Achievements & Challenges in Breeding Perennial Forage Crops in Western Canada

Bill Biligetu

Dec 6 2022

Outline

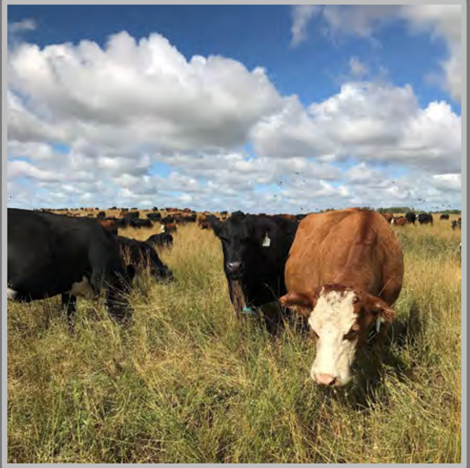
- Forage breeding
- Some grass breeding projects
- New grass variety update
- Legume (alfalfa) breeding (salt-grazing)
- Future perspectives

Forage breeding in Canada

Saskatchewan's Growth Plan
The Next Decade of Growth | 2020-2030

Perennial forage

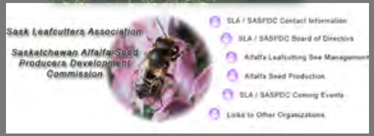
Beef and dairy industry



Increase livestock cash receipts to $3 billion

Forage Seed industry

SFSDC



SASPDC

$ 30-40 millions in 2022

Environment & Carbon economy

Amount of N fixed in Western Canada

	lbs N / acre
Alfalfa	100 - 250
Fababean	80 - 160
Pea	50 - 150
Soybean	70 - 120
Lentil	30 - 100
Dry Bean	5 - 70

UofS Forage Breeding Program

Hybrid brome Meadow brome

Crested wheatgrass

Hybrid wheatgrass

Minor species

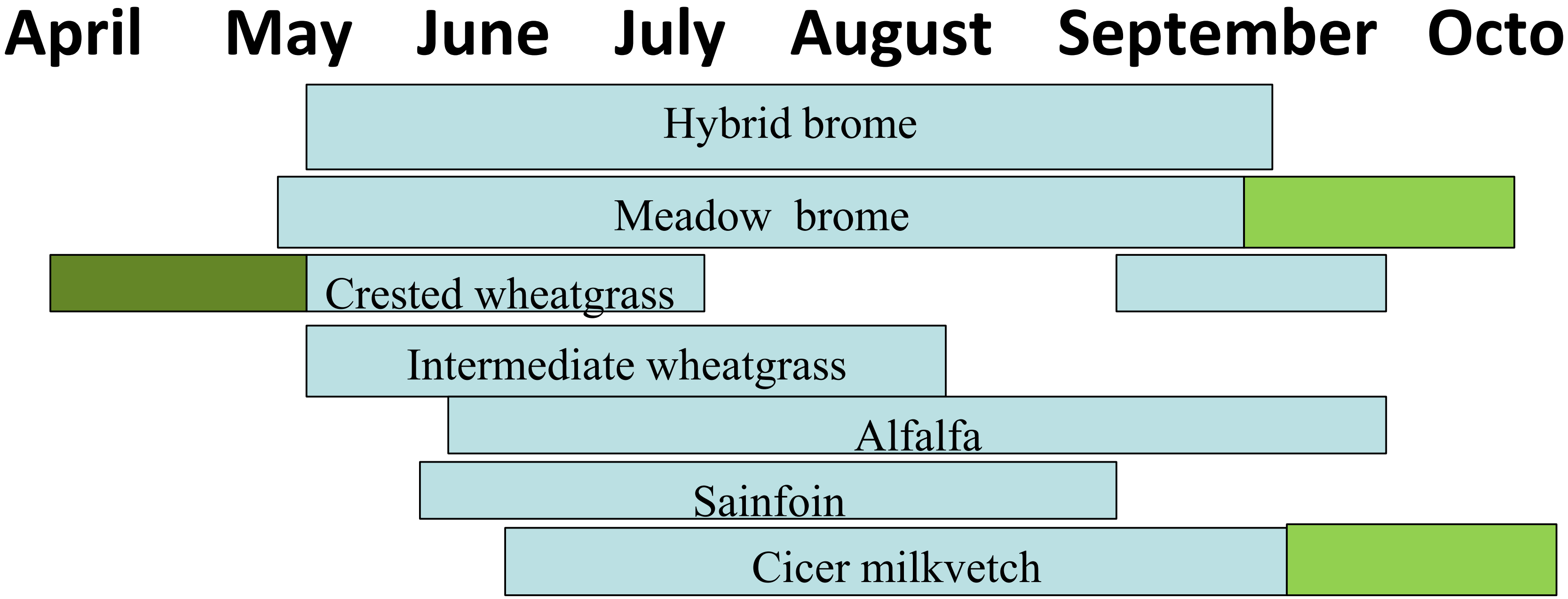
- Orchardgrass
- Tall fescue
- Timothy

alfalfa

sainfoin

Dr. Andrew Sharpe's on-going research on reference genome development of bromegrass

Species fit to the forage system



Timothy – water logging tolerance, major hay for horses
Hybrid wheatgrass – salinity tolerance

Saskatoon grass breeding

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- **Hybrid brome – high fiber (NDF) digestibility**
- Collaborative research with Dr. Greg Penner's lab
  - NDFd – forage intake, total energy, high animal gain/milk production
  - NDF is about 50-60% of forage dry matter

Forage yield + regrowth
Tiller number
Plant height
Disease rating

200 plants x 2 yrs samples

In situ NDF digestibility

High NDF S9624

Low NDF S9625

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S9624 – high NDF digestibility

Table 1. Vigor score of hybrid brome breeding lines and cultivars in a spaced nursery in 2020 and 221 near Clavet, SK.

Cultivar	Spring vigor (1-5 scale)			Hay vigor (1-5 scale)		
	2020	2021	2-yr means	2020	2021	2-yr means
AC Knowles	2.8b	2.9ab	2.8b	2.7b	3.1b	2.9c
AC Success	2.9b	2.6c	2.7b	3.0a	3.2ab	3.1b
S9624HB	**2.7b**	**2.7bc**	**2.7b**	**2.9ab**	**3.0b**	**3.0bc**
S9625HB	3.1a	3.0a	3.1a	3.1a	3.5a	3.3a
P value	0.01	0.007	<0.01	0.004	0.002	< 0.001
SEM	0.11	0.1	0.08	0.09	0.12	0.09

Note: Each value represents means of 90 individual plants

Table 2. Forage yield (kg/ha) of hybrid breeding lines selected for high and low NDF digestibility in sward density trial near Clavet, SK

Cultivar	2020 DM Yield					2021 DM Yield				
	Height /cm/	Cut 1 June 29	Cut 2 Sep 10	Total Yield kg/ha	Yield % of AC Success	Height /cm/	Cut 1 June 28	Cut 2 (no cut)	Total Yield kg/ha	Yield % of AC Success
AC Knowles	114	4634	287	5238	93	63	1727	-	1727	86
AC Success	115	5447	228	5630	100	65	2002	-	2002	100
S9624HB	**116**	**5088**	**181**	**5291**	**94**	**61**	**1660**	**-**	**1660**	**83**
S9625HB	116	5063	221	5269	94	65	1792	-	1792	90
P-value	0.96	0.61	0.35	0.69	-	0.82	0.62	-	0.62	-
SEM	2.51	337	53.9	365	-	3.23	173	-	173	-

Table 3. Nutritive value of hybrid breeding lines selected for high and low NDF digestibility at early anthesis stage near Clavet, SK

Cultivar	2020			2021		
	% NDF	% ADF	% Protein	% NDF	% ADF	% Protein
AC Knowles	61.5	37.2	9.4	59.3	31.4	11.6
AC Success	61.5	36.1	10.0	58.9	30.6	12.4
S9624HB	**60.8**	**36.9**	**11.2**	**57.2**	**30.2**	**12.8**
S9625HB	62.4	37.9	9.1	59.4	31.6	11.8
P-value	0.11	0.16	0.34	0.12	0.11	0.44
SEM	0.65	0.59	0.58	0.45	0.38	0.48

- Highly digestible hybrid brome development is possible, but there is a potential risk of yield reduction. Further selection is underway…

Saskatoon grass breeding

- **Crested wheatgrass**

Diploid

Tetraploid

Hexaploid

Dr. Alison Ferrie's (NRC Saskatoon) on-going research on developing **DH lines** of crested wheatgrass

- Value to genomic and molecular study
- Cross to produce new genotypes

Crested wheatgrass

Value: drought tolerance, early greenness for early grazing

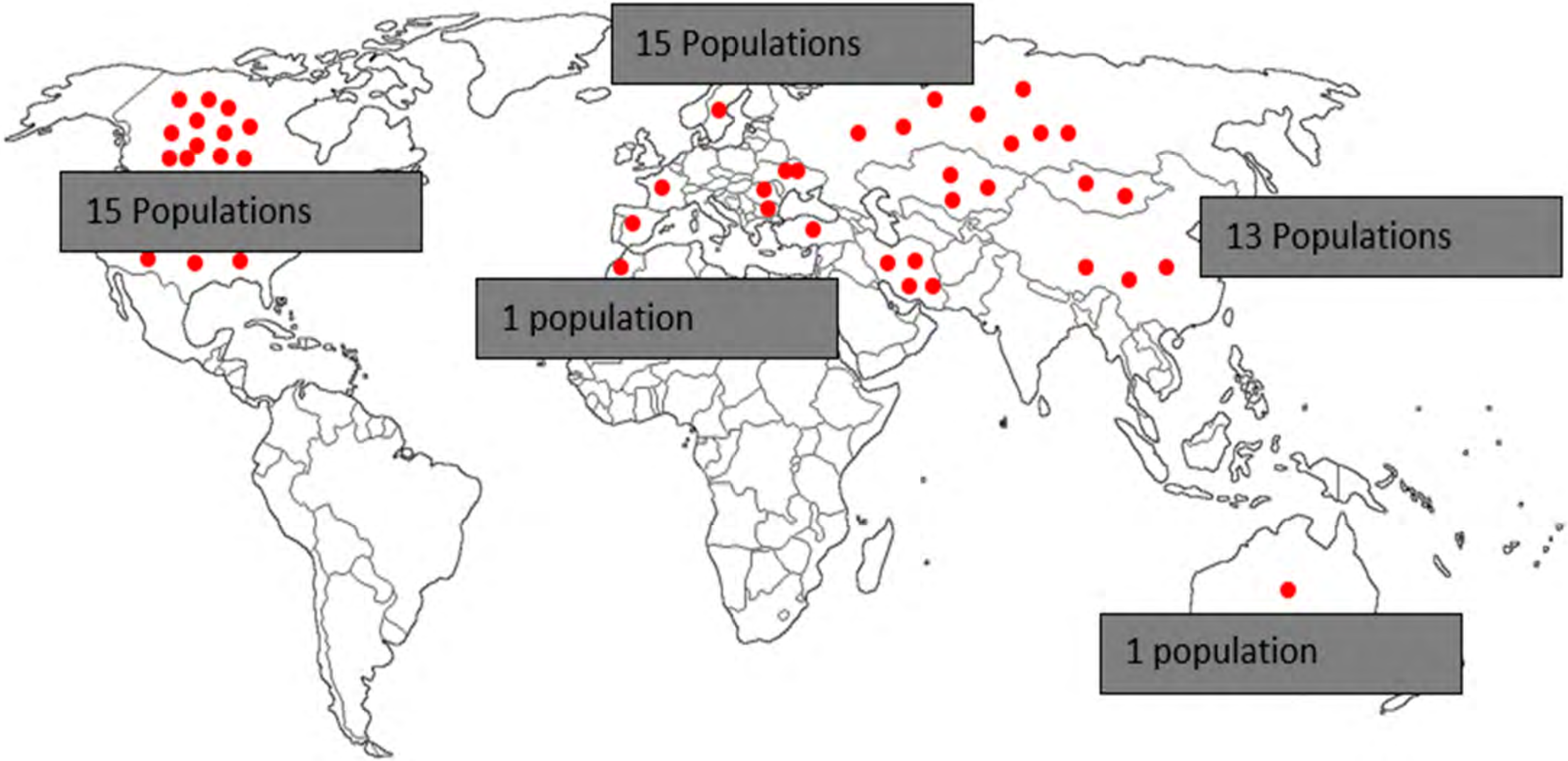
Breeding goal: Late maturity

April 21 2015

Alfalfa CWG

Seed head development

Germplasm collection



Total: 45 populations

Selection for late maturity in crested wheatgrass

- **Genetically very diverse**
- **Maturity**
  - 5 days on average
- **Later maturing lines**
  - A few days
  - Leaf-to-stem ratio better choice?

RNA-Seq Study –DEGs
Collaborative research with Dr. Yong Bi Fu's lab at AAFC Saskatoon

Table 3. List of 22 (out of 5246) differentially expressed genes (DEGs) associated with flowering at the stem elongation stage (VS) between the early and late maturing lines of crested wheatgrass.

Gene ID [a]	Putative Function	Nr ID	$\log_2$FC	FDR
DN67303-c0-g12	flowering locus T [*Lolium perenne*]	AIE58042.1	−11.5	1.90×10^{-6}
DN59102-c0-g1	timing of cab expression 1	AMK48976.1	−10.9	7.50×10^{-6}
DN74264-c0-g1	SYD isoform X1	EMT16433.1	−10.7	1.10×10^{-5}
DN74350-c1-g11	flowering locus T [*Lolium perenne*]	AAW23034.1	−10.4	2.40×10^{-5}
DN60639-c0-g1	transcriptional corepressor SEUSS [*Oryza sativa*]	BAJ98061.1	−10.3	2.70×10^{-5}
DN73561-c0-g2	GIGANTEA [*Oryza sativa*]	CDM81775.1	−10.3	3.30×10^{-5}
DN73410-c0-g1	nucleic acid binding [*Zea mays*]	BAJ87586.1	−10.0	6.50×10^{-5}
DN75888-c2-g3	cryptochrome 2	ABX58030.1	−9.9	8.10×10^{-5}
DN66158-c0-g2	HD3A_ORYSJ	BAH30246.1	−9.9	8.20×10^{-5}
DN76849-c0-g1	phosphatidylinositol 4-phosphate 5-kinase 1-like	EMS61702.1	−9.7	1.23×10^{-4}
DN62519-c0-g1	spotted leaf 11	BAJ85648.1	−9.6	1.61×10^{-4}
DN67113-c2-g1	auxin response factor	EMT32630.1	−9.2	3.60×10^{-4}
DN66104-c0-g1	COL10_ARATH	Q9LUA9.1	−9.2	1.12×10^{-5}
DN68702-c0-g2	SWI SNF complex subunit SWI3B	EMT14023.1	−9.1	4.87×10^{-4}
DN69810-c0-g1	CONSTANS CO6 [*Zea mays*]	BAJ98422.1	−9.1	4.74×10^{-4}
DN77293-c0-g2	probable serine threonine- kinase vps15 isoform	EMT17455.1	−9.1	4.74×10^{-4}
DN70348-c0-g2	MADS-box transcription factor 18	XP_006657934	−9.1	1.28×10^{-5}
DN48214-c0-g5	constans-like 1 [*Picea abies*]	EMT25416.1	−9.0	5.74×10^{-4}
DN64729-c0-g4	MADS-domain transcription factor [*Zea mays*]	ABF57916.1	−8.8	9.36×10^{-4}
DN74533-c0-g2	phragmoplast-associated kinesin [*Oryza sativa*]	EMT21759.1	−8.8	8.80×10^{-4}
DN66288-c0-g1	Os01g0687700 [*Oryza sativa*]	CDM83875.1	8.9	7.84×10^{-4}
DN64871-c0-g1	gamma-glutamylcysteine synthetase	BAJ84988.1	9.3	2.75×10^{-4}

[a] The underlined DEGs are related to photoperiod pathway. FDR: false discovery rate.

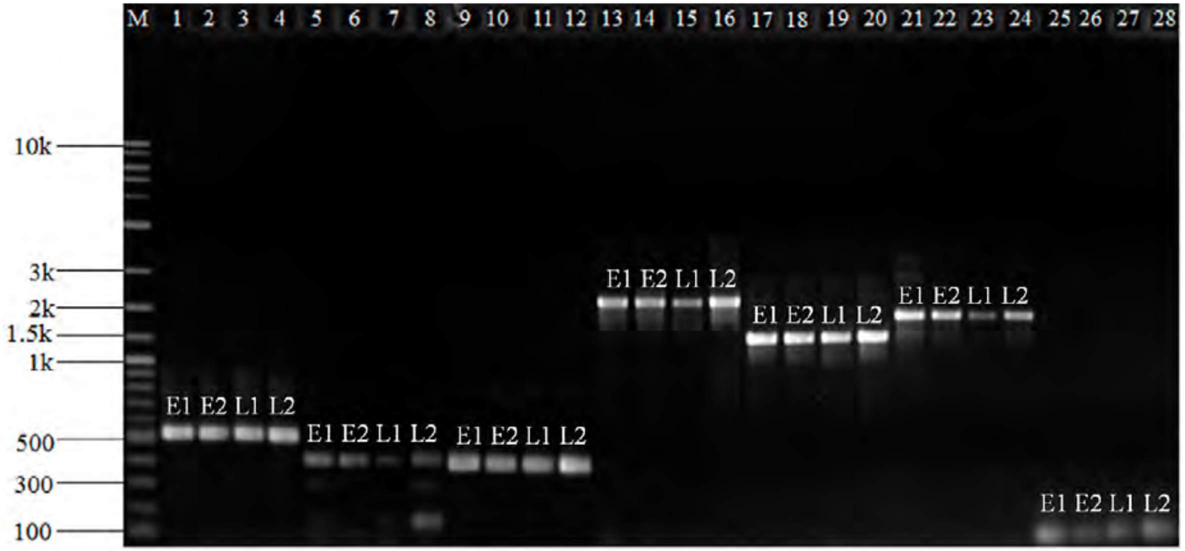


Figure 3. The PCR amplifications of the seven (out of nine) selected flowering-associated differentially expressed genes at the stem elongation stage of crested wheatgrass. M: DNA ladder; Lane 1–4:

qRT-PCR analysis of the gene expression of 12 DEGs at stem elongation

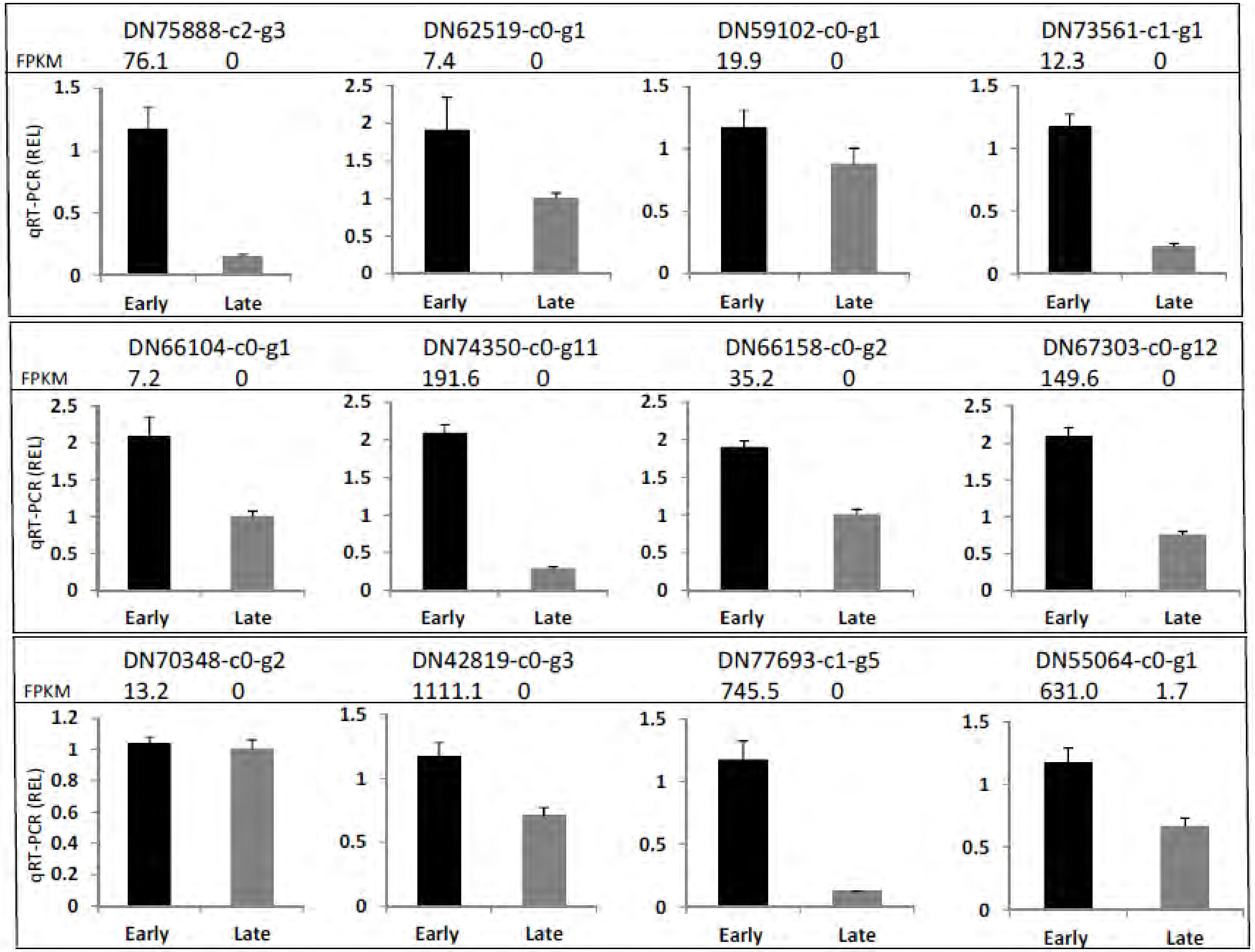


Figure 4. qRT-PCR analysis of the gene expression of 12 differentially expressed genes selected at the stem elongation stage between the early and late maturing lines of crested wheatgrass. Each panel shows gene ID and RNA-Seq readings of fragments per kilobase of transcript per million mapped reads (FPKM) for both lines. REL: relative expression level.

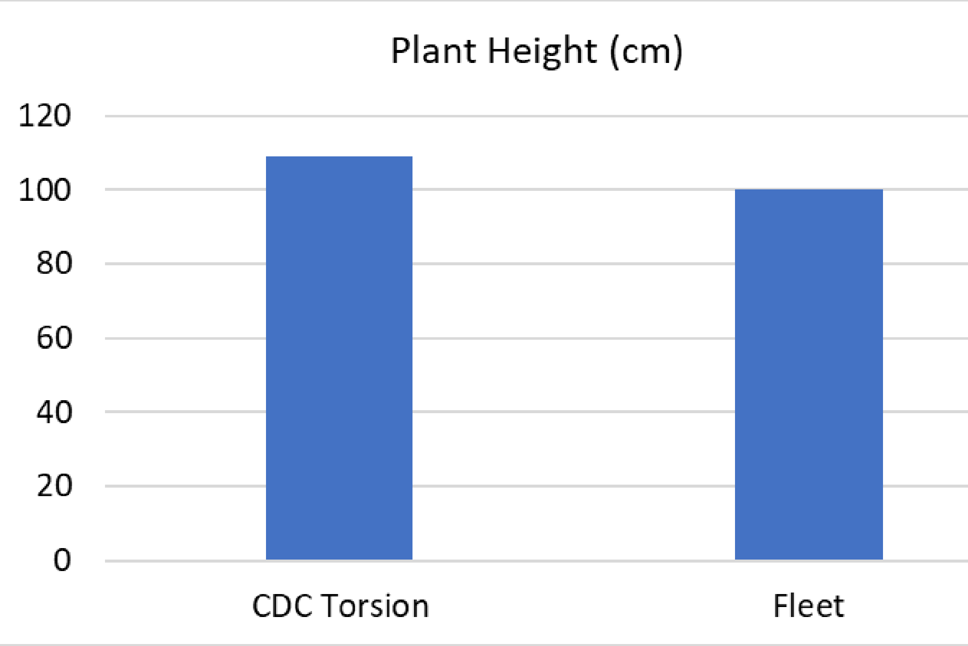
Marker assisted screening might be important for the development of Later maturing crested wheatgrass

New Grass Cultivars

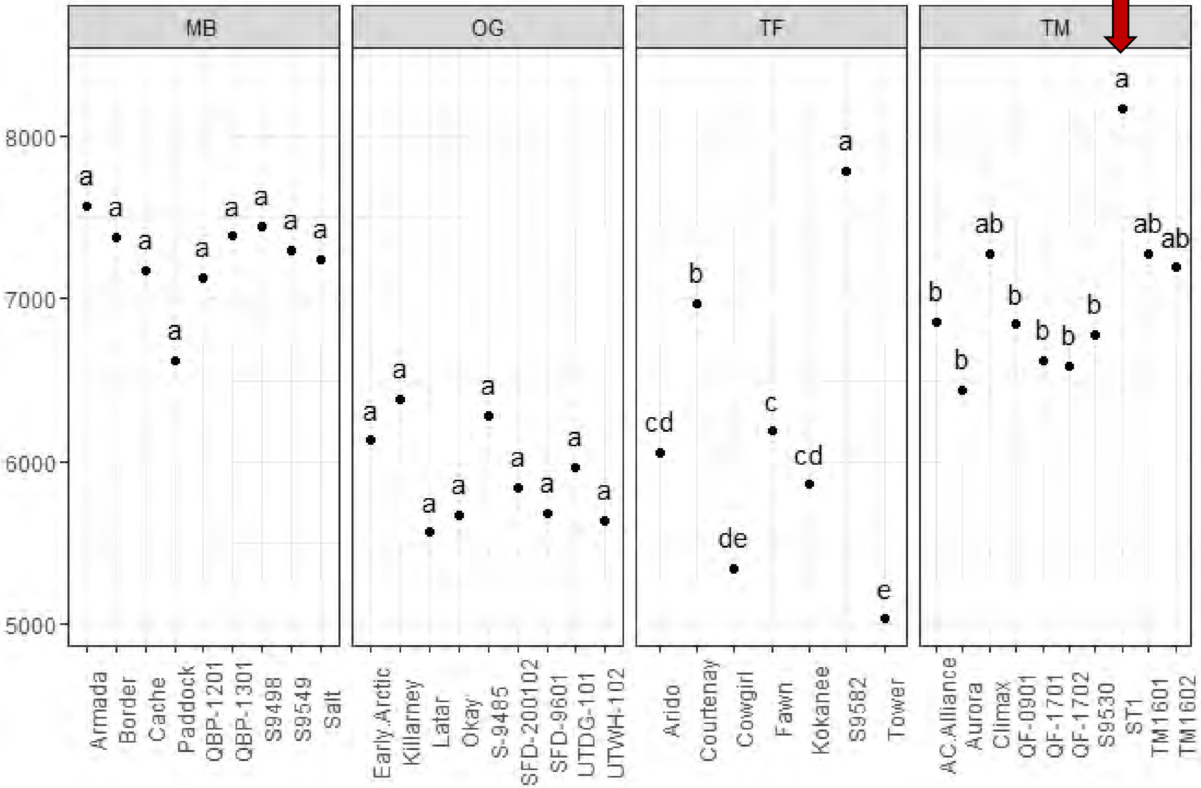
- 'CDC Torsion' **Meadow brome: released in 2020**
  - Licensed to Brett Young Seed
  - improved biomass/regrowth yield

Table 1. Forage dry matter yield (kg/ha) of S9549 meadow brome at various sites

Zone	Location	Year	Fleet	S9549
Brown	Swift Current	2018	1787	1965
		2019	2246	2228
Dark Brown	Saskatoon	2018	5225	5153
		2019	7370	7871
Black	Melfort	2018	4203	4039
		2019	6044	7165
Mean	Station-year	6	4479	4737
	% Fleet		**100**	**106**



- **CDC Tiznow Timothy (ST1)**
  - 7% higher biomass yield than ‘Climax’ (15 site –year)
  - 45% higher seed yield than ‘Climax’ (4 site –year)



Hybrid wheatgrass S9615

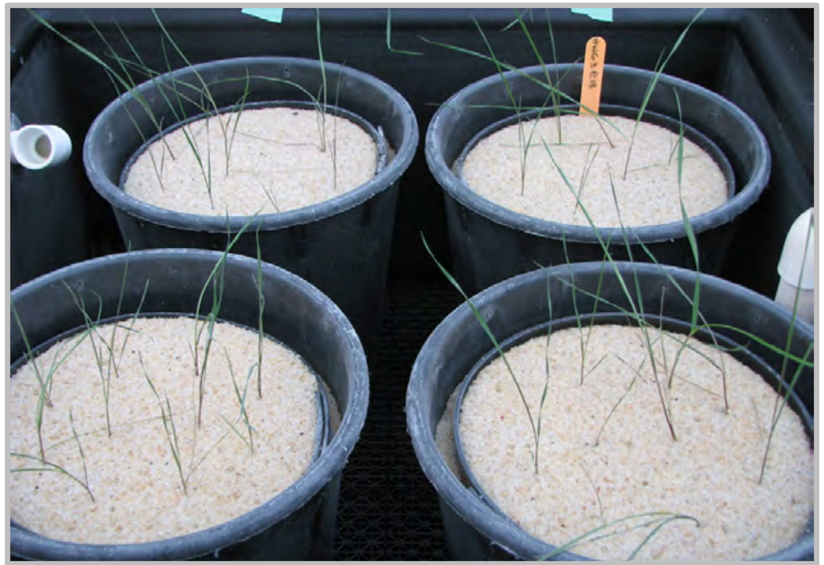
- Proposed variety name: CDC Salt King
- Main use: saline area
- Selected for higher seed yield without reducing salt tolerance
- 4 site-year test: 17.6% higher seed yield than AC Saltlander
- Slightly early maturing (2-3 days) compared to AC Saltlander
- Shorter rhizome than AC Saltlander
- Good salt tolerance
- No major disease issue

Hybrid wheatgrass S9615 – Breeder seed plot (2021)

New Grass Cultivars

Hybrid wheatgrass

EC=16dS/m



AAFC Swift Current Salt lab

18 ds/m E.C.

Hybrid wheatgrass

EC= 9 dS/m

Strongfield SK 2019

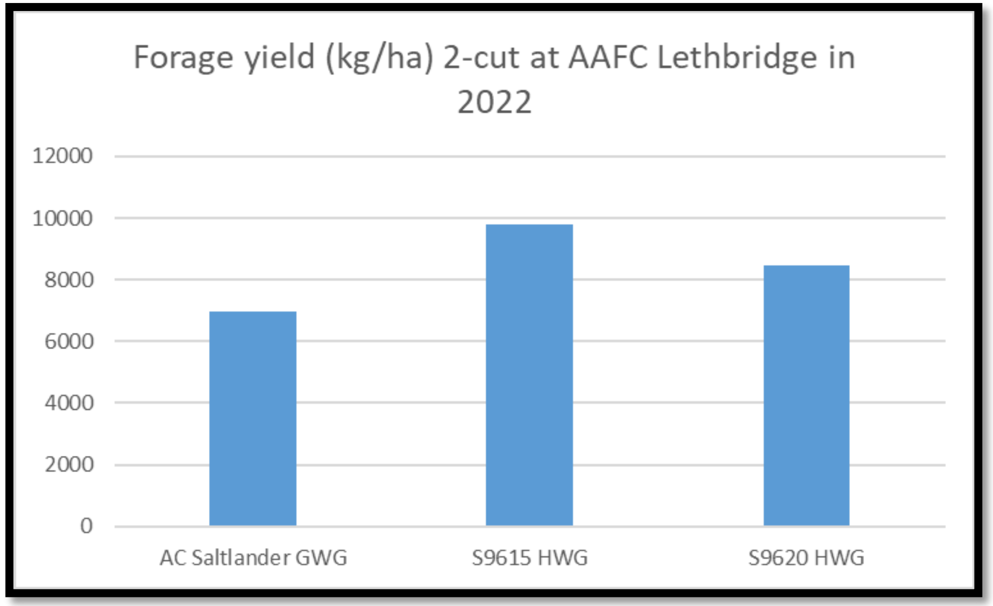
EC=9.8 dS/m

Table 2. Performance of hybrid wheatgrass S9615 at 18 dS/m E.C. salinity level at Agriculture and Agri-Food Canada Salt lab, Swift Current, SK

Variety	Test ID	7-d Emergence (%)	14-d Emergence (%)	Survival (%) at 70th day	Plant Height (cm)	Biomass (g/plant)	Tiller/plant	crude protein (% DM)	ADF (% DM)	ND (% D
S9615	S015	83	88	90	65.4	4.0	18	20.8	27.7	5
AC Saltlander	S001	81	83	89	60.1	3.3	12	20.9	27.5	5
S9604	S004	69	73	79	65.2	2.7	12	19.9	29.1	5

Table 3. Seed yield (kg/ha) of S9615 in non-saline soil

		S9615	AC Saltlander	S9600
Saskatoon SK	2019	509	446	445
	2020	434	297	314
Clavet SK	2021	420	414	360
Outlook SK	2021	84	77	65
	Mean	362	308	296
	% AC Saltlander	117	100	96



Forage legume breeding

Evaluation of saline-tolerant forage mixtures for establishment, forage yield, and saline soil remediation.

Alex Waldner
M.Sc. Candidate Plant Sciences

Collaborative research with Dr. Jeff Schoenau’ s lab (Soil Science)

Soil Microbial Biomass

Pollinator Activity

CDC Salt King hybrid wheatgrass
Salt tolerant alfalfa

Forage Legume breeding

Alfalfa salt tolerance

- Moderate tolerance
- over 50 varieties in USA
- Germination under salt
- Bridgeview
- Halo
- Rugged

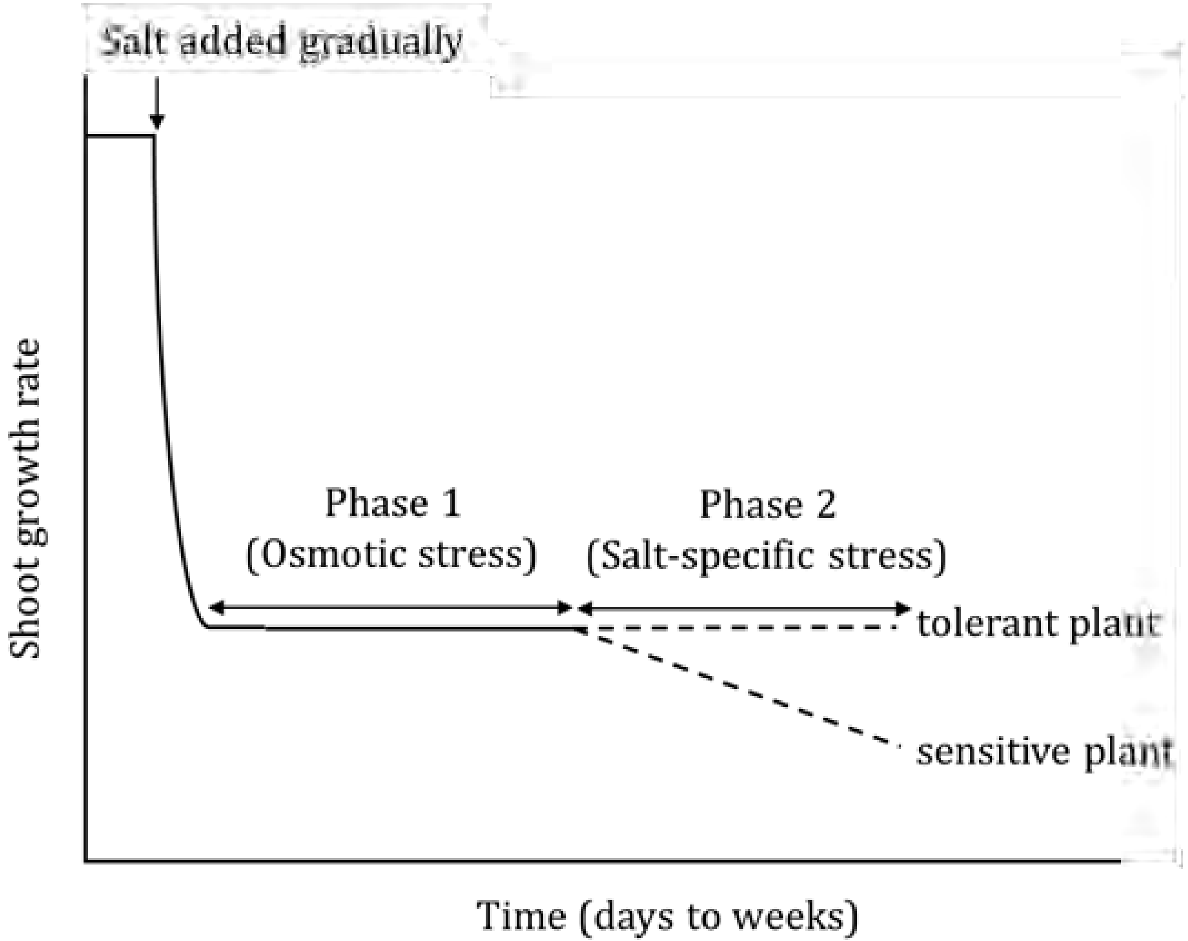


Figure 2. Two-phase growth response to salinity (From Munns, R. 2005. New Phytologist; 167:645–663.)

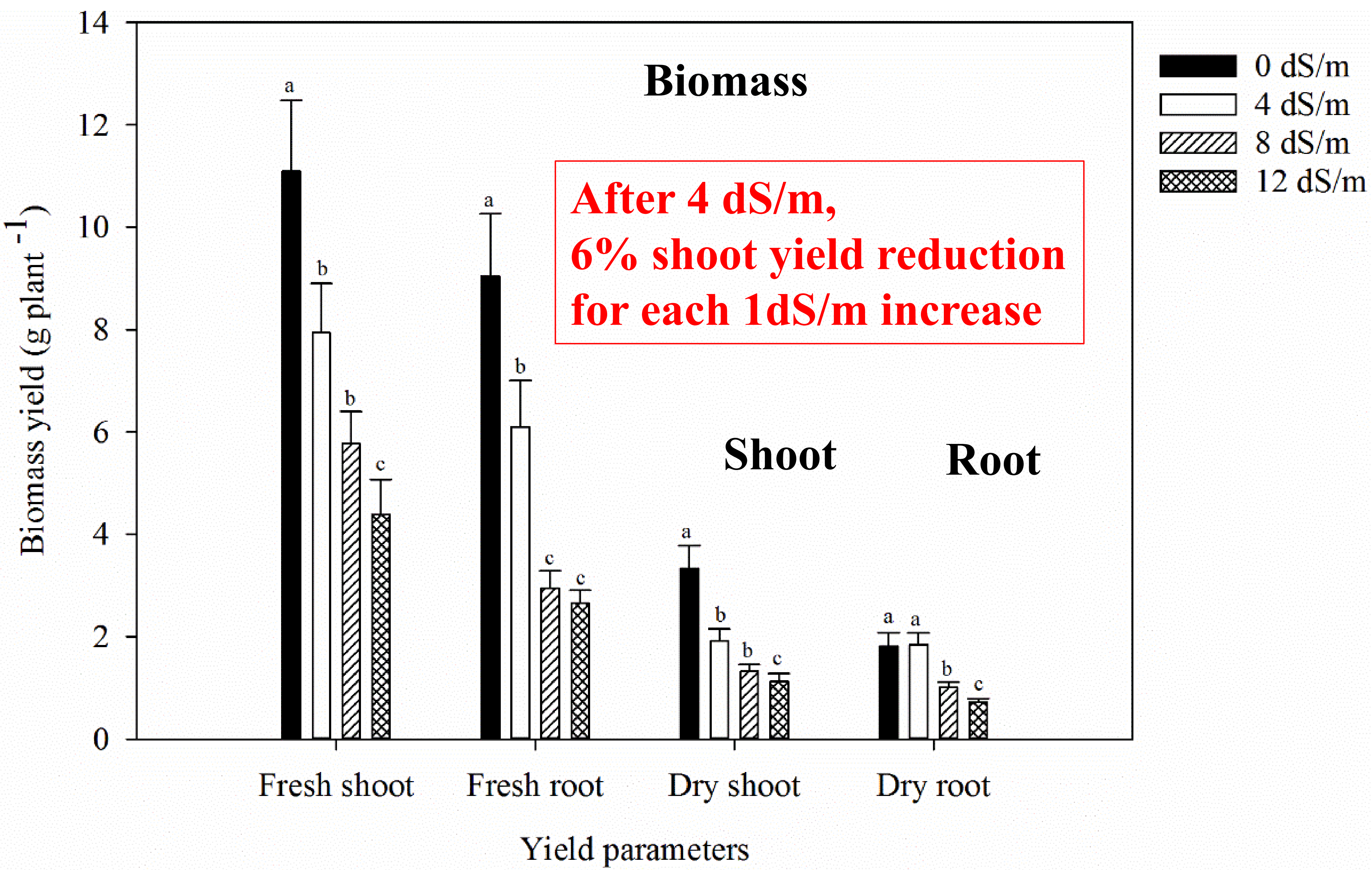
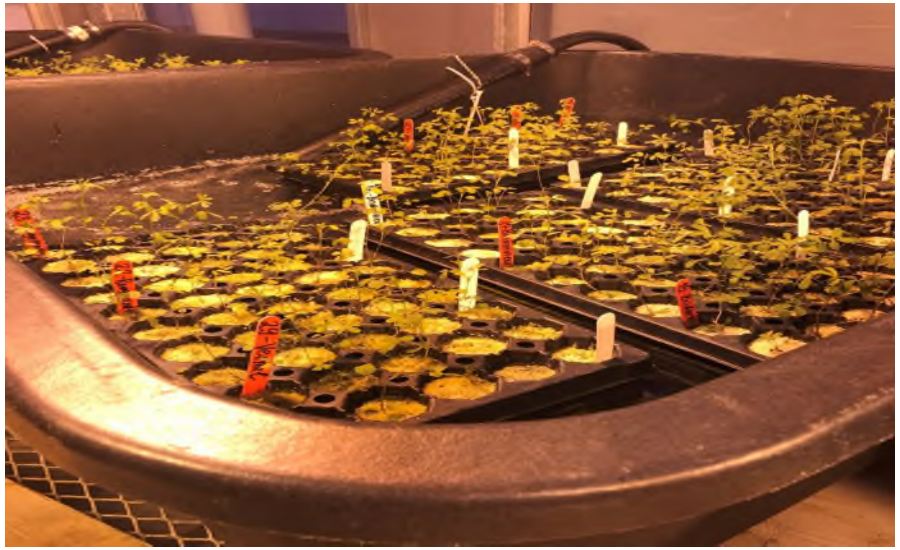


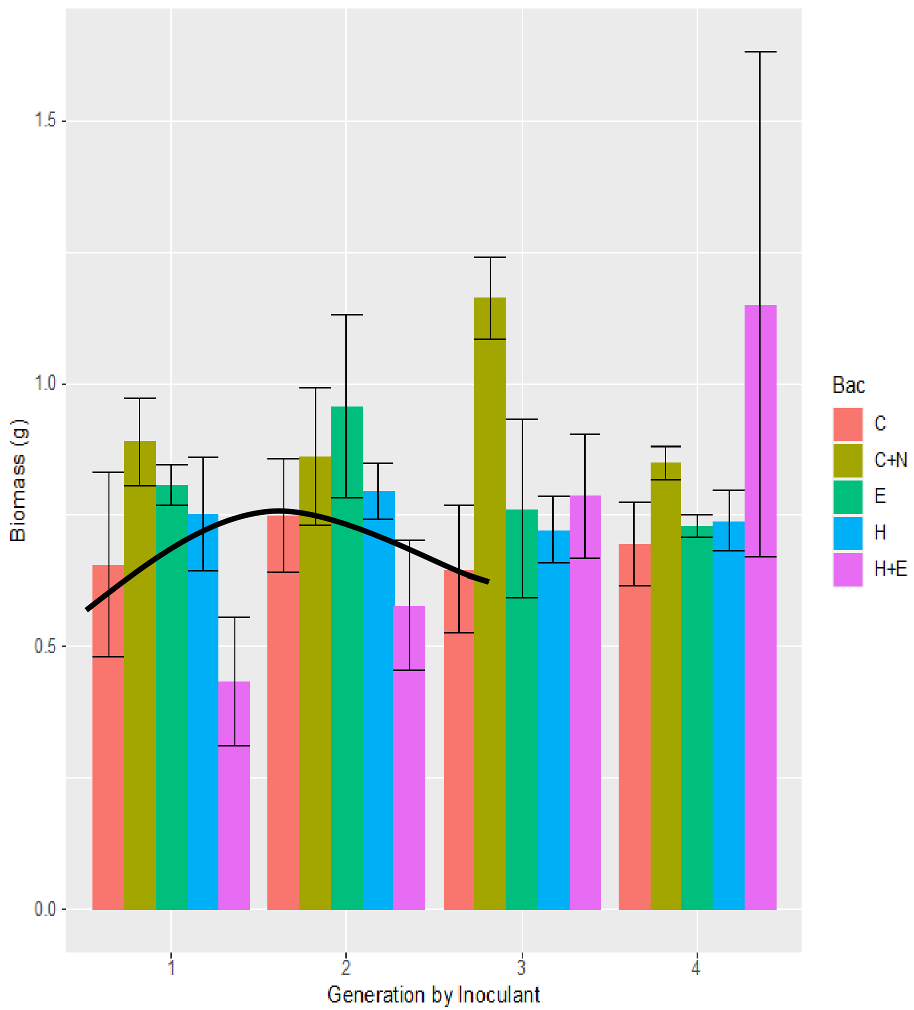
Figure 4. Fresh and dry root and shoot biomass yield of five alfalfa varieties under four gradient of salt stress

Selection for salt tolerance (Current project)

- Diverse genetic background
- Test breeding lines in saline areas
  - Germination
  - Germination stage tolerance ≠ mature plant tolerance
  - Growth
  - Winter hardiness
- Populations: S906(1), S906 (2), S906 (3), S906 (4), S906(5)



Shoot Biomass vs Alfalfa Generation by Inoculant at 60 days <u>in 8ds/m</u>



S906(1) S906 (2) S906 (3) Halo

M.Sc candidate Seth Lundell's Research

C =Control

C+ N= 100kg/ha N fertilizer

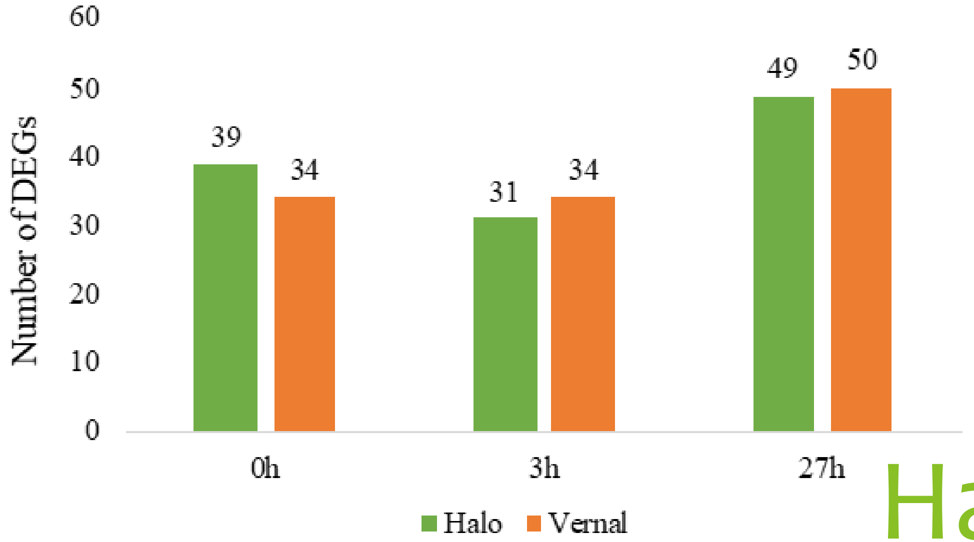
E=rhizobia for N fixation

H= Halomonas maura

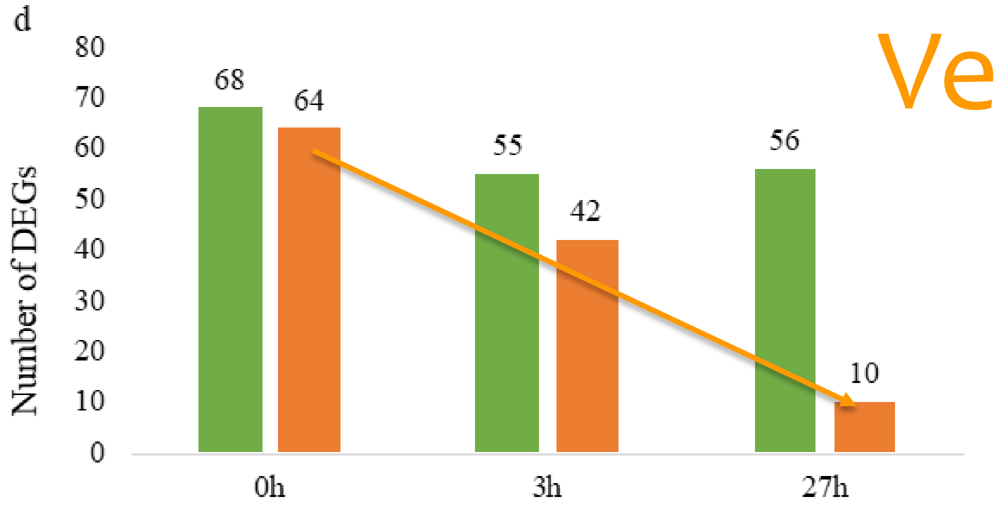
E + H= rhizobia + salt tolerant bacteria

Number of differently expressed gene (DEGs)

Leaf



Root



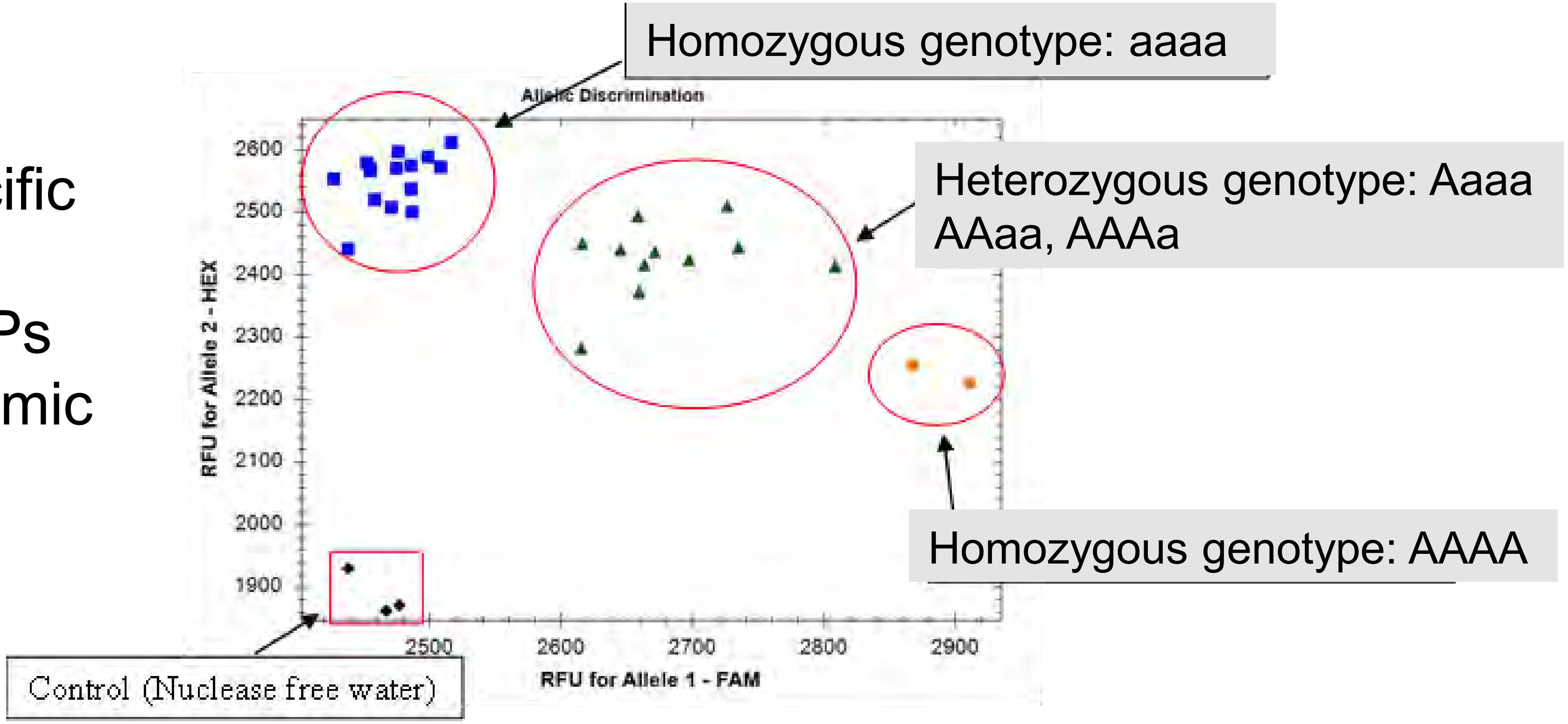
Halo – salt tolerant

Vernal -sensitive

Bhattarai et al. 2021
BMC Plant Biology

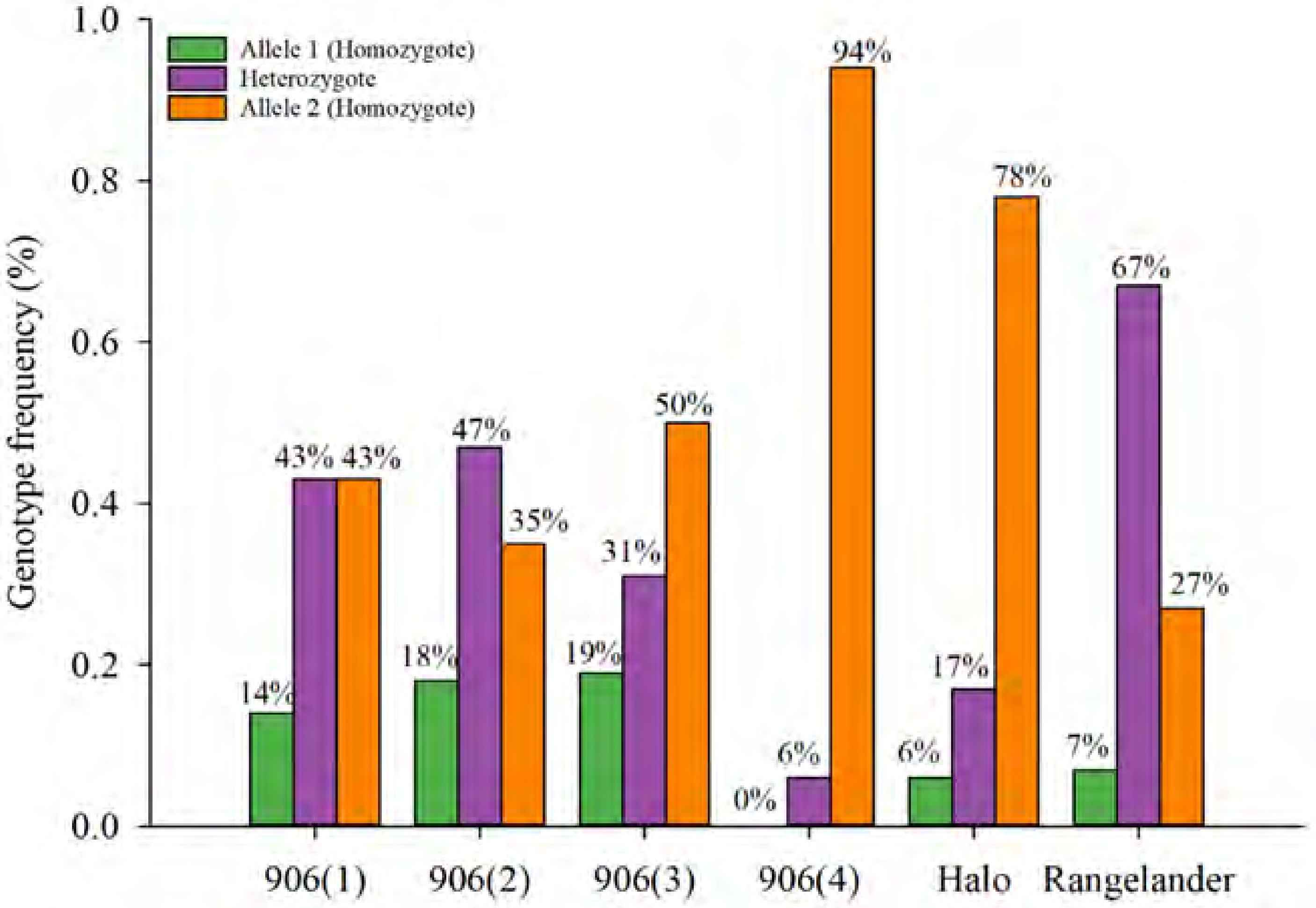
Application of KASP method for validating SNPs in alfalfa salt tolerance

- Kompetitive allele-specific PCR (KASP) assay
- Validate significant SNPs associated with agronomic traits



- SNP chr1.1_32922152
- MS.gene037960: abiotic stress tolerance (Dong et al. 2006)

- 35 markers
- 20 random plants/population



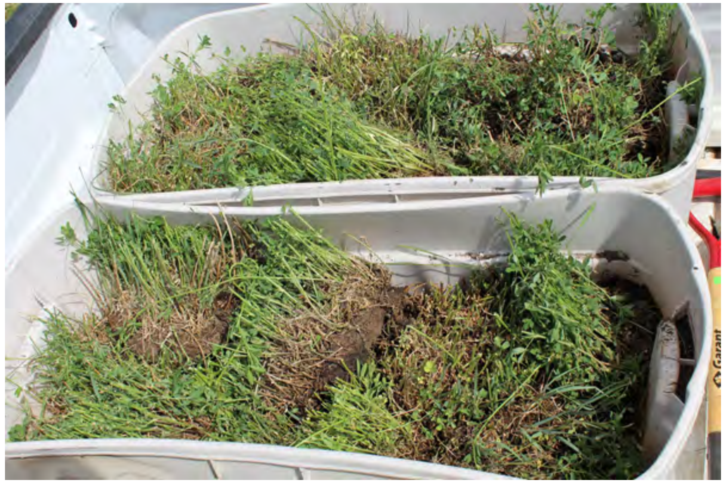
Selection of Legumes under intensive grazing

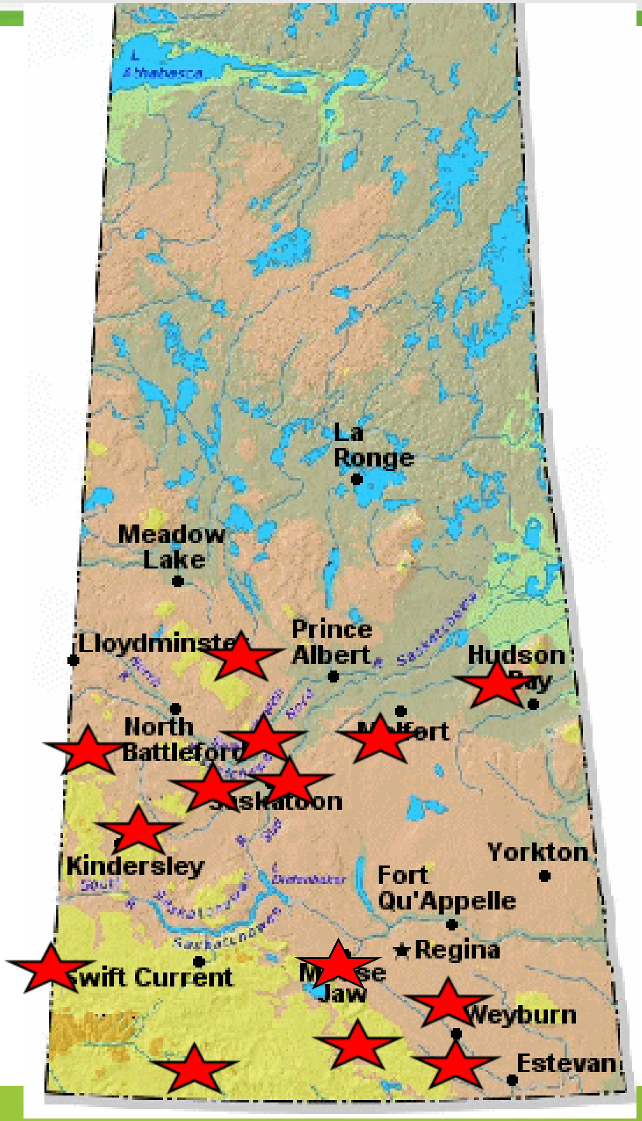
- Dr. Bart Lardner's group
- ADF/SCA funded project
- 3- yrs of grazing pressure at LFCE
- 0.3 ha x 4 replications
- Sainfoin - grass
- Alfalfa –grass
- Crossed 1 breeding population/each species
- More results to come….

Photo by: Cassidy Sim and Lana Height

Saskatoon program- Development of grazing tolerant alfalfa

- **Alfalfa stand 25+yr long grazing history**
- **4 Soil zones**
- **14 sites**
- **30 plants/site**





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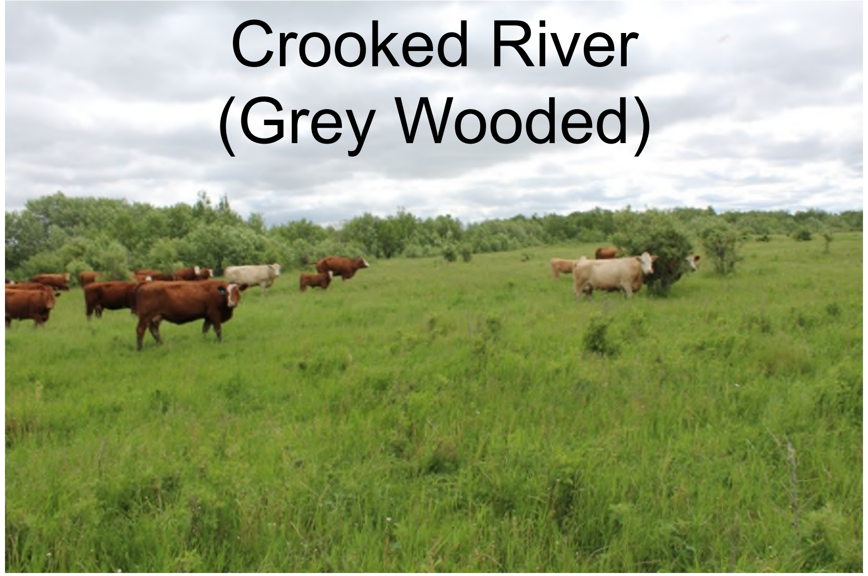
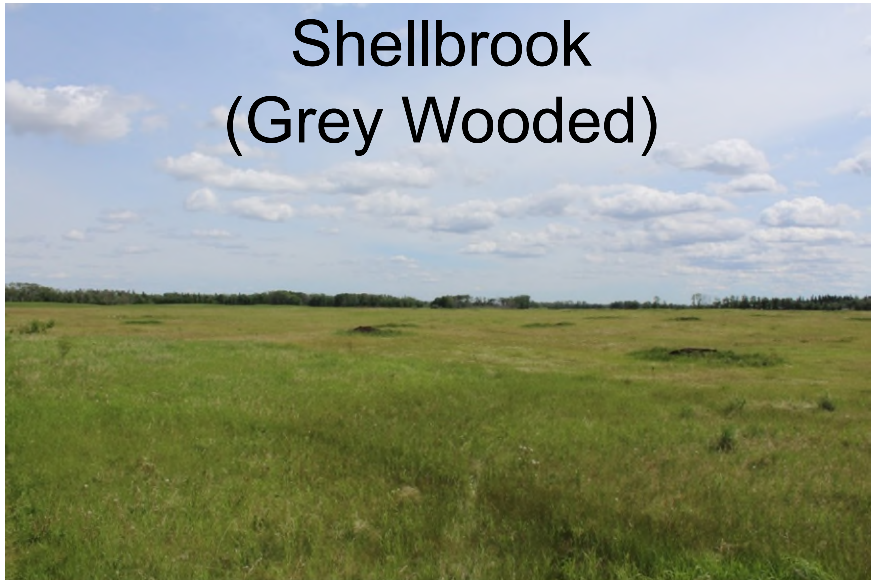
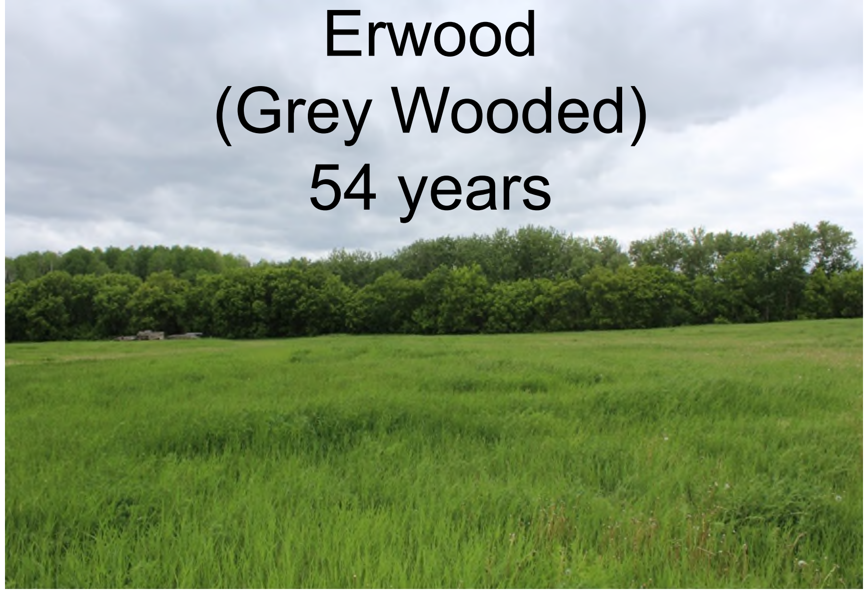
Long-term grazing sites (> 25 years)







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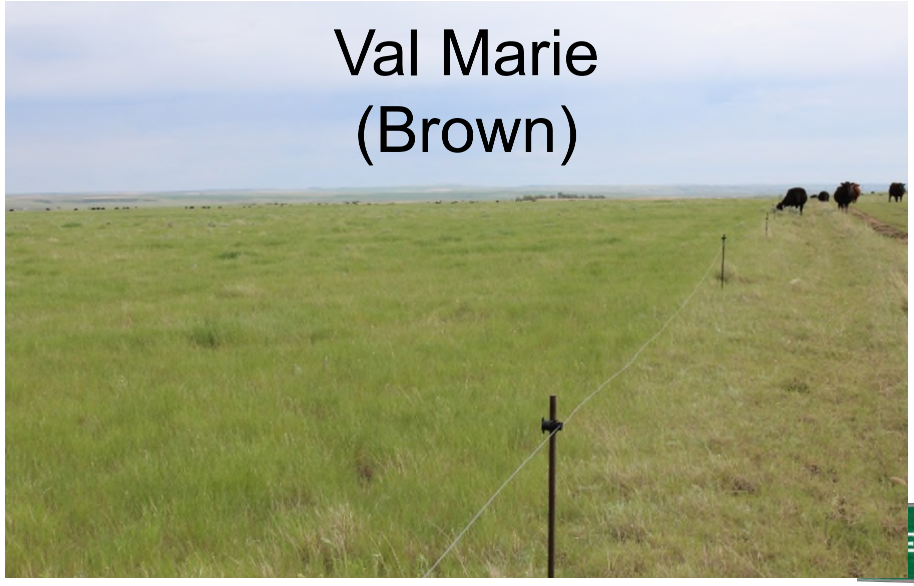
Long-term grazing sites (> 25 years)

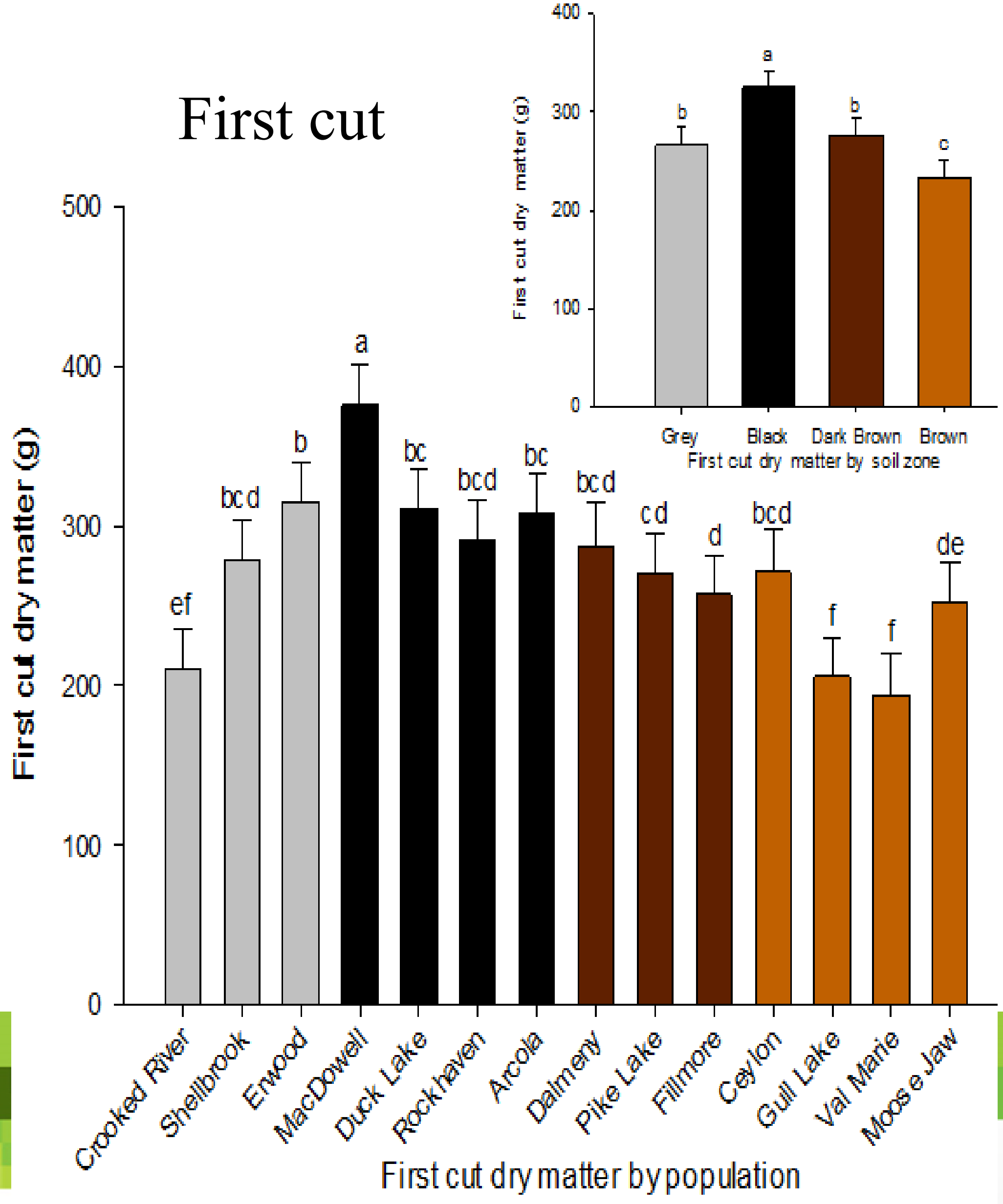


E WHAT THE WORLD NEEDS

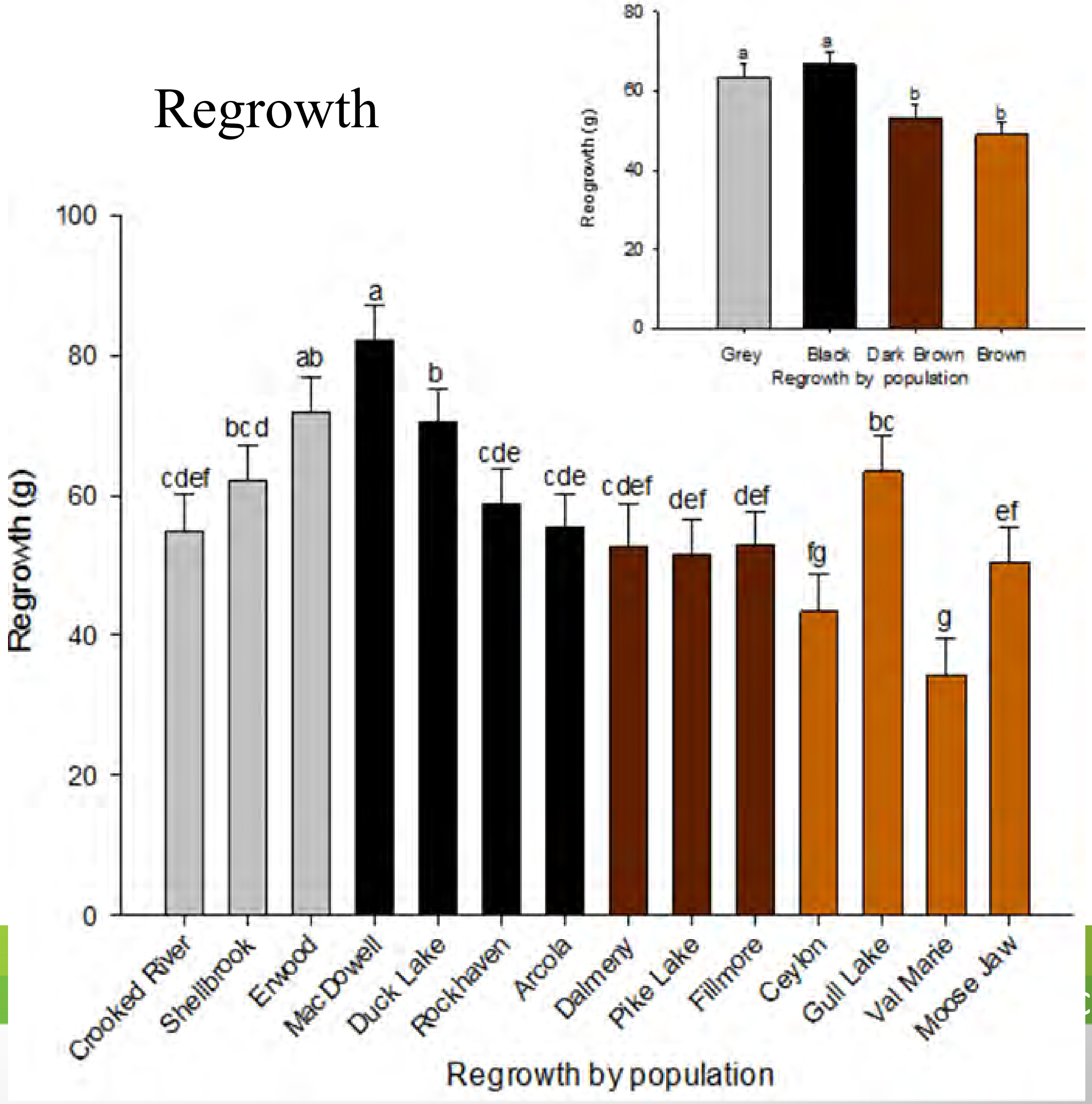
2017 summer

Forage yield

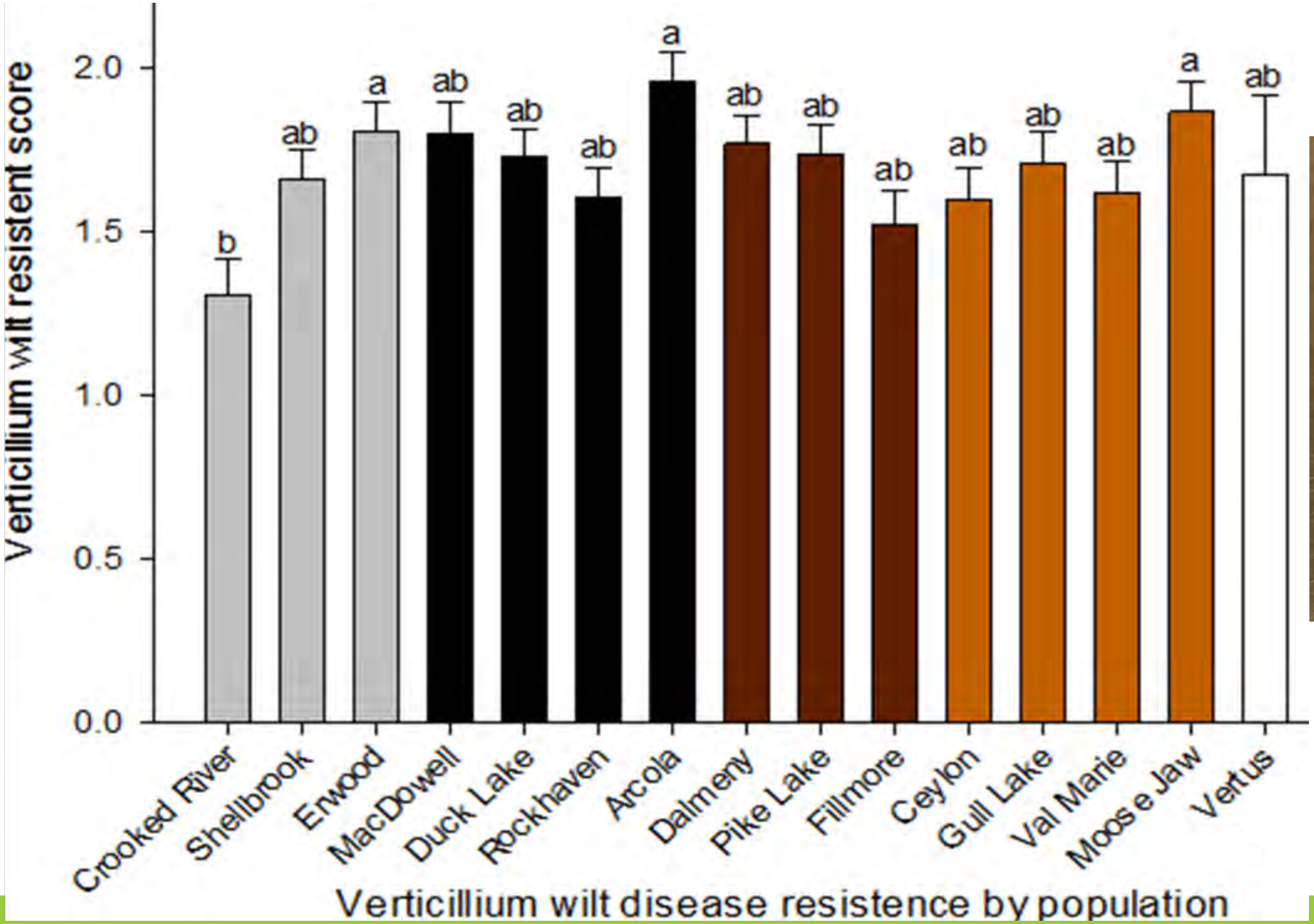
First cut



Regrowth



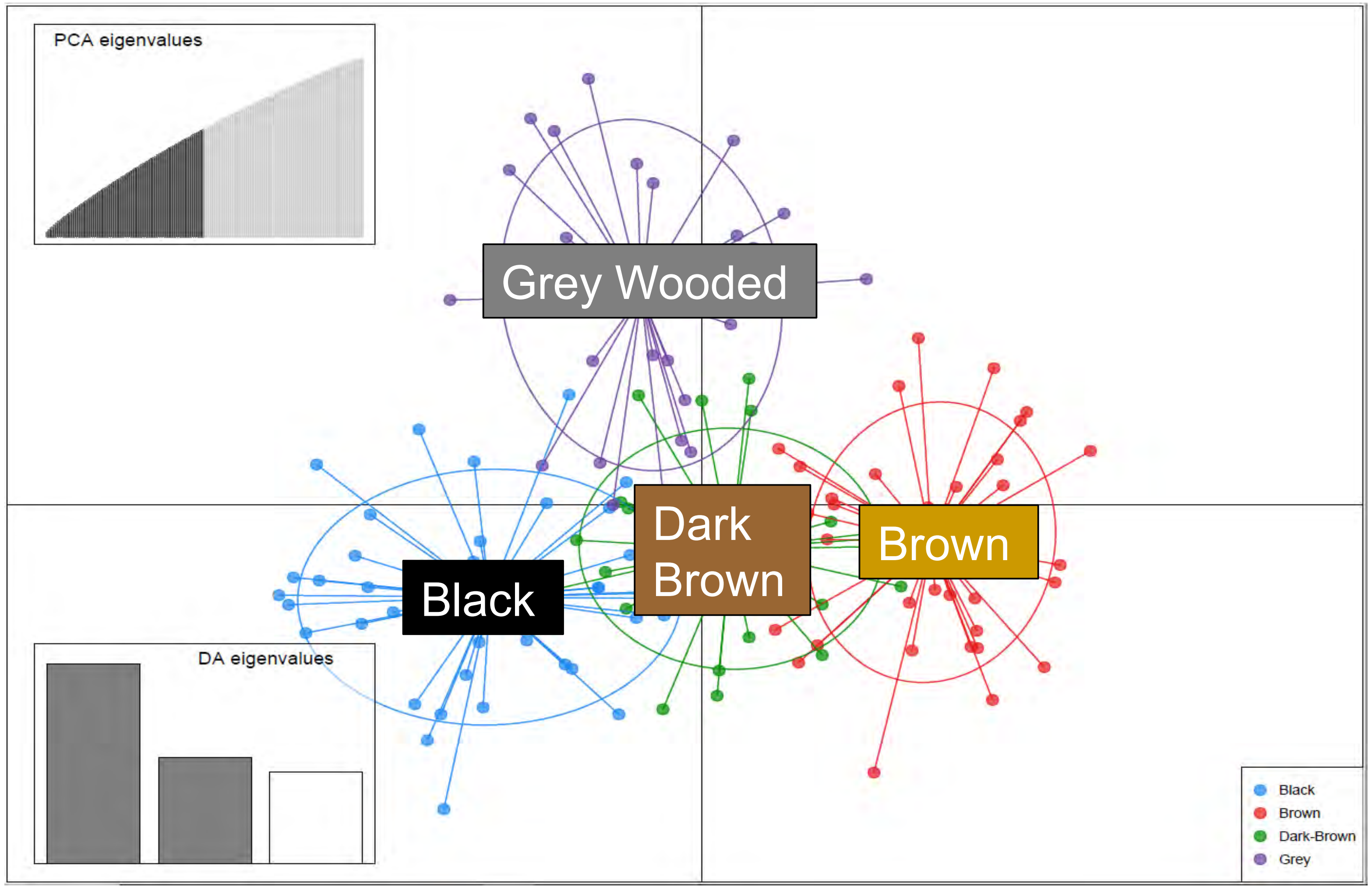
Verticillium wilt disease evaluation



Verticillium wilt spore under 40X microscope

University of Saskatchewan

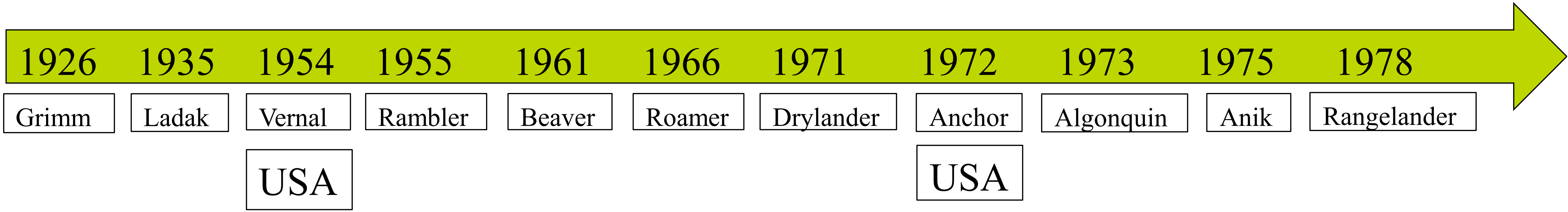
Genetic variation by four soil zones based on **19,853 SNPs** of 142 alfalfa genotypes representing 14 alfalfa populations from long-term grazing sites



Wang et al. Scientific report 2022

Plant materials:

- Alfalfa populations from long-term grazing (> 25 years) sites
- 11 commercial alfalfa cultivars released from 1926 to 1980 in Western Canada

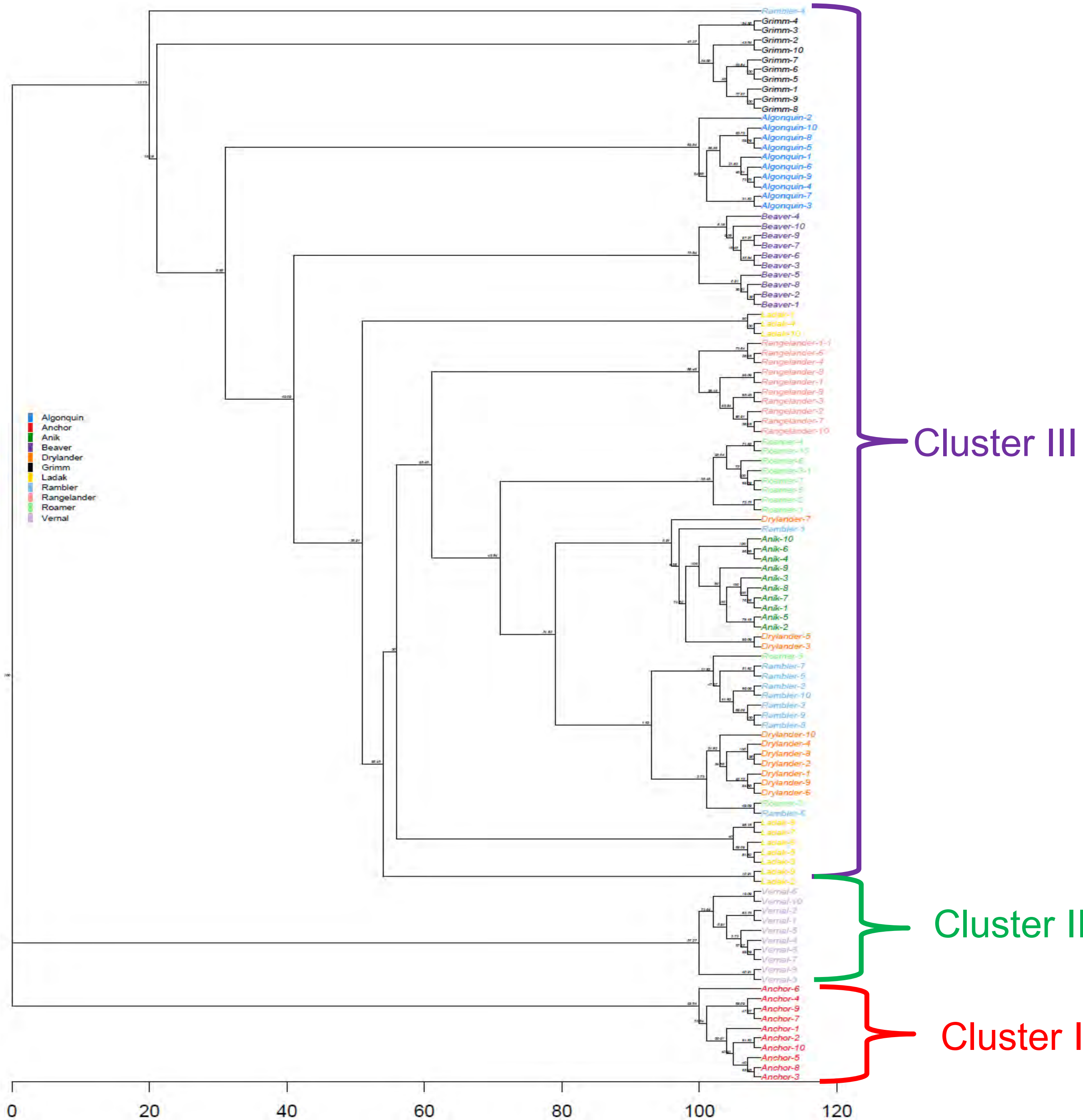


Methods:

- Genotyping-by-sequencing (GBS)

UNIVERSITY OF SASKATCHEWAN

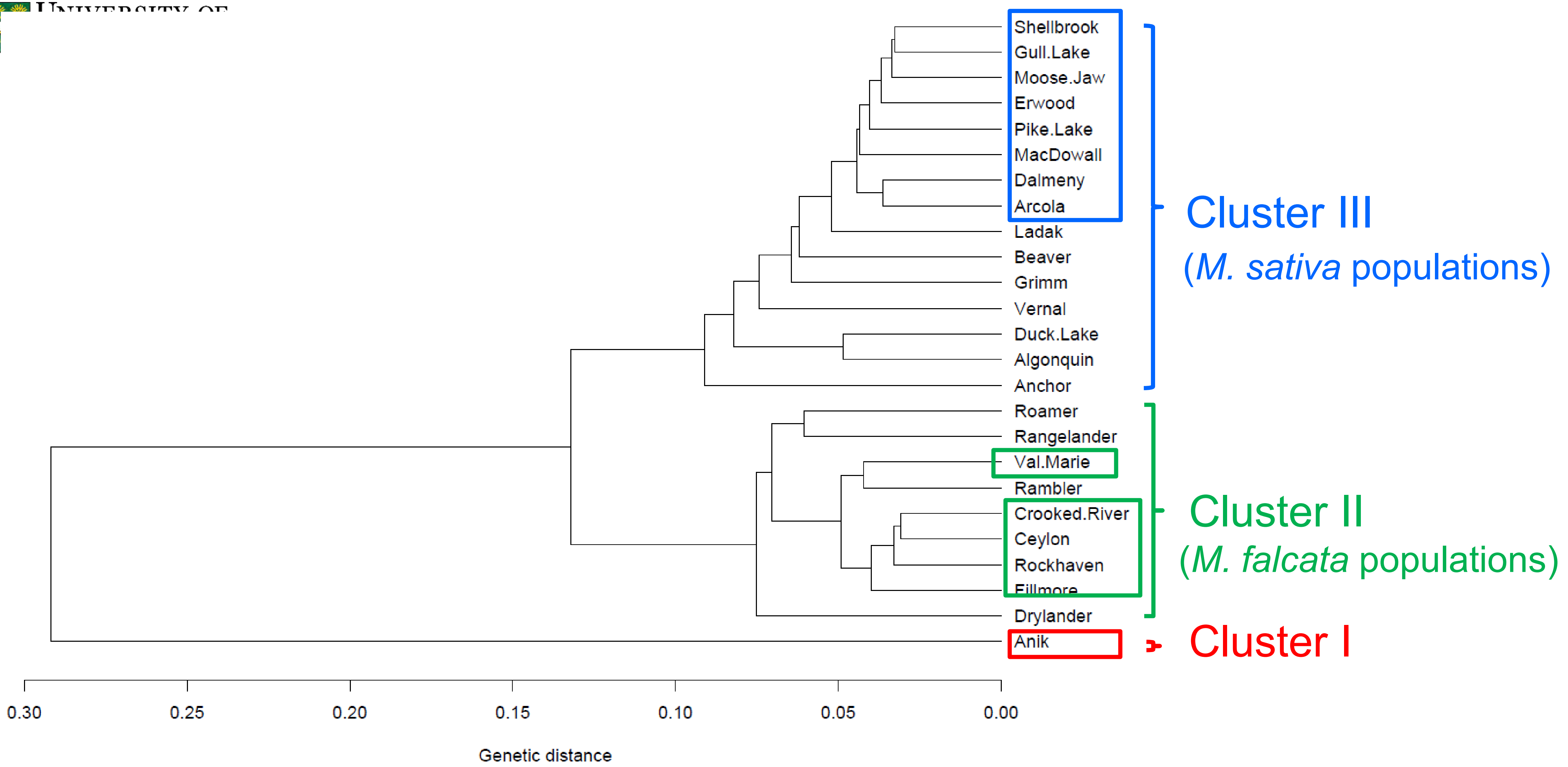
Genetic relationship of 110 alfalfa genotypes representing 11 commercial alfalfa cultivars released from 1926 to 1980 based on 19,853 SNPs



Cultivars from Canada:

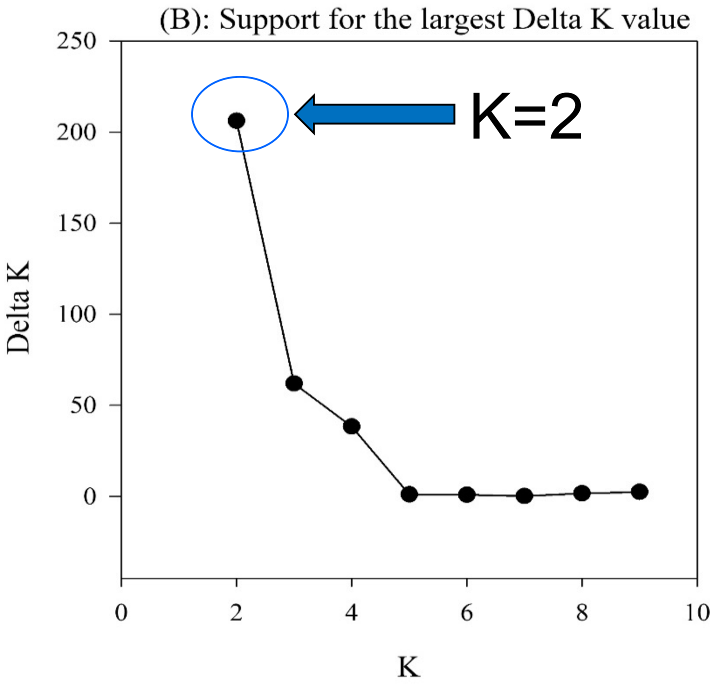
- Grimm
- Algonquin
- Beaver
- Ladak
- Anik
- Rangelander
- Roamer
- Rambler
- Drylander

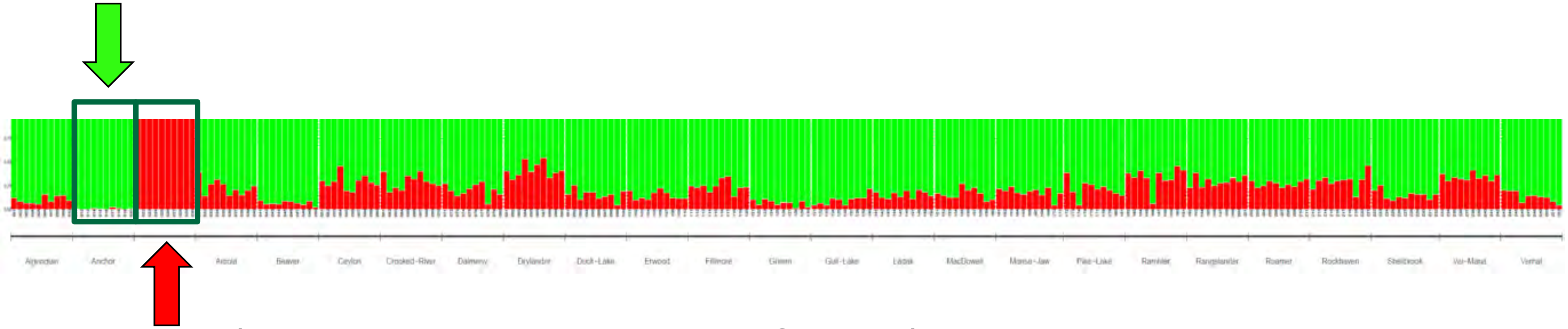
- Vernal from USA
- Anchor from USA



Genetic relationship of 14 alfalfa populations from long-term grazing sites with 11 commercial alfalfa cultivars released from 1926 to 1980 in Western Canada

Structure analysis of genetic background





University of Saskatchewan

Alfalfa selection from Long-term grazing sites

Alfalfa forage yield (kg/ha) trial at Clavet SK						
Cultivar	2020		2021		2022	
	Forage yield	% of Beaver	Forage yield	% of Beaver	Forage yield	% of Beaver
Beaver	7,789	100	3,175	100	6,727	100
Unicorn	6,684	86	3,531	111	6,094	91
Rangelander	7,174	92	3,464	109	6,002	89
SL905AF	6,456	83	3,743	118	6,779	101
CRS1001	6,865	88	2,813	89	5,397	80
AC Grazeland	7,268	93	3,308	104	5,319	79
LSD	1114.42		248.02		1479.00	
P-value	0.20		0.002		0.05	
CV %	6.80		9.65		10.09	

BE WHAT THE WORLD NEEDS

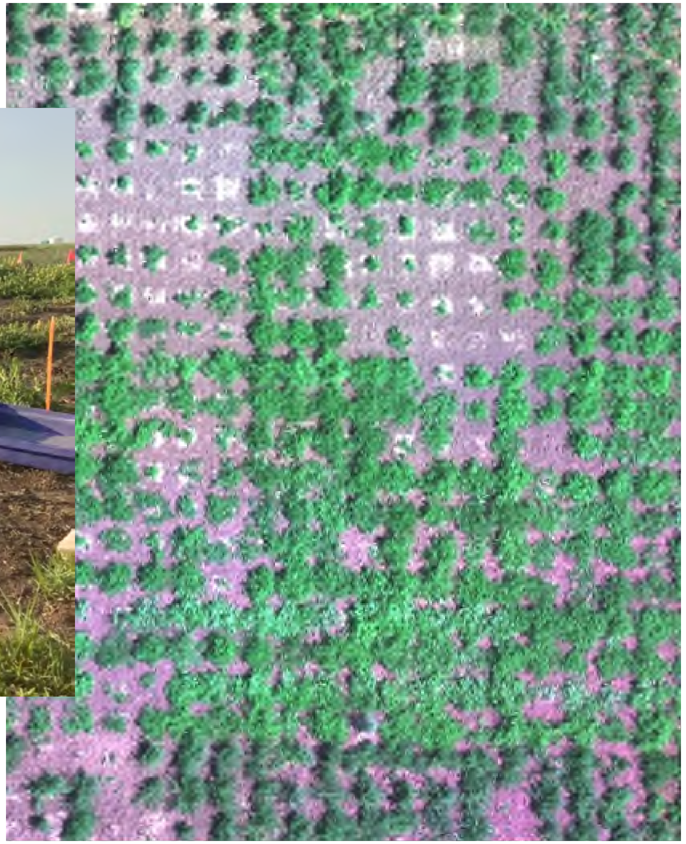
Forage breeding: future perspectives

Application of newer technologies

Precision phenotyping

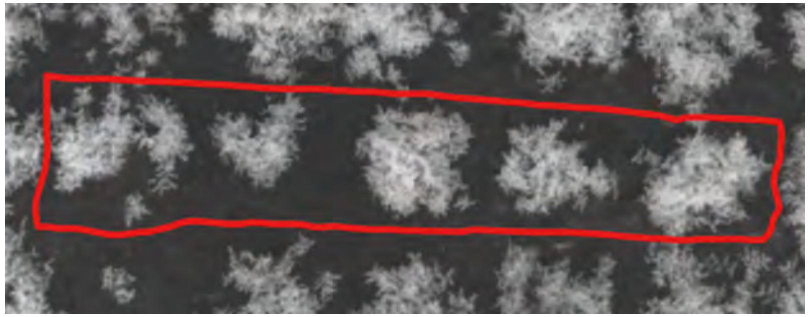
- UAV-Multi-spectral (Camera) phenotyping
- 10-15 min/flight
- NDVI
- Plant height
- Density

David MacTaggart (M.Sc. Candidate)



NDVI CANOPY VOLUME

NDVI Green Area



CORRELATIONS WITH FORAGE YIELD

1st Harvest

	Forage DMY	
Forage DMY	1	
Max Stem Length	0.69	***
Leaves/Stem	0.60	***
Stem Density	0.68	***
NDVI Green Area	0.92	***
NDVI Canopy Vol.	0.87	***

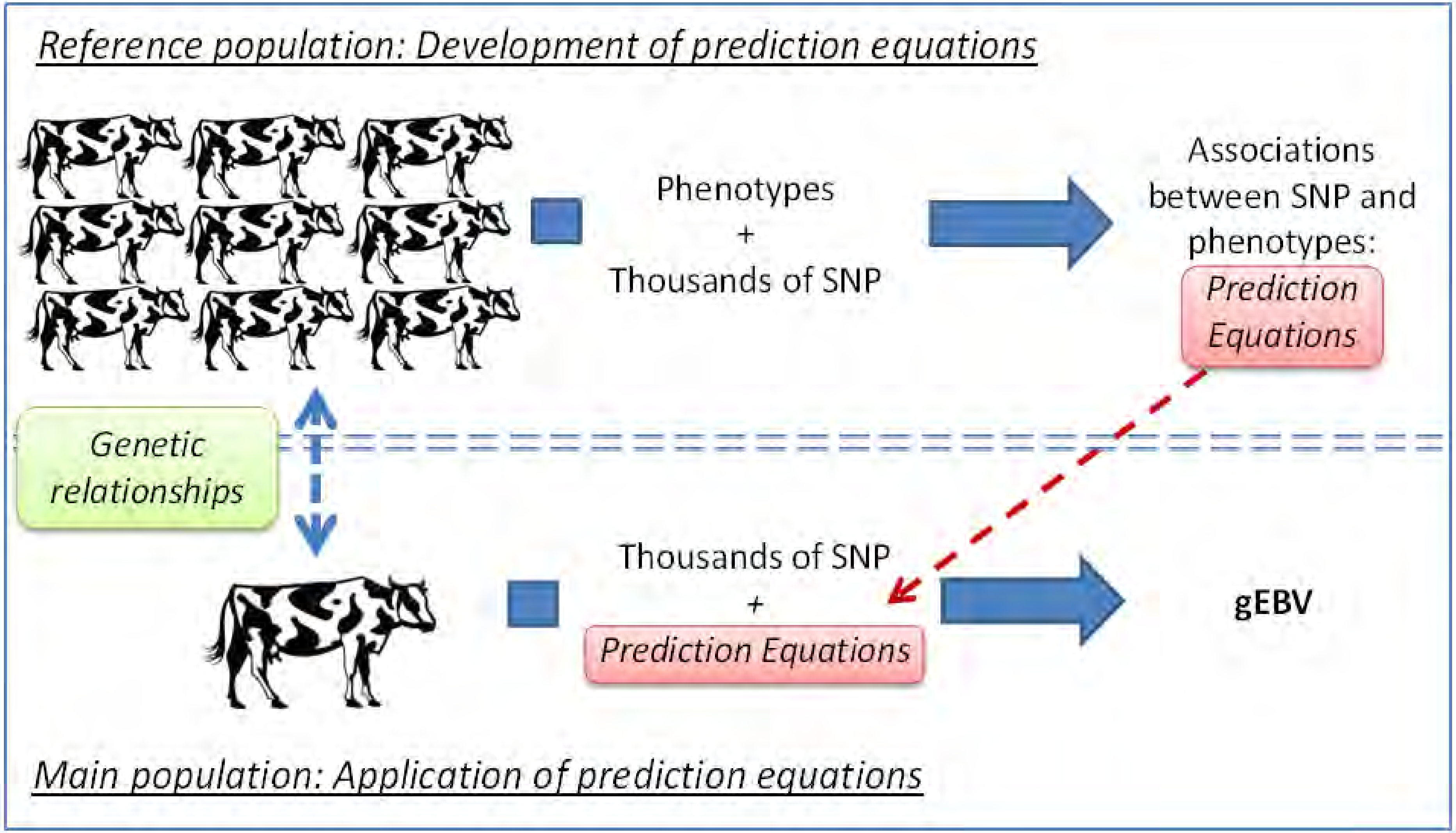
Stockpile Harvest

	Forage DMY
Forage DMY	1
Max. Stem Length	0.60***
Leaves per Stem	-0.07
Stem Density	0.40***
Rhizome Spread	0.39***
NDVI Green Area	0.66***
NDVI Canopy Volume	0.44***

Application of marker assisted selection

- KASP method is an example
- RNA-Seq can generate lots of SNPs
- Genomic selection

Genomic selection: Alfalfa



https://wiki.groenkennisnet.nl/display/TAB/Chapter+8.15%3A+Genomic+selection

Genomic prediction of plant height at Saskatoon in 2021 and 2022

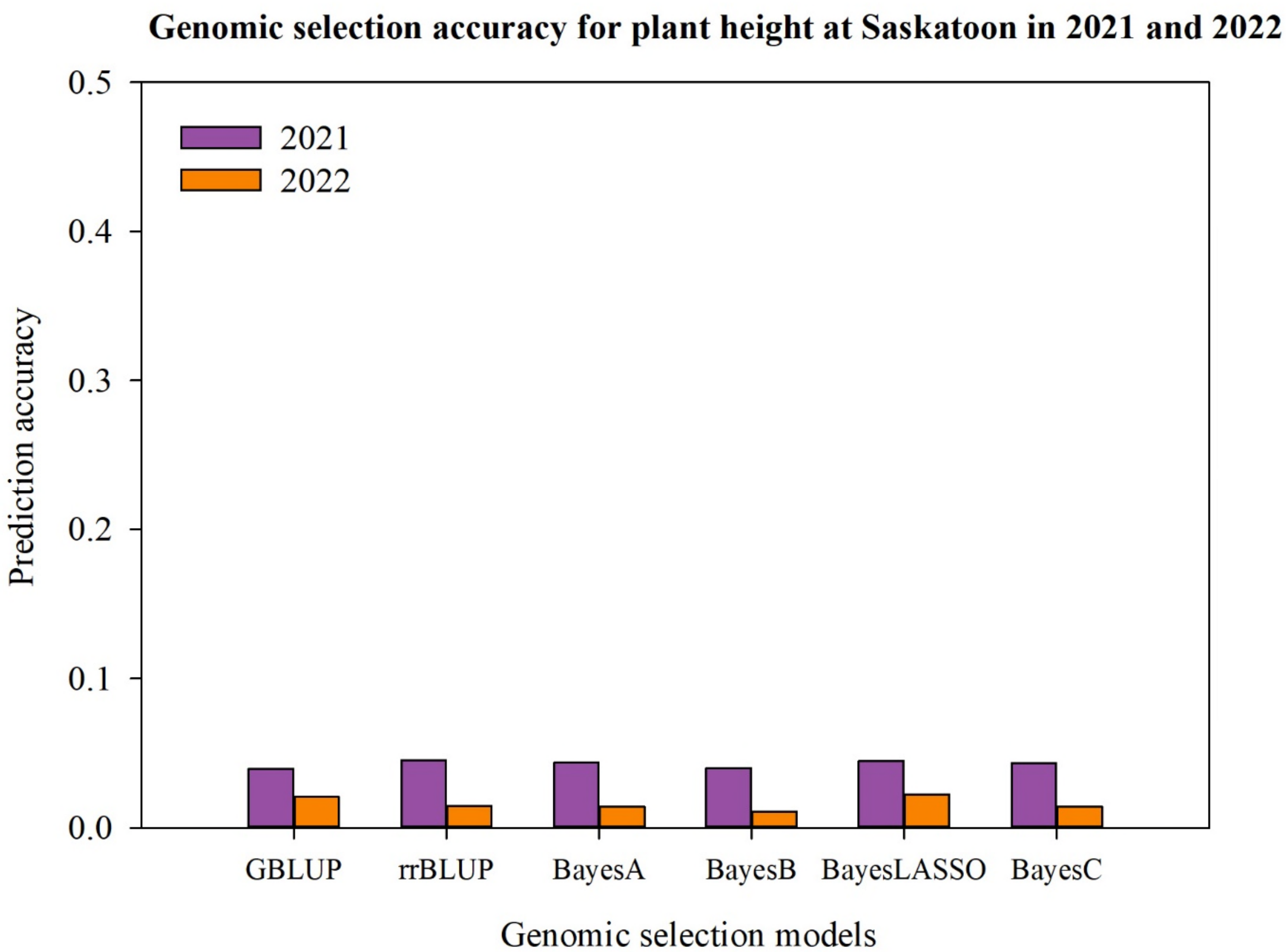
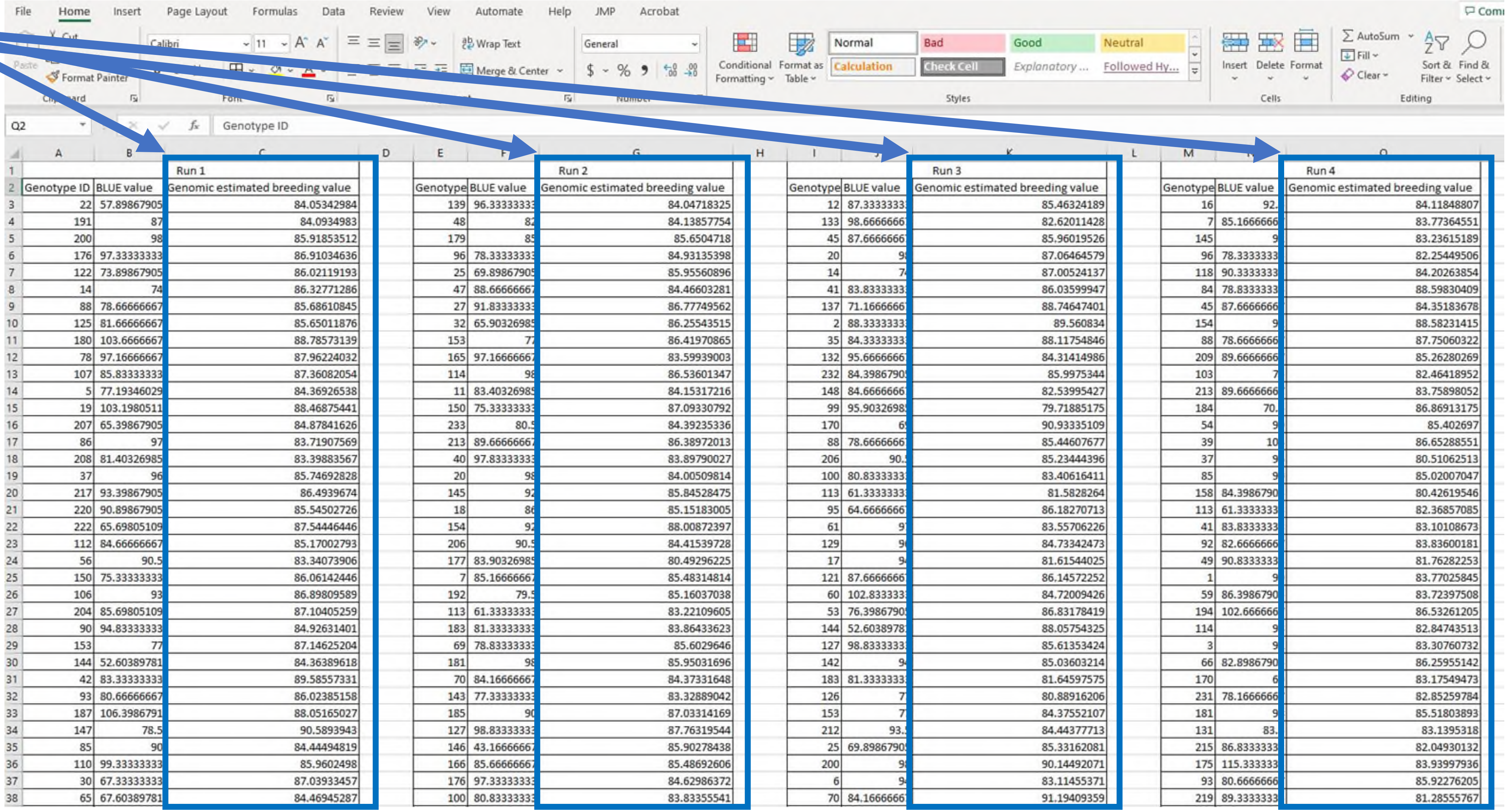


Figure. Comparison of prediction accuracy of six additive genomic selection models for plant height at Saskatoon in 2021 and 2022.

Then the genomic selection model with the highest prediction accuracy was used to calculate the GEBV of plant height for testing populations (117 genotypes) at 1000 random runs

GEBV at each run



	A	B	C
1			Run 1
2	Genotype ID	BLUE value	Genomic estimated breeding value
3	22	57.89867905	84.05342984
4	191	87	84.0934983
5	200	98	85.91853512
6	176	97.33333333	86.91034636
7	122	73.89867905	86.02119193
8	14	74	86.32771286
9	88	78.66666667	85.68610845
10	125	81.66666667	85.65011876
11	180	103.6666667	88.78573139
12	78	97.16666667	87.96224032
13	107	85.83333333	87.36082054
14	5	77.19346029	84.36926538
15	19	103.1980511	88.46875441
16	207	65.39867905	84.87841626
17	86	97	83.71907569
18	208	81.40326985	83.39883567
19	37	96	85.74692828
20	217	93.39867905	86.4939674
21	220	90.89867905	85.54502726
22	222	65.69805109	87.54446446
23	112	84.66666667	85.17002793
24	56	90.5	83.34073906
25	150	75.33333333	86.06142446
26	106	93	86.89809589
27	204	85.69805109	87.10405259
28	90	94.83333333	84.92631401
29	153	77	87.14625204
30	144	52.60389781	84.36389618
31	42	83.33333333	89.58557331
32	93	80.66666667	86.02385158
33	187	106.3986791	88.05165027
34	147	78.5	90.5893943
35	85	90	84.44494819
36	110	99.33333333	85.9602498
37	30	67.33333333	87.03933457
38	65	67.60389781	84.46945287

E	F	G
		Run 2
Genotype	BLUE value	Genomic estimated breeding value
139	96.3333333	84.04718325
48	82	84.13857754
179	85	85.6504718
96	78.3333333	84.93135398
25	69.8986790	85.95560896
47	88.6666666	84.46603281
27	91.8333333	86.77749562
32	65.9032698	86.25543515
153	77	86.41970865
165	97.1666666	83.59939003
114	98	86.53601347
11	83.4032698	84.15317216
150	75.3333333	87.09330792
233	80.5	84.39235336
213	89.6666666	86.38972013
40	97.8333333	83.89790027
20	98	84.00509814
145	92	85.84528475
18	86	85.15183005
154	92	88.00872397
206	90.5	84.41539728
177	83.9032698	80.49296225
7	85.1666666	85.48314814
192	79.5	85.16037038
113	61.3333333	83.22109605
183	81.3333333	83.86433623
69	78.8333333	85.6029646
181	98	85.95031696
70	84.1666666	84.37331648
143	77.3333333	83.32889042
185	90	87.03314169
127	98.8333333	87.76319544
146	43.1666666	85.90278438
166	85.6666666	85.48692606
176	97.3333333	84.62986372
100	80.8333333	83.83355541

I	J	K
		Run 3
Genotype	BLUE value	Genomic estimated breeding value
12	87.3333333	85.46324189
133	98.6666666	82.62011428
45	87.6666666	85.96019526
20	98	87.06464579
14	74	87.00524137
41	83.8333333	86.03599947
137	71.1666666	88.74647401
2	88.3333333	89.560834
35	84.3333333	88.11754846
132	95.6666666	84.31414986
232	84.3986790	85.9975344
148	84.6666666	82.53995427
99	95.9032698	79.71885175
170	69	90.93335109
88	78.6666666	85.44607677
206	90.5	85.23444396
100	80.8333333	83.40616411
113	61.3333333	81.5828264
95	64.6666666	86.18270713
61	97	83.55706226
129	96	84.73342473
17	94	81.61544025
121	87.6666666	86.14572252
60	102.833333	84.72009426
53	76.3986790	86.83178419
144	52.6038978	88.05754325
127	98.8333333	85.61353424
142	94	85.03603214
183	81.3333333	81.64597575
126	77	80.88916206
153	77	84.37552107
212	93.5	84.44377713
25	69.8986790	85.33162081
200	98	90.14492071
6	94	83.11455371
70	84.1666666	91.19409359

M	N	O
		Run 4
Genotype	BLUE value	Genomic estimated breeding value
16	92.	84.11848807
7	85.1666666	83.77364551
145	9	83.23615189
96	78.3333333	82.25449506
118	90.3333333	84.20263854
84	78.8333333	88.59830409
45	87.6666666	84.35183678
154	9	88.58231415
88	78.6666666	87.75060322
209	89.6666666	85.26280269
103	7	82.46418952
213	89.6666666	83.75898052
184	70.	86.86913175
54	9	85.402697
39	10	86.65288551
37	9	80.51062513
85	9	85.02007047
158	84.3986790	80.42619546
113	61.3333333	82.36857085
41	83.8333333	83.10108673
92	82.6666666	83.83600181
49	90.8333333	81.76282253
1	9	83.77025845
59	86.3986790	83.72397508
194	102.666666	86.53261205
114	9	82.84743513
3	9	83.30760732
66	82.8986790	86.25955142
170	6	83.17549473
231	78.1666666	82.85259784
181	9	85.51803893
131	83.	83.1395318
215	86.8333333	82.04930132
175	115.333333	83.93997936
93	80.6666666	85.92276205
219	89.3333333	81.28555767

Highest 20 genotypes for plant height at 1000 random runs

ID	Count at 1000 random runs	Frequency in 1000 random runs	The average GEBV of 1000 random runs	BLUE value
Gen_272	458	0.458	90.5	63.2
Gen_271	423	0.423	89.8	79
Gen_87	408	0.408	89.5	72.3
Gen_256	376	0.376	89.7	78.5
Gen_167	361	0.361	89.1	68.7
Gen_260	361	0.361	89.3	82.7
Gen_95	354	0.354	89	83.3
Gen_248	339	0.339	88.9	84.3
Gen_114	332	0.332	88.6	95.7
Gen_328	328	0.328	88.9	72.7
Gen_270	328	0.328	88.7	92
Gen_116	324	0.324	90	81.8
Gen_34	320	0.32	89	92.5
Gen_274	318	0.318	89	81
Gen_223	314	0.314	89	98.8
Gen_236	290	0.29	88.8	83.2
Gen_178	284	0.284	89.3	79
Gen_288	279	0.279	88.8	87.8
Gen_240	274	0.274	88.8	71.2
Gen_63	271	0.271	88.8	85.9

Acknowledgements

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BCRC
Beef Cattle Research Council

SFSDC

NSERC
CRSNG

SCA
Saskatchewan Cattlemen's Association

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