

# **Accelerated Grape Breeding**

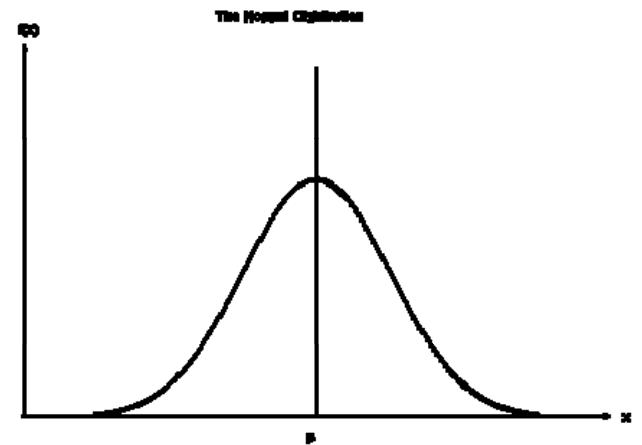
**Daryl J. Somers**

**Director of Applied Genomics  
Vineland Research and Innovation Centre**

- Complex traits and molecular breeding
- Lessons learned from wheat molecular breeding
- Strategies and tools for grape breeding

## Complex traits:

- Typically controlled by multiple genes
  - various modes of inheritance
- Often influenced by the environment
  - temperature
  - nutrition
  - moisture
- Testing in multiple environments if essential to quantify the genetics component of trait expression.

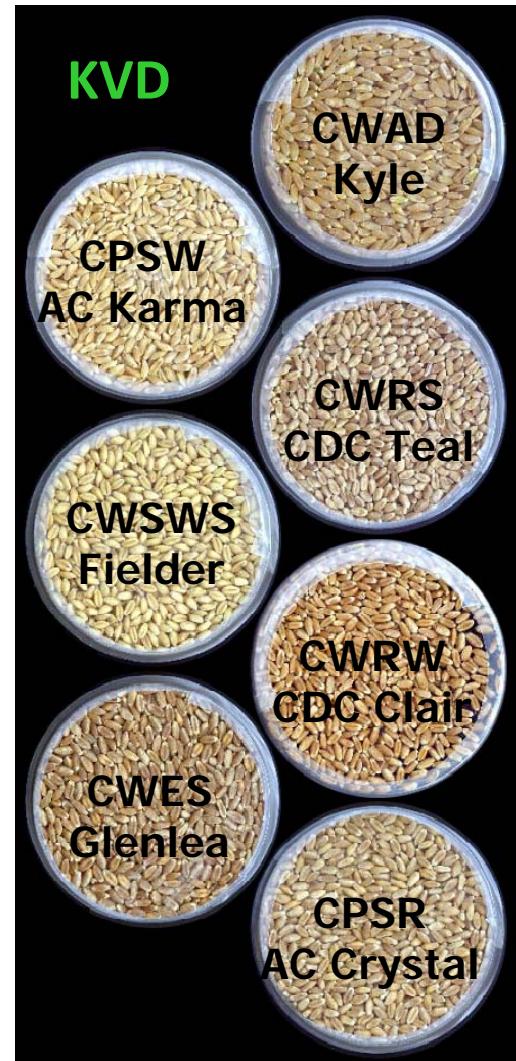


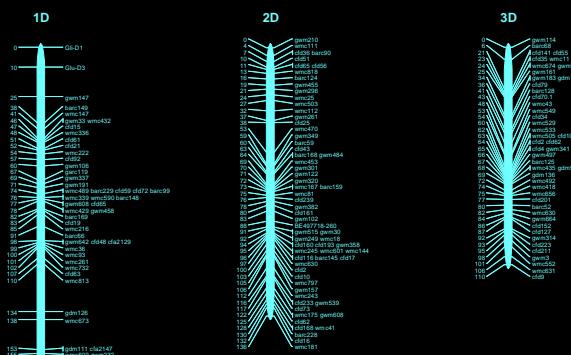
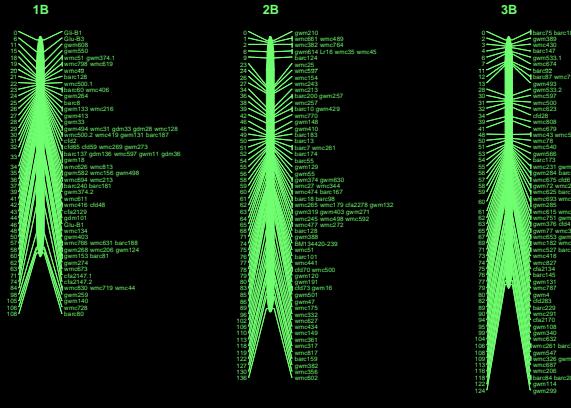
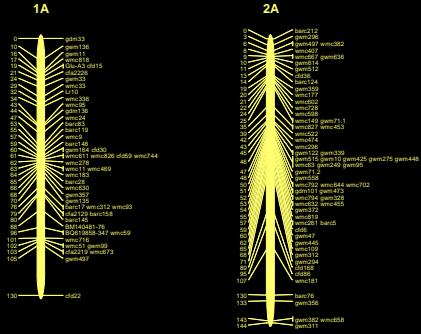
## Tools to characterize complex traits

- Genetic map – underpins most of the research
- High throughput DNA diagnostic methods
  - DNA extraction
  - SSRs, DaRT, SNPs
  - next Gen Seq
- Space, labour, time – to accurately phenotype
- Statistical analysis software
  - merges genotypes with phenotypes
  - maps QTLs.

# Cereal Quality and Segregation

- Seed quality testing will begin at F4 (micro tests on 50-100g)
- Continues through F6, F8
  - Quadramat mill – flour yield
  - NIR – protein content
  - mixograph – dough strength
- “A” test – F9 Advanced testing begins
  - Buhler mill – flour yield
  - NIR – protein content
  - Chemical/physiological tests
  - Dough strength (multiple methods)
  - Baking (multiple methods)
- “B” test – F10
- “C” test – yr 1-3 – F11-13
- Registration





Daryl J. Somers · Peter Isaac · Keith Edwards

## A high-density microsatellite consensus map for bread wheat (*Triticum aestivum L.*)

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**Abstract** A microsatellite consensus map was constructed by joining four independent genetic maps of bread wheat. Three of the maps were F<sub>1</sub>-derived, doubled-haploid line populations and the fourth population was 'Synthetic' × 'Opata', an F<sub>6</sub>-derived, recombinant-inbred line population. Microsatellite markers from different research groups including the Wheat Microsatellite Consortium, GWM, GDM, CFA, CFD, and BARC were used in the mapping. A sufficient number of common loci between genetic maps, ranging from 52 to 232 loci, were mapped on different populations to facilitate joining the maps. Four genetic maps were developed using MapMaker V3.0 and JoinMap V3.0. The software CMap, a comparative map viewer, was used to align the four maps and identify potential errors based on consensus. JoinMap V3.0 was used to calculate marker order and recombination distances based on the consensus of the four maps. A total of 1,235 microsatellite loci were mapped, covering 2,569 cM, giving an average interval distance of 2.2 cM. This consensus map represents the highest-density public microsatellite map of wheat and is accompanied by an allele database showing the parent allele sizes for every

marker mapped. This enables users to predict allele sizes in new breeding populations and develop molecular breeding and genomics strategies.

### Introduction

The value of crop species genetic maps has steadily increased from when they were first introduced in the 1980s. Wheat molecular genetic maps first comprised RFLP markers (Chao et al. 1989; Devos et al. 1993; Devos and Gale 1997) and over time, PCR-based markers became the dominant marker type for genetic map construction, including RAPDs (Williams et al. 1990), AFLPs (Vos et al. 1995), and microsatellites (SSRs; Röder et al. 1998; Pestsova et al. 2000; Gupta et al. 2002). The primary reason to shift toward PCR-based markers and particularly SSR marker maps is the potential to use the maps in plant breeding (Gupta and Varshney 2000). Conventional plant breeding requires the analysis of thousands of plants in a short time period at low cost. Microsatellite markers and high-throughput capillary electrophoresis are good platforms upon which to implement marker-assisted selection (MAS) in breeding programs.

Molecular breeding is more effective if the molecular map is densely populated with markers. This provides molecular breeding strategies with more choice in the quality of markers and more probability of polymorphic markers in an important chromosome interval. The first microsatellite map in wheat possessed 279 microsatellites (Röder et al. 1998). This marker density is useful for QTL and gene mapping, but is limiting for the precise transfer of QTLs between different genetic backgrounds. Specifically, the limitation comes from the lack of polymorphic markers immediately flanking QTLs.

Wheat genomics research is increasing the use of genetic maps, particularly in map-based gene cloning efforts. Map-based cloning requires an accurate, fine genetic map to correctly position a gene of interest between close flanking markers (Peters et al. 2003).

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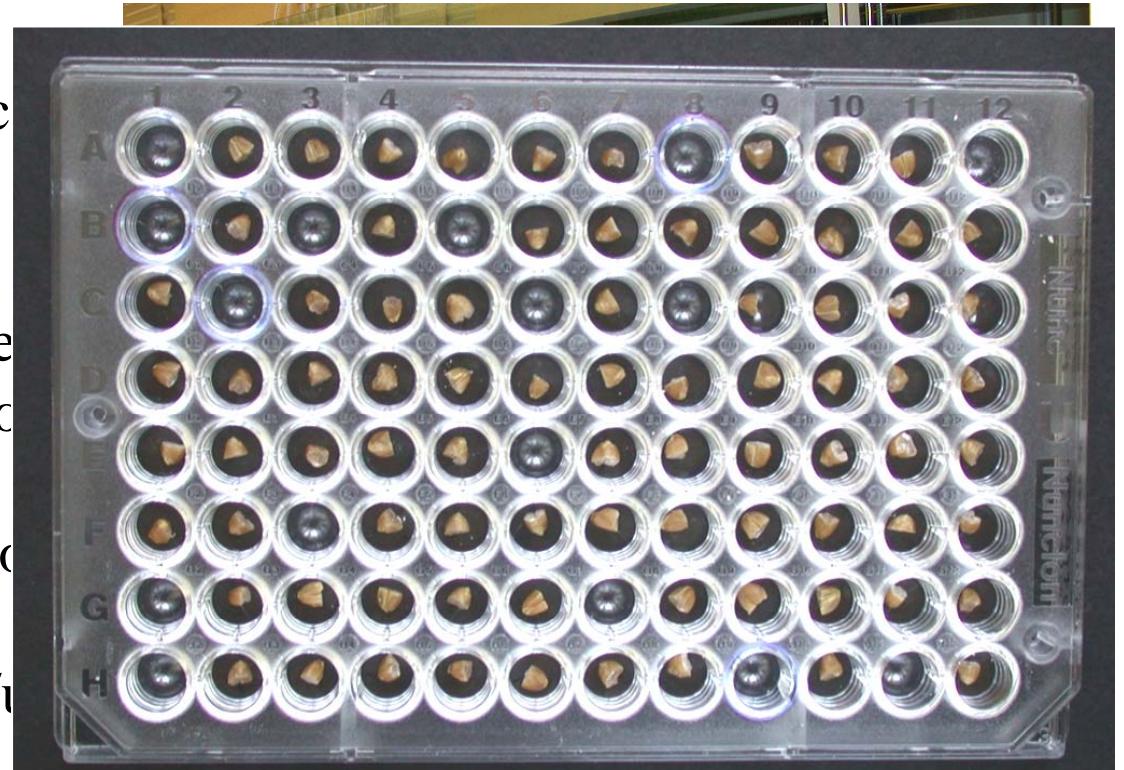
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Woodland Rd.,  
Bristol, BS8-1UG, UK

## HT – MAS techniques and c

### DNA extraction

96 well format, free

Non-organic, ammo



### DNA quantification with Ho

96 well fomat

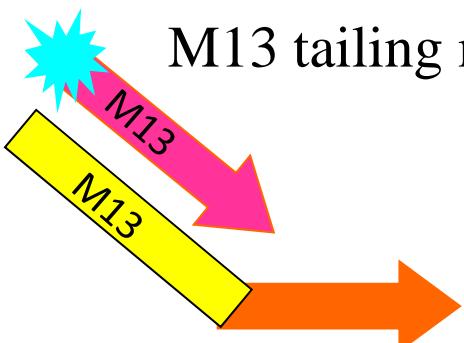
dilute DNA to 6ng/ul

### PCR set up

384 well format on Teca

10 uL rxn with 24 ng gL...

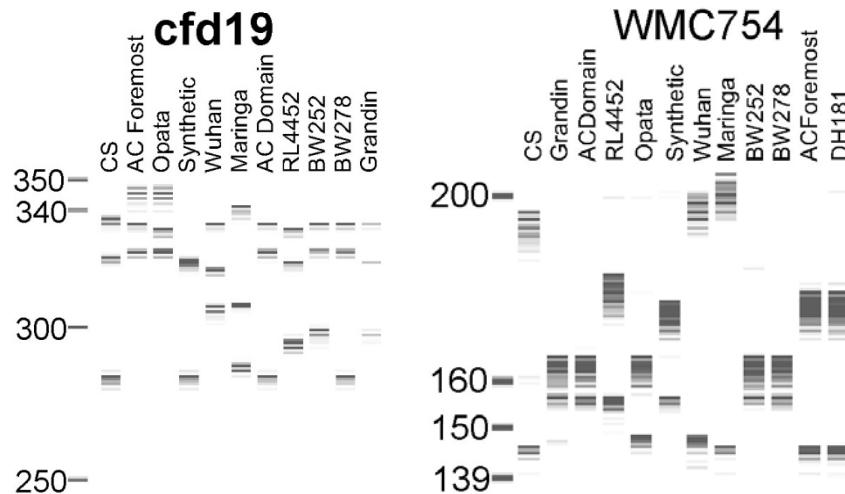
M13 tailing method for primer design



Canada

# Microsatellite Allele Database and Image Archive

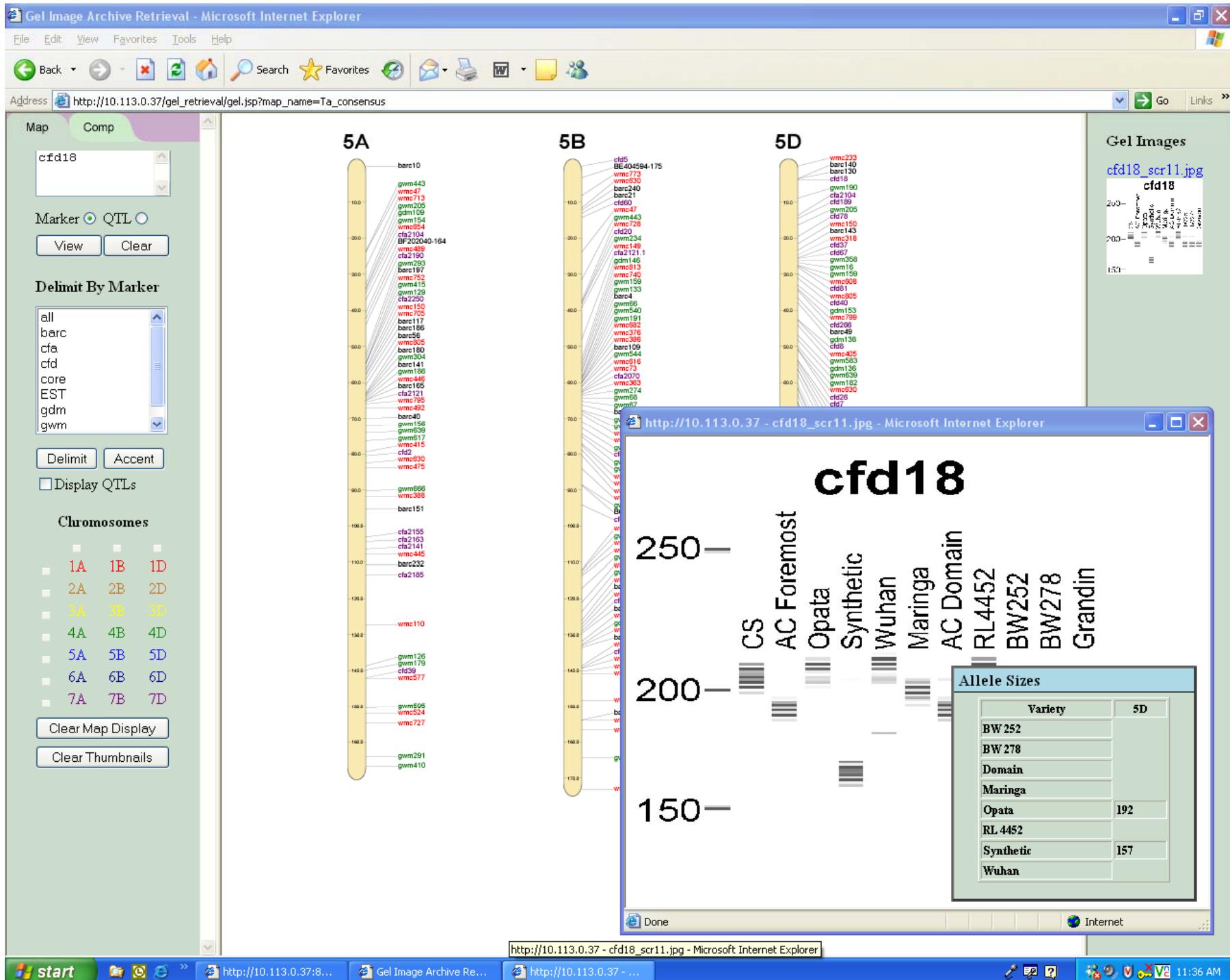
Many wheat microsatellites are not locus specific.



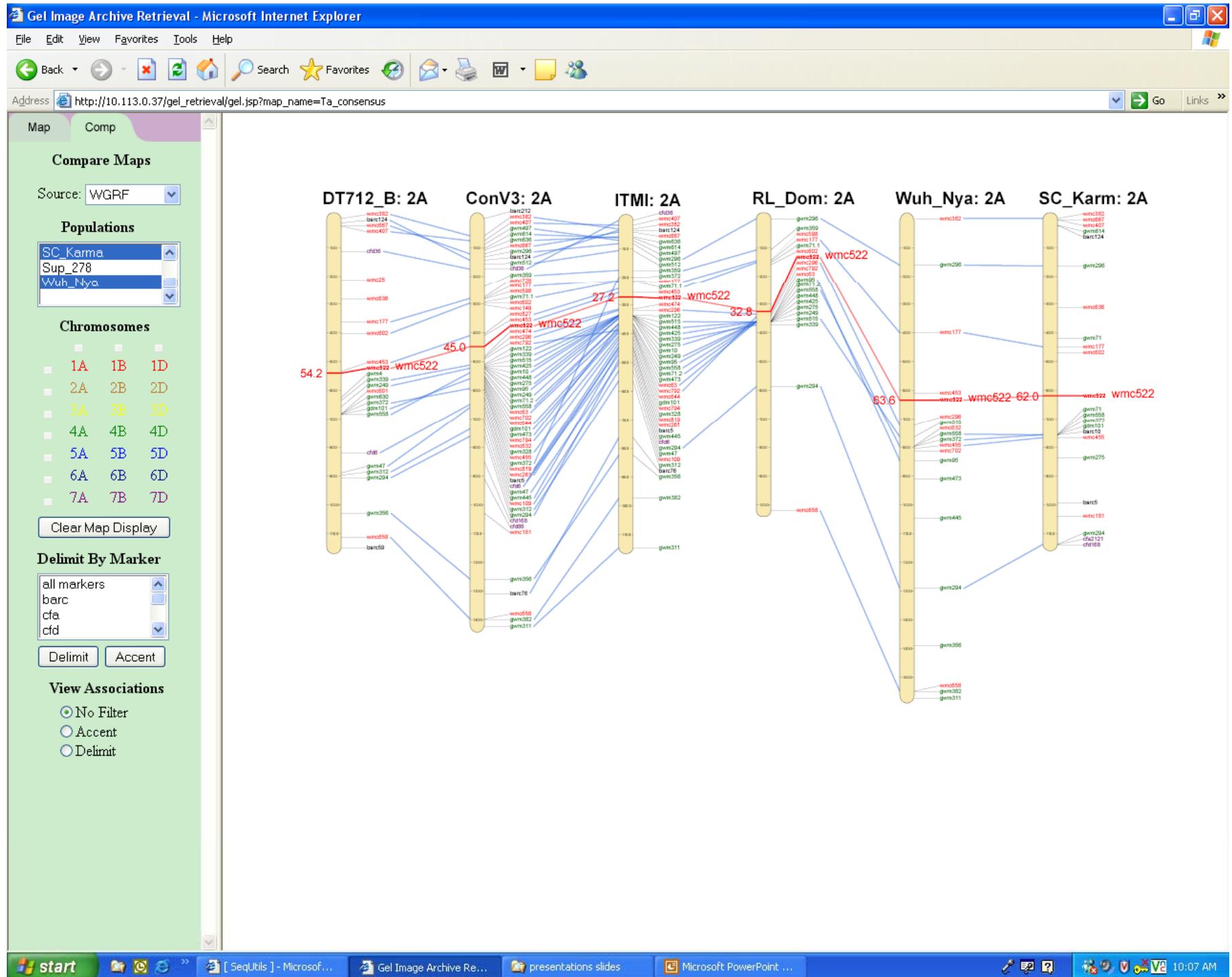
Knowledge of allele size and image quality is important to apply markers.

Allele database records allele size of 330 accessions at 125 loci/genome.

Chromosome	Distance	Difference	Locus	# of alleles	AC Barrie	AC Cadillac	AC Cora	AC Domain	AC Elsa	AC Intrepid	AC Majestic	AC Minto	AC Splendor	AC Superb (BW252)	Alikat	CDC Teal	Columbus	Invader	Katepwa	Lancer	Laura	McKenzie	Neepawa	Park	Pasqua	Prodigy	Robin
1A	6.1		gdm33	8	178	178	180	178	178	183	180	183	178	180	180	180	182	180	180	183	180	180	180	180	178		
1A	23.8		cfa2226	2	189	189	189	189	191	191	189	189	189	189	189	189	189	189	189	189	191	189	189	191	189		
1A	24.6	0.8	cfd15	3	195	N	N	195	195	N	N	N	195	N	N	N	195	N	212	195	195	N	N	N	195		
1A	28.9	4.3	wmc818	9	168	138	162	138	168	168	138	F	162	138	162	162	162	168	162	195	168	156	162	162	162	168	
1A	35.2	6.3	wmc336	3	144	146	144	144	144	144	146	144	144	144	144	F	144	144	144	144	144	N	144	144	144	144	
1A	47.6	12.4	barc83	4	285	285	285	285	285	285	285	285	285	275	285	285	285	285	285	285	285	285	273	285	285	285	
1A	18.1	0.8	wmc241	7	150	168	150	172	150	150	150	150	F	172	150	150	150	150	150	150	150	172	150	150	150	150	

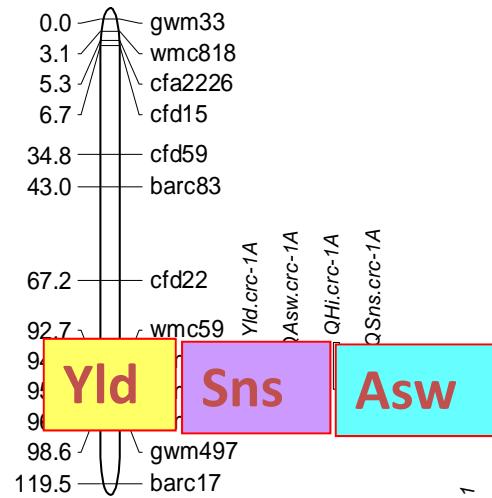
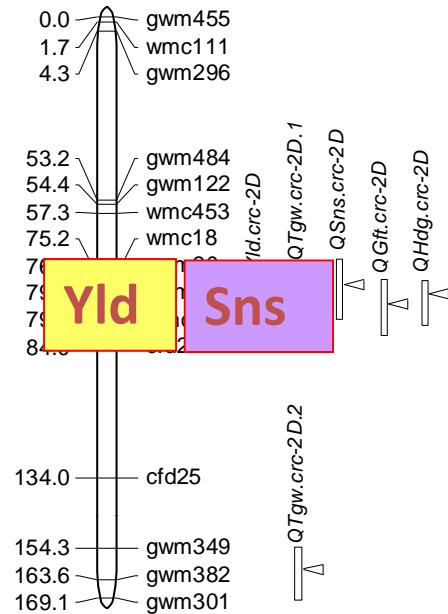
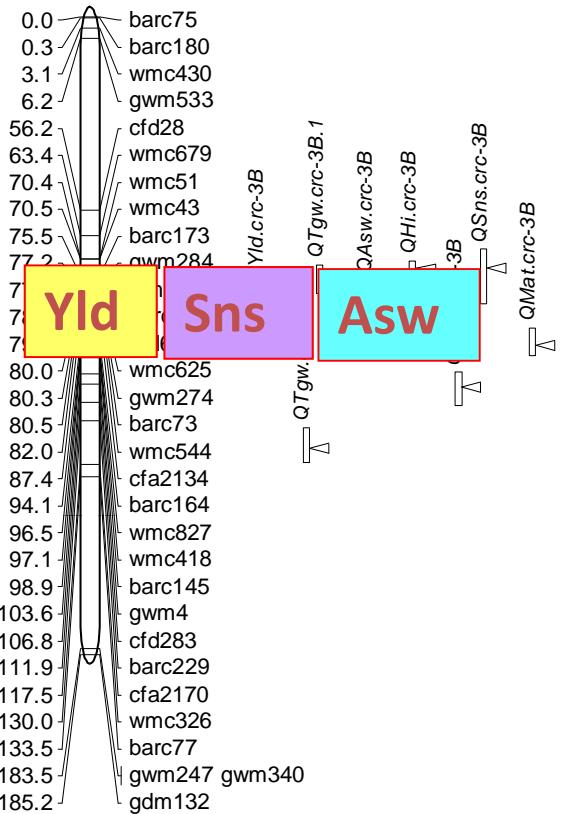
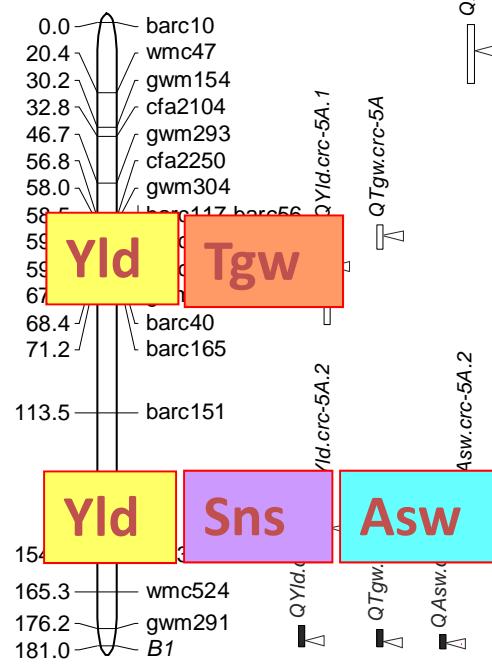


11:36 AM



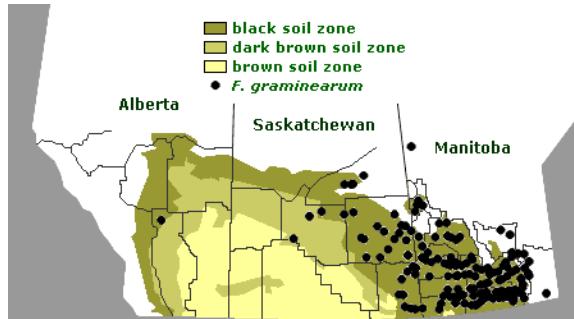


>3,000 plots processed

**1A****2D****3B****5A**

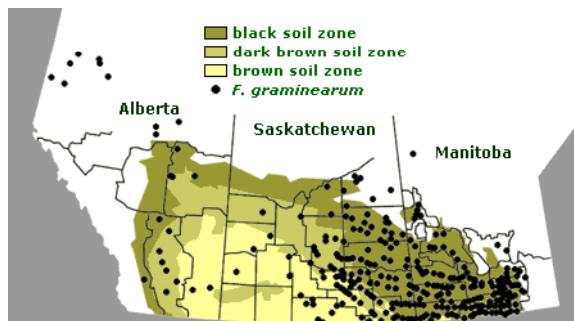
# FUSARIUM HEAD BLIGHT





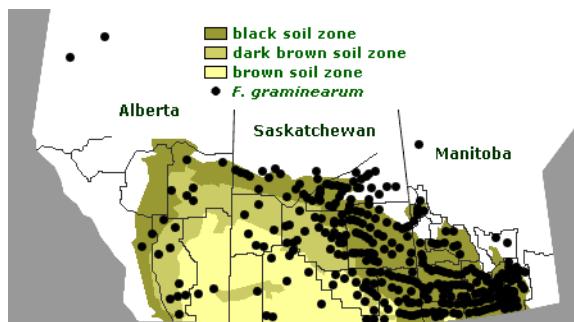
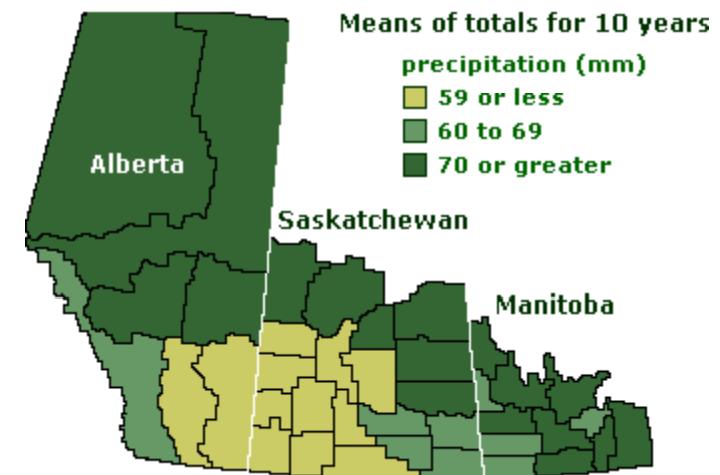
1994

10 years spread of FHB on the prairies.



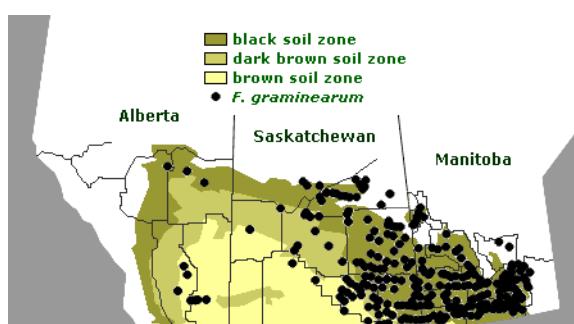
1996

## Precipitation

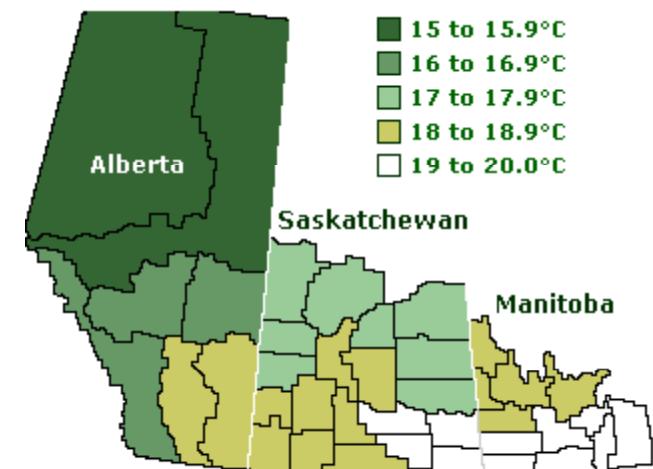


1999

## Temperature



2003



## Fusarium Head Blight

*Fusarium graminearum*

Polygenic resistance from Asian sources

G x E interaction impacts disease reaction and impairs selection

Affects all level of grain industry from production – marketing - consumer

Good candidate for MAS!



# Molecular mapping of novel genes controlling *Fusarium* head blight resistance and deoxynivalenol accumulation in spring wheat

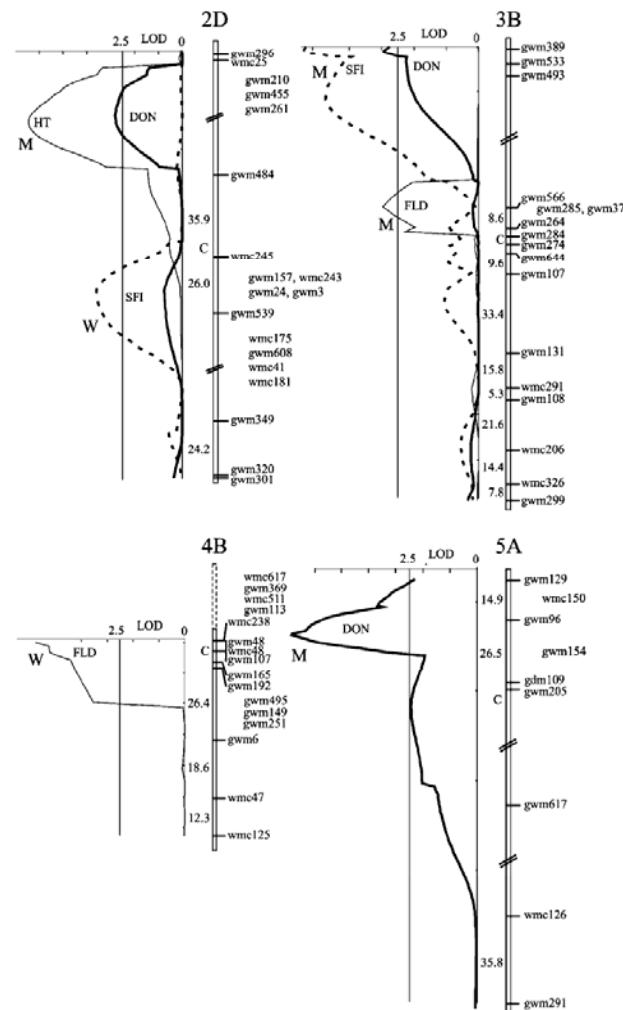
Daryl J. Somers, George Fedak, and Marc Savard

**Abstract:** *Fusarium* head blight of wheat is an extremely damaging disease, causing severe losses in seed yield and quality. The objective of the current study was to examine and characterize alternate sources of resistance to *Fusarium* head blight (FHB). Ninety-one  $F_1$ -derived doubled haploid lines from the cross *Triticum aestivum* 'Wuhan-1'  $\times$  *Triticum aestivum* 'Maringa' were examined for disease reaction to *Fusarium graminearum* by single-floret injection in replicated greenhouse trials and by spray inoculation in replicated field trials. Field and greenhouse experiments were also used to collect agronomic and spike morphology characteristics. Seed samples from field plots were used for deoxynivalenol (DON) determination. A total of 328 polymorphic microsatellite loci were used to construct a genetic linkage map in this population and together these data were used to identify QTL controlling FHB resistance, accumulation of DON, and agronomic and spike morphology traits. The analysis identified QTL for different types of FHB resistance in four intervals on chromosomes 2DL, 3BS, and 4B. The QTLs on 4B and 3BS proximal to the centromere are novel and not reported elsewhere. QTL controlling accumulation of DON independent of FHB resistance were located on chromosomes 2DS and 5AS. Lines carrying FHB resistance alleles on 2DL and 3BS showed a 32% decrease in disease spread after single-floret injection. Lines carrying FHB resistance alleles on 3BS and 4B showed a 27% decrease from the mean in field infection. Finally, lines carrying favourable alleles on 3BS and 5AS, showed a 17% reduction in DON accumulation. The results support a polygenic and quantitative mode of inheritance and report novel FHB resistance loci. The data also suggest that resistance to FHB infection and DON accumulation may be controlled, in part, by independent loci and (or) genes.

**Key words:** marker-assisted selection, *Fusarium*, wheat, microsatellite.

**Résumé :** La fusariose de l'épi chez le blé est une maladie très grave qui entraîne une diminution importante du rendement et de la qualité des grains. L'objectif de ce travail était d'examiner et de caractériser des sources alternatives de résistance à la fusariose de l'épi (FHB). Quarante-vingt-onze lignées haploïdes doublées dérivées de la  $F_1$  d'un croisement *Triticum aestivum* 'Wuhan-1'  $\times$  *Triticum aestivum* 'Maringa' ont été inoculées au *Fusarium graminearum* par injection dans une seule fleur lors d'essais en serre et par aspiration lors d'essais au champ. Les travaux en serre et au champ ont également été employés pour déterminer la valeur agronomique et caractériser la morphologie de l'épi de ces lignées. Des échantillons de grains ont été récoltés au champ pour doser la teneur en déoxynivalénol (DON). Au total, 328 marqueurs microsatellites ont été employés pour établir une carte génétique pour cette population et ces données ont été employées afin d'identifier des QTL contrôlant la résistance à la FHB, l'accumulation en DON ainsi que ceux qui déterminent certains caractères agronomiques et morphologiques. L'analyse a identifié des QTL pour différents types de résistance à la FHB sur quatre intervalles des chromosomes 2DL, 3BS et 4B. Les QTL situés sur 4B et 3BS à proximité du centromère sont nouveaux et n'ont pas été rapportés par d'autres. Des QTL contrôlant l'accumulation de DON indépendamment de la résistance à la FHB ont été localisés sur les chromosomes 2DS et 5AS. Les lignées portant les allèles de résistance pour les locus situés sur 2DL et 3BS affichaient une réduction de 32 % de la progression de l'infection suite à une inoculation florale. Les lignées portant les allèles de résistance aux locus sur 3BS et 4B montraient une réduction de 27 % des symptômes au champ. Finalement, les lignées ayant les allèles favorables sur 3BS et 5AS affichaient une réduction de 17 % de la teneur en DON. Ces résultats viennent appuyer le modèle d'une hérédité polygénique et quantitative et permettent d'identifier de nouveaux locus de résistance à la FHB.

Somers et al.



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<sup>1</sup>Corresponding author (e-mail: somersd@em.agr.ca).

fection in 2000 based on the 1997 and 2000 SFI experiments (Table 2). Both FLD and SFI data showed poor correlations between years. The DON measurements were better correlated over the two years ( $r = 0.60$ ) (Table 3). Four agronomic and head morphology traits were measured in the

field and the average values over both years for each line were compared with FHB infection and DON levels. In general, there was poor correlation between these traits with the exception of plant height and days to heading versus FLD FHB infection. Taller and later plants showed less FHB in-

# Accelerated development of FHB resistant bread wheat using Marker-Assisted Selection

Version 2.0 - April/2003

## Phase 1: Parental Genotyping

6 seeds of each parent lines are screened with 3 AFLP primer combinations and 12 Mstas to detect off types and select a single "normal" plant of each accession.

The list of parents for the project includes:

- 98B08-A47
- 98B08-A228
- Prodigy
- BW301
- AC Elsa
- PT205
- BW264
- BW263
- HCT24
- HCT26
- HCS14
- 98B08-A111
- 98B08-A41

Additional genotype checks include:

- Wuhan
- Maringa
- Opata
- Synthetic
- Chinese Spring
- BW252
- BW278
- DH18
- AC Foremost
- Sumai 3

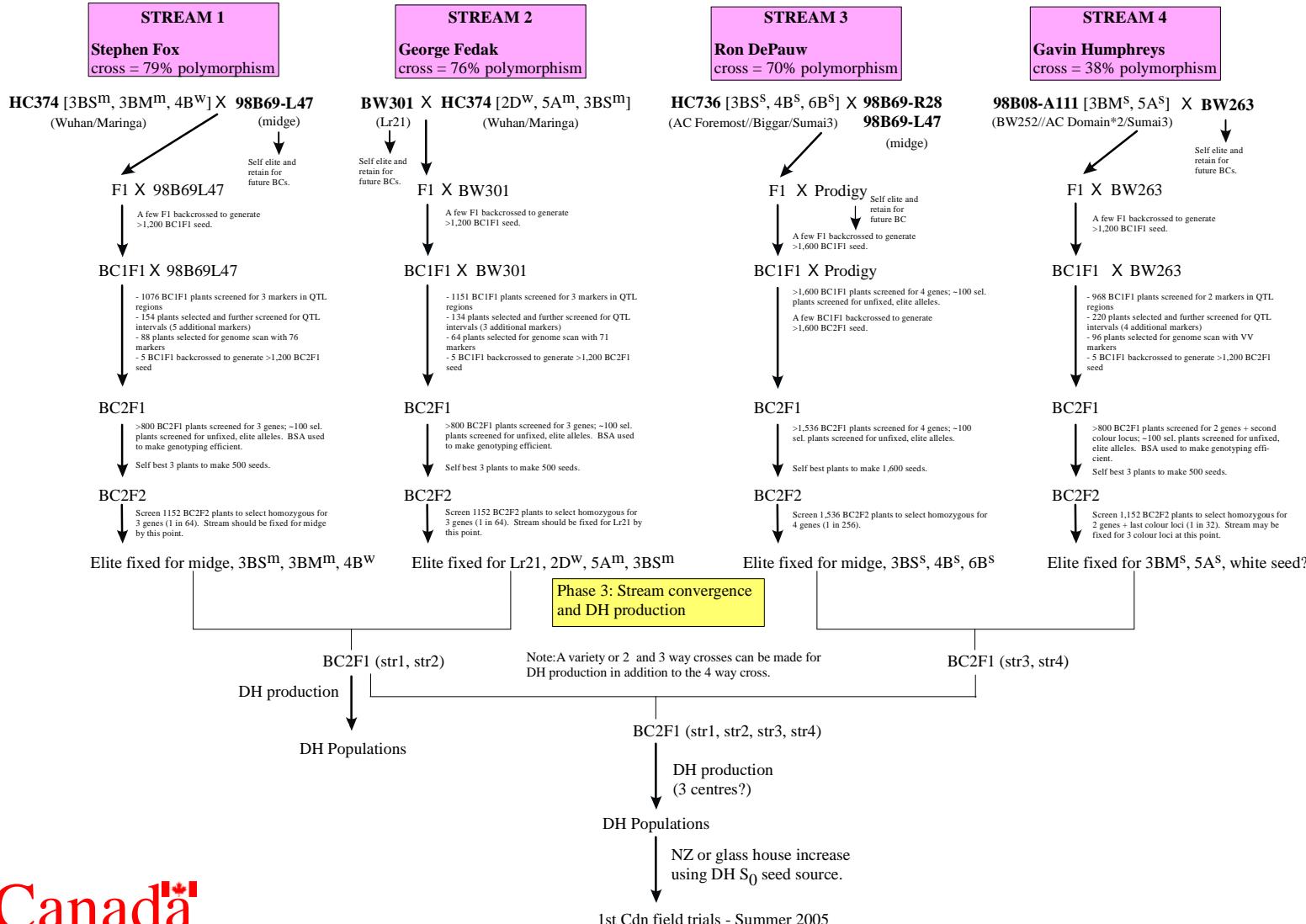
The selected parent plants and genotype checks are genotyped at 700 Mstas loci with ~550 Mstas.

During parental genotyping, the elite lines and pest resistance donors will be intercrossed in multiple combinations, using elite cytoplasm to generate F1 seed. The genotyping information will be used to select 4 streams beginning with a single cross.

## Investigators:

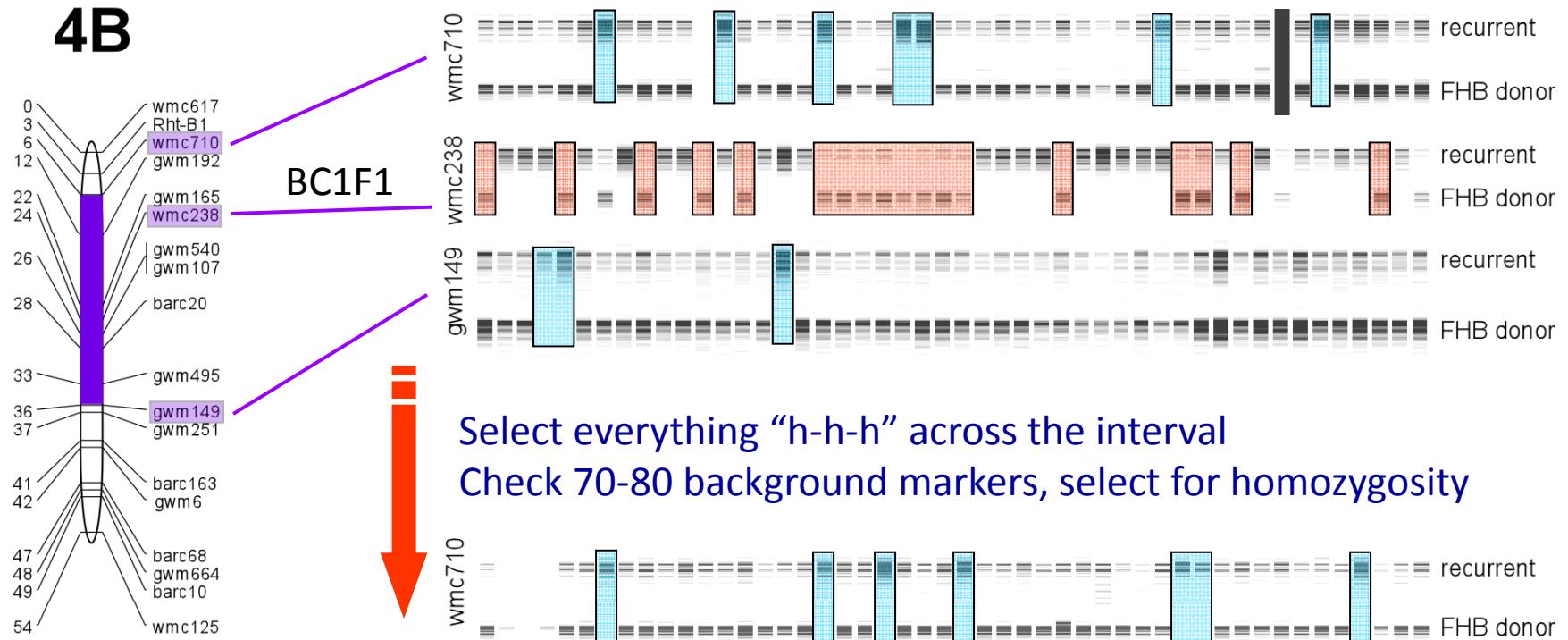
Daryl Somers	Molecular breeding
Julian Thomas	Genetics
Stephen Fox	Breeding
Gavin Humphreys	Breeding
Ron DePauw	Breeding
George Fedak	Breeding
Jeannie Gilbert	FHB Pathology
Brent McCallum	Lr Pathology
Bob Lamb	Entomology

## Phase 2: Marker-assisted selection of BC1F1



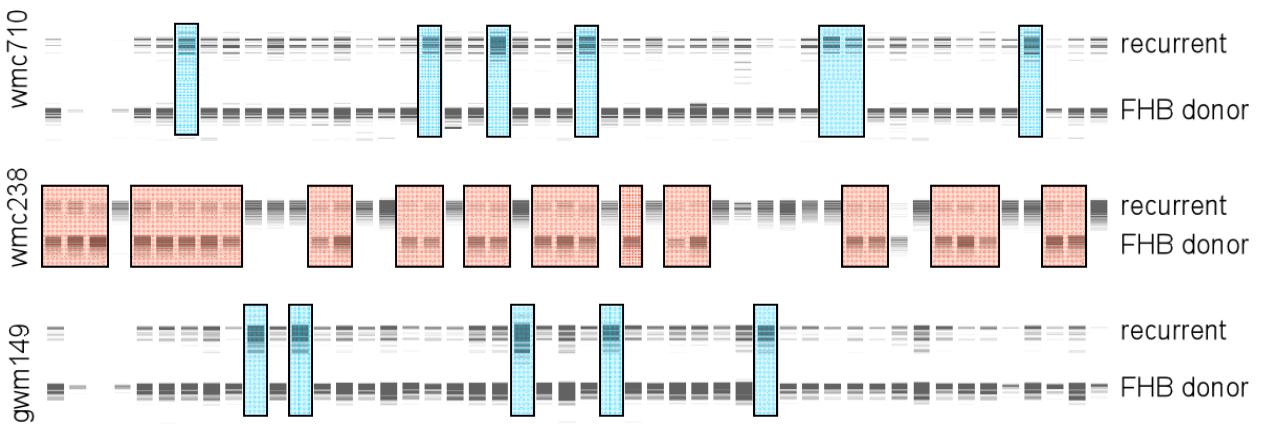
## QTL introgression – the details

**4B**



Select everything “h-h-h” across the interval  
Check 70-80 background markers, select for homozygosity

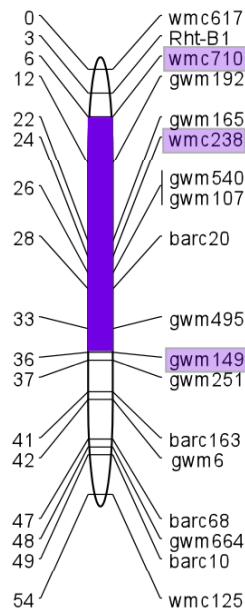
**BC2F1**



Select everything “h-h-h” across the interval  
Check unfixed background markers, select for homozygosity

## QTL introgression – the details

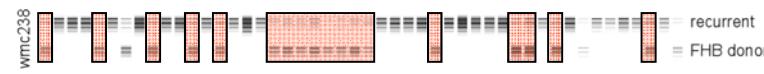
**4B**



BC1F1

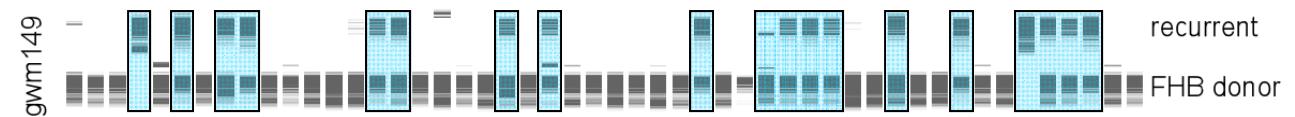
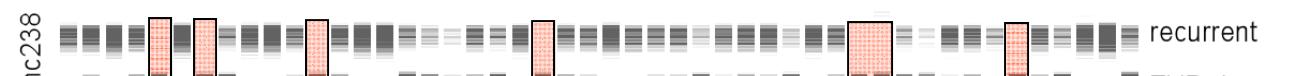


BC2F1



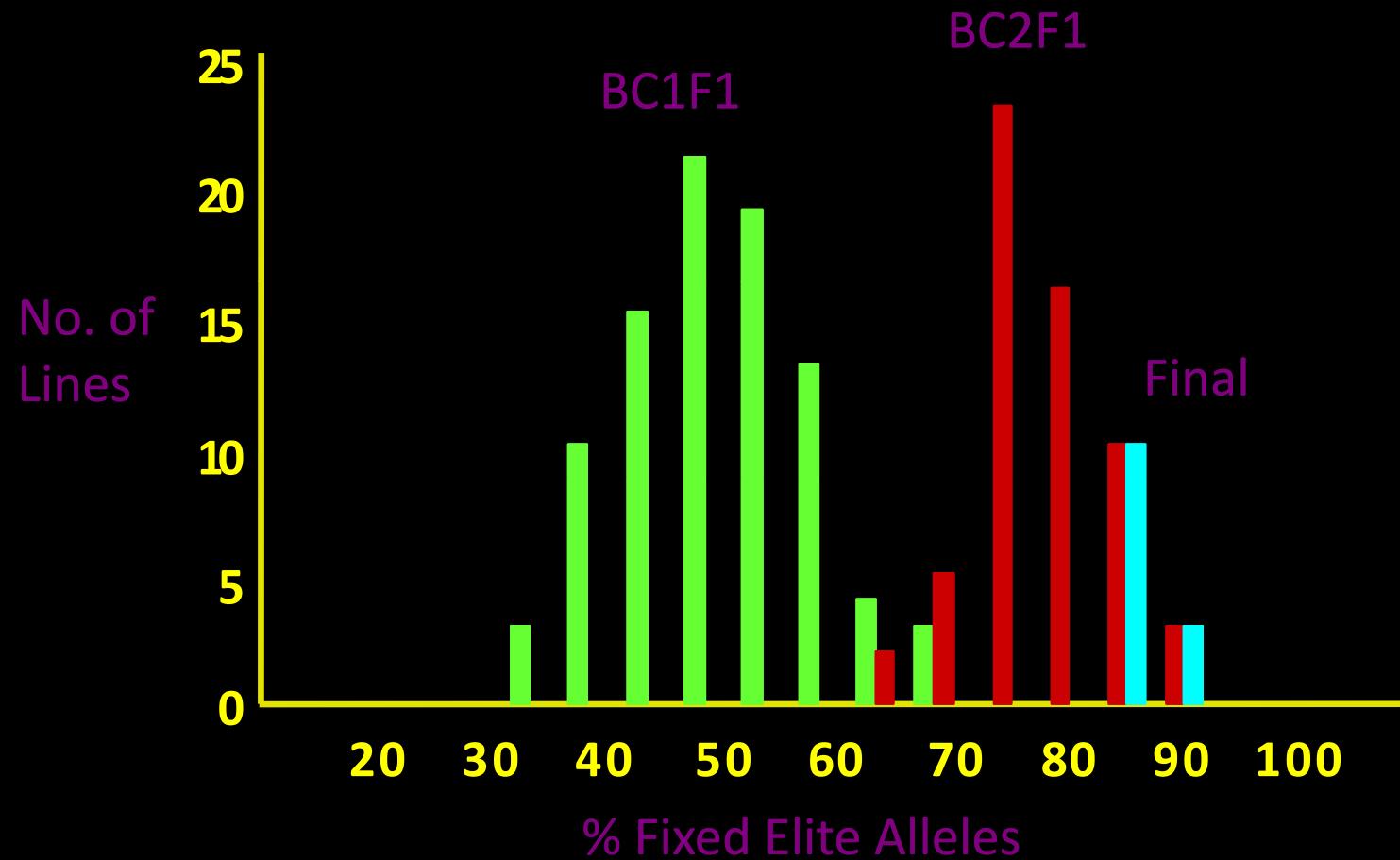
Self the elite, heterozygous plants

BC2F2



Select plants homozygous donor across the interval

# Fixation of recurrent parent using molecular breeding.

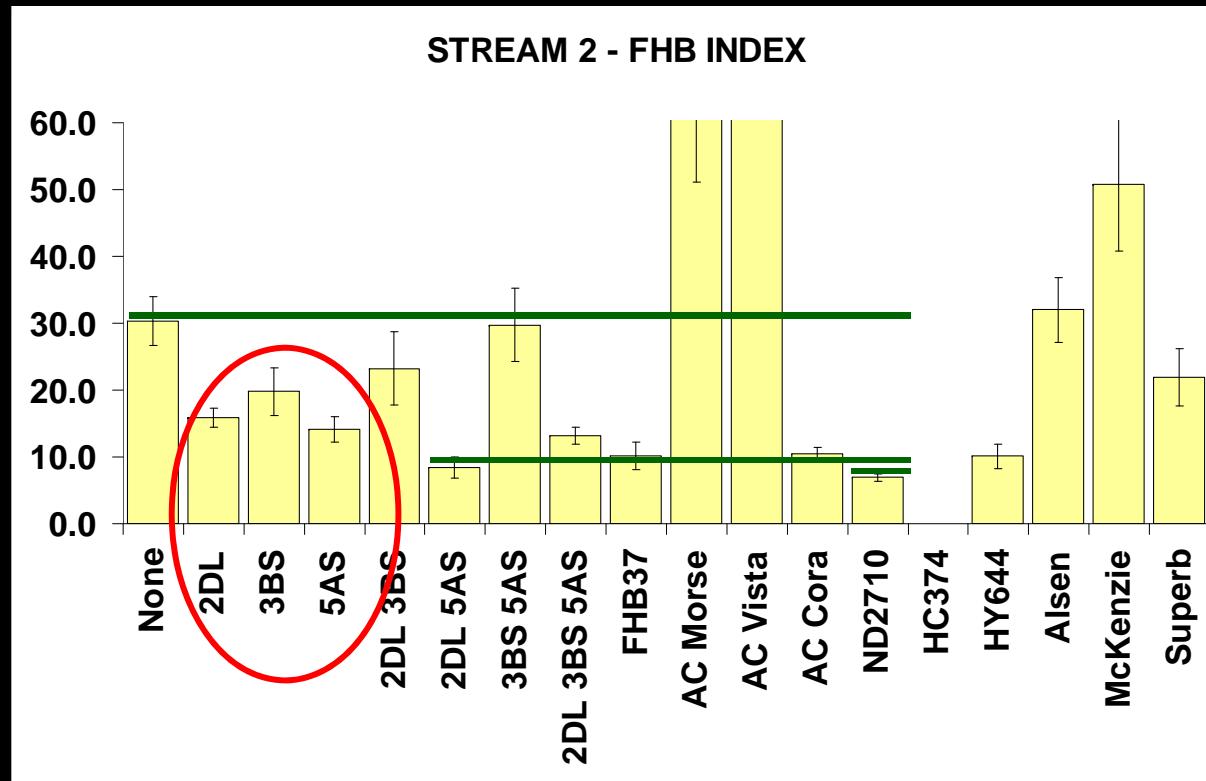


120 BC2F3 entries x 3 reps – Ottawa ON – 2004



Canada

## BC2F3 (homozygous rows)



2 years of replicated data - Ottawa ON – Fedak  
Clear effect of single genes.

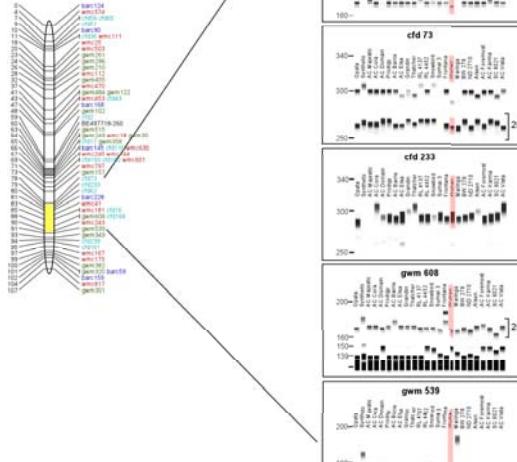
# FHB

Complex traits + diverse genetics require haplotype information.

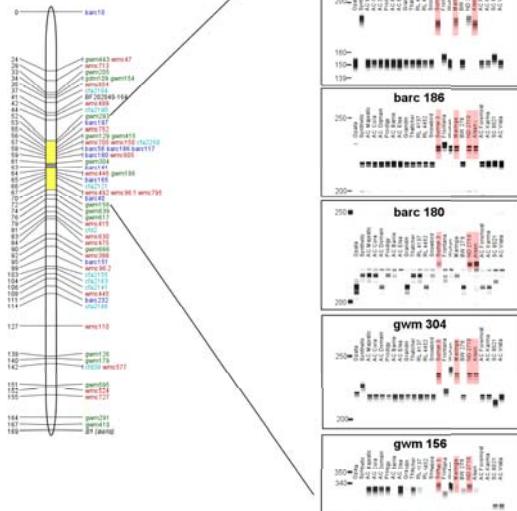
Developed haplotypes images for major FHB loci.

Routinely used to screen:  
Parents, F1, ... advanced.

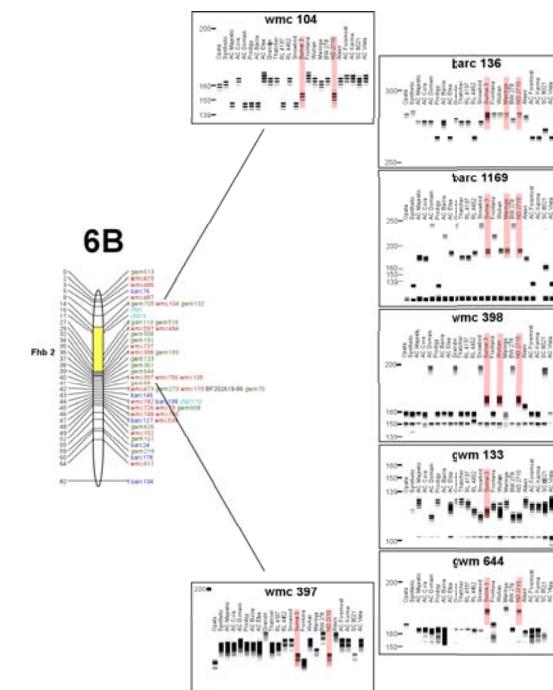
**2D**



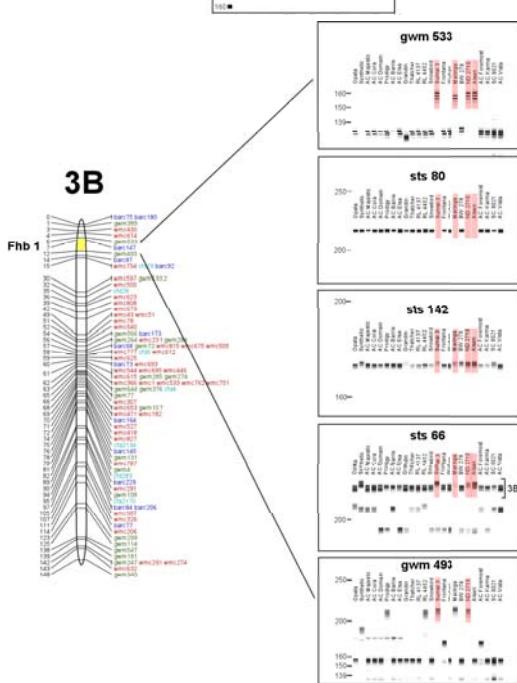
**5A**



**6B**



**3B**



# Grape Breeding

- Grape is a hybrid crop
  - high degree of heterozygosity
- Perennial, vegetatively propagated
- Buds and Seeds require a cold / dormant period for growth/germination
  - stratification

Freezing temperatures (-20C to -25C)

- stress dormant wood and buds
- kill buds, no renewed growth
- vines lose productivity for years

# Freezing tolerance

- Some elements of freezing tolerance can be controlled
  - vineyard management
  - rootstocks
  - variety
- Bud freeze tolerance can be measured after acclimation
  - low temperature exotherm – ice formation
  - Tenny DTA freezer system
  - Good sample throughput
- Niagara varieties show LT50 at -20C to -25C

*Vitis riparia* will survive to -40C.  
strategy is to BC *V. riparia* into *V. vinifera*

# Genetic markers and Genetic maps

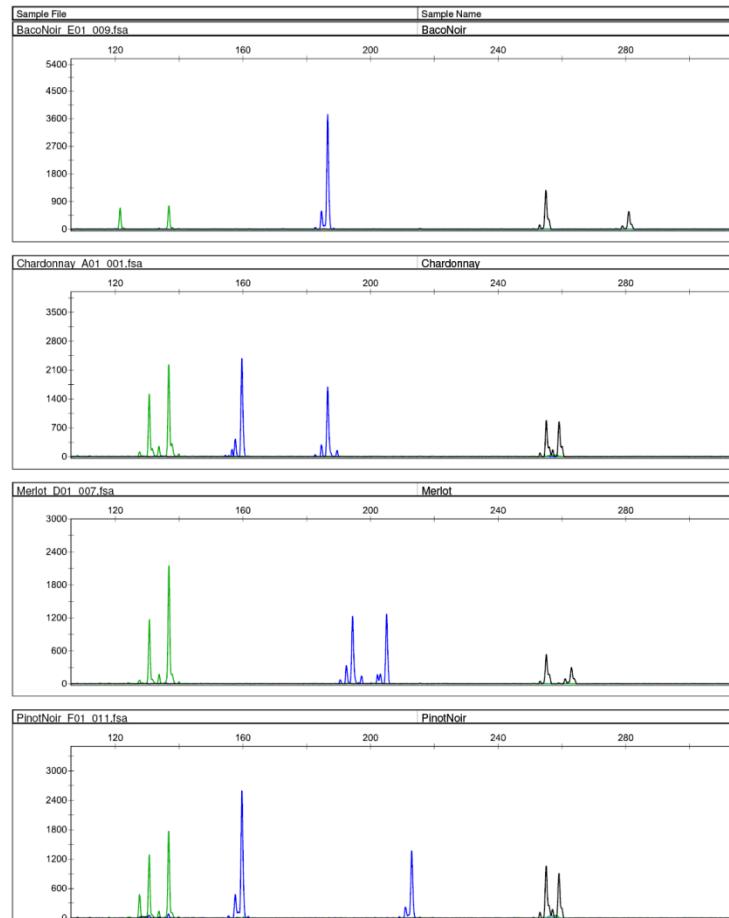
Microsatellite markers (simple sequence repeats)



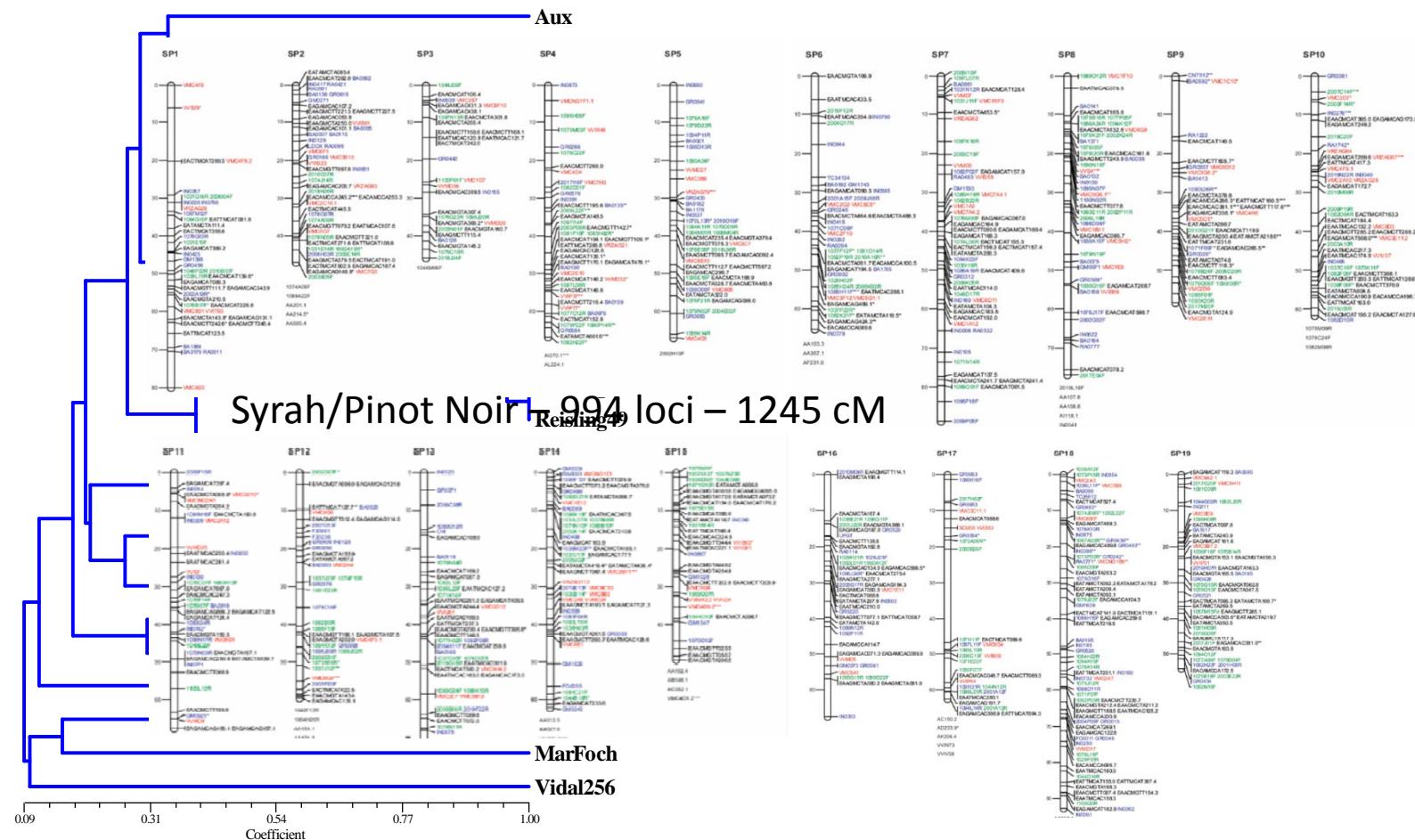
- International community uses 6 markers to distinguish varieties.
- **Vineland is set up to generate and deliver this information.**

# Grape Genotyping

- ABI3130 XL genotyper
- Established HT DNA extraction
- Screened >800 SSRs on 24 Niagara *Vitis* cvs.
- Adapting DNA melt point tech for SNP detection.
- DaRT marker technology from Triticarte (Australia).

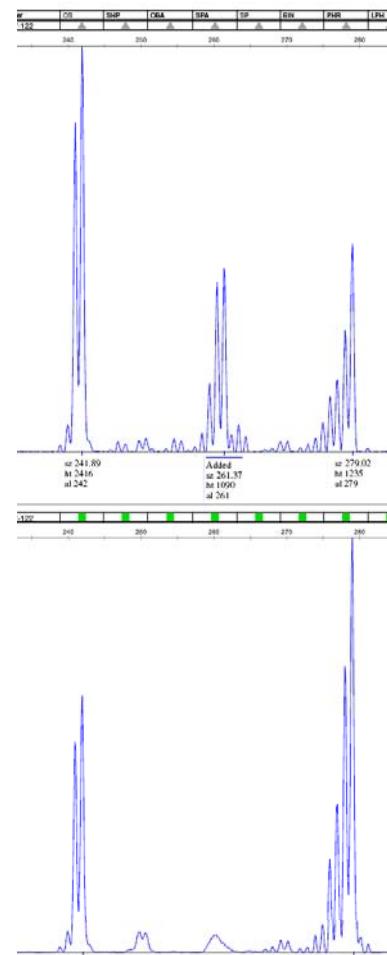


# Genetic Analysis



# Grape Genotyping

- Discover chimerism within leaf tissue.
- May be derived from L1, L2 cell layers.
- Gives rise to clonal variation.

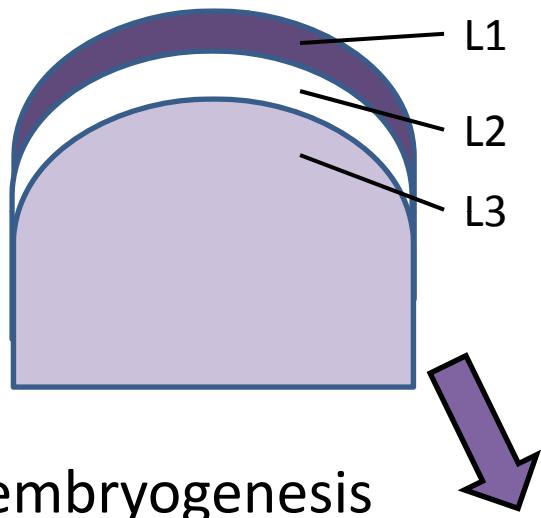


Limberger

Pinot noir

# Periclinal chimera

Plant meristems are generally composed of 3 cell layers.



Cell layers generally do not mix.

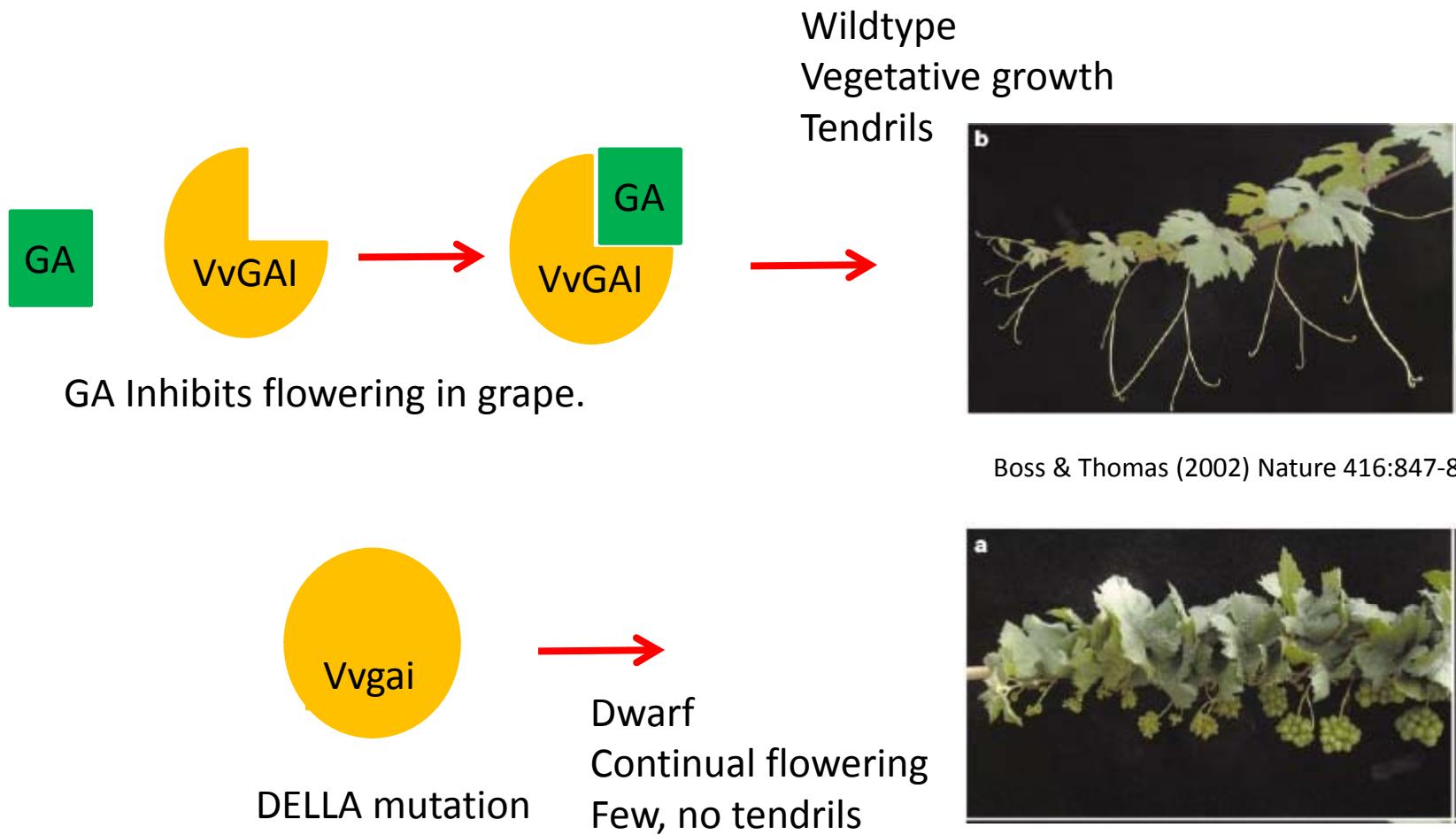
Configuration was discovered by observing mutations in each cell layer.

Derive plants from individual cell layers



Genetically distinct cell layers (mutants) derive phenotypically distinct plants

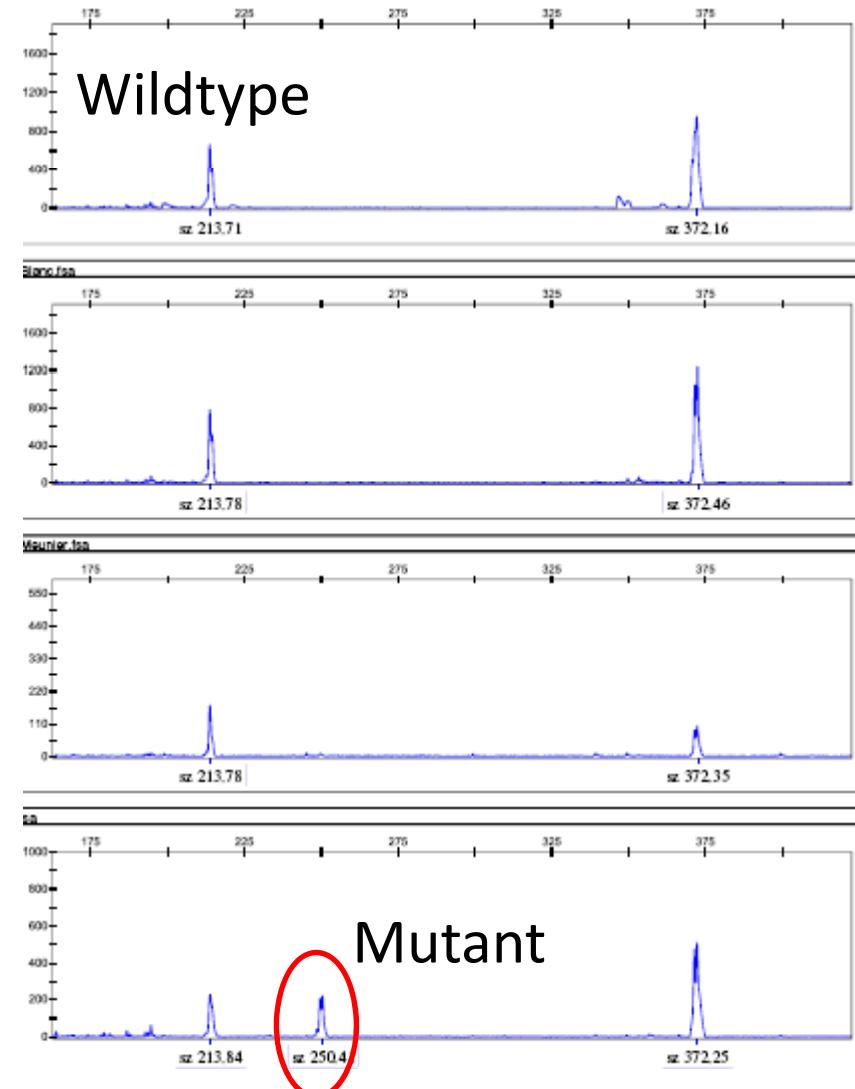
# Dwarf Grape – rapid cycling



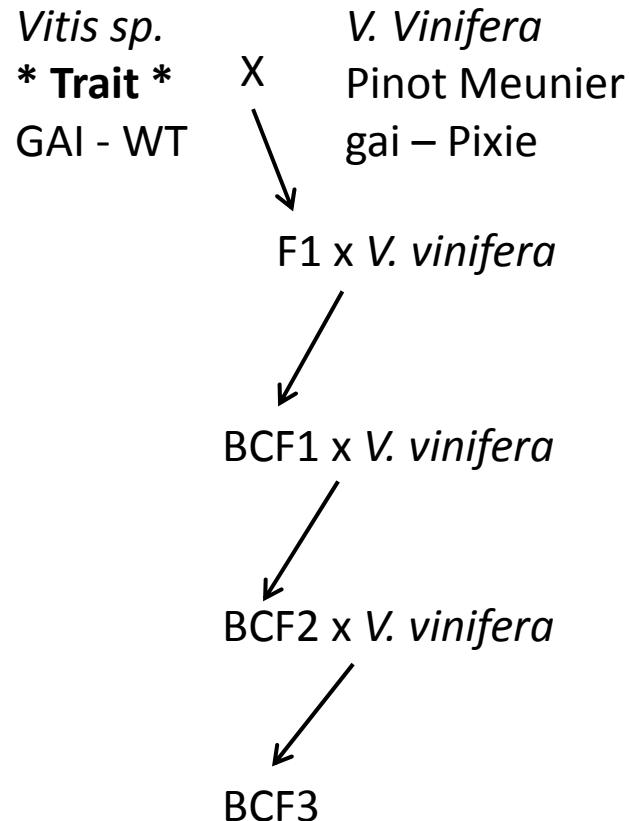
# GAI/gai detection with PCR

## Vitis vinifera GAI-like protein 1 (GAI1) gene

TTGGGTTCCGAGAGACCCACCCTCTGCAACTCCTCCTTTC  
ATCTCTCCCTCCTCCAATACAAGCGGCACTAGTATTCAA  
CATACCCACCGCCTGATTCCCATCAACCC CCCCTCACAC  
TATGAAGAGGGAGTATCATCATCCTCATCACCCAACTTG  
CTCCACGTCCCCACCGGCAAGGGTAAGATGTGGGATG  
CCGACCCCCAGCAAGACGCCGGCATGGATGAGCTT(T)  
GCTGTTTGGGCTACAACGTCAAGGCCTCCGACATGGC  
TGAGGTCGCTCAGAAGCTTGAACAGCTTGAGGAAGTTA  
TTGTTAATGCTCAGGAGGATGGCCTCTCTCATCTCGCTT  
CCGAGACTGTTATTACAACCCCTCCGATCTGTCTAACT  
GGCTTGGAAAGCATGCTCTCCGAGTTCAACCC ACTCCC  
AATTGCGCCCTTGAC

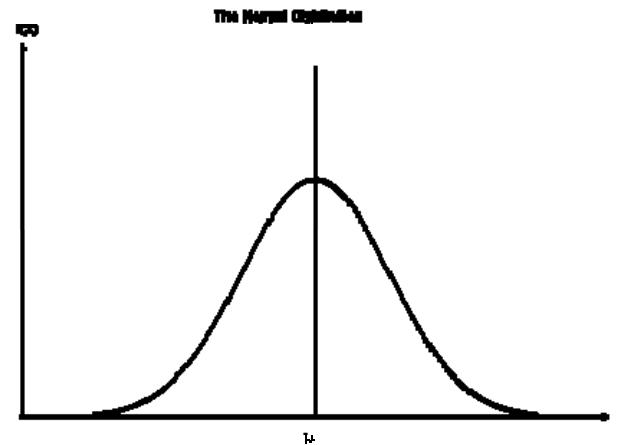


# Accelerated Grape Breeding



Can use SSR or DaRT  
markers to track progress.

Accelerates restoration of  
elite genetic background.



# Risky aspects of research

## Freeze tolerance testing

- Cold acclimate seedlings at 4C – 2-3 weeks
- Determine bud hardiness in programmable freezer.

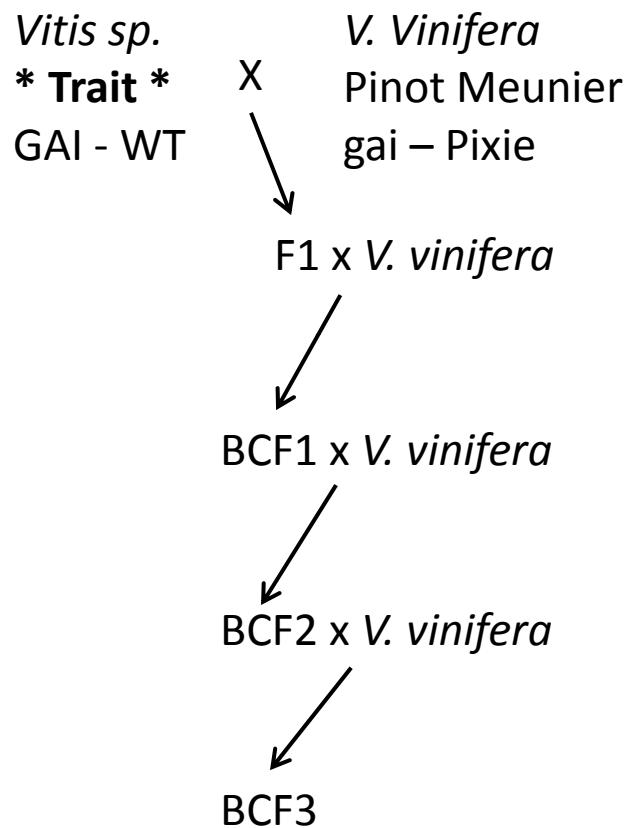
## Fruit ripening and seed germination

- Plant maintenance
- Stratification and GA applications
- Embryo rescue

## Performance of new grape genotypes

- What can be selected in the GH?

# Future Planning



Create dwarf versions of all current and future varietals to be produced in the region

This creates a resource to cross future traits into multiple varietals within 1 or 2 generations.

Collect pollen from important accessions worldwide that may contribute traits to Canadian vines.