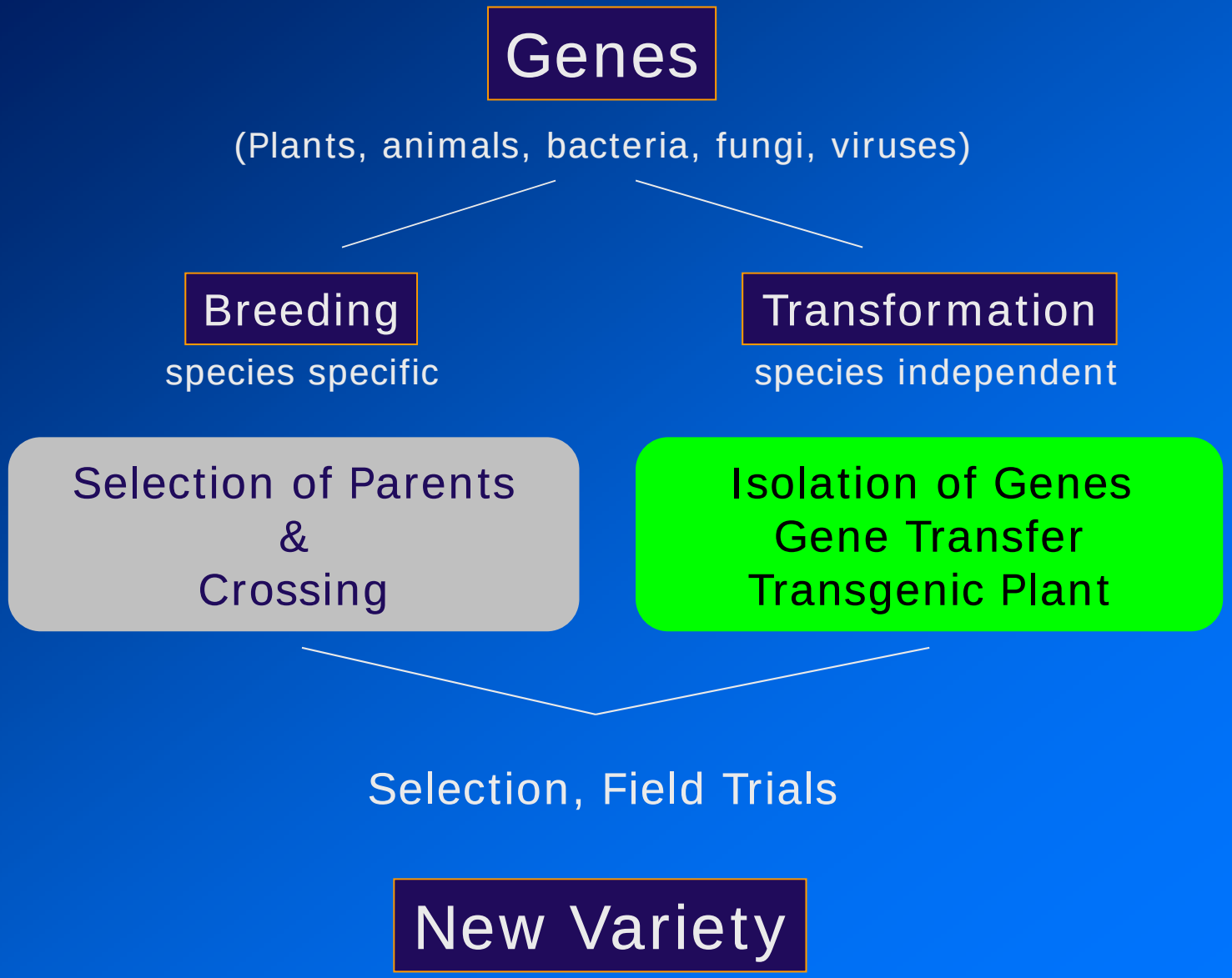
Applications of Biotechnology in Horticulture

Mahran El Nagar

Theusday, 12/5/2015

Biotechnology in Breeding



Plant Biotechnology

... is the *in vitro* manipulation of plants (or plant material) for enhanced productivity.

التقنية الحيوية

الإستخدام التقني الموجه للكائنات الحية على المستوى الخلوي والجزيئي للحصول على نواتج مفيدة .

I.Tissue culture techniques in plant biotechnology:

1. propagation *in vitro* (clonal propagation of identical plants)
2. production of secondary plant products
3. development of new variants (generation of new varieties)

II. Molecular biological techniques in plant biotechnology:

1. marker assisted breeding
2. genetic engineering

III. Genetically modified (GM) crops:

1. new traits
2. public concerns, risks
3. legal aspects, regulations

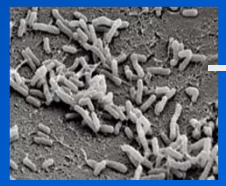
III. Genetically modified (GM) crops:

11. new traits

- herbicide tolerance
- insect resistance
- virus resistance
- male sterility
- pathogen resistance, stress tolerance
- nutritional enhancement: production of provitamin A
- specialty products: oils
- specialty products: plastics
- genetic manipulation of fruit ripening

Delivering the Gene to the Plant

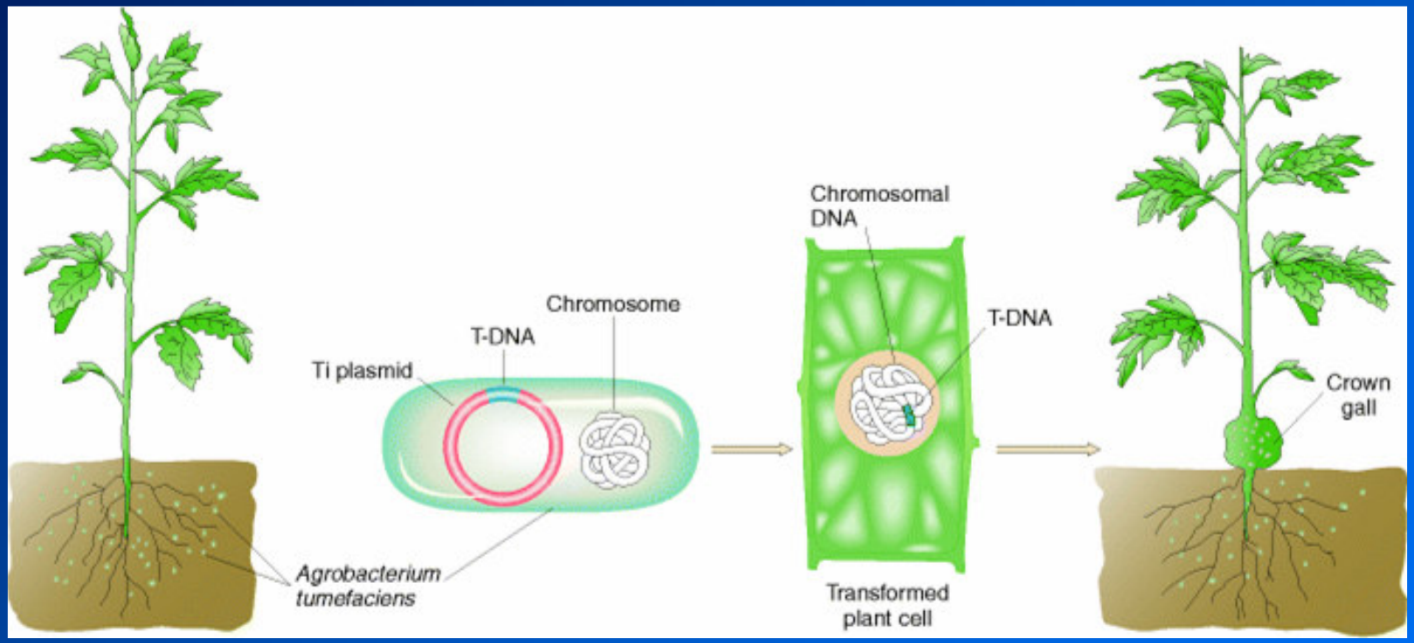
- Two major delivery methods
  - *Agrobacterium*



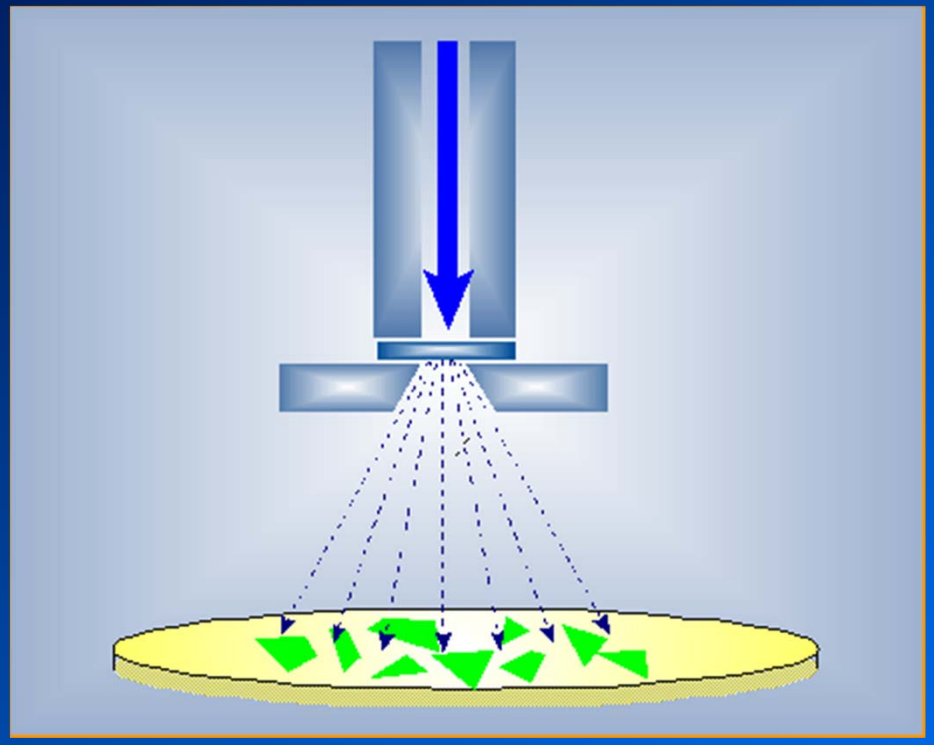
  - Gene Gun

Tissue culture required to generate transgenic plants

Agrobacterium tumefaciens



Particle Gun

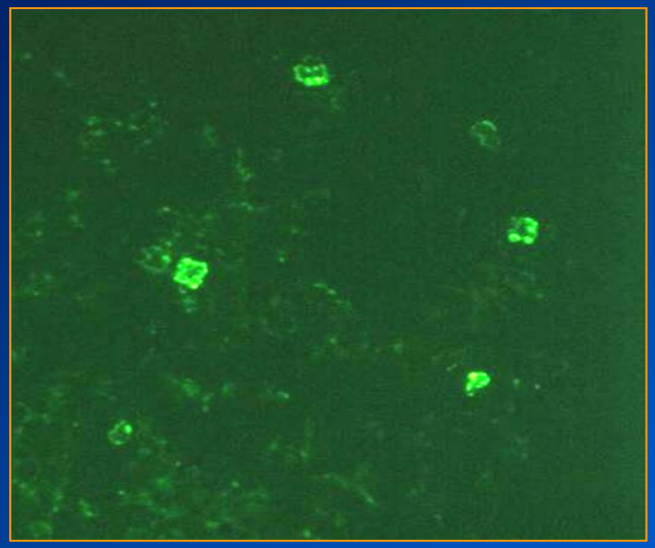


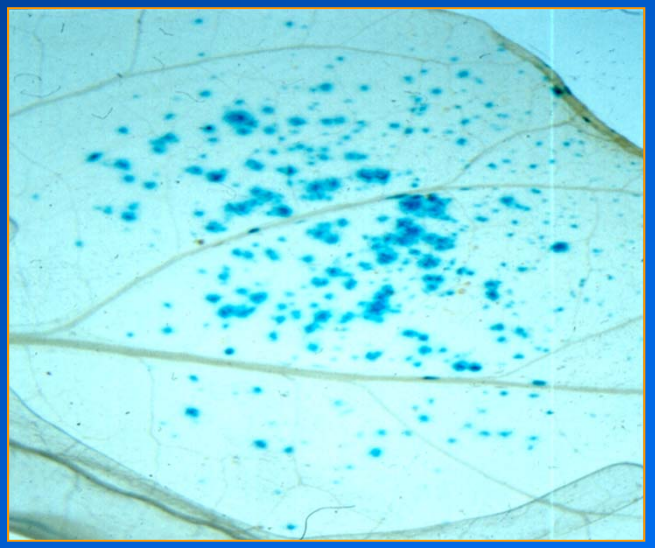
Reporter gene expression

Green Fluorescent Protein

ß-Glucuronidase

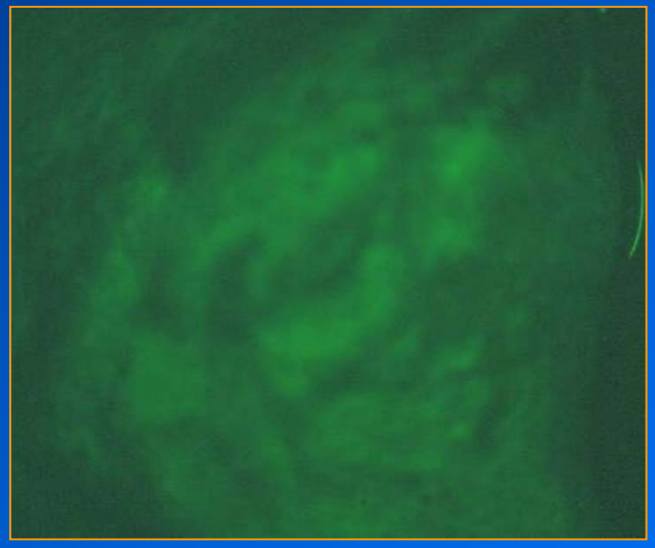
GFP





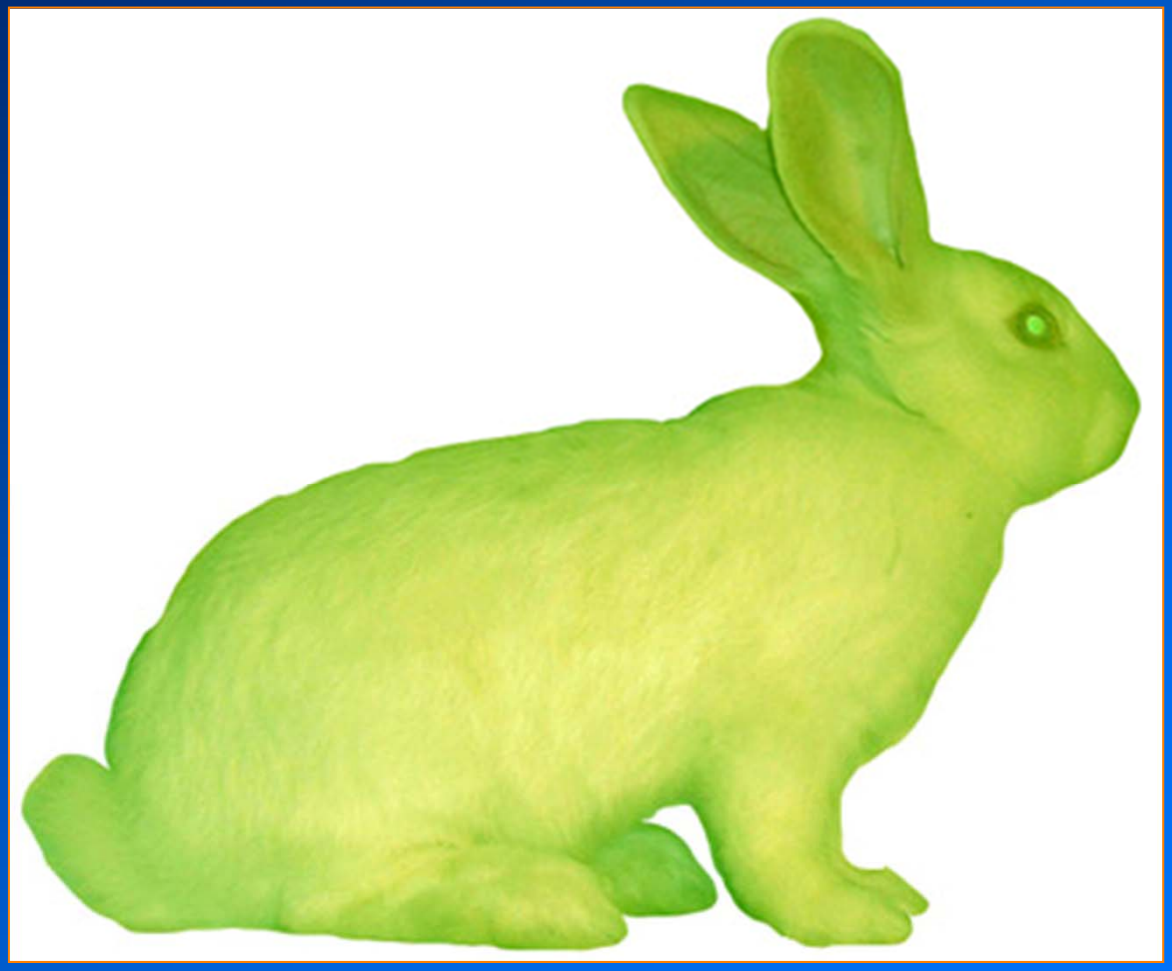
GUS

Control



Control

Application: GFP rabbit



What is Gene Silencing?

- None or only weakly expression for a gene which in the normal condition give expression.
- Gene remains present , it is not mutated but it is silent

* Down regulated or inactive

Transgene in Petunia

- Rich Jorgensen
- Key enzyme for flower pigmentaion:Chalcon-Synthase (CHS)
- Overexpression of Chalcon Synthase in Petunia

- Expected:

Transformation

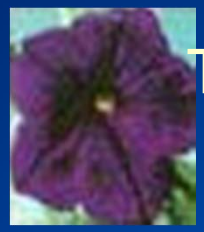
Wild type
(normal expression)

Transgene
(more intense colour)

Real out come from the Transformation

Transgene

Wild type



Transformation

Colourless regions

Large colourless regions

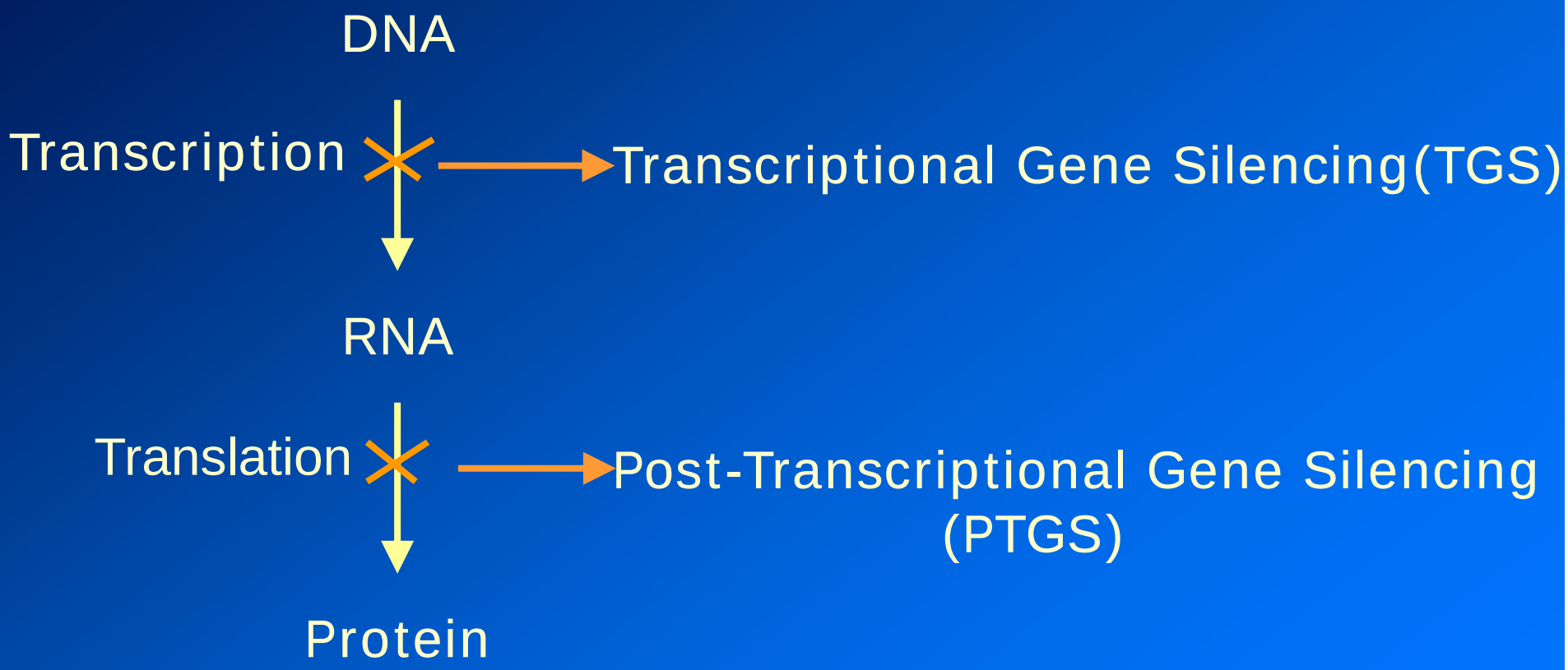
Colourless

Real out come from the Transformation

- No expression from the gene construct
- Transgene : an additional gene introduced (in contrast to endogenous gene)

- Name of phenomenon: Cosuppression

Types of Gene Silencing



Transcriptional Gene Silencing (TGS)

DNA methylation

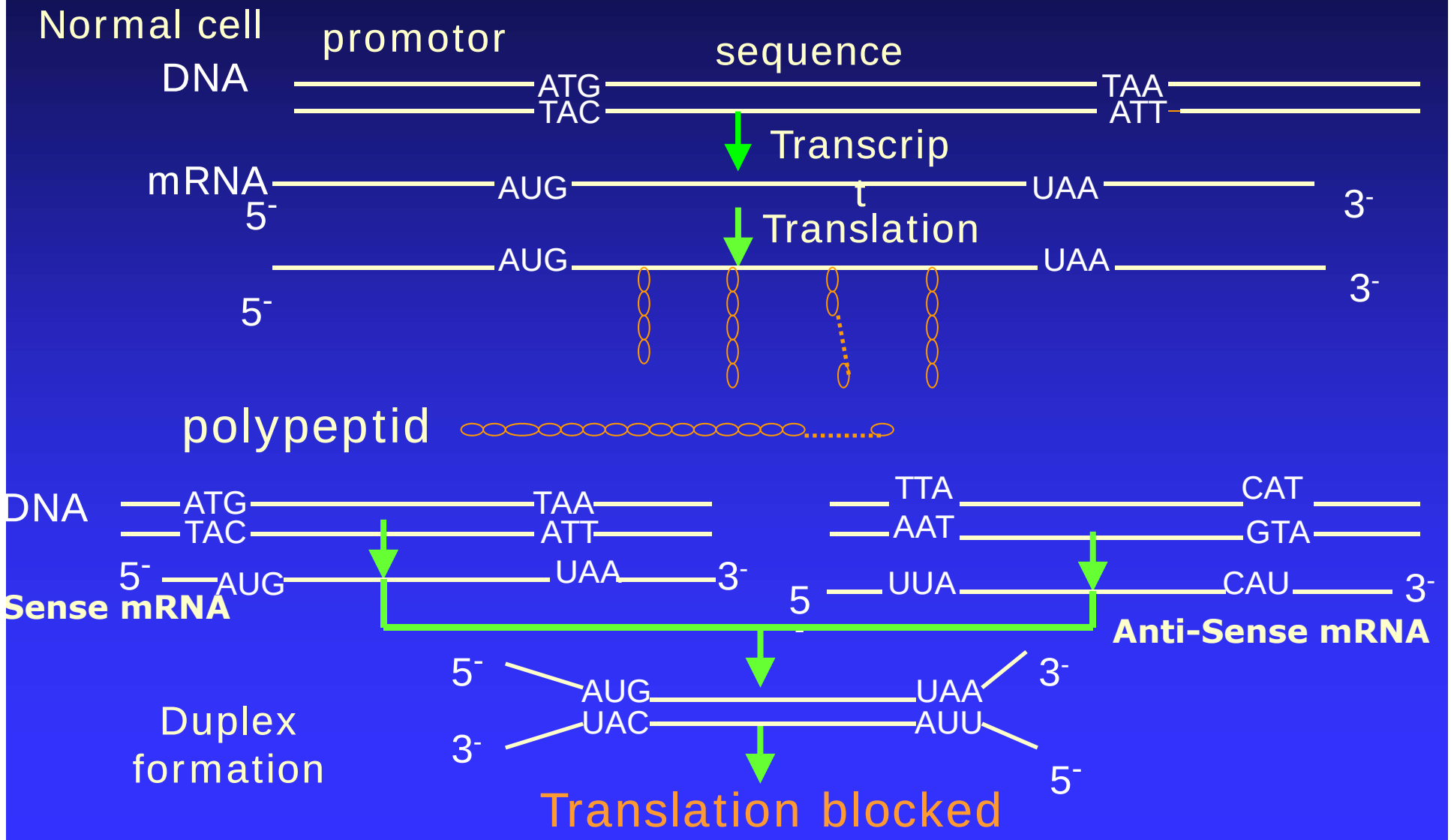
* modification of DNA sequences by addition of methyl groups
* methylation of the promotor region blocks transcription initiation
* methylation within the coding sequence of genes

Thus lower Transcriptions rates

Post-transcriptional Gene Silencing (PTGS)

- Aberrant RNA (antisense RNA, dsRNA)
- Untranslatable sequence, lack of introns, self-complementarity
- Methylation of regions that are duplicated in the transgene
- Methylation occurs as a consequence of pairing between homologus regions of the endogenous genes and the transgenes

Anti-sense RNA and regulation of gene expression



The Roles of gene silencing

- Protection against invasive RNAs especially RNA viruses
- Regulation of endogenous gene
- Studying the function of genes

High ß-Carotene content in Tomato

- Arabidopsis ß-Lcy cDNA
- Tomato fruit-specific pds-promoto

GGDP

↓

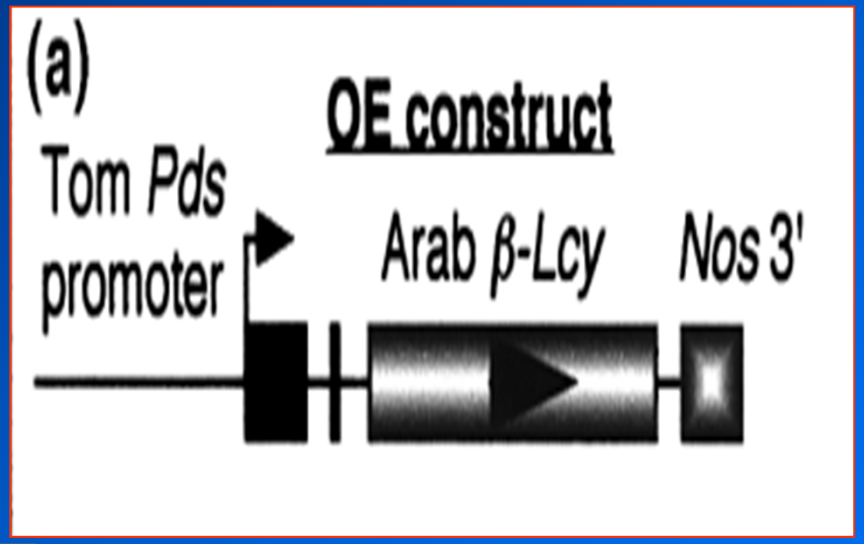
Phytoene

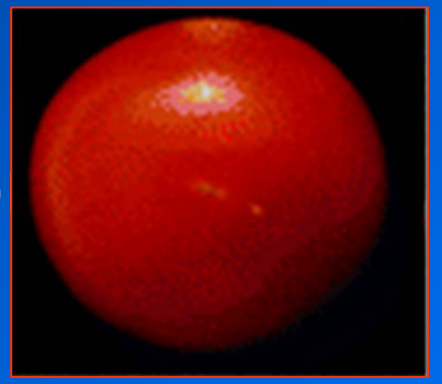
↓

Lycopene

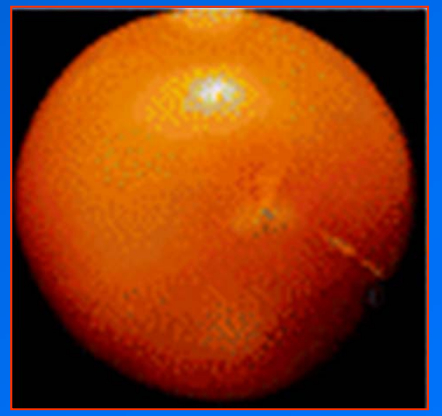
↓ **Lcy**

ß-carotene





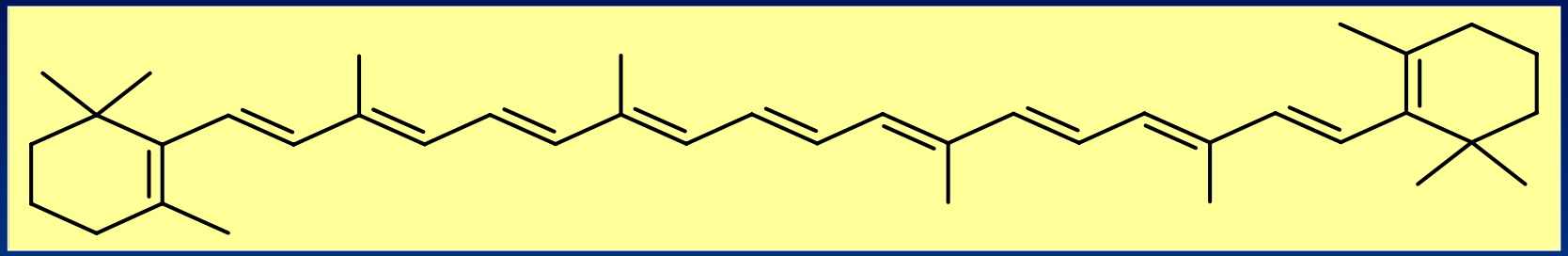
Wild type



Overexpression

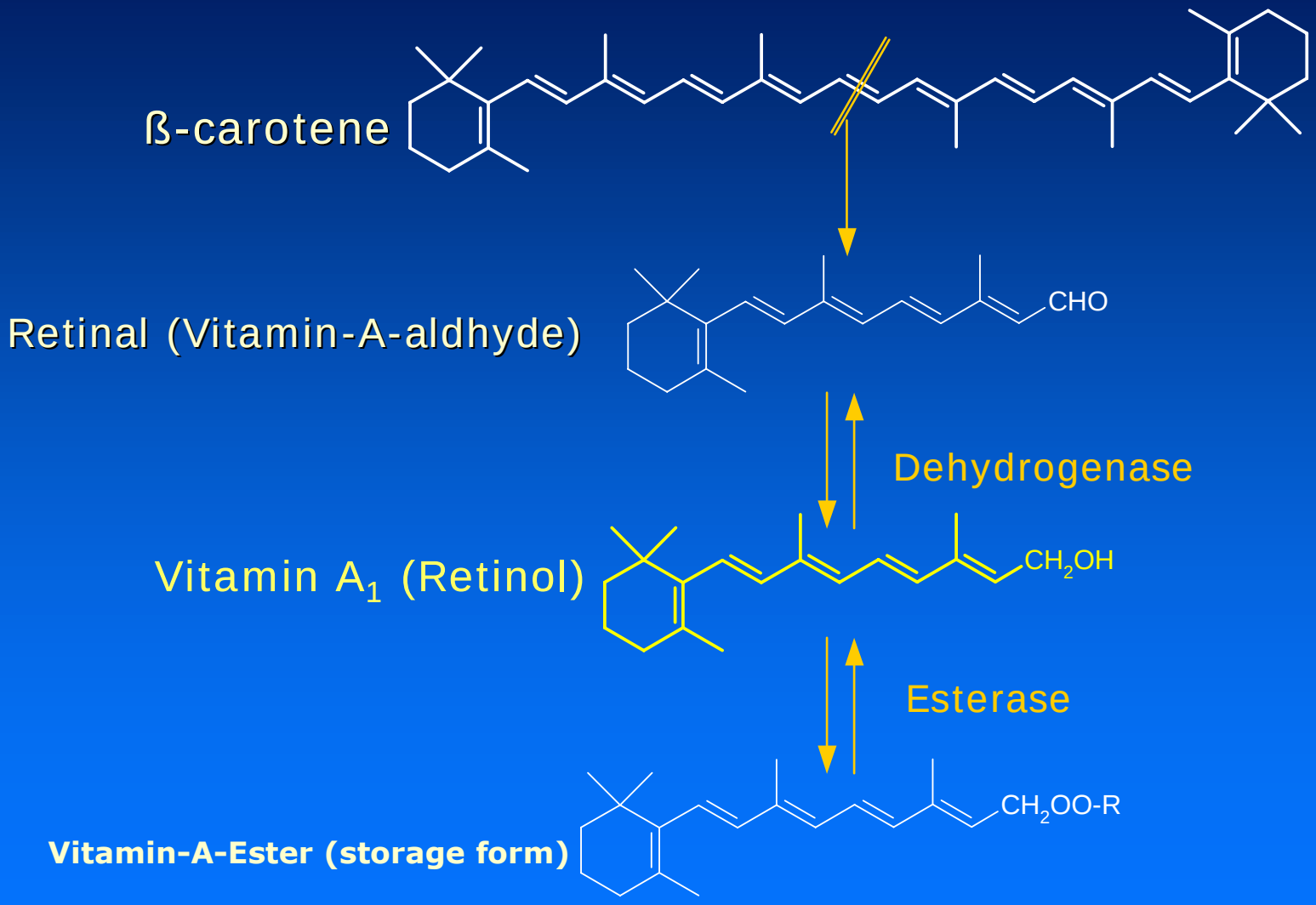
- 7-fold increase in ß-carotene content

ß-carotene

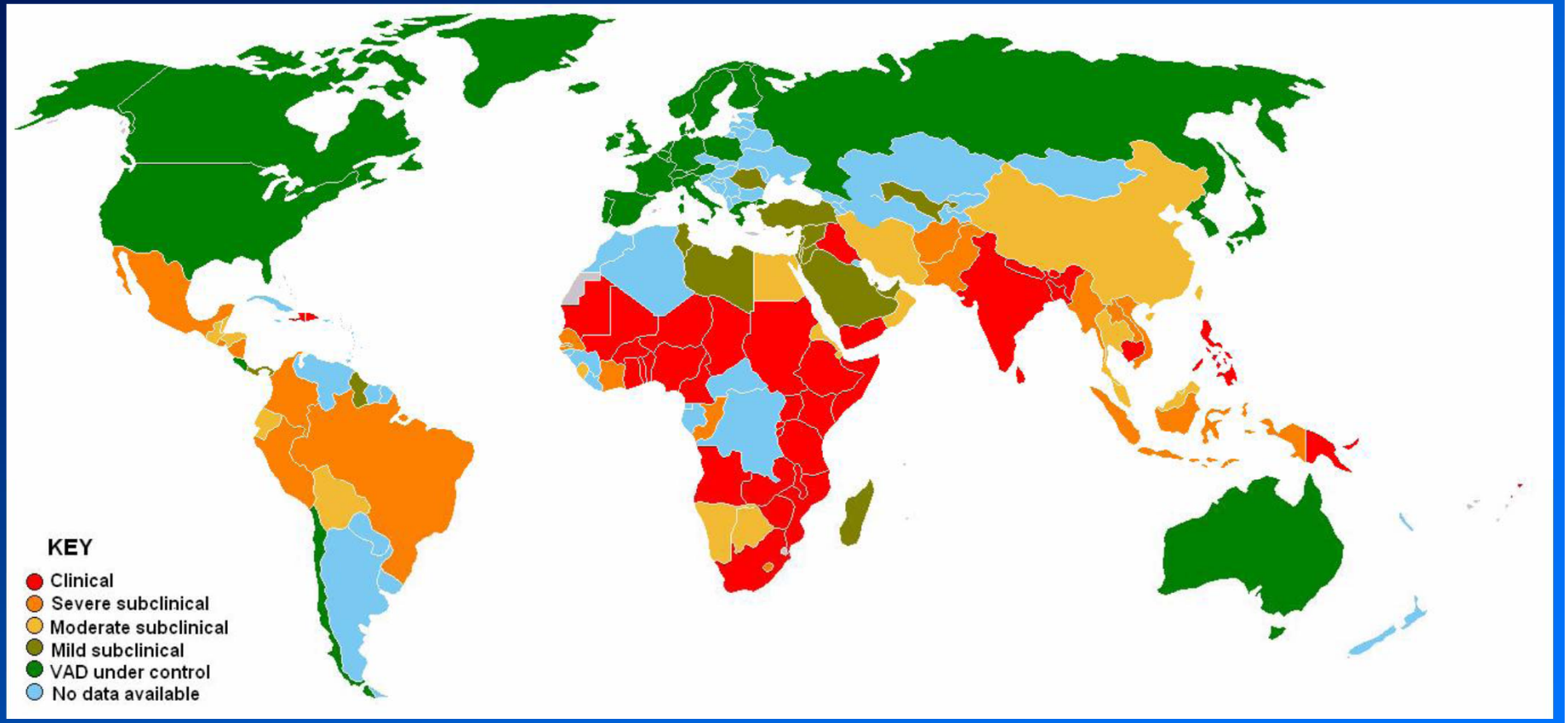


- ß-carotene (precursor of vitamin A)
  one of 600 carotenoids
- Natural sources:
  * All green plants
  * Various fruits
- Uses
  * Pharmaceutical products
  * Cancer preventive
  * Positive effects on immune system
  * Vitamin supplements
  * Supplement for fish and poultry feed
  * Colorants for cosmeticals and food

Vitamin A_1 - Retinol

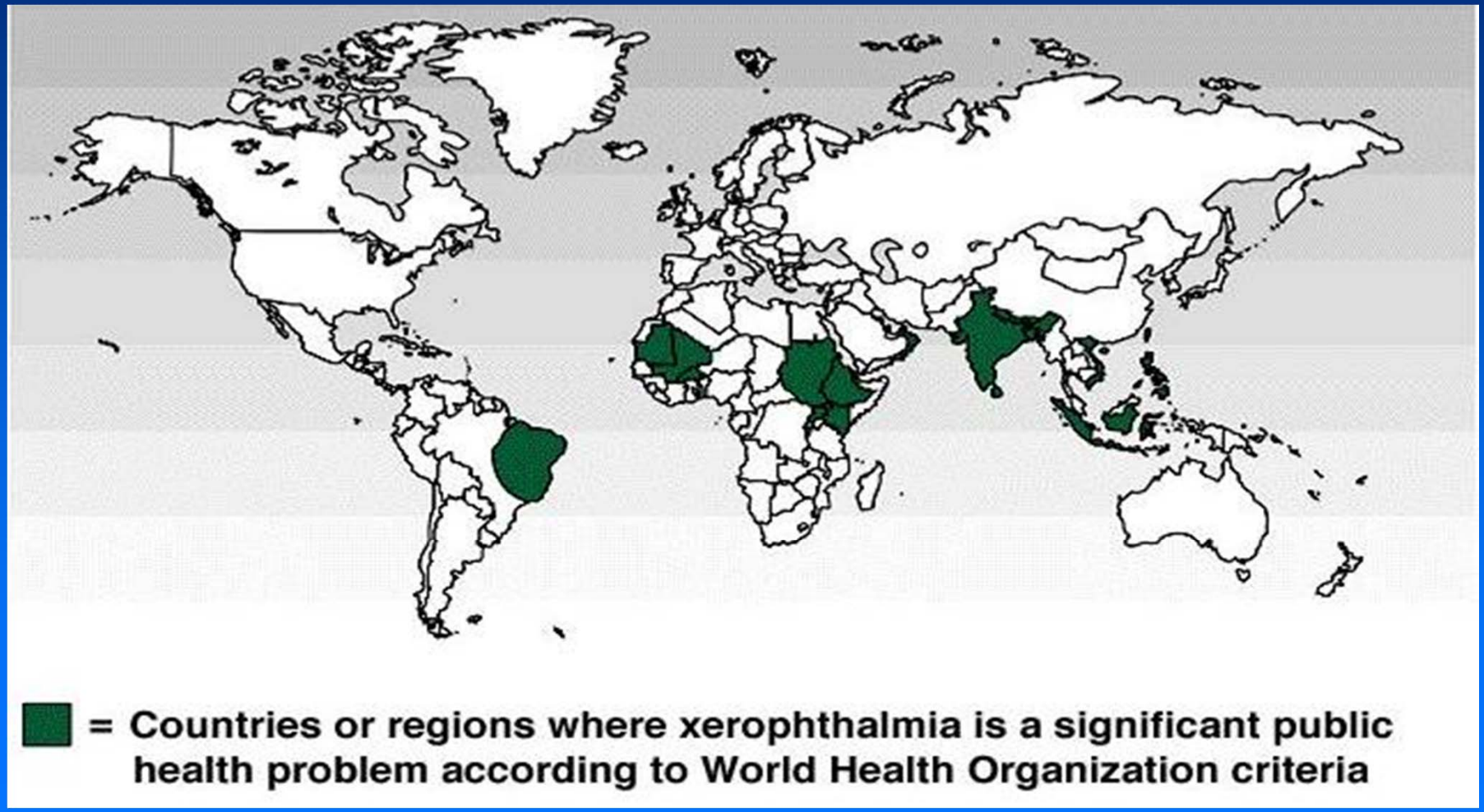


Vitamin A deficiency

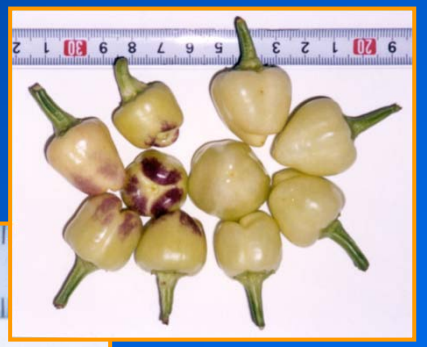


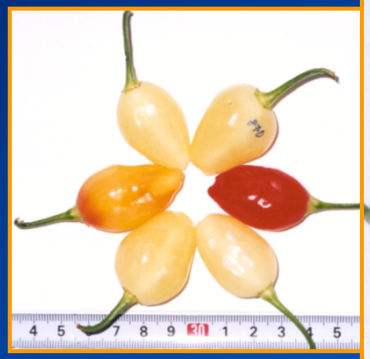
Data from the World Health Organization

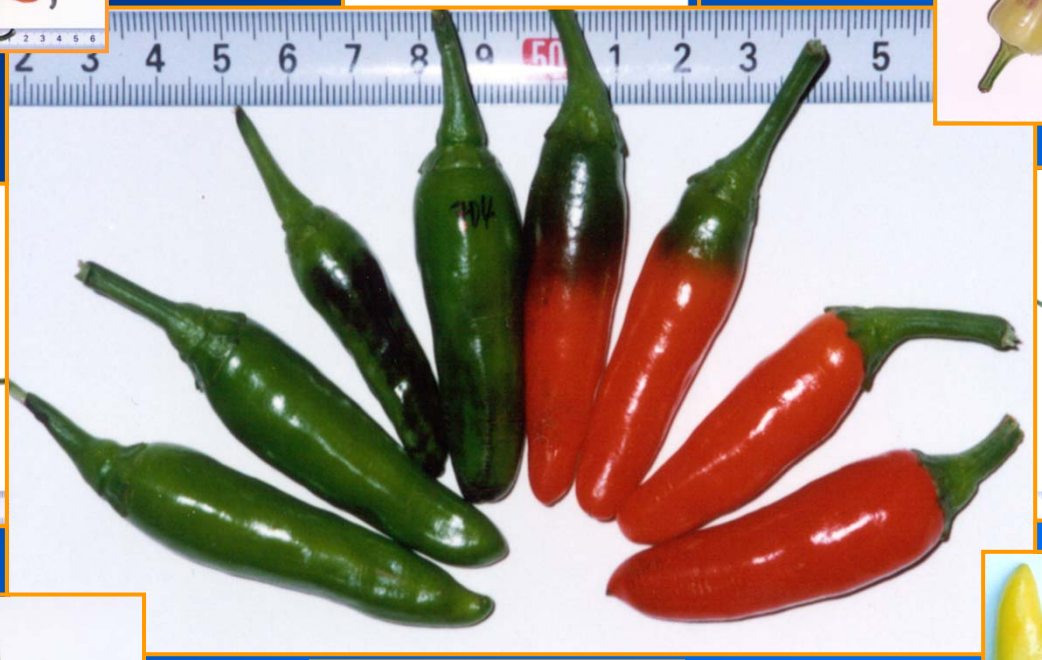
Areas of Blindness Caused by Vitamin A Deficiency

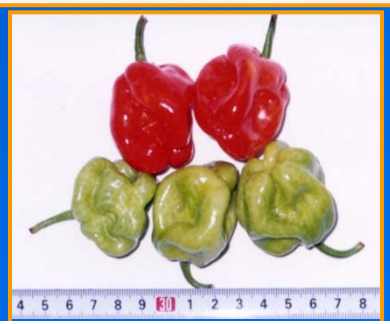


Genetic Polymorphism in Chili Peppers









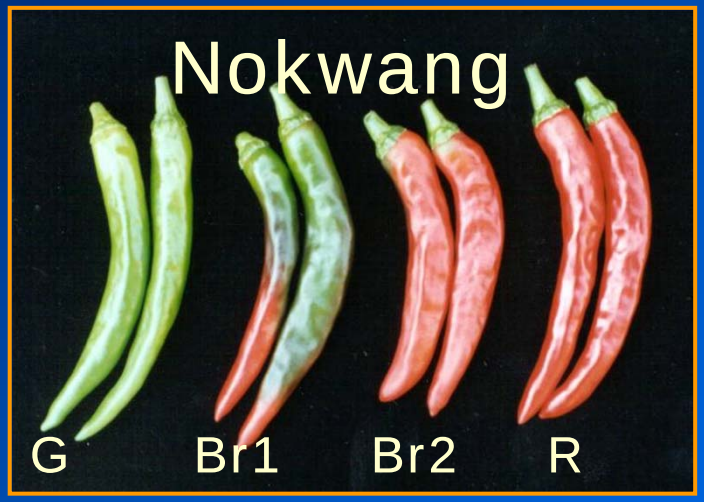
Capsicum in Perspective

Comparison of different Plant Species

Plant/Tissue	total carotenoids	ß-carotene
capsicum fruit	12000	2000
tomato fruit	2850	271
sweet potato	1130	
carrot	600	600

All values in mg/kg DW

Carotenoid biosynthesis during fruit ripening



	total Carotenoids	Lutein	Zeaxanthin	ß-Carotene	Capsanthin
G	10.57	4.32	3.36	2.90	-
		40.85	31.75	27.40	
Br1	55.61	1.25	6.54	3.84	43.35
		2.26	11.77	6.90	77.95
Br2	119.14	0.90	8.38	3.67	104.33
		0.76	7.03	3.08	87.58
R	406.08	0.79	16.65	8.97	372.15
		0.20	4.10	2.21	91.65

values in mg/kg DW of fruit (Percent)

Why Capsicum?

- Modification of carotenoid content and composition
  * Major carotenoids capsanthin and capsorubin with no nutritional value
  * ß-carotene, zeaxanthin, lycopene are desired
  * Reducing capsanthin and capsorubin by breeding also reduced overall carotenoids

Genes used for increasing ß-Carotene in *Capsicum annuum*

Geranylgeranyldiphosphate (GGDP)

Psy

Phytoene

PDS

Lycopene

LCYE | **LCYB**

α- Carotene → **Lutein**

ß-Carotene → **Zeaxanthin** → **Antheraxanthin** → **Violaxanthin**

Antheraxanthin → **Capsanthin**

CCS

Violaxanthin → **Capsorubin**

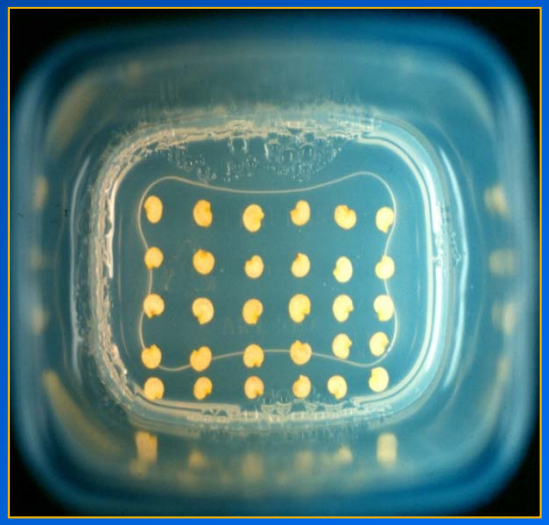
* **Overexpression of LCYB**
* **Antisense of CCS**
* **Combination of both**

Establishment of aseptic cultures

Seed

Sterilization

Clorix



Seed culture

Ten days

Seed germination

Starting material

Flow diagram of Cotyledons regeneration

Explant

?

> 8 months

Mature plant

Shoot induction

4 weeks

Shoot elongation

4 weeks

Rooting and adaptation

8 weeks

Rooting

Transformation of tomato using *A. tumefaciens*

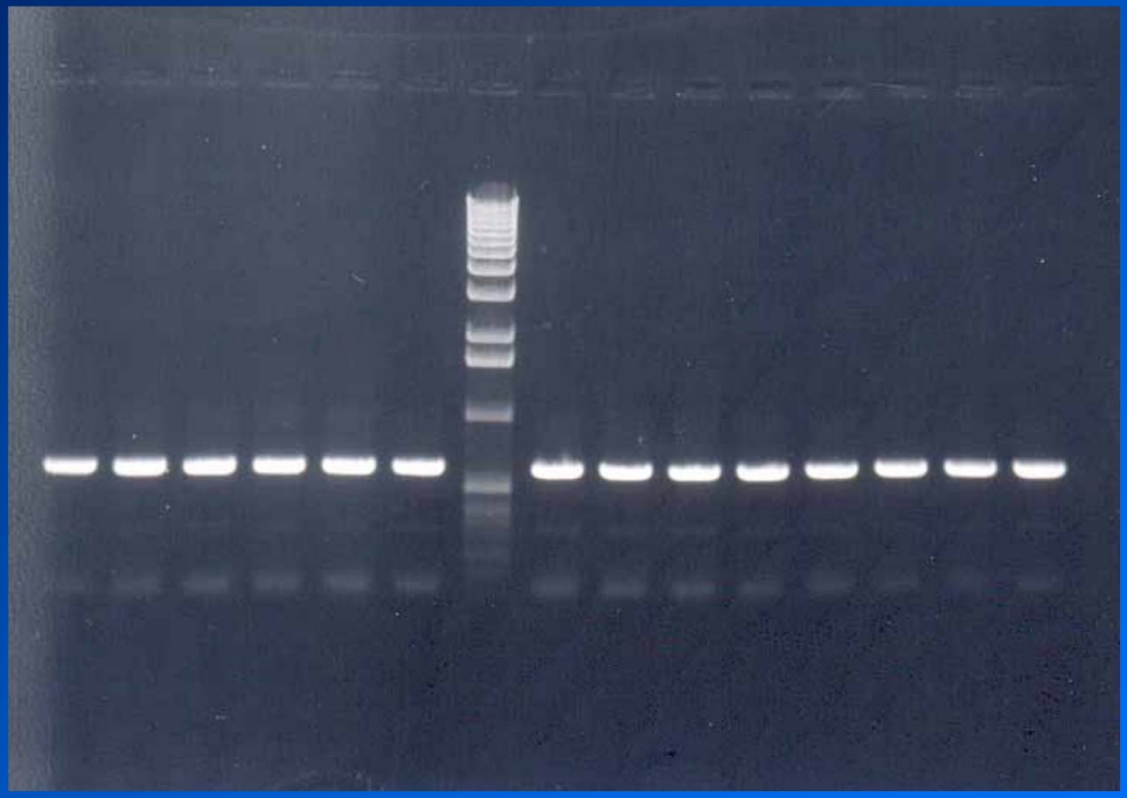
tomato seedlings, 10 days old

cotyledon explants placed on conditioning medium

Transformation of tomato using *A. tumefaciens*

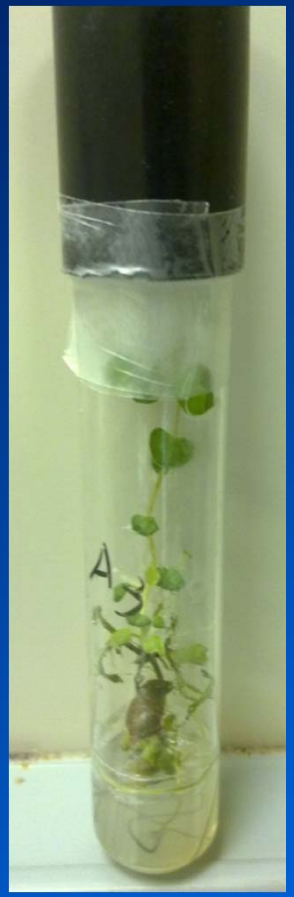
Transformation of tomato using *A. tumefaciens*

PCR amplification of introduced construct in putative transgenic tomato plants



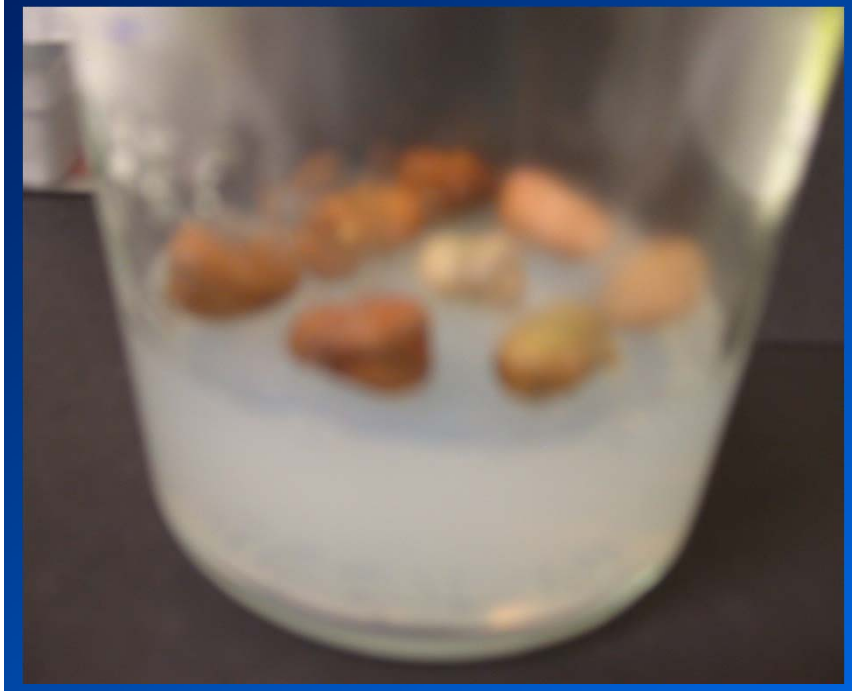
•**El Nagar, M. M. (2013).** Genetic engineering for increasing salinity tolerance in tomato. Australian Journal of Basic &Applied Science. Australian Journal of Basic and Applied Sciences, 7 (1):433-440.

Potato

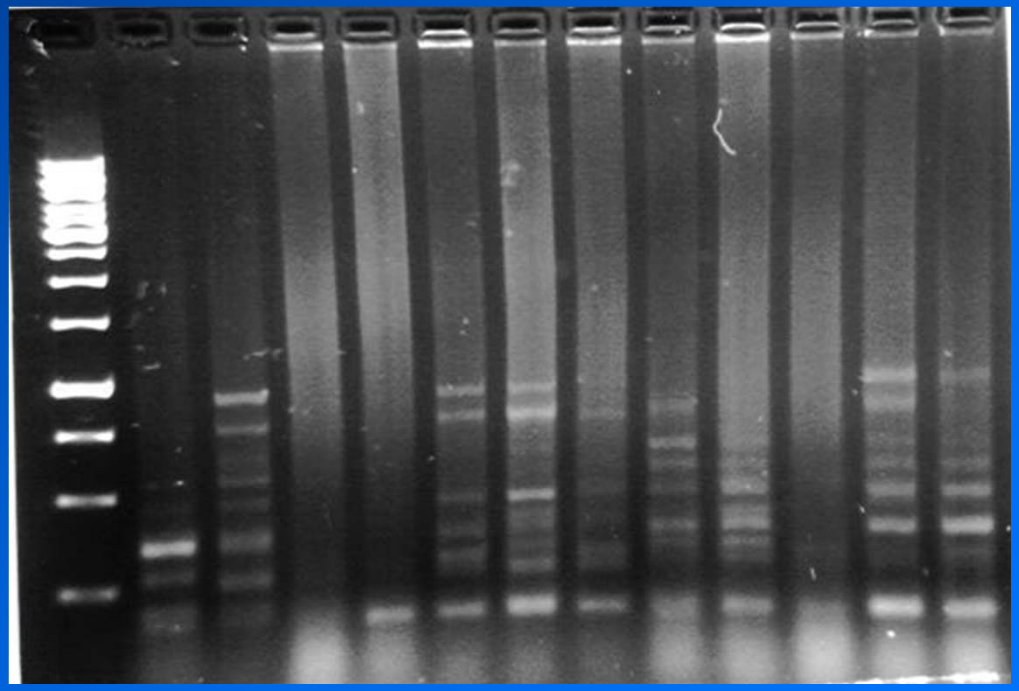


•**El Nagar, M. M. and Mekawi, E. M. (2012).** Yield components and chemical compositions of some potato cultivars: *in vitro* microtubers production and field performance. Research Journal of Agriculture and Biological Sciences, 8 (2): 235-244**.**

Broad bean



In vitro Evaluation



ISSR

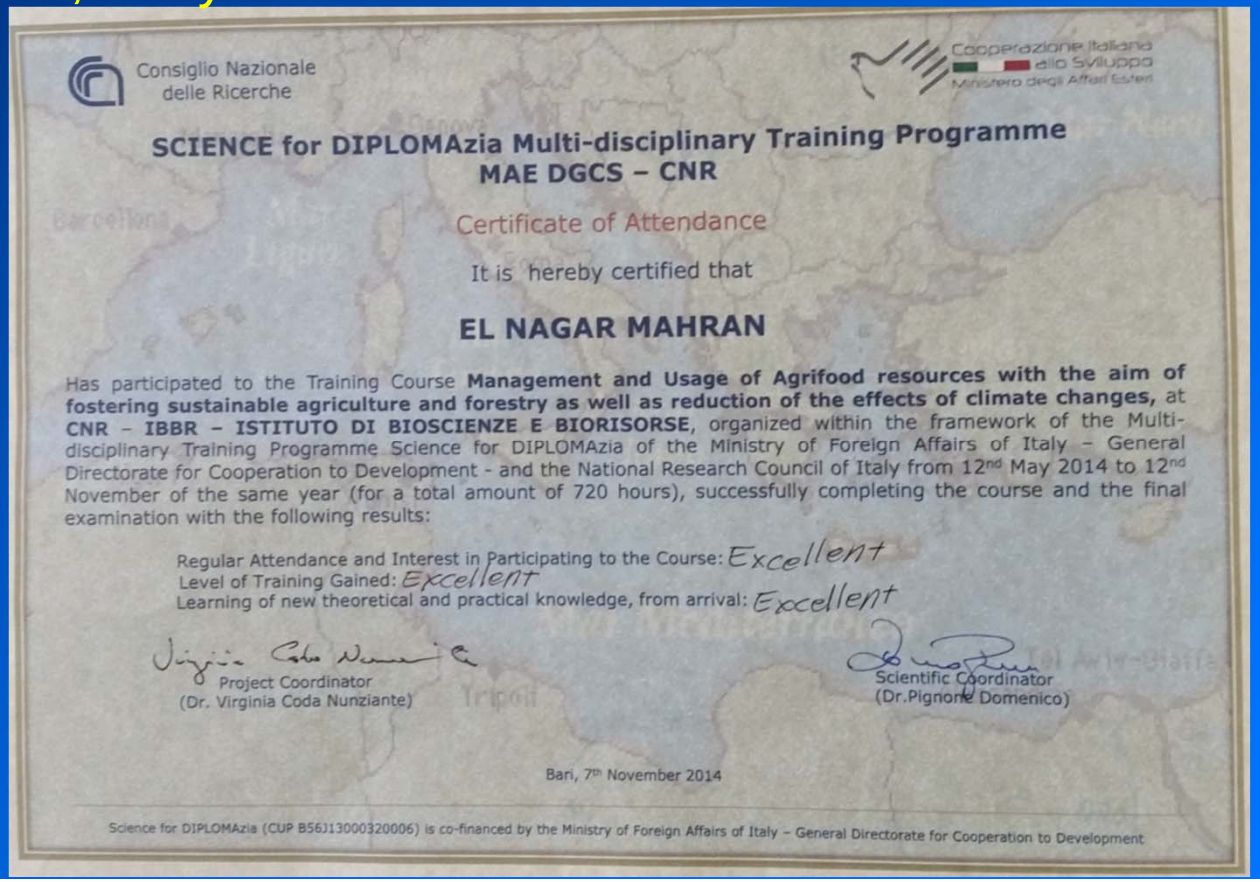
Micro grafting

Grants

- Post-Doctoral Scholarship from Institute of Biosciences and BioResources (IBBR), U.O.S. Palermo, Italy www.ibbr.cnr.it. 12/05/2014 – till 12.11.2014

International training

(Management and Usage of agrifood resources with the aim of fostering sustainable agriculture and forestry as well as reduction of the effects of climate changes) within training program Science for Diplomacy “DIPLOMAzia” 19 May:18 June 2014, Palermo, Italy.



Consiglio Nazionale delle Ricerche

Cooperazione Italiana allo Sviluppo
Ministero degli Affari Esteri

SCIENCE for DIPLOMAzia Multi-disciplinary Training Programme
MAE DGCS – CNR

Certificate of Attendance

It is hereby certified that

EL NAGAR MAHRAN

Has participated to the Training Course **Management and Usage of Agrifood resources with the aim of fostering sustainable agriculture and forestry as well as reduction of the effects of climate changes,** at **CNR – IBBR – ISTITUTO DI BIOSCIENZE E BIORISORSE**, organized within the framework of the Multi-disciplinary Training Programme Science for DIPLOMAzia of the Ministry of Foreign Affairs of Italy – General Directorate for Cooperation to Development - and the National Research Council of Italy from 12nd May 2014 to 12nd November of the same year (for a total amount of 720 hours), successfully completing the course and the final examination with the following results:

Regular Attendance and Interest in Participating to the Course: Excellent
Level of Training Gained: Excellent
Learning of new theoretical and practical knowledge, from arrival: Excellent

Project Coordinator
(Dr. Virginia Coda Nunziante)

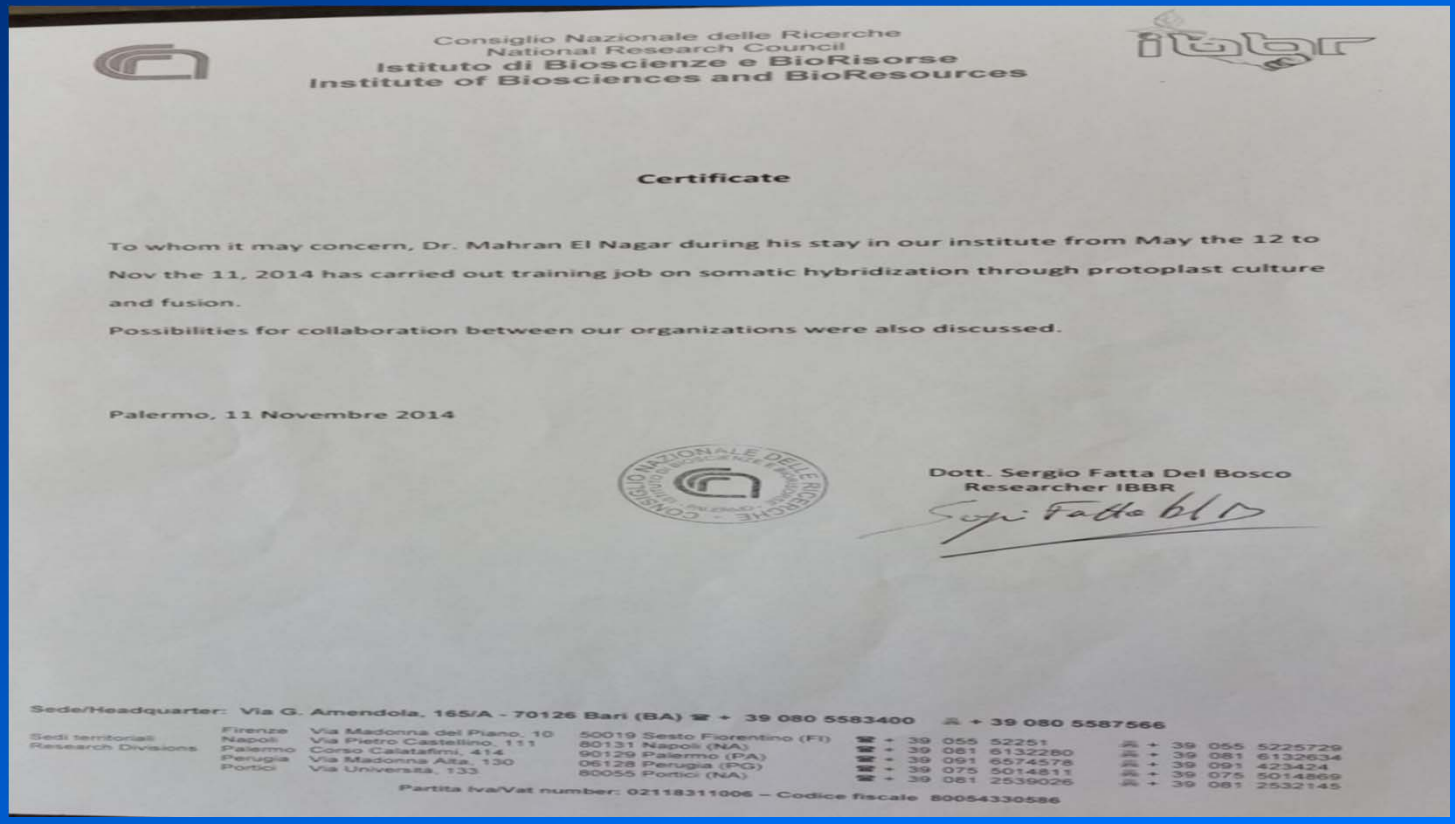
Scientific Coordinator
(Dr.Pignone Domenico)

Bari, 7th November 2014

Science for DIPLOMAzia (CUP B56J13000320006) is co-financed by the Ministry of Foreign Affairs of Italy – General Directorate for Cooperation to Development

International training

- "Somatic hybridization through protoplast culture and fusion" September 19th- October 20th, 2014. Institute of Biosciences and BioResources (IBBR), Division of Palermo, National Research council. Corso Calatafimi, 414-I-90129, Palermo, Italy.



Consiglio Nazionale delle Ricerche
National Research Council
Istituto di Bioscienze e BioRisorse
Institute of Biosciences and BioResources

Certificate

To whom it may concern, Dr. Mahran El Nagar during his stay in our institute from May the 12 to Nov the 11, 2014 has carried out training job on somatic hybridization through protoplast culture and fusion.
Possibilities for collaboration between our organizations were also discussed.

Palermo, 11 Novembre 2014

Dott. Sergio Fatta Del Bosco
Researcher IBBR

Sede/Headquarter: Via G. Amendola, 165/A - 70126 Bari (BA) ☎ + 39 080 5583400 🖷 + 39 080 5587566

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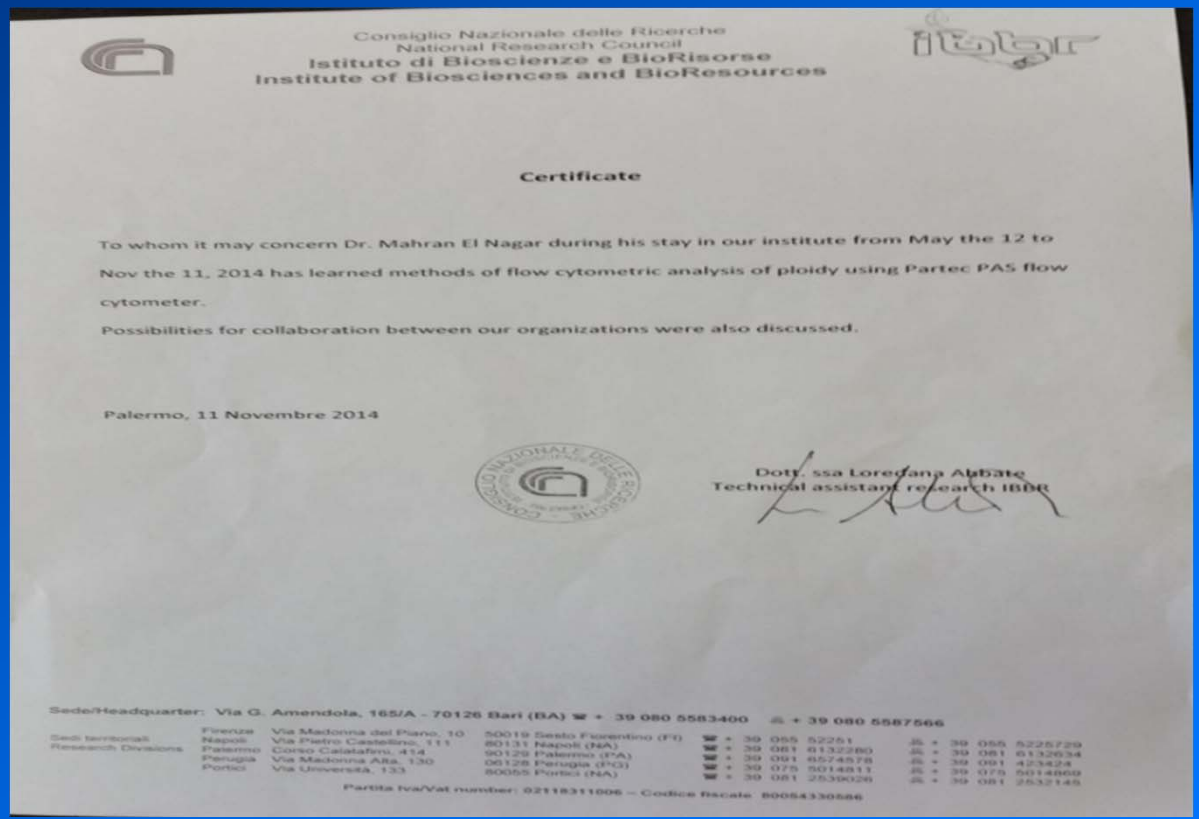
Partita Iva/Vat number: 02118311006 – Codice fiscale 80054330586

Protoplast

International training

- "Methods of flow cytometric analysis of ploidy using Partec PAS flow cytometer" October 21th - November 11th, 2014. Institute of Biosciences and BioResources (IBBR), Division of Palermo, National Research council. Corso Calatafimi, 414-I-90129, Palermo, Italy.



Consiglio Nazionale delle Ricerche
National Research Council
Istituto di Bioscienze e BioRisorse
Institute of Biosciences and BioResources

Certificate

To whom it may concern Dr. Mahran El Nagar during his stay in our institute from May the 12 to Nov the 11, 2014 has learned methods of flow cytometric analysis of ploidy using Partec PAS flow cytometer.

Possibilities for collaboration between our organizations were also discussed.

Palermo, 11 Novembre 2014

Dott. ssa Loredana Abbate
Technical assistant research IBBR

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Portici Via Università, 133 80055 Portici (NA) + 39 081 2539026 + 39 081 2532145

Partita Iva/Vat number: 02118311006 – Codice fiscale 80054330586

Conferences

- **European Biotechnology week, conference in Bari, Italy, 8-12 October 2014.**

Consiglio Nazionale delle Ricerche

CNR EXPO

CIHEAM

EUROPEAN BIOTECH WEEK

EUROPEAN BIOTECH WEEK (SECONDA EDIZIONE)
6 -12 OTTOBRE 2014

ATTESTATO

Si certifica che EL NAGAR MAHRAN, ha partecipato alla conferenza ***Biodiversità e Biotecnologie : due risorse per l'agricoltura del mediterraneo*** nell'ambito della European Biotech Week (Seconda Edizione) 6-12 ottobre 2014 organizzata dal CNR, CIHEAM-Bari e ASSOBIOTECH presso lo CIHEAM – Bari (Istituto Agronomico Mediterraneo) in data 10 ottobre 2014.

Si rilascia il presente attestato su richiesta dell'interessato/a per tutti gli usi consentiti dalla legge.

Valenzano, 10/10/2014

"IL COMITATO ORGANIZZATORE"

Publications

Publications

- **Mercati, F.; Fontana, I.; El Nagar, M.; Gristina A. and Carimi F. (2015).** Transcriptome analysis of *Capparis spinosa* L. by Illumina paired-end RNA Sequencing and SSR marker discovery. Plos One 1 (15): 2455-2465.
- **El Nagar, M.; Mercati, F.; Gristina A.; Martorana, A. and Carimi F. (2015).** Comparative molecular analysis between wild and cultivated *Capparis spinosa* L. collection from Mediterranean Island complex. MolecularBreeding3:245-254.
- **Mercati, F.; El Nagar, M., Martorana, A. and Carimi, F. (2015).** Shelf Life improvement evaluation in artichoke using chemical and molecular approach. Sent to Journal of Agricultural and Food Chemistry**.**
- **El Nagar, M.; Mercati, F.; Gristina A.; Martorana, A. and Carimi F. (2015).** Simulation of Fungal-Mediated Cell Death by Fumonisin B1 and Selection of Fumonisin B1–Resistant (*fb*) Sweet Maize Mutants Sent to Journal of Plant Cell Reports.

Grants

- Post-Doctoral scholarship from the Minister of Higher Eductaion, Department of Cultural Affairs and Missions, Egypt in the Institute of plant physiology and biotechnology, University of Hohenheim, Stuttgart, Germany (Genetic engineering for increasing drought tolerance of tomato). www.uni-hohenheim.de. 2015

Research projects

- Improvement of Antioxidant Content in pepper (*Capsicum annuum* L). Through Genetic Transformation. Fund from Scientific Research Fund, Benha University, Egypt.
- Attempts to enhance abiotic stress tolerance ability of some potato plants via *Agrobacterium* mediated genetic transformation by *OPR1*. Fund from Scientific Research Fund, Benha University, Egypt.
- Enhancing Food Security Through Improved Yield and Local Seed Production of Potato and Sweet potato. Canadian International Food Security Research Fund (CIFSRF)

Publications

- **EL Nagar, M.M. and Mekawi, E. (2014).** Comparison of different genotypes of rocket (*Eruca sativa*) in terms of chemical compounds extracted from seeds and in the callus induced from tissue culture. Middle East Journal of Agriculture Research , 3(4): 1074-1082.

- **EL Nagar, M.M. and Mekawi, E. (2015).** Regeneration of pepper (*Capsicum annuum* L.) and evaluation of antioxidant compounds at various ripening stages. Sent to Molecular Breeding.

Thanks