

Understanding raspberry using 'Omics' technologies

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Assisting the raspberry breeding process

- Breeding is a slow process (highly heterozygous, juvenile)
- Difficult to achieve a lot of objectives
- Personal preferences
- Environment/weather
- Reduced actives
- New challenges

Moving targets

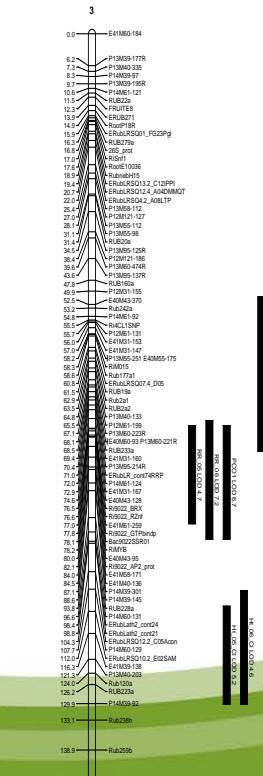
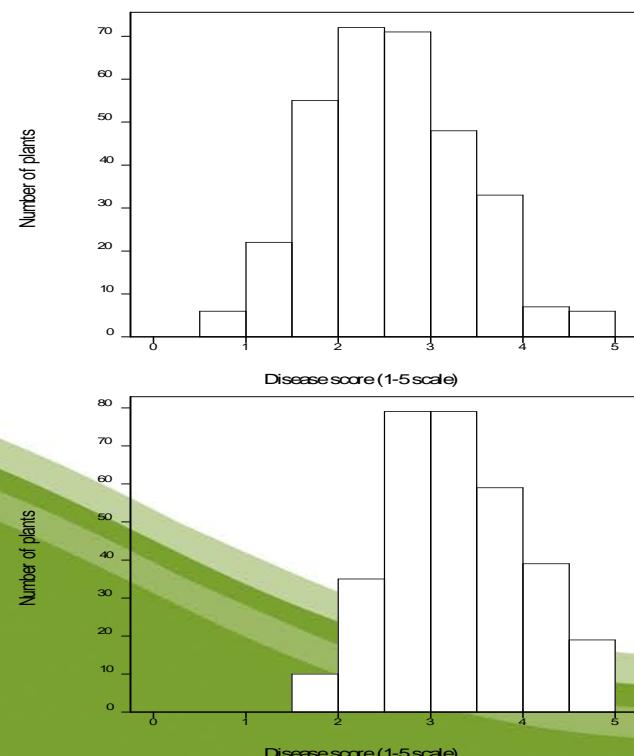


Progress with marker assisted breeding eg. root rot



Some progress understanding phenotype – Mapping raspberry root rot resistance/tolerance

- Linkage map development
- QTL Mapping LG3&6
- Identified marker
- Validation in germplasm
- Marker deployment
- Rub118b for breeding

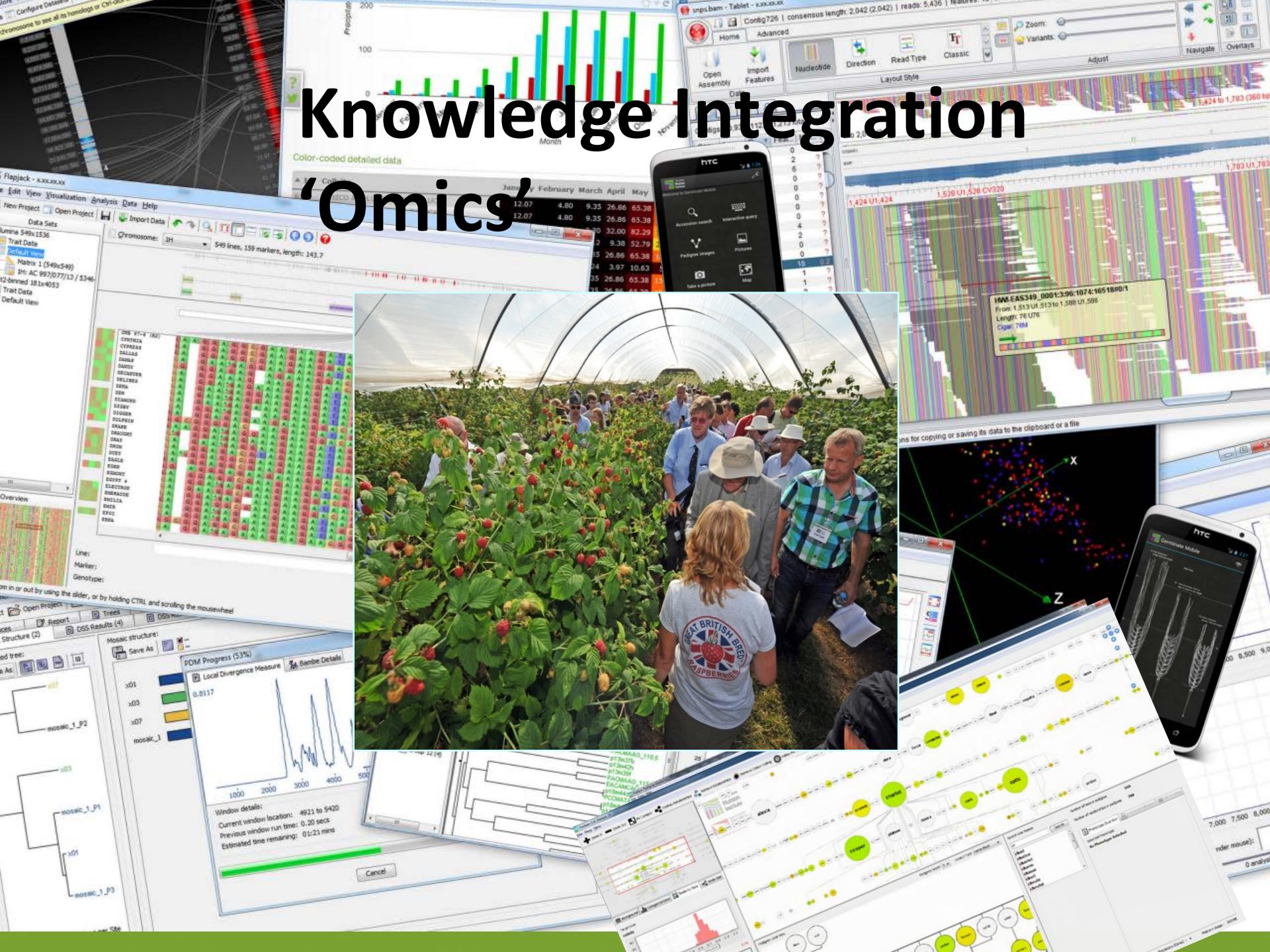


Deployment of markers for resistance to *Phytophthora* root rot

- Markers identified in 2008 (Hortlink 0169)
- Parents with root rot resistance marker identified for crossing in 2009 (25 families)
- Currently >40 selections identified with the marker
- 10 selections fruiting in 5-plant plots in 2013
 - Exceptional root vigour, Two selections with good fruit size, quality and yield
 - Resistance holding in infestation plot
- Better understanding of phenotype



Knowledge Integration 'Omics'



Development, integration and use of “omics” technologies in raspberry breeding



● Genomics

- Genome assembly and annotation – DNA sequence - **information**

● Transcriptomics

- Genes: mRNA transcriptome, alternative splicing, miRNA transcriptome - **active part**

● Metabolomics

- Compounds: sugars, acids, volatiles etc – **flavour, colour etc**

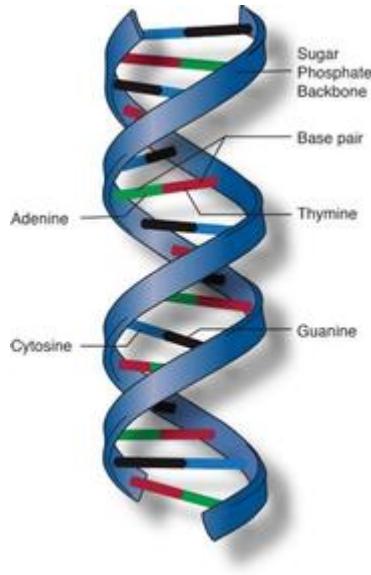
● Phenomics

Response of plant traits to environment/stresses and use of high throughput imaging systems – **physical plant traits**

Genomics

■ **Genomics** - sequencing and analysis of the **genome**.

Actual DNA sequence

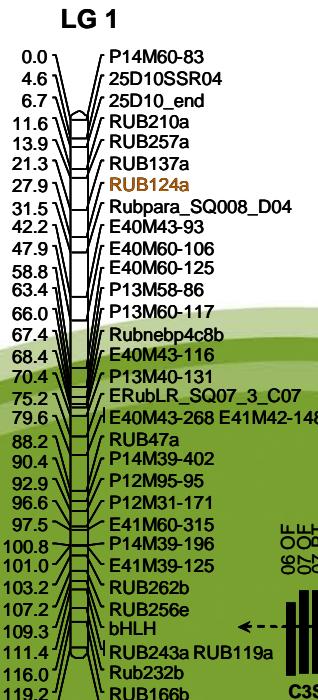


TCGGGGTCCGAGTTGTAATTGTTAGAGGAAGCTCG
ACAGCTCAAATTGAAATCTGGCTCCTCGGGGCGCG
GTACGGGTGCCCTACTGAGTTCCCTGGAACGGGACCG
GGGTGAGAGCCCCGTCTGGTAGGACACCCAGCCCCGT
GTGCGGGTTCCCTCCGAGTTCCCTGGAACGGGACCG
GGTGCGGGTTCCCTCCGAGTTCCCTGGAACGGGACCG
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GGTGCAGGGTTCCCTCCGAGTTCCCTGGAACGGGACCG
AGTTCCCTGGAACGGGACGCCATAGAGGGTGAGAGGCC
AATTGAAATCTGGCTCCCTGGGGCCCGAGTTGAA
GGGTGAGAGCCCCGTCTGGTCGGAAACCCAGCCCCGT
CCACGCCATAGAGGGTGAGAGCCCCGTCTGGTCGGAA

Raspberry Glen Moy genome sequence

- Allows us to make a physical map of the actual sequence and link to the genetic map and to search regions of genetic map for genes related to traits

Genetic map



Loci
Rub124a

QTL Peak
 $5 \geq LOD \geq 20$



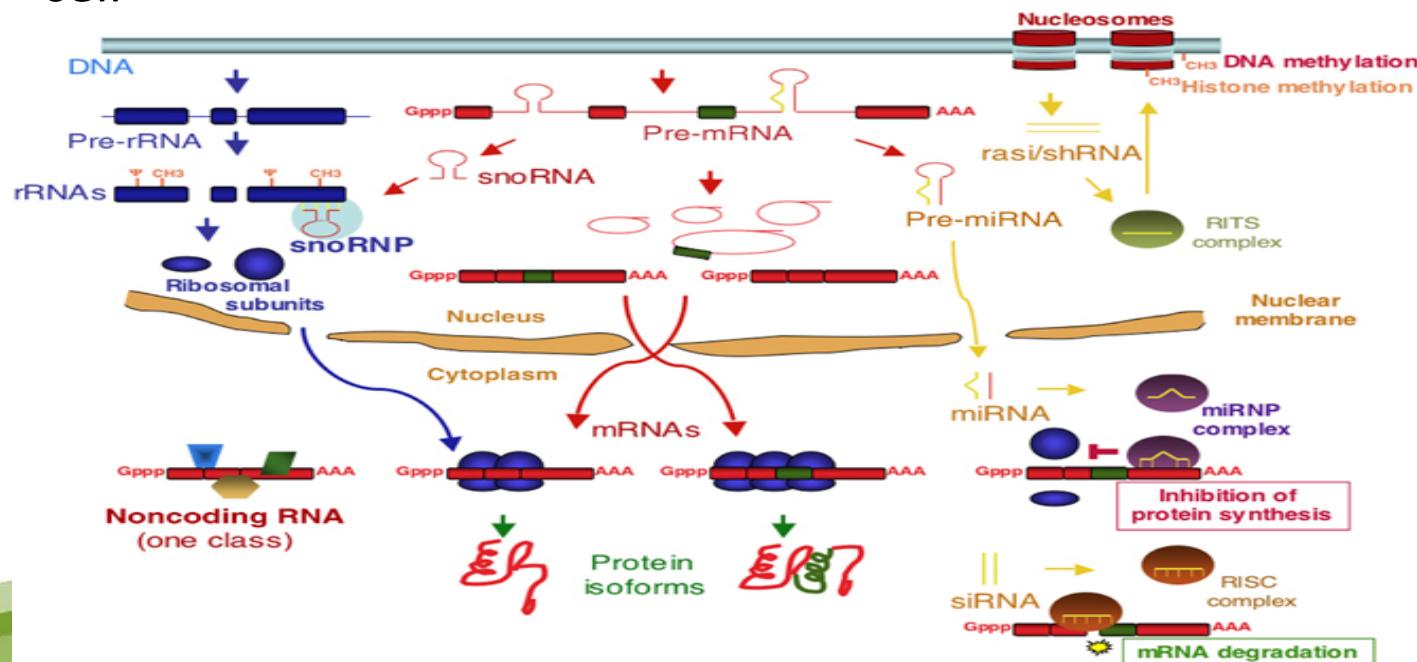
A vertical sequence of DNA bases (A, T, C, G) represented by colored boxes (red, green, blue, yellow). An orange arrow points from the genetic map to this sequence. A legend on the right identifies the colors: Red = Adenine, Green = Thymine, Blue = Cytosine, Yellow = Guanine. Labels include: Step, Phosphate Backbone, Base pair, Adenine, Thymine, Cytosine, Guanine.

Physical map

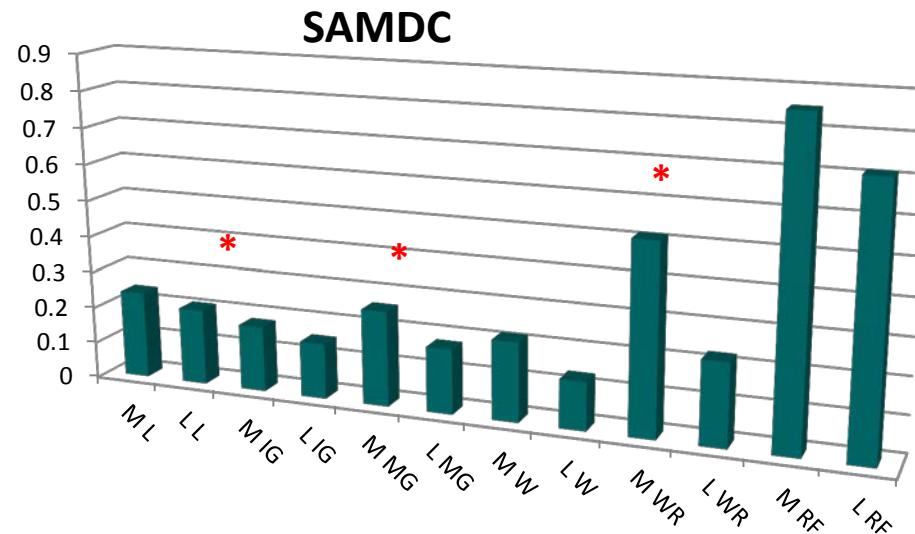
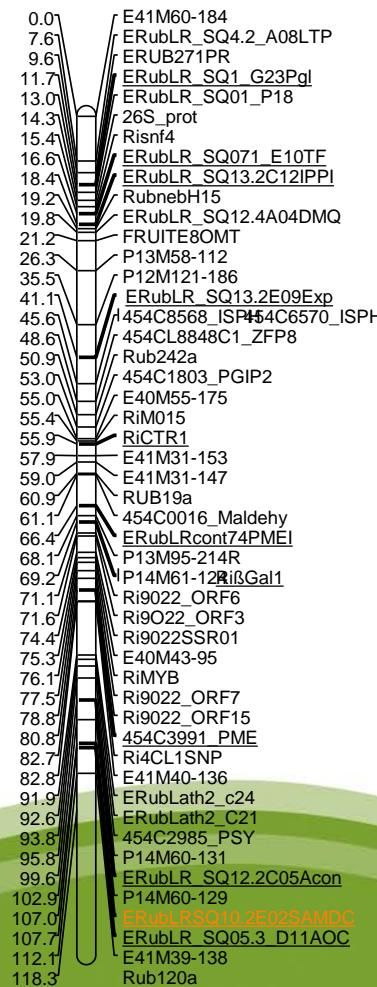


Transcriptomics

- Transcriptomics —RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell

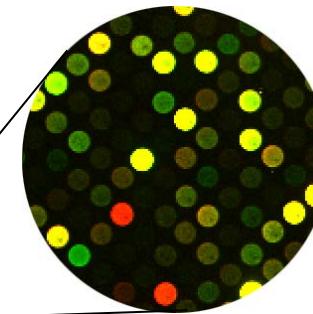
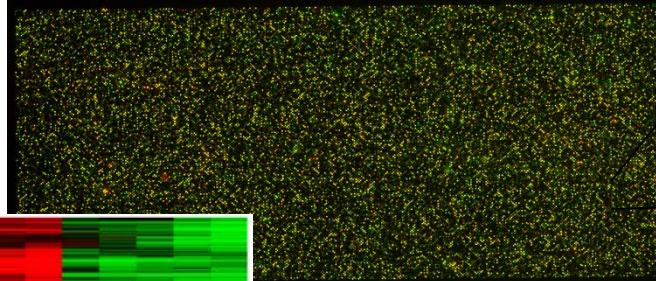
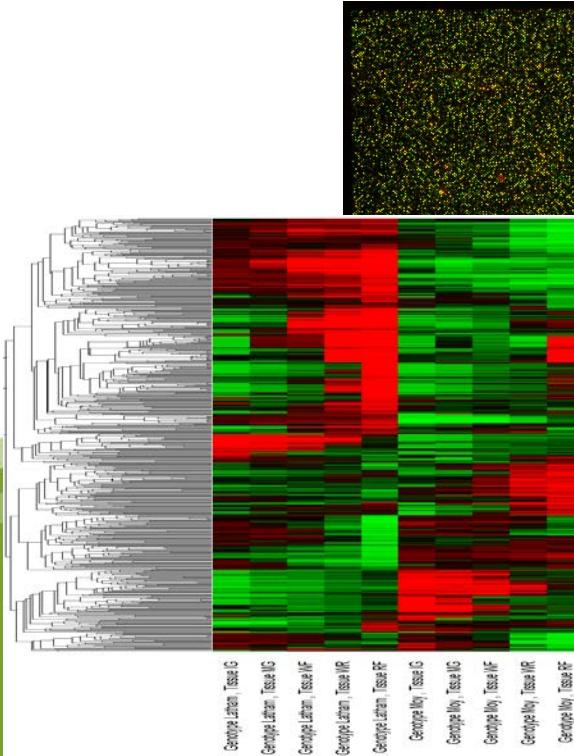


Gene expression and shelf life/softening



Gene expression - microarray

- 60, 000 genes available on a microarray for gene expression studies
- Allows us to determine which genes are switched on at a particular location/time/event

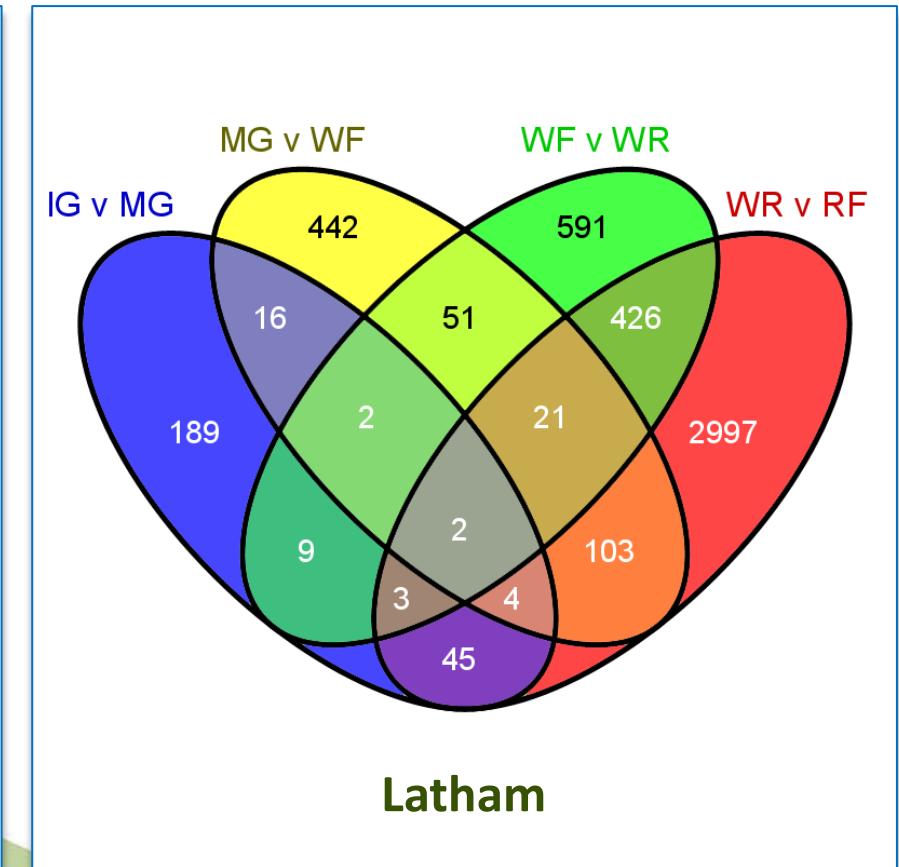
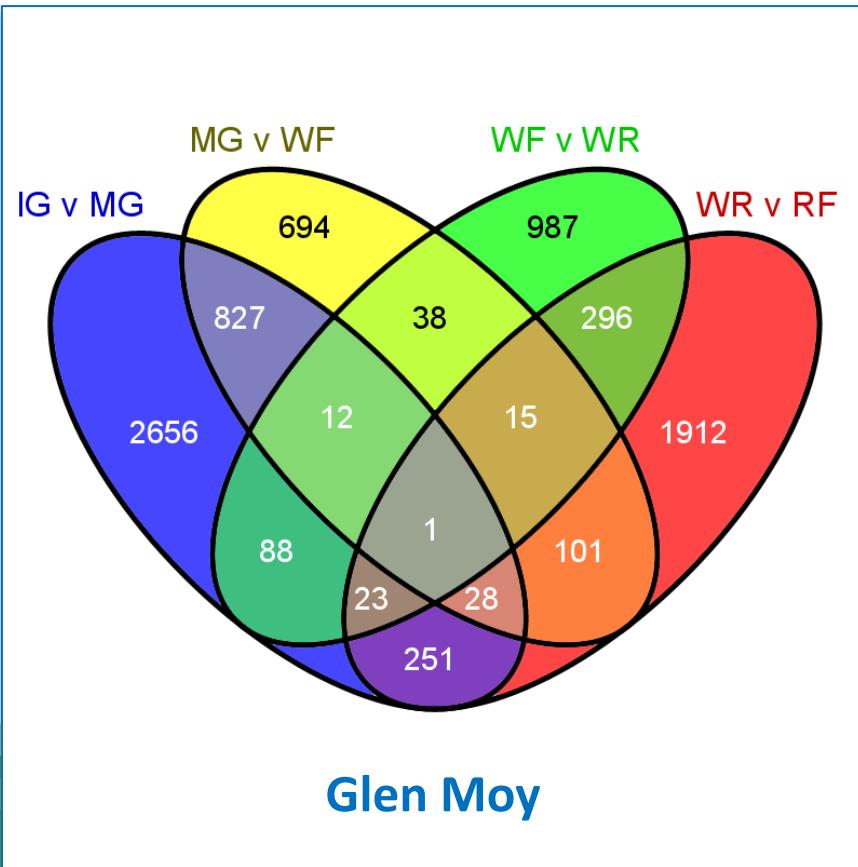


Yellow: active in A

Green: active in B

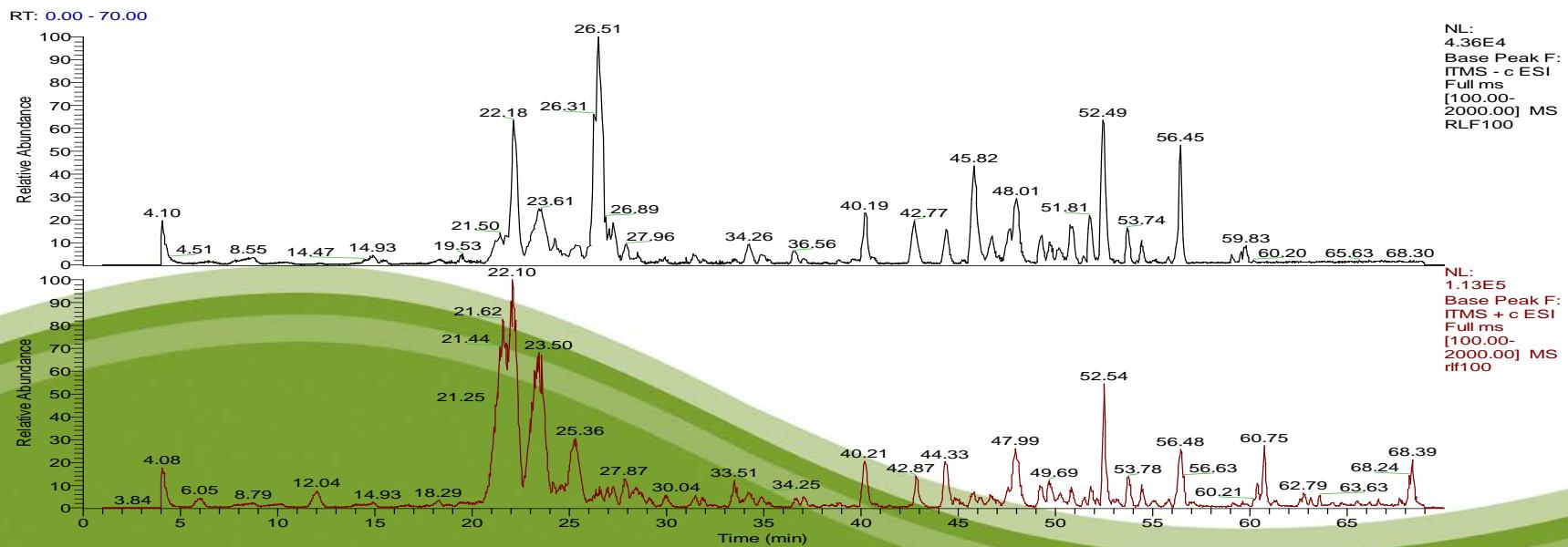
Orange: active in both

Fruit development



Metabolomics

- Metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes.
- Metabolic profiling can give an instantaneous snapshot of the physiology of that cell.



Composition

Season Sample	Field 2006 Progeny		Field 2007 Progeny		Polytunnel 2007 Progeny	
	Volatile	Mean ± SEM	Min-Max	Mean ± SEM	Min-Max	Mean ± SEM
b-damascenone	72.29 ± 4.02	0 – 463.8	87.10 ± 6.82	0 – 872.10	64.88 ± 4.71	3.17 - 368.8
b-ionone	13.65 ± 0.53*	0.46 – 47.88	9.83 ± 0.44	0.61 – 44.33	6.43 ± 0.36*	0.25 - 33.02
a-ionone	7.31 ± 0.19*	1.65 – 17.16	3.86 ± 0.14	0.79 – 13.82	5.16 ± 0.24*	0.22 - 21.61
a-ionol	2.24 ± 0.09*	0.16 – 7.28	1.83 ± 0.09	0.24 – 11.68	5.96 ± 0.30*	0.16 - 29.97
Linalool	4.72 ± 0.32*	0.67 – 22.26	2.90 ± 0.17	0.33 – 14.52	4.39 ± 0.20*	0.90 - 29.28
Geraniol	2.64 ± 0.08*	0.68 – 8.87	1.82 ± 0.07	0.44 – 10.74	3.68 ± 0.14*	0.11 - 15.46
(Z)-3-hexenol	22.35 ± 0.34*	0.71 – 28.15	9.06 ± 0.29	0.63 – 28.18	16.70 ± 0.67*	0.07 - 54.03
Acetic acid	1.39 ± 0.06*	0.06 – 8.26	0.64 ± 0.05	0.01 – 4.05	0.72 ± 0.03	0.01 - 3.54
Hexanoic acid	6.54 ± 0.35*	0.89 – 41.68	7.97 ± 0.30	1.71 – 22.88	7.04 ± 0.34	0.28 - 30.84
Acetoin	1.02 ± 0.05	0.09 – 4.74	1.03 ± 0.04	0.10 – 4.79	0.80 ± 0.05*	0.02 – 4.95
Benzyl alcohol	0.59 ± 0.03*	0.15 – 2.18	1.07 ± 0.04	0.09 – 3.16	2.67 ± 0.11*	0 - 9.94

Metabolites & gene expression –ve correlations at 1 stage of fruit development

Glen
Moy

Latham



76 metabolites
8939 probes

26 metabolites
2091 probes

60 metabolites
5378 probes

Phenomics

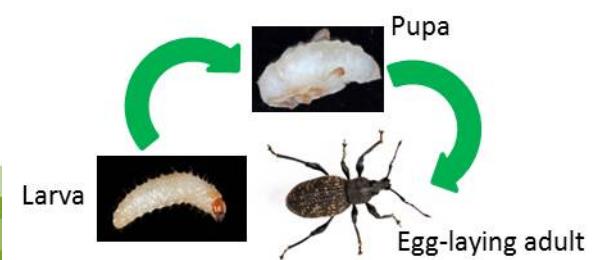
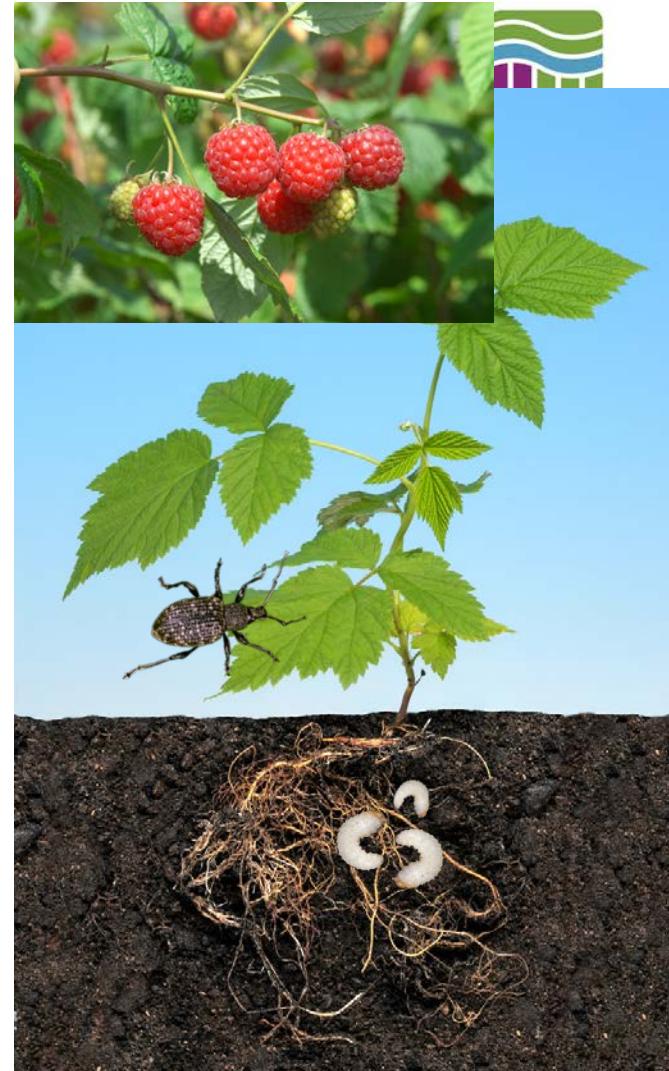
- Plant physical and biochemical traits as they change in response to genetic mutation and/or environmental influences
- Need a way to identify changes induced by stresses – imaging technologies



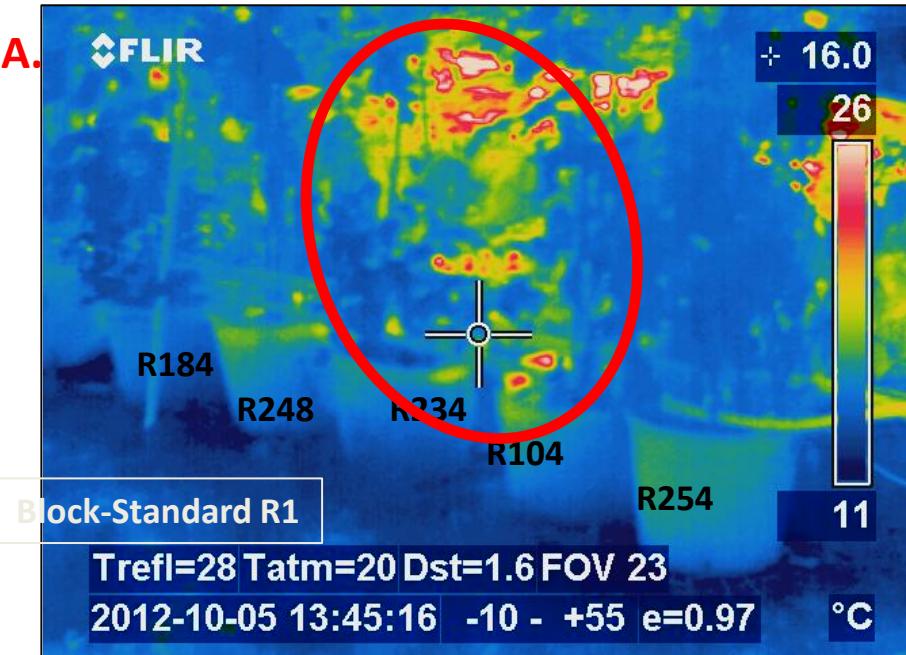
Vine weevil damage to raspberry

Vine weevil (*Otiorhynchus sulcatus*) is an intractable pest

- Difficult to detect (root-feeding larvae)
- Difficult to treat
- Leads to yield loss
- Economically-damaging



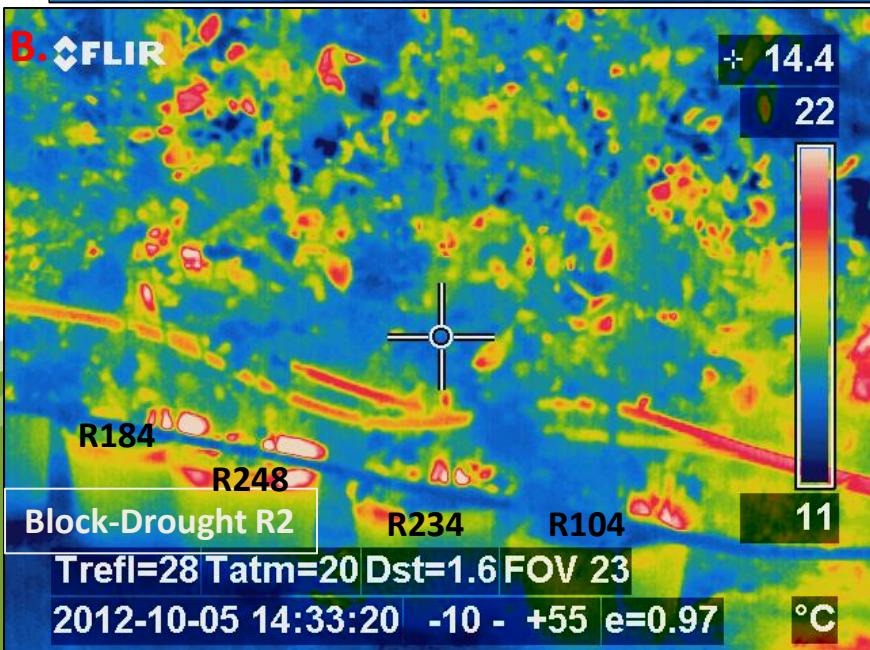
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Water stress

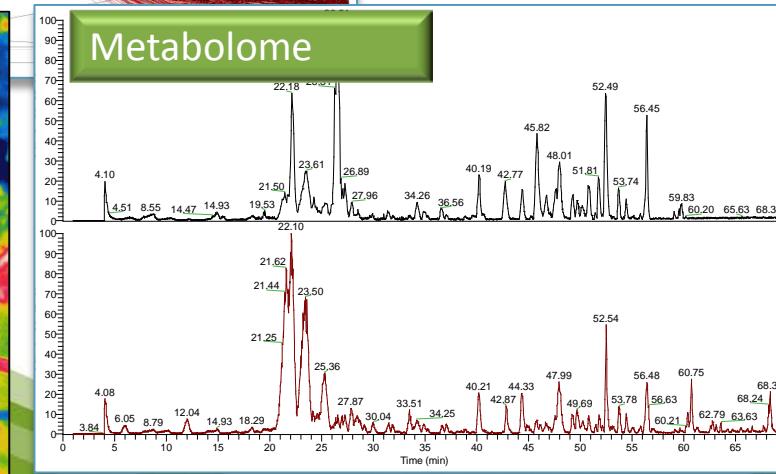
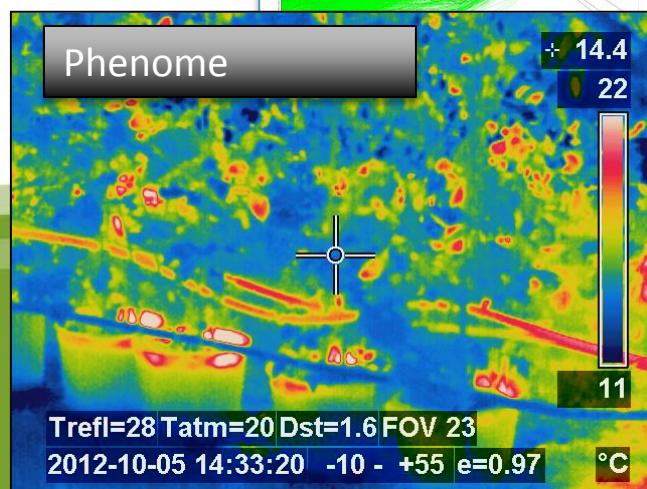
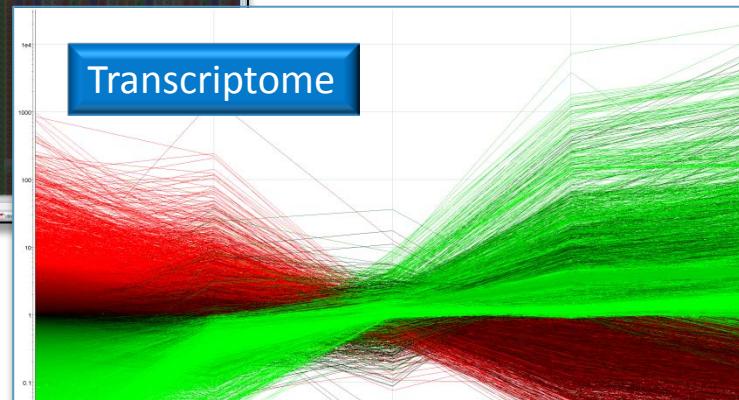
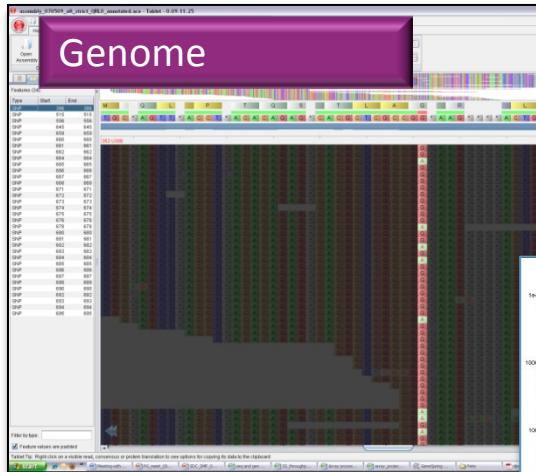
- Infrared thermography detects notable differences to water stress in raspberry plants
- R234 has greater water requirement than R254

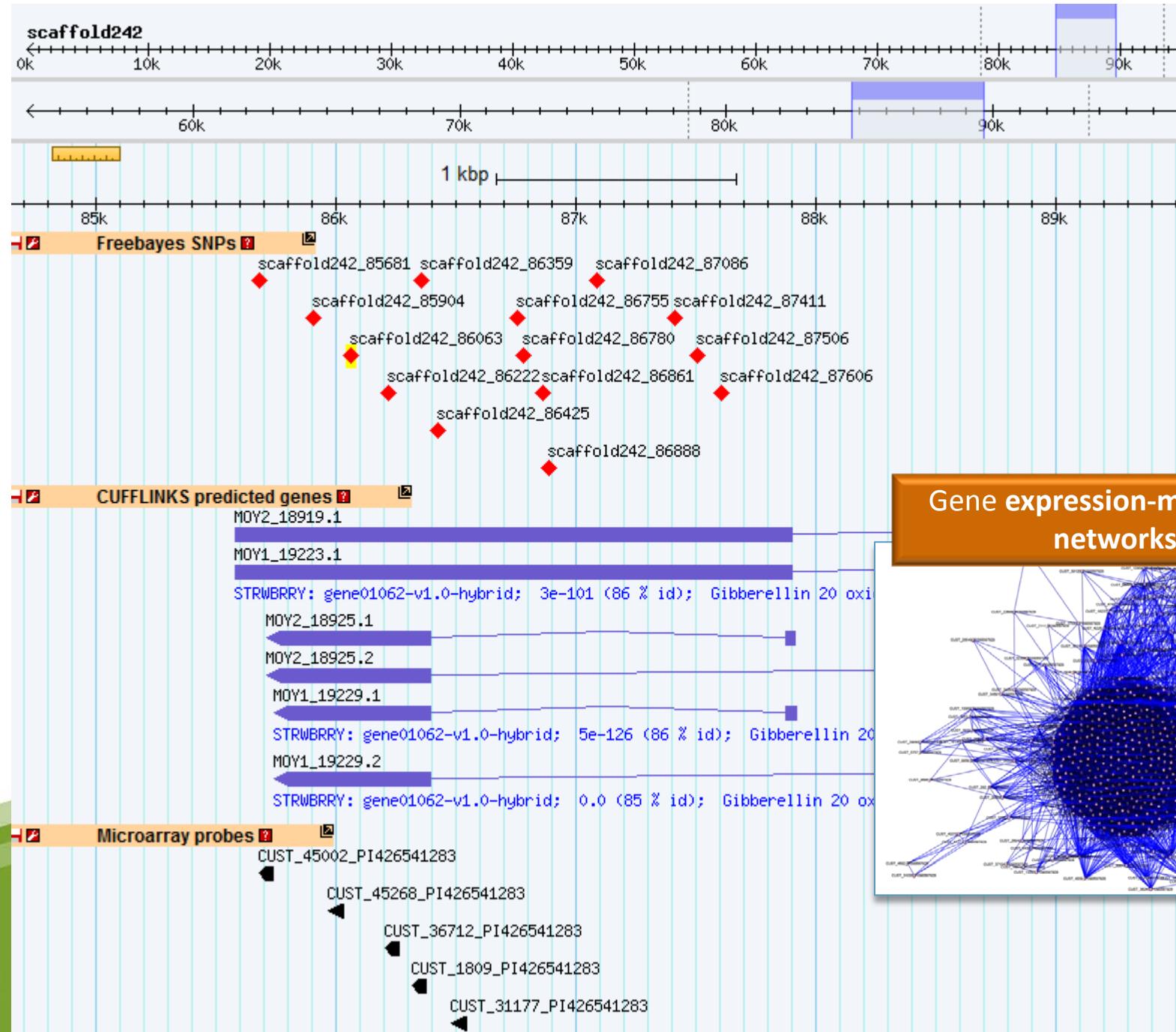
B.



Using imaging technology to identify water stress +/- pest and disease pressures – data points

Integration of knowledge





Knowledge and tools for breeding



Kay Smith
Susan McCallum
Chris Hackett
Rob Hancock
Ankush Prashar
Ali Karley
Craig Simpson
Linda Milne
Pete Hedley
Runxuan Zhang
Nikki Jennings
Dominic Williams
Avril Britten
Carolyn Mitchell

Innovate UK
Scottish Government
Industry Consortia