Generation Challenge Program

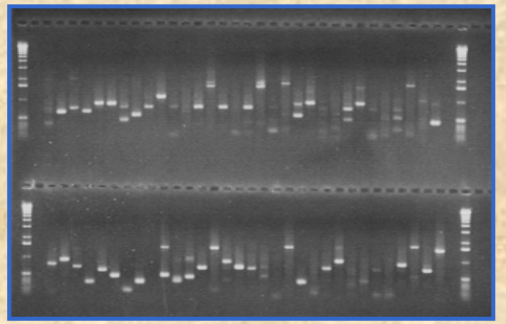
Plans for phenotypic screening of a T-DNA mutant collection of rice

T-DNA mutants collection

- Genome sequencing of rice completed in 2002
- 35,000 lines by the Génoplante consortium
  - Functional genomics
  - T-DNA insertions (*Agrobacterium*-mediated transformation)
  - Nipponbare (*Oryza sativa* japonica)
- Flanking sequence determined (FST database)
- Collaboration for rice functional genomic projects

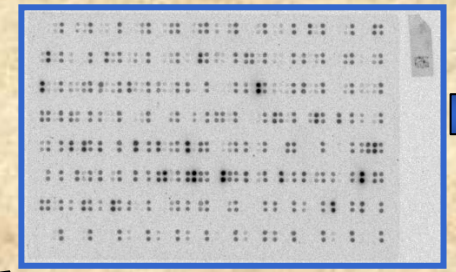
Agrobacterium-mediated production of T-DNA plants

Walk PCR-based amplification of flanking regions (T-DNA, *Tos17*)

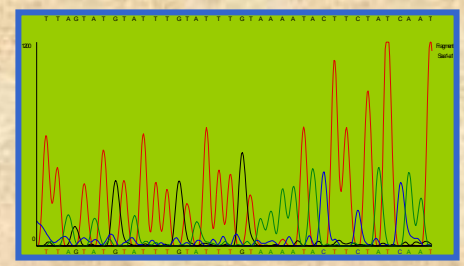


Sequencing T-DNA left border flanking regions

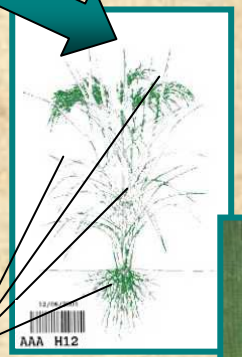
Display of flanking regions (T-DNA, *Tos17*) on filters

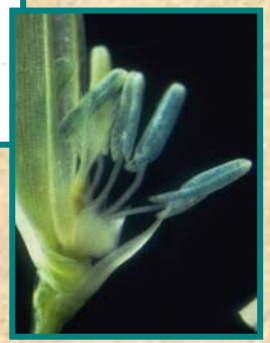


Screening through hybridization with candidate sequences or cDNA populations



Blast T-DNA FSTs against rice genome, ESTs, proteins..

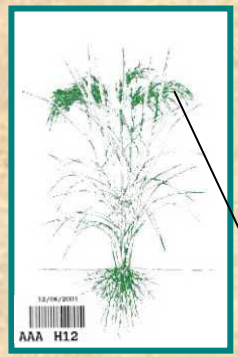




GUS assays on vegetative and reproductive organs of T0 plants

Evaluation of T1 progeny for :

- Abiotic stress
- Embryogenesis
- Grain filling
- Biotic stress
- Non targeted phenotypes



Cold storage and distribution of T2 seeds

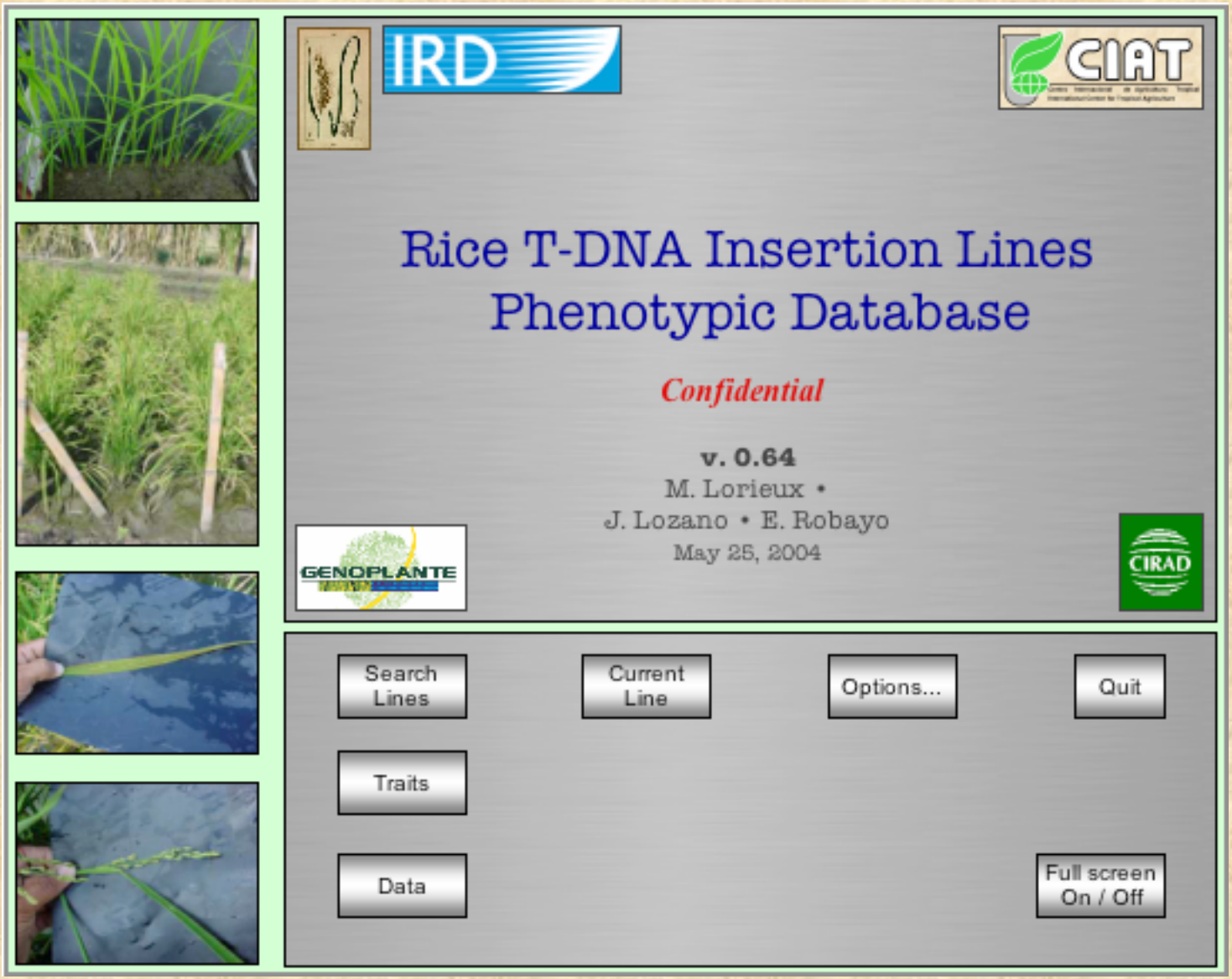
Phenotypic Characterization at CIAT

- Phenotypic characterization of the entire collection
  - Extensive, non-targeted
  - Screenhouse & field conditions
  - Field & lab facilities
  - Climate/diseases
  - CIAT as model for Colombian laws on GMOs
- Phenotypic database coupled with FST database
- Seed multiplication

Screenhouse observations

Field screening

Phenotypic Database



Strategy for screening rice insertion lines for drought response

- Aim: functional genomics of drought response in rice
- Need to address problem of big numbers
- How to narrow down the number of lines to be screened efficiently?
- Which kind of phenotypic screening related to numbers?

Case 1: Field screening under controlled conditions

- Setting up of facility for drought screening
- Roof & controlled irrigation
- Medium throughput (~ 300 lines/3 years)
- Adapted to screening of lines based on a reverse genetics approach (typically 100 – 150 genes)
- Phenotypic description of drought response (leaf rolling; sterility/grain filling; tillering/height; leaf color)
- Canopy temperature (IR camera)
- Complementary/correlated to more in-depth greenhouse screening based on physiological parameters and modeling

Case 2: Field screening under uncontrolled conditions

- Narrow down number of lines using information from
  - phenotypic data from database
  - redundancy
  - FST availability & quality
- Still high number of lines (e.g. forward genetic screening) (500-2,000 lines or +)
- High throughput
- Simplified phenotypic screening
- GMOs outside CIAT HQs
- Pb of replications over years and uncontrolled conditions

Other remarks

- Unified protocols with other GCP projects on drought screening in rice
- Phenotyping workshop & year 1 outputs lessons

Other candidate traits under consideration

- In partnership with Agropolis ARGEBIOS group
- Strong expertise in phenotypic screening
- Transversal approach to exploit the potential of T-DNA insertional mutant collection for deciphering rice broad spectrum resistance to important diseases

Bacterial blight	Bacteria
Rice Blast	Fungus
RHBV	Virus