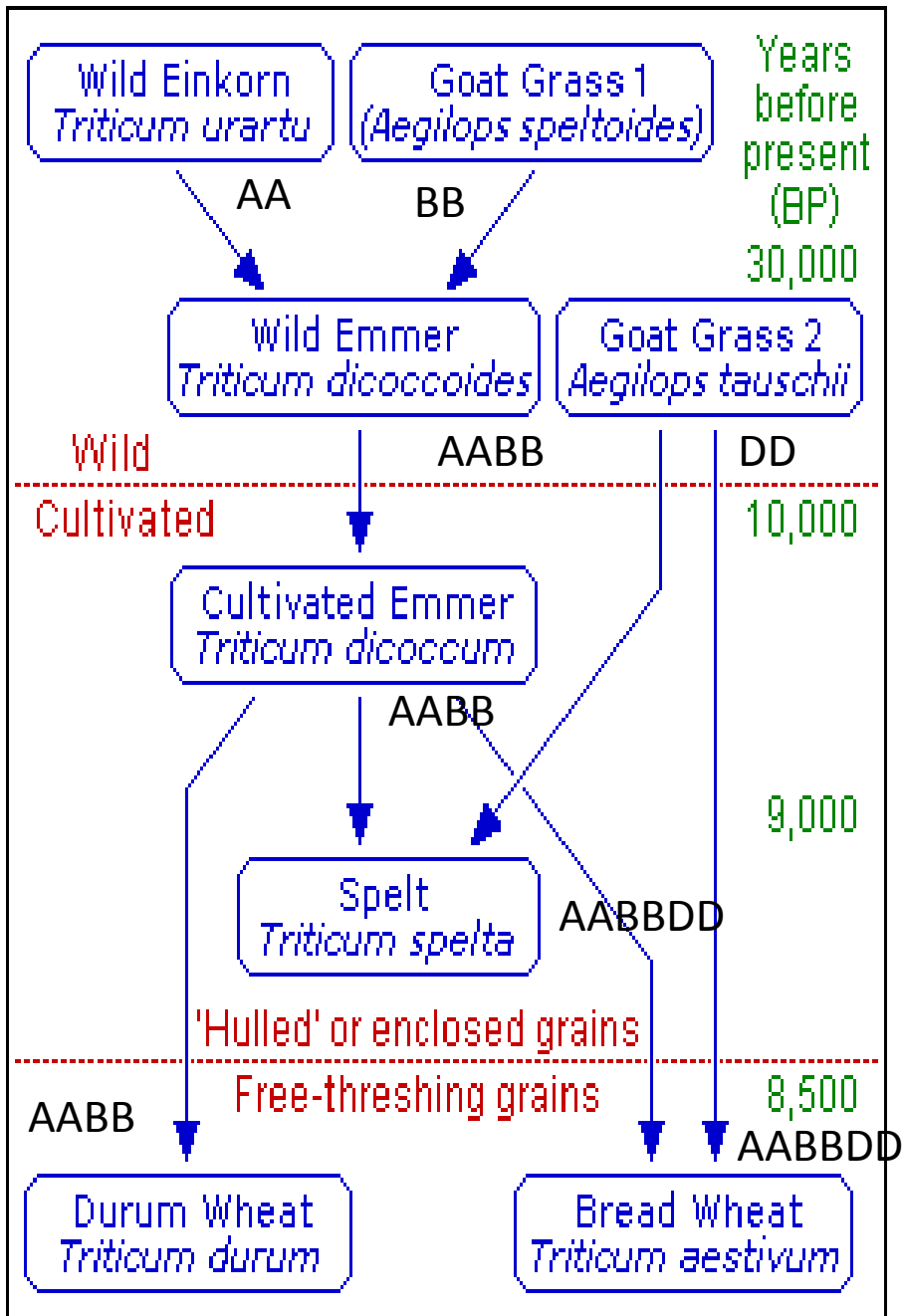


Wheat rust genetics

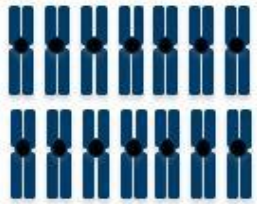


Evolution of Wheat, from the prehistoric Stone Age grasses to Spelt, Durum Wheat and Bread Wheat.

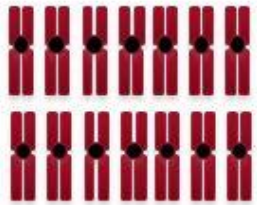
- Wild Einkorn (AA) hybridised with a Goat Grass (BB) at least 30,000 years ago to produce Wild Emmer (AABB). About 10,000 years ago, when this began to be cultivated by hunter-gatherers for food, their **subconscious plant selection** slowly created Cultivated Emmer with larger grains.
- Later, as this became more widely cultivated, it spread into the natural habitat of another wild Goat Grass (DD). Random hybridisations between the Cultivated Emmer (AABB) and the Goat Grass (DD) produced some early forms of hard shelled Spelt (AABBDD).
- Another similar hybridisation occurred later but with a mutation that changed the ears from having the grain enclosed within a hard shell to an ear that would release the grain more easily.
- The hunter-gatherers would naturally have selected this easier threshing form to collect so that, assisted by this human selection, it slowly evolved into free-threshing Bread Wheat.
- Over time, Emmer Wheat also mutated and evolved to create Durum Wheat, now used for making a wide range of pasta products such as macaroni, spaghetti and lasagna.

The Three Wheat Genomes

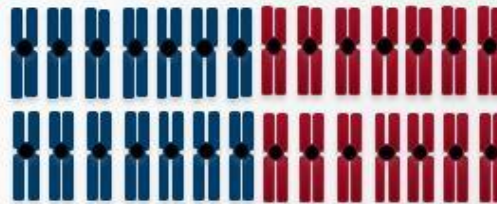
Diploid AA
Triticum urartu



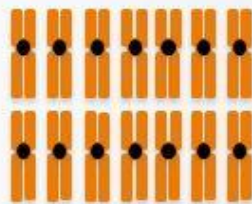
Diploid BB
Aegilops sp.



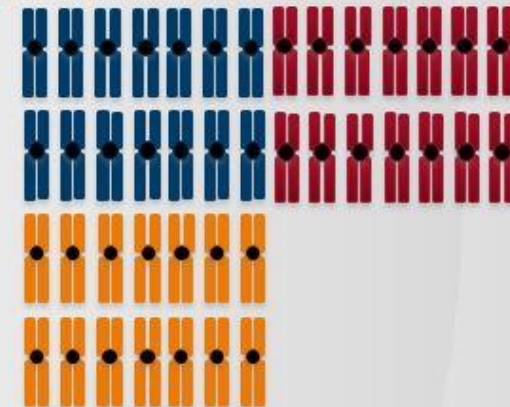
Tetraploid AABB
Triticum dicoccoides



Diploid DD
Aegilops tauschii



Hexaploid AABBDD
Triticum aestivum



The Three Wheat Genomes

Diploid AA
Triticum urartu

Diploid BB
Aegilops sp.

Secondary/
Tertiary
Gene Pool

Tetraploid AABB
Triticum dicoccoides

Primary Gene Pool

Diploid DD
Aegilops tauschii

Hexaploid AABBDD
Triticum aestivum

Bread Wheat



Gene pools of the Triticeae *and gene transfer from wild relatives*

Primary Gene Pool:

Progenitor species with homologous genomes. These cross easily with common wheat.

- *Ae. tauschii* **DD**
- *T. turgidum* **AADD**
- Landraces **AABBDD**

Secondary Gene Pool:

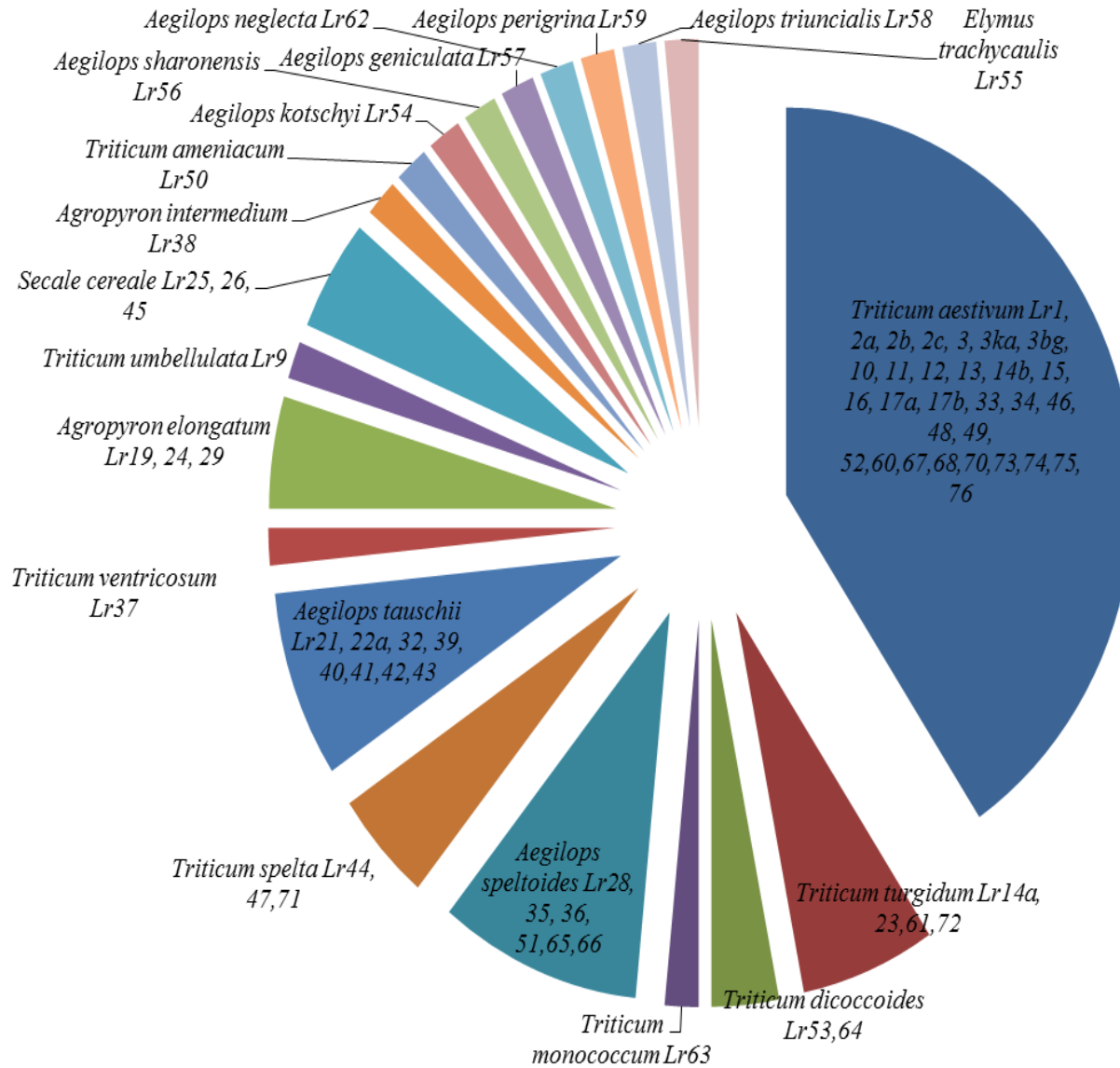
Progenitor species with homoeologous genomes related to A, B, and D. These cross with some difficulty with common wheat.

- *T. monococcum* **AA**
- *T. urartu* **A^uA^u**
- *T. timopheevii* **AAGG**
- *Ae. speltoides* **SS**
- D-genome *Aegilops*

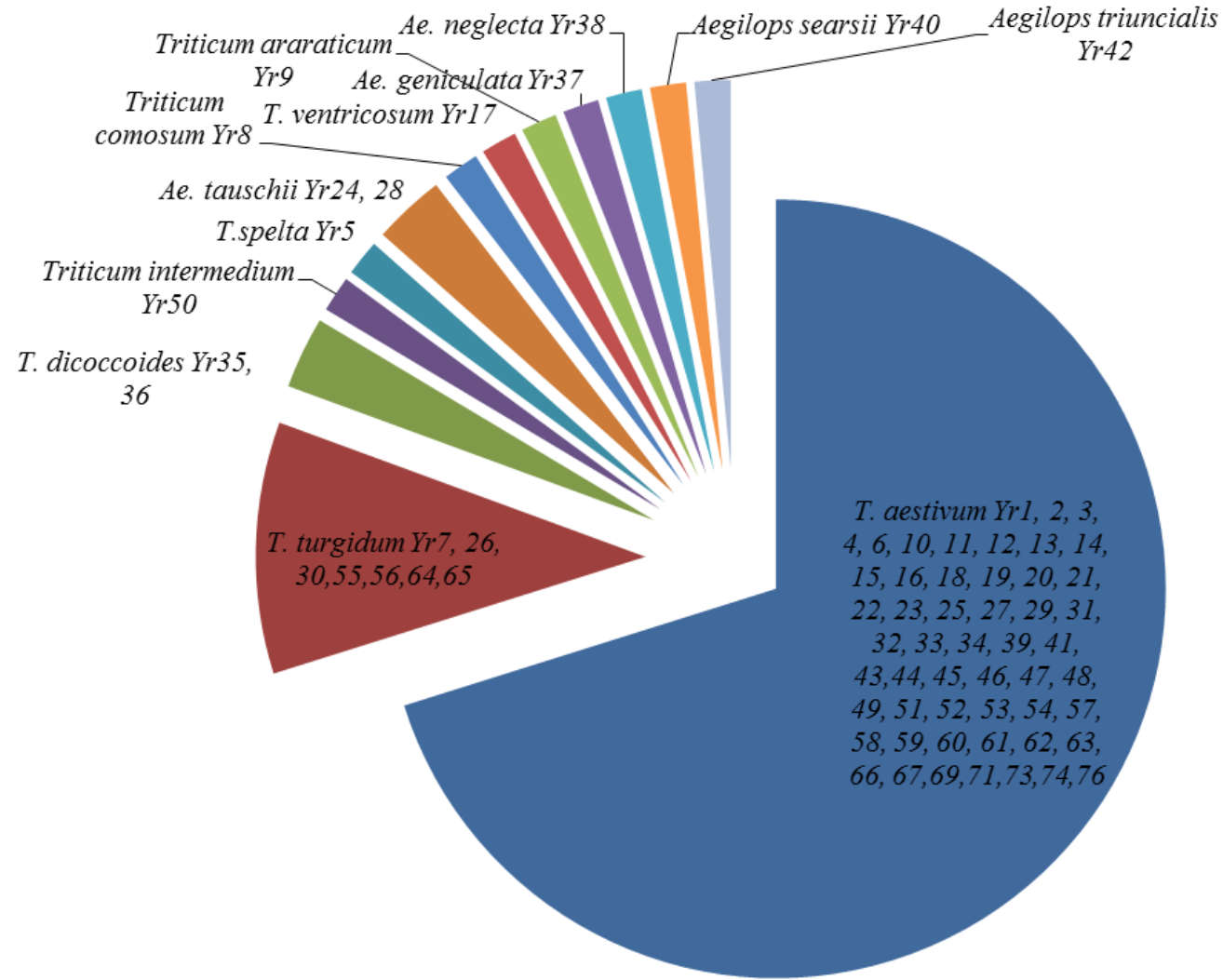
Tertiary Gene Pool:

Triticeae species with homoeologous genomes **not** closely related to A, B and D diploid and polyploid *Aegilops* species, *Thinopyrum*, *Secale*, and *Hordeum*. These cross only with considerable difficulty with common wheat.

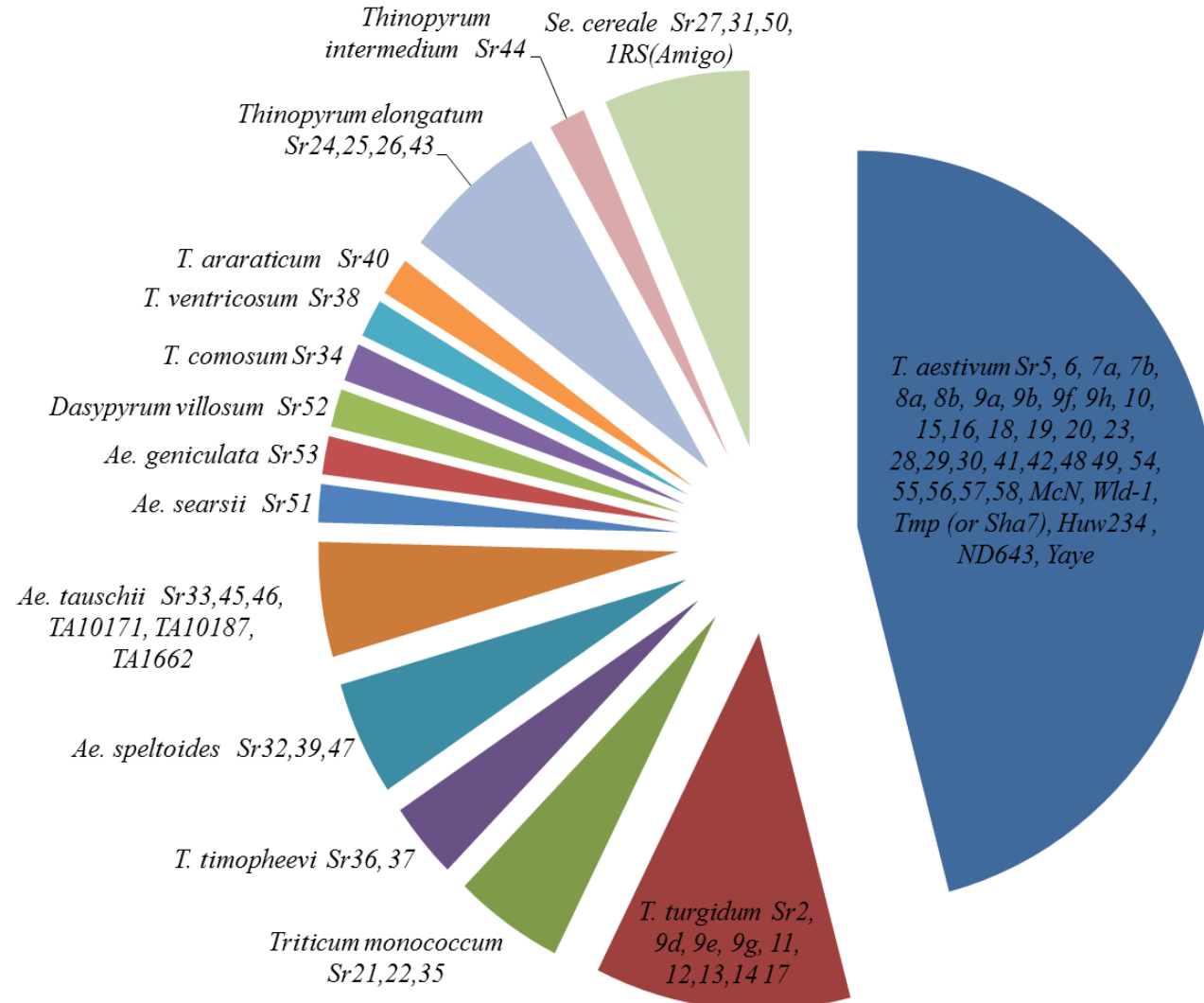
Characterized Leaf rust resistance genes



Characterized Stripe rust resistance genes



Characterized Stem rust resistance genes



Resistance genes for Ug99 race group (APR and all-stage)

- Genomic regions for APR and other genes in context of Ug99 stem rust were published by Yu et al. (2014)
- Total number of resistance loci:
 - A genome with 37 loci [Origin: *T. urartu*]
 - B genome with 86 loci [Origin: *Ae. speltoides*]
 - D genome with 18 loci [Origin: *Ae. tauschii*]
- Hotspots of resistance loci across the genome:
 - QTL: 3BS (19), 6BS (9), 5BL (9), and 2BL (7)
 - Qualitative genes: 3BS, 5BL, and 2BL

Yu et al. (2014) Theoretical & Applied Genetics, DOI
[10.1007/s00122-014-2326-7](https://doi.org/10.1007/s00122-014-2326-7)

A consensus map for Ug99 stem rust resistance loci in wheat.

The genomes of wheat:

- **Common wheat ($2n=6x=42$ chromosomes)**

- **AABBDD**

- 16 billion base pairs organized in 21 pairs of chromosomes

- Vs. humans with 3.2 billion bp
 - Vs. maize with 2.5 billion bp
 - Vs. rice with 430 million bp

**It's
HUGE!**

- **Durum wheat ($2n=4x= 28$ chromosomes)**

- **AABB**

- 10 billion base pairs organized in 14 pairs of chromosomes

- ***Triticum monococcum* ($2n=2x=14$ chromosomes)**

- **AA**

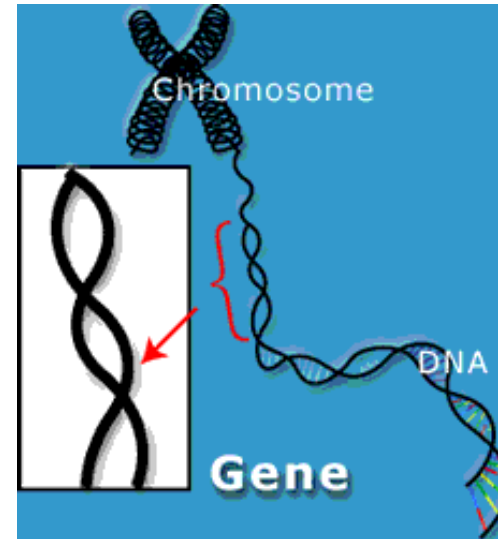
- 5 billion base pairs organized in 7 pairs of chromosomes

Why study genetics

- For breeders to use resistance it helps to know something about the **resistance phenotype** and how it is **inherited**
 - **how many genes**
 - **are they dominant**
 - **do they interact**
 - **do they work against all races**
 - **How much protection do they provide**

Genetics terms you need to know:

- **Gene** – a unit of heredity; a section of DNA sequence encoding a single protein
- **Genome** – the entire set of genes in an organism



- **Alleles** – two genes that occupy the same position on homologous chromosomes and that cover the same trait (like 'flavors' of a trait).
- **Locus** – a fixed location on a strand of DNA where a gene or one of its alleles is located.

- **Homozygous** – having identical genes (one from each parent) for a particular characteristic.
- **Heterozygous** – having two different genes for a particular characteristic.

- **Dominant** – the allele of a gene that masks or suppresses the expression of an alternate allele; the trait appears in the heterozygous condition.
- **Recessive** – an allele that is masked by a dominant allele; does not appear in the heterozygous condition, only in homozygous.

- **Genotype** – the genetic makeup of an organisms
- **Phenotype** – the physical appearance of an organism (Genotype + environment)

- **Monohybrid cross:** a genetic cross involving a single pair of genes (one trait); parents differ by a single trait.
- **P** = Parental generation
- **F₁** = First filial generation; offspring from a genetic cross.
- **F₂** = Second filial generation of a genetic cross

Classical gene-for-gene theory

RR = homozygous resistant ; Rr = heterozygous resistant; rr = homozygous susceptible
AVR AVR = homozygous avirulent; AVR avr = heterozygous avirulent; avr avr = homozygous virulent

		HOST GENOTYPE	
		RR or Rr	rr
PATHOGEN GENOTYPE	AVR avr or AVR AVR	Disease-resistant	Susceptible to disease
	avr avr	Susceptible to disease	Susceptible to disease

“For every resistance gene in the host there is a corresponding gene for avirulence in the pathogen conferring resistance and vice versa”- H. Flor 1942.
Phytopathology

Genetics of Resistance

- **Types of crosses**
 - R x S or S x R
 - R x R – tests of allelism
- **What generations to study**
 - F1, F2, F3,.....
 - BC, TC F1 or F2
- **Homozygous lines – DH, SSD, RIL**
- **Others??**

Genetics of Resistance

- No. of individuals or lines
- No. of individuals within lines
- What pathotypes?
- Should pathogen cultures be absolutely pure?

Interpreting Genetic Data

- Where to partition between R & S
- Hypothesis making
- Testing the hypothesis
- Validation of the hypothesis
 - progeny testing;
3 : 1 becomes 1 : 2 : 1
 - larger populations
 - more crosses

Independent Segregation at Two Loci

With selfing - by phenotype

- **15:1 or 9:3:3:1**

- **by genotype**

- **15:1 or 1:2:1:2:4:2:1:2:1**

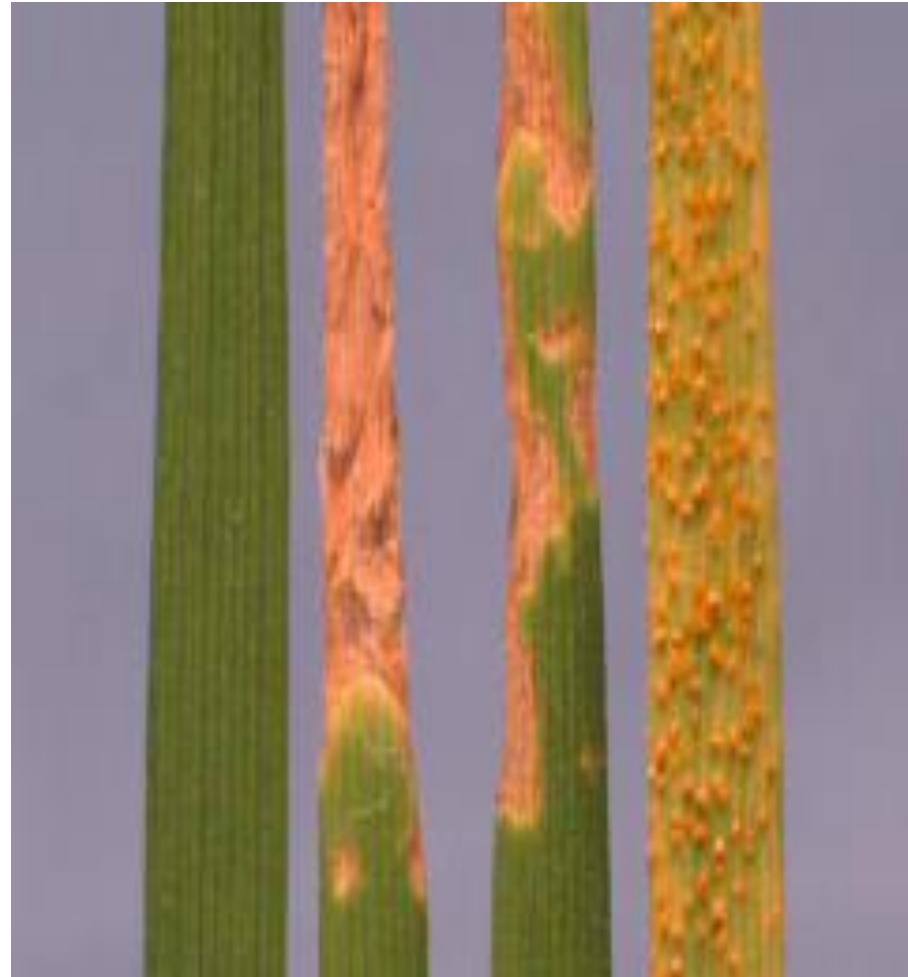
With testcrossing – by phenotype

- **3:1 or 1:1:1:1**

- **by genotype**

- **3:1 or 1:1:1:1**

If low infection types conferred by two genes are different then genetic ratios can be subdivided



The interaction of genes may be of following types

- 1) Two gene pairs affecting same character – 9:3:3:1
- 2) Epistasis, one gene hides effect of other
 - a) Recessive Epistasis - 9:3:4
 - b) Dominant epistasis - 12:3:1
- 3) Complementary genes - 9:7 (2 genes responsible for production of a particular phenotype)
- 4) Duplicate genes – 15:1 (same effect given by either of two genes)

Genetics of rust resistance

Both Qualitative and Quantitative genetic variation exist for rust resistance

Qualitative vs Quantitative resistance

Qualitative resistance

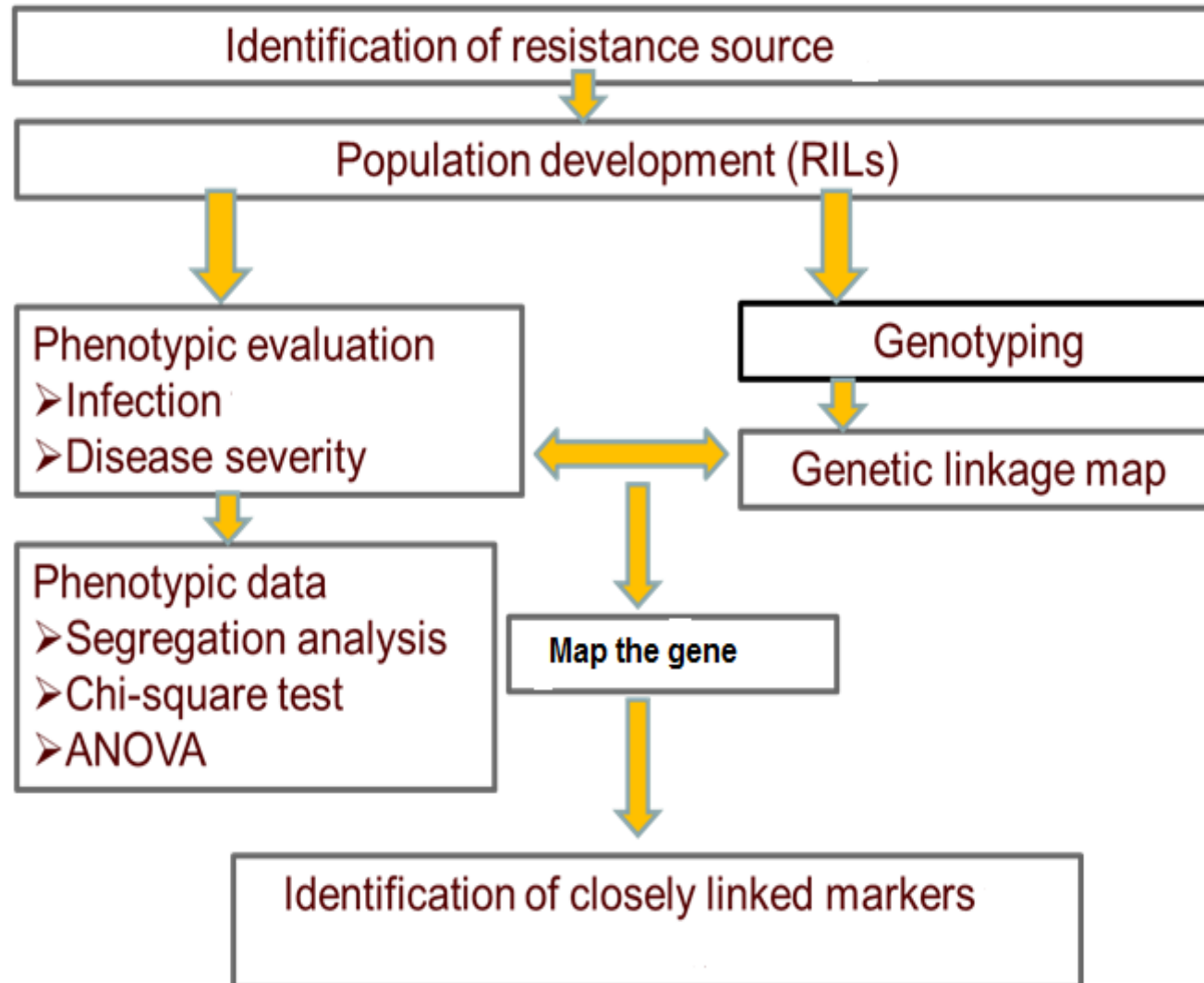
- Based on gene-for-gene interaction
- Phenotypes can be classified as binary trait (R vs S)
- Hypersensitive reaction is visible (clear necrotic/chlorotic area around the infection point)
- Also called race specific, seedling or all stage resistance

Qualitative vs Quantitative resistance

Quantitative resistance

- Interaction between host and pathogen usually governed by multiple minor genes but amount of infection (severity) can be QUANTIFIED
- Phenotypes can be observed as continuous distribution (% disease severity)
- No hypersensitive reaction
- Broadly: also called as race non-specific, adult plant resistance (APR)

Characterizing new genes



Resistance source

- CIMMYT Line: ND643/2*Weebil carries moderately effective all stage resistance against Ug99
- Postulated as *SrND643* (resistance is derived from ND643, a spring wheat from NDSU, USA)
- ND643/2*Weebil is common in new CIMMYT lines
- *SrND643* shows:
 - ❖ Seedling IT: 2 to 2+ with TTKSK
 - ❖ Field: 5-20 MR or M

Materials and Methods

Phenotypic evaluation:

1. Population:

❖ 150 F_{4:5} Recombinant inbred lines (RILs) derived from Cacuke and ND643/2*Weebil

2. SR evaluation (Inoculated rust nurseries):

❖ KARI, Njoro, Kenya (Main and Off-seasons, 2010)

3. Disease scores:

❖ Disease severity (DS) : 0-100% visual score

❖ Infection response (IT): R, MR, M, MS, S

Phenotypic evaluation

Strategy for single gene mapping:

- Mendelize the phenotypic trait into discrete classes
- R-type: RILs with R, MR or M response
- S-type: RILs with MS or S response
- All the RILs has phenotype of R or S (binary trait)
- Chi-Square test for 1:1 segregation

Molecular Marker analysis

Selective genotyping:

- Extract DNA from 22 R-type and 22 S-type RILs
- Genotype with DArT markers (629 polymorphic)
- 12 DArT markers (Chr 4A) showed significant association with *SrND643* locus

Molecular Marker Analysis

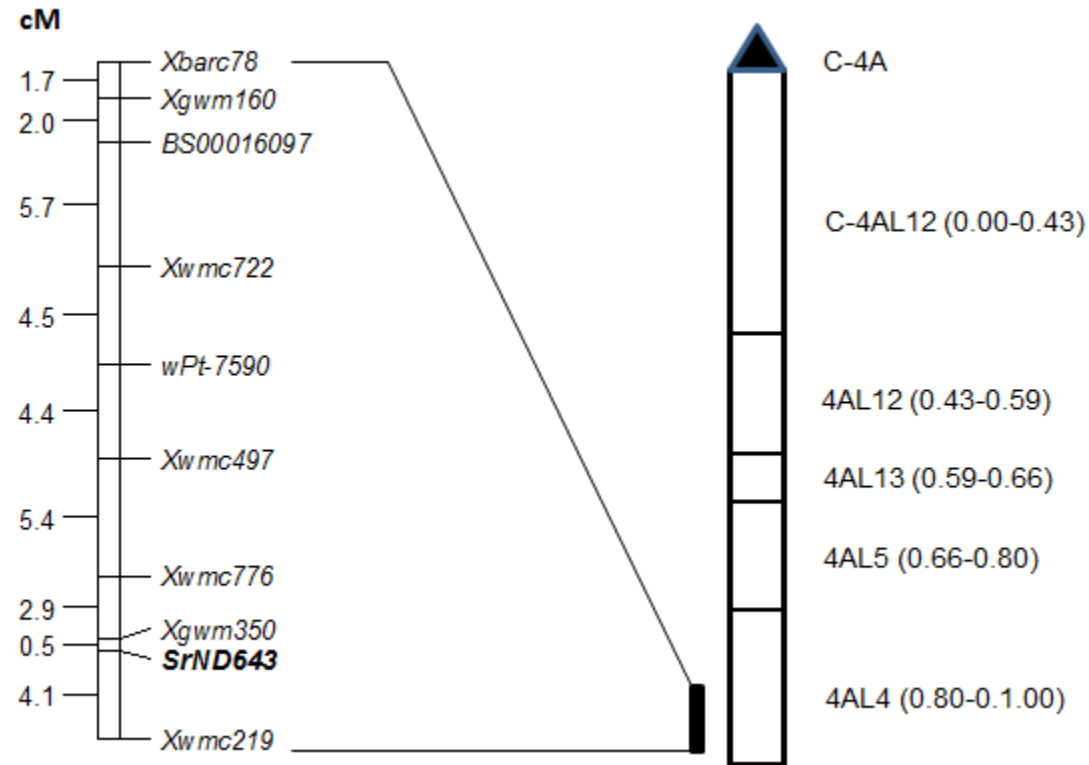
Bulk Segregant analysis (BSA):

- Equal amount of DNA from 10 R-type and 10 S-type RILs mixed to prepare R and S bulks
- Two parents and bulks screened for 48 SSR and 4 SNP markers on chromosome 4A
- 7 SSR and 1 SNP marker were clearly polymorphic between parents and bulks

Genetic Mapping

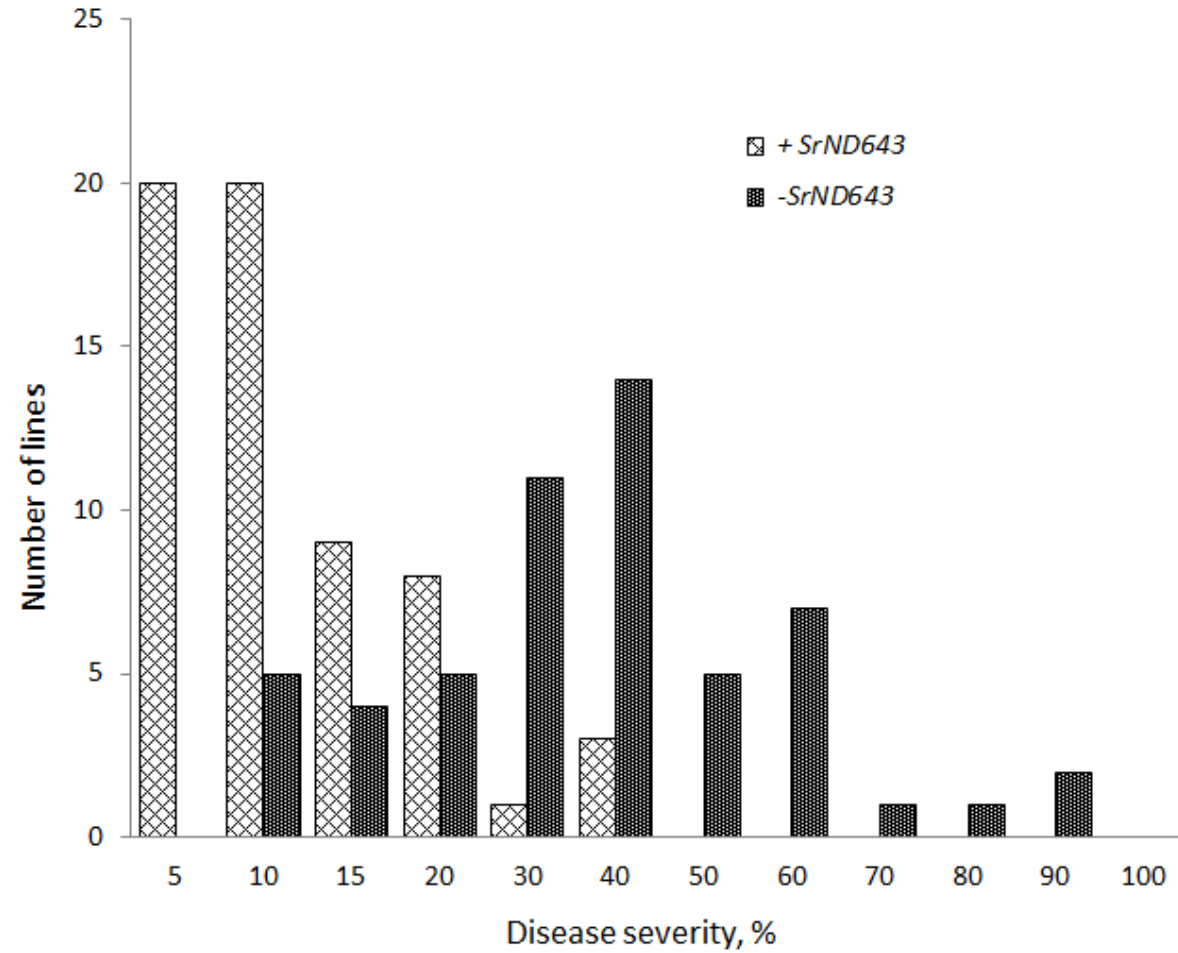
- All the polymorphic markers were genotyped in whole population
- Linkage mapping carried out using inclusive composite interval mapping (ICIM) software
- Kosambi mapping function

Genetic Mapping



Genetic position of *SrND643* on partial linkage map of 4AL (to the left) with corresponding bin in physical bin map (to the right). The genetic distances between the markers are given in centimorgans.

Effect of SrND643 in Disease severity



Validation of Molecular markers for MAS

- About 50 advanced from CIMMYT nurseries, SRRSN, IBWSN, HRWSN
- All of them carry ND643 in the pedigree
- Evaluated in the seedling or field condition against TTKSK (Ug99)
- All lines were genotyped with SrND643 flanking markers (*Xwmc219* and *Xgwm350*)
- Predictability of Markers were compared

Genetics of slow rusting resistance in the field

F1 generation

**Rust progress on F1 plants
compared to the resistant and
susceptible parent**

Genetics of slow rusting resistance in the field

F2 generation

**Rust rating on individual plants
compared to the resistant and
susceptible parent at appropriate time**

Genetics of slow rusting resistance in the field

F3 or higher generation

**Classification of small plots of individual
F2-plant derived F3 lines along with the
resistant and susceptible parents**

Segregation Ratios (%)

Assumption: single partially dominant gene (R_1) for resistance

Cross:	Susceptible (r_1r_1)	X	Resistant (R_1R_1)
F_1		R_1r_1	Intermediate in resistance
F_2	25 R_1R_1	50 R_1r_1	25 r_1r_1
F_3	25 HR	50 Seg	25 HS
F_4	37.5 HR	25 Seg	37.5 HS
F_5	43.75 HR	12.5 Seg	43.75 HS
F_6	46.875 HR	6.25 Seg	46.875 HS
<i>Doubled</i>			
<i>haploid</i>	50 HR		50 HS

F₃ Segregation Ratios

Genes With Minor/intermediate but Additive Effects on Disease Severity

No. of Genes	Lines (%)			
	HPTR	HPTS	SegI	SegS
2	6.3	6.3	37.5	50.0
3	1.6	1.6	56.3	40.6
4	0.4	0.4	68.0	31.3
5	0.1	0.1	76.2	23.6

HPTR = Homozygous Parental Type Resistant

HPTS = Homozygous Parental Type Susceptible

SegI = Segregating, or intermediate, but no completely susceptible plant

SegS = Segregating with completely susceptible plants

F₅ and F₆ Segregation Ratios

Genes With Minor/intermediate but Additive Effects on Disease Severity

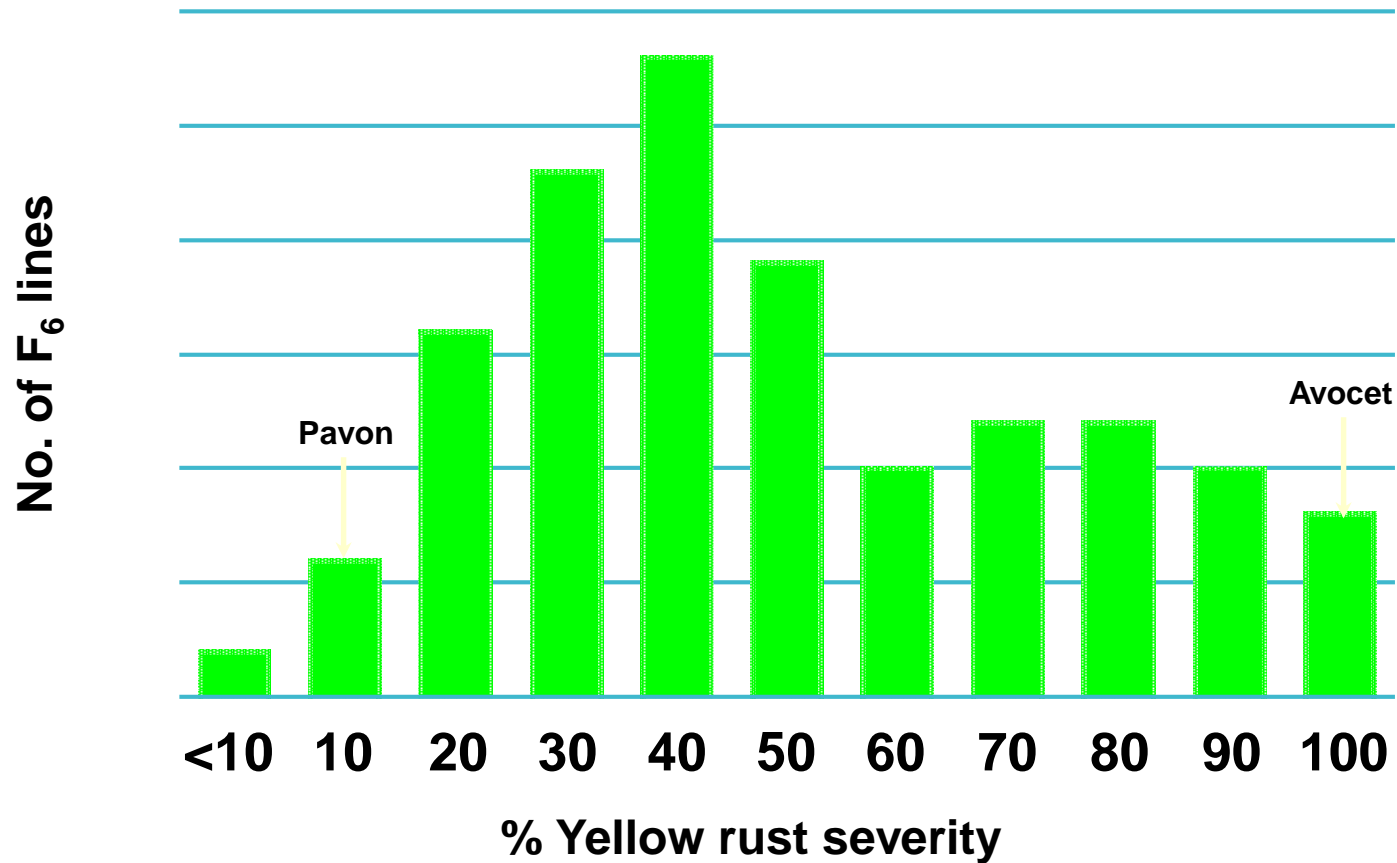
No. of Genes	Generation	Lines (%)		
		HPTR	HPTS	Other
2	F ₅	19.1	19.1	61.8
	F ₆	22.0	22.0	56.0
3	F ₅	8.4	8.4	83.2
	F ₆	10.3	10.3	79.4
4	F ₅	3.7	3.7	92.6
	F ₆	4.8	4.8	90.4
5	F ₅	1.6	1.6	97.4
	F ₆	2.3	2.3	95.4

HPTR = Homozygous Parental Type Resistant

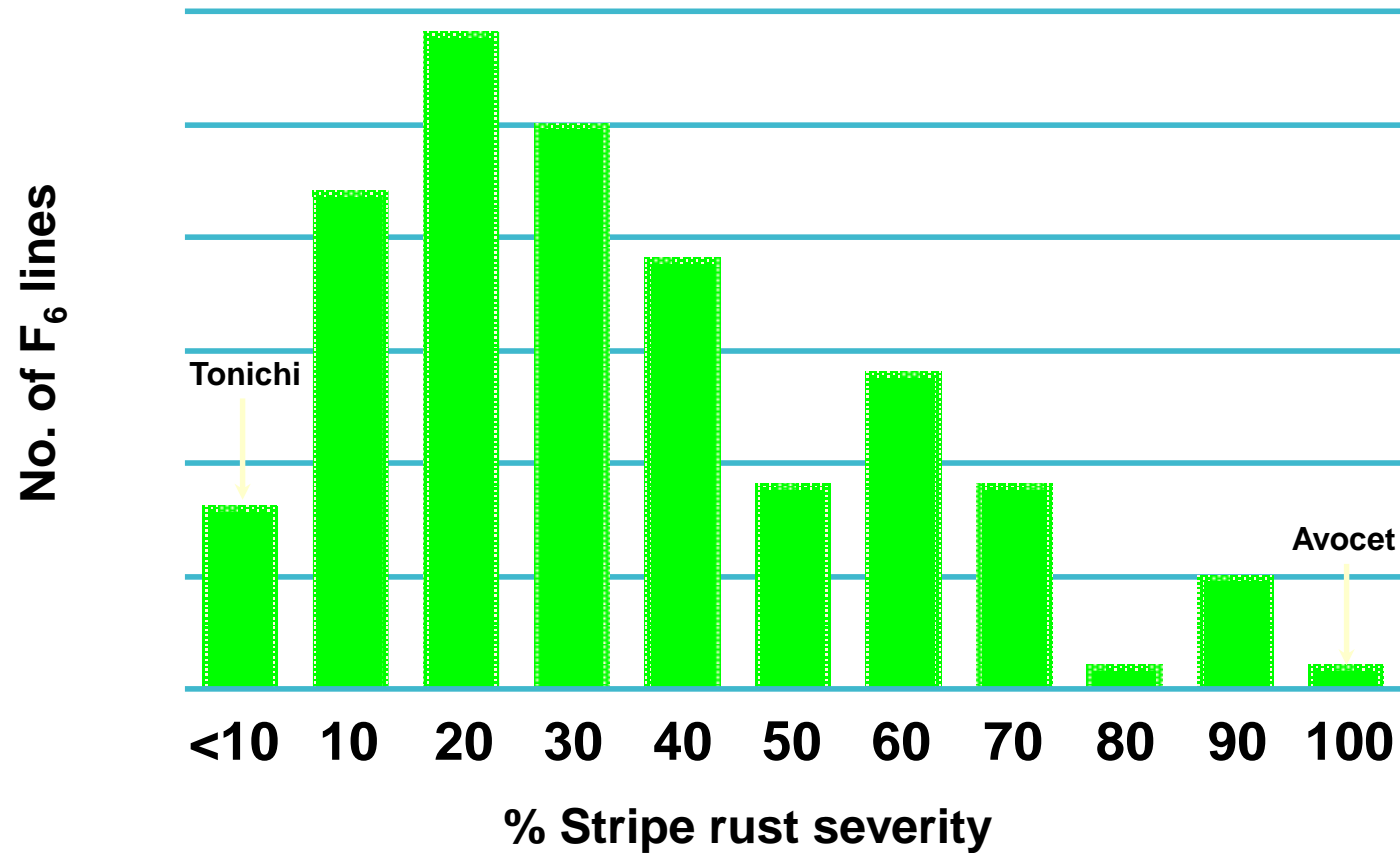
HPTS = Homozygous Parental Type Susceptible

Other = Lines with intermediate levels of disease severities

Distribution of 146 F₆ lines for stripe rust severity in Avocet S/Pavon 76 cross evaluated in field at Toluca, Mexico during 1997 (estimated no. of additive genes = 3)



Distribution of 142 F₆ lines for stripe rust severity in Avocet S/Tonichi 81 cross evaluated in field at Toluca, Mexico during 1997 (estimated no. of additive genes = 4)



Mapping of Adult Plant Resistance to Stem Rust

- 15 RIL mapping populations developed and phenotyped in Kenya for at least 2 seasons
- Genetic studies suggest the involvement of 3 or 4 minor genes in each resistant parent
- Initial molecular mapping of six populations completed
- Other populations being assigned to graduate students

Methodology

- Phenotyping conducted in 2009, 2010 field seasons
- Artificial epidemics with Ug99+Sr24 (TTKST)
- SR severity recorded when 80-100% disease on most susceptible RILs- Modified Cobb scale

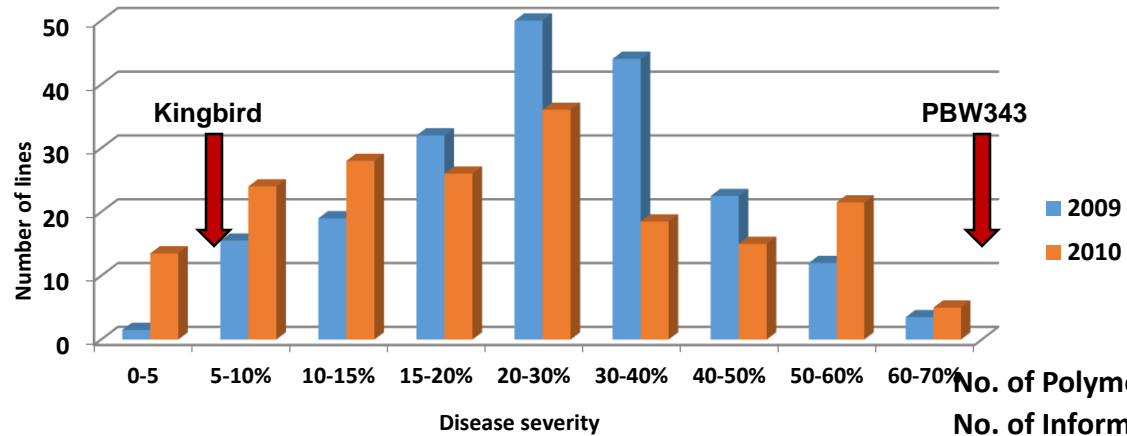


- **DArT and SSR markers**
- **Inclusive Composite Interval Mapping (ICIM), Q-gene, QTL Cartographer**

- **ICIM- <http://www.isbreeding.net/>**

- **Increased power of detection**
- **Additive and Epistatic Effects**
- **LOD Scores > 2.5, Probability in step wise regression 0.001**
- **User friendly**

PBW343 X Kingbird



No. of Polymorphic DArT Markers

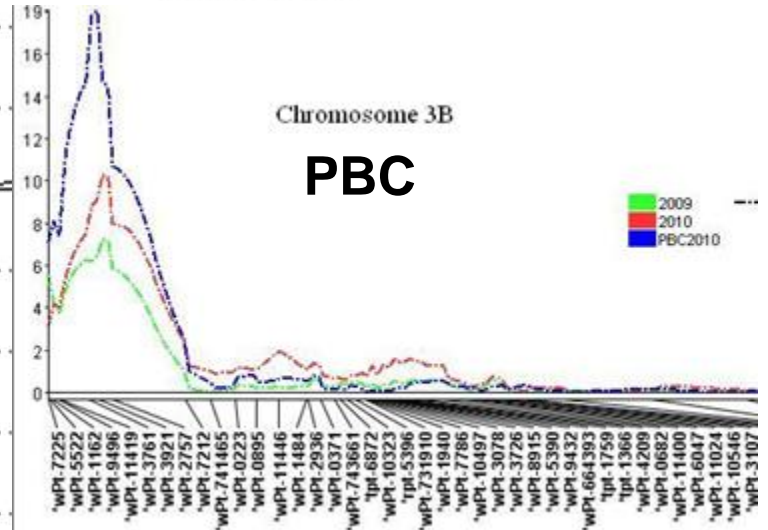
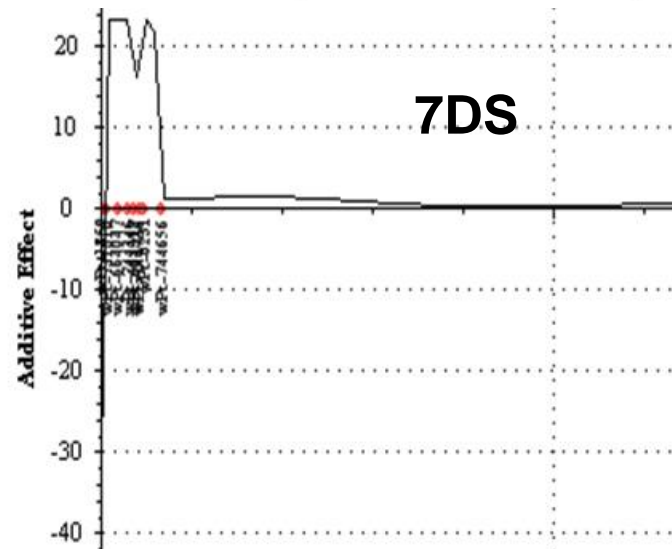
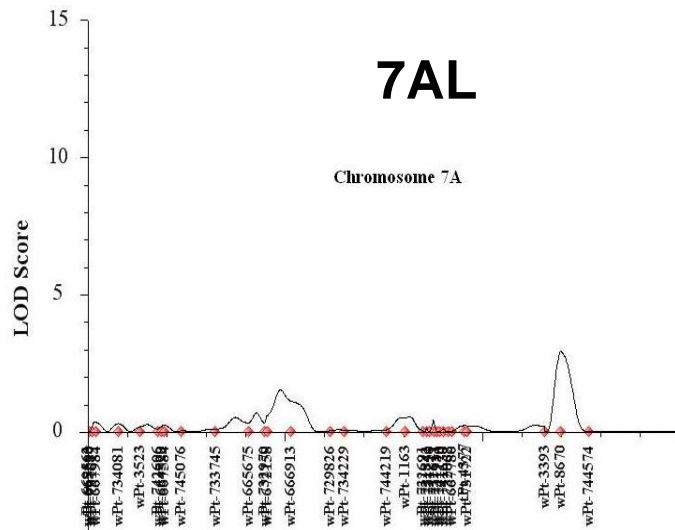
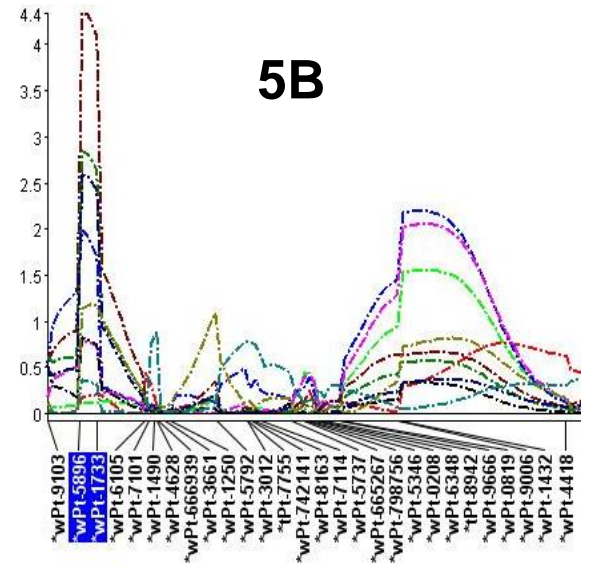
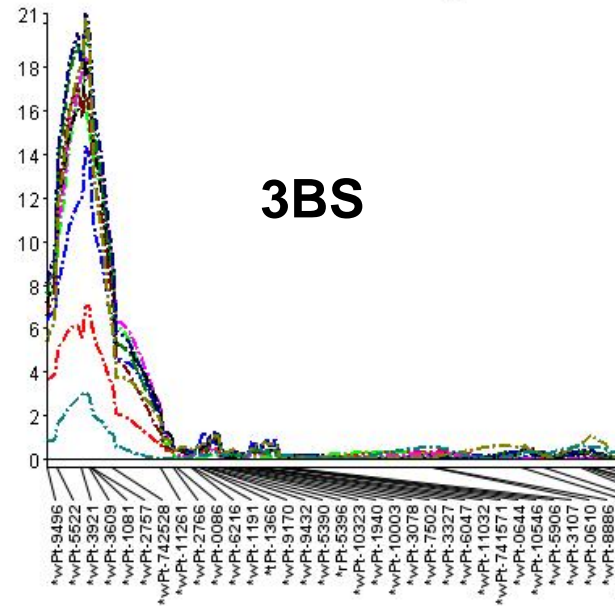
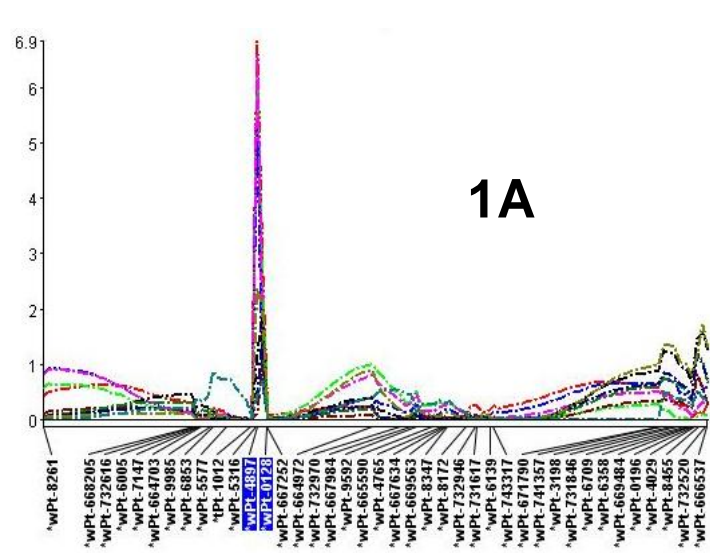
736

No. of Informative DArT Markers

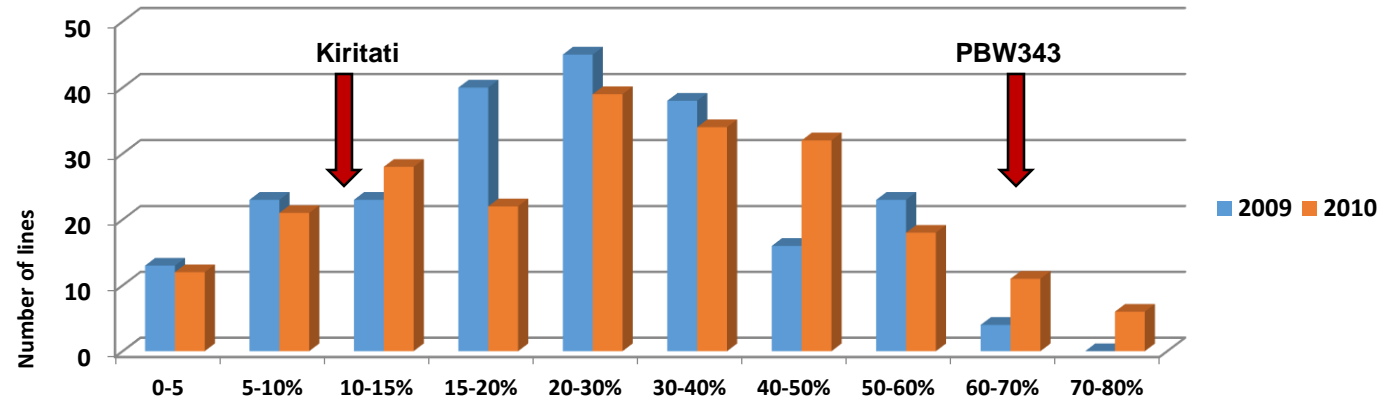
508

Year	Chromosome	Marker Position	Left Marker	Right Marker	LOD	PVE(%)	Est. Add	R ²
2009, 10	1A	251	<i>XwPt-0128</i>	<i>XwPt-4987</i>	6.9	17.5	-11.2	
	3BS	21	<i>XwPt-3921</i>	<i>XwPt-2757</i>	21	41.5	11.0	
	5B	191	<i>XwPt-2607</i>	<i>XwPt-1733</i>	4.4	13.7	5.6	51.2
	7AL	1201	<i>XwPt-8670</i>	<i>XwPt-744574</i>	3.2	10.1	-5.4	
	7DS	0	<i>XwPt-1859</i>	<i>XwPt-731810</i>	3.4	9.3	-31.8	

PBW343 X Kingbird



PBW343 X Kiritati



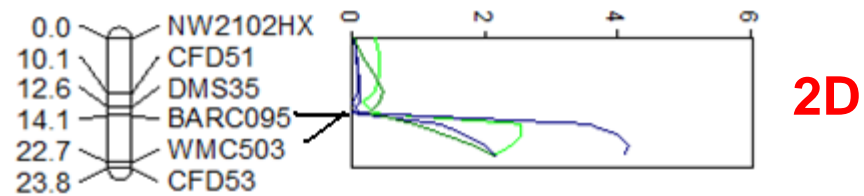
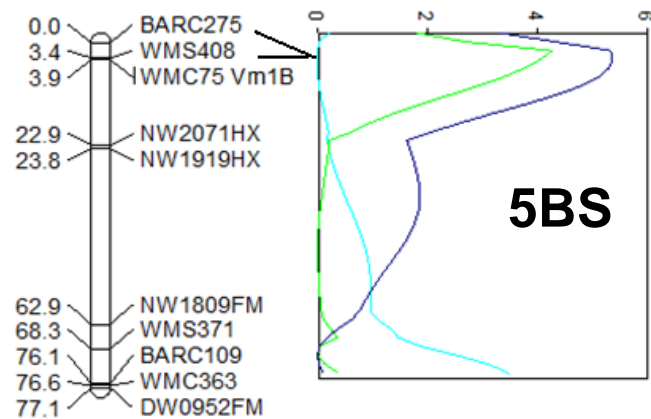
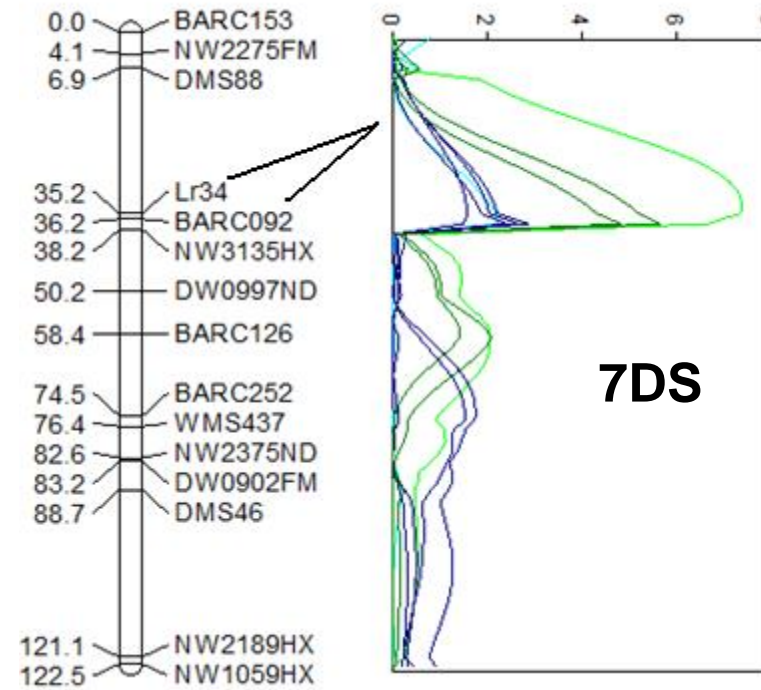
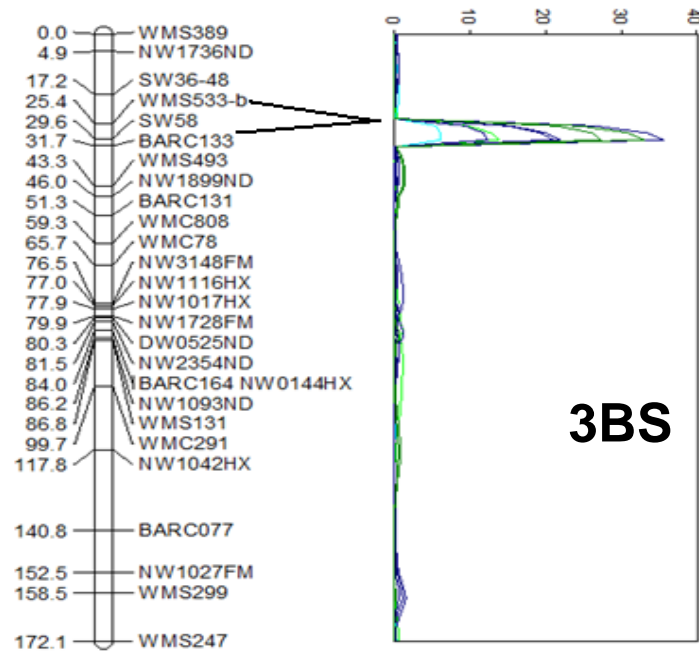
Disease severity

No. of Polymorphic DArT Markers
No. of Informative DArT Markers

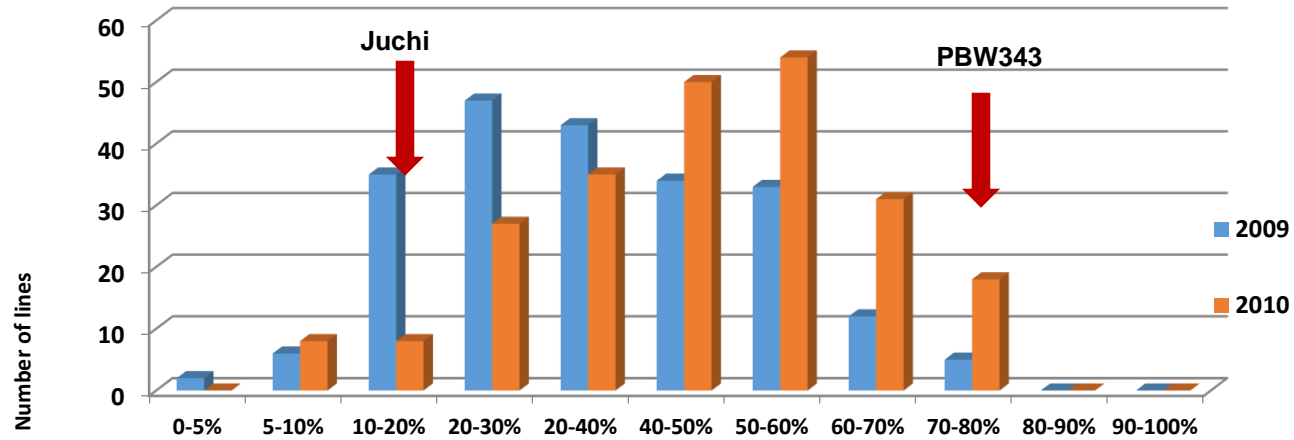
271
271

Year	Chromosome	Marker Position	Left Marker	Right Marker	LOD	PVE(%)	Est.ADD	R ²
2009, 10	2D	20	<i>Xbarc095</i>	<i>Xwmc503</i>	3.6	N/A	-3.7	6.0
	3BS	30	SW58	<i>Xbarc133</i>	17.3	N/A	7.6	25.0
	5BS	76	<i>Xwms371</i>	<i>Xbarc109</i>	5	N/A	3	8.0
	7DS	36	<i>Lr34</i> -linked	<i>Xbarc092</i>	7	N/A	5.3	12.0

PBW343 X Kiritati



PBW343 X Juchi



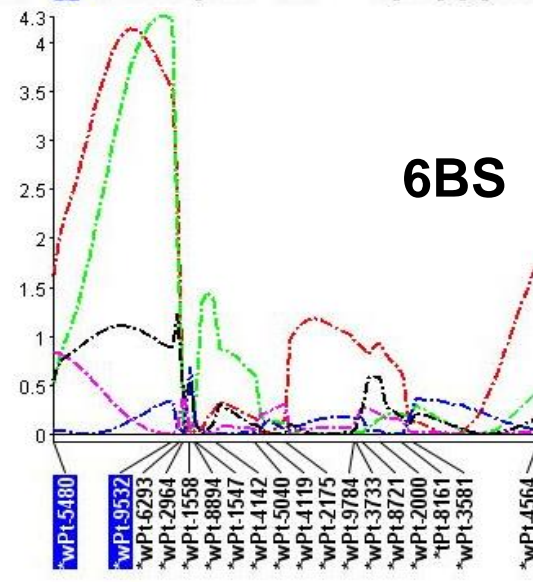
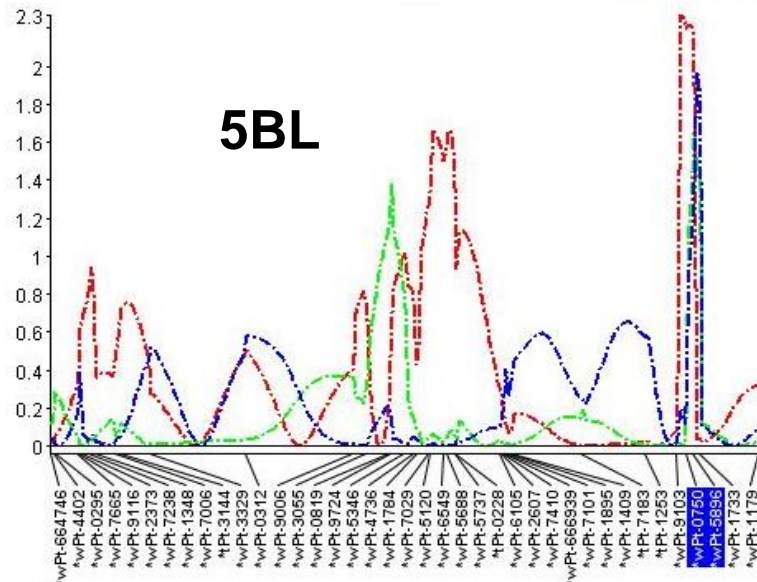
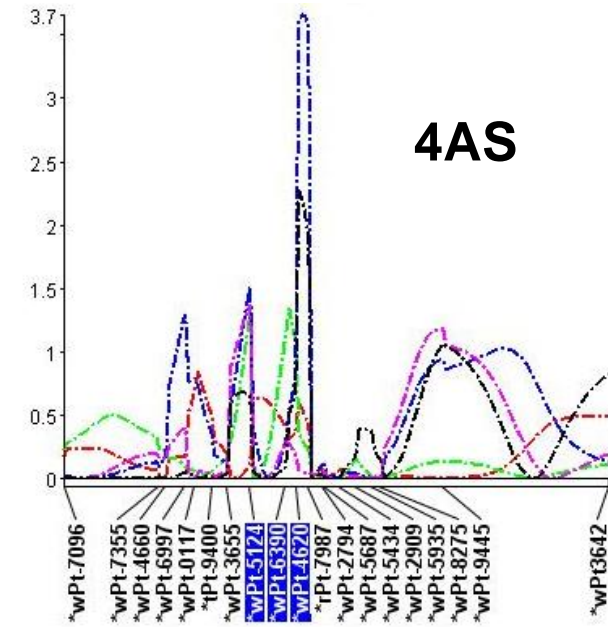
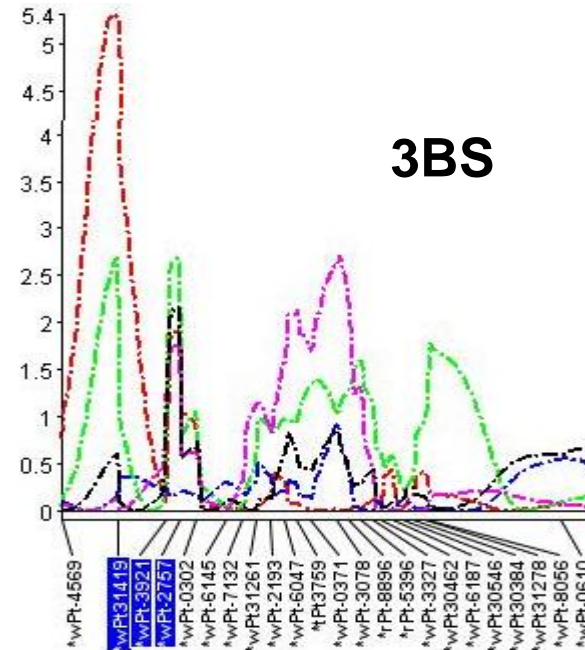
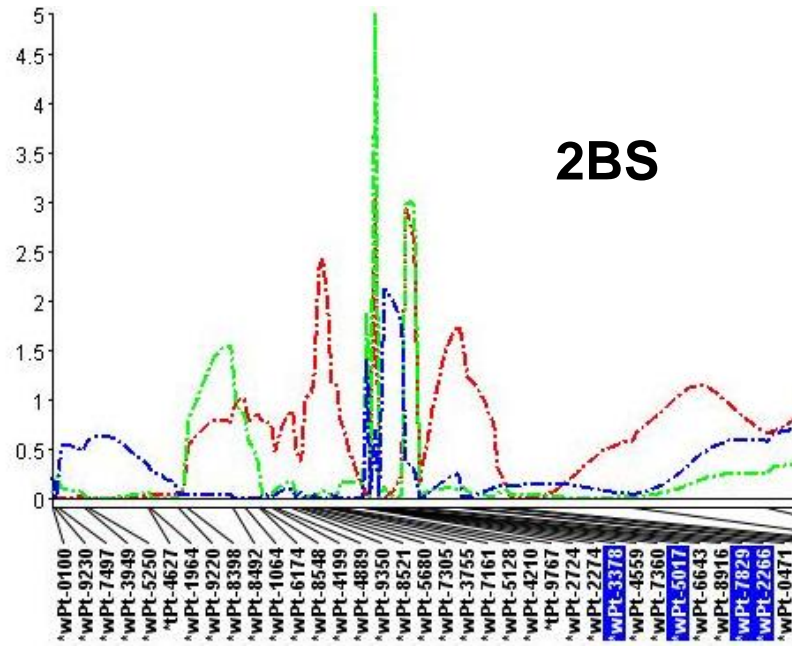
No. of Polymorphic DArT Markers 734

Disease severity

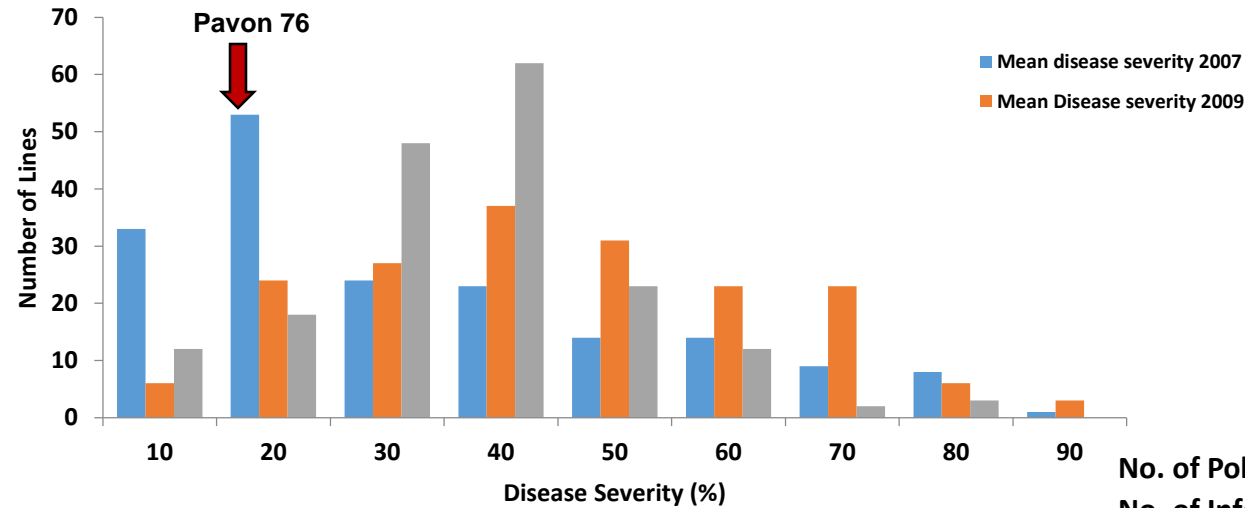
No. of Informative DArT Markers 371

Year	Chromosome	Marker		LOD	PVE(%)	Est. Add	R ²	
		Position	Left Marker					Right Marker
2009, 10	2BS	152	<i>XwPt-7829</i>	<i>XwPt-2266</i>	4.6	16.6	7.5	
	3BS	28	<i>XwPt-8056</i>	<i>XwPt-800213</i>	5.4	8.3	5.1	
	4AS	123	<i>XwPt-5124</i>	<i>XwPt-6390</i>	3.7	12.8	6.9	42.4
	5BL	472	<i>XwPt-0750</i>	<i>XwPt-5896</i>	2.3	16.5	-7.5	
	6BS	21	<i>XwPt-5480</i>	<i>XwPt-9532</i>	4.3	23.5	8.5	

PBW343 X Juchi



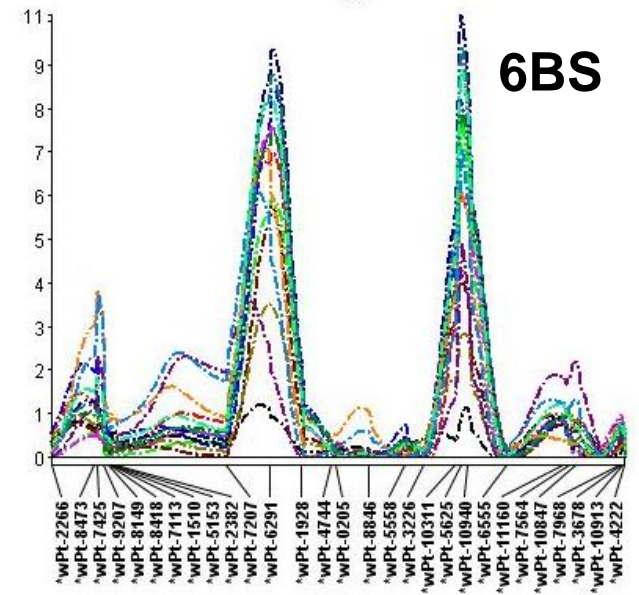
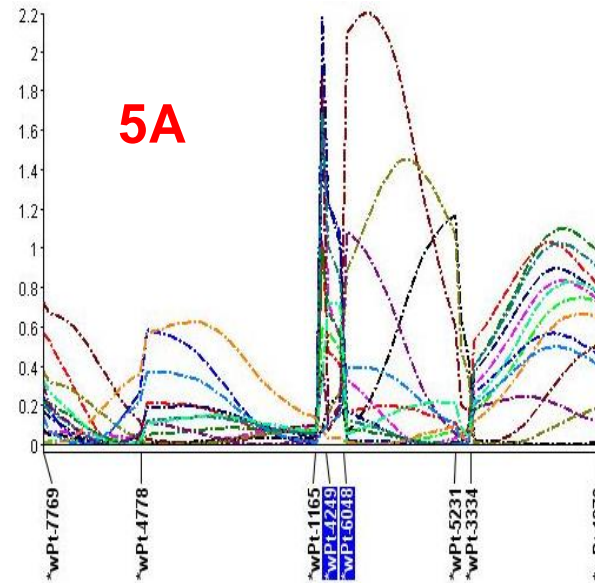
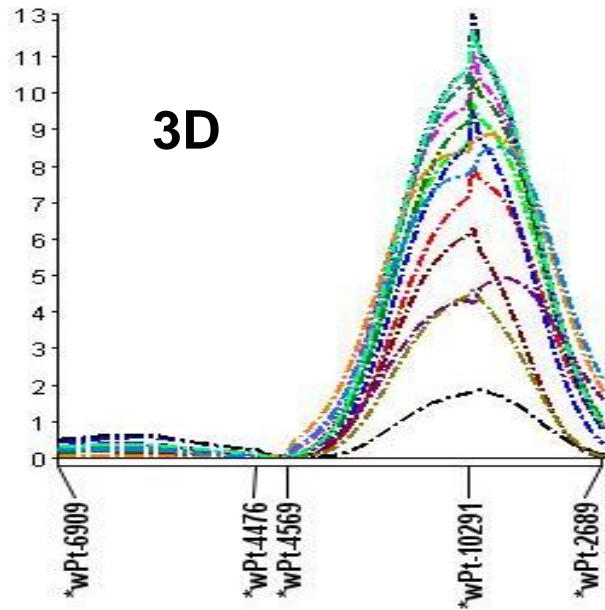
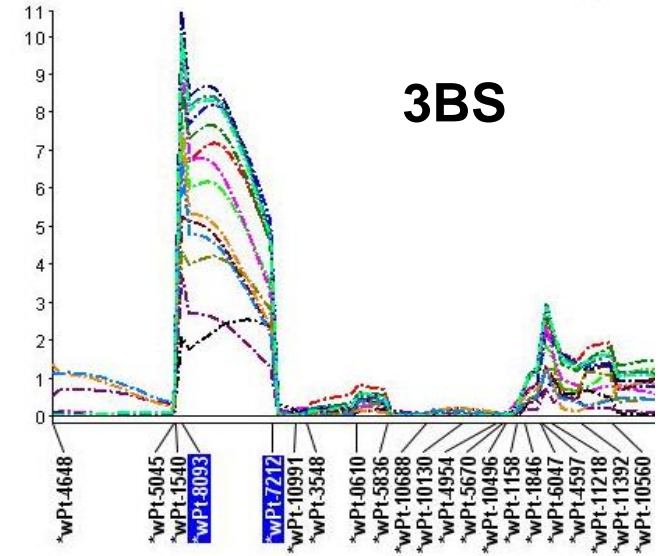
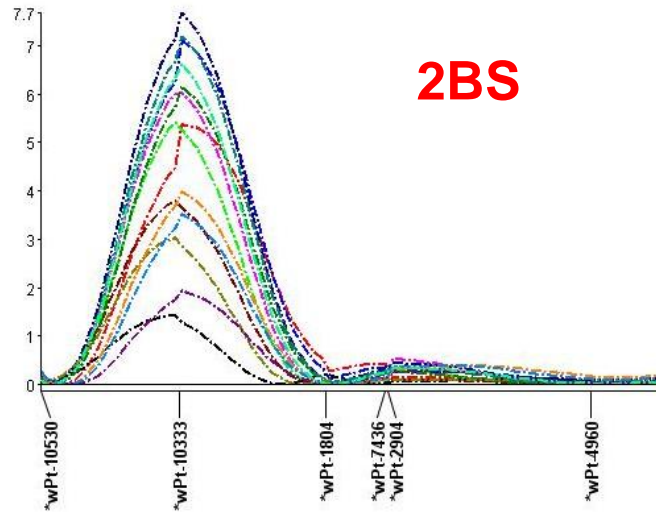
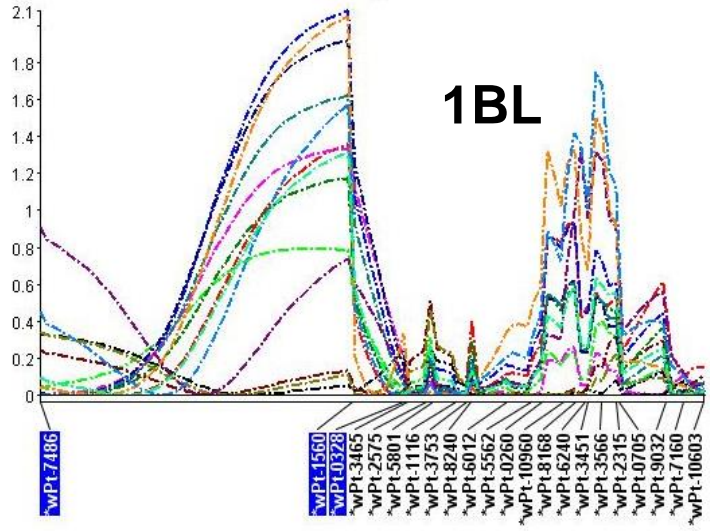
Avocet X Pavon 76 (RILs without Sr26)



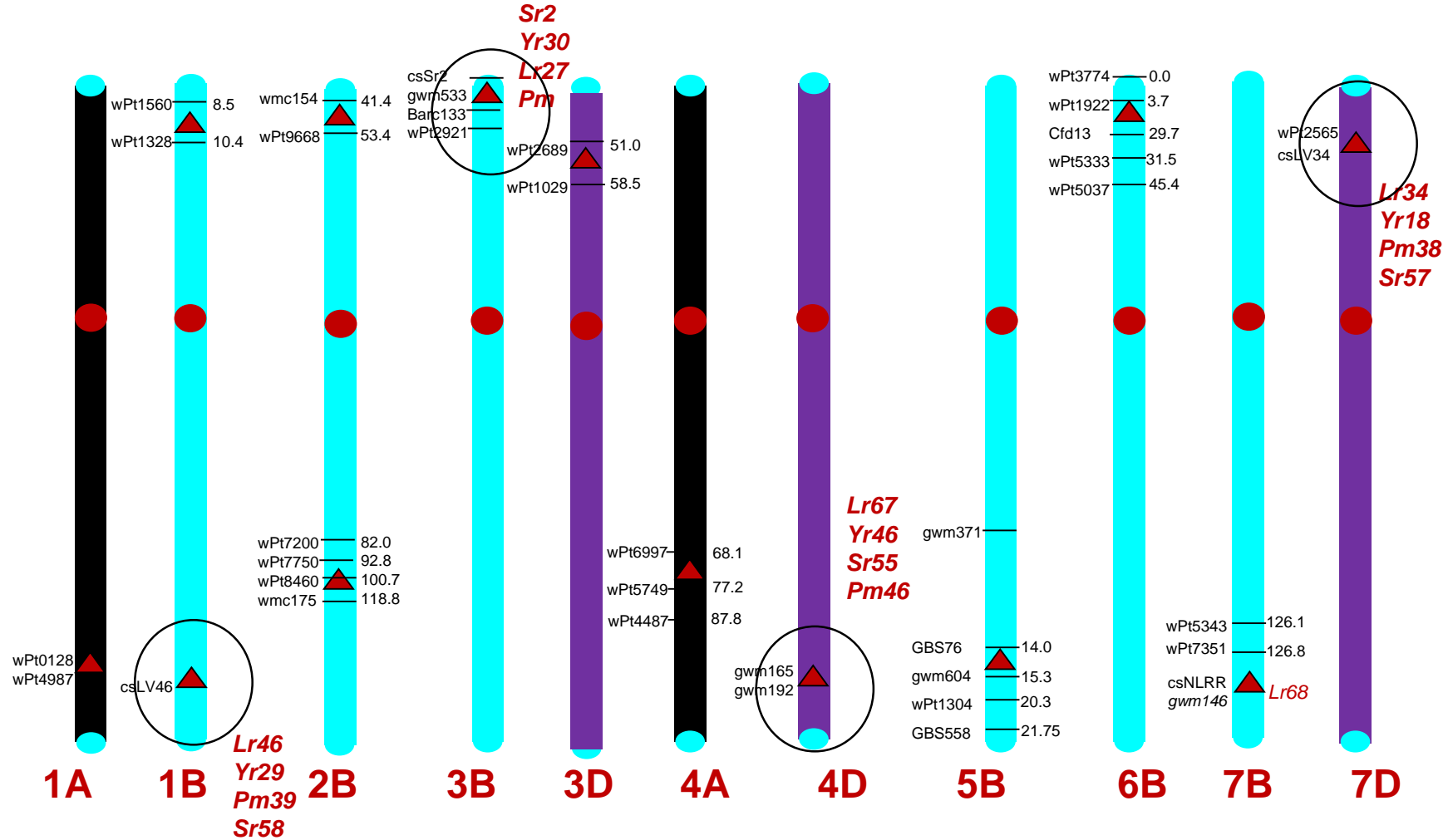
No. of Polymorphic DArT Markers 510
 No. of Informative DArT Markers 359

Year	Chromosome	Marker		LOD	PVE(%)	Est.ADD	R ²
		Position	Left Marker				
2007, 09, 10	1BL	278	<i>XwPt-1560</i>	<i>XwPt-7486</i>	2.1	23.8	N/A
	3BS	52	<i>XwPt-8093</i>	<i>XwPt-7212</i>	11	18.9	N/A
	5A	8	<i>XwPt-6048</i>	<i>XwPt-4249</i>	2.2	6.3	N/A
	6BS	8	<i>XwPt-1541</i>	<i>XwPt-0171</i>	11	13.4	3.2

Avocet X Pavon 76



Diversity for slow rusting, minor genes: 13 genomic regions identified in CIMMYT wheat through bi-parental and association mapping studies



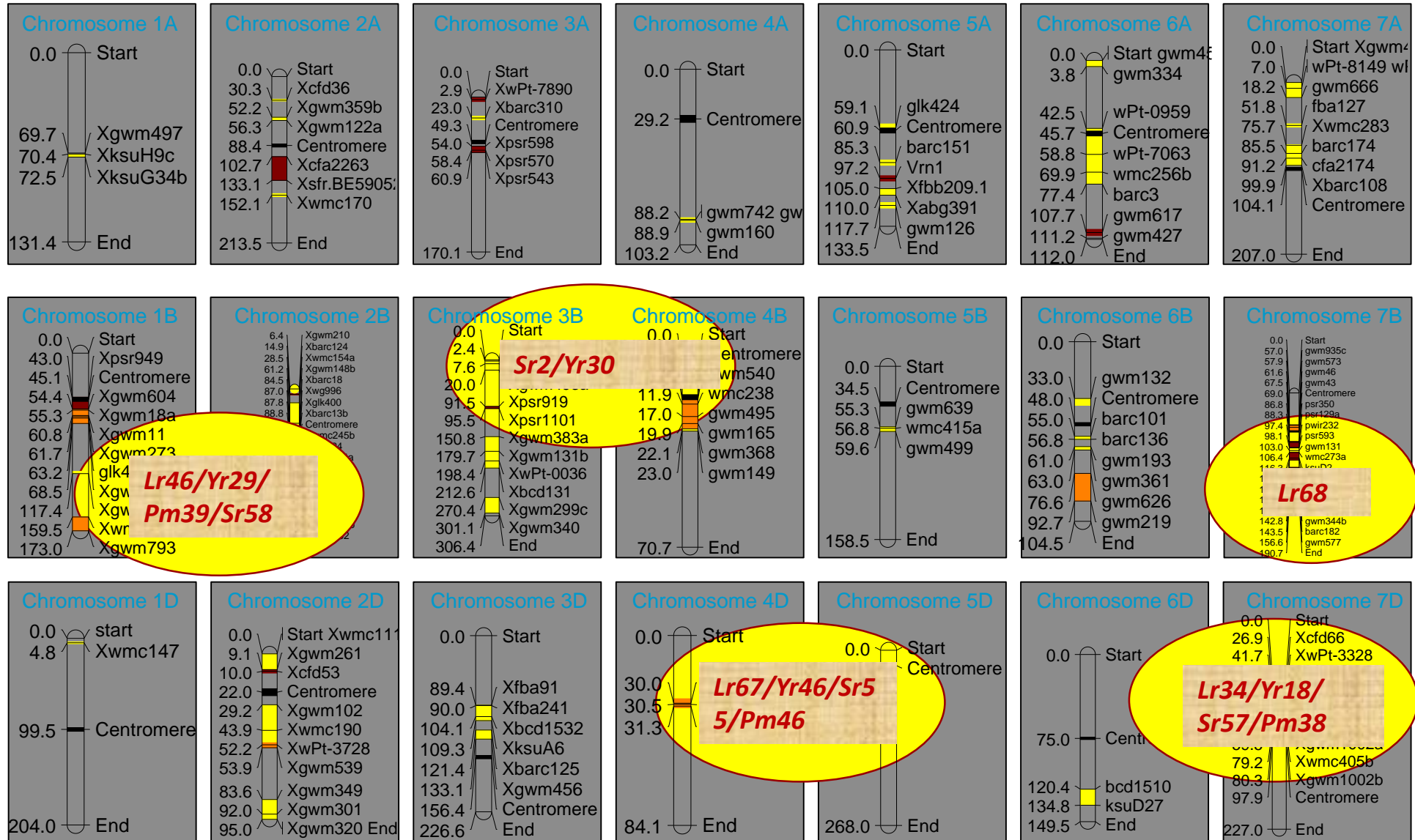
Resistance genes for Ug99 race group (APR and all-stage)

- Genomic regions for APR and other genes in context of Ug99 stem rust were published by Yu et al. (2014)
- Total number of resistance loci:
 - A genome with 37 loci [Origin: *T. urartu*]
 - B genome with 86 loci [Origin: *Ae. speltoides*]
 - D genome with 18 loci [Origin: *Ae. tauschii*]
- Hotspots of resistance loci across the genome:
 - QTL: 3BS (19), 6BS (9), 5BL (9), and 2BL (7)
 - Qualitative genes: 3BS, 5BL, and 2BL

Yu et al. (2014) *Theoretical & Applied Genetics*, DOI
[10.1007/s00122-014-2326-7](https://doi.org/10.1007/s00122-014-2326-7)

A consensus map for Ug99 stem rust resistance loci in wheat.

Leaf and Yellow Rust QTL and designated slow rusting resistance genes

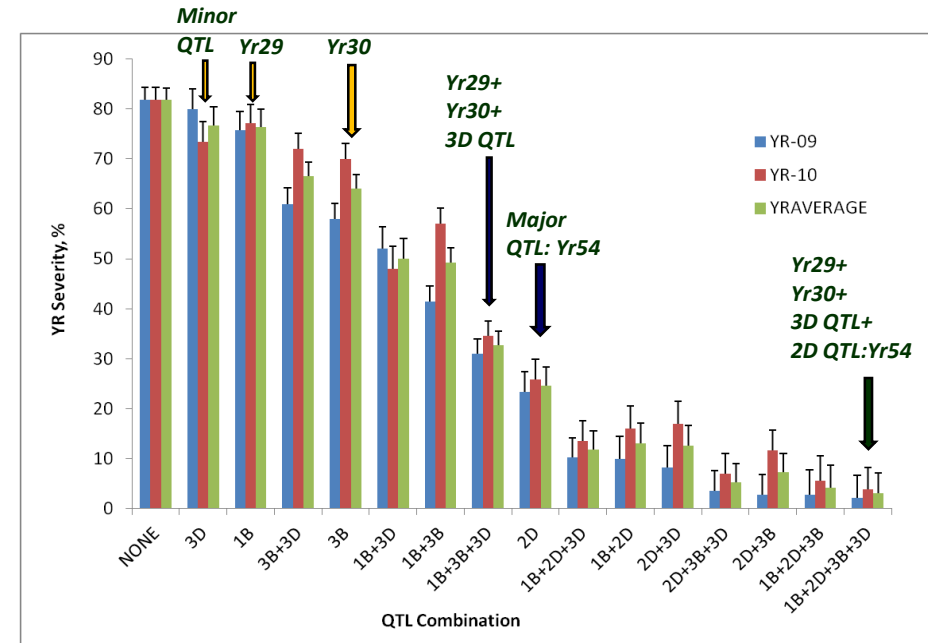


New APR QTL continue to be identified

APR QTL interaction in enhancing yellow rust resistance of Avocet x Quaiu3 RILs

- A large effect APR QTL on chromosome 2B for YR (designated as *Yr54*)
- A small effect APR QTL on 3D for LR/YR
- Slow rusting APR genes *Lr46/Yr29* and *Sr2/Yr30*

Combination of above genes results in immunity to YR in Quaiu3



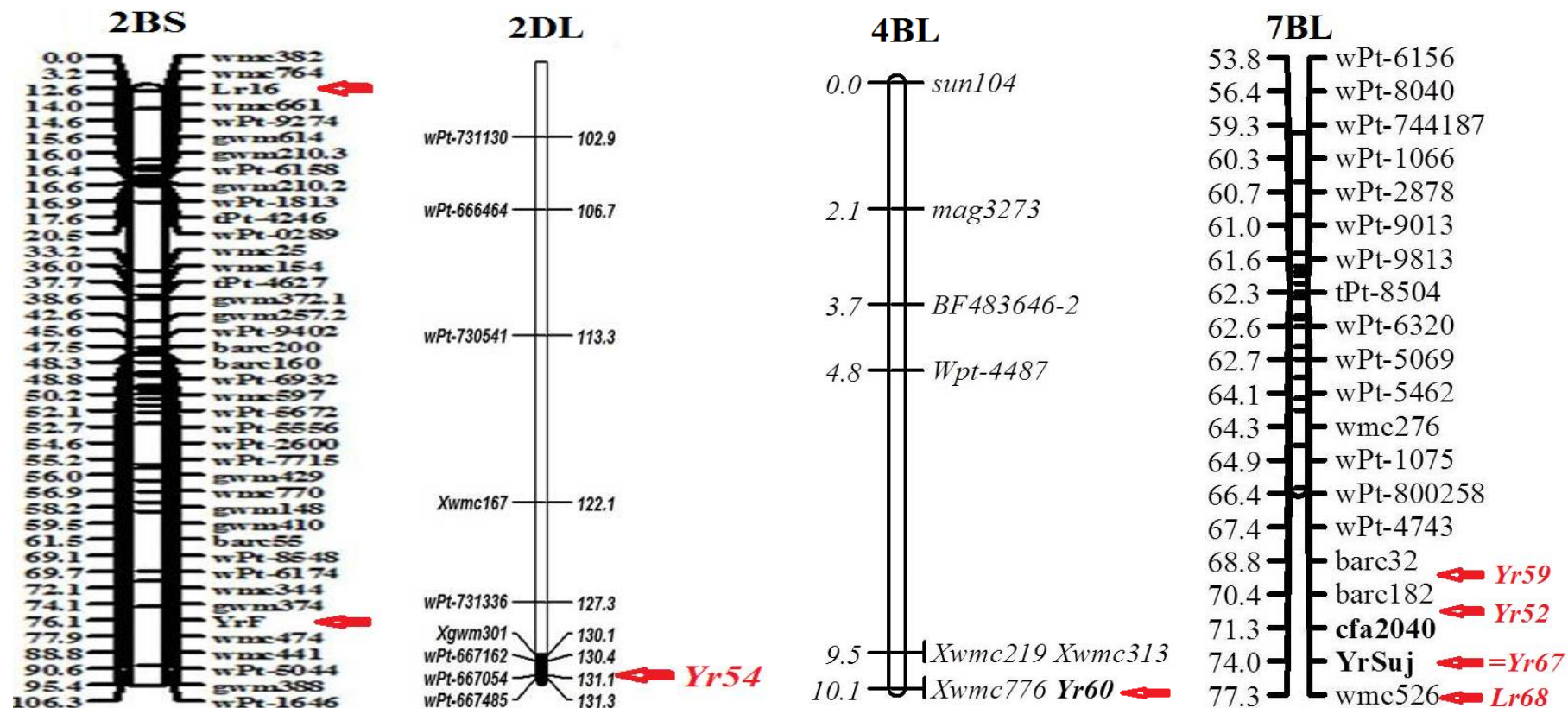
Examples of small/moderate effect race-specific resistance genes characterized recently

YrF in Francolin on 2BS

Yr54 in Quaiuz on 2DL

Yr60 in Lalbahadur on 4BL

Yr67 in tall variety Sujata on 7BL- pleiotropic effect on LR



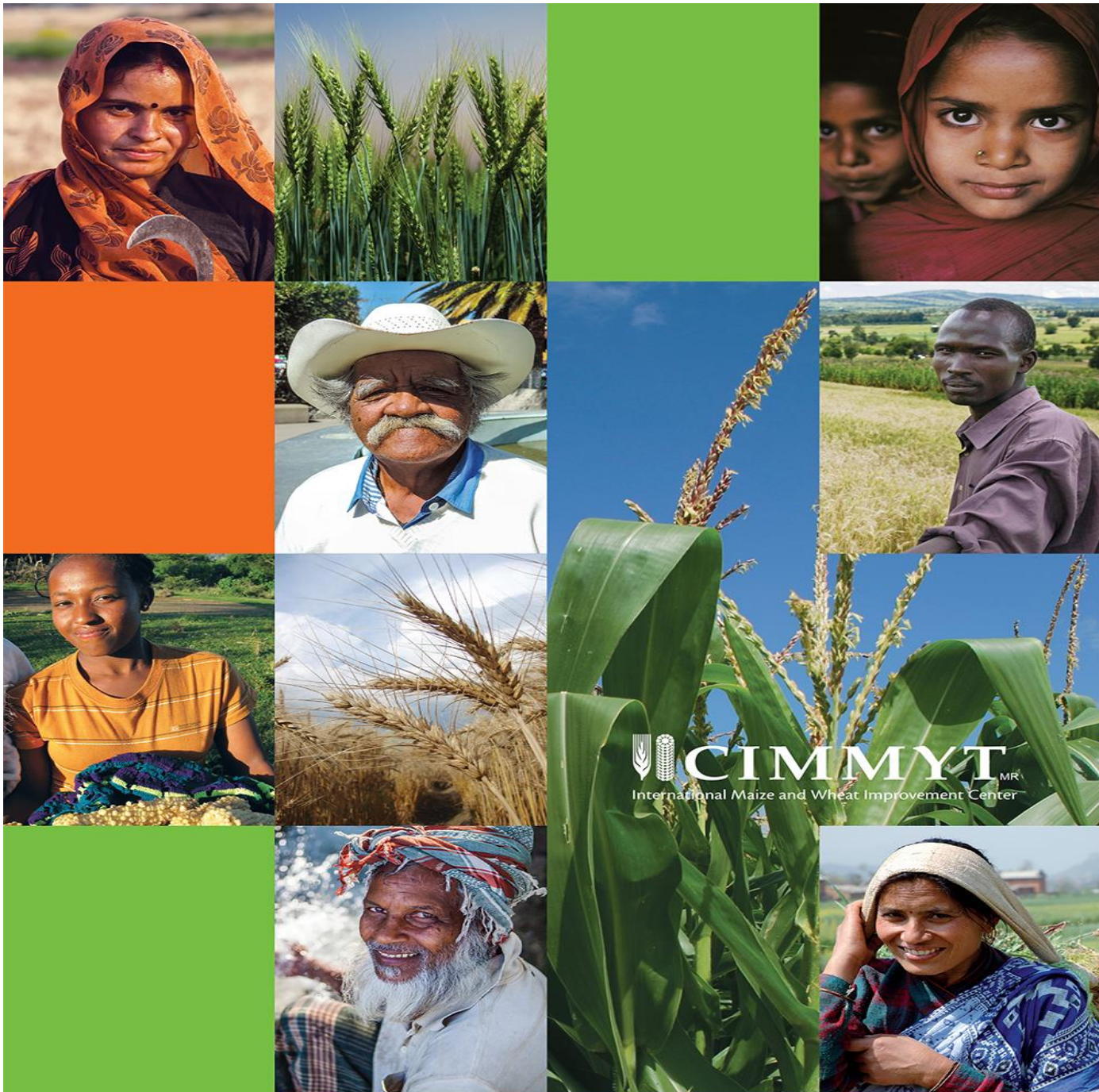
Sources:

Lan et al.2014

Basnet et al. 2014

Herrera et al. 2015

Lan et al. 2015



**Thank you for
your interest!**

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