

Populations for legume genetics and genomics



GCP ARM 2007

Crop Genetics

John Innes Centre

Populations for legume genetics and genomics

People :

Mike Ambrose

Julie Hofer

Carol Moreau

Lynda Turner

Catherine Rameau

Giles Oldroyd

Trevor Wang

Pascal Ratet

Richard Thompson

Abdelhafid Bendahmane

Pea FN population

Pea FN population

Medicago FN population

Lotus Tilling (+info)

Medicago Tnt1 population

Medicago Tilling

Pea Tilling

JIC

INRA Versailles

JIC

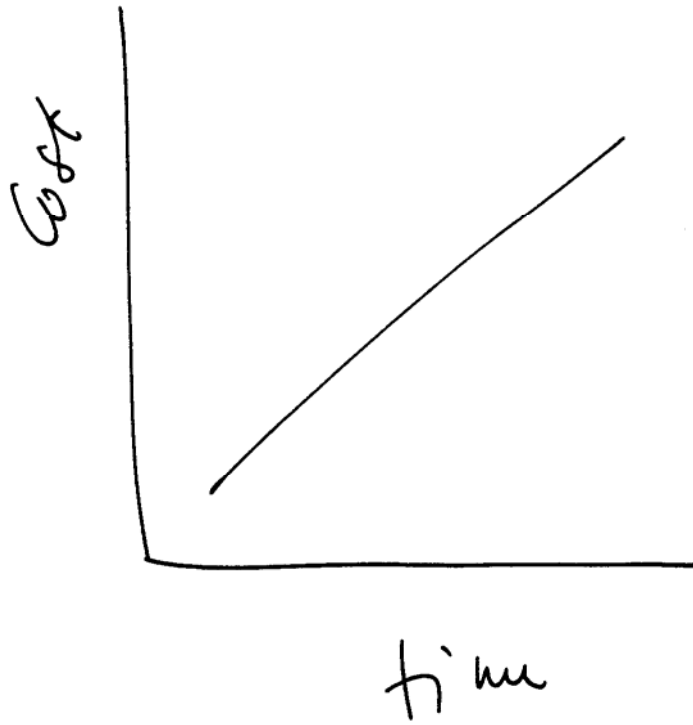
JIC

CNRS Gif-sur-Yvette

INRA Dijon

INRA (Evry)

Populations for legume genetics and genomics



Populations for legume genetics and genomics

- diversity panels
- reference mapping populations
- trait mapping populations

- mutant populations for:
forward screens
reverse genetics

Forward genetics

J12822 FN population (20 Gy):
3000 M3 lines available for genotyping, 125 phenotypic mutants
identified: estimate of 10,000 – 20,000 independent deletions



Térèse FN population (4 Gy) 3000 lines (C. Rameau INRA Versailles
& Da Luo SIPPE Shanghai):

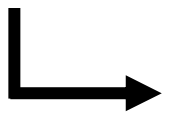
Forward genetics

Deletion analysis *TL* cloning strategy:

Fast neutron mutagenesis



- Line JI 2822
- RI line derived from mapping parents JI 15 x JI 399
- well marked genetically
- small
- rapid cycler
- +/- phenotype visible
- 7 new alleles obtained (expected deletions)



AFLP screening



What do we know about the deletions?

one *cri* allele



one *uni* allele



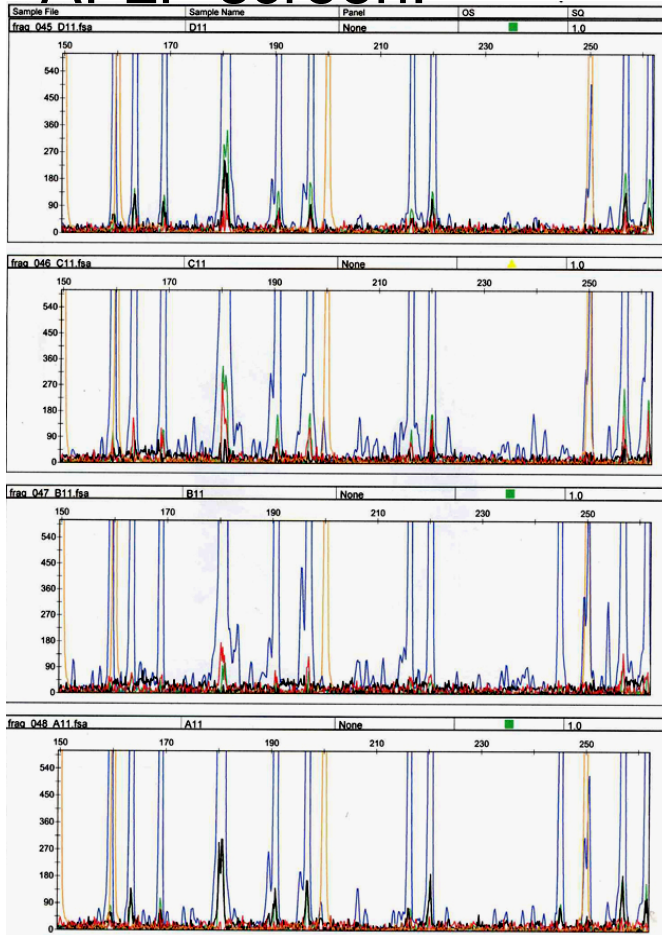
Seven (+) *tl* alleles



Forward genetics



AFLP screen:



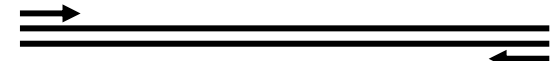
bulked
mutants

bulked
mutants

wild type

wild type

Pst + 2
primer



Mse + 3
primer

~~1024~~ primer combinations
512



Reverse Genetics

Medicago truncatula fast neutron Platform

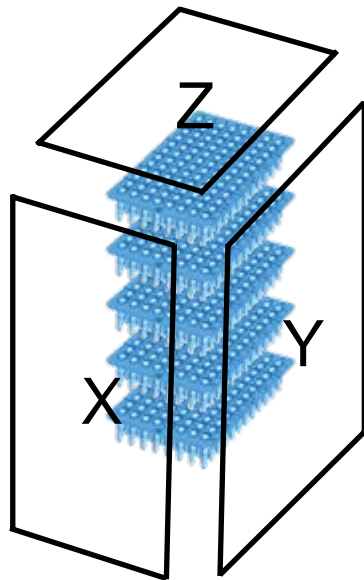


Fast Neutron bombardment (25-30-32.5 Gy)

Knock Out deletion mutants

Mutant population: Jemalong A17

Test screen:	60,000 M2	12,000 M1	5 towers
By Feb 2007	180,000 M2	36,000 M1	15 towers
2008 (+NF)	~400,000 M2	~80,000 M1	~30 towers



High-throughput detection system

3D-pooling system

High sensitivity guaranteed by RE

Elimination of false positives by 2-stage screening

Rate of success so far:

So far 40% (4/10 genes screened)

Will increase with size of population

[Collaboration with the Noble Foundation](#)

Medicago truncatula Tnt1 insertion Platform



Tnt1 insertion mutagenesis provides knock-out mutants for forward and reverse genetics

Needs an *in vitro* culture step: labour consuming
Work shared between 10 laboratories:

Gif (CNRS-F),
Toulouse (INRA/CNRS-F),
Dijon (INRA-F)
Valencia (CSIC-S),
Perrugia (CNR-I),
Sofia (ABI-Bu)
Gent (VIB-Be),
Bruxelles (VUB-Be),
Szeged (BRC-H)
York (UYo-UK)

Medicago truncatula *Tnt1* insertion Platform



Tnt1: LTR retrotransposon from tobacco

Transpose during tissue culture

Stable insertions

Knock-Out insertion mutants

Reverse and Forward Genetics possible

Tnt1 insertion population: from R108 to Jemalong H2A

	Regeneration	Transformation	Total	Insertions
Current	2,600	400	> 3,000	> 60,000
GLIP objective	▽▽▽	▽	5,000	100,000

Tnt1 border sequence database (proof of concept)

Current	700 BS	300 in genes
GLIP objective	1,000 BS	~ 450 genes



wt



Mtstp



Mtpim

TILLING platforms

<i>Species</i>	<i>Location</i>	<i>Personnel</i>
<i>Lotus japonicus</i>	JIC and SL	Trevor Wang, Martin Parniske
<i>Medicago truncatula</i>	U C Davis INRA-Evry-Dijon and JIC	Doug Cook/Abdel Bendahmane, Richard Thompson, Jon Clarke
<i>Glycine max</i>	STP and Purdue/ Missouri/Southern Illinois	Brad Till and Niels Nielsen/Kristin Bilyeu/Khalid Meksem
<i>Cicer arietinum</i>	STP and WSU	Brad Till and Fred Muehlbauer
<i>Phaseolus vulgaris</i>	Geneva and USDA- ARS-TARS Puerto Rico; CIAT	Bill Broughton and Tim Porch, Matthew Blair
<i>Pisum sativum</i>	INRA-Evry and Dijon	Abdel Bendahmane and Richard Thompson

TILLING platforms

Species	Population size	hits / kb	(genome)
<i>Lotus japonicus</i>	5300 (+3500)	12	(1100)
<i>Medicago truncatula</i> UCD	4032	9.9	(1330)
GLIP	4000 (4500)	13	(1750)
<i>Glycine max</i> Forrest & Williams	6150	1/150 - 1/200	
<i>Cicer arietinum</i> (ICC12004 Desi)	4608 (target)	1/165 (n=25)	
<i>Phaseolus vulgaris</i> (BAT93)	5000 (target)		
<i>Pisum sativum</i> Caméor Térèse	4700 (5000) 3000	16	(~16,000)

GLIP TILLING platforms



Objectives: Development and management of

- ***Medicago* and pea mutant collections**
- HTP pea and *Medicago* **TILLING platforms**

- **URLEG INRA Dijon**
Richard Thompson
- **JI Genome Lab**
Jonathan Clarke
- **URGV CNRS-INRA Evry**
Abdel Bendahmane

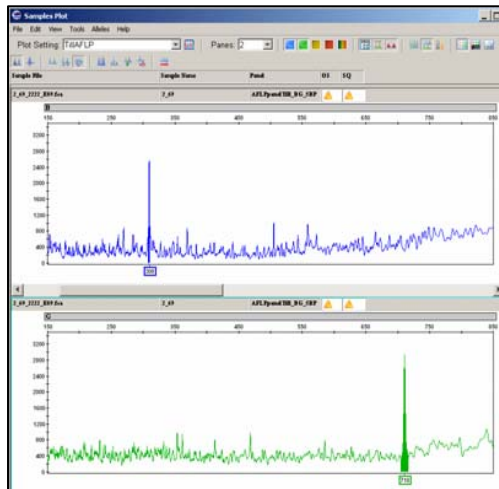


Medicago truncatula TILLING platform



EMS chemical mutagenesis (point mutations)
Mutant population: Jemalong A17

Current	4,000 M2	4,000 M1
Objective	4,500 M2	4,500 M1



- Efficiency: 13 mutant alleles / kb
Requests treated in chronological order
Priority to GL-TTP members

Tilling request for 61 genes received, 42 already done.

Pea TILLING platform



Mutant population:
cv. Cameor

Current	4,700 M2	4,700 M1
Objective	5,000 M2	5,000 M1

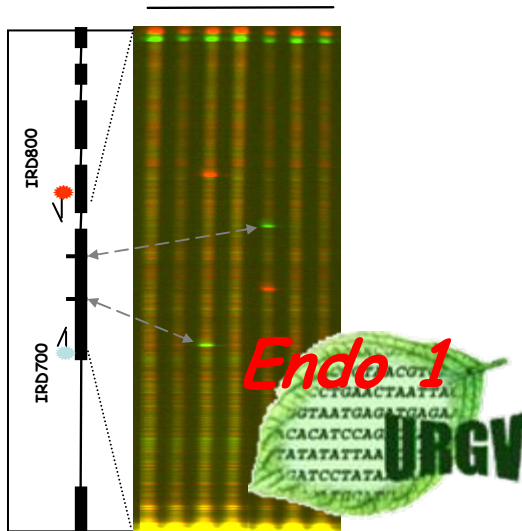
Efficiency: 16 (10-40) mutant alleles / kb

1-2 weeks to obtain mutant allele sequence

- Requests treated in chronological order
- Priority to GL-TTP members

21 genes already Tilled. Include genes of agronomic interest.

TILLING database (M2 and M3 families, genes and phenotypes)



Hear more about legumes at:



6th European Conference
on *grain legumes*

12th – 16th November 2007

Lisbon Conference Centre, Portugal

see:

www.eugrainlegumes.org