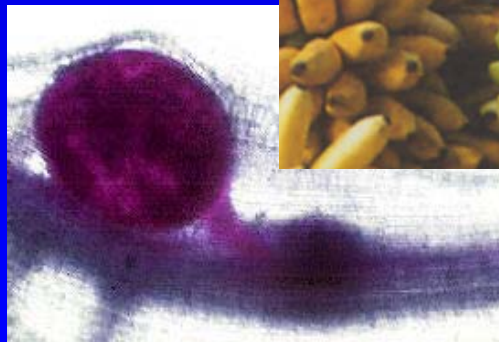
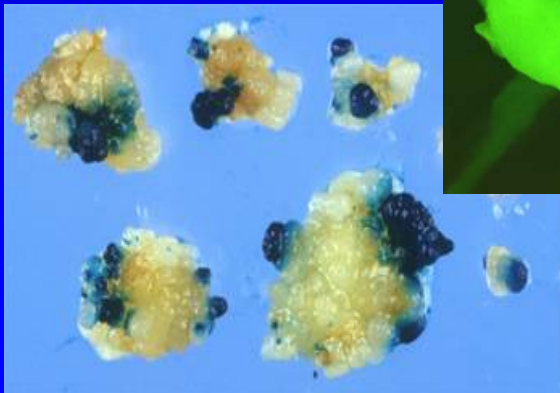
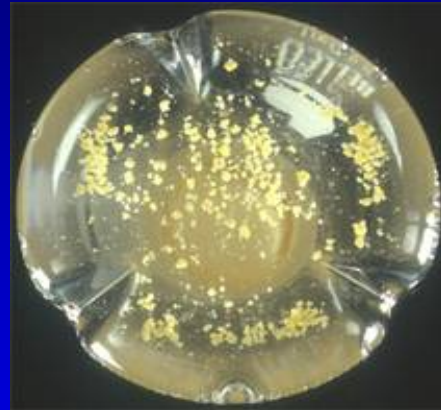
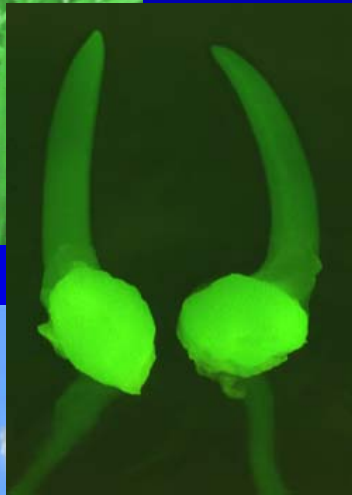


# Rice and cooking banana transformation for nematode resistance

*(John Innes Centre - DFID)*





Department for International Development

# Nematode Resistance programme (1995-2005)



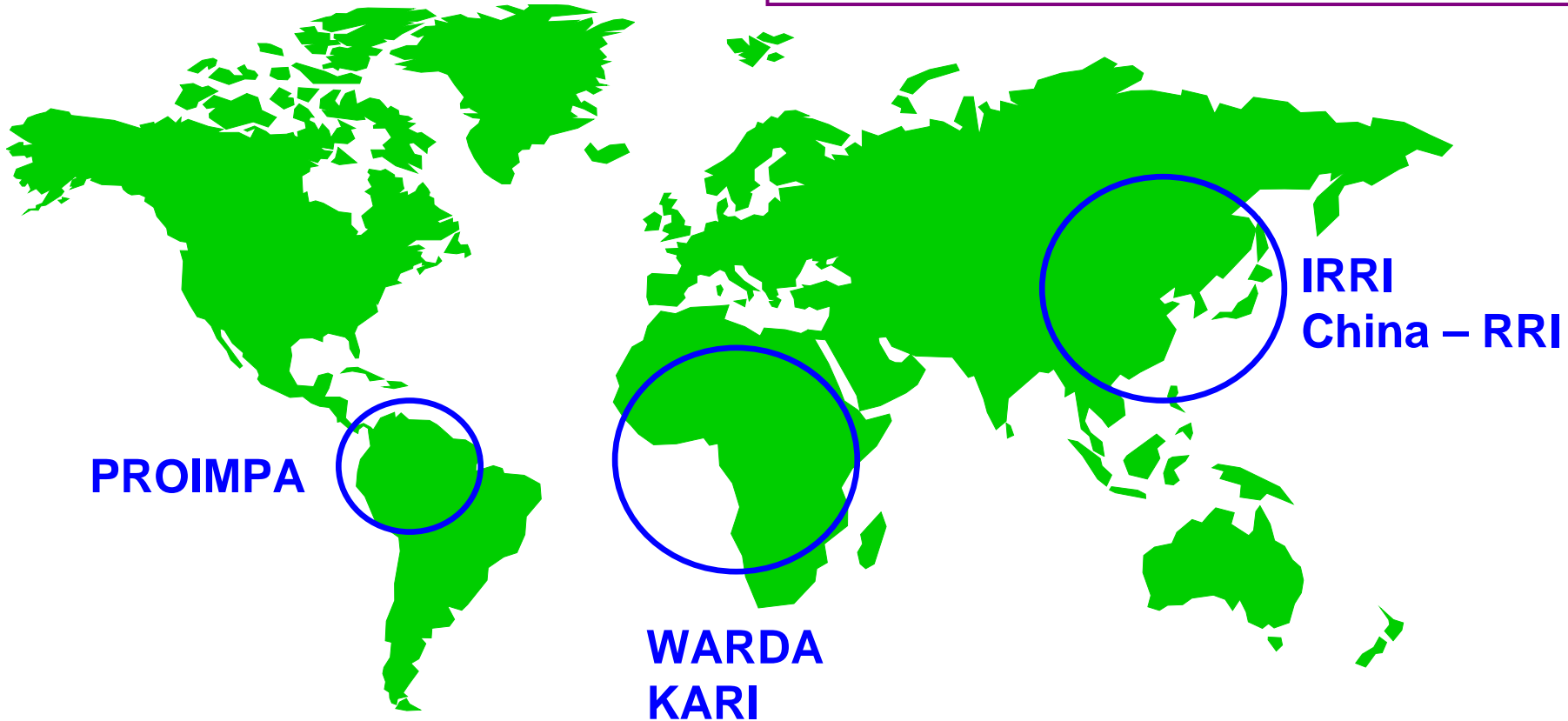
Potato  
Rice  
Banana



John Innes Centre



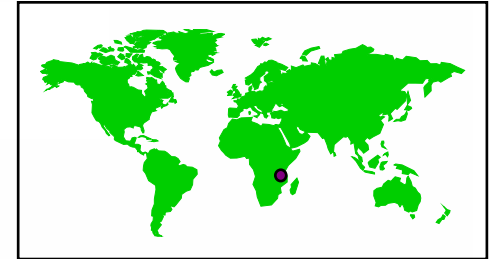
- Rice & banana transformation
- Transgene delivery, integration, expression, stability
- Nematode resistance
- Potato transformation





**Banana transformation**  
*John Innes Centre - DFID R8031*

# Strategy for banana transformation



AAA

Cooking banana from Uganda



→ **E. callus**

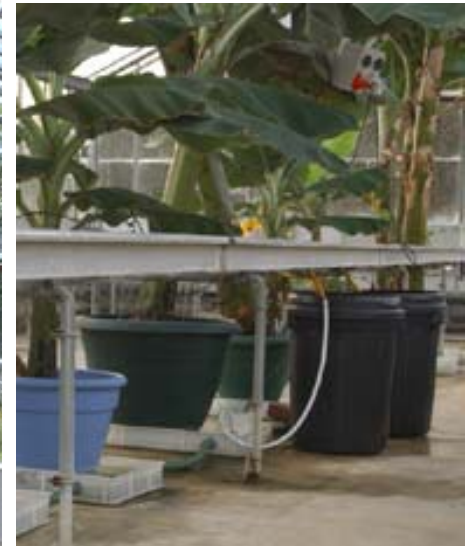
↓  
**E. cell culture**

↓  
**DNA delivery**  
**Selection**  
**Regeneration**

***Production of AAA Ugandan cooking banana plants from shoot tip cultures at John Innes Centre***



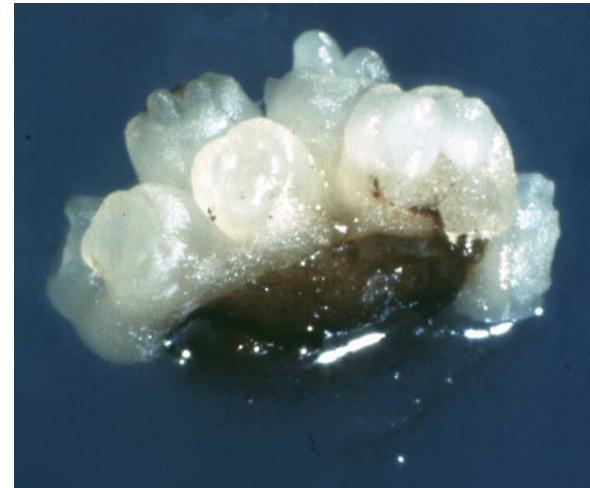
## *Nursery (hydroponics)*



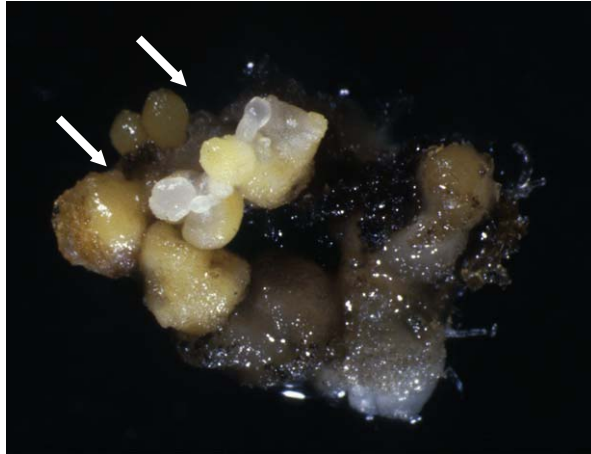
## *Isolation of immature flowers*



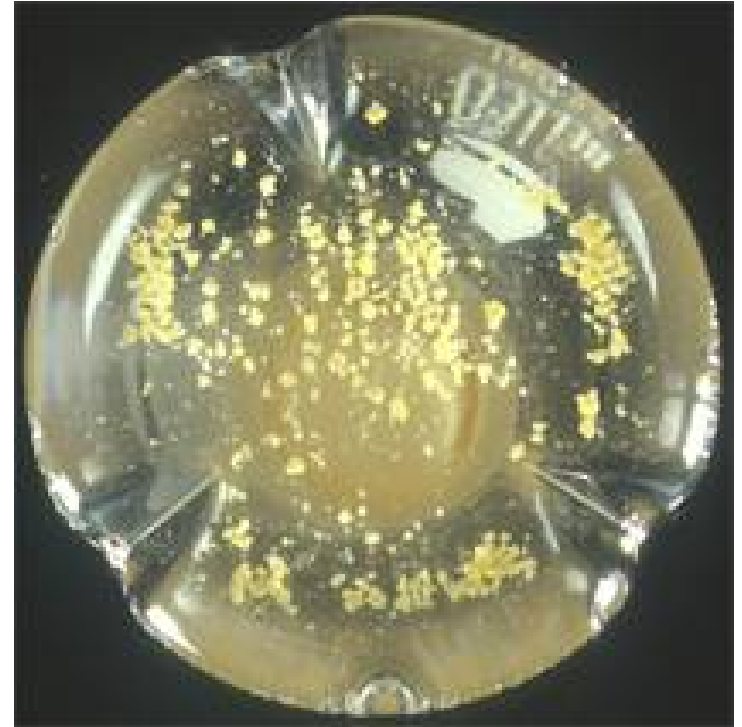
12 months



# *Production of embryogenic cell suspension cultures*



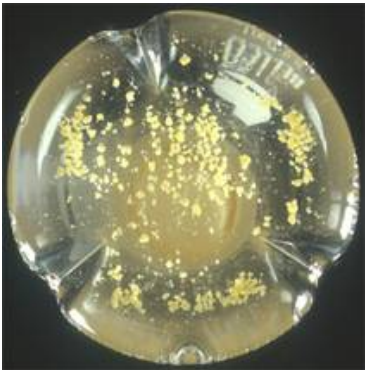
3-6 months



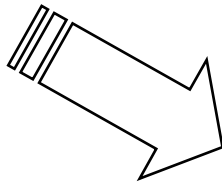
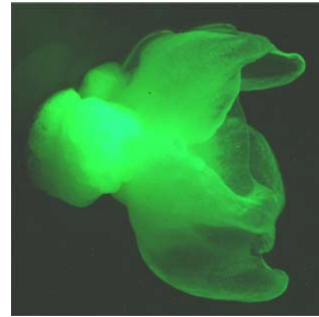
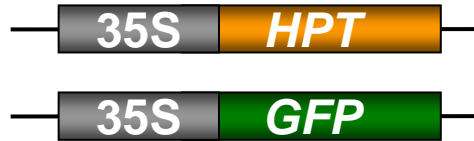
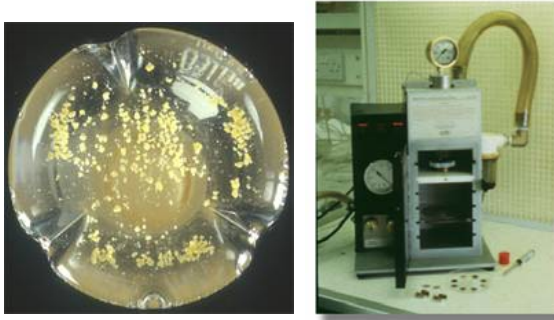
3-6 months



***Regeneration of banana plants  
from embryogenic cell suspension cultures***



## Regeneration of transformed banana plants



Selection



## ***Transformed banana plants (independent clones)***



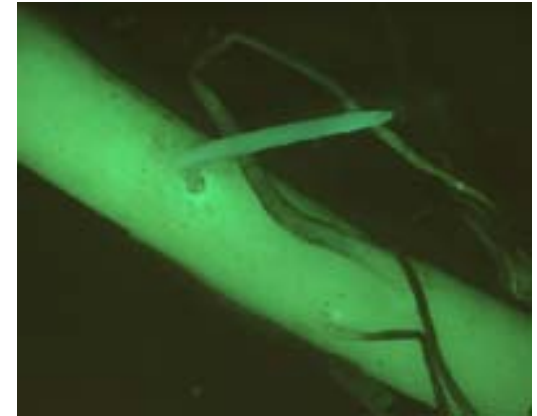
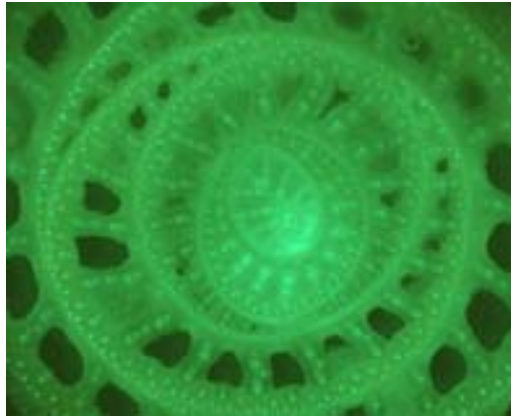
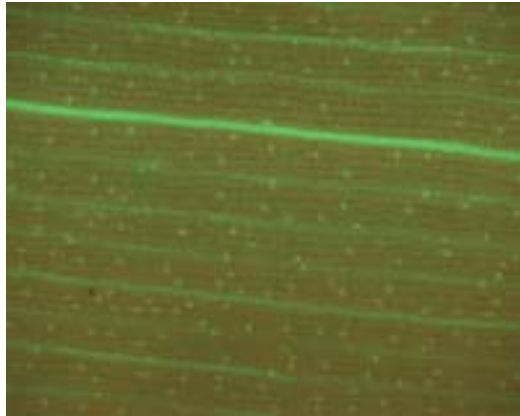
# *GFP expression in transformed banana plants*

Leaf

Stem

Root

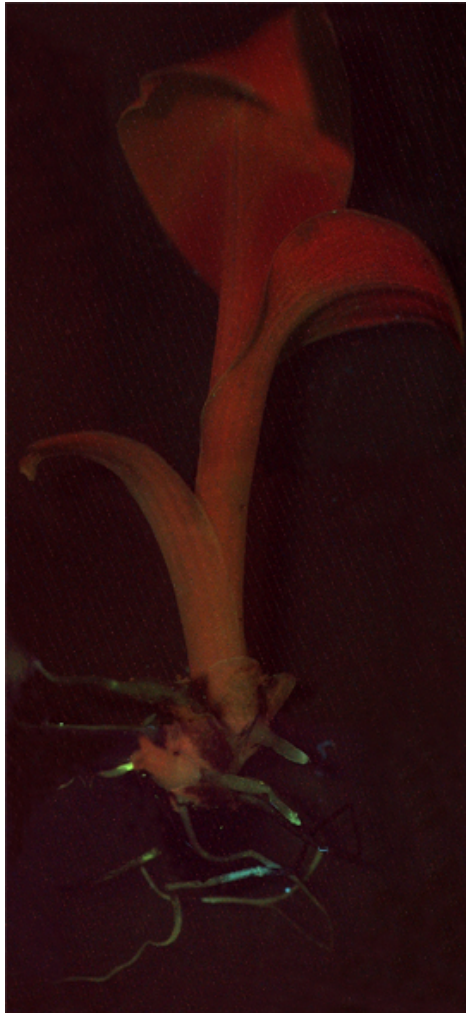
NA5



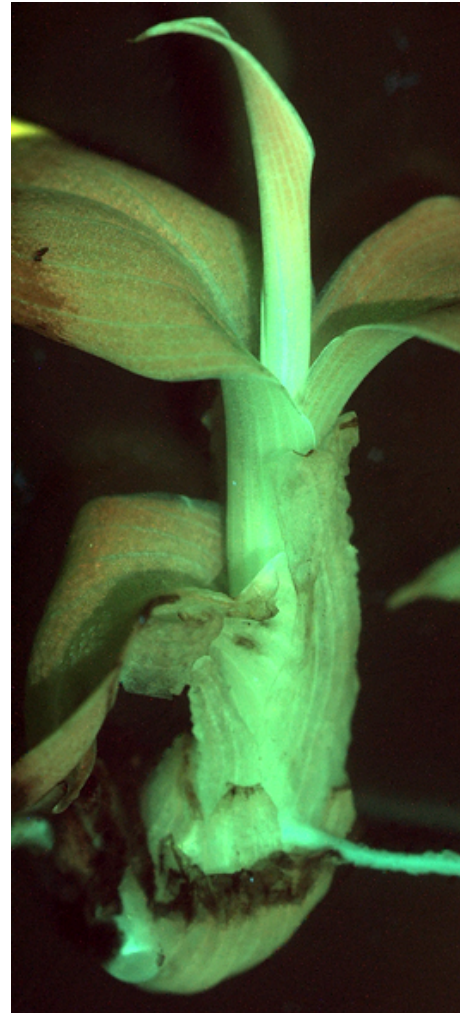
w.t.



***GFP expression in transformed banana plants  
after 25 cycles of shoot tip culture (more than 2 years)***

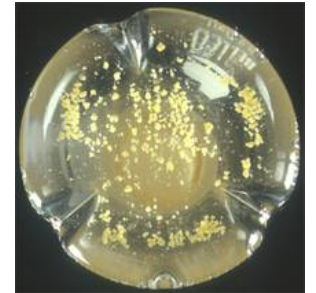
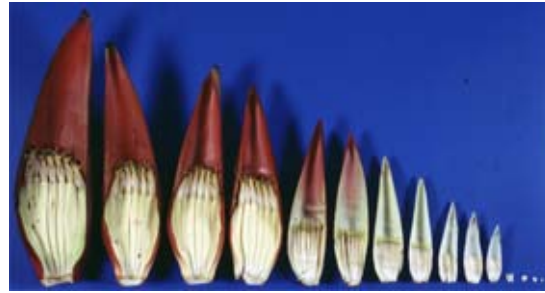
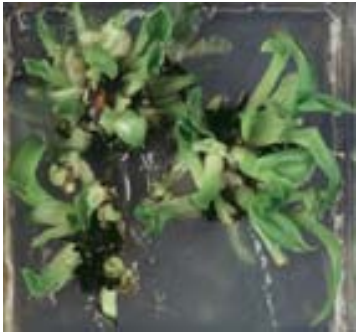


**w.t.**



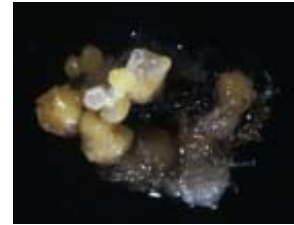
**NA5**

# Banana transformation



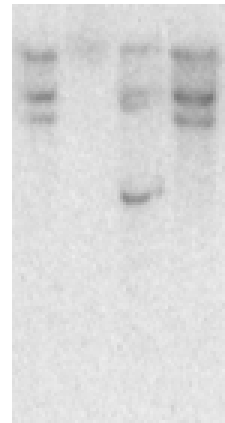
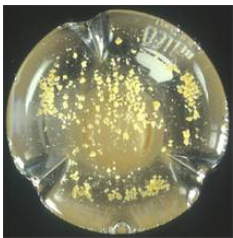
Since  
2000

since  
2002



## Past Results

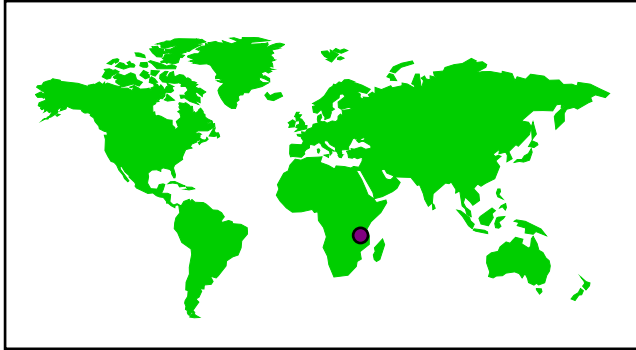
since  
2003



24 - 30  
months



## *Transfer of technology to KARI-NARO (Kampala, Uganda)*





# Banana transformation with cystatin genes for nematode resistance

## Bioassay (U. Leeds)



Clone

No. plants

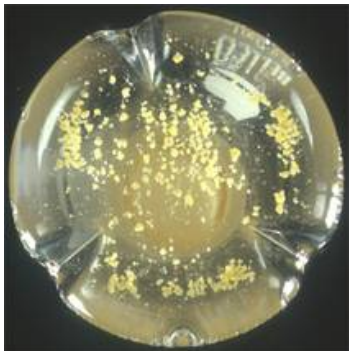
BI5

9



BF3

10





***Future work:***



- ❖ **New banana nursery, embryogenic calli, embryogenic cell suspension**
- ❖ **Continue banana transformation (with UBI:CC?)**
- ❖ **Attend RF meeting in Nairobi, Jan 24-27<sup>th</sup> 2004**



# **Rice transformation**

*John Innes Centre*

*DFID R8031*

*RF-DFID R7548*

- ❖ **Clean gene technology**
- ❖ **Root specific promoter**
- ❖ **Future work**

- ❖ **Clean gene technology**
- ❖ Root specific promoter
- ❖ Future work

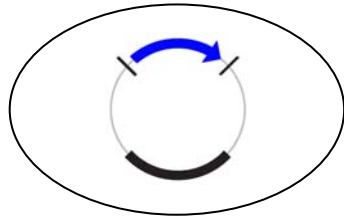


## **Genotype independent rice transformation** *(particle bombardment - Immature embryos)*

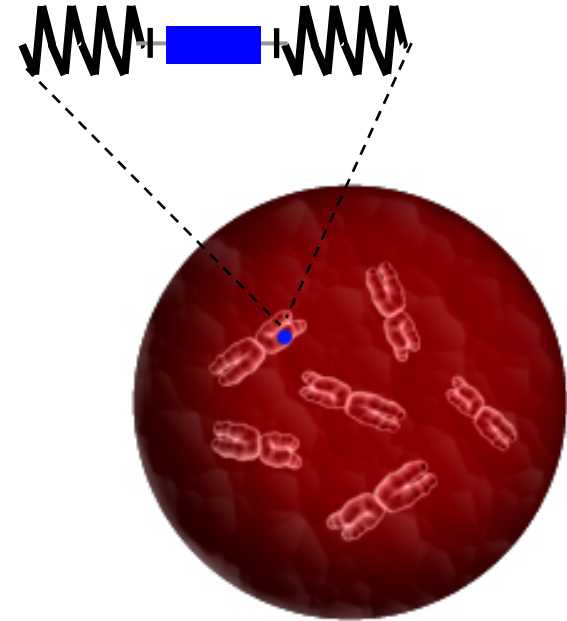
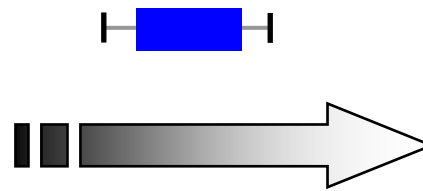
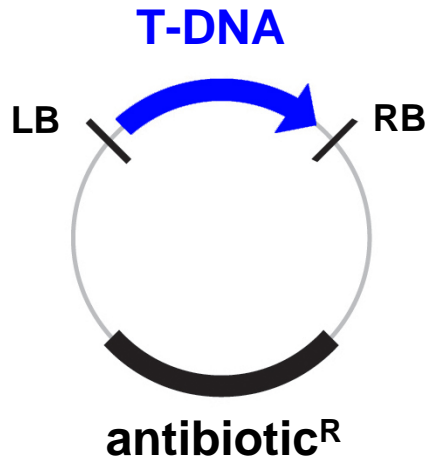


## **High throughput rice transformation** *(Agrobacterium – callus derived from mature embryos)*

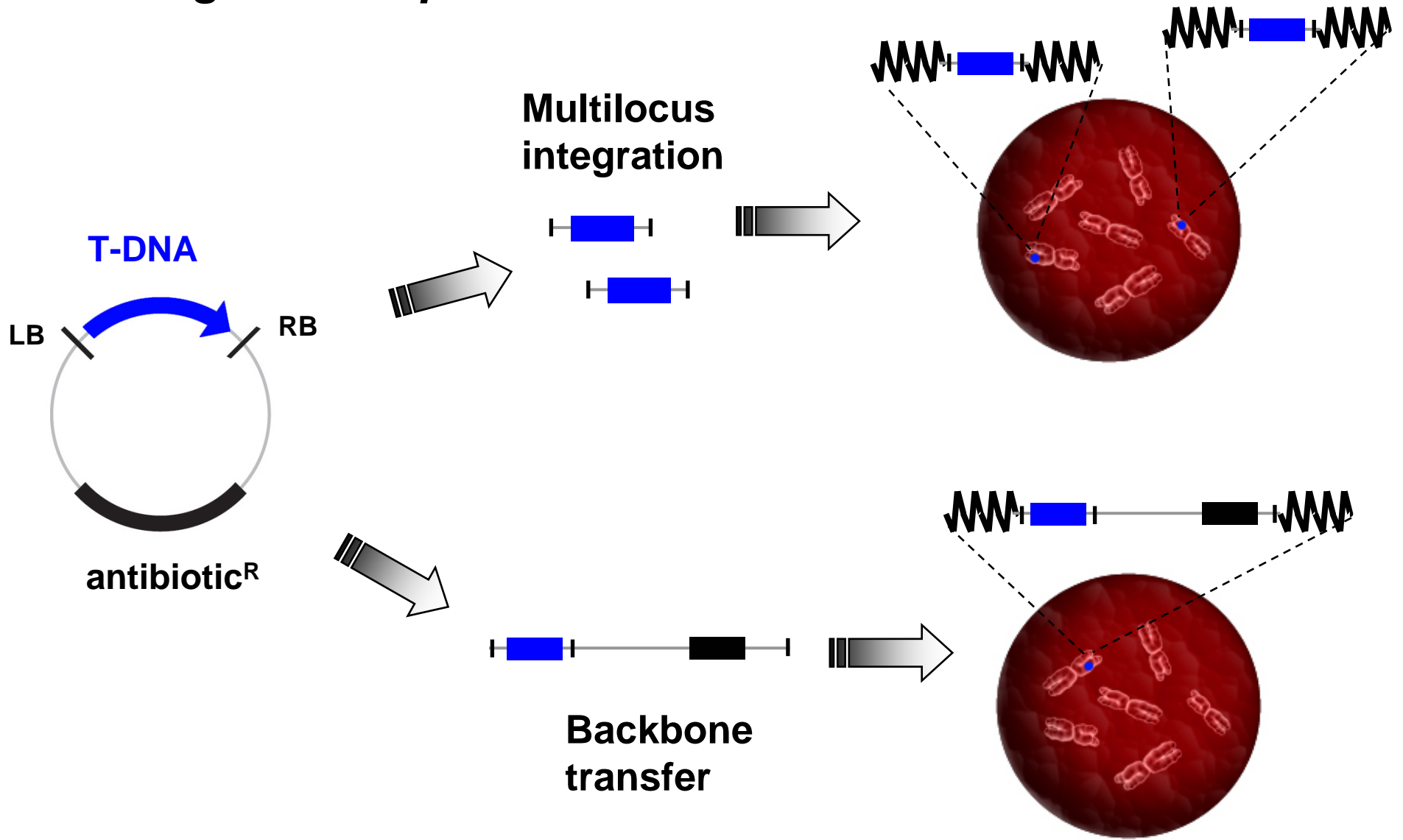
## *T-DNA integration in plants*



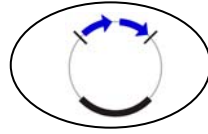
*Agrobacterium*



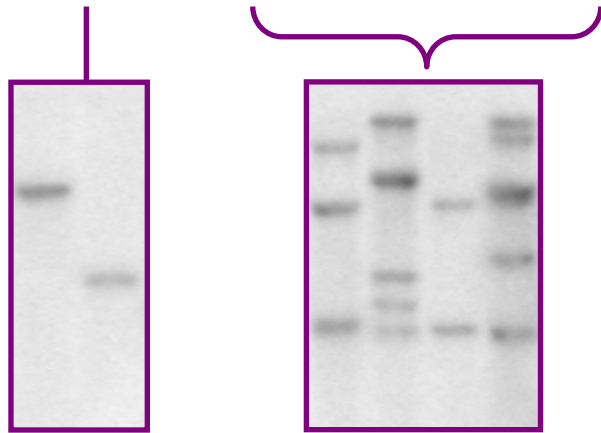
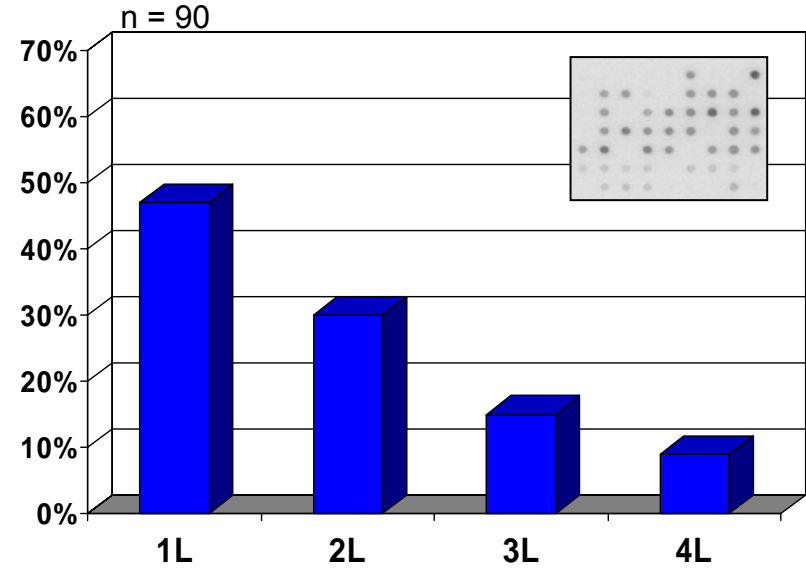
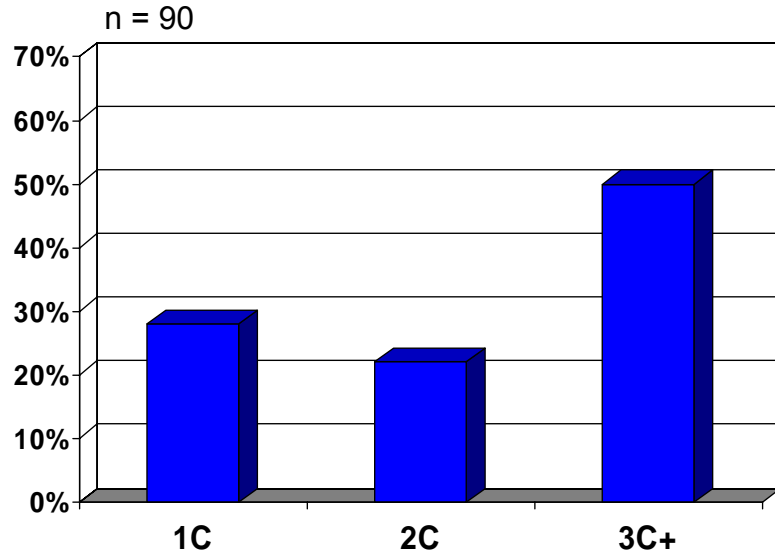
## *T-DNA integration in plants*



## T-DNA integration in rice



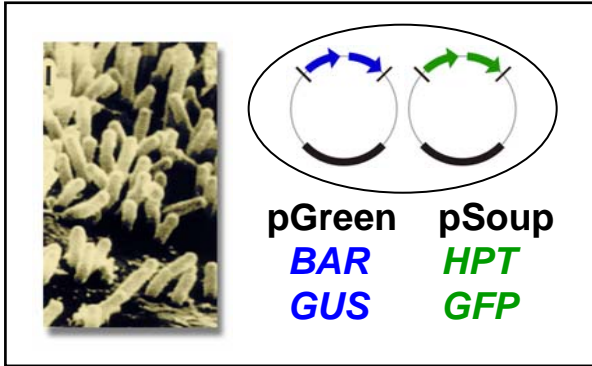
BAR + GUS



Up to 50% multiloci integration  
Up to 50% backbone transfer



## Dual T-DNA integration



62 plant lines  
coT- coE

62x7 = 434 genot.

62x4 = 248 phenot.

$T_0$

64  $T_1$  per line  
~ 4000 plants

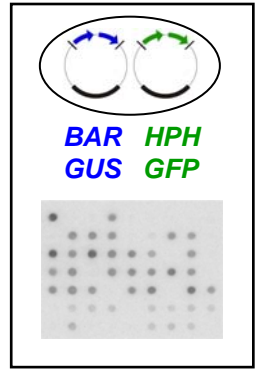
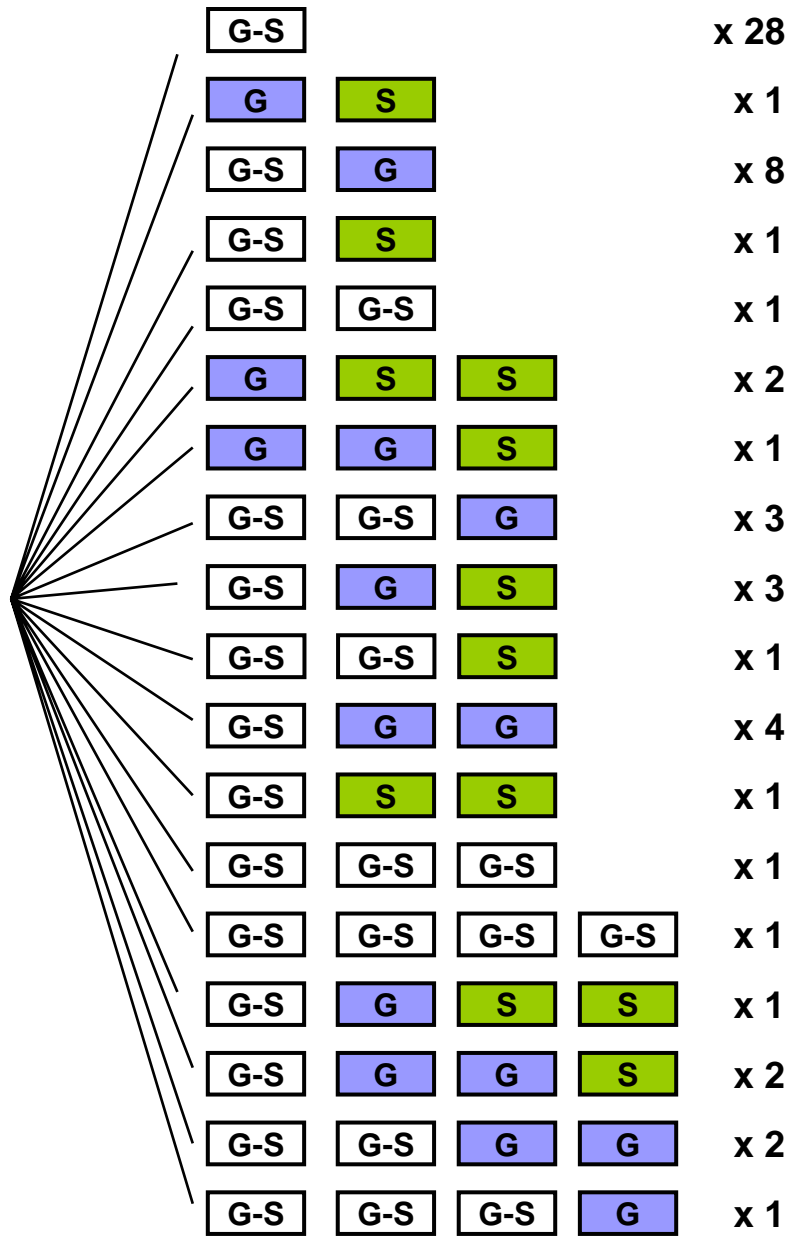
395x5 = 1975 genot.

~4000x2 = ~8000 phenot.

$T_1$

Observed  
segregation  
ratios

## Dual T-DNA integration

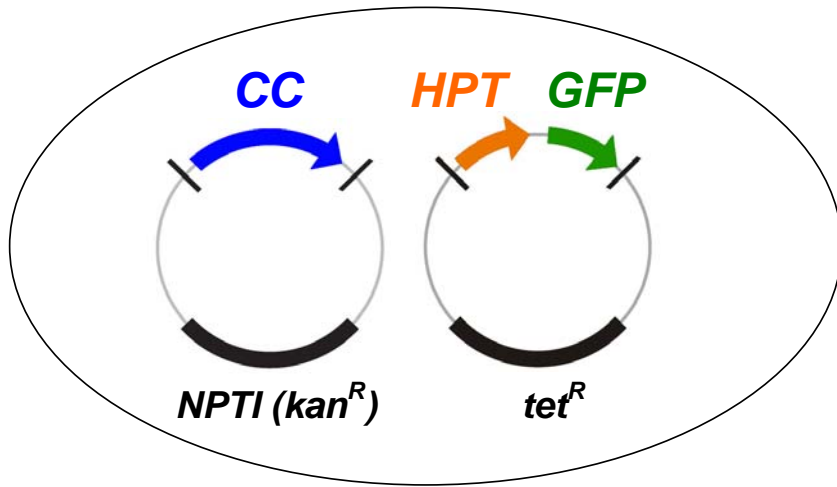


Genotype

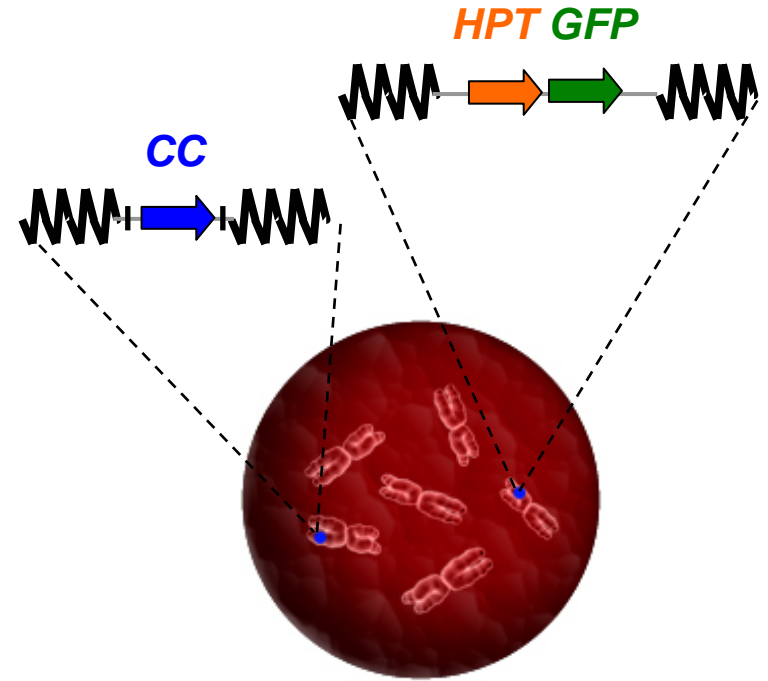
62 plant lines  
127 loci

- G-S 57%
- G 29%
- S 14%

## “Clean gene” rice plants containing *TUB::corn cystatin*



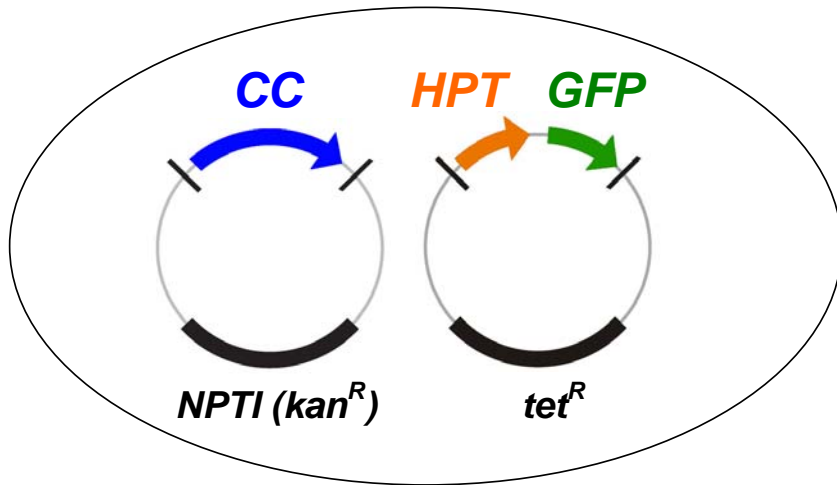
*Agrobacterium*



Unlinked integration

T<sub>0</sub>

## “Clean gene” rice plants containing *TUB::corn cystatin*



*Agrobacterium*

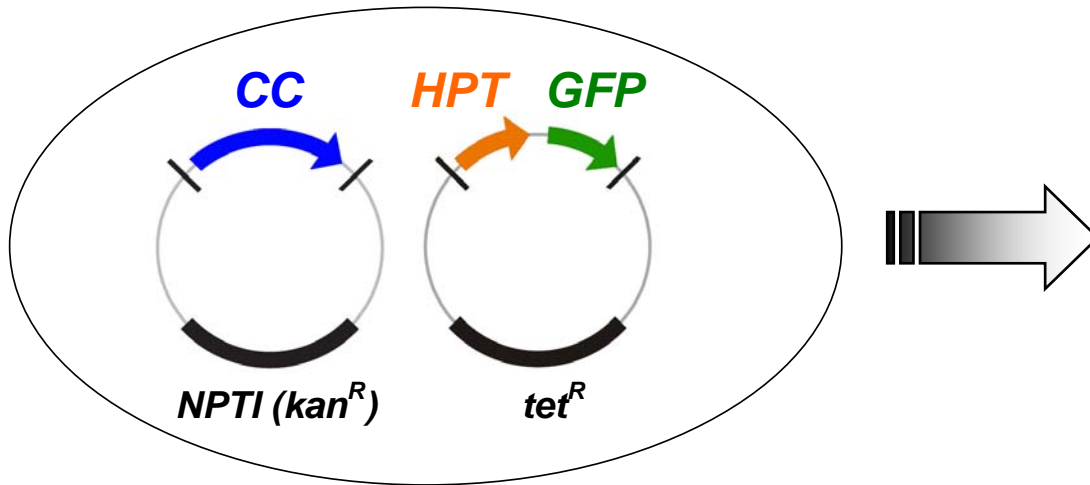


T<sub>1</sub>

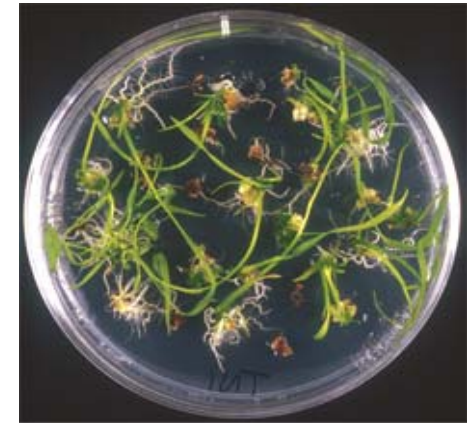
	● ○	● ●	○ ●	○ ○
● ○	● ●			● ●
● ●				
○ ●				
○ ○	● ●			

Progeny plants containing **CC**  
 - free of selectable marker gene (no **HPT-GFP**)  
 - free of backbone (no *NPTI*)

## “Clean gene” rice plants containing *TUB::corn cystatin*

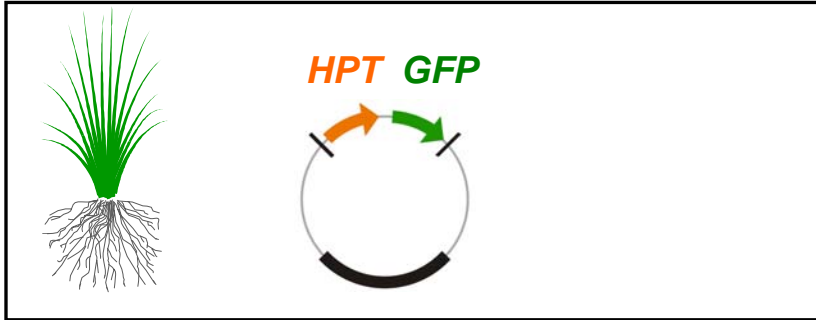


*Agrobacterium*

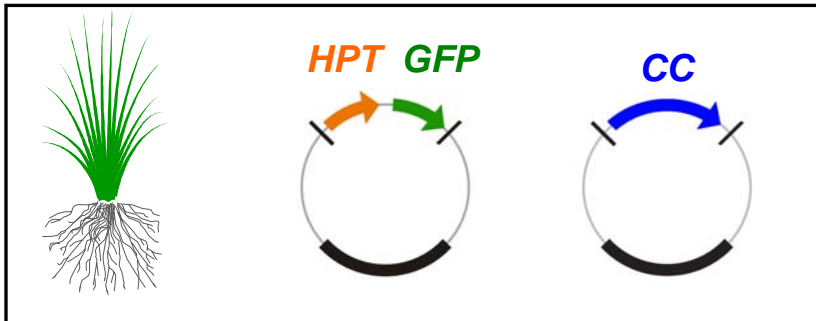


98 independently transformed rice plant lines

## “Clean gene” rice plants containing *TUB::corn cystatin*

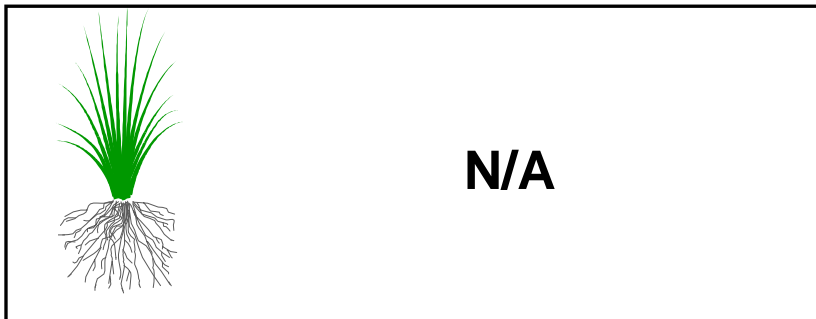


35 plant lines



47 plant lines

57%  
Co-transformation



16 plant lines

## “Clean gene” rice plants containing *TUB::corn cystatin*

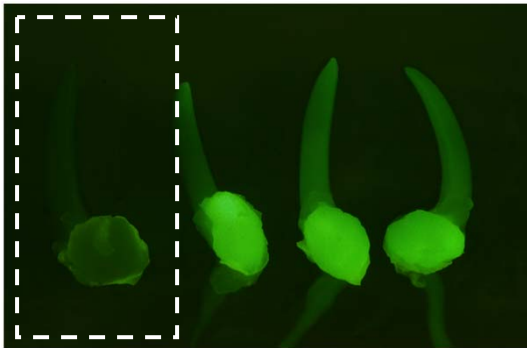
Clone No.	Plant No.	PCR cc	No. of seeds
NN1	NBNN1454	+	170
NN2	NBNN1455	+	200+
NN8	NBNN1509	+	11
NN15	NBNN1510	+	374
NN16	NBNN1530	+	182
NN24	NBNN1500	+	29
NN25	NBNN1501	+	78
NN31	NBNN1526	+	387
NN32	NBNN1527	+	132
NN33	NBNN1528	+	129
NN34	NBNN1512	+	182
NN36	NBNN1513	+	63
NN39	NBNN1529	+	7
NN40	NBNN1475	+	283
NN42	NBNN1477	+	200
NN44	NBNN1479	+	100
NN45	NBNN1520	+	54
NN48	NBNN1522	+	149
NN57	NBNN1446	+	294

Clone No.	Plant No.	PCR cc	No. of seeds
NN61	NBNN1486	+	193
NN62	NBNN1450	+	219
NN67	NBNN1453	+	224
NN80	NBNN1467	+	226
NN85	NBNN1494	+	348
NN92	NBNN1499	+	135
NN96	NBNN1536	+	133
NN71	NBNN1481	+	119
NN98	NBNN1537	+	240
NN100	NBNN1503	+	200+
NN106	NBNN1517	+	175
NN108	NBNN1518	+	152
NN109	NBNN1506	+	134
NN113	NBNN1473	+	328
NN114	NBNN1474	+	172
NN120	NBNN1534	+	476
NN121	NBNN1505	+	106
NN126	NBNN1535	+	119
NN130	NBNN1491	+	234

38 Co-T lines

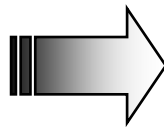
## “Clean gene” rice plants containing *TUB::corn cystatin*

For each Co-T plant line

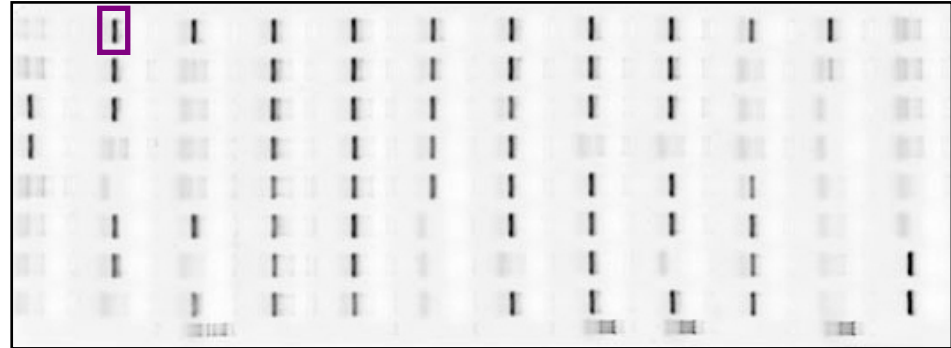


Progeny plants **GFP-**

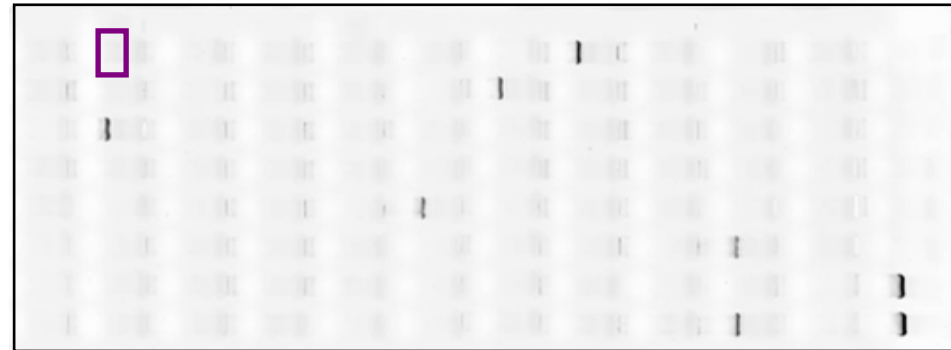
**T<sub>1</sub>**



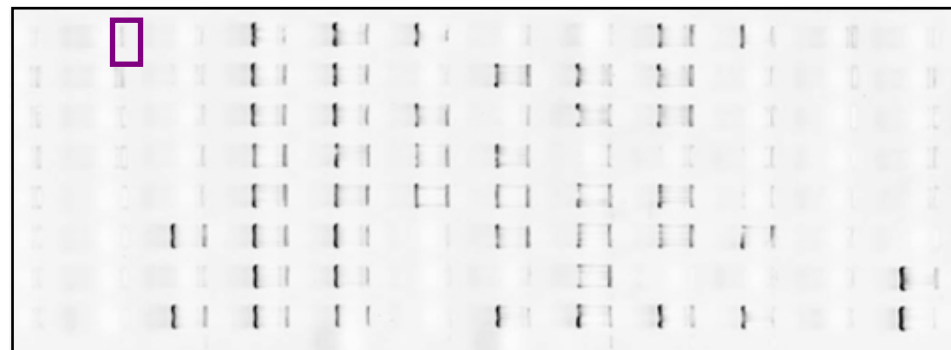
**DNA  
PCR**



**CC**



**HPT**



**NPTI**

**CC+, HPT-, NPTI-**



## “Clean gene” rice plants containing *TUB::corn cystatin*

### BATCH 1:

12 plant lines (1161 seeds)  
2 lines silenced for *gfp*  
4 lines producing CGT plants

1061 plants ( $T_1$ )  
190 plants **GFP-**  
175 plants DNA extraction  
97 plants **CC+**  
72 plants **CC+ HPT-**  
16 plants **CC+ HPT- NPTI-**

22%

20%

9%

1.5%

« clean gene »  
corn cystatin  
rice plants

Bioassay at Leeds U.

## “Clean gene” rice plants containing *TUB::corn cystatin*

### BATCH 1:

12 plant lines (1161 seeds)  
2 lines silenced for *gfp*  
4 lines producing CGT plants

1061 plants ( $T_1$ )  
190 plants **GFP-**  
175 plants DNA extraction  
97 plants **CC+**  
72 plants **CC+ HPT-**  
16 plants **CC+ HPT- NPTI-**

Bioassay at Leeds U.

### BATCH 2:

25 plant lines (2388 seeds)  
5 lines silenced for *gfp*  
*ongoing*

1988 plants ( $T_1$ )  
417 plants **GFP-**  
370 plants DNA extraction  
*ongoing*  
*ongoing*  
*ongoing*

To Leeds U. Dec 13<sup>th</sup> 2004

- ❖ Clean gene technology
- ❖ **Root specific promoter**
- ❖ Future work

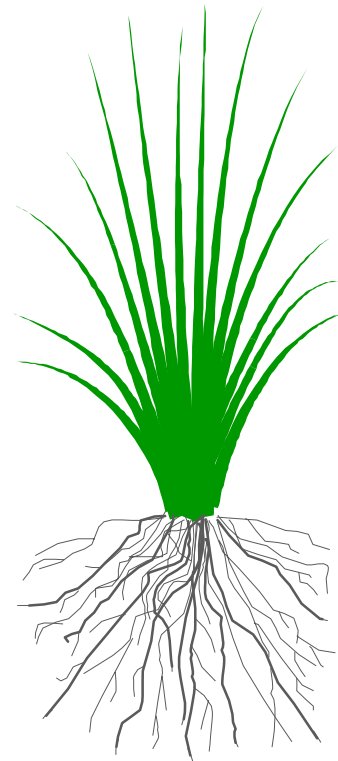
## *Expression pattern of root-specific promoters in rice*



44 indep. plant lines



6 indep. plant lines



GUS-

GUS-

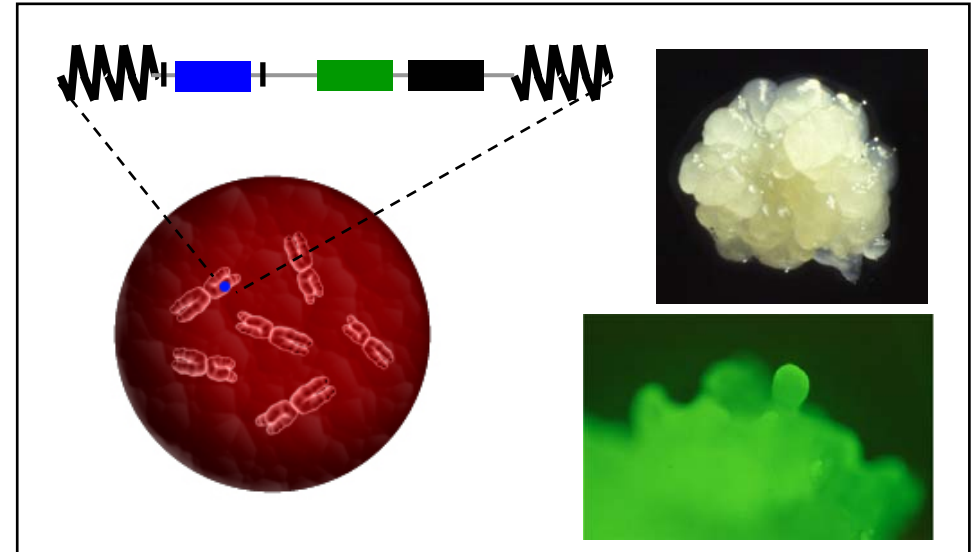
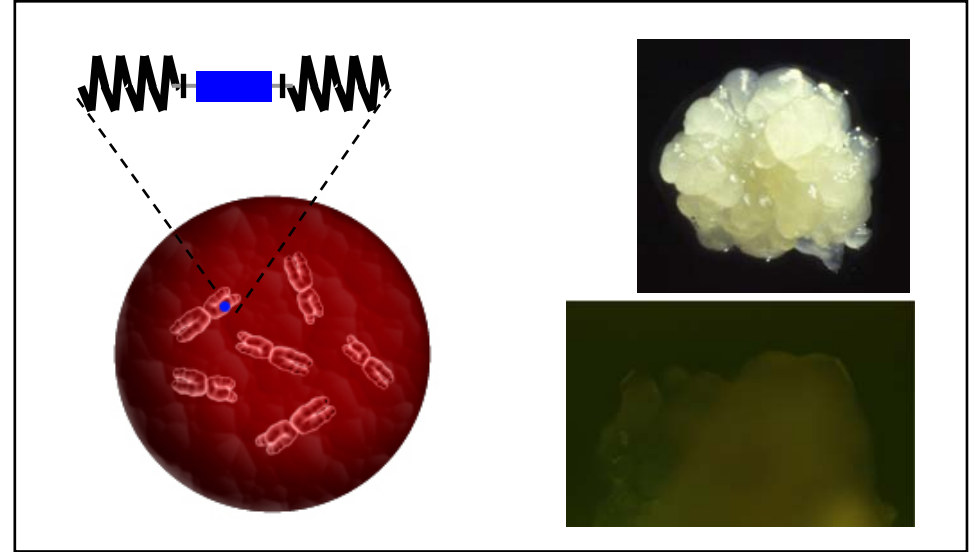
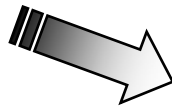
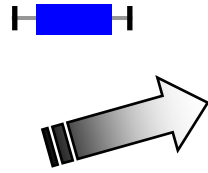
- ❖ Clean gene technology
- ❖ Root specific promoter
- ❖ **Future work**

## ***Future work:***

- ❖ **Molecular analysis / homzygous lines of “clean gene” rice plants resistant to nematodes**
- ❖ **Further root-specific promoter testing?**
- ❖ **Expression of *PsMTA:gus* & *ARSK:gus* in the presence of nematodes?**
- ❖ **Molecular (& bioassay) of *RYMV-MPI* plants from R7415**
- ❖ **Design of new binary vectors**



## Design of new binary vectors



# ***Group in Crop Genetics Department***

## **Current members:**

**B. Worland (DFID)**

**A. Derevier (DFID)**

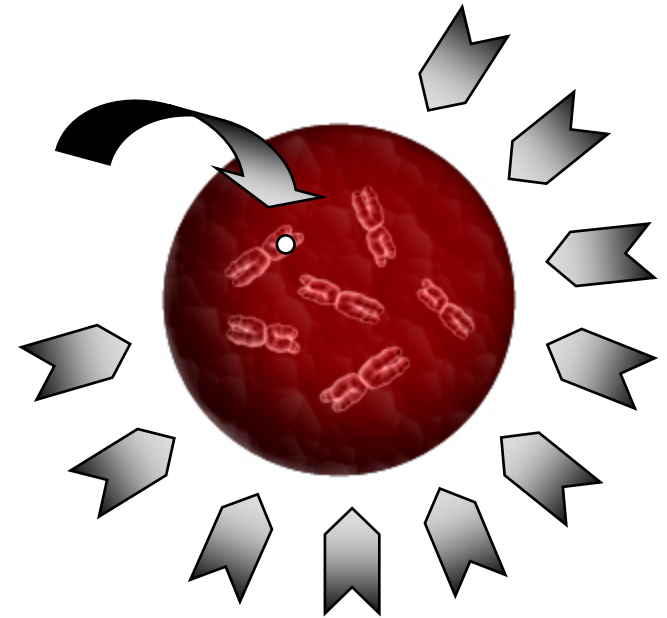
**P. Vain (DFID)**

**J.W. Snape (JIC)**

## **Past members:**

**A. Afolabi (DFID - RF)**

**S. Ross (DFID)**



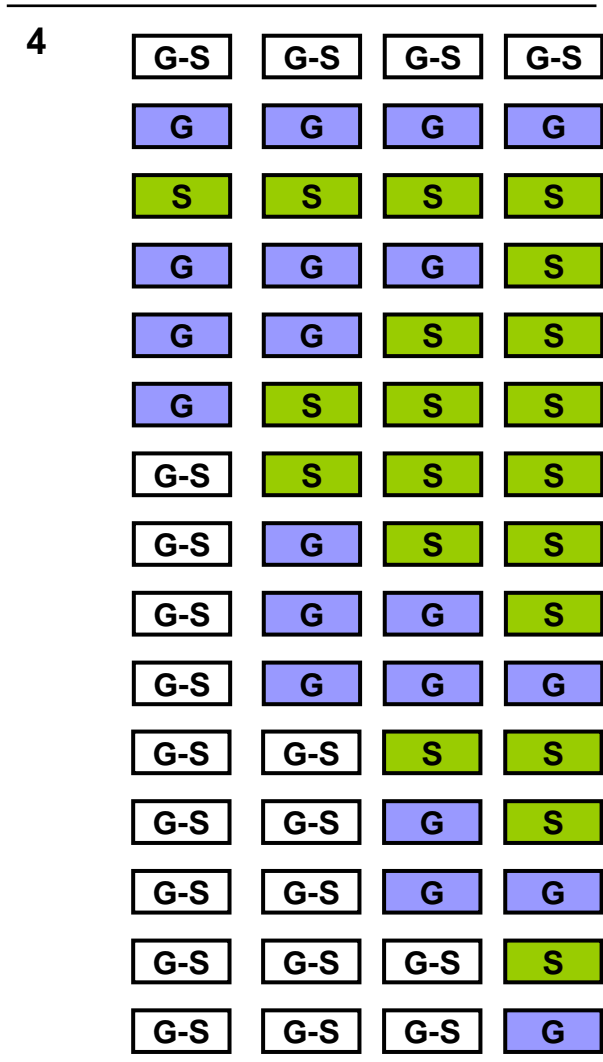
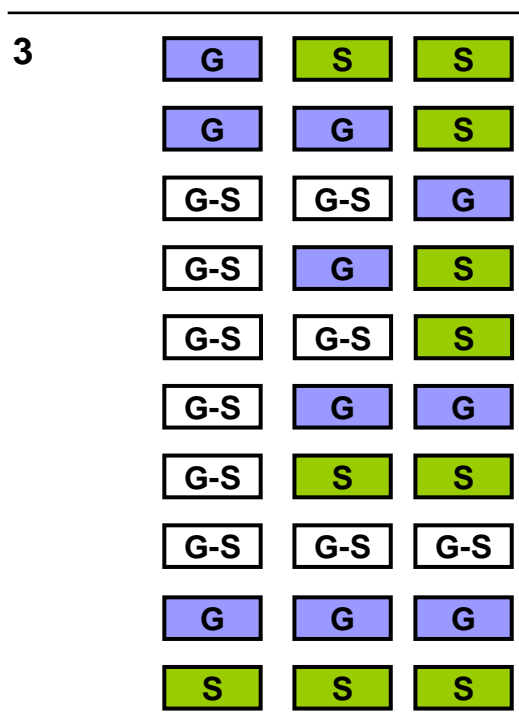
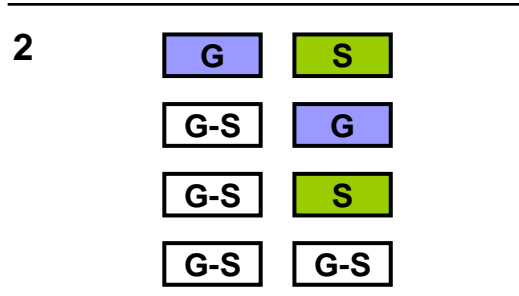
## **Funding:**

**DFID** Department for  
International  
Development





## Models of possible T-DNA linkages



G-S
-----

pGreen/pSoup T-DNAs linked

G
---

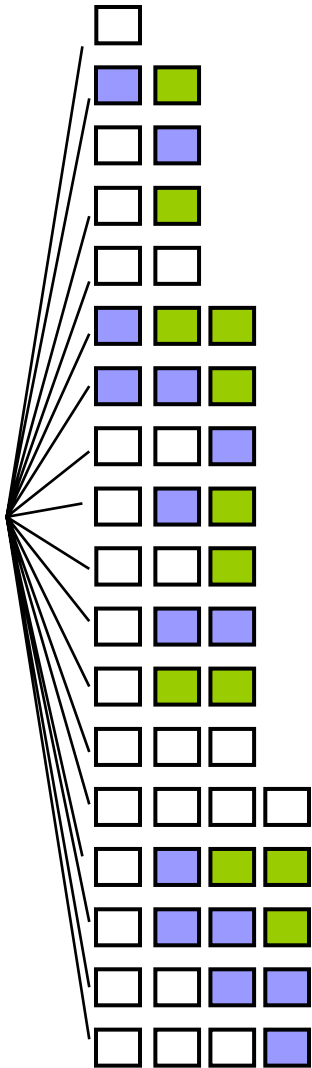
pGreen T-DNA(s) only

S
---

pSoup T-DNA(s) only

**Theoretical Segregation ratios**

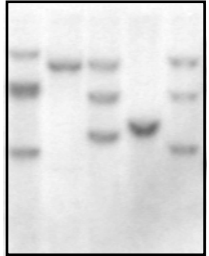
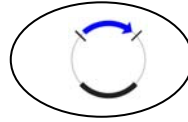
## Dual T-DNA integration



- Segregation of transgene phenotype should not be used to estimate loci number (30% of loci undetected) or T-DNA linkage
- Clean gene technology ~ 10% of loci  
(71% coTrans. x 28% coExp. X 43% single T-DNA loci)
- Multiple T-DNA copies often results from the integration of different T-DNA molecules
- New opportunity to study true random T-DNA integration
- Backbone transfer (53-66% of loci +antibiotic<sup>R</sup> gene)

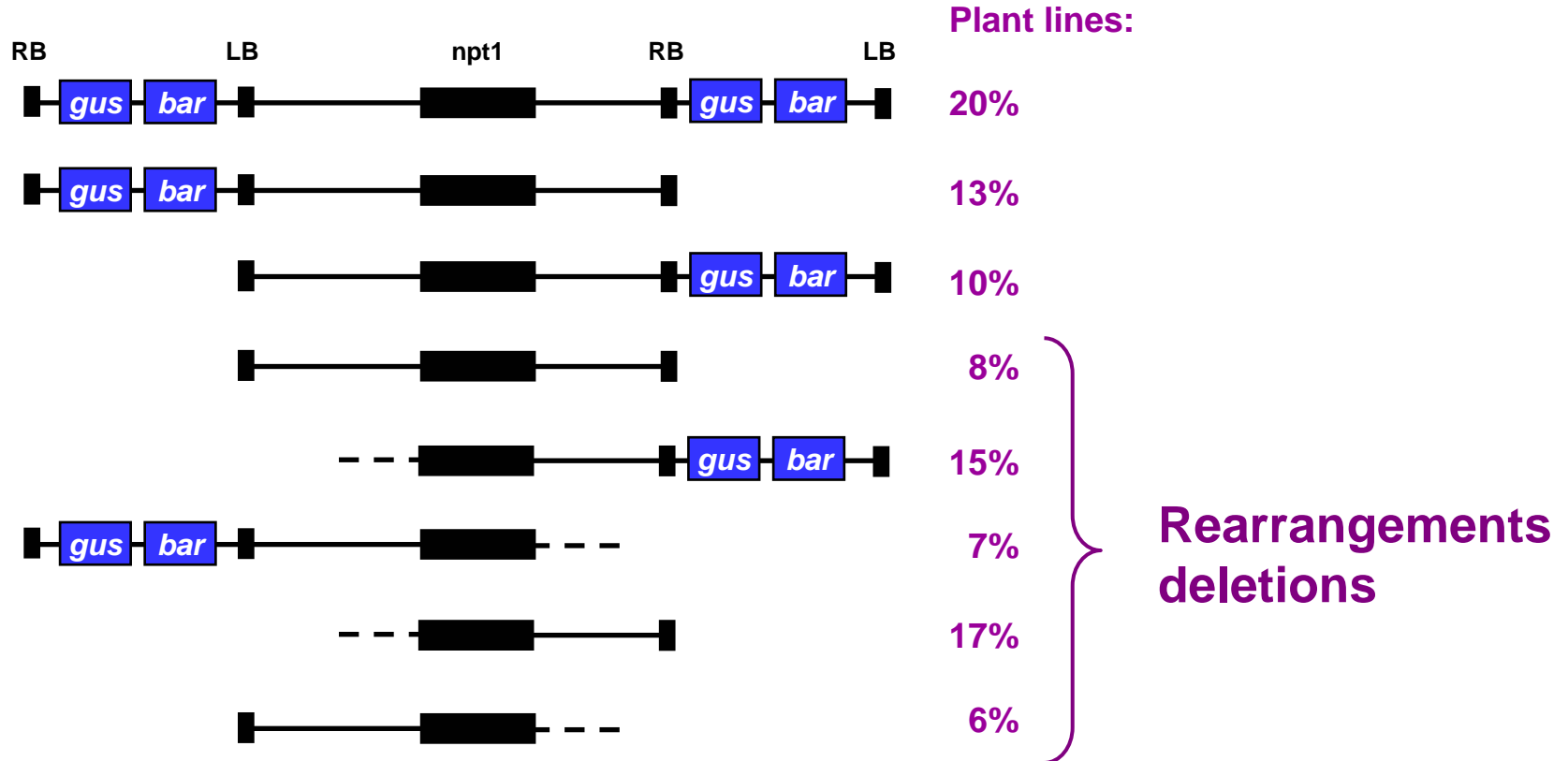
# Transgene integration and behaviour

## Backbone transfer in rice

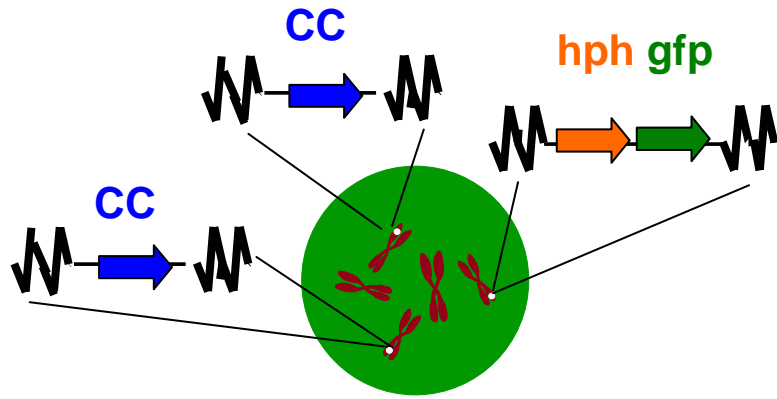


*bar*  
*gus*  
oLB  
oRB  
*npt1*

- Frequent in rice: 53-66% of loci (+antibiotic<sup>R</sup> gene)
- in 7% of 1C lines, in 47% of 2C+ lines
- Different types of integration:

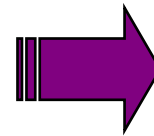


## “Clean gene” rice plants containing *TUB::corn cystatin*



Unlinked integration

T<sub>0</sub>



Progeny plants GFP-  
PCR: *hph*-, *gfp*- & *CC*+

	● ○	● ●	○ ●	○ ○
● ○	● ●	● ●	○ ●	○ ●
● ●	● ●	● ●	○ ●	○ ●
○ ●	○ ●	○ ●	○ ●	○ ●
○ ○	○ ○	○ ○	○ ○	

T<sub>1</sub>