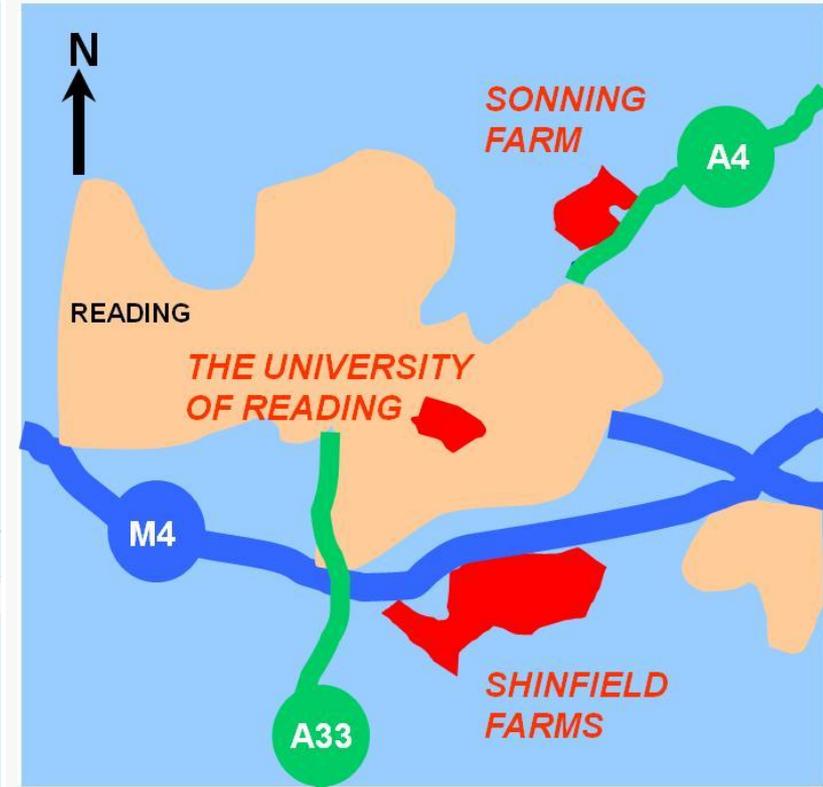
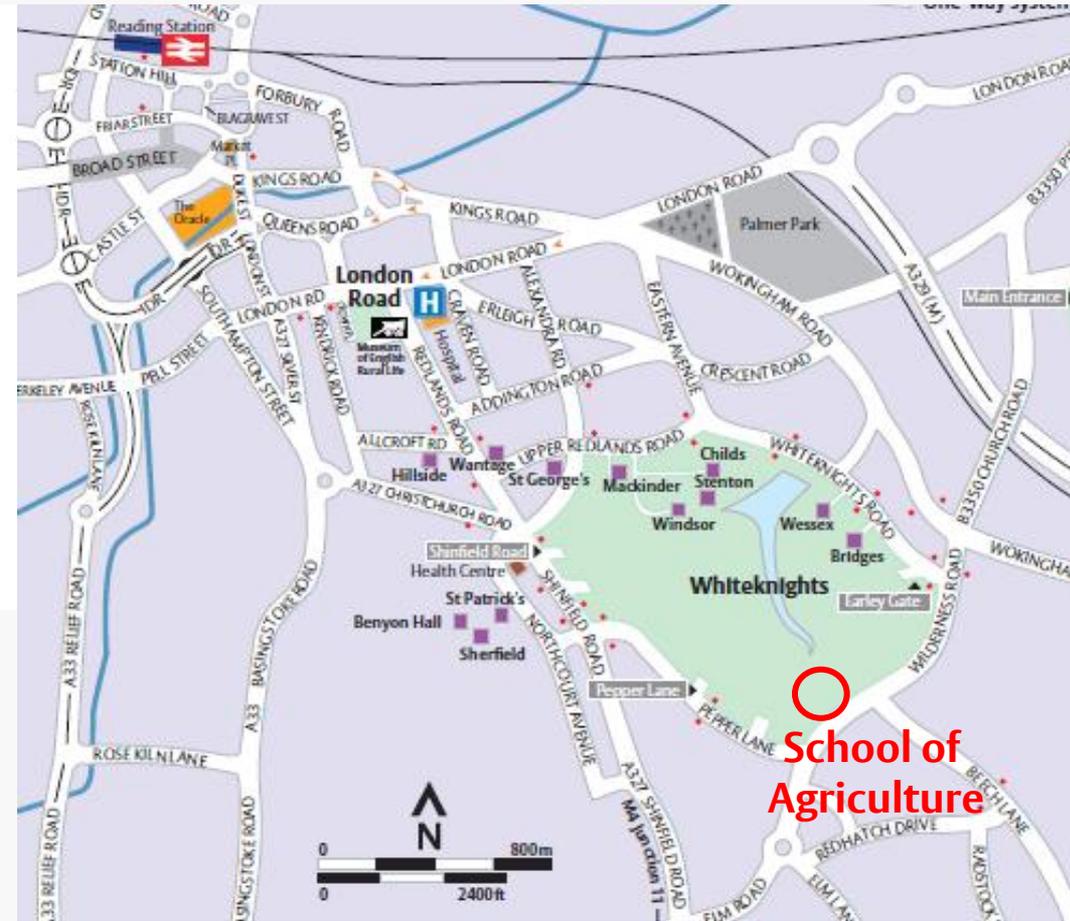




SNP discovery and validation for genomic-assisted breeding of faba bean (*Vicia faba* L.)

Donal O'Sullivan
8th Oct 2013, Rabat

School of Agriculture, Reading



Ag research supported by 2 farms

All-faculty campus near town centre

Sonning farm (180ha)



Acknowledgements:

NIAB Colleagues – Jane Thomas, Amanda Cottage, Anne Webb, Krystyna Gostciewicz, Douglas Hobbs

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MergeMap (UC-Davis, Close Lab), Strudel (JHI, Matthews et al)



Technology Strategy Board
Driving Innovation



Why faba bean?

1. Faba bean is the broadacre grain legume best placed (in UK) to provide:
 - sustainably produced protein for food and feed
 - return N to soil in a sustainable rotation
2. We have identified several researchable topics that can be readily addressed IF we have the right tools to do so.

SNP DISCOVERY



ALBUS
nematode-susceptible
“white” flower



BPL10
nematode resistant
“normal” flower

23.2k contigs

454 sequence of RNA
From 10-day old seedlings

26.7k contigs

Alignment of 14k gene
transcripts present in
both lines to Mt3.5
predicted CDSs

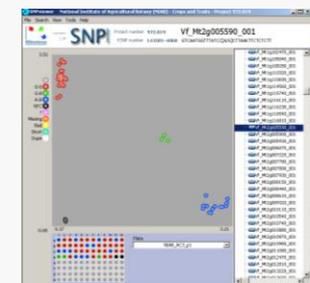


40,000 putative SNPs

```
>ALBUS AGGACTTGTACGTACG
>BPL10 AGGACTATGTACGTACG
>Mt3.5 ACGACTTGTAGGTACG
```

888 new
KASPAR
assays
designed

Validation on 37
reference lines



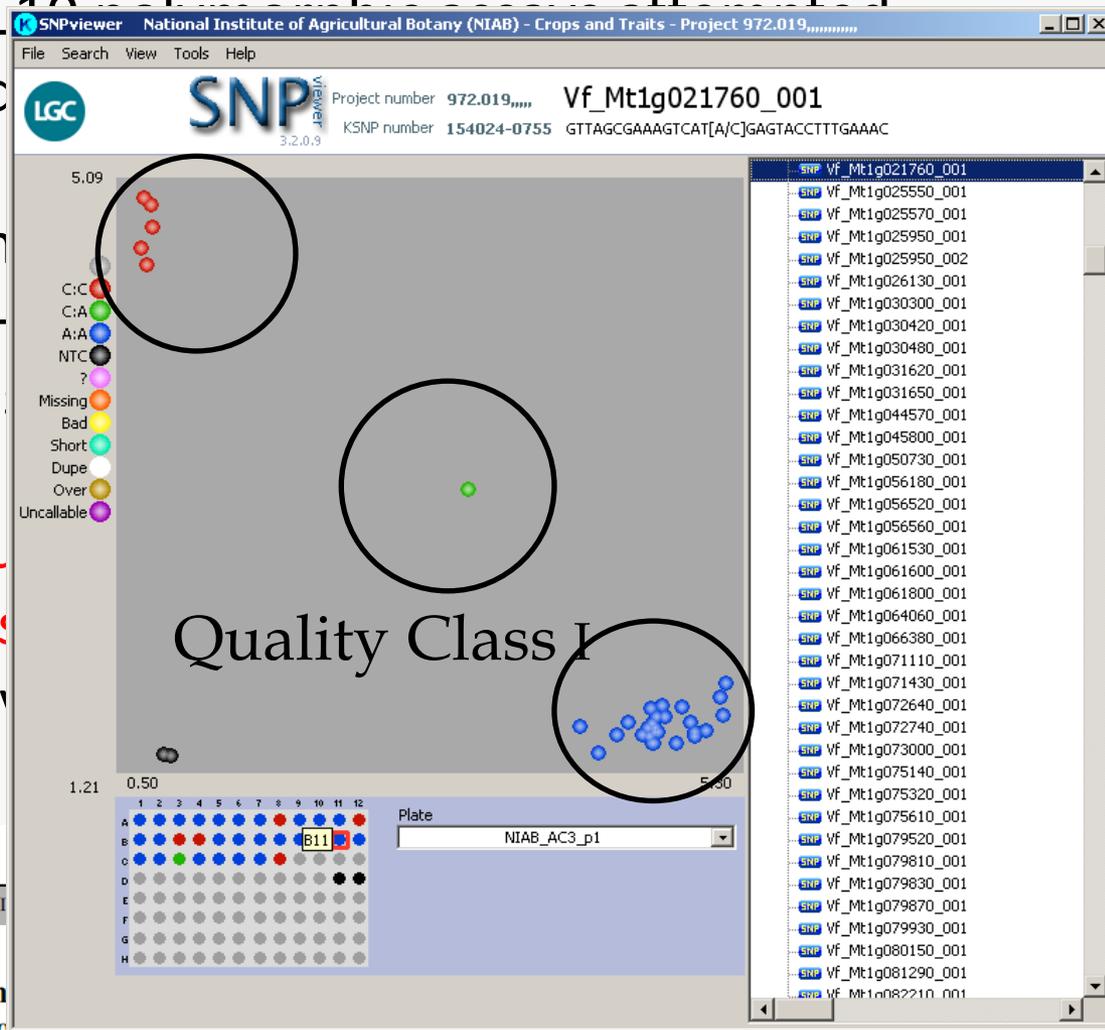
SNP VALIDATION

KASPar assay validation summary

- 888 new Albus-BPL10
- Genotyped on 37 c
- Results:

Class I - High
 Class II & III -
 Class IV - Les
 Class V - Jun

- Paid for by 5 institu
 - Validation panel als
 - Added SNP assays v
- Cottage et al



Mol Breeding
 DOI 10.1007/s11032-012-9745-4

SHORT COMMUNICATI

Heterozygosity an
 nucelotide polym
 programme

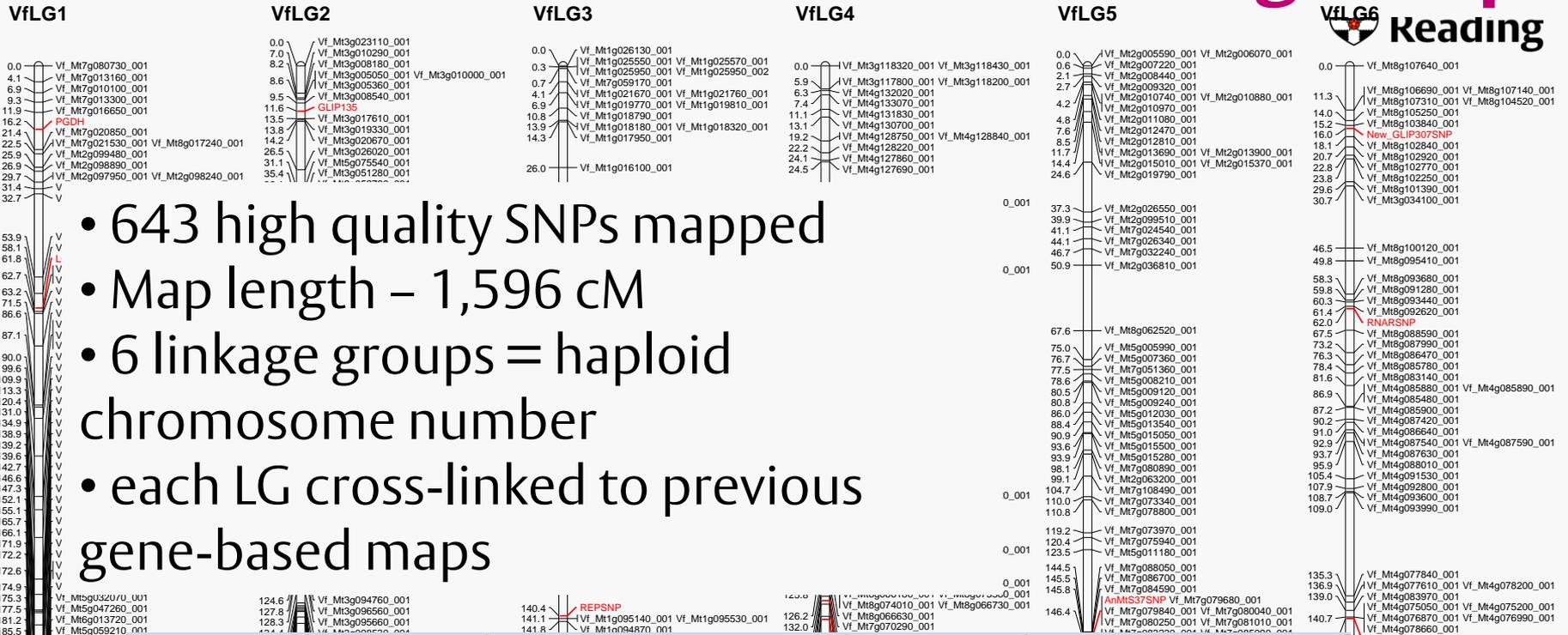
A. Cottage · K. Gostkiewicz · J. E. Thomas ·
 R. Borrows · A.-M. Torres · D. M. O'Sullivan

MAPPING

Populations used

- ALBUS x BPL10 (anchor) – 136 F₂s
 - Segregates for white flower (zero tannin)
- NV657 x NV643 – 170 F₂s
 - Segregates for ZT
- NV644 x NV153 – 125 F₂s
 - Segregates for dwarfism, hilum colour, ZT
- NV639 x NV658 – 50 F₂s
 - Segregates for closed flower mutation
- 128,615 good datapoints
- Call rate across pops ranged from 98-99%

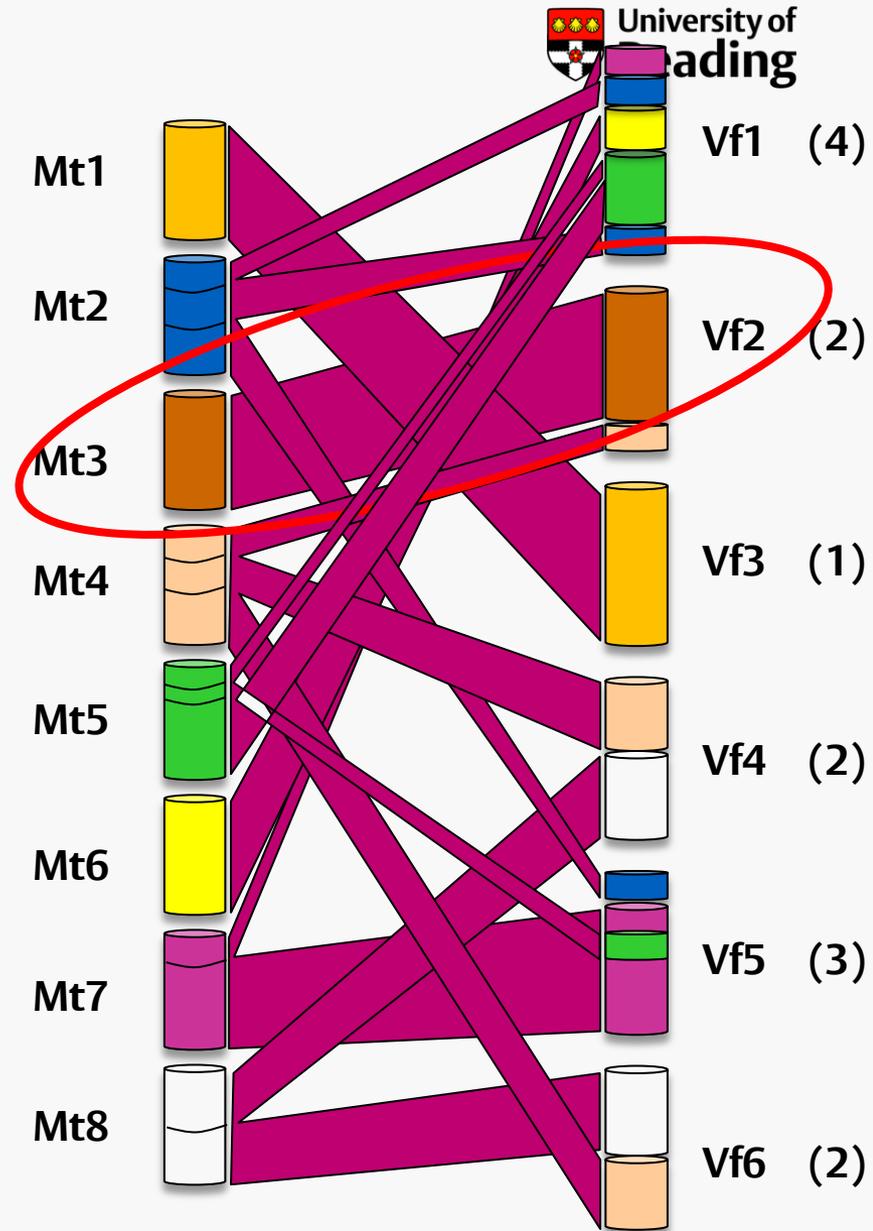
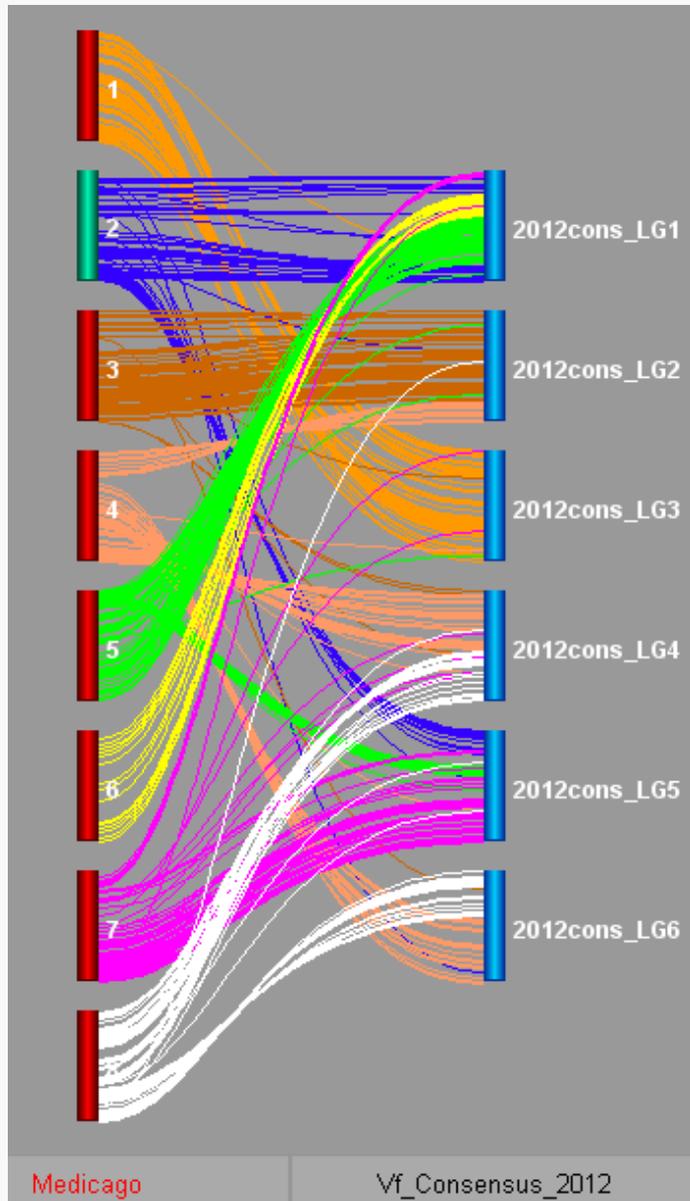
A consensus faba bean SNP linkage map

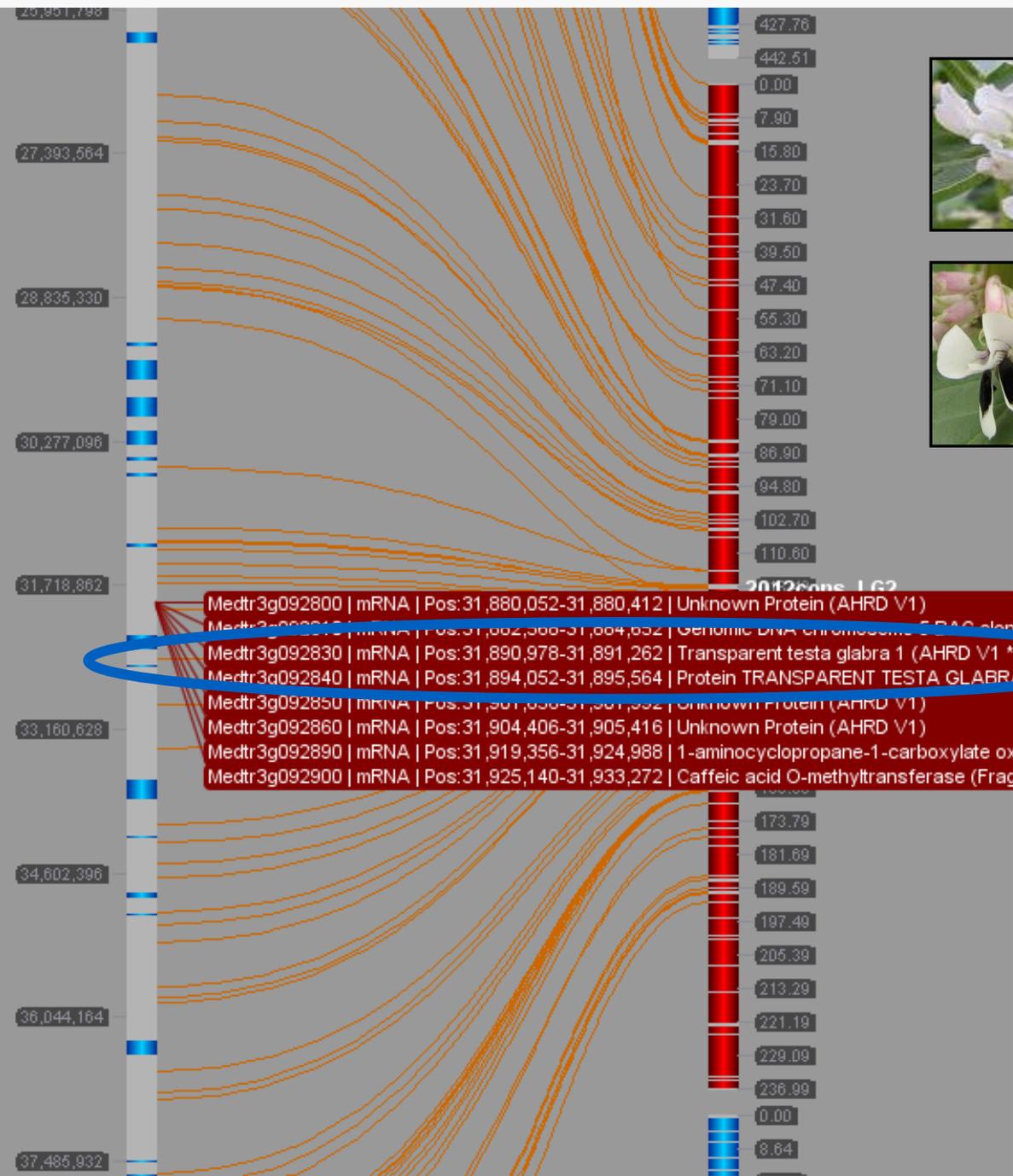


- 643 high quality SNPs mapped
- Map length – 1,596 cM
- 6 linkage groups = haploid chromosome number
- each LG cross-linked to previous gene-based maps

Chr	LG	Mt	# markers	length	mapped traits
I	1	2>7>6>5	187	443 cM	<i>Cf</i>
II	2	3>4	119	237 cM	<i>Zt</i>
III	3	1	81	259 cM	
IV	4	4>8>3	91	218 cM	
V	5	7>5>2	96	234 cM	<i>Dwf1, Hc</i>
	6	8>4	69	205 cM	
			643	1,596 cM	

SYNTENY





ALBUS
“white” flower



BPL10
“normal” flower

The *Vicia faba* orthologue of the WD40 TF *AtTTG1/Pisum A2* gene is a good positional and biological candidate for *ZT*

MUTAGENESIS

Hedin/2 inbred line

- ~Medium-small seeds
- Spring habit
- Highly autofertile
- Highly inbred
- Summer 2013 multiplication
 - 2x pollinator-free cages
 - Isolation distance
 - 2 x single inbred plant progenies
 - 300 plants total
 - c. 25,000 seeds



Mutagenesis 2013/14

- Mutagen dose response and herbicide growth inhibition tests over winter
- Spring 2014 – $>20,000 M_0$ – harvest bulk of $>2,000,000 M_1$
- Winter 2014 – screen batches of 50,000 using a high density seedling screen every 10 days
- Herbicide targets TBC

Sebastien 1992

sulfonylurea-resistant soybean patent

TABLE III

**Number and Type of Soybean Mutants
Selected from Eight M2 Populations**

Population code	Estimated Number of M2 Plants Screened	Mean number of M2 per M1 plant	Number of Putative Mutants saved	
			Resistant	Tolerant
A3205-EMS	100,000	2.5	0	3
Williams-EMS-1	42,000	5.4	0	6
Williams-EMS-2	88,000	11.7	2	10
Williams-NMU-1	55,000	8.9	21	6
Williams 82-NMU-A	26,000	17.3	0	2
Williams 82-NMU-B	30,000	24.0	0	0
Williams	18,000	18.0	0	1

Beware ease of evolving resistance

Table 1. Site of action and Weed Science Society of America mode of action group for herbicide-resistant weeds in the United States based on the number of resistant weed biotypes (summed across states) and number of the weed species (Heap 2012).

Site of action	Group	No. of biotypes	Weed species
ACCase	1	34	13
ALS	2	121	37
Auxins	4	12	8
Carotenoid biosynthesis	28	1	1
Chloroacetamides	15	1	1
Dinitroaniline	3	12	5
EPSPS	9	39	9
Not classified	27	3	1
Organic arsenicals	17	7	1
Protox	14	3	1
PS I	22	5	4
PS II (nitriles)	6	1	1
PS II (triazines)	5	91	25
PS II (ureas)	7	11	7
Thiocarbamates	8	6	5
Total		347	119

Abbreviations: ACCase, acetyl-coenzyme A carboxylase; ALS, acetolactate synthase; EPSPS, enolpyruvyl shikimate-3-phosphate synthase; PS, photosystem.

THANK YOU!
SHOKRUN!
MERCI!