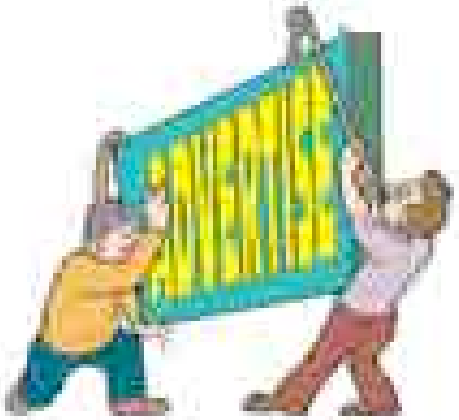
South China Botanical Garden,
The Chinese Academy of Sciences

Efficient regeneration system and *Agrobacterium*-mediated transformation of Vetiver

Speaker: Bing Yang
Doctoral candidate

Advisers: Dr. Hanping Xia
Dr. Guojiang Wu

24-10-2006



Outline:

- **Part I: Why (Background)**
- **Part II: Some concrete work we have done**
- **Part III: Prospects to further this research**

Part I

Research Background

WHY?

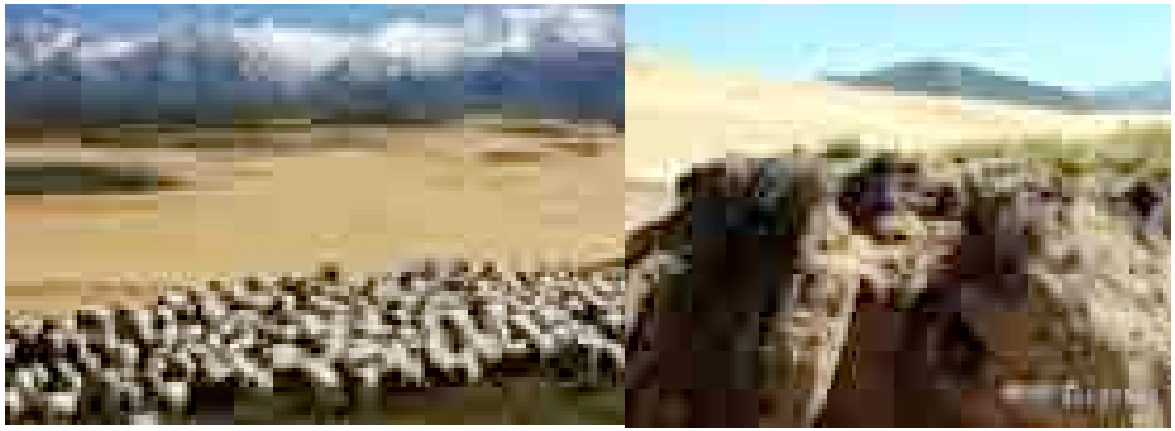

Beijing 2008
www.6m9m.com

Water and soil erosion

The quantity of soil and water erosion in china is above 8 billion tons annually.

(Peng Keshan,2004)

Desertification



Deforestation

Water contamination

How to do it?

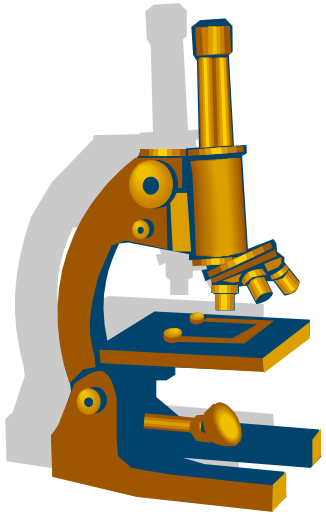

Magical Plant -- Vetiver
2005 6 30

Slope protection and stabilization

March 20, 2002

June 28, 2003


Pollution
rehabilitation

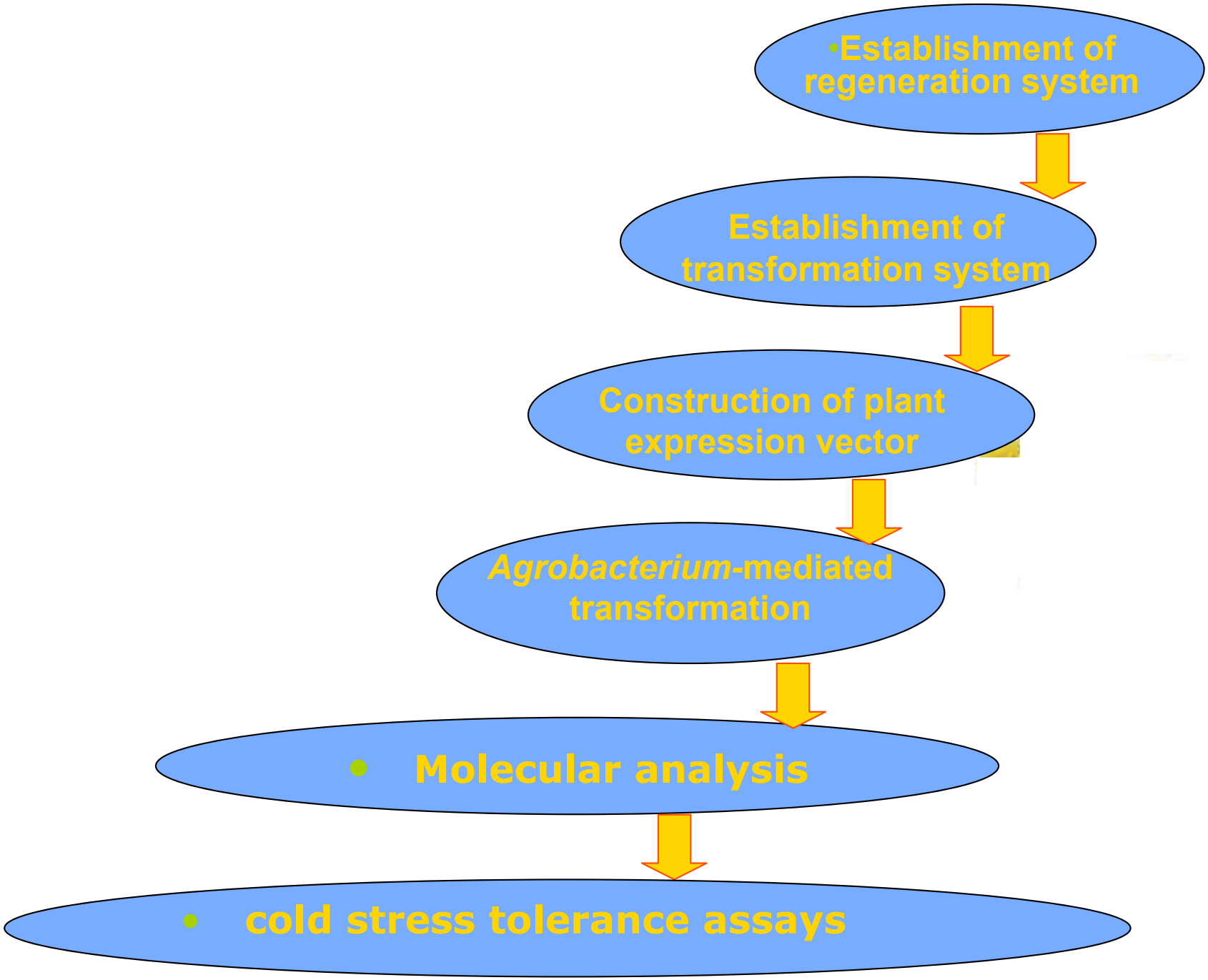


- **Poor tolerance to low temperature**

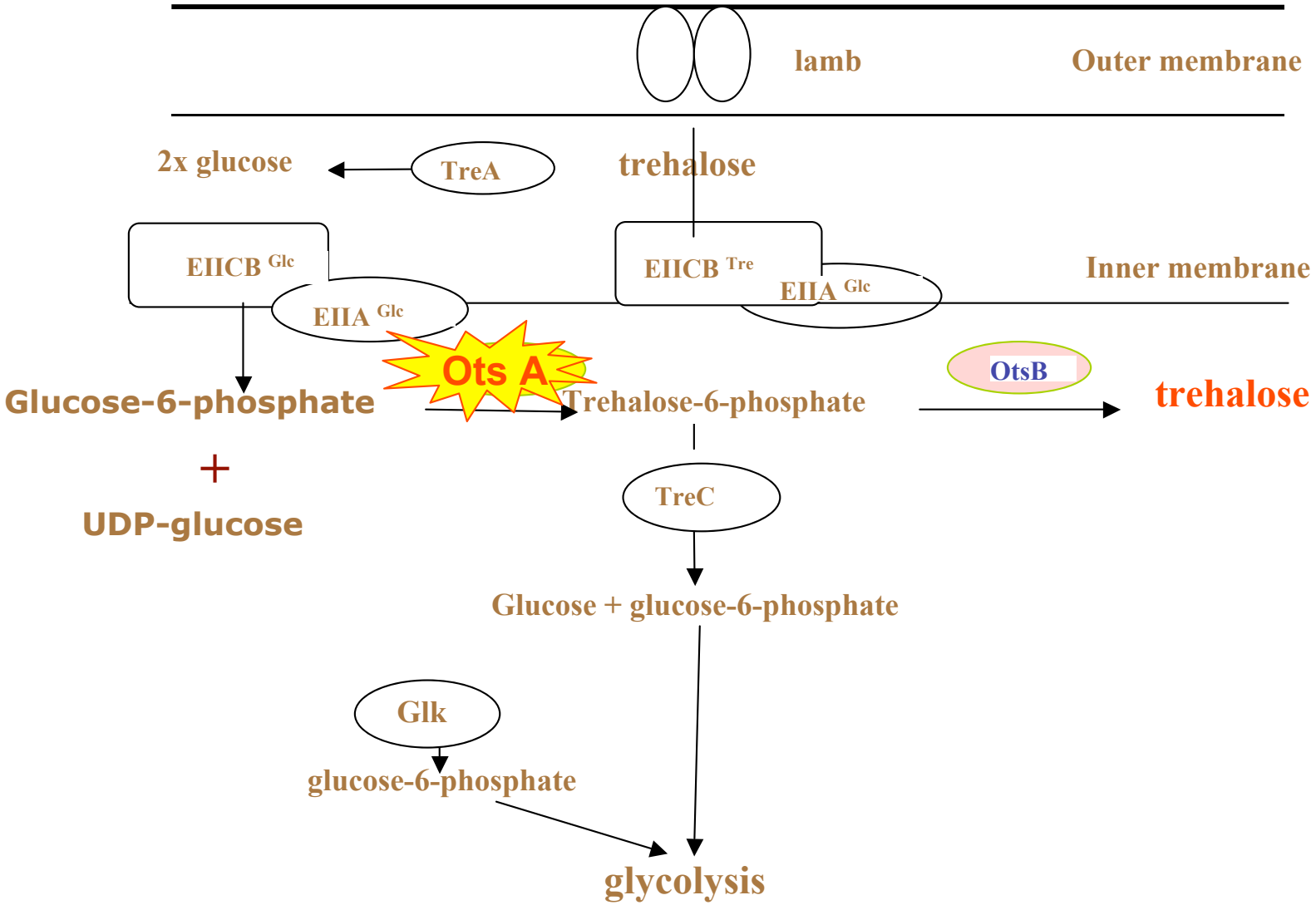
Application distributing map of vetiver in China

Research objective

To enhance cold tolerance, a cold-resistant gene is introduced into vetiver cells using molecular technology of gene transformation, and as a result, make this excellent grass play a more positive role in environment restoration in North China and other parts of the world.


•Establishment of regeneration system
Establishment of transformation system
Construction of plant expression vector
Agrobacterium-mediated transformation
• Molecular analysis
• cold stress tolerance assays

Trehalose-6-phosphate synthase gene
otsA gene



Metabolic Pathway of trehalose in E.Coli

(Crowe et al., 1990; Strom et al., 1993)

Trehalose

- **Protectant of protein and membrane**
- **Osmoprotectant**
- ---Crowe et al., 1990; Strom et al., 1993.

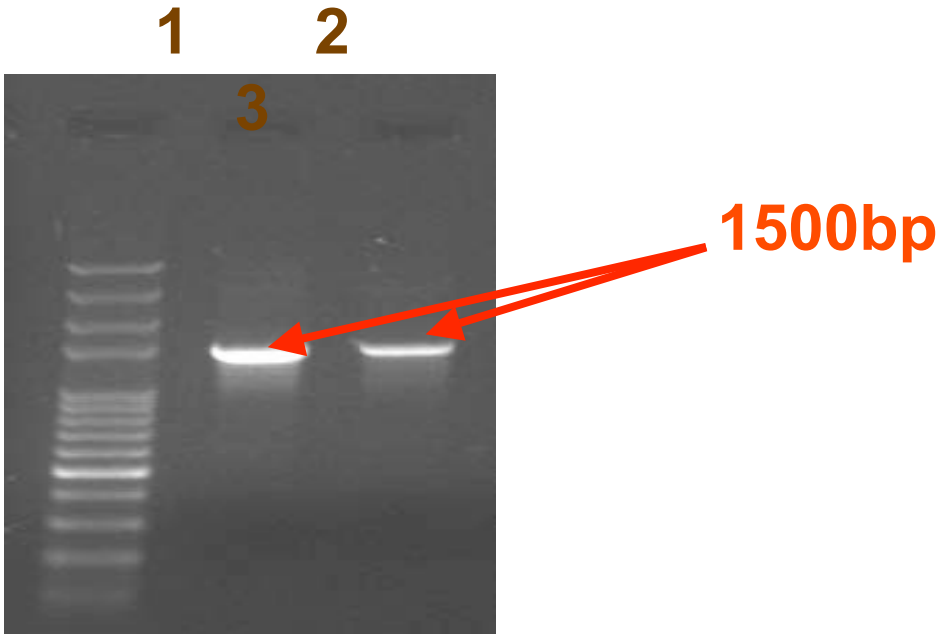
Tobacco

otsA gene

Potato

Sugarcane

---Goddijn *et al.*, 1997; Yeo *et al.*, 2000; Wang *et al.*, 2003



A

PCR amplification and restriction analysis of *otsA* gene

- 100 bp ladder: 5,000, 3,000, 2,000, 1,500, 1,000, 900, 800, 700, 600, 500, 400, 300, 200, 100

Part II

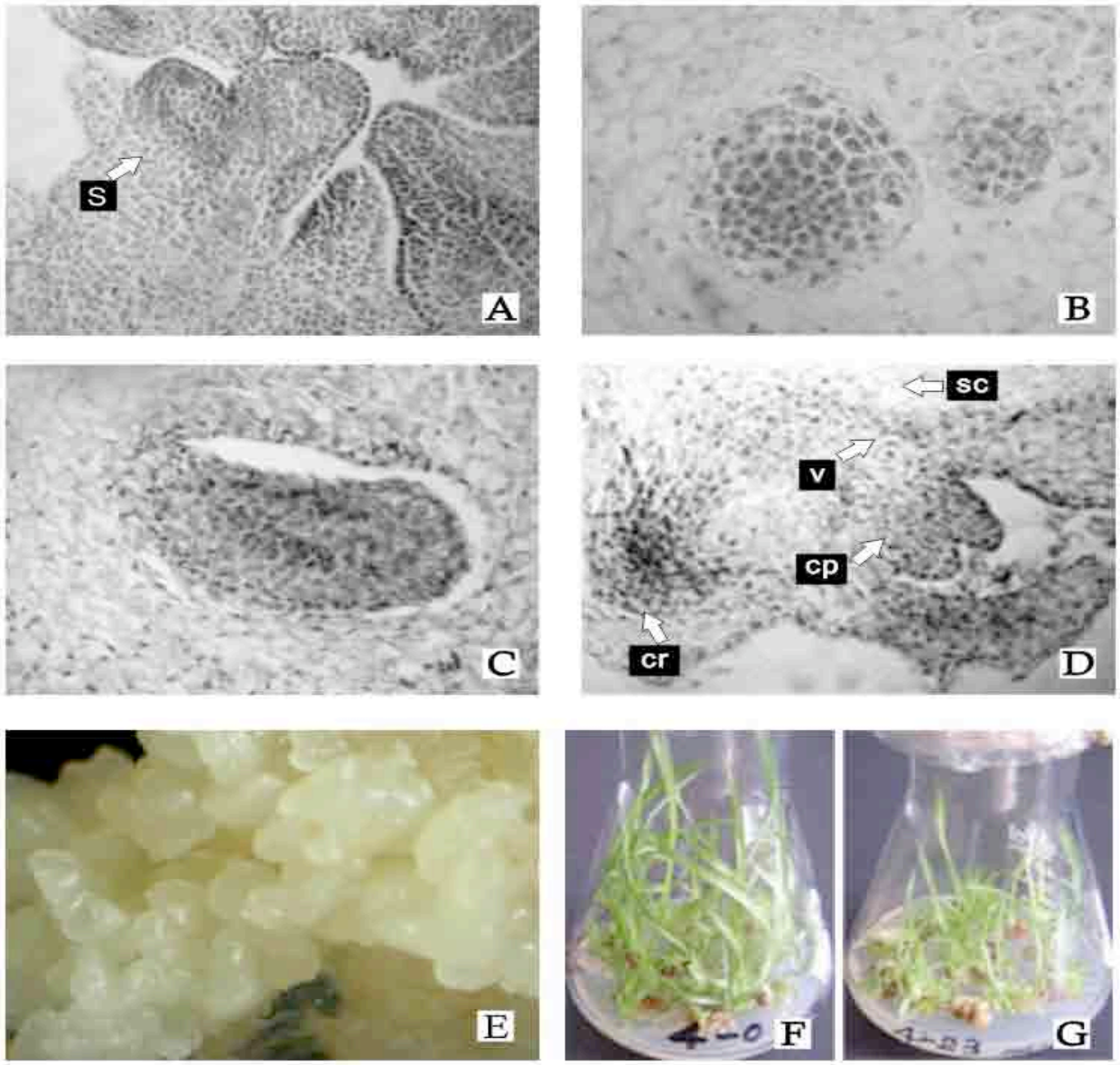
What concrete work have we done?

2.1 Establishment of an efficient regeneration system of vetiver

- **Induction medium:**
  MS + 2.0 mg /L 2,4-D + 0.5 mg /L KT

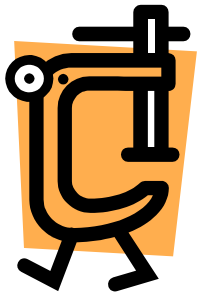
- **Differentiation medium :**
  MS + 1.0 mg/ L 6BA

- **Rooting medium:**
  ½ MS + 0.1mg/L IBA + 0.1 mg/ L PP333

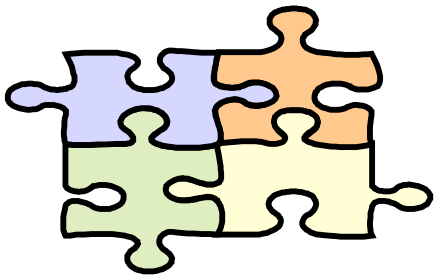


Development process of a somatic embryo of Vetiver

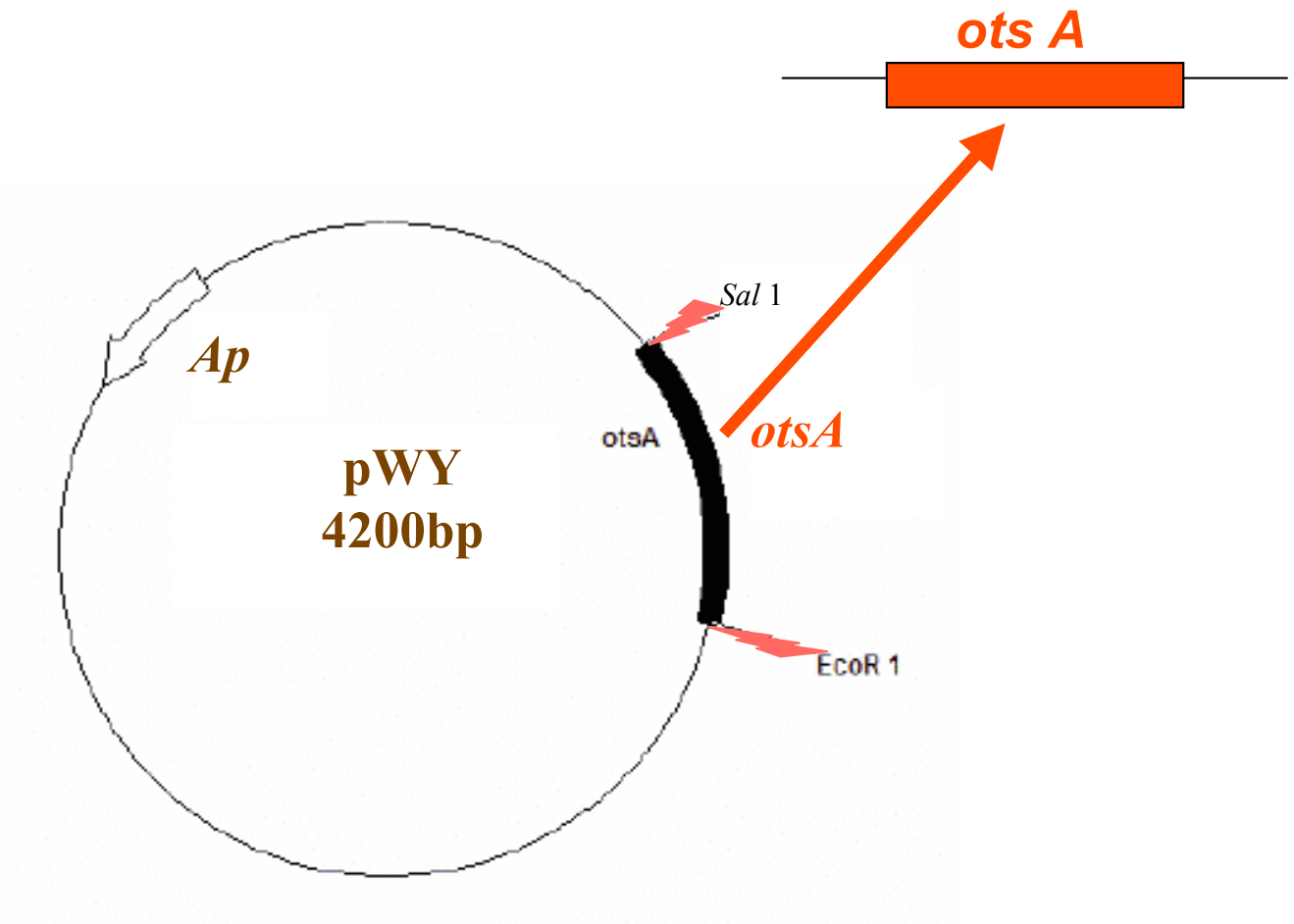
2.2 Establishment of genetic transformation system for vetiver grass

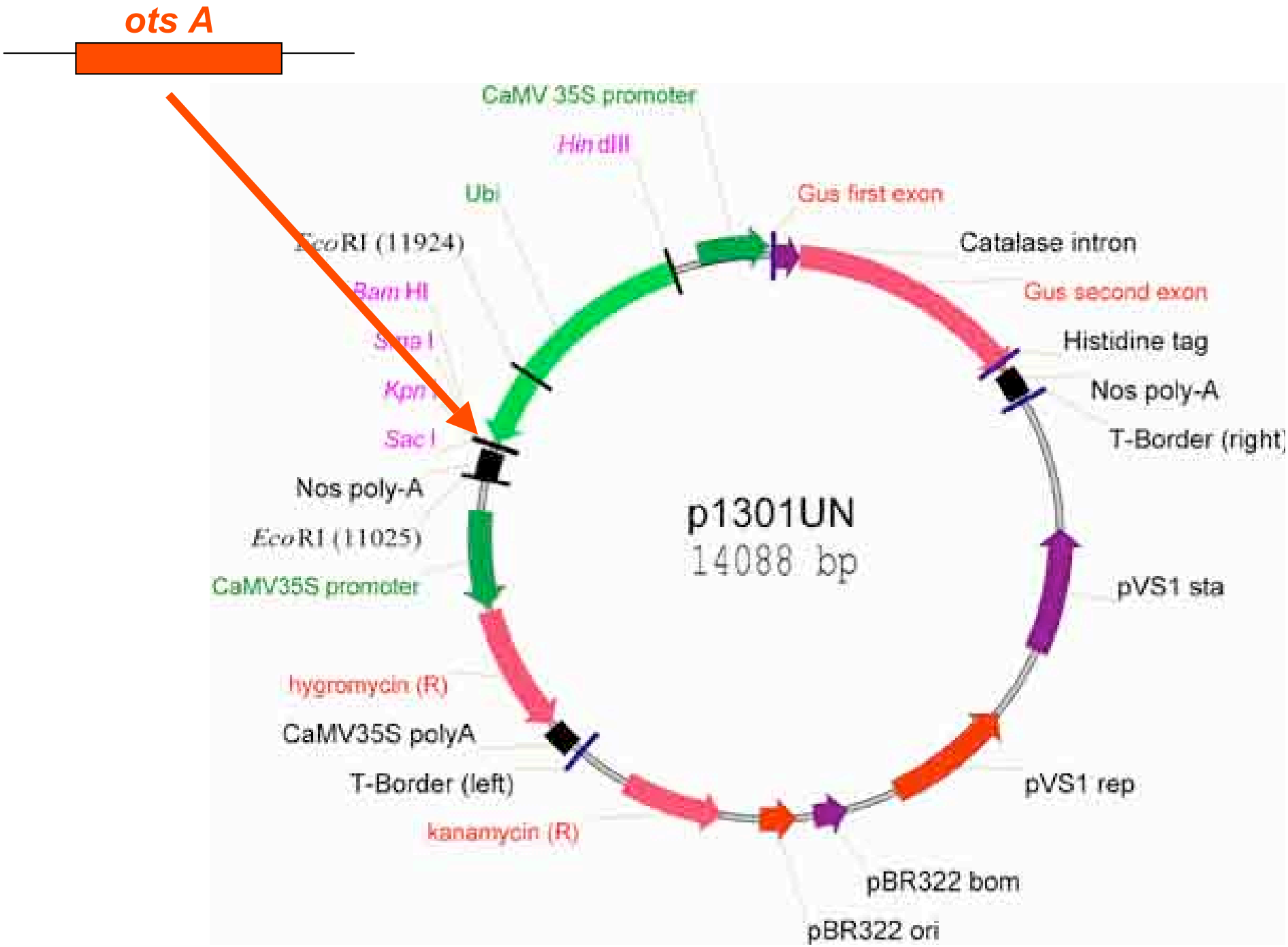


- **Con. of Agr.tumefaciens 0.4-0.5 OD**
- **Con. of acetosyringone (AS) 200 μM**
- **Co-cultivation temperature 22-25℃**
- **Duration of co-cultivation 3-4 d**

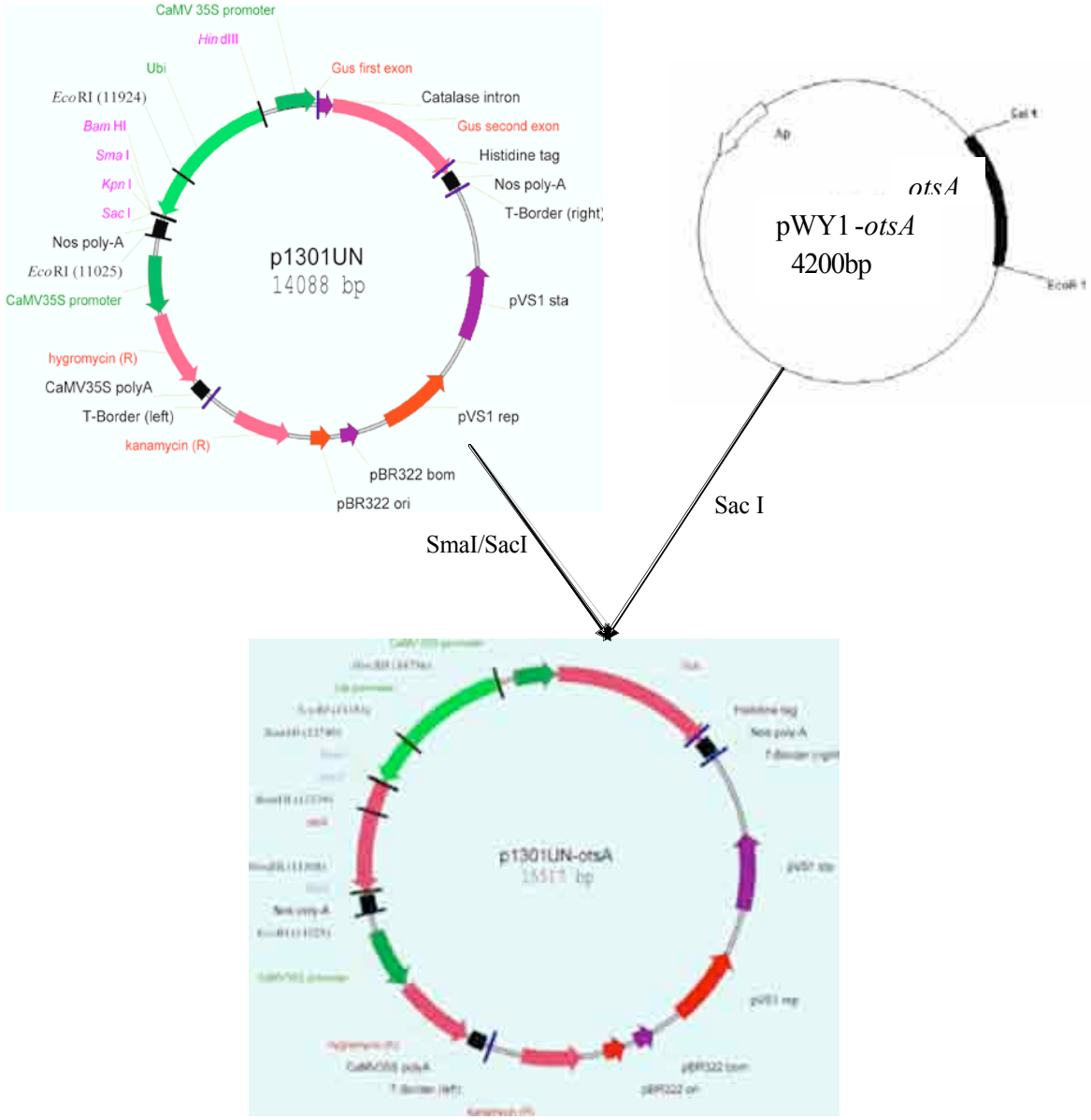


2.3 Construction of plant expression vector p1301UN-*otsA*


ots A
Sal 1
Ap
otsA
otsA
pWY
4200bp
EcoR 1


ots A
CaMV 35S promoter
Hin dIII
Ubi
Gus first exon
EcoRI (11924)
Catalase intron
Bam HI
Gus second exon
Histidine tag
Kpn I
Nos poly-A
Sac I
T-Border (right)
Nos poly-A
p1301UN
14088 bp
EcoRI (11025)
CaMV35S promoter
pVS1 sta
hygromycin (R)
CaMV35S polyA
T-Border (left)
pVS1 rep
kanamycin (R)
pBR322 bom
pBR322 ori

Construction of plant expression vector p1301UN-*otsA*

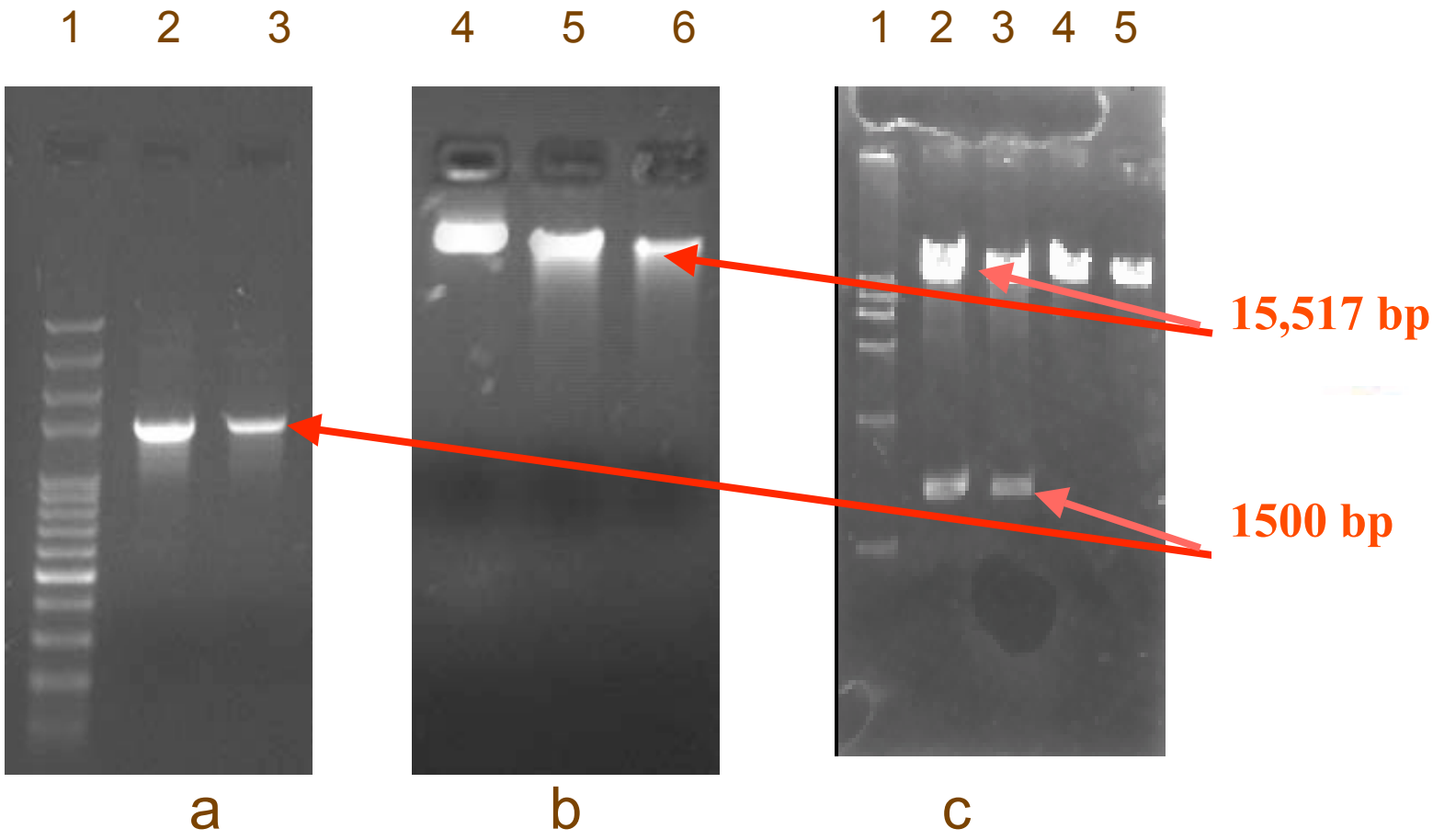


PCR amplification

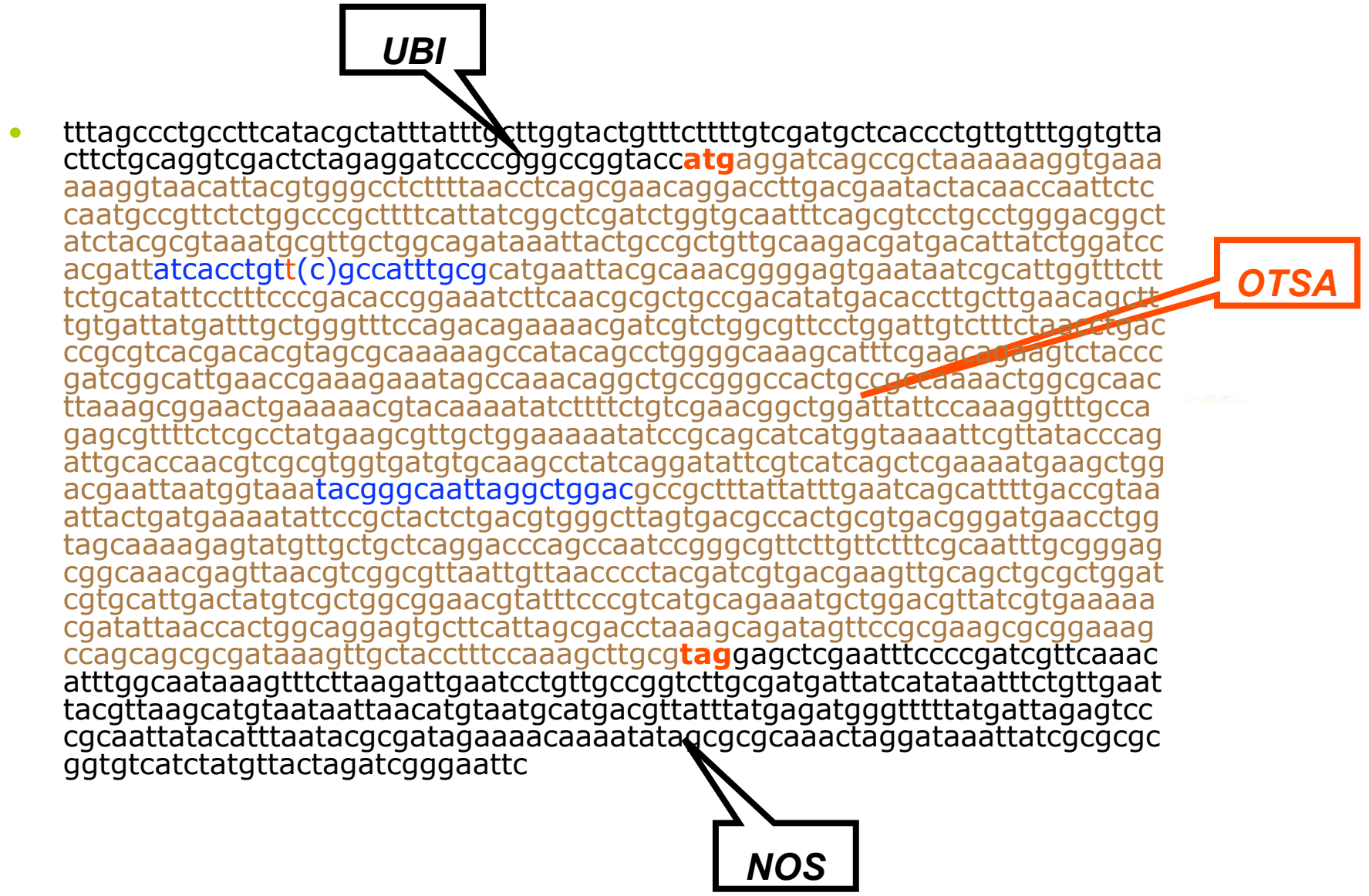
Restriction digestion

Ligation

Transformation



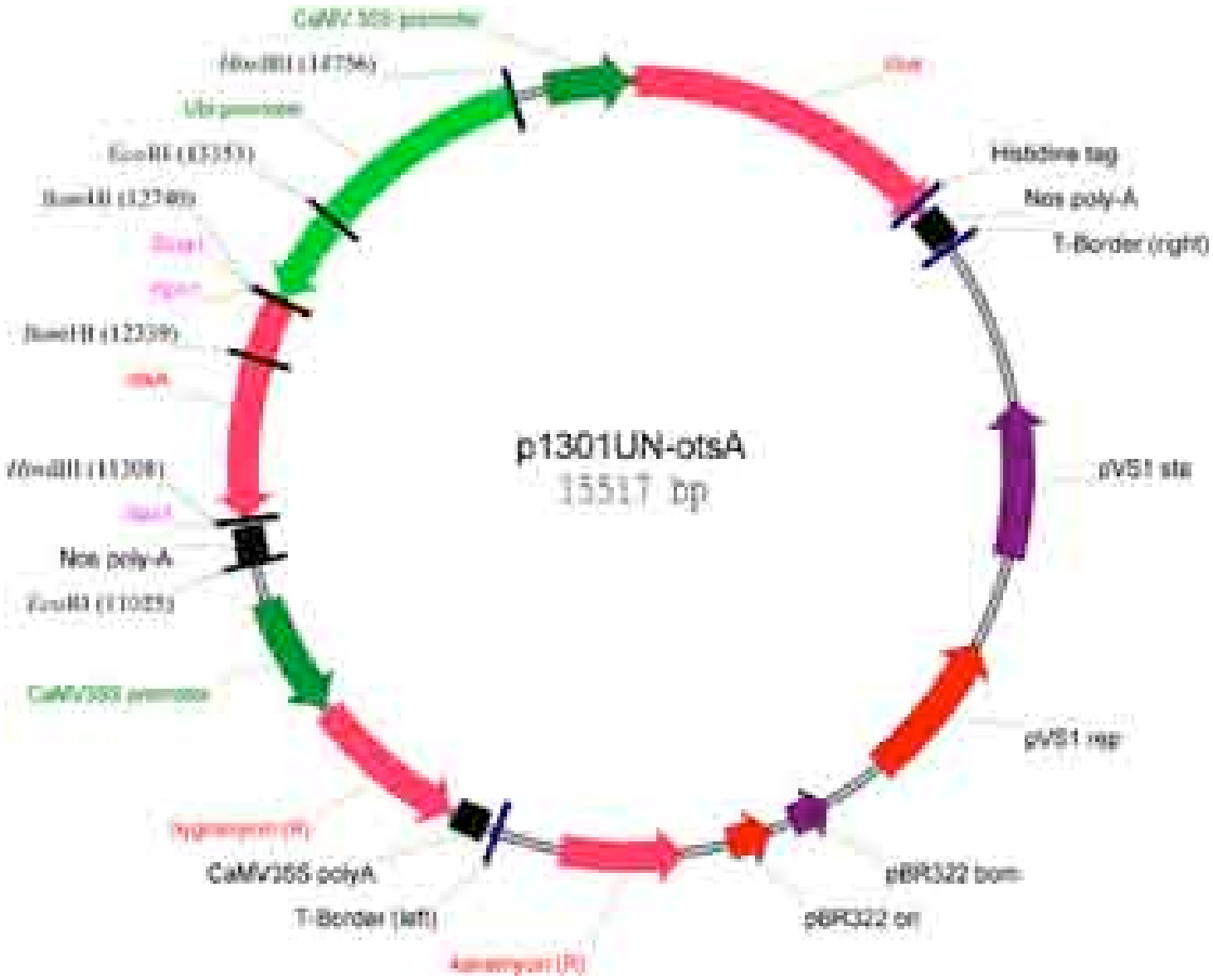
(a) PCR amplification and restriction analysis of otsA gene
(b) Restriction analysis of plasmid p1301UN
(c) Restriction analysis of recombinant plasmid p1301UN-otsA



- tttagccctgccttcatacgctatttatttgccttggtactgtttcttttgtcgatgctcaccctgttgtttggtgtta
cttctgcaggtcgactctagaggatccccgggccggtaccatgaggatcagccgctaaaaaaggtgaaa
aaaggtaacattacgtgggcctcttttaacctcagcgaacaggaccttgacgaatactacaaccaattctc
caatgccgttctctggcccgcttttcattatcggctcgatctggtgcaatttcagcgtcctgcctgggacggct
atctacgcgtaaatgcgttgctggcagataaattactgccgctgttgcaagacgatgacattatctggatcc
acgattatcacctgtt(c)gccatttgcgcatgaattacgcaaacggggagtgaataatcgcattggtttctt
tctgcatattcctttcccgacaccggaaatcttcaacgcgctgccgacatatgacaccttgcttgaacagctt
tgtgattatgatttgctgggtttccagacagaaaacgatcgtctggcgttcctggattgtctttctaacctgac
ccgcgtcacgacacgtagcgcaaaaagccatacagcctggggcaaagcatttcgaacaggaagtctaccc
gatcggcattgaaccgaaagaaatagccaaacaggctgccgggccactgccggccaaaactggcgcaac
ttaaagcggaactgaaaaacgtacaaaatatcttttctgtcgaacggctggattattccaaaggtttgcca
gagcgttttctcgcctatgaagcgttgctggaaaaatatccgcagcatcatggtaaaattcgttatacccag
attgcaccaacgtcgcgtggtgatgtgcaagcctatcaggatattcgtcatcagctcgaaaatgaagctgg
acgaattaatggtaaatacgggcaattaggctggacgccgctttattatttgaatcagcattttgaccgtaa
attactgatgaaaatattccgctactctgacgtgggcttagtgacgccactgcgtgacgggatgaacctgg
tagcaaaagagtatgttgctgctcaggacccagccaatccgggcgttcttgttctttcgcaatttgcgggag
cggcaaacgagttaacgtcggcgttaattgttaacccctacgatcgtgacgaagttgcagctgcgctggat
cgtgcattgactatgtcgctggcggaacgtatttcccgtcatgcagaaatgctggacgttatcgtgaaaaa
cgatattaaccactggcaggagtgcttcattagcgacctaaagcagatagttccgcgaagcgcggaaag
ccagcagcgcgataaagttgctacctttccaaagcttgcgtaggagctcgaatttccccgatcgttcaaac
atttggcaataaagtttcttaagattgaatcctgttgccggtcttgcgatgattatcatataatttctgttgaat
tacgttaagcatgtaataattaacatgtaatgcatgacgttatttatgagatgggtttttatgattagagtcc
cgcaattatacatttaatacgcgatagaaaacaaaatatagcgcgcaaactaggataaattatcgcgcgc
ggtgtcatctatgttactagatcgggaattc

DNA sequence analysis of p1301UN-otsA

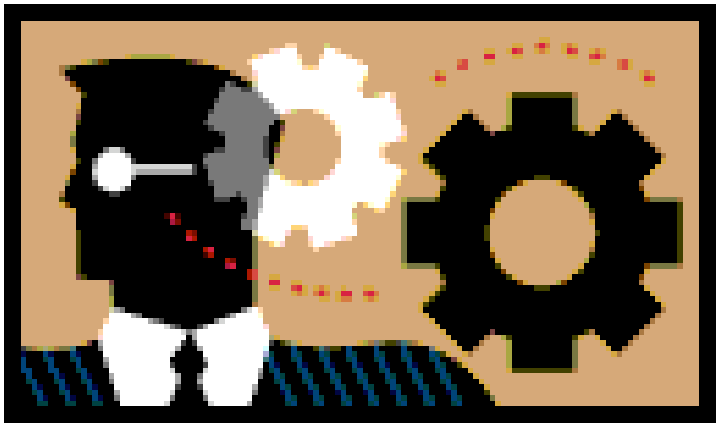
(*Ubi-otsA-Nos*)



Plant expression vector p1301UN-*otsA*

2.4 Genetic transformation mediated by EHA105/p1301UN-*otsA*

Co-cultivation

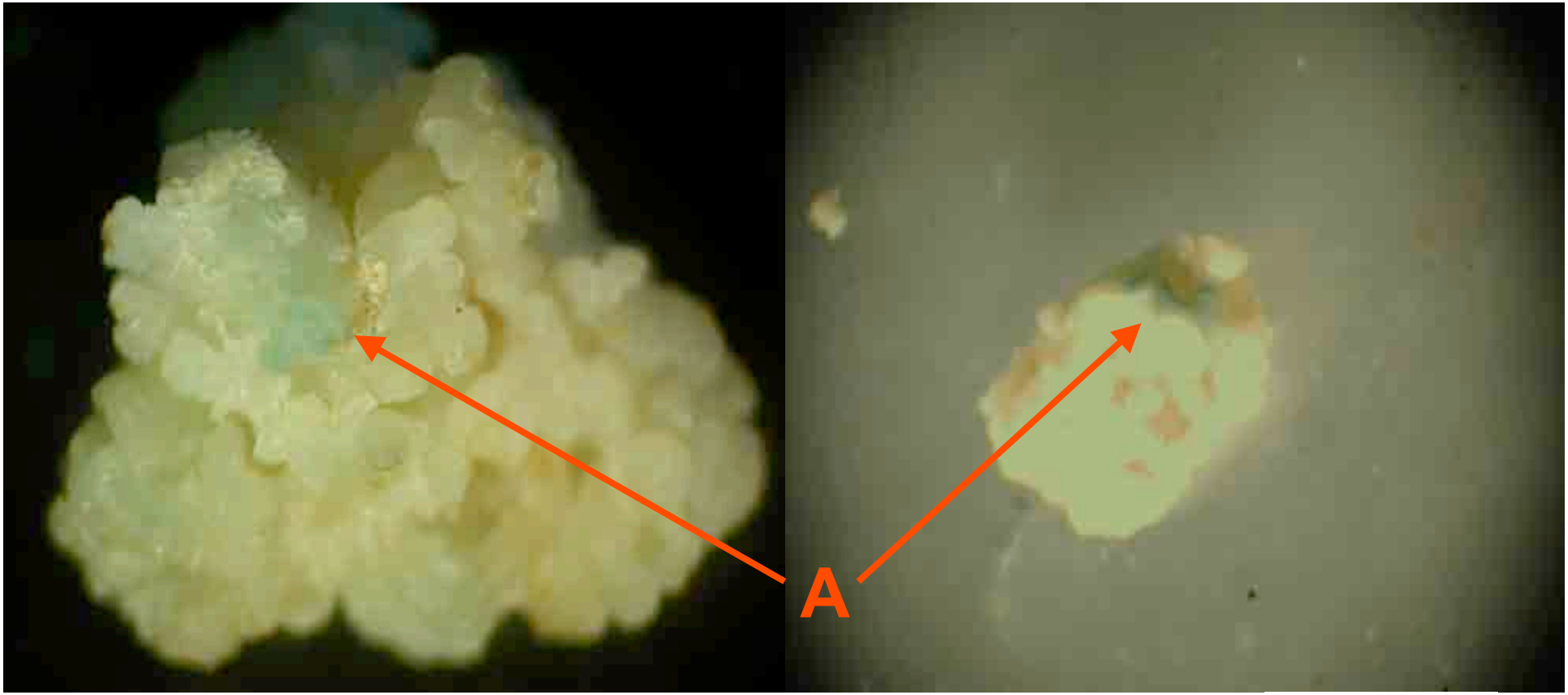


Resistance selection

Molecular analysis

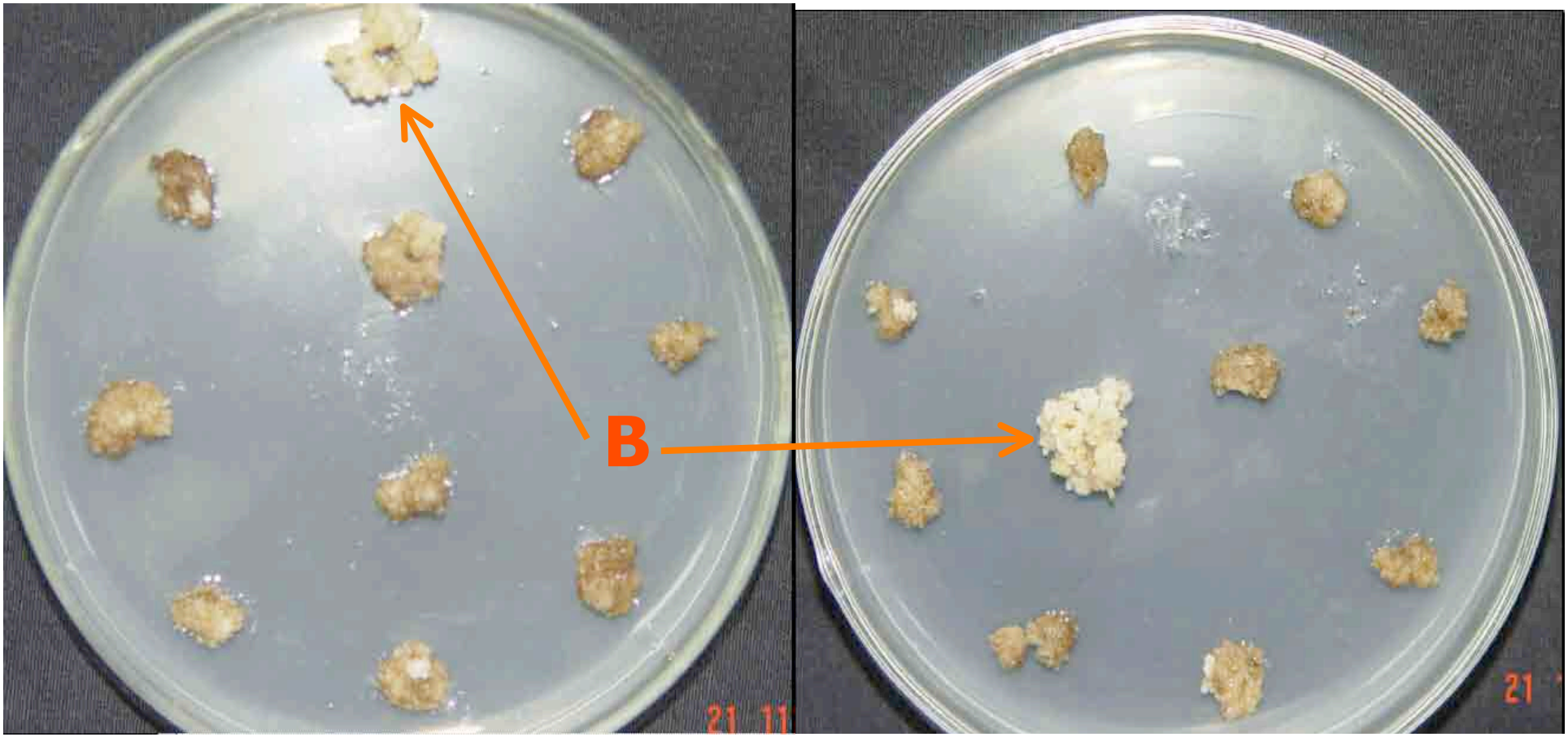
Cold stress tolerance assays

GUS assay



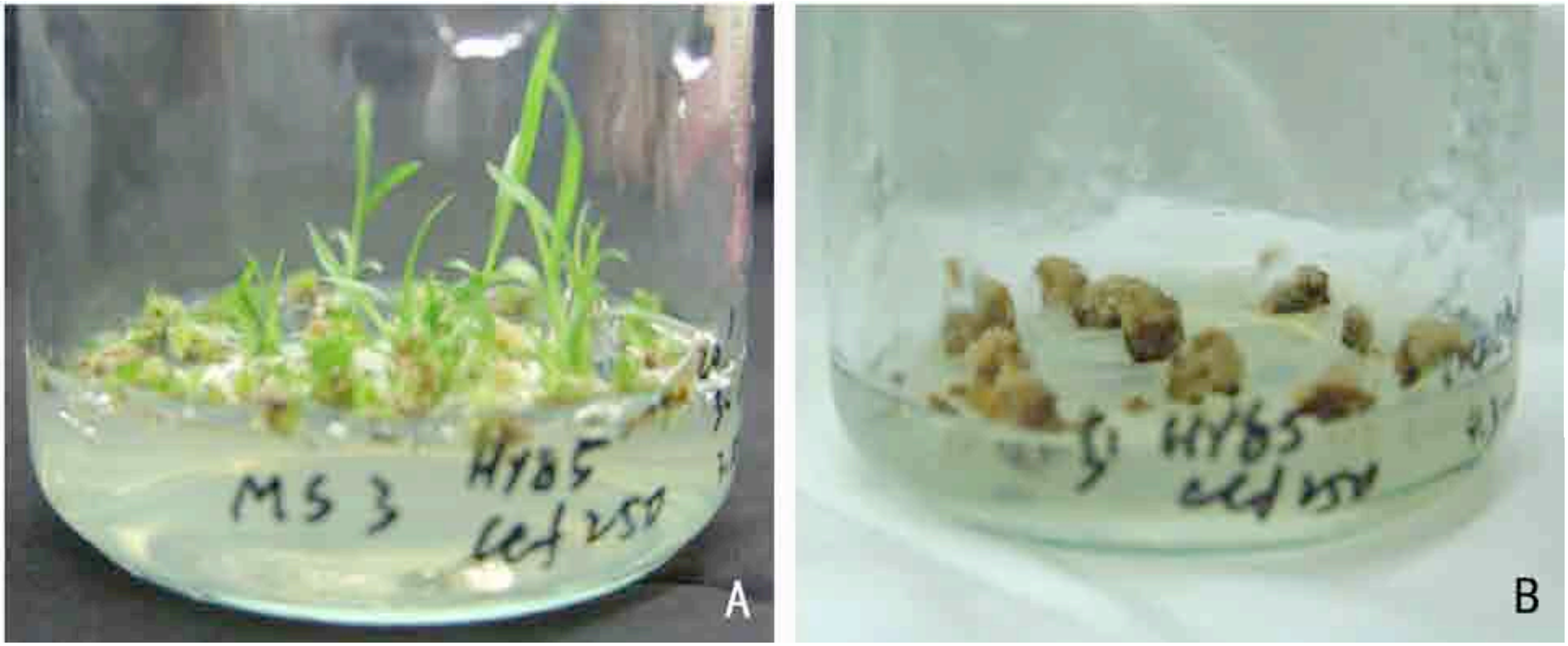
Calli co-cultivation after 3 days

hyg B resistant calli



Calli selection after 4 weeks

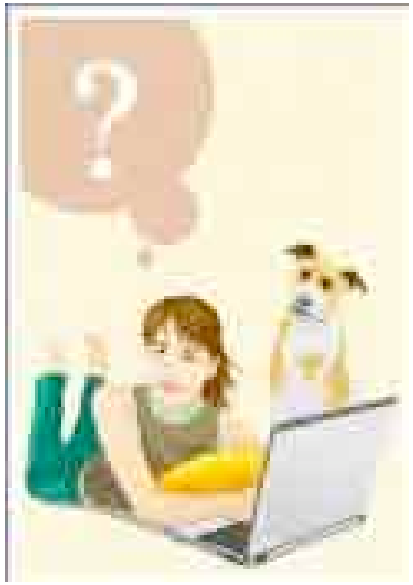
Hyg B resistant buds



A: Resistant buds cultivation after 4 weeks
B: Control

Hyg B resistant plantlets

Resistant plantlets after 2 months



Part III
Further research plan

- **Southern analysis of the putative transformants**

- **Cold tolerance analysis of transgenic plants**

Research goal

To screen a new cultivar of vetiver with cold tolerance or other resistance ability by gene engineering, and make this excellent grass playing more effective role in environment restoration and protection in the world.


My dream

A new beginning

Acknowledgments

This research was supported by the Natural Science Foundation of China and the Wallace Genetic Foundation through the Vetiver Network.

This paper was conferred the Innovation Prize by the Vetiver Network, so we wish to thank the Vetiver Network for encouragement to us.

Mr. Frank Mason from Australia provided me with parts of expenses for attending the conference, thereby I would like to express my heartfelt thanks for his generosity and kindness.

Thanks for your attention!