P R O C E E D I N G S

Nineteenth Hard Red Winter Wheat Workers Conference

January 21 - 23, 1992

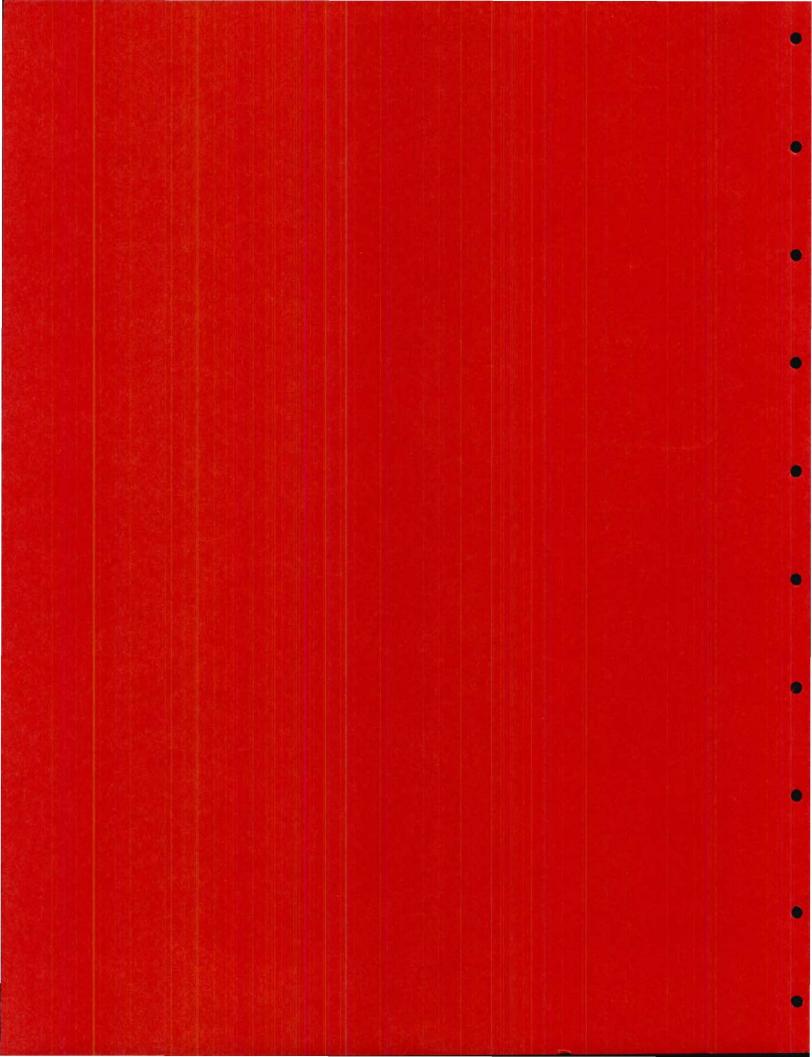


U. S. Department of Agriculture Agricultural Research Service Lincoln, Nebraska

and

Department of Agronomy Nebraska Agricultural Experiment Station University of Nebraska

Sponsored by The Hard Red Winter Wheat Improvement Committee



UNITED STATES DEPARTMENT OF AGRICULTURE

Agricultural Research Service

and

State Agricultural Experiment Stations

in the

Hard Red Winter Wheat Region

PROCEEDINGS

of the

NINETEENTH HARD RED WINTER WHEAT

WORKERS CONFERENCE

Lincoln, Nebraska January 21 - 23, 1992

Report Not For Publication¹

U. S. Department of Agriculture Agricultural Research Service

and

Department of Agronomy Nebraska Agricultural Experiment Station University of Nebraska

December, 1992

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FOREWORD

Approximately 125 wheat workers representing public and private wheat research programs from throughout the U.S. participated in the Hard Red Winter Wheat Workers Conference held in Lincoln, Nebraska, on January 21-23, 1992. This was the nineteenth such conference since the initiation of the cooperative state-federal hard red winter wheat investigations in 1929. The conference has been held on three year intervals and is organized and sponsored by the Hard Red Winter Wheat Improvement Committee (HRWWIC).

The format of this conference follows the loose and unwritten traditions established by the HRWWIC for past Regional Conferences. Research areas of interest were identified by the officers of the HRWWIC and the Conference Organizing Committee, then discussion leaders were drafted to organize the respective sessions. Our appreciation goes out to the discussion leaders and Organizing Committee, as well as to the many speakers involved, who deserve credit for the overall success of this conference.

Submission of written material for this Proceedings was optional and the format for submissions was left up to the authors. As such, the Proceedings do not reflect the complete scope of the presentations, nor the scope or intensity of discussions. A business meeting of the HRWWIC was held during the conference and minutes of that meeting are included in this proceedings.

The HRWWIC and conference organizers wish to express their sincere appreciation for financial support provided by the Nebraska Crop Improvement Association; Nebraska Wheat Board; AgriPro Bioscience, Inc.; ConAgra Grain Processing Company; Hege Equipment, Inc.; and HybriTech Seeds, Inc.

Special thanks go out to Joyce Kovar for her kind help with both organization of the conference and these proceedings.

C. James Peterson, USDA-ARS Secretary, Hard Red Winter Wheat Improvement Committee

CONFERENCE PROGRAM

January 20, 1992

Registration Opening Mixer

January 21, 1992

Welcome and Opening Remarks

Darrell Nelson, Dean and Director, University of Nebraska

Leaf Rust Resistance Session

Chair, Stan Cox, USDA-ARS, Manhattan, KS

Specific LR Genes Utilized in Great Plains Breeding Programs
 ▶ Don McVey, USDA-ARS, St. Paul, MN

Leaf Rust Gene Deployment

David Marshall, Texas A&M, Dallas, TX

Incorporating New Genes from Alien Progenitor Species

Stan Cox, USDA-ARS, Manhattan, KS

Discussion of Strategies and Approaches

Wheat Diseases Session

Chair, Charlie Rush, Texas A&M, Bushland, TX

Virus Diseases

Roy French, USDA-ARS, Lincoln, NE

Pythium

Larry Singleton, Oklahoma State University, Stillwater, OK

Rhizoctonia

Charlie Rush, Texas A&M, Bushland, TX

Conference Luncheon

Speaker, Rob Bruns, AgriPro, Berthoud, CO

Entomology Session

Chair, Jim Hatchett, USDA-ARS, Manhattan, KS

Breeding for RWA Resistance in the West Central Great Plains
 Jim Quick, Colorado State University, Ft. Collins, CO

Sources of RWA Resistance in North African Durums and Wheat Alien Species

Gerald Wilde, Kansas State University, Manhattan, KS

Current Status of Greenbug Resistance

Dave Porter, USDA-ARS, Stillwater, OK

Resistance to Wheat Curl Mite

Tom Harvey and Joe Martin, Kansas State University, Hays, KS

Transfer of Hessian Fly Resistance Genes from Rye to Wheat

Jim Hatchett, USDA-ARS, Manhattan, KS

Wheat Quality Session

Chair, Bob Graybosch, USDA-ARS, Lincoln, NE

Stability of Kernel Hardness for Hard Red Winter Wheats

Rollie Sears and Stan Cox, Kansas State University, Manhattan, KS

Feasibility of Using Near-Infrared and Visible Light Spectrophotometry to Measure Bread-making Potential of Commercial Wheat Flours

Steve Delwiche, USDA-ARS, Beltsville, MD

Genotype and Environment influences on Quality and Biochemical Composition of Hard Red Winter Wheat

Jim Peterson, USDA-ARS, Lincoln, NE

Detecting 1B/1R Wheat-Rye Translocations Using Monoclonal Antibodies

► Neil Howes, Ag. Canada, Winnipeg, Canada

Flour Biochemical Consequences of Wheat-Translocations

Bob Graybosch, USDA-ARS, Lincoln, NE

January 22, 1992

Poster Session

Breeding and Genetics Session

Chairs, Ed Smith and Arron Guenzi, Oklahoma State University, Stillwater, OK

Development and Utilization of a RFLP Based Genetic Map of Wheat

Kulvinder Gill, Kansas State University, Manhattan, KS

Use of Molecular Markers in Analyzing Quantitative Traits in Wheat

 Stan Cox, USDA-ARS, Manhattan, KS

79,274

Doubled Haploids in Wheat Breeding

Stephen Baenziger, University of Nebraska, Lincoln, NE

Transformation of Cereals

Mike Fromm, Monsanto, St. Louis, MO

Status of Wheat Cell and Tissue Culture Systems

Arron Guenzi, Oklahoma State University, Stillwater, OK

Hybrid Wheat: Current World Status and Future Prospects

John Erickson, HybriTech, Wichita, KS

Creating a Heterotic Group in Hard Red Winter Wheat

Rollie Sears, Kansas State University, Manhattan, KS

Regional Business Meeting

Chair, Dave Worrall, Texas A&M, Vernon, TX

Statistical Applications Session

Chair, Jim Peterson, USDA-ARS, Lincoln, NE

Probability Approach to Selection for Quality Traits

Kent Eskridge, University of Nebraska, Lincoln, NE

Using Spacial Correlations in Designed Experiments

David Marx, University of Nebraska, Lincoln, NE

Statistical Considerations in Development and Utilization of Genome Maps

Steve Knapp, Oregon State University, Corvallis, OR

Conference Banquet

Dr. Byrd Curtis, CIMMYT, Ft. Collins, CO

January 23, 1992

National Issues Forum: PVP and Plant Patenting

Chair, Virgil Smail, USDA-ARS, Manhattan, KS

PVP Versus Patents: A Legal Description of Their Relative Roles

 Robert J. Jondle, Venable, Baetjer, Howard and Civiletti, Washington, D.C.

Genetic Distance and PVP/Patent Issues (ASTA Breeders Subcommittee Report)

Stephen Baenziger, University of Nebraska, Lincoln, NE

AOSCA's Position on PVP Versus Patenting

John Erickson, HybriTech, Wichita, KS

International Regulations and Trends Toward Varietal Protection

Ian Edwards, Pioneer Overseas Corp., Johnston, IA

ASTA and Seedmans Perspective on PVP Versus Patenting

Jim Girardin, Arrow Seeds, Broken Bow, NE

National Wheat Improvement Committee: Update and Initiatives

► Ian Edwards, Pioneer Overseas Corp., Johnston, IA

Status of Germplasm Screening Efforts, GRIN, and Small Grains Collection Darrell Wesenberg, USDA-ARS, Aberdeen, ID

v

Plans for Regional Quality Testing Program

O. K. Chung, USDA-ARS, Manhattan, KS

Germplasm Exchange Issues, Conference Wrap-up

 David Worrall, Texas A&M, Vernon, TX Jim Peterson, USDA-ARS, Lincoln, NE

POSTULATED GENES FOR RESISTANCE TO LEAF RUST IN PARENTAL LINES OF NINE HARD RED WINTER WHEAT BREEDING PROGRAMS

D. V. McVey and D. L. Long USDA-ARS St. Paul, MN

The breeding lines provided were evaluated for their reaction to leaf rust by inoculating them with 19 isolates of leaf rust. The leaf rust isolates differed for the avirulence/virulence formulae. Using the gene-for-gene theory, the <u>Lr</u> genes were postulated. The <u>Lr</u> genes used and the coding system are given in Table 1. The isolates used are given in Table 2. The infection type data is presented in Table 3, and the postulated <u>Lr</u> genes in Table 4.

Set 1 Set 2 Set 3 Set 4	1 9 3KA 10	2A 16 11 18	2C 24 17 14A	3 26 30 14B	LETTERS in CODE
BCDFGHJKLZZPQR%F	R ♦ S ♦	R> S> R> S>	R R S S R R S S R R S S R R S S	R S R S R S R S R S R S R S R S R S R S	first letter = Set 1 second letter = Set 2 third letter = Set 3 fourth letter = Set 4

Table 1. Lr genes used and the system used to define isolate code.

No.	ld. Number	Code	No.	ld. Number	Code
1.	87-21-731	ТСВК		88-1-314	MLFT
2.	89-16-762	TLGK	12.	88-30-629	LBGT
З.	87-14-377	TDBP	13.	90-14-285	MGMJ
4.	90-14-4 94	KFBP	14.	84-21-9C	MGCP
5.	89-12-516	MCDJ	15.	82-21-95C	CLLJ
6.	81-PER-SCD	SCDJ	16.	82-21-30C	CBMJ
7.	90-1-158	TCBM	17.	84-21-126B	PGBL
8.	89-39-554	KBGM	18.	84-32-122C	TGBP
9.	86-41-76	MFLP	19.	82-21-OQ	
10.	88-34-387	KDBP			

Table 2. Leaf rust isolates used to evaluate some hard red winter wheat parental lines.

Line					-			solates											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
TX89D9233	;,X	;1,S	;	;,S	0;	0;	;	;	;,S	;	;1	;1	0;	0;	X-	;1,X	0;	0;	0;,S
TX90D9377	0;	;1-C	;	:	0;	0;	0;	0;	0;	0;	x	0;	0;	0;	x	x	;	0;	0;
TX90D9716	;	;1C	;	;	0;	0;	0;	0;	0;	0	x	0;	0;	0	x	x	;	0;	0
TX86D1332		;	1C	 ;	0;	;	;	;	;1C	;	1CN	1CN		23	0;	0;	2C		0;
TX88D3424		;	:		0;	;	;	:	;1C	;1-	0;	0;	0;	;	0;	0;	;	;1-	0;
TX896435		;	:		0;	:	1	1:	;1	;	0;	0;		;	0;	;	;	:	:
TX90D9277	0;	;,S	:	;	;,S	;,S	:	0;	0;	0;	**	0;	0;	0	;,S	;,S	1:	0;	0;
TX89D9627			;1C	;1C	;1-		;	;1-	;1C	;1-	;1-	1:	;1-	1:	:	;	:	;1-	0;
																		<u>т</u>	
18083					;	:						<u> ;</u>							
19236	20	x	x	1	0;	:	;1-	;	;1	0;	0;	0;	;1	;1C	0;	0;	;1		0;
30223W		x	X	X+	:	;					x	:	x		x	x	X+		
654	;,S	;,S	;,S	; . S	:	;,S	;,S		x			;,S		;,S	;,S	;,S		;,S	;,S
9146059	2C	;1C	23	;	:	:	;1C	;	1	0;	:	;	;1	;10	0;	;	;1		:
9146025	;	:			;	;	;	:			;	0;	:	:	0;	0;	;	:	
1085	;	;	:	:	0;	:	;	:	:	0;	0;	;	;1-	;	0;	0;	;1	;1-	0;
906	1P;	1P;	2P0;	2P;	3P;	4P;	2P;	1P;	2P0;	3P;1	4P0	3P0	3P0	1P;	2P;	SP;	1P;	3P;	2P0;
TX86V154	;	;	:	:	0;	0;	1:	0;	0;	0;		0;	0;	0;	X-	21C		1:	0;

Table 3. Reaction of hard red winter wheat parental breeding lines to 19 selected isolates of leaf rust.

·		<u> </u>				<u> </u>					r								
Line	1	2	3	4	5	6	7	6	9	10	11	12	13	14	15	18	17	18	19
Arapahoe	;1	;	1CN	1CN	;	0;	0;	:	;1	1CN	;	0;	0;	:	;	<u> </u>	:	:	;
NE89504		:	1CN	12CN	0;	:	;1	;	;1	1CN	;	0;	0;	;	;		:	:	:
NE89526	;1-	;1-	S.;		:	:	;	:			:	:	;	;1-	;1	;1-	;10	;	:
NE67615	2CN	1CN	1CN	1CN	;	;	10	;1CN	;1	1CN	1CN	:			2CN	2CN			2C
N87V106	;1C	;1-	1C	23	;	;	;1C	1C	12C	;1	;1,S	;	12	;,S	;	;1C	1C	2	?
CO860086	;1-	8P;	<u> </u>		9P;	10P;	;			<u> </u>					;1C	;1C		<u> </u>	6P
CO860235	;	;			0;	:	;								;12-	;1C			;
CO850061		23			21C	21C	1C				X+	21	;1		x	X.	x		;
CO870449	;1-	;	23	23	0;	0;	;	:	x	23	:	0;	:	;	;1-	:	:	:	
CO860094	;	;	_		0;	;	••	. :			;1-	0;	;1-	;	;	:	;	;1	;
										<u>г</u>		T				r		T	
W87-018	12C	6P;1			5P;1	5P;1	;10				9P;	;1	;1	6PX		;1	; 1		╂───-
W190-108	xc	21C	X	-i	0;	<u>;1</u>		↓∶	<u>×</u>	0;	┣──-	<u> </u>	┣━━	X	0;	0;	<u>×</u>	X	;
WI90-162	×	↓ ;	X	;1	:	;		;	Υ	X	:	10	23	<u>x</u>	X	;1C	X-	;1	;1
W188-028	; 1-	<u> ;</u>		L	0;	0;	0;	;1	·		:	0;	<u> ;</u>	0;	; <u> </u>	0;	0;	;	
W190-108	8P;	7P;			9P;	8P;	6P;	X			6P;	;	6P;	5P;	;1-	;1-	;	:	
JRE154199								[·				<u> </u>		[1
JRE926008	;,\$;,\$;,S	;,S	;,S	;,S	;,S	;,S			;.S	;,\$;,S	23	;1	;	;,S	1
JRE926036			;	0;	0;	0;	0;	0;	0;	0;		0;	0;	0		1	0	;	0;
JRE154224	X+C			;	;	X+	X+C	:		0;	[;	:			;
JRE926027	0;	;1C	;	0;	0;	0;	Ó;	0;	0;	0;	1	0;	0;	0		1		;	0;
JRE154201	;	;1	;,S	;,\$;	:	;	:	;,\$:	;	;	:	;	;	0;	:	;
JRE928588	12C	;,1C	;1-C	;1C	;1-C	;1-C	1C	;1-	;1-	;1.	;1	0;	0;	;	;1-C	;1C	0	;	:
JRE926050	0;	1:	1:		0;	0;	:	0;	0;	0;	0;	0;	0;	0	0	0:	0	0;	0;

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																	•		
Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
HBF0290-144		21C	2	x	;	:	1		21		1	;	;1			;1C	0;	x	21C
HBF0303-156	;1C	;			:	0;	;	x	X+	x	X	0;	x	x				x	
HBF551-137	XC,S	;	;		:	;	;12	;1-		;1-C	;1-C	0;	0;	;	;	0;	:	;	;
KS84W063-9-18	x	;1C	;1C	20	;1-C	;1-C	21C	;1C	;1	12	x	;1			x	;12-	1C	;1C	21C
KS86509-1-1	;1-	;1-	;1-	;	:	:	;1-C	;	;1-	0;	;1-	;1-	;1	:	0;	0;	X	;1C	0;
KS85W663-3-2	21C	21C	;1C	;1C	:	;1C	X 5	;1-	21C	;	;1	12	x	;12C	0;	;	;1-	21C	;1C
HBC302E	;1-	;	;1C	;1C	;	;1-C	;	1	Y21	1C	;1	1	1	;10	;1-C	;1C	;1-C	;1	;
KS84170E8-2	;1-C	;	;		;	;1C	;10	;1-C	;1-C	;1-	;	0;	0;	:	0;	0;	;	;1-	;
KS87809-6-1			-;		;	-:			;1C	0;	;1-	0;	0;	;	0;	;	;1-C];	0;
	<u> </u>	γ			1	г [.]					1	···	1.			1	1	1	r –
KS87H325-2	×	ļ	;1C	;1C	;	;	21C	;1	X+	×	×	0; `		×	<u>×</u>	;		X,S	
KS89H20	2	<u> </u>	;1C	;10	;	;	21C		Y21	;1-	;1	;1- -	;1	;10	:	;	;	;1	;1
KS89H48-1	;1C	;1C	;1C	10	;10	;10	;1C	;1C	;1C	;1C	1	21	;1	;1-C	;1-	;1-C	;	;10	;1-C
KS89HW53	_		X+	X	x	X+			X+		;12	21	;12		x	x	x		
KS82H238-1	23	;1C	10	23	21C	21C	2C	1	21	21	x	x	1	x	x	X.	X	10	;1
KS89H192	;1-	;1-	23	:	;	:	;	0;	0;	0;	0;	0;	0;	0;	0;	:	;	;	0;
KS87H6	;	;	1CN	1CN	0;	:	;	;	;1	;1C	0;	;1-C	;	;	;	;	;	;	;
Blue Dame	2CN	2CN	2CN	2CN	2CN	12CN	2CN	2CN	2CN	2CN	1CN	1CN			2CN	2CN			2CN

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Line	Postulated Lr gene	Line	Postulated <u>Lr</u> gene
D. S. Marshall		J. R. Erickson	
TX86D1332	1,10,16	154199	None
TX88D3424	2A,24,26	154201	10,24,Seg2A
TX896435	2A,3,26	154224	1,+
TX89D9233	1,24,26	926008	+
TX89D9627	1,2A,10, 2 6	926027	3,10,+
TX90D9277	1,2 A,24,2 6	926036	+
TX90D9377	9?,10,14A,+	926050	1,2A,24,26
TX90D9716	9?,10,14A,+	926586	2A ,10,18
T. J. Martin		S. W. Perry	
KS87H6	10,16,24	654	+, Seg1 0
KS89H20	3,11	1085	9,24
KS87H325-2	3,+	9146025	24
KS89H48-1	17,18,30?	9146059	1,2A,3,1 7
KS89HW53	14 A, +	906	+
KS89H192	1,2A,24	30223W	3,14A,+
KS82H238-1	14A,30	19236	3,+
Blue Dame	16	18083	3
R. Bruns		J. S. Quick	
W87-018	+	CO850061	14A,+
W188-028	24	CO860086	Seg10,+
WI90-106	1,+	CO860094	10,24
WI90-162	14A,+	CO86235	10
WI90-108	24?	CO870449	3KA,24
R. G. Sears		C. J. Peterson	
KS84170E8-2	2A,24,26	Arapahoe	10,16,24
KS84W063-9-18	3KA,+	NE87615	3,16
KS87809-6-1	9,24	NE89504	10,16,24
KS86509-1-1	1,17	NE89526	10,24,Seg26?
KS85W663-3-2	1,2A,3,30	N87V106	3,30?
KBF0290-144	3,+		
HBC302E	24,30	W. D. Worrall	
JBF551-137	3,26	TX86V1540	9,10,+
HBF0303-156	3+		

 Table 4.
 Postulated Lr genes for resistance to leaf rust in some hard red winter wheat parental lines.

Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Lr3					;10	;1-C		_				;							
Lr10	;10_	;1C			;1-	;1	;	L											;
Lr30	23	2C	;1C	1C	1C		;1	;10	;1C	;1C		;10			2		;10	1C	;1-
Lr16	2CN	2 CN	1CN	2 CN	1CN	2 CN	2 CN	2CN	1CN	1CN	2CN	2CN			2 CN	2 CN			2CN
Lr24	;	;1-			0;	0;	;	;			;	;1-	;	0;	;	;	;	;	
Lr10+16+24	;	;1-	1CN	2CN	0;	0;	;	 ;	1CN	1CN	;	;1-	;	0;	;	;	 ;	;	- ;
Arapahoe	;1	;	1CN	1CN			0;	;	;1	1CN	;	0;	0;	;	;	;	;	;	;
NE89504	;	;	1CN	12CN	0;	;	;1	;	;1	1 CN	;	0;	0;	;	;	;	;	;	_;
Lr10+24+26	;	;1-	;1-24		0;	0;	;	;		;1024	;	;1-	;	0;	;	;	;	;	<u> </u>
NE89526	;1-	;1-	s,;		;	;	;	;			;	;	;	;1-	;1-	;1-	;10	;	;
Lr3+Lr16	2CN	2CN	1CN	2CN	;1C	;1C	2 CN	2CN	1CN	1CN	2 CN	;			2CN	2CN			2CN
NE87615	2CN	1CN	1CN	1CN	;	;	1C	;1CN	;1	1CN	1 CN	;			2CN	2CN			2CN
Lr3+30	2 3	2C	;1C	2	;10	;10	;1	23	;10	23		;	<u> </u>		2		;10	1C	;1-
N87V106	;10	;1-	1C	23	;	;	;10	1C	12C	;1	;1,S	;	12	;,s	;	;10	1C	2	?
																<u> </u>			+
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Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17_	18	19
JRE154199										L									
JRE926008	• •			;,S		. c	;,s	;,S											
JRE926036	;,s	;,S		1			,, <u>s</u> 0;	0;	0;	0;	;,S	;,S 0;	0;	;,S_ 0	23	;1	0	;,5	0;
Lr1				;	0;	 		;		;		┥	<u> </u>		;	;	┨───	╀	;
JRE154224	X+C			;		X+	X+C	;		0;					;	;			;
Lr3+10	;1C	;10			;1-	;1	;	· · ·		<u> </u>		;					<u> </u>	<u> </u>	;1
JRE926027	0;	;1C	;	0;	0;	0;	0;	0;	0;	0;	1	0;	0;	0			┨	<u>;</u>	<u>o;</u>
Lr2A+10+24	;	;1-			0;	0;	;	;	;	<u> </u>	;	;1-	;	0;	;	;	;	;	0;
JRE154201	;	;1	;,s	;,s_	;	;	;	;	;,s_	┨────	<u> ;</u>	;	;	;	;	;	0;	<u>; </u>	;
Lr2A+10+18	;1C	;1C	;10	12C	;1	;1	;	;1	;	;10	;1	;1C	;10	0;	;	;	;1-	10	0;
JRE926586	12C	;,10	;1-C	;1C	;1-C	;1-C	1C	;1-	;1-	;1	;1	0;	0;	;	;1-C	;10	o	;	;
Lr1+2A+24+ 26	;	;1-	;1-	;	0;	0;	;	;	;	;	;	0;	;	0;	;	0;	;	;	;
JRE926050	0;	;	;	;	0;	0;	;	0;	0;	0;	0;	0;	0;	0	0	0;	0	0;	0;
		┣──		┝───	┼───	┨────	╂		┨───	┨───	╂	┨	╂ ──			╂───	┨	┨───	╆

Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Lr1				;	0;			;		;					;	;		
Lr14A					_								X?		x-	x-	x	
Lr24	;	;1-			0;	0;	;	;	+		;	;1-	;	0;	;	;	;	;
W87-018	12C	6P;1	·		5P;1	5P;1	;1C				9P;	;1	;1	6PX	;	;1	;1	
Lr1				;	0;			;		;					;	;		
<u>WI90-106</u>	хс	21C	x	;	0;	;1		;	x	0;		<u> </u>		x	0;	0;	x	x
Lr14A						· · · · · ·							x?		x-	x-	x	
WI90-162	x	;	<u>x</u>	;1.	;	;	23	;	<u>Y</u>	x	;	1C	23	x	x	;1C	x -	;1
Lr24	;	;1-			0;	0;	;	;			;	;1-	;	0;	;	;	;	;
WI88-028	;1-	;			0;	0;	0;	;1			;	0;	;	0;	;	0;	0;	;
WI90-108	8P;	7P;		_	9P;	8P;	6P;	x			6P;	;	8P;	5P;	;1-	;1-	;	;
	╂-──			_	<u>+</u>					_				┨───			╂──	
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Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Lr3_					;1C	;1-C						;							
18083					;	;						;							
19236	2C	x	x	;	0;	;	;1-	;	;1	0;	0;	0;	;1	;1C	0;	0;	;1		0;
Lr3+14A					;1C	;1-C						;	x?		x-	x-	x		
30223W		x	x	<u>x+</u>	;	;					x	;	x		x	x	X+		
Lr10	;10	;10			;1-	;1	;												;1
654	;,s	;,S	;,s	;, <u>s</u>	;	;,s	;,s		x			;,5		;,5	;,5	;,5		;,s	;,s
Lr1+2A+3+ 17	2C	2C	;1-C	;	0;	;1-C	;1C	;	;	;	;1	;	; 1C	0;	;	;	;1-		0;
9146059	2C	;1C	23	;	;	;	;1C	;	1	0;	;	;	;1	;10	0;	;	;1	ļ	;
Lr24		;1-			0;	0;					;	;1-	;	0;		;	;	<u> </u>	
9146025	;	;			;	;	;	;			;	0;	;	;	0;	0;	;	;	
Lr9+24	;	;1-	;	;	;	;	;	;	;	;	;	0;	0;	0;	 ;	0;	0;	;	;
1085	;	;	;	;	0;	;	;	;	;	0;	0;	;	;1-	;	0;	0;	;1	;1-	0;
906	1P;	1P;	2P0;	2P;	3P;	4P;	2P;	1P;	2P0;	3P;1	4P0	300	3P0	1P;	2P;	3P;	1P;	3P;	2P0;

Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Lr3					;1C	;1-C						;							1
KS87H325-2	x		;1C	;1C	;	;	21C	;1	X+	х	x	0;		x	x	;		x,s	
			-				-					-							
	23 2	-	1C ;1C	23 ;1C	;1C	;1-C	23 21C		23 Y21	23	23 ;1	;1-	23	23	1C	;1C	;1C	23	21
<u> </u>			, 10	, 10	/	,	210		121	, 1	<u>, 1</u>	11	/ <u> </u>	,10	,	/	<u> </u>	/	<u>//</u>
LR17+18	2C	2C	;1C	12C	2C	2C	;1C	;1	;1C	;1C		23	12	;1C	;12	23	;1C	1C	;1-0
KS89H48-1	;1C	;1C	;10	1C	;1C	;1C	;1C	;10	;10	;10	1	21	;1	;1-C	;1-	;1-C	;	;1C	;1-0
Lr14A													x?		x-	x-	x		-
KS89HW53			x+	x	x	x+			X+		;12	21	;12		x	-	x		\square
													· ·						
Lr14A+30	23	2C	;10	1C	1C		;1	23	;1C	;10		;1C	;1Ċ	5	x	2	;1C	1C	;1-
KS82H238-1	23	;10	1C	23	21C	21C	2 C	1	21	21	x	<u>x</u>	1	x	x	x-	x ·	1C	;1
Lr1+2A+24	;	;1-		;	0;	0;	;	;	;	;	;	;1-	;	0;	;	;	;	;	0;
KS89H192	;1-	;1-	23	;	;	;	;	0;	0;	0;	0;	0;	0;	0;	0;	i	;	;	0;
Lr10+16+24	•	;1-	1CN	2 CN	0;	0;			1CN	1CN		;1-		0;		 :	:	:	;1
KS87H6	;	;	1CN	1CN	0;	;	;	;	;1	1	, o;	;1-C	;	;	;	;	;	;	;
Lr16	2CN	2CN	1CN	2CN	1CN	2CN	2 C N	2CN	1CN	1CN	2CN	2CN			2CN	2CN		<u> </u>	2CN
	2CN 2CN	2CN 2CN	2CN	2CN 2CN	2CN		2 CN 2 CN	2CN 2CN	2CN	2CN	1CN	1CN			2CN	2CN		-	2CN
June Dame						12011	2.011	2.0.1			1.0								<u> </u>
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Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Lr3				L	;1C	;1-C	.		1			;							
HBF0290-144		21C	2	x	;	;	1		21		1	;	;1			;1C	0;	x	21C
HBF0303-156	;10	;			;	0;	;	x	X+	X -	x	0;	x	x			;	x	<u> </u>
Lr3+26	_		;1-`		;1C	;1-C		;1-		;1C	;1C	0;	;	;	;	0;	;1-	;	;
HBF551-137	xc,s	;	;		;	;	;12	;1-		;1-C	;1-Ĉ	0;	0;) ;		0;	;	;	;
Lr3KA	23	23	21C	23	23	2	23	23		223		21		23			23	23	
KS84W063-9- 18	X	;1C	;1C	2C	;1-C	;1-C	21C	;1C	;1	12	x	;1			x	;12-	1C	;1C	21C
Lr1+17	2C	2C	;1-C	;	0;		;1C	;	;12-	;	;12	2C	23	23	;	;	23	23	;
KS86509-1-1	;1-"	;1- ''	;1-	;	;	;	;1-C	;	;1-	0;	;1-"	;1-	;1	; "	0;	0;	x ″	;10	0;
Lr1+2A+3+ 30	23	2C	;1C	;	0;	;10	;1	;	;	;	;1	;	;10	0;	;	;	;1-	10	;
KS85W663-3-2	21C [″]	21C	;10	;10_	;	;1C	<u>x</u>	;1-	21C	;	;1	12	<u>x</u>	;12C	0;	;	;1-	21C	;10
Lr24+30	;	;1-	;10	1C	0;	0;	;	;	;1-C	;10	;	;1-	;	;	;	;	;	;	;1-
HBC302E	;1-	;	;10	;10	;	;1-C	;	1	¥21	1C	;1	1	1	;1C	;1-C	;1C	;1-C	;1	;
Lr2A+24+26	;	;1-	;1-			0;	;	;	;	;10	;	0;	;	;	;	0;	;	;	;
KS84170E8-2	;1-C	;	;	·	; ;	;1C ["]	;10	;1-C	;1-C	;1-	;	0;	0;	;	0;	0;	;	;1-	;
Lr9+24	;	;1-	;	;	;	;	;	;	;	;	;	;	0;	0;	0;	;	;	;	;
KS87809-6-1	;	i 17	; '	;	;	;	;	;	;1C [']	0; _′	;1-"	0;	0;	;	0;	;	;1-C	;	0;

Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Lr1				;	0;	1		;		;					;	;			;
Lr2A						_			;		;1	;10	;1C	0;	;	;	;1-		0;
Lr3					;1C	;1-C						;							
Lr9	;		;	;	;	;	;	;	;	;		0;	0;	0;		0;	0;	;	;_
Lr10	;1C	;10			;1-	;1	;		•	Ţ									;1
Lr14A							_						X?		x-	x-	x		
Lr24	;	;1-			0;	0;	;	;			;	;1-	;	0;	;	;	;	;	
Lr26	-		;1-					;1-		;1C	;10	0;	;	;	;	0;	;1-	;	;
Lr1+24+26		;1-	;1-	;	:10	;1-C	:				:		 	 :			 ;		<u> </u>
TX89D9233	;, x [~]	;1,5	·	<u> </u>	0;	0;	:	;	;,s	;	;1	;1	0;	0;	x-	;1,X	, 0;	0;	0;,5
	1	<u>, , , _</u>	,	<u> </u>			<u>i</u>	<u> </u>		ľ		<u> </u>	<u>·</u>	<u> </u>					
Lr9+10+14A	;	;1C	;	;	;	;	;	;	;	;	· ·	;	;	0;	x-	0;	0;	;	;
TX90D9377	0;	;1-C	;	;	0;	0;	0;	0;	0;	0;	x	0;	0;	0;	х	x	;	0;	0;
TX90D9716	;	;10	;	;	0;	0;	0;	0;	0;	0	x	0;	0;	0	X	x *	;	0;	0
 Lr1+10+16	:1C	;1C	1CN		0;		2 CN	:	1CN		2CN	2CN			 :				
TX86D1332	;	;	1C ″	;	0;	;	;	;	;1C	;	1CN	1CN ⁴		23	0;	0;	2C	<u> </u>	0;
	<u> </u>	;1-	;1-		0;	0;		;1-		;1C		0;		;			:	<u> </u>	<u> </u>
TX88D3424	•	, <u> </u>	, <u>-</u> , ²¹	<u> </u>	0;	•	•		;1C	<u> </u>	, 0;	0;	, 0;	;	0;.	0;	;	;1-	0;
	<u> </u>	,	/			<u> </u>	<u> </u>	<u> </u>	<u>, 10</u>	<u>, -</u>	<u> </u>	<i>r</i>					Ľ.		
Lr2A+3+26			;1-		;1C	;1-C		;1-	;	;10	;1	0;	;	;	;	0;	;1-	;	0;
TX896435		• *	• •		0;	. `		 .	;1	•	0;	0;	l.	1.	0;				;

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Lr1+2A+24+26	;	;1-	;1-	;	0;	0;	;	;	;	;	;	;1-	;	0;	;	;	;	;	;
TX90D9277	0;	;,s	;	;	;,s	;,s	;	0;	0;	0;	**	0;	0;	0	;,5	;,s	;	0;	<u>o;</u>
Lr1+2A+10+26	;1C	;10	;1-	;	0;	;1	;	;	;	;	;10	0;	;	;	;	0;	;1-	;	;
TX89D9627	;	;	;10	;10	;1-	;	 ;	;1-	;10	;1-	;1-	<u> ;</u>	;1-	;	_ ;	;	<u>;</u>	;1-	0;
Lr24	;	;1-	<u> </u>		0;	0;	;	;			;	;1-	;	0;		;	;	;	
Lr10	;1C	;1C	<u> </u>		;1-	;1	;			·									;
Lr9	;		;	;	;	;	;	;	;	;		0;	0;	0;		0;	0;	;	;
Lr9+10	i	;1C	;	;	;	;	;	;	;	;		0;	0;	0;		0;	0;	;	;
TX86V154	ļ;	;	;	;	0;	0;	;	0;	0;	0;		0;	0;	0;	x-	21C	;	;	0;

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Postulated <u>Lr</u> genes for resistance to leaf rust in some hard red winter wheat cultivars

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演 德

Cultivar	Postulated	Cultivar	Postulated
Abilene	10,24	Newton	1
Agseco 7846	None	Redland	3,16
Agseco 7853	None	Sandy	10
Amigo	24	Siouxland	24,26
Arapahoe	10,16,24	TAM 105	10,+
Arkan	24	TAM 106	10,+
Brule	Seg 3,16	TAM 107	None
Chisholm	None	TAM 200	24
Collin	24	Thunderbird	1,24
Eagle	None	Vona	14A, +
Hail	3,+	Victory	14A, +
Karl	1,+	2157	14A, +
Larned	14a, +	2163	14A, +
Mit	1,10	2172	14A, +
		2180	3,14A,+

Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	1
Agseco7846				*								-							;
Agesco7853																			
Eagle										_								3	
Hawk																			
TAM 107	X+C																		
	<u> </u>	1.1					г. –												
Lr10	;1	;1			;1	;1	li	· ·											i
TAM 105	;	;	1		;1	;1	;		†—–	-		1		†	¥2;	Y2;		1	1;
TAM 106	;	;			;	;	;	;			; .		1		;	;		-	1;
SANDY	;	;1		1	;	;	;1								;1	;1			;
Lr10	;1	;1			;1	;1	 ;								<u> </u>			╂──	+;
Lr1	1			;	0;	Ē		;	<u> </u>	;				<u> </u>	;	;	1		1;
Lr10+LR1	;1	;1		17	0;	;1	;	;		;		1			;	;		1	;
MIT	;	;	X	;	0;	;	;	;		0;					0;	;		1	0
Lr1		<u> </u>	+-	;	0;		+	;	<u> </u>	 ;	┼───	╂───	+-	┼──	;	;	┼──		
NEWTON	1			;	;		<u> </u>	;	1 '	0;	1	1	1	t	0;	0;		<u> </u>	0;
KARL		;	2	;	0;	;	1	0;		0;	;1	;1	;1		0;	0;	;1	Y	0;

		- · · · · · · · · · · · · · · · · · · ·																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Lr24	;	;1-			0;	0;	;	;			;	;1-	;	0;	;	;	;	;	
ABILENE	;1-	;1-			0;	;	;	;			0;	;	0;	0;	;1-	;1-	;1-	;1-	
AMIGO	;1	;1-			; _	;	;1	;			0;	0;	;1-	;1-	;1-	;1	;1	;1	
ARKAN	;1	;1-			0;	0;	;	;			0;	;1	0;	0;	;	;	;	;	
CIMMARON	;	;			;	;	;	;			0;	0;	;1-	;	;	;	;	;	
COLLIN	;1-	;			;	;	;	;			;1-	;1-	0;	0;	;1-	;	;	;	
TAM 200	;	;			;	;	;	;			0;	0;	0;	0;	;	;	;	;1-	
								T								_			
Lr24	;	;1-			0;	0;	;	;			;	;1-	;	0;	;	;	;	;	
Lr1				;	0;			;		;					;	;			;
Lr24+Lr1	;	;1-		;	0;	0;	;	;		;	;	;1-	;	0;	;	;	;	;	;
THUNDERBIRD	;1-	;1-		;	;	0;	0;	0;		0;	0;	0;	;1-	;	0;	;	;	;	0;
	<u>Ľ</u>	<u> </u>	<u> </u>	<u>.</u>	<u>.</u>		1 <u></u>						<u> </u>	<u>.</u>					
Lr14A				l								-	X+?		x-	x	x		
Larned				23	23	23		23	23	23	1		;1		1-CN	x-	x		
Victor	23		x		x	;1C		X+	x	x	;1	;1	;1C	x	;1-C	x	x-		
Vona		;1C			23	2	;1C				X+		X+		х	x	x		;
2172			X+		;	;		X+							х	x			
2157	;12C		x	х	2	;10	;1C	23	23	23		;	;1	10	x	x	;	23	;1 C
2163	x	xc			;	;	;1-C								;1	x	;1		
<u> </u>	t				-					[<u> </u>								Γ
						<u> </u>	<u> </u>	<u>+ – – – </u>		· · · ·	1				<u> </u>			1	
			1		;10	;1-C						;							
Lr3 Lr3+Lr14A					;1C ;1C	;1-C ;1-C						;;	X+?		x-	x-	x		<u> </u>

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Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Lr3					;1C	;1-C						;							
Hail		21C			;	;	2-					;	;1		x	x	<u> </u>		
Lr16	2CN	2CN	1CN	2 CN	1CN	2CN	2 CN	2CN	1CN	1CN	2CN	2CN	-		2CN	2CN			2CN
Lr16+Lr3	2CN	2 CN	1CN	2 CN	;10	;1-C	2CN	2CN	1CN	1CN	2 CN	;			2 CN	2CN			2CN
Redland	2CN	;1C	1CN	2CN	;	;	;1-C	1CN	1CN	1CN	;1-	;1-			2CN	2CN	-		2CN
Lr10	;10	;1C	<u> </u>		;1-	;1	;												;1
Lr24	;	;1-			0;	0;	;	;			;	;1-	;	0;	;	;	;	;	
Lr10+16+24	;	;1	1CN	2CN	0;	0;	;.	;	1CN	1CN	;1-	;	;	0;	;	;	;	;	;1
Arapahoe	;	;	1CN	2CN	;	;	;	;	;	;1-	;	;	0;	0;	0;	;	;	;	;
Lr24	;	;1-			0;	0;	;	;			;	;1-	;	0;	;	;	;	;	
Lr26			;1-					;1-		;10	;1C	0;	;	;	;	0;	;1-	;	;
Lr24+Lr26	;	;1-	;1-		0;	0;	;	;		;1C	;1C	0;	;	;	;	;	;	;	;
Siouxland	;	;	;		;	;	;	;		0;	0;	0;	0;	0;	0;	;	;	;	;
						<u> </u>		 							+		<u>}</u>		<u>}</u>

LEAF RUST GENE DEPLOYMENT AND DURABILITY

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Leaf rust of wheat (caused by *Puccinia recondita*) is an endemic disease in the Great Plains, yet has the potential of being epidemic in any growing season (Marshall, 1988). Inoculum from oversummering sources typically infects fall-sown wheat in the southern plains and sporulating pustules can be found on seedling leaves as early as 4 wk after planting. Even though the infection cycle of *P. recondita* slows with cooler temperatures, large quantities of spores are transported north by wind during the spring and summer. Breeding wheats with durable resistance to leaf rust and determining the best methods to strategically use resistant germplasm are high priorities of the Texas Agricultural Experiment Station. About 36 individual genes for leaf rust resistance (*Lr* genes) have been identified. Of these, about seven *Lr* genes are most effective when expressed in adult plants. The remaining *Lr* genes are expressed throughout the life of the plant (typically referred to as seedling resistance genes). The phenotype of the *Lr* genes varies and several of the genes respond differentially to temperature changes.

Studies of the pathogen population in Texas (Marshall, 1988, 1989b, & 1992), the USA (Long *et al*, 1985, 1989), and Canada (Kolmer, 1988, 1990) have demonstrated the high level of variability in virulence of *P. recondita*. The fungus has adapted rapidly to wheat varieties which have been released with singly effective Lr genes (Kolmer, 1989; Marshall, 1989b). It is clear that indiscriminant incorporation and release of Lr genes in wheat varieties is an unwise strategy. Identification and diversification of Lr genes will most likely lead to sustainable leaf rust resistance (Marshall, 1989a).

The genes that have been heavily used and subsequently lost in hard red winter wheats include Lr1, Lr3, Lr10, and Lr24. Those that are in the process of being lost include Lr2a, Lr2c, Lr11, and Lr26. Of the remaining seedling resistance genes, Lr14a, Lr15, and Lr20 have not been widely used, but are ineffective because of their association with other fitness traits in *P. recondita*. This leaves about 17 Lr genes having potential usefulness. However, these 17 are not equal in terms of how they are expressed and their effectiveness in controlling *P. recondita*. For example, it is known that Lr16 (and possibly Lr9) have been used in the past, yet can be 'recycled' because in the absence of selection, the corresponding virulences tend to be lost from the pathogen population (Marshall, 1989b). Also, some genes (like Lr27 and Lr31) are expressed better when present together, rather than separately.

It is probable that the adult plant Lr genes (mainly Lr12, Lr13, Lr22a, Lr22b, Lr33, Lr34, and Lr35) have not been widely used in the hard red winter wheat germplasm. 'Sturdy' is known to possess Lr12 and Lr34 (Dyck, 1991), however lines derived from 'Sturdy' probably do not have this gene combination unless specifically selected for. Adult plant Lr genes in combination with themselves or in combination with seedling Lr genes tend to produce resistance phenotypes enhanced beyond that of any one Lr gene working alone.

In general, it is most likely that uniformity tends to promote the increase of diseases specific to a uniform plant population and that diversity tends to decrease those diseases (if the diversity is planned, and the genes that are diversified are those that 'count') (Marshall, 1989a). Diversity also tends to give protection against the 'unknown' disease, insect, or environmental stress. *Lr* genes can be diversified within a field (intrafield; using varietal mixtures or multilines), between fields within an area (interfield), and/or between areas within a region. Studies in Texas (Mahmood *et al*, 1991) and elsewhere have demonstrated unequivocally that leaf rust can be suppressed using intrafield diversification. However, grain yield may not be maintained or increased in proportion to the decrease in rust severity in some cases. Thus, in north-central Texas, we have studied and advocated the use of interfield varietal diversification. This program was based on the planting of different varieties (containing different *Lr* genes) in adjacent fields. This program was rapidly adopted by producers in north-central Texas and has been rather successful.

The potential exists for researchers in the hard red winter wheat region to breed and deploy wheat varieties for leaf rust resistance. However, much work and cooperation must first be done. The major obstacle to an effective leaf rust gene deployment program is the lack of information concerning which Lrgenes and Lr gene combinations are in our wheat varieties, lines, and germplasm. If this information was available, then a flexible plan, with equitable distribution of Lr genes could be accomplished. The identification of Lr genes must be an on-going program and must be accompanied with on-going programs in virulence determination and the breeding of durably resistant wheat germplasm.

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NEW LEAF RUST RESISTANCE GENES FROM WHEAT'S PROGENITOR SPECIES

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Whether or not strategies for reducing the evolution of virulence in Great Plains leaf rust populations are adopted, and regardless of the course taken, a supply of yet-unused resistance genes is a necessity. The donors of wheat's A and D genomes represent very accessible sources of a potentially large number of such genes.

The first collection of *Triticum tauschii*, the D genome donor, acquired by the Wheat Genetics Resource Center (WGRC) at Manhattan, KS originated primarily in the USSR and Iran. Of its 60 accessions, 31.6% had low, and 50% intermediate, infection types in leaf rust screening.

We have since obtained a larger collection of *T. tauschii* from Kyoto University. Of 183 accessions of var. *typica* collected from throughout the species range (Turkey to Pakistan), only 3.2% had low, and 2.7% intermediate, infection types. But among 24 accessions of vars. *strangulata* and *meyeri*, all from the Caspian coasts of Azerbaijan and Iran, 20.8% had low, and 33.3% had intermediate, infection types. Obviously, there is a strong geographical determinant of leaf rust resistance in this species.

At the WGRC, we have transferred resistance from 10 accessions and have resistance from 5 more sources still segregating. We have released 5 leaf rust-resistance germplasms derived from *T. aestivum* x *T. tauschii* backcrosses: KS86WGRC2, KS89WGRC7, KS90WGRC10, KS91WGRC11, and KS91WGRC12. The germplasm KS91WGRC12 carries adult-plant resistance from an accession collected in far-eastern Afghanistan, where no seedling-resistant accessions have been found.

Our philosophy has been to introgress these genes directly into current, elite hard red winter genotypes in order to make them highly accessible to breeders. Good agronomic type can be recovered quickly, and under heavy leaf rust infection, introgressed lines can be superior to their recurrent parents. For example, in a replicated test at Hutchinson, KS in 1991, 16 sister lines of KS90WGRC10 exceeded their recurrent parent, TAM 107, by 8 to 55% in grain yield, 6 to 28% in thousand-kernel weight, and 0.6 to 1.3 percentage points in grain protein.

Although direct introgression has worked fine with *T. tauschii*, it has been much more difficult with the A-genome species because of severe sterility and plant vigor problems in the direct hexaploid x diploid crosses.

Nevertheless, we have transferred resistance from three A-genome species: *T. urartu, T. boeoticum, and T. monococcum.* In *T. monococcum* crosses, only a single accession, PI 355520, produced female-fertile hybrids with hexaploid wheats we have used. We have used PI 355520 as a "bridge" parent to transfer resistance from other *T. monococcums.* Resistance from A-genome species appears to be more quantitative in nature than that from D-genome accessions.

WHEAT VIRUS DETECTION USING THE POLYMERASE CHAIN REACTION

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Barley yellow dwarf is the most damaging viral disease of wheat and other cereal crops worldwide, yet its diagnosis remains quite difficult. The causal agent, barley yellow dwarf virus (BYDV), is a complex of at least five different phloem-limited luteoviruses which can be distinguished by their aphid vector specificities, serological properties, and nucleic acid sequences. The virus is not mechanically transmissible and occurs in very low concentrations in infected plants. All current diagnostic methods used for BYDV, such as ELISA, hybridization with cDNA probes, and bioassays with aphid vectors, require multiple tests in order to detect all five BYDV serotypes.

Our objective was to develop a single assay capable of detecting most BYDV isolates. Four potentially luteovirus group-specific primers for use in the polymerase chain reaction (PCR) were synthesized based on RNA sequence data of three luteoviruses. One primer pair (designated LU1 and LU4) amplifying *ca.* 530 bp cDNA fragments spanning the virion capsid protein genes of potato leafroll virus (PLRV), beet western yellows virus (BWYV), New York barley yellow dwarf (BYDV) serotype PAV also amplified the corresponding regions of New York BYDV serotypes MAV, RMV, RPV, and SGV, a Montana RMV isolate, as well as many isolates collected in the Northern Great Plains. The different luteoviruses were distinguished by restriction analysis of the PCR cDNA products. An inherent advantage of PCR is its extreme sensitivity. Virus-specific products were readily detected in PCR assays of crude extracts containing as little as 5 ng of BYDV-infected tissue. Strong positive signals were also obtained in PCR assays of individual aphids.

Similar PCR assays have also been developed for soil-borne wheat mosaic, wheat streak mosaic, hordeum mosaic, and agropyron mosaic viruses. PCR will be most useful as a diagnostic tool for those viruses and strains for which high quality antisera are not widely available and for laboratories not routinely using ELISA. Because of the ability to differentiate closely related strains with detailed "fingerprints", PCR will be a very useful tool in epidemiological studies of wheat virus diseases.

PYTHIUM DISEASES OF CEREALS

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INTRODUCTION

DEFINING THE PROBLEM - <u>Pythium</u> species, as pathogens of cereals, have long been recognized as important in the culture of wheat in the United States (Vanterpool, 1940). The disease caused by this group of organisms is commonly referred to as Pythium Root Rot, or Browning Root Rot. Several <u>Pythium</u> species are currently regarded as important pathogens of cereals worldwide (Wiese, 1987). However, there are many questions that need to be addressed relative to their role as pathogens, and their interaction with other soilborne pathogens.

In general, soilborne diseases can be characterized by the following definition:

Diseases associated with inhibition and/or dysfunction of the root system (anonymous).

Although this is a simplistic definition of the damage that occurs as a result of soilborne pathogens, the actual determination that soilborne pathogens are causal agents is difficult, and not straight forward. Historically, research programs to determine the significance of soilborne diseases have not been given adequate because of more obvious disease problems. Diseases such as Loose Smut, Tan Spot, Powdery Mildew, and others have always attracted major attention for funding because of their more obvious symptomology. This is not an unexpected situation because the more obvious problems will be addressed first. Although with soilborne diseases even with their obscured symptomology. they are a very significant component of cereal production. Bolley in 1913 placed emphasis on this aspect with the following quote "When a valuable fertilizer is present and the roots are dead by disease, the wheat plant cannot make use of it. If the roots are healthy, they can make use of it." (Bolley, 1913). His statement applies to most soilborne pathogens as it relates to root health. Thus, it is because of the soilborne pathologist's concern about root health that we emphasize the importance of root health in regard to soilborne pathogens. It should be pointed out that a factory with a weakened foundation can be likened to that of a plant with diseased root system. Neither unit of production can be expected to attain maximum productivity with these limitations.

FIELD SITUATION - Typically, grower concern over root health increases when he encounters a field where "Whiteheads" occur at a high level. This situation usually occurs in root rot prone areas sometime after heading as the crop is approaching maturity. Individual wheat heads die prematurely resulting in the condition referred to as "whitehead". For example, the "whiteheads" represent a distinct contrast compared to the normal green healthy heads that are still developing normally. Healthy vs. Whitehead comparisons of dissected heads of each reveals the following. For a normal green head, the developing kernels are plump and green and contrast markedly with the smaller shriveled kernels in the prematurely ripened head. In the whiteheaded heads, the kernels cease to develop as a result of the destruction of the lower internodal tissue by the pathogen <u>Fusarium graminearum</u> in this example. Other pathogens can also produce in this type of damage. In all cases, there is a direct loss to the producer in proportion to the percentage of whiteheads that are present.

ROOT ROT DISEASES OF WHEAT - The following diseases are common part of our soilborne disease complex in Oklahoma: Common root rot [causal agent <u>Bipolaris sorokiniana</u>], Dryland foot rot [causal agent(s) <u>Fusarium spp.</u>], Pythium root rot [causal agent(s) <u>Pythium spp.</u>], Take-all [causal agent <u>Gaeumannomyces graminis</u>], and Sharp eye-spot [causal agent know as <u>Rhizoctonia cerealis</u>]. Common root rot and Dryland foot rot have long been known to occur throughout the wheat growing areas of the state. Pythium root rot also occurs commonly throughout the wheat growing areas. Both take-all and sharp eye-spot are more sporadic in their occurrence, the damage they cause, and distribution.

PYTHIUM DISEASES - Field symptoms of Pythium spp. on wheat are characterized by the yellowing, sloughing of lower leaves, and stunting in extreme cases. The field symptoms can resemble and can be mistaken for a nutrient deficient plant primarily because the damaged root system is not capable of providing sufficient nutrient and water uptake. Pythium diseased plants are generally unthrifty and stunted in appearance, and sometimes individual plants die. After extracting such plants from the soil, damage will be evident with the presence of dark brown to reddish and possibly water soaked lesions on the roots. Isolations from this root tissue can be accomplished by using a "baiting method" that involves germinating wheat seed in a mass of field collected roots. After 6-7 days, the "bait" plants roots can be separated from the root mass and may exhibit varying degrees of damage, and symptoms similar to those previously described. After being isolated on an appropriate medium, various asexual and sexual fruiting structures can be observed and used for identifying the Pythium spp. that are present. The primary survival structure for Pythium spp. in field soils is the thick walled oospore.

Population of Pythlum in Soil - As pathologists and breeders, we should be concerned about the following questions: how much <u>Pythium</u> inoculum is

present in a soil planted to wheat?, and when does <u>Pythium</u> infection of seedlings occur? For question number 1, we know from soil dilution planting that the <u>Pythium</u> spp. can range in populations up to 80-100 propagules per gram of soil.

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From embryo infection studies with various concentrations of field soil (1.0X - 0.01X; diluted in sterile sand), we have found that up to 70% of the wheat germs will be infected in all soil dilutions including the lowest of 10% field soil. Even at 1% field soil, we still found that 6% of the germs were infected with Pythium. This type of germ infection of wheat seed planted in field soils is not an uncommon event although the level of infestation >70% or <70% may vary with the location and soil type. We have commonly found that Pythium spp. are prevalent in soils from wheat fields. Also, the infection occurs rapidly within a 24 hr period. This rapid infection of the germs of these plants is important. Transplant studies from germ infected plants compared to healthy have shown that the germ infected plants do not recover their normal growth characteristics as seedlings when compared to non-embryo infected seedlings. In replicated tests, germ infected plants exhibit significantly lower root and shoot weights as compared to healthy seedlings. Thus, germ infected plants are never going to be as vigorous and productive as non-germ infected seedlings. Thus the infection of these seedlings with Pythium is continuously stressing and affecting the growth of the wheat plants.

Effects of soil temperature on germ infection - The effect of soil temperature on embryo infection within 24 hour was examined over a range of soil temperatures. Embryo infection of >70% occurred over a range of soil temperatures from 15-30C. The amount of germ infection was considerably reduced at 10C and at 37C. This suggests that a number of <u>Pythium</u> species are involved in this germ infection. <u>Pythium irregulare</u>, <u>P. arrhenomanes</u>, <u>P. ultimum</u>, and <u>P. aphanidermatum</u> have been commonly associated with this type of infection in our wheat soils. Thus <u>Pythium</u> spp. are very aggressive in their initial invasion of wheat seedlings in field soil which occurs over a wide range of soil temperatures.

In our lab, the Most Probable Number Method from Pfender et al, 1981 was adapted to obtain estimates of Infectious Propagules of <u>Pythium</u> species per gram of root tissue (Singleton et al, 1991). Standard soil dilution procedures already allow for estimating the number of <u>Pythium</u> propagules per gram of soil. How representative are these estimates of the <u>Pythium</u> spp. that are pathogenic in our soils? Two methods were used to determine the infectious propagules per gram of field collected wheat roots, and from the soil associated with the root at four field locations. We found that on a gram for gram basis, from the roots we were recovering only 1-8% of the population that was indicated was present by the soil dilution method. This is not unexpected in that the host roots are being attacked by the more pathogenic portion of the total Pythium species

population of the soil. As too the question about the pathogenic composition as shown here we were recovering a greater proportion of the pathogenic species of <u>Pythium</u> when we were assaying the field collected wheat roots. Thus it was concluded that this method was a more suitable method for identifying the more pathogenic portion of our field populations of <u>Pythium</u>. The impact of these findings on embryo infection will be determined in future research relative to the general health and productivity of mature plants.

Cultivar Responses to Embryo Infection - Ten cultivars were evaluated in replicated tests for in the amount of embryo infection in a field soil. Two of the cultivars Kenya and Wichita exhibited only 50% embryo infection as contrasted to Chisholm and some other cultivars with >80% levels of germ infection. This is an aspect of germ infection that needs further research to determine if this type of response is genetically controlled.

CONCLUSIONS - Other studies in our laboratories have shown that some species of *Pythium* produce toxic compounds in <u>vitro</u> that have been demonstrated to cause a significant portion of the disease symptomology that we see associated actual <u>Pythium</u> infection in the field (Mojdehi, et al, 1990, 1991).

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AG CHARACTERIZATION OF RHIZOCTONIA SOLANI ISOLATES FROM WHEAT AND SUGARBEET IN THE TEXAS PANHANDLE

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Rhizoctonia solani has been increasingly recognized over the last two years as an important pathogen of wheat in the Texas Panhandle. Disease caused by this pathogen is usually observed on seedlings from early plantings of hard red winter wheat. It is characterized by reduced stands and weak nonvigorous growth of individual plants. Infected plants typically lose lower leaves, and lower leaf sheaths become dark brown and necrotic. Roots exhibit individual, discrete, dark brown, water soaked lesions or may be completely pruned off. Severely infected plants often die, leaving gaps of varying lengths in rows.

Although Rhizoctonia has long been recognized as a pathogen of wheat, there is considerable discrepancy in reported species and anastomosing groups responsible for disease. In the Pacific Northwest, *R. oryzae* and *R. solani* Ag8 have both been associated with diseased wheat, *Rhizoctonia oryzae*, with a root rot of wheat, and *R. solani* Ag8, with bare patch (Weller et al. 1986, Smiley et al. 1990). Bare patch is a disease previously recognized in Australia which kills young plants leaving irregular "bare" patches throughout the field. Lipps and Herr (1982) reported sharp eyespot of wheat in Ohio was caused by *R. cerealis*, a binucleate species, but the same disease in Arkansas was reportedly caused by *R. solani* Ag4 (Sterne and Jones 1978). Different Ag4 isolates which were recovered from sharp eyespot lesions on wheat stems did not infect roots, although these isolates did kill seedlings in greenhouse studies. *Rhizoctonia cerealis*, which also killed seedlings, did not typically cause root damage.

Because of the uncertainty about species and anastomosing groups of Rhizoctonia which cause disease on wheat, a survey was conducted to determine which ones predominate in the Texas Panhandle. Also, since sugarbeets are grown in rotation with wheat in this area and are parasitized by Rhizoctonia, isolations from sugarbeets were included in this study.

Collections of diseased wheat and sugarbeet plants were made in the fall of 1990. Diseased plant tissue was washed and plated onto potato dextrose agar. Ninety-eight fungal colonies which resembled Rhizoctonia spp. in color and morphology were subcultured and subsequently speciated and paired with known *R. solani* Ag tester isolates. Results of the anastomosis pairing are presented in Table 1. Eighty-nine percent of the isolates collected from mature sugarbeets were *R. solani* Ag2-2, and 95 percent of the wheat isolates were *R. solani* Ag4. No binucleate cultures of Rhizoctonia were isolated from wheat.

After all cultures had been Ag typed, selected isolates from each Ag group were tested for pathogenicity to wheat seedlings, and linear growth at six temperature regimes, ranging from 10-35C, was measured after 48 hr (Tables 2 and 3). None of the isolates tested grew well at temperatures below 20C (Table 2). All isolates had maximum growth at 25-30C, and at every temperature, Ag4 and Ag5 were the most vigorous. Ag4 was especially suited for warm temperatures and grew relatively well even at 35C. The binucleate isolate was the least vigorous at every temperature tested.

The binucleate isolates of Rhizoctonia, in addition to having nonvigorous growth, were also nonpathogenic (Table 3). *Rhizoctonia solani* isolates in Ag groups 2-2 and 5 were also nonpathogenic or only weakly so. No isolate from these two groups significantly reduced seedling emergence when compared to the control, and the disease ratings on seedlings were also low. Conversely, both *R. solani* Ag4 isolates were highly pathogenic. Both greatly reduced seedling emergence and also caused significant disease symptoms on emerged seedlings. Fortunately, the Ag2-2 isolates from sugarbeets were nonpathogenic to wheat. However, the Ag4 isolates from wheat were highly pathogenic to sugarbeet seedlings, confirming previously published reports (Windels and Nabben 1989).

One can conclude from the results of this study that Ag4 is the predominant anastomosing group of *R. solani* which is strongly pathogenic to wheat in the Texas Panhandle. Furthermore, temperature studies suggest disease might be avoided if wheat is planted when soil temperatures are less than 20C.

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	Ag grouping ⁴					
Crop & No. of isolates	Ag4	Ag2-2	Ag5	Binucleate		
Beet (46)	3	41	1	1		
Wheat (45)	43	1	1	-		
Beet seedling (7)	2	1	3	1		

Table 1. Ag grouping of 98 *Rhizoctonia solani* isolates collected from wheat and sugarbeets.

 Anastomosis group was determined by challenging each isolate against known tester isolates.

	Anastomosing group				
Temperature (C)	Ag4	Ag2-2	Ag5	BI	
10	17.2 d	14.1 d	18.7 b	17.4 cb	
15	26.6 cd	21.2 cd	27.0 b	23.7 b	
20	52.4 b	34.0 b	47.6 a	37.2 a	
25	61.7 a b	40.3 ab	52.3 a	42.2 a	
30	66.4 a	45.9 a	52.6 a	39.0 a	
35	31.7 c	22.9 c	18.3 b	11.9 c	

Table 2. Growth (mm) of Rhizoctonia solani isolates at 6 temps.*

* Colony diameter after 48 hr, measured in mm.

AG group ^a	% Emer	rgence	Disease index ⁶
Ag2-2 WS	87	a	1.0 b
Ag2-2 B	75	ab	0.3 c
Ag4 WS	56	ЪС	1.5 b
Ag4 BS	37	С	2.5 a
Ag5 WS	93	a	0.0 c
Ag5 BS	100	a	1.0 b
Ag-BI-B	87	a	0.0 c
Ag-BI-BS	93	a	0.0 c
	100	a	0.0 c

Table 3. Pathogenicity of eight Rhizoctonia isolates on wheat seedlings.

- Anastomosis group was determined by challenging each isolate against known tester isolates. Upper case letter following each Ag designation indicates host from which the isolate was taken. B = mature beet, BS beet seedling, and WS = wheat seedling;
- ^b Disease index ranged from 0-4, with 0 = no disease and 4 = dead seedling. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

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BREEDING WHEAT FOR RUSSIAN WHEAT APHID RESISTANCE IN THE WEST CENTRAL GREAT PLAINS

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Breeding for host plant resistance has been one of the most important objectives in the effort to reduce losses by the Russian wheat aphid (RWA). The development of resistant cultivars involves consideration of genes in the wheat plant, genes in the pest, and their interaction with the environment. The purpose of this paper is to describe the economic justification, regional program efforts, sources of resistance genes, and breeding progress for the development of Russian wheat aphid resistant varieties.

Since the initial detection of the Russian wheat aphid (*Diuraphis noxia*, Mordvilko) in the Texas Panhandle of the USA in 1986, it has been found in 16 western states of the U.S. and three provinces in western Canada. Economic losses due to lower production and costs for insecticide use in the U.S. have been estimated at more than \$660 million during 1986-1990. In the United States, the first significant level of resistance found in wheat was in PI 372129 (Turcikum 57 = T-57) in Colorado (2,5). T-57 is an introduction from Russia and possesses several undesirable traits for a hard red winter or spring wheat breeding program. Subsequently, 12 other wheats from various countries have expressed significant resistance levels in regional uniform seedling screening programs (4).

Research reports presented at the 1989 Wheat Workers Conference summarized the following:

<u>Webster</u>: described greenhouse screening technique, indicated no resistance in wheats, and significant resistance in triticales

Baker, et al.:	described	resistance	in re	lated	species	5
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Quick, et al.: described resistance in PI 372129 wheat

Souza, et al.: described field screening techniques

Scott, et al.: compared screening techniques

Worrall, et al.: described variation among wheats and RWA colonies

Burd and <u>Burton</u>: described detailed plant symptoms

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Regional research efforts currently underway in the west central Great Plains region are summarized as follows:

<u>Colorado</u>: breeding and genetics of host plant resistance

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Kansas: breeding for both RWA and wheat curl mite resistance (KSU-Hays); screening and breeding (Hybritech)

Oklahoma: developing a core collection of resistant materials, genetics of resistance, and mechanisms of resistance

<u>Texas</u>: breeding using triticale resistance sources

Evaluation of F2 populations of crosses among six wheats, PI 137739 (Dn1), PI 262660 (Dn2), PI 372129 (T-57 and tentatively Dn4), PI 294994, PI 243781, and PI 262605 was done to determine dominance and allelic relationships. Segregation in the F2 indicates that different genes condition resistance. The inheritance of resistance in PI 137739, PI 262660 and T-57 are all monogenic dominant (1,3). The results show that the gene in T-57 is not allelic to either Dn1 or Dn2. It has been suggested by the South African workers (personal communication) that PI 294994 has two genes for resistance. Our results show that at least one of these genes is allelic to the gene in T-57, PI 243781, and PI 262605. The gene in PI 262605 is allelic to the gene in T-57.

Variety development is proceeding well using the T-57 source. Results from selection in the F5 generation are shown in Table 1. Population sizes of various generations are shown in Table 2. Trials of F6 lines will be evaluated in eastern Colorado in 1992 to test the RWA-resistant progeny of T-57 crosses. Improved field screening techniques and additional crosses are being evaluated.

The Colorado Agricultural Experiment Station at Colorado State University announced the release of CORWA1 hard red winter wheat (*Triticum aestivum* L.) germplasm for breeding and experimental purposes in December 1991. CORWA1 (Sumner/CO820026,F1//PI372129,F1/3/TAM107) is resistant to the biotype of the Russian wheat aphid present in Colorado. T-57 is the source of resistance, and CORWA1 has been similar to T-57 in reaction to the RWA in seedling tests.

The first cross to T-57 was made in June 1987 and the topcross to TAM107 made in December 1987. The topcross seeds (designated F2) were screened for RWA reaction in February 1989. Survivors were transplanted and vernalized in March-April 1989, and F2 plants grown in the greenhouse during May-June 1989 (Table 2). The F3 seeds were germinated, vernalized and transplanted to the greenhouse in October 1989. F4 seed was harvested separately from each F3 plant and data were collected on height, days to flower,

grain color and grain hardness. Seed from each F3 plant was simultaneously vernalized and screened for RWA resistance. Selected F4 lines were increased in the greenhouse during May-August 1990. F3-derived F5 lines were evaluated in the field at Akron, Julesburg, and Fort Collins during the 1990-1991 season, and for RWA reaction in the greenhouse in February 1991 (Table 1).

CORWA1 is similar to TAM107 grain color and hardness, plant height, days to heading, straw strength, leaf and stem rust reactions, winter survival, grain volume weight and grain yield. It has white glume color and marginal gluten quality as measured by the sodium dodecyl sulfate test (Dick and Quick, Cereal Chem. 60:315-318).

Small quantities (3 g) of seed of CORWA1 are available upon written request to J. S. Quick. It is requested that appropriate recognition of source be given when this germplasm contributes to research or development of new cultivars.

Research underway will make breeding for resistance more efficient: 1) improved field screening techniques, 2) determination of the level of 'field resistance' required, 3) more information on the mechanisms of resistance, and 4) isolation of a toxin and its use in screening programs.

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	Fort Collins 1-row	Julesburg 2-row	Akron 6-row
NO. LINES PLANTED:	797	650	126
PERCENT DISC. FOR:	_%	<u>%</u>	_%_
RWA	38	-	•
WINTER KILL (<70%)	. -	28	16
LEAF RUST (> LAMAR)	26	-	-
STEM RUST (> 10%)	-	11	2
GENETIC NECROSIS	6	-	-
SDS SEDIMENTATION (<80 mm)	-	-	6
HEADING (> LAMAR)	9	-	33
HEIGHT (<60 OR >90 cm)	12	26	24
LODGING (>50%)	1	0	0
TEST WT (<tam107)< td=""><td>-</td><td>-</td><td>56</td></tam107)<>	-	-	56
G. YIELD (< TAM107)	-	55	3 5
Advanced (%)	112(14)	91(14)	38(30)

Table 1.	Results of selection in the F	5 generation at three sites in eastern
	Colorado in 1991.	· ·

	PLA	PREVIOUS	
GEN.	SCREENED ADVANCED		GENERATION
F2*	2000	220 (46)**	-
F3	2259	800	46 F2
F4	-	800	800 F3 36 F2
F5	800	120	800 F3, F4 21 F2

Population sizes of T-57 materials evaluated for resistance to the Russian wheat aphid. Table 2.

×

Includes seed from F1 and 3-way crosses. Only 46 survived vernalization following RWA screening. **

SOURCES OF RUSSIAN WHEAT APHID RESISTANCE IN NORTH AFRICAN DURUMS AND WHEAT ALIEN SPECIES

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The Russian wheat aphid (RWA) is a serious pest of wheat in the Great Plains of the United States. Damage symptoms by this pest include leaf rolling, leaf chlorosis and plant stunting. Current control measures include the use of insecticides and eradication of volunteer wheat. A concerted effort is being made to identify sources of resistance in wheat for use in breeding resistant cultivars.

Triticum species have three ploidy levels comprised of diploids, tetraploids and hexaploids. So far, sources of resistance to the RWA have been found only in the diploid wheat, *T. monococum* and hexaploid wheats, *T. aestivum*. No resistance has been reported in tetraploid wheat, *T. turgidum*.

The objectives of this study were to evaluate a group of 219 Tunisian durum wheats comprising both cultivars and land races for RWA resistance and to determine the mechanisms of such resistance identified in seven accessions. In addition, 550 entries from the Sando collection and 691 entries from a wild barley collection were evaluated for their resistance to RWA.

Resistance was detected in 18 or 0.08% of the durum wheats evaluated. This is the first detection of Russian wheat aphid resistance in *T. turgidum*. Antibiosis and antixenosis were the mechanisms governing resistance in the 7 accessions showing the highest levels of resistance.

Resistance to RWA was detected in 37 or 0.07% of the 550 Sando entries screened and 36 or 0.05% of the wild barley entries tested. Antibiosis was the resistance mechanism detected in the resistant Sando entries. Mechanisms of resistance studies are currently underway with the resistant wild barley material.

Resistance found in these materials could be used for the development of durum and bread wheat cultivars with resistance to the RWA.

CURRENT STATUS OF GREENBUG RESISTANCE EFFORTS

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Development of greenbug (Schizaphis graminum (Rondani) resistant germplasm dates back to the detection of resistance in 'Dickinson' Selection 28A (DS 28A) in the 1950s (Hatchett et al., 1987). Since that time four additional sources of resistance for various greenbug biotypes have been detected and characterized. Five different genes (Gb1, Gb2, Gb3, Gb4, and Gb5) have been identified in five different genotypes: DS 28A, 'Amigo', 'Largo', CI 17959, and Cl 17882, respectively (Tyler et al., 1987). These genes have conditioned resistance to greenbug biotypes A, B, C, and E. Since its detection in 1980, biotype E has been the predominant biotype in most wheat-producing areas (Porter et al., 1982). However, Puterka et al. (1988) have since detected a biotype, designated "G" (GbG), that damages all previously known sources of greenbug resistance in wheat detected to date. Mass screening efforts conducted at the USDA-ARS Plant Science Research Laboratory, Stillwater, OK, have resulted in the detection of several germplasms resistant to GbG (Porter et al., 1991). Germplasm selections, originating from hybridizations using x-ray irradiated pollen of wheat/ rye (Secale cereale L.) hybrids to pollinate hard red winter wheat females, were screened en masse with greenhouse cultures of biotype G. The performance of five GBG-resistant selections (GRS1201-GRS1205) from this test, along with three resistant checks ['DS28A', 'Amigo', and 'Largo' (designated Gb1, Gb2, and Gb3, respectively)], and two susceptible checks, 'TAM W-101' and Pl 140207, were compared under infestations of biotypes B, C, E, F, and G. The GBG-resistant wheat germplasms exhibited high levels of resistance to all economically important greenbug biotypes (i.e., B, C, E, and G) at the seedling stage. Comparison of the damage caused by each of the five greenbug biotypes to the GBG-resistant germplasms with the differential damage sustained by the resistant checks (i.e., DS28A, Amigo, and Largo), indicates that the source of resistance in the GBG-resistant germplasms is different from all previously described resistance genes (i.e., Gb1, Gb2, Gb3, Gb4, and Gb5). Table 1 summarizes the relationship between greenbug biotypes and sources of resistance in wheat.

One of the germplasm lines (GRS1201) is a 1AL/1RS wheat/rye (Secale cereale L.) translocation line developed by the late Emil Sebesta. Dr. Sebesta x-ray irradiated mature pollen of an alien substitution wheat X rye hybrid (short wheat selection/'Scout' (TX69A345-2)//'Insave F.A.', obtained from Neal Tuleen, Texas A&M, College Station, TX) and pollinated 'TAM W-101' hard red winter wheat. X_1 plants were selected for fertility and underwent seven generations of selfing and selecting for fertility. In the X_7 generation, seedlings were identified

as resistant to greenbug biotype G during routine screening tests. Greenbug resistance of GRS1201 is conditioned by a single dominant gene located, presumably, on the 1RS chromosome derived from 'Insave F.A.' rye. Additional tests by Tom Harvey, KSU, Fort Hays, KS revealed that GRS1201 is also resistant to greenbug biotype I. Cytological analysis was performed by Bernd Frebe, KSU, Manhattan, KS.

This germplasm should be extremely valuable in the development of new multibiotype greenbug-resistant cultivars.

Germplasm and		Greenbug biotype						
gene designations	Origin	В	С	Ε	F	G	Н	
			Re	actio	n to I	pioty	[†]	
DS 28A (Gb1)	T. turgidum durum	S	S	S	R	S	S	NT
Amigo (Gb2)	S. cereale	R	R	S	S	S	S	NT
Largo (Gb3)	T. tauschii	S	R	R	S	S	R	R
Cl 17959 (Gb4)	T. tauschii	S	R	R	S	S	S	R
CI 17882 (Gb5)	T. speltoides	S	R	R	S	S	S	NT
GRS1201-1205	S. cereale	R	R	R	S	R	S	R

Table 1. Wheat germplasm sources of resistance to greenbug biotypes.

^TR and S indicates resistant and susceptible reactions, respectively; NT indicates not tested.

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RESISTANCE TO THE WHEAT CURL MITE IN COMMON WHEAT

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Wheat streak mosaic (WSM), which is transmitted by the wheat curl mite (WCM) <u>Eriophyes tulipae</u> Keifer, is a serious threat to wheat production throughout the Great Plains. During the past five years it has been the most important disease of wheat in Kansas causing an average annual loss of 15.5 million bushels.

Resistance to the WCM is effective in reducing the incidence of WSM in the field, but the only available resistance to the mite has been from rye, wheatgrass, and goatgrass. Previously, over 3,500 accessions of common wheat were screened for WCM resistance, but all were found to be susceptible.

During 1988-89, we screened 5,082 common wheats for resistance to the Russian wheat aphid, <u>Diuraphis noxia</u>, and identified 43 resistant accessions. While growing these accessions in the greenhouse, they were accidentally infested with WCM, and we noted that some of the lines did not have the typical leaf curling and trapping that is characteristic of WCM-infested wheat. This prompted us to test the 43 accessions for resistance to WCM.

The following eight accessions proved to be resistant to WCM when infested manually and with airborne mites in the greenhouse: PI 222679, PI 222682, PI 222655, PI 222651, PI 222661, PI 222680, PI 221699, and CI 9355. These accessions appeared to be slightly more resistant than PI 475772 (rye-derived resistant check).

These are the first common wheats found to be resistant to WCM. All of the resistant accessions originated from Iran or Russia, except CI 9355 which was from Sweden. Breeding with the resistant selections is underway.

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TRANSFER OF HESSIAN FLY RESISTANCE GENES FROM RYE TO WHEAT

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Cultivated rye, Secale cereale L, is an important source of disease and insect resistance genes for the improvement of common wheat, *Triticum* aestivum L (Riley and Macer 1966; Zeller and Hsam 1983, for reviews).

The Hessian fly, *Mayetiola destructor* (Say), is a destructive pest of wheat throughout most of the production areas of the world. In the U.S., genetic resistance has been used for many years to protect wheat cultivars from damage caused by the insect. More than 20 resistance genes have been identified in *Triticum* species for use in breeding resistant cultivars (Gallun 1977 for review; Amri et al. 1990). However, breeding for resistance has become more complex because of the development of host-specific biotypes of the insect. Virulence genes corresponding to the resistance genes H3, H5, H6, and H7 H8 in combination are now prevalent in Hessian fly populations in areas where those genes have been deployed for several years. Thus, germplasm of wheat and its relatives is being searched continually for new diverse sources of resistance to virulent biotypes of the Hessian fly.

Among the genetic resources available, rye offers great potential as a source of resistance to Hessian fly. Although this resistance has been known for many years, only in the last decade have efforts been made to utilize rye genes in the development of resistant wheats. Several years ago, two cooperative research projects were initiated with Kansas State University and Oklahoma State University in an attempt to introgress resistance genes of rye into the wheat genome. As a result, two genes that condition resistance (antibiosis) to all known Hessian fly biotypes were transferred to common wheat via wheat-rye chromosomal translocations (Friebe et al. 1990; Friebe et al. 1991), A resistance gene from 'Chaupon' rye was transferred to the wheat genome by either a spontaneous or tissue-culture induced 2BS-2RL wheat-rye translocation. The C-banding pattern of this chromosome showed that the break point of the translocation was within the centromeric region, indicating that the translocation originated by centric breakage and fusion. Germplasm of a 2BS·2RL translocation line, named 'Hamlet', was released in 1989 as KSWGRC8 (Sears et al. 1992).

A second Hessian fly resistance gene derived from 'Balbo' rye was transferred to common wheat by radiation-induced terminal and intercalary chromosomal translocations, involving rye chromosome 6RL and wheat chromosomes 6B, 4B, and 4A. Almost the complete 6RL arm is present in the 6BS·6BL-6RL translocation. Only the distal half of 6RL is present in the 4BS·4BL-6RL translocation, which locates the resistance gene in the distal half of 6RL. Only a very small segment (ca 1.0 um) of the distal region of 6RL is present in the intercalary translocation 4AS·4AL-6RL-4AL. The 6RL segment is inserted in the intercalary region between the centromere of 4A and a large proximal C-band of 4AL. The break points of all three translocations are outside the centromere region, indicating they were induced by the x-ray treatment. Germplasm containing the 6RL translocations will be released in 1992.

The 2RL and 6RL translocations are cytologically stable and can be used directly in wheat breeding programs. The development of wheat cultivars carrying these chromosomal translocations will provide a broader base of genetic resistance to all known biotypes of the Hessian fly.

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STABILITY OF KERNEL HARDNESS IN HARD RED WINTER WHEATS

1.10

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Single kernel hardness using both the Norris instrument and the USDA-ARS single kernel hardness tester (SKHT) was determined on selected varieties within the Kansas State University Variety Testing Program during 1987, 1988, and 1989. The USDA-ARS SKHT is currently being developed as a prototype for future objective classification of hard and soft wheats by the Federal Grain Inspection Service (FGIS).

For the varieties examined (Table 1 and 2), none had been previously selected for hardness based upon single kernel hardness determinations. Each had been determined uniformly hard based upon experimental milling of composite samples and comparison of milling performance against historically described hard winter wheats. The objective of this study was to determine if mean hardness and stability of hardness varied significantly from year to year or from location to location. We also wanted to determine the levels of variation within each variety for single kernel hardness. In the proposed FGIS objective classification system, varieties with either low or medium mean hardness values but with large standard deviations should be avoided because an unacceptable number of softer than average kernels would be classified soft.

Both year and location affect hardness values obtained by the SKHT. The environmental effects of 1987 produced a much harder wheat crop than either 1988 or 1989. The mean average hardness was 80 in 1987, 63 in 1988, and 59 in 1989. Irrigated locations were generally softer (66) than dryland locations (70), although not significantly. Western Kansas locations were harder (70) than north central locations (65) which were harder than south central locations (61). In general the standard deviations of the respective varieties did not change appreciably.

Stability statistics can be found in Table 1. SAFE, a safety-first index (1) is a conservative measure of stability. It is the estimated hardness value below which a variety's mean hardness will fall 5% of the time. Therefore, varieties with larger mean values or confidence limits would be preferred using this test. Varieties with SAFE values below 50 would most likely cause classification problems in softer than average years or environments. Varieties in the future, we believe, should be nearer 55-60, thus avoiding potential classification problems. Care should be taken not to select too severely for kernel hardness within winter wheats. Hardness values above 80 are negatively correlated with flour yield, and mean kernel hardness values between 65-75 should be targeted

for winter wheats grown in the Great Plains. The safety-first stability statistic used in this manner appears to be an excellent estimate of hardness stability for varieties. The wheat breeder in this case is most interested in stability of hardness across a range of diverse environments. Regression coefficients based upon Eberhart and Russell stability analysis (2) provide information on responsiveness of each variety but would not clearly predict the chance a variety could be misclassified based upon hardness. Using the regression coefficient, varieties with a high average hardness (75) and a low b-value (<.50) would be good. Chisholm in this study had a very low b-value, but also a low mean hardness value, which in some cases would lead to classification problems. Deviations from regression (S_d^2) would be valuable used in conjunction with mean hardness values. Varieties with low mean values and high S_d^2 should also be avoided. In this study, most of the varieties were similar in stability, with the exception of Tam 107, which tended to get very hard in hard environments and Mesa, which was more stable across environments.

In Table 2 are the results from 1988 and 1989 combined across locations. Several varieties grown in these two years were not grown in 1987. The low cutoff, (the hardness value below which the softest 5% of kernels fall within each sample), also provides a reasonable estimate of how often a cultivar could cause classification problems. It is also a safety-first type of stability statistic. This type of statistic could be associated with the eventual definition of a soft and hard kernel. Currently, a soft kernel will be defined as having a soft value between 20-30 and a hard kernel defined as having a measured value between 40-50. A dead zone of at least 15 will have to be maintained to avoid misclassification and also insure a high probability of detecting soft and hard mixtures in the grain. The normal standard deviation for wheat samples run in the SKHT ranges from 15-17 units. In Table 2 a variety with a mean lower than 30 indicates that a variety has a larger number of kernels that would be defined as soft than varieties with means above 30. In this study, varieties with low mean hardness also tended to have predicted low means of less than 30. In most cases the distributions were normal and standard deviations similar (±17). This linear relationship should allow wheat breeders to use mean hardness values at least as a good initial value for hardness.

In this study, significant genotype x environment interactions were detected but the interactions were not caused by changes in rank. Tam 107 was highly responsive, and Chisholm not responsive across environments, for example. The lack of GxE interaction without changes in rank is important and indicates that mean hardness values are of use in early generation selection. Varieties "at risk" in the future classification system were both identified by low means and safety-first stability statistics. Varieties with above-average hardness values but low safety-first stability statistics were rare but identified. Tam 200 had an average mean hardness but a large standard deviation and the lowest low cutoff of all the varieties examined.

It appears from this data that mean hardness values will be of benefit for breeders to examine in early generation yield trials. Low average hardness values (\leq 55) would signal a potential classification problem. These values could be obtained from either SKHT instruments or NIR machines in conjunction with protein measurements. SKHT measurements will be necessary as lines advance to elite yield trials and ultimately toward release. Optimum values to select appear to be between 65-75 with as low a standard deviation as possible. The safety-first stability parameters described by Eskridge (1) appear to be very useful in determining whether a variety could have problems with classification based upon the SKHT.

19. S. C.

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Table 1.	Average hardness values, and stability statistics for selected winter
	wheat varieties grown at 16 testing locations throughout Kansas in
	1987, 1988 and 1989.

Cultivar	Mean Hardness	SAFE	b	S d ²
Abilene	66.4	54.0	1.18	4.0
Arkan	61.2	48.7	1.01	3.9
Chisholm	57.2	46.0	0.45	5.6
Karl	64.7	51.6	1.00	5.6
Mesa	69.6	57.9	0.92	3.8
Newton	67.6	52.0	0.78	5.2
Norkan	65.7	53.0	1.05	4.4
2157	63.4	47.5	0.97	5.9
RioBlanco	66.6	51.4	0.98	5.4
Siouxland	68.5	54.2	1.02	4.1
Tam 107	74.5	62.2	1.28	7.1
Thunderbird	72.2	58.2	1.21	5.1
Victory	69.6	56.8	1.24	5.0

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Table 2.	1988 and 1989 mean hardness values and cutoff for the lowest 5%
	of the frequency distribution of 300 kernels within each variety. Each
	variety was grown at 16 locations throughout Kansas.

Variety	Mean Hardness	Low Cutoff ¹
		•
AGSECO 7846	61.4	30.1
Abilene	59.0	29.6
Arkan	55.5	24.5
Century	68.8	36.5
Chisholm	56.2	23.8
Dodge	56.9	26.0
Karl	58.5	26.7
Mesa	62.9	31.5
Newton	62.9	30.7
Norkan	59.2	27.8
2157	58.0	25.7
2163	57.5	25.9
2172	69.6	35.4
RioBlanco	60.7	28.6
Siouxland	62.0	30.4
Sierra	67.0	33.2
Tam 200	59.5	20.2
Tam 105	62.7	31.0
Tam 107	65.0	34.2
Tam 108	58.3	26.9
Thunderbird	64.1	33.6
Victory	62.2	30.5
-		

¹The hardness value below which the softest 5% of kernels fall within each sample of 300 kernels.

FEASIBILITY OF USING NEAR-INFRARED AND VISIBLE LIGHT SPECTROPHOTOMETRY TO MEASURE BREAD-MAKING POTENTIAL OF COMMERCIAL WHEAT FLOURS

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Increasing processing speeds in commercial bakeries have placed added demands on the need for online measurement of flour quality. Quality to the baker can be defined in terms of the dough rheological properties during mixing, the shape and appearance of the baked product, and equally important, the time-based uniformity of these properties. Though several of the major baking companies have quality control laboratories for monitoring flour, turn-aroundtime, often requiring hours, is often too long for operators who wish to make real-time adjustments. Consequently, millers and bakers are actively searching for online instrumentation for process control.

We are currently examining the possibility of using near-infrared (NIR) or visible-light spectrophotometry for online flour measurement. Such an instrument would be positioned in the dry flour stream directly ahead of the water addition stage. To the wheat breeder, a flour quality instrument that is reliable, inexpensive, and fast would be very useful for determining the baking potential of new wheat lines, early in the breeding program.

The objectives of this study were to (1) examine the possibility of using spectrophotometry to determine flour performance, (2) determine the better wavelength region (NIR or visible-light) for such analysis, (3) decide upon a modeling technique (multiple linear regression [MLR] or partial least squares [PLS], and (4) determine, when possible, the relationships between the spectral readings and the intrinsic chemical properties of the flour that contribute to good bread.

Procedure

Flour samples were obtained from one major milling company (milling company A) and two major bakeries (baking companies A and B). Companyfurnished sample attributes were water absorption, loaf volume, mixing time, mixing tolerance, grain score, and overall baking score. From each sample, three 5 g subsamples were drawn and scanned on an NIRSystems Model 6500 spectrophotometer in the diffuse reflectance mode over a wavelength range of 400- to 2500-nm. Subsample scans were averaged prior to numerical modeling. Each company's samples were analyzed separately from those of the other two, owing to company-to-company differences in dough handling and baking equipment. MLR analysis was applied separately both wavelength regions. Generally, the MLR program searched for the best combination of one or more terms that would provide the best model for each of the six attributes. Terms examined were in the form of log(1/R), d(log(1/R)/d λ , d²(log(1/R))/d λ^2 , and single term ratios thereof. PLS analysis consisted of using up to ten factors (i.e. eigenvectors), and developing a separate model for each attribute, having first applied a particle size correction treatment to each spectrum.

Results

Highlighting the analysis on the samples of milling company A, MLR statistics are shown in Table 1. The ratio of two second derivatives provided the best compromise between model simplicity and performance; however, only the NIR model for water absorption yielded an r^2 value greater than 0.5. On the whole, the NIR region was sightly better than the visible region.

Parameter	Visible region			NIR region			
	@ nm/nm <u>λ</u> 1/ <u>λ</u> 2	r²	SEC	@ nm/nm <u>λ</u> 1 / <u>λ</u> 2	r ²	SEC	
Abs. (%)	718/1020	0.351	2.18	2260/1894	0.733	1.40	
Hgt. (in)	1038/784	0.304	0.14	2354/2184	0.440	0.12	
M. time, m	1030/874	0.409	3.43	2112/2402	0.381	2.87	
Grn. scr.	926/962	0.162	0.90	1240/2266	0.228	0.86	
M. toi, m	568/614	0.116	0.57	2148/1602	0.100	0.57	

Table 1 Calibration statistics of MLR models applied to Milling Company A. Conditions for models are $d^{2}(\underline{\lambda}_{1})/d^{2}(\underline{\lambda}_{2})$, n=102.

Calibration and validation statistics from the PLS models of milling company A are shown in Table 2. Generally, the PLS models were better than the corresponding MLR models, albeit at added complexity.

Parameter	range	mean	r²	SEC	r ²	SEP	Bias
Abs. (%)	52.0 - 68 .5	61.0	0.860	1. 06	0.772	1.24	-0.04
Hgt. (in)	4.5 - 5.5	5.0	0.558	0.10	0.134	0.14	-0.01
M. time, m	2 - 35	9.2	0.506	3.28	0.305	5.85	-1.14
Grn. scr.	4 - 7	5.4	0.332	0.84	0.190	0.96	0.08
M. tol, m	0 - 3	1.7	0.156	0.58	0.002	0.81	06
Bk. scr.	0 - 7	4.5	0.535	1.19	0.196	1.46	20

Table 2Calibration and validation statistics from 10-factor PLS models of milling companyA. The calibration set had n=102 samples, the validation set had n=306 samples.

Referring to Table 2, it is noted that only the water absorption model yielded a coefficient of determination greater than 0.5 when applied to validation samples. (The statistics of MLR and PLS models of baking companies A and B are comparable to those shown for milling company A and are therefore omitted.) Some of the difficulty with obtaining high correlations for some attributes is attributed to the discrete nature in which some of the chemical data was reported. For example, mixing times and tolerances were reported to the nearest minute, which is a course measurement unit, since most of the samples had times that were within a few minutes of each other. Another potential contributor to poor model performance is the fact that flour samples were not identified by wheat class and/or cultivar. Most often, each sample was a blend of hard red winter and hard red spring classes, however the exact proportion of these two classes was also unknown.

Upon examining the factors, factor 1 demonstrated that the preponderance of spectral variation was due to water. Factors 2 and 3 demonstrated an interdependence between starch and endosperm proteins, however the precise relationships between these two broad chemical groups is confounded by the blending of classes and cultivars. Interpretation of factors 4 through 10 is difficult at the present time. Future research will entail the study of pure-cultivar flours through the use of laboratory mixing and baking equipment. Such controlled studies will hopefully better demonstrate possibility (or rule out the use) of reflectance spectrophotometry on determining bread quality.

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GENOTYPE AND ENVIRONMENT INFLUENCES ON QUALITY AND BIOCHEMICAL COMPOSITION OF HARD RED WINTER WHEAT

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Introduction

The milling and baking industry has recently voiced concern over a perceived decline in hard red winter wheat baking quality. Much of the criticism is directed to the variability, or inconsistency, observed in grain and flour milling and baking quality. Strategies to improve consistency of end-use quality need to be considered and developed. However, the role of genotype, environment, and their interactions in contributing to quality variation must be determined in order to provide realistic and attainable goals for improvement.

Methods for screening and selection of baking quality have changed little in breeding programs over the last 30 years. Mixograph and pup loaf bake tests remain the primary tools for quality evaluation. However, these evaluations are time and resource consuming, making it difficult for breeders to perform adequate multilocation evaluations for quality. Little information is available documenting the differential responses of varieties to environmental conditions.

This study was initiated with the goal of characterizing interactions of genotype and environmental effects on end-use quality parameters. Concurrently, grain biochemical analyses were conducted to understand the basis for observed variation in quality. Understanding biochemical variation in grain as it relates to performance in quality evaluations may provide new directions, both for development of rapid screening methods and deployment of new strategies to improve wheat quality.

Materials and Methods

Thirty winter wheats were grown in 10 Nebraska locations during harvest years 1990 and 1991. The 30 varieties included advanced lines and varieties developed by breeding programs throughout the region. Grain from a total of 18 environments was harvested and used for quality analyses.

Grain samples of varieties from two replications at each environment were micromilled and flour used for mixograph and biochemical analyses. Flour protein concentration (NIR), SDS sedimentation volume, and soluble pentosan concentration were determined. Flour protein molecular size distribution and solubility characterizations were determined by size-exclusion high-performance liquid chromatography (SE-HPLC). Protein components measured by SE-HPLC included levels of glutenin and gliadin, and three groups of non-gluten proteins that were differentiated into 'light' albumins and globulins, 'heavy' albumins and globulins, and non-gluten residue proteins. Grain samples of the varieties were composited by location, Buhler milled, and resulting flour evaluated in a 100 gm pup loaf bake procedure.

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Results and Discussion

Extensive variation was found in all measured quality attributes. Flour protein concentration among samples ranged from 7.8 to 22.7% with a mean of 12.9%, mixograph peak time varied from 1.3 to 9.25 with a mean of 3.9 minutes, and mixograph tolerance (measured as width of curve two minutes after peak) 5.8 to 39.7 with a mean of 19 mm. Loaf volumes ranged from 615 to 1135 cc with both grain and texture ratings ranging from excellent to very poor.

The magnitudes of variation in quality parameters that could be attributed to environment, genotype and genotype x environment interaction (GxE) effects were compared by using ratios of variances (Table 1). Variances attributed to environments were mostly two to four times larger than that attributed to genotypes for each quality parameter. Variances attributed to genotypes were 1.6 to 2.7 times larger than GxE interaction variances for protein, absorption, and mixograph parameters. Genotypic variance and GxE interaction variance were of similar magnitude for loaf volume, but GxE variance components were notably larger for loaf external, grain, and texture scores. On average, approximately 50% of the variation in a given quality parameter was attributed to environmental effects and 25% each to genotypic effects and GxE interactions. Loaf grain and texture were exceptions to this generalizations with substantial GxE effects.

There were large differences among varieties for stability of quality traits. In general, varieties with longer average mixing times and tolerances expressed more variation in their response to environment than those with short mixing time and low mixing tolerances. However, stability for quality traits must be interpreted in terms of minimum levels of acceptability for each parameter, rather than overall ranges in variation. As such, varieties with longer average mixing times and tolerances were more likely to remain at acceptable levels over environments. Variation in varietal stability for quality suggests that improvement in stability is possible and that quality evaluations should be conducted from multiple locations to identify lines of questionable performance.

Contributions of genotype and environment to variation in protein composition and pentosans were significant. GxE effects on protein composition and pentosans were either low or non-existent. Glutenin, measured as a percent of total protein, did not vary over environments. Percent of flour protein found in gliadin fractions showed positive relationships to mixograph tolerance, mixograph peak height, absorption, and loaf volume, while the amount of protein found in two water-soluble fractions (primarily albumin and globulin proteins) displayed negative relationships with most important quality variables. However, few simple correlations of protein fractions with quality parameters were greater than r=0.60. Genetic correlations were generally higher than simple correlations and there were strong positive correlations of glutenin with loaf external, grain and texture. Loaf attributes were negatively correlated with levels of albumins and globulins. However, changes in flour protein composition were highly dependent upon total flour protein concentrations, making it difficult to separate the two effects. Pentosan concentrations were negatively correlated to loaf grain and texture.

The inability to identify a single protein or biochemical component that can explain the majority of variation in mixing or baking properties suggests the need to apply multivariate approaches to quality analyses. Canonical correlation analyses were used to determine the proportion of variation in a quality parameter that can be accounted for by using all biochemical measurements. By combining biochemical parameters into optimized linear models, we could account for up to 60% of the variation in mixograph tolerance and loaf volume. However, even optimized relationships of biochemical parameters did not adequately explain variation in loaf grain and texture. Relationships between protein components and quality parameters often appeared to be non-linear. Consideration of threshold effects and non-linear analyses may provide additional understanding of the biochemical bases for quality.

Several of the wheats included in the study carry rye chromosome 1RS in the form of either 1AL/1RS (e.g. TAM107) or 1BL/1RS (e.g. Siouxland) translocations. These varieties showed significantly lower scores for mixing tolerances and loaf characteristics. Both 1AL/1RS and 1BL/1RS lines suffered from a lack of glutenin protein (the protein primarily responsible for wheat flour elasticity) and the lower glutenin concentration was evident in all environments. Also, 1AL/1RS wheats possessed significantly higher levels of soluble pentosans, a factor likely contributing to poor loaf grain and texture in 1RS wheats.

Potential exists for improvement in consistency of wheat quality. Meeting this goal, however, will require allocations of resources to increased numbers of quality evaluations. Samples from earlier stages in the breeding program and from multiple locations will need to be evaluated. Measurements of protein composition may provide enhanced understanding of inherent deficiencies in some genotypes with unique quality attributes. The goal of development of effective, rapid biochemical screens for quality, at least at the present time, appears difficult to obtain. Table 1.Ratios of variance components attributed to environment, genotype,
and interaction effects for wheat quality parameters.

Parameter	Environment/ Genotype	Genotype/ GxE	Environment/ GxE	
Flour protein	2.30	2.71	6.29	
Mixograph peak time	4.03	1.85	7.46	
Mixograph tolerance	0.56	1.95	1.10	
Absorption	2.13	1.59	3.39	
Loaf volume	3.19	0.96	3.07	
Loaf external	4.52	0.41	1.86	
Loaf grain	1.96	0.30	0.58	
Loaf texture	2.17	0.27	0.58	

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DETECTION OF THE 1BL/1RS WHEAT-RYE TRANSLOCATION IN HEXAPLOID WHEATS USING A MONOCLONAL ANTIBODY BASED TEST

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In hexaploid wheats, both 1AL/1RS and 1BL/1RS wheat/rye translocations have been used to confer improved disease resistance and improved agronomic performance. Secalins coded by the 1RS chromosome arm can be detected by gel electrophoresis, but it is difficult to distinguish between 1AL/1RS and 1BL/1RS translocations. We have published ⁽¹⁾ a monoclonal antibody (MAb) based method which relies upon the presence/absence of 1BS coded gliadins. Successful transfer of this technology to breeding programs involves (a) using one third of a kernel (brush end) and examining five kernels for each line to detect segregating lines, (b) extracting with 50% propan-2-ol at 40°C to soften kernels (c) binding protein extracts onto ELISA microtitre plates and exposing to a 1BS gliadin specific first antibody followed by an alkaline phosphatase coupled second antibody to detect the amount of first MAb bound.

The major advantages are (i) 500-1000 samples can be assayed per day, (ii) inexpensive reagents (iii) non-hazardous (iv) gives a qualitative (presence/absence) result relatively independent of kernel size or protein content.

This test does not detect 1AL/1RS wheat-rye translocations. Antibodies specific to 1RS rye secalins that could detect either 1AL/1RS or 1BL/1RS translocations are being developed by others (R. Graybosch, personal communication) that may be able to be combined with the 1BL/1RS test for positive identification of either translocation.

Additional antibody based tests are being developed to detect 6AL/6AgS and 6DL/6AgS wheat/agropyron translocations conferring resistance to the leaf curl mite.

⁽¹⁾ Howes, N. K., Lukow, O. M., Dawood, M. R. and Bushuk, W. J. Cereal Sci. <u>10</u> (1989) 1-4.

FLOUR BIOCHEMICAL CONSEQUENCES OF WHEAT-RYE TRANSLOCATIONS

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Chromosome arm 1RS has been transferred to wheat in the form of several different translocations. Two translocations, 1AL/1RS and 1BL/1RS, have been used in hard red winter wheat breeding programs. 1BL/1RS occurs in the cultivar 'Siouxland' while 1AL/1RS is found in 'TAM107', 'TAM200', 'TAM201', 'TAM202' and 'Century'. These translocations carry a variety of genes conditioning disease and pest resistance; preliminary studies also suggest advantages in terms of grain yield and yield stability under conditions of stress. Unfortunately, deleterious quality effects also have been associated with the presence of these translocations.

Several experiments have been conducted to attempt to discover the biochemical basis for the deleterious quality effects. Flour protein composition, as measured by size-exclusion high-performance liquid chromatography, was determined in both translocation and normal lines. Three protein fractions were discussed: glutenin (polymers of MW greater than 100K), high-molecular-weight (HMW) gliadin (water-insoluble monomers of MW between 25K and 100K) and high-molecular-weight (HMW) albumin + globulin (monomers of MW between 25K and 100K) soluble in 0.04M NaCl). Protein concentrations of each fraction were expressed in terms of percent of total extracted protein. Three different sets of plant materials were studied: 1) a collection of thirty wheat cultivars, seven carrying 1RS, grown in nine Nebraska environments in 1990, 2) a group of sister lines derived from a cross between a 1AL/1RS parent and a 1BL/1RS parent, and 3) 726 experimental lines selected from 12 breeding populations segregating for either 1AL/1RS or 1BL/1RS.

In all nine Nebraska locations, wheats carrying 1RS averaged significantly lower amounts of glutenin and significantly higher amounts of HMW albumin + globulin than normal wheats. The HMW gliadin fraction of 1RS wheats was lower than that of normal wheats in low protein environments, but not in high protein environments. With increasing amounts of total flour protein, the amount of HMW albumin + globulin either increased or remained constant in 1RS lines. In normal wheats, the amount of protein in averaged higher glutenin concentrations, and lower HMW albumin + globulin concentrations, than 1BL/1RS lines.

The effects of 1AL/1RS and 1BL/1RS wheat-rye chromosomal translocations on flour quality and protein composition were compared in a common genetic background. Four classes of sister lines derived from a cross

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between the experimental line TX81V6610 (1AL/1RS) and the cultivar 'Siouxland' (1BL/1RS) were identified: 1AL/1RS, 1BL/1RS, double-translocation (both translocations present), and normal (neither translocation present). Significant differences were observed among classes in SDS sedimentation volume, mixograph time and mixograph tolerance; no differences in flour protein concentration were observed. SDS sedimentation volumes and mixograph tolerance scores were higher among 1AL/1RS lines than among 1BL/1RS sister lines. Significant changes in flour protein composition, determined by size-exclusion high performance liquid chromatography, were associated with differences in quality parameters. Changes in flour protein composition were consistent with those observed in the previously discussed study.

To assess the effects of genetic background on end-use quality of wheats carrying wheat-rye translocations, mixograph properties, SDSS volumes and flour protein concentrations of 1RS wheats were compared to those of non-1RS and heterogeneous sister lines obtained from 12 breeding populations (total 726 lines). Quality characteristics were examined in relation to genetic background as measured by size-exclusion chromatography. In 10 of 12 populations, quality characteristics were significantly higher in non-1RS lines than in 1RS wheats; in the remaining two populations, no differences in quality parameters were observed. Heterogeneous lines were intermediate in quality between 1RS and non-1RS lines. In 11 of 12 populations, the amount of glutenin protein was higher in non-1RS wheats, and, in all populations, levels of salt-water soluble proteins (HMW albumin + globulin) were higher in 1RS wheats. Twelve lines were selected as possessing both enhanced end-use quality (when compared to currently grown 1AL/1RS and 1BL/1RS cultivars) and suitable agronomic attributes. These lines have been planted for additional testing/observation in 1992.

USE OF MOLECULAR MARKERS FOR ANALYZING QUANTITATIVE TRAITS IN WHEAT

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The idea of detecting linkage between polygenes and Mendelian marker loci is almost as old as genetics itself. But until recently, technology for detecting segregating markers at large enough numbers of loci to cover large parts of the genome was not available. Of course, our newly acquired ability to detect seemingly limitless numbers of restriction fragment length polymorphisms (RFLPs) in segregating populations has opened up a whole new field in quantitative genetics: analysis of individual quantitative trait loci, or QTLs (Edwards et al., 1987; Knapp et al., 1990; Knapp, 1991; Lander and Botstein, 1989; Patterson et al., 1991; Reiter et al., 1991).

QTL analysis has advanced much more quickly in certain species - most notably, maize and tomato - than in wheat. The primary reason is the very low level of marker polymorphism among wheat cultivars. Polyploidy in cultivated wheats compounds the problem. Furthermore, except for aneuploid analyses of the chromosomal locations of QTLs, quantitative genetic analysis has never been very prominent in wheat research.

Use of crosses with wild species may be an area in which marker-assisted analysis of quantitative traits in wheat can be exploited immediately. For example, hexaploid BC_2 -derived progeny of direct crosses between *Triticum aestivum* (AABBDD) and the wild diploid *T. tauschii* (DD) have several advantages as populations for QTL research:

- (1) high levels of polymorphism at RFLP loci
- (2) presumably large QTL effects
- (3) diploid segregation
- (4) mean agronomic performance similar to that of elite lines.

In the Wheat Genetics Resource Center at Manhattan, we are pursuing QTL analysis in such populations. I believe that, for most crop species, markerassisted analysis of quantitative traits will have its largest impact in generation of basic knowledge and in germplasm enhancement. The direct utility of marker-assisted selection for improvement of quantitative traits in cultivar development, however, has been badly oversold. Early predictions of a revolutionary increase in the speed and progress of plant breeding through use of RFLPs will not be confirmed, because breeders will still need to conduct extensive field evaluations to contend with the effects of genetic background, negative correlations among important traits, and genotype x environment (GxE) interaction.

Lande and Thompson (1990) showed that molecular markers can enhance progress from selection if (1) heritability of the trait is relatively low and (2) marker genotypes account for a relatively large proportion of the genetic variance. This results in a "Catch 22" situation: To get reliable estimates of marker locus effects on traits of low heritability, one must grow replicated experiments in different environments. And since heritability is a plastic value that increases with increasing replication, the researcher has increased the heritability by the time QTL effects and locations have been estimated. Although Lande and Thompson (1990) showed that by combining marker and field data, one can improve breeding progress significantly, RFLP analysis cannot reduce significantly the amount of field evaluation that is done. Rather, RFLP analysis must be superimposed on existing yield-testing procedures, something that only a very well-financed breeding program can afford.

I should emphasize that marker-assisted analysis holds great potential for giving us a better scientific understanding of the above problems. Although the impact on crop improvement of such knowledge is much more difficult to quantify than the impact of an advance in breeding methodology, it is extremely important. We can look to one example in wheat to illustrate this. Selection for gliadin or high molecular-weight glutenin variants probably will never be used routinely for quality breeding in the hard red winter wheat region. However, our knowledge of the effects of storage-protein genes on quality has a long-term, positive impact that is impossible to measure.

Many QTL studies have shown, as expected, that the distribution of locus effects is very highly skewed, with a small number of loci having large effects and a much larger number having small but detectable effects (Edwards et al., 1987; Paterson et al., 1991; Reiter et al., 1991). Since these latter loci lie at the threshold of detectability, we can be fairly confident that there are an even larger number of loci with real but individually invisible effects on the quantitative trait. Loci with large effects will be detected by any statistical methodology, whereas those traditional polygenes with smaller effects will lie above or below the threshold of detectability in different environments, depending on the experimental design and statistical analysis used.

Detectable QTLs have generally accounted for less than half of the genetic variation in most populations. Even these R^2 values are probably overestimates, since in most studies, identification of QTLs and estimation of their effects are done in the same experiments, inflating estimates of genetic effects with genotype x environment interaction effects.

Breeders will need to continue working with those QTLs whose effects are too small to detect reliably with markers but which account for a majority of the genetic variance in most crosses studied. If this part of the genome is ignored, breeding could become profoundly conservative, a matter of simply reshuffling QTLs with large effects, disease and insect resistance genes, reduced height genes, and a few others, in narrow, well-characterized genetic backgrounds.

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In summary:

- (1) RFLP methodology provides a tool that will allow a tremendous expansion of our knowledge of genetics and genome organization.
- (2) RFLPs linked to other individually detectable loci will permit much more efficient genetic manipulation, by, for example, identifying heterozygotes for dominant genes, identifying different resistance genes that cannot be distinguished using different isolates of the pathogen or insect, and selecting in environments in which the trait affected by the gene is not expressed.
- (3) The primary effect of RFLP methodology on improvement of quantitative traits will be indirect, through expansion of our basic knowledge of plant genetics.
- (4) Where RFLPs are used directly in wheat, their greatest impact will be in germplasm enhancement, where there is a much higher prior investment per cross and a greater probability of finding large allelic effects than in cultivar development programs.

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UPDATE ON DOUBLED HAPLOID IN WHEAT BREEDING

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While numerous wheat cultivars have been reported as being developed by doubled haploid breeding methods, the majority of cultivars have been developed in countries where it is difficult to determine their relative advantage and to have direct comparisons with conventionally derived breeding methods. In Europe, only 'Florin' has been developed by anther culture and its impact has so far been small. Wheat doubled haploid breeding can be divided into two philosophies: 1. those who have committed to the technology and are actively breeding using anther culture without making comparisons to conventionally derived lines, and 2. those who are doing efficacy testing of the method but have not committed to using doubled haploids in their breeding programs.

Current research in wheat anther culture is concentrating on the role of sugars in media. Sucrose has been commonly used for most tissue culture media, however, barley workers have reported greater success with maltose and glucose. Maltose is a disaccharide containing two glucose molecules. Sucrose is a disaccharide containing glucose and fructose. It is believed that maltose may have different degradative properties in the media, as well as benefit from not having a fructose moiety. Fructose is often detrimental when added to tissue culture media. Maltose appears to be superior to sucrose for wheat anther culture also. Perhaps more importantly, maltose allows previously recalcitrant genotypes to respond better in anther culture, thus lessening the genotype specificity for which wheat anther culture is known. Genotype specificity was a major limitation in previous wheat media for creating the diverse, anther culture derived doubled haploid lines needed by plant breeders. A second area of active research is in 'gelling' agents. Barley anther culture was enhanced by the use of barley starch as a gelling agent replacing agar or Ficoll. Wheat starch appears also to be beneficial for wheat anther culture in both initiation and regeneration media. Wheat starch is very inexpensive when compared to Ficoll and less expensive than agar. As starch is a polymer of glucose molecules (hence also of maltose molecules), the starch and sugar research may both relate to a beneficial degradation of maltose and its polymers.

A second method of developing wheat haploids using intergeneric hybrids has recently been developed. As opposed to previous attempts using *Hordeum bulbosum* which required the crossability genes and greatly limited the number of lines that could be used, the new intergeneric method uses maize, sorghum, or pearl millet pollen and wheat ovules. The male chromosomes are rapidly eliminated (after 48 hrs.) and the maternal haploid embryo would die without intervention, either embryo rescue or hormonal treatments to prolong the endosperm development. This method has little to no genotype specificity (male or female) and appears to be quite promising. The critical aspects are: 1. having healthy female plants, 2. vigorous pollen, and 3. successful intervention. The method is new, so little is known about the field performance of the derived doubled haploids. However, previous studies using *H. bulbosum* as a pollen source did identify method-related induced variation. The induced variation was less than that induced with anther culture.

Relatively few experiments have reported on the field performance of anther culture derived doubled haploid plants. The research groups that are routinely using anther culture have reported preliminary data from field experiments involving hybrid parent materials which indicated little gametoclonal variation. The groups involved with efficacy testing have larger experiments and have identified gametoclonal variation in doubled haploid lines derived from 'pure line' parent materials. Hence it is possible that the discrepancy between results could be due to the source of parent material. In doubled haploids from a hybrid, the genetic variation in the cross may much greater than gametoclonal variation induced by the technique, hence the gametoclonal variation becomes lost within the total 'genetic' variation. In doubled haploids from a 'pure line', all of the variation should be due to intravarietal heterogeneity or the doubled haploidy method. Hence gametoclonal variation would be proportionately larger (more measurable) in these experiments. Unfortunately, this explanation does not seem correct in that recent work comparing SSD and doubled haploid lines derived from Pavon 76 x Chris indicate the doubled haploid lines on average were lower yielding than the SSD derived lines. This result could be due to gamete selection or to gametoclonal variation. One cross is insufficient to differentiate between these two hypotheses and additional research involving other crosses is needed.

A problem with field efficacy testing is that it takes three to four years to develop and test the lines. Hence the field efficacy tests are of doubled haploids created using older methods. The tests are always of where you have been and not where you are. The newest culture improvements are three to four years away from completed field testing.

HYBRID WHEAT Current World Status and Future Prospects

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INTRODUCTION

Interest in developing wheat hybrids was initiated in the early 1950's with the discovery of cytoplasmic male sterility (CMS) by Kihara and co-workers in Japan. Neither the *Aegilops ovata* nor *Aegilops caudata* cytoplasms were suitable for commercial use and no fertility restorer sources were available. Discovery of *Triticum timopheevi* as a useful source of CMS in 1959 by Wilson and Ross in Kansas and subsequent development of fertility restoration by Wilson and Ross and Schmidt, et. al. at Nebraska provided the genetic basis for hybrid wheat development.

The 1960's was a decade of enthusiastic hybrid wheat research. Hybrid wheat development programs were initiated by all of the major commercial seed companies and most public breeders were involved in basic research. As the complexity of the *T. timopheevi* system became apparent some programs reduced their efforts. Alternative systems of CMS were investigated by Maan and Lucken in North Dakota, Gotsov and Panayotov in Bulgaria, Tsunewaki in Japan, and Driscoll in Australia. None of the alternate genetic systems have proven superior to the original system based on *T. timopheevi*. The *T. timopheevi* system was refined and incorporated into the emerging semi-dwarf germplasm in the late 1960's.

Chemical hybridizing agents were under development in the 1970's, primarily by Rohm & Haas and Shell. The CHA's were viewed as a simple alternative to the more complex CMS system. Early compounds had problems with phytotoxicity, genotype specificity, and environmental interactions.

Several prototype hybrids were released commercially by DeKalb and Pioneer in the mid 1970's. Hybrid performance and seed production problems caused withdrawal of these products from the market. Many public programs and some private companies reduced or terminated their hybrid wheat development efforts.

The 1980's saw commercial introduction of CMS hybrids by HybriTech and Cargill. Rohm & Haas produced commercial hybrids using a CHA. The Shell CHA was nearing commercial utilization before animal toxicity problems forced its withdrawal. Depressed wheat prices, technical problems, and marginal or variable hybrid performance resulted in the sale of the Rohm & Haas wheat program to HybriTech and the withdrawal of Cargill from commercial sales.

CURRENT STATUS

Experimental hybrids have been evaluated in the USDA regional nurseries since the mid-1980's. Data for yield, response, and stability from the SRPN and NRPN for the 5-year period of 1986-1990 are presented in the following tables. Averaged over five years, the hybrids have yielded 7 to 10% more than the pureline entries. The hybrids were more responsive to changes in environment than the pure lines and exhibited greater stability also.

Data from HybriTech trials were analyzed for response and stability. Hybrids were more responsive than inbreds, with two parent hybrids more responsive than 3-way hybrids. The hybrids were more stable than the inbreds.

Heterosis information is normally not generated since including parents in yield trials would triple a large testing effort. Data from a special trial show 2way hybrids about 19% better than the parents, 3-way hybrids were 13% above the parents. F2's of 2-way hybrids were 9% above the parents, while 3-way F2's were 3% below the parents.

Comparisons between identical hybrids produced both by CMS and CHA have given virtually identical results. There were some indications of better winter survival for CMS hybrids having *T. timopheevi* cytoplasm.

Improvements in hybrid performance relative to new and popular varieties have been occurring at a rate of about 2% per year. Current levels of performance are about 12% above the best check and 20% above the average of several check varieties.

The most consistent performance in a public test has been Quantum 542 in Montana state trials. Quantum 542 finished first each year from 1987 to 1990 with about a 4 bu/A yield advantage over two popular varieties, Neeley and Judith. An indication of future improvements is HybriTech data from two years of testing in Montana. Two experimental hybrids have outyielded Quantum 542 by about 10 bu/A or about 27% above the best varietal check. One hybrid is produced via CMS and the other by CHA. In addition to hybrid performance, efficient seed production is an essential element in hybrid wheat economics. Data from the past four years and longer term show hybrid seed yields at about 80% of the pollinator with a range of 50% to over 100%. Seed fields are normally seeded in a 3 female: 1 male ratio with female widths ranging from 40 to 60 feet. Any environmental factors which influence the relative flowering dates of the parents have the most effect on seed yields.

Hybrid wheat research continues in most of the major wheat producing areas of the world. Most research in First World countries is being done by private companies. In Western Europe there are many private plant breeding firms that are cooperating in association with one of several larger multinational companies. Former and currently centrally planned economies continue to work on hybrid wheat development. China recently announced the initiation of commercial production. Commercial hybrids are being sold in Argentina, Australia, South Africa and the United Western Europe is awaiting registration of chemical hybridizing agents expected within the next year or two. Registration of these compounds in the United States is expected by 1994.

Source	1986 (#)	1987 (#)	1988 (#)	<u>1989 (</u> #)	1990 (#)	× (2)		
		•						
Public	118 (27)	114 (27)	116 (28)	103 (24)	120 (26)	114 (132)		
Private	116 (7)	115 (6)	119 (9)	104 (12)	108 (4)	112 (38)		
Hybrid	127 (8)	119 (7)	119 (5)	113 (6)	126 (4)	121 (30)		
	Response (b)							
Public	1.01	0.99	1.00	1.01	1.00	1.00		
Private	0.98	1.03	1.03	0.97	0.98	1.00		
Hybrid	1.04	1.08	1.0 9	1.12	1.07	1.08		
			Stability	(r²)				
Public	0.88	0.90	0.88	0.89	0.92	0.89		
Private	0.86	0.91	0.91	0.90	0.93	0.90		
Hybrid	0.92	0. 9 4	0.93	0.94	0.95	0.93		
					-			

Table 1. Performance data from the SRPNYield (% of Checks)

Source	1986 (#)	1987 (#)	1988 (#)	1989 (#)	_1990 (#)	<u>× (Σ)</u>
Public	111 (20)	108 (24)	107 (20)	108 (13)	110 (18)	109 (95)
Private	121 (1)	102 (5)	127 (1)	100 (2)	- (0)	106 (9)
Hybrid	116 (6)	124 (4)	118 (2)	117 (4)	119 (4)	119 (20)
	·		Response	(b)		
Public	1.03	0.99	0.99	0.97	0.99	1.00
Private	1.15	0.91	1.16	0.94	•	0.97
Hybrid	1.00	1.31	1.24	1.11	1.23	1.15
			Stability (r [*])		
Public	0.83	0.86	0.90	0.89	0.94	0.88
Private	0.91	0.83	0.88	0.89	-	0.86
Hybrid	0.83	0.93	0.89	0.95	0.96	0.91
-						

Table 2. Performance data from the NRPNYield (% of Checks)

FUTURE DEVELOPMENTS

Commercial registration of chemical hybridizing agents will provide access to hybrid wheat development for some plant breeding organizations currently developing pure lines. The CHA's are a complement to CMS development, shortening development time and permitting improved efficiency. Both systems have advantages and disadvantages, but both are merely tools to achieve a goal.

Other genetic systems for inducing sterility and restoring fertility are being researched by various biotechnology groups. Transfer to wheat depends upon transformation systems being developed. If effective in wheat they probably will not be used for commercial production before the year 2000.

As wheat transformation systems are perfected, various specific traits will be inserted into wheat. In most cases these traits or the genes controlling them will be patented. An obvious means of protecting an expensive investment is to market it through hybrids with built-in protection.

The future for hybrid wheat appears promising. Progress in performance relative to pure-line varieties is being made. New systems of hybrid production are nearing the commercial stage. Progress is wheat transformation will permit incorporation of new traits into a self-protecting system. Adoption of hybrid wheat by farmers will depend upon economic considerations. These factors may restrict hybrid wheats to the more productive areas. A current example of this was the production of Quantum 555 in northwestern New Mexico under pivot irrigation. In 1991 this hybrid yielded an average of 136 bu/A on nearly 2000 acres with a top yield of 162 bu/A in one 144 acre field.

Hybrid Wheat Development

Decade	Events
1950	Discovery of CMS in Aegilops cytoplasms
1960	Discovery of CMS in T. timopheevi cytoplasm Discovery of Rf genes in T. timopheevi Initiation of commercial hybrid breeding Initiation of public basic research
1970	Refinement in CMS Agronomic improvements - semi-dwarf CHA development Commercial prototypes released Reduced public research
1980	Commercial hybrids sold Germplasm diversification Registration of commercial CHA
1990	Improved hybrid performance Registration of improved CHA Alternate genetic hybridization systems Wheat transformation
2000	Specific trait insertion Economic protection via hybrids

Туре	Number	Response `b´	Stability
Hybrid	147	1.02	0.90
3-way	115	1.00	0.90
2-way	30	1.08	0.92
Check	2	1.24	0.96
inbred	261	0.91	0.71
B-line	132	0.92	0.75
R-line	115	0.89	0.67
Check	14	1.02	0.78

Table 4. Hybrid vs. Inbred Results

F₂ HYBRID TRIAL

Туре	N	Yield Mean	Percent Parent X
Single Cross Hybrid	28	72.1a	118.8
3-Way Cross Hybrid	24	68.4 b	112.7
Checks	16	67.3 b	110.9
Single Cross F ₂ 's	28	65.9 b	108.6
Females	44	61.2 c	100.8
Males	28	60.2 c	99.2
3-Way Cross F₂'s	24	59.0 c	97.2

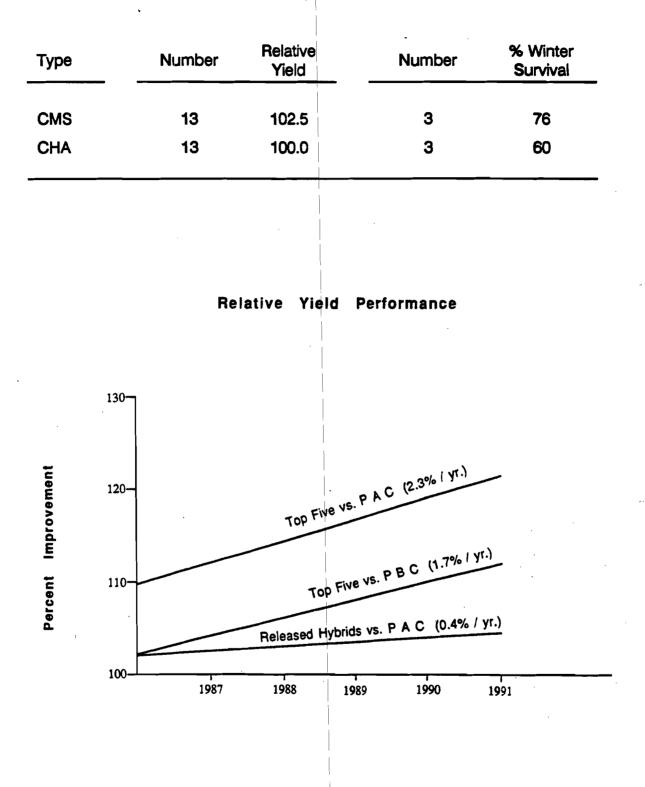


Table 5. CMS vs. CHA Hybrid Results

Entry	1987	1988	1989	1990	Overail Average
Quantum 542	74.4	41.7	57.7	68.3	60.7
Rocky	65.7	41.3	53.2	58.3	54.7
Neeley	6 8.1	40.9	52.3	62.1	56.1
Judith	70.4	38.3	52.9	62.5	56.3
Redwin	64.2	34.9	48.0	57.1	51.3
Tiber	65.8	34.3	51.2	59.1	52.7
Winalta	58.1	34.1	50.1	52.1	48.5

Montana State University Trials for 1987-1990

HybriTech Montana Triais

Entry	1990A (4)	1990B (4)	1991 (2)	₹A (6)	⊼В (6)
NH 1609	81.1		87.1	83.1	
NH 1643		76.6	89.8		81.0
QT 542	72.0	68.7	77.5	73.8	71.6
Neeley	65.3	62.5	66.3	65.6	63.8
Rocky	60.0	62.3	66.1	62.0	63 .6
C.V.% L.S.D05	7.4 3.9	9.5 5.1	7.7 7.0	· .	

Percent Yield Increase Required to Provide 8x Return

i.

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Added Yield	Seeding Rate (lbs)	Present Yield Base (Bu)			I)	
		30	45	60	75	90
6 bu.	45	20	13	10	8	6
8 bu.	60	26	17	13	11	9
10 bu.	75	33	22	17	13	11
12 bu.	90	40	26	20	16	13

Hybrid Seed Yields 1988-91

	No. Fields	No. Hybrids	Yield Average Bu/A		Range (%)	
			F	M	%	
'88	7	5	58	68	85	6 6-11 3
'89	7	6	61	73	84	59-106
'90	22	9	52	65	80	50-119
' 91	15	10	41	55	75	35-106
x	13	8	51	64	80	49-113
					<u> </u>	

Worldwide Hybrid Wheat Research

Country	Program	CMS	СНА
United States	Agripro	a-	X
и и .	Cargill	x	
	HybriTech	x	X
	Sogetal		X
	Trio	X	
* *	Arizona Plant Breeders	X	
Australia	Cargill	x	
Argentina	Cargill	x	
South Africa	Sensako	X	
н н	Bethlehem	x	
China	Various	x	X
Bulgaria	Tolbukhin	x	
Hungary	Martonvasar		x
Germany	Various	x	x
France	HybriTech		x
"			x
"	Pioneer	X	x
		~	~
Russia			X
Canada	Agripro		x
	· · · · · · · · · · · · · · · · · · ·		

CREATING A HETEROTIC GROUP IN HARD WINTER WHEAT

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Hybrid wheat grown in the southern Great Plains of the United States has been, at least to date, unsuccessful in competing for a significant share of the commercial acreage. There are many reasons for this development including: 1) the generally low market price for wheat during the past decade, 2) the high cost of borrowing money, 3) the high cost of hybrid seed wheat, 4) availability of pure line varieties with near equal performance records, 5) and the reluctance of farmers to invest large amounts of money into their seeding operations. Never the less, hybrid wheat still represents a significant research effort in the southern Great Plains, primarily from private industry. Hybrid wheat has the potential to provide not only increased yields, but increased stability across a range of environments famous for its instability (1,5,11). Before hybrid wheat can compete with the best selection of varieties however, the level of heterosis above the best wheat varieties must approach 20% or 10 bu/acre in higher vielding environ-ments. Although in specific years or environments this level of performance has been achieved, the average performance of hybrid wheat has been closer to the best varieties, not attractive enough for farmers to invest large amounts of money into planting hybrid wheat. The difference in performance between hybrids and varieties can be attained either by developing superior inbred parental lines or by increasing the level of heterosis observed in wheat F, hybrids.

This research is focused on improving the heterosis in wheat. The concept of developing or identifying a heterotic group in wheat is not new. In fact, several cross combinations such as North American hard red spring wheats crossed with either Argentine or Spanish derived spring wheats have been reported to be superior compared to normal F_1 spring wheat hybrid performance (3). Improving heterosis above what has already been reported is unlikely within the current germplasm pool. Heterosis in corn has not changed significantly in 50 years (2). Corn hybrids have continued to improve because the inbreds involved in the hybrid combinations have been improved dramatically.

In wheat however, we have a unique opportunity to modifying the genome to enhance specific interactions of its allopolyploidy nature. Sears (1954) reported several wheat chromosomes to be less critical when lost from monosomic or nullisomic plants. Specifically group 1 and group 7 chromosomes are less critical than the chromosome groups when missing in an aneuploid. In addition specific chromosomes within a group are less critical than other homologous chromosomes or chromosome arms. These chromosomes or chromosome arms essentially represent neutral gene blocks in the genome. They can be present or absent without having large negative impacts on the plant's appearance or genetic balance. The successful use of two wheat-rye translocations 1BS/1RL and 1AS/1RL in wheat and their association with group 1 chromosomes appear to be likely examples of this concept. Positive gene blocks from rye (1RS) have replaced the neutral gene blocks 1AS and 1BS. The opportunity to substitute parts of group 7 chromosomes with rye or other species could result in additional vigor. This could also be true for specific homologous chromosome arms in groups 2-6 as well. We have the knowledge and the techniques using monosomic instability to specifically create these unique types of translocations or chromosome substitutions (9). The likelihood that neutral or negative gene blocks will be identified is expected. To search for positive gene blocks is exciting.

The wheat-rye translocation 1BL/1RS carries new genes for disease resistance, improves adaptation in wheat, and has been reported to increase yield in hard winter wheat germplasm (7). Similarly the wheat-rye translocation 1AL/1RS carries genes for disease and wheat curlmite resistance (5), and also appears to improve adaptation. Rarely had wheats performed well in the International Wheat Performance Nursery outside the Great Plains until the introduction of the 1AL/1RS translocation into released varieties Century and Tam 107.

Recently a new wheat-rye translocation, 2BS/2RL has been released. Resistance to Hessian fly and tan spot have been contributed from rye. In addition, preliminary data suggests that this translocation may also contribute increased vigor (4). F₁ hybrids involving the 2BS/2RL translocation as a heterozygote have greater biomass, larger heads, more seeds/spike, lower harvest index, and higher grain yields than controls. All characteristics of increased heterosis in successful hybrid combinations.

The purpose of this work is to systematically combine wheat-rye translocations initially, then other gene blocks later that contribute to increased heterosis in a wheat background. These blocks of genes are stable and can be easily manipulated by breeding because of their inheritance patterns. We hope to capitalize on the heterosis provided by wheat-rye translocations in an additive approach. Combining 1AL/1RS with 2BS/2RL could possibly contribute an increase to the level of heterosis previously reported in wheat. Preliminary data suggests that this in fact may be the case. Hybrids involving these two translocations yielded 36% and 37% heterosis above their parents in space planted field tests at Berthoud, CO in 1990. Larger field tests will be necessary to verify the accuracy of these preliminary results.

We feel it may be possible to improve the heterosis in wheat by substituting specific gene blocks into wheat, in effect replacing neutral gene

blocks that have existed since wheat evolved some 10,000 years ago. These neutral blocks have been identified by Dr. E. R. Sears in his initial descriptions of the aneuploid genetic stocks of wheat. Chromosome groups 1 and 7 appear likely to be the most responsive to this approach. Chromosome group 1 already has provided increased stability, disease resistance and heterosis in wheat. That other chromosome arms can yield additional positive results may hold promise in improving the performance and economic success of hybrid wheat for the future.

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SELECTION FOR QUALITY TRAITS IN WHEAT BASED ON THE PROBABILITY OF THE TRAITS FALLING WITHIN ESTABLISHED LIMITS

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Introduction

The ability to identify and select hard red winter wheat varieties with enhanced consistency and stability over environments for end-use quality traits is important for the production and marketing of a high quality product. Significant variation in end-use guality occurs among wheats due to the interaction of Genotype and Environment. However, stability analyses, such as regression and variance approaches commonly used for grain yield, are inadequate for treatment of end-use quality measurements. Mixing and baking analyses provide large numbers of parameters for consideration, requiring multivariate solutions. Quality parameters often are not normally distributed and often intercorrelated. Also, the definition of stability for end-use quality depends on ones perspective. The milling and baking industry desires varieties that have high probability of multiple quality traits falling within acceptable, specified bounds, regardless of differences among varieties or the inherent nature of the environmental response. Wheat breeders need estimates of stability for selection that take into account environmental response in relation to performance of known, established varieties.

The problem is: how to identify and select varieties grown over multiple environments with enhanced stability and high probability to provide 'acceptable values' when traits are intercorrelated.

Statistical Approaches

Determine the probability that 'acceptable' end-use quality values are achieved for each variety under consideration from multiple environments. Two definitions of 'acceptable' were used:

1) Industry perspective: probability that quality traits for a variety grown over multiple environments fall within specified, acceptable bounds for baking quality.

2) Breeder's perspective: probability that quality parameters for a given variety equals, or exceeds, values for an established check variety at each environment.

However, calculation of these probabilities is not trivial as: 1) quality traits are intercorrelated and multivariate in nature; 2) it is not obvious what probability distribution to use or assume; 3) multivariate probabilities are generally not easy to compute. These problems can be solved if the multivariate normal probability distribution may be considered as an adequate approximation.

Multivariate probabilities of traits falling within acceptable limits were computed using a FORTRAN program available from Schervish (1984). Calculations can be made assuming that bounds for each parameter are available, traits are approximately multivariate normal in distribution, and variety covariance matrices could be pooled based on homogeneity tests. Multivariate normality was determined using the Shapiro-Wilk test. Univariate probabilities also were computed for each quality parameter to determine which individual traits contributed to low multivariate probabilities of acceptance among the varieties.

Database for Analyses

Grain samples were obtained from eighteen hard red winter wheat genotypes grown in replicated trials at seven locations in both 1988 and 1989. Locations used each year were Lincoln, Clay Center, North Platte, Sidney, and Alliance, Nebraska; and Yuma, Arizona. Thirteen released varieties and five experimental lines were evaluated.

Grain samples were micro-milled and flour quality evaluated for: protein concentration (%) by macro-Kjeldahl; mixing time (min) and mixing tolerance (0-9) using 10 gm National Manufacturing mixograph; SDS sedimentation volume using standard method modified to test 2 gm flour samples; and kernel hardness (0-9) using microscopic evaluation of individual crushed grains. SDS sedimentation provides estimate of protein quality based on solubility in SDS solution and is a reflection of loaf volume potential.

Industry acceptability bounds for each quality parameter were arbitrarily chosen as minimum to maximum values:

Flour protein - 12.5 to 19% Mixing time - 3.5 to 8.0 min Mixing tolerance - minimum of 2.5, no upper bound SDS sedimentation - 30 to 40 cc Kernel hardness - 4.9 to 8.0

Breeder's acceptability limits for quality parameters were determined using values for the variety 'Scout 66' as minimally acceptable at each environment.

Results

Although individual quality traits were not all normally distributed, the traits were determined to be approximately multivariate normally distributed. Variety covariance matrices were pooled based on failure of test to reject homogeneity. Multivariate probabilities were then calculated.

Probabilities of individual varieties meeting industry acceptability for all quality parameters ranged from 31% to near 0. Centurk 78, Karl, and Bennett show the highest probabilities when grown over multiple locations. Their probabilities of 26 to 31% may appear low, but one must consider that five traits are involved and this reflects frequency for ALL traits to simultaneously fall within acceptable bounds. Probability values were less than 4% for OK83396, N87U113, Siouxland, and N87U110, indicating that these lines have extremely low probability of meeting industry standards even under the most favorable environmental conditions.

Univariate probabilities for the varieties were calculated to indicate the relative contribution of individual quality traits to the multivariate probability value. In several cases, varieties had a low multivariate probability as a result of only 1 or 2 parameters with low probability of acceptance. For example, the low multivariate value obtained for Redland was the result of the low univariate probabilities for flour protein and kernel hardness. Siouxland and N87U113 had low probability values for mixing tolerance and SDS sedimentation, but relatively high values for other parameters.

Probabilities also were calculated in terms of quality traits falling above those for an established check variety (Scout 66) for each of 18 wheats. Karl, Centurk 78, and Bennett show the highest probabilities (34 to 40%) of all quality parameters meeting or exceeding values for Scout 66 when grown over multiple environments. The remaining varieties all have less than 12% probability of exceeding quality of Scout 66. Five of the varieties having essentially no chance (<1%).

Univariate probabilities for the varieties relative to Scout 66 were used to indicate relative contributions of individual traits to the multivariate value. Redland shows relatively low probability of exceeding Scout 66 for flour protein, SDS sedimentation and kernel hardness. N87U110, N87U113, N87U102, TX86V1110, and OK83396 each had high probability of exceeding flour protein (>93%) and kernel hardness (>54%) levels of Scout 66 over environments, but each had lower probabilities for mixing characteristics and/or SDS sedimentation values that reduced the multivariate probability and overall acceptability of these lines.

Conclusions

Calculation of multivariate and univariate probabilities provides a useful alternative in characterizing stability and acceptability of varietal end-use quality. The approach allows analyses of intercorrelated traits, as are commonly found in quality analyses, which may not be normally distributed. Resulting probabilities can be readily calculated in terms of either fixed bounds, as might be based on industry standards, or relative to differences from a known check variety. Univariate analyses complements the multivariate probability approach by showing relative influence of individual traits on meeting overall acceptability. Selections of upper and lower bounds or check varieties are flexible and easily modified. The probability results are easily interpretable, providing a versatile measure of varietal stability, industry acceptability, and selection value for complex quality traits.

DESIGNED EXPERIMENTS IN THE PRESENCE OF SPATIAL CORRELATION

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Soil heterogeneity is generally the major cause of variation in plot yield data and the difficulty of its interpretation. If a large degree of variability is present at a test site, some method of controlling it must be found. Controlling experimental variability can be achieved either by good experimental design or by analysis procedures which account for the spatial correlation. Classical designs are only moderately equipped to adjust for spatially correlated data. More complex designs including nearest and certain restricted Latin square designs are developed for field experimentation when spatial correlation causes classical designs to be less desirable.

However, complex designs are sometimes difficult to implement in the field due to constraints on randomization and are often difficult to analyze. Measuring related variables and using analysis of covariance has been shown to be a good analysis technique to improve a design's efficiency. If a plot is surrounded by neighbors who are doing well, it can be expected that it will do well also. If this plot does in fact do well, that fact does not necessarily mean that the treatment which was applied to that plot is doing well. Conversely, if a plot is surrounded by plots who are doing poorly, then the treatment applied to that plot might be considered relatively good even if the response to the treatment is inferior when viewing that plot in isolation.

Nearest neighbor (NN) analysis was first introduced by Papadakis and then reviewed by Bartlett in a more mathematically critical paper. NN allows for the recovery of information from replicated field experiments with large blocks. This is especially useful in cultivar trials or other experiments where large numbers of treatments or cultivars are used. Generally, the procedure calls for the development of a covariate for a particular plot by averaging the yields of surrounding plots.

Researchers can also adjust the analysis of spatially dependent variables through the theory of regionalized variables developed by Georges Matheron. The application of Matheron's theory to problems originating in mining and geology led to the more popular name of geostatistics. The quantifying of the spatial correlation is done by constructing a semivariogram which describes the spatial relationship through the parameters of nugget, range, and sill. Using the geostatistical representation of the spatial data in conjunction with general mixed model theory in statistics allows for a more general solution of the spatial analysis problem. The statistical formulation will be presented in abbreviated form for background. The mixed model is characterized as:

$\underline{\mathbf{y}} = \mathbf{X}\underline{\mathbf{b}} + \mathbf{Z}\underline{\mathbf{u}} + \underline{\mathbf{e}}$

where \underline{b} is a vector of fixed effects and X its associated matrix of design or regression constants, \underline{u} is a vector of random effects and Z its associated design matrix, and \underline{e} is a vector of errors. \underline{u} and \underline{e} each are assumed to have zero means and variance-covariance matrices G and R respectively. G and R can be completely general, to account for various patterns of correlations among observations. These correlations may take the form of spatial correlations. Our interest is in estimating \underline{b} accurately when the structure in R is of the variance-covariance form attributable to spatial correlation. Thus one of the various semivariogram models is assumed. Thus although in geostatistics it is difficult to estimate the spatial correlation unless the "drift" or in this case the treatment means are known and vise versa, by using the general mixed model approach we may iteratively solve for both. Consequentially we decide upon a semivariogram model and obtain the BLUP estimators of the nugget, range, and sill. The estimators of the treatment effects are BLUE.

The designs, both classical and nearest neighbor type designs, are analyzed using the classical statistical analysis approach and a strategy using general linear mixed models which takes into account that there is spatial correlation present. The results indicate that properly designed experiments may be analyzed either by the usual statistical techniques or more complex methods which adjust for spatial correlation. However, if no serious thought is used in constructing the design of the experiment then the usual analysis techniques are no longer valid.

MINIMUM DISTANCE: ASTA SUBCOMMITTEE REPORT

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The American Seed Trade Association (ASTA) empaneled a subcommittee to develop the concept of minimum distance for wheat breeding. Similar subcommittees were empaneled for most major crops. The issue of minimum distance was important to the ASTA because new technologies, specifically genetic transformation, were viewed as a quick way to add a new trait to an existing cultivar. The new trait would satisfy the novelty requirement of the Plant Variety Protection Act (PVP). Hence companies and institutions that have devoted 10 to 15 years to develop a new cultivar, could lose their ownership rights to a genetic engineering group. As the genetically engineered cultivar would not be possible without having an existing cultivar, ASTA felt that there was a dependency relationship between the existing cultivar and the genetically engineered cultivar. The later cultivar being essentially derived from the former. Of course many other breeding methods similarly rely upon a previously existing cultivar and their products also would be considered as being dependent or essentially derived.

The subcommittee tried to develop minimum distance concepts with the goal of developing a comprehensive report that would not be perceived as potentially being antagonistic to new technologies and with a clearer understanding of the appropriate use of backcrossing. A secondary goal was to develop a list of primary descriptors that could lead to streamlining the PVP form.

The subcommittee recommended that the minimum distance between two novel lines be defined as those two lines differing in a statistically significant manner for at least one trait. The subcommittee devoted considerable time to developing the concept of dependent lines as there exist a number of techniques (selection within an existing cultivar, backcrossing, mutation, genetic transformation, somaclonal and gametoclonal variation, etc.) that can be used to develop lines that meet the criterion of minimum distance or essentially derived. However, lines developed by the above techniques were considered to be "dependent" upon having a previously existing line. As the concept of dependence was difficult to precisely describe, an alternative approach was used to describe the requirements for two lines to be independent (dependent lines are those lines which are not independent). Independence of two lines was defined as being:

Differences from Parents

Breeding Procedure

Single cross line development First backcross Second backcross Third backcross Fourth backcross Fifth backcross one trait¹ two traits² three traits four traits five traits six traits

¹ In addition to nuclearly controlled traits, the introduction of an alien cytoplasm is considered as one trait.

² Where the line must differ from the parent by multiple traits, each trait should be linked no closer than 10 map units to any other trait used for independence when crossed to Chinese Spring.

For genetic improvement techniques that require only one parent line (i.e. selection within a cultivar, mutation breeding, etc.), to establish independence, the new line would be considered as being equivalent to a backcross five derived line, hence must differ by at least six traits linked no closer than 10 map units to any other trait when crossed to Chinese Spring.

It should be understood that similar lines can be independently developed from different parents (a rare occurrence) and these lines need only differ by one trait. The incentive should be for development of independent lines (discovery) and a lesser incentive for lines which are dependent upon the availability of the original line.

A list of primary trait descriptors was developed. All lines should be characterized for these traits which are the minimum and obligatory characteristics that need to be measured for PVP and patent applications. All comparisons for describing a new wheat line must be to a commercially available line that has been approved for PVP. If two lines are similar for their primary descriptors, any other descriptive trait can be used to differentiate between the lines.

The following protocol was recommended for determining a possible dependency relationship:

- 1. Similarity for the primary descriptor traits.
- 2. Similarity for secondary traits.
- 3. Comparison and verification of breeding records.
- 4. The two parties use the established grow-out procedure which should include parents if possible.
- 5. The two parties negotiate a royalty.

Implicit in these recommendations are that ASTA establish protocols to 1. authenticate breeding records, and 2. regulate grow-out procedures. The subcommittee also felt that it would be best if the parties involved in an implied dependency case tried to resolve the issue with as little government intervention as possible.

While the subcommittee recognizes that seed for commercial (public) sale can be used for crossing except in such instances where legal restrictions require prior consultations, the subcommittee recommends the above policy should be changed as follows: Seed that is for commercial (public) sale should be available for crossing on a reciprocal basis. Persons opting not to exchange or allow germplasm for crossing must inform all other breeders of their decision and must not use the other breeders' germplasm for crossing without written permission.

The subcommittee recommends the continuation of the policy that an inbred line cannot be used as a parent for commercial hybrid wheat unless written permission is obtained from the line originator or their institution. These recommendations are applicable only to commercial wheat cultivars and not to research lines that are used in research experimentation only.

Since the ASTA Wheat Minimum Distance Subcommittee completed its work, UPOV has proposed a new treaty to protect plant breeders' rights. In addition, the perceived threat from genetic engineering has greatly lessened. Current government regulations concerning genetic engineering are stringent and the genetic engineering companies, once they have successfully inserted a trait, are using backcrossing to move the trait into other cultivars. Backcrossing is and has always been one method to breed new cultivars. The time required for backcrossing, selection within a line, and many of the other breeding methods which were suggested as being harmful to plant breeders' rights, as well as the time required for seed increase prior to commercial sale have greatly lessened the concern over rapid loss of ownership rights. However, the concept of minimum distance and dependent (essentially derived) cultivars will still be useful if the UPOV conventions as applied in Europe are adopted in the United States.

INTERNATIONAL REGULATIONS AND TRENDS TOWARD VARIETAL PROTECTION

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INTRODUCTION

Plant Variety Protection has been the subject of numerous conferences in recent years; it has sparked substantial international debate; it has impacted the access to germplasm from the centers of origin of major crop species; and it has had a profound impact on the manner in which international seed companies do business. Today we witness a large diversity of viewpoints, some based on science and business, others based on emotion but stated with passion and eloquence. Plant Variety Protection determines the manner in which seed companies do business, the crop species in which they invest research funds, the countries in which they choose to do business, and the germplasm that they are willing to expose.

The need to protect intellectual property rights is not new: At the Paris Convention in 1883, the philosophical importance of protecting biological inventions was recognized. However, it was not until 1952 that the International Association of Plant Breeders for the Protection of Plant Varieties (or ASSINSEL as it was named) was finally instrumental in getting the matter of plant variety protection onto the agenda of APPI (The International Association for the Protection of Industrial Property). They failed for act decisively, and it was in 1961 that the International Convention for the Protection of New Varieties of Plants (UPOV) established the basic principles of plant variety protection. Actual implementation took several more years for the member countries.

In the U.S., a Congressional report dated April 3, 1930, on the U.S. Plant Patent Act recognized the problems facing breeders of self-pollinated species. The statement was interesting; let me quote a small portion:

"Today the plant breeder has no adequate financial incentive to enter upon his work. A new variety, once it has left the hands of the breeder, may be reproduced in unlimited quantity by all. The originator's only hope for financial reimbursement is through high prices for the comparatively few reproductions that he may dispose of during the first two or three years. After that time, depending on the speed with which the plants may be reproduced, the breeder loses all control of his discovery." Does this statement sound familia? It took another 40 years before the Plant Variety Protection Act of 1970 finally provided an impetus for companies to breed self-pollinated crops.

Many believe that the U.S. seed industry is at the crossroads. On the one hand it could be argued that, with the current reduction in public plant breeding each year, there are increased opportunities - and a responsibility - to breed, produce, and deliver improved seeds to U.S. farmers. On the other hand, the industry sees real threats to profitability because of difficulties in maintaining proprietary rights to the products of its research and development. The cost per unit of gain through traditional breeding become harder to beat. Biotechnology research costs are rapidly escalating as this becomes an essential, but expensive, aid to plant breeding. Additional costs in time and money are incurred through compliance with new federal and state regulations for safety and comfort in the workplace.

How then do international regulations and trends towards varietal protection affect our research and the way in which we do business? I do not propose to go into details on plant variety protection law; this has been well addressed already. I propose to focus on how PVP and patent law has impacted on the investment in research on self-pollinated crops in the U.S. and in Europe, using wheat as our example. Finally, I propose to take a look at developing countries and explore some of the concerns and perceptions that surround current trends in varietal and germplasm protection.

2. THE U.S. PLANT VARIETY PROTECTION ACT

Commercial wheat breeding has been practiced for over 100 years. When the value of selecting improved strains from within landrace varieties was demonstrated, and the value of identifying superior progeny in segregating populations from single crosses was shown, seed companies appeared on the scene to market these products. However, variety <u>breeding</u> in the U.S. was left to the Land Grant Colleges and the state and federal experiment stations. There were two main reasons for companies to stay out of breeding; firstly, the public sector did a very good job in making genetic advances in a self-pollinated crop like wheat; and secondly, on-farm multiplication of wheat cultivars was a compelling reason for discouraging the development of proprietary cultivars.

In the early 1960's, a possible mechanism for developing hybrid wheat was discovered by Japanese scientists. Shortly thereafter, a restoration system for cytoplasmic male sterility was discovered by public researchers in Nebraska and Kansas. At last, there appeared to be a means by which companies might offer genetically superior wheats to the farmer, but still recoup their investment

in genetic research ... since new seed would have to be planted each year. This led to a number of companies, including Pioneer, DeKalb, Cargill, and Northrup King entering the hybrid wheat arena, and they made a significant investment in wheat breeding research.

When the Plant Variety Protection Act was passed on December 24, 1970, it did two important things for companies; first, for those already engaged in hybrid wheat research, it provided a means by which varietal seed could be sold and generate revenues prior to hybrids coming on stream; and, second, for smaller companies that could not afford the larger research investment needed for developing hybrid wheat, it provided a means by which they could enter the wheat seed business.

It should be pointed out that PVP certificates have both a crop exemption (usually called a farmers exemption) and a research exemption. The original intent of allowing a grower to save his own seed or sell some to neighbors has been subject to flagrant abuse, and it has led to the practice known as "brownbagging." The Federal Seed Act allows the term VNS (variety not stated) to go on a bag. Customers are then told verbally what the bag contains. Interestingly, VNS is not possible under the new UPOV Convention of March 1991, and should not have been possible under the 1978 Convention. The U.S. is a member of UPOV, and we have probably never been in full conformity with the treaty.

Two key rulings were handed down last October in favor of Asgrow Seed Company that could affect future PVP cases. The first case was Asgrow versus Dennis and Becky Winterboer of Milford, Iowa. The defendants argued that they had the right to sell 49% of their soybean crop as seed. Asgrow countered that the PVP Crop Exemption allows farmers to save only enough seed to meet all of their possible planting needs. Judge O'Brien ruled in favor of Asgrow, saying that farmers have the right to save one bushel of soybeans for every acre that they could potentially plant the following year. If, on a 1500 acre farm only 750 acres were planted, then 750 bu. of surplus seed could be sold as long as all other requirements of PVP law are met. The second major ruling was also handed down in October 1991 against Lake Village Seed Company for willfully violating Asgrow's rights under PVP. The 12 member jury decided that Asgrow was entitled to royalties of \$4.51, \$4.45, and \$5.83 for each 50 lb. unit of soybean seed sold in 1987, 1988, and 1989, respectively. The significance of this case is that the PVP law was upheld before a jury of the defendant's peers.

Not withstanding the above successes, we have witnessed a cycle in the entry and exit of companies from the wheat seed industry between 1970 and 1990. The period of 1970 to the early 1980's saw the peak in commercial breeding activity. Never before had the grower been offered such a choice of wheat cultivars. Several public institutions with a previous track record of turning out one or two new cultivars per decade were suddenly stimulated to release new cultivars every other year! Competition has been good for our industry, and the farmer has been the principal beneficiary of this competition. But, as we view certain of our wheat producing regions today, one must ask the question: "Where have all the companies gone?"

From the mid 1980's, we have seen a decline in private wheat breeding activity. Unfortunately, this has coincided with a period of decline in the net funding of public wheat breeding research. State programs have faced funding cuts, and the U.S.D.A. has changed its emphasis from traditional plant breeding to biotechnology and more basic areas of research. The net result is that there are fewer new crosses being made today and less genetic input into our industry.

Violations of PVP law, weaknesses in the Act itself, and our own lack of ability to catch the violators, have been at the heart of company decisions to withdraw from variety development in certain regions. Furthermore, hard economic times for our growers have encouraged the practice of farm-saved seed. In May 1990 testimony was given before the Department Operations, Research and Foreign Agriculture Subcommittee of the House Agriculture Committee regarding "Amendments to Strengthen the Plant Variety Protection Act." However, strong opposition to the proposed changes was voiced by several commodity groups.

To gain a sense of perspective, let us examine the size of the U.S. wheat seed market. In a 1990 survey of major crop seeds in the U.S., Duvick estimated the gross turnover of wheat seed at \$200 million. This compares with about \$250 million in France, \$140 million in Germany, and \$100 million in the U.K. Farmer purchases of new seed range from about 20% in the Hard Red Winter wheat area, through about 30% in the Hard Red Spring wheat area to about 55-60% in the Soft Red Winter wheat area. Companies in pureline variety seed development will tend to focus on markets that have a higher percentage of repurchased seed. Companies remaining in the Hard Red Winter wheat area are, in several instances, hoping for hybrid wheat breakthroughs to restore profitability to their wheat product line.

It is an obvious truism that, in the private sector, genetic research will flow to the more profitable markets, and the growers will be the beneficiaries. In this regard, the Soft Red Winter wheat region will probably fare better that the HRW or HRS regions. In France, the wheat acreage is roughly the same size as the U.S. SRW wheat acreage. Currently, the government program (INRA) and 15 companies (10 french / 5 multi-national) compete; on average 72 new varieties enter first year registration each year; 32 pass into second year registration each year; and 10-12 new varieties are registered. Plant variety protection laws are rigidly enforced, and there is an association responsible for royalty fee collections. This has a strong impact on the rate of genetic gain for yield and other traits.

I would not want to leave this discussion on the U.S. without addressing the changing nature of wheat research today. Budget constraints have forced state breeding programs to become more entrepreneurial, and there is less of a distinction between private and public breeding today. Several states collect royalties on their varieties, and these may be shared between the state seed association, the university, and the breeding program. In a few cases, breeders now have discretionary power on how they divide or disburse the funds between their program and the supporting services. The full impact of these changes on germplasm movement between state programs has yet to be determined. However, it could lead to a more uniform approach to the enforcement of PVP laws.

3. PLANT VARIETY PROTECTION IN EUROPE - U.P.O.V.

In Europe a variety must be protected in order to prevent its use by others, and it must be registered if it is to be sold in commerce. In general, the same standards used to determine whether it can be protected are also used to determine its uniqueness for registration. Thus, protectability has come to be equivalent to non-infringement. It follows that breeders would like to see acceptance of a narrow minimum distance between the varieties they seek to register, but a broad minimum distance when other breeders seek to register varieties. This issue was considered and resolved to a greater or lesser extent decades ago under patent law in what is termed the Doctrine of Equivalents.

When the Union for the Protection of New Varieties of Plants (UPOV) was first formed in 1961, only five countries were represented. Until 1978, membership was restricted to European countries, but today there are 20 member countries including the U.S., Canada, Australia, New Zealand, South Africa, Israel, and Japan. In Europe it has not been possible to date to patent plant varieties and this, coupled with some variation in legislation on varieties from country-to-country, has caused a certain amount of confusion. Currently, the new proposed UPOV legislation is based on the March 19, 1991, Convention, and it contains a number of improvements in PVP for breeders. It will become official once five member countries have ratified it by enacting their own legislation to conform to the changes. The first important change is that the new Convention introduces the concept of "essentially derived" varieties to protect breeders from plagiarized varieties. Any new variety developed in such a manner as to conserve the essential characteristics of the initial (or recurrent parent) variety is considered to be essentially derived. It is eligible to receive protection, but it may not be commercialized without the consent of the breeder of the original variety. As an example, this could include backcrossing genes for disease resistance into a variety, substituting the variety into a source of cytoplasmic male sterility to develop an inbred, or genes affecting quality components that are introduced through transformation into an existing variety. The details are not settled, but clearly germplasm source, breeding intent, and method and genetic minimum distance will all play a part.

The second change is that the <u>duration</u> of <u>protection</u> is now extended from 18 to 20 years. Thus, the principle of "essentially derived" will be in effect for the 20 year duration of PVP on the original variety.

The third change is that it is now possible for a breeder to exercise his right, in special cases, over the <u>harvested material</u> from a crop planted to a protected variety using unauthorized seed. The law applies only when the breeder cannot exercise his first right over the seed. Thus, if a variety is grown in a country with no PVP, it is now possible to prevent the grain being exported into a UPOV member country. Farm-saved seed is excluded from this provision. This change will help the cut flower industry in particular.

Fourthly, the convention clearly defines <u>farm-saved seed</u>. A farmer's use of saved seed is now limited to his own holdings. This is similar to the O'Brien ruling in favor of Asgrow (Oct. 1991) but goes a step further; no farm-saved seed may be sold to others. The convention accommodated the ASTA amendment that a conditioner must return conditioned farm-saved seed of a protected variety to the grower from whom he originally received it.

Fifthly, the new convention excludes the earlier provision that limited the forms of protection available to varieties in UPOV member countries. Thus, there is now more flexibility, and each country can choose what limitations there should be in the forms of protection.

It now remains for the U.S. to enact enabling legislation so that our PVP Act conforms with the 1991 UPOV Convention. The USDA will shoulder responsibility for this with help from ASTA. If the necessary legislation is not put through, the U.S. can remain a UPOV member by adhering to the 1978 Convention.

In Europe the percentage of certified (R1) wheat seed use each year ranges from lows of 20-30% in Spain and Greece to highs of over 60% in the U.K. and Germany. France, the largest seed market has 54% certified seed use. The farmers' privilege remains a hotly debated issue in Europe. Both the UPOV Convention and the Community Plant Variety Rights (CPVR) proposal, currently before the European parliament, allows the farmers' privilege for saved seed, but the precise conditions are left to the member countries. It is up to them to enact legislation to minimize abuse of the system. In the fall of 1990, the European Community proposed new regulations on plant variety rights that go beyond marketed seed to include all replication. They have seen the damaging results of farm-saved seed on the U.S. plant breeding industry and have resolved to not let this happen in Europe.

In the U.K. National Farmers Union members met with the British Society of Plant Breeders. Cereal seed is divided into three classes: 1) certified seed; 2) processed non-certified seed (PNCS) or what we term "custom-cleaned"; and 3) farm-saved seed. A compromise scheme was worked out whereby normal royalties are paid on certified seed, and a royalty equal to 53% of the second generation certified rate would be paid on PNCS or custom-cleaned seed. This would raise a further \$3.5 million in royalties. Farmer groups in France have also responded favorably to a sliding scale of royalties. In general, there is a recognition that both growers and seed companies need to survive.

In Austria a wheat grower who registers to grow the quality class of wheat (as opposed to feed wheat) is required to produce documentary evidence of seed purchase in order to market his crop. Thus, there is 100% use of certified seed in the quality wheat class. Spain is currently considering similar legislation for the durum wheat class.

In Europe there is much variation from country to country but, in general, it would be true to say that PVP has been more effective than in the U.S., royalties are collected in a more efficient manner, and there is a greater willingness to accept legislation within the seed industry and among growers. The issue of utility patents has yet to be resolved, and a proposal for the protection of biotechnology inventions is still before the European parliament.

European plant breeding occurs predominantly within the private sector. The privatization of the Plant Breeding Institute at Cambridge, England, in 1987 was the final major transfer in Western Europe. Today, in Central Europe, a number of research institutes are in various stages of privatization as economies change from a centralized to an open market system. How has germplasm exchange fared under private plant breeding? In general, quite well! The breeder exemption has been operative under PVP, and many "strategic alliances" are formed that usually involve germplasm exchange and reciprocal testing of varieties between companies. The assignment of marketing rights to others on a royalty fee basis usually ensures maximum leverage on varieties that can move to countries where the originating company is not strongly represented.

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4. THE IMPACT OF PVP AND GERMPLASM PROTECTION ON VARIETAL DEVELOPMENT IN DEVELOPING COUNTRIES

One challenge that has persisted over the years is to communicate the advantages of PVP to developing countries. As of 1986, only Argentina, Chile, Kenya, the Republic of Korea, and Zimbabwe had adopted a form of variety protection. The first point to make is that PVP attracts investment in plant breeding research; secondly, PVP fosters sound research by permitting companies to take a long-range view of developing new products, rather than extracting quick profits from marginally advantageous varieties; thirdly, it provides reciprocal protection for local companies seeking to market their products abroad; and fourthly, PVP permits access to elite pools of proprietary germplasm that are used elsewhere in the world.

In countries that have no PVP legislation, it is typical for multi-national companies to market hybrid seed that is produced offshore in a protected market. Where local production is mandatory, public inbreds are used in the hybrids, and three and four-way hybrids are common. Elite hybrids, where both inbreds are proprietary, are simply not sold. With self-pollinated crops like wheat, elite purebred varieties are also not sold, and marketing is limited to public domain varieties and research overheads are kept to a minimum. We have been willing to go one step further in Pioneer by marketing proprietary varieties that have been in commercial production for a period of time and could, therefore, be obtained from on-farm storage in a country where PVP exists. However, as these examples illustrate in countries where no PVP exists, there is little incentive to develop proprietary material locally. In contrast, in Zimbabwe where PVP legislation exists, we are developing proprietary hybrids of corn, sorghum, and sunflower locally.

In developing countries without PVP, CIMMYT has played a major role in distributing wheat germplasm via their international nurseries program. Over the years, a range of material has been supplied ranging from F2 segregating populations to near-finished lines in screening and yield nurseries. However, experience has shown that, in general, the segregating populations have not been managed effectively due to limited local plant breeding expertise, and the greatest success has come from identifying and increasing finished lines. It was, therefore, decided to discontinue sending F2 nurseries. The general tendency has, therefore, been to rely upon material broadly adapted to a mega-

environment, and forgo the added genetic gains that could potentially be made through a local selection program.

It would be true to say that over the last decade, we have seen dramatic increases in germplasm control, and an intensification of the international debate. However, at this time, it is important that we view matters in perspective. Pitted against each other in this debate are the so called "genepoor" developed countries that depend on the diversity found in the world's germplasm for breeding elite hybrids and varieties of crops, and the "gene-rich," primarily underdeveloped countries, home to the vast majority of the remaining genetic base for most of the world's major crops.

The 1983 meeting of the Food and Agriculture Organization of the United Nations (FAO) was an acrimonious event that brought the issue of germplasm control to the world's attention. Several developing nations led 77 countries to sign an "international undertaking" declaring that, in principle, plant genetic resources, even advanced on elite lines, should be considered a common heritage and shared among all countries of the world. Thirteen countries, including the U.S., found the position wholly unacceptable, and a statement has occurred on the issue. The seed industry's position was summed up by the late Dr. William Brown, then chairman emeritus of Pioneer Hi-Bred International, when he stated that, "To ask that an elite strain costing companies hundred of thousands of dollars be exchanged with a primitive cultivar is simply not reasonable."

It is interesting to note that, at the time this debate surfaced, of the \$32 billion spent worldwide on buying seeds in 1984, U.S. companies sold only \$328 million abroad, and of this only 12% went to all the developing countries. The key issue has been cost; many developing countries simply cannot afford improved seed. Even seed sold for a minimal profit can prove prohibitive, and countries find that they are better off growing what they have. Some had suggested that multi-national seed companies take seeds from developing countries, make a few genetic "improvements" in them, patent them, and then turn around and sell them at vastly inflated prices back to the countries where they got them. In reality, profits are minimal and a long-term perspective is required. Those who have been involved in the laborious process of alien gene transfer from wild species are well aware of the time and expense and backcrossing work required to enhance the germplasm. The further steps in then moving it to a marketable product are also significant and the costs substantial. For this reason, using wild species is not a common practice. However, most of this work has been done by public institutions in developed countries, and the germplasm has been stored in collections that are accessible to all.

The International Board for Plant Genetic Resources (IBPGR) was created to act as a catalyst in the collection, preservation, and free exchange of germplasm throughout the world. It has a network of over 600 scientists working in more than 100 countries and includes 177 base collections of germplasm in 43 gene banks (22 in developed countries and 21 in developing countries). The CGIAR centers, such as CIMMYT in Mexico and IRRI in the Philippines, are included on this roster and play a significant role.

As the perceived value of genetic resources increases, the question of how to resolve the international impasse over who owns genetic resources is clearly not going to go away. The U.S. Board of Appeals' decision in 1985, stating that plants are patentable material, paved the way for commercializing agricultural biotechnology. This was considered essential in order to protect innovation and insure continuing investment.

Human and animal genetics have led the way in the patenting of specific genes. The NIH has a current project to sequence the entire human genome. Techniques for the rapid sequencing of genetic material have been refined to the point where currently about 2,000 genes per month are being sequenced. Patents are being filed prior to the utility of these genes being established. This has led to concerns being expressed that this precedent could be very damaging. For example, a gene present in a line obtained from an international wheat collection could be sequenced, patented, and become the intellectual property of the patent holder. It has raised the question, "Could genes fundamental to a nation's ability to feed itself be at risk?" The answer is yes, in theory. However, the patents filed by the NIH have not issued, to date, and in the case of a wheat gene in use in a developing country, patent enforcement would be extremely difficult and politically disastrous. Nevertheless, the NIH action has led to recent meetings involving the NIH, the NSF, the Department of Commerce, the State Department, and industry representatives. Our company's position is that it is a bad idea, since it does not involve a new invention or utility. Japan currently has a project to sequence the rice genome. The consequences could be far reaching. A number of people now feel that an international agreement is needed that defines some limits to patenting and the restriction of germplasm use. However, this could take time, and the possibility remains that patents could issue during the interim.

5. SUMMARY

The above synopsis of international regulations, and trends towards varietal protection, probably serves to demonstrate how complex life has become! There are probably many plant breeders who look back on the "good old days" when germplasm exchange was simple, funding was adequate, and good science could be pursued in an atmosphere of collaboration. However, there are probably a number of us who look forward with anticipation, recognizing that despite the complexities, the challenges of the future will bring out the best in us. There will be a better focus and, ultimately, a better return on our research investment.

These times of budgetary constraints will probably bring private and public plant breeding closer together than ever before. Research partnerships, or strategic alliances, will be needed to move our science forward and solve specific problems. The issues such as "essentially derived," "minimum distance," the protection of intellectual property rights, and access to germplasm, will constantly challenge us.

We will need to effectively communicate our position on PVP to developing countries, and support for international germplasm networks such as the IBPGR will be critical. We need to also recognize the diversity of opinion that exists among developing countries on plant variety protection. Some feel quite comfortable with the concept and have come to recognize the benefits that it has brought to their own seed industries. These countries will ultimately be the most effective advocates for PVP among their peers. The development of a new variety is the consequence of good science and significant investment. The magnitude of this investment is often poorly understood in the U.S. and other developed countries. Self-pollinated crops, such as wheat, will have an on-going challenge to protect intellectual property and recover investment. However, recent changes in the PVP laws give some cause for optimism.

STATUS OF GERMPLASM EVALUATIONS, GRIN, AND NATIONAL SMALL GRAINS COLLECTION

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The USDA-ARS National Small Grains Collection (NSGC) is one of the several components of the National Plant Germplasm System. The NSGC is a working collection in contrast to the base collection at the National Seed Storage Laboratory (NSSL) at Fort Collins, Colorado. The NSSL is the only long-term seed storage facility in the United States. The numbers of accessions in the NSGC and other small grains working collections in the world are summarized below:

SMALL GRAINS GERMPLASM WORKING COLLECTIONS Significant Collections (> 200 Accessions)*

Taxonomy	No. Collections	Total Accessions	NSGC Accessions+
Triticum	37	401,500	43,505
Hordeum	51	280,300	26,295
Avena	22	37,000	22,545
Oryza	29	212,000	· 16,131
All Species			113,769
* Holden, 1 + Aberdeen			

The systematic evaluation of accessions in the NSGC and other elite germplasm continued to be coordinated or conducted by National Small Grains Germplasm Research Facility (NSGGRF) staff at Aberdeen during 1991. Cooperative evaluations continued for reaction to Russian Wheat Aphid; Hessian fly; barley yellow dwarf virus; barley stripe mosaic virus; spot and net blotch of barley; stripe, leaf, and stem rust of wheat; crown rust of oats; dwarf bunt; beta-glucan, protein, and oil content of oats; beta-glucan and protein content of barley; and ploidy analysis of *Triticum* species. Recently initiated cooperative evaluations included testing of over 12,000 NSGC barley accessions and other elite barley germplasm for reaction to stem rust race QCC in North Dakota,

Puerto Rico, and Minnesota and testing of over 8,000 NSGC barley accessions and other elite germplasm for reaction to barley stripe rust race 24 in Bolivia under the direction of Colorado State University staff. The Aberdeen staff has been directly involved in the entry of NSGC evaluation data into the GRIN system; the evaluation of growth habit of 15,000 NSGC wheat accessions; the maintenance, evaluation, and distribution of oat germplasm donated by the Coker Pedigreed Seed Company; and taxonomic classification of NSGC oat and barley accessions.

Under the direction of H.E. Bockelman, the NSGC staff distributed over 170,000 accessions in 1991. Maintenance and evaluation of NSGC small grains germplasm, including quarantine entries, also continued at Maricopa, Arizona in 1991 under the supervision of S. Nieto. In dwarf bunt screening trials conducted in 1990-91, B.J. Goates identified 50 *T. aestivum* and 27 *T. durum* lines from Turkey with high resistance.

The increase and cooperative evaluation of a wheat germplasm collection derived from a series of interspecific crosses completed by W.J. Sando in the 1930s and previously last grown in the 1960s, continued in 1991. Location funds were also used in 1991 to partially support the evaluation of Pioneer Seed Company developed hard red winter wheat germplasm at Manhattan, Kansas. Specific Cooperative Agreements or within ARS Fund Transfers involving such cooperative evaluations and related research for all small grains typically now involve over 20 University and ARS projects in at least 16 states. Descriptors appropriate for each of the principal small grain crop species - wheat, barley, oats. and rice - have been established in collaboration with the appropriate Crop Advisory Committees. Field evaluation data are recorded on such descriptors as growth habit, number of days from planting to anthesis (heading), plant height, spike or panicle density, lodging, straw breakage, shattering, and awn and glume characteristics, including color. Spikes or panicles are collected from each evaluation or nursery plot at maturity to facilitate detailed laboratory analysis for seed characters and for more precise spike or panicle descriptors than can be obtained under field conditions. Yield data are also recorded for each accession. Data on field descriptors have been obtained on approximately 35,500 wheat accessions, 11,000 oat accessions, and 9,000 barley accessions during the 1983-91 period. Special nurseries were grown for that purpose at Aberdeen, Idaho and Maricopa, Arizona, with grain being harvested from each field evaluation nursery to replenish NSGC seed stocks.

Evaluations for disease and insect resistance were initiated in 1983 along with the agronomic evaluations. Accessions of *Triticum* submitted for formal NSGC disease, insect, and other evaluations to date include the following:

NSGC DISEASE EVALUATIONS - WHEAT

an garan

Barley Yellow Dwarf Virus	1983-91	Davis, CA	27,300*
	1983-91	Urbana, IL	33,000+
Soilborne Mosaic Virus	1985-89	Urbana, IL	10,000
Leaf Rust	1983-89, 91	Manhattan, KS	34,900#
Stripe Rust	1984-91	Pullman, WA	25,575+
Stem Rust	1987-91	St. Paul, MN	19,692
Common Bunt	1985-86	Pendleton, OR	5,000
Dwarf Bunt	1990-91	Aberdeen, ID	1,570
Karnal Bunt	1988-90	Ludhiana, India	1,522

* Plus Iranian Wheat Collection, Triticale, and Sando Series.

+ Plus Sando Series.

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Plus Pioneer Germplasm.

NSGC INSECT EVALUATIONS - WHEAT

1983-91 1990-91	West Lafayette, IN Stillwater, OK	30,605 10,803*
8 TAYONOM		AT
		AI
	1990-91	1990-91 Stillwater, OK & TAXONOMIC EVALUATIONS - WHE

1987-91	Aberdeen, ID	31,595
1988-91	Columbia, MO	625
1983-91	Aberdeen, ID	*
1983-91	Maricopa, AZ	*
	1988-91 1983-91	1988-91Columbia, MO1983-91Aberdeen, ID

* Data entered in GRIN for 14 characters, involving from 8,553 to 19,044 accessions each.

The Germplasm Resources Information Network (GRIN) is a database containing the characteristics and availability of all genetic resources included in the National Plant Germplasm System. The Database Manager is J.D. Mowder, Beltsville, Maryland. Data obtained from evaluations of NSGC germplasm are entered in the GRIN system by the NSGGRF staff in cooperation with the ARS Germplasm Services Laboratory, Beltsville, Maryland. The NSGGRF staff interacts with the GRIN system in recording NSGC orders (seed requests), entering a variety of data, and conducting information searches. Data for systematic evaluations for a number of descriptors, not currently available on GRIN, are being prepared for entry into the system. No evaluations have been conducted to date for descriptors such as drought tolerance, salt tolerance, winterhardiness, Cephalosporium stripe, flag smut, leaf blight, loose smut, powdery mildew, snow mold, take all, tan spot, wheat streak mosaic, green bug, cereal leaf beetle, and protein. Data currently available on GRIN for wheat includes the following:

NATIONAL SMALL GRAINS COLLECTION DISEASE EVALUATION DATA ON GRIN - WHEAT

Barley Yellow Dwarf Virus	Davis, CA	2,012
	Urbana, IL	4,199
Soilborne Mosaic Virus	Urbana, IL	6,589
Leaf Rust	Manhattan, KS	24,446
Stem Rust	St. Paul, MN	10,000
Common Bunt	Pendleton, OR	12,900
Dwarf Bunt	Logan, UT/Aberdeen, ID	6,400
Septoria	Bozeman, MT	8,095

NATIONAL SMALL GRAINS COLLECTION INSECT EVALUATION DATA ON GRIN - WHEAT

Hessian Fly (Biotype-B)	West Lafayette, IN	448
Hessian Fly (Biotype-C)	West Lafayette, IN	24,226
Hessian Fly (Biotype-E)	West Lafayette, IN	24,409
Russian Wheat Aphid	Stillwater, OK	9,214

NATIONAL SMALL GRAINS COLLECTION AGRONOMIC & TAXONOMIC EVALUATION DATA ON GRIN - WHEAT

Growth HabitAberdeen, ID31,434Ploidy AnalysisColumbia, MO520Agronomic DescriptorsAberdeen, ID*Agronomic DescriptorsMaricopa, AZ*

* Data entered in GRIN for 14 characters, involving from 8,553 to 19,044 accessions each.

Similar evaluations not reported in detail here are currently underway for other major NSGC components, including barley, oats, rice, and triticale. Other important cooperative projects include the "Conservation of North American Genetic Resources of Triticale" (University of California, Davis - C.O. Qualset); "Recalcitrance in Wheat Protoplast Regeneration: Genetic and Genomic Effects" (Alabama A&M University, Normal - G.C. Sharma); and "Evaluation of Yugoslav Wheat Collections for Drought" (USDA-ARS, Aberdeen - H.E. Bockelman). Related research concerned with wheat germplasm is conducted at Aberdeen under the CRIS project entitled "Molecular Biology of Cereal Genome and Improvement of Stress Tolerance in Wheat Germplasm" under the direction of S. Ramagopal. B.J. Goates conducts evaluations of wheat germplasm for bunt resistance at Aberdeen, Idaho and Logan, Utah. Additional CRIS projects concerned with small grains germplasm at Aberdeen include "Evaluation and Enhancement of Oat Accessions in the NSGC (D.L. Hoffman); "Coordination and Conduct of National Oat Germplasm Enhancement (D.M. Wesenberg); and "Coordination and Conduct of Small Grains Germplasm Evaluation & Enhancement" (primarily barley under the direction of P. Bregitzer, A. Hang, and D.M. Wesenberg).

* The authors wish to acknowledge the important contributions of the NSGGRF staff in this effort, with special thanks to Glenda B. Rutger, A. Lee Urie, John F. Connett, Kathy E. Burrup, Dave E. Burrup, Kay B. Calzada, Vicki Gamble, Evalyne McLean, Judy Bradley, Carol S. Truman, M.A. Bohning, and L.W. Briggle.

USDA/ARS REGIONAL WHEAT QUALITY TESTING LABORATORY: HARD WINTER WHEAT QUALITY LABORATORY (HWWQL)

Okkyung Kim Chung and George Lookhart Research Leader and Lead Scientist Grain Quality and Structure Research Unit (HWWQL) U.S. Grain Marketing Research Laboratory Manhattan, KS

The HWWQL, one of the four USDA/ARS Regional Wheat Quality Testing Laboratories, was first established in 1937 by Congress to work with wheat breeders of the Great Plains to determine the breadmaking qualities of hard winter wheat varieties for release. The lab was located at the Kansas Agricultural Experiment Station, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas. In 1972, the HWWQL became part of the U.S. Grain Marketing Research Laboratory. The HWWQL evaluates intrinsic quality parameters of breeders' lines from the regional nurseries (Southern, Northern, and Western Plains Regional Performance Nurseries: SRPN, NRPN, and WPRPN), state and private nurseries, and Wheat Council, etc. Evaluations include physical and chemical characteristics of wheats and their milled flours. milling properties, dough and gluten characteristics and bread characteristics. For the earlier generation samples, evaluation is limited to micromilling and dough properties, proximate analyses, and kernel hardness. We are proposing to include about 600 samples for complete testing (both milling and baking): 100 lines from the regional nurseries; 200-250 samples from the Great Plains state nurseries (approximately 30-40 samples/year from each of CO, KS, NE, OK, TX, SD, etc.), 100 samples from other state and private nurseries; and 150-200 samples for research purposes. Micromilling and mixograph evaluation for early generation research will be limited to about 200-300 samples, and for the G x E studies to 400 samples from the SRPN entries at multiple locations. The HWWQL also proposes to offer check sample services on experimental milling. NIR, mixograph, and/or straight-dough pup loaf breadmaking to the various state hard winter wheat testing laboratories. This service will be provided yearly by supplying 3 to 4 wheat samples and/or flours and will help each participating lab keep a check on their methodology.

ANALYSIS OF WHEAT SEED STORAGE PROTEIN GENE PROMOTERS IN A TRANSIENT ASSAY

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We are utilizing a suspension cell line derived from developing maize endosperm as a transient assay system for promoter function of genes expressed in cereal endosperm tissue. Protoplasts derived from these cells are electroporated in the presence of plasmids containing hybrid genes consisting of test promoters transcriptionally fused to either the Chloramphenicol Acetyltransferase (CAT) or luciferase coding sequences. After systematic optimization of each step of the assay protocol, levels of CAT or luciferase expression driven by maize ADHI promoter and first intron are 1000x those of promoterless control plasmids. We have tested the relative strength of several wheat seed storage protein gene promoters in this system. Using luciferase as the reporter, an α -gliadin gene region extending from 2800 to 45 bp upstream of the start codon supported expression levels about 2x background. Regions from the High Molecular Weight (HMW) Glutenin Subunit genes Glu-1Dx5 and Glu-1Dy10 extending from 434 to 5 bp and 450 to 24 bp relative to their start codons, respectively, were assayed using the CAT reporter. These promoters support equal levels of expression, 30-40 times a promoterless CAT control. Constructs including 2800 and 1400 bp of Dy10 5' flanking regions are no more active than the -450 construct. Small deletions from the 5' end of the -434 Dx5 promoter significantly lower its expression. The precise sequences missing in these deletions will be presented. The results of these analyses will define the cis-acting elements necessary for expression of the HMW Glutenin genes in this maize endosperm-derived suspension cell line.

VARIETAL AND ENVIRONMENTAL EFFECTS ON PHENOTYPIC STABILITY IN HARDNESS OF HARD RED WINTER WHEAT PROGENIES

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Wheat hardness score (HS) was measured by near-infrared reflectance spectroscopy (NIR) in 3,282 hard red winter wheat progenies grown in 1987-1989 from the Northern and Southern Regional Performance Nurseries (NRPN & SRPN) representing 24-45 genotypes from 9-19 locations across thirteen states. Phenotypic stability (PS), a nonparametric statistic, expressed as the mean of the absolute rank differences of a genotypes' HS over the N environments, was calculated for each progeny within each nursery for each growing season. PS values of the most stable progenies in the NRPN were 5.1 (1988), 5.6 (1989), and 7.5 (1987); the least stable progenies were 15.7 (1987), 11.7 (1988), and 11.1 (1989). PS values of the most stable progenies in the SRPN were 9.7 (1987), 10.3 (1988), and 10.9 (1989); the least stable progenies were 20.1 (1987), 19.8 (1988), and 18.8 (1989). Step-wise linear regression of HS versus temperature and moisture data from each growing season indicated a temperature x moisture interaction for wheat hardness. HS for a given variety tended to be harder when grown in Idaho, Montana, and Wyoming, and softer when grown in Oklahoma, Texas, and Kansas.

CHROMOSOME SPECIFIC MARKERS IN GENETIC STUDIES OF DISEASES RESISTANCE IN WHEAT

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A first step in understanding and utilizing sources of resistance to disease requires knowledge of the chromosome location of different sources of resistance. Resistance of wheat to viruses, loose smut and bunt are difficult to determine on single plant tests, making analysis of F_2 progeny from crosses to a monosomic series unmanageable. A simpler genetic test involves test crosses of resistant plants to nullisomic or alien chromosome substitutions in conjunction with chromosome specific markers to identify which progeny have the resistant parent chromosome.

The test cross method can be applied where there is a Monoclonal antibody (MAb) that can distinguish monosomic from euploid kernels. A disease resistant euploid line can be crossed to a susceptible monosomic or nullisomic plant and monosomic F_1 plants test crossed with pollen from a susceptible plant.

Where chromosome specific MAbs or different alleles of chromosome specific proteins are not available, alien chromosome substitutions and translocations can be used. Selfed F_2 seeds from a cross involving the 6AgS:6DL translocation (conferring leaf curl mite resistance), can be sorted into kernels homozygous for the translocation (absent 6DS gliadin) and those homozygous or heterozygous for the 6DS chromosome arm.

Kernel endosperm proteins coded by specific chromosomes are very convenient chromosome specific markers. Gliadins and glutenins as markers for group 1 and group 6 chromosome have been well characterized. We have studied markers for wheat chromosomes using monoclonal antibodies to α -amylase inhibitor (group 2) exogenous inhibitors (group 3), purple endosperm (group 4), albumins (group 5) and non-gliadin 70% ethanol soluble proteins (group 7). The main advantages with these markers are that only a small number of progeny families (<20) must be evaluated, the assays are rapid non-destructive and inexpensive.

IMMUNOELECTRON MICROSCOPY OF VIRUSES INFECTING WHEAT

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In spite of decades of research little is known of how viruses initiate infection in plant cells. One of the many ways by which the virus infection process can be studied is by visualization of virus and virus-directed proteins inside cells by conventional electron microscopy. The virions, and sometimes some of their virus-directed proteins, can be seen when one is dealing with a stable virus. Many viruses have not been seen by electron microscopy inside cells although it is known that they exist and must be there. It took approximately 30 years to develop better techniques and better electron microscopes. With the new techniques it is now possible to trace the exact intracellular location of virions or some of the proteins they code for by immunoelectron microscopy (a combination of antibody reactions and electron microscopy). Interactions between different viruses infecting the same cell can also be studied. It is expected that results of these studies will lead to the design of new strategies for virus disease control in wheat and other crop plants. Examples of conventional and immunoelectron microscopy will be displayed of the following viruses: wheat streak mosaic virus, wheat spindle streak mosaic virus, soil-borne wheat mosaic virus, hordeum mosaic virus, agropyron mosaic virus and barley stripe mosaic virus.

PURIFICATION OF THE HIGH MOLECULAR WEIGHT GLUTENIN SUBUNITS OF WHEAT

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The high molecular weight glutenin subunits (HMW-GS) of the varieties Chevenne and Chinese Spring were extracted and purified via two different methods. In the first method (Khelifi and Branlard, 1991), the HMW-GS were purified through the use of an acid PAGE system followed by an SDS-PAGE gel. The collected HMW fractions had reduced background proteins, but were not completely pure. The second method involved a DMSO extraction of flour followed by reduction and alkylation of the proteins (Burnouf and Bietz, 1989). The subunits were collected from an SDS-PAGE gel. The HMW-GS collected from this procedure were also shown to have fewer background proteins, and were also not completely pure. When these subunits were run on RP-HPLC, they eluted at approximately 50% acetonitrile indicating that they were much more hydrophobic than previously reported (Wieser and Belitz, 1990). The peaks were collected and run on mini SDS-PAGE gels and silver stained. The collected peaks contained only the HMW-GS purified. Our data show that each HMW-GS remains unchanged during purification by the initial SDS-PAGE gel, electroelution, separation on RP-HPLC, collection and re-analysis on SDS-PAGE.

RYE-WHEAT TRANSLOCATIONS TO DOUBLE THE DOSAGE OF GLU-D1 GENE IN WHEAT

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It has been demonstrated recently in nulli-tetrasomics of 'Chinese Spring' wheat that doubling the dosage of chromosome 1D at the expense of chromosome 1A significantly improves bread-making quality. Nulli-tetrasomics cannot be used in commercial wheat to increase the dosage of glutenin genes because of their inherent meiotic instability. However, doubling of the glutenin genes can be accomplished by a translocation. Wheats with a pair of translocated chromosomes and a pair of normal 1D chromosomes would have four doses of *Glu-D1* gene. The translocated segment of 1D with the *Glu-D1* gene would need to be small to prevent any possibility of meiotic pairing with the normal chromosome 1D, and the translocated chromosome would need to replace chromosome 1A.

Two translocations which meet these requirements were produced in hexaploid triticale. Chromosome 1D from cv. 'Wheaton' was allowed to misdivide in the presence of rye chromosome 1R and a 1RS.1DL translocation was recovered. Homoeologous recombination between 1DL in the translocation and the long arm of normal 1R was induced by the removal of chromosome 5B. After the first round of recombination, chromosome designated $1R.1D_{5+10}$ -1 was obtained. It has a normal short arm of chromosome 1R, a proximal segment of chromosome 1D, and a distal segment of chromosome 1R. Following the second round of recombination, chromosome designated $1R.1D_{5+10}$ -2 was identified. Cytologically it appears as normal rye chromosome 1R. The exact length of the 1DL segment in this chromosome is not known but its maximum length cannot exceed 16.5% of 1DL.

Both chromosomes carry the Glu-D1 gene from Wheaton encoding for the HMW glutenin subunits 5+10. Because pairing initiation in wheat is telomeric and the inserts are in interstitial positions, they would not be expected to pair with complete chromosome 1D under normal conditions.

Both chromosomes can be used in wheat, triticale and rye breeding. In wheat, as a substitution for chromosome 1A the $1R.1D_{5+10}$ -1 or $1R.1D_{5+10}$ -2 chromosomes would perhaps combine the benefits of increased dosage of *Glu-D1* with the heterotic effect associated with the presence of 1RS. The extra dosage of *Glu-D1* may compensate for the detrimental effects of 1RS on quality. Chromosome $1R.1D_{5+10}$ -1 would appear more suitable for wheat because the

length of the 1DL segment is longer and consequently, less rye chromatin would be introduced. Either of the translocated chromosomes would allow to develop stable combinations of different HMW glutenin subunits (5+10 with 2+12, 5+10 with others).

In triticale either of the chromosomes replacing normal chromosome 1R would result in a load of *Glu* genes similar to that of breadwheats with 1RS translocations. Both chromosomes are being transferred to diploid rye. It is likely, however, that only the chromosome $1R.1D_{5+10}$ -2 has a chance of being incorporated into the rye genome because the amount of wheat chromatin in it is less and it should be better tolerated than chromosome $1R.1D_{5+10}$ -1. It is not clear at the moment what effects, if any, *Glu-D1* may have on breadmaking properties of rye.

EFFECT OF THE 1B/1R TRANSLOCATION ON AGRONOMIC PERFORMANCE OF HARD RED WINTER WHEAT IN NEBRASKA

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The 1BL/1RS wheat-rye translocation has been shown, using cultivar comparisons, to enhance agronomic performance in wheat. The performance advantage has been attributed either to disease resistance genes or genes contributing to improved adaptation on the 1RS segment. The potential advantage of 1RS in hard red winter wheat was evaluated using 59 randomly F.derived F, and F, lines from the cross Siouxland x Ram, thus minimizing the confounding effect of background. The lines were tested in seven environments in Nebraska using a randomized complete block design with three replications. Yield, components of yield, test weight, plant height, anthesis date, and leaf rust severity were measured. Significant differences among chromosome types (1BL/1RS, 1B, or heterogeneous-mixture of 1BL/1RS and 1B genotypes) were found for yield, seed spike¹, seed weight, plant height, test weight, and leaf rust. On average, lines with 1BL/1RS (2099 kg ha⁻¹) were significantly higher yielding than the 1B (1924 kg ha1) and the heterogeneous lines (1942 kg ha1). Chromosome type by environment interaction was significant for yield, seed weight, and tiller m⁻² and was caused by changes in magnitude. The 1BL/1RS lines were equal to or higher yielding than the 1B lines in every environment. The average yield advantage of the 1BL/1RS was 9% above 1B lines which was partially explained by improved average seed weight (3%) of 1BL/1RS lines. The other yield components varied with the environment and in specific environments explained the yield advantage. Leaf rust was not correlated with vield. not present in every environment, and did not explain the 1BL/1RS advantage.

A COMPARISON OF METHODS TO ACCOUNT FOR SPATIAL VARIATION IN WHEAT YIELD TRIALS

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<u>Abstract.</u> Accurate differentiation among cultivars is critical to plant breeding programs. Field trials generally use some form of blocked experimental design, e.g. a randomized complete block design (RCB). Standard analysis of such designs depends on the assumption that variability among plots within a block is small relative to variability between blocks (see Figure 1). Failure of this assumption results in biased estimates of cultivar means and inflated estimate of error variance.

Large sets of plots whose behavior conforms the RCB assumption are uncommon in nature; hence it is rarely satisfied in large agronomic field trials (e.g. greater than 8-12 treatments or cultivars). *Spatial variability*, depicted in Figure 2, in far more common; its existence and its effects on data analysis have been widely discussed (Jensen and Federer, 1964; Kempton, 1981; Kempton and Lockwood, 1984; Pearce, 1978, 1980; Fowler, 1979), yet blocked designs and their analysis continue to be standard practice in many field trials in the United States.

Nearest neighbor adjustment (NNA, Wilkinson, et. al., 1983) is a relatively simple, yet effective method of accounting for spatial variablity in field trials. Two methods of implementing NNA analysis are compared with RCB analysis to assess NNA's potential benefit in identifying superior cultivars.

MATERIALS AND METHODS

Data from three winter wheat breeding nurseries - Nebraska Intrastate (NIN), Nebraska Triplicate (NTN), and Uniform Hard Red Winter Wheat Southern Regional Performance (SRPN) - grown in 1988-89 at four locations in Nebraska - Lincoln, Clay Center, North Platte, and Alliance - were used in this study. These nurseries were chosen to represent diverse, elite germplasm that would be evaluated in a breeding program.

NTN, a preliminary yield trial, had 58 experimental lines and 8 check cultivars. NIN, an advanced trial, had 19 released cultivars, 35 experimental lines, and 2 triticale experimental lines. SRPN, an elite regional nursery, had 3 check cultivars, 36 experimental lines, and 6 experimental hybrids.

All nurseries were planted using randomized complete block designs with three (NTN, SRPN) or 4 (NIN) replications. A complete block was not planted linearly, but was broken into groups of 20-30 plots and planted in consecutive tiers in the field. Plots consisted of 4 rows (0.3 m between rows), each 3 m long. Recommended seeding rates and cultural practices were used.

Data were analyzed using three procedures. The first is the standard analysis for a randomized complete block design. The second and third were NNA analyses using two difference approaches (explained below).

Nearest Neighbor Adjusted (NNA) Analysis -- Basic Idea Observations arranged spatially as follows:

		e _{i-1 j}	
Latitude	e _{i,ŀ1}	У _{іјк}	e _{i,j+1}
		e _{i+1,j}	

Longitude

where y_{ijk} is the observation at the ith latitude and jth longitude, and kth entry. $e_{i+1,j}$ denotes the residual at the (i-1)st latitude and jth longitude $e_{i+1,j} = y_{i+1,j} - y_{i+1,j}$, where $y_{i+1,j}$ is the mean of the entry on the (i-1,j)th plot.

Other residuals (eii+1, etc.) defined analogously

Nearest Neighbor Adjustments calculated from residuals

e.g. East-West NNA $EW_{ij} = \frac{1}{2} (e_{i,j+1} + e_{i,j+1})$ North-South NNA $NS_{ij} = \frac{1}{2} (e_{i+1,j} + e_{i+1,j})$

NEAREST NEIGHBOR ADJUSTED ANALYSIS I Adjustment Using Adjacent Residuals Implemented Using SAS Program

Procedure Hereafter Referred to as "NNA-SAS"

1. Define NNA terms

 $EW_{ij} = \frac{1}{2}(e_{i,j+1} + e_{i,j+1}) \qquad NS_{ij} = \frac{1}{2}(e_{i+1,j} + e_{i+1,j})$

 $\{ \gamma_{i}, \gamma_{i} \} \in \mathbb{R}^{d}$

2. Estimate parameters of model $y_{ijk} = \mu_k + \beta_1 EW_{ij} + \beta_2 NS_{ij} + \epsilon_{ijk}$

where μ_k is entry mean, B_1 and B_2 are NNA parameters, and ϵ_{ijk} is residual

- 3. Compute adjusted entry means, μ_k NOTE: procedure is essentially analysis of covariance with NNA terms, EW_{ij} and NS_{ij}, as covariates. May use EW covariate only, NS covariate only, or both, depending on pattern of spatial variability.
- 4. ITERATIVE PROCEDURE: Wilkinson, et. al. (1983) suggestion: e_{ij} 's from raw data may be imprecise. Solution: recompute e_{ij} using adjusted mean (i.e. $y_{ijk} \mu_k$), then repeat steps (2) and (3). Repeat until μ_k stabilizes.

NEAREST NEIGHBOR ADJUSTED ANALYSIS II From Schwarzbach (1984) Analysis of Field Trial (ANOFT) Procedure

Referred to hereafter as NNA-ANOFT

- 1. For each plot, compute "nearest neighbor difference": $NND_1 = y_{ii} - \frac{1}{2}(y_{i,i+1} + y_{i,i+1})$
- 2. Compute mean of NND, for each entry. Denote as NND,
- 3. Compute "expected nearest neighbor difference": $NND_2 = y_{ii} - \frac{1}{2}(y_{i,l+1} + y_{i,i+1})$
- 4. Compute mean of NND₂ for each entry. Denote as NND₄.
- 5. Compute adjusted cultivar means

 $\mu_{k} = \text{cultivar mean} + (3/4)(\text{NND}_{a} - \text{NND}_{b})$

6. Substitute appropriate μ_k 's for y_{ij} 's in step (3), recompute NND₂, and repeat steps (4) and (5). Iterate until μ_k 's stabilize.

Table 1. Coefficients of Variation from Various Analyses

NIN	Lincoln	Clay Ctr.	N. Platte	Alliance	
RCB	6.1	16.1	19.0	27.6	
NNA-SAS ew	6.0	15.4	19.2	21.6	
NNA-SAS best	5.3 (ewns)	15.4 (ew)	15.9 (ns)	15.8 (ewns)	
NNA-ANOFT	5.2	14.7	19.2	14.7	
NTN	Lincoin	Clay Ctr.	N. Platte	Alliance	ļ
RCB	9.0	17.7	19.1	16.0	•
NNA-SAS ew	7.9	17.0	17.3	16.4	
NNA-SAS best	6.9 (ewns)	14.2 (ewns)	17.5 (ew)	13.4 (ewns)	
NNA-ANOFT	7.2	13.8	17.6	13.9	l
SRPN	Lincoln	Clay Ctr.	N. Platto	Alliance	2
RCB	6.4	23.4	21.8	17.0	
NNA-SAS ew	6.3	23.7	22.4	15.3	
NNA-SAS best	5.8 (ewns)	20.0 (ns)	21.5 (ewns)	13.2 (ewns)	
NNA-ANOFT	6.0	17.5	22.8	12.6	

Table 4. Effect of various analyses on entry means ranks for selected trials.

N. Platte	RCB	SAS	ANOFT	Alliance	RCB	SAS	ANOFT
NE87643	1	3	2	NE86503	1	12	12
NE87612	2	1	1	NE87619	2	4	5
Bruie	3	2	3	NE86501	3	24	28
Arapahoe	4	9	4	Redland	4	6	4
Rediand	5	12	5	Centurk 78	5	14	21
NE67499	6	14	7	Rawhide	6	2	2
Roughrider	6	8	6	Siouxland	7	27	22
NE83404	8	4	8	NESCOG	8	11	13
NE86501	9	11	9	Arapahoe	•	17	9
NE83406	10	7	10	NE87613	10	9	11
NE87615	13	5	13	Buckskin	28	1	1
NE87613	22	6	23	NE85556	23	3	3
NE83432	17	10	16	Kari	37	5	7
				Bruie	25	7	14
				NE86527	44	8	8
				NE86507	30	10	10
				Scout 66	17	14	6

SUMMARY AND CONCLUSIONS

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- 1. Spatial variability was present all yield trials observed. It was substantial (resulted in > 10% reduction in CV) in 7 of the 12 trials.
- 2. Standard RCB analysis resulted in inflated coefficients of variation and, in several instances, unrealistic estimates of entry means.

The data from the NIN - Alliance trial provide the most dramatic example.

3. Both NNA procedures were effective in adjusting for spatial variability effects.

Both resulted in reduced CV's. In cases where substantial re-ranking of entry means occurred relative to the RCB analysis, both sets of NNA ranks were consistent.

4. The analysis of covariance procedure (NNA-SAS) appears to be more versatile, in the sense that it can account for spatial variability in more than one direction. This was required in 8 of the 12 yield trials observed.

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LATE PLANTED WINTER WHEAT RESULTS

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Wheat in the Great Plains is not always planted at the optimum time for a variety of reasons. Sometimes unwanted causes occur including replanting following stand loss to wind, pests, or winter killing. In other cases the seedbed may be too dry or too wet to plant at a normal time. Additionally, planting may be purposely delayed in order to avoid diseases or insects, in order to preirrigate, or to accommodate a double-cropping sequence. In order to identify wheat responses to delayed establishment, sequential monthly planting dates from October 1 to March 1 were undertaken during the seven years 1985-1991 at Garden City, Kansas. TAM 107 was seeded at a constant heavy rate in bordered drill strip plots in RCB design. Resulting relative grain yields tapered off with progressive planting dates as follows: Oct. 1 = 100%, Nov. 1 = 77%, Dec. 1 = 59%, Jan. 1 = 57%, Feb. 1 = 45%, Mar. 1 = 16%, Apr. 1 = 0%. The April date did not vernalize or reproduce. Relative to the optimum planting date on Oct. 1, the March 1 date was the last planting timing to produce heads and grain but was the lowest yielding, gave the most delay in heading (26 days later), was the latest to ripen (17 days later), the shortest (5" less), and produced the smallest seed (43% less weight), the lowest test weight (21% less), the fewest heads/plant (63% fewer), the fewest kernels/head (33% fewer), the fewest number of kernels per plant (76% fewer), and had the shortest grain filling period (9 fewer days). Little variation occurred through the range of dates for stand emergence or number of spikelets/spike. These results can assist farmers, seed sellers, crop insurers, and administrators of Farm Programs to make cropping decisions on "how late is too late" for planting winter wheat in the Central Great Plains.

TEAMWORK & COORDINATION IN WHEAT GERMPLASM IMPROVEMENT IN THE HARD RED WINTER WHEAT REGION

Byrd C. Curtis CIMMYT (Retired)

Development of the splendid array of winter wheat cultivars now available to growers in the Hard Red Winter Wheat Region of the Great Plains can be largely attributed to the spirit of teamwork, coordination and cooperation that has existed among wheat scientists in the region during the past 60 plus years. This presentation is a brief review of that unusual effort and some of the factors that now threaten its continuation.

Production of hard red winter wheat has been underway in the Great Plains since 1974 or about 118 years and was mostly based on germplasm originating from the Crimea. Introductions or selections therefrom comprised the cultivars grown for more than the initial half of this period. Hybridization became a serious method of improvement about 1920 when it was realized that progress via mere selection was limited. From the beginning of the hybridization process to develop improved germplasm, multilocation testing or cooperative approach of evaluation was involved. For example, Reitz* reported that breeding generations leading to 'Yogo', an important Montana cultivar originating from a Kansas cross made in 1919, were evaluated in several states. Part of the F1 seed was grown in California, the F2 in Kansas, and F3 and later generations in North Dakota, Montana and Colorado. The final selection of 'Yogo' was made at Moccasin, Montana. Similarly, selections from the Kansas cross, P1066 X Marquis, that led to 'Tenmarq' were tested in half a dozen states before the final ones were chosen for the 1932 release.

These and similar experiences confirmed the importance of a cooperative attack on problems of wheat improvement and prompted a request from the states for a coordinated program. Consequently, a conference was held on November 8, 1928 at Manhattan, Kansas comprising about 200 interested persons from the Southwest to discuss the problems of wheat improvement, production and marketing. The conference recommended (1) more research on biotic and abiotic stresses, soil fertility and marketing; (2) increased funding from the USDA and elsewhere be provided to coordinate wheat improvement work; and (3) that a permanent wheat research committee be appointed. The cooperative HRWW program was set up at worker's conferences in 1929 and 1930 and the first field tests were harvested in 1931. A mimeographed report on the results were distributed by the program leader in April, 1932.

* Reitz, L.P. and S.C Salmon. 1959. Hard Red Winter Wheat Improvement in the Plains, a 20-Year Summary. USDA Tech. Bul. 1192.

Four coordinators have served the HRWW Region, funded by the USDA, and are listed as follows:

Karl S. Quisenberry	1930 - 1946
Louis P. Reitz	1946 - 1954
Virgil A. Johnson	1954 - 1986
C. James Peterson	1986 - present

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Although I lack first-hand knowledge about Quisenberry as a regional coordinator, it is apparent from the quality of germplasm developed during his tenure that he succeeded in garnering a high degree of cooperation among HRWW states. I personally witnessed the effectiveness of Reitz and Johnson during their duty tours although I was around only during the latter years of the Reitz regime before he moved to the ARS/USDA National Program Staff (earlier titles were different) for cereal grains. From this position Reitz heavily supported the HRWW regional program as well as other regional programs.

At the time I entered the regional scene at Oklahoma State University in 1953, Virgil Johnson was about to assume the Regional Coordinator position. The environment for wheat breeding at that time was far different than now, to wit:

Lack of mechanica	aids for	- N	ursery	planting
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- Nursery culture
- Plot harvesting
- Seed cleaning and processing

Mechanical calculating machines were slow and inefficient (Monroe-Matic calculator was in vogue but punch-card computers were on the immediate horizon)

Phones were still in the "number please" stage

Airlines had only prop planes - DCs 3, 4, etc.

Germplasm base being used for HRWW improvement was narrow

No standard cultivar abbreviations were available

Good farmer interest with good attendance at field days

Much student interest in applied breeding and genetics

There were outstanding examples of teamwork and productivity in some wheat research programs during the 1950s and 1960s. Outside the region, the combined federal and state team led by Orville Vogel in Washington was superb and led to development of high yielding semi-dwarf cultivars, the most notable being 'Gaines'. Similar success was realized from the wheat research team at Purdue University in Indiana. And we must not forgot the magnificent productivity of Norman Borlaug's team at CIMMYT in Mexico. Interestingly, there was considerable interaction and cooperation between and among these particular teams.

Within the heart of the HRWW Region there existed some powerful wheat research teams, some being more productive of useful cultivars than others. The Nebraska team led by John Schmidt and Virgil Johnson are recognized for development of successful cultivars for the harsh environments of the central and northern areas. Some Nebraska varieties were widely grown throughout the region. The Texas team were similarly effective for the southern portion of the HRWW region and in recent time some of their cultivars have shown adaptation as far north as Kansas and Nebraska. The Kansas team, comprised of the widest array in the region of strong disciplinary units of plant pathology, entomology, plant physiology, milling and baking science, biochemistry, etc., produced some good cultivars but not up to expectations based on the available scientific base. The coordination or perhaps I should say cooperation never quite "jelled" to effect maximum productivity. Nevertheless, the Kansas institution played a vital role in supporting the remainder of the region. When compared with above teams the Oklahoma team clearly lacked success in cultivar development, although considerable change for the better has occurred in recent years. Oklahoma excelled in the transfer of biotic resistances from closely related wheat species, particularly to greenbug and to leaf rust, and in developing screening techniques for insect resistance.

Several private companies entered wheat breeding in the 1960s and early 1970s following the discovery of a male sterile/restorer system offering promise of hybrid wheat. This intensified breeding in the region, although much of the added effort was obviously on the hybrid mechanism, particularly by private companies. Public agency scientists were concerned that the private sector entry would result in diminished public funding. Much of this concern was justified since it was obvious that private research would not provide germplasm for all important niches of wheat production. Fortunately the state programs survived, though squeezed for research funds, since difficulties in profit realization forced significant reduction of private sector effort. Except for a limited amount of private effort, wheat breeding in the US is now largely back in the hands of public agencies. It should not go unrecognized that hybrid wheat as a crop was developed during the 1970-1990 period and may yet become an important crop should the economic situation change. Some private companies with strong breeding teams have been successful in the development and marketing of pure line wheat cultivars.

Several key organizations and activities contributing to teamwork and cooperation in the region, particularly in the last 40 years are listed below (not necessarily in order of importance):

Organizations and Conferences:

HRWW Improvement Committee USDA Hard Winter wheat Quality Laboratory Hard Winter Wheat Quality Advisory Committee HRWW Worker's Conferences Special Committees (e.g. Nomenclature and Gene Symbols) Disciplinary Worker's Groups (e.g. Insects, Leaf Rust) National Wheat Improvement Committee USDA Small Grains Collections State Crop Improvement Associations Great Plains Wheat, Inc. International Wheat Genetics Symposium State Wheat Commissions and Foundations USDA Crops Research Division USDA Cooperative Rust Laboratory USDA/ARS Grain Marketing Research Laboratory

Cooperative Nurseries (Regional, National, International)

Publications:

Wheat Newsletter - Volume 1 was published in 1954 (Congratulations to Elmer Heyne and Jim Quick)

Wheat Monograph, original and revised (again thanks to Elmer Heyne for his dedication and work on this)

Wheat Abbreviations (USDA, Oregon State Univ. & CIMMYT)

Without exception, when one examines reasons for success among the most productive institutes in germplasm improvement it is readily apparent that the leadership were highly field oriented, strongly encouraged teamwork, and fostered cooperation both within and with outside agencies. Further, they had intimate knowledge of their germplasm. Outstanding examples of this were the teams at Washington, Nebraska and CIMMYT.

Maintaining the high degree of field orientation, teamwork and cooperation has become increasingly difficult as result of several significant happenings in the region during the past 30 years or so. These are discussed in the following paragraphs.

a. Adoption by public employers of the "Public or Perish" attitude, becoming apparent in the 1950s, led to restricted and/or delayed exchange of information and germplasm since scientists needed to publish before revealing the secrets of their efforts. Further, the degree of precision research necessary for publication is not required for germplasm improvement and thusly, detracted from the breeding effort. I am fully aware that science developments need documentation but not on the scale that has excessively fattened the literature with mundane "slick papers" over the past three decades.

b. Discovery of the mechanism for development of hybrid wheat was disruptive of the good teamwork and cooperative attitude that prevailed in the region. First was the concern of some institutes and individual scientists about credit for scientific discoveries making hybrid wheat possible and subsequent reduction in information and germplasm flow among institutions. Even institutions not directly involved in the rift were affected since most had access to germplasm belonging to quarreling parties and were cautioned about distributing same to organizations having hybrid wheat or other profit motivated objectives. Public agencies lost staff to private agencies that often caused friction and ultimately reduced germplasm exchange and cooperation in the region.

c. Although the "jury is still out", the effect of new plant patent laws that emerged in the early 1970s did seem to have a damaging effect on teamwork and cooperation. Whether or not real damage occurred, the patent laws caused some anxiety among states and much anxiety between the US and foreign countries. The once easy task of obtaining germplasm from foreign countries is not that simple anymore.

d. The "biotechnology era", initiated in the 1980s, has caused dramatic reduction in funding of applied breeding at public institutions. Too often, vacated applied breeder positions are being filled by scientists who are expected or are likely to spend an inordinate amount of time in sophisticated laboratories. Eventually, biotechnology may partially supplant breeder activity that produced the excellent cultivars of today but excessive employment of biotechnologists (with heavy funding requirements) today is ill-timed. Unfortunately, most institutions feel the need to "jump on the band wagon" for fear of being left behind. Surely a more prudent approach could be something like a regional biotechnology laboratory catering to all institutions in the region; to serve in a fashion similar to that of the USDA wheat quality laboratory at Kansas State University. Combining of states resources now spent on smaller biotechnology

programs joined with a USDA effort would allow a "critical mass" of scientists using pooled expensive laboratory equipment.

e. Funding of state wheat research programs through proceeds from sale of university developed cultivars, now being considered, is a scary proposition. This idea might be feasible if implemented by private industry, ala the European system, but for universities to attempt such would be disastrous to information and germplasm exchange and cooperation. Private companies can enter into all kinds of financial arrangements and germplasm exchanges as they, in fact, do in Europe but for universities to mix seed marketing on the scale anticipated with other normal public supported university activities seems foolish and counter-productive.

Our objective should be to overcome these obstacles and continue development of high performance cultivars of acceptable quality.

In closing, I wish to say that if I have done nothing more than make you aware of the necessity and importance of teamwork, coordination and cooperation in the endeavor of wheat cultivar improvement in the HRWW region, I have been successful. I strongly urge newcomer scientists in the region to make themselves aware of the regional system that has developed those magnificent cultivars that our farmers are growing today. Economics aside, there is going to be another billion people arriving for lunch in the world between now and the year 2000!

REGIONAL BUSINESS MEETING

Hard Red Winter Wheat Improvement Committee January 22, 1992 Lincoln, Nebraska

MINUTES

The meeting was called to order by Chairman Worrall at 1:30 p.m. Committee members in attendance were:

P. S. Baenziger J. Hatchett D. Porter R. Bequette R. Hunger J. Reeder R. Bruns S. Kuhr R. Sears O. K. Chung M. Lazar T. S. Cox G. Lookhart V. Smail E. Donaldson D. Marshall E. Smith J. Erickson J. Martin J. Gellner J. Moffatt R. Graybosch M. Olewnick M. Witt A. Guenzi C. J. Peterson

Committee members not present:

C. Baker B. Carver N. Christensen B. Cooper D. Cox S. Curren R. French K. Frev B. Gill W. Heer

G. Hockett

B. McDonald J. Michels H. Nguyen

J. P. Hill

J. Krali

D. Johnston

J. Lawless

D. Mathre

G. Paulsen

S. Perry

D. Shelton J. Webster J. Wilson D. Worrall

J. Quick

- C. Roozeboom
- C. Rush
- P. Sebesta
- D. Seifers
- A. Scharen
- J. Shanahan
- L. Singleton
- E. Souza
- N. Tuleen
- J. Watkins

Members voted to approve minutes of the last meeting held at Dallas, TX on February 2, 1989, and dispense with reading of the minutes. The minutes are printed in the Proceedings of the Eighteenth Hard Red Winter Wheat Workers Conference, January 30 to February 2, 1989, Dallas, TX.

Regional Nurseries

SRPN -- Maximum number of entries (45) and check varieties Kharkof, Scout 66, and TAM-107 to remain the same.

- NRPN -- A motion to replace the check variety Colt with Abilene and retain Kharkof and Roughrider was passed. The maximum number of entries (45) is to remain the same.
- UWHN -- (Southern and Northern Sections) -- Check varieties will remain and maximum number of entries for each section will remain at 300. Warrior, Scout 66, and Vona are currently used as checks in the Southern Section and Warrior, Centurk 78, and Norstar in the Northern Section.
- Soilborne Mosaic Nursery -- A motion to replace the check varieties Pawnee, Bison, and Concho with Larned, Mustang, and Karl was passed and maximum number of entries will remain at 200.

Cooperating states and companies are not limited to a specified maximum number of entries in the SRPN or NRPN; rather they are instructed to prioritize candidate entries to provide guidance to the regional coordinator in the event that the total number of candidate varieties exceeds the nursery limit.

Seed requirements for the regional nurseries are currently 15 lb/entry in the SRPN; 11 lb/entry in NRPN; 120 gm/entry in UWHN; and 100 gm/entry in the Soilborne Mosaic Nursery. Seed is to be untreated. Seed of check varieties are increased and distributed with new entries each year from Lincoln, NE. The current format of the Regional Report is to be retained.

National Wheat Improvement Committee Report

Dr. Rollie Sears was elected Chair of the National Wheat Improvement Committee at the NWIC meeting in November, 1991. Dr. Sears reported on the NWIC issues and efforts to establish the committee as a source of information to national legislators. Current areas of NWIC concern include: the need for support of leaf rust research at Manhattan; replacement of the Ernie Sears position at Columbia, MO; restoration of the national wheat variety surveys; support for the U.S. Grain Marketing Research Laboratory; development of standardized terminology for grain quality definitions to improve communications among the wheat industry and policymakers.

Regional Germplasm Exchange issues

Members of the HRWWIC expressed concern over recent trends toward privatization of public research programs and increasing protection of plant germplasm in the U.S. A Germplasm Subcommittee was established, chaired by Dr. Stan Cox, to address issues affecting germplasm exchange in the region. Drs. Bob Graybosch, Ed Smith, and Dave Worrall will serve on the subcommittee with Rob Bruns and Jim Peterson serving as ex-officio members. The mission of the subcommittee is to provide information to public administrators and the HRWWIC related to the importance of germplasm exchange, regional collaboration, and the regional nursery program to wheat improvement in the Great Plains. The subcommittee is to begin by surveying public institutions regarding current policies and attitudes toward: PVP and other forms of plant variety protection; germplasm exchange and availability of germplasm; use of royalties and research fees. The subcommittee will then develop informational materials and papers on the potential impacts of: privatization of wheat variety development; intellectual property rights; plant variety protection; alternative release mechanisms; and administrative decisions on wheat germplasm exchange and wheat improvement.

International Germplasm Exchange

For several years, Dr. Peterson has coordinated importation of wheat germplasm from the CIMMYT programs in Turkey and Mexico, guarantine increase, and distributed seed to regional and U.S. research programs. With the closing of the International Nursery Program, this service has been discontinued due to budget constraints. Dr. Kronstadt, Oregon State University, has tentatively agreed to provide interested U.S. researchers with seed of the CIMMYT-Turkish Screening Nursery. Drs. Worrall and Peterson are continuing to work with APHIS, CIMMYT, and USDA-ARS to find mechanisms for importation, increase, and U.S. distribution of CIMMYT spring wheat germplasm from Mexico. The CIMMYT program has tentatively agreed to coordinate the International Winter Wheat Performance Nursery out of Mexico. However, U.S. researchers will be unable to receive seed or grow field trials of the new IWWPN without an initial quarantine increase cycle. The NWIC has recently established an International Germplasm Exchange Subcommittee to examine methods and means of obtaining germplasm from international breeding programs. The Committee will work with the USDA-ARS and National Small Grains Collection to address germplasm exchange priorities and needs.

Status of Pioneer Hard Winter Wheat Germplasm Evaluations

Dr. Joe Martin reported on the status of Pioneer germplasm which was donated to Kansas State University in 1990. Results of the 1991 Pioneer Observation Nursery have been compiled and a summary of data is to be included in the 1991 Regional Nursery Report. The second Pioneer Observation Nursery is now planted at eight locations in the region. A third and final observation nursery is planned to be distributed for fall 1992 plantings.

Evaluation of Regional Nursery Samples for Baking Quality

Dr. Okky Chung reported on efforts by the U.S. Grain Marketing Research Laboratory to increase the number of quality evaluations of experimental lines from regional breeding programs. Of particular interest was evaluation of SRPN samples from several locations each year to obtain estimates of stability and genotype by environment interactions for quality traits. A regional subcommittee was formed to serve in an advisory capacity to the Grain Marketing Research Laboratory. Dr. Jeff Gellner agreed to serve as chair, and Drs. Ed Smith, Dave Worrall, and Jim Quick as members. The subcommittee will work with the Laboratory to prioritize regional quality evaluations and propose appropriate quality analyses to be conducted.

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Election of Regional Officers

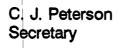
Rob Bruns was elected as Chair of the Hard Red Winter Wheat Improvement Committee. David Worrall and Stan Cox were elected representatives to the National Wheat Improvement Committee. They, together with the Chairman and Secretary, will represent the Hard Red Winter Wheat Region on the National Committee.

Site of Next Wheat Breeders Field Day

The 1992 Wheat Breeders Field Day is to be held at Bushland, Texas. A tentative date of May 27 was proposed.

Site of Next Regional Conference

An invitation from Oklahoma State University researchers to hold the 1995 Regional Conference at Stillwater, OK, was accepted. The next conference will be held sometime in January or February as has been done in the past. A motion was passed to contribute funds remaining from the 19th HRWWW Conference to the Annual Wheat Newsletter fund. The National Wheat Improvement Committee has proposed that a North American Wheat Workers Conference be held in March, 1994. Details and location have yet to be determined.



RESOLUTIONS

The following resolutions were unanimously adopted:

No. 1. Whereas, wheat end-use quality, storage, and classification are vital components of a healthy and prosperous wheat industry; and

Whereas, the U.S. Grain Marketing Research Laboratory is an integrated laboratory and a leader in providing new research for these important areas;

Be it resolved, that the Hard Red Winter Wheat Improvement Committee supports the expansion of both the facilities and mission of the U.S. Grain Marketing Research Laboratory through its proposal of a Total Quality-Based Grain Marketing System for U.S. grain.

No. 2. Whereas, Tom Roberts, as Executive Director of the Wheat Quality Council for over 40 years, has served the winter wheat industry admirably and has provided an important communications bridge between the milling and baking industry and the Hard Red Winter Wheat Improvement Committee; and

> Whereas, Tom Roberts has so freely and willingly given his time and efforts to pursuing the goals of improving hard red winter wheat;

> Be it resolved, that the Hard Red Winter Wheat Improvement Committee congratulates Tom on his efforts and achievements. We extend our appreciation and heartfelt thanks for his leadership in improving winter wheat quality and wish him the best in his retirement.

No. 3. Whereas, the Hard Red Winter Wheat Improvement Committee recognizes the distinguished professional contributions of Dr. Ian Edwards to wheat and wheat improvement as chairman of the National Wheat Improvement Committee during the last seven years; and

Whereas, the National Wheat Improvement Committee has addressed issues affecting the Hard Red Winter Wheat Region accurately and fairly under the guidance and leadership of Dr. Edwards; Be it resolved, that the Hard Red Winter Wheat Improvement Committee commends Dr. Edwards for his efforts and contributions to the National Wheat Improvement Committee and expresses its sincere appreciation to him for his many contributions to U.S. wheat improvement.

No. 4. Whereas, Dr. David Worrall has provided superior and active leadership to the Hard Red Winter Wheat Improvement Committee; and

Whereas, Dr. Stan Cox and Rob Bruns, along with Dr. Worrall, have served as excellent and conscientious representatives of the Hard Red Winter Wheat Improvement Committee to the National Wheat Improvement Committee;

Be it therefore resolved, that the Hard Red Winter Wheat Improvement Committee expresses its sincere appreciation to past-Chairman Worrall, Stan Cox and Rob Bruns for their efforts and superior contributions on behalf of the committee.

No. 5. Whereas, the 19th Hard Red Winter Wheat Workers Conference has been an excellent and informative meeting and our hosts have expended much time and effort to ensure the success of the conference;

Be it therefore resolved, the Hard Red Winter Wheat Workers express their sincere appreciation to Dr. Darrell Nelson, Dean and Director of the Nebraska Agricultural Experiment Station, and Dr. Jim Peterson for serving as hosts in this conference; to Drs. Jim Peterson and Bob Graybosch for directing local arrangements; to Drs. David Worrall, Stan Cox, Charlie Rush, Bob Graybosch, Arron Guenzi, Ed Smith, and Virgil Smail for program arrangements; and to Joyce Kovar, Laura Oberthur, Marizan Hugo, Ben Moreno, Bob Divoky, and Kyle Ditch for their aid in local arrangements;

Be it further resolved, the Hard Red Winter Wheat Workers express their sincere appreciation for financial support of the conference from the Nebraska Crop Improvement Association; Nebraska Wheat Board; AgriPro Bioscience, Inc.; ConAgra Grain Processing Company; Hege Equipment, Inc.; and HybriTech Seeds, Inc.

WHEAT WORKERS CODE OF ETHICS

Adopted by the National Wheat Improvement Committee 10/27/76

The originating breeder, station, or company has certain rights to the unreleased material. These rights are not waived with the distribution of seeds or plant materials but remain with the originator for disposal at his initiative.

The recipient of unreleased seeds or plant materials shall make no secondary distributions of the germplasm without the permission of the owner/breeder.

The owner/breeder in distributing unreleased seeds or other propagating materials, grants permission for use (1) in tests under the recipient's control, (2) as a parent for making crosses from which selections will be made, and (3) for induction for mutations. All other uses, such as testing in regional nurseries, increase and release as a cultivar, selection from the stock, use as parents in commercial F_1 hybrids or synthetic or multiline cultivars, require the written approval of the owner/breeder.

Plant materials of this nature entered in crop cultivar trials shall not be used for seed increase. Reasonable precautions to insure retention or recovery of plant materials at harvest shall be taken.

The distributor of wheat germplasm stocks may impose additional restrictions on use or may waive any of the above.

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