

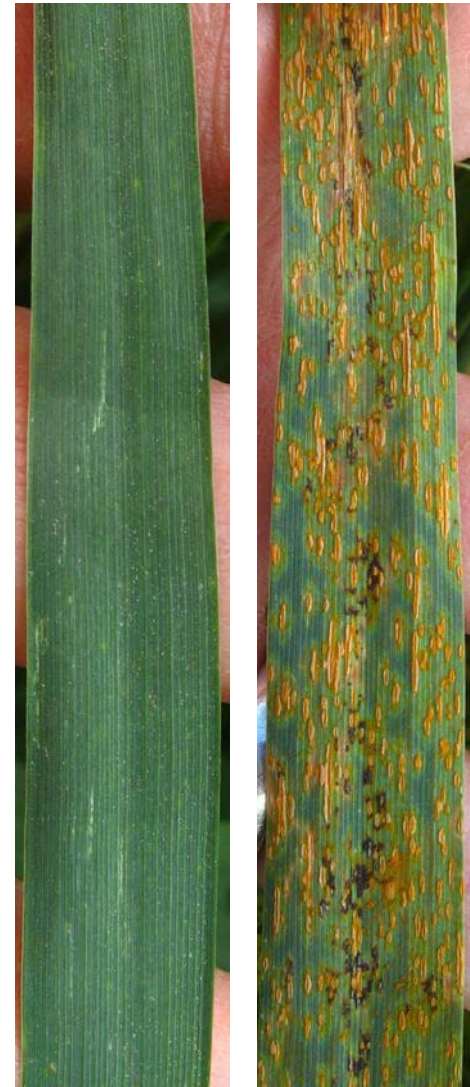


Development of SNP Markers for Breeding Disease Resistant Oat Varieties

Belaghihalli N Gnanesh, Curt A McCartney, Jennifer W
Mitchell-Fetch, Yang Lin, James Chong, Aaron D Beattie,
Pete E Eckstein, Jim G Menzies, Taye Zegeye, Tom Fetch,
Randy Kutcher and Eric W Jackson

Diseases of oats

- Crown rust
 - *Puccinia coronata* f.sp. *avenae*
 - 100+ described R genes
- Stem rust
 - *Puccinia graminis* f.sp. *avenae*
 - 17 described R genes

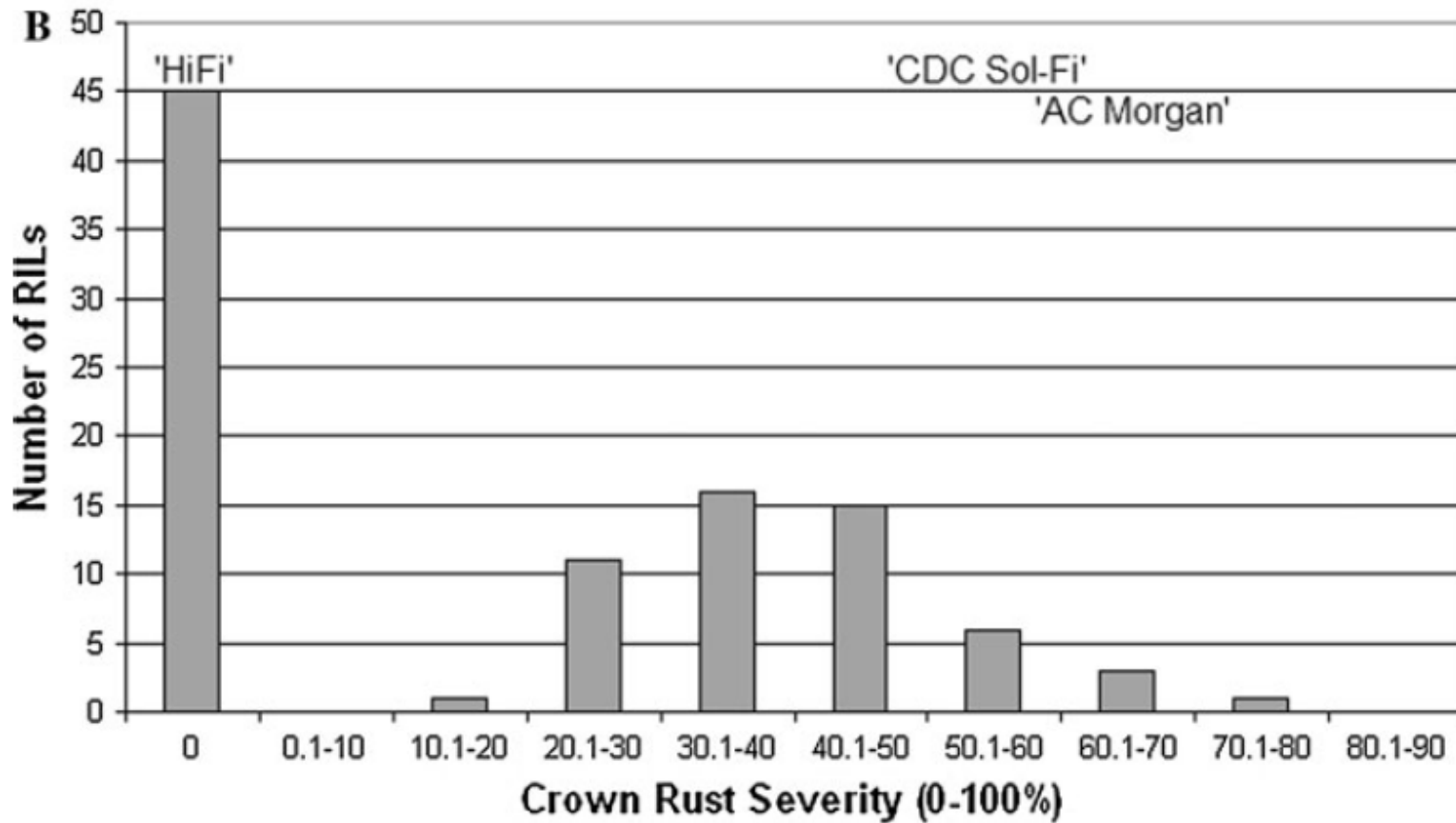
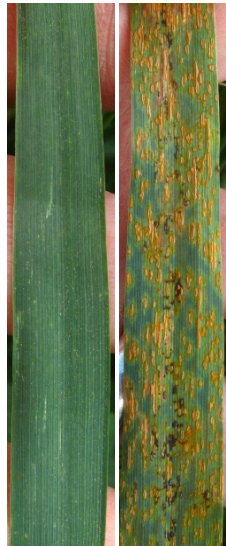


Marker Development

- Crown rust
 - *Pc91*
 - MN841801 (APR)
 - *Pc68*
 - *Pc94*
 - Kame, Morton (*PcKM*)
- Stem rust
 - OT3090 (*Pg12?*)



Pc91



McCartney et al. 2011

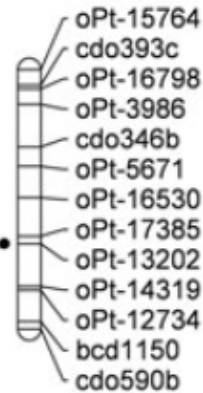
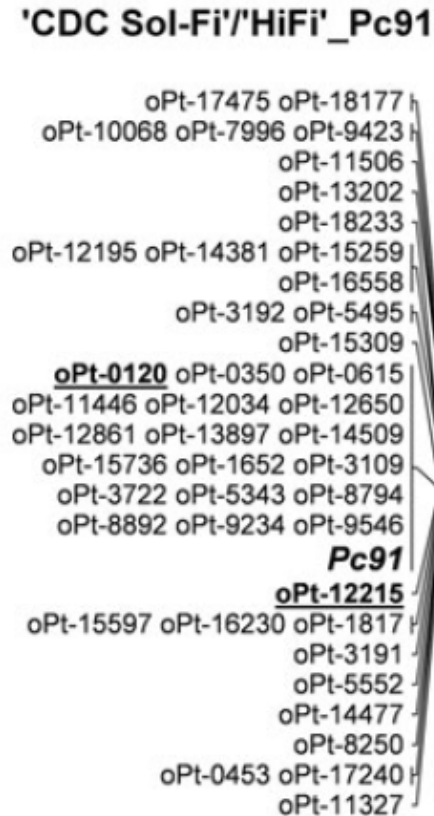
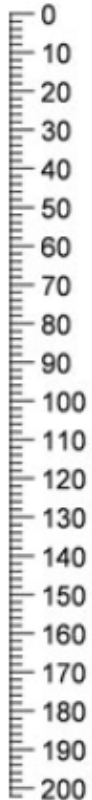


Pc91

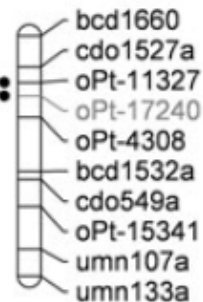
KOD_1_3_38_X3

7C-17A

'CDC Sol-Fi'/'HiFi'_Pc91



KOD_1_3_38_breakpoint



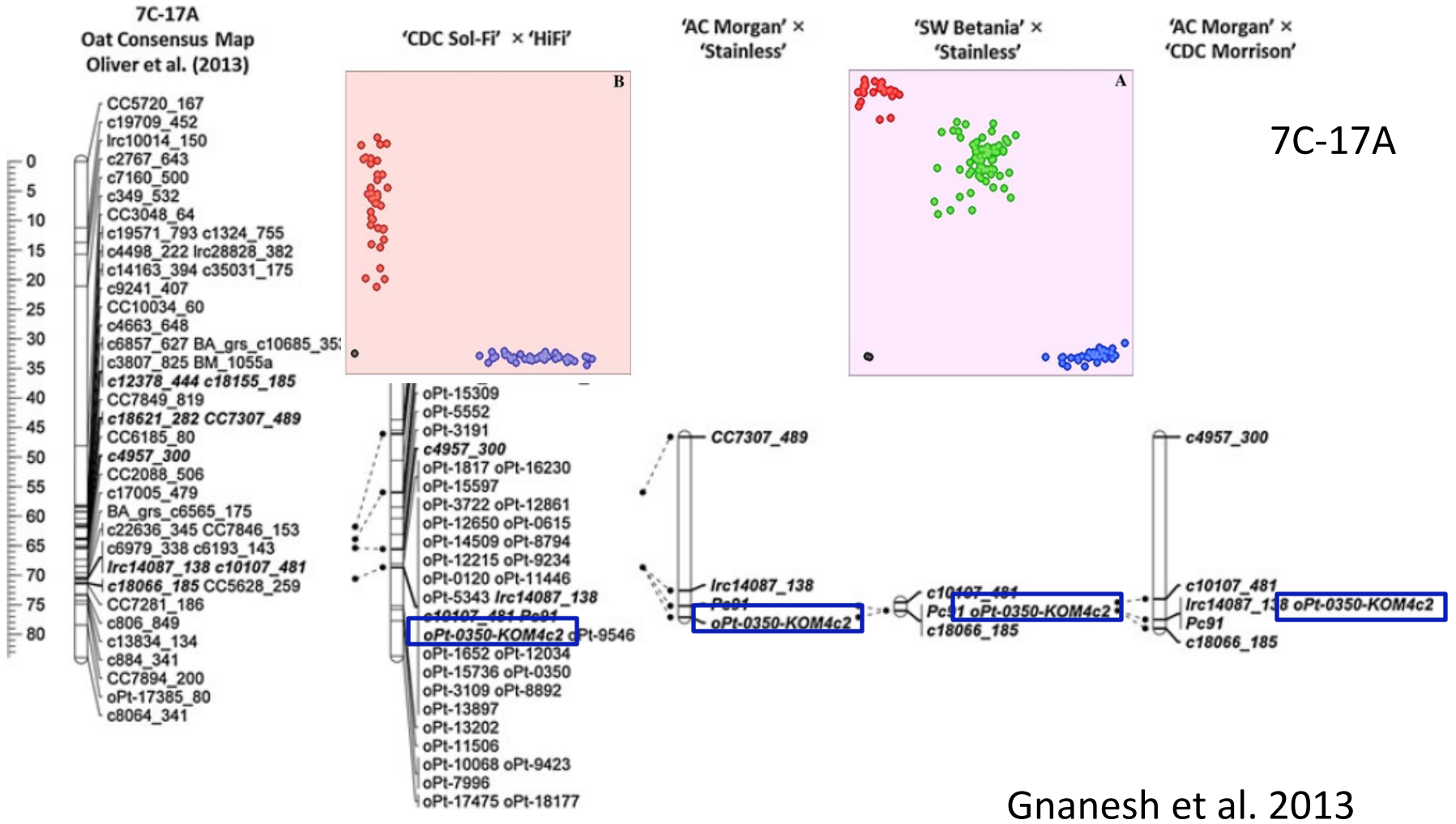
KOD_1_3_38_X1

McCartney et al. 2011



Pc91

Not all SNPs are diagnostic



Gnanesh et al. 2013

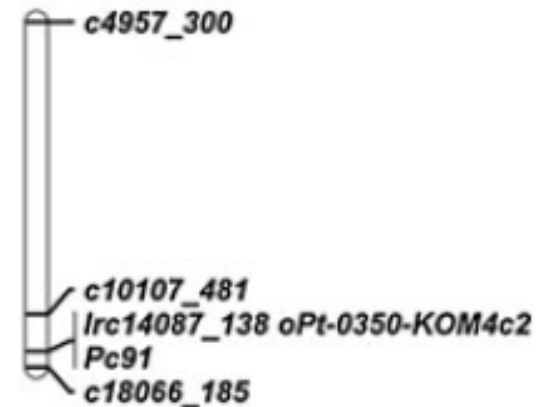


Pc91: Follow-up

7C-17A

- AC Morgan / CDC Morrison
 - Segregates for stem rust resistance
 - Likely *Pg2*, *Pg13*
 - *Pg13* should be linked to *Pc91* based on comparative mapping
 - Coupling of *Pc91* and *Pg13* is valuable for breeding

'AC Morgan' ×
'CDC Morrison'



Adult Plant Resistance

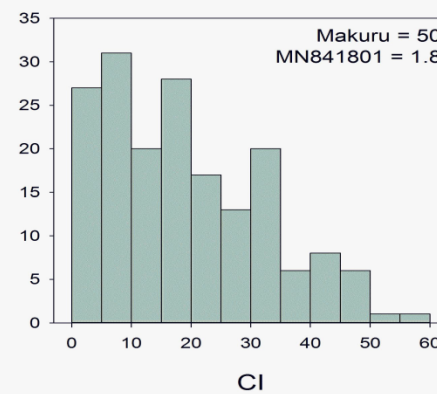
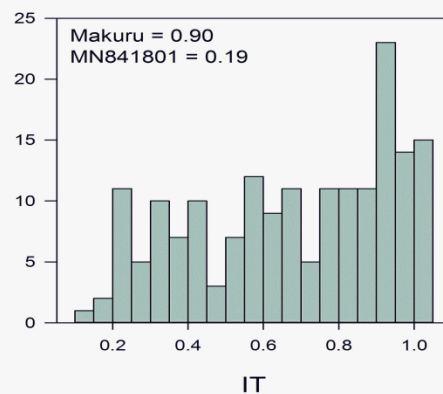
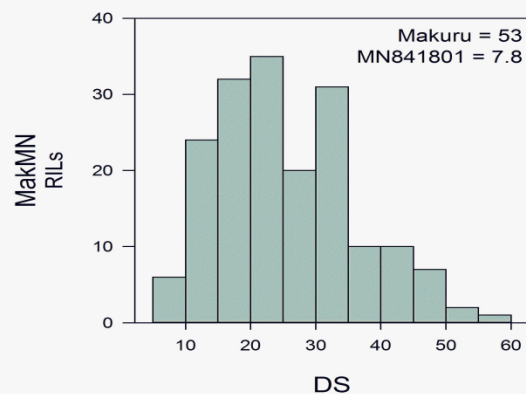
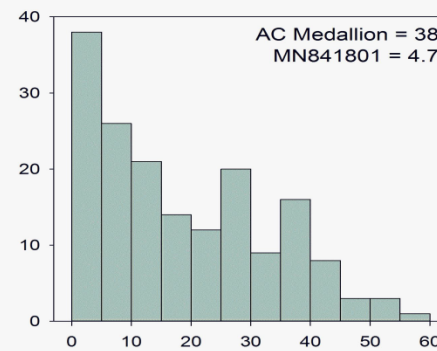
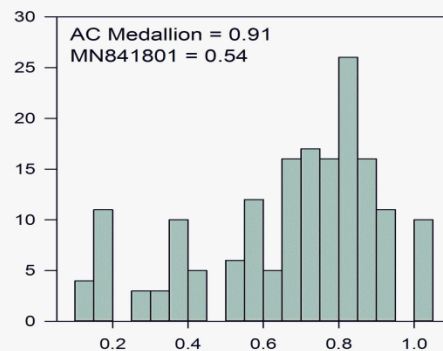
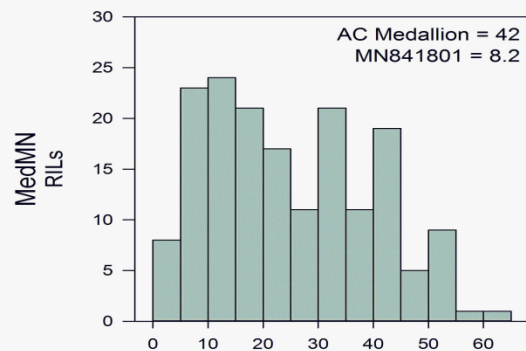
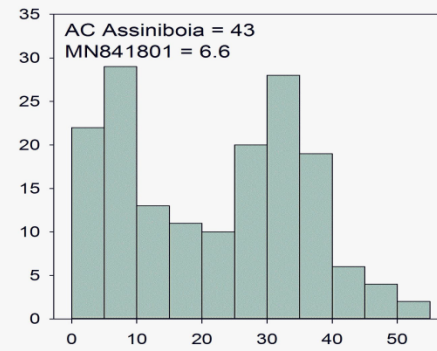
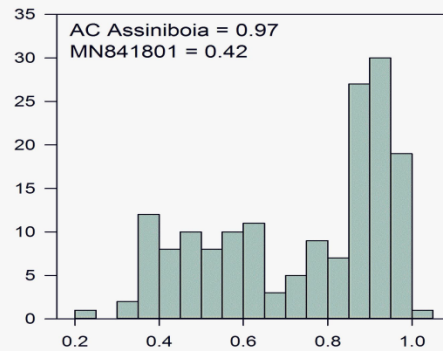
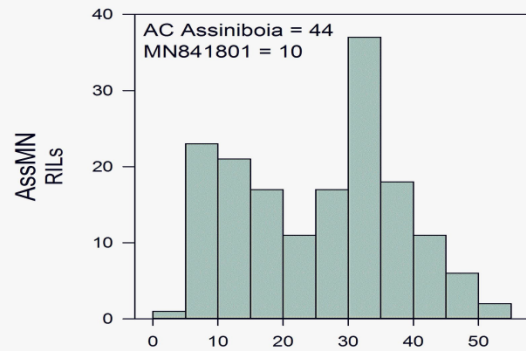
- MN841801
 - Adult plant, crown rust resistance
 - Partial resistance
 - Non-race-specific...
 - Previous studies identified multiple QTL (Acevedo et al. 2010, Portyanko et al. 2005)
- James Chong
 - AC Assiniboia / MN841801 (AssMN)
 - AC Medallion / MN841801 (MedMN)
 - Makuru/MN841801(MakMN)
 - James expected two genes



Adult Plant Resistance

- AssMN
 - 163 F₈-derived RILs
 - Six field tests inoculated with CR251
 - Virulent on all seedling genes in AC Assiniboia and MN841801
- MedMN
 - 156 F₆-derived RILs
 - One field tests inoculated with CR251
- MakMN
 - 160 F₇-derived RILs
 - Two field tests inoculated with CR251





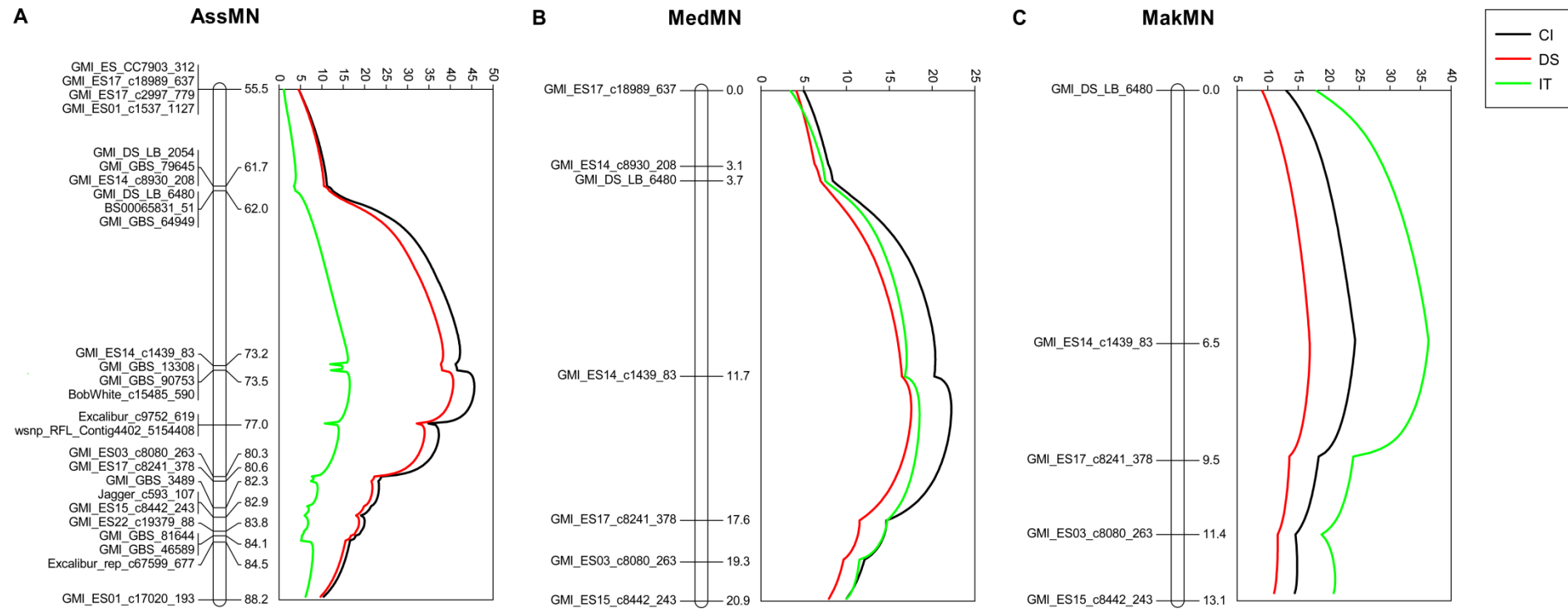
Mean flag leaf crown rust disease severity (DS), infection type (IT), coefficient of infection (CI) pooled over six, one, and two environments.



Linkage Mapping

- Marker Testing
 - DArTs: 90 RILs
 - 6K Oat SNPs: 163 RILs
 - 90K Wheat SNPs: 163 RILs
- Linkage map
 - 45 linkage groups (Rec. Frac. = 0.2)
 - 1539 cM
 - 1,684 SNPs





Simple interval mapping QTL scans revealing *Q_{Pc.crc-14D}* on oat chromosome 14D. QTL analysis of APR is based upon data averaged environments for DS, IT, and CI.



Consensus Map

AssMN

MedMN

MakMN



Oliver et al. 2013



Comparative Mapping

Locus name	Oat Chromosome	AssMN Position (cM)	Wheat Survey Sequence Arm	E-value	Bit Score
GMI_ES15_c12071_400	14D	48.56	2DS	3.00E-27	125
GMI_ES_CC7903_312	14D	55.48	2DS	1.00E-100	371
BS00065831_51	14D	62.03	2BS	1.00E-36	158
GMI_GBS_64949	14D	62.03	2AS	7.00E-11	69.9
BobWhite_c15485_590	14D	73.51	2BS	1.00E-36	158
Excalibur_c9752_619	14D	76.96	2BL	1.00E-36	158
w SNP_RFL_Contig4402_5154408	14D	76.96	2DL	8.00E-28	128
GMI_ES17_c8241_378	14D	80.63	2DL	4.00E-11	71.9
GMI_ES22_c19379_88	14D	83.83	2AL	4.00E-45	184
GMI_GBS_46589	14D	84.14	2DL	4.00E-15	83.8
Excalibur_rep_c67599_677	14D	84.45	2AL	1.00E-36	158

- Centromeric region of wheat Group 2 chromosomes
- Wheat APR genes
 - *Lr34/Pm38/Sr_/Yr18* (7DS)
 - *Lr46/Yr29* (1BL)
 - *Lr67/Sr55/Yr46* (4DL)
 - *Sr2* (3BS)
 - ***Yr16* (2DS/2DL)**



Pc68

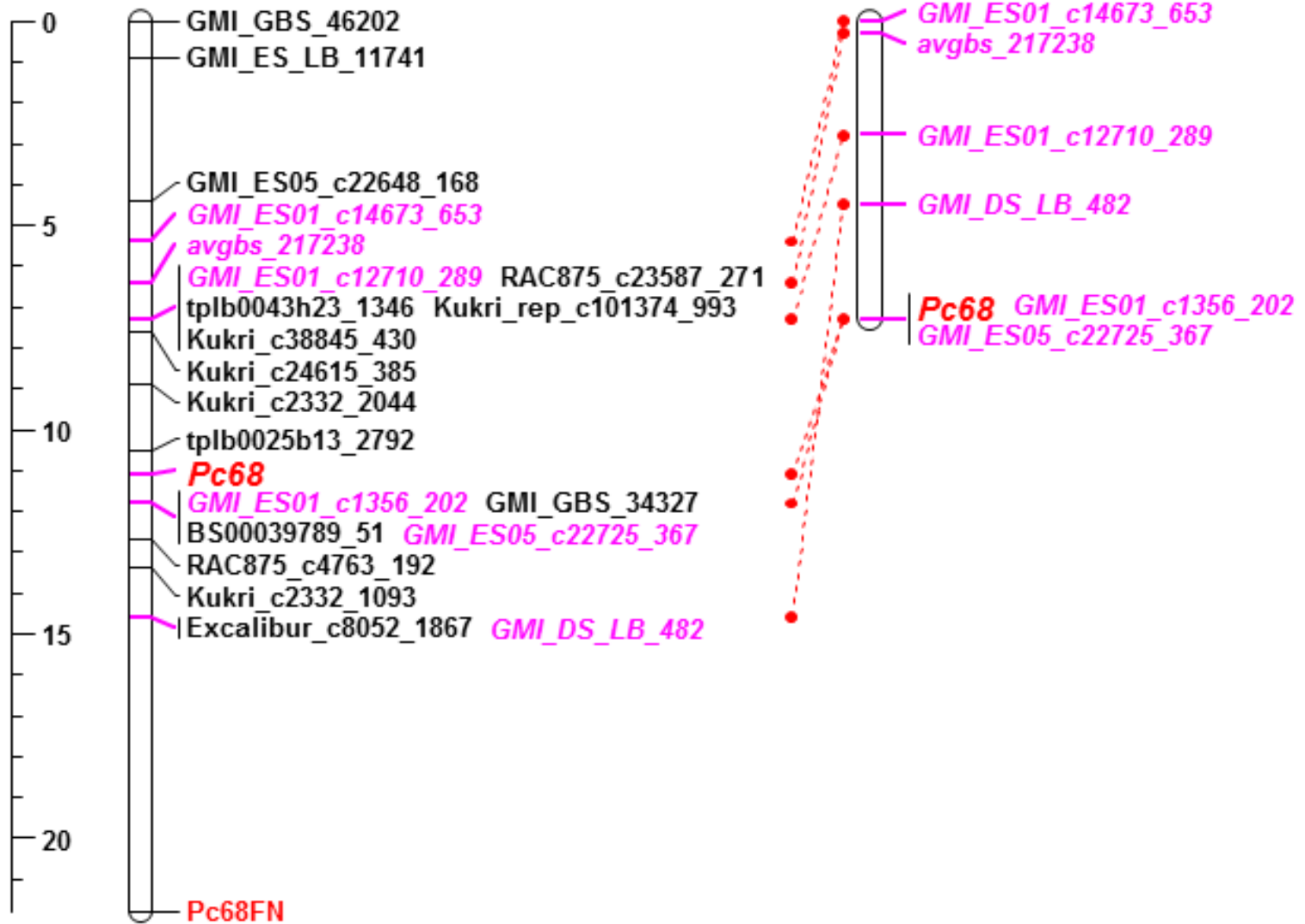
- Strong resistance
- Frequent virulence to *Pc68*
- Segregates in two populations
 - AC Assiniboia / MN841801
 - AC Medallion / MN841801



Pc68

AC Assiniboia / MN841801

AC Medallion / MN841801



Pc94

- Strong resistance
- Little virulence to *Pc94*
- Chong et al. (2007) SNPs not diagnostic in all germplasm



Pc94

- OT3033 × OT3024
 - 186 F₄-derived RILs
 - Tested with crown rust race CR 254
 - Also 4 environment field test (90 RILs)

Population	Gen ^a	Total	Res ^b	Sus ^c	Seg ^d	ER ^e	χ ²	P Value
OT3033 × OT3024	P ₁	25	-	25				
	P ₂	24	24	-				
	F ₄₋₇	186	77	96	13	1:1	2.08	0.1486



Pc94

- Tested with DArTs (Aaron Beattie & Pete Eckstein)
- DArT-derived SNPs
 - Comparative mapping identified DArTs for sequencing on parents (Aaron Beattie & Pete Eckstein)
 - Converted to KASP
- Bulk Segregant Analysis using 6K Oat Infinium Assay
 - Converted to KASP



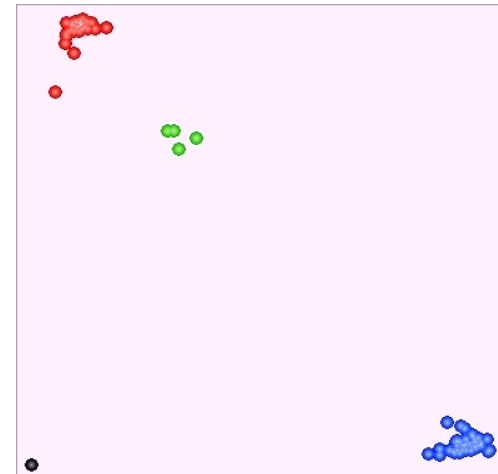
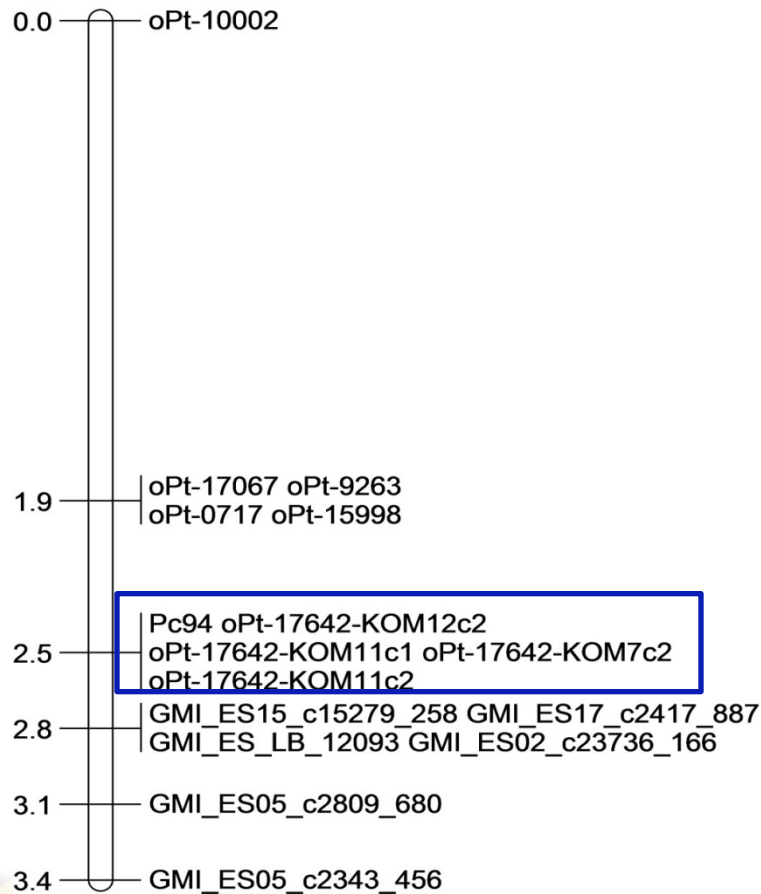
Pc94

SNP	OT3024					OT3033					Pc94_R_Ri108_09					Pc94_R_Ri125_26					Pc94_R_Ri136_37					Pc94_R_Ri155_62					Pc94_R_Ri195_96					Pc94_S_Ri103_104					Pc94_S_Ri111_114					Pc94_S_Ri125_128					Pc94_S_Ri132_136					Pc94_S_Ri148_154					-	Hits
	A	H	B	-	A	H	B	-	A	H	B	-	A	H	B	-	A	H	B	-	A	H	B	-	A	H	B	-	A	H	B	-	A	H	B	-	A	H	B	-	A	H	B	-																		
GMI_ES05_c2343_456	A	5	0	0	0	0	5	0	0	H	A	A	A	A	A	H	H	H	H	H	0	Yes																																								
GMI_ES15_c15279_258	A	5	0	0	0	0	5	0	0	H	A	A	A	A	A	H	H	H	H	H	0	Yes																																								
GMI_ES05_c2809_680	B	0	0	5	0	5	0	0	0	A	B	B	B	B	B	A	A	A	A	A	0	Yes																																								
GMI_ES15_c1401_507	H	0	5	0	0	4	0	0	1	A	H	H	H	H	H	A	A	A	-	A	1	Yes																																								
GMI_ES_LB_12093	H	0	5	0	0	0	0	5	0	B	H	H	H	H	H	B	B	B	B	B	0	Yes																																								
GMI_ES02_c23736_166	H	0	5	0	0	0	0	5	0	B	H	H	H	H	H	B	B	B	B	B	0	Yes																																								
GMI_ES17_c2417_887	H	0	5	0	0	0	0	5	0	B	H	H	H	H	H	B	B	B	B	B	0	Yes																																								
GMI_DS_LB_4454	H	0	4	0	1	0	0	5	0	B	H	H	H	-	H	B	B	B	B	B	1	Yes																																								
GMI_ES05_c2441_293	A	5	0	0	0	1	0	1	3	B	A	A	A	A	A	B	-	-	A	-	3	Yes?																																								
GMI_ES01_c16302_758	A	5	0	0	0	1	1	0	3	H	A	A	A	A	A	H	-	-	A	-	3	Yes?																																								
GMI_ES01_c24747_317	A	1	0	0	4	0	5	0	0	H	-	-	-	-	A	H	H	H	H	H	4	Yes?																																								
GMI_ES22_c7848_332	B	0	0	5	0	0	2	1	2	-	B	B	B	B	B	-	H	H	B	-	3	Yes?																																								
GMI_ES05_c15473_315	B	0	0	5	0	0	4	1	0	H	B	B	B	B	B	H	H	H	B	H	0	Yes?																																								
GMI_ES22_lrc11958_292	B	0	0	5	0	0	4	1	0	H	B	B	B	B	B	H	H	H	B	H	0	Yes?																																								
GMI_DS_LB_602	B	0	0	5	0	0	3	1	1	H	B	B	B	B	B	H	H	H	B	-	1	Yes?																																								
GMI_ES14_c16054_391	B	0	0	5	0	0	3	1	1	H	B	B	B	B	B	H	H	H	B	-	1	Yes?																																								
GMI_ES15_c4675_465	H	0	5	0	0	4	1	0	0	A	H	H	H	H	H	A	A	A	H	A	0	Yes?																																								
GMI_ES02_c11775_206	H	0	5	0	0	2	1	0	2	A	H	H	H	H	H	A	-	A	H	-	2	Yes?																																								
GMI_DS_CC1800_254	H	0	4	0	1	2	1	0	2	A	H	H	H	-	H	A	A	H	-	-	3	Yes?																																								
GMI_ES15_c5522_677	H	0	5	0	0	0	1	3	1	B	H	H	H	H	H	B	B	H	-	B	1	Yes?																																								



Pc94

OT3033 / OT3024
9D

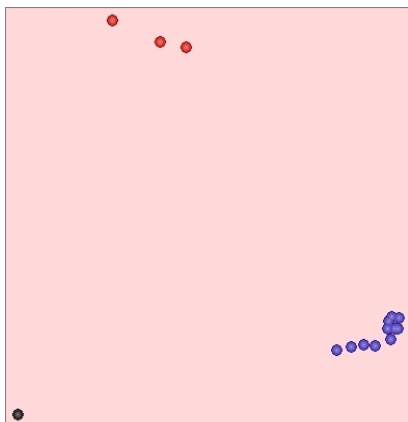


oPt-17642-KOM11c2

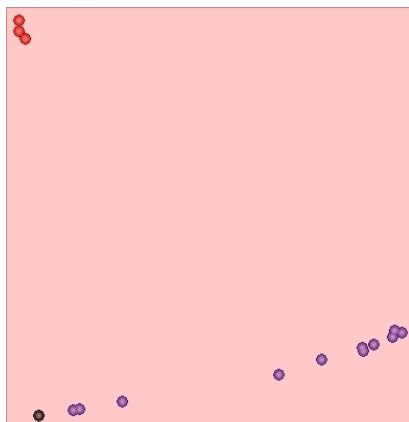


Pc94

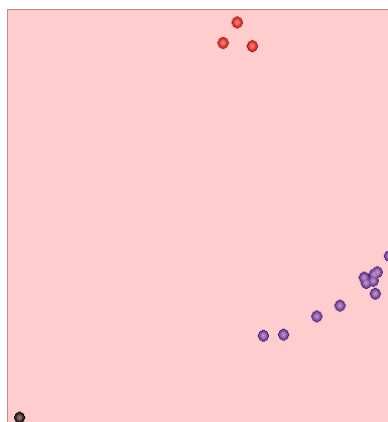
oPt-17642-KOM7c2



oPt-17642-KOM11c2



oPt-17642-KOM12c2



Carriers
Not carrier
Controls

Genotypes	OT3033	OT3024	Leggett	S42	OT7030	AC Morgan	CDC Sol-Fi	CDC Morrison	HiFi	D921-643	AC Assiniboia	Summit	CDC Dancer	Ronald	Souris	Stainless	OT2041	Newburg	CDC Minstrel	CIAV-9212	AC Gwen	HiFi-reselected	
Pc94	A	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
oPt-17642-KOM11c1	A	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
oPt-17642-KOM12c2	A	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B



PcKM

- Crown rust resistance in Kame and Morton
 - Kame (B605X//Dane/Newdak)
 - Morton (ND880922/B605X)
 - B605X is from Iowa
 - Effective resistance in field tests
 - Virulence to gene?



PcKM

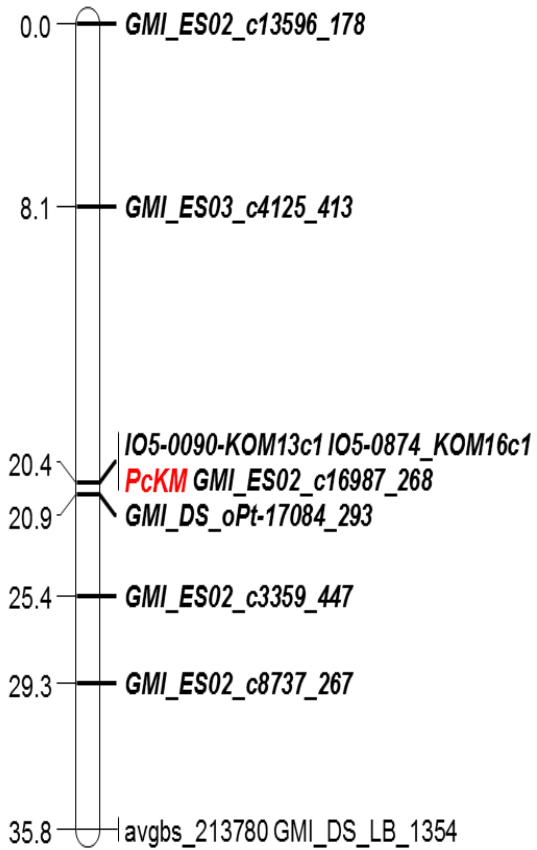
Crown Rust Reaction

PcKM Segregation

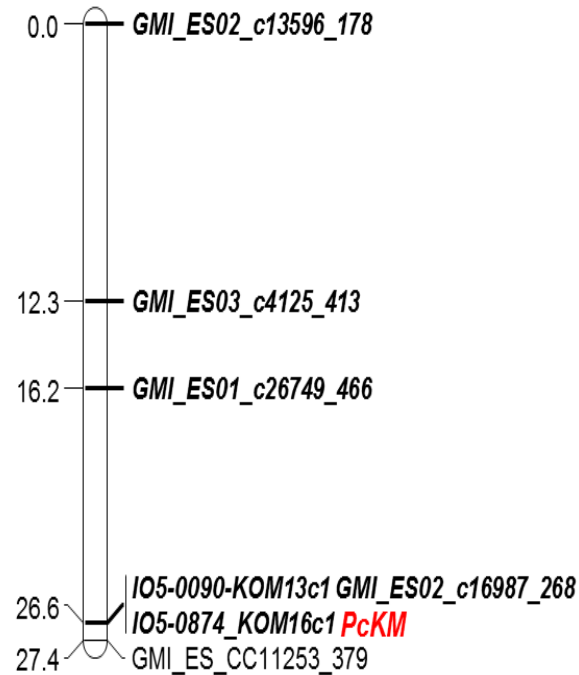
Population (S × R)	Nursery	Nursery Type	Susceptible Parent	Resistant Parent	RIL Population	Resistant	Susceptible	Segregating	χ^2 ^b	P value
OT3019 × 'Morton'	Guelph 2008	Field	7	1	0 - 9	50	50	-	0.00	1.00
	Guelph 2009	Field	6	1	1 - 8	48	50	-	0.04	0.84
	Saskatoon 2009	Field	10MRMS	0	0 - 85S	50	50	-	0.00	1.00
	Guelph 2010	Field	6	1	0 - 8	48	52	-	0.16	0.69
	Winnipeg 2013	Greenhouse	4	0	0 - 4	49	50	1	4.44	0.11
'CDC Weaver' × 'Kame'	Guelph 2008	Field	8	2	0 - 8	32	40	-	0.89	0.35
	Guelph 2009	Field	5	2	1 - 8	28	44	-	3.56	0.06
	Saskatoon 2009	Field	trS	0	0 - 85S	28	44	-	3.56	0.06
	Winnipeg 2013	Greenhouse	4	0	0 - 4	31	35	6	0.41	0.61
OT9001 × OT3060	Winnipeg 2013	Greenhouse	4	0	0 - 4	34	31	7	2.51	0.63



12D
OT3019 × Morton



12D
OT9001 × OT3060



12D
CDC Weaver × Kame



Stem Rust Resistance

- Stem rust races NA67 and NA76
 - Virulent on *Pg2* and *Pg13*
 - First identified in 1998



Marker Development

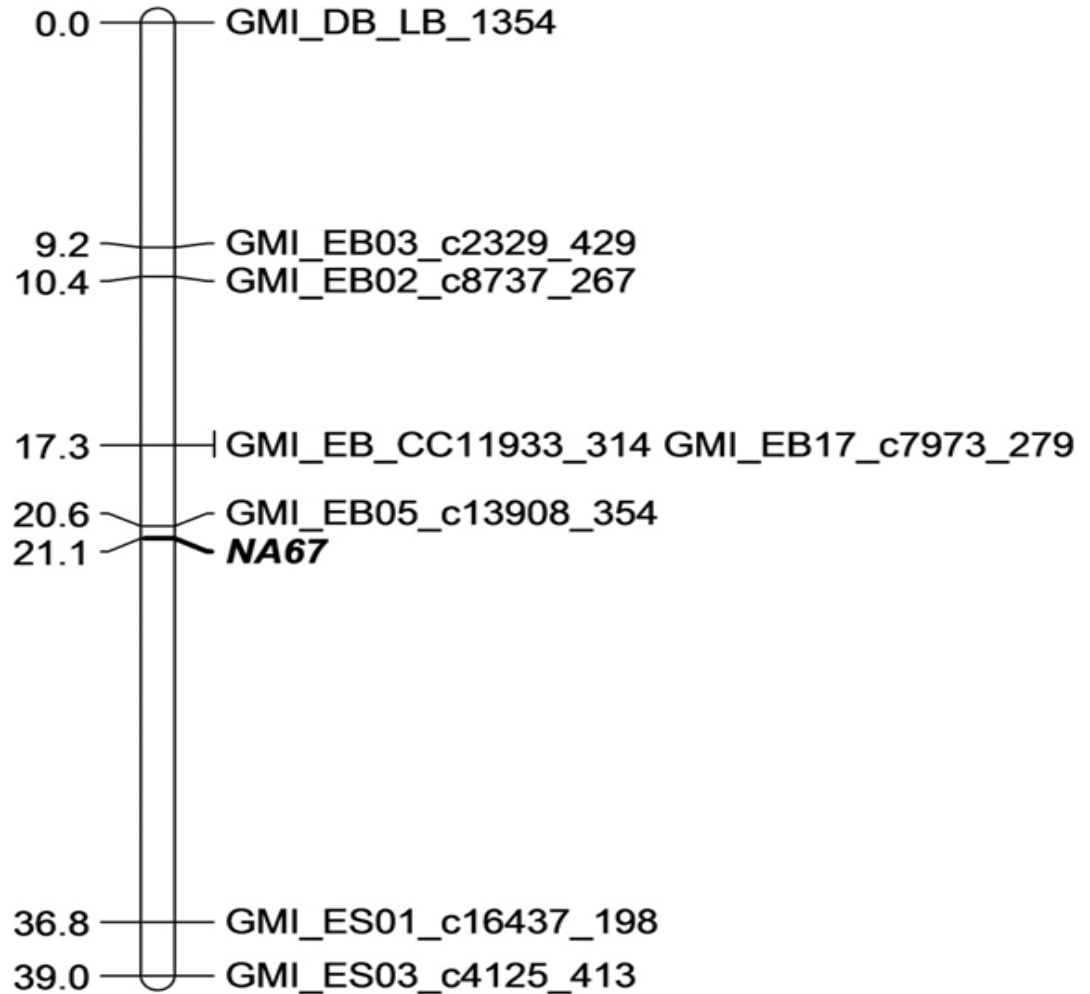
Trait	Race	Populations	Gena	Total	Resb	Susc	Segd	ERe	χ^2	P Value	
Stem rust (NA67)	NA67_GH_WP	OT7030 × Leggett (2011)	P1	30	30						
			P2	30	-	30					
			F _{5:6}	225	101	118	6	1:1	1.32	0.25	
	NA67_GH_WP	OT7030 × Leggett (2012)	P1	30	30						
			P2	30	-	30					
			F _{5:6}	226	105	116	3	1:1	0.54	0.45	

BSA



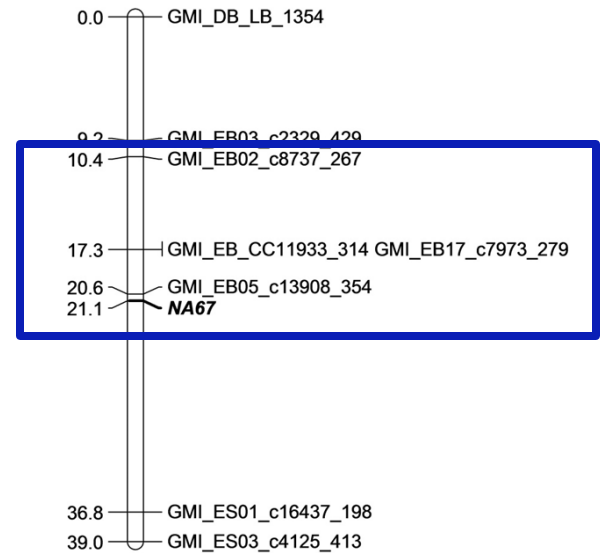
12D

OT7030 × Leggett



Differentials	Weaver	Stainless	Leggett	OT7030	Pg6/7	Pg8	Pg9	Pg10	Pg12	Pg13	Pg15	Pg16	Pga	Alpha	Omega	Wisc
GMI_ES02_c8737_267	A	B	A	B	-	-	B	A	B	A	A	B	B	-	B	A
GMI_ES_CC11933_314	A	B	A	B	-	A	A	B	B	B	A	A	B	B	B	B
GMI_ES17_c7973_279	A	B	A	B	A	A	A	B	B	A	A	A	B	B	B	B
GMI_ES05_c13908_354	A	B	A	B	A	A	A	A	B	A	A	A	B	B	B	A
NA67	A	B	A	B	B	A	A	A	B	A	A	B	B	B	B	B

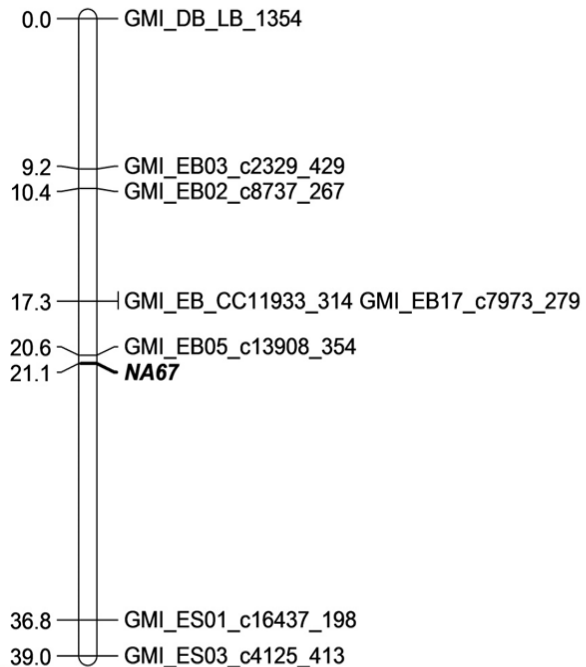
12D
OT7030 × Leggett



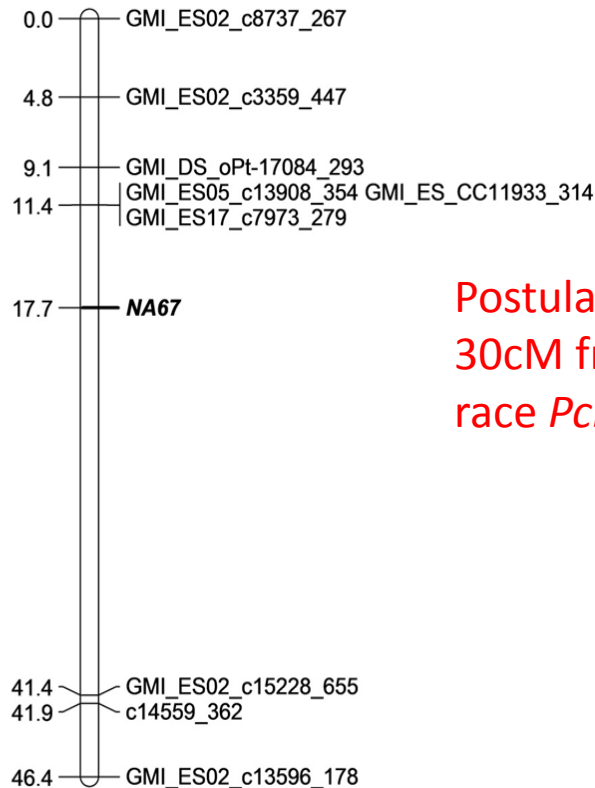
CDC Weaver × Stainless

Race	Populations	Gen	Total	Res	Sus	Seg	ER	χ^2	P Value
NA67_GH_WP	CDC Weaver × Stainless (2013)	P1	30		30				
		P2	30	30					
		F6:7	99	46	28	25	1:1	4.37	0.036

12D
OT7030 × Leggett



12D
CDC Weaver × Stainless



Postulated gene is approximately 30cM from the new crown rust race *PcKM*

Closing Thoughts

- 6K oat SNPs are great genetics tools
 - Close markers are not always diagnostic
 - Causes:
 - Emphasis on polymorphic SNPs in variety panel
 - Good for genetics though
 - Variety panel may not have varied for gene of interest

Marker	Position (cM)	Lines with Gene					Lines without Gene				
		1	2	3	4	5	6	7	8	9	10
SNP 1	0	A	A	A	A	A	A	B	A	B	B
SNP 2	1	A	A	A	A	A	B	B	B	B	B
Gene	2	A	A	A	A	A	B	B	B	B	B
SNP 3	2	A	B	A	B	A	B	B	B	B	B
SNP 4	4	A	A	B	A	A	B	A	B	B	A



Closing Thoughts

- Association mapping & genome-wide selection
 - Excellent markers from winter oats might not work in spring oat
- Targeted SNP development
 - SNP database
 - Synteny: brachypodium, rice, barley, wheat
 - Order oat gene-based SNPs on reference sequence (genome zipper)
 - Exome capture sequencing paired with BSA
 - Roche NimbleGen SeqCap EZ Exome



Summary

- SNP Markers for:
 - *Pc91*
 - *Pc94*
 - MN841801 adult plant crown rust resistance
 - *Pc68*
 - *PcKM*
 - NA67 stem rust resistance



Future Work

- All the resistant genes (stem rust and crown rust) should be assigned to the oat chromosome
- Demonstrate *Pg13* and *Pc91* are linked in coupling in CDC Morrison
- More research needed on *Pga*, *Pg2*
- Other crown rust resistance genes



Publications

- Chromosome location and allele-specific PCR markers for marker-assisted selection of the oat crown rust resistance gene *Pc91*. *Molecular Breeding*, 32 : 679-686, 2013.
- A major quantitative trait locus conferring adult plant partial resistance to crown rust in oat. *BMC Plant Biology* (Submitted), 2014.
- Oat, In A. Pratap and J. Kumar (eds.), *Alien Gene Transfer in Crop Plants, Volume 2: Achievements and Impacts*, DOI 10.1007/978-1-4614-9572-7_3, Springer, 2014. (**Book Chapter**)
- Oat Fungal Diseases and the Application of Molecular Marker Technology for Their Control, In A. Goyal and C. Manoharachary (eds.), *Future Challenges in Crop Protection Against Fungal Pathogens, Fungal Biology*, DOI 10.1007/978-1-4939-1188-2_12, Springer, 2014. (**Book Chapter**)

In preparation

- Molecular mapping and validation of an allele-specific marker for the *Pc94* oat crown rust resistance gene.
- Genetic analysis and molecular mapping of a seedling crown rust resistance gene (*PcKM*) in oat.
- Molecular mapping and validation of oat crown rust resistance gene *Pc68* using allele-specific PCR markers.
- Identification and mapping of resistance to stem rust gene in oat

CORE

- Consensus map
- Population structure
- **Association mapping (Crown and Stem rust) ????**

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