TILLING mutagenesis for common bean (*Phaseolus vulgaris* L.):

A tropical legume mutant resource

M. Blair

GCP Annual Meeting

Rome, Sept 29 - Oct 1

Why Beans?

- Produced as a dry grain and as a vegetable
- Dry grain is an important source of protein and minerals in diets of the poor.
- Highest level of direct human consumption of the grain legumes.
- Over 20 million ha of dry beans produced worldwide (8.5 M ha in LAC, 4 M ha in Africa)

Characteristics of the Bean Genome

650 MBp/haploid genome

True diploid

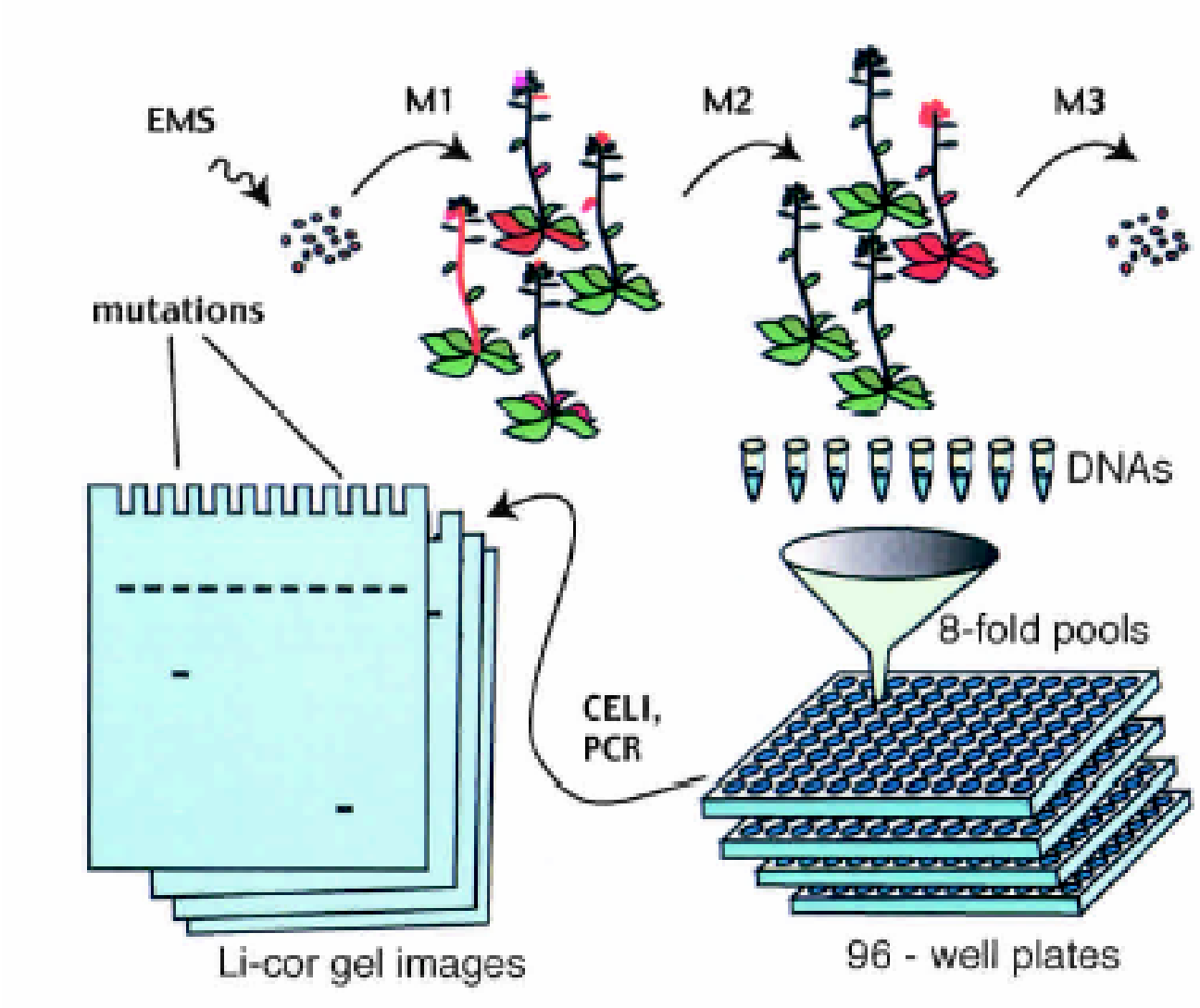
N = 11 chromosomes

Self-pollinating

Project Goal

To create a TILLING population for forward or reverse genetics in common bean.

TILLING (Targeting Induced Local Lesions in Genomes)



Colbert et al., 2003

Specific Objectives:

- Develop a reverse genetics tool for common bean that can be used to discover genes that are important determinants of agronomic traits for the crop.
- Analyze candidate genes for drought tolerance and other traits (N-fixation or Al tolerance) as a proof of concept study to show the value of the TILLING populations.
- Objective for year 1 is to perfect the mutagenesis protocols and develop the mutant stocks.
- Objective for year 2 are to multiply the mutant stocks and carry out the proof of concept study with drought responsive genes.

Mutagenesis in Common Bean: Early results

- Ethyl methane sulphonate (EMS) was used at CIAT (Davis et al., 1988) and elsewhere (Gautam et al., 1998) in the 80s and 90s and shown to be efficient and reliable.

- Chemical mutagenesis was applied to common bean principally to develop a number of nodulation mutants for a limited number of genotypes:

(eg Davis et al., 1988 used EMS (80 mM for 4 hrs) to mutate RIZ 30 and RIZ 36, finding 112 non-nodulating mutants in the M2 generation following inoculation with Rhizobium strains, CIAT632 and CIAT899)

Mutagenesis in Common Bean: Recent results

- Appropriate concentrations of EMS to use for large-scale common bean seed mutagenesis were worked out at the Univ. of Geneva (Pankhurst et al., 2003).
- A collection of aproximately 1000 fertile M1 mutant plants has been generated.
- We have grown four to eight seeds from each of 350 M1 plant to begin phenotypic screening and DNA extraction of mutants.
- Plant architecture, Leaf variegation and Seed quality (size and color) have been observed to vary in the mutants.
- Simultaneously M2 plants are being tested for nodulation defects at the University of Geneva.

CIAT

Leaf mutants observed so far:

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

Architecture mutants observed so far:



Plant architecture – severe stunting, dwarf and semi-dwarf phenotypes found along with some spindly (elongated shoot) mutants.

CIAT
Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

N-fixation mutants observed so far:

Screening plants for non-nodulation

Non-nodulation mutants observed so far:

Pv 366

Pv 354

Pv 341

Pv 418

Reduced nodulation mutant:

Expected Outputs

- A mutant collection of common bean amenable for forward and reverse genetics.
- Demonstrated capability to apply reverse genetics using mutant population.
- Demonstrated trait evaluation using mutant stocks.
- Training of researchers in common bean mutagenesis and mutant screening procedures.
- Systems in place to produce and distribute mutant seeds.

Future Targets

Drought stress. 73% of the Latin American and 40-50% of African bean production occurs under water-deficits

Al tolerance. Major problem of East Africa and parts of lowland Central America.

EST sequencing

CIAT libraries

4,000 Leaf ESTs – G19833
3,000 Root ESTs – DOR364

UNAM - Univ. Minnesota libraries

3,000 Leaf ESTs – Jamapa
4,000 Root ESTs – Jamapa
4,000 Nodule ESTs – Jamapa
4,000 Pod ESTs - Jamapa

TILLING

Total: 22,000 ESTs from *P. vulgaris*

Ramirez et al. 2005

Drought mapping in common bean:

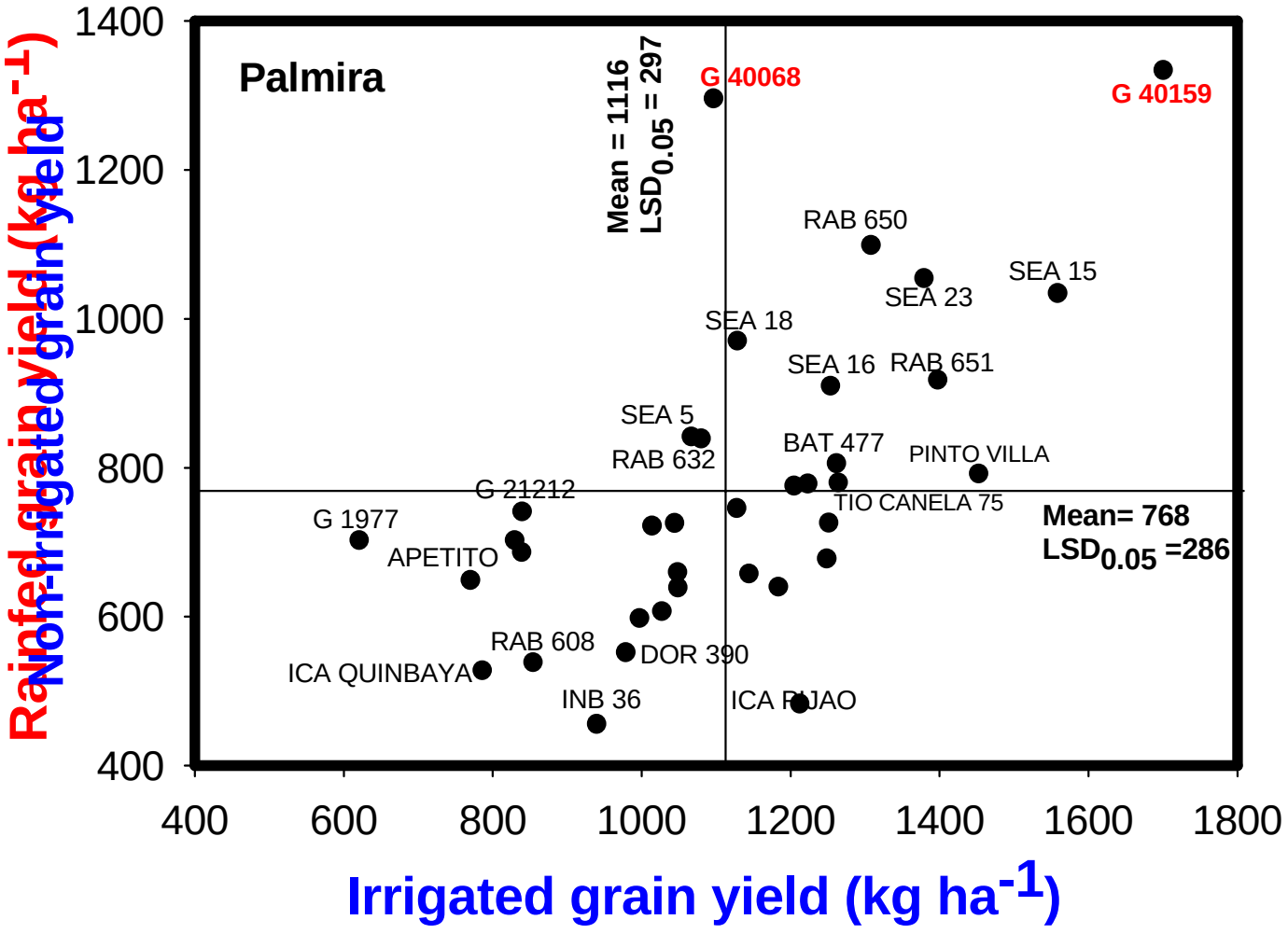
M. Blair

GCP Annual Meeting

Rome, Sept 29 - Oct 1

Yield under drought:

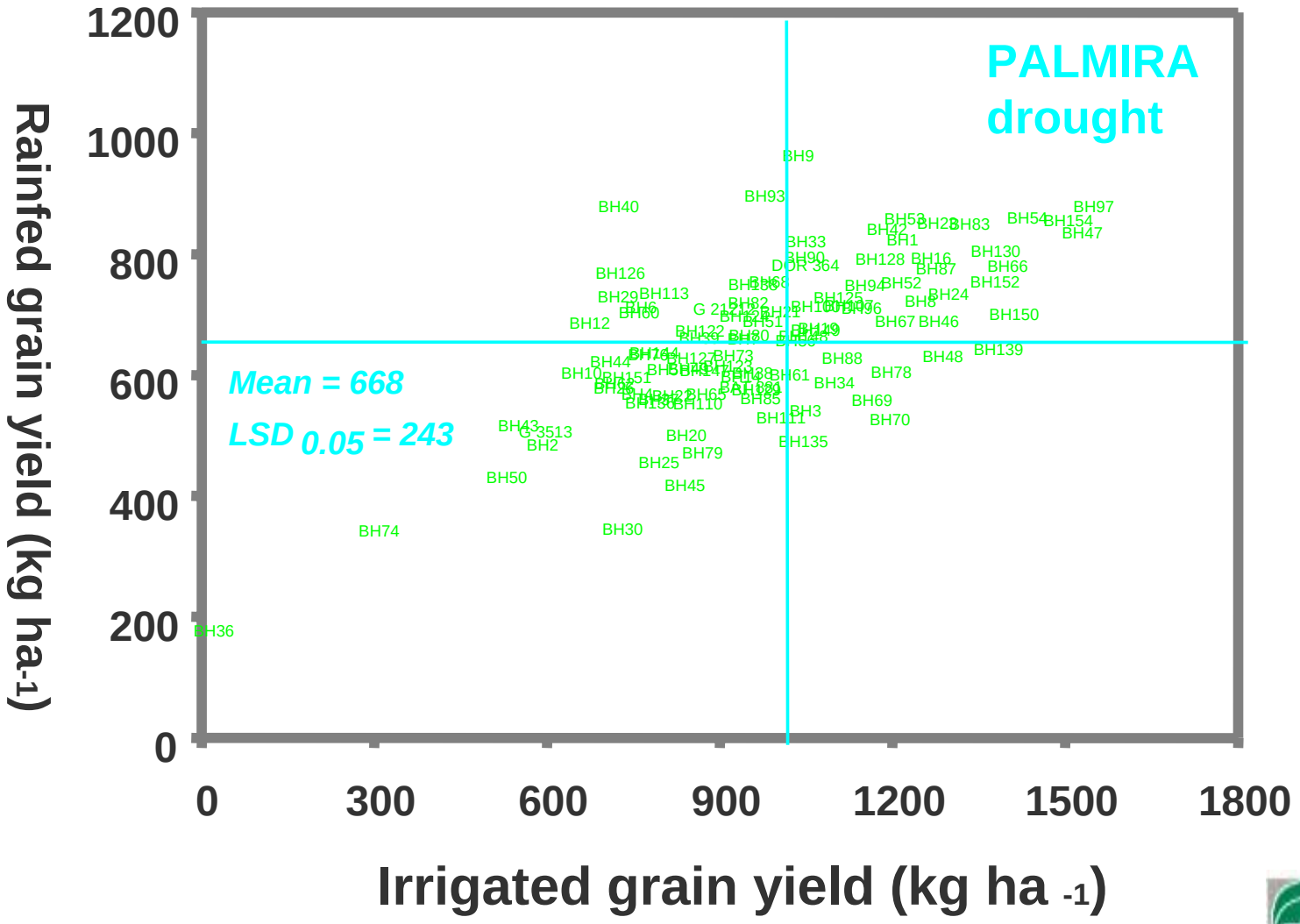
Drought adapted advanced lines



Development of RIL populations

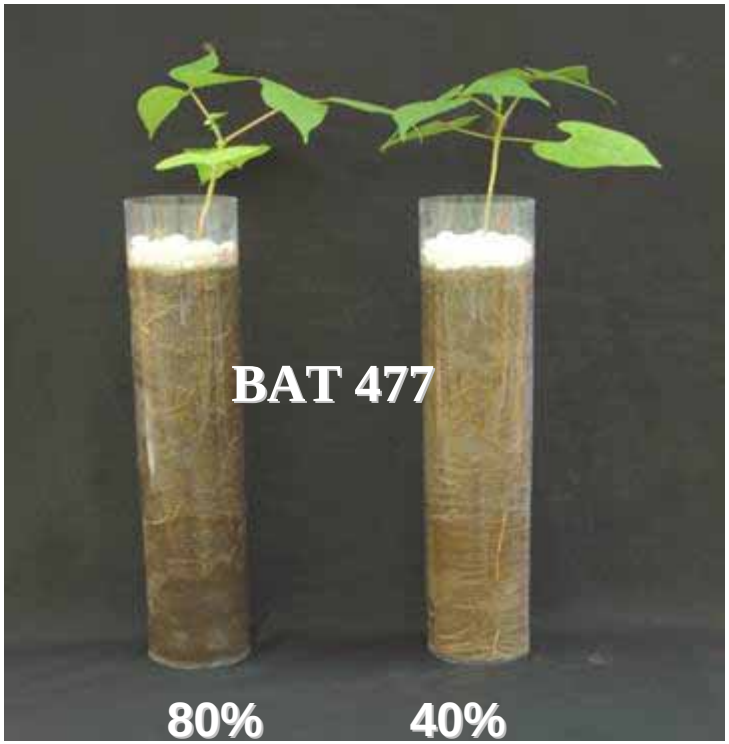
Population	No RILs	Locations
DOR364 x BAT477	96	CIAT-GH/F, Nicaragua, Mexico
BAT881 x G21212	89	CIAT-F, Nicaragua
MD 2324 x SEA5	115	CIAT-F, Nicaragua
A686 x SEA15	140	CIAT-F
Tio Canela x SEA21	140	CIAT-F
G5273 x MAM38	120	CIAT-F

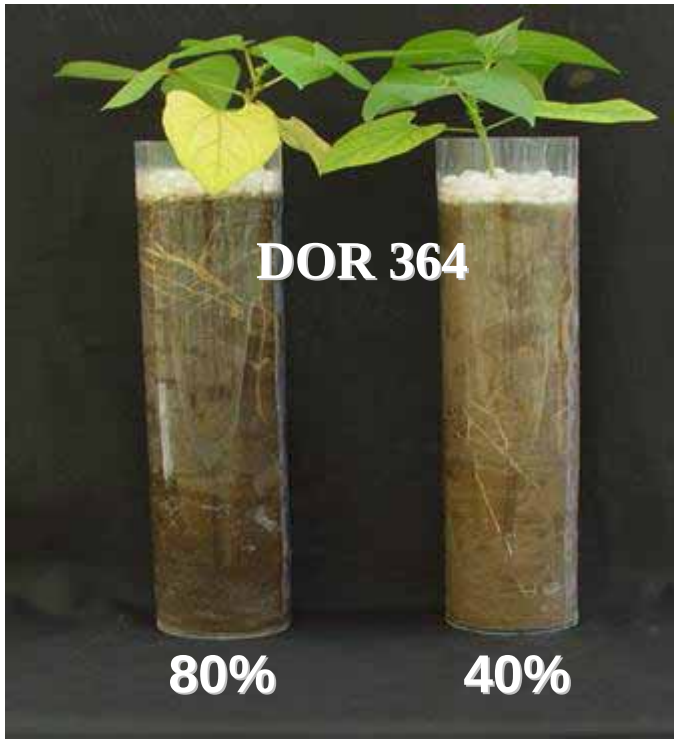
Drought adapted and irrigation responsive RILs of BAT 881 x G 21212



Screening for root architecture

Beans grown in 50 cm soil cylinders





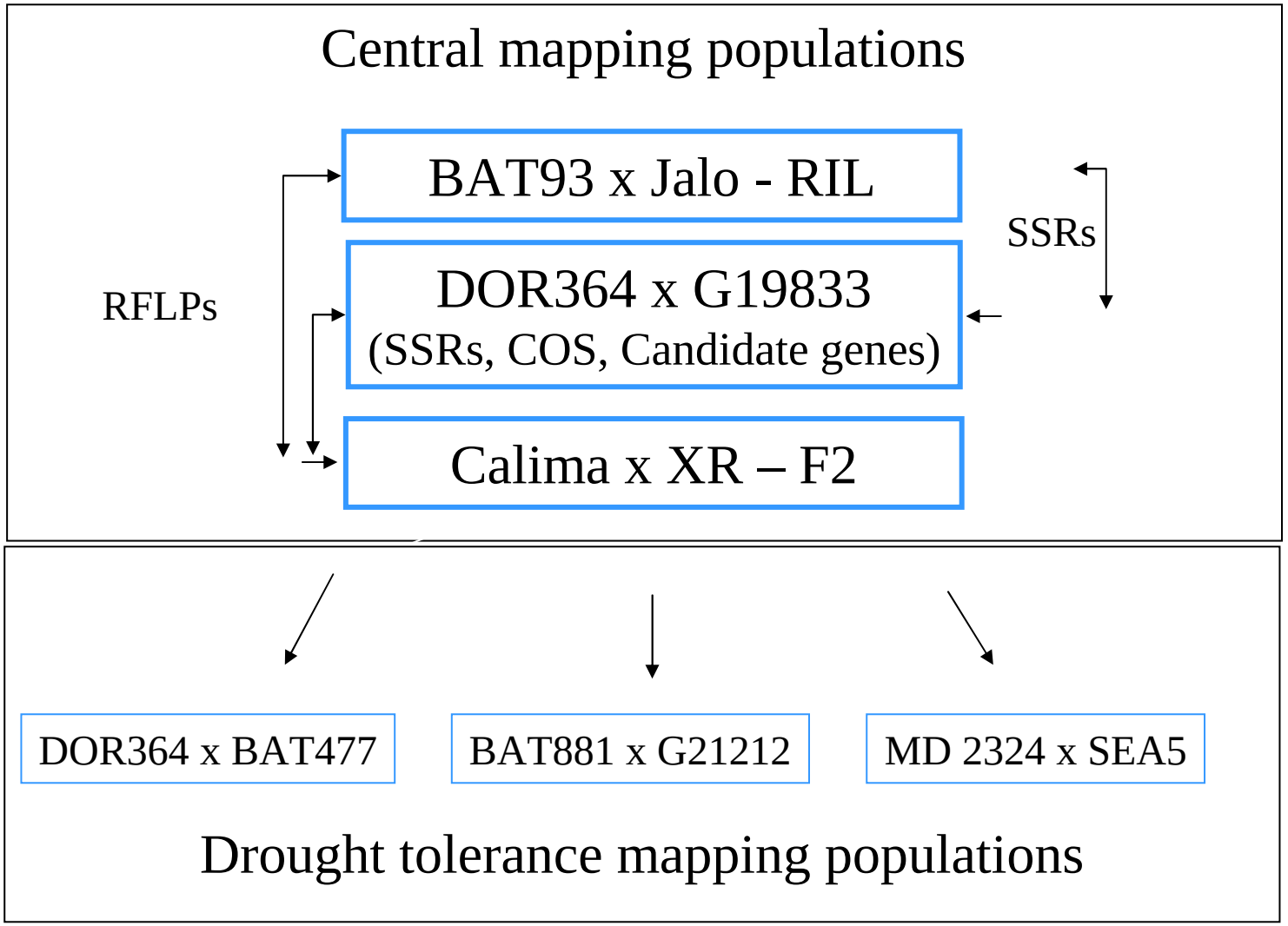
Evaluation of first set of RILs is underway

RIL population
Mapping

Population	Marker Type	Polym. Level	Map Status
1) DOR364 x BAT477	SSR, RAPD	low	700 cM
2) BAT881 x G21212	SSR, AFLP,RAPD	Low-mod	800 cM
3) MD2324 x SEA5	SSRs	low	na
4) A686 x SEA15	--	low	na
5) Tio Canela x SEA21	--	low	na
6) G5273 x MAM38	SSRs	high	na

CIAT

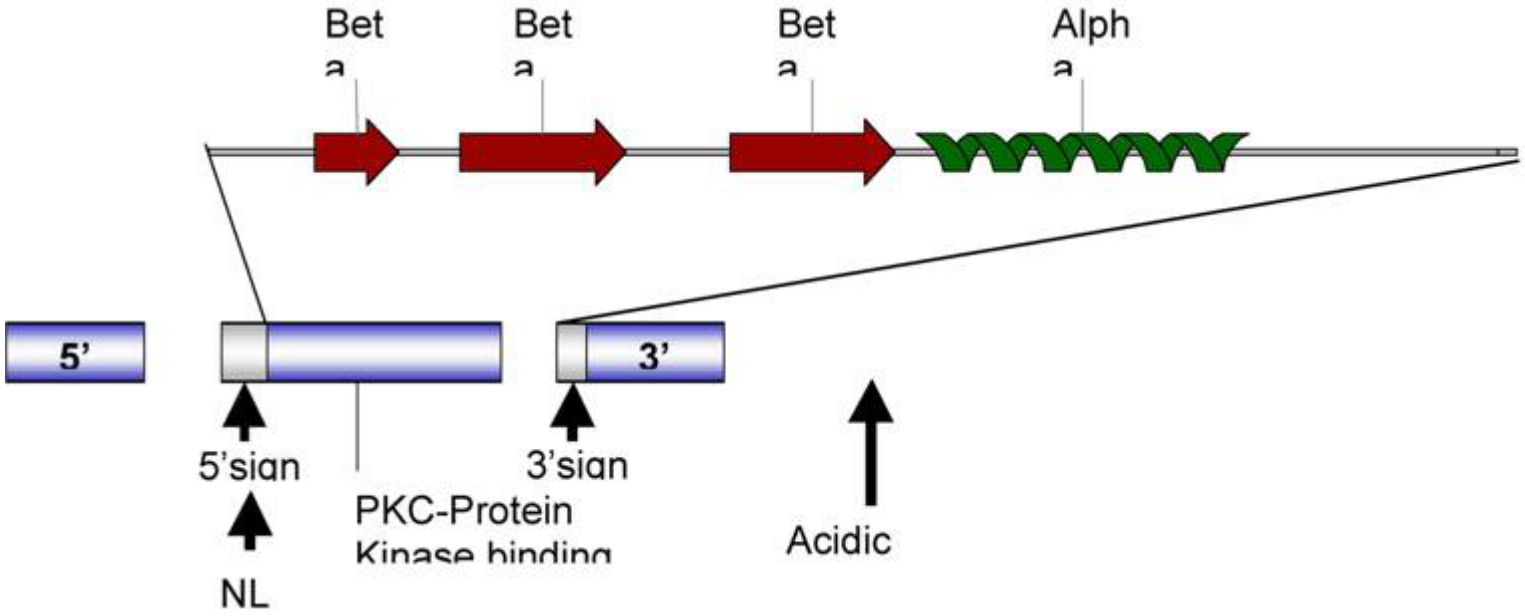
Need for Comparative Mapping



Candidate Gene Mapping

We plan to map three DREB genes that have been identified for common bean.

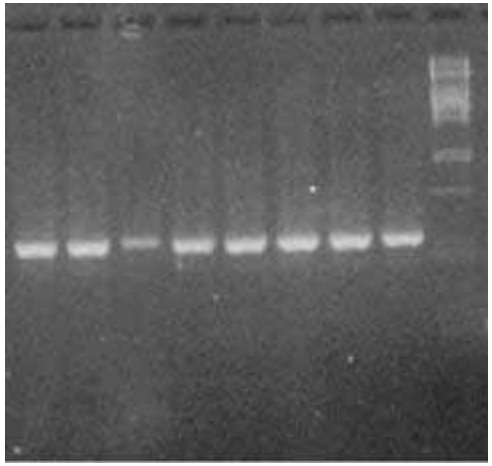
Full length seq. PvDREB2 / PvDREB3 and partial PvDREB1 having high sequence identity with other DREB genes were isolated. All have AP2/EREBP domains with 3 beta chains / 1 alpha helix that are necessary for specific binding to DNA. DREB specific boxes found in the flanking regions of the AP2/EREBP domain. Putative nuclear localization signal (NLS) and Protein Kinase binding sites also found.



Cross Legume Markers

We have analyzed a set of 19 cross legume markers from Univ. California - Davis that amplify for common bean and plan to analyze a further set of 25 of these same markers to link the central and drought maps to genetic maps made for other legumes inlcuding Medicago truncatula and soybean.

HRIP



AAT	Alanine Amino transferase	CAPS
ACCO	1- ACC Oxidase	CAPS
ACL	ATP Citrate lyase	CAPS
AGT	Putative 4alfa-glucano transferase	SNP
AIGP	Auxin independent growth promoter	SNP
APX	Ascorbate Peroxidase	CAPS
APYR1	Mt apyrase 1	CAPS
ASNEP	Asparaginyl endopeptidase	SNP
ASPP	Aspartic protease precursor	SNP
ATCP	Aquaporin like channel protein	SNP
ATP2	ATP synthase ~-chain, mitochondrial precursor	SNP
BGAL	gene analog~-Galactoidase	SNP
CAF	Caffeoyl-CoA-O-methyl-transferase	CAPS
CAK	Calcium dependent protein kinase	CAPS
CALTL	Calreticulin	SNP
CDC16	Cell division control protein 16	CAPS
CDC2	Putative cdc2 kinase	SNP
cgO008F	Gibberelin 3~-hydroxylase, Vr	SNP
cgP137F	Unknown protein	SNP
chit1	Mt chitinase 1	Length
CNGC4	Cyclic nucleotide regulated ion channel	SNP
CPCB2	Putative coatomer protein complex, ~2	SNP
CrS	Cystathionine gamma-synthase precursor	Length
CYSK	Cysteine synthase	SNP
CYSP	Cysteine synthase	SNP

Acknowledgements:

CIAT - TILLING, maping and DREB genes

Carlos Hernando Galeano
Claritza Muñoz
Leonardo Galindo
Eduardo Tovar
Lina Rodriguez
Manabu Ishitani
Idupulapati Rao
Steve Beebe

And lots of advice from John Innes Center; Univ. of Washington and others…

Univ. of Geneva - mutagenesis

Clive Pankhurst
Patricia Lariquet
Bill Broughton