Genetic analysis of developmental traits associated with enhanced winter survival in autumn-seeded rye (*Secale cereale* L.)

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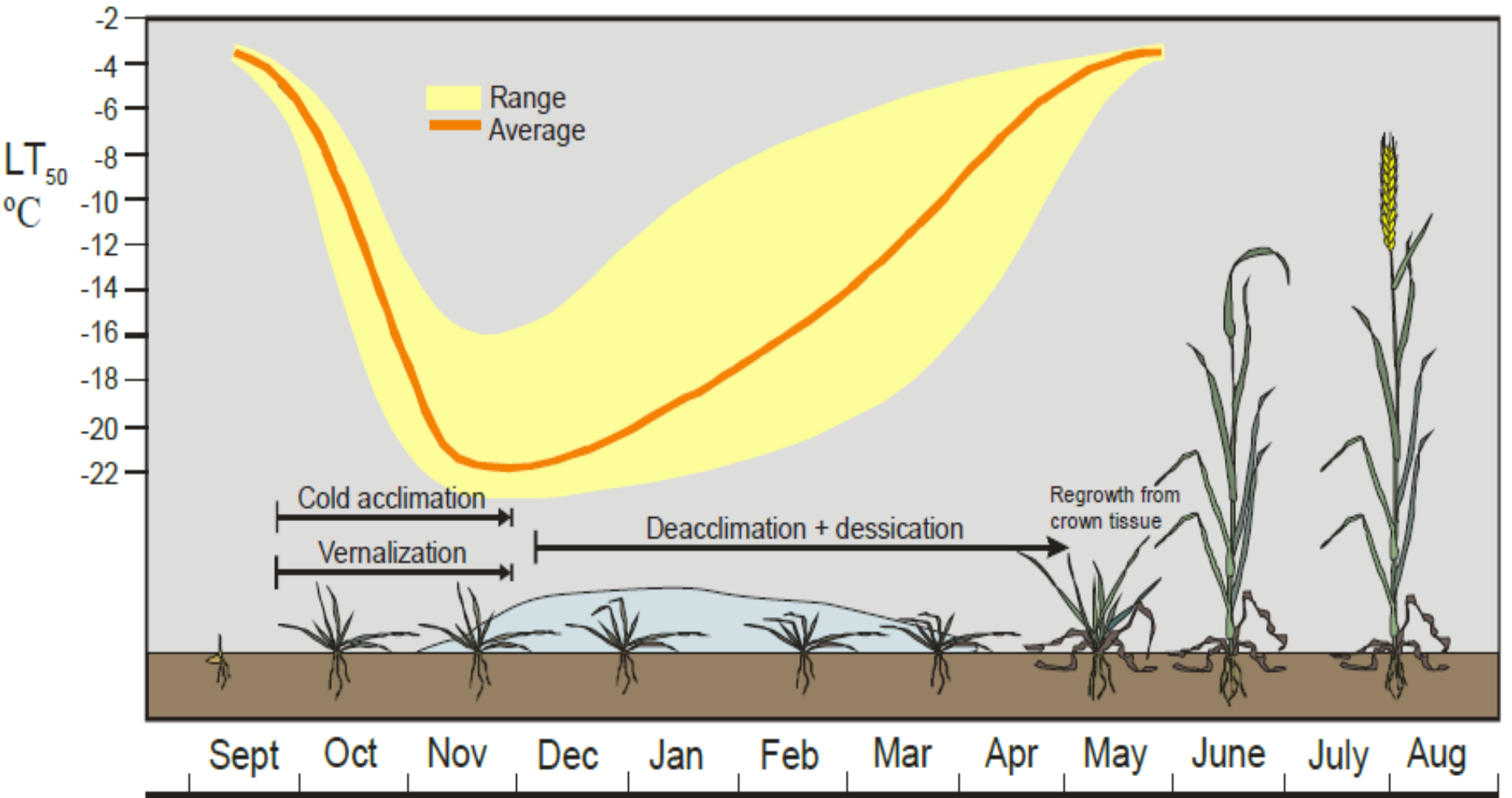1. Department of Plant Science, University of Saskatchewan, Saskatoon, SK, S7N 5A8, Canada
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Outline

- Introduction

- Phenotyping of traits

- Genotyping by sequencing

- Association mapping of traits studied

- Conclusions

Cold acclimation prepares plants for over-wintering

Vernalization

Vegetative ⟶ Reproductive phase

Spring Genotype

Winter genotype

Winter Field Survival Index (FSI)

Genotype	Spring cereals	FSI	LT_{50}
Bonanza	barley	115	-7.7
Manitou	wheat	160	-9.6
Gazelle	rye	210	-11.6
Genotype	Winter cereals	FSI	LT_{50}
Dover	barley	300	-15.3
Novamichurinka	durum wheat	370	-18.1
Ulianovkia × Kodiak	triticale (8X)	480	-22.6
CDC Kerstel	Wheat	497	-23.3
Karkov 22MC	wheat	499	-23.5
Ulianovkia	wheat	530	-24.7
Sangaste	rye	550	-25.5
Kodiak	rye	575	-26.5
Cougar	rye	620	-28.3
Frontier	rye	735	-33.0
Puma	rye	735	-33.0

Developmental traits associated with winter survival

- Final leaf number

- Prostrate growth

Erect   Inter mediate   Prostrate

- Anthocyanin production

Hypotheses

➢ Winter field survival in fall-seeded cereals is an interaction between freezing tolerance and the developmental traits of plants.

➢ Genes that affect the developmental traits have a role in determining the winter survival potential of plants.
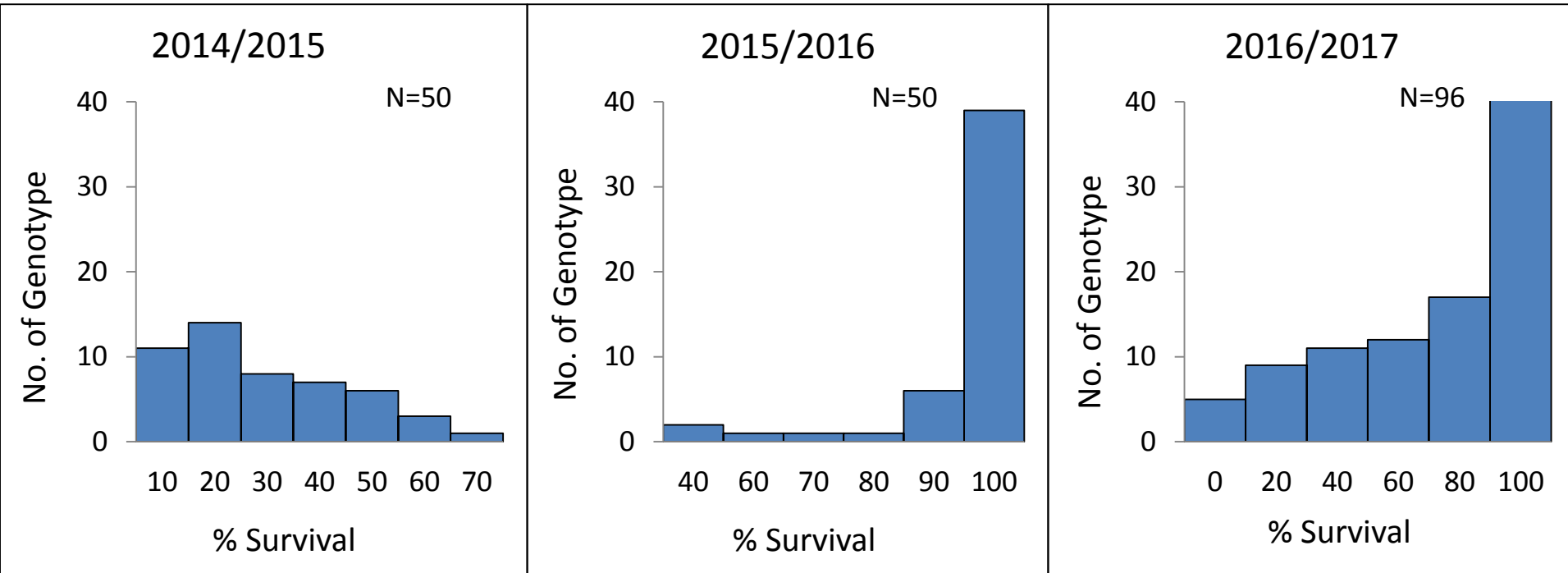
Objectives

➢ Determination of winter survival for a panel of 96 rye genotypes.

➢ Analyze rye panel for developmental traits associated with winter hardiness.

➢ Genotype rye panel by Genotyping by Sequencing (GBS).

➢ Association mapping of rye panel to identify genomic markers associated with winter survival.

Origin of rye genotypes studied

Winter Field Survival (WFS) in different trials

Correlation coefficients for 50 genotypes

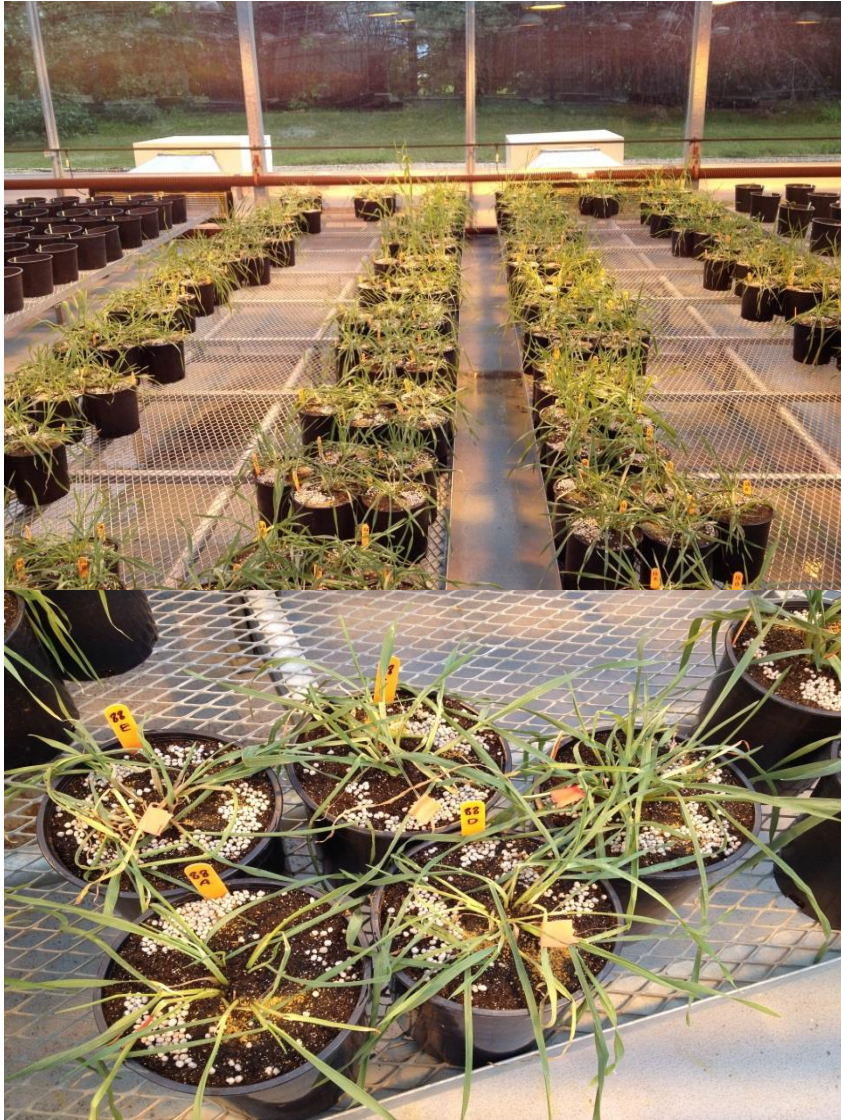|	WFS % 2016/2017	WFS % 2015/2016
WFS % 2015/2016	0.65**	
WFS % 2014/2015	0.54**	0.44**

**Significance of Pearson correlation coefficient P ≤0.001
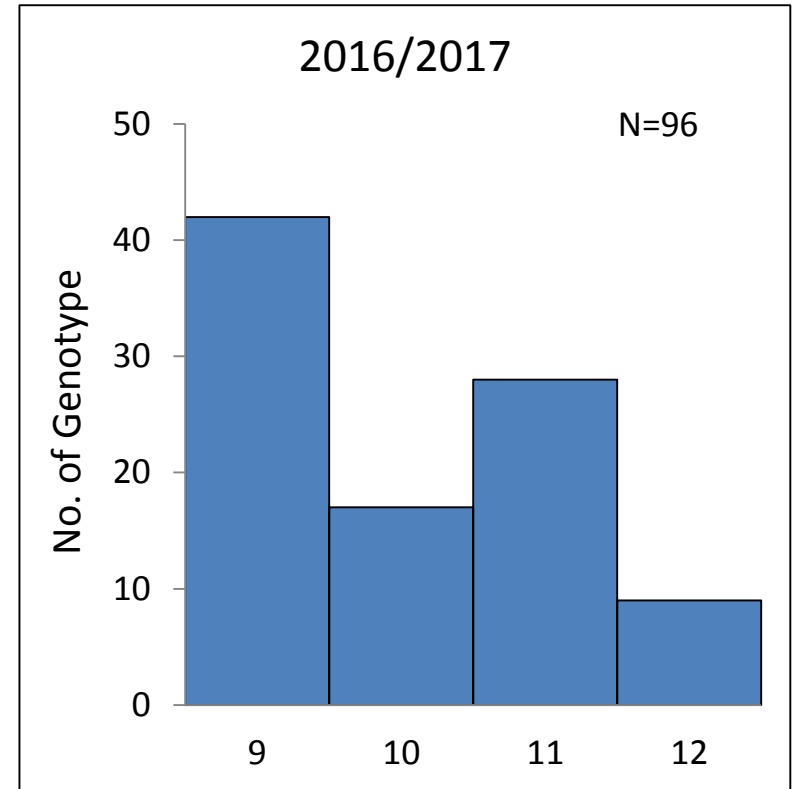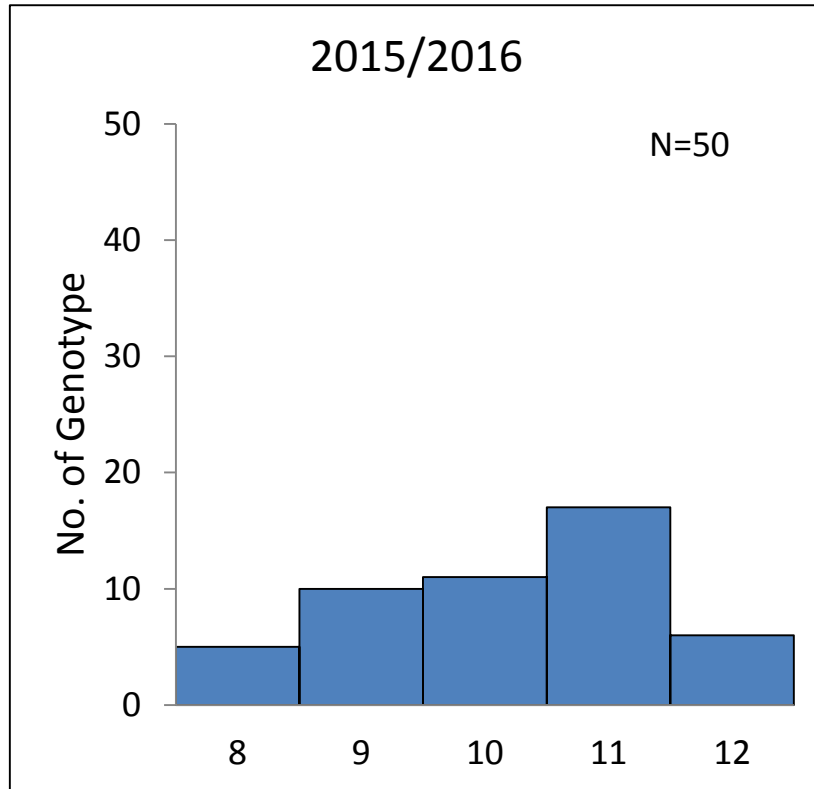
Developmental traits

- Final leaf number
- Prostrate growth habit
- Anthocyanin profile
- Days to Anthesis

- Top internode elongation
- Flag leaf area
- Plant height
- Awn length

Greenhouse rye seed production
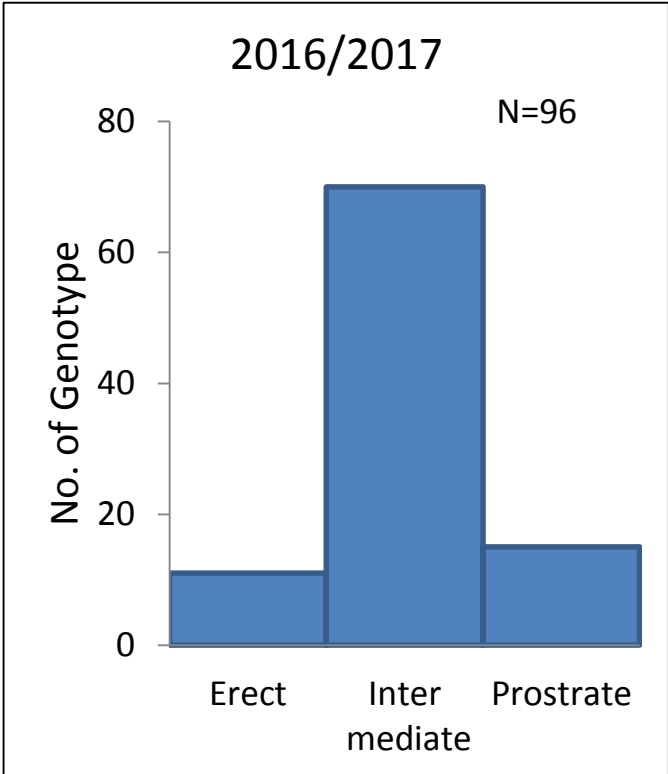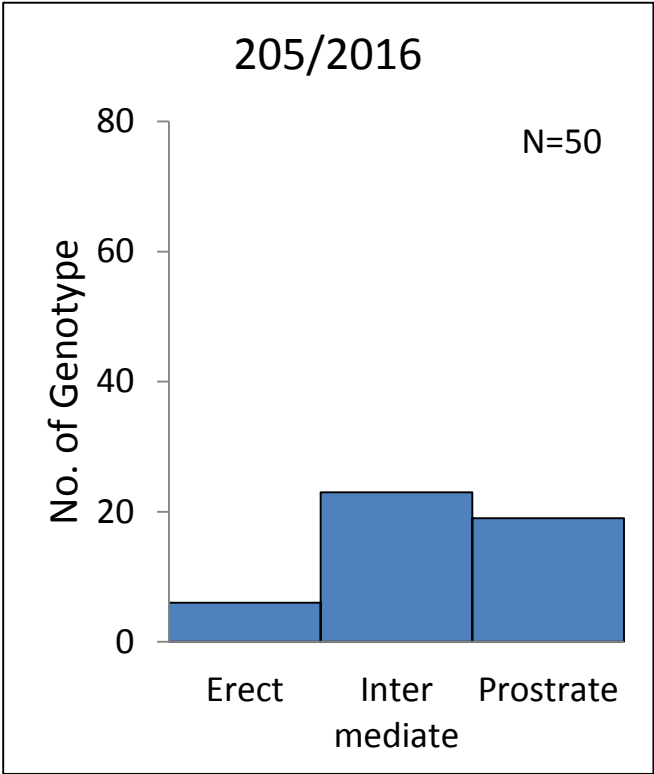
Final Leaf Number (FLN) in different trials

Correlation coefficients for 50 genotypes

	FLN 2016/2017
FLN 2015/2016	0.80**

**Significance of Pearson correlation coefficient P ≤0.001

Prostrate Growth Habit (PGH) in different trials

Correlation coefficients for 50 genotypes

	PGH 2016/2017
PGH 2015/2016	0.56**
**Significance of Pearson correlation coefficient P ≤0.001	

Correlation coefficients between traits
2015-2016 trial

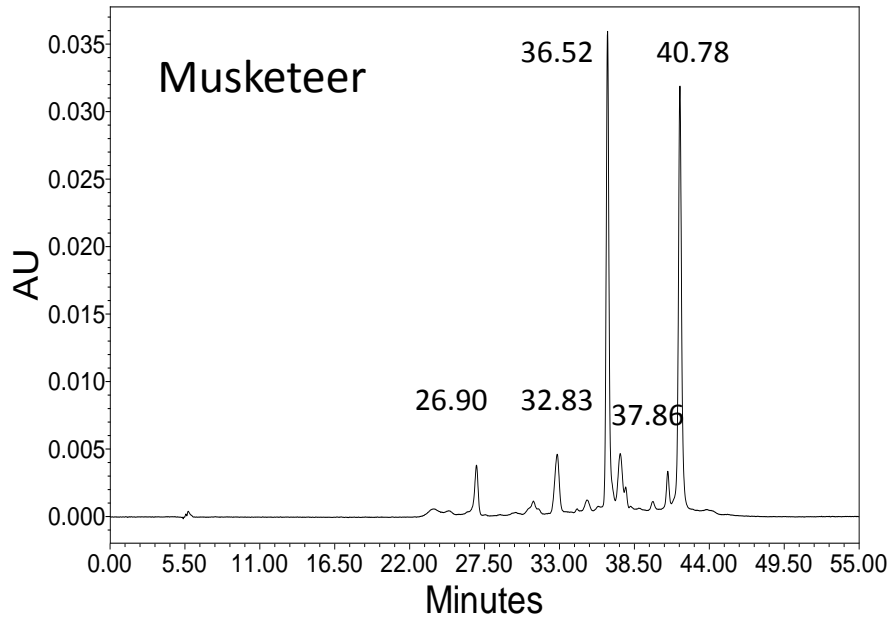|	Winter Survival	Final Leaf Number	Plant Height	Prostrate Growth Habit	Awn Length	Top Internode Elongation	Days to Anthesis
Final Leaf Number	0.53**						
Plant Height	0.15	0.16					
Prostrate Growth Habit	0.41*	0.32	0.20				
Awn Length	0.17	0.29	0.37*	0.29			
Top Internode Elongation	0.60**	0.59**	0.27	0.28	0.18		
Days to Anthesis	0.51**	0.34	-0.27	0.16	0.06	0.31	
Flag Leaf Area	0.44*	0.39*	0.006	0.23	0.33	0.48**	0.50**

**Significance of Pearson correlation coefficient P ≤0.001; *Significance of Pearson correlation coefficient P ≤0.01
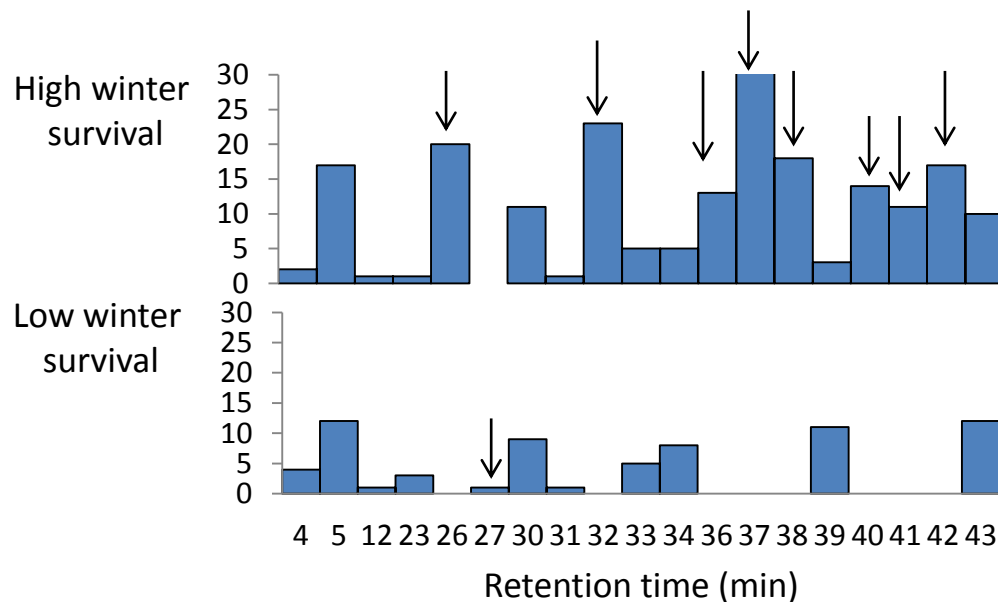
Correlation coefficients between traits 2016-2017 trial

	Winter Survival	Final Leaf Number	Plant Height	Prostrate Growth Habit	Awn Length	Top Internode Elongation	Days to Anthesis
Final Leaf Number	0.84**						
Plant Height	0.25	0.17					
Prostrate Growth Habit	0.36**	0.28*	-0.007				
Awn Length	0.13	0.21	0.29*	0.22			
Top Internode Elongation	0.68**	0.49**	0.11	0.17	0.33**		
Days to Anthesis	0.29*	0.26	-0.22	0.32*	-0.07	0.1	
Flag Leaf Area	0.64**	0.66**	0.15	0.27*	0.28*	0.61**	0.19

**Significance of Pearson correlation coefficient $P \leq 0.001$; *Significance of Pearson correlation coefficient $P \leq 0.01$

Analysis of anthocyanin in rye genotypes

- HPLC

- C18 column

- Mobile phase:
  A: 5 % Formic acid
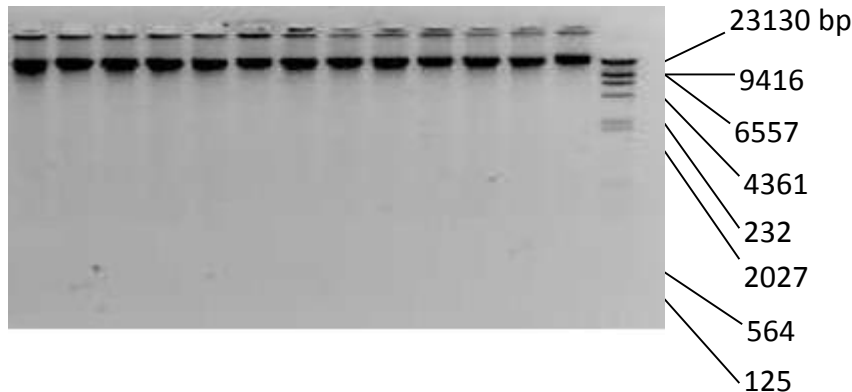 B: 22.5 % Methanol &
      22.5 % Acetonitrile

- 8 peaks associated with winter hardy genotypes

  - Cyanidin-3-O-rutinoside

  - Peonidin-3-Oglucoside

Genotyping by Sequencing (GBS)

- Isolation of high-quality genomic DNA

DNA absorbance ratio	
A260/A280	A230/A260
1.8 - 2	2 - 2.2

23130 bp
9416
6557
4361
232
2027
564
125

20 ng/µl DNA quantified by PicoGreen

- Sequencing library construction

DNA digestion
PstI/*MsPI*

↓

Size selection

↓

Add adaptors
with barcodes

- DNA sequencing
  Ion Torrent

↓

FastQ files

Bioinformatics analyses

Remove adaptor/barcodes from FastQ files (Trimmomatic)
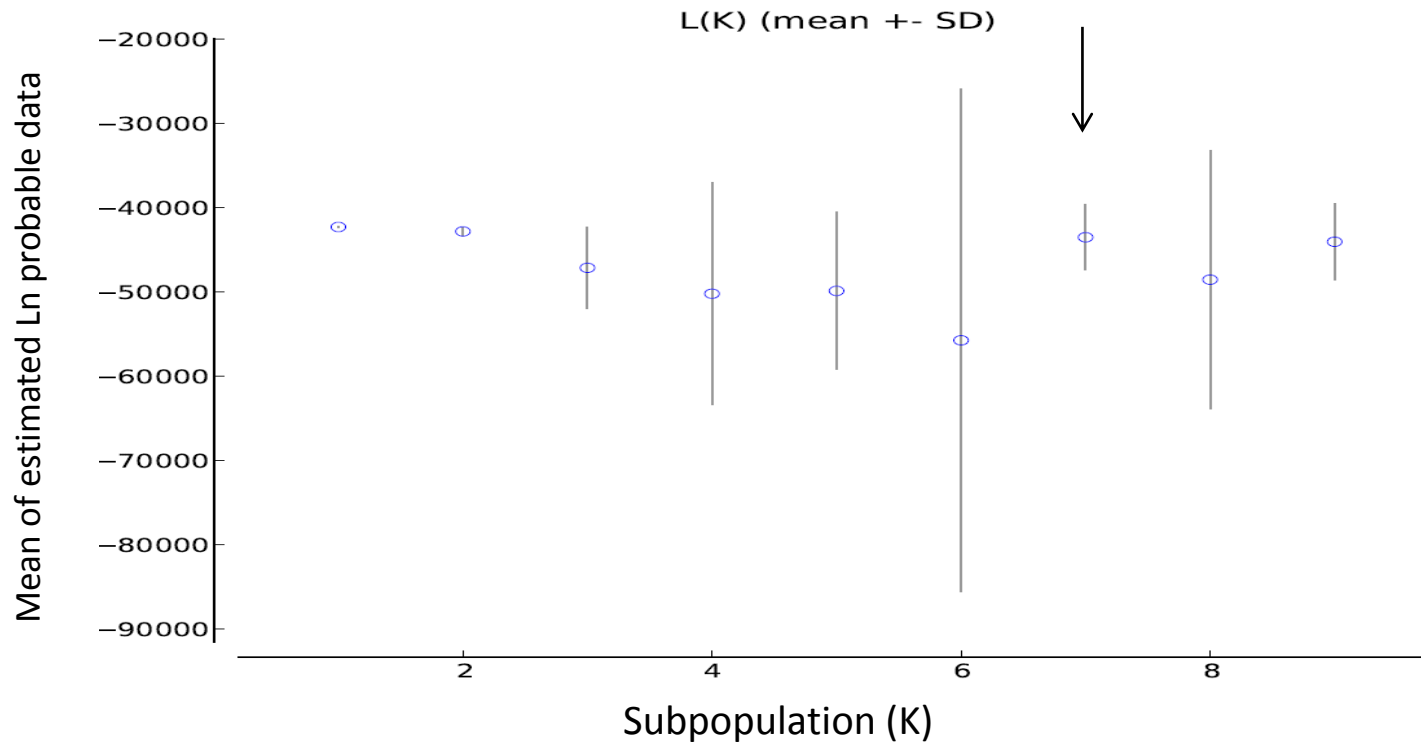
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Aligned to rye ref genome (Bowtie)
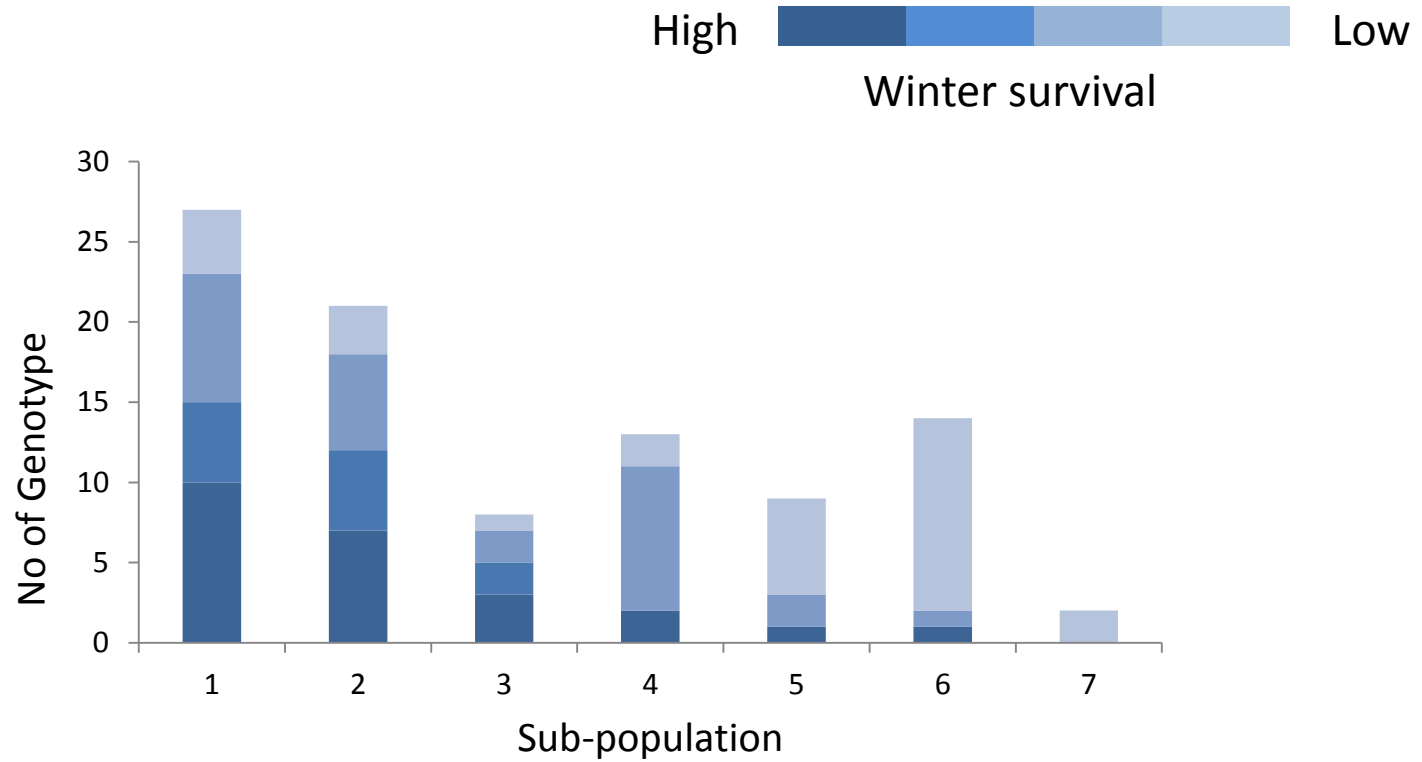
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Determine and filter SNPs (SAM, VCF)

↓

Genotype	Total reads Mb	Average read length bp	No. of SNPs
Rye	357	108	3444

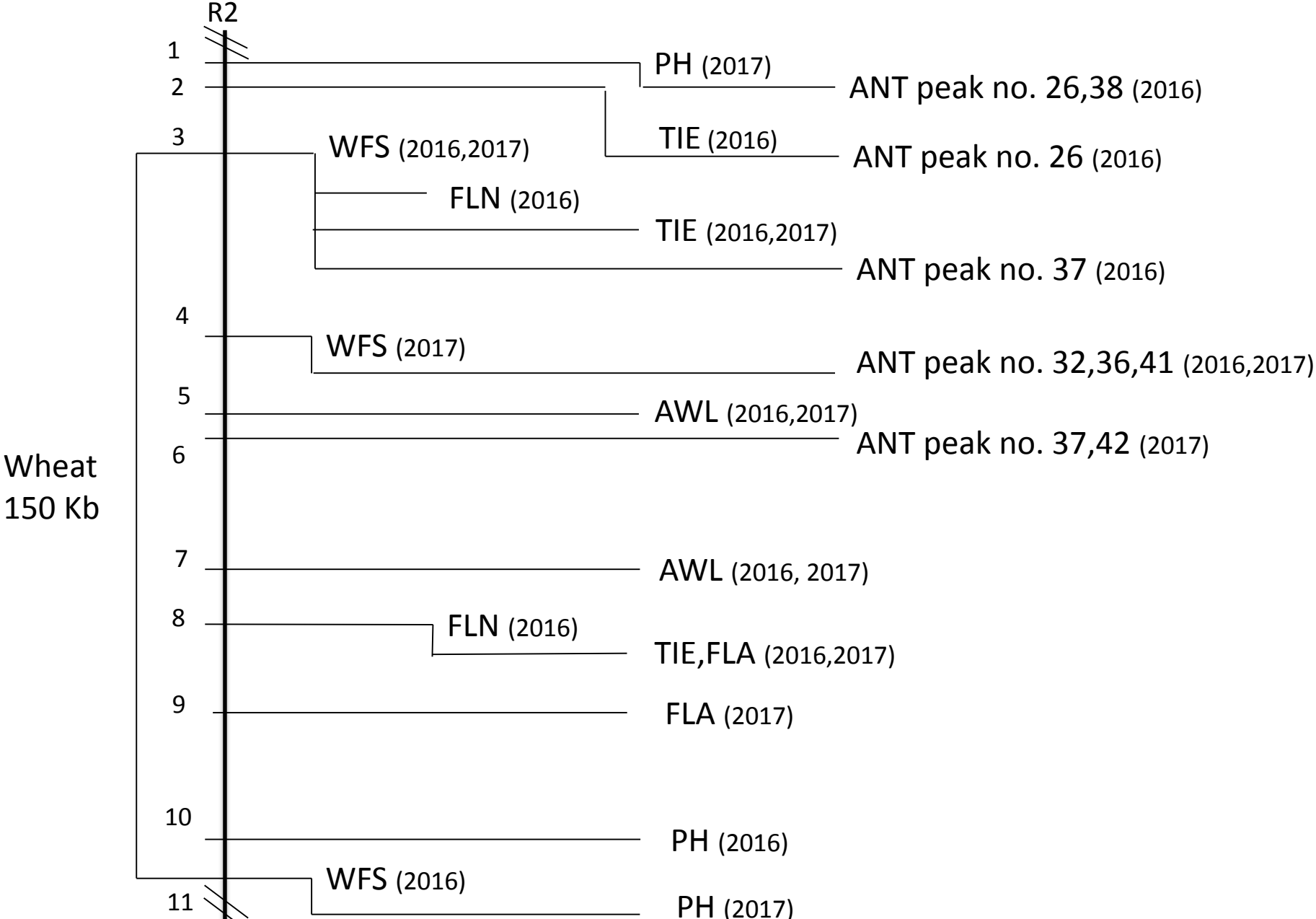# Analysis of GBS data by Structure

L(K) (mean +- SD)

Winter Field Survival in sub-populations of rye genotype

SNPs associated with developmental traits by association mapping

Developmental traits	SNPs No. 2016	SNPs No. 2017
Winter survival	14	16
Final leaf number	15	21
Prostrate growth habit	7	10
Anthocyanin peaks	21	27
Days to anthesis	20	13
Top internode elongation	24	13
Flag leaf area	20	30
Awn length	11	14
Plant height	20	29

QTL cluster associated with developmental traits, winter field survival and anthocyanin production

Conclusions

➢ Rye genotypes in panel studied show large variation for winter survival

➢ Strong correlations between final leaf number, prostrate growth and winter survival

➢ Rye genotypes segregated into seven subpopulations from extremely high to very low winter survival

➢ Specific Anthocyanin profile associated with winter hardy rye genotypes

➢ The region on rye genome was associated with QTL for several developmental traits, anthocyanin production and winter survival

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