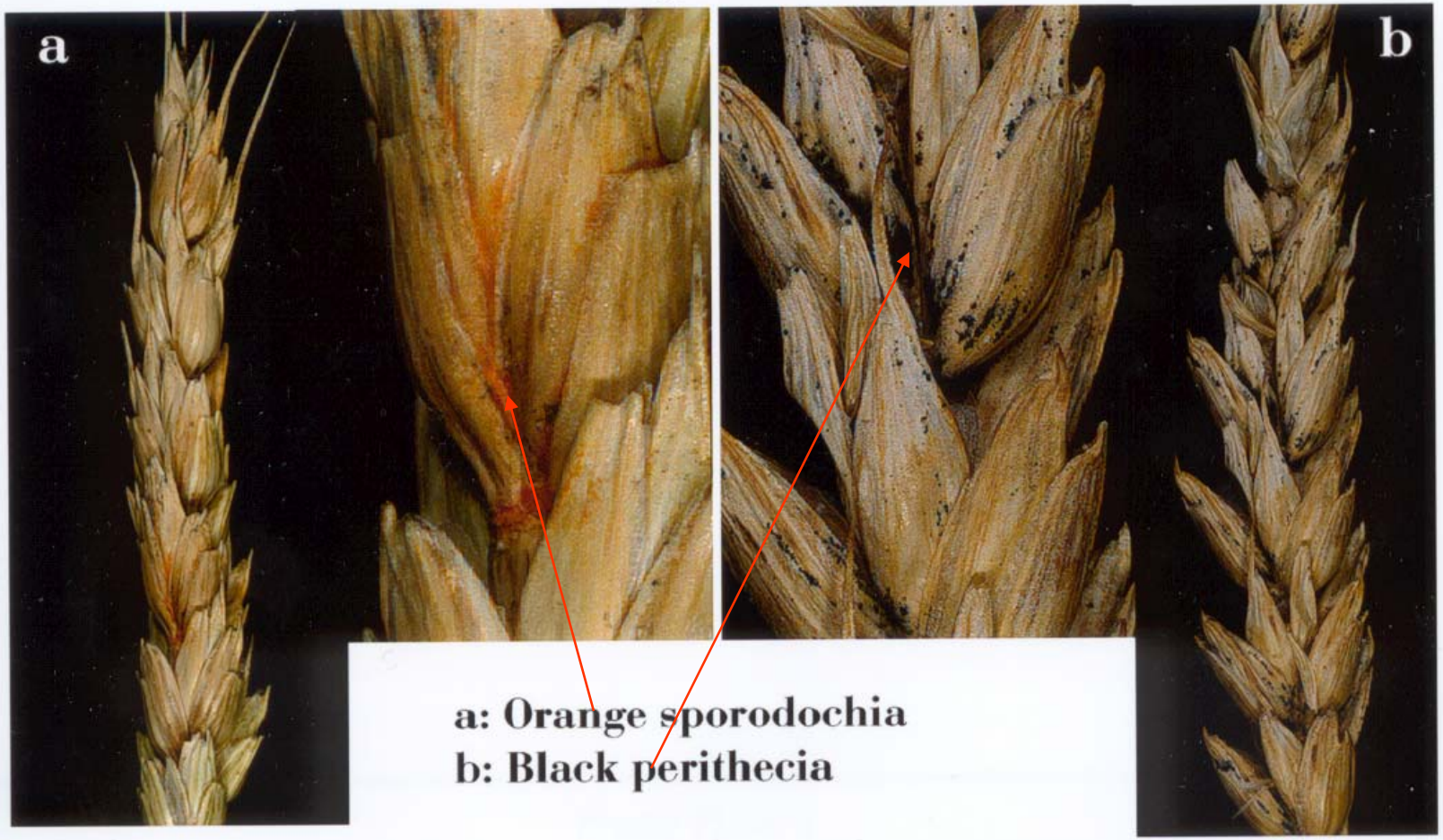
Genetics and Selective Breeding for *Fusarium* Head Blight Rsistance in Common Wheat

Jianli Chen

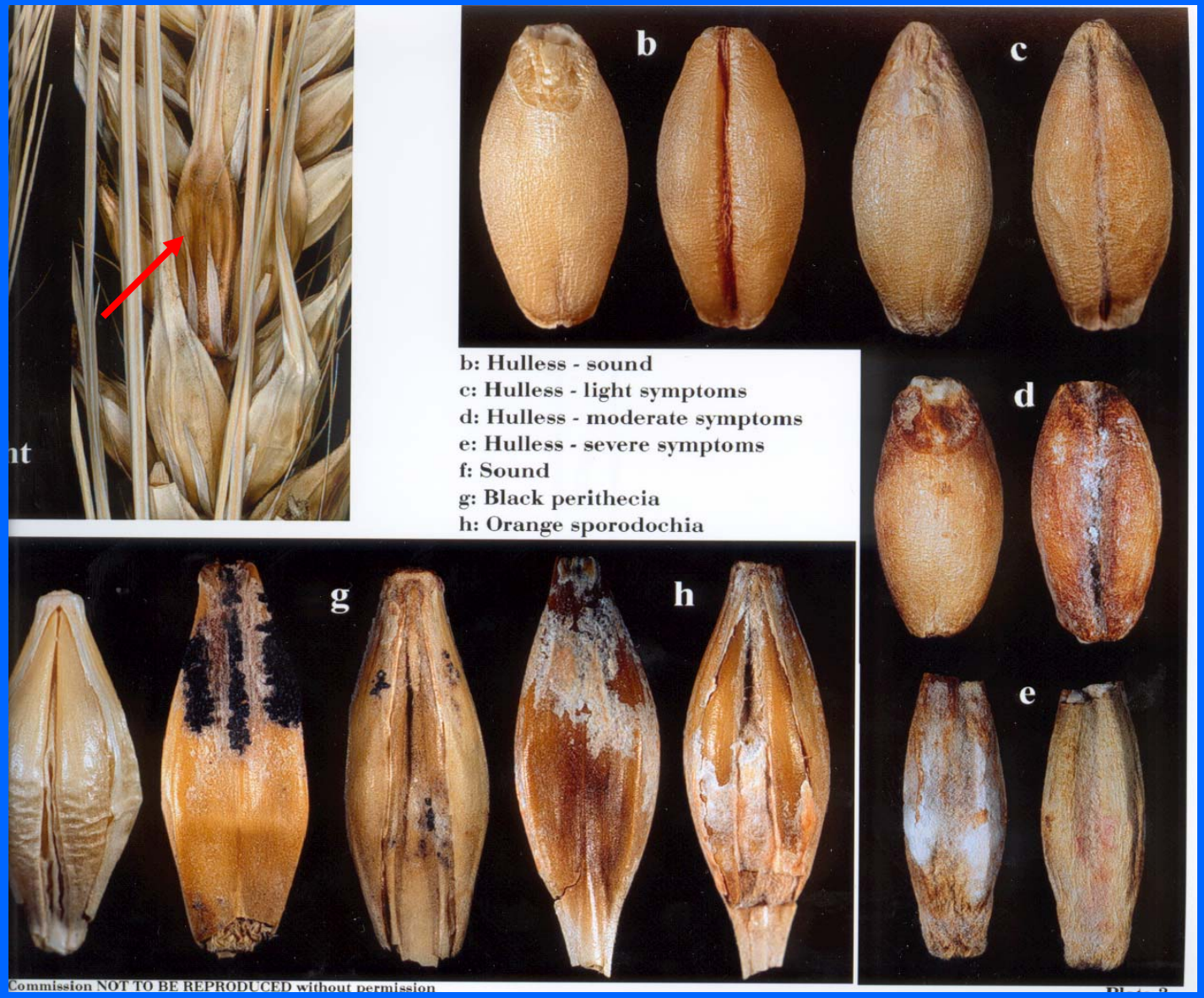
Head Blight on Wheat

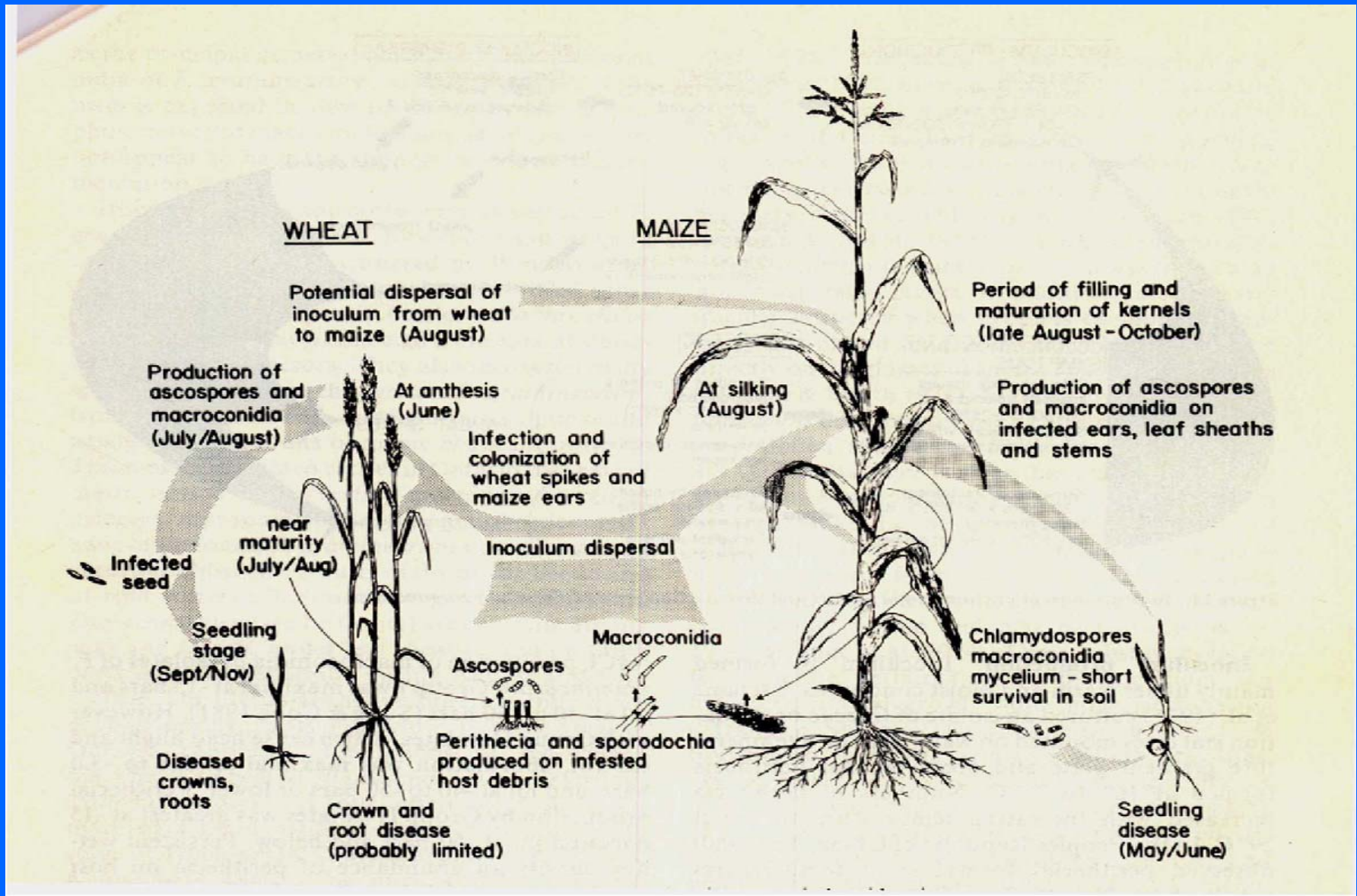
Plate 1



Scab Colonized Wheat Seeds

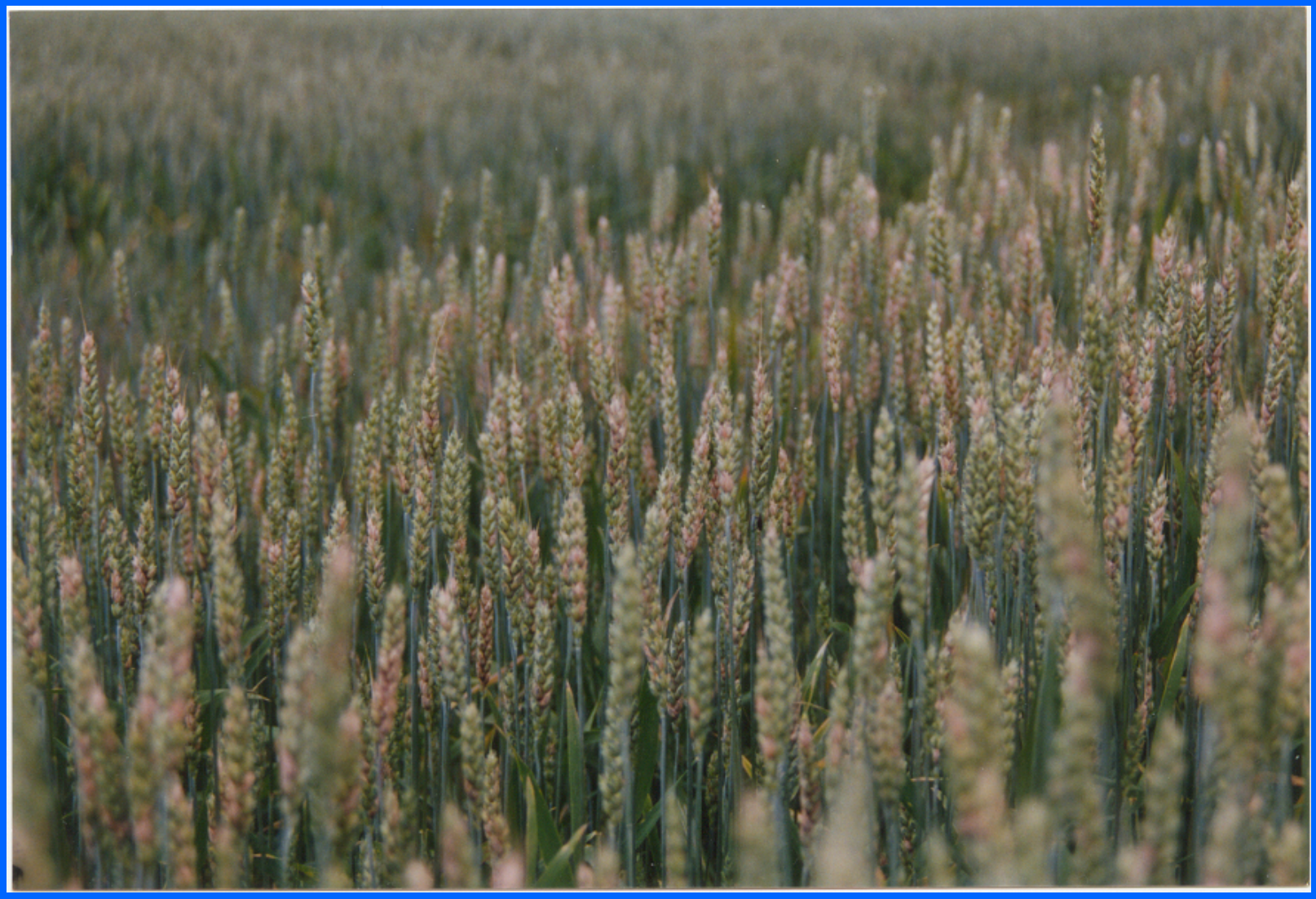
Head Blight on Barley





Disease cycle of F. graminearum in wheat and maize grown in humid regions (J.C. Sutton, 1982)

Infection Occurred in Field Nursery



Importance of Scab Research in Small Grain Crop

- A devastating disease prevalent in warm, humid regions throughout the world.
- Resulting in farm losses in at least 18 States cumulatively valued at over $2 billion in the US since 1990s (Scab News, 2001).
- Virginia farmers lost 5 million bushes of grain, worth $12.5 million in revenue in 1998 (Estimated loss).
- The safety problem is threatening both food and beer industries because of the mycotoxins.

Causal Organism and Epidemiology

- **Over 18 *Fusarium* species were reported to be pathogenic to wheat, but *F. graminerum* is the principal one responsible for scab in many countries.**
- **Crop residues and infected grains are important innoculum sources in the field.**

Causal Organism and Epidemiology

- Macro conidia or ascospores dispersed by the wind and rain are the main forms of innoculum.
- Anthers have been suggested as being a saprophytic base for primary infection. Infection occurs primarily during the flowering stage.

Causal Organism and Epidemiology

- **High relative humidity seems to be more important than high temperatures for scab development.**
- **Temperature of 25-28 ^{o}C and relative humidity above 85% for 72 hours are usually enough for the spike to be successfully infected.**

Types of Resistance to Scab In Common Wheat

- Type I, resistance to initial infection (Schroeder & Christensen, 1963);
- Type II, resistance to disease spread (Schroeder & Christensen, 1963);
- Type III, resistance to toxin accumulation in seed (Miller et al.,1985);
- Type IV, resistance to kernel infection and damage (Mesterhazy,1995& 1997);
- Type V, resistance to yield loss (Mesterhazy, 1995 &1997).

Types of Resistance

- There are no immune materials for type I resistance.
- Type II is the main resistance in wheat; however, no adapted varieties or resources possess type II resistance.
- Some sources were reported having type III, IV and V resistance in commercial varieties.

Sources for Type II Resistance in Wheat

- Chinese sources: Sumai 3, Wang Shuibai
- Japanese sources: Yan Gang Fang-Zhu, Xin Zhong-Chang, Wenzhou Hong He-Shang and Nyu Bai
- Brazilian source: Frontana
- Italian sources: Funo and Mentana (Nanda 2419)

Characteristics of Type II Resistance Sources

- Good resistance: 1-3 infected florets per spike with little or no rachis infection
- Spring type
- Low yield potential
- Other disease problems

Sources for Type II Resistance in Wheat

- **Improved sources: W14, Shaan85, Ning7840, Shanghai 4, and Saikai 165**

- **Wild sources: T. turgidum var. Diciccoides (II), A. squarrosa (I&II), Roegneria Ciliaris Kosh and Kamoji, Leymus racemosus**

Sources for Tolerance to Scab in Wheat

ERNIE

ROANE

VA96W-326

Freedom

INW 9824

Characteristics of Scab Tolerant Sources

- **Susceptible to initial infection**
- **Some tolerance to spread and reduced scabby seeds**
- **Low yield loss, test weight reduction and toxin content**
- **Escape disease damage (Rapid grain filling stage)**
- **High yield potential**

Inheritance of Scab Resistance

Major gene assumption

- One dominant resistance gene and some minor modifiers for scab resistance in Sumai 3 (J. Chen, 1989).
- Two major genes (Zhou et al., 1987 and T. Ban, 1997) and three major genes (Bai, 1995) in Sumai 3.

Major Gene Assumption

- Two major genes in Ning 7840 (Bai,1995; Ginkel et al., 1996) and three major genes in Saikai 165 (T.Ban,1997).
- Two genes (Ginkel et al., 1996) and three genes (Singh et al, 1995) in Frontana.
- Genes in Ning 7840 and Frontana are all different (Ginkel et al., 1996).

Mutigene Assumption

- **Scab resistance is controlled by poligenetic inheritance with additive effects and components of dominance (Gu, 1984; Liu, 1985; Snidjers, 1990; Wilcoxson et al., 1992; Zhang and Pan, 1984).**

Chromosome Locations

Aneuploid Analysis		Marker Analysis	
Sumai 3	Other	Sumai 3	Other
1B(2),	1B(3), 1D(2)		
2A(2), 2B(1)	2A(1), 2B(3)	2AL	2AS
3B(1), 3D(1)	3A(2), 3B(2), 3D(3)	3AL, 3BS	3AL, 3BS
	4A(1), 4B(2), 4D(2)	4BS	
5A(2)	5A(4), 5B(3), 5D(3)		5AL
6B(1), 6D(2)	6A(1), 6B(5), 6D(1)	6AS	6BS
7A(1), 7D(2)	7A(5), 7B(2), 7D(1)		7BS

Need for Genetic Studies

- Genetic studies on type II resistance have been conducted extensively on Sumai 3, Frontana, and related sources such as Ning 7840, Saikai 165, and UNG-229.
- Results of these studies are inconsistent with regard to the mode of inheritance of resistance, chromosome locations and the number of genes conferring resistance.
- Genetic studies on other types of resistance have not been addressed; the relationship between type II and other types of resistance is unclear.

Need for Molecular Markers

- Confounding environmental effects on screening and selection efficiency.
- Marker assisted selection performed prior to flowering such as in the seedling stage may allow the breeder to identify resistant plants prior to making crosses.
- Selections for resistance could be made in large populations and even in early generations, and among progeny at any stage and without the need for disease evaluation.
- Genetic mapping will likely facilitate a better understanding of the genetic control of scab resistance, but only a few markers have been reported

Need for Selective Breeding

- How can we reduce the time required for developing resistant varieties ?
- Which techniques are practical and most effective?
- When and how should the new techniques be used?

Accelerating Development of Resistant Varieties

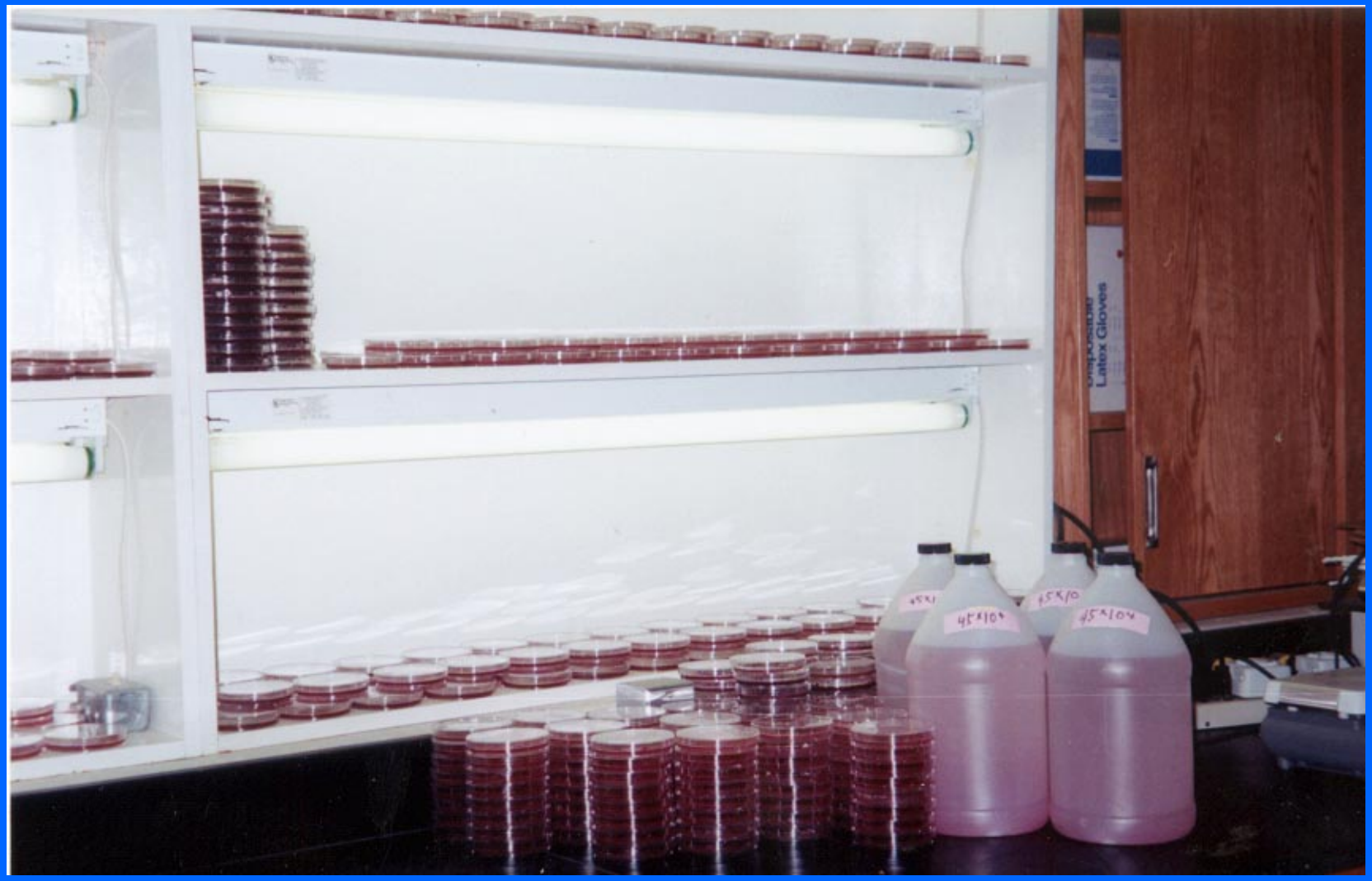
- Techniques for establishment and assessment of *Fusarium* head blight
- Identification of resistance among both exotic germplasm and adapted wheat cultivars or lines
- Identification and development of scab resistant wheat lines or varieties with top-crossing, backcrossing and doubled haploid techniques

Major Problem Addressed

- **Fast, economical and practical way for inoculum production.**
- **Fast, efficient and reliable protocols for disease establishment and assessment of resistance in both field and greenhouse tests.**

Field Inoculum Production: Mason Jar Method

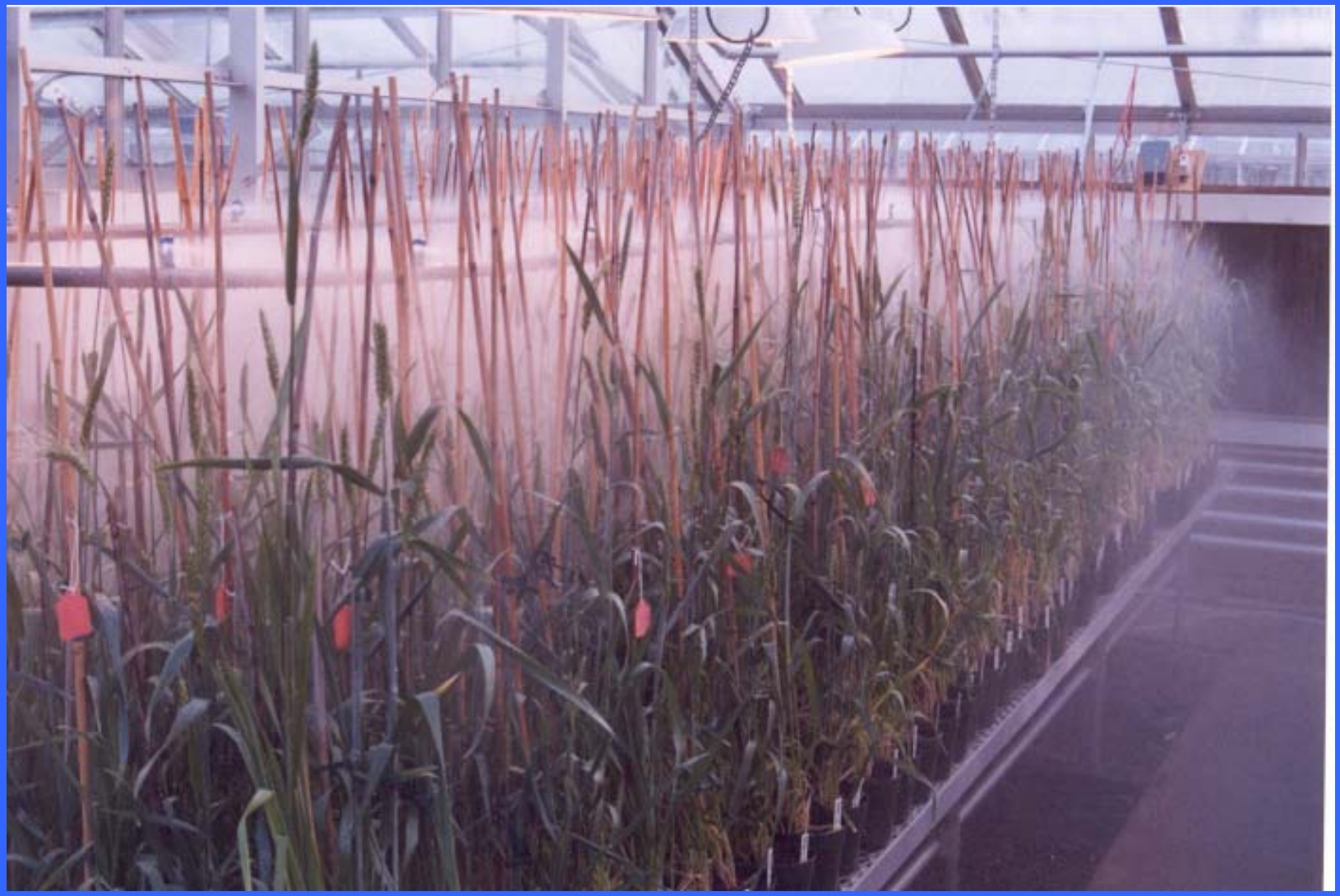
Coniadial Production: Petridish Method



Tools for Greenhouse Inoculation

Floret Inoculation

Screening for Type II Scab Resistance



1 2 3 3-3.8 4 5

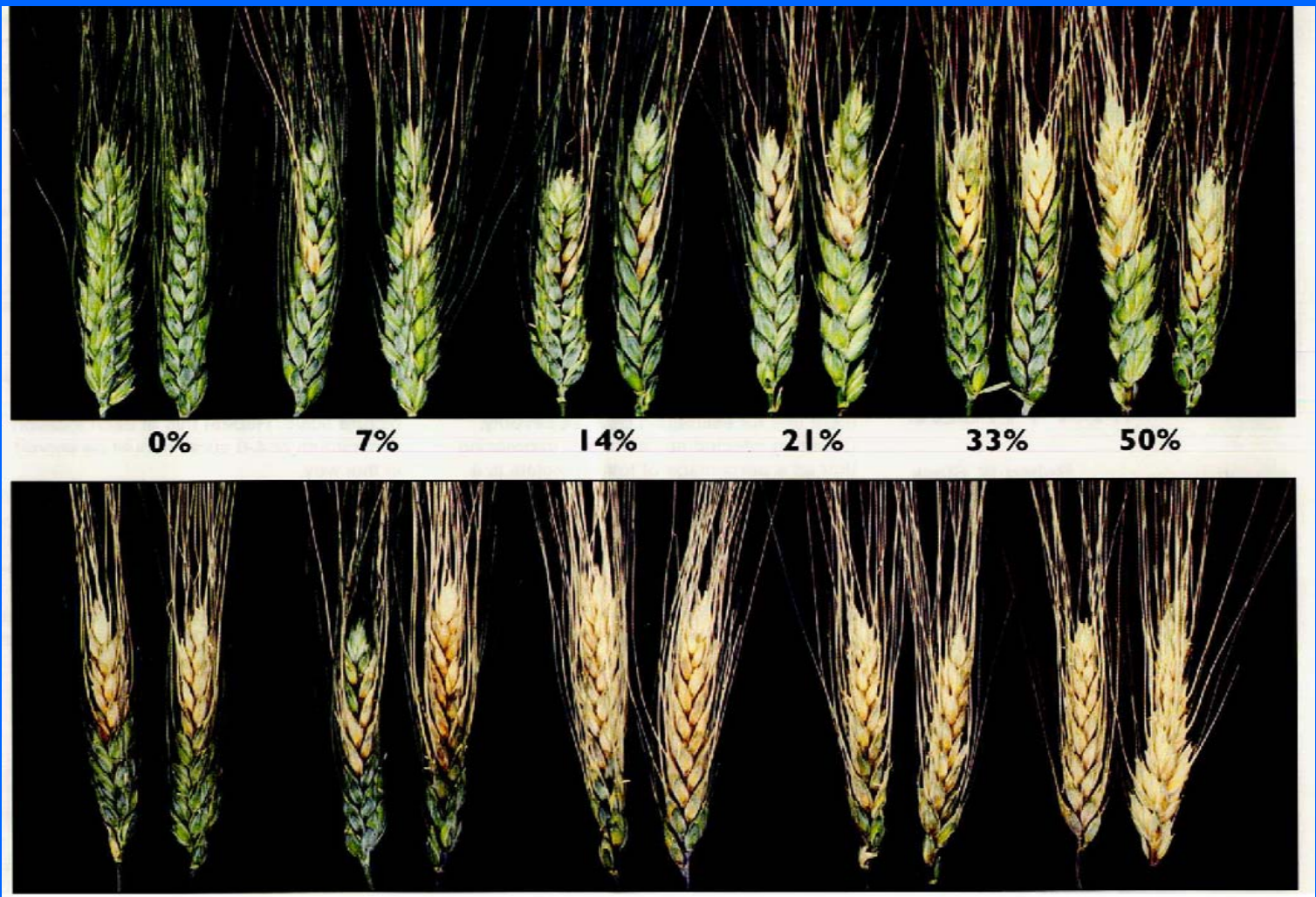
A five-grade rating method for single floret inoculation

Scab Research in Field Nurseries

Early Generation Selection for Scab Resistance

Early Generation Selection for Scab Resistance

Early Generation Selection for Scab Resistance



A Visual Scale to Estimate Severity of Fusarium Head Blight in Wheat
(as described in Stack & McMullen, 1997)

Conventional Breeding System

Year	Population	Processes
1st	Initial crosses made	AABB x aabb
2nd	F1	AaBb
	Gametes	AB Ab aB ab
3rd	F2	**1AABB 2AABb 2AaBB 4AaBb 1AAbb 2Aabb**
		6.25% 1aaBB 2aaBb 1aabb
4th-5th	F3-F4	Advance Progeny: Bulk head selections
6th-7th	F5-F6	Head Row Test
8th-14th		Testing and Cultivar Release

Breeding Using Doubled Haploid System

Year	Population	Processes
1st	Initial crosses made	AABB x aabb
		↓
2nd	F1	AaBb
	Gametes	AB Ab aB ab
		↓ **Haploid production** **Cochicine treatment**
	Doubled Haploid	**(25%)** AABB AAbb aaBB aabb
		↓
3rd	H1	Head Row Test
		↓
4th-10th		Testing and Cultivar Release

Haploid Production: Wheat x Maize Hybridization

Breeding Method: ***Backcrossing or Pyramiding***

- **Parent** **aabb x AABB**
- **F_1** **aabb x AaBb (50%)**
- BC_1F_1 aabb x AaBb (75%) Aabb aaBb aabb
- BC_2F_1 aabb x AaBb (87.5%) Aabb aaBb aabb
- BC_3F_1 aabb x AaBb (93.75) Aabb aaBb aabb
- **BC_3F_2** **1/16 AABB (6.25)**
- **BC_3F_3** **AABB (6.25)**

Breeding Method for FHB Resistance

- **Parent** **aabb x AABB**
- **F_1** **aabb x AaBb (50%)** → **Doubled haploid**
- BC_1F_1 aabb x AaBb (75%) Aabb aaBb aabb
- BC_2F_1 aabb x AaBb (87.5%) Aabb aaBb aabb
- BC_3F_1 aabb x AaBb (93.75) Aabb aaBb aabb
- **BC_3F_2** **1/16 AABB (6.25)** **Doubled haploid**
- **BC_3F_3** **AABB (6.25)** **Marker-assisted selection**

Inbreeding and Population Advancement

- **Backcross population: BC_1F_1 to BC_4F_1, floret inoculation of 1-3 heads and harvest seeds from corresponding resistant plants in greenhouse.**
- **Segregating population: F_2 or F_3, inoculation with colonized scabby seeds, mass selection based on plant type and resistance.**
- **Pure lines: F_4 or F_5, spray conidial suspension, selection of individual plants or progeny rows for both resistance and agronomic traits.**
- **Advanced line development: F_6 or F_7, spray conidial suspension, plot evaluation and yield loss estimation in field test; Floret inoculation and assessment of type II resistance in greenhouse.**

Progress on Breeding in Wheat

- We have found and confirmed high levels of type II resistance
- We have also identified tolerance to scab in soft red winter (SRW) wheat varieties, such as Roane, Freedom, Ernie and INW 9824.
- Nearly six hundreds crosses have been produced including one or more type II resistance sources or type II resistance combined with other types of resistance.
- Advanced lines are being developed with the combination of scab resistance and good agronomic traits.

The Most Significant Accomplishments

- VA00W562 and VA00W566 have shown good scab resistance in Scab Uniform Tests and good agronomic traits in preliminary tests in multi-locations.
- Some new lines were developed via different breeding methods.

VA00W566: Newly Developed Scab Resistant Line

Table 1. Summary of doubled haploid lines produced in 1999-2001 tests.

Year	No. of Crosses Used	Doubled Haploid Lines Obtained	No. of Lines in Observation Test
1999	12	413	12
2000	10 + 2	109 + 169	/
2001	9	101	/
Total	19 + 14	109 + 582	


Wheat Headrows
Doubled Haploid Lines

Doubled Haploid Line: Field Infection

Table 2. Summary of backcrossing test made in 00-01 greenhouse test.

Recurrent Parents	Resistant parents and the number of crosses made to their recurrent parents in BC_3F_1					
	Fuati 8944	Shaan 85-2	W14	VR95B 717	Shaan 85-15	Futai 8945
Pion2684	16	21	23	19		
Madison	14 (BC4)			12		11(BC4)
Ernie	22	21	20	24		
Roane	7		3	19		
VA95W326	14		15			
Apripro Mason					7	
GA891283LE					65	

Breeding Method
Disease Screening and Backcrossing

- Backcross populations: BC_1F_1 to BC_4F_1
- Floret inoculation of 1-3 heads
- Harvest crosses from corresponding resistant plants


Disease Screening and
Backcrossing in Greenhouse


Disease Screening and Backcrossing
in Greenhouse

Genetic Study

- Three resistance sources, W14, Shaan 85 and Ernie were crossed with susceptible soft red winter (SRW) wheat variety Madison and/or Pioneer 2684.
- One to three heads per individual in F_2 populations, 10 to 30 individuals per family in $F_{2:3}$ populations, and 28 to 51 individuals per parent were inoculated via floret inoculation procedures.
- Ratings of severity were assessed three times at 7, 14 and 21 days after inoculation. Percentage of scabby seeds for each individual was measured based on the mean of hand-threshed single heads. DON content was analyzed as ppm by a SIM Shimadzu QP5000 GC/MS system. Severity, scabby seeds and DON content were evaluated for F_2 populations and only severity was evaluated for $F_{2:3}$ lines.
- The disease data were analyzed along with the marker data to identify QTLs controlling FHB resistance in the cross of Pioneer 2684 x W14.

Mapping Study

- One F_2 **Population Pioneer2684 x W14 (156 DNA samples)**
- **Six resistance sources (Funo, Sumai 3, W14, Shaan85, VR95B717, Ernie) and two susceptible parents were used in the genotyping study.**
- **300 SSR primer pairs known to be located on 1B, 2B, 3B, 3A, 5A, 6A, 6B, 6D, and 7B were selected.**
- **DNA extraction, PCR amplification and SSR assays were as described by Saghai Maroof et al. 1984,1994; Bryan et al. 1997; Roder et al. 1998.**

Identification and Characterization of Scab Resistance in Four F_2 Populations

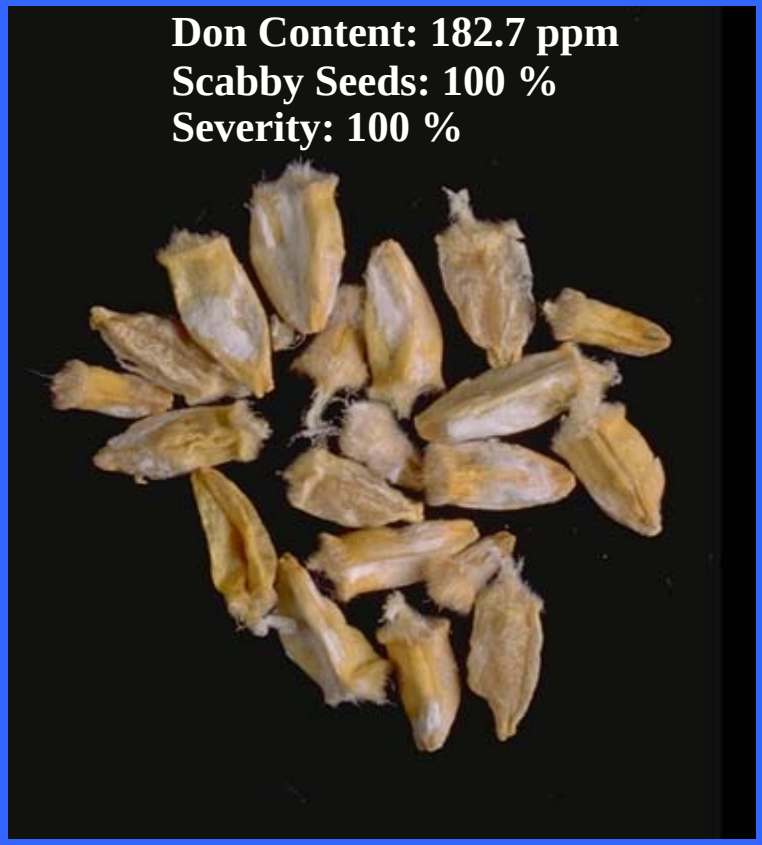
- Significant differences in type and level of resistance were found between parents and among individuals in F_2 populations.
- Highly-resistant individuals with type II resistance were found to also possess type III and type IV resistance, having consistently lower severity, scabby seeds, and less than 10 ppm toxin accumulation.
- Highly susceptible individuals were found to have variable or consistently high ratings for these parameters.
- About 25 percent of individuals with type III & IV resistance didn't show resistance of type II.
- Individuals with type IV resistance were found to have resistance with type III in most cases.

Individuals with Type II, III and IV Resistance

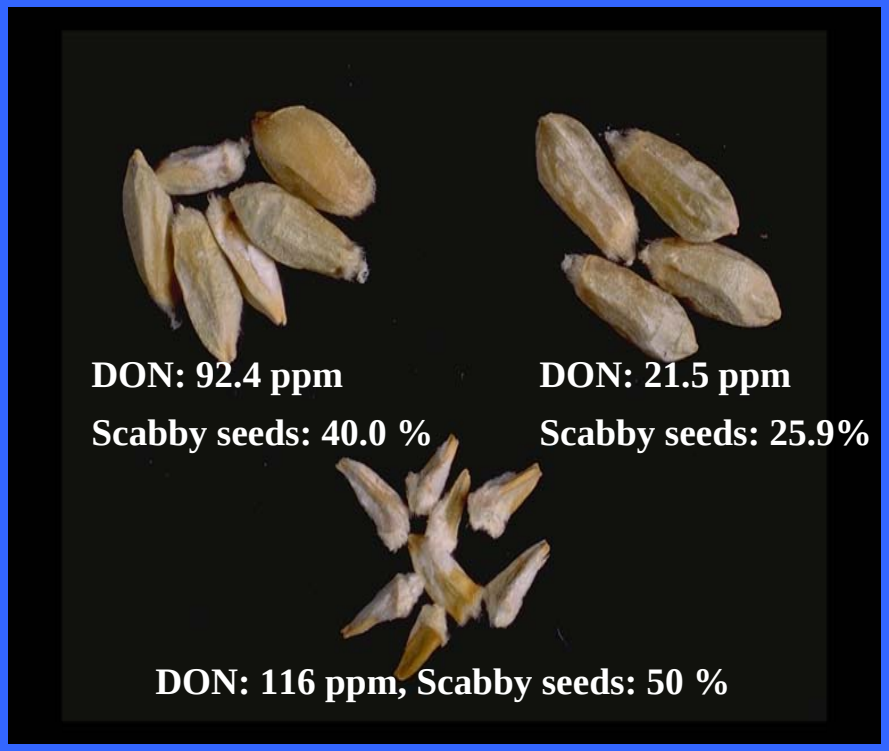
DON Content: 3.0 ppm

Scabby Seeds: 6.1 % Severity: 14.3 %

Individuals Susceptible to Type II, III and IV Resistance



Moderately Resistant to Susceptible Individuals with Varied Scabby Seeds and Varied DON Content



Genetic Relationship Between Type II, III and IV Resistance in Wheat

- **Significant positive correlations were found between scab severity (type II) scabby seeds (Type IV resistance), and DON content (type III).**
- **Correlation value between scabby seeds and DON content (r = 0.8456) was much higher than those between severity and DON content (r = 0.5388), and severity and scabby seeds (r = 0.5911), which suggests that DON content could be predicted by percentage of scabby seeds in most cases.**
- **Assessing severity before harvest, and scabby seeds after harvest may be an effective and economical way to select for resistance as a whole.**

Inheritance of Resistance to Disease Spread, Seed Colonization and DON Accumulation in Four F_2 Populations Inoculated with *Fusarium graminearum*

- Different frequency distributions were observed in four populations for parameters of severity, scabby seeds and DON content.
- A normal distribution with transgressive segregation for both susceptibility and resistance was observed in cross Pioneer2684 x Ernie for all parameters. Four genes were estimated for resistance in Ernie based on a quantitative approach (Wright, 1968).
- Right skewed distribution with two peaks coinciding with both parents was observed in the crosses with W14 and Shaan 85 as the resistance sources, and suggests that the resistance of W14 and Shaan 85 is controlled by major genes.
- Two major genes with complementary effect were estimated by $\chi 2$ analysis, and two to three genes were estimated for resistance conferred in W14 and Shaan 85 based on a quantitative approach (Wright, 1968).
- Transgressive segregants were more frequently identified for scabby seeds and toxin content than scab severity in each of the crosses.

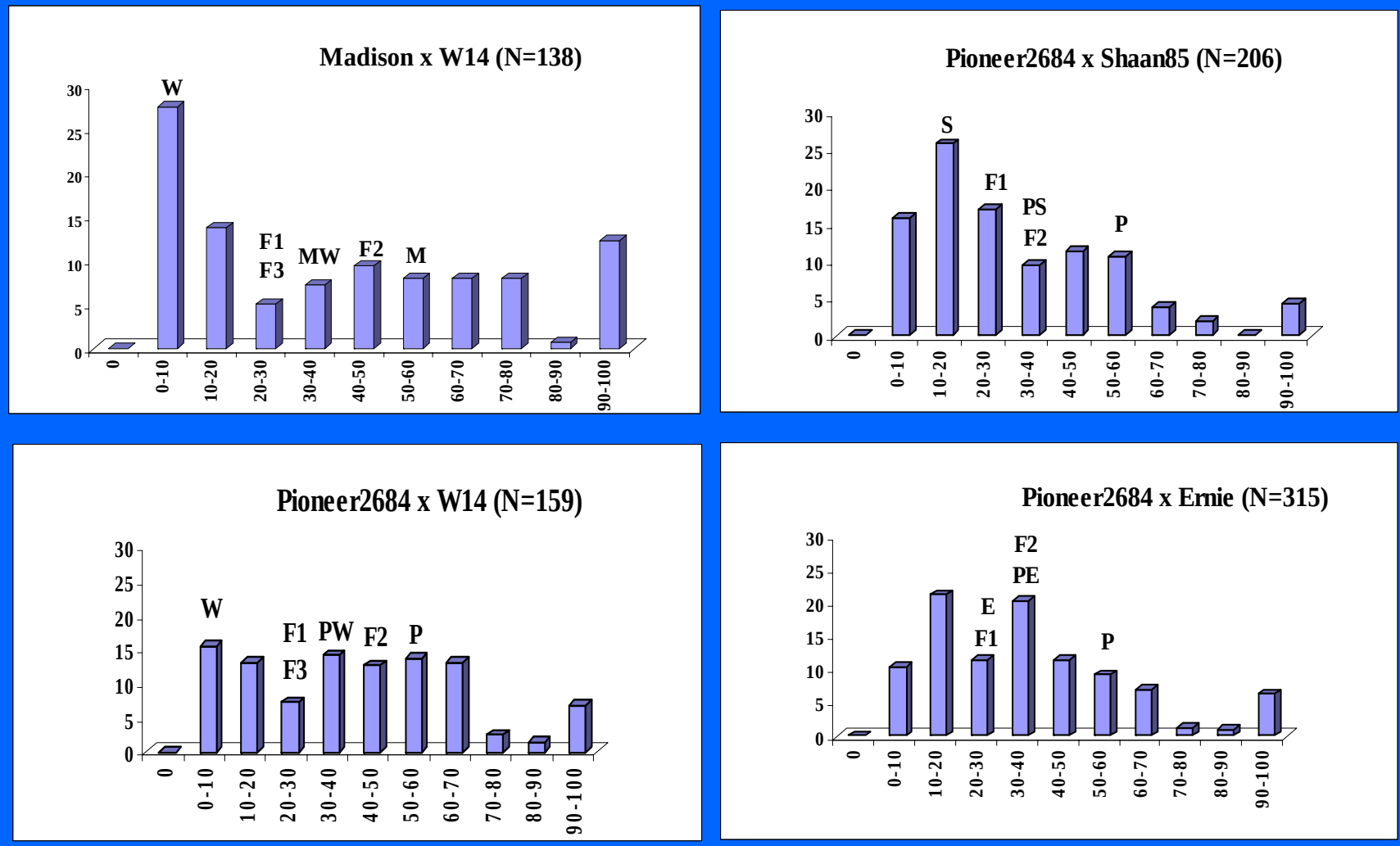
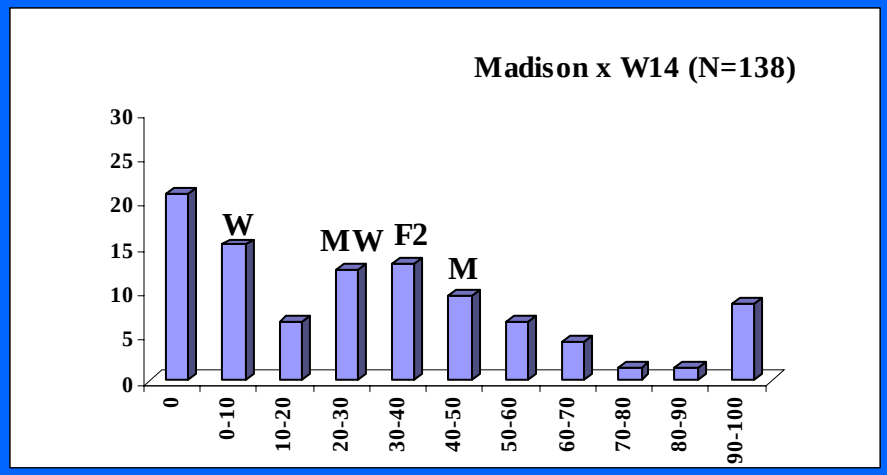
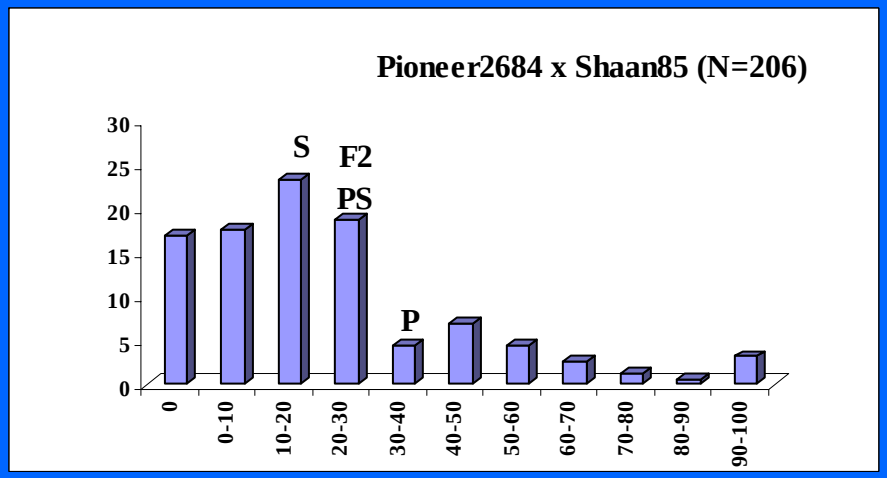
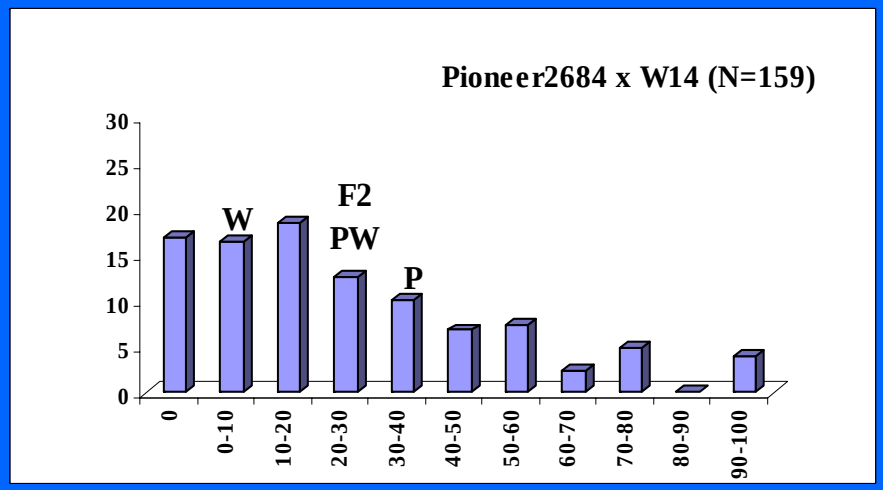


Fig 1. Comparison of distribution for disease severity (%) in four F_2 populations.







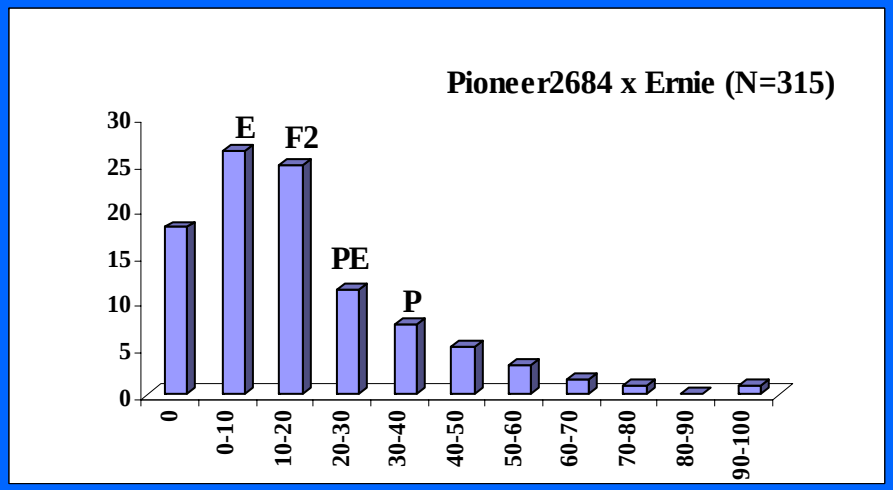


Fig 2. Comparison of distribution for scabby seeds (%) in four F_2 populations.

Scab QTLs Found in Current Mapping Population

- A total of 45 loci were mapped to five chromosomal regions in current population and two major QTLs in addition to the 3BS QTL were identified, one potentially on 2BS.
- Fifteen markers were identified in three QTL regions to be significantly ($p < 0.05$) associated with scab resistance, and explained 23, 28, 21, and 36 % of total variation in percentage of scabby seeds, DON content, and severity in 82 F_2 individuals and severity in 82 corresponding $F_{2:3}$ families, respectively.

Table 3. Coefficients of determination and *P* values for DNA markers in 2BS QTL region associated with FHB resistance in a F_2 population Pioneer x W14.

Makers	DF	Coefficient of Determination (%)				Source of Resistance Allele
		Disease Severity (F_2, %)	Disease Severity ($F_{2:3}$, %)	Scabby Seeds (%)	DON Content (ppm)	
Q_{FHB-2B}	76	**10.72 (0.031)**	**12.52 (0.015)**	**11.63 (0.021)**	**16.04 (0.003)**	**W14**
BARC13	80	6.22 (0.024)	7.34 (0.014)	5.00 (0.043)	4.45 (0.057)	**W14**
GMS410	80	9.78 (0.004)	11.77 (0.002)	10.21 (0.003)	12.33 (0.001)	**W14**
PSP3030s	80	4.99 (0.044)	6.18 (0.024)	4.27 (0.063)	3.87 (0.076)	**W14**

Table 4. Coefficients of determination and *P* values for DNA markers in 3B QTL region associated with FHB resistance in a F_2 population Pioneer x W14.

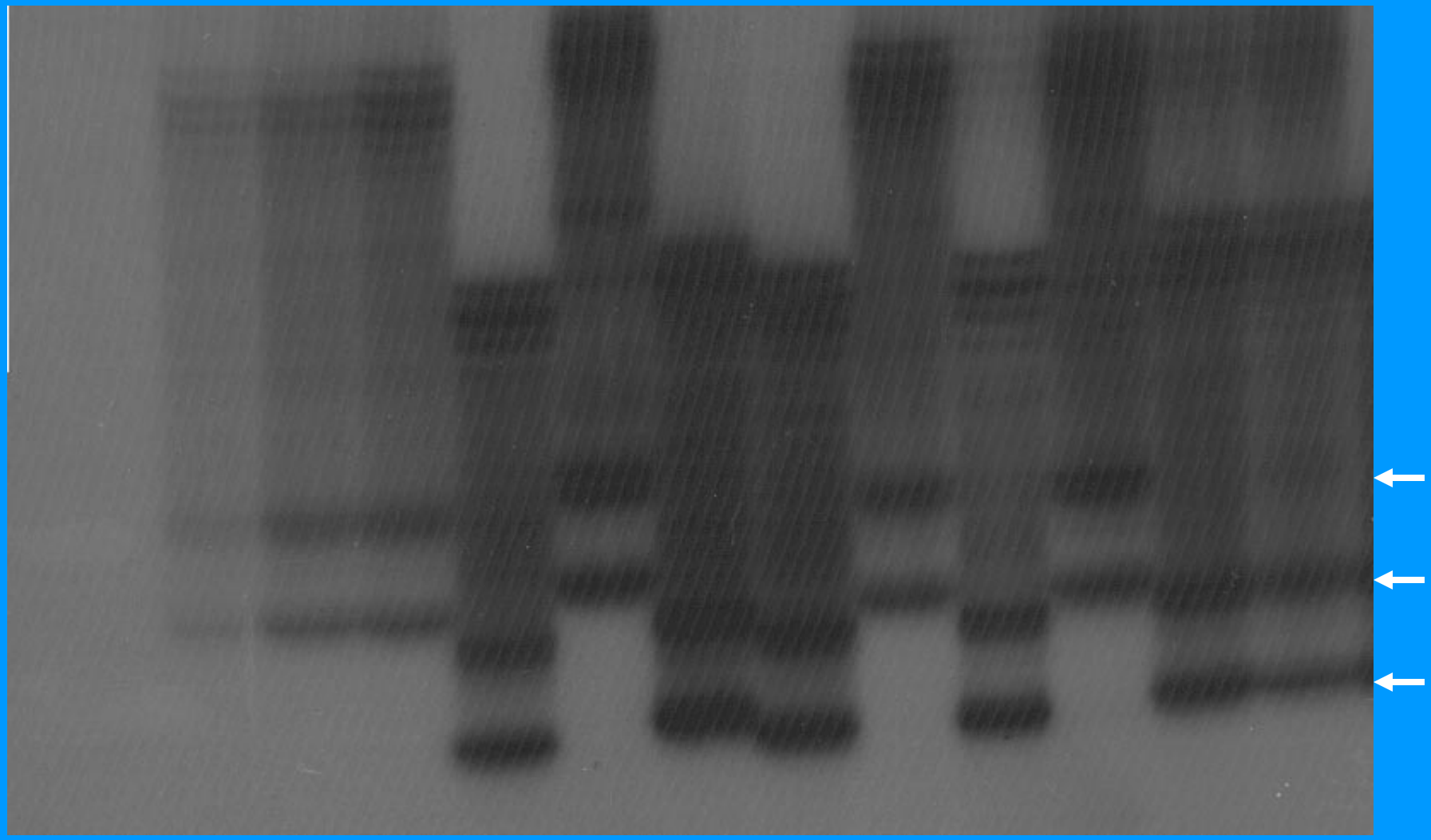
Makers	DF	Coefficient of Determination (%)				Source of Resistance Allele
		Disease Severity (F_2, %)	Disease Severity ($F_{2:3}$, %)	Scabby Seeds (%)	DON Content (ppm)	
GMS533a	80	5.09 (0.042)	17.38 (0.000)	9.21 (0.006)	4.63 (0.052)	W14
GMS533B	80	6.39 (0.022)	15.41 (0.000)	8.51 (0.008)	3.86 (0.077)	W14
GMS389	80	5.68 (0.031)	7.46 (0.013)	4.04 (0.070)	5.33 (0.037)	W14
GMS247	80	4.98 (0.044)	/	/	/	W14
BARC84	80	/	9.37 (0.005)	/	4.97 (0.044)	W14
BARC77	80	/	4.78 (0.048)	/	4.69 (0.051)	W14

Table 5. Genotyping of six resistance sources and two susceptible varieties with eight SSR markers that associated with resistance to scab.

Markers	Genotype							
	Funo	Sumai3	W14	Shaan85	VR95B717	Ernie	Pion2684	Madison
GMS429 (2BS)	0	4	1	1	1	0	0	0
GMS148b (2BS)	1	1	1	4	4	0	0	0
GMS410a (2BS)	3	3	3	3	4	1	1	1
GMS389 (3BS)	0	3	3	3	5	1	1	4
GMS533 (3BS)	4	3	3	3	3	0	1	0
BARC77 (3BL)	5	5	3	4	4	5	1	3
BARC84 (3BL)	1	1	1	1	0	1	0	1
GMS274b	0	0	0	0	0	1	1	1

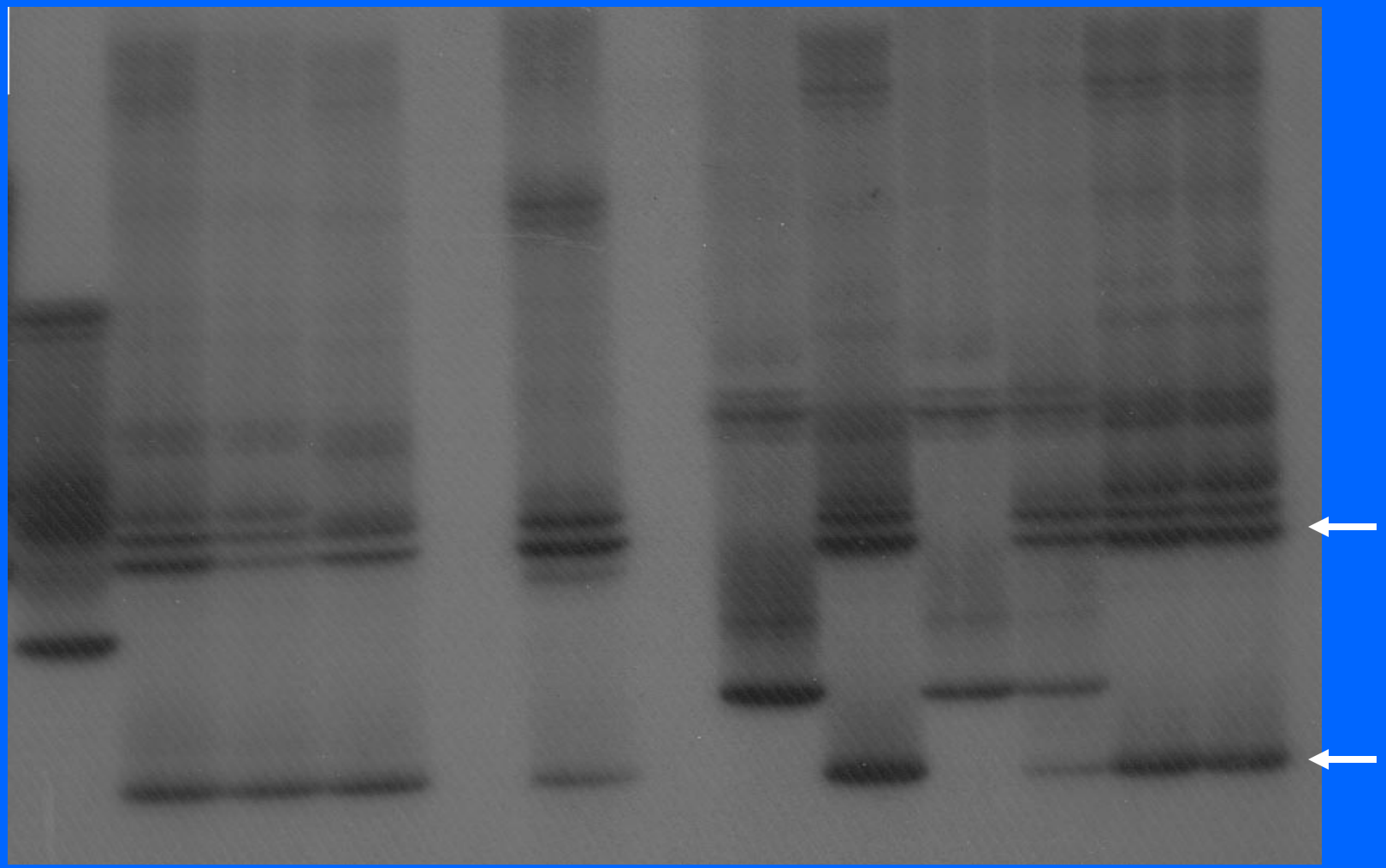
SSR Marker: GMS389

F Su S W E V M P W Ps_1 Rs_1 Ps_2 Rs_2



SSR Marker: GMS533

F Su S W E V M P W P_{S1} R_{S1} P_{S2} R_{S2}



Conclusions

- Resistance to disease spread, DON production, and seed colonization could be defined as three types of resistance in wheat.
- Different types of resistance may have some gene(s) or allele in common.
- Major gene action confers resistance in type II resistance sources, while minor gene action confers resistance in the SRW wheat cultivar Ernie.
- More than one resistant parents should be included in the cross, and comprehensive breeding techniques should be applied for selective breeding.

Acknowledgement

- **Funding was provided by USDA Scab and Barley Initiative, Virginia Small Grain Board and Virginia Agricultural Council.**
- **C. Griffey, M.A.Saghai Maroof, Eric Stromberg, D. Brann, X. Weiping*, R. Biyashev.**
- **T. Pridgen, M. Chappell, W.Rohrer, J. Shaw, W. Zhao, D. Nabati, J.Wilson, and B. Robinson.**
- **J. Mammadov, S. Jeong.**