

Improving the Genetic Merit of Forage via Molecular Breeding

**Brent Barrett, Team Leader
Forage Breeding**

**AgResearch Grasslands
Palmerston North, New Zealand**

11 July 2012 @ NAFA



The Genetic Gain Race

Species	Genetic gain DM (% per annum)	Reference
Maize	1.5 - 2%	Duvick 1992; Tollenaar & Lee 2002
Lucerne	0.2%, 0.2%, 0%	Hill et al. 1998; Holland & Bingham 1994; Wiersma 1997
Smooth brome grass	0.15%	Vogel et al. 1996
White clover	0.6%, 1.3%	Woodfield & Caradus 1994; Woodfield 1999
Perennial ryegrass	0.2%, 0.4%	Sampoux et al. 2010 Easton et al. 2002

Pastoral Agriculture's Central Role in New Zealand

USA



NZ



■ Sheep ■ Dairy cows ■ People

Half of our land area is under grazing in temperate and sub-tropical zones, primarily improved pastures

Agriculture generates a fifth of our economic activity, and half of our export earnings

Forage Breeding Team

- Grasses, Legumes, Herbs
- Novel Endophyte
- Marker-Aided Breeding
- Seed Production
- Physiology – Seed & Plant



Breeding Targets

Grass

- Endophyte Performance
- DM Yield
- Persistence
- Quality/Intake
- Drought/Abiotic Stress
- Heading / Aftermath

Legume

- Legume Content / Mixed Sward
- Persistence / Pest Tolerance
- Drought/Abiotic Stress
- Quality
- Seed Yield

Forage Breeding System





Goal

Red Clover
with Grazing
Tolerance

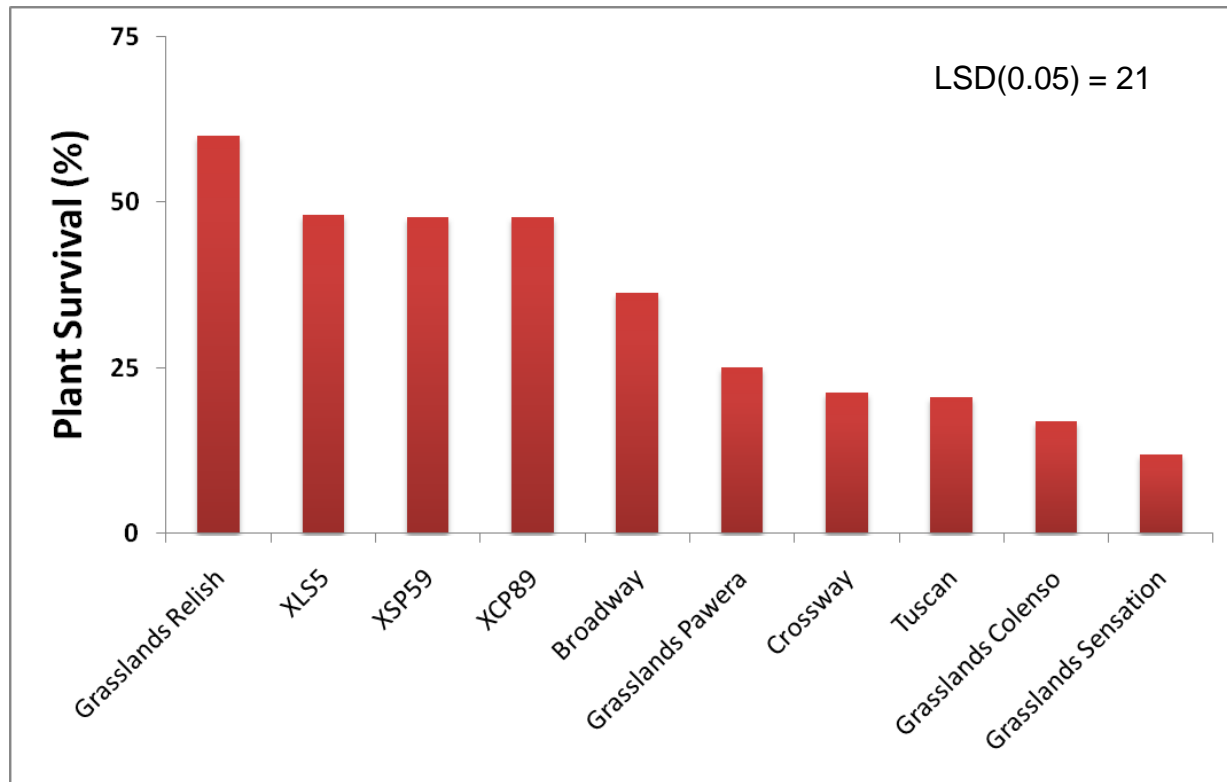
Approach

Test a Global
Collection in
New Zealand



Four Summers Later...

Plant survival after rotational grazing by cattle at
Aorangi Research Farm, Manawatu, 2007-2011



Ford & Barrett (2011) NZGA

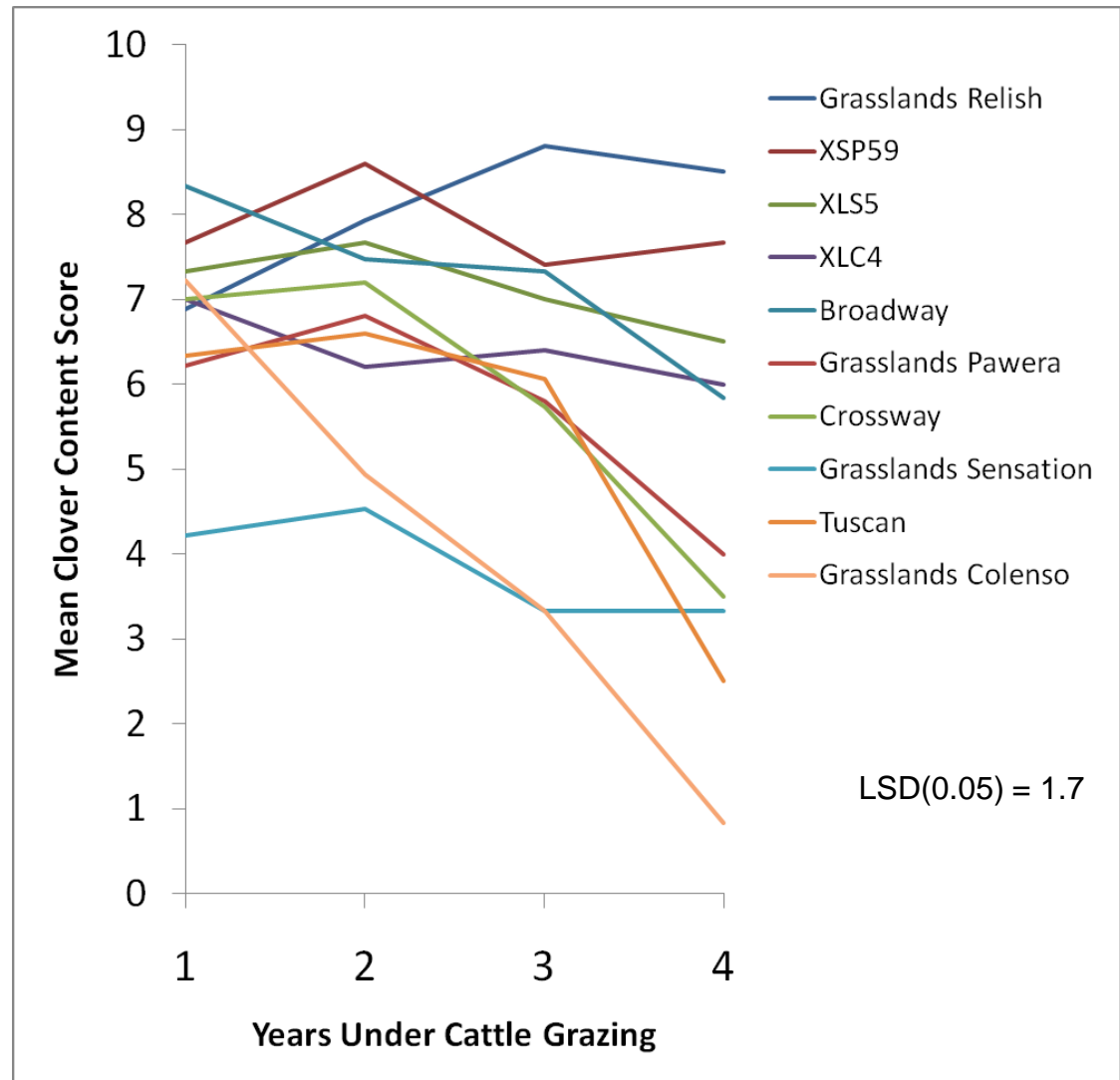
Persistence & Yield Under Grazing

Release of
Grasslands Relish

4 Experimental Lines
Topped the Trial
After 4 Summers

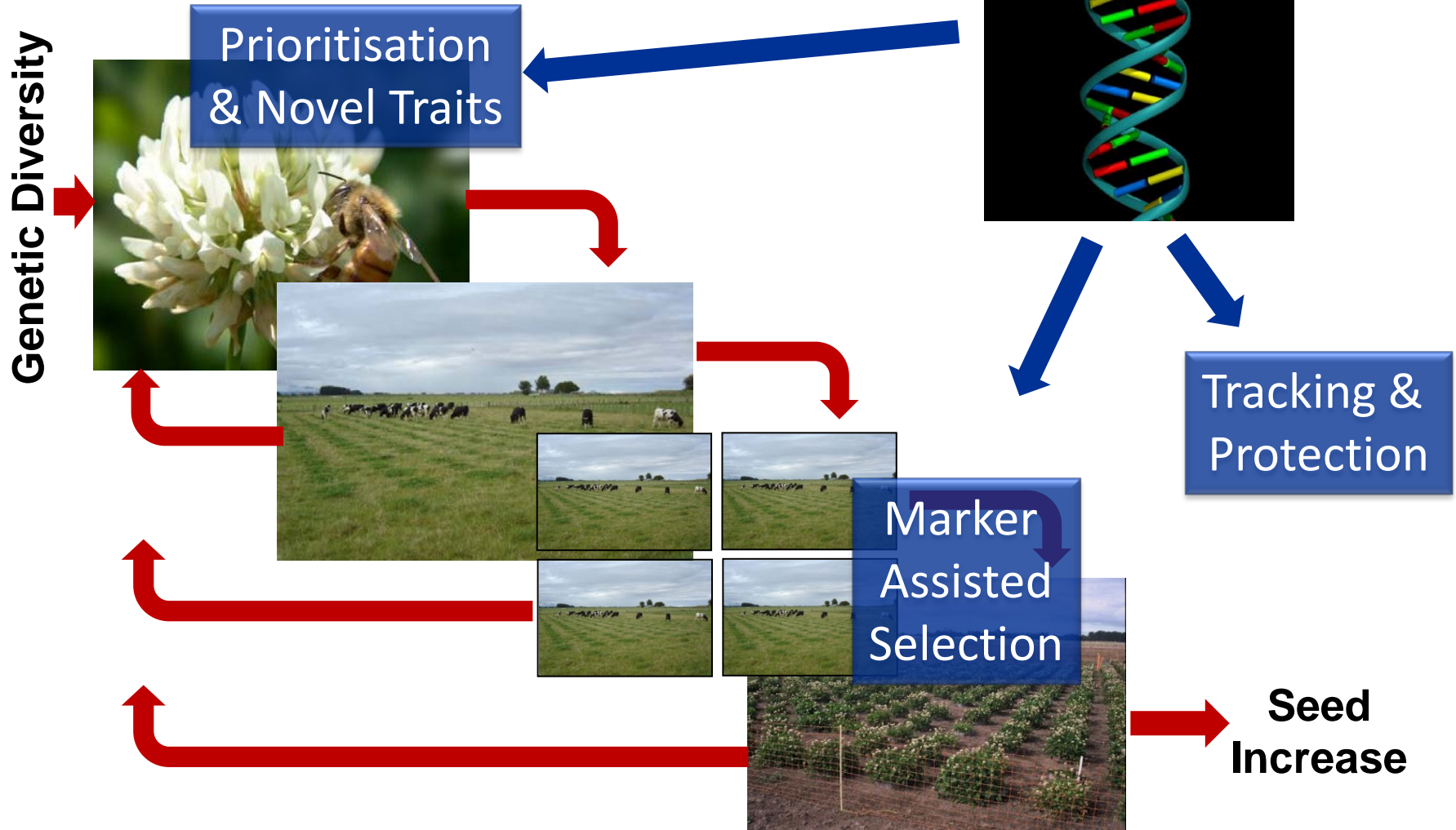
Not a molecular
marker in sight

Genetic resources and
relevant phenotyping

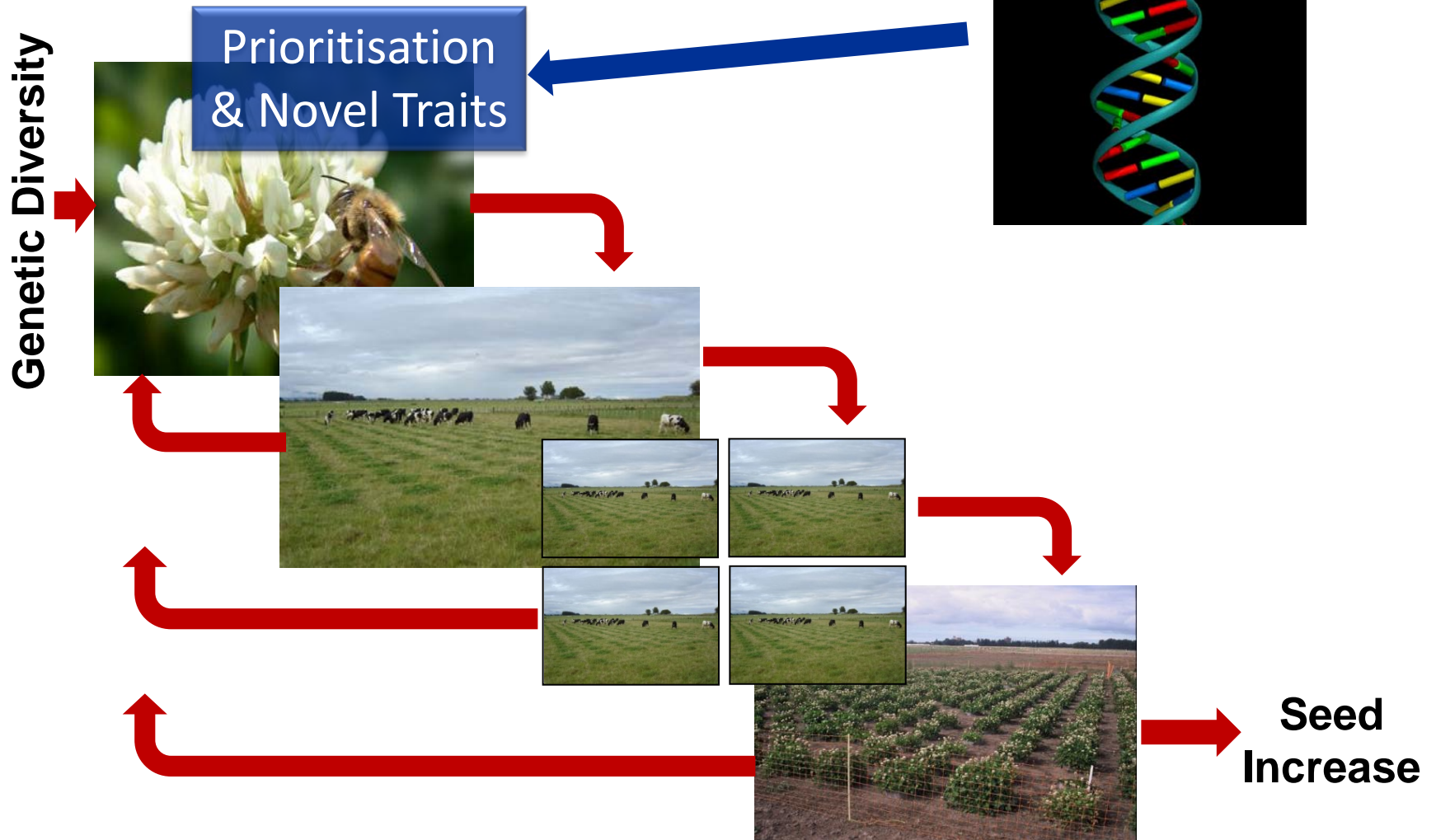


Ford & Barrett (2011) NZGA

Molecular Breeding

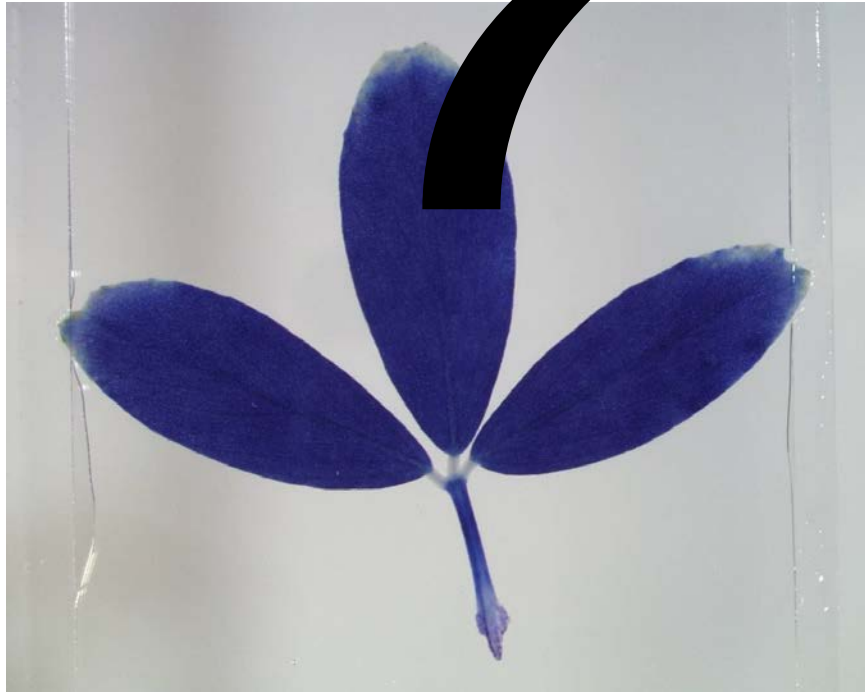


Molecular Breeding

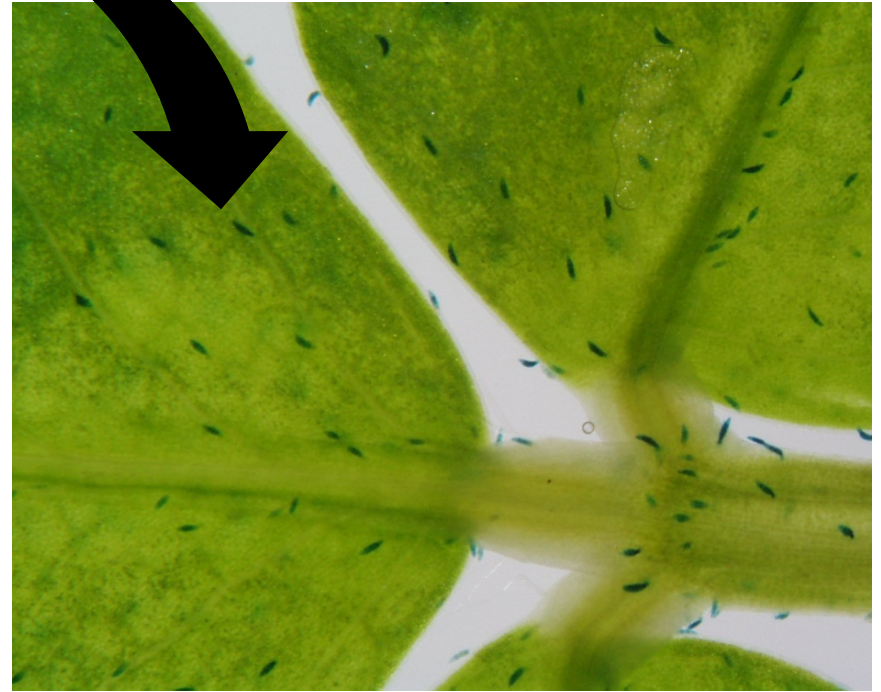




Activating Condensed Tannin Accumulation in White Clover and Alfalfa Leaves

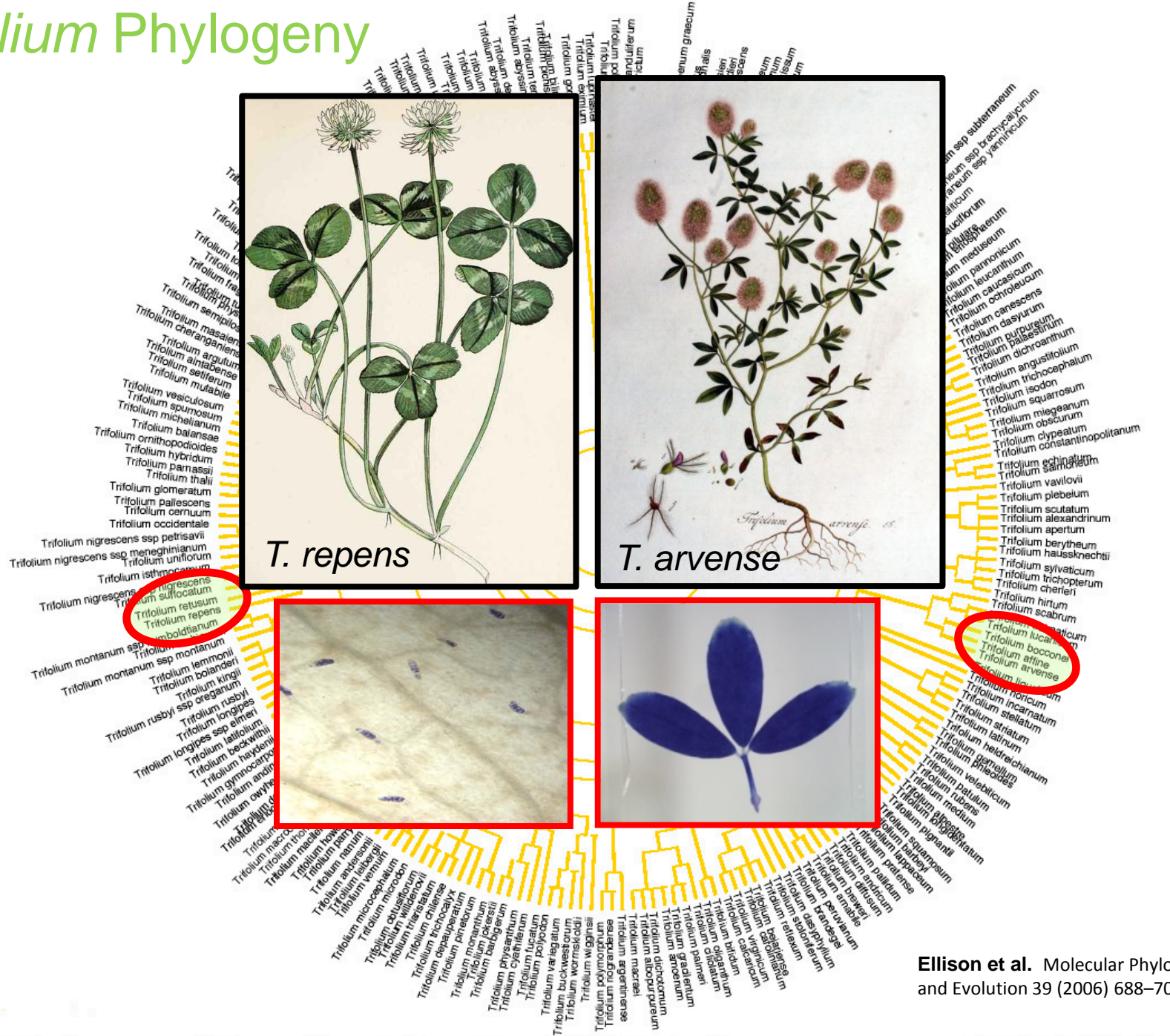


T. repens



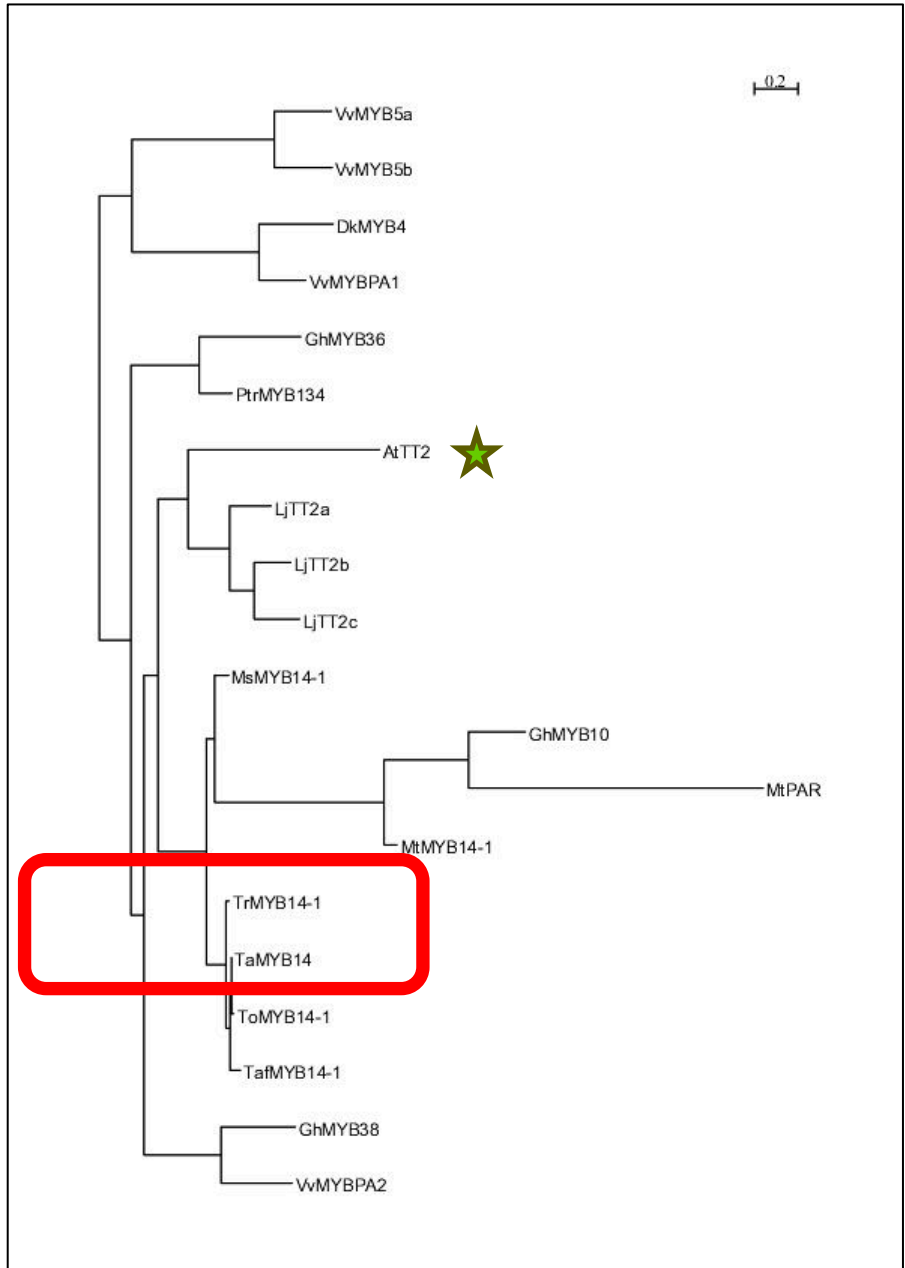
Hancock et al. 2012

Trifolium Phylogeny



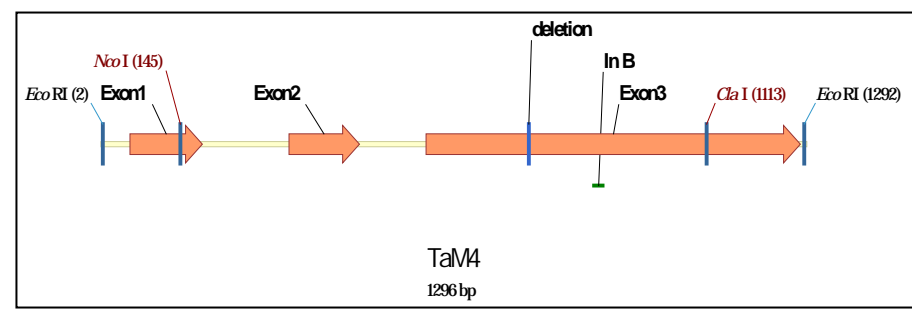


TaMYB14- a Leaf Specific MYB Factor

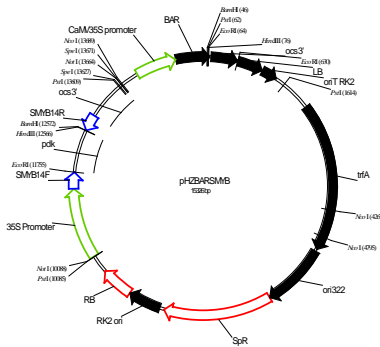
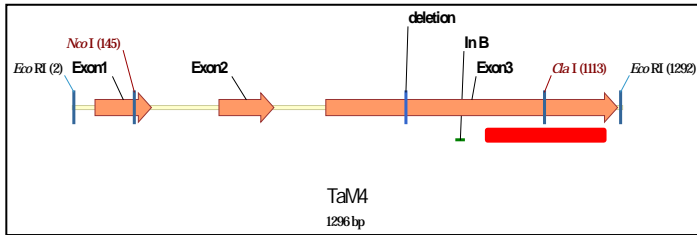


- Transcription factor
- Isolated from *T. arvense*
- 942 bp coding region
- 314 amino acids

TaMYB14 Structure

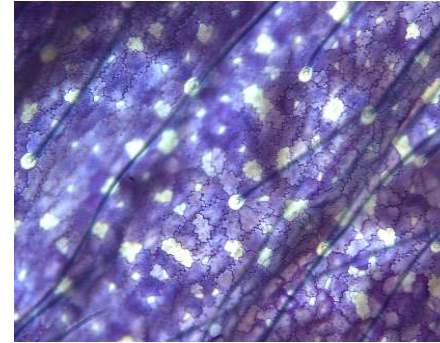


Deactivating CTs in *T. arvensis*

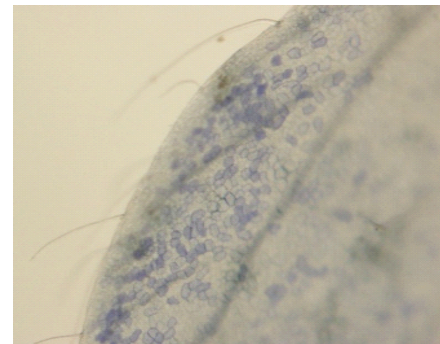


RNAi Gene Silencing
of TAMYB14

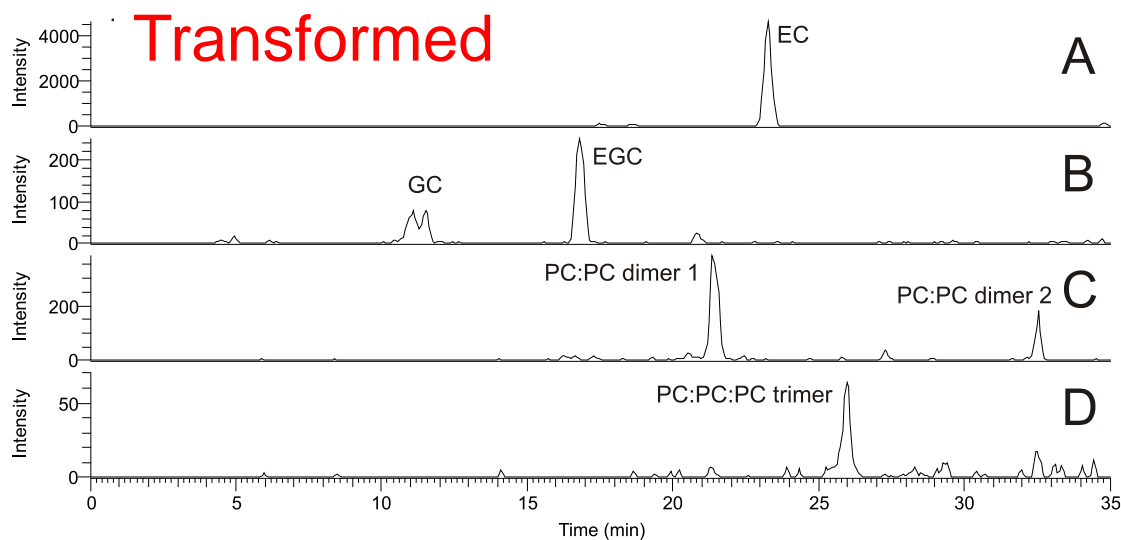
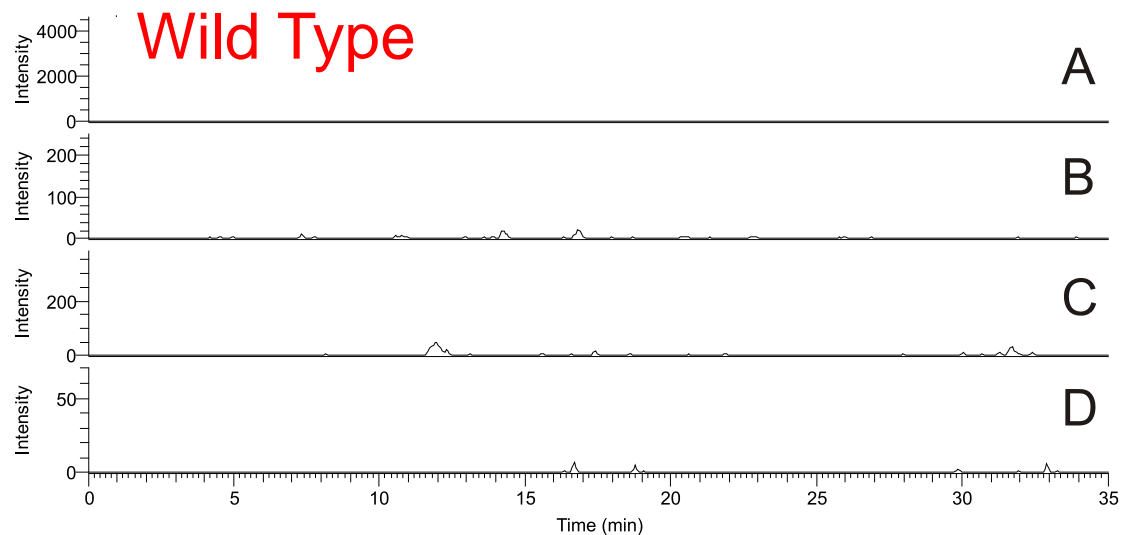
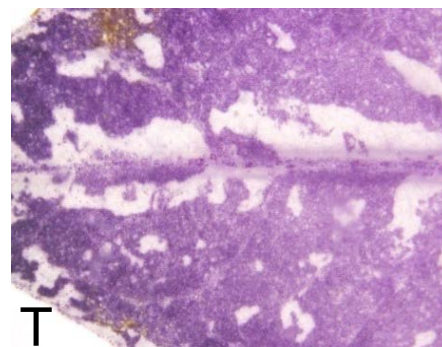
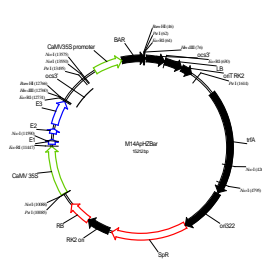
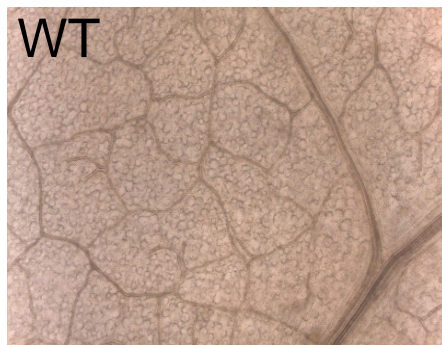
Wild Type



Silenced

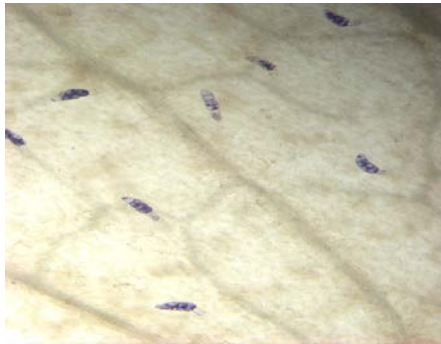


Activating CT Accumulation in Tobacco Leaf

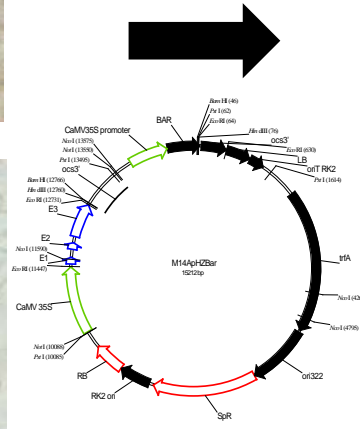
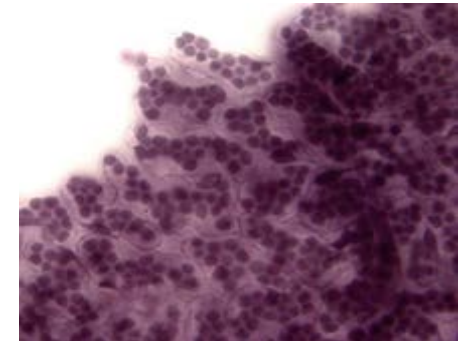
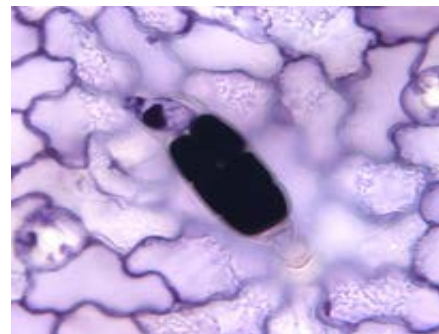
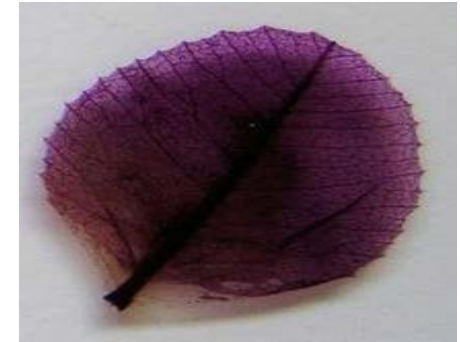
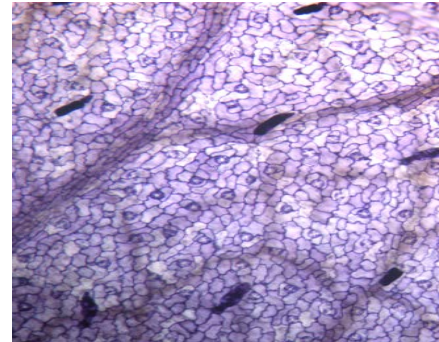


....and in White Clover

Wild Type

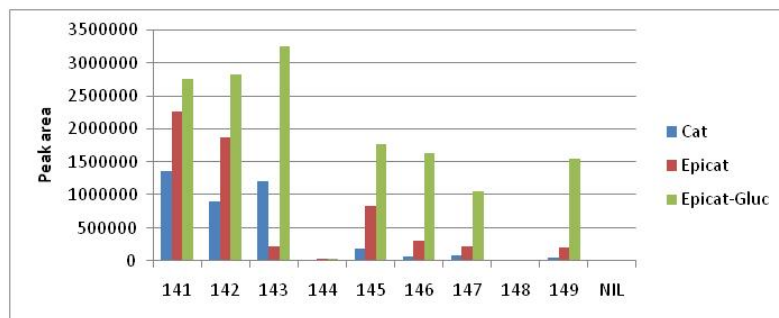
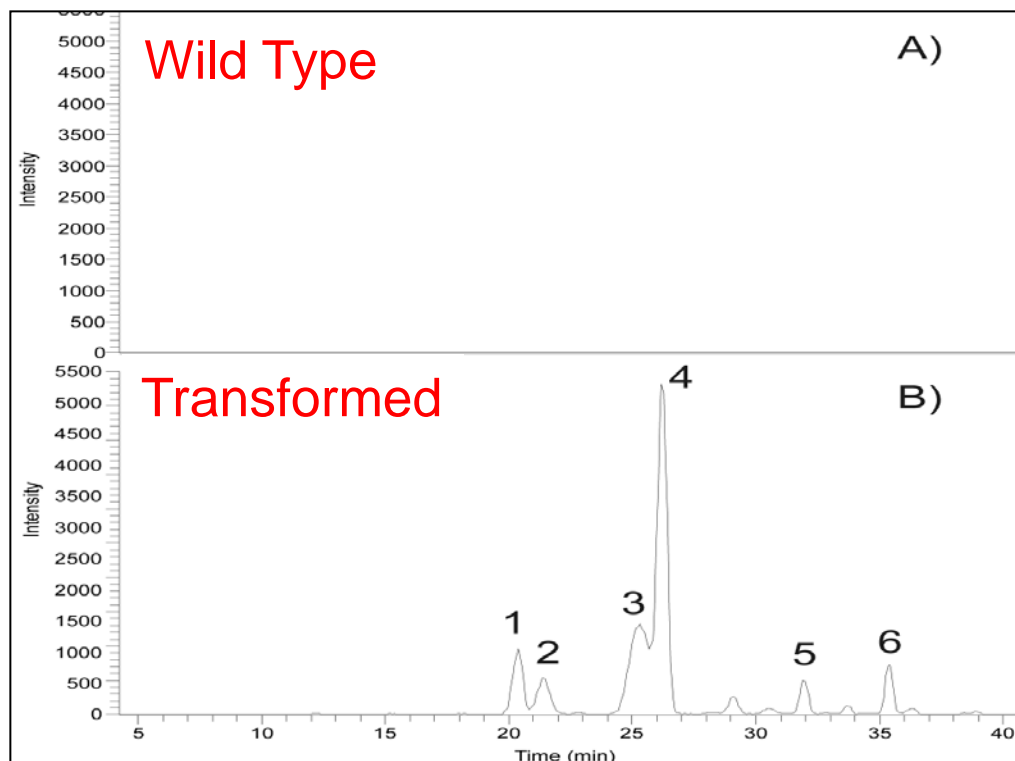
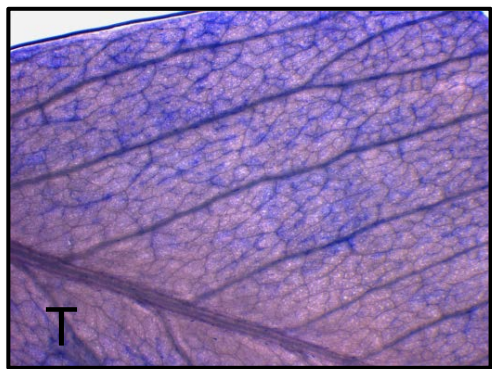
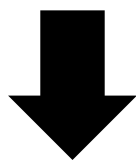
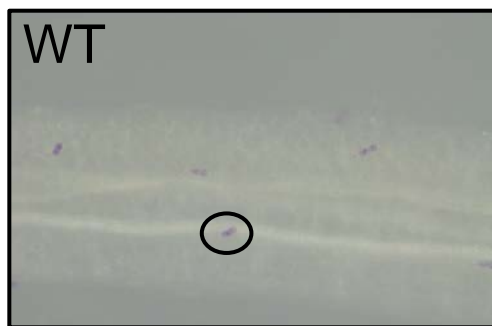


Transformed





.....and in Alfalfa

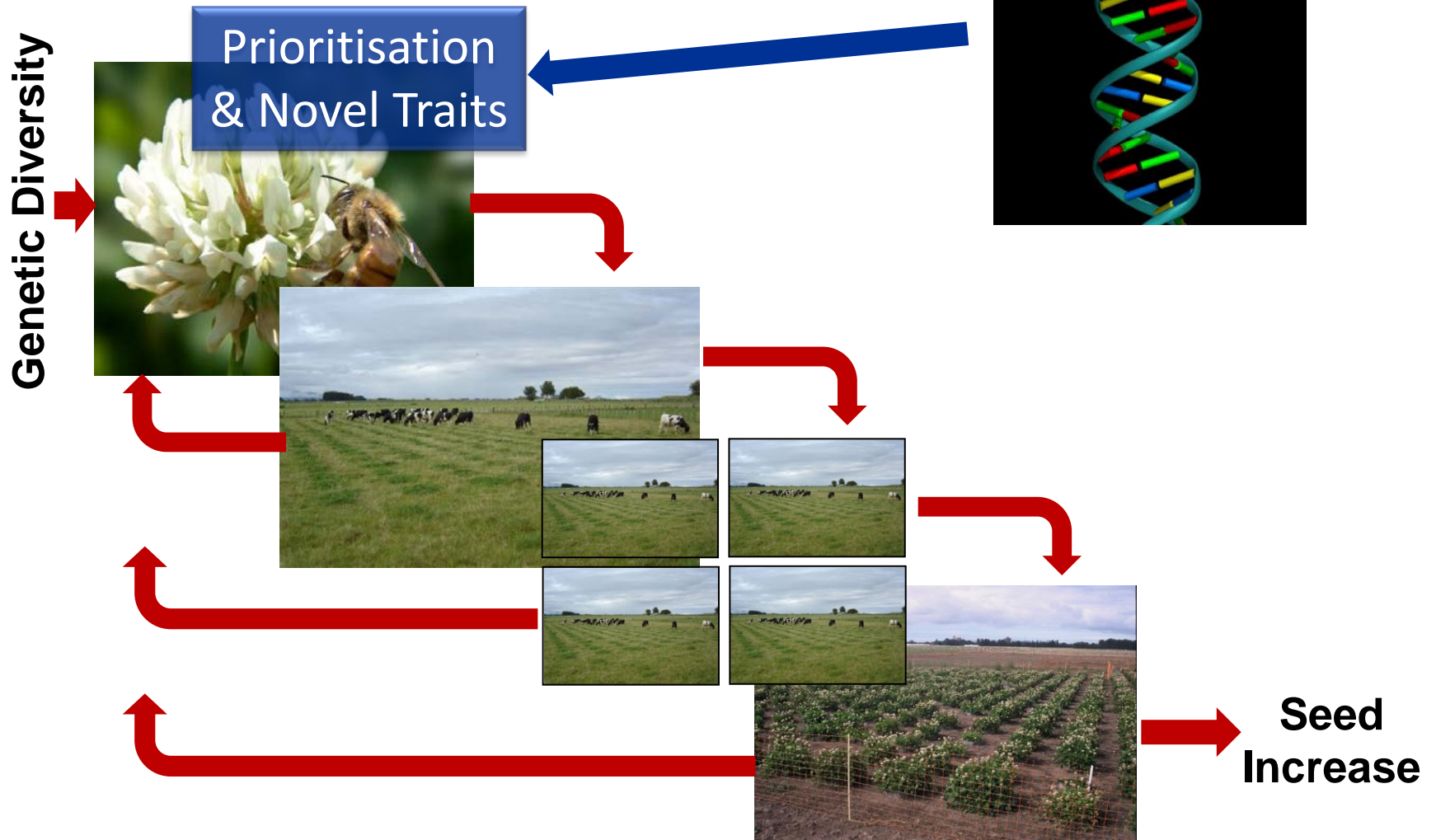


Providing Novel Traits to Farmers

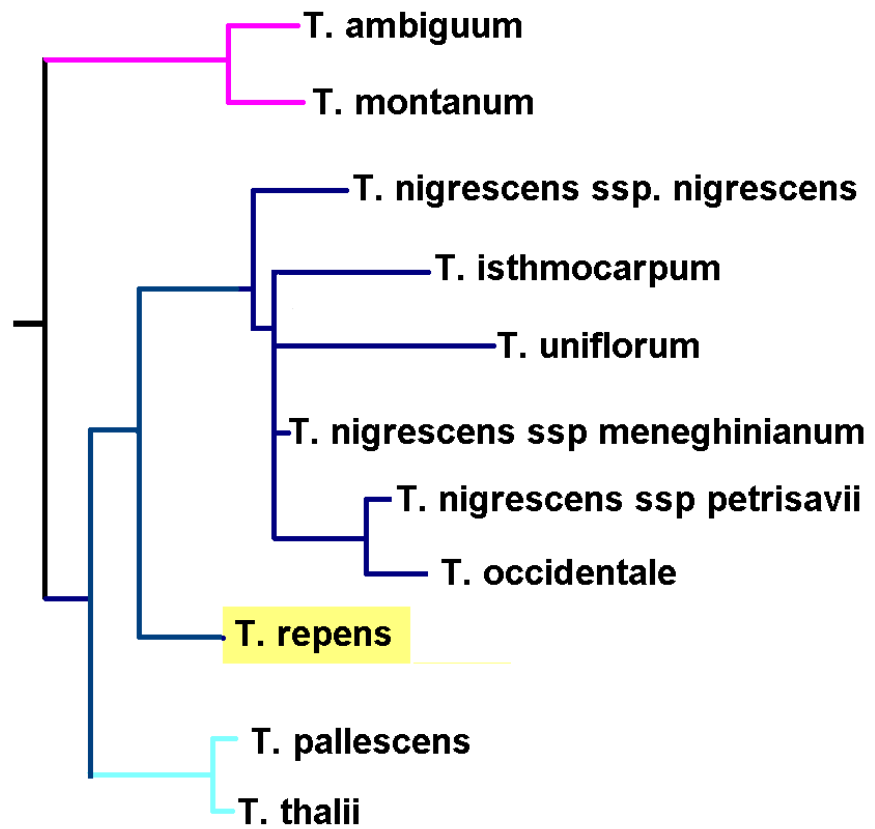
- Patent assignee is Grasslanz Technology Ltd
 - Alfalfa: Licensed to Forage Genetics USA
 - White Clover: further in-house development



Molecular Breeding



Molecular Breeding: Genetic Resource Prioritisation



The White Clover Complex

Williams et al.

Ellison et al. Molecular Phylogenetics and Evolution 39 (2006) 688–705

Diversity of the White Clover Complex

Habitats from the Atlantic coast of Europe to the Caucasian mountains

Habits from prostrate, creeping to 1.5 metres tall, non-creeping

Life cycles from annual to long-lived perennial

Adaptations maritime to alpine

Resistances to diverse diseases and pests



Trifolium uniflorum

Mediterranean

Natural tetraploid ($2n=4x=32$)

Very robust tap-root system + nodal roots

Probably drought resistant

Sturdy (durable?) stolons

Chromosomes pair – we aim to introgress traits into *T. repens*



Photo SW Hussain

Nodal Root Systems of Adjacent *T. repens* (L) and *T. repens* X *T. uniflorum* (R)



Photo SW Hussain

Trifolium Hybrids

Inter-Specific
Hybrid

White Clover



Double Bridge Breeding Scheme

***T. occidentale* crosses with white clover to produce seeds (no embryo rescue needed)**



Hybrids between *T. ambiguum* and *T. occidentale* are fertile and produce seeds

***T. occidentale* and *T. ambiguum* can potentially now be used as bridges to transfer traits from 11 other taxa into white clover**



Synthetic White Clover

T. pallescens X *T. occidentale*

May be the closest living species to the ancestors of white clover

One partially fertile diploid (*po*) hybrid (*pp* x *oo*)

5% pollen stainability

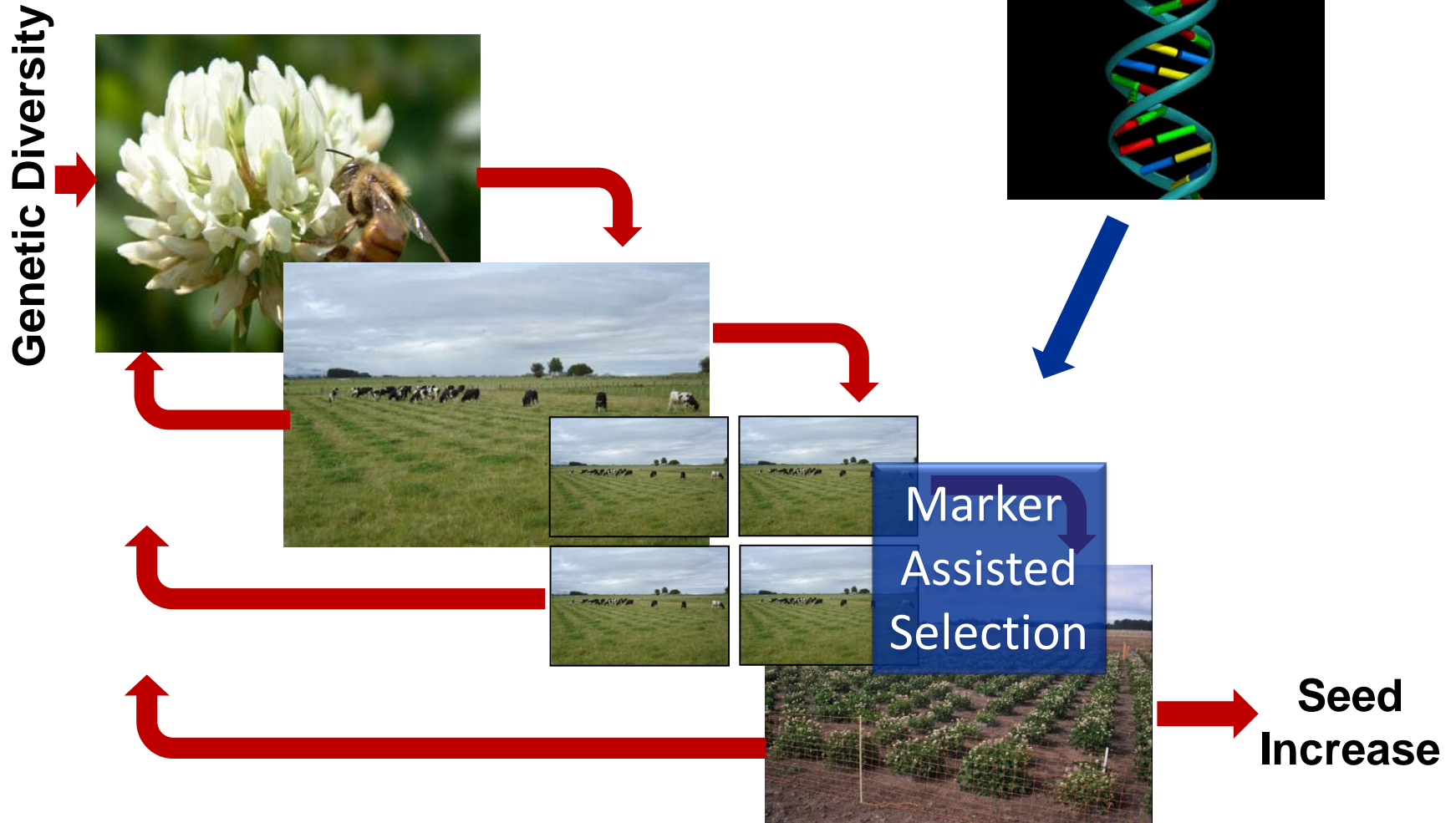
Seeds set and progeny established

F₁ is stoloniferous with weak nodal rooting



Williams et al. (2012) BMC Plant Biology

Molecular Breeding



Markers and Genomic Resources

Current platform

Sequence Resources

White clover and ryegrass EST (>50,000 each)

White clover GeneThresher® (>364,000)

Full genome for white clover and perennial ryegrass (in progress)

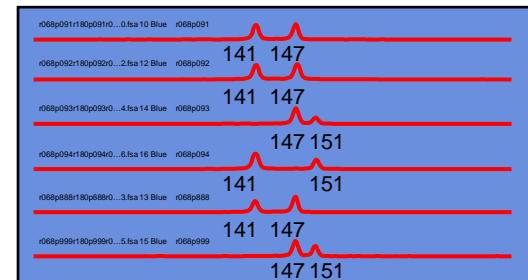


Bioinformatics – *in silico* alignment with model species

Marker resources

EST and GeneThresher®-derived SSRs

Candidate Gene based SNP



Linkage maps

QTL: Herbage yield, seed yield, drought tolerance, root morphology, stolon morphology, shoot morphology, leaf morphogenesis, endophyte compatibility



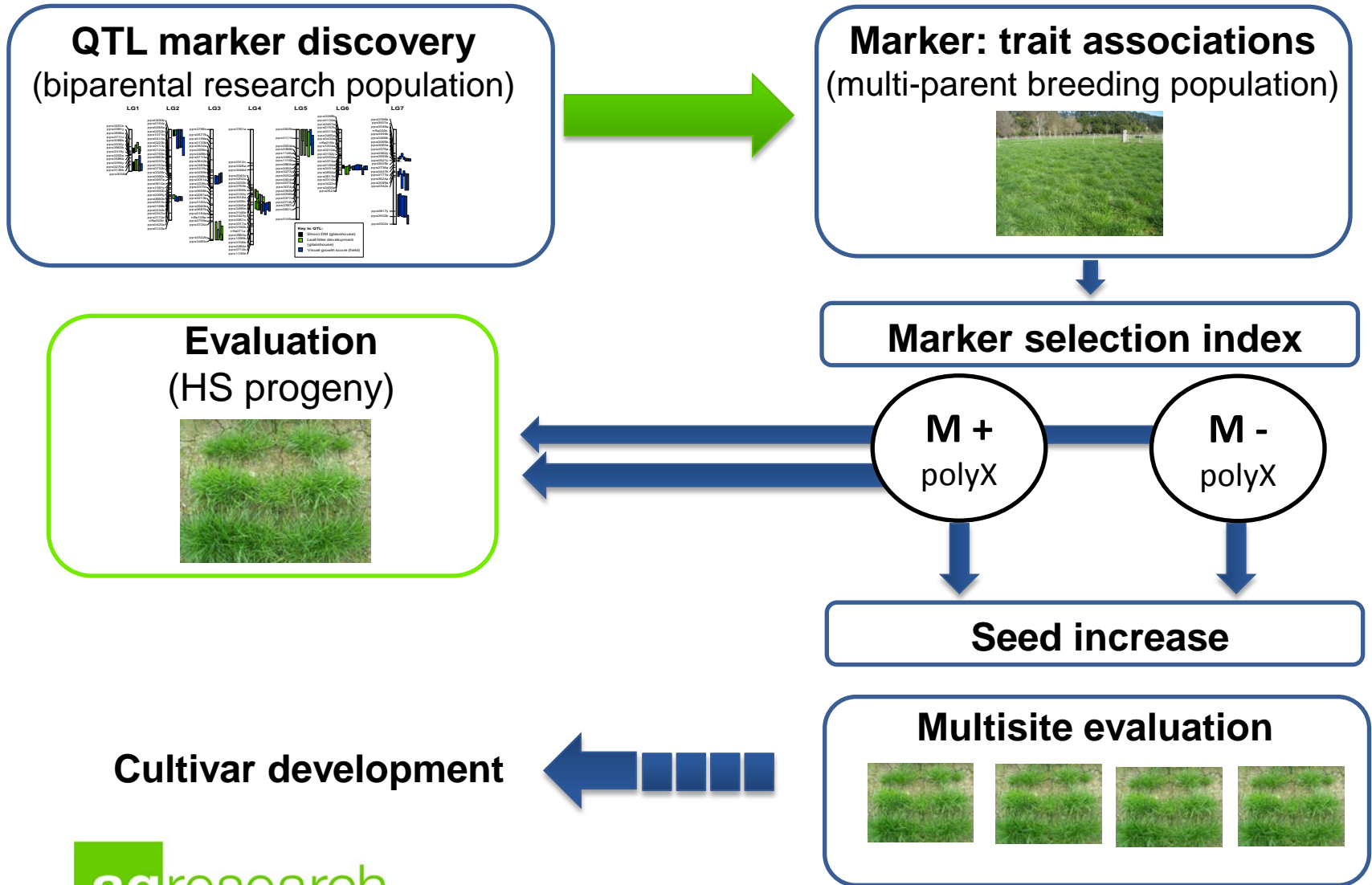
Current Platform: Of What Value in Breeding?

Goal: utilise existing information and markers for MAS, accelerating gain in existing field breeding systems.

Specifically: what can be achieved with QTL-associated SSRs in our elite breeding populations of complex parentage??



Method





MAS with SSR Markers in Forage Breeding Populations

Species	Trait	Status
White clover	Seed yield	In use
	Herbage yield	In development
	Stolon Branching	In development
Perennial ryegrass	Herbage yield	In development

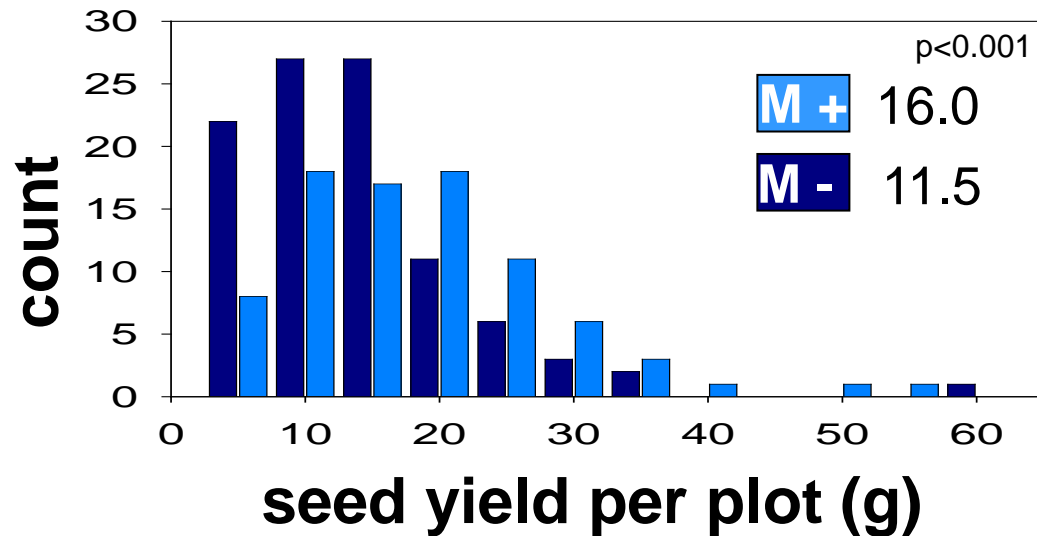
White Clover Seed Yield

Breeding Populations with Complex Parentage

Top Performers Under Grazing, Highly Variable Seed Yield

Single Marker Effects ($p < 0.001$) in 8 of 12 Populations Tested

Mean 38% Differential in Seed Yield



Herbage Yield in Perennial Ryegrass

Population	DM Yield (% Ultra AR1)
GA208 AR37	108
Alto AR37	104
Extreme AR37	103
Arrow AR37	102
One50 AR1	101
Samson AR37	101
Ultra AR1	100
Helix AR1	99
GA207 AR37	96
Samson AR1	96
Stellar AR1	85



Herbage QTL Identification

Glasshouse

Dry matter yield (DM)
Morphogenetic & structural traits



Sartie et al. 2011 Euphytica.

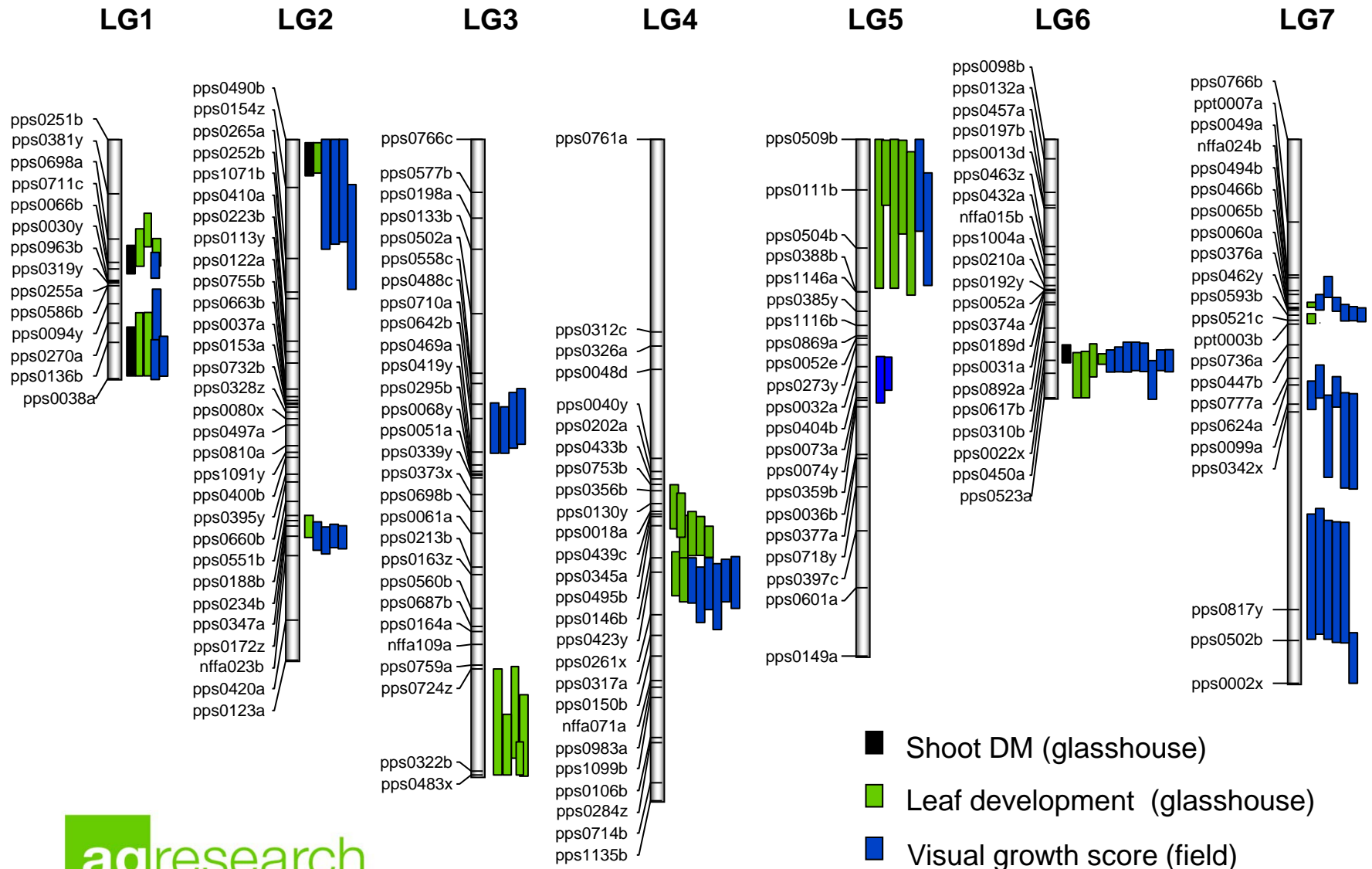
Field

Multi-site, -year, -season
Visual growth score



Faville et al. 2012 NZJAR.

QTL For Yield And Related Traits In Ryegrass



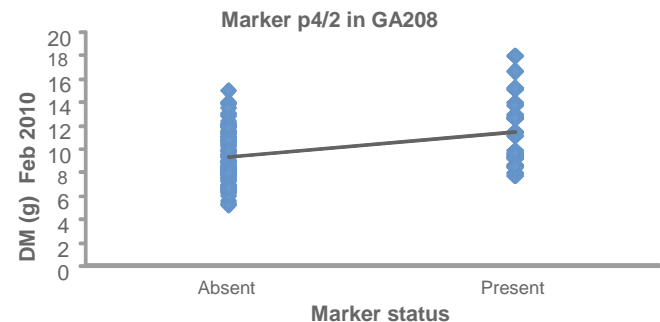
Yield Markers in GA208

Field trial Plants (n=130) in simulated sward, replicated spatial design, single site over two years

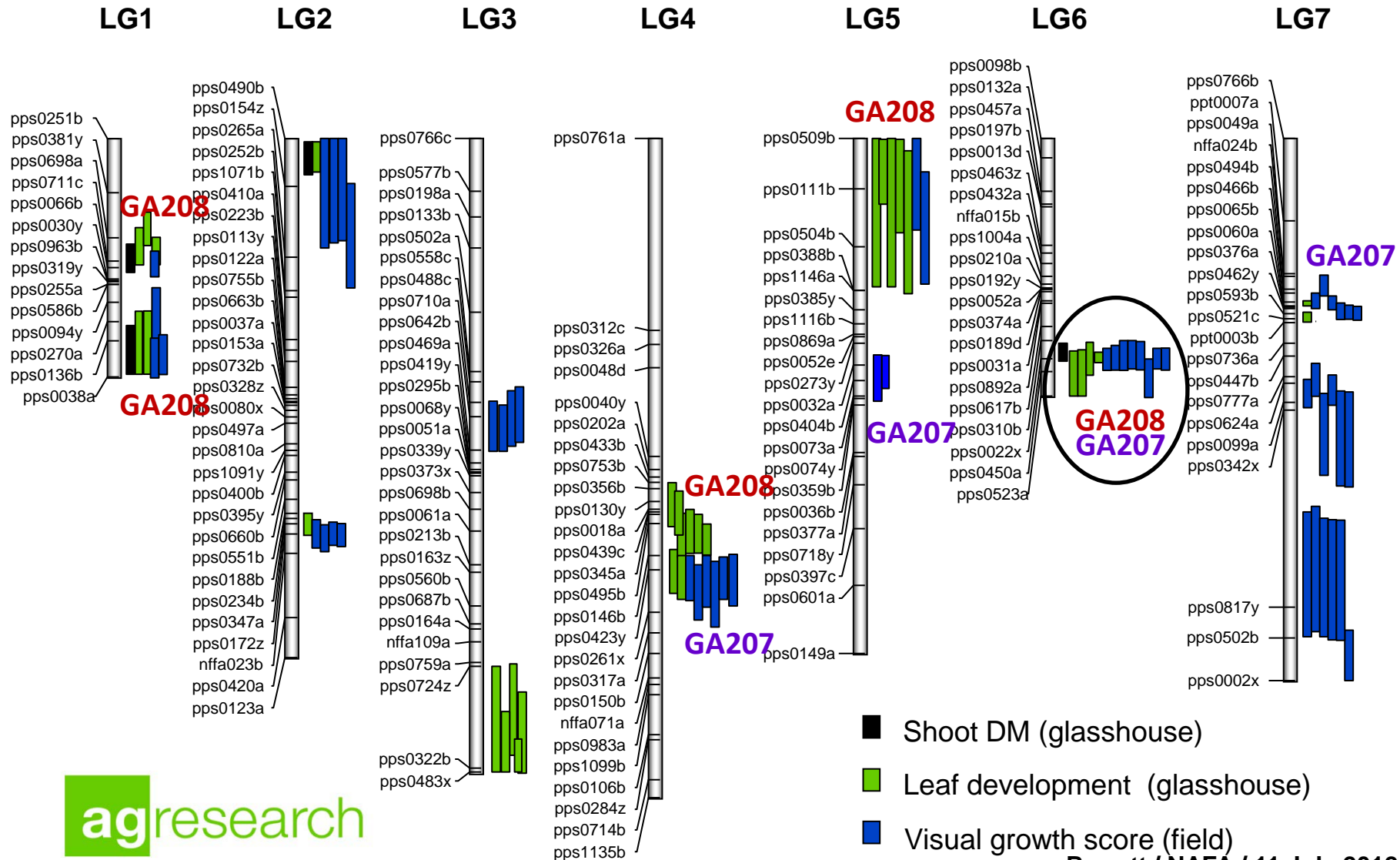
BLUPs: Growth scores prior to each grazing, DM four times per annum

24 SSR markers subtending 14 QTL

8 markers significant ($P < 0.001$) by regression in GA208

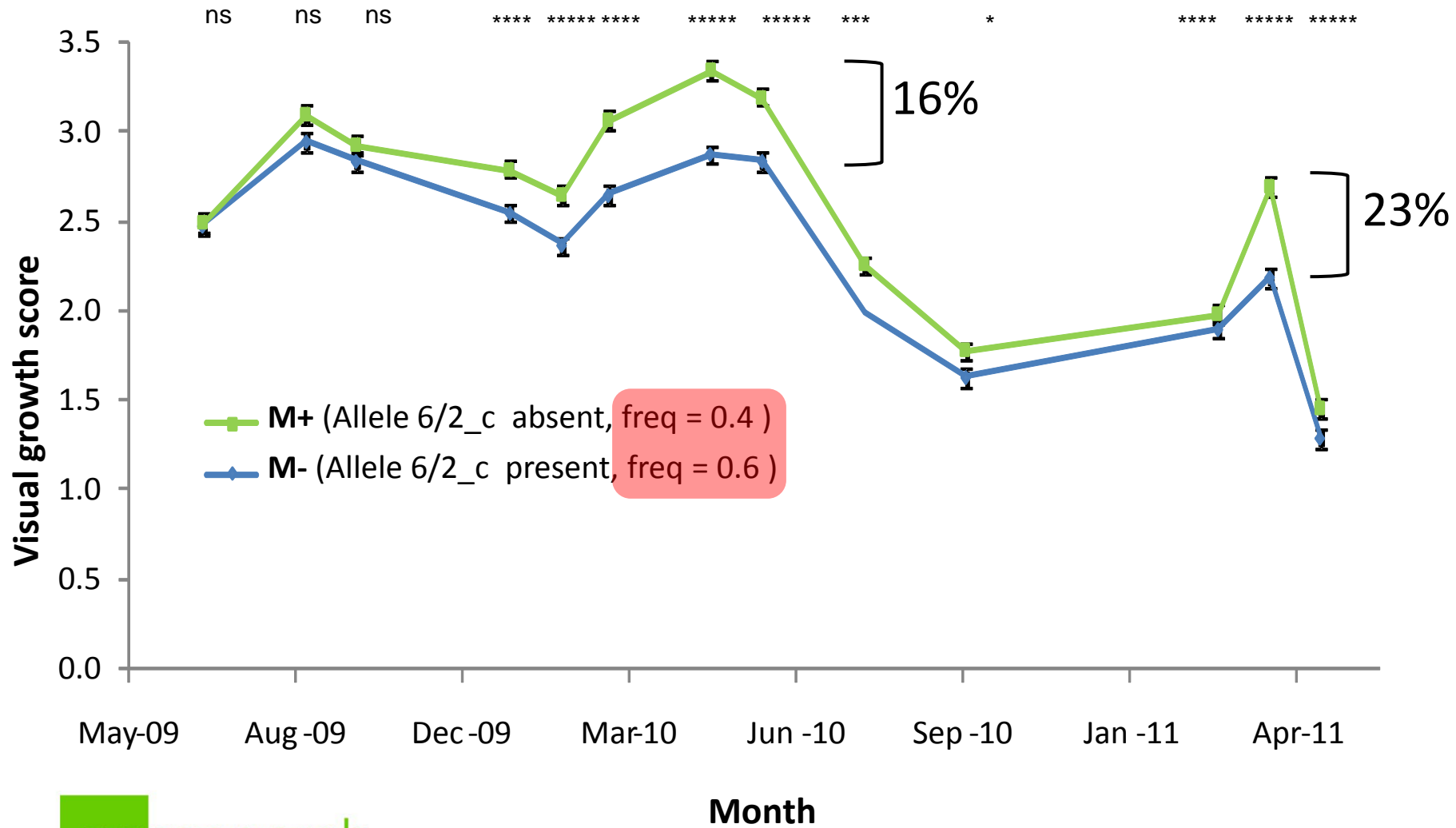


Associations Generally Population-specific

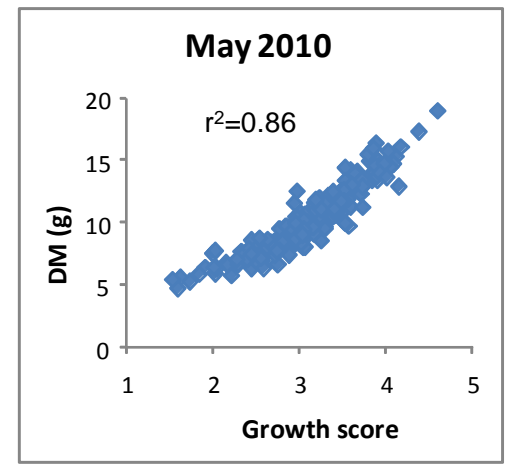
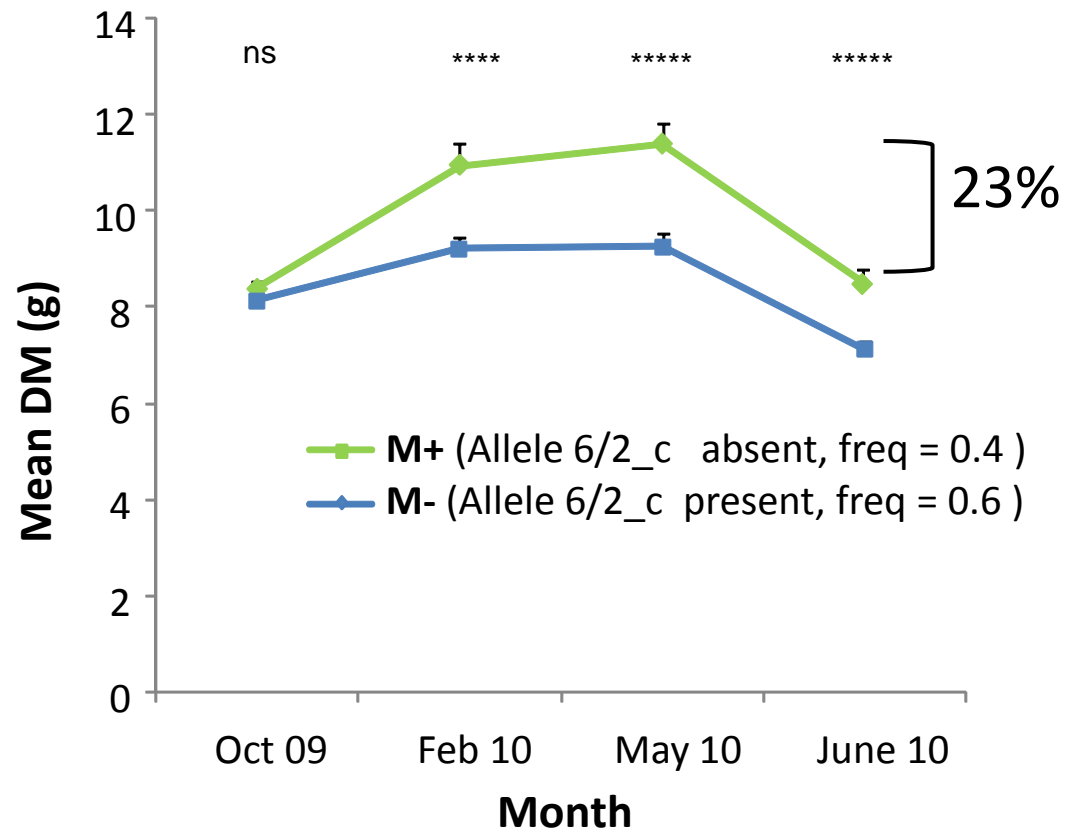




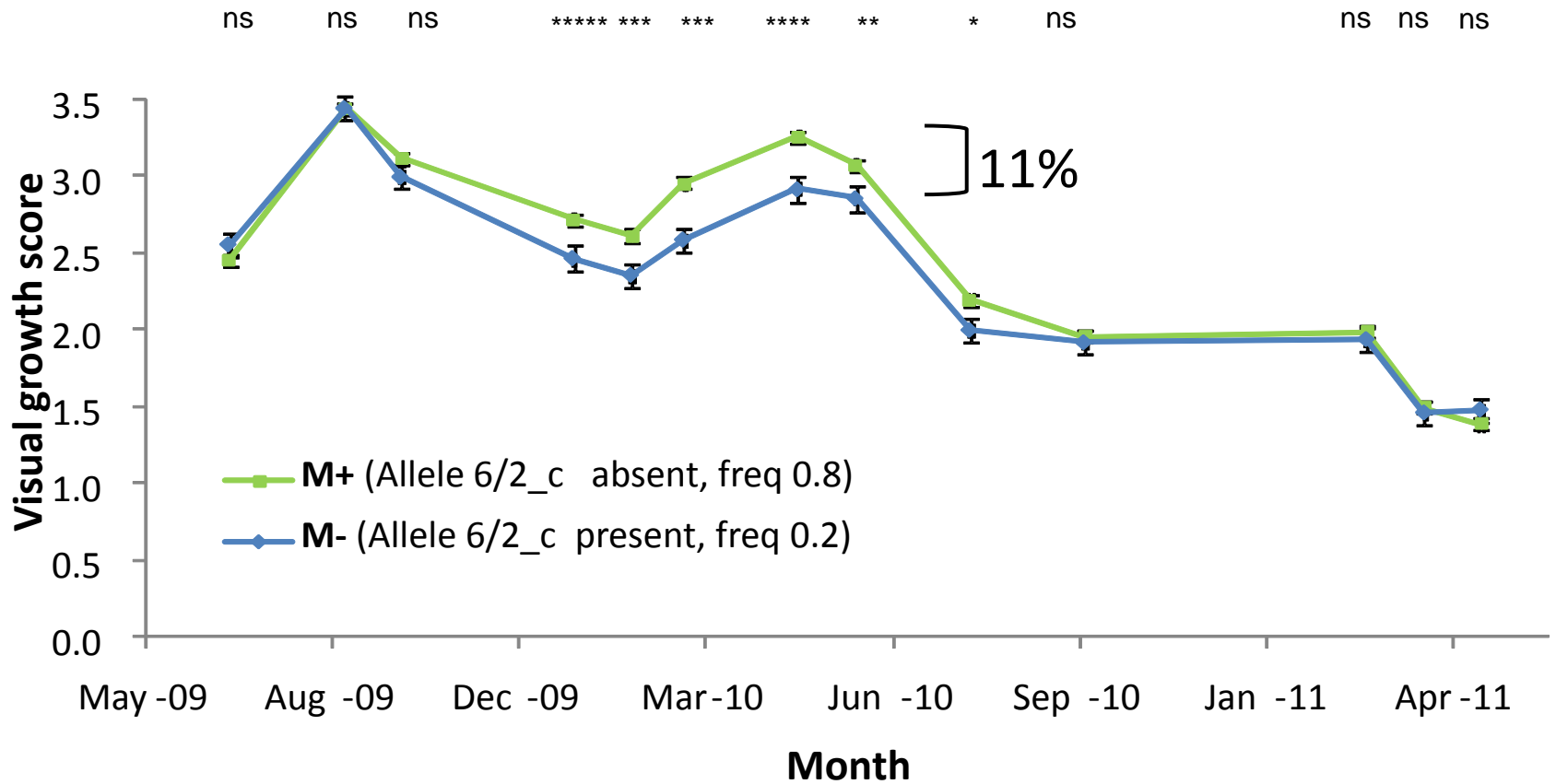
A Single Marker Detects a Substantial Herbage Yield Increase in GA208



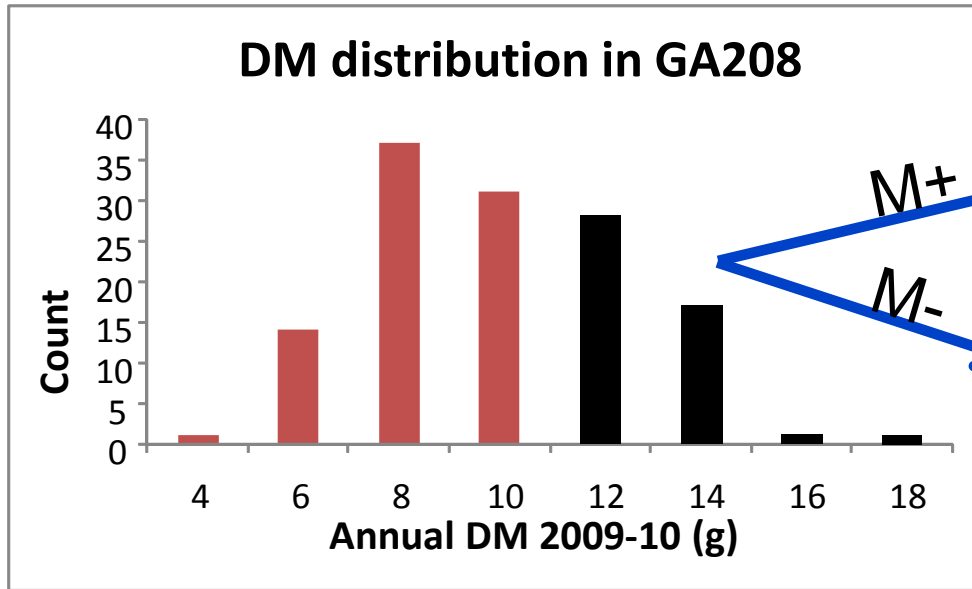
Dry Matter Yield effects in GA208



Similar Effect With Same Marker in GA207



GA208 Single Marker Selections



Mean DM

12.8 g (± 1.10 SD)
n = 10

M +
poly
cross

12.2 g (± 0.61 SD)
n = 10

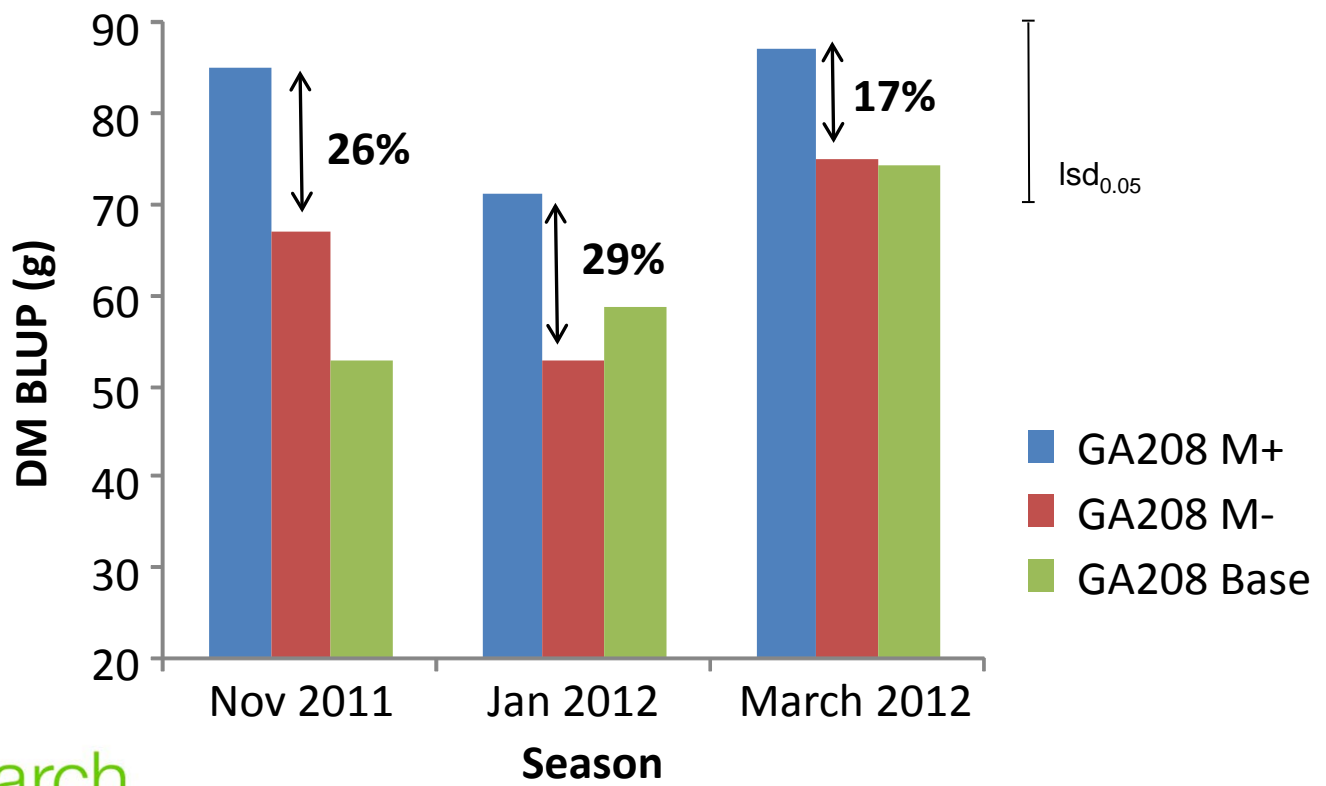
M -
poly
cross



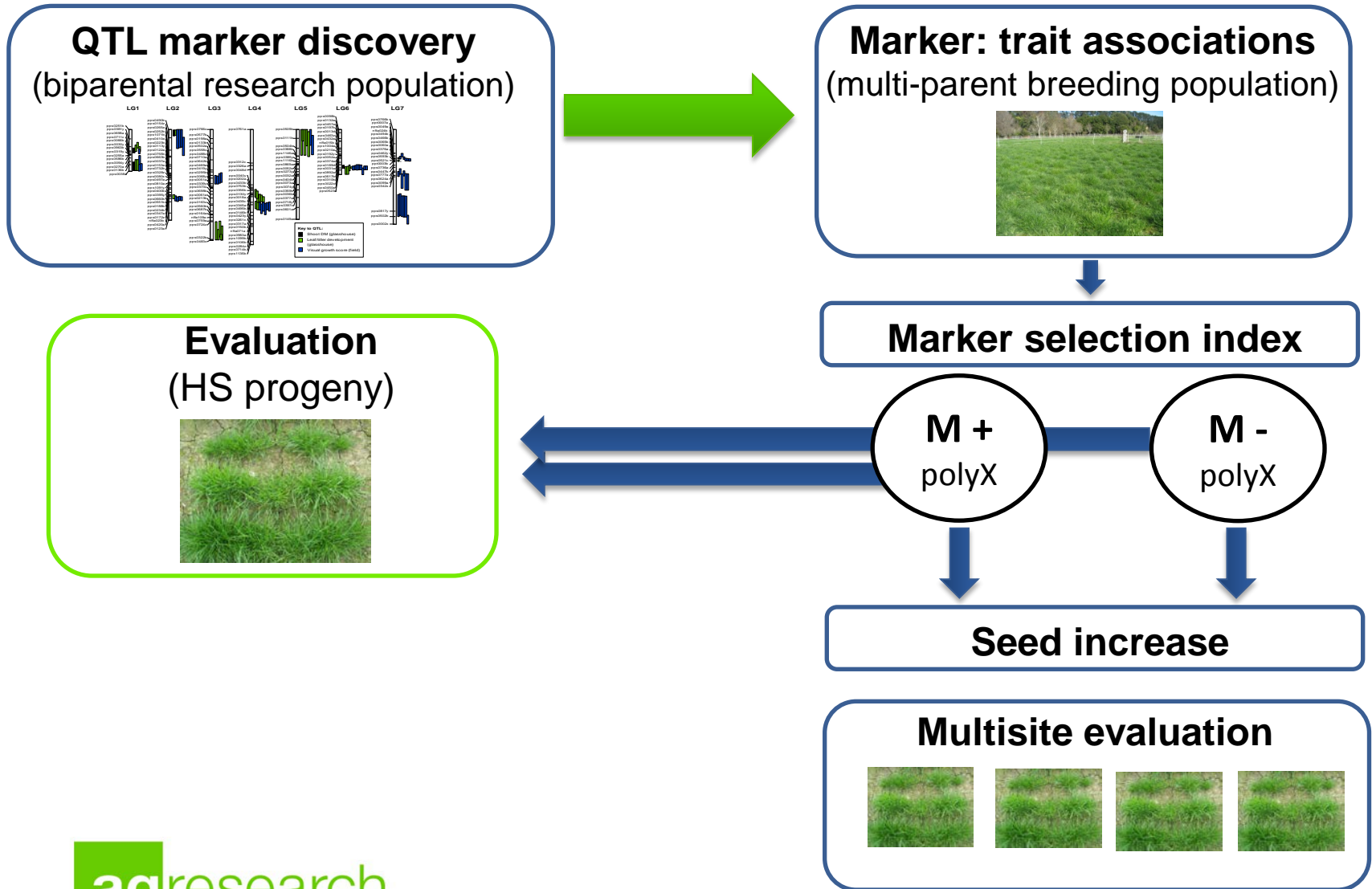
Evaluation Of Single Marker Selections



HS progeny (M+ and M- composites) testing in rows
DM and growth score data
Single site



Method





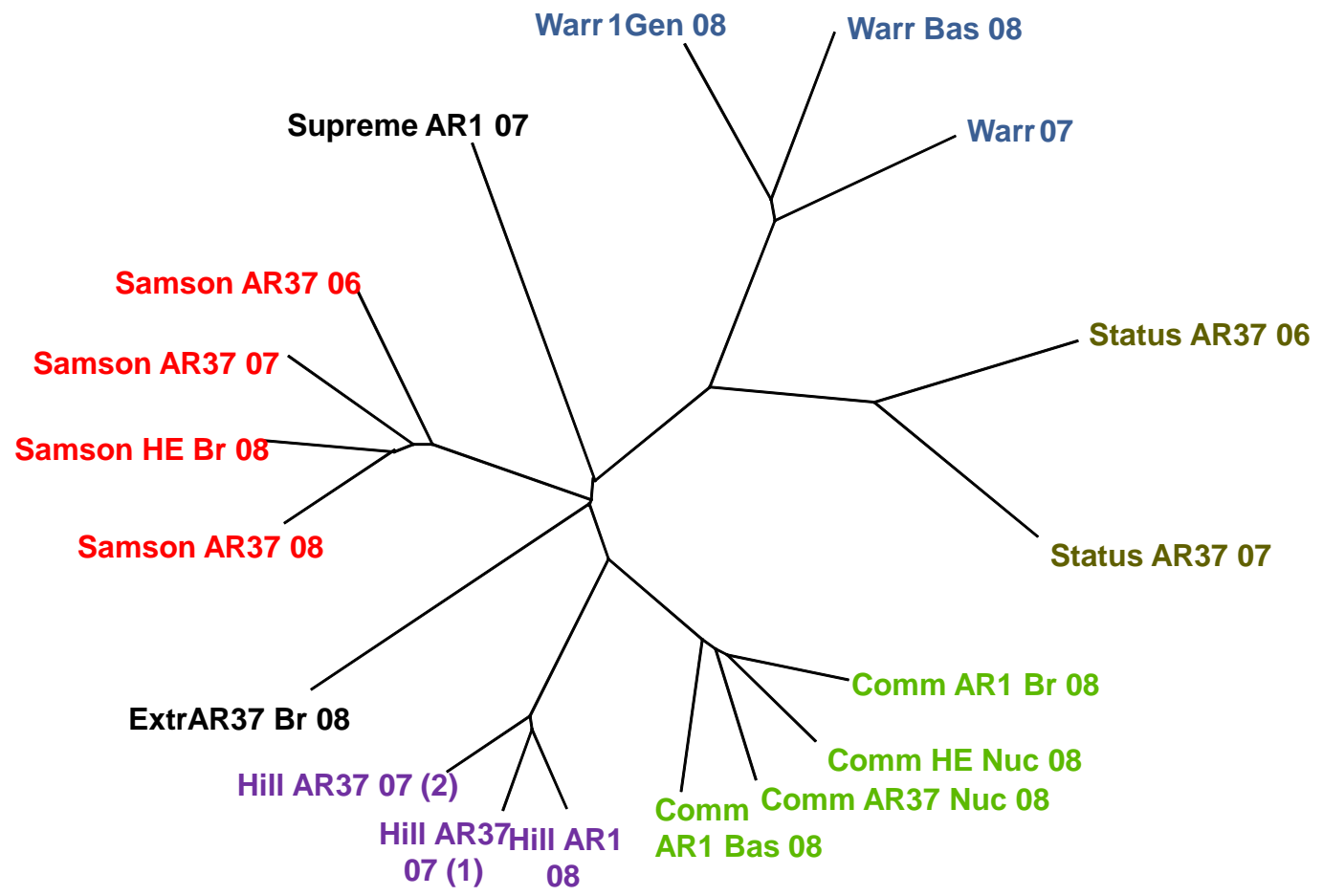
Molecular Breeding







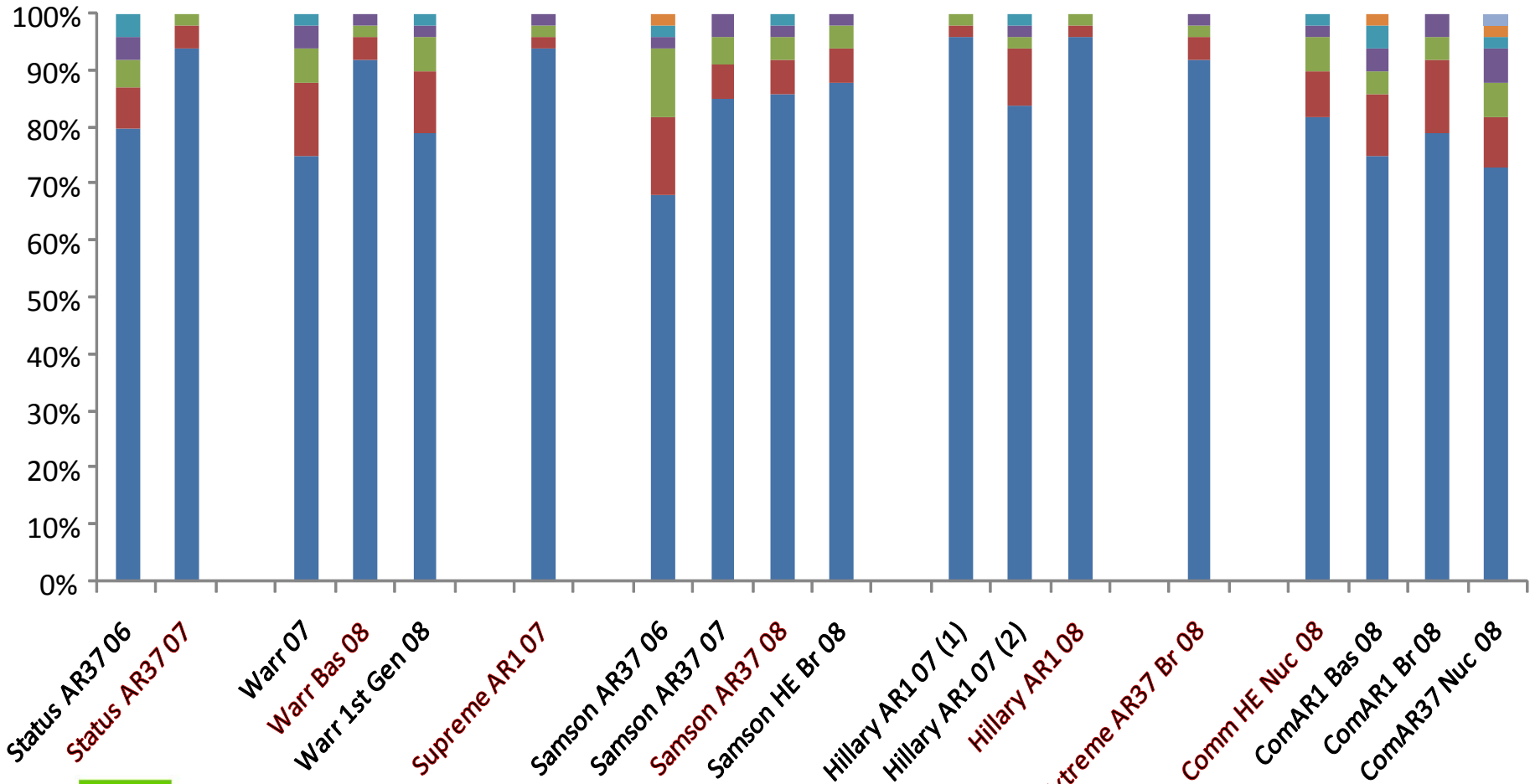
DNA Fingerprinting: Cultivar Tracking & IP Protection



Rogers GD 0.02 ———

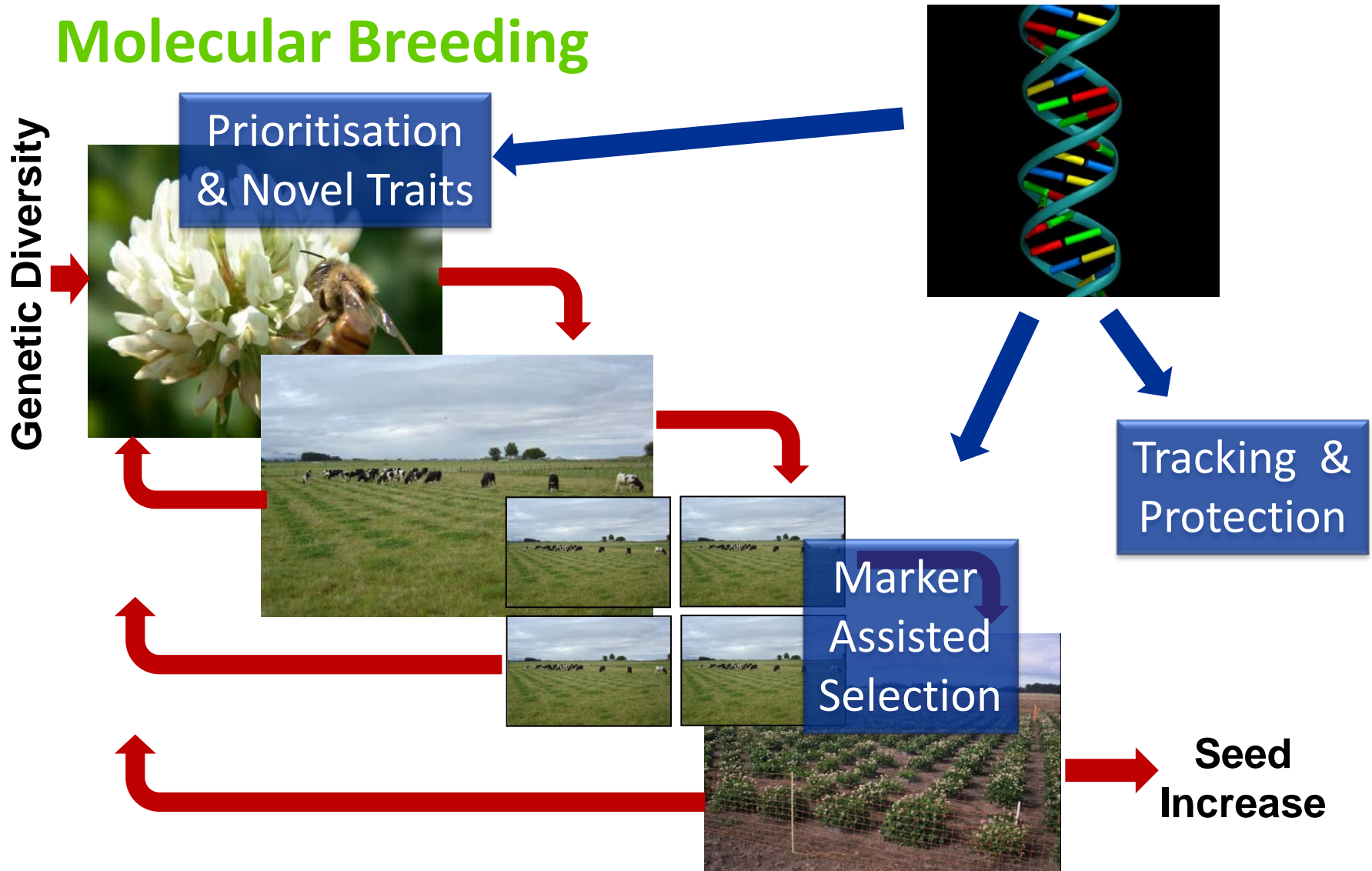


Frequency-Based Assignment Testing





Molecular Breeding



Marker-Assisted Selection: Looking Ahead

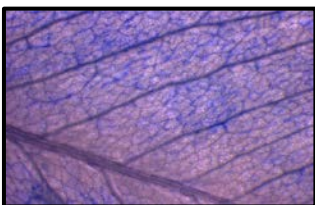
- ❖ Continued focus on utility for breeding and relevant phenotyping
- ❖ Ramp up application of herbage yield and other single marker tests in forage breeding populations
- ❖ Paternity testing in white clover and perennial ryegrass
- ❖ Beyond single locus selection, grappling with (& reaping the rewards of?) genomic selection
- ❖ Marker:trait association via spatial-temporal trends in allele frequencies for grazed plots & swards
- ❖ Beyond SSRs, implementation of high density marker system; Genotyping by Sequencing

Acknowledgements



Grazing Tolerant Red Clover

John Ford
Zulfi Jahufer
Derek Woodfield



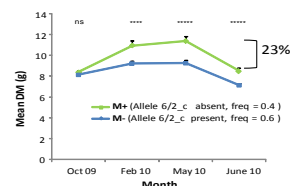
Alfalfa & Clover Condensed Tannins

Kerry Hancock
Vern Collette
Anar Khan



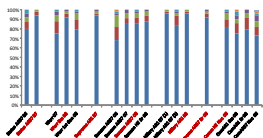
Trifolium Inter-Specific Hybrids

Warren Williams
Isabelle Williams
Nick Ellison



Marker-Assisted Selection

Marty Faville
Andrew Griffiths
Zulfi Jahufer



DNA Fingerprinting

Marty Faville
Jana Schmidt



Investment

