Advances in Forage Research and Breeding at the Noble Foundation

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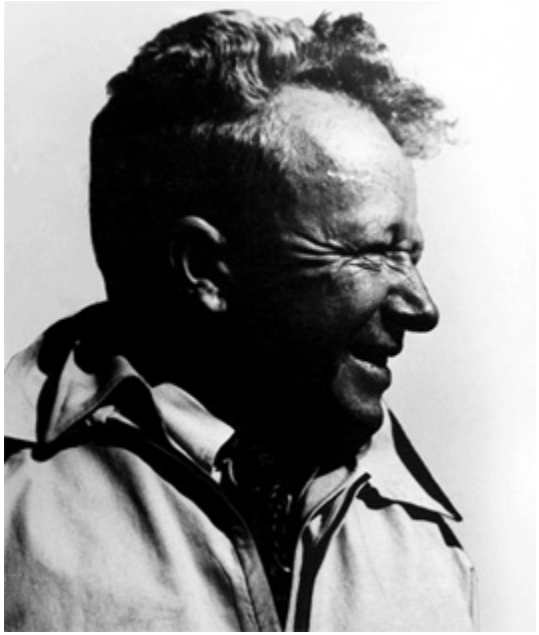
July 9, 2014

NAAIC, Trifolium and Grass Breeders Conference

Lethbridge, Canada

THE SAMUEL ROBERTS NOBLE FOUNDATION

The Noble Foundation - Values



THE SAMUEL ROBERTS
NOBLE
FOUNDATION

Mission Statement

The purpose of the NF is to **advance agricultural science** and practice by conducting field and laboratory research.

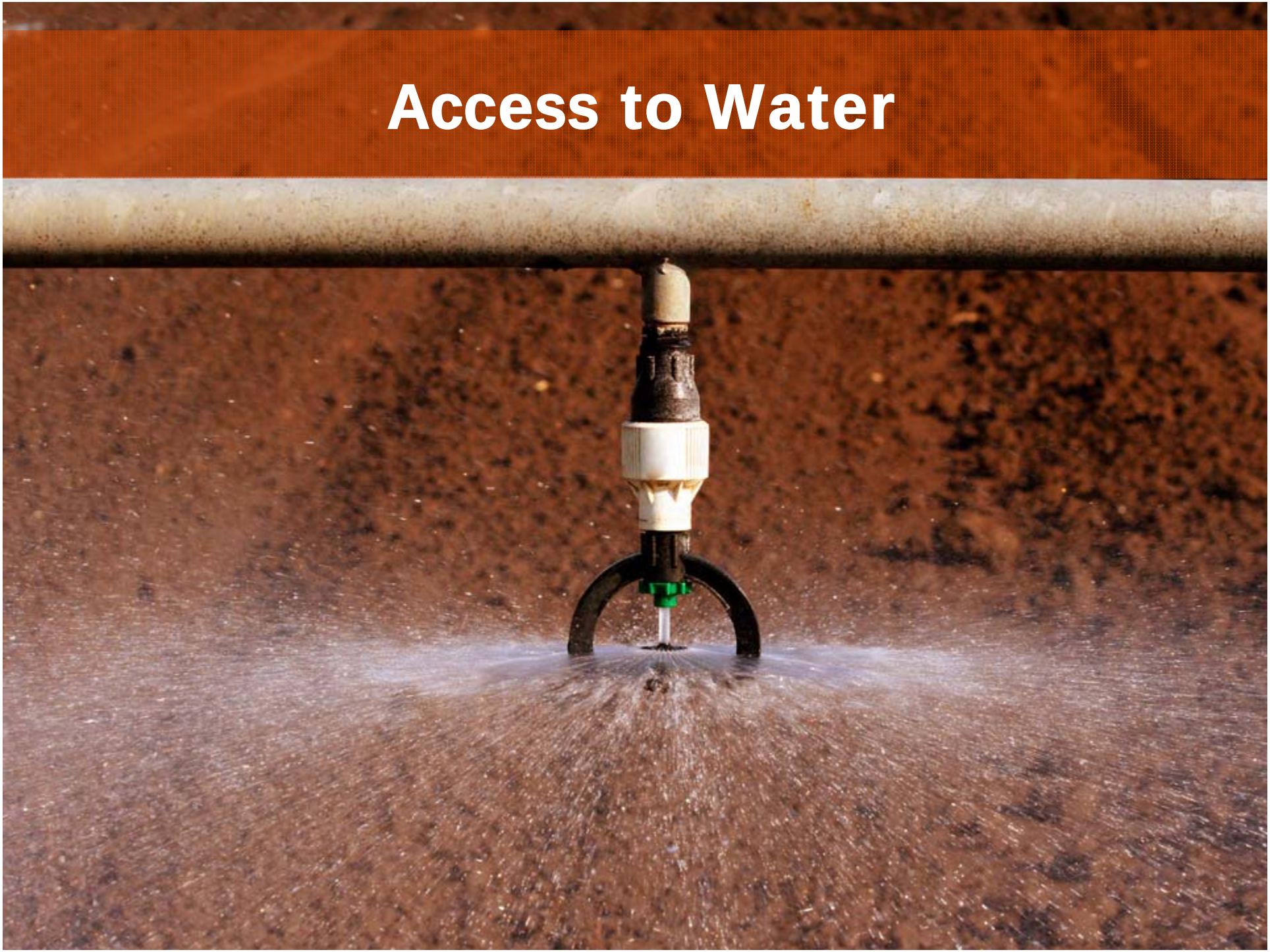
Vision Statement

The NF will provide **solutions to the agriculture challenges** facing the region, nation and world, thereby contributing to improved global food security.

The NF will generate **purposeful outcomes** of **knowledge, technology and products** that benefit agricultural producers and consumers

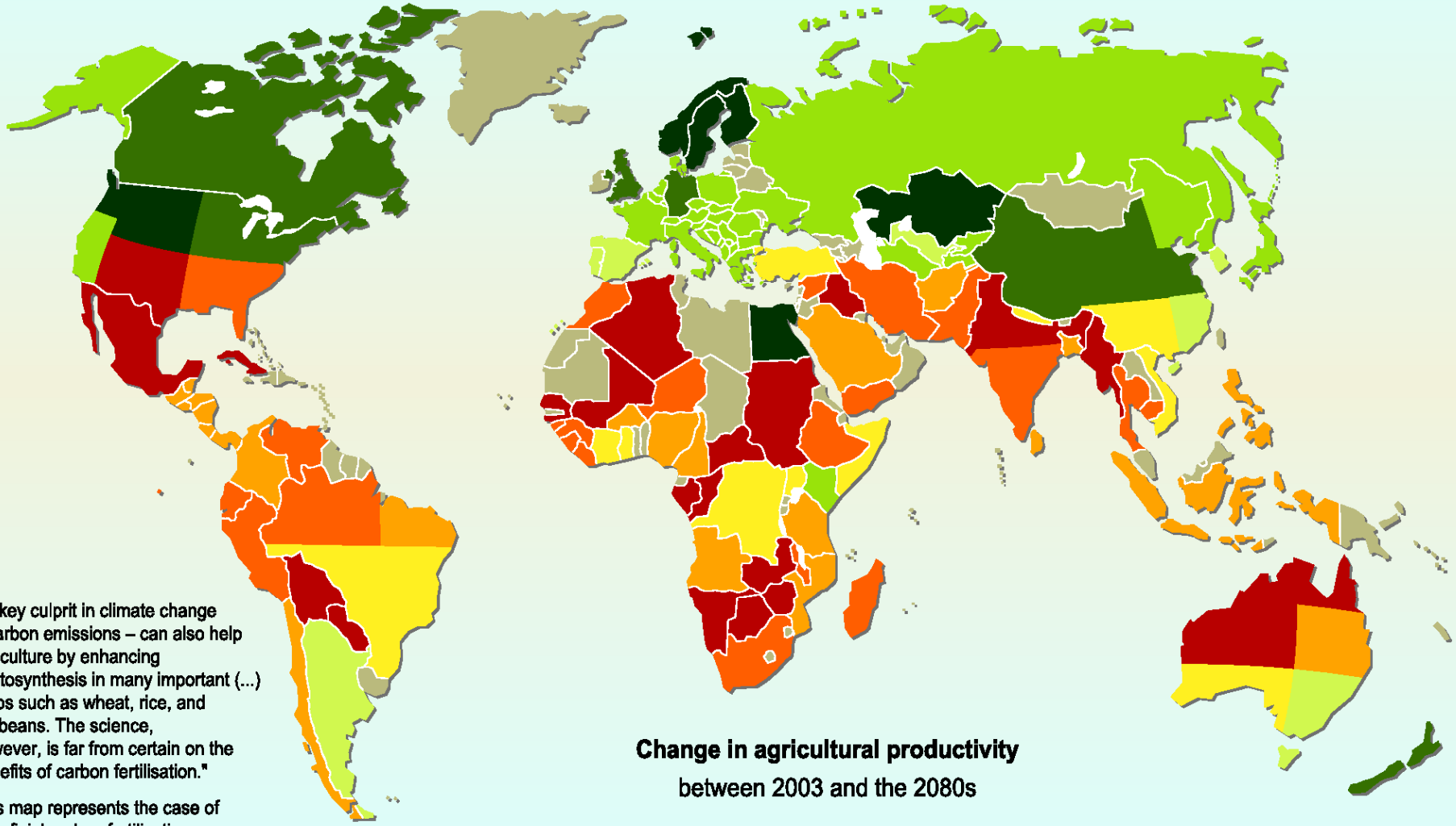
Population Growth

Access to Water



Soil Amendments and Fertilizers

Impact of Climate Change on Agricultural Yields



" A key culprit in climate change – carbon emissions – can also help agriculture by enhancing photosynthesis in many important (...) crops such as wheat, rice, and soybeans. The science, however, is far from certain on the benefits of carbon fertilisation."

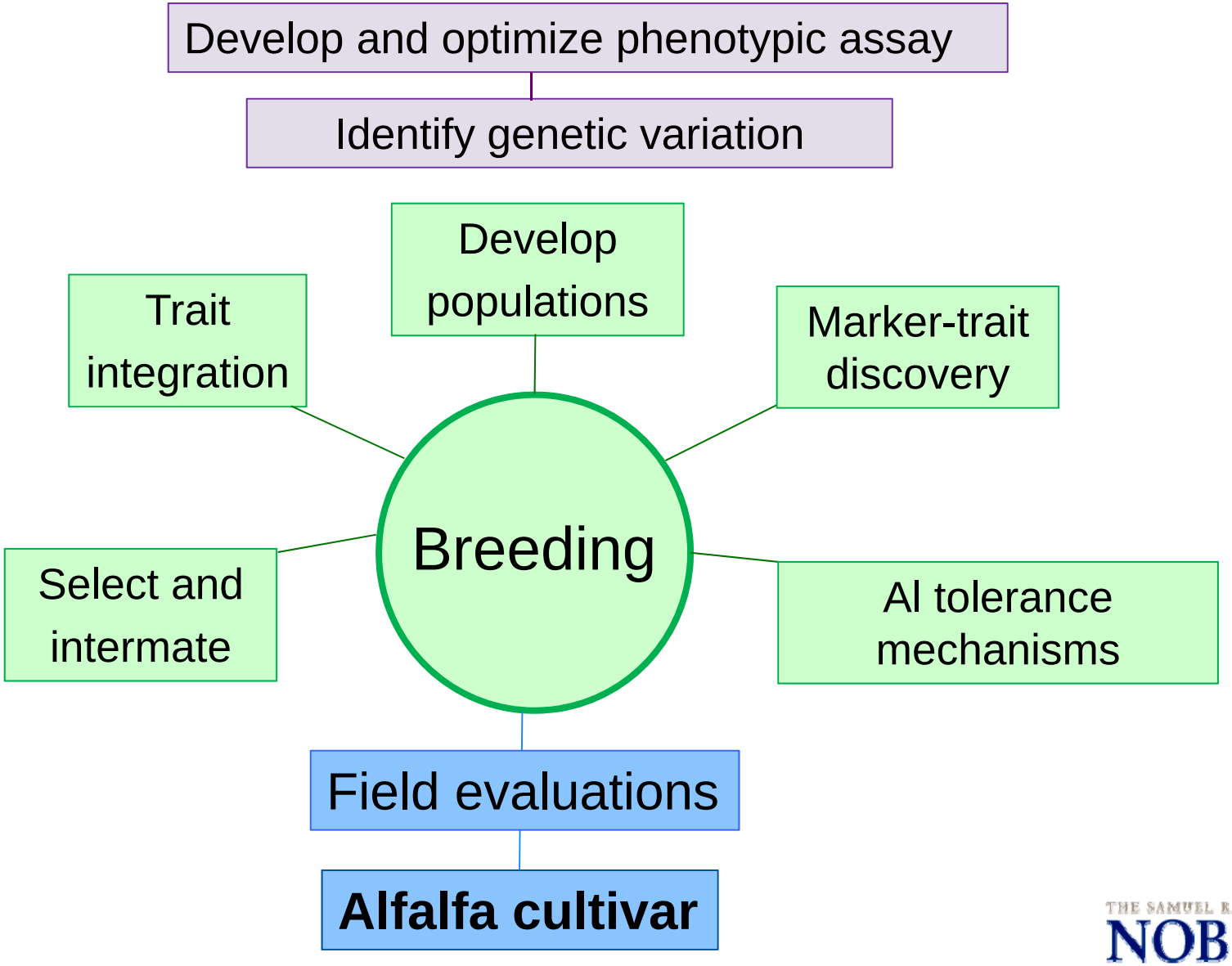
This map represents the case of beneficial carbon fertilisation processes.

Source: Cline W., 2007, *Global Warming and Agriculture.*

Sustainable Agriculture Initiatives

Target Traits for Improvement

Acid and Al Tolerance in Alfalfa - Overview



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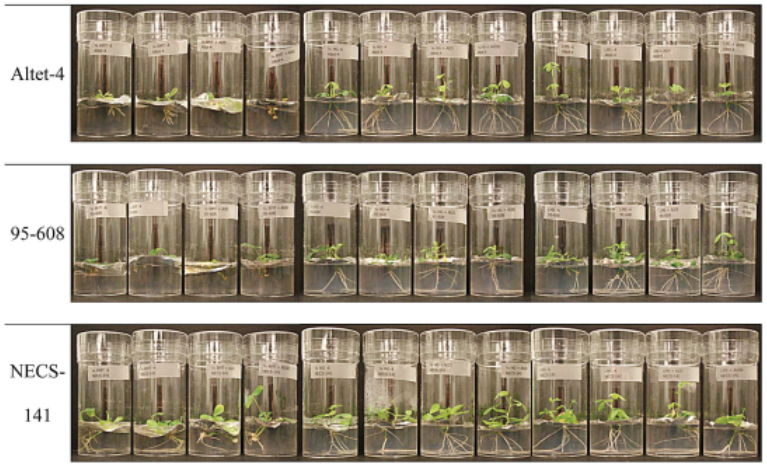
Acid and Al Tolerance in Alfalfa - Phenotype

Develop and optimize phenotypic assay

Identify genetic variation

	Modified Blaydes Media (MBD)				Modified Hoagland Media (MHG)				Least Macro Salt Media (LMS)			
pH	7	4	4	4	7	4	4	4	7	4	4	4
Al (µM)	0	0	100	400	0	0	50	100	0	0	50	100



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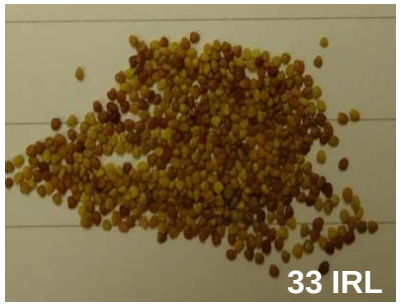
Seedling Assay - Al Tolerance in White Clover

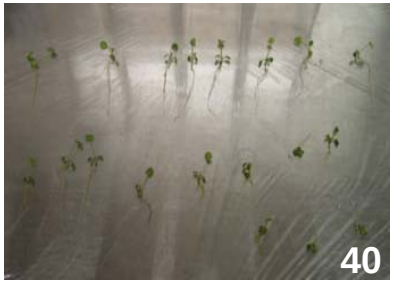
Base population



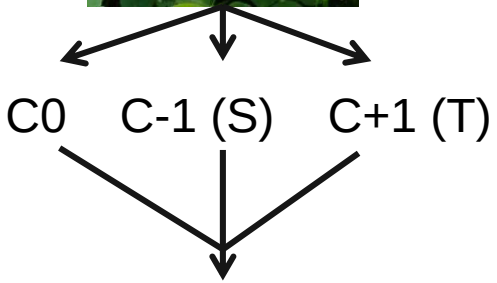
Phenotype

500 plants at 1200 µM $AlCl_3$

Selection



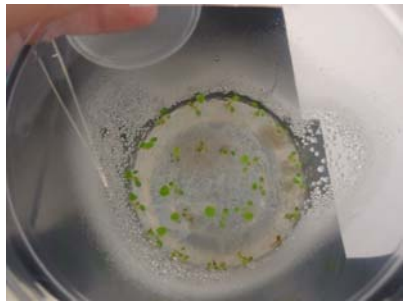
Seed production



Field trials (BZ)

Population	Root length (cm)
Sus (C-1)	3.65^{c}
Cycle 0	4.29^{bc}
Tol (C+1)	5.00^{a}

Phenotype



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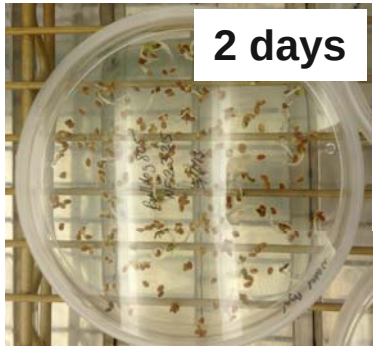
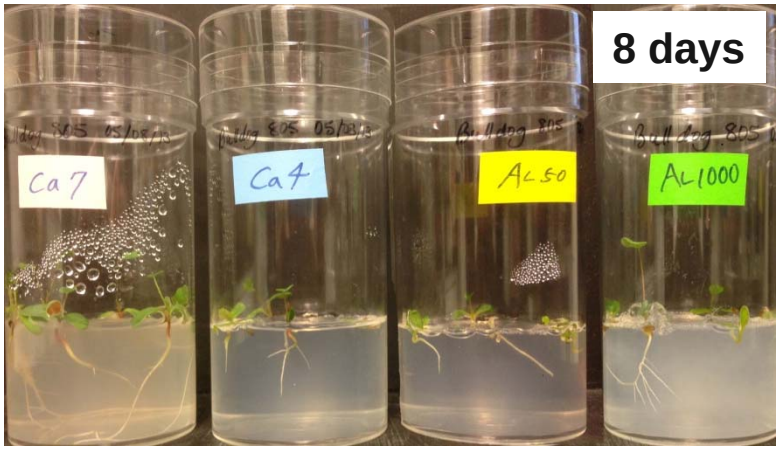
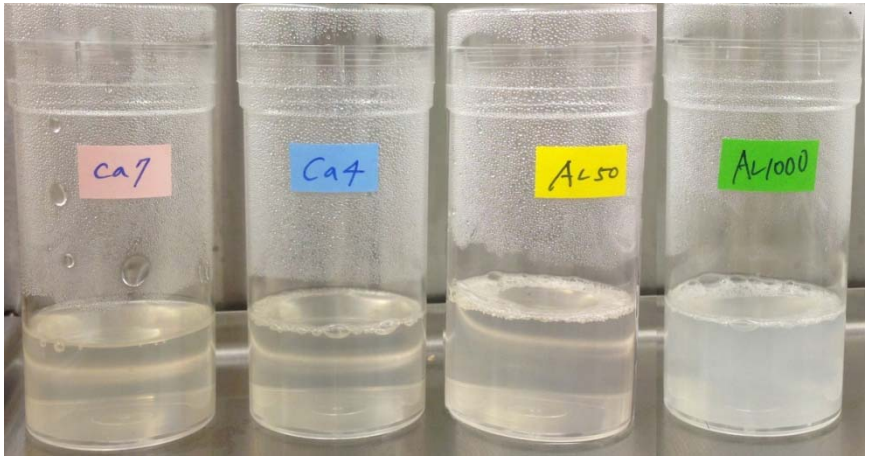
Al Tolerance Seedling Assay – Bulldog805

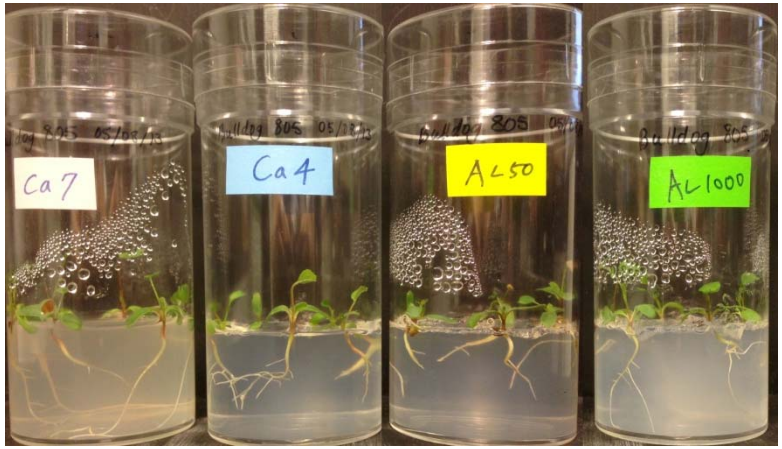
Develop and optimize phenotypic assay

Germplasm Selection

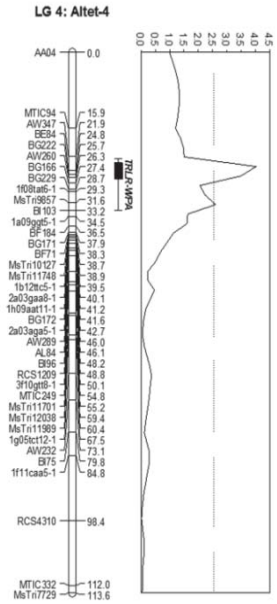








Acid and Al Tolerance in Alfalfa - Populations



Develop populations

Trait integration

Marker-trait discovery

Breeding

Select and intermate

Al tolerance mechanisms

Khu et al., 2013. Crop Sci.

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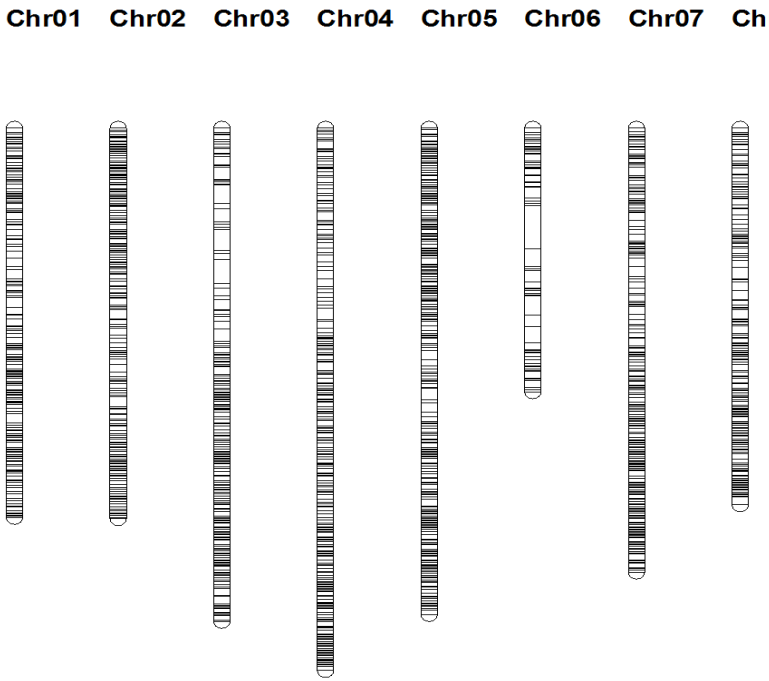
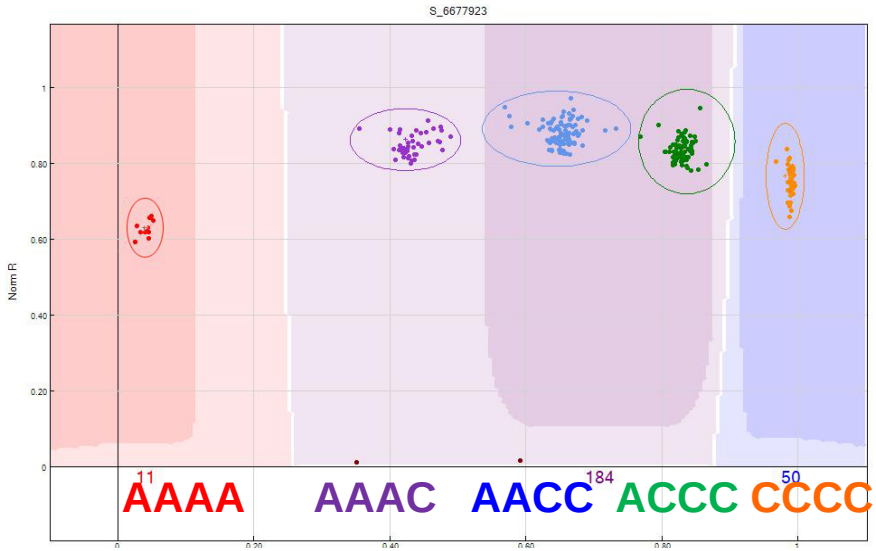
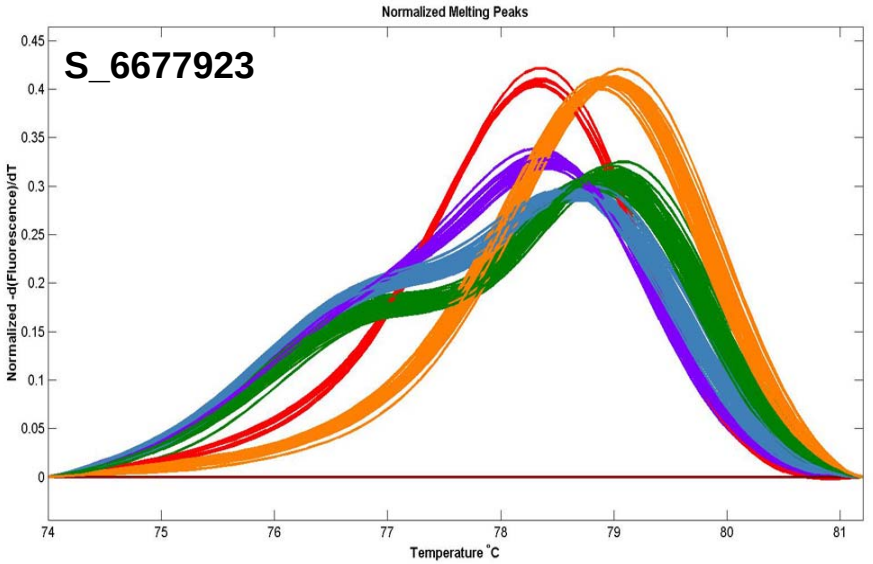
Marker-Trait Discovery (SNP Genotyping)





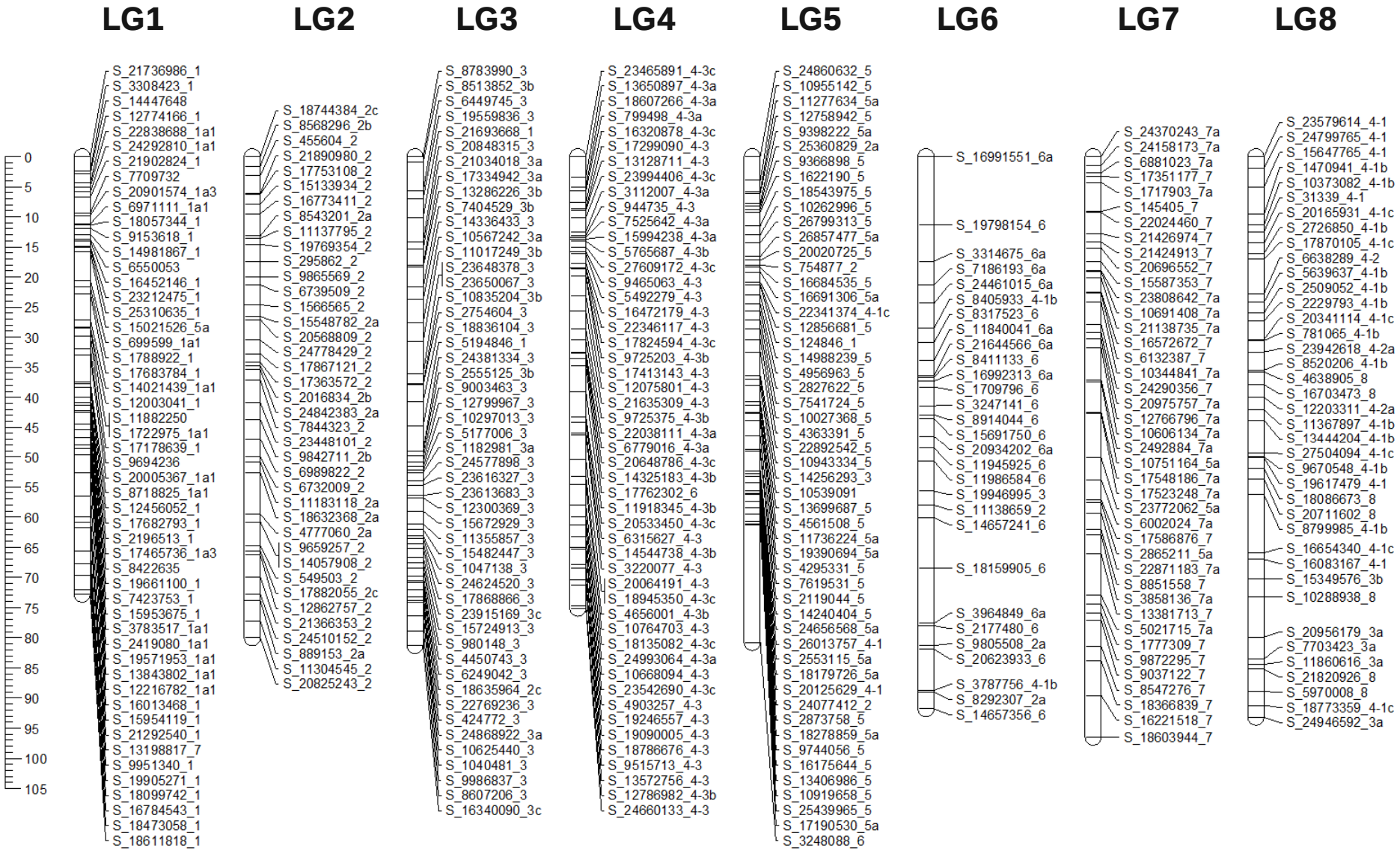


- Infinium iSelect Chip
- 9,277 SNP in CG
- 576 alfalfa genotypes

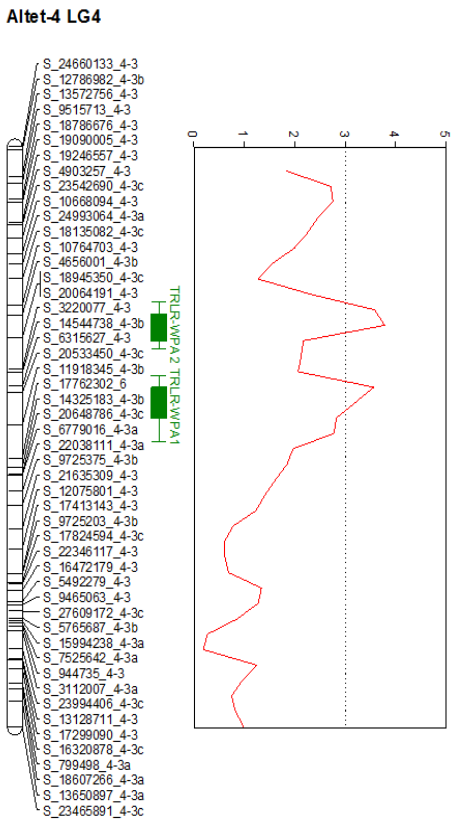
Han et al., 2011. BMC Genomics; Han et al., 2012. Mol. Breeding.

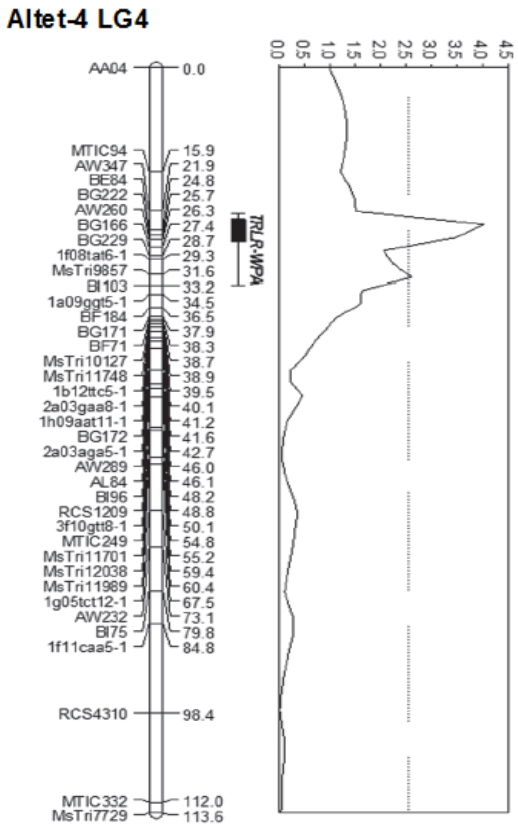
Alfalfa Linkage Map - Infinium SNPs

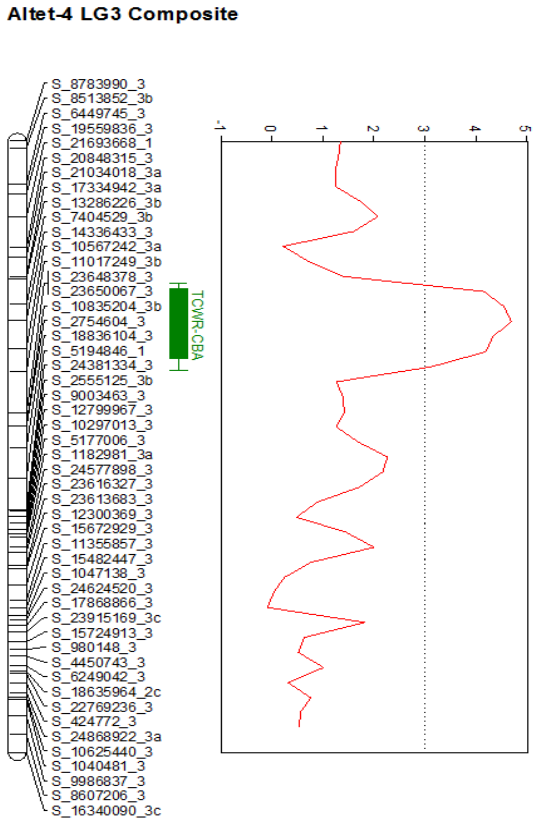
Altet-4

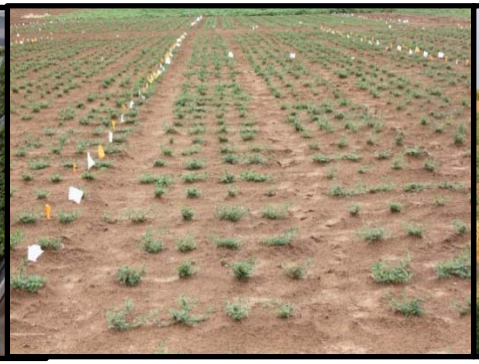


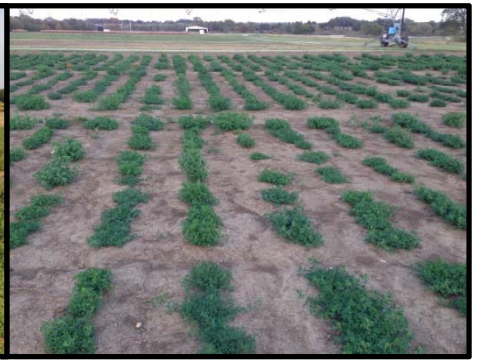
Al Tol QTL (Refine and Discover)











Acid and Al Tolerance in Alfalfa – Mechanisms

NAAIC Poster #1

Develop populations

Trait integration

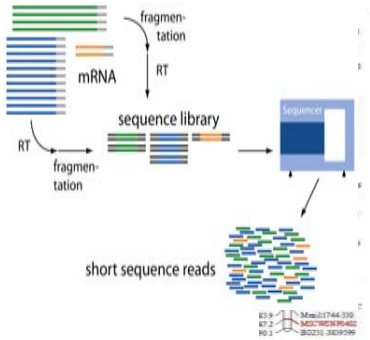
Marker-trait discovery

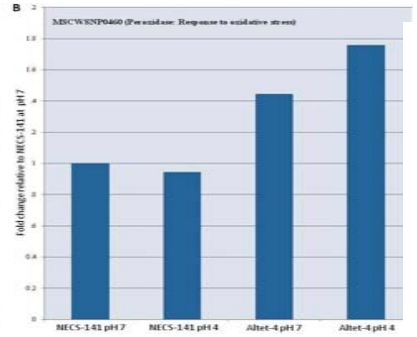
Breeding

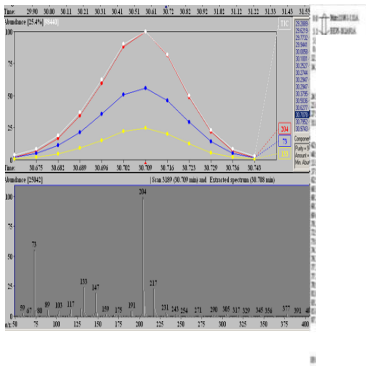
Select and intermate

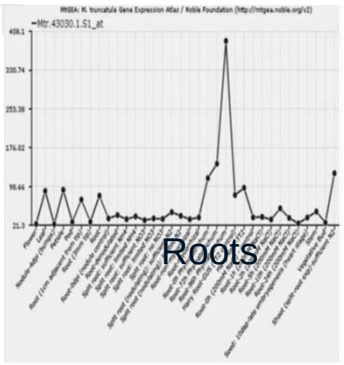
Al tolerance mechanisms

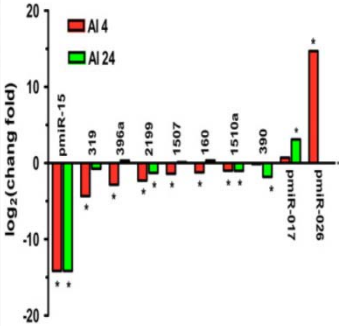
RNA Seq qRT-PCR Metabolomics Gene Atlas miRNA





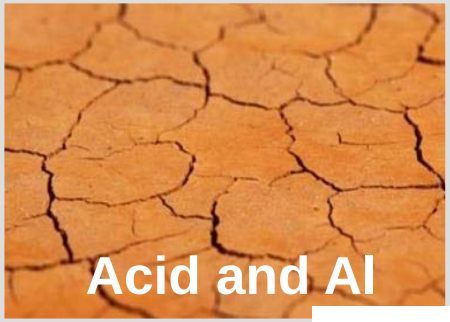




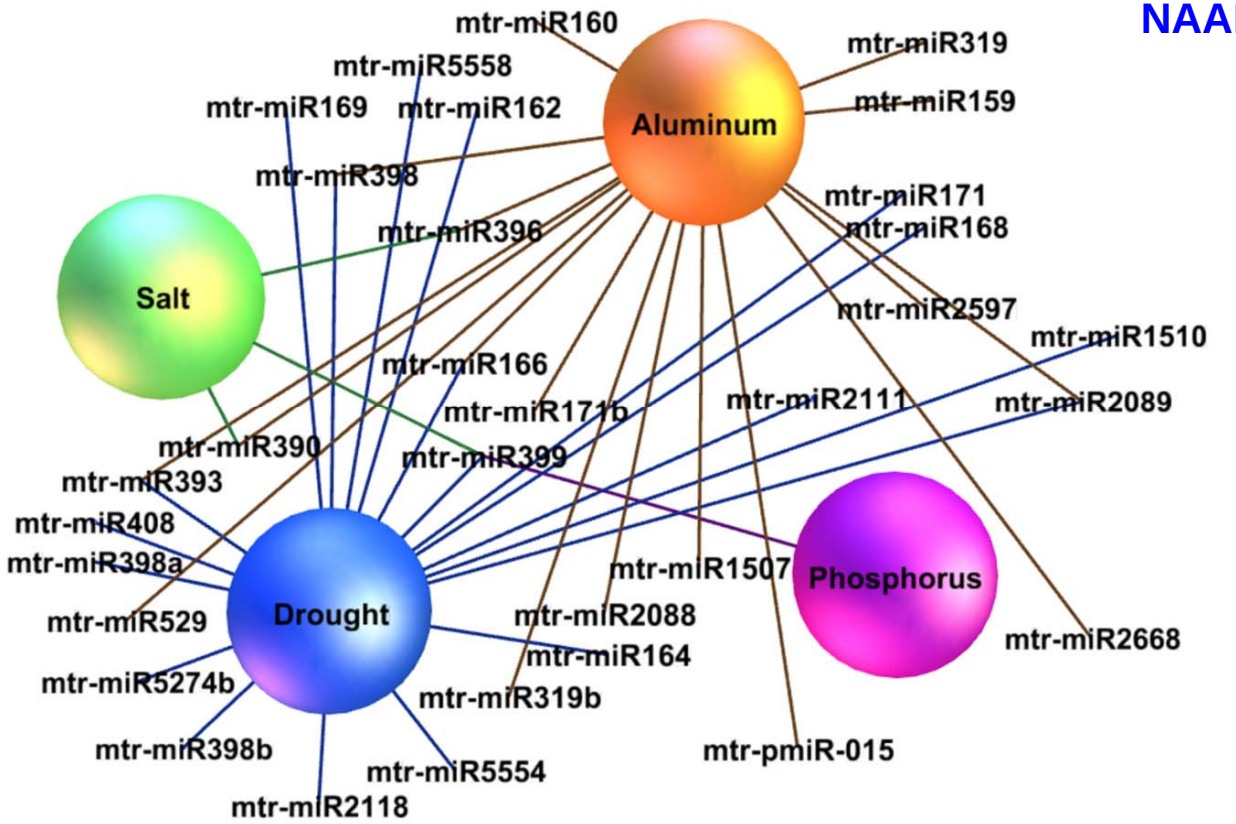


Discovery of miRNAs in Alfalfa

Small, non-coding RNAs that regulate gene expression



NAAIC Poster #24



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Acid and Al Tolerance in Alfalfa - Breeding

Develop populations

Trait integration

Select and intermate

Breeding

Altet-4 × NECS-141

F_1 Phenotype

Al Tol F_1 × Bulldog805 / NFAA07-6

Phenotype: field and WPA

T = C+1

S = C-1

Tolerant

×

BC Bulldog805 (Nd+2)

Sensitive

×

BC Bulldog805 (Nd+2)

T = C+2

S = C-2

Phenotype

Recombine

Select

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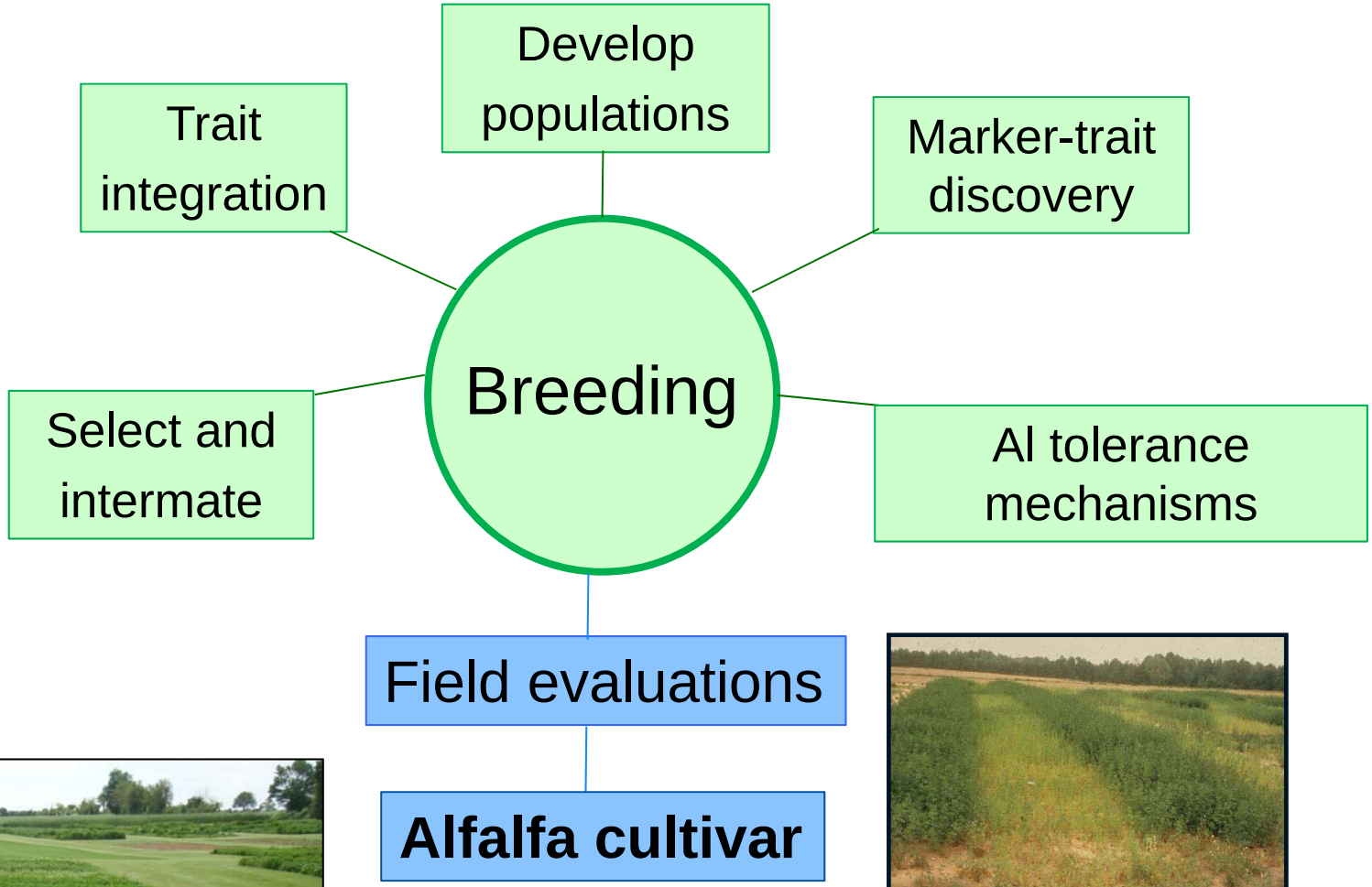
Acid and Al Tolerance in Alfalfa - Testing



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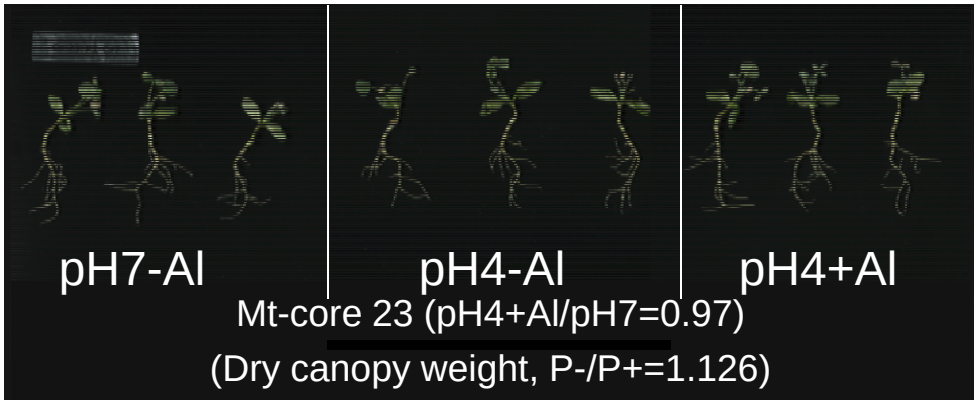
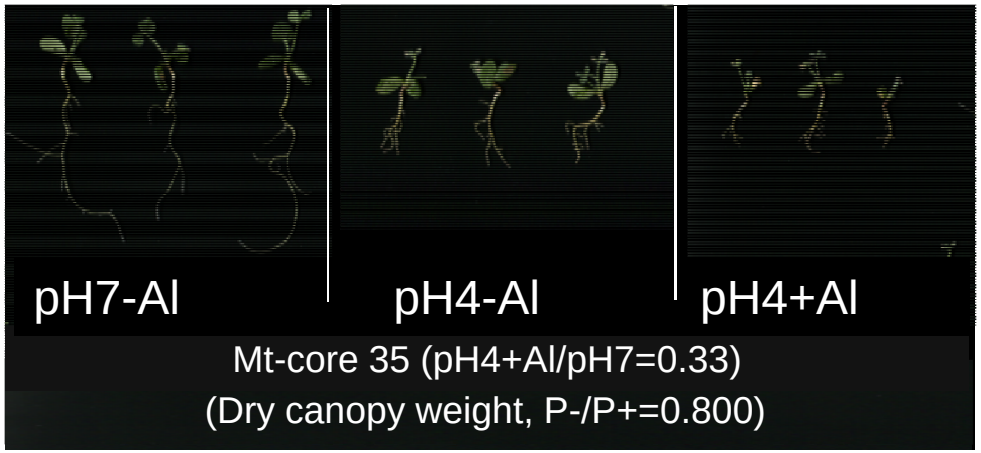
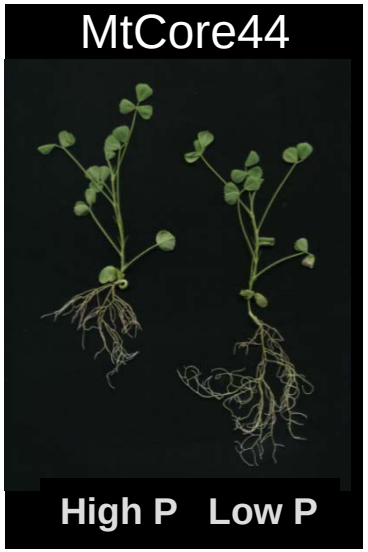
Phosphorus Utilization (*M. truncatula* & Alfalfa)

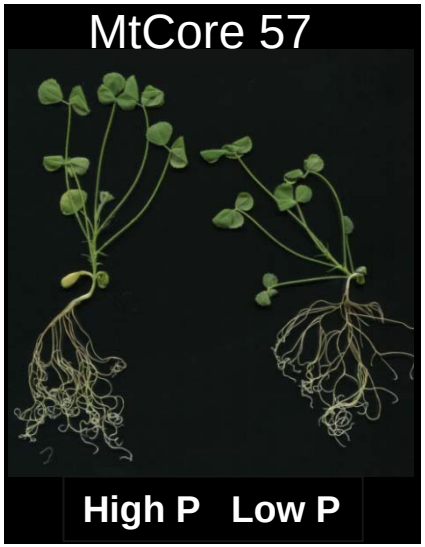


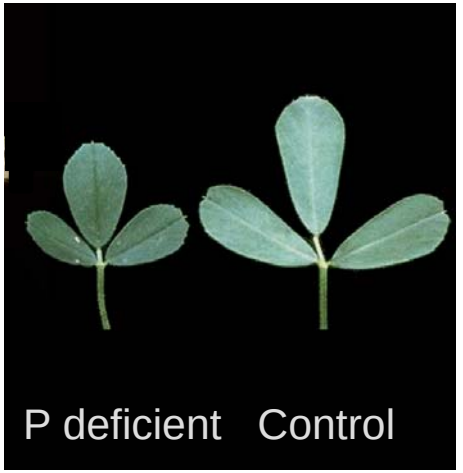


High P: KH_2PO_4 1000 µM

Low P: KH_2PO_4 0.5µM







Root growth

Biomass

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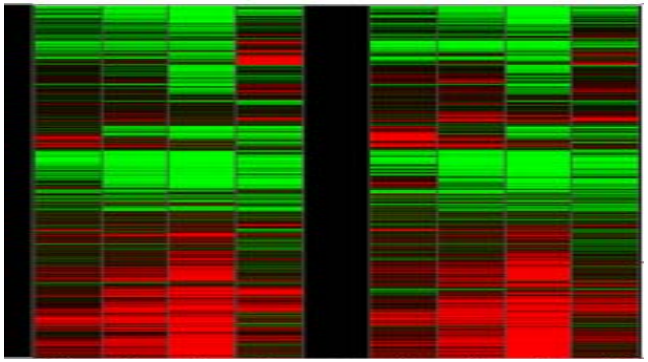
Drought Tolerance in Alfalfa

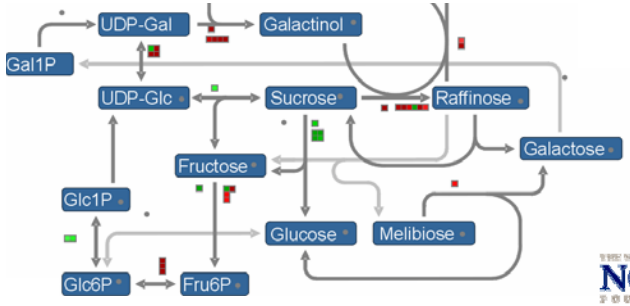
Germplasm characterization (field)

Identify desirable genotypes (pop. dev.)

Value of population for stress (Al tol)

Drought responses





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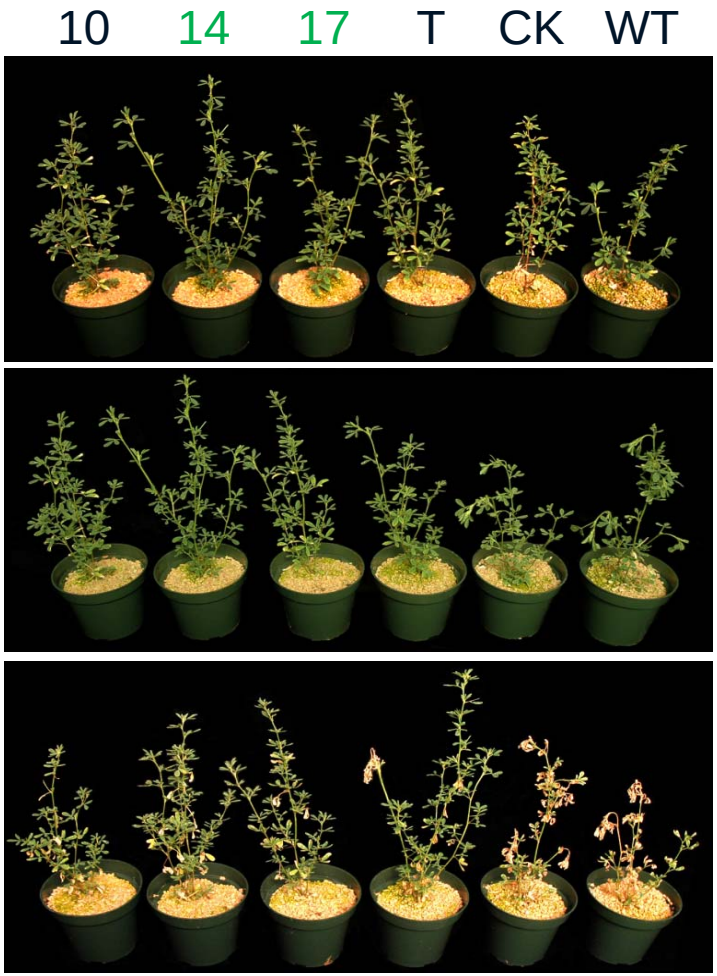
Over-expression of *WXP1* for Drought Tolerance



T = *WXP1* construct: CK = empty vector control

Forage Genetics International

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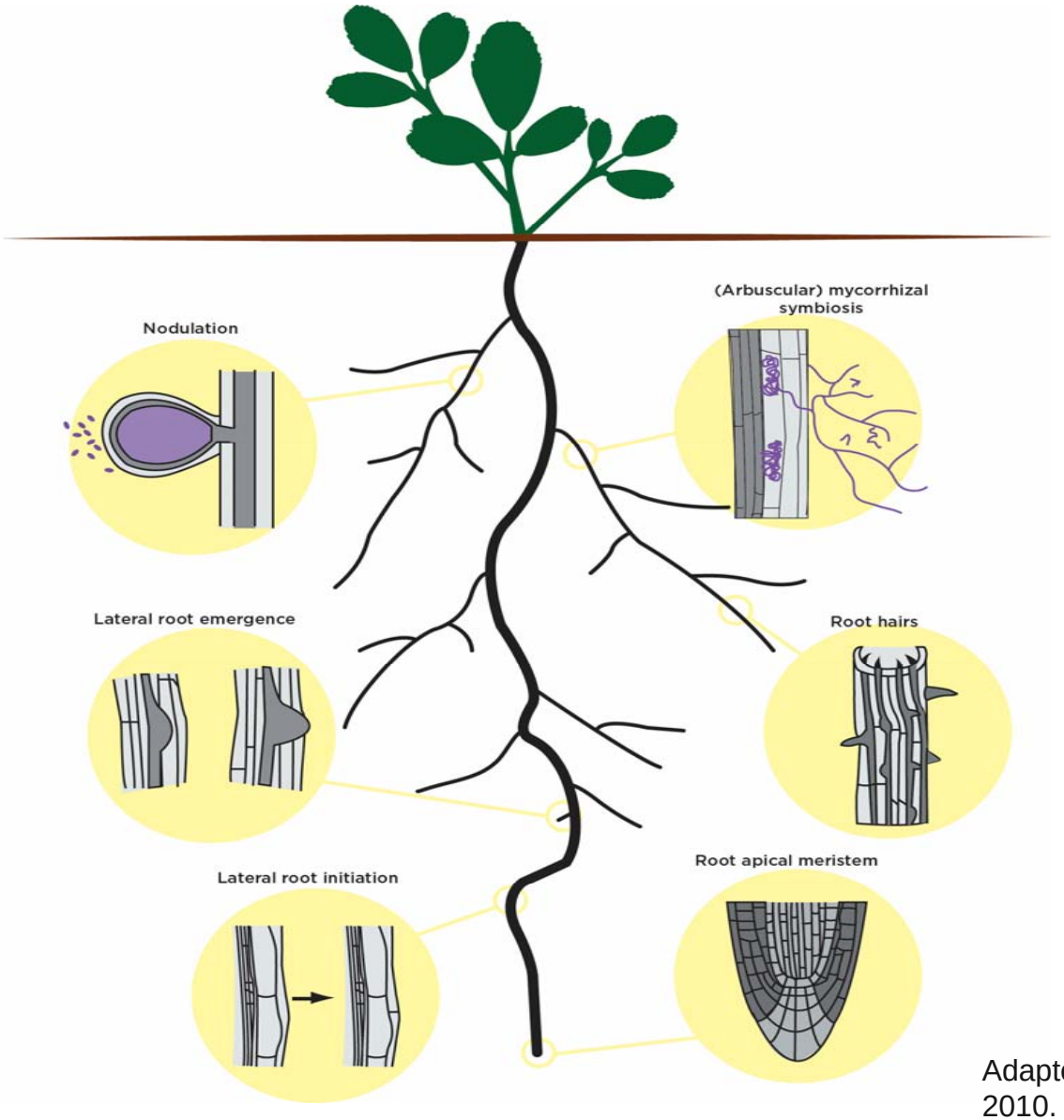
Growth and Development of Alfalfa Roots



- Nutrient and water-use-efficiency
- Beneficial microbes
- Site of Al toxicity
- Root structure affects capacity to access water
- Opportunity to understand root architecture in alfalfa
- Winterhardiness, persistence
- “Root breeding”

Adapted from Den Herder et al., 2010. Trends Plant Sci. 15:600-607.

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Root Phenotyping in the Field

Challenges:

- labor intensive
- large variability
- underestimation of fine roots
- lost 3-D, spatial distribution
- High throughput is difficult
- Roots in space and time

‘Shovelomics’ in corn

- Visual vs. measured traits
- Numbers, angles and patterns

Trachsel et al., 2010. Plant and Soil. DOI 10.1007/s11104-010-0623-8

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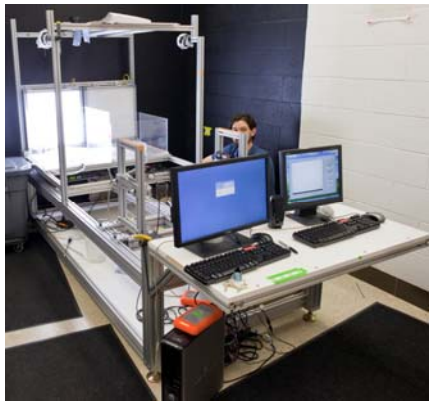
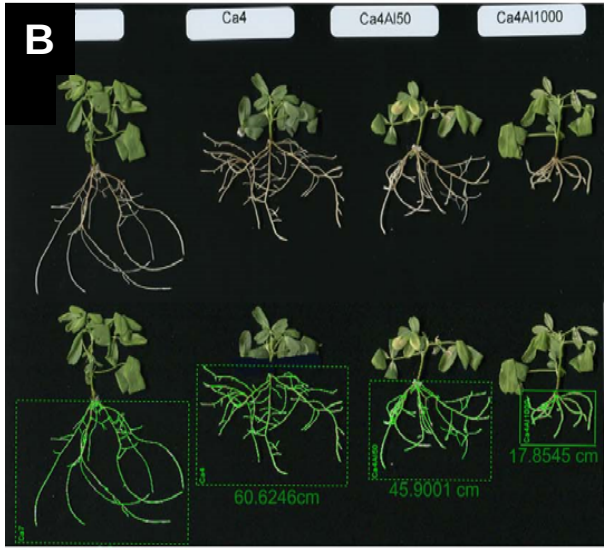
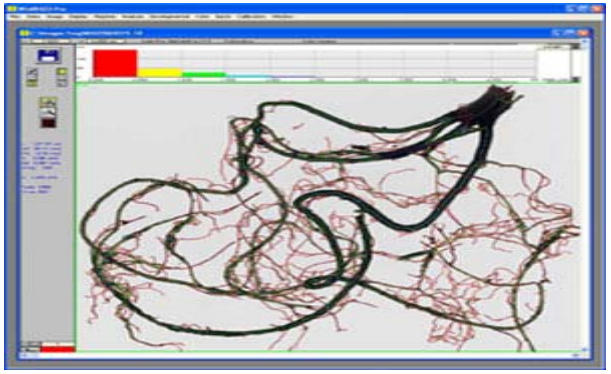
Evaluation of Root Growth in Alfalfa







A. Containers in media (3D) seed vs. clones

B. Root growth in response to pH and Al

C. Harvesting roots

D. Root growth in the field

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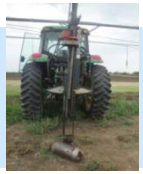
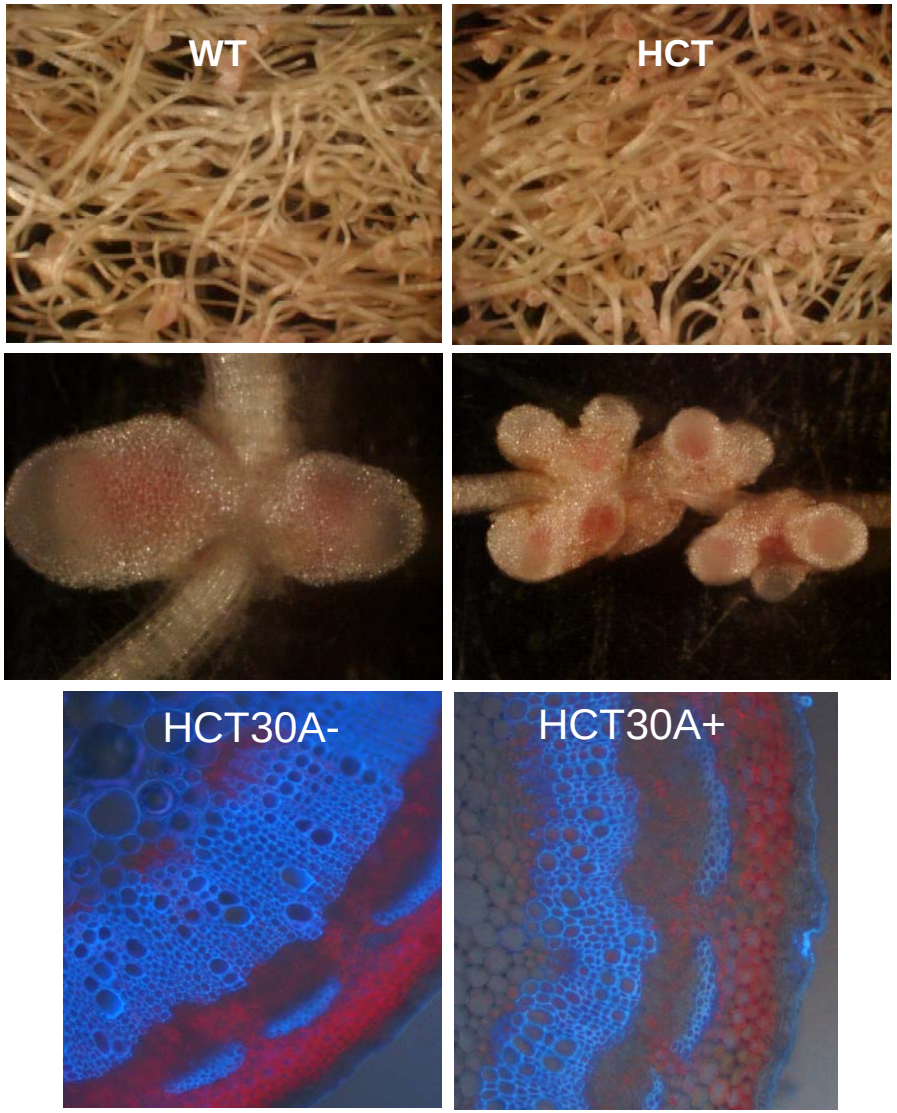
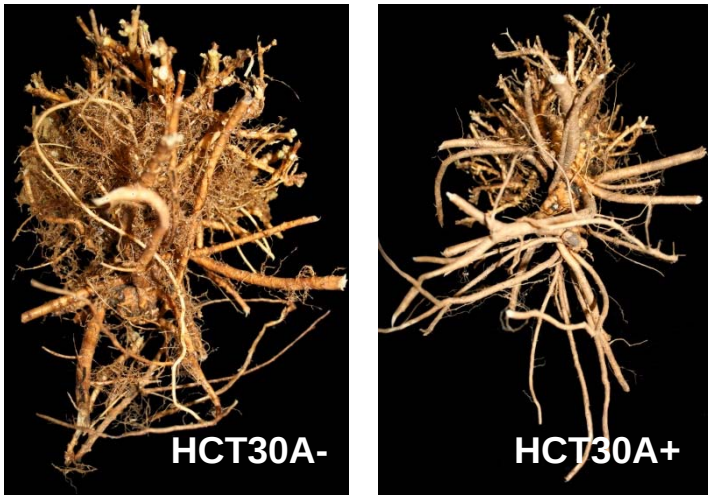
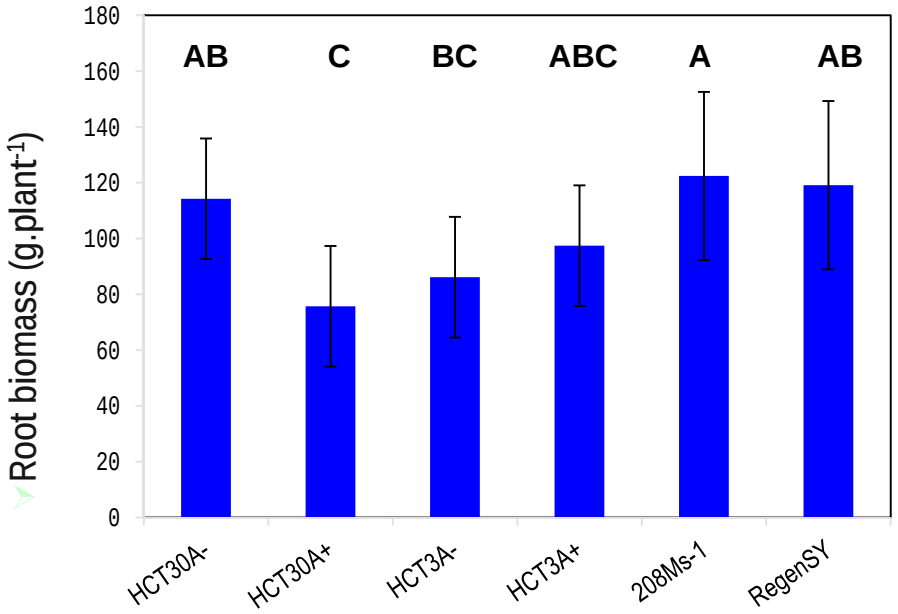
Reduced Lignin Alfalfa Root Growth





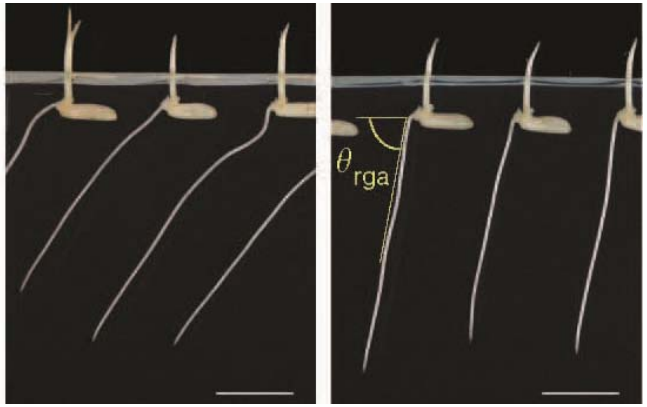


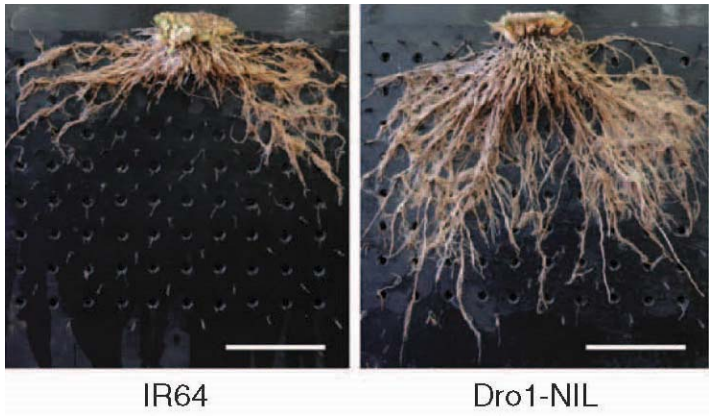


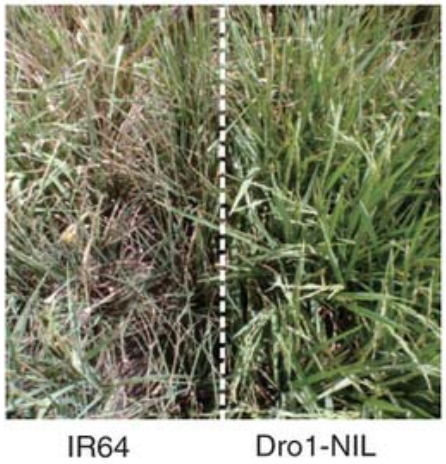
Gallego-Giraldo et al., 2013. Plant Physiology.

Root Traits to Enhance WUE (Yield) in the Field

Are root traits in the laboratory and greenhouse correlated with field-grown performance?

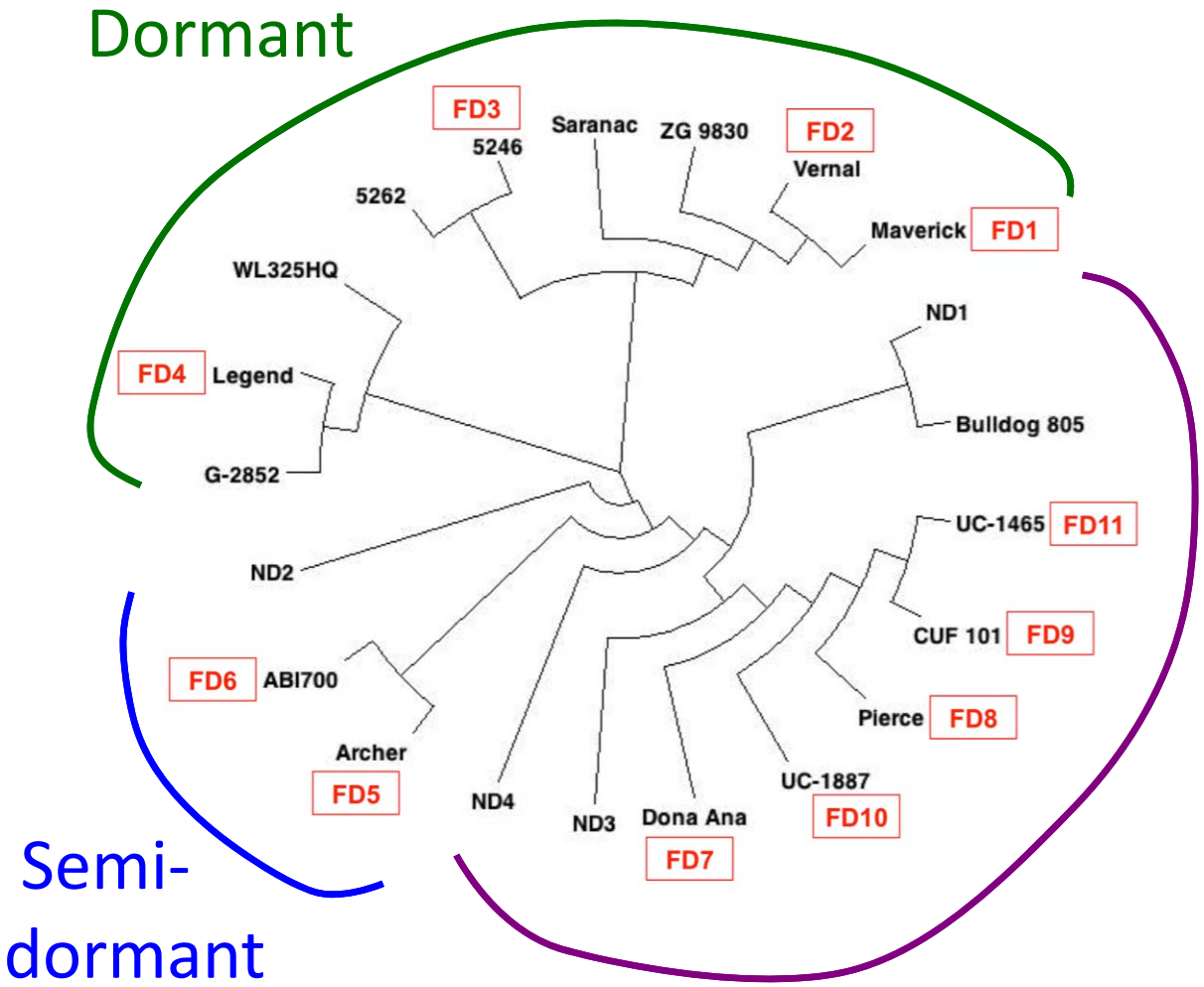






Uga et al., 2013. Nature Genetics. doi:10.1038/ng.2725.

Cultivated Alfalfa Structured by Dormancy

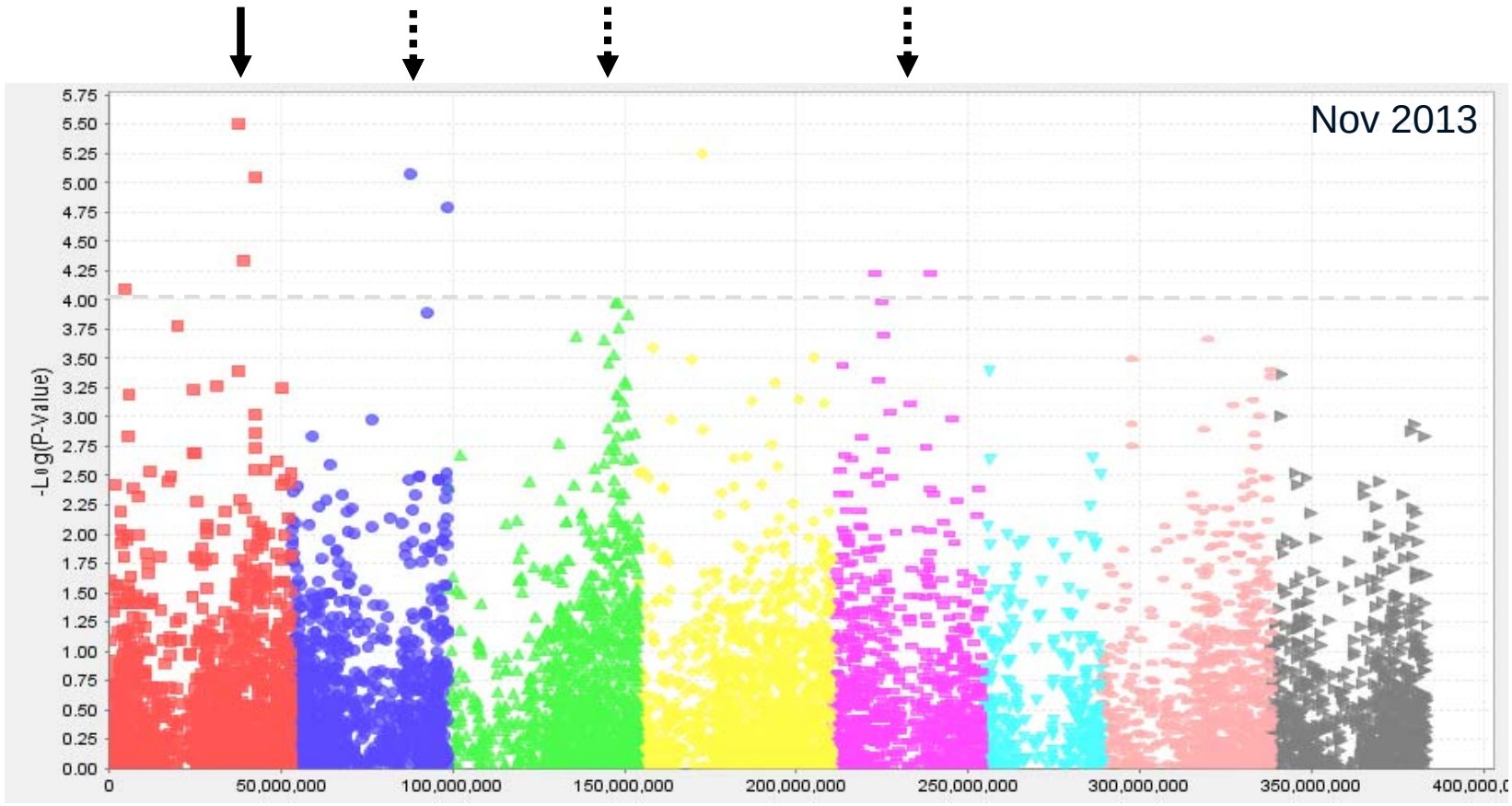


FD GWAS population

- 16 cultivars
- 5 ND breeding populations
- 217 genotypes
- Infinium SNP array

Li, X., Y. Han, Y. Wei, A. Acharya, A.D. Farmer, J. Ho, M.J. Monteros, E.C. Brummer. 2014. SNP array. PLoS One 9:e84329. doi:10.1371/journal.pone.0084329.

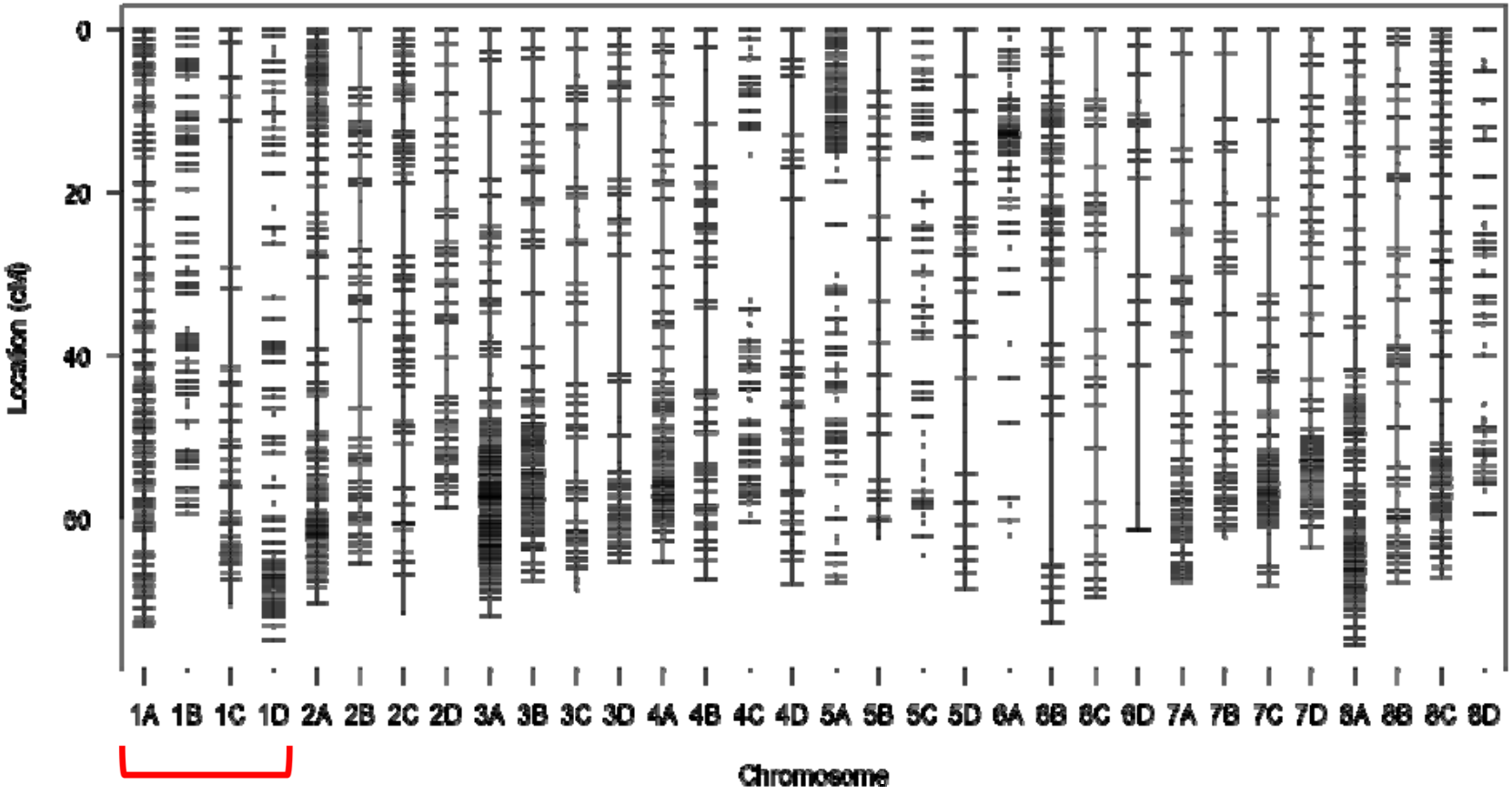
QTL for Fall Dormancy in Alfalfa (Stem length)



High Density Alfalfa Linkage Map (4X) - GBS

GBS with 100-plex; $20 per sample; 8,922 SNPs

DM3 x **DM5** linkage map, 2,154 SNPs mapped on 32 LGs, total length 2,133 cM



4 haplotypes per chromosome

(Li et al., 2014, G3 *Accepted*)

Phenotyping of DM35 Populations

Dupy farm, OK 2012

Noble Headquarters farm, OK 2013

Insect Resistance

Insect (Whitefly) Resistance in Alfalfa

UC-356
Source of WF resistance

One cycle of selection

UC-1872
Highly susceptible
C-1

4 cycles of selection for WF^R

UC-2458
Resistant population

3 cycles of selection for WF^R

Selection for aphid ^{R}, Phytothphora^R, nematode ^{R}, anthracnose ^{R}

UC-2598
Highly resistant
C+7

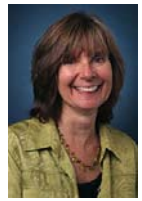
Elite subset of UC-2458 (1/2 sib families) planted

Resistant genotypes (#3, 27, 30, 37) chosen (Jiang et al 2007)

Expansion of seed

UC-2933
Highly resistant
C+4

L. Teuber



L. Walling

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GBS to Identify Changes in Allele Frequency

Ratio of allele frequency in C+7 to C-1 (Mt V3.5)

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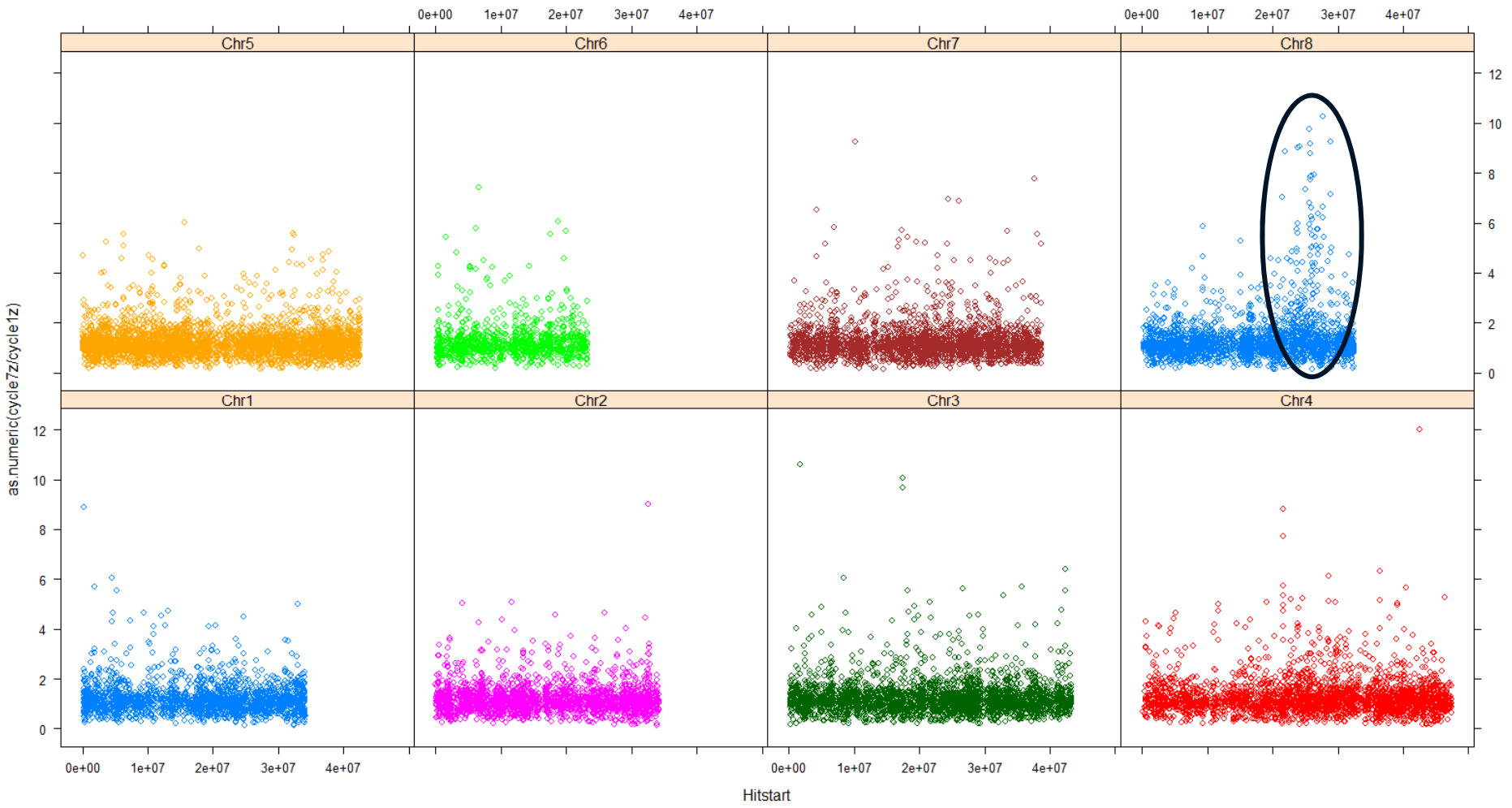

Chromosome 8 = likely location of a WF R locus

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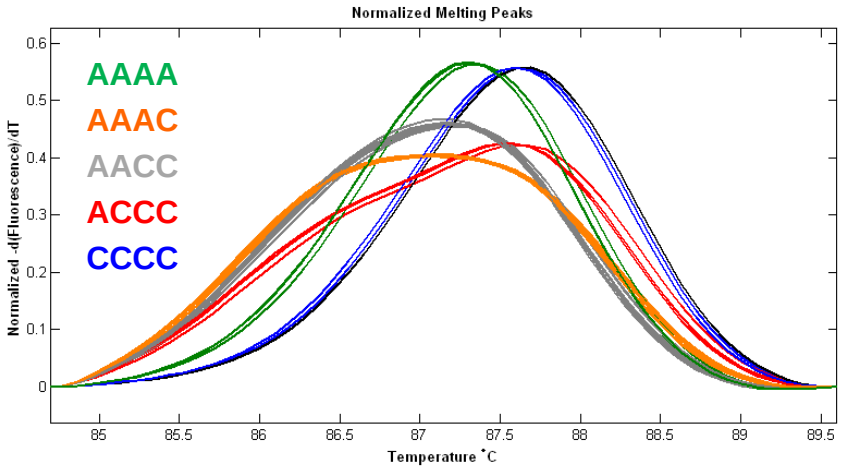
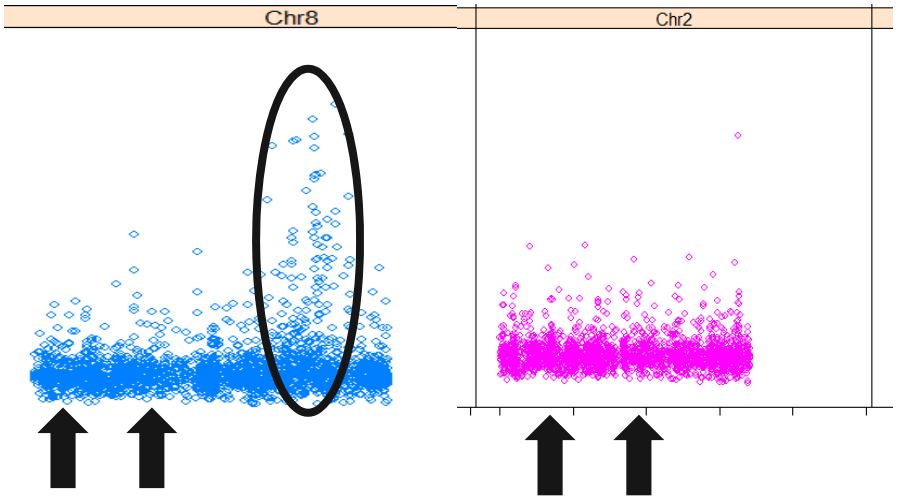
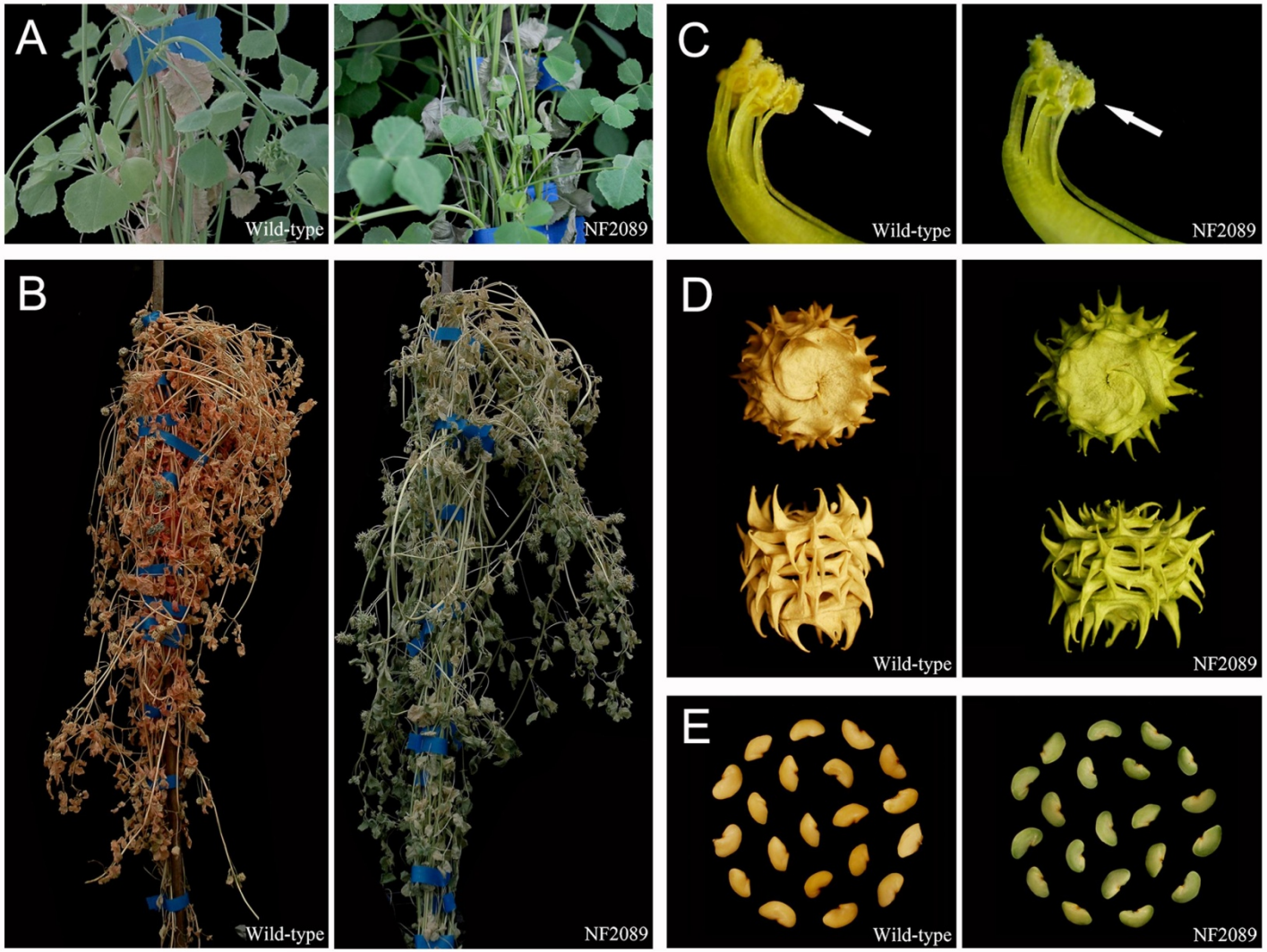
Target vs. non-target Allele Frequency Changes





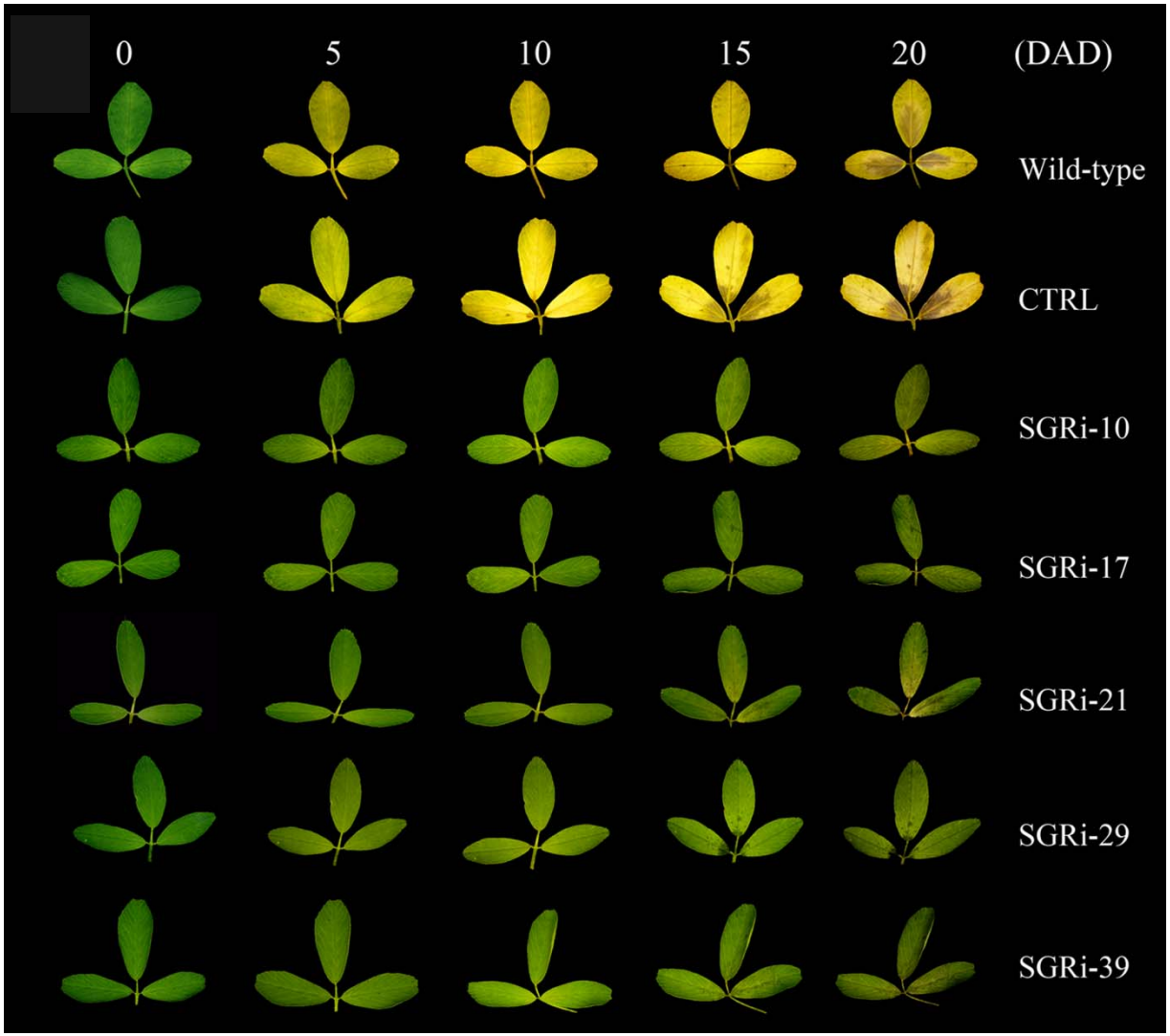
	Target Region			Non-target		Non-target	
SNP	SNP0335	SNP1632	SNP0947	SNP0693	SNP0065	SNP0128	SNP1257
Chr	8	8	8	8	8	2	2
Position (Mbp)	22.4	22.7	26.1	6.0	9.2	17.5	31.5
Allele Frequency Change							
C+4 vs. C-1	0.18	0.21	0.18	0.04	0.02	0.04	0.08
C+7 vs. C-1	**0.22**	**0.25**	**0.33**	0.07	0.03	0.01	0.05

Identification and phenotypic characterization of a *M. truncatula* staygreen mutant



Zhou et al., 2011. Plant Physiology. 157:1483-1496.

Dark induced senescence of alfalfa *MsSGR*-RNAi transgenic leaves



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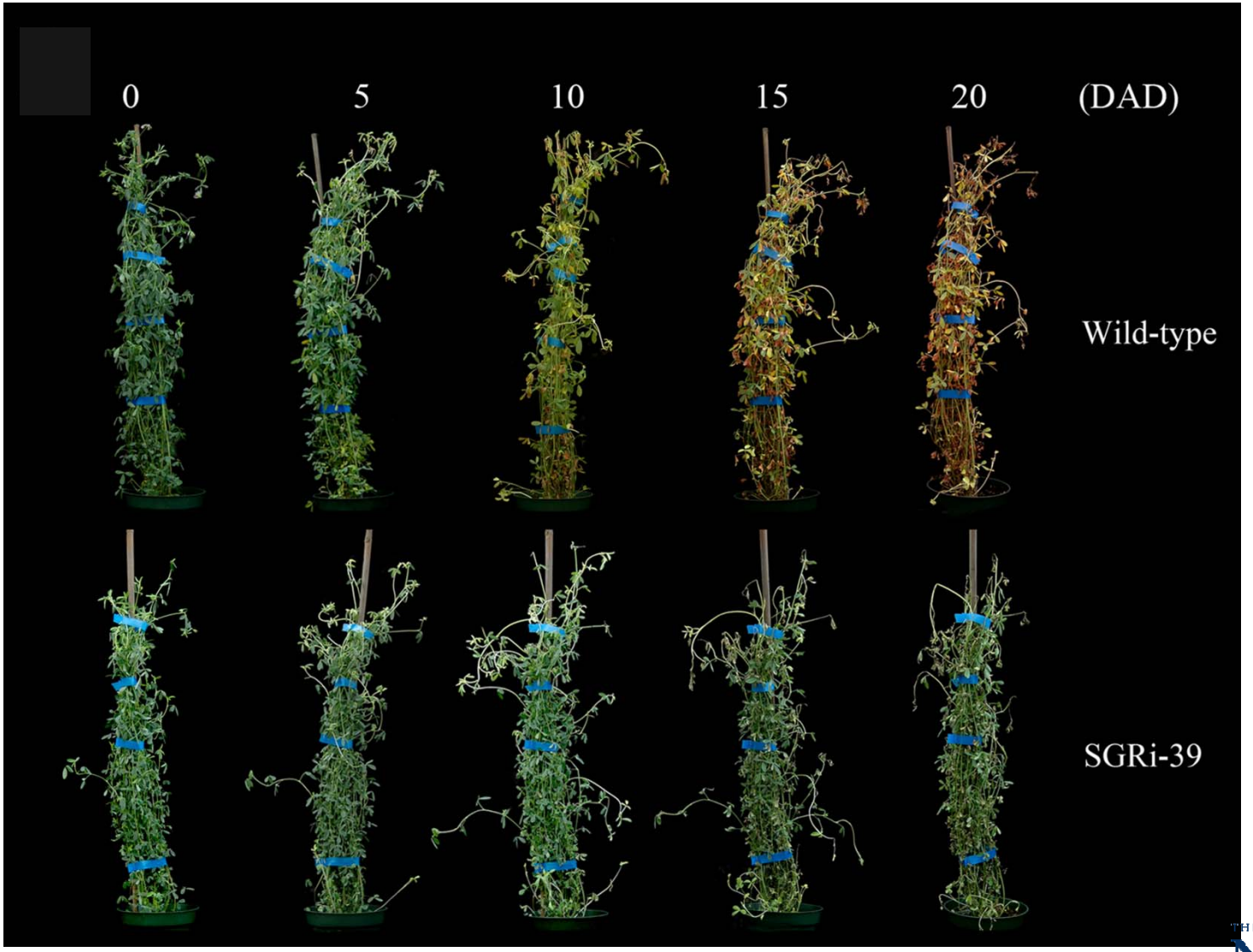
Dark induced senescence of alfalfa *MsSGR*-RNAi transgenic plants



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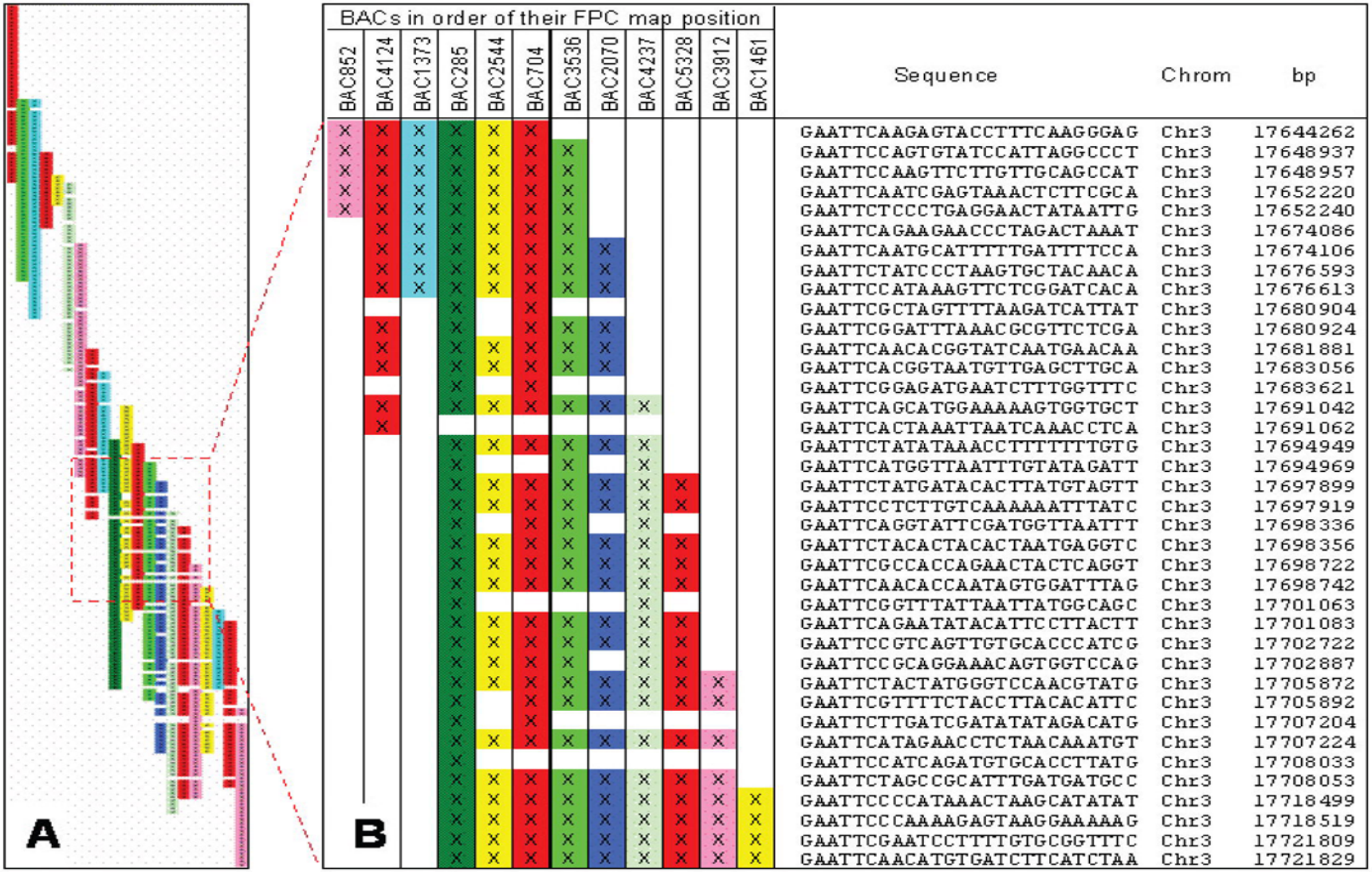
Tetraploid Alfalfa Genome - WGP



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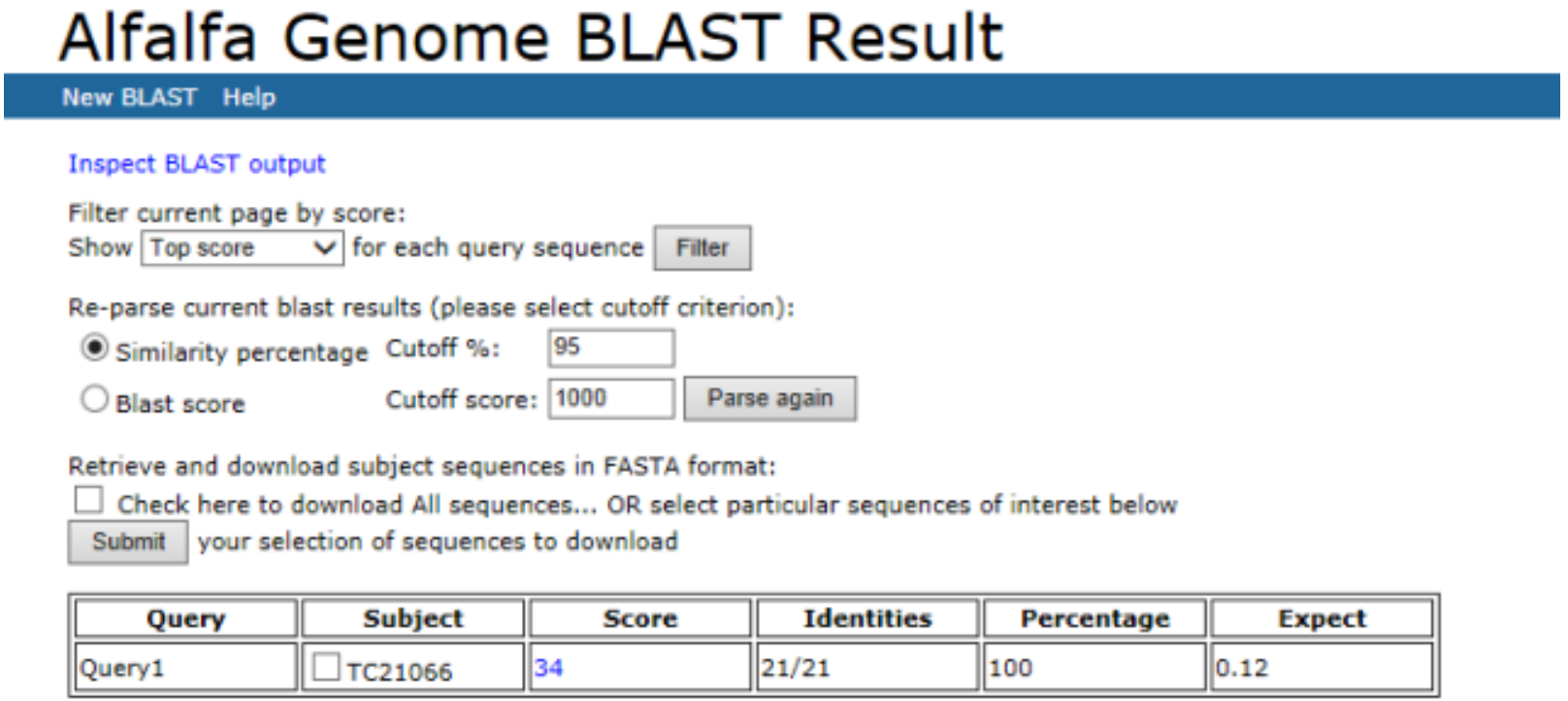
Alfalfa Genome BLAST Server



Alfalfa Genome BLAST Result

New BLAST Help

Inspect BLAST output

Filter current page by score:
Show Top score for each query sequence Filter

Re-parse current blast results (please select cutoff criterion):
Similarity percentage Cutoff %: 95
Blast score Cutoff score: 1000 Parse again

Retrieve and download subject sequences in FASTA format:
Check here to download All sequences... OR select particular sequences of interest below
Submit your selection of sequences to download

Query	Subject	Score	Identities	Percentage	Expect
Query1	TC21066	34	21/21	100	0.12

1. 454 assembly = 24,144 contigs
2. LIPE libraries (3) + SIPE libraries (5) = 272,573 contigs
3. PacBio sequencing planned

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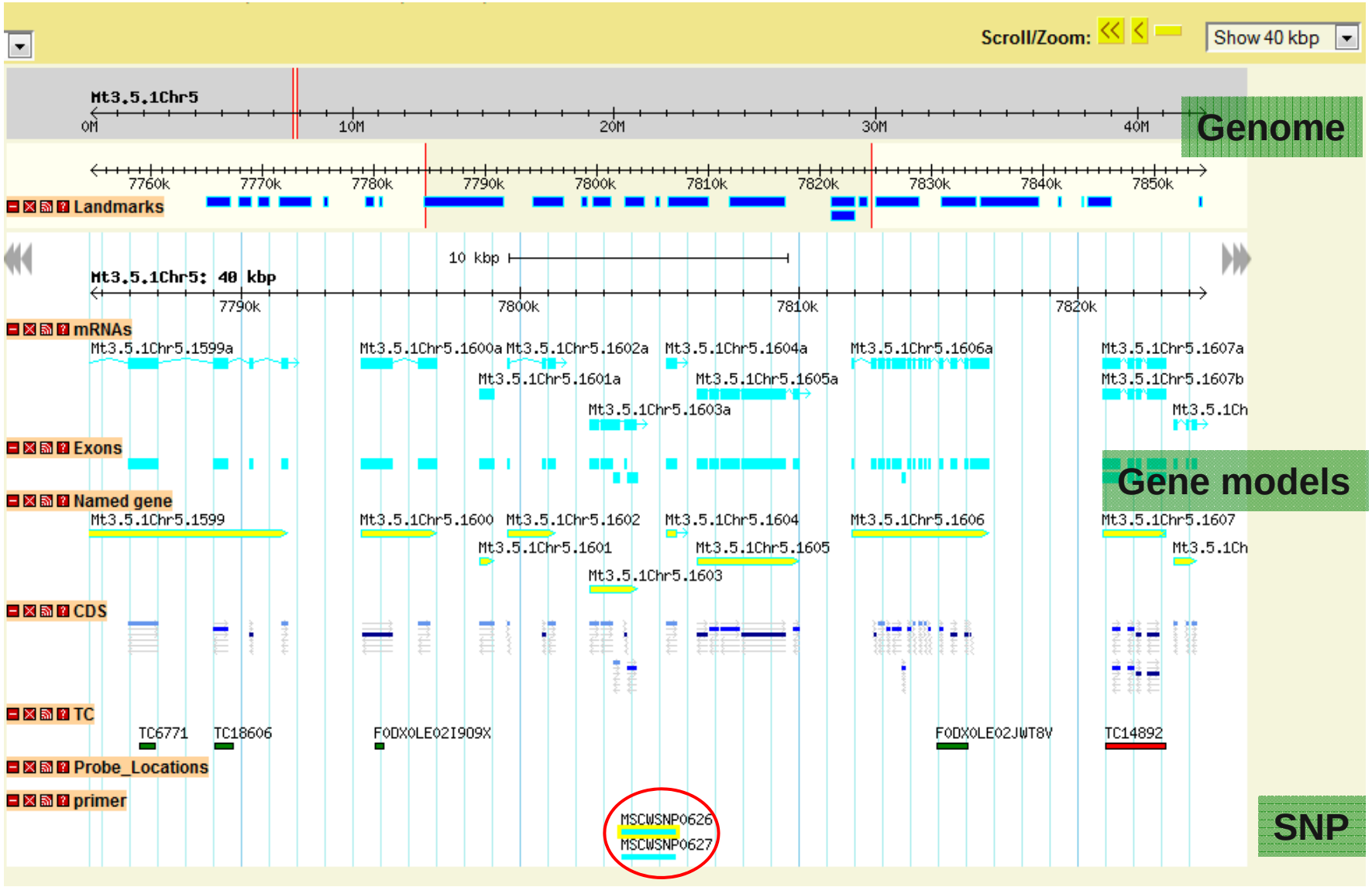
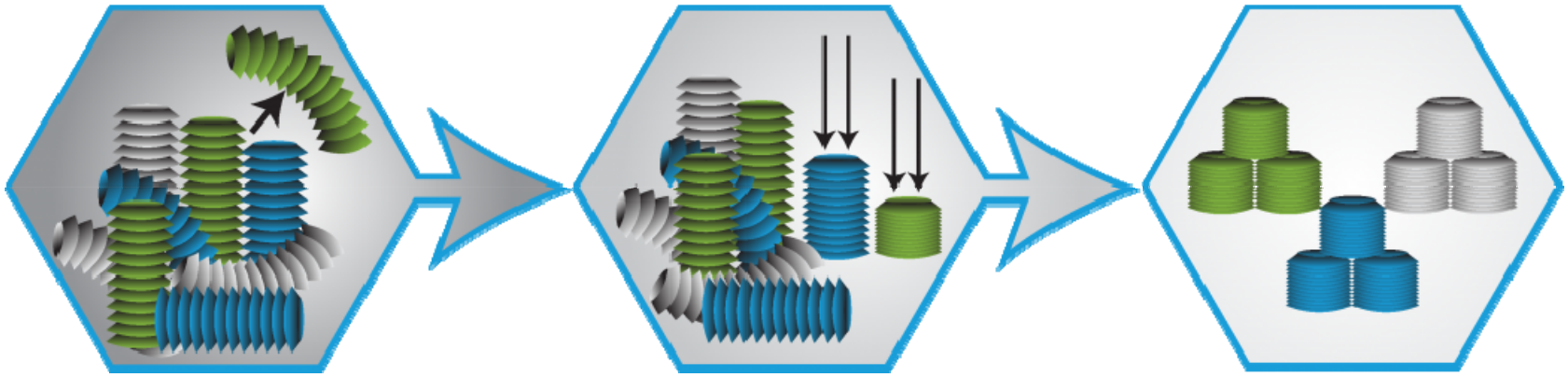
Integrated Databases - Alfalfa



From Available Datasets to Meaningful Information



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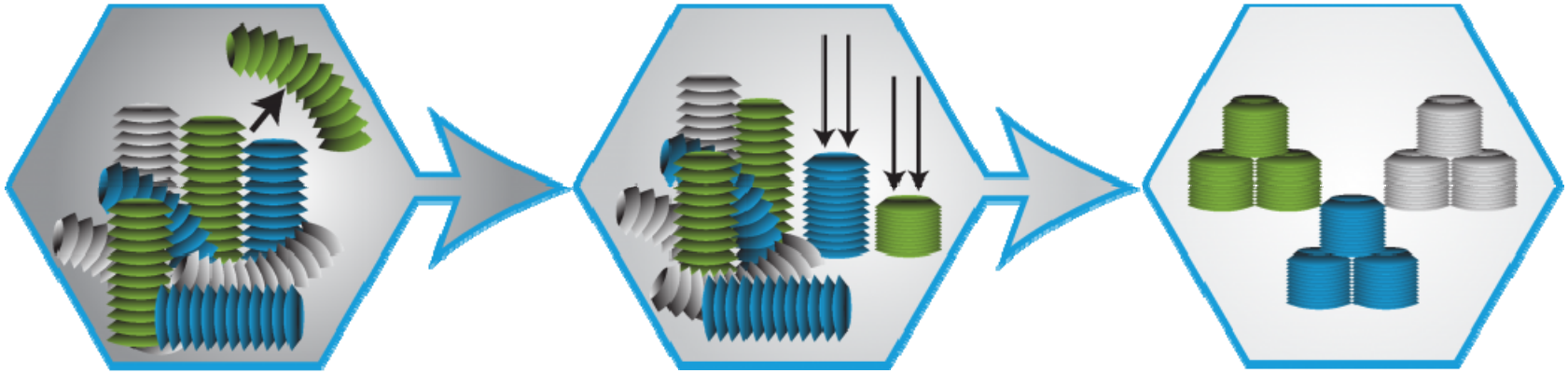
Alfalfa Breeder's Toolbox



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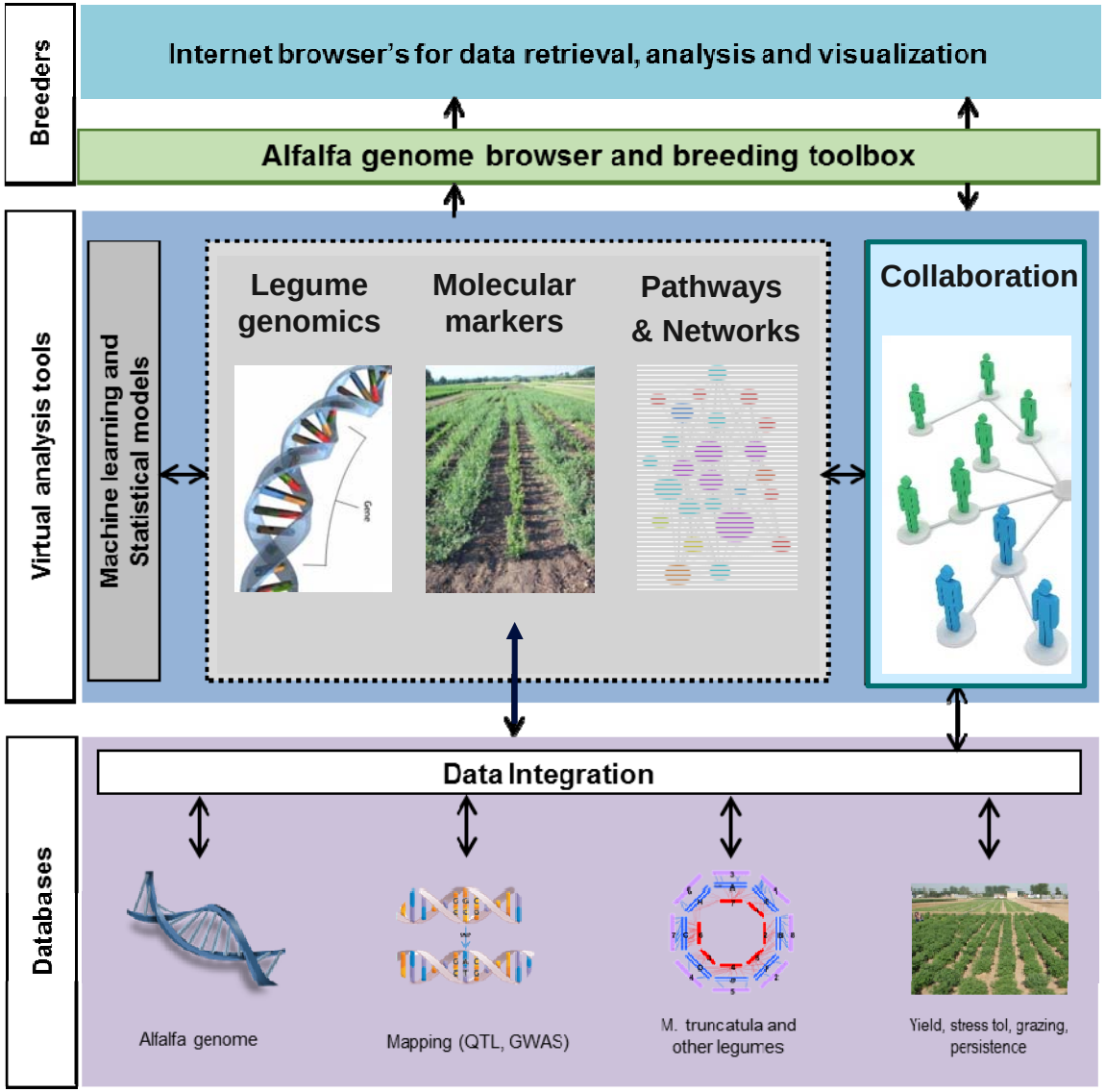
Alfalfa Breeder's Toolbox



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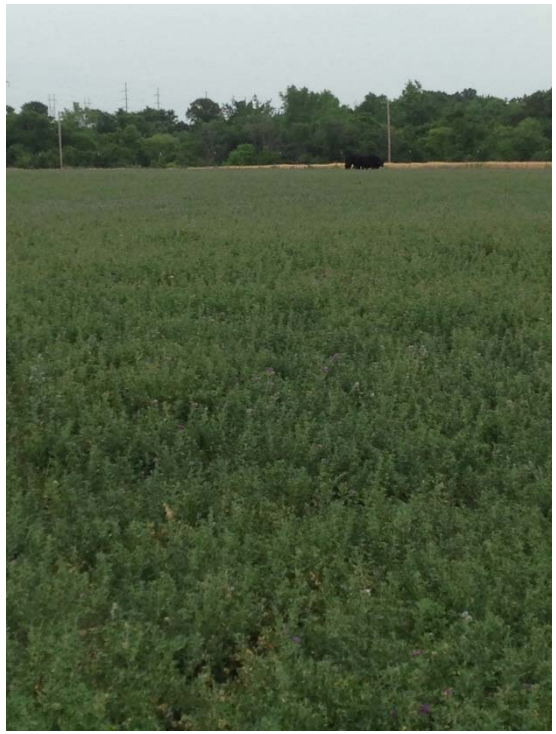
Alfalfa in Forage Based Systems



- Production and economics of grazing alfalfa-tall fescue mixtures
- Production and economics of alfalfa-wheat/crabgrass rotation
- Establishment of alfalfa in existing bermudagrass (3 planting dates, 3 seedbed preparations, 7 fungicide/insecticide seed treatments).
- Estimating alfalfa forage mass and nutritive value with mobile sensors

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Forage Legume Improvement Strategies - Summary

Genetic variation

Molecular technologies
Phenotyping

Selection
Evaluation

Cultivar development

Base germplasm pool

Characterized with markers to identify novel germplasm

Phenotyping to identify/select best populations or genotypes

Mapping traits for marker toolbox

Phenotyping

Develop/update GS model and introduce new genotypes/traits into population

Integrate phenotypic or marker screen for diseases/insects prior to GS

Marker-only Genomic Selection

Yield, composition, stress tolerance

Evaluate for potential release

Products

Forage Legume Research Team



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Xuehui Li
Yanling Wei
Will Chaney
Postdoctoral Fellow


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Stephanie Greene & Ted Kisha

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Dave Miller - Pioneer
Dan Gardner - Dairyland
David Johnson – Calwest

OSU
Raman Sunkar

France
Bernadette Julier
Maria Cruz de Carvalho


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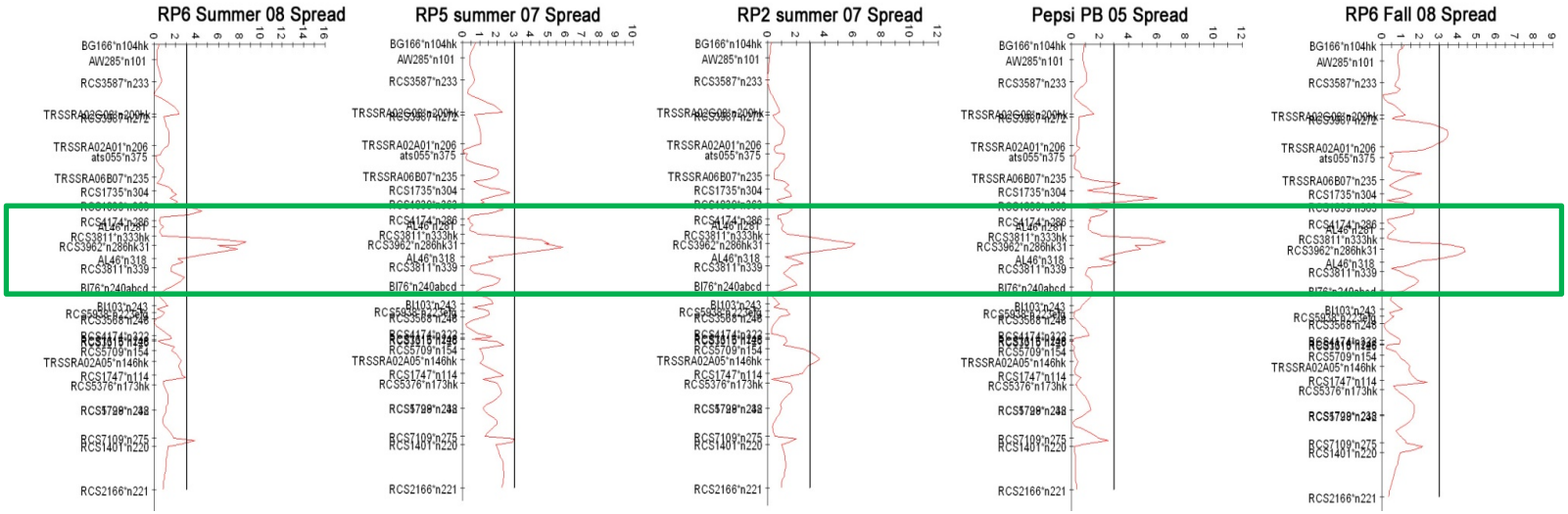
OCAST
Oklahoma Center for the Advancement of Science and Technology
oklahoma
bioenergy center
NSF
USDA
Forage
Genetics
International

White Clover Breeding Strategies

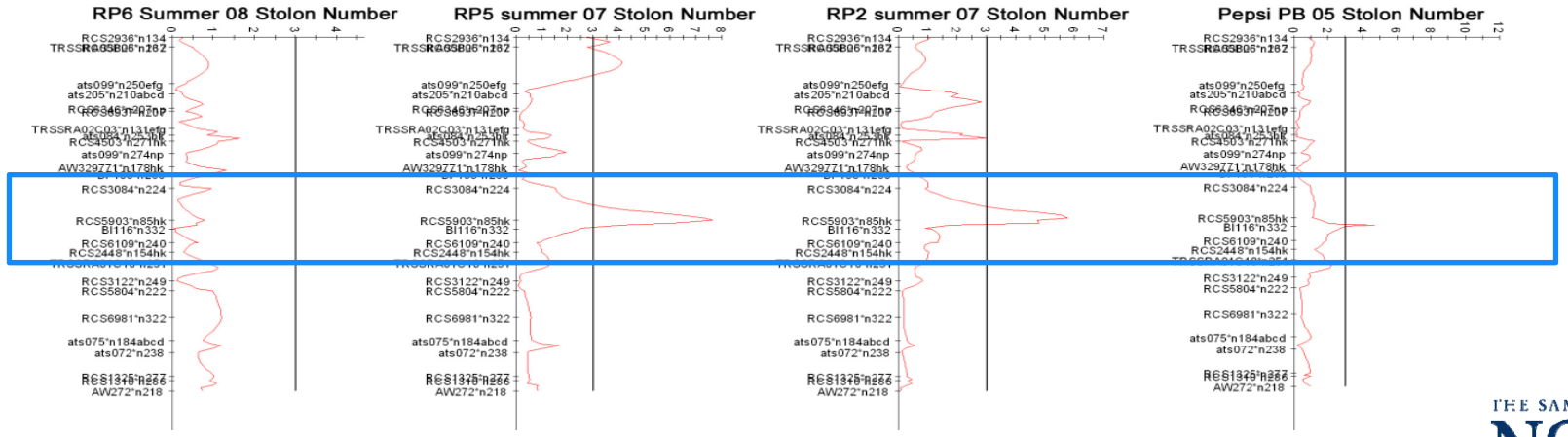
- Multi-year and multi-location field trials
- Target agronomic traits
  - Leaf characteristics and stolon number
  - Persistence
  - Plant flowering
- Evaluate molecular markers in a different genetic background (broad-based white clover germplasm)
- Target traits: stolon number and leaf size
- White clover and tall fescue grazing study (Dupy Farm)

Multi-Loc & Year QTL Identification

LG D1 Plant Spread



LG B1 Stolon Number



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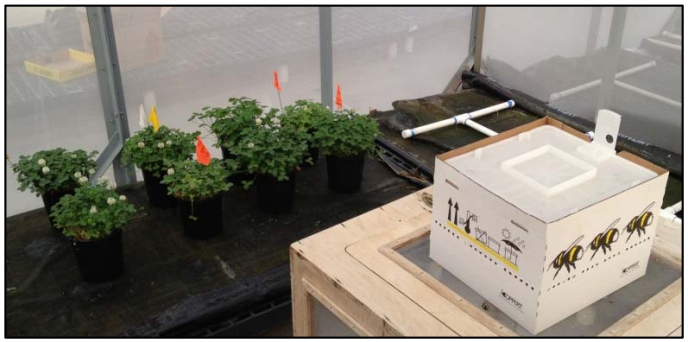
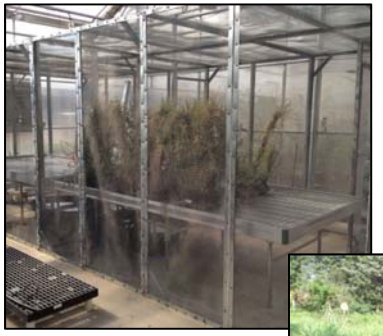
WC Divergent Selection Strategy

Phenotype-based Selection	Marker-based Selection	Propagation and seed production
Small-medium leaf High stolon density	Stolon High density	
Large leaf High stolon density	Leaf size Large	
Large leaf Low stolon density	Stolon Low density	
Small-medium leaf Low stolon density	Leaf size Small-medium	





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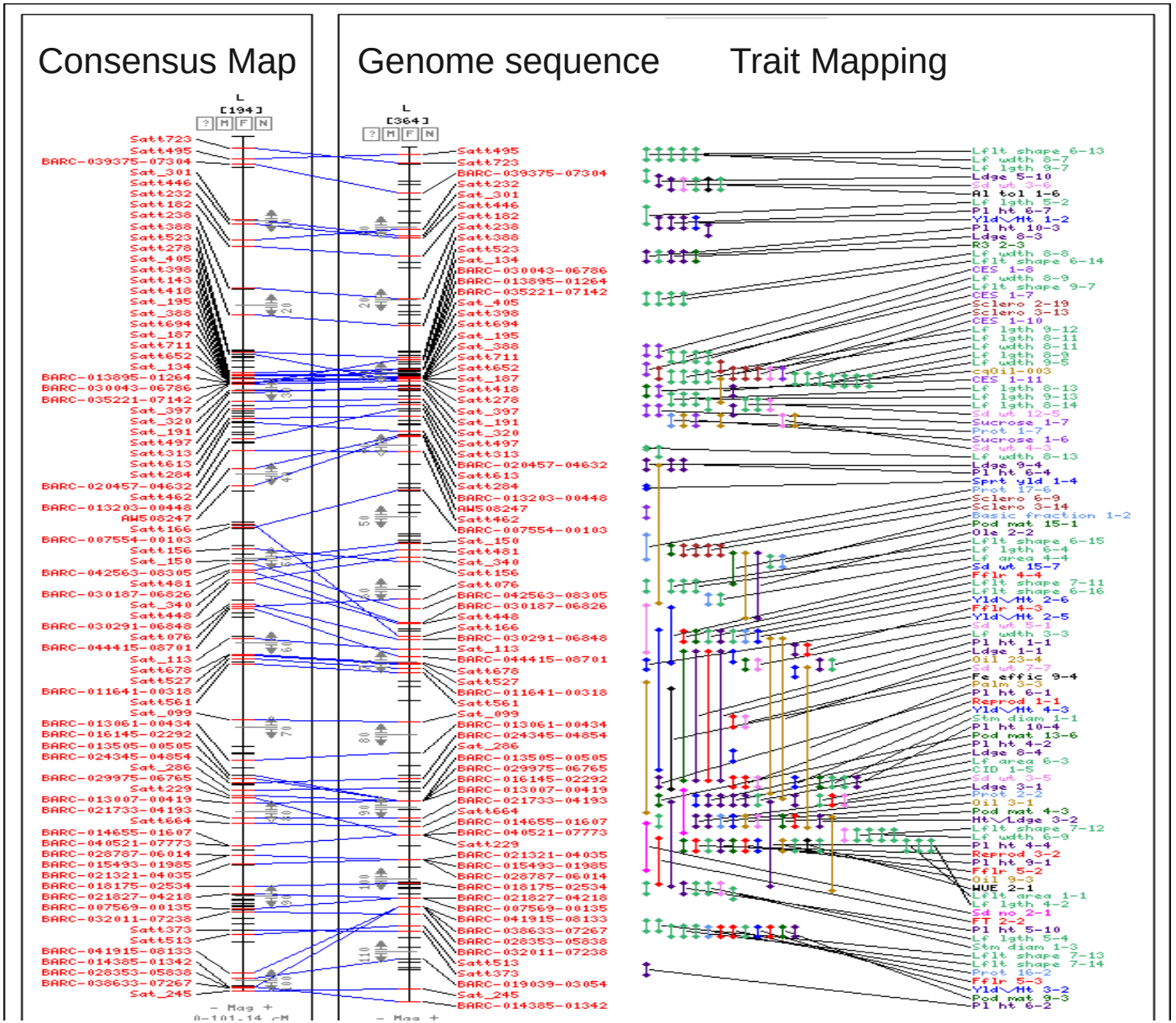
Developing a Breeder's Toolbox



QTL_fungal
QTL_inorganic
QTL_misc
QTL_nematode
QTL_oil
QTL_other-seed
QTL_pod
QTL_protein
QTL_reprod-period
QTL_whole-plant
QTL_yield