Improving common bean productivity for drought prone environments in sub-Saharan Africa

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Centro Internacional de Agricultura Tropical
International Center for Tropical Agriculture

(Objective 3 – Tropical Legumes I)

Generation

Talk Outline

- Marker development
- QTL detection
- Further population development
- Reference collection

Marker Development

- Goal has been to develop molecular tools for the efficient pyramiding of drought tolerance with other traits.
- New genetic markers are being developed based on simple sequence repeat (SSR) and single-nucleotide (SNP) polymorphisms.
- SSR marker development based on multiple sources, such as small insert genomic libraries, ESTs and BAC end sequences.
- SNP markers have been based on cDNA sequences from subtractive libraries for drought tolerant/susceptible genotypes, candidate genes and cross-legume sequences.

Small Insert – SSR

Involved the construction of various genomic libraries

Subcloning method	Restriction enzyme	Bean Genotype	Vector	Insert Size (kb)
Enzyme digestion - ligation	*Alu*I	DOR364	pBS	0.5
	*Hae*III	DOR364	pBS	0.5
	*Rsa*I	DOR364	pGEM	0.5
	*Taq*I	DOR364	pJV1	0.7
	*Dra*I/*Alu*I	G19833	pBS	0.3
	*Dra*I/*Hae*III	G19833	pBS	0.4
Sonication – blunt ending - ligation	NA	DOR364	pBS	0.5

Small Insert - SSR

Probe type	Exposure time[a]	Library			Total
		*Alu*I	*Hae*III	*Rsa*I	
2nt	1	6	2	29	37
	2	78	57	11	146
	3	25	46	28	99
total		109	105	68	282
3nt	1	1	0	6	7
	2	21	10	4	35
	3	8	32	53	93
total		30	42	63	135
Overall total		139	147	131	417

Total : 417 SSRs discovered in 18,000 small insert clones from 3 libraries screened with di- and tri-nucleotide repeat motif probes

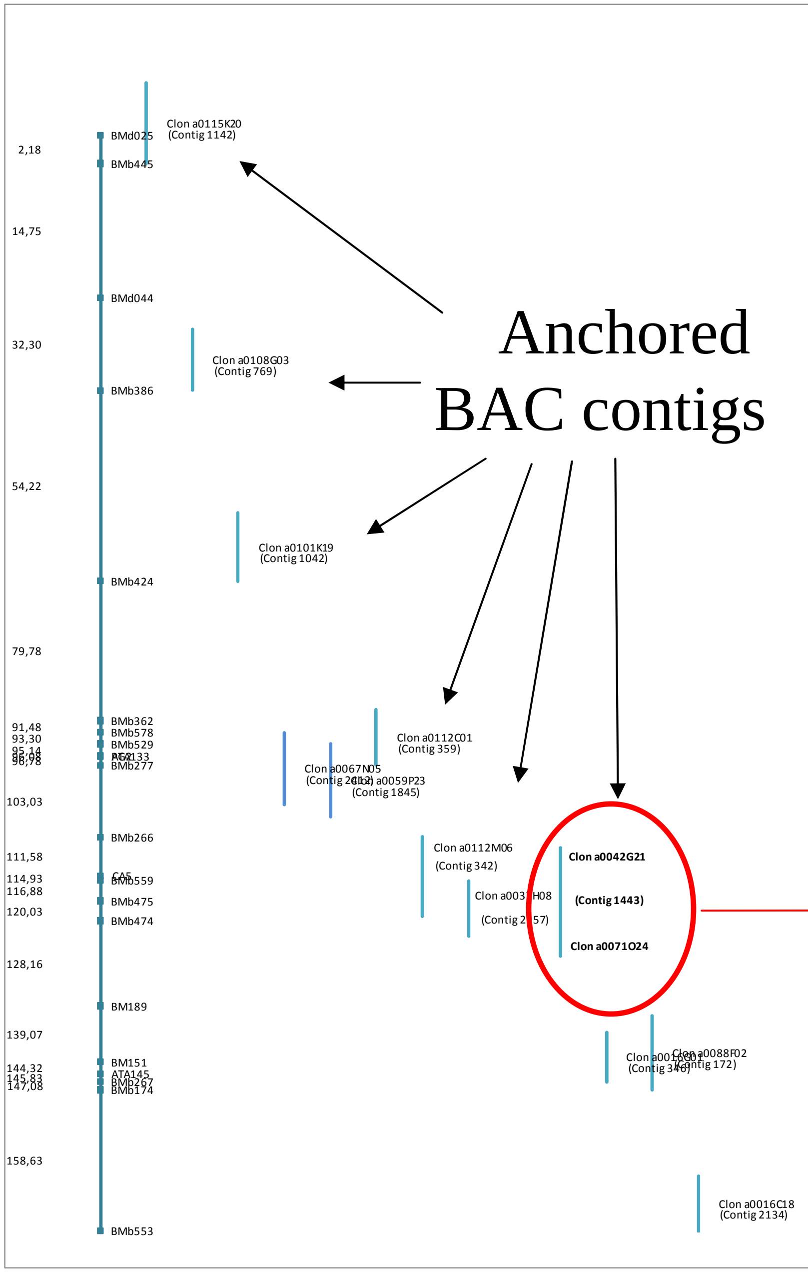
Source: Blair et al. (2009b) Genome

EST-SSRs / motif frequency

Discovered through hybridization screening of 18,000 cDNA clones and sequencing of positive hits at 5' and 3' ends

SSR type[1]	5' sequences	3' sequences	total	Percentage of total	Proportion 5' end
Di-nucleotides	181	28	209	42.1	86.6
ac/gt/ca/tg	44	4	48	9.7	91.7
ag/ct/ga/tc	124	13	137	27.6	90.5
at/ta	13	11	24	4.8	54.2
gc/cg	0	0	0	0.0	--
Tri-nucleotides	194	30	224	45.1	86.6
aag/aga/gaa/ttc/tct/ctt	24	1	25	5.0	96.0
aat/ata/taa/tta/tat/att	2	7	9	1.8	22.2
aac/aca/caa/ttg/tgt/gtt	34	2	36	7.2	94.4
acc/cac/cca/tgg/gtg/ggt	19	2	21	4.2	90.5
agc/cag/gca/tcg/gtc/cgt	68	15	83	16.7	81.9
agg/gag/gga/tcc/ctc/cct	30	2	32	6.4	93.8
atc/cat/tca/tag/gta/agt	4	1	5	1.0	80.0
ccg/gcc/cgc/ggc/cgg/gcg	8	0	8	1.6	100.0
gac/cga/acg/ctg/gct/tgc	5	0	5	1.0	100.0
Tetra-nucleotides	31	20	51	10.3	60.8
Penta-nucleotide	11	2	13	2.6	84.6
Total	417	80	497	100.0	-

Source: Blair et al. (2009a) BMC Plant Bio



Linkage Group b08

BAC end SSR Linkages to Physical Map of the Bean Genome

Based on a CIAT/CUGI BAC library with an average insert size of 142 kb and 12 X coverage.

Possible to order contigs in many cases

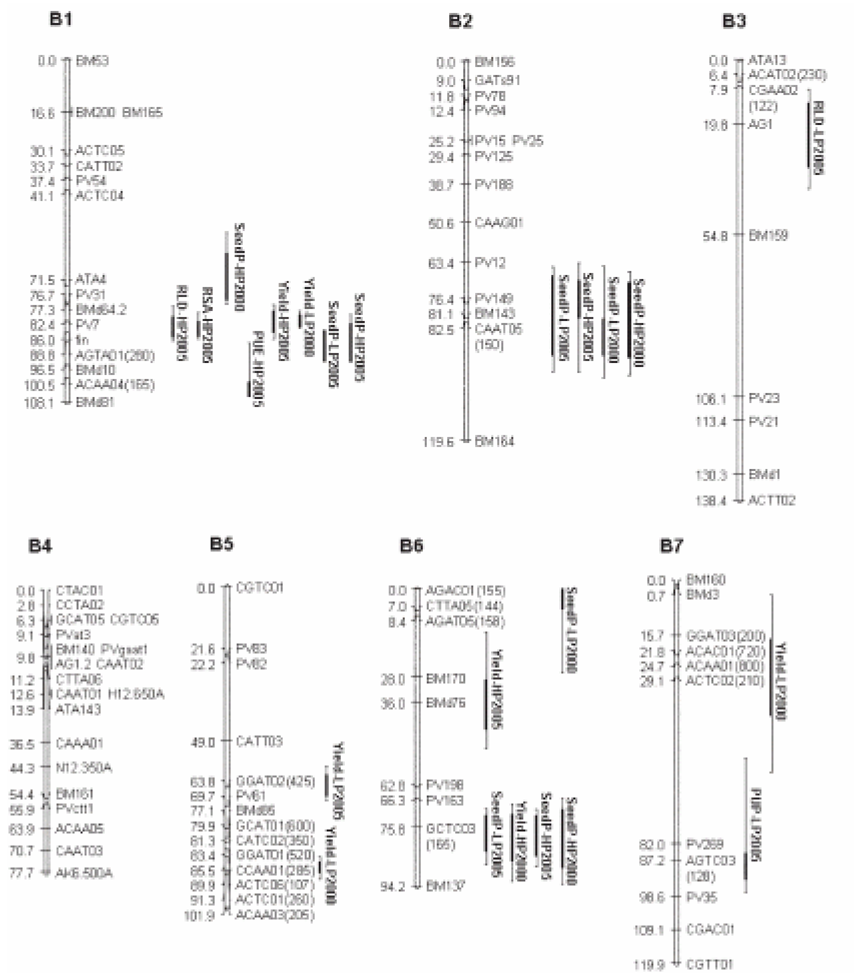
Possible to orient contigs in some cases

Result is a more saturated genetic map and greater utility of the Purdue physical map

Improvement of Genetic Maps Especially Intra-genepool

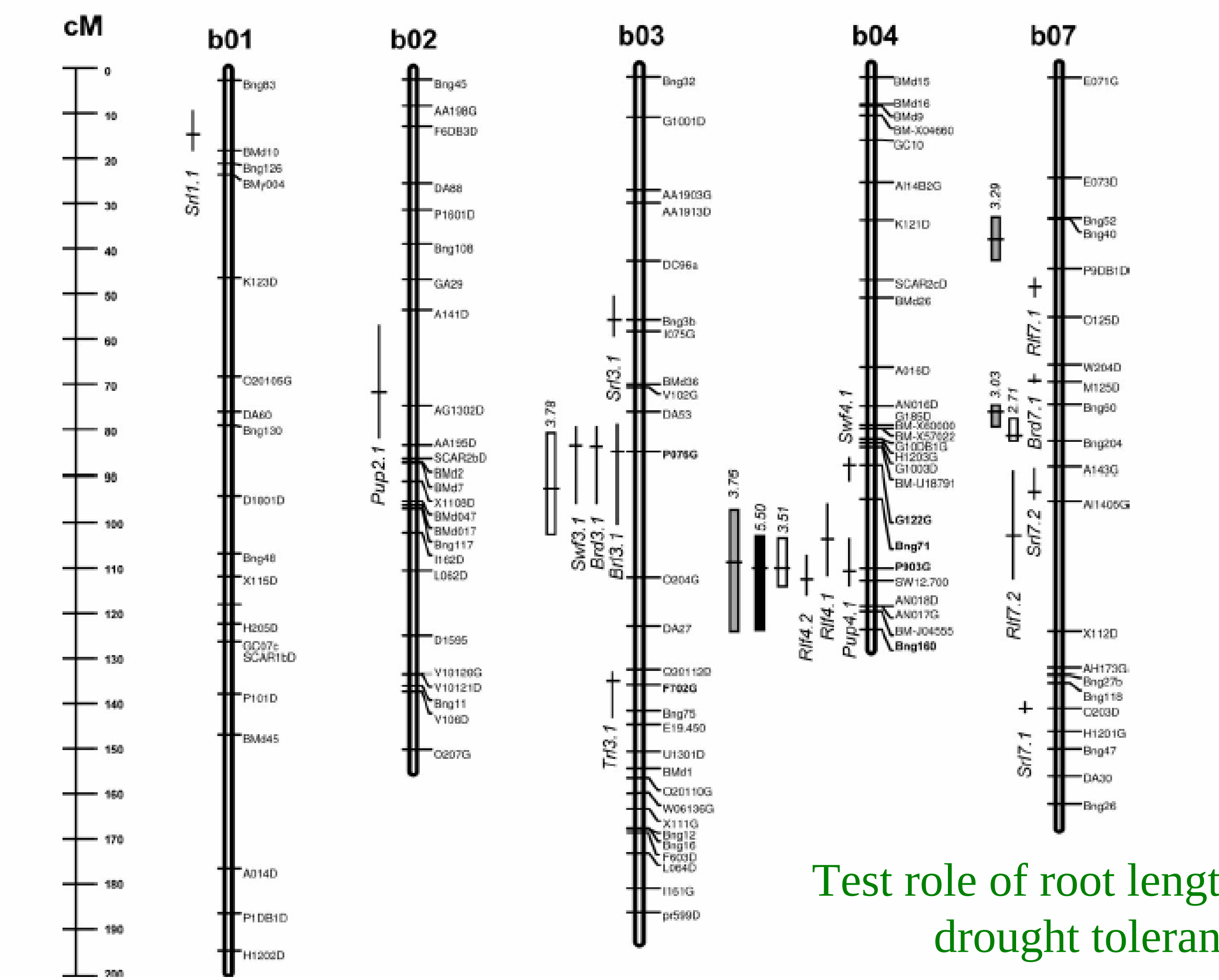
- The many genomic and gene-based SSR markers are allowing us to genetically map in narrow crosses.

- This has been very important for QTL detection in the Andean and Mesoamerican genepools



Source: Cichy et al. (2009) Crop Science

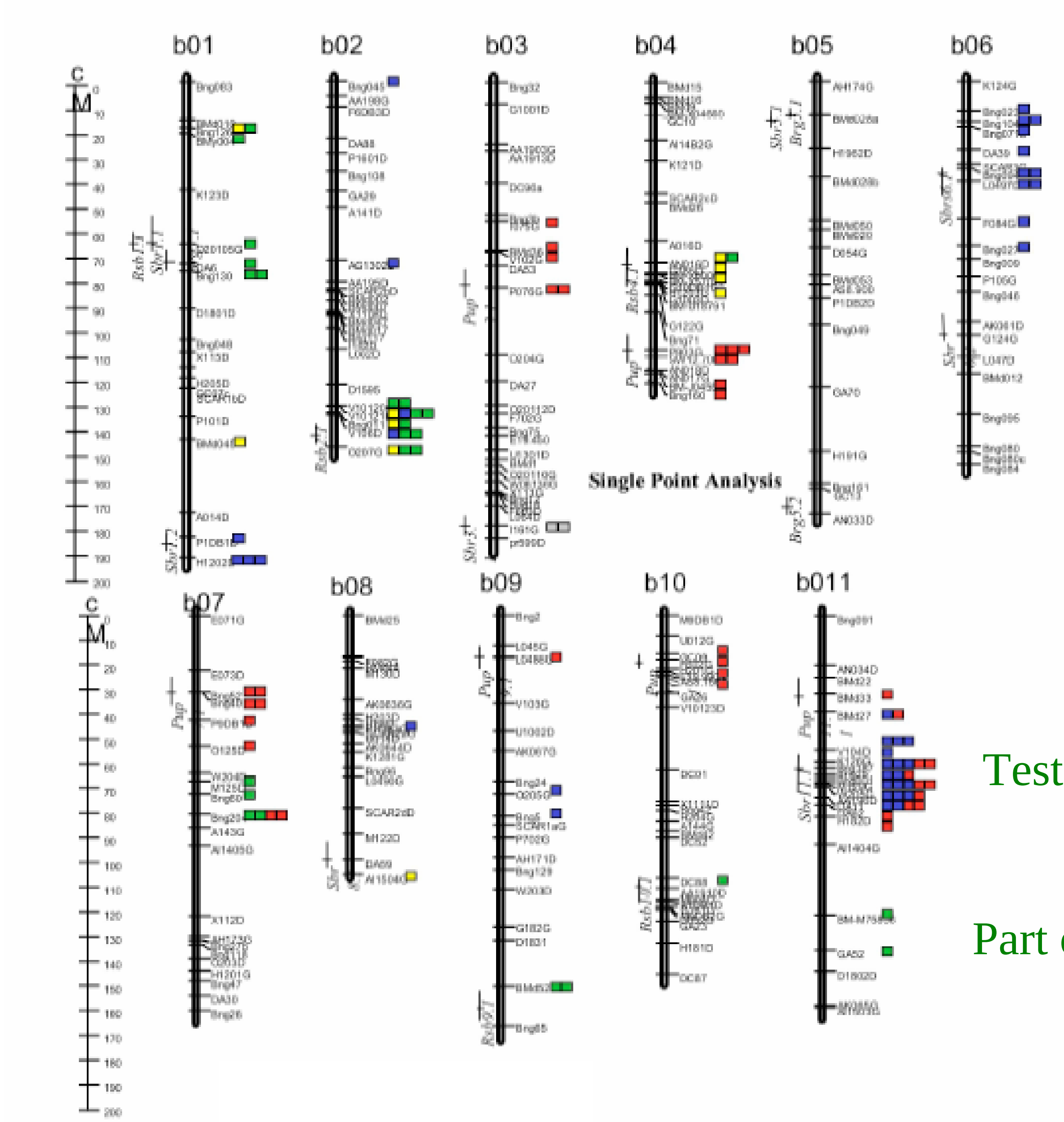
Root length QTL



Test role of root length QTL on drought tolerance?

Source: Beebe et al. (2006) Crop Science

Low P root gravitropism QTL



Test the role of basal root angle on drought tolerance

Part of PSU-CIAT-IIAM project

Population development

• new good x good recombinant inbred line populations have been made for both mapping and crop improvement.

• will be used for marker assisted recurrent selection (MARS)

• emphasize less well-studied drought tolerance sources such as Durango derived breeding lines crossed with commercial varieties

SEA5 = cream colored
SEA15 = medium red seeded

CAL96 = K132 (Ugandan release, high marketability)
CAL143 = Napilira (Malawi release, low P tolerant)
BRB191 = BCMNV resistance source

Inter/ra-genepool crosses

- Derived from 49 populations from partial diallel combining commercial var. from Southern Africa and 10 drought tolerance sources (5 Andean, 5 Mesoamer.)
- Advanced lines produced at CIAT and in Southern Africa
- Most promising populations chosen on the basis of analysis of each population's combining ability, as well as the plant ideotype, adaptation to drought stress and yield potential.
- Will be used for marker assisted recurrent selection (MARS) as well.

Advanced Backcrosses

- Two drought tolerant donor parents: SER16 and SER48 (both good combiners within the Mesoamerican gene pool) crossed with
- Three commercial Andean cultivars from Eastern and Southern Africa used as recurrent parents: CAL 96, CAL 143, and CIM 9314-36.
- Over 500 F1-BC2 families generated.
- Importance of Calima-Red Mottled, Sugar and Red Kidney types in target countries

  - o Eastern Africa (D.R. Congo, Kenya, Rwanda-Burundi, Uganda, N. Tanzania)
  - o Southern Africa (Malawi, Mozambique, South Africa, S. Highlands Tanzania, Zambia, Zimbabwe)

Reference collection

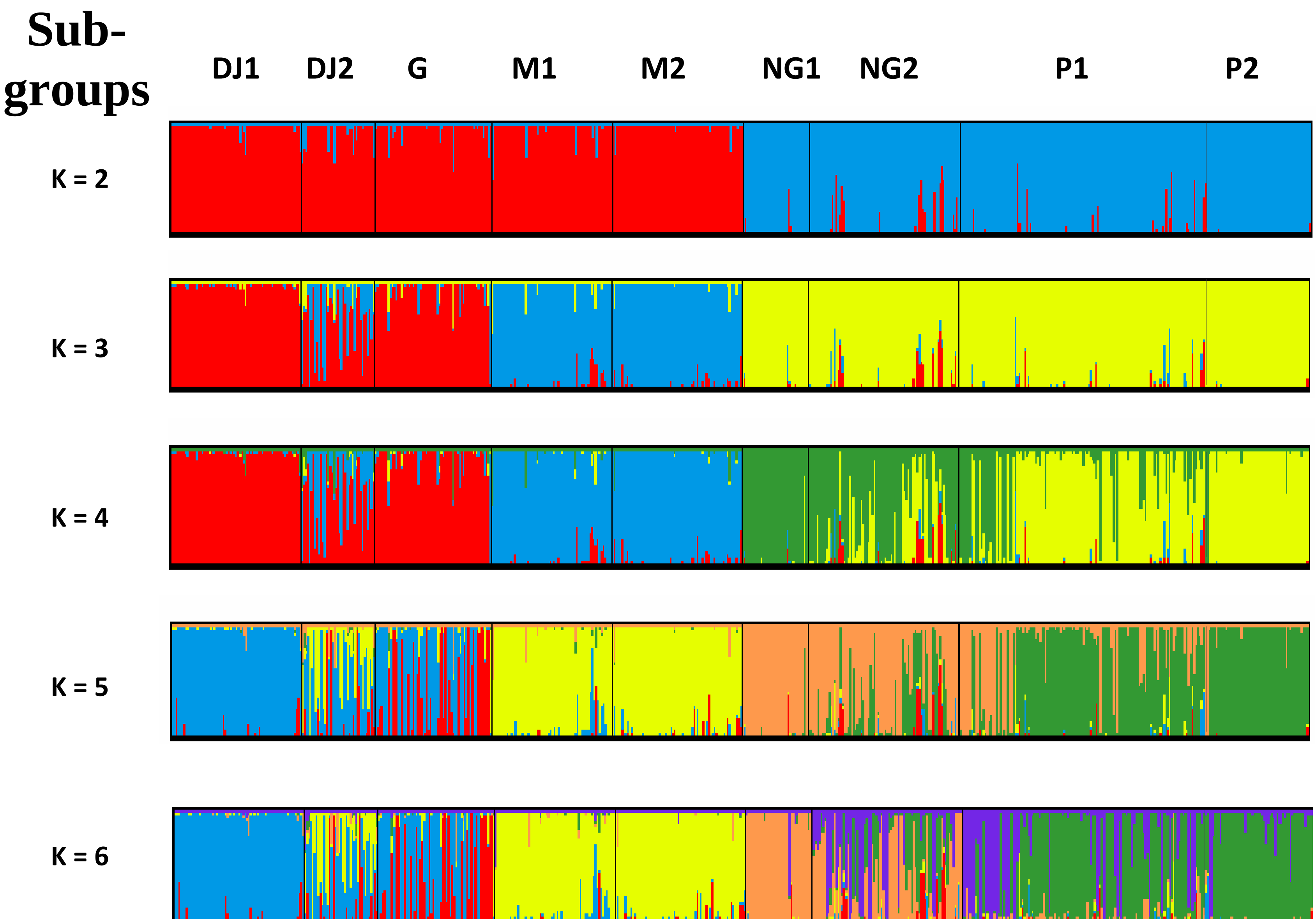
• subset of CIAT core collection (200 genotypes) with population structure well understood through molecular marker analysis

• useful for comparison of drought tolerance sources within each genepool and comparison of subgroups within each genepool.

• analyzed in Lattice design experiments with stratification by genepool origin (Mesoamerican : 11 x 11, Andean : 9 x 9) and drought or irrigated treatments

• phenotypic data useful for association analysis with genetic markers

Population Structure



Source: Blair et al. (2009c); Theor Appl Genet

Phenotyping Sites I - CIAT

Colombia – 2 sites

Darien (1500 masl)

Palmira (1000 masl)

Phenotyping Sites II – East Africa

Ethiopia – 1 site
Awassa (1200 masl)
(8° N latitude)

Kenya – 2 sites
Kabete (1800 masl)
Thika (1600 masl)
(3° S latitude)

Phenotyping Sites III – Southern Africa

Malawi – 2 sites
Chitedze (1100 masl)
Kasinthula (750 masl)

Zimbabwe – 2 sites
Gwebi
Harare

part of PhD thesis by Zimbabwean bean breeder

Additional sites planned:
Tanzania – 1 site (Arusha)
Mozambique - 1 site (Susandega)

Phenotypic traits measured

- Shoot traits measured at mid-podfill including

1. leaf chlorophyll content (SPAD),
2. canopy temperature and canopy temperature depression (CTD),
3. leaf area index (LAI)
4. canopy dry weight per plant
5. shoot and immature seed total nonstructural carbohydrates (TNC).

- Shoot traits measured at harvest including:

1. Yield and yield components (number of pods per plant, number of seeds per pod, and 100 seed weight),
2. pod harvest index (dry wt of pods/dry wt of total biomass at mid-podfill x 100)
3. grain filling index (100 seed weight of rainfed/100 seed weight of irrigated).
4. seed total nonstructural carbohydrates (TNC) for translocation effic.

- Root traits to be measured in deep rooting cylinders

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Association mapping

Partial core collection has been shown to be useful for association mapping of seed size, weight, height and length across genepools

Tests will be undertaken to see if there are associations of drought tolerance shoot and root traits with markers in the reference collection.

Linkage group[a]	Durango-Jalisco				Mesoamerica			
	SW[b]	W	H	L	SW	W	H	L
B01								
BM200	***			*	*			
PV-ag003								*
B02								
BM139					*			
BM143								*
BM156					***			
BMd02								
BMd17					*			
BMd18							*	
BMd47								
PV-cct001							*	
B03								
AG01					***			
BM172					*			
BMd01			*		*			
GATs54					*			
B04								
BM140					*			
BMd08					***			*
BMd15					*			
BMd16					***			
PV-ctt001					*	*	*	*
PV-at003					*			
PV-at001								
B05								
BM175								
BMd20								
B06								
BM137		*						*
BM187								*
B07								
BM160					*			
BM183					***	*		***
BM201								*
BM205	*				***			
Phs		*			***			
B09								
BM141								
BM188A								
B011								
BM188B								

Source: Blair et al. (2009c); Theor Appl Genet

Conclusions

- New marker development to speed up the identification of QTL for drought tolerance.
- QTL detection in multiple intra-genepool and inter-genepool mapping populations.
- Establishment of reference collection as a baseline for association mapping.

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Thanks for your Attention