GENETIC TRANSFORMATION AND EVOLUTION OF BIOTECH CROPS

BT COTTON, BT BRINJAL AND GOLDEN RICE

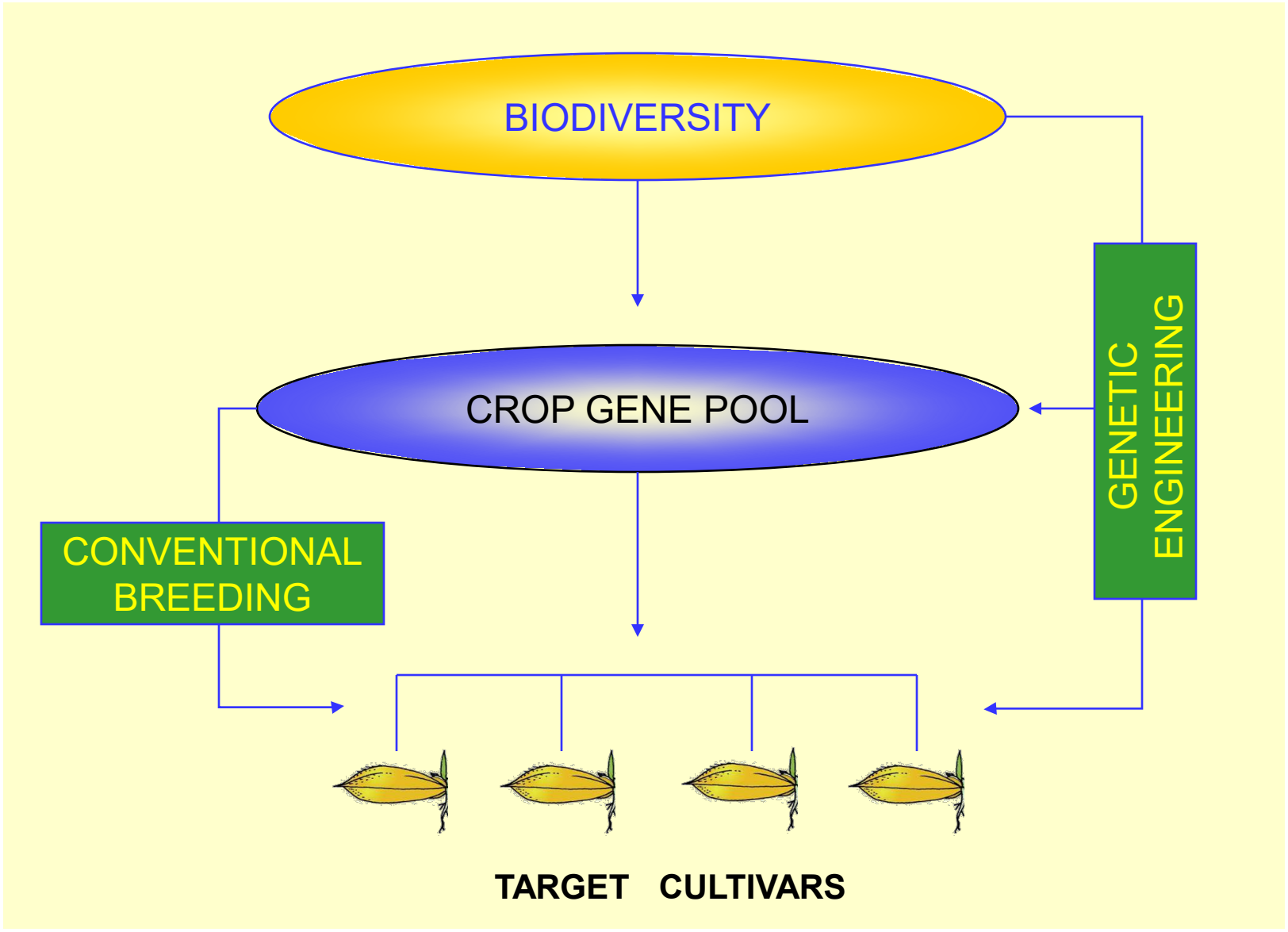
Manish Kumar Jain

(PhD, CSIR-UGC-NET 2010)

Department of Zoology

Govt. Auto. Girls P.G. College of Excellence, Sagar, MP

ENLARGING THE CANVAS OF PLANT BREEDING



RECOMBINANT DNA TECHNOLOGY

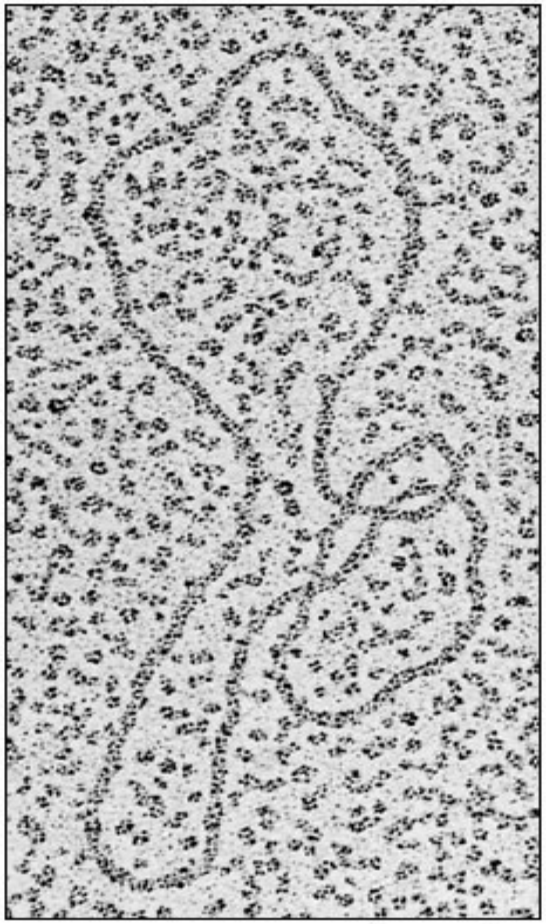
TRANSGENIC CROPS

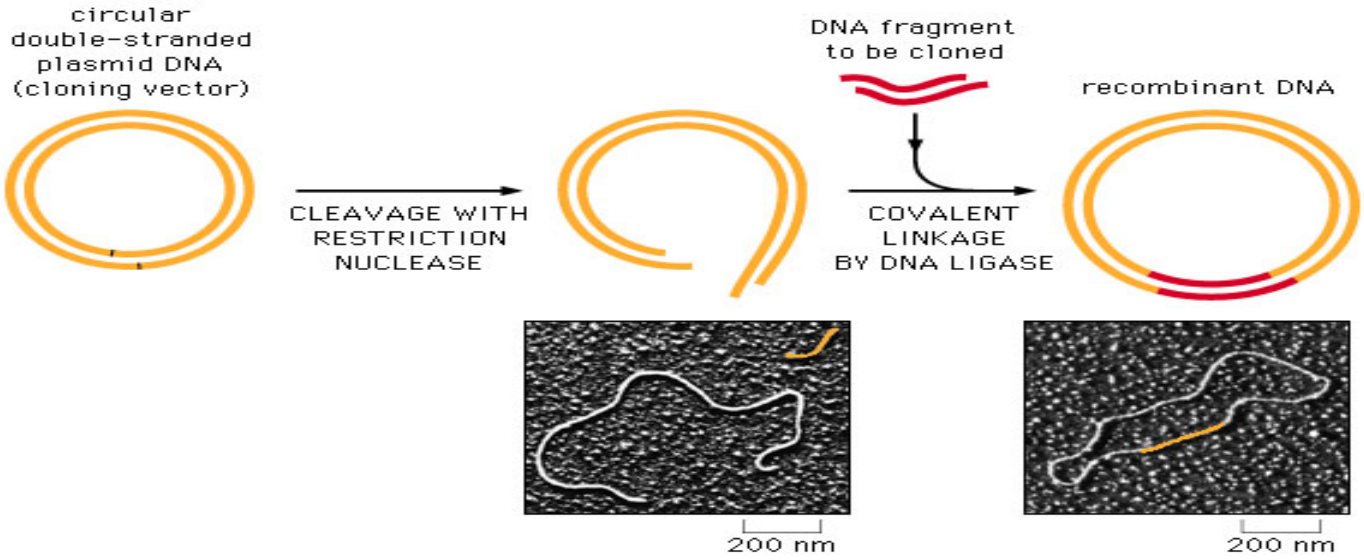
A transgenic crop plant contains a gene or genes which have been artificially inserted instead of the plant acquiring them through pollination. The inserted gene (known as the **transgene**) may come from another unrelated plant, or from a completely different species: transgenic Bt cotton, for example, which produces its own insecticide, contains a gene from a bacterium. Plants containing transgenes are called **genetically modified** or **GM crops**

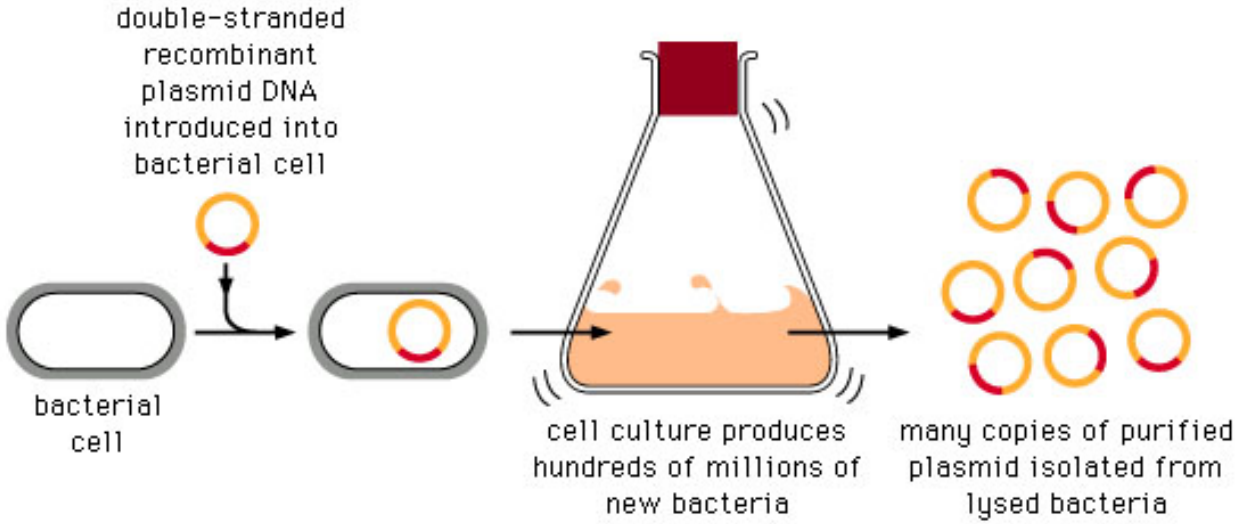
STEPS IN GENETIC ENGINEERING

- Isolation of gene from a foreign organism
- Cloning of the gene into a vector (gene construct)
- Gene transfer through vector into target plant
- Screening of transformants to identify plants having the foreign gene

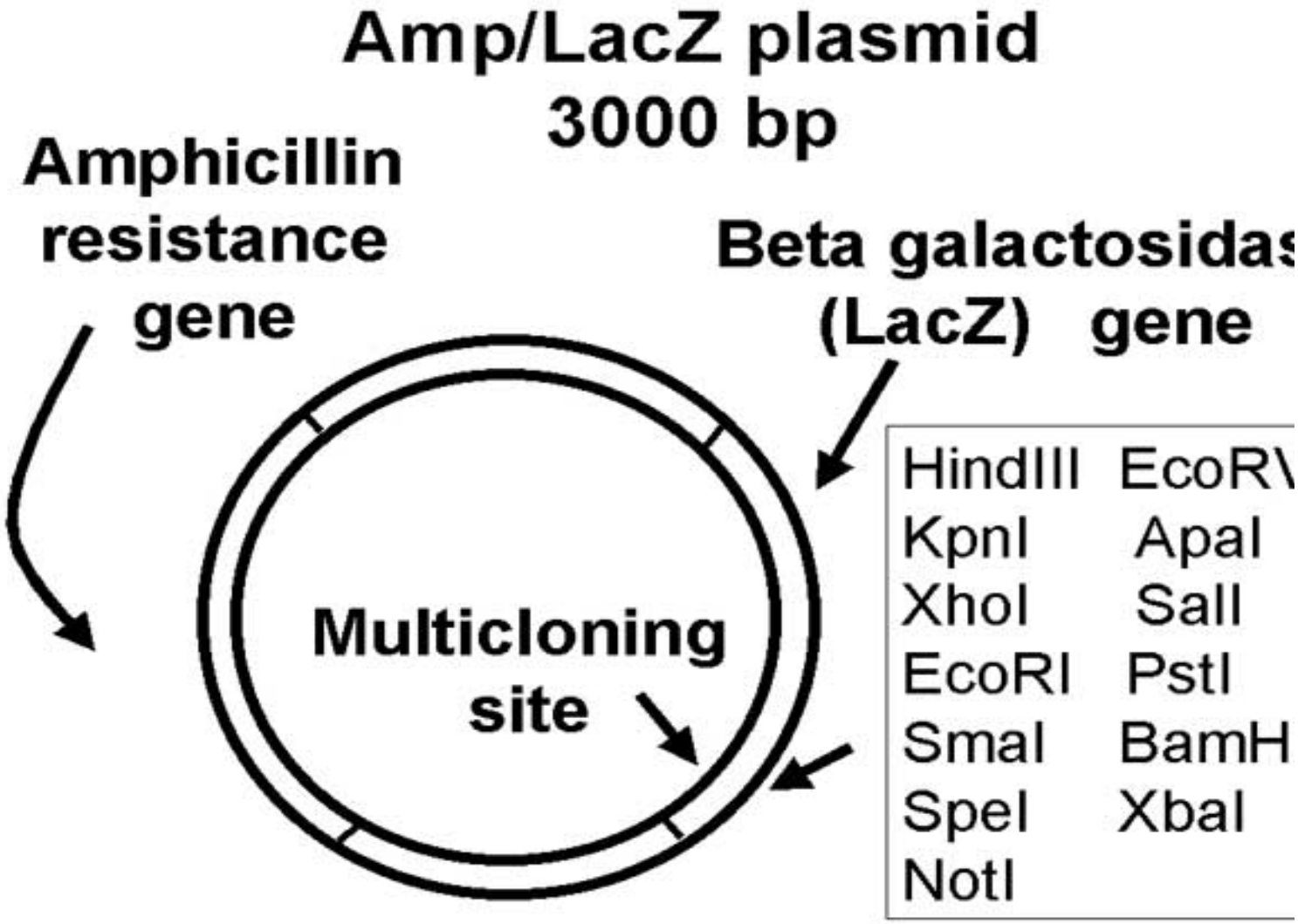
BACTERIAL PLASMID



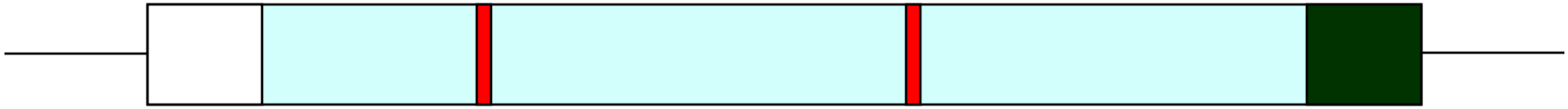




TYPICAL BACTERIAL DNA CLONING VECTOR (PLASMID)



GENE EXPRESSION CASETTE



Promoter

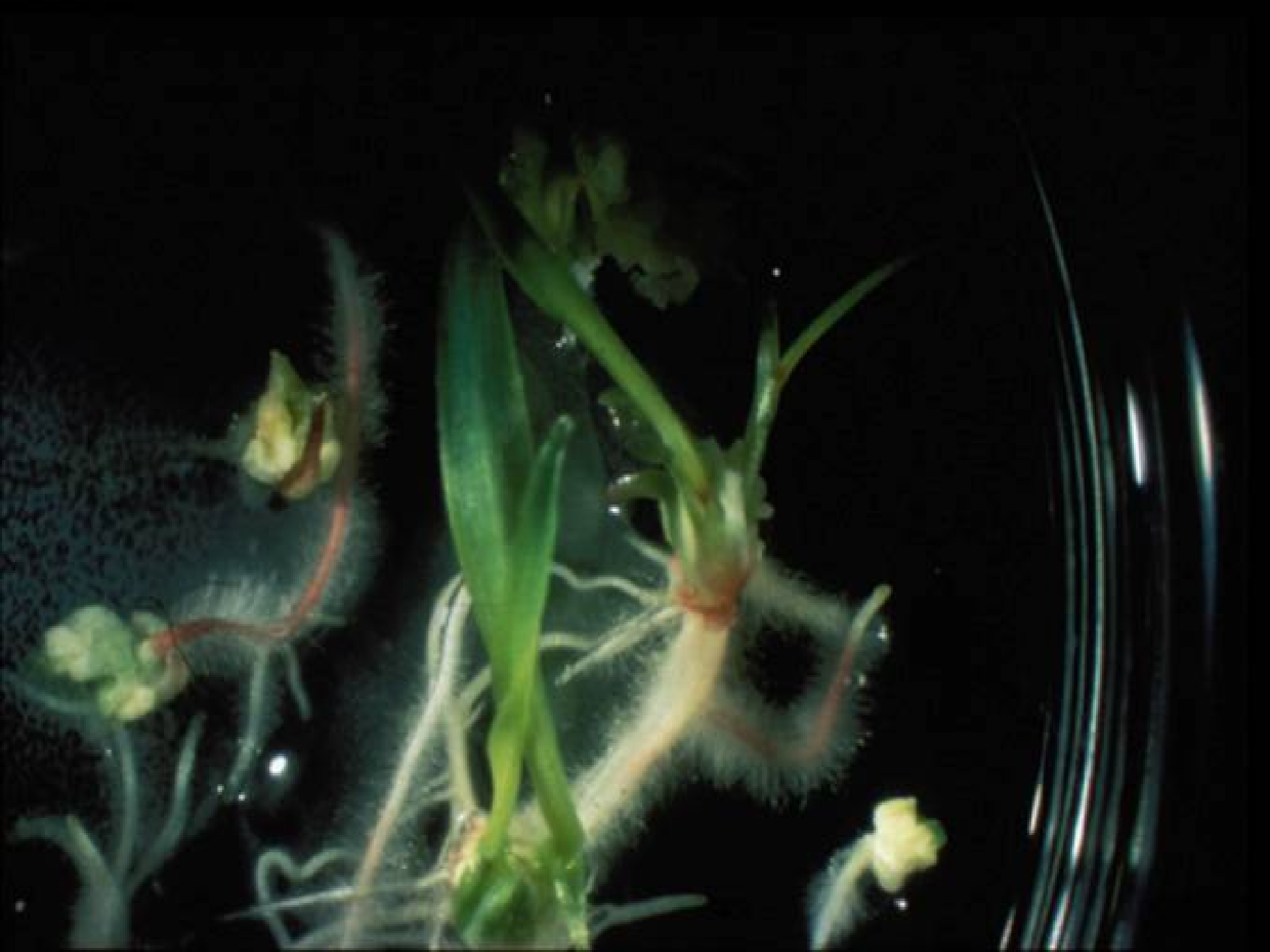
Gene of interest (exon-coding region)

Intron

Terminator

REQUIREMENTS FOR PLANT TRANSFORMATION

A. **Cell culture and plant regeneration system**
B. **Plant expression vector**
C. **Method of delivery of DNA into the cell**
D. **Proof of transformation of plant**



BASIC ELEMENTS IN A PLANT EXPRESSION VECTOR

A. **Promoter**
B. **Selectable marker gene**
C. **Gene of interest**
D. **Poly Adenylation signals / transcription terminators**

SELECTABLE/SCREENABLE MARKERS COMMONLY USED IN TRANSFORMATION

Gene	Source
nptII	*Tn5*
Cat	*E. coli*
gusA	*E.coli*
Gfp	*A. victoria*
Luc	*firefly, bacteria*

SELECTABLE MARKERS USED IN VECTORS

Two kinds of plant selection systems are currently deployed – Negative selection and Positive selection

- Negative selection – done in the presence of an antibiotic or herbicide (eg., hygromycin resistance, kanamycine resistance) – Patent rights for these are owned by Multinational companies
- Positive selection – A novel system called Mannose-6-phosphate transferase selection system employed nowadays- IP rights with Syngenta

PROMOTERS

A. Constitutive

All tissues, all the time independent of development and environment

B. Tissue-specific/Inducible

Regulated with signals

EXAMPLES OF PROMOTERS USED IN PLANT TRANSFORMATION

Constitutive

35S	**CAMV**
Actin-1	**Rice**
Ubi-1	**Maize**
Adh1/Emu	**Maize**
mas	***Agrobacterium***
ocs	***Agrobacterium***
nos	***Agrobacterium***

Ubi 1 > Act 1 > Adh 1 > 35S

EXAMPLES OF PROMOTERS USED IN PLANT TRANSFORMATION

Tissue-specific

Glutelin	**Rice**	**Seed**
Globulin	**Rice**	**Seed**
Prolamin	**Rice**	**Seed**
Vicillin	**Bean**	**Seed**
PHA-L	**Pea**	**Seed**
Patatin	**Potato**	**Tuber**
Alpha-amylase	**barley**	**aleurone**
PEPC/RUBISCO	**Maize**	**green-tissue**

EXAMPLES OF PROMOTERS USED IN PLANT TRANSFORMATION

Inducibe

Adh1	**Ethanol**
cab	**Light (LRE)**
rd29	**Osmotic stress (*DRE*)**
Ubi	**Osmotic stress**
Pin2	**wound**
HSP	**Heat shock**

CONSTRUCTION OF PLANT TRANSFORMATION VECTOR

Requirements

- **Vector**
- **Insert (promoter-cds-PolyA+)**
- **Unique restriction enzymes**
- **Primer pair (for PCR cloning)**
- **DNA ligase (T4/*E.coli*)**
- **DNA elution/clean up reagents (kit)**

TRANSCRIPTION TERMINATORS

Exact mechanism not well understood

- **Model signals contain AATAAA sequence –10 to –30 of the PolyA site (not universal)**

- **And a GT-rich region(s) upstream or downstream contribute to the efficiency of the signal**

Introns

Increase the efficiency of the gene expression especially when placed between the 5'UTR of promoter and the ATG start codon of the plant gene

Examples: ubi, act, adh, shrunken gene-introns

METODS OF DNA DELIVERY TO CELLS

- **Agrobacterium mediated**
- **Biolistic transformation**
- **Electroporation**
- **Microinjection**
- **Protoplast transformation**

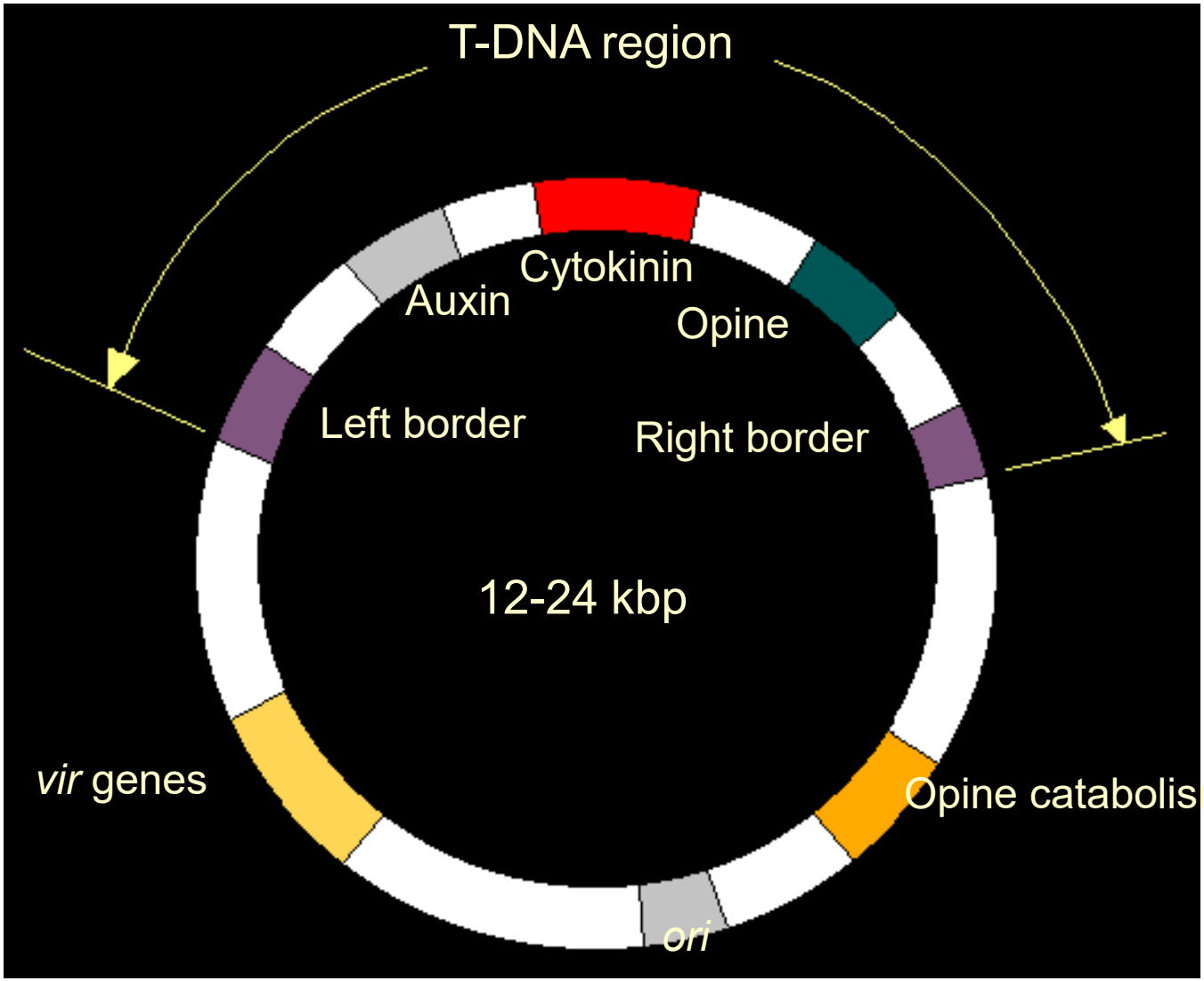
AGROBACTERIUM MEDIATED TRANSFORMATION

Tumor or crown gall formation is a process of transformation in which plant cells receive a transferred DNA fragment from the Ti-plasmid that resides in the bacterium

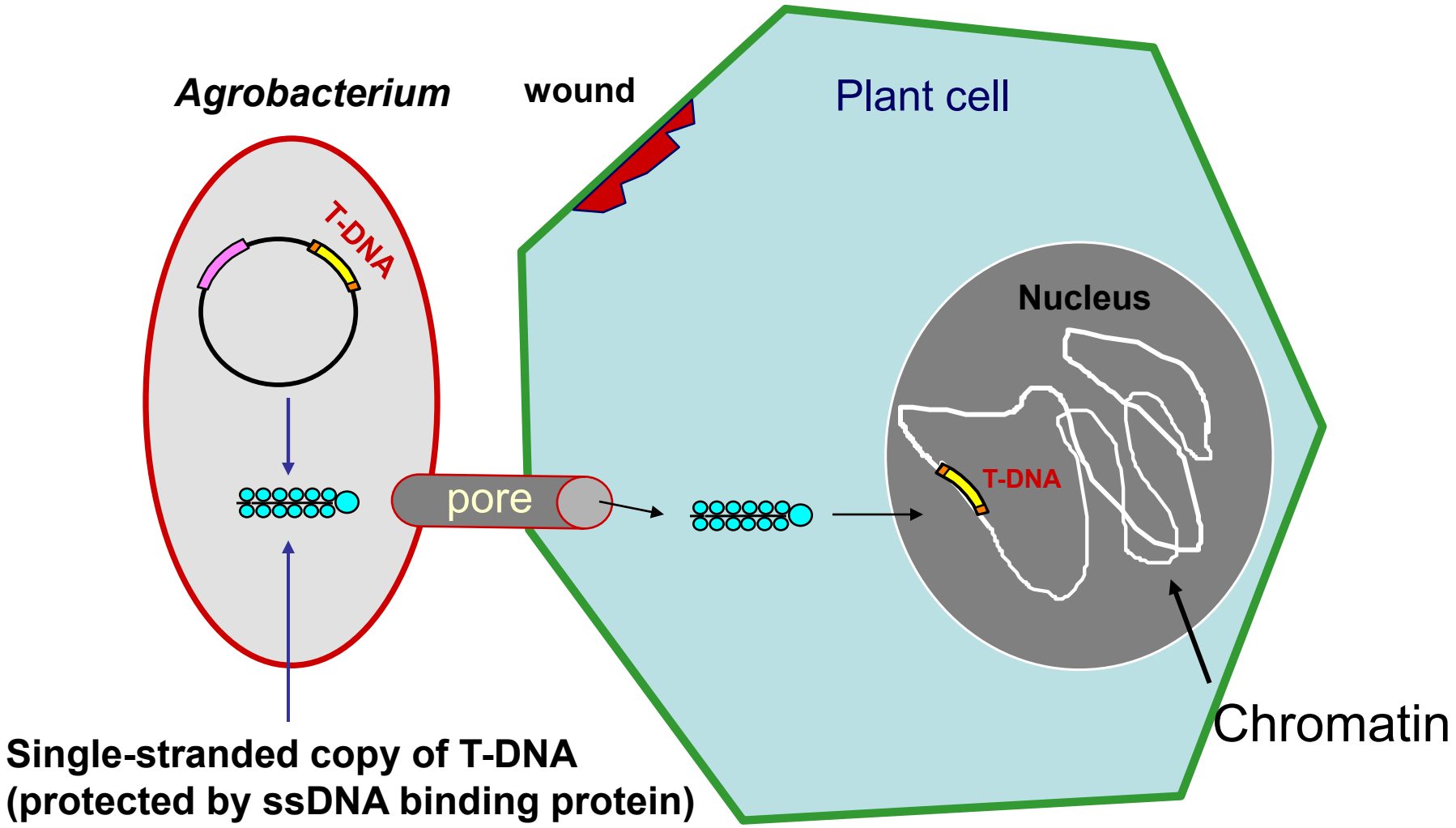
Genes present on T-DNA do not have any role in the transfer process; rather they get transcribed in the plant cell after getting integrated into the genome

Foreign DNA positioned between T-DNA borders also can get transferred to the plant cell.

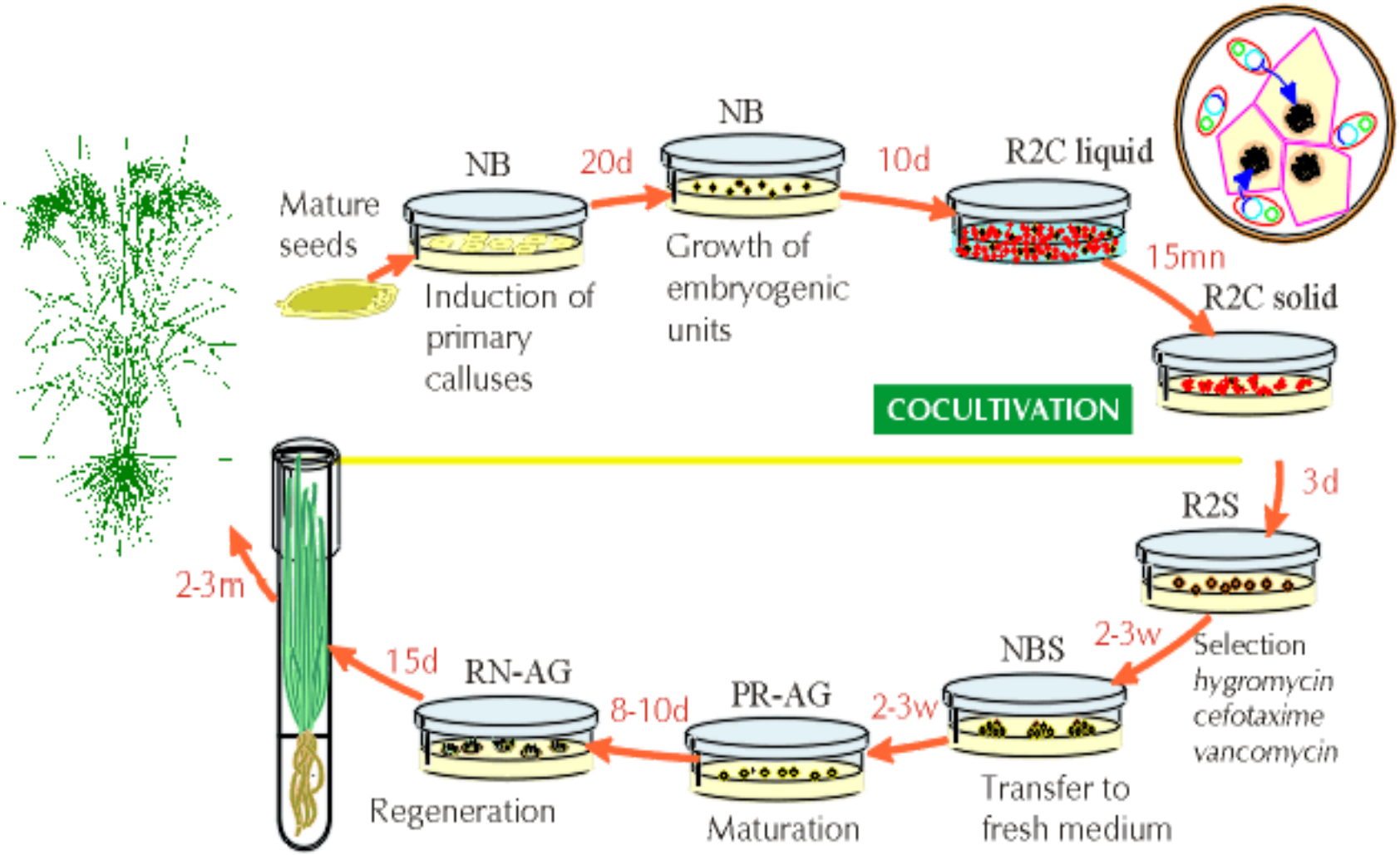
Ti PLASMID


T-DNA region
Cytokinin
Auxin
Opine
Left border
Right border
12-24 kbp
vir genes
Opine catabolism
ori

TRANSFER OF T-DNA FROM *AGROBACTERIUM* TO PLANT CELL

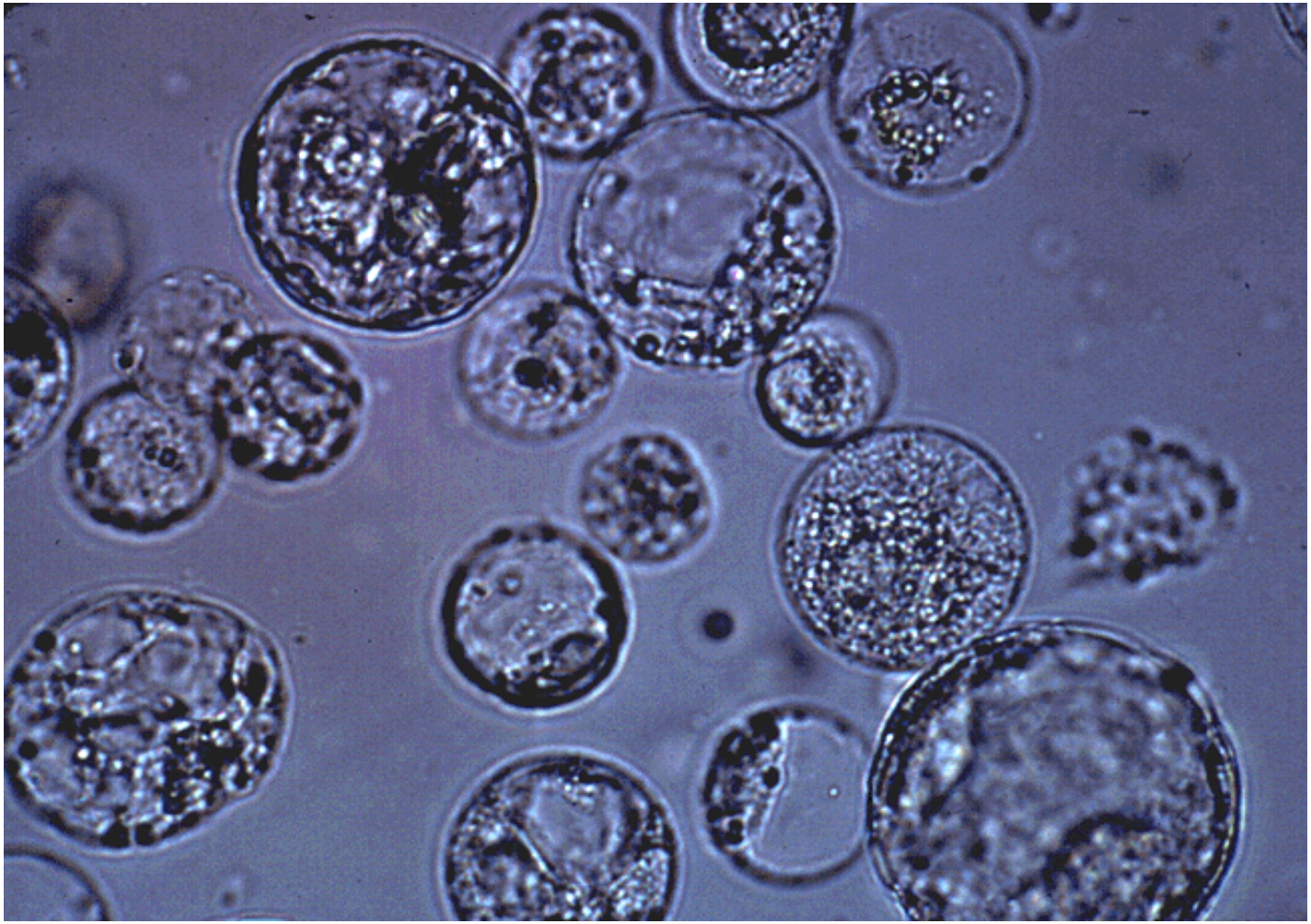


AGROBACTERIUM TUMEFACIENS - MEDIATED TRANSFORMATION OF RICE

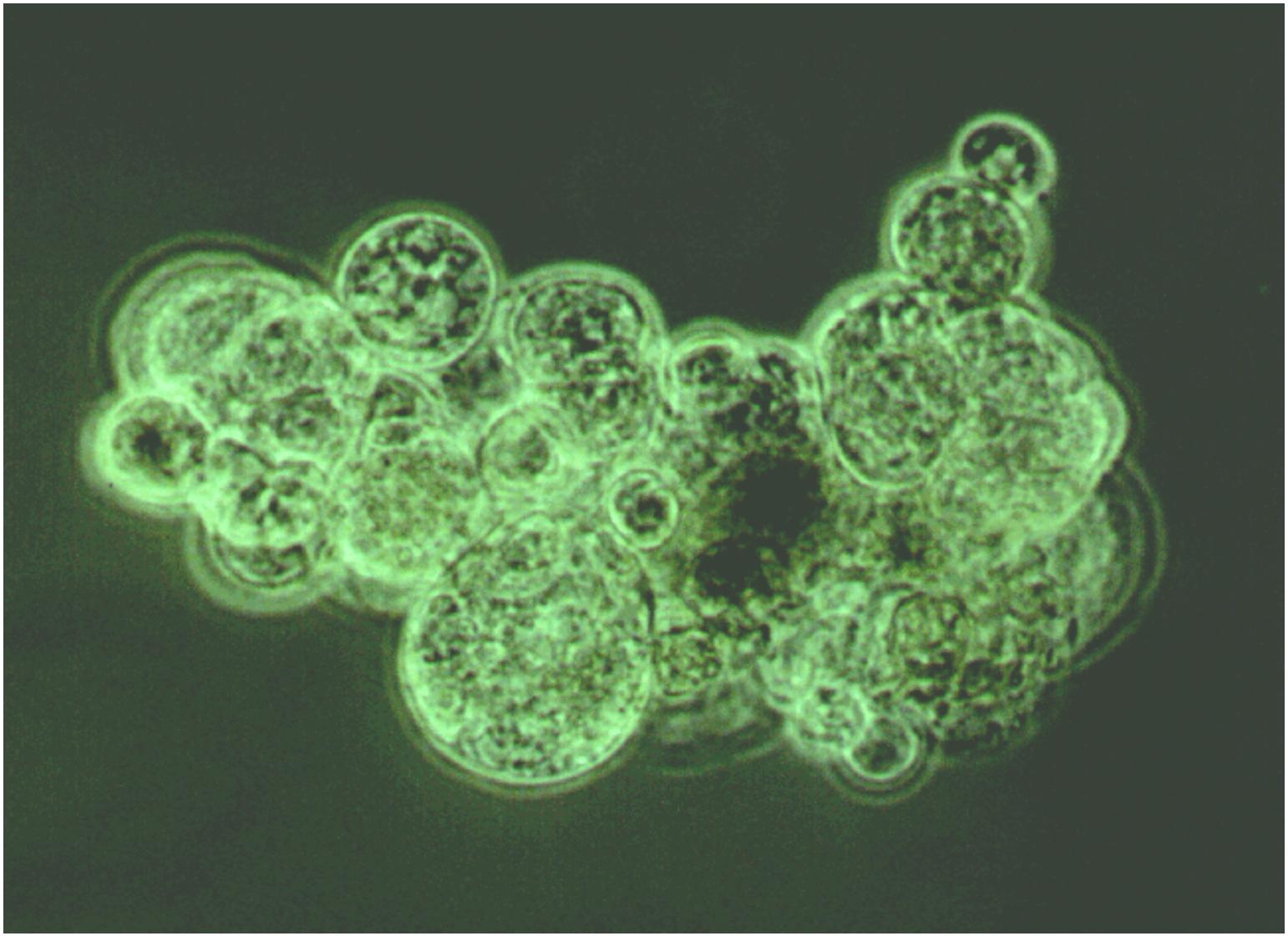


“GENE GUN”

PROTOPLAST CULTURE

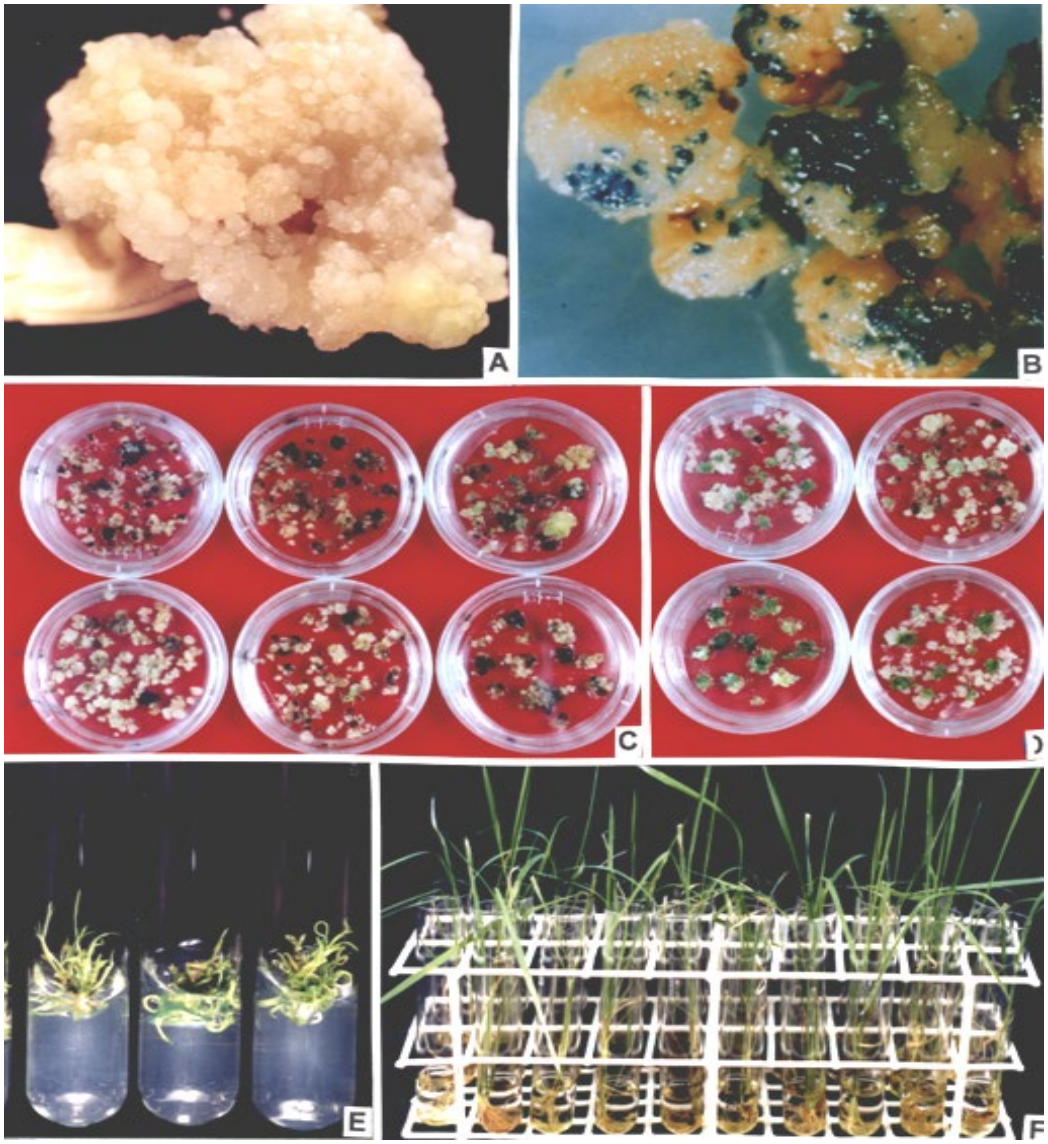


PROTOPLAST CULTURE

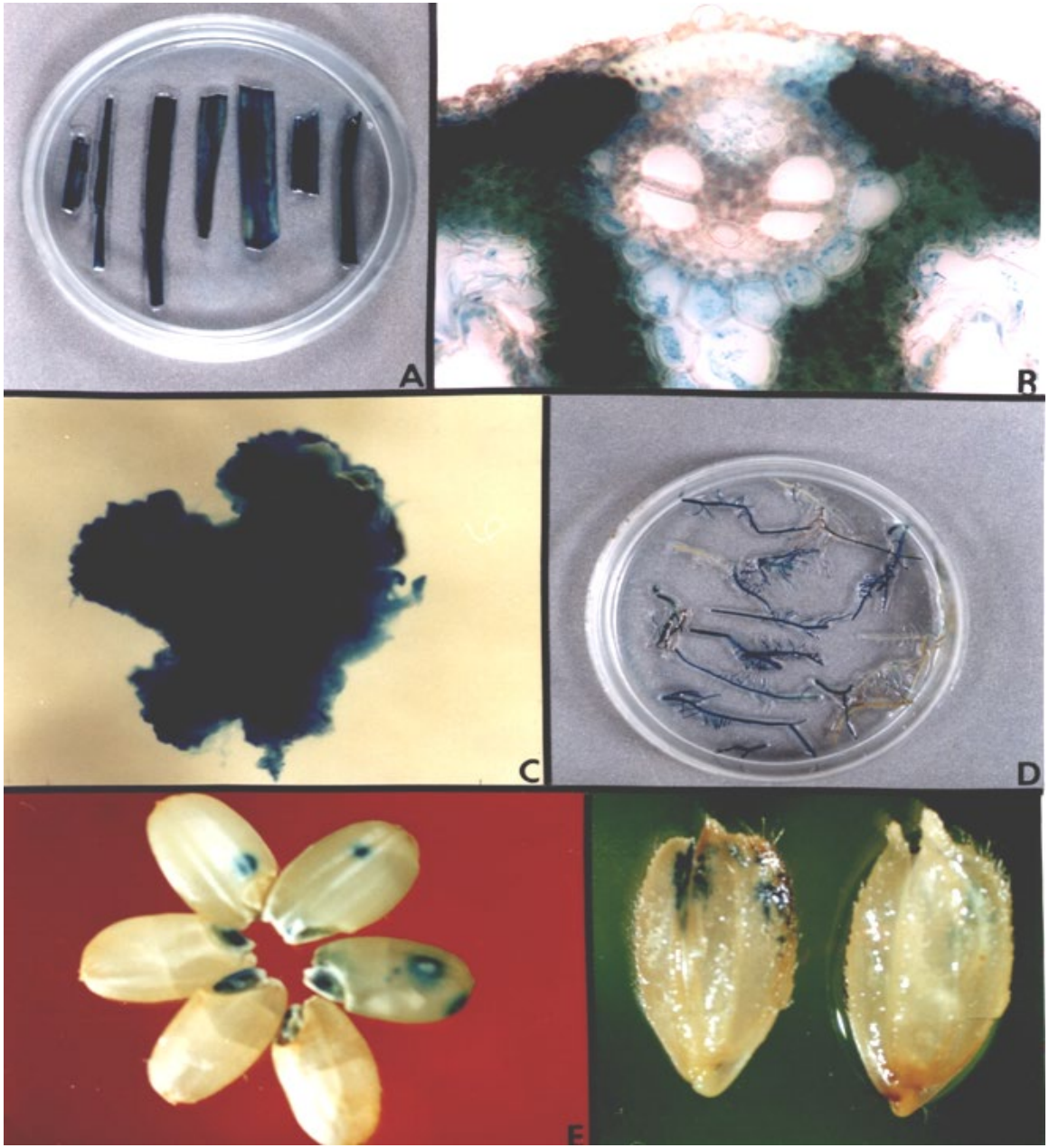


PROOF OF TRANSFORMATION

AGROBACTERIUM MEDIATED TRANSFORMATION IN TAIPEI 309

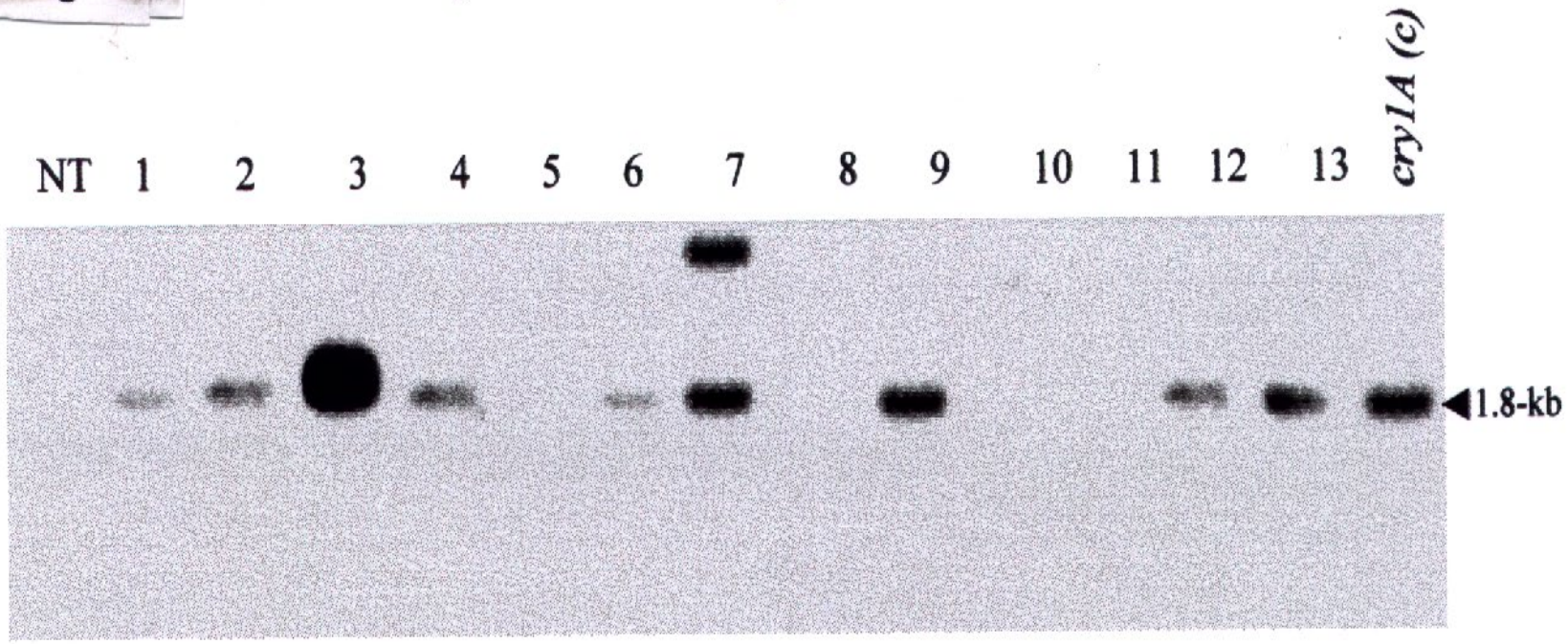


GUS EXPRESSION IN DIFFERENT PLANT ORGANS OF TRANSGENICS



SOUTHERN ANALYSIS OF WU10 B TRANSFORMANTS

Fig-4.17 Southern hybridization analysis of WU10B transformants



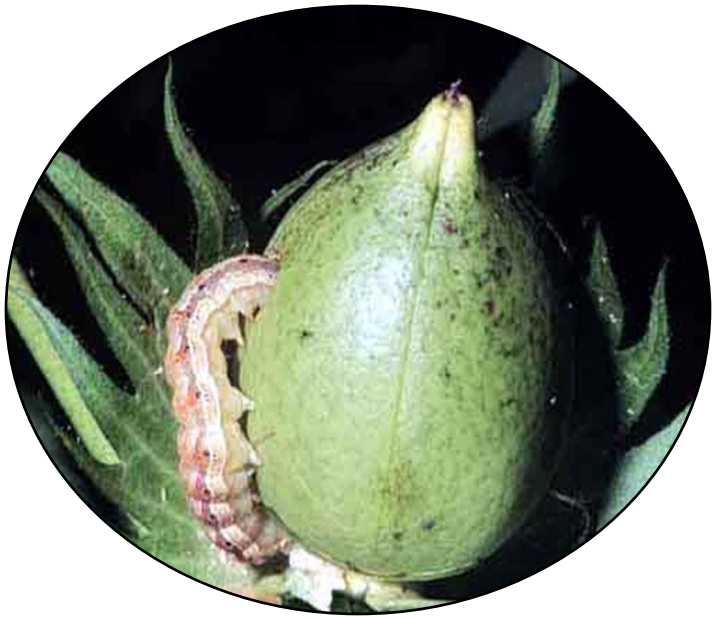
NT = non-transformed control; Numbers (1 - 4 and 6,7,9,12 and 13) corresponding to lanes represent the *CrylA(c)* +ve, lane 5,10 and 11 *CrylA(c)* - ve

BIOTECH CROPS

Bt COTTON

BOLL WORM DAMAGE

Boll damage



Bt COTTON

- Resistant to Boll Worms
- First Transgenic crop released in India in 2002
- Resistance is conferred by *Cry1 Ac* gene from Bt

CHRONOLOGY OF BT COTTON DEVELOPMENT

1994	Formation of IBSC and application for transgenic Bt cottonseed import.
1995	Permit from DBT received to import 100 g. Bt Cotton seed of Coker 312 from Monsanto, USA.
1996	Imported seed and Green House trial initiated.
1996	Limited field trial (1 Location) to assess pollen escape.
1996	Back crossing (Ongoing) breeding for transfer of Bt gene into elite parental lines in green house.
1997/98	Limited field trials (5 locations) to assess pollen escape.
1998	Toxicological (Ruminant goat model) and Allergenicity studies.

Bt COTTON DEVELOPMENT cont..

1998-99		Multi-centric research trials (15 +25 locations) to assess efficacy of Bt gene in Indian elite germplasm.
2000-01	(a)	Large-scale trials (100ha) to assess efficacy of Bt gene in Indian elite germplasm
	(b)	Hybrid seed production (150 ha)
	(c)	Various biosafety studies.
	(d)	ICAR trials at 6 locations.
2001-02	(a)	Large-scale trials (100ha) to assess efficacy Bt gene in Indian elite germplasm and the performance of Bt hybrids,
	(b)	Hybrid seed production (300 ha)
	(c)	Biosafety studies.
	(d)	ICAR Trials at 11 locations.
2002		**Commercial cultivation in Six States (Andhra Pradesh, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu)**

Bt COTTON DEVELOPMENT cont..

- The *Cry1Ac* gene, which encodes for an insecticidal protein, Cry1Ac, derived from the common soil microbe *Bacillus thuringiensis* subsp. K*urstaki*

- The *nptII* gene, which encodes the selectable marker enzyme neomycin phosphotransferase II (NPTII), was used to identify transformed cells that contained the Cry1Ac protein.
  The *aad* gene which encodes the bacterial selectable marker enzyme aminoglycoside adenyltransferase (AAD)
  allowed for the selection of bacteria

  NPTII and AAD proteins are used as a selectable marker and have no pesticidal activity and are not known to be toxic to any species.

BT COTTON EVENTS APPROVED FOR CULTIVATION IN INDIA

Event name	Event number	Source company/ institution	Genes	Year of approval
Bollgard I	MON 531	Monsanto	*cry1Ac*	2002
Bollgard II	MON 15985	Monsanto	*cry1A*c and *cry2Ab*	2006
Event 1	Event 1	IIT, Kharagpur	Truncated *cry1Ac*	2006
GFM Cry1A	GFM Cry1A	Chinese Academy of Sciences	*cry1Ab+cry1Ac*	2006
Dharwad Event	Dharwad Event	UAS, Dharwad	Truncated *cry1Ac*	2008
9124	9124	Metahelix	*cry1C*	2009

NUMBER OF HYBRIDS/VARIETIES PER EVENT APPROVED FOR CULTIVATION IN INDIA (AUGUST 2009)

Event number	Source company/Institution	#
MON 531	Monsanto	205
MON 15985	Monsanto	309
Event 1	IIT, Kharagpur	33
GFM Cry1A	Chinese Academy of Sciences	69
Dharwad Event	UAS, Dharwad	1
Event 9124	Metahelix	2

COMMERCIAL RELEASE OF DIFFERENT BT COTTON EVENTS IN INDIA, 2002 - 2008

S. No.	Crop	Event	Developer	Year of approval
1	Cotton*	MON 531	Mahyco/Monsanto	2002
2	Cotton*	MON 15985	Mahyco/Monsanto	2006
3	Cotton*	Event-1	JK Agri-Genetics	2006
4	Cotton*	GFM Event	Nath Seeds	2006
5	Cotton**	Cry1Ac Event	CICR (ICAR) & UAS, Dharwad	2008
6	Cotton*	Cry1c 9124 MH 5125 & 5174	Metahelix	2009

BT COTTON EVENTS CURRENTLY UNDERGOING FIELD TESTS IN INDIA

Event name	Event number	Company/Institution	Genes
Event 1 + Event 24	Event 1 + Event 24	JK Agri	*cry 1Ac and cry 1EC*
Wide strike	Event 3006-210-23 +Event 281-24-236	Dow Agro	*cry 1Ac and cry 1F*
Roundup Ready Flex Bt	MON 15985 + MON 88913	Monsanto	*cry 1Ac, cry2Ab, CP4EPSPS*

Hindu 6/3/10

Bt cotton ineffective against pest in parts of Gujarat, admits Monsanto

Firm asks farmers to switch to its second-generation product to delay resistance further

Priscilla Jebaraj

NEW DELHI: For the first time anywhere in the world, biotech agriculture giant Monsanto has admitted that insects have developed resistance to its Bt cotton crop. Field monitoring in parts of

In November 2009, Monsanto's scientists detected unusual survival of the pink bollworm pest while monitoring the Bt cotton crop in Gujarat. In January and February, samples taken from the field were tested in Monsanto's laboratories. It has

- **Pink bollworm resistant to pest-killing protein of Bt cotton in four districts**
- **Monsanto's advice ridiculous, say scientists**

laboratory, Monsanto held that "field resistance is the cri-

santo India's Director of Scientific Affairs Rashmi Nair. She also recommends that

II will take longer for the pest to develop resistance. Anyway, the Bt toxin is active only for 90 days, while pink bollworm is a late season pest, he adds.

"All the hype about the effectiveness of Bt against pests is bogus ...This proves that you can't stay ahead of the pest

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However, the
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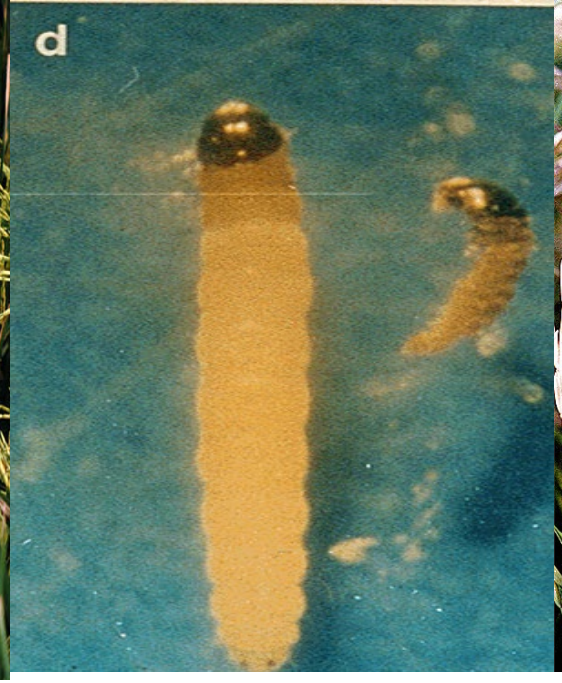
Bt BRINJAL

Bt BRIJAL

- Bt brinjal was developed by transforming the brinjal proprietary line of Mahyco.
- Bt brinjal contains the following three genes
- The *cry1Ac* gene, which encodes for an insecticidal protein, Cry1Ac, derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k*). The *cry1Ac* gene is driven by enhanced CaMV 35S promoter.
- The *nptII* gene which encodes the selectable marker enzyme neomycin phospho transferase II (NPTII) was used to identify transformed cells that contained the Cry1Ac protein. It has no pesticidal properties. The *nptII* gene is derived from the prokaryotic transposon Tn5.
- The *aad* gene which encodes for the bacterial selectable marker enzyme aminnoglycoside adenyl transferase (AAD) allowed for the selection of bacteria containing the pMON 10518 plasmid on media containing spectinomycin or streptomycin. The *aad* gene is under the control of a bacterial promoter and hence not expressed in Bt brinjal. The *aad* gene was isolated from transposon *Tn7*.

GM RICE

- GM Rice for Stem Borer Resistance
- GM Rice for Sheath Blight and BLB Resistance
- GM Rice for Nutrition- Golden Rice and Iron rich Rice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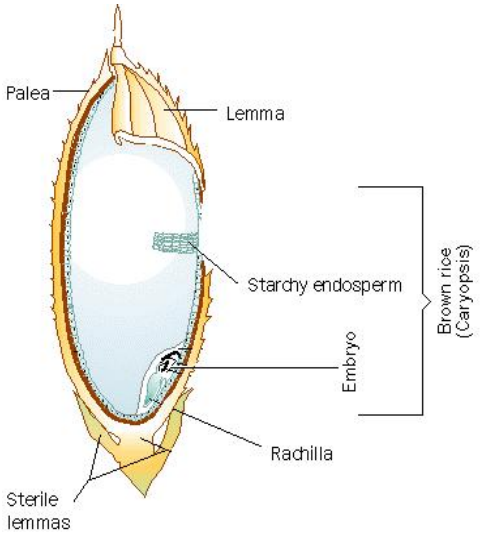
BACTERIAL BLIGHT RESISTANT GM RICE

Transgenic Rice in Field--Fujian, 2002

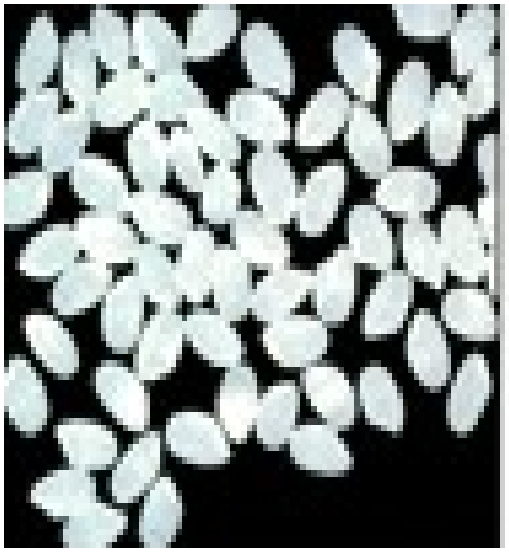
GOLDEN RICE

GOLDEN RICE

The term Golden rice refers to the genetically engineered rice capable of producing β-Carotene (Provitamin-A) inside rice endosperm



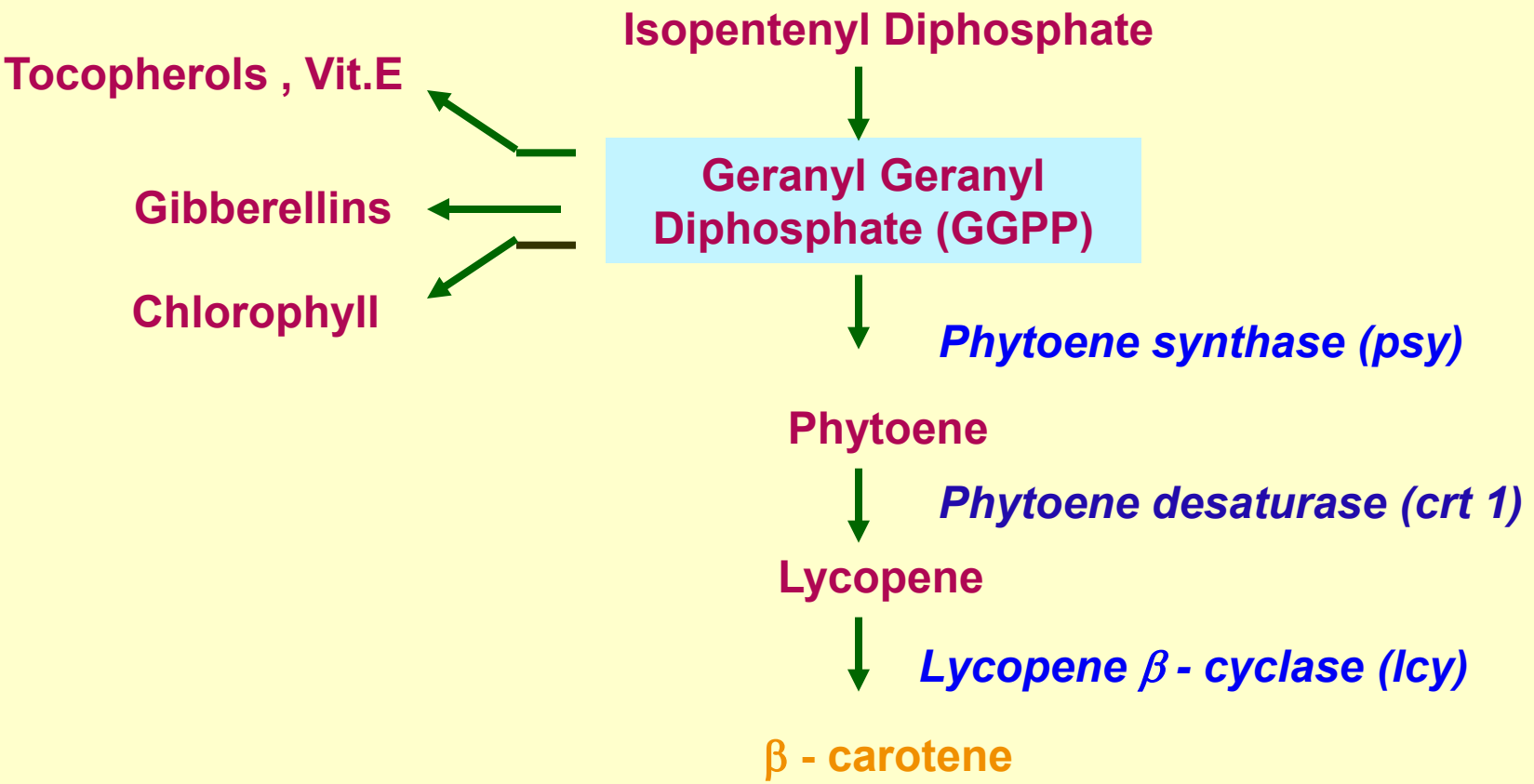
Rice seed



white rice

Golden rice

PATHWAY ENGINEERING FOR DEVELOPMENT OF GOLDEN RICE



Gene Source: – ***psy*** **and** ***lcy*** **from** ***Narcissus pseudonarcissus***
– ***crt1*** **from** ***Erwinia uredovora*****.**

PATHWAY ENGINEERING FOR DEVELOPMENT OF GOLDEN RICE

Erwinia uredovora

Geranyl geranyl Pyro Phosphate

Phytoene synthase (psy)

Phytoene

Phytoene desaturase (crt 1)

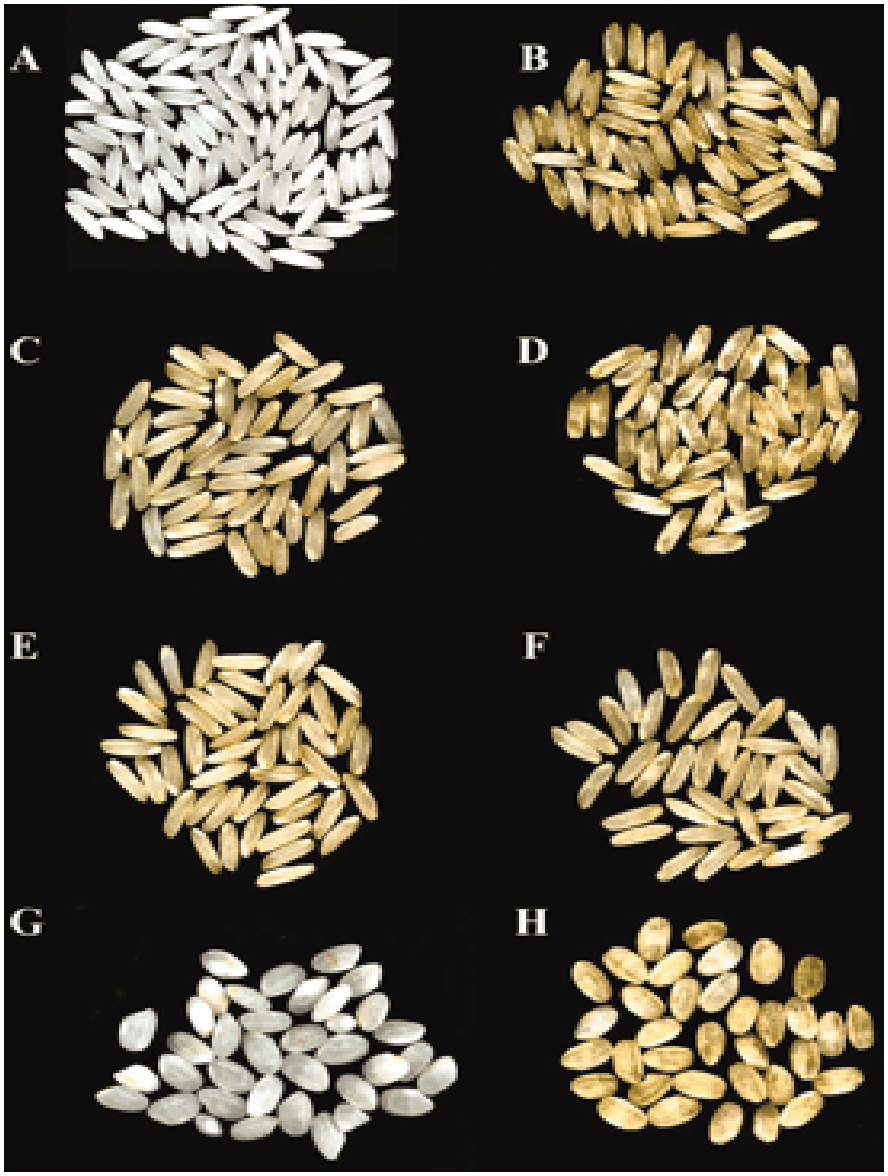
Lycopene

Lycopene β - cyclase (lcy)

β - carotene

Daffodil

JAPONICA & INDICA MARKER FREE GOLDEN RICE LINES



GOLDEN RICE – SUMMARY OF DEVELOPMENT

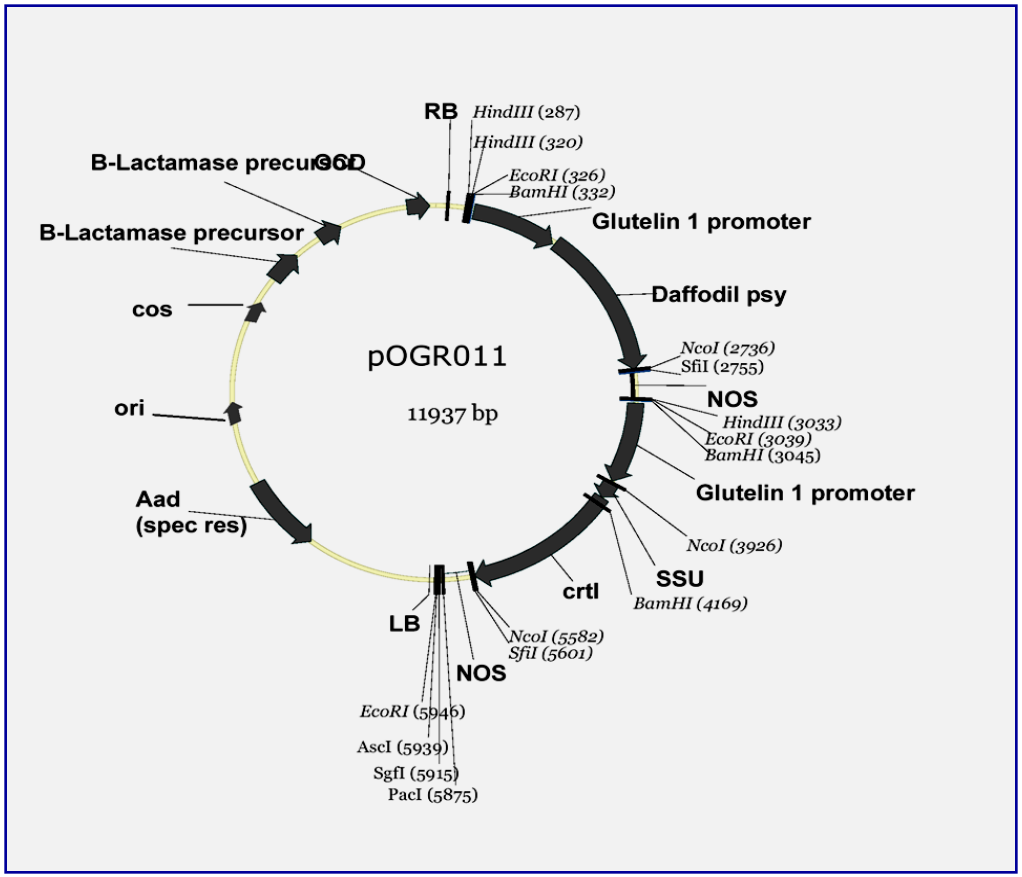
Version 1 – Taipei309 – developed in Prof. Potrykus's lab with *hyg*^{R} gene – *psy* + *crt1* + *lcy* - 1.7 µg/ g of carotenoids - received in 2003 - work abandoned

Version 2 – Taipei309 & IR64 – developed in Prof. Peter Beyer's lab through mannose-6-phosphate selection system – *psy* + *crt1* - 1.2 µg/ g of carotenoids - received in 2004 – Backcross breeding work in progress

Version 3 – SGR1- Cocodrie – developed by Golden rice Humanitarian Board-Syngenta collaboration – *psy* + *crt1* - 7 µg/ g of carotenoids – "marker free" – developed through a co-transformation system - recently received – Backcross breeding work initiated

Version 4 – SGR2 – Kaybonnet - developed by Syngenta – maize *psy* + *crt1* - 30 µg/ g of carotenoids –developed through mannose-6-phosphate selection system – expected to be received in India by the end of 2005

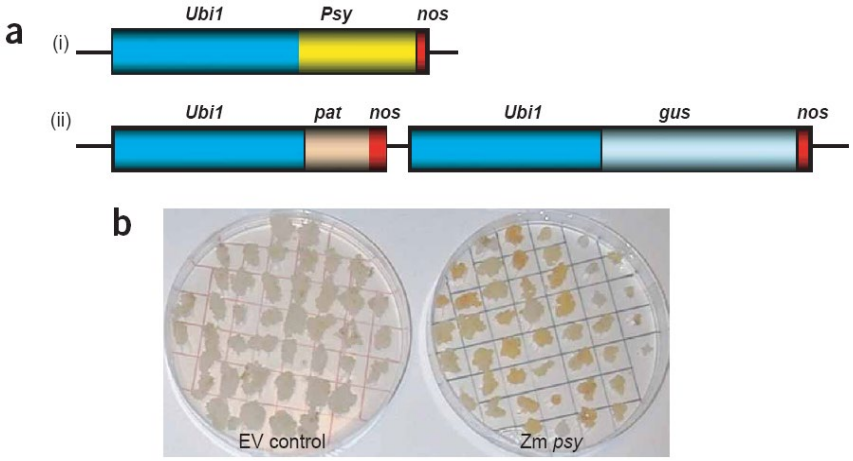
GOLDEN RICE VERSION 3.0 - SYNGENTA



Golden Rice developed in the background of American rice variety Cocodrie – contains up to 7 μg per gram dry weight

Vector map of the gene construct used for development of Version 3 of Golden rice – Syngenta Golden Rice 1 (SGR1)

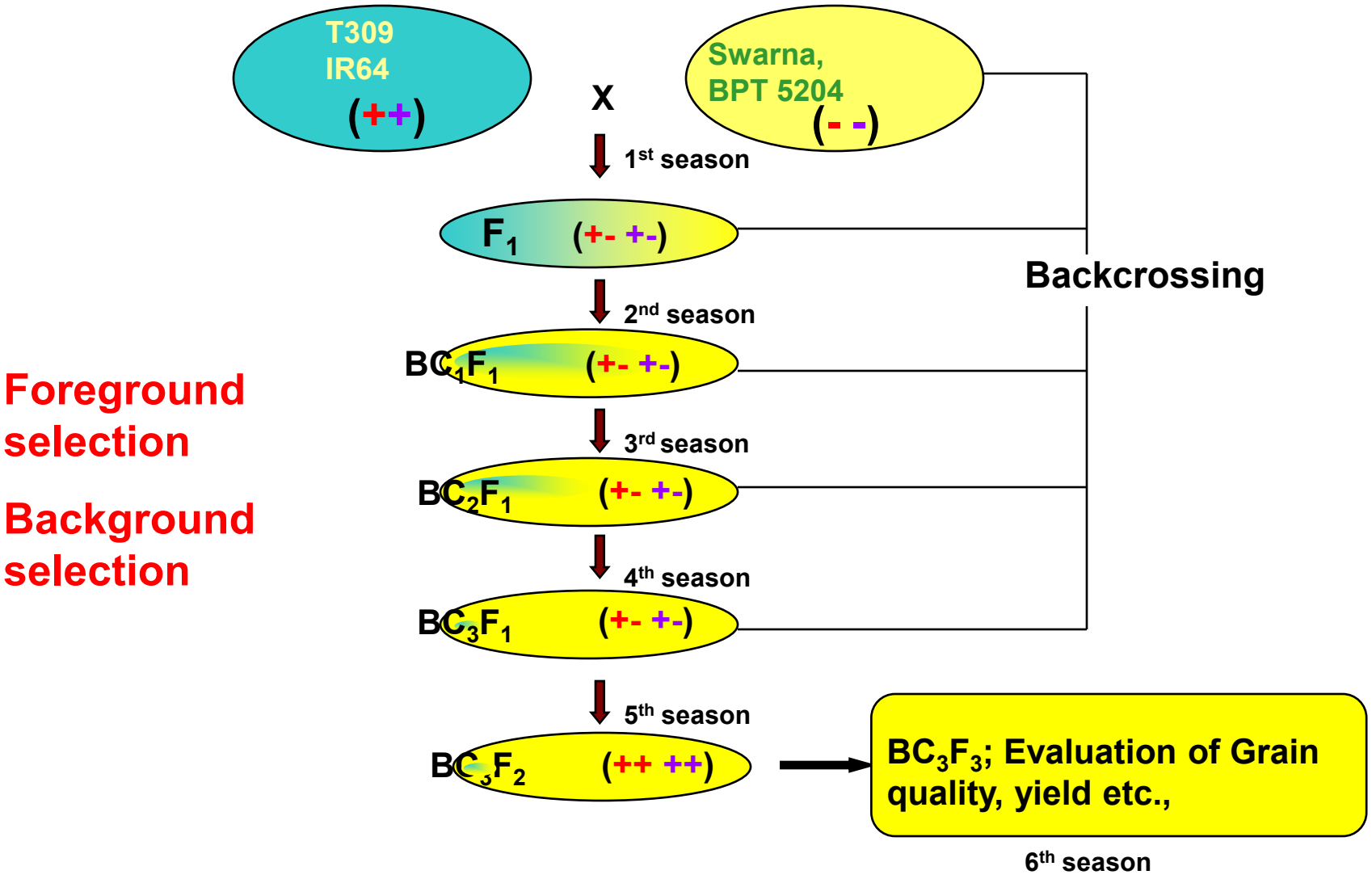
GOLDEN RICE VERSION 4.0 - SYNGENTA



How to increase β-carotene levels in rice endosperm?

Vector map of the gene construct used for development of Version 4 of Golden rice –Syngenta Golden Rice 2 (SGR2)

MARKER ASSISTED STRATEGY FOR TRANSFER OF GOLDEN RICE TRAIT




Journey to be continue


Clothing & Textile


Clothing & Textile

GOVT. M. H. COLLEGE OF HOME SCIE
& SCIENCE FOR WOMEN (AUTO.)

शासकीय स्वशासी कन्या स्नातकोत्तर उत्कृष्टता महाविद्यालय
सागर (म.प्र.)
गृह विज्ञान विभाग
शैक्षणिक भ्रमण-जबलपुर क्षेत्र

शासकीय स्वशासी कन्या स्नातकोत्तर उत्कृष्टता महाविद्यालय
सागर (म.प्र.)

गृह विज्ञान विभाग

शैक्षणिक भ्रमण-जबलपुर के

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के संयुक्त तत्वाधान में

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गृह विज्ञान विभाग

शैक्षणिक भ्रमण–जबलपुर क्षेत्र

दिनांक - 15 मार्च 2023

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