


PHASEOLUSGENES
Bean Breeder's Molecular Marker Toolbox

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About PhaseolusGenes



Phaseolus beans (navy and black beans; great northern, small red, and pinto beans; red kidney, yellow or azufrado, and scarlet/red or haricoto beans; green or stringless beans) represent the most important legume species for direct human consumption. They fill an important role in human nutrition as a source of protein, fibers, minerals, and vitamins. They also include a much-needed complement in rotations and cropping systems as a source of nitrogen (Broughton et al. 2003).

Why a genetic and genome database for Phaseolus? Bean breeders were among the early adopters of marker technology to facilitate the mapping and transfer of genes of agronomic interest, particularly disease resistance (e.g., Haley et al. 1993; Miklas et al. 1993; Nodwin et al. 1993).

Since then, bean breeders have added many additional markers to their toolbox (Kear et al. 2003; Miklas et al. 2006), but the results and information have remained dispersed among journals, databases and, yes, laboratory notebooks and cabinets. Furthermore, the lack of DNA sequences has limited the development of additional markers for...

In recent years, additional tools and sources of genomic information about the bean genome have become available, including molecular linkage maps, EST collections, BAC libraries, a physical map, and, soon, a whole-genome sequence for *Phaseolus vulgaris* (McClean et al. 2006; Gepts et al. 2005).

Concurrently, genomics efforts are well under way in other species, including legume species (soybean, lotus and Medicago). Using DNA as the lingua franca of genetics, results such as gene location and expression of individual and their phenotypes can be extrapolated for mutual gene discovery.

These additional genomic resources, especially the mapped sequences, provide an exciting opportunity to design additional markers that complement the existing markers and speed up the application of genomic information to bean breeding.

The main goals of this genome and genetic database are to facilitate the use of existing markers and the development of new markers by breeders ("bean breeder's marker toolbox"), to associate genetic and phenotypic information useful to breeders; to coordinate information with other legume databases (especially soybean), and, eventually, to assure its integration into the Legume Information System (www.comparative-legumes.org).

We are grateful to our funders, the Kirkhouse Trust in the U.K., who provided the impetus for this database, specifically in support of the African Bean Consortium project to introduce the capability for marker-assisted selection (MAS) among East African bean breeders. The MAS effort will focus specifically on five diseases: SCN/BCMV, anthracnose, angular leafspot, common bacterial blight, and Pythium root rot. A 1x sequencing of the methyl-filtered bean genome DNA will provide much-needed additional sequence resources.

The BeanCAP project, funded by USDA/NIFA will strengthen the PhaseolusGenes database by integrating of the Phaseolus whole-genome sequence developed by Purdue Univ. and the Joint Genome Institute of the Department of Energy; SNP, CAPS, and dCAPS markers; and phenotypic information related to field performance and nutritional quality traits, from the main U.S. market classes.

Search markers

Search term:

Search fields:

Search

Blast search (soybean)

(blastn w/ e-value cutoff 0.0001)

Enter one sequence in simple text

This search may take up to a minute

Search Reset

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Paul Gepts (Dept. of Plant Science) and Doreen Lu (Bioinformatics Core) at UC Davis

Contribution of UC Davis to the BeanCAP Further Development of PhaseolusGenes, Bean Breeder's Marker Toolbox



Contributors

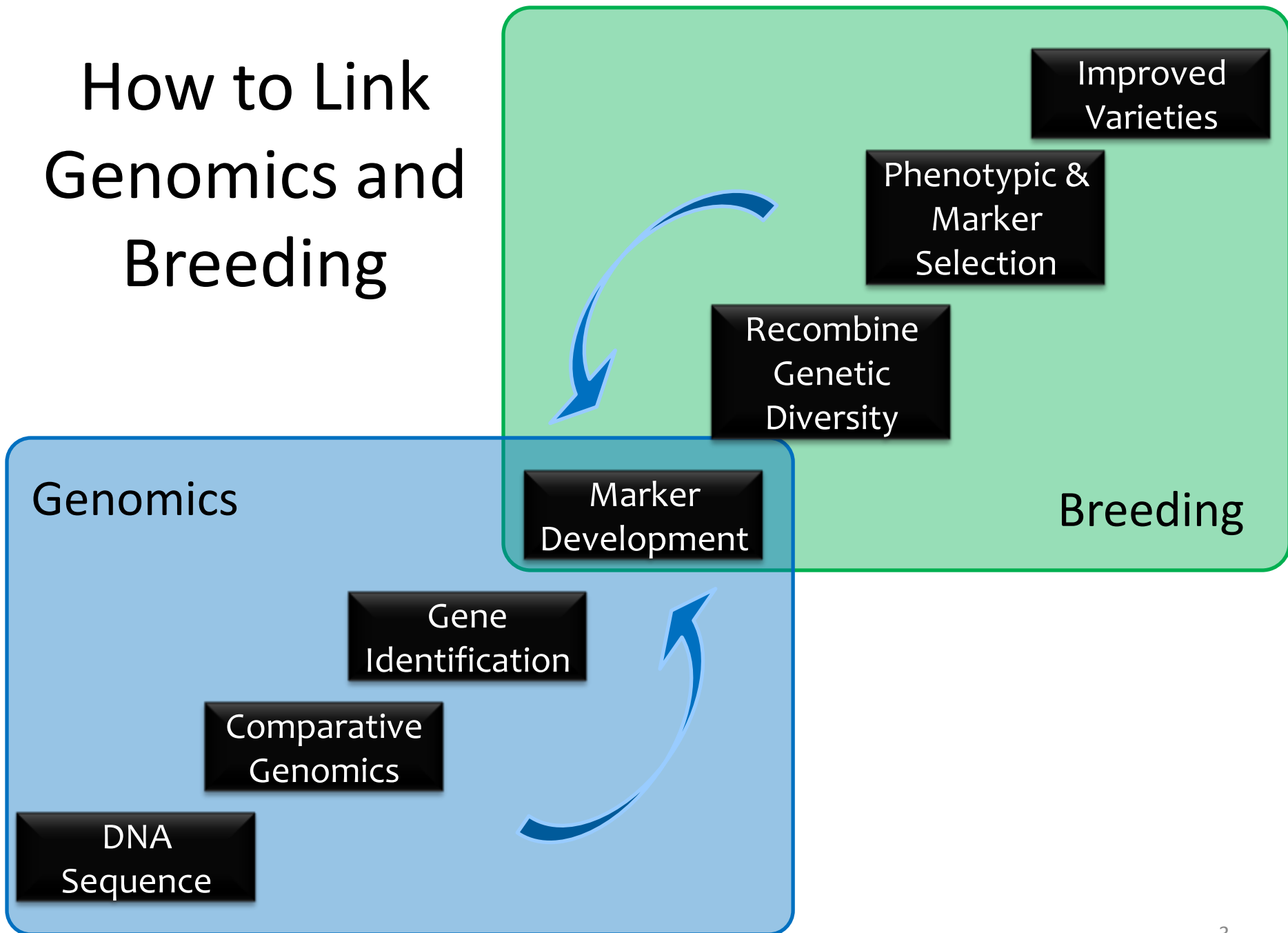
- Dawei Lin
- Jose Boveda
- Monica Britton
- Joe Fass
- Nikhil Joshi
- Zhi-Wei Lu
- Shelby Repinski
- Adriana Navarro Gómez
- Paul Gepts

Outline

- Introduction: Paul
- Database structure: Dawei
- Microsatellite marker integration: Paul
- QTL survey: Adriana
- Conclusion: Paul



How to Link Genomics and Breeding



Antecedents (I)

- Bean researchers have actively developed markers since early 1990s:
 - currently 76 SCAR markers;
 - 80-90% of released bean varieties in last decade have a MAS component
 - main limiting factor: **absence of sequence**
- Information about markers is **dispersed**
- African Bean Consortium project: early version of database; **limited** by its objectives:
 - Introduce MAS
 - 5 major diseases



Markers in *Phaseolus*

Marker Type	Examples	Number
SCAR markers	ALS, ANT, BCM, BCT BGYM, CBB, HB, Rust, WM, FW, Apion, Seed or Floral Pigmentation/pattern	76
Genes of putative agronomic interest	P metabolism, polyphenolics, flowering time, ...	38
RAPD	Rust	1
STSs	RFLP, Leg, g	633
SSRs	BM, GATS, PvBR, BMd, BMc, SSR-IAC, PV, FJ, ...	795
Total		~ 1540



Antecedents (II)

- **New** types of data:
 - ESTs
 - Genome survey sequence
 - Whole-genome sequence
 - SNP
 - Comparative genomics: soybean WGS, Arabidopsis
- **Rapid** addition of new information



Dawei Lin:
Director, Bioinformatics Core,
Genome Center, UC Davis



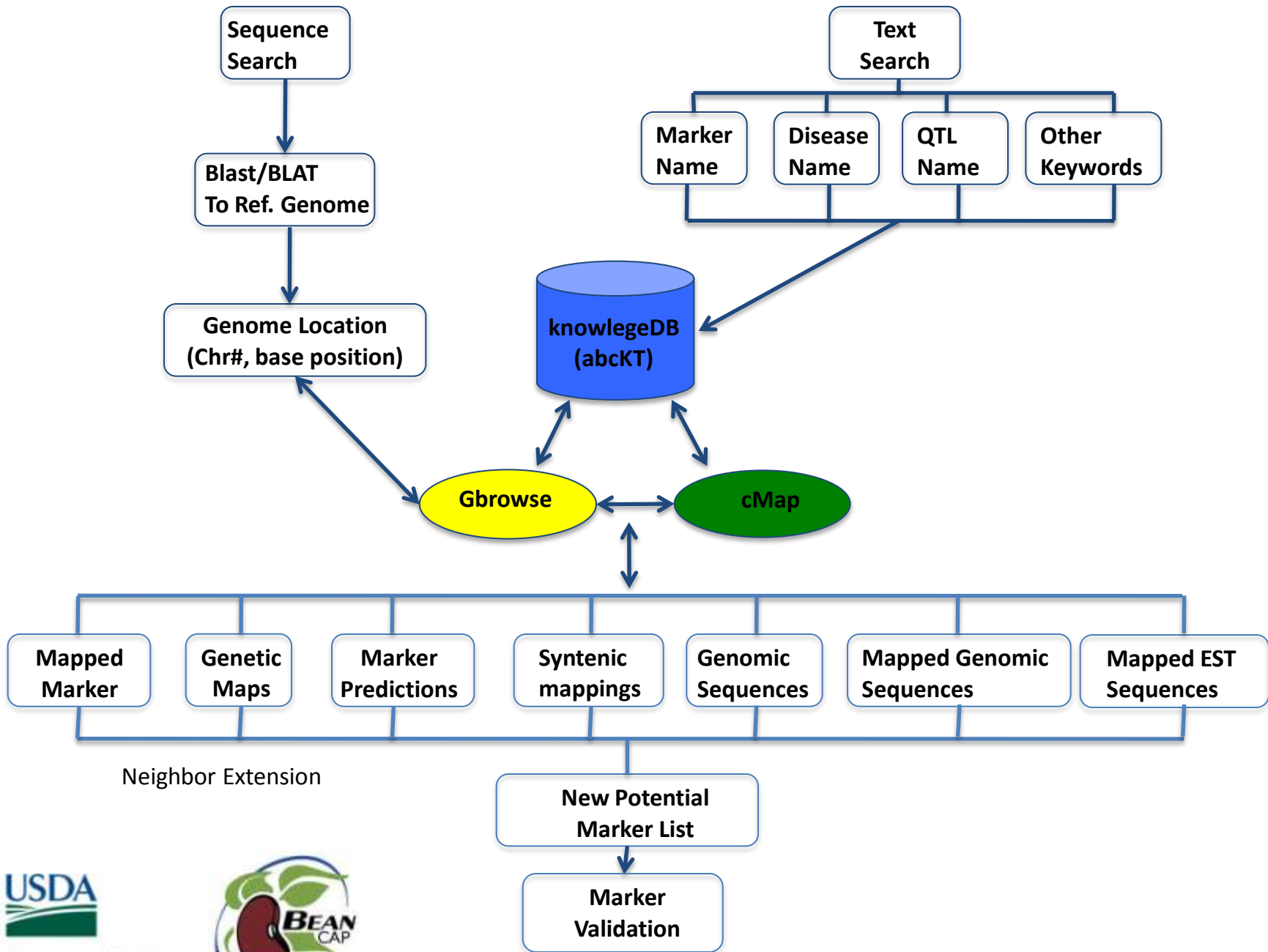
United States Department of Agriculture
National Institute of Food and Agriculture



Bioinformatics in Marker Discovery

- Genomics
 - Genome Browsing
 - Comparative Genomics
 - Large Scale Functional Predictions
- Curated Functional Information
- Genetic Mapping







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breeders were among the early adopters of marker technology to facilitate the mapping and transfer of genes of agronomic interest, particularly disease resistance (e.g., Haley et al. 1993, Miklas et al. 1993; Nodari et al. 1993).

Since then, bean breeders have added many additional markers to their toolbox (Kelly et al. 2003; Miklas et al. 2006), but the results and information have remained dispersed among journals, databases and, yes, laboratory notebooks and cabinets. Furthermore, the lack of DNA sequences has limited the development of additional markers for

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Search markers

Search term:

Search fields:

Blast search (soybean)

(blastn w/ e-value cutoff 0.0001)

Enter one sequence in simple text

This search may take up to a minute





Best *A thaliana* hit: *At5g136301*; Annotation (inheri...

Gene type: marker | Gbrowse mappings (2): [link](#) [link](#)

REFERENCE ID

112

GENE (PHENOTYPE)

Best *A thaliana* hit: *At5g136301*; Annotation (inherited from *A thaliana*) : nobelamin biosynthesis protein; Jalo EEP558 GenBank accession : DX823442; BAT93 GenBank accession : DX823442; Forward primer : GAAGGCATTGATCGTTGAACTTCATAGC; Reverse primer : TATTGTTCACAGGGGTTGGAGTT; BAT93 result : larger; Jalo EEP558 result : smaller; Polymorphic populations : A55/G122; Source : Genomic; feature_aliases : Pv-2490;

MARKER

q2490

SEQUENCE

```
TCGTGAGATTGAGAGAGACTAACCCACACAGTGGGATGGAGTSCAACTTCAGGCCAAGT
TGACAACTGGGTGTACGAAAGAGCCCACTACATTCATTCTAGATGAGAAATGCTGTG
CAAGCTCATGAACTAATCCAACTCCTTCAGGAGCTGGTGCAGACTTCTTGGAGGC
CAATGGACGTGTTACTGGGAACTTCAGAGACAAATATTGAGAAGCTCAAGCASTTGTA
TTCGGAGSTGGAGACAAAATTGAAGGCATTGATCGTTGAACTTCATAGCTACTATTGAS
AAGACTATACACAGTGAAGAGAGAAAAACATTTTCTAATGTGATTTTGTACTTT
```

GENBANK CODE

DX823442

FORWARD PRIMER

GAAGGCATTGATCGTTGAACTTCATAGC

REVERSE PRIMER

TATTGTTCACAGGGGTTGGAGTT

LINKAGE GROUP

1

REFERENCE

P. McClean, pers. comm.

HYPERLINK

[u'http://lis.comparative-legumes.org/cgi-bin/cmap/viewer?mapMenu=amp;featureMenu=amp;corrMenu=amp;displayMenu=amp;advancedMenu=amp;ref_species_acc=Pvs&ref_map_set_acc=PvMcCleanNDSU2007&sub=Show+Selected+Set&27s+Map&prev_ref_species_acc=Pvs&prev_ref_map_set_acc=0&highlight=amp;pixel_height=amp;image_type=png&date_source=cmap_preview,']

Search Markers

Search term:

Search fields:

Results

[EXPORT ALL](#)

1. Best *A thaliana* hit AT23434
2. Best *A thaliana* hit AT23432
3. (None)
4. Pfe-1: halo blight (HB) resist



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Search

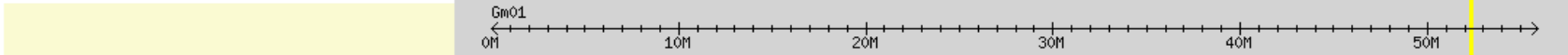
Landmark or Region:

Gm01:52340090..5234500 Search

Data Source

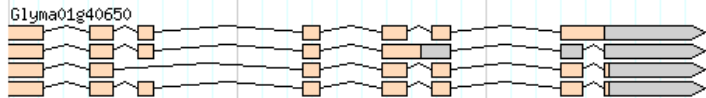
Common Bean Gbrowse based on Soybean (Glyma1, Dec2008, ftp.jgi-psf.org)

Overview

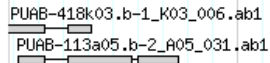


Details

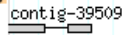
Soybean Gene



Phaseolus vulgaris BAT93 MF GSR reads



Phaseolus vulgaris BAT93 MF GSR assembly



Alignments of Pv reads containing predicted SSRs (SSR finder)

Alignments of Pv reads containing predicted SSRs (ssrit)

Alignments of Pv reads containing predicted SSRs (QDD)

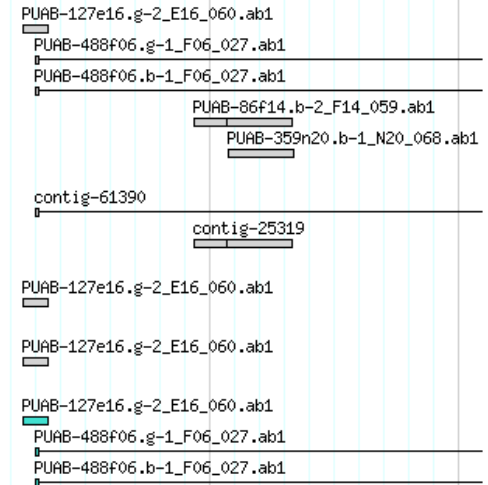
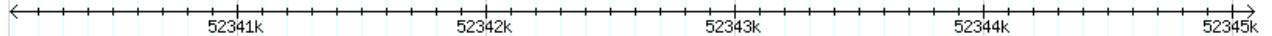
STS markers



Reports & Analysis:

Annotate Restriction Sites Configure... Go

Scroll/Zoom: << < > >> Show 5 kbp + >>> Flip



SSR Motif Discovery

1. Genome survey sequence (funded by the ABC project)
 - 1x methyl-filtrated DNA of breeding line BAT93
 - Good quality reads: 414,923
 - Average length: 635 bp
 - Total sequence: 263.5 Mbp (~ 44% of genome)
 - Probable or possible cytoplasmic sequences: 31,985
 - Hits to *Phaseolus*: 51% (note: *Phaseolus* BLAST database: all available markers, BES, all nucleotide/EST sequences in NCBI (E-value cutoff: E-10))



SSR Motif Discovery (II)

2. SSR detection *in silico*:

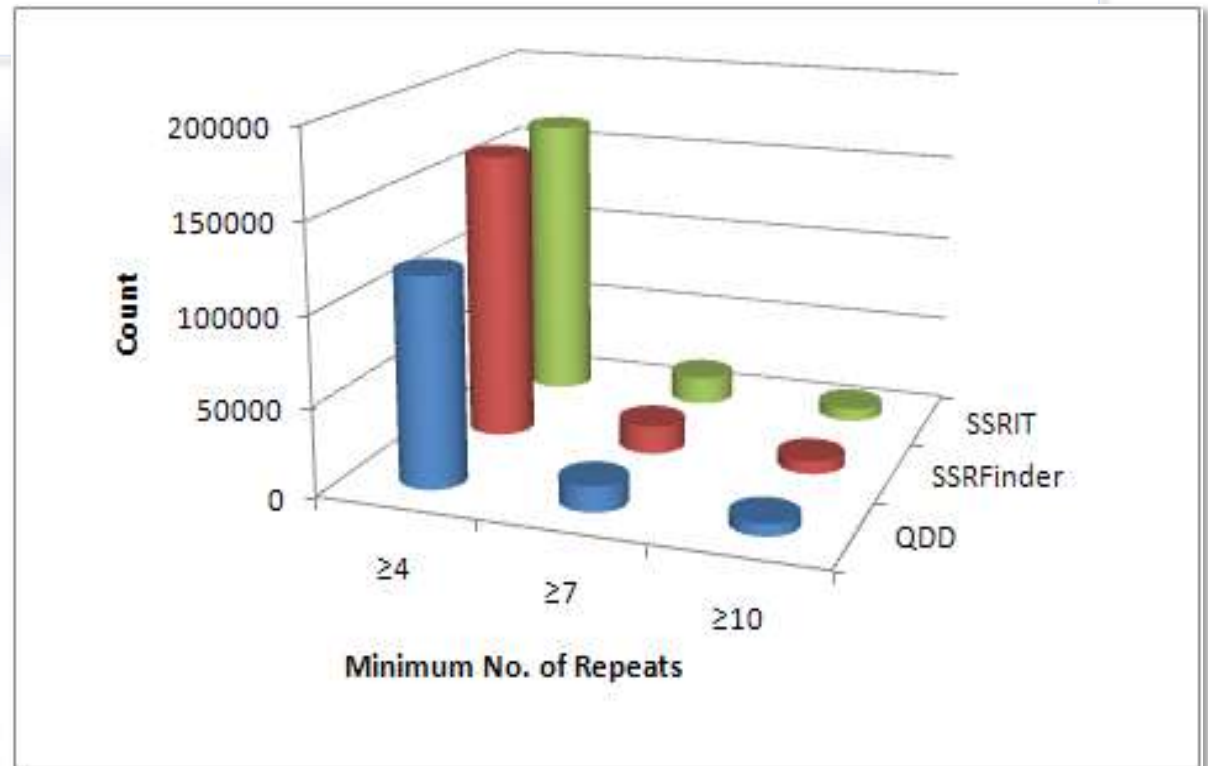
- Three programs:
 - SSRFinder (Sanchez-Villeda et al. 2003, http://maizemap.org/bioinformatics/SSRFINDER/SSR_Finder_Download.html)
 - SSRIT (Temnykh et al. 2001, <http://www.gramene.org/db/markers/ssrtool>)
 - QDD (Megléczy et al. 2010, <http://gsite.univ-provence.fr/gsite/Local/egee/dir/megleczy/QDD.html>)
- Common set of parameters:
 - Repeat lengths: 2 to 6
 - Minimum number of repeats: 4
 - No multiples of repeats



SSR Motif Discovery (III)

Result 1:

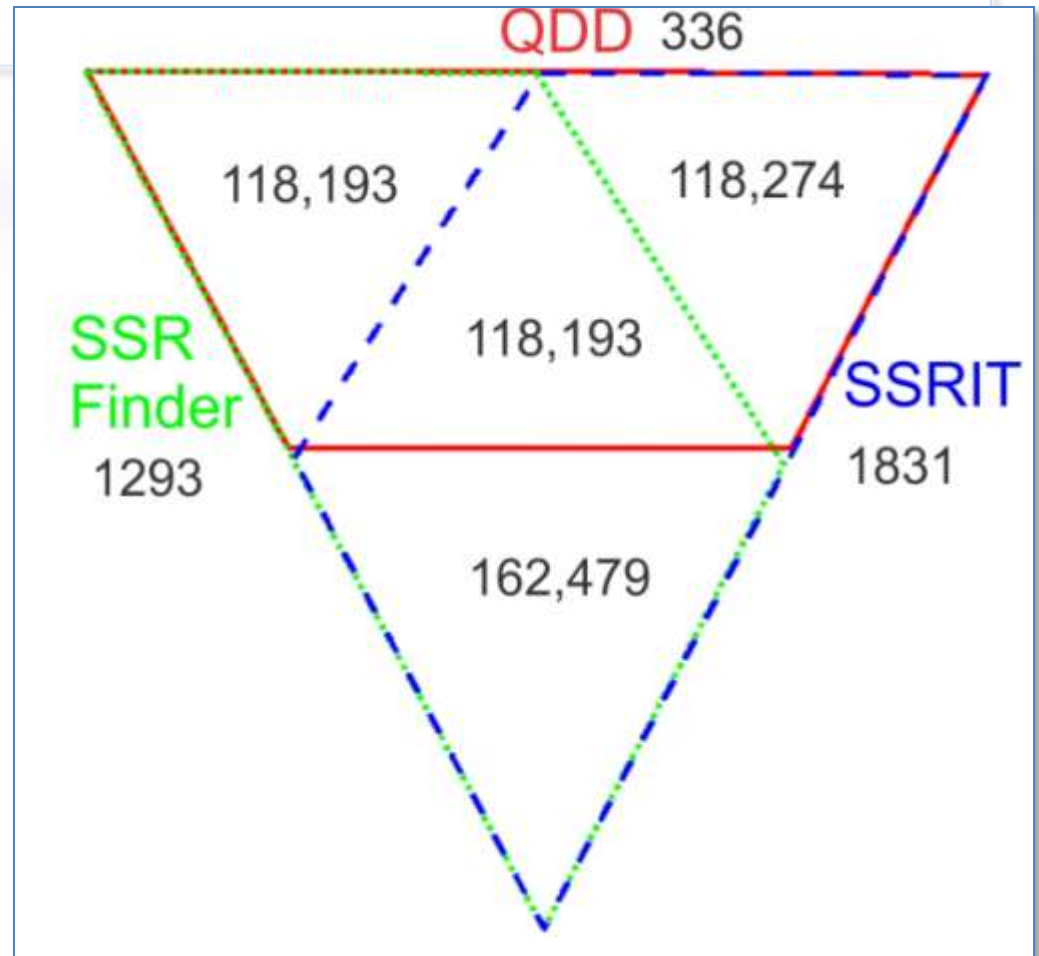
- There is a large number of SSRs: >150,000; comparable between SSRFinder and SSRIT, 1/3 less in QDD
- Sharp drop-off after filtering for repeat number



SSR Motif Discovery (IV)

Result 2:

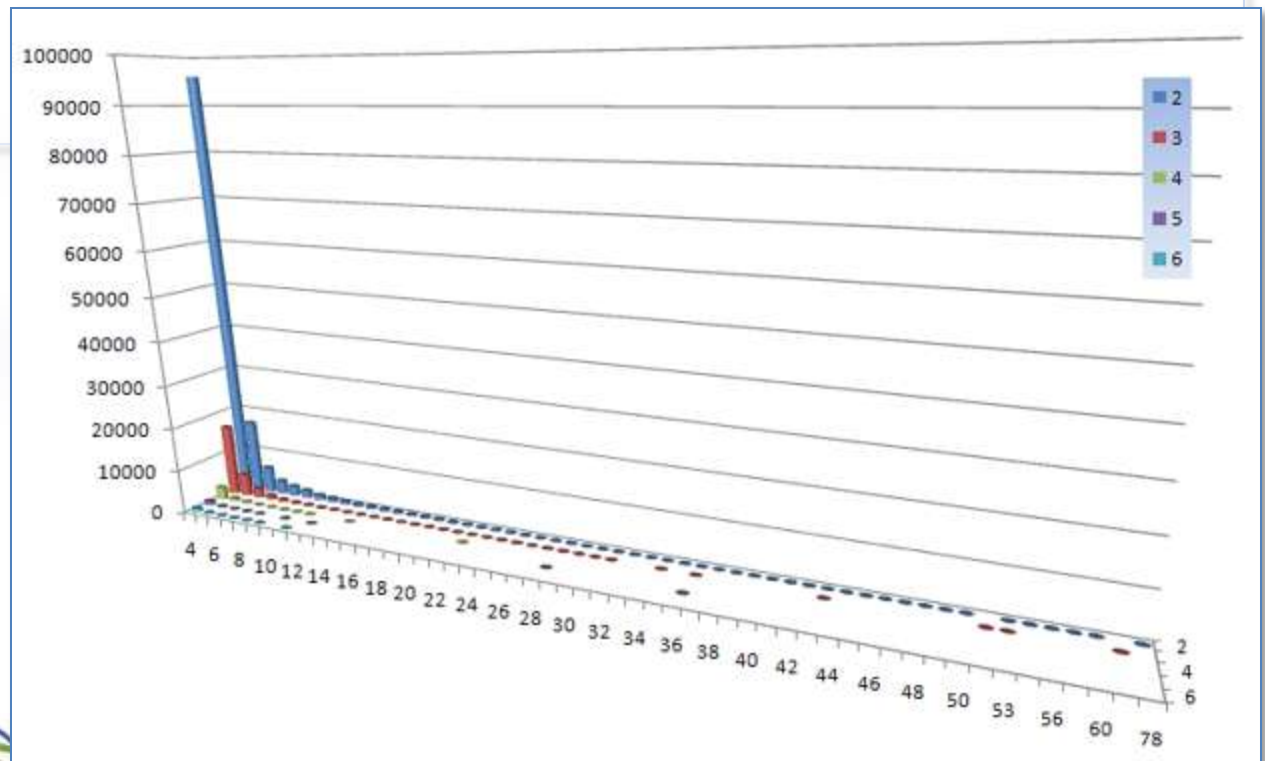
There is a large overlap among programs, with few unique SSRs identified by individual programs



SSR Motif Discovery (V)

Result 3:

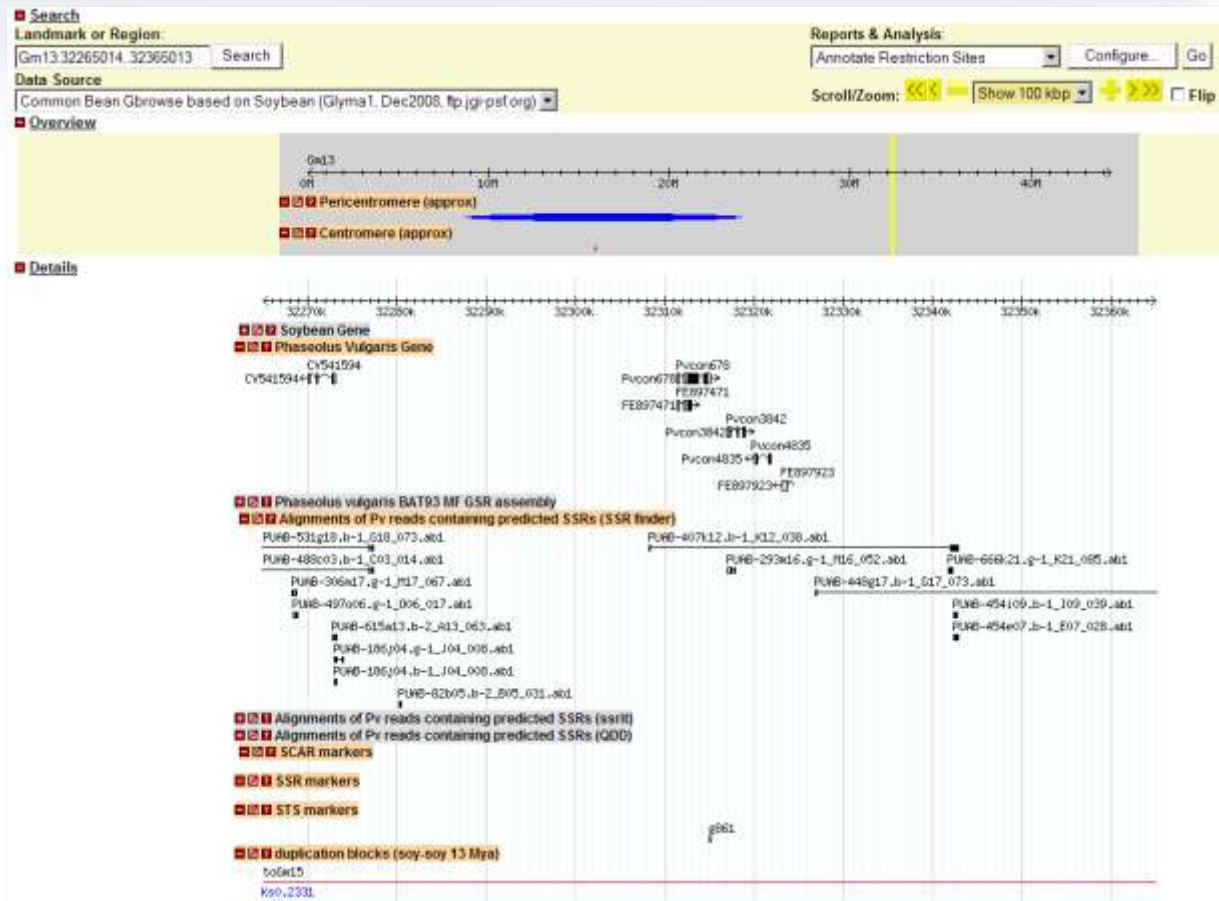
The majority of SSRs are di-nucleotides, with few repeats; the polymorphism levels of the different classes remains to be determined.



SSR Motif Discovery (VI)

Result 4:

The sequence reads containing SSRs have been mapped onto the soybean whole-genome sequence displayed in PhaseolusGenes



Adriana Navarro Gómez



United States Department of Agriculture
National Institute of Food and Agriculture



QTLs-Literature review

2500 papers (abstracts and whole papers) read:

- Bean Improvement Cooperative (BIC)
- Google Scholar
- ISI Web of Knowledge
- Crop Science
(<https://www.crops.org/publications/cs>)
- Journal of the American Society for Horticultural Science (<http://journal.ashspublications.org/>)
- Other Journal's web sites



> 45 papers used
From 1993 to 2011



Example 1

QTL for seed iron and zinc concentration and content in a Mesoamerican common bean (*Phaseolus vulgaris* L.) population

Matthew W. Blair · Juliana I. Medina ·
 Carolina Astudillo · Judith Rengifo ·
 Steve E. Beebe · Gloria Machado · Robin Graham

Theor Appl Genet

Table 4 Quantitative trait loci (QTL) for iron and zinc concentrations (parts per million, ppm) or contents (µg/seed) and seed size with associated markers and linkage groups (LG) identified by composite interval mapping in the G14519 × G4825 population

Trait	Location	Method ^a	QTL ^b	LG	Marker	LOD ^c	R ²	TR ²	Additivity	Source
Iron concentration (ppm)	Darién	AA	QFeDaAA6.1	6	BM239	4.71	23.27	34.66	4.78	G14519
	Palmira	AAS	QFePaAA7.1	7	BM248	2.99	9.57	41.74	3.88	G4825
	Popayán	AAS	QFePoAA6.1	6	BM158	5.10	19.80	34.30	3.83	G14519
	Popayán	ICP	QFePoICP6.1	6	BM158	5.42	18.26	36.05	3.26	G14519
	Darién	AA	QZnDaAA6.2	6	V1001B	5.24	38.42	65.10	8.77	G4825
	Darién	AAS	QZnDaAA6.1	6	H1201A	4.44	17.83	26.68	3.30	G14519
Zinc concentration (ppm)	Palmira	AAS	QZnPaAA6.1	6	BM158	4.87	14.39	36.96	1.29	G14519
	Palmira	AAS	QZnPaAA6.2	6	H1201A	2.85	10.05	37.02	1.18	G4825
	Popayán	AAS	QZnPoAA2.1	2	PV15	3.92	11.94	43.11	1.41	G4825
	Popayán	AAS	QZnPoAA3.1	3	BMd1	2.92	10.52	46.24	1.32	G4825
	Popayán	AAS	QZnPoAA6.1	6	BM158	5.34	17.36	43.98	1.72	G14519
	Popayán	ICP	QZnPoICP6.1	6	BM158	4.92	28.91	50.36	2.27	G14519
Seed weight (g/100 s)	Darién	100SW	SWS.1	5	K1201B	2.76	10.32	26.47	1.94	G14519
	Darién	100SW	SWT.1	7	BM248	2.53	10.58	28.52	1.82	G14519
Iron content (µg/seed)	Darién	AAS	QFe_contDaAA6.1	6	BM239	7.54	55.17	56.20	0.53	G4825
	Palmira	AAS	QFe_contPaAA1.1	1	W0901B	3.56	20.13	41.73	0.28	G14519
Zinc content (µg/seed)	Palmira	AAS	QZn_contPaAA7.1	7	BM201	2.80	11.14	30.76	0.25	G4825
	Darién	AAS	QZn_contDaAA1.1	1	W0901B	2.73	14.16	30.61	0.08	G14519

^a Methods refer to atomic absorption spectroscopy (AAS or AA) and inductively coupled plasma-optical emission spectroscopy (ICP).
^b QTL name based on method and association with iron (Fe) or zinc (Zn) as well as location of experiment in Darién (Da), Palmira (Pa) or Popayán (Po) and QTL order on linkage group.
^c QTL surpassing empirical LOD thresholds based on 1,000 permutations recommended by Churchill and Doerge (1994).

Table 5 Markers significantly associated with iron and zinc content (in µg/seed) and seed size identified with single point regression analysis in the G14519 × G4825 population

QTL ^a	Chromosome	Marker	LOD	R ²	Significance	Source
qSW_po2.1	2	ATA16	0.96	40.31	0.047*	G14519
qSW_po11.1	11	BM239	2.14	13.80	0.002**	G14519
qSW_pa5.1	5	K1201B	0.96	4.52	0.038*	G14519
qSW_pa6.1	6	ATA173	0.89	5.68	0.046*	G14519
qSW_pa10.1	10	ATA216	1.17	5.41	0.022*	G14519
qFeCont_poICP	11	BM239	2.07	11.90	0.002**	G14519
qFeCont_poAA	11	BM239	1.86	9.96	0.004**	G14519
qZnCont_poAA	3	BMd1	0.97	6.54	0.037*	G14519
qZnCont_poAA	11	BM239	1.80	12.23	0.005**	G14519
qZnCont_poICP	11	BM239	1.80	10.90	0.005**	G14519

^a QTL name based on method either atomic absorption spectroscopy (AA) or inductively coupled plasma-optical emission spectroscopy (ICP) and association with iron (Fe) or zinc (Zn) as well as location of experiment in Darién (Da), Palmira (Pa) or Popayán (Po) and QTL order on linkage group.
^b Level of significance corresponding to $P < 0.05$ (*) and $P < 0.01$ (**).



Example 2

QTL Mapping of Resistance to *Thrips palmi* Karny in Common Bean

A. Frei, M. W. Blair,* C. Cardona, S. E. Beebe, H. Gu, and S. Dorn

QTL Analysis for Thrips Resistance Traits

In the IM analysis, LOD thresholds of 2.6 to 2.8 (LR 11.82–12.83) were calculated based on the result of a 1000-fold permutation test for each trait analyzed and were used to declare a putative QTL as significant. One major thrips-resistance QTL was identified on LG b06 for both damage and RA in the season 1999B (Fig. 2 and 3). This QTL was named *Tpr6.1*, and the highest LOD at the terminal marker BMc128 was 5.8 (LR 27.2) for damage in 1999B and 4.6 (LR 21.6) for RA in 1999B. This QTL explained 26.8% of variance for damage in 1999B (total R^2 of 0.278), and 21.9% of variance for RA in 1999B (total R^2 of 0.230). The *Tpr6.1* QTL was also associated with RA and damage in 2000B, but at LOD values slightly lower (<2.8 and <2.1, respectively) than the calculated threshold for this season (Fig. 2).

As the correlation between seasons was significant, JIM analysis was conducted and revealed QTLs on LGs b02, b03, and b08 (Fig. 2). Joint analysis for damage and RA within the same season confirmed the QTL for season 1999B on LG b06 in the BMc128-O1505 interval and identified a peak LOD (6.3) that was also found at the terminal end of this interval. Meanwhile, joint analysis for both damage and RA scores analyzed jointly across three seasons also revealed a QTL for both dam-

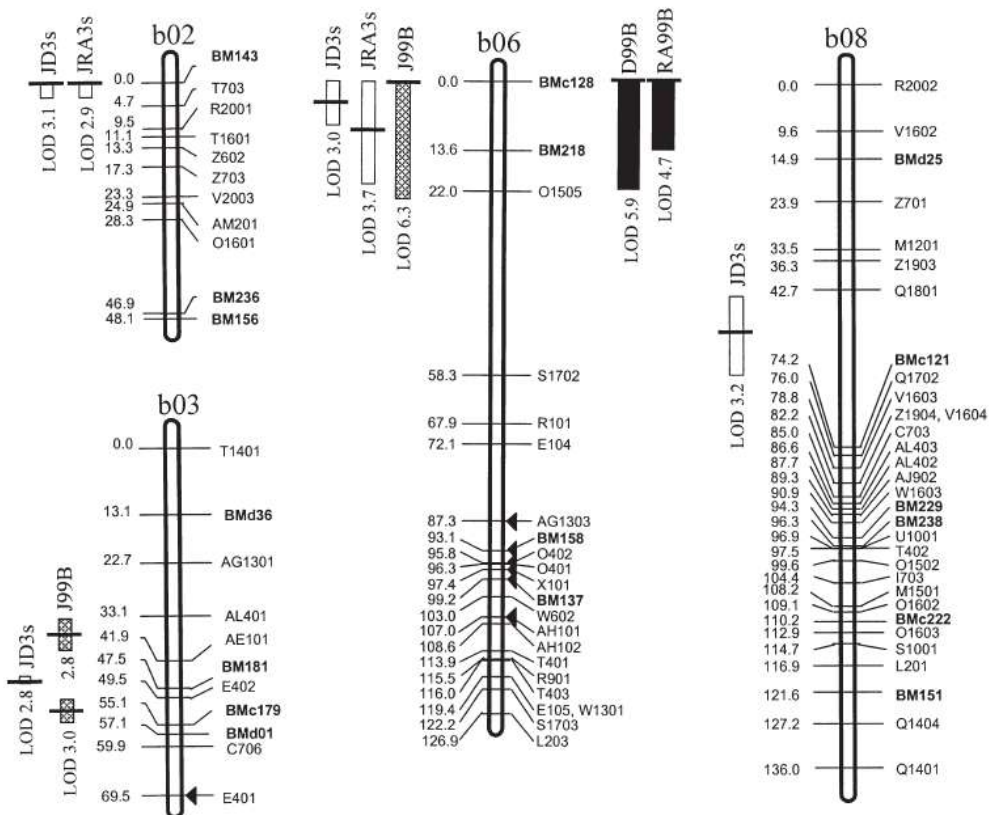


Fig. 2. Genetic linkage map for the BAT 881 × G 21212 population, showing linkage groups that contained significant QTLs for *Thrips palmi* resistance. The QTLs were identified by single interval mapping (black boxes, D99B = damage 1999B, RA99B = reproductive adaptation 1999B), by joint interval mapping for resistance traits within the same season (gray boxes, J99B = joint for damage and RA in 1999B) and by joint interval mapping for each resistance trait across three seasons (white boxes, JD3s = joint for damage across three seasons, JRA3s = joint for RA across three seasons). The LOD peaks are indicated by black horizontal bars, and their values are shown below the QTL box.



QTL-survey

Information on QTLs was collected for addition to PhaseolusGenes (Table 1) from > 45 papers.

Table 1. QTL information collected

Trait	Female, male parent	R^2	Markers, Flanking markers
Year	QTL	LOD	Source of allele
Location	Population size, type	P value	Type of analysis
Literature source	URL		Trait ontology



United States Department of Agriculture
National Institute of Food and Agriculture



More Information Associated with Each QTL than with Major Genes or Markers → Adjust Structure of Database

QTLs ADRGARIA - Microsoft Excel

	H	I	J	K	L	M	N	O	P	Q	R	
	Location	Year	Citation	Fem parent	Male parent	Source	Additivity	R2 (%)	LOD	P value	Pop size	And
1			Remans et al., 2008	BAT477	DOR364	BAT477	0.08	10.9	3		90	
2			Remans et al., 2008	BAT477	DOR364	BAT477	0.17	36.1	3		90	
3	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-4.85	15.00			157	
4	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-2.19	6.00			157	
5	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-2.37	16.00			157	
6	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-2.75	18.00			157	
7	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-2.36	22.00			157	
8	Popayán A (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-1.46	13.00			157	
9	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-1.46	13.00			157	
10	Popayán A (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-2.41	22.00			157	
11	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-1.67	16.00			157	
12	Popayán A (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-2.15	11.00			157	
13	Villaviciosa (Spain)	2004	Pérez-Vega et al., 2010	Xana	Cornell49242	Cornell49242	-8.17	55.00	7.1		104	
14	Villaviciosa (Spain)	2004	Pérez-Vega et al., 2010	Xana	Cornell49242	Xana	4.96	19.00	3.8		104	
15	Villaviciosa (Spain)	2004	Pérez-Vega et al., 2010	Xana	Cornell49242	Xana	2.69	11.00	3		104	
16	Villaviciosa (Spain)	2005	Pérez-Vega et al., 2010	Xana	Cornell49242	Cornell49242	-3.54	14.90	4.6		104	
17	Villaviciosa (Spain)	2005	Pérez-Vega et al., 2010	Xana	Cornell49242				n.s.		104	
18	Villaviciosa (Spain)	2005	Pérez-Vega et al., 2010	Xana	Cornell49242	Xana	3.91	18.70	5.2		104	
19	Villaviciosa (Spain)	2006	Pérez-Vega et al., 2010	Xana	Cornell49242				n.s.		104	
20	Villaviciosa (Spain)	2006	Pérez-Vega et al., 2010	Xana	Cornell49242	Cornell49242	-2.45	11.00	3.8		104	
21	Villaviciosa (Spain)	2006	Pérez-Vega et al., 2010	Xana	Cornell49242				n.s.		104	
22	Popayán A (Colombia)		Blair et al., 2006	Cerinza	G24404	Cerinza	4.08	37.00			157	
23	Popayán A (Colombia)		Blair et al., 2006	Cerinza	G24404	Cerinza	3.93	14.00			157	
24			Beattie et al., 2003	WO3391	OAC Speedvale				2.55		110	
25			Beattie et al., 2003	WO3391	OAC Speedvale				5.72		110	
26			Beattie et al., 2003	WO3391	OAC Speedvale				8.08		110	
27			Pérez-Vega et al., 2010	Xana	Cornell49242	Cornell49242	-4.22	12.30	4.4		104	
28			Pérez-Vega et al., 2010	Xana	Cornell49242	Xana	5.7	24.30	3.8		104	
29			Pérez-Vega et al., 2010	Xana	Cornell49242	Xana	4.98	18.00	6.3		104	
30			Pérez-Vega et al., 2010	Xana	Cornell49242	Cornell49242	-4.37	11.00	4.1		104	
31			Pérez-Vega et al., 2010	Xana	Cornell49242	Xana	6.14	22.00	7.6		104	
32			Johnson and Gepts 2002	California Dark red Kidney	Yolano	California Dark red Kidney	4.7	14.00			150	
33	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-9.8	9.00			157	
34	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	Cerinza	6.7	10.00			157	
35	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	G24404	-6.09	8.00			157	
36	Danién (Colombia)		Blair et al., 2006	Cerinza	G24404	Cerinza	5.82	13.00			157	
37			Beattie et al., 2003	WO3391	OAC Speedvale				2.5		110	
38			Beattie et al., 2003	WO3391	OAC Speedvale				2.58		110	

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QTL-survey

925 QTLs (trait x population x environment) have been reported.
They concerned all major organs (roots, stem, leaves, pods, and seeds)

Plant 364

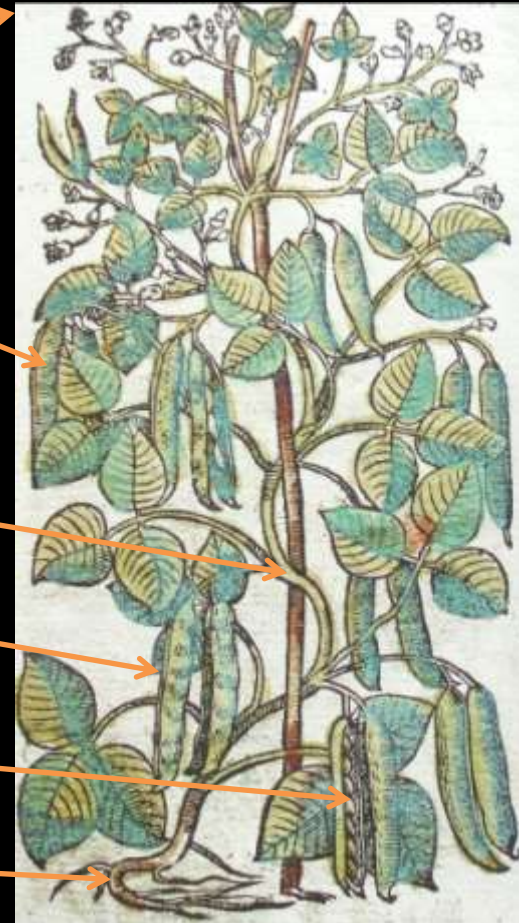
Leaf 264

Stem 19

Pod 32

Seed 142

Root 104



QTL-survey

And agronomically important trait categories: development and performance, biotic and abiotic stresses, nutritional composition

Trait category	QTL number
Abiotic stress T	98
Biotic stress R	521
Composition	66
Development/performance	240
Total	925



United States Department of Agriculture
National Institute of Food and Agriculture



Average Genetic Parameters for QTLs

33 populations; average pop. size: 100; 28 QTLs per population

	R ² (%)	LOD	P value
Average (925 QTLs)	17.2	5.0	0.03



What is Next for QTLs?

- Integrate the information into PhaseolusGenes
 - Searchable table
 - Links with:
 - Gbrowse (for markers)
 - Cmap
- Downloadable table with all QTL information

What is Next for PhaseolusGenes?

- QTL information
- Complete mapping information in BJ (and other populations)
- Add other markers:
 - SNPs: Hyten et al. 2010, BeanCAP, etc.
 - COS markers
 - BAC ends
- Integration of whole-genome sequences
- Trait ontogenies

