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# PROCEEDINGS

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## **Twentieth Hard Red Winter Wheat Workers Conference**

**January 25-27, 1995  
Oklahoma City, OK**

**Departments of Agronomy and Plant Pathology  
Oklahoma Agricultural Experiment Station  
Oklahoma State University**

**and**

**Plant Science Research Laboratory  
U.S. Department of Agriculture  
Stillwater, Oklahoma**

**Sponsored by  
The Hard Red Winter Wheat Improvement Committee**



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## Twentieth Hard Red Winter Wheat Workers Conference

January 25-27, 1995  
Oklahoma City, OK

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Department of Agronomy, Oklahoma State University

Robert M. Hunger  
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Stan Cox, USDA-ARS, Manhattan, KS  
Jim Peterson, USDA-ARS, Lincoln, NE  
David Porter, USDA-ARS, Stillwater, OK  
David Worrall, Texas A&M



**Sponsored By:**

The Hard Red Winter Wheat Improvement Committee

Oklahoma State University

USDA-ARS, Plant Science Research Laboratory, Stillwater, OK

Oklahoma Wheat Commission

Shawnee Mills

American White Wheat Producers Association

Johnston Seed Company

Oklahoma Crop Improvement Committee

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# PROGRAM

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*20th*  
*Hard Red Winter*  
*Wheat Workers*  
*Workshop*

January 25-27, 1995  
Oklahoma City, OK

*Embassy Suites*  
*1815 South Meridian*  
*Oklahoma City, OK 73108*  
*Phone: 405-682-6000*

*Sponsored by:*

*Hard Red Winter Wheat Improvement Committee,*

*Oklahoma State University,*

*and USDA-ARS*

The Hard Red Winter Wheat Improvement Committee is proud to host the 20th Regional Wheat Workers Workshop with a program that should be of interest to all Wheat Workers. The workshop will explore key issues and new developments impacting wheat improvement in the Great Plains through key invited presentations, roundtable discussions, and a volunteered poster session. The meeting will provide a relaxed and casual atmosphere to encourage interaction among colleagues and professionals dedicated to wheat improvement.

## **TUESDAY, JANUARY 24**

3:00-7:00 Registration  
5:00-7:00 Workshop mixer

## **WEDNESDAY, JANUARY 25**

7:00-3:30 Registration

**8:30 Opening Remarks**  
Rob Bruns, Agripro Seeds

**8:40-10:00 Wheat Breeding**  
**Methodology:**  
Chair: David Worrall,  
Texas A&M

International perspective  
S. Rajaram, CIMMYT, Mexico

Private sector perspective  
John Moffatt, Agripro Seeds

Public sector perspective  
Rollie Sears, Kansas State Univ.

Roundtable discussion

**10:00-10:30 Break - Sponsored by  
Oklahoma Wheat Commission**

**10:30-12:00 Future of Wheat  
Breeding:**

Chair: David Worrall,  
Texas A&M

Changing times  
Ed Hiller, Texas A&M

Wheat breeding and the Land  
Grant mission  
Ed Smith,  
Oklahoma State Univ.

Roundtable discussion

**12:00 Conference Luncheon:**  
Future funding opportunities for  
international development activities  
Guest Speaker: Art Klatt,  
Oklahoma State Univ.

**1:30-3:00 Gene Deployment:**  
Chair: Stan Cox, USDA-ARS,  
Manhattan, KS

Progress on molecular mapping of  
disease and insect resistance genes  
in wheat  
Bikram Gill, Kansas State Univ.

Panel Discussion - Slowing the  
evolution of new, more virulent,  
leaf rust races  
Dave Marshall,  
Texas A&M, Dallas  
Stephen Baenziger,  
Univ. of Nebraska  
Bob Bowden,  
Kansas State Univ.

**3:00-3:30 Break-Sponsored by  
Shawne Mills**

**3:30-5:00 Germplasm Enhancement:**

Chair: David Porter,  
USDA-ARS, Stillwater, OK

Impact of breeding and genetics on  
reducing RWA damage in the  
Western U.S.

Jim Quick,  
Colorado State Univ.

Germplasm development for  
enhanced end-use quality

Bob Graybosch,  
USDA-ARS, Lincoln, NE

Status of the wheat database

Olin Anderson,  
USDA-ARS, Albany, CA

Adaptation of the wheat curl mite to  
resistance in wheat

Tom Harvey,  
Kansas State Univ.

**THURSDAY, JANUARY 26**

**8:00-5:00 Poster session**

Chair: Bob Hunger, Oklahoma  
State Univ.

**8:30-10:00 Impact of changing  
management practices:**

Chair: Gene Krenzer,  
Oklahoma State Univ.

Variety selection:

A producer's perspective  
Don Oswald, Apache, OK

Management practices and net returns  
in a wheat-stocker cattle enterprise

Gene Krenzer,  
Oklahoma State Univ.

Possible effects of management  
practices associated with sustainable  
agriculture on diseases

Bill Bockus, Kansas State Univ.

**10:00-10:30 Break - Sponsored by  
American White Wheat Producers  
Association**

**10:30-12:00 Transgenic wheat**

Chair: Arron Guenzi,  
Oklahoma State Univ.

From transformation to commercial  
wheat products

Joyce Fry, Monsanto

Genetic engineering of wheat for  
drought resistance

Troy Weeks,  
USDA-ARS, Albany, CA

Using transgenic wheat to explore  
protein contributions to bread-making  
quality

Olin Anderson,  
USDA-ARS, Albany, CA

**12:00 Workshop Luncheon**

**1:30-3:00 Regulatory issues (TBA)**

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

**3:00-3:30 Break - Sponsored by  
Johnston Seed Company**

**3:30-5:00 Regional business meeting**

Chair: Rob Bruns,  
Agripro Seeds  
Jim Peterson,  
USDA-ARS, Lincoln, NE

**FRIDAY, JANUARY 27**

**8:30-12:00 Hard white wheat  
development**

Chair: Brett Carver,  
Oklahoma State Univ.

Introducing hard white wheat:  
Challenges to plant breeders,  
agronomists, and growers  
Cal Qualset,  
Univ. of California, Davis

**Marketing hard white wheat -  
Domestic issues**

Glenn Weaver,  
ConAgra, Omaha, NE

**Marketing hard white wheat -  
International issues**

John Oades,  
U.S. Wheat Associates,  
Portland, OR

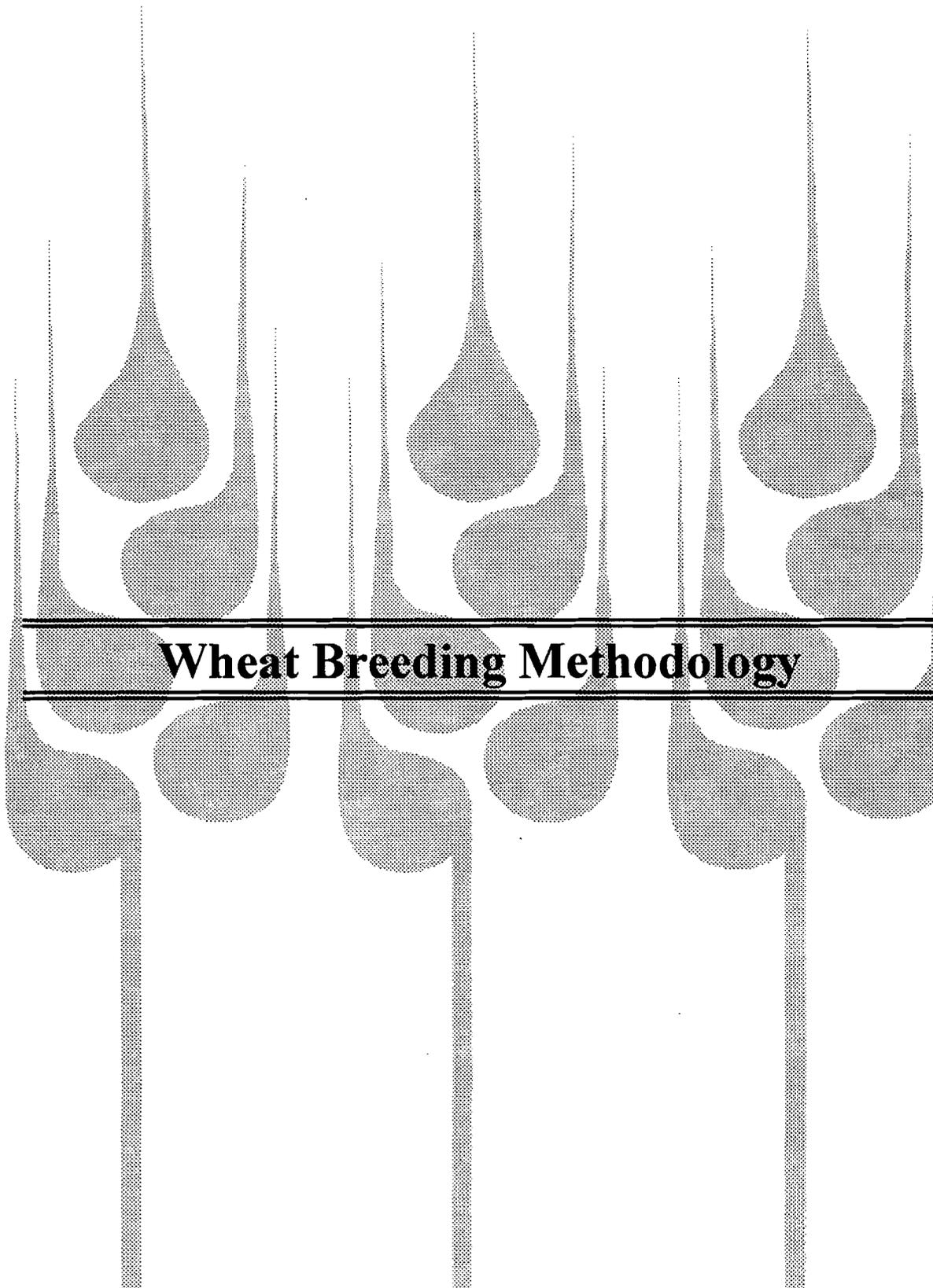
**10:00-10:30 Break - Sponsored by  
Oklahoma Crop Improvement  
Association**

Improvement of kernel color and pre-  
harvest sprouting in hard white wheat  
Joe Martin, Kansas State Univ.

**End-use quality testing during hard  
white wheat development**

Dave Shelton,  
Univ. of Nebraska

**12:00 Conference Wrap-up and  
closing remarks**

The background of the page features a stylized, halftone-style illustration of wheat grains. The grains are arranged in a repeating pattern, with each grain having a long, thin awn extending upwards. The grains are rendered in a light gray color with a fine dot pattern, giving them a textured appearance. The title is centered over this background.

# **Wheat Breeding Methodology**



# Wheat Breeding Methodology: International Perspectives

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S. Rajaram and M. van Ginkel, Wheat Breeders  
CIMMYT, Apartado Postal 6-641, 06600, Mexico D.F., Mexico

## Shuttle Breeding and International Multilocation Testing.

### *Is the Wide Adaptation/Stability Concept Still Valid?*

In the last decade of the 20<sup>th</sup> Century, when the mainstream debate in agricultural sciences has centered on biotechnology - a new methodology (or even a new science?), and its application in plant breeding, - it is considered both awkward and old fashioned to reiterate the importance of old but proven methodologies such as shuttle breeding and multilocation testing. Do we have enough data on biotechnology and proof of its achievements, to decide whether funding of and research on major methodologies in conventional plant breeding ought to be discarded in favor of pursuing these new methodologies? Ten years ago, we were told by many learned scientists that biotechnology would be "delivering" 10 - 15 years from then. It has not happened today, at least not in wheat. The basic methodologies remain intact, and have been augmented by a few new ones; all is an evolutionary process.

The shuttle breeding methodology is uniquely CIMMYT's; it was proposed 50 years ago, and implemented by Borlaug (1968), initially accompanied by much criticism, but finally to wide acclaim. This methodology has been responsible for the production of photoperiod insensitive and

otherwise widely adapted germplasm. In particular, the shuttle breeding process involving contrasting locations has proven a most efficient way to introduce and select genes for photoperiod insensitivity. The photoperiod insensitive genes, *Ppd1* and *Ppd2*, abound in CIMMYT's spring wheats and along with the dwarfing genes, *Rht1* and *Rht2*, resulted into a new plant type; not just lodging tolerant (the initial aim), but dramatically higher yielding with high biomass (due to pleiotropic effects/close linkage; Hoogendoorn et al., 1988). When superimposed with rust resistance (Borlaug, 1968), the new genetic combination provided adaptation to most irrigated wheat-growing areas of the subtropics.

In the last 10 years, CIMMYT and the Oregon State University Wheat Program, have launched a joint shuttle breeding enterprise between Pendleton/Hyslop (Oregon) and Toluca (Mexico), for the selection of widely adapted facultative/winter wheat germplasm derived from spring x winter wheat crosses (Kronstad and Rajaram, 1990).

The resulting progenies have shown remarkable wide adaptation in such far away and distinct regions as the Anatolian Plateau of Turkey, Afghanistan, Iran and Uruguay. The original base-germplasm pool bred only at sites in Oregon, lacked such alleles as *Ppd1* or *Ppd2*, while the shuttle operation permitted a combination

of photoperiod insensitivity due to selection at Toluca, plus a high yield base identified at Pendleton/Hyslop. In addition, resistances were combined.

We are pleased to note that in the last 20 years, most major wheat breeding programs in the world have adopted multilocation testing in contrasting environments as an integral part of their philosophy, including those in the Great Plains of the USA, North Western Europe, Eastern and Southern Africa, the Southern Cone of South America and the Indian Sub-continent. These programs are cooperative ventures with many contributing components, and are not directly or easily influenced by CIMMYT. The widespread adoption of this methodology, therefore, has lead us to believe that it has wider application in choosing suitable parents and developing germplasm for release and recommendation to farmers, and deserves to be newly brought to our attention.

We do not want to appear to be critical of biotechnology; indeed, we are eagerly looking forward to advances in transformation technology for application in our own program and have ourselves actively started out on this road within CIMMYT. However, we believe it can not be justified to divert an overly large proportion of resources from conventional, proven plant breeding methodologies and their further study to biotechnology, especially in developing countries. We must continuously monitor the limits, advantages and draw backs, of both conventional plant breeding and new methodologies, and seek a responsible complementarity between the two, incorporating that what is effective and efficient.

### **Concept of Megaenvironments**

CIMMYT has never sought nor proposed a single variety for the whole world. However, certain critics of our program have unduly emphasized this as our intent. Wide adaptation is defined by us as the ability of a variety to produce high yields in many similar environments. Such germplasm needs critical and essential diversity/variability for disease resistance, while carrying certain elements of homogeneity such as photoperiod insensitivity and dwarfing genes. Uniformity of certain traits should not in and of itself be equated with genetic vulnerability.

The concept of incorporating diversity for disease resistance, combined with homogeneity for those agronomic traits which impart high yields, adaptability and stability, has been our objective through the 1950's, when the Bread Wheat Breeding Program was managed by the Rockefeller Foundation/Office of Special Studies within the framework of a bilateral mission within Mexico, through the 1960's and 1970's, led by CIMMYT with an international mandate, and into the 1980's and 1990's, achieving a global focus.

Since 1988, the Bread Wheat Breeding Program has made more than 150,000 crosses, globally distributed 10,000 advanced lines, and received recognition and acknowledgment from the world's NARSs (National Agricultural Research System), which released more than 500 advanced lines as varieties to the farmers, grown on roughly 40 million hectares in most wheat growing regions of the developing world. This huge area must convince the world's science community in both the NARSs, the CGIAR and developed countries of our intent to achieve stability of yield, broaden the genetic base, while narrowing the

probability of genetic vulnerability due to major wheat diseases and pests, and of the value of shuttle breeding and multilocation testing in actually achieving these objectives.

In 1988, CIMMYT's Strategic Plan (CIMMYT, 1989) proposed the term Mega-environment (ME) to subdivide global wheat domains. We must, however, state that our breeding program's objectives have continually been evolving over the past 50 years, seeking to combine superior agronomic traits with essential and specific abiotic and biotic tolerances, in order to address millions of hectares of very diverse wheat-growing conditions. At the time of the proposed ME-based breeding, CIMMYT's Bread Wheat Breeding Program was already strategically and distinctly addressing the issues involving adaptation to such varied environments as irrigated regions, high rainfall areas, acid soils, semiarid zones, tropical areas and winter wheat zones. From a publicity standpoint, the ME-based breeding approach has been a powerful communication/media success; suddenly the reviewers of the 3<sup>rd</sup> Quinquennial Review of CIMMYT in 1989 embraced the concept and congratulated CIMMYT's new upper management for providing a new vision and philosophy. False pride among administrators abounded, due to a lack of knowledge of the history of their own institute, CIMMYT. We equated our evolved biological/breeding terminology along the lines of ME's, and delineated and equated our biological definitions to describe a total of 12 ME's (Rajaram et al., 1993). These are illustrated in Table 1. However, in actual fact, the new classification had evolved through a long process of exploiting and learning from shuttle breeding and multilocation testing.

Currently CIMMYT's Bread Wheat Breeding Program emphasizes the regions of ME<sub>1</sub>, ME<sub>2</sub>, ME<sub>3</sub>, ME<sub>4</sub>, ME<sub>5</sub>, ME<sub>6</sub>, ME<sub>7</sub>, ME<sub>9</sub>, ME<sub>10</sub>, and ME<sub>12</sub>. All spring wheats are addressed from Mexico, and most of our winter wheat breeding is done at Turkey in collaboration with Oregon State University, the Turkish National Program and ICARDA. The following traits/genes are considered essential for the different ME's.

### Spring Wheat

#### *ME<sub>1</sub> (Irrigated):*

*Rht1 + Rht2; Ppd1 or Ppd2; high yield potential; input responsive and -efficient; Sr2-complex and Lr34-complex; better balance of HMW glutenins (1 or 2\*,7+8, 5+10); some heat tolerance; lodging tolerance; largely white/amber-grained.*

#### *ME<sub>2</sub> (High Rainfall):*

*Rht1 or Rht2, and sometimes Rht8; Ppd1 or Ppd2; Sr2 complex and Lr34 complex; HMW glutenins (1 or 2\*,7+8, 5+10); better resistances/tolerances to *Septoria tritici*, BYDV, stripe rust and *Fusarium head scab*; sometimes resistance to powdery mildew, *Septoria nodorum*, tan spot, bacterial leaf blight (*X. translucens*), and root rots; sprouting tolerance; largely red-grained.*

#### *ME<sub>3</sub> (Acid Soil):*

*Same as for ME<sub>2</sub>, + tolerance to Al/Mn toxicity; efficient 'P' uptake/utilization.*

#### *ME<sub>4</sub> (Semiarid):*

*"Tall dwarf" in stature (*Rht1* or *Rht2* without modifiers); combination of input responsiveness (yield potential) and input efficiency (drought tolerance); *Sr2 + Lr34* complexes; sometimes stripe rust and bunt resistance needed; some heat tolerance; some cold tolerance; both white/amber and red grained.*

### *ME<sub>5</sub> (Tropical):*

A: Low humidity tropics: ME<sub>1</sub> characteristics superimposed with high temperature tolerance; targeted for countries such as Sudan and Peninsular India.

B: High humidity tropics: ME<sub>2</sub> characteristics superimposed with high temperature and *Helminthosporium sativum* tolerance; sometimes sprouting tolerance needed; targeted for such areas as Bangladesh, Eastern India and Paraguay.

### *ME<sub>6</sub> (High Latitude):*

A: High Rainfall: Same as for ME<sub>2</sub>, with *ppd1* or *ppd2* allele(s).

B: Semiarid: Same as for ME<sub>4</sub>, with *ppd1* or *ppd2* allele(s).

## **Facultative wheat**

### *ME<sub>7-8</sub> (Irrigated/High Rainfall):*

Moderate level of vernalization requirement (either *vrn1*, *vrn2* or *vrn3*); sometimes rapid grain fill; cold tolerance; most other traits as for ME<sub>1</sub> or ME<sub>2</sub>.

### *ME<sub>9</sub> (Semiarid):*

Moderate level of vernalization requirement; cold tolerance; most other traits are the same as for ME<sub>4</sub>.

## **Winter Wheat**

### *ME<sub>10/11</sub> (Irrigated/High Rainfall):*

High level of vernalization requirement with either *vrn1 + vrn2*; *vrn1 + vrn3*, *vrn2 + vrn3*, or *vrn1+2+3*; eye spot resistance needed; most other traits are the same for ME<sub>1</sub> or ME<sub>2</sub>.

### *ME<sub>12</sub> (Semiarid):*

High level of vernalization requirement needed; some bunt resistance needed; other traits are the same as for ME<sub>4</sub>.

## **Genetic Diversity**

In this decade, primarily due to 1992 Rio Biodiversity Conference, the issue of genetic vulnerability has been brought to the forefront on a large scale. Unfortunately, the new financial resources have not been coming to plant breeding, but are being allocated to gene resources stored in gene banks. Most of these banks are sterile storage buildings; at most those in charge have only a vague idea of what is stored inside. In order for gene resources to help future plant breeding, the entire strategic thinking by the people who control the banks, needs to be changed to meet the criteria of plant breeding.

At the present rate of genetic resource utilization in breeding, the variability stored in current advanced lines in most breeding programs is adequate for the fore-foreseeable future. Only in the case that a rare genetic vulnerability issue arises, for example of the magnitude of sudden widespread occurrence of Karnal bunt or extensive wheat blast epidemics in Brazil, should a genetic search of large dimensions be needed. Even then I am not sure the banks would be able to respond, because they lack information on such unforeseen catastrophes.

Here we describe what the CIMMYT Bread Wheat Breeding Program is doing to thwart epidemics due to prioritized, major and minor, well-known pathogens globally, through gene accumulation, gene deployment, and particularly through providing access to large-scale, operable genetic variability to NARSs.

Our breeding program products are based on annually executed 10,000 simple, top and limited back crosses, utilizing known variability from spring wheats, winter/facultative wheats, durum wheats,

*Aegilops squarrosa*, rye and *Agropyron* spp. in a mega-environmental setting. Products are made available to NARSs as follows:

1. International Bread Wheat Screening Nursery (IBWSN):  $\pm$  400 entries for ME1. At times, base advanced lines (1500 PC's) are made available to certain NARSs in need of dire variability.
2. High Rainfall Wheat Screening Nursery (HRWSN):  $\pm$  400 entries for ME2/ME3. Also advanced lines (PC's) can be made available, if a NARS elects to have request them.
3. Semi-Arid Wheat Screening Nursery (SAWSN):  $\pm$  400 entries for ME4. 1000 lines are screened to select these entries and the original source is available for limited distribution.
4. Warmer Areas Wheat Screening Nursery (WAWSN):  $\pm$  100 entries for Humid Tropical ME5.
5. PC's WW/FW. Available on request a set of  $\pm$  500 lines of WW/FW habit suitable mostly to E7 and ME10. These materials are non-repetitive and complementary to our WW/FW efforts in Turkey, and available every 2<sup>nd</sup> or 3<sup>rd</sup> year.
6. Special Genetic Stocks with rare combinations of resistances, yield and quality.
7. Also available to NARS is the variability of all unselected and unexploited 7000 crosses as F2 segregating populations. In recent years, and specially after the 1988 Strategic Plan, this variability has not been distributed to NARSs in response

to their stated preference and CIMMYT's policy to encourage local crossing.

Because of the above dynamism of germplasm use and distribution, CIMMYT and the NARSs can be considered to be well prepared to control any expected and unexpected evolutionary forces in the pathogens. But we can not be complacent, and therefore we continue to introgress genetic variability. Because of the dynamics on the pathogen side, and at times due to shifting interpretations of gene pool management, advanced lines must contain unexplored variability unwanted today, but valuable in the future. This phenomenon is best illustrated in Australia when CIMMYT germplasm proved valuable on two occasions: first for cereal cyst nematode and second for stripe rust resistance when most Australian varieties succumbed to supposedly European or African introduced stripe rust races.

Chinese germplasm has helped CIMMYT to win Karnal bunt battle in Mexico. Similarly the Brazilian cultivar, FRONTANA, has been critical in launching the conquest of leaf rust. Involved here are complex genetic resource utilization questions; CGIAR/TAC will have to address these. One of the logical alternatives to the present scenario is the recognition and encouragement of active partnerships between bank managers and plant breeders at the IARC level. This had not happened at the NARSs level and most banks have measurably failed to deliver to plant breeders what they wanted. There is no reason to think the situation is presently any better within the CGIAR. Consequently, we of the IARCs have been sitting on the largest collection of gene

diversity, falsely championing the course of humanity, without really doing anything.

### **Modified Pedigree-Bulk Selection Method, Combined with Mechanization and Computerization of Bread Wheat Breeding**

With the globalization of CIMMYT's Bread Wheat Breeding Program in the 1980's and the evolution of the concept of 12 ME's, the number of crosses made annually increased dramatically from 2000 in the early 1970's to 10,000 in the 1980's. The total number of segregating populations (F2-F7) grew from 20,000 lines to 150,000. Similarly, the number of entries in yield trials increased from 1000 to 5000 annually. The total acreage in breeding and testing expanded from 30 ha to 100 ha in the same period.

To accommodate this increase in breeding populations, the methodology of selection was changed from a pure pedigree system to a modified pedigree-bulk selection approach. The new method allowed one experienced CIMMYT breeder to evaluate all segregating populations, except the F2's, in a timely fashion. Simultaneously, total mechanization of planting and harvesting, and the computerization of field books have allowed a limited group of support staff and technicians to carry out all responsibilities as before. These three major changes introduced in our operation increased the ability to introgress variability by significantly increasing the number of crosses directed for specific MEs, while keeping the selection program highly efficient, and without sacrificing population size per cross.

The breeding program in the 1970's traditionally made double and top crosses in equal proportion. Subsequently the

double cross was eliminated due to poor output, and the limited backcross (one backcross) was introduced for most ME's, in which a limited amount of variability was allowed compared to top and double crosses. This strategy permitted the introduction of known genes or traits in a highly productive agronomic background. This practice has begun to be accepted by breeding programs in developing and developed countries alike.

The modified pedigree-bulk method practiced at CIMMYT is described below:

- F1** Based on simple cross, limited backcross or top cross.
- F2** 2000 spaced plants per cross. Individual plant selection based on agronomic type, disease resistance, and seed health and -type.
- F3** Selected F2 plant progeny planted individually as F3, at a normal seeding rate of 100 kg/ha in 3 rows of 2m per line, in order to observe and evaluate competitive ability within the line. The selection of lines is thus based on visual assessment of agronomic performance and disease resistance in a plot rather than on an individual plant. Ten to 15 heads are selected per selected F3 line, threshed in bulk, and promoted to the F4 generation.
- F4-F5** Same as F3

- F6** Planted as in the F3, but 5-10 heads are selected and threshed individually from selected F6 plots.
- F7** Individual heads are planted, and plots are selected for subsequent complete bulk-harvest.
- YT** The bulked lines are included in preliminary yield trials and subsequently replicated yield trials, before industrial quality test are carried out. Finally, the superior lines are included in one of CIMMYT's International Nurseries or Yield Trials, classified by ME.

### ***Handling of Pathogen Populations in Breeding***

Needless to say, that we believe strongly in partial resistance to pathogens based on non-differential, non-specific host-pathogen interaction expressed as low severity with a susceptible to moderately susceptible infection type, which is best selected under heavy epidemic pressure in field conditions. Epidemiologically, this resistance is of a dilatory type (slow rusting in the case of rust; Caldwell, 1968), probably requiring no more than 3-4 accumulated minor genes for most diseases. It has been hypothesized that this resistance would possibly be of a durable nature. We must also emphasize, that we have little faith, and consider it a waste of time and resources, to breed for resistance based on hypersensitive, major genes, which historically have proven to be of a specific nature, differentially interacting with pathogen biotypes or

racess. The recent breakdown of *Lr19* of alien origin, a major gene that so many researchers thought would be an exception, to a new leaf rust race in central Mexico (Huerta, pers. comm.), reminds us of the risks involved with this type of resistance.

CIMMYT's work on breeding for slow rusting (partial) resistance started in 1950 with the Hope-type of stem rust resistance, and in 1970 with Frontana-based leaf rust resistance. Genetic studies recently carried out at CIMMYT identified the genes, *Sr2* and *Lr34* (Singh and Rajaram, 1991, 1992) respectively, as major components of these resistances. But the best level of partial, slow-rusting type of leaf rust resistance is based on the interaction of 3-4 such genes (Singh et al ). Therefore the selection strategy should be based on epidemiological phenomena (in this case slow rusting), rather than the on the tracking of individual genes which cause slow rusting. The technique of selecting slow rusting genotypes is well known (Van Ginkel and Rajaram, 1993).

Partial resistance is naturally abundant in most wheat-pathogen interactions such as: wheat-stem rust, wheat-leaf rust, wheat-stripe rust, wheat-powdery mildew, wheat-Septoria leaf blotch etc. It "only" requires long-term commitment on the part of the breeder, and proper pathological backup, to make this important breeding venture successful.

### ***Abiotic Stresses in Marginal Environments***

Drought, heat, cold, Al-toxicity, N and P utilization efficiency, waterlogging, Boron toxicity and Zn deficiency are some of the most immediate issues receiving attention in CIMMYT's Bread Wheat Breeding Program across all 12 ME's.

Genetic variability has been identified for most of these topics.

Our breeding philosophy on how to combine the genes for these characteristics with stable yields, differs from that promoted by other International Centers. We have noted that the genes for yield potential and the traits for adaptation to marginal environments are, for most practical purposes, different and hence can be blended together (Rajaram, 1991; Calhoun et al., 1994; Van Ginkel et al., in press). This hypothesis differs significantly from that published by ICARDA (Ceccarelli et al., 1987), where land-races, as base materials rather than gene donors, are further re-selected or crossed among themselves, to develop tolerance to abiotic stresses. In the case of our stated methodology, high yielding varieties with good agronomic type and disease resistance must constitute one of two parents in breeding and selecting germplasm for superior performance under abiotic stress; with the other parent being germplasm possessing abiotic stress tolerance traits. The latter can be a land-race, exotic stock or an alien gene source, but may also be other high yielding advanced lines containing a different genetic constitution, from which superior transsegregants can be identified during a well-focused selection process.

To permit the recombination of the high yield base and stress tolerance traits, we conduct selection of segregating populations in alternate environments representing optimum and stress growing conditions. Based on this methodology, we have now available germplasm with high yield, combined with individual tolerances to such stresses as drought, heat, cold, Al toxicity, and waterlogging. We find satisfaction in the fact that national programs have started releasing

varieties to their farmers in marginal areas, that were developed using the above described CIMMYT philosophy.

What are the advantages of having stress tolerance in a high to moderate yielding background? Germplasm of this kind can be exploited in variable conditions as is the case in most stress environments, where input responsiveness pays off in the better years. Figure 4 describes typical germplasm for stress environments, where obviously input responsiveness and -efficiency genes have effectively been combined (Braun et al., 1992).

### *Yield Potential*

Continuing to increase yield potential is one of the major concerns of CIMMYT's Bread Wheat Breeding Program, along with that of expanding genetic diversity. After the introduction of the Norin 10 dwarfing genes (*Rht1* and *Rht2*), there has been a continuous gain in yield potential at the rate of 0.91% per year (=58 kg/ha/year) between 1962-1988 (Table 2). Physiologically, the most recent varieties have cool canopy temperatures (Figure 1), high stomatal conductance (Figure 2), and an elevated photosynthetic rate (Figure 3) (Rees et al 1993). Genetically, these lines possess the 1BL/1RS translocation. The 1BL/1RS translocation has been implicated to impart high yields in optimum, irrigated conditions (Table 3), and in marginal, drought environments (Table 4) (Villareal et al., 1994). Recent studies also indicated high N-utilization and -efficiency (Ortiz-Monasterio et al. 1990), high P-efficiency (Rajaram et al, 1991), and heat tolerance (Hu and Rajaram, 1994) in these lines. If the 1BL/1RS translocated wheats possess these characteristics of drought tolerance, heat tolerance, and N- and P-efficiency, this should partly explain the overall

superiority of these varieties in marginal environments as shown in Figure 4, and in addition their wide adaptation and stability across MEs in general.

In the 1980's, an effort was made to produce ideotypes with semi-erect leaves and tightly arranged tillers. In Table 5, the effect of this morphological character is compared to the original droopy leaf habit, with the erect leaf type showing a yield advantage of 4% (Apichart, 1990). Our highest yielding lines, such as Super Kauz (Table 2), possess this characteristic.

What are our future plans? We have been able to demonstrate the superiority of the 1AL/1RS translocation wheats both in optimum, irrigated conditions (Table 5) (Del Toro et al., 1993), and in reduced irrigated environments (Table 6). This translocation has been widely exploited in programs in the Great Plains, but not yet in CIMMYT. The interaction between 1BL/1RS and 1AL/1RS is not yet known.

We also plan to blend the synthetic wheats (*Ae. squarrosa* x Durum wheat) into the normal germplasm, in order to increase 1000 grain weight, possibly without seriously affecting other yield components such as spike/m<sup>2</sup> and no. of grains/m<sup>2</sup>.

### **Conclusion**

Breeders with a responsibility to develop superior germplasm, be they employed at Universities or at International Centers, must not become so short-sighted to automatically join fashionable bandwagons (Simmonds, 1991), just to attract short-term funding. The strategic decisions involved in issues such as the balance of funding between conventional breeding and biotechnology

should involve weighing against one another the advantages and disadvantages of both technologies, with an eye towards the efficiency of generating long-term output. It is clear that, a superior technology, once identified and proven, must replace an old one, but likewise older methodologies that work should be valued.

The current CIMMYT breeding methodology is based on the principles of increasing yield potential, and incorporating biotic and abiotic stress tolerances, through the use of such methodologies as shuttle breeding, international multilocation testing, modified pedigree-bulk selection, and heavily mechanizing and computerizing operations.

The CIMMYT Bread Wheat Breeding Program serves the poor of the developing countries (both rural and urban) as part of the CGIAR mandate. In doing so, it has developed close links with almost all of the world's wheat scientists on the issues of germplasm exchange and knowledge generation, but also most assuredly on the continuous development of superior breeding technologies, including biotechnology in a proper proportion.

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**Table 1. Mega-environments used by the CIMMYT Bread Wheat Breeding Program**

ME		Moisture regime	Temperature regime	Wheat type	Area (%)	Production (m.tons)
ME <sub>1</sub>	FE	Irrigated	Temperate	Spring	36.1	83
ME <sub>2</sub>	HR	High Rainfall (>500mm)	Temperate	Spring	8.5	25
ME <sub>3</sub>	AS	High Rainfall (>500mm) Acid Soils	Temperate	Spring	1.9	3
ME <sub>4</sub>	SA	Low Rainfall (<500mm)	Temperate/hot	Spring	14.6	20
ME <sub>5</sub>	TE	Irrigated/ High Rainfall	Hot	Spring	7.1	12
ME <sub>6</sub>	HL	Semiarid	Temperate	Spring	6.2	13
ME <sub>7</sub>	FE	Irrigated	Cool	Facultative	]	
ME <sub>8</sub>	HR	High Rainfall	Cool	Facultative	] 10.0	23
ME <sub>9</sub>	SA	Semiarid	Cool	Facultative	]	
ME <sub>10</sub>	FE	Irrigated	Cold	Winter	]	
ME <sub>11</sub>	HR	High Rainfall	Cold	Winter	] 15.0	30
ME <sub>12</sub>	SA	Semiarid	Cold	Winter	]	
Total		m hectares				

FE = Favorable Environment; HR = High Rainfall; AS = Acid Soil; SA = Semiarid;  
TE = Tropical Environment; HL = High Latitude

**Table 2.** Yields for the historical series of bread wheats for the period of 4 years, 1990-93. Cd. Obregon, Sonora, Mexico

Variety	Year of release	Average grain yield (kg/ha)	Average biomass yield (kg/ha)	Harvest index (%)
Pitic 62	1962	6240 ± 273 E	17337 ± 889 BC	0.360 D
Siete Cerros CD	1966	6414 ± 390 DE	17331 ± 1047 BC	0.370
Yecora 70	1970	6982 ± 608 CD	15656 ± 1387 D	0.446 A
Nacozari 76 CD	1976	7035 ± 420 BCD	18211 ± 730 AB	0.386
Ciano 79	1979	7329 ± 478 ABC	18917 ± 1008 A	0.387 C
Seri 82	1982	7400 ± 334 ABC	16729 ± 905 CD	0.442 A
Oasis 88	1988	7656 ± 540 AB	16434 ± 1625 CD	0.466 A
Super Kauz	1988	7729 ± 370 A	18583 ± 970 AB	0.416 B

Genetic Gain 0.95% per year or 58 kg/ha/year

Source: Rees et al. 1993

**Table 3.** The effect of the 1BL/1RS chromosome translocation on yield characteristics of 28 random F2-derived F6 lines (14 1BL/1RS and 14 1B) from the cross of *Triticum aestivum* L. cvs Nacozari76/Seri82, under optimum irrigated conditions during the 1991-92 and 1992-93 crop cycles. Cd. Obregon, Sonora, Mexico.

Plant characteristics	1BL/1RS	1B	Mean difference.	CV (%)
Grain yield (k/ha)	6266	6006	260*	6.0
Above-ground biomass at maturity (k/ha)	15200	14700	500*	9.0
Grains/m <sup>2</sup>	15906	15634	272*	5.8
Grains/spike	44.3	42.6	1.7**	9.7
1000-grain weight(g)	40.19	39.87	0.32**	2.9

Source: Villareal et al. 1994

\*, \*\*: Significant at the 0.05 and 0.01 respectively

**Table 4.** The effect of the 1BL/1RS chromosome translocation on yield characteristics of 28 random F2-derived F6 lines (14 1BL/1RS and 14 1B) from the cross of *Triticum aestivum* L. cvs Nacozari76/Seri82, under reduced irrigated condition during the 1991-92 and 1992-93 crop cycles. Cd. Obregon, Sonora, Mexico.

Plant characteristics	1BL/1RS	1B	Mean diff.
Grain yield (kg/ha)	4945	4743	202*
Above-ground biomass at maturity (t/ha)	12600	12100	500*
Grains/m <sup>2</sup>	14074	13922	152NS
Grains/spike	43.5	40.6	2.9*
1000-grain weight(g)	37.05	36.53	0.52*

Source: Villareal et al. 1994; NS : Not significant  
 \* Significant at the 0.05

**Table 5.** Means of paired F2-derived F6 lines with contrasting leaf types for grain and straw yield and grains/m<sup>2</sup> under optimum management condition during 1988-89 and 1989-90 crop cycles. Cd. Obregon, Sonora, Mexico.

Plant characteristics	Droopy leaf	Erect leaf	SE
Grain yield (kg/ha)	8216 <sup>B</sup>	8584 <sup>A</sup>	± 64.68
Straw yield (kg/ha)	7580 <sup>A</sup>	7364 <sup>B</sup>	± 114.56
Harvest index (%)	52 <sup>B</sup>	54 <sup>A</sup>	± 0.33
Grains/m <sup>2</sup>	25059 <sup>B</sup>	2671 <sup>A</sup>	± 251.74

Source: Apichart V. Ph.D. thesis, OSU, Oregon; Values followed by a different letter are different at Pμ 0.05.

**Table 6.** The effect of the 1AL/1RS chromosome translocation on yield characteristics of 85 individual random F2-derived F6 lines from 3 1AL/1RS x 1A bread wheat crosses under optimum irrigation conditions during the 1991-92 and 1992-93 crop cycles. Cd. Obregon, Sonora, Mexico.

Plant characteristics	1A/1R (39)	1A (46)	Pr>F
Grain yield (kg/ha)	5585	5348	***
Above-ground biomass at maturity (t/ha)	14.8	14.3	**
Grains/m <sup>2</sup>	14341	14310	NS
Spike/m <sup>2</sup>	474	441	***
1000-grain weight(g)	40.2	38.5	***

Source: Villareal et al. 1994. NS: Not significant  
 \*\*,\*\*\*: Significant at the 0.05

**Table 7.** The effect of the 1AL/1RS chromosome translocation on yield characteristics of 85 individual random F2-derived F6 lines from three 1AL/1RS x 1A bread wheat crosses under reduced irrigation conditions during the 1991-92 and 1992-93 crop cycles. Cd. Obregon, Sonora, Mexico.

Plant characteristics	1A/1R (39)	1A (46)	Pr>F
Grain yield (kg/ha)	4388	4282	*
Above-ground biomass maturity (k/ha)	11600	11200	*
Grains/m <sup>2</sup>	12884	12576	*
Spikes/m <sup>2</sup>	414	389	***
1000-grain weight(g)	34.3	33.9	NS

Source: Villareal et al. 1994. NS: Not significant  
 \*,\*\*\*: Significant at the 0.05 and 0.001 respectively

# Wheat Breeding Methodology: A Private Sector Perspective

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John Moffatt  
AgriPro Seeds

## *What is Plant Breeding?*

Plant breeding is the genetic adjustment of plants to the service of man, Frankel, 1958. This is a definition that fits very well into the thinking and philosophy of all breeders whether public or private. The definition remains true whether your involved in a large breeding effort with global impact or a more modest, regional effort. This is the common ground for breeders that I want firmly established before proceeding further.

## *Who is AgriPro Seeds, Inc?*

AgriPro Seeds, Inc. is a private company initiated in 1973 and, as of July 1, 1994 is a wholly owned subsidiary of Helena Chemical Company. Our business revolves around the sale of agricultural crop seed products. The wheat product line is supported by an in-house research and development team that is dedicated to the development of high yielding, high quality, disease resistant hybrids and varieties for the southern Great Plains region including Kansas, Nebraska, Colorado, Texas and Oklahoma. Over the past twenty years AgriPro has developed and released 23 Hard Winter Wheats for the southern Great Plains of the United States. Some of those varieties may be more prominent

in the minds of farmers, and breeders for that matter, than the name AgriPro.

## AgriPro Brand Variety:

1977	WINGS
1978	ROCKY
1981	HAWK
	ARCHER
1983	WRANGLER
	MUSTANG
	RAM
1986	VICTORY
	THUNDERBIRD
	STALLION
1987	TRAILBLAZER
1988	MESA
	ABILENE
1989	RIO BLANCO (hard white)
	WACO
1990	BRONCO
	SIERRA
1992	LONGHORN
	TOMAHAWK
1993	PECOS
	LAREDO
1994	PONDEROSA
	OGALLALA
1995	HICKOK

## *What is our Purpose?*

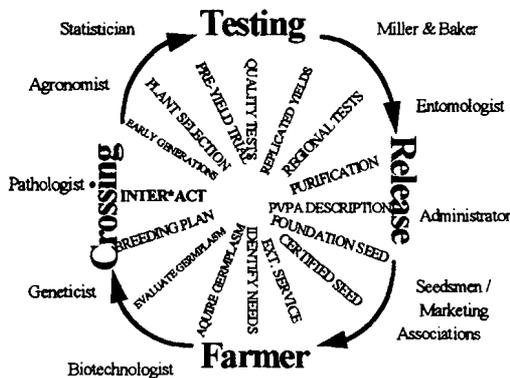
We will spend some time on methodology but I need to stress our purpose which necessarily focuses our efforts and separates us from other

breeding endeavors. Our purpose, as private breeders, is to generate a return on investment through the release and sale of proprietary varieties and hybrids. As a result, our efforts must be directed toward that end.

### ***What is the Process?***

The breeding project is the focus of all activity and the breeder serves as a hub for that activity. The following figure is an attempt to graphically portray what the process of varietal release involves.

### **The Role of Wheat Breeder in a Multidisciplinary Team Breeding Approach**



There is very little in this process that differentiates public from private. In either case the process is complex and involves many different support roles. Perhaps the only significant differences would lie in release committee process but this is true even among public programs. In both public and private breeding the breeder plays a central role. However, due to budgetary restraints in many instances the private breeder is called upon to fill many more of the support roles. In either case the release of a new variety is no small feat and requires a team effort. At Agripro there

is heavy emphasis on the breeder and as a matter of policy and philosophy one breeder is responsible for the region.

### ***What is the Scope?***

The Agripro Hard Winter Wheat breeding program is regional in scope with test locations in five different states. While varietal adaptation generally ignores state boundaries, the public breeder is only responsible and answerable to the constituents of his state. The private breeder is responsible for a broader geographic area and must routinely provide good varietal material to his seed distributors across the region. In many cases these distributors' operations span several states.

Across the five state region Agripro has 19 locations designed to provide yield and adaptation information. We have five additional locations selected primarily for disease observation. Virtually all 24 locations are on-farm-tests with private cooperators. In most cases these cooperators are seedsmen that have been farming the same ground for many years. They know how to minimize production risks on their farm and give us optimum performance estimates for our genetics in their area. They serve as our eyes on the ground and we have timed many note-taking trips on the basis of their recommendations.

We have five main breeding locations. Nardin, OK is a continuous wheat nursery in northcentral Oklahoma where our F2 populations and bulks are grown. Salina, KS is a continuous wheat nursery in northcentral Kansas. Goodland, KS is a dryland site in northwestern Kansas. Dumas, TX is an

irrigated nursery in the panhandle of Texas. Berthoud, CO is our main location and is used for final population work, seed multiplication, and yield potential estimates. In addition, we plant seven irrigated locations running on a line north to south from Dumas, TX to Imperial, NE and seven continuous/fallow wheat locations from Rome, KS to Broken Bow, NE.

We place great emphasis on disease and observation single-row plot planted with precision bubble trays. At those locations which contain only advanced materials in trial we generally plant a set of disease and observation trays which contain a representative row from every hybrid, inbred, variety and parent that we have used in the past four years. Each of our planters is equipped to plant both seven row plots and tray material with minimal change over time. Planting the row materials requires very little land resource and only about 2 hrs to plant. These rows allow the breeder to expose his entire program to many different and unique environments in the same year and to derive maximum benefit from any environment. The benefits lie not only in the verification and selection of parental material but also in moving an entire program in a favorable direction.

### ***Germplasm Makeup***

What factors or methodology sets Agripro apart from other breeding programs? Perhaps the most obvious difference would be germplasm makeup. There are four major components the Agripro Hard Winter Wheat program. The early nucleus of the program came from Colorado State University, a public program best characterized at the time as having a heavy CIMMYT influence (pre-

VEERY). Western European materials also had a heavy early influence tracing primarily to the Nickerson Programs. A little later on Eastern European materials, particularly from Martonvasar, had a major impact on our germplasm base. And last but probably most important is Agripro's Mid-west Hard Red Spring Wheat program. Each source has played an important role in shaping the distinct nature of the Agripro germplasm today.

### ***Computer Assisted Crossing Program***

Another aspect of the Agripro breeding program that may be unique is the use of a parent matching computer program to assist in the identification of key crosses. The program was developed primarily for hybrid test seed production blocks but is applicable to varietal crossing schemes. Fifty times more efficient than random crossing, the program uses the breeder's input of parental strengths and weaknesses and his acceptance criteria to look at thousands of potential crosses, in a matter of minutes, and identify only those that meet the breeder's acceptance levels for all traits being considered.

It is a powerful tool in maintaining the focus of the breeding program and in controlling its direction. Successful use of such a program is based on an accurate, up-to-date computer data base on all parental stocks. Creating the data base is a time consuming process but well worth the effort.

## *Breeding Schemes*

We use three different breeding schemes in the program. The majority of our crossing effort flows through a pedigree program. These crosses would be primarily made up of elite x elite or elite x adapted combinations. New or unique germplasm is generally pushed through single-seed descent to speed incorporation into the main stream. The bulk method is reserved for populations that show limited promise. Populations directed into the bulk program are combined on the basis of a common parent or trait. Five of our released varieties derive from our bulk breeding effort. Our basic breeding philosophy is to use whatever works.

## *Program Flow*

The program flow is not too different from other programs in the region. We make between 1000 to 1200 crosses each year. Twenty-five to forty per cent of our crossing effort is dedicated to germplasm development involving elite x trait or elite x unadapted combinations. As mentioned earlier, our population work is conducted at Nardin, OK and Berthoud, CO. Fifteen to twenty-five thousand individual plants are harvested out of F3 populations and planted as single rows at three locations. These rows are evaluated for general agronomics and inbred characteristics. Selected rows are given a preliminary quality evaluation. Five hundred to six hundred F3:5 inbred lines are then tested at five locations. The following year advanced lines are moved into test hybrid production and/or at least three more years of in-house varietal testing followed by university testing and the SRPN.

## *Selection Criteria*

It has been our experience that the best inbreds are generally the best lines in the program. So, in addition to good inbred characteristics we also look for yield potential, broad adaptation, good test weight patterns, and good pathology. Adding something new or unique to the mix of available varieties is also an important consideration on the varietal side of the program. What we have to offer to the customer must be clearly distinguishable from other wheats that are available. The general philosophy of selection criteria is similar to that for breeding schemes ... if it works, do it!

## *Hybrid Wheat*

Hybrid wheat development is an area that for the most part has been a private industry driven project. Hybrid wheat offers obvious advantages over varietal wheat to private companies, but there are also some terrific advantages to be gained by wheat farmers. We have been working on hybrid wheat for over 13 years and have seen a true yield advantage from the outset. Year-in and year-out the hybrids have displayed more vigorous growth and a greater ability to handle what ever stress the season had to offer. In our efforts over the years to capture a good agronomic type, we have become convinced that hybrid wheat's most important offering to the farmer is not vigor or yield but stability in production. We are in the early stages of test marketing three new hybrids which are first to be released from the Agripro breeding effort. We believe the farmers will see the same advantages to hybrids that we see and will include hybrid wheat as one of his more important management choices in avoiding production risk.

In summary, let me remind you of the private sector's purpose -- to garner a return on investment through the release of proprietary hybrids or varieties. All activity is necessarily directed to a breeding project that is regional in scope and is responsible for a continual flow of state-of-the-art product.

Agripro has a high level of commitment to germplasm development. We have attempted to maximize the benefits of the computer as it applies to a breeding program to the extent of developing a trait matching crossing

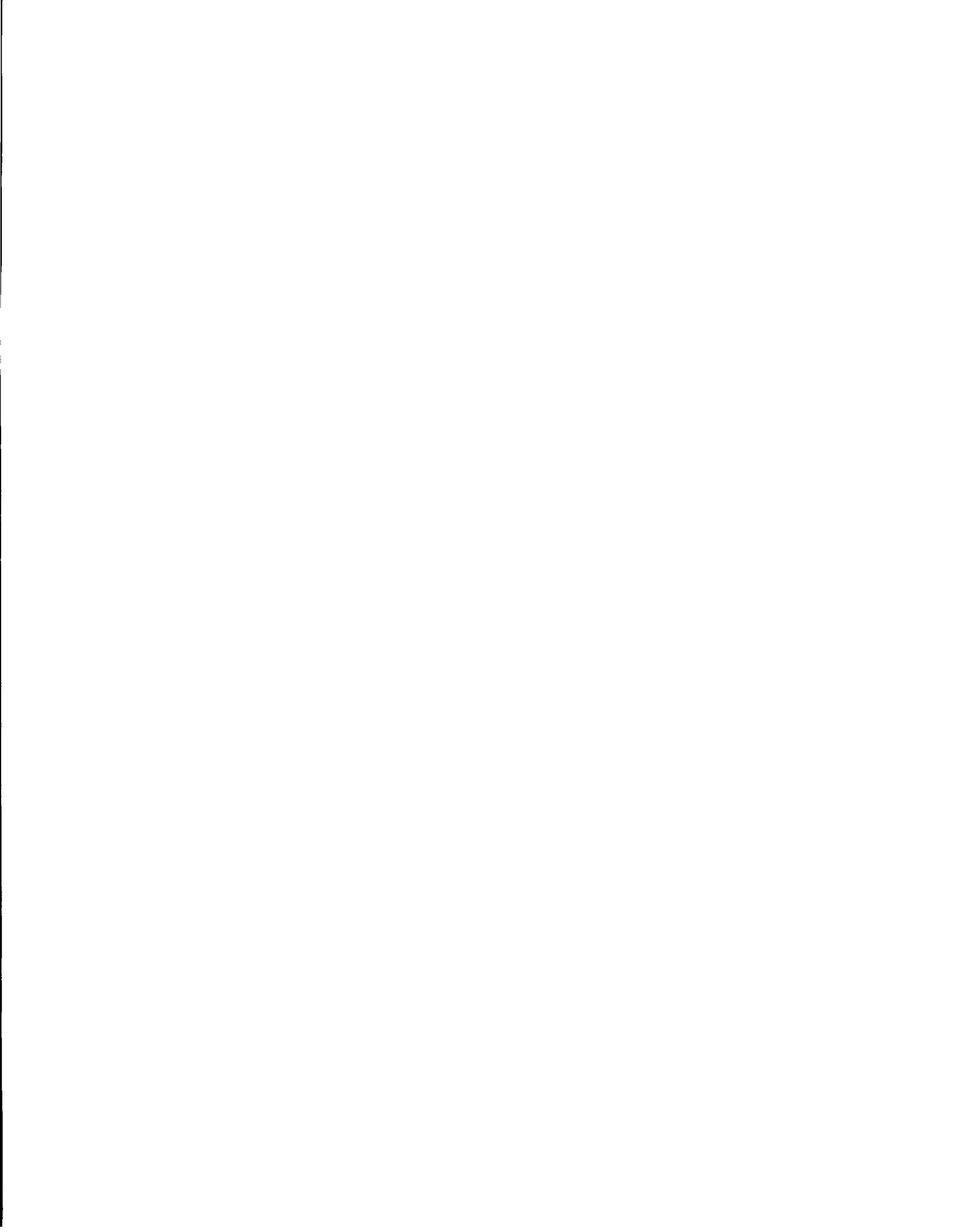
program. Breeding schemes, program flow, selection criteria are not fixed in stone -- **if it works ... do it!**

We rely very heavily on single row disease and observation plots at many locations to gain maximum benefit from what any year give us. Always try to add something new to the varietal mix, give the farmer the **NEW INGREDIENT**.

Hybrid wheat, with the improved vigor, yield and greater overall **production stability** is the future of many farmers in the southern Great Plains.



**Future of Wheat Breeding**



# Future Of Wheat Breeding With Reference To University Programs

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Edward L. Smith  
Oklahoma State University

## *Introduction*

My topic is the Future of Wheat Breeding with Reference to University Programs and my view of the future will be tempered by 30 years experience as a university wheat breeder. I wish to thank David Worrall for asking me to address this topic on your program. He mentioned that with all my years of experience, I should have something worthwhile to say. It's true that I have some seniority among regional wheat breeders. It's also true that I have some definite opinions in regard to wheat improvement. I hope you will find something worthwhile in what I have to report and I'll try to keep my biases in check.

Since my considerations about the future of wheat breeding is conditioned by my experiences in wheat breeding, I would like to review briefly a few things that happened or didn't happen in the region during the past 30 years or so.

1. Semi-dwarf stature. This was a new concept in the region 30 years ago. The first HRW semi-dwarf variety was Sturdy (Seu Seun 27) released by TAMU in 1966. We have come a long way with short stature since then.
2. Hybrid wheat. Interest in the development of hybrid wheat began in the early 1960's. Some thought it would take only a few years to make it work. Efforts have waxed and waned and programs have come and gone. Still, considerable effort is underway at present.
3. Advent of private sector. Private wheat breeding programs came on strong in the region about 25 years ago with work on purelines as well as hybrids. All wheat workers benefited, I believe, when public and private workers joined as colleagues in regional activities (see membership list of HRWW committee).
4. PVPA of 1970. This Plant Variety Protection Act stimulated private sector research and development in wheat by providing a system of 'Breeder's Rights'. 'Brown Bagging' eventually became a problem of serious proportions, and the act was recently amended.
5. Biotechnology. There was a big push starting 15 years ago with regard to the application of biotechnology to plant improvement. Biotechnology was to reduce the time required for variety development as well as to make better genotypes by DNA transfer. However, wheat has proven to be more difficult to

- manipulate than most other crops. So far biotechnology has had little impact on wheat variety development.
6. Specialized wheat research equipment. A good, dependable plot combine harvester arrived on the scene 25 years ago. This was followed by the spin planter and the seed indexer. The adoption of this equipment greatly facilitated field operations in breeding programs everywhere. We all began to move up the numbers.
  7. Publication of the Wheat Workers Code of Ethics. Generally, we don't think of this as very important, but I believe it is. It formalizes guidelines for germplasm exchange and ensures that appropriate credit is given to the owner/originator (NWIC meeting, 1976).
  8. Chromosome engineering. Pioneering work on the use of alien gene complexes (chromosome translocations) for wheat improvement was done by E. R. Sears, USDA - University of MO, with *Aegilops umbellulata*. Later, others made useful transfers from secale and agropyron.
  9. Finlay-Wilkinson GE interaction studies. This concept was popularized by their 1963 paper. This led us to classify environments as well as genotypes and to think about stability parameters.
  10. Computer analysis. The application of computer systems to plant breeding brought significant benefits to the wheat breeder. Statistical analysis of data improved. A large number of items could be dealt with speedily and field book pages and harvest labels could be printed.
  11. Plant architecture. The ideo-type breeding system proposed by Donald in 1968 has been tried by many breeders in the region in one form or another. This approach has not panned out for yield improvement. The exchange of one complex trait (grain yield) for 10-20 simpler traits (components of yield) has not been very effective probably due to invalid assumptions about genetic systems involved.
  12. Disease and insect resistance. We have all been preoccupied with resistance breeding in the region for a lot of years. Leaf rust resistance, for example, has been successful by virtue of diligence and good cooperative programs. Resistance to greenbugs has not been nearly as successful but the work goes on.
  13. Funding. The traditional funding for university wheat breeding programs has been recurring state and federal allocations, along with grower check-off programs. Now we are in a time of change with static or reduced budgets from traditional sources. Many breeders are being encouraged to consider research fees or royalties from new varieties.

So what does the future hold for wheat breeding? I would like to consider this in terms of four of the topics mentioned above. These are: Hybrid wheat, Biotechnology, PVPA, and Funding.

### *Hybrid Wheat*

You may question the inclusion of hybrid wheat in reference to university breeding programs. I believe, however, that what happens to hybrid wheat will have a significant bearing on public breeding programs. Research on hybrid wheat has been going on for three decades. Some success has been achieved. At present hybrid varieties occupy a very small acreage in the region. Acreage devoted to hybrids is unlikely to increase substantially until the relative cost of producing hybrid seed is reduced.

Although the level of heterosis is generally assumed to be greater in outbreeders than inbreeders, substantial levels of heterosis are known to occur in wheat. So far in wheat, heterosis has been difficult to exploit because of the lack of commercially viable hybrid seed production systems. The advent of an effective chemical hybridizing agent has greatly facilitated the making of experiment hybrids for testing and evaluation. The use of CHA as a method for pollination control is likely to be more attractive to breeders in the future. My own impression about the future of hybrid wheat is positive rather than negative (within limits, that is). For one thing, hybrids may be intrinsically better buffered than purelines against certain types of production stresses (increased stability).

Even with effective CHA systems, hybrid seed production remains a problem

of concern. The concern is due primarily to the relatively low rate of seed multiplication (seed sown to seed harvested). This is 50 to 100 for wheat compared to 500 to 1,000 for maize, sorghum, and sunflower. This is a difference of an order of magnitude. The difference is due to plant morphology and no amount of breeding is likely to change this to any great extent.

Some wheat workers have suggested that hybrids, if successfully commercialized, will be limited to high production situations, since higher yield levels obtained by the growers would tend to offset hybrid seed production costs (fixed costs). In any event, seed production problems will tend to limit the adoption rate of hybrids. Perhaps 15 percent of the wheat acreage is the maximum area in the region that would be dedicated to the production of hybrid varieties in the future.

### *Biotechnology and Plant Breeding*

Certain authorities said 15 years ago that biotechnology was just around the corner. Well, it's still around the corner with regard to wheat improvement. Early on, the popular press got into the act. Traditional plant breeding was described as imprecise, slow, halting, error-prone, second class. Biotechnology on the other hand was described as cutting-edge-science, fast, precise, glamorous, first class. The hype was almost overwhelming. Over the years these distinctions have blurred somewhat so that now plant breeding and biotechnology are considered by some researchers and most administrations as partners in plant improvement. Even so, the partnership is an uneasy one with many points of disagreement. But biotechnology and

plant breeding share common goals and objectives and often compete for funding from a common source. They share a common vocabulary, up to a point. Some divergence occurs in the interpretation of the word "success". When a biotechnologist says that he has achieved success, the traditional plant breeder thinks he is talking about a new cultivar ready for release to growers. What the biotechnologist means is that he has successfully made a callus culture, or regenerated a whole plant (perfecting a step in the process). Perhaps we should be more generous and more objective in our outlook. For better or worse, biotechnology and traditional plant breeding must travel the road together. We need to remember that both systems have limitations. What biotechnology cannot do is bring about new multigene recombinations of characters to build a completely new range of adaptation. Traditional plant breeding can do this. In fact, this is the essence of traditional plant breeding. This point seems to be overlooked by those whose job is to publicize biotechnology. Traditional plant breeding is limited to gene exchange within compatible matings. Biotechnology can range farther a field.

Certainly, efforts in biotechnology have resulted in substantial progress in the basic understanding of molecular genetics and cell culture. We have learned to appreciate the potential of biolistics as a method of moving DNA about and of 'BOBWHITE' as a laboratory responsive wheat variety. However, the application of biotechnology to wheat breeding and variety development is not yet a viable enterprise. Biotechnology will not be a panacea. It should, when techniques are perfected, permit researchers to reach certain goals faster and possibly at less

cost as compared to traditional wheat breeding.

In the future, I believe, there will be a need for both the traditional wheat breeder and the biotechnologist, for each will complement the other. For wheat breeding positions in the university setting, where funding is a problem, administrators are likely to seek an individual with training and capabilities in both systems. In this type of position with dual expectations, neither component is likely to turn out as well as expected. Usually the dual situation is slanted towards biotechnology, and traditional plant breeding suffers the most.

#### *PVPA*

The original Plant Variety Protection Act (PVPA) became law in 1970 (PL91-577). The primary feature of the act was to provide developers of 'novel' varieties of sexually reproduced plants exclusive rights to those varieties for 17 years. Protection was intended to have the attributes of ownership of personal property. The rights were assignable and formed the basis for the collection of royalties. The breeder/owner could protect his rights by privately filing suit in civil court. With such a system of protection of "breeders rights" private seed companies were willing to invest significant resources in variety improvement in certain crops including wheat and other small grains. Up to that time, variety development in the small grain crops was largely carried out by the public sector. However 20 years of experience indicated that the PVPA as originally structured failed to provide adequate protection to the originator/owner of wheat varieties. In the HRWW region there evolved a brisk business in "brown-bag" sales of protected

varieties. Pressure by the commercial seed trade led to a revision of the PVPA in an attempt to plug some of the gaps in the original act.

The PVPA act of 1994 (signed by President Clinton on September 21) contains several important amendments. Probably the most important one for wheat workers in the region is the absence of the so-called farmers exemption. Under the 1994 amendment, it will be a violation of the act for farmers to sell saved seed of protected varieties without authorization from the owner. Another change is that the act will now contain language to meet standards of UPOV (Union for Protection of New Varieties of Plants) which is the international plant breeders rights organization. Only those applications submitted for protection after the act becomes law will be covered by the new law. Varieties being reviewed now or those submitted before the effective date must be withdrawn and re-filed in order to receive amended protection. Varieties which possess a certificate awarded under the existing law (PVPA of 1970) cannot be reconsidered under the revised law and cannot claim such protection. The revised law will take effect 180 days after being signed by the President.

With the new Act of 1994, certain questions remain: 1) Will the new act be more strictly enforced, 2) Will the act restrict germplasm exchange, 3) Will the act tend to alter the present balance of private vs public breeding programs, 4) Will the act affect decisions on research and development of hybrid wheat, 5) Will the act increase commitment of public breeding but decrease exchange of germplasm in order to maximize profits? We will have to wait and see. In any event, the original PVPA was not doing

the job for wheat workers. Perhaps the new act will do a better job. For plant breeders, there will still be much concern regarding germplasm exchange.

### *Funding*

Oh, for the good old days! Not long ago, times were better, or so it seemed to public plant breeders. It seemed that the work we were doing was worthwhile and that we were appreciated by everyone. We had strong support for our breeding programs, and we were not held accountable for our efforts. At least we tend to remember it in that way.

No longer. Now, budgets are reduced, and we are held accountable for the way we spend the tax-payer's money. We are expected to do more with less. The funding base is fragile and we are preoccupied about how to protect our funding sources in the future. One writer recently summed up the situation: "The public no longer wishes to support plant breeding and genetic research with tax dollars, in increasing amounts, unconditionally for all time, without question".

So, amidst funding short falls and fragile budgets university researchers and administrators are looking more closely into the commercialization of plant breeding programs. In the university setting, administrators are considering royalties, research fees, license arrangements and other means of deriving income from the products of plant breeding. Some have stated publicly that university plant breeding programs must pay their own way or walk the plank.

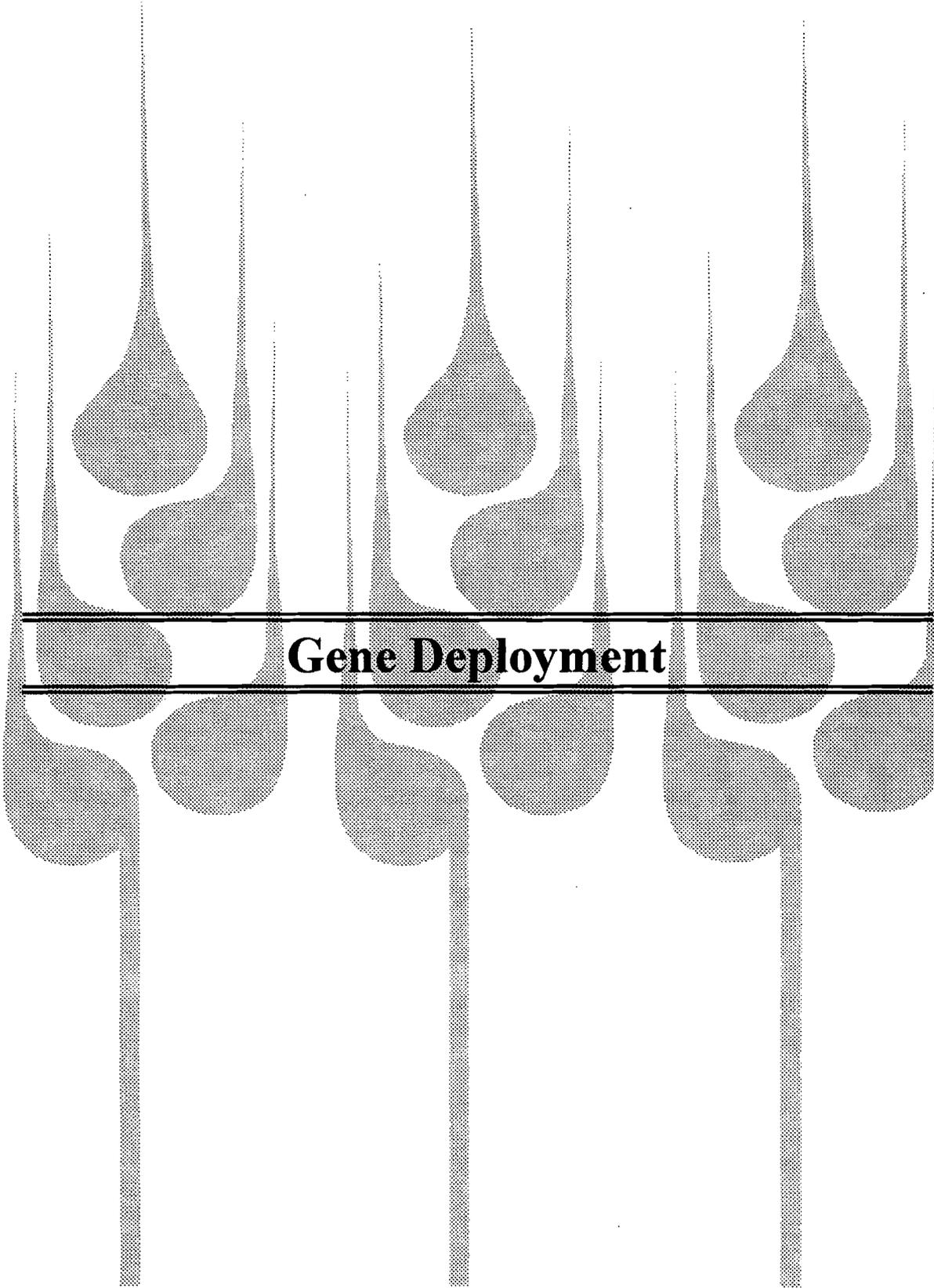
Universities may seek partnership arrangements with other universities and/or the USDA to keep plant breeding programs viable. In all probability, university programs more and more will seek out partnerships with private industry. In the near future, a possible scenario is that the university will gradually transform itself into a tax-payer-subsidized business, doing research and development work in a number of areas, including plant breeding.

For the university, an acceptable underlying principle should be that the research program must maintain quality of service to traditional clientele without compromise. Publicly funded plant breeding research programs must be relevant to the public needs, and must be responsive, accountable, and equitable if they are to be sustained. It must not forsake traditional values for expediency.

### *Conclusion*

The message is clear. We are under increasing pressure to do more with less. In the university system it is becoming more and more difficult to justify tax-supported increases for wheat variety development and other production oriented research projects. Will the universities still be in the wheat breeding business 25 years from now? Yes, I think so, but perhaps with a biotechnology flavor and with much more preoccupation on how to derive income from plant breeding efforts. Continuation of the present level of wheat breeding in the university system is not a foregone conclusion. Breeding programs on some crop species are being discontinued at certain universities. In the region wheat appears to be safe at the moment but the axe could fall on some programs facing serious budget shortfalls.

Certainly, in the future, we should strive to strengthen the interaction between private and public sector breeding programs. Each can and should complement the other with such things as germplasm exchange, graduate student training, regional workshops and variety testing. One authority recently stated that in the future there will likely be more concern about efficient administration of wheat breeding programs and less emphasis on the technical aspects of plant breeding. In spite of all this, I believe there will be adequate time and funding for the practice of the science and art of wheat breeding at the university.



**Gene Deployment**



# How Can We Slow Evolution of New, More Virulent Leaf Rust Races?

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## A. Background - Rust Epidemiology

How does rust epidemiology "work". The hard winter wheat (HWW) region of North America stretches nearly 2,500 miles from Texas, northeastern New Mexico, Oklahoma, Colorado, Kansas, Nebraska, to eastern Wyoming, then continuing in a narrow band in western South Dakota, southwestern North Dakota, northcentral Montana, and southern Alberta. In terms of rust epidemiology, the hard spring wheat areas of Mexico, the Dakotas, Minnesota, Saskatchewan and Manitoba must also be taken into account. It has been shown repeatedly that there are about three epidemiological "units" (geographic regions) for leaf rust in the hard wheat area. {An epidemiological unit is an area within which rust inoculum moves freely and the rust races are very similar. Some inoculum moves from one epidemiological unit to another, but the amount is very small compared to inoculum movement within an epidemiological unit.} These three units for leaf rust are (1) southern Mexico; (2) northern Mexico and south Texas; and (3) central Texas to Alberta. For stem rust, there are about six or seven epidemiological units over the same area. The epidemiological units for leaf and stem rust differ mainly because leaf rust can persist over a wider range of environmental conditions than can stem rust (mainly interpreted as the ability of leaf rust to overwinter over a much wider

area than stem rust). The similar race composition of leaf rust throughout the HWW area is consistent with rapid, long-distance spread annually, therefore the selective effects of resistance genes must be taken into consideration as a whole when accounting for leaf rust virulence frequencies in the HWW area.

The overwintering ability of leaf rust is a very important key to understanding leaf rust spread and persistence. It also has a significant impact on how we should use host resistance to manage the disease. Over the entire hard wheat region, the distribution and intensity of overwintering should be viewed as a gradation going from essentially no overwintering in Alberta, to common overwintering in Texas. Overwintering has been reported as far north as Alberta, but is quite rare. In most years, leaf rust will overwinter from about central Kansas through Texas. In years having severe winters, overwintering may be limited to south-central Texas. Because the growth of the disease is more or less exponential, the more disease there is at the "start", the greater the amount added in a short period of time. Overwintering sites usually occur as foci and can be found on older leaves early in the growing season. Infections caused by spores deposited by wind or rain from exogenous sources are usually more dispersed and tend to occur on the younger leaves, nearer the top of the canopy. Once rust has "taken hold" in a

field (established either through overwintering foci or exogenous inoculum), then further increase is a function mainly of the field's inoculum.

Rust spores are airborne, and are generally spread in a south-to-north direction beginning in late winter and continuing through the summer months. In late summer and early fall, some spores are transported in a north-to-south direction. These spore movements coincide with wheat development. Rust spores can travel great distances, yet most (about 90%) are deposited within about 100 ft or less of a given inoculum source. Most of the remaining 10% are deposited within moderate distances from a source, and a small percentage get taken up to high altitudes, where long distance spread can occur. {These percentages can be somewhat deceiving due to the tremendous numbers of spores produced.} In reality, the traditional "*Puccinia* Pathway" is a rather simplified view of rust spread. In addition, there is some west-to-east spore movement, and late summer - early fall inoculum in the south may come from oversummering sources, as well as a small amount from Mexico (inter-epidemiologic unit spread). Nevertheless, in a given growing season, rust typically begins to increase in the overwintering areas (usually the south) and then spreads north. Initial rust development north of overwintering sites must depend upon inoculum produced in the south. If there was little or no exchange of inoculum within the epidemiological unit, then local management practices should be enough to limit rust development.

The factors affecting rust epidemics are host, pathogen, environment, and time. The first requisite for development of rust epidemics is a

large number of spores early in the season. In general, the environmental conditions needed for the development of a good wheat crop are also conducive to leaf rust development. Other than overwintering, perhaps the next most influential factor in pathogen development is the duration and amount of sporulation, because large numbers of spores produced over a long period of time serves to bridge periods during the season that are adverse to infection. For epidemics to occur, you need large acreages of susceptible varieties and adapted races. In order for a great deal of inoculum to build up in the southern parts of the region, there needs to be an extended period of cool nights and warm days in the overwintering areas (the time for this to occur is about February in central Texas, Feb.-March in north Texas, March in Oklahoma, and perhaps April in northern Kansas.

## **B. Pathogen Variation**

Without question, the chief cause of impermanence of disease resistance is the appearance and rapid distribution of new races of a pathogen. What are the driving forces behind the development of new races? There are two basic forces operating. The first is the hard-learned force of "man-guided evolution" where new resistance genes are matched by new virulence genes (or the absence of avirulence genes). This produces rust races that may not necessarily be the "fittest" overall, rather they are simply the best adapted to a given variety (resistance gene combination). The second force is more subtle and is often masked by the first. It can be termed "nature-guided evolution." It can be viewed as the background evolution that is always occurring (mostly at random), and on which is superimposed wheat variety-

guided selection. This background level of evolution can explain why some races carry virulences to which they have never been exposed. It can also explain why some virulences appear seemingly “out of nowhere.” Given that the background variation is virtually unpredictable, what should be our strategy to lessen the effects of man-guided evolution?

- *Stock Market Analogy*

The occurrence of leaf rust races is very much analogous to companies in the stock market. At any given time you have some companies (races) coming into the stock market (wheat crop), other companies disappearing. The companies tend to wax and wane over varying periods of time. Occasionally brand new companies appear. In a given time period, the number of companies in the market fluctuates. Sometimes nearly all of the companies are on the increase; at other times, some companies are increasing and others are decreasing. The performance of all the companies is affected by internal and external factors. However, it is virtually impossible to predict which companies will do best in the future.

The analogy can be taken even further. The stock market today looks very different from how it looked 10, 20, or 30 years ago. Some of the companies in the market today did not exist 30 years ago. Yet the market seems to be “progressing” in the sense that the types of companies that are most frequent and which are doing well individually, are those companies that are more contemporary (for example telecommunications, computers, etc.). Similarly, leaf rust races seem to “progress” in response to modern wheat varieties.

What then, is the best strategy to achieve long-term gains in the stock market? The answer, as any financial advisor will tell you, is to diversify. Yet what has been the answer to achieve durable leaf rust resistance --- *the sequential deployment of single resistance genes*.

**C. What are the Facts about Leaf Rust Epidemiology?**

- (1) The pathogen is highly variable and the variability is unpredictable.
- (2) The hard winter wheat area is a single epidemiological unit.
- (3) It is easier to manage leaf rust when its populations are low.
- (4) Resistance genes that confer complete control (immunity or near-immunity) are rapidly rendered ineffective when deployed “singly”.
- (5) Spaciogenic (spatial phenomena of germplasm diffusion) uniformity for leaf rust resistance is an invitation for an epidemic.

In the face of these facts, what can we do locally and as a region? Locally of course, many steps may be taken to diversify (or narrow in some cases) germplasm for leaf rust resistance.

Several steps for leaf rust resistance can also be taken on a regional basis. Some of the measures are already being done well; others are being done, yet need to be modified or increased; and some are new initiatives. Unfortunately, a “regional initiative for the control of leaf rust” is often equated to “gene deployment” in its traditional sense (delimitation of geographic areas in which selected resistance genes would be bred

into varieties). A “regional initiative” should be defined in much broader terms.

At the North American Wheat Workers Workshop (7-9 March 1994, Kansas City, MO), a proposal for the North American Program for Cereal Diversity (NAPCD) was put forth. The proposal was to initially develop a policy or set of guidelines for the management of leaf rust resistance in wheat. The proposed program outlined six steps that could be operational in a 3-5 year period. They were:

- (1) Diversification of the use of *Lr* genes.
- (2) Release unexposed *Lr* genes only in combinations.
- (3) Eliminate the release of varieties possessing single, immunity (or near immunity) conferring resistance genes, particularly in rust-prone areas.
- (4) Better identification of *Lr* genes in released and potential varieties.
- (5) Coordination of federal, state, and private organizations involved in developing and releasing rust resistance varieties.
- (6) Timely exchange of information between all involved.

In terms of spaciogenic diversity, and what we know about leaf rust epidemiology, a south-north separation of *Lr* genes appears intuitively attractive. Of course, there are many details that complicate the problem: such as which genes go where; where should the “dividing line” be; what about widely-adapted varieties; etc. Most of the problems associated with *Lr* gene diversification would be rendered insignificant if there were, say 20

unexposed, equally effective, different *Lr* genes available for incorporation into varieties. The fact of the matter is the number available is few (see attachments). More importantly, even if 10 *Lr* genes were available to “southern” breeders and another 10 available to “northern” breeders, the sequential release of single *Lr* genes still needs to be rigorously avoided.

We, as representatives of the hard wheat region, could support and strive to reach the goals of the proposed NAPCD, in the form the following (as examples): (i) a revived Uniform Rust Nursery; (ii) increased participation in the annual rust survey conducted by the USDA-Cereal Rust Lab; (iii) generate more information concerning the types and identification of leaf rust resistance factors in released and potential wheat varieties; (iv) through the NWIC, support increased funding for the USDA-Cereal Rust Lab to continue rust survey and germplasm identification activities; (v) through the NWIC, support increased funding for the transfer and incorporation of new *Lr* gene combinations into adapted varieties and breeding lines (such as the USDA component of the Wheat Genetics Resource Center at Kansas State); (vi) increase efforts to identify and incorporate forms of general resistance into wheat germplasm and varieties; (vii) freely exchange information concerning rust resistance in varieties and germplasm in a timely manner; and (viii) seek-out ways to implement and promote varietal diversification at a local level.

# Thoughts on the Concept of Gene Deployment for Improved Leaf Rust Resistance

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University of Nebraska

Gene Deployment intuitively makes sense. However, it is a relatively old idea and one must wonder where it has been used and has it been successful. If it has been successful, could that success be duplicated in the Great Plains? Is this *deja vu* all over again? One should also wonder what are the tradeoffs of using a gene deployment strategy especially as that strategy affects alternative resistance strategies (gene pyramids, chemical control, adult plant resistance, slowed infection, or integrated disease management).

A possible example of a gene deployment success has been the widespread use of Lr16 in predominant Nebraska cultivars (Brule, Redland, Arapahoe, and Vista). Few cultivars in the southern Great Plains use this gene and the resistance of the above lines seems to have lasted longer than other leaf rust (incited by *Puccinia recondita*) resistance genes. It should be noted however that the leaf rust resistance in the differs, hence Lr16 is part of a pyramid of genes. Also, virtually all of the cultivars show some level of infection (meaning the resistance may achieve durability by slow rusting or delayed susceptibility). Finally, Nebraska does not seem to have the severe leaf rust epidemics that are found further south where *P. recondita* is able to over winter. Leaf rust must be reintroduced into

Nebraska every year. Finally, it should also be recognized that individual spore cultures of *P. recondita* in Nebraska are virulent on numerous genes. For example, the most frequent *P. recondita* phenotype in Nebraska in 1993 was virulent on Lr1, Lr2a, Lr2c, Lr3, Lr10, Lr11, Lr18, Lr24, Lr26, and Lr30 (J. E. Watkins, personal communication). With this mind, what are some of the ramifications of a gene deployment strategy?

## Specifically:

1. A decision would have to be made as to what are deployment regions-these regions would be biologically defined and not governmentally(country and state borders are meaningless). We know the existing field based deployment pattern of growing cultivars in separate fields does not work. At what scale would it work? The current concept of having a deployment region from northern Texas to Alberta intuitively seems too large, particularly as *P. recondita* is often not a significant problem in Nebraska, and resistance to the disease is not vital for our decision to release wheat cultivars (Rawhide, Alliance, Nekota, and Niobrara are susceptible, whereas Arapahoe and Vista are resistant).

2. Similarly, how deployment regions interact needs to be known (i.e. could the same gene be allocated to two different deployment regions if the two regions were considered as being "independent").
3. A gene allocation strategy would have to be developed with some understanding that genes may need to be allocated on the basis of turn-over. For example, parts of Texas may be allocated more genes than Nebraska because Texas is "hotter" for virulence changes than Nebraska.
4. It is also important to develop reasonable expectations for a gene deployment strategy. For example, if Texas or Kansas currently have all of the *P. recondita* resistance genes available and *P. recondita* is not being controlled genetically, would gene deployment with fewer genes available to either state, make a difference? It may be that hot spots for *P. recondita* are such that gene deployment does not affect them, but may help in regions away from the hot spots.
5. An understanding of how allocated genes can be used within the region would need to be developed. For example, should allocated genes be used singly or in pyramids. *P. recondita* pyramids its virulence genes which may mean that once a race develops with virulence on the genes in adjacent regions, that the race can readily mutate in the next region to attack the allocated genes. Would there be a "cascade" effect where races develop in the hot spots on all of the available genes and then mutate for virulence in the next region and in the next region, etc.?
6. If we had gene deployment systems, would we also have cultivar deployment systems? Would a broadly adapted cultivar like Siouxland, TAM107, or Karl not be allowed to be grown outside of the gene deployment region? What good is it to deploy genes and then allow cultivars that contain those genes be grown anywhere.
7. A gene deployment system would be voluntary among breeders, seedspersons, and producers?

#### Some other considerations:

1. A gene deployment strategy would either restrict germplasm exchange (lines are not used as parents outside the deployment region) or require selection against lines containing genes that were not allocated in a region. The latter is a problem that patented genes also poses and has been of considerable concern for breeders as they approach biotechnology. Similarly, resistance genes often come in clusters of useful genes, for example the genes on 1BL/1RS. If Lr26 were allocated to one region, that could preclude Sr31 from being deployed where it is most needed and similarly the powdery mildew gene.
2. As mentioned above, it would restrict commerce.
3. It may violate institutional or governmental policies of freedom of access. The Plant Variety Protection Act requires protected lines to be available as parents for the development of new lines. The gene pyramid and its component parts

would be available without restriction of use.

4. Fewer genes would be available within a deployment region for use and for pyramiding.
5. Maybe there are limitations to genetic-based resistance. Efforts involving fungicide, genetic-based resistance, and avoidance should be attempted.

6. Some consideration should be given to the concept of gene pyramiding. First, to be successful, it would require unused genes to avoid the step by step loss of resistance genes when genes are deployed singly and multiply. The unused genes would be unavailable to other programs which means that there will be fewer genes generally available. Three genes probably would be needed to avoid the problems with rapid gene loss as is the case in single and even two gene resistance. Secondly, the probable mechanism of incorporating the genes will be through backcrossing each gene singly into an elite line and then intermating those single gene resistant lines and selecting the three gene pyramid. The difficulties with backcross breeding are well known-namely by the time an elite line is identified and used as a backcross parent, and the seed increased to commercial scale; the genetic background has become obsolete. The next concern would be the use of the three gene line as a parent with the implicit understanding that the three genes are to be kept together in the resulting progeny if they are released. Keeping the three genes together will depend on the generation of selection.

Generation of selection	Homozygous Resistance 3 genes	Phenotypic Resistant (1 to 3 genes)	Susceptible (no genes)
F2	1/64	63/64 (27/64)*	1/64
F3	27/512	485/512 (125/512)	27/512
F4	343/4096	3753/4096 (729/4096)	343/4096
F <sub>o</sub>	1/8	7/8 (1/8)	1/8

\*Number listed in parentheses is the frequency of lines that contain all three genes in a homozygous or heterozygous condition. Selection of these lines would allow the possible selection of three gene homozygous lines in their progeny.

The selection of three gene resistance in a segregating population will probably begin by eliminating the susceptible types and then screening for the resistant genes. For example, if one selected in the F<sub>2</sub>, 63/64 of the population would be screened with markers and 1/64 would be selected as three gene homozygotes. If the interest was in selecting lines that contain the three resistance genes in a homozygous or heterozygous form, then 27/64 would be selected. Of course, further selection would be required in later generations. The advantage of including heterozygous lines would be the maintenance of a sufficient population size to select for the other traits of interest (such as yield and quality). However, the difficulty or the amount of work of needed to identify lines having three homozygous genes from lines that are resistant should not be underestimated. For example, it may be difficult to distinguish a line with three homozygous genes from one that has two homozygous genes and is heterogeneous for the third gene, even with good markers. It becomes a population/sample size question. To increase the proportion

of phenotypically resistant lines, selection could be delayed until later generations. However, the best case would be 12.5% of the population (14.3% of the phenotypically resistant lines) would have three homozygous resistant genes. Again the population size would have to be large to effectively work with other traits of interest.

One final concern with gene pyramiding is that random mutations to virulence occur in *P. recondita*. Every resistance gene breakdown is due to these random virulence mutations which is then selected for by the deployment of a leaf rust resistance gene. These random mutations may develop for the three unused genes during the backcross and gene pyramiding phase. Obviously, a gene pyramid with one defeated gene and two

effective resistance genes will greatly reduce the power/effectiveness of the gene pyramid. However, it would be doubtful that another effective, unused leaf rust gene would be allocated to the pyramid project because genes randomly become defeated indicating the pyramid theory is flawed, and only a few genes should be allocated for an untested theory as each allocated gene reduces the genes available to others. In conclusion, the conundrum is that breeding for resistance is caught between practices that have often failed and theories with obvious flaws. Perhaps the best approach is begin the experiments that test the theories so that the necessary information can be obtained to weight the different approaches.

# How Can We Slow the Evolution of New More Virulent Wheat Leaf Rust Races?

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## Statement of Problem

The “boom and bust cycle” of wheat leaf rust resistance genes causes undesirable fluctuations in yield and quality for farmers and end-users. For example, estimated yield losses in Kansas were 11% in 1992 and 1993. Many fields suffered in excess of 20% loss. Because new cultivars typically lose their resistance after several years of large scale production, there is a constant burden on wheat breeders to develop new resistant cultivars. This reduces effort that could be applied to increasing yield potential or quality. Under current conditions, new resistance genes have a limited useful lifetime when used as a single gene. It is possible that we may run out of new genes some day.

## Objective

Develop methods to avoid boom and bust cycle; prolong the usefulness of resistance genes.

## Background

### *Boom and bust cycle.*

A boom happens when a new cultivar is released with good rust resistance, usually relying on a single gene for protection (though additional defeated genes may also be present). The new variety has good yields and it becomes popular. After a few years, it occupies

large acreages. The bust is due to genetic shifts in the rust population. A virulent race arises either by mutation of existing races or by immigration from some other region. Selection increases the frequency of the new virulent races in the rust population. Yields of the formerly resistant cultivar are then reduced and more variable so acreage decreases. Some recent examples in Kansas include Newton, Arkan, Siouxland, AGSECO 7846, TAM 200, Abilene, Mesa, and Karl. Based on current information, Jagger could follow the same pattern.

### *Gene-for-gene interactions between rust and host.*

Most genetic interactions between leaf rust and wheat are probably governed by Flor's gene-for-gene (GFG) theory. Although the underlying mechanisms of GFG interactions are not understood, GFG interactions are common for biotrophic pathogens. For each (usually dominant) gene for resistance in the host, there is a corresponding (usually dominant) gene for avirulence in the pathogen which is somehow recognized by the host resistance gene. This results in incompatibility between the host and the pathogen. It is manifested as a lower pustule infection type and/or reduced reproduction of the pathogen. The interaction is compatible when either the avirulence gene is lacking or the resistance

gene is lacking or both. Corresponding gene pairs are usually independent in action. However, only one incompatible gene pair is required to condition avirulence. Any incompatible gene pair is epistatic over compatible gene pairs.

Because the pathogen must be able to simultaneously defeat all of the resistance genes in a cultivar in order to be compatible, old defeated genes can still be useful in certain combinations. For example, there are races which are virulent on *Lr9* and other races which are virulent on *Lr16*. However, there are no known races which are virulent on both. Therefore the combination of *Lr9* and *Lr16* should be resistant in the field.

Avirulence and resistance are not always dominant traits. Kolmer and Dyck (1994) found a range from complete dominance to complete recessiveness for resistance genes in the host and from complete dominance to complete recessiveness for avirulence genes in the rust. Pathogen and host genes can interact in complex ways. For example, a resistance gene may appear dominant with a homozygous avirulent rust isolate, then appear recessive with a heterozygous isolate (Samborski, 1963).

A few exceptions to GFG have been found in this system. *Lr27* and *Lr31* are only functional when both are present. Genetic background can affect the expression of some resistance genes (Johnson, 1984). Also, certain genes are synergistic such as certain combinations with *Lr13* (Kolmer, 1992b). Finally, there may be some genes that provide nonrace-specific resistance.

### *Coevolution of rust and wheat.*

Our understanding of the coevolution of leaf rust and wheat is fairly good. The pathogen has no significant sexual cycle in North America (Kolmer, 1992a) and there is no evidence of parasexual genetic exchange. Therefore the only known means of genetic change is through mutation. Apparently, immigration from other continents is also minimal. Our current rust population apparently consists of a limited number of clonal lineages which have mutated into different virulence phenotypes.

Major sources of information on rust evolution are rust virulence surveys and changes in cultivar field reactions to rust. Numerous races have been detected using sets of cultivars with different single gene resistances. The leaf rust population seems to be divided into Pacific Northwest, Midwest, Northeast, and Southeast populations with limited mixing between the populations (Leonard et al., 1992). *Aegilops cylindrica* (jointed goatgrass) may also have its own distinct subpopulation (Dave Long, pers. comm.) Within a biogeographic area, race changes have been documented over time (Kolmer, 1989). Most of these changes are attributable to changes in the frequency of various host resistance genes in commercial wheat fields. One problem is that we have incomplete information on resistance genes in current cultivars (McVey and Long, 1993). Typically, new cultivars are protected by only one or two effective genes.

In almost every case, virulence changes seem to occur one gene at a time. The recent simultaneous appearance of virulence to *Lr3ka* and *Lr30* appears to be an exception. The two corresponding

avirulence loci are tightly linked in the pathogen (Jim Kolmer, pers. comm.). It is not known if the linkage accounts for the simultaneous occurrence. A deletion mutation could possibly explain it, but it would have to occur in both nuclei if avirulence was dominant. Tight linkage between some avirulence genes has been observed in leaf rust, but most pairs are unlinked (Kolmer, 1992c). Deletion mutants may also suffer a fitness penalty.

After defeated host resistance genes decrease in frequency, the corresponding specific virulence usually remains at high frequency in the rust population. It is common for current rust isolates to have virulence to six or more resistance genes. This indicates that there is no strong selection pressure against rust races with multiple virulences. Exceptions to this rule seem to be *Lr9* and *Lr16* which have decreased in frequency after resistance gene frequency was reduced. However, it is dangerous to extrapolate rust survey data into fitness estimates for individual genes since all genes in the rust genome are effectively linked due to lack of sexual recombination (Kolmer, 1989, 1992c). There is not much evidence for residual efficacy of defeated host resistance genes against compatible pathogen races (Johnson, 1984).

*The pathogen has three major weaknesses.*

First, there is little or no recombination among races of wheat leaf rust in N. America (Kolmer, 1992a). Mutation is the only proven mechanism for genetic change. Every character is in linkage disequilibrium so deleterious mutations cannot be easily shed. Also, the pathogen cannot bring together virulence from different races. Therefore, defeated genes can remain useful if used in

particular combinations. This would not be true in a sexual population.

Second, the pathogen is dikaryotic which is equivalent to diploid. Avirulence is usually dominant; therefore mutations to virulence usually must occur in both nuclei before compatibility is achieved. The pathogen may be able to achieve compatibility in several ways. There is a finite probability (somewhere around  $10^{-12}$  per spore) of simultaneous double mutations. Many loci seem to be heterozygous even before selection pressure is applied (Kolmer, 1992c). In that case, only one nucleus must mutate. In other cases, the heterozygote is partially virulent and has a large advantage over the homozygous avirulent races. In that case, mutations can occur sequentially.

Third, the population level regularly crashes during early summer and again in late winter. This should increase genetic drift and the probability that favorable mutants will be lost by chance.

*Wheat and wheat breeding programs also have handicaps.*

Wheat tends to be grown in large, genetically homogeneous fields. A few cultivars account for the majority of the acreage in a region. This favors specialization by the pathogen.

Wheat breeding programs have limited resources. There are many breeding objectives and resistance to leaf rust is just one of these. Until recently, most efforts to breed against leaf rust in the Midwest have been totally empirical. In many cases, the genes which give low rust reactions in new materials are not named or even known; the genes we know are often not the important ones. Breeding

for rust resistance often must be done in the field. This has the advantage of detecting certain kinds of resistance (e.g. slow rusting or adult plant resistance) that might be missed in seedling assays. It also allows detection of rare races that might be virulent on new germplasms. However, field tests suffer from variable disease pressure and there is a risk that the population of races in a given nursery may not be a representative sample. Another handicap for breeding programs is the long lag time between "designing" a cultivar and its availability to a large number of producers. This almost precludes rapid responses to shifts in pathogen races.

There are also several technical problems which make breeding for rust resistance difficult. It is difficult to make desired resistance gene combinations because one resistance gene can mask all others unless suitable rust cultures are available to break all but the gene of interest. It is also difficult to breed for multiple unlinked genes and maintain good agronomic characteristics. There may be genetic drag when introgressing alien chromosome segments which contain rust resistance genes.

A final problem is lack of control of resistance gene resources. It is difficult to tell breeders which genes they can use. It is also difficult to tell farmers which cultivars they can plant. Legal methods of control such as patents are not desirable for many reasons. A consensus to actively cooperate has not yet developed among breeders or farmers.

### **Strategies for slowing evolution of new races**

There are numerous possible ways to slow the evolution of new rust races and thus avoid the boom and bust cycle. Several popular strategies are outlined here for comparison.

#### *Destroy volunteer wheat in the summer*

If all volunteer wheat could be destroyed during the summer in Kansas, Oklahoma, and Texas, the leaf rust would have great difficulty in surviving. This bottleneck would reduce the population size and slow evolution of new races. Unfortunately, farmers are not easily motivated to control volunteer wheat. We have had extensive campaigns to eradicate volunteer for wheat streak mosaic control. A surprising fraction of farmers do not respond positively due to cost, desire to use volunteer for pasture, or indifference.

#### *Physiological tolerance and/or early maturity to escape*

Triumph 64 may be a good example. It is early and often matures before leaf rust gets severe. If leaves are lost, it fills from stem. It consistently produces mediocre yields and above-average test weights with or without leaves. Since the variety is susceptible or moderately susceptible, there is little selection pressure on the rust. In any case, it is hard to imagine how the rust could adapt to earliness and the ability to fill from the stem. Ability to fill from the stem is a desirable character, but stems don't capture much light compared to leaves. Therefore, this strategy may limit yield potential. In severe rust years, even early varieties may suffer significant yield losses.

### *Gene deployment.*

The basic idea is to create greater diversity in the host population. This causes the pathogen to frequently land on an incompatible host and thus waste a large proportion of its inoculum. This then slows the progress of the epidemic. Gene deployment can be the result of a plan or as a fortunate consequence of large numbers of genes available from different breeding programs. It can occur at different scales including within field (blends or multilines), within farm (several cultivars), within region (several breeding programs with different *Lr* genes), or between regions.

Gene deployment has two opposing effects on leaf rust evolution. First, it should decrease the effective population size of the pathogen which decreases the chances for rare mutational events. Second, it constantly challenges the pathogen to adapt to new hosts and could promote development of a super-race with broad virulence. The details of the effects will depend greatly on the scale of deployment and whether the genes are deployed singly or in combinations. Between-region gene deployment will be of less use if the pathogen survives year round in the locality (e.g. southern hard red winter wheat region). However, it could be of use to regions which receive exogenous inoculum (e.g. northern spring wheat region).

A good gene deployment plan would require extensive knowledge of the resistance genes in our breeding programs. More data is needed about the resistance genes in current commercial cultivars. There are probably some important unnamed genes in use that need to be

described. Gene deployment would also require a high degree of cooperation among breeders.

### *Genetic engineering (designer genes).*

In the future, it is likely that virulence mechanisms and resistance mechanisms will be understood at the molecular level. We may then be able to design an inexpensive solution to the rust problem that would be both effective and durable. For example, we might introduce a protease that cleaves an essential protein present in all races of the pathogen. Alternatively, we might learn how to transfer resistance genes from corn or rice to wheat. In any case, this approach is speculative and long-term. Although it could provide the ultimate solution, we need to pursue a more immediate answer.

### *Pre-emptive breeding.*

In Australia, they anticipate changes in pathogen populations one step ahead of time (R. McIntosh, pers. comm.). They actually create an anticipated new rust race, then use it to screen their next generation of cultivars. This allows them to build gene combinations that would otherwise require some type of marker-assisted selection. The downside is that this requires a secure containment facility to prevent accidental release of the new race. In Australia they do it by geographic isolation. It is probably too risky for breeders in the USA.

### *Prehaustorial resistance.*

Prehaustorial resistance inhibits the development of rust germlings prior to the formation of haustoria. There is often no visible sign of the aborted infection. This

type of resistance is different from the classic posthaustorial hypersensitive necrotic response conditioned by many *Lr* genes. Prehaustorial resistance is often observed when rust isolates are inoculated onto nonhost plant species. Niks and Dekens (1991) suggested that prehaustorial resistance indicated basic incompatibility between host and parasite and should therefore be more durable than hypersensitive type genes. *T. monococcum* was suggested as a source of prehaustorial resistance. Germplasm release WGRC23 is thought to possess prehaustorial resistance from *T. monococcum* (Stan Cox, pers. comm.). The hypothesis that prehaustorial resistance is more durable has not been adequately tested.

#### *Polygenic race nonspecific resistance.*

Vanderplank originated the concept of "horizontal" race nonspecific resistance in contrast to "vertical" race specific resistance. Horizontal resistance is supposed to be polygenic, incomplete (partial resistance), and durable. Clearly, the characteristics of horizontal resistance are not always correlated so the term is potentially ambiguous (Johnson, 1984). Another problem with the concept is that race nonspecificity is not a fixed character. It only means that virulent isolates have not yet been detected. It does not preclude their future detection. Nevertheless, the narrower concept of polygenic race nonspecific resistance is probably useful.

Roelfs et al. (1992) cited many cultivars with putative race nonspecific resistance, although the inheritance of resistance in most has not been determined. Rollie Sears (pers. comm.) has argued that old varieties like Scout

possessed a useful level of background, race nonspecific resistance which has been lost in many modern cultivars. This background resistance was presumed to be polygenic. If such quantitative genes exist, each gene would exert only a minor selection pressure on the rust and therefore resistance should be durable.

Background polygenic resistance would not offer much protection in a severe rust year like 1992. Also, it would be difficult to maintain polygenic resistance in a breeding program if major genes were also present due to masking by the major genes. It is difficult to breed for polygenic traits, especially when there are many other breeding objectives. Some cases of slow-rusting may be a particular type of polygenic, race nonspecific resistance.

#### *Slow-rusting*

Slow-rusting is a type of resistance where a susceptible infection type is observed, but the rate of disease development is slower than fully susceptible cultivars. It is usually considered to be race nonspecific and durable (Das et al., 1993). Slow-rusting may not be a particularly useful term. Slow-rusting can be attributed to several effects including lower receptivity (number of infections), longer latent period, or lower spore production (smaller uredinia or shorter sporulation period). *Lr13* has been reported to increase the latent period. Since *Lr13* has been broken by certain pathogen races, it cannot be considered race nonspecific and therefore confounds our definition of slow-rusting. Combinations of known race specific genes can produce race nonspecific slow-rusting phenotypes.

Theoretically, slow-rusting should reduce the selection pressure on the rust. However, it also increases the number of spores available to challenge resistance genes compared to more complete types of resistance. There is little direct evidence that slow-rusting per se reduces the rate of rust evolution. Slow-rusting combinations of genes could be durable for other reasons (see below). Slow-rusting is difficult to work with since tedious measurements of latent period, uredinium size, or uredinium numbers must be used on seedlings. In the field, slow-rusting is detected by lower area under the disease progress curve (AUDPC) or by lower final rust severity. Field experiments with slow-rusting are fraught with pitfalls for the unwary researcher. For example, a cultivar could appear slow-rusting simply because virulent races were initially rare in the nursery (Johnson, 1984).

#### *Adult plant resistance.*

Adult plant resistance has the interesting property of not being expressed in the seedling stage. *Lr12*, *Lr22a*, *Lr22b*, and *Lr35* have been identified as adult plant resistance genes (Roelfs et al., 1992). Little is known about pathogen virulence on these genes since rust survey screening is typically done only on seedlings. However, virulence has been found against *Lr12* and *Lr22b*. Although it might be expected that adult plant resistance would decrease the selection pressure on the rust, there is little data to support such a conclusion. Like slow-rusting, the number of spores challenging adult plant resistance may be large because populations can build up on young plants. Adult plant resistance is difficult to work with since it cannot be detected reliably in seedlings.

#### *Empirically discovered durable gene combinations.*

Certain combinations of genes have proved durable after considerable exposure in commercial cultivars. Most of these involve *Lr13* or *Lr34*. These genes have individually been associated with slow-rusting. The combination of *Lr13* and *Lr34* is apparently special. Each gene has been defeated individually, but the combination has performed well in CIMMYT germplasm all around the world. Since both genes have been defeated individually, presumably only one locus in the pathogen needs to mutate to become compatible. There is some risk that the pathogen will eventually adapt. However, it is possible that the double mutant has a heavy fitness penalty. The combination is already the basis of resistance in most N. American spring wheats. It may not be wise for the entire continent (or world) to depend on the same gene combination for protection. *Lr13* and *Lr34* plus one or two additional effective genes would be safer. This type of combination would be difficult to detect without proper markers.

#### *Designed durable gene combinations (pyramids).*

Pyramids are just a name for combinations of several resistance genes. Hopefully the pyramid would provide protection against a wider range of races than single genes. It might also be more durable. Most of our commercial cultivars already have combinations of *Lr* genes. However, all combinations of genes are not the same. Many combinations have already been defeated. Pyramid effectiveness depends on how many pathogen loci would have to mutate to

break the combination. The answer depends on the virulence formula of the particular pathogen race of interest. The effective pyramid size equals the minimum number of virulence mutations required for any known race in the pathogen population to adapt to the pyramid. A pyramid of 2 undefeated genes and one defeated gene would have an effective pyramid size of 2.

One way to make a pyramid is to combine older, defeated genes in novel combinations. For example, the combination of *Lr9* + *Lr16* + *Lr24* should provide resistance even though the genes are defeated individually. The advantage of recycling old genes is that they are often well known and pathogen cultures may exist to help in the screening of the pyramid. The disadvantage is that the effective pyramid size will usually be lower than desired when old genes are used. In the example of 9+16+24, virulence already exists to *Lr9*+*Lr24*. Therefore the effective pyramid size is only one. Another major disadvantage is that older genes may be deployed singly or in small combinations in commercial cultivars. This can provide the pathogen with a pathway to defeat the pyramid in several sequential single-mutation steps. Finally, if the pathogen actually does have mechanisms for genetic recombination (as yet undiscovered), it could conquer a pyramid of defeated genes in a single recombination event.

Another approach is to create pyramids of new undefeated genes. Many new genes have been found in *T. tauschii* and other wheat relatives. A pyramid of several new (i.e. effective against all extant races) leaf rust resistance genes should be durable if each of the genes is strong (prevents reproduction of pathogen) and genes are not exposed singly or in subsets

of the pyramid. For the rest of the paper, the term pyramid will refer to combinations of undefeated, strong genes that are not exposed singly. The rationale for pyramids is that stepwise mutation will be prevented and the probability of simultaneous mutation to multiple virulence is low.

These are the assumptions: 1) the gene-for-gene relationship applies as described above; 2) the probability of pathogen mutation to virulence is low for each locus (roughly  $10^{-6}$  per spore if avirulence is recessive,  $10^{-6}$  if avirulence is dominant and pathogen is heterozygous,  $10^{-12}$  if avirulence is dominant and pathogen is homozygous avirulent); 3) frequency of pre-existing virulence is zero; 4) mutations at different loci are essentially independent; therefore probability of multiple simultaneous mutations to virulence is multiplicative. Given the assumptions, the probability of multiple mutation with three pyramid genes would range from  $10^{-18}$  to  $10^{-36}$ . Occasionally, certain combinations of virulences in the pathogen may have associated fitness costs which further reduces the probability of finding new virulent races.

Assumption 1 is well founded as discussed in the Background section. Assumption 2 is also empirically and theoretically sound. Kolmer (1992c) suggested that heterozygosity was less common for genes derived from alien sources. Therefore, dominant genes from *T. tauschii* might tend toward the  $10^{-12}$  probability estimate. (*Lr41* is obviously an exception since virulence was detected before large scale production). Assumption 3 can be empirically tested with trap plots. Assumption 4 is the most important and the most controversial (Schafer and Roelfs, 1985; Mundt, 1990;

Kolmer et al., 1991; Mundt, 1991). The major evidence against independence comes from artificial mutagenesis studies where simultaneous mutation to multiple virulence has been reported (Statler, 1987). Possible mechanisms would include 1) deletion mutations that affected several linked avirulence loci, or 2) suppressor genes that turn off expression of multiple avirulence genes. Presumably major changes such as these would carry a fitness cost. Other than the *Lr3ka* and *Lr30* connection, there is little evidence for simultaneous virulence switches in virulence surveys.

The number of genes in the pyramid to achieve a desired probability of breakdown also depends on the effective size of the pathogen population. Using a worst-case scenario of a continental stem rust epidemic, Schafer and Roelfs (1985) estimated the population of stem rust to be  $7.5 \times 10^{21}$  urediniospores per year. Using very conservative mutation rates, they estimated that a six gene pyramid would be needed to provide durable resistance. In reality the effective population size is probably much smaller. For leaf rust, huge epidemics are rare. In any case, only a small fraction (less than  $10^{-4}$ , M. Eversmeyer, pers. comm.) of spores would likely be transported from fields of susceptible cultivars and deposited on adjacent fields of pyramid cultivars. Under normal conditions, only 10% of virulent spores would successfully infect and conditions are often sub-optimal. In addition, Schafer and Roelfs did not factor in the effect of frequent population crashes. Therefore their estimates can probably be reduced by five orders of magnitude and still remain conservative for leaf rust. That would put the effective population size around  $10^{17}$  per year. Assuming the probability of breaking a

three gene pyramid is  $10^{-18}$  in the worst case scenario, then  $10^{-1}$  spores per year would be expected to successfully infect the pyramid cultivar in a severe rust year. An additional margin of safety would exist if the pyramid was introduced into a cultivar that already had some leaf rust resistance genes (even if they were defeated singly). The pyramid could be increased to four genes to provide a worst-case probability of defeat at  $10^{-7}$ .

The advantage of a resistance gene pyramid is that it should be more durable than releasing the same genes singly. In fact, it has the potential to prevent evolution of new virulent races permanently. Even if the pyramid is not ultimately durable, the cultivar should last longer than cultivars with a single effective gene. Although the initial cost is high (roughly \$15,000 per cultivar for molecular marker screening once they have been developed), long range expenses are very low. The strategy is compatible with gene deployment by using different gene combinations in different regions.

The disadvantages include the requirement for many new, strong resistance genes. Second, it is difficult to keep new genes from being deployed singly. Third, it is difficult to verify presence of components of a pyramid due to genes masking each other. This must be overcome with marker-assisted selection and that requires development of appropriate flanking markers for each gene. Fourth, it is difficult to breed for multiple unlinked genes, especially if each must be screened with markers. Fifth, by the time a pyramid is backcrossed into elite germplasm, it will no longer be elite.

## The Pyramid Plan

The Pyramid Plan will evolve over the next decade. Each stage prepares the way for moving to the next stage. It is an open plan and anyone can participate. It is compatible with other plans, particularly regional gene deployment.

### *Short term: develop infrastructure*

In the short-term, we will continue to control leaf rust through cultivar diversity and conventional breeding. We'll boom when we can and possibly use fungicides when we bust. We will support the concept of regional gene deployment. We will fight the temptation to use genes that are already in use in other regions such as *Lr13+Lr34* (spring wheats) or *Lr16* (Nebraska).

We will develop a consensus among breeders to reserve a set of pyramid genes with the ultimate payoff being that pyramid technology can be shared. In order to serve those who need new genes immediately, the WGRC will continue to release new single genes in a "shareware" pool for those who want them. Probably all currently released WGRC material including WGRC2 (Lr39), WGRC7 (Lr40), WGRC10 (Lr41), WGRC11 (Lr42), WGRC12 (unnamed), WGRC15 (unnamed), WGRC16 (Lr43), WGRC23 (unnamed) will be in the shareware pool. Of course, all previously named *Lr* genes are also available to everyone.

The WGRC will begin to develop a special reserved set of 3-4 new, undefeated, highly effective genes from wild wheat relatives. These will not be public germplasm releases. Each new gene will be placed singly in a common

background for developing markers and verification that the gene is undefeated in regional nurseries.

### *Medium term: test of concept*

We will backcross three reserved pyramid genes into KSU elite germplasm (such as Jagger). We will backcross each gene individually and screen with a rust isolate that is virulent to any background genes. After isogenization, the three backcross lines will be intercrossed and progeny will be screened with flanking molecular markers for each gene.

We will offer the same pyramid genes and molecular markers to those who want to put the pyramid in their own backgrounds. However, recipients must agree that genes will only be used in the full pyramid. It would be nice if we could do marker screening at KSU as service and to maintain quality control.

### *Long term: make technology user friendly*

We will clone the pyramid genes and chromosome engineer all three into a tightly linked cassette. This will then be placed either on an alien chromosome segment or an artificial chromosome to avoid recombination. It will segregate as a unit and avoid unintentional fractionation of the pyramid. This will greatly ease screening because it could then be done with a single rust culture which is virulent on the background rust resistance genes in the recurrent parent. The proportion of progeny with the desired trait will be much higher, thus easing the burden on the breeder. Other desirable genes like stem rust resistance can be added to the linkage group as needed. We can also increase the

size of the pyramid if increased security is needed.

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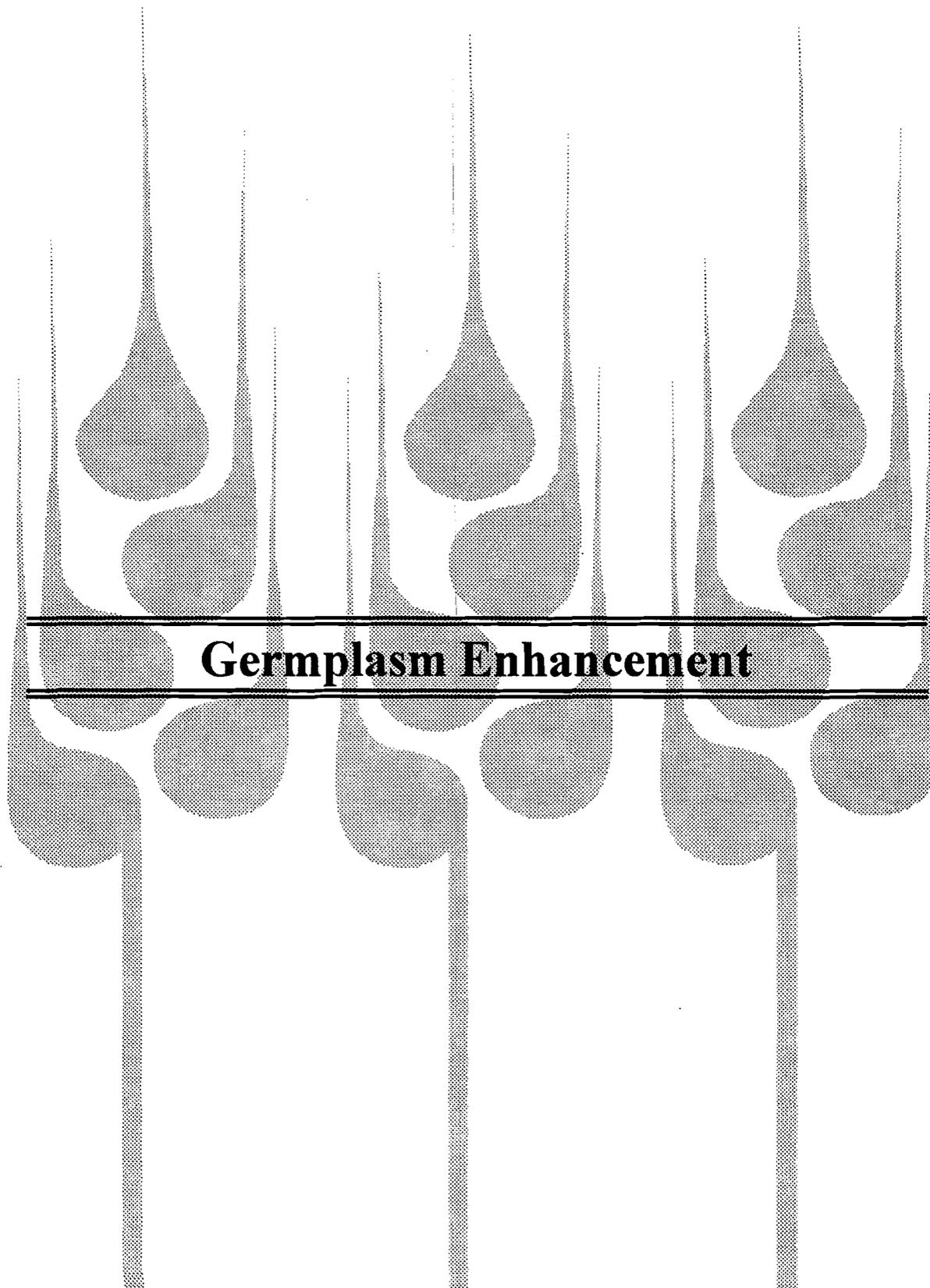
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# **Germplasm Enhancement**

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# Impact on Breeding and Genetics on Reducing RWA Damage in the Western USA

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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 (i) describe the economic justification, (ii) breeding progress for the development of Russian wheat aphid resistant cultivars, (iii) sources of resistance genes, and (iv) regional program efforts.

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 17 western states of the US and three provinces in western Canada. The economic impact during 1986-1992 in the US have been estimated at more than \$850 million. Losses caused by the RWA during 1990-93 were small and variable compared to 1986-1989, but when favorable conditions for the aphid occurred, losses increased dramatically in 1994 (Table 1). In the United States, the first significant level of resistance found in wheat was in PI 372129 (Turcikum 57 = T-57) in Colorado (12). Subsequently, 12 other wheats from various countries expressed significant resistance levels in regional uniform seedling screening

programs (9,10), and many additional resistant wheats have recently been reported by workers in California, Idaho, Kansas, Mexico, and Oklahoma. All introductions from the regions of RWA origin possess several undesirable traits for a hard red winter or spring wheat breeding program. Selected introductions and new breeding lines were evaluated in a regional uniform field test at 5 locations in 1993 (13). New breeding lines were as resistant as their resistant parents, confirming greenhouse seedling tests.

Research on breeding for resistance to the RWA was recently summarized by Quick (11). Cultivar development is proceeding well using the T-57 source. An elite line, CO910927, was tested in the 1993-94 Southern Regional Performance Nursery, and was released as 'Halt' in August 1994 (14). Halt is an awned, semidwarf height, white-glumed cultivar which has been most similar to 'Yuma' in appearance at maturity (Table 2). The spikes are semi-lax, and it is similar in maturity, straw strength, and height to 'TAM 107'. Halt has averaged about 5% lower grain yield than Yuma and TAM 107 over all eastern Colorado dryland trials. In the SRPN, Halt ranked higher than TAM 107 in grain yield in 3 of 5 locations in the RWA-affected area (Table 3). Milling

and baking quality have been superior to TAM 107 and equal to 'Lamar'. Halt is the first Russian wheat aphid-resistant cultivar developed in the USA.

Screening procedures developed by entomologists for screening breeding materials are very efficient. At least seven different major genes (1-8) have been associated with RWA resistance (Table 4). Significant breeding advances have been made and host plant resistance will soon be the key to integrated management of the RWA. An understanding of the mechanisms of resistance associated with the major resistance genes, and/or molecular markers associated with them will be very valuable in developing durable resistance through gene pyramiding and deployment.

Information on the regional breeding effort for RWA resistance was obtained through a survey conducted by the author in December 1994. The sources of resistance being used to develop resistant wheats for the southern Great Plains region are shown in Table 5. The regional effort on size and type of program, anticipated germplasm and variety release, genetic sources and studies, and technical needs are shown in Tables 6, 7, and 8.

During the past three years, germplasms have been released by programs in Colorado, Montana, Oklahoma (USDA-ARS), and Kansas (Table 9) and were tested in uniform field screening tests (13). Small quantities (3 g) of seed of these lines are available upon written request to the originator. It is requested that appropriate recognition of source be given when this germplasm contributes to research or development of new cultivars.

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**Table 1.** Economic impact of Russian Wheat Aphid in Colorado: 1986 - 1994.

Year	Production Lost	Acres Sprayed	Impact \$\$	% Sprayed	% Grown
1986	5.50	90	13.2	NA	NA
1987	7.10	1150	27.1	48.0	5.03
1988	3.00	350	14.0	15.0	4.35
1989	4.00	850	22.8	38.0	4.63
1990	3.00	475	10.8	25.0	6.77
1991	0.73	115	3.5	42.0	5.98
1992	1.70	100	5.8	15.5	4.93
1993	0.03	6	0.1	NA	NA
1994	2.02	430	12.1	NA	NA
TOTALS	27.07	3566	109.4	30.6	5.28

Production lost in millions of bushels, acres sprayed x 1000, impact in \$ millions, % of total acres sprayed, and % of acres grown. Source: Frank Peairs, Dep. of Entomology, CSU.

**Table 2.** New hard red winter wheat performance in Colorado; relative data.

Trait	Cultivar				
	Halt	Akron	TAM 107	Yuma	Lamar
Yield(D),%	95	100	100	98	95
Yield(I),%	-	107	100	102	
TW, lb/bu	60	60	60	60	61
HT, in	30	31	30	30	36
LG, 0-9	2.0	1.0	1.0	2.0	4.0
DH	142	144	142	144	146
Hail Loss,%	40	30	40	40	50
W. Surv.,%	60	80	90	50	80
L Rust, 0-9	5	2	7	1	1
RWA	R	S	S	S	S
WSMV	R	S	R	S	S
Col., cm	85	85	95	80	105
Qual, ml	VG	VG	VG	VG	VG
Qual, mx	EX	EX	AC	VG	EX
Qual, Bk	EX	EX	AC	VG	EX

**Table 3.** Performance ranking for grain yield of Halt in the 1993-94 Southern Regional Performance Nursery.

Location	Halt	TAM 107
Colorado (4)	22	27
Kansas (5)	29	16
Colby, KS	14	20
Nebraska (5)	28	20
Hemingford, NE	15	26
Oklahoma (4)	41	20
Goodwell, OK	35	30
Texas (4)	40	21
Bushland, TX (D)	37	30

**Table 4.** Genetics of resistance to the Russian Wheat Aphid

PI/SEL	Gene Symbol	Class	Genetics Reference
137739	Dn1	HWS	SA, CO
262660	Dn2 ?	HWW	SA, CO
SQ 24	dn3	T. taus.	CO
262605	Dn1	HRW	CO
372129	Dn4	SWW	CO
294994	Dn1,4,5,6 ??	HRW	SA, CO, OR
243781	Dn6	HWW	CO
CORWA1	Dn4	HRW	CO
KS92WGRC24	Dn6	HRW	CO
STARS9302W	Dn5	HRW	CO
CI 2401	Dn4, *	HRW	CO
CI 6501	Dn6	HRW	CO
151918	Dn4	winter	CO
94355	*	winter	CO
94365	*	winter	CO
222666	*	HRW	CO
222668	*	HRW	CO
225245	*	HWW	CO
225262	*, *	HWW	CO
225271	*	HRW	CO
149898	Dn_, Dn_	winter	OK
225217	Dn_	winter	OK
245462	Dn_, Dn_	winter	OK
386148	Dn_	triticale	CO
AUS-VAV1	Dn5	spring	CO
140207	Dn_	spring	OK
366515	Dn_, Dn_	spring	OK
366616	Dn_, Dn_	spring	OK

\*: allelism unknown, but not Dn4, Dn5, or Dn6

Dn\_: allelism unknown

CO, OK, OR, SA: Colorado, USDA/Oklahoma, Oregon, South Africa,

**Table 5.** Sources of resistance used by regional wheat breeding programs.

Program	Sources
Colorado	PI372129 (T-57), PI243781, PI294994
Idaho	T-57, PI137739, PI294994, PI94365
Kansas	YILMAZ-10
Montana	PI372129, PI294994
Nebraska	CORWA1, PI137739, PI262660
Oklahoma	PI149898, PI140207, PI366616, PI245462, PI225217, CI2401, PI366520, PI366525, PI366515
Oregon	PI294994
Cargill	PI372129, PI149898
Hybritech	T-57, PI137739, PI294994

**Table 6.** Regional breeding efforts for RWA resistance.

Program	% Of Program	Type of Screening	Wheat Classes
CO	100	GH*, F**	HRW, HWW
ID	10	GH, F	FIVE
KS	5	GH	HRW, HWW
MT	20	GH, F	HRW, HWW, HRS
NE	5	GH	HRW, HWW
OK/OSU	15	GH, F	HRW
OK/ARS	90	GH, F	HRW, HRS, HWS, SWS
OR	10	GH, F	CLUB
Cargill	5	F	HRW, HWW
Hybritech	5	GH, F	HRW, HWW, HRS

\*GF = Greenhouse; \*\*F = Field

**Table 7. Regional breeding efforts for RWA Rresistance.**

Program	Germplasm Release	CultivarRelease	Resistance Source(s)	Allelism Studies	Mechanism Studies
CO	1991	1994	- No. - 4	Y	Y
ID	1996	1998	7	Y	Y
KS	1993	1998	6	N	N
MT	1992	1998	4	N	N
NE	2004	2004	4	N	N
OK/OSU	?	2001	4	N	N
OK/ARS	1993	--	28	Y	Y
OR	?	?	4	Y	N
TX	?	?	CORWA1	N	N
Cargill	?	?	4	N	N
Hybritech	?	?	10	N	N

**Table 8. Regional breeding efforts for RWA resistance.**

Program	Technical Needs
CO	Molecular Tags, Allelism, Mechanisms
ID	Molecular Tags, Allelism, Mechanisms
KS	None
MT	Allelism, Mechanisms, Molecular Tags
NE	Molecular Tags
OK	RWA Biotype Survey, Allelism, Mechanisms
OR	Mechanisms
TX	Test Condition Standards
Cargill	Mechanisms
Hybritech	Pyramiding Information

**Table 9. Regional germplasm releases.**

Year	Location	Name	Gene
1991	Colorado	CORWA1	DN4
1992	Montana	14 HRSW	DN4
1993	Oklahoma-ARS	STARS-9302W	DN5
		STARS-9303W	DN5
1993	Kansas	KS92WGRC24	DN6
		KS92WGRC25	DN6

# Germplasm Development Of Enhanced End-Use Quality

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## Introduction

A number of factors argue in favor of increased emphasis on germplasm enhancement for improved wheat end-use quality. The majority of hard winter wheat continues to be used in the production of traditional leavened bakery products. The industry, however, has moved to more highly automated systems that place additional performance demands on raw materials. The increasing use of hard white wheat, and increased demand for whole grain products, also may require enhanced quality attributes, especially increased protein quality and dough strength. Secondly, wheat breeders continue, either unknowingly or intentionally, to develop and release cultivars carrying rye chromosome arm 1RS. 1RS has a negative impact on dough strength and bake performance. Finally, alternative uses of hard winter wheat may require the development of new quality types, not yet present in the Great Plains gene pool.

An examination of past performance of cultivars and elite germplasm developed in the Great Plains was conducted in order to determine whether additional attention need be focused on the development of novel germplasm with altered and improved quality. Mean performance of all lines entered in each year of the Southern Regional Performance Nursery (SRPN)

and the Northern Regional Performance Nursery (NRPN) was expressed as proportions (%) of long-term check cultivars (Scout 66 for the SRPN, Kharkof for the NRPN), and plotted as a function of time (Figures 1 and 2). Data were obtained from yearly reports published by the USDA-ARS Grain Marketing Research Lab, Manhattan, Kansas. Of the traits examined, the only significant deviation from the performance of the long-term check cultivars was in Mixograph time. In the SRPN, mean Mixograph times of entries were at least 130% of Scout 66 from 1974 through 1983, though from 1983 to 1990 the figure was only 110%. Since 1990, Mixograph times, relative to Scout 66, have been increasing once again. In the NRPN a similar trend was observed, although there has been no recent increase in mix times relative to Kharkof. All remaining traits, flour extraction, flour ash content, flour protein, absorption, and loaf volume, have remained at, or near, 100% of the long-term checks. It is interesting to note that flour protein content has not dropped over this time frame, even though grain yield has risen from 125% of checks to 140-170%.

Wheat breeders, therefore, have been able to steadily improve grain yield **without** a corresponding decrease in flour protein content, or quality in general. At the same time, however, no net improvement in overall quality has

occurred. While processes requiring flour as a raw material are in a state of flux, the raw materials themselves appear to be in a state of homeostasis. Perceived changes in the quality of the raw materials by industry end-users are more likely the result of short-term environmental events, and do not appear to arise from changes in the average quality of wheat in the Great Plains breeding populations. Strategies to achieve an increase in the average genetic performance of wheat are discussed below.

### **Enhancing Flour Protein Quality**

In general it appears the quality of hard winter wheat has changed little over the past two decades. Exploitation of new markets and uses of wheat, as well as adjustments to shifting demands in traditional industries, may depend upon the availability of wheat with new quality attributes. Maintenance of fairly consistent quality over the past two decades has resulted in, or been the result of, a lack of variation in, important biochemical determinants of quality. Protein content has shown little change over this time period (Figures 1 and 2). Protein composition, however, may be a more important factor than protein content alone, in the determination of end-use quality. Gluten proteins, gliadins and glutenins, are primarily responsible for flour functionality. Specifically, it is the glutenin proteins, which possess the ability to polymerize through intermolecular disulfide bonds, that impart strength and elasticity to dough. Quantitative loss of glutenin, either due to the presence of wheat-rye chromosomal translocations (Graybosch et al., 1993) or deletion of genes encoding glutenin proteins (Lawrence et al., 1988) always results in a loss of

dough strength. It is logical to assume, therefore, that an increase in the amount of glutenin, relative to gliadin, would result in an increase in dough strength. Little quantitative variation appears to exist in glutenin content among hard winter wheats; what variation has been detected, however, seems to be important. Cultivars (e.g. Chisholm, Cimarron and Redland) with relatively higher amounts of glutenin tend to perform well in bake tests. In addition, it is now clear that one possible means of reversing the undesirable effects of 1RS is to increase flour glutenin content.

Increased glutenin will only be achieved by the introduction of novel genes. Three strategies for the development of wheat with increased glutenin content are being pursued. All cultivated hexaploid bread wheats possess no more than five active genes encoding high-molecular-weight glutenin subunits. Two active genes typically are found on both chromosomes 1B and 1D; chromosome 1A, in contrast, will carry only one or zero active genes, although inactive genes are apparently present. In some accessions of the tetraploid *Triticum dicoccoides*, two active 1A genes occur. In the hexaploid landrace TAA36, a gene duplication has occurred that has increased the number of 1B encoded genes to three (Lukow et al., 1992). Through backcrossing, these additional glutenin genes have been introduced to both tetraploid (durum) and hexaploid bread wheats. Field testing, to identify quantitative changes in glutenin and in quality, is pending. In the future, glutenin genes introduced to spring wheats via genetic engineering (see paper by O. Anderson, this volume) also will be introgressed to Great Plains wheats.

Scores of genes encoding gluten proteins operate in a typical cultivar. Even though they function quite differently in the breadmaking process, gliadin and glutenin genes possess nearly identical amino-acid profiles. Thus, addition of glutenin genes could result in no increase in glutenin content if resource allocation between gliadin and glutenin genes is unaltered. Reduction of the number of gliadin genes, concomitant with increased numbers of glutenin genes, might be necessary. Within the soft white cultivar 'Raeder' a deletion has been detected which results in the loss of 1/6 of all gliadin genes (D'Ovidio et al., 1991). Both "wild-type" and mutant biotypes exist within the cultivar; comparison of the amount of gliadin in the two types via high-performance liquid chromatography has revealed a significant quantitative loss of gliadin in the mutant form. This trait has been introduced to adapted materials, and will be combined with the additional glutenin genes. The goal is to alter the profile of gluten proteins so that glutenin production is favored relative to gliadins.

### **Improving Quality of IRS Wheats**

Wheat breeders in the region continue to release lines that carry rye chromosome arm IRS. In 1994, 35% of the lines in the SRPN carried either a 1AL/IRS or a 1BL/IRS chromosomal translocation; the figure for the NRPN was 6.5%. IRS is known to reduce quality, especially in terms of diminished dough strength, reduced loaf volumes, and poor bake performance. The availability of germplasm with stronger doughs would offset some of these negative effects. Increasing the proportion of flour protein found as glutenin is an approach being used to improve quality of both IRS and non-

IRS wheats. However, such directed and rational approaches might not be the sole means of improving the quality of IRS wheats. The strong gluten characteristics of 'Plainsman V' and its descendant 'Karl' do not appear to be dependent upon enhanced glutenin. By backcrossing IRS into these strong gluten types, the deleterious effects should be overcome. Indeed, after a single cross to Plainsman V, the mean Mixograph tolerance of IRS progeny was significantly higher than the IRS parent, 'Siouxland'. Thus, an improvement has been achieved with no understanding of the underlying biochemical mechanism.

### **Improved Wheat Starch**

Variation in starch properties is an important component of breeding for wheats acceptable in Asian noodle production. Breeding programs in Australia routinely screen breeding lines for starch properties, using procedures such as the rapid viscoanalyzer, and starch swelling assays (Crosbie, 1991). The ratio of amylose/ amylopectin is an important factor determining starch gelatinization and pasting (Davis, 1994), and, consequently, acceptability in certain Asian noodle products. Little quantitative variation in the ratio of the primary components of starch, amylose and amylopectin, is known to exist among hard winter wheats.

Amylose/amylopectin ratios are influenced by the activity of the waxy protein, or granule-bound starch synthase. Wheat possesses three forms of this enzyme, each with a molecular weight of  $\approx 60,000$ . Genes encoding the waxy proteins occur on chromosomes 7A, 4A and 7D (Nakamura et al., 1992). Recently, semi-waxy mutants of wheat

have been described in which either one or two of the waxy proteins have been deleted (Nakamura et al., 1993). The waxy mutants possessed enhanced amylopectin contents, reduced amylose, and improved quality in Japanese Udon noodles. A number of single mutants have been discovered in the USDA germplasm collection, and are being combined, and introgressed, to hard winter wheats.

### Summary

Germplasm development for improved end-use quality is based upon the goal of restructuring the biochemical organization of wheat flour. Addition of glutenin genes, and removal of gliadin genes, will result in wheats with stronger gluten, improving both the bake performance of non-1RS wheats, and alleviating some of the quality defects of 1RS wheats. Altering the starch amylose/amylopectin ratios through use of partial waxy mutants will result in the development of enhanced germplasm for use in Asian noodle production, expanding the markets for Great Plains hard winter wheat.

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# Status of the Wheat Database

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The United States Department of Agriculture's national research initiative in plant genomes has the goal of developing new technologies and genes for enhancing U.S. agriculture production. A major focus is the use of modern genetic mapping techniques to identify and isolate agronomically important genes. However, the identification and isolation of genes is of limited utility unless there exists an efficient mechanism for disseminating to researchers the increasing amounts of molecular and genetic information. Thus, an integral part of the plant genome initiative is the establishment and maintenance of computer databases. These databases will serve both as a repository of information and as a research tool containing interrelated data types.

The initial focus of the Plant Genome Database Program (Dr. Jerome Miksche, director) was to develop prototype databases using four model plant systems: maize, soybeans, trees, and wheat. As the database models have stabilized, additional crops have been included, including barley, rice, sorghum, cotton, the other legumes, and the solanaceae. Separate databases for a crop, or a crop group, are assembled at sites around the United States. Data is then transferred to the National Agricultural Library (NAL) in Washington, D. C., which is the intended

primary access point for database users. Individual databases may also be accessible directly from different locations, but the general access to all crops will be at NAL.

It was immediately obvious in the wheat database project that important data needed to be included from crops other than wheat. The ability to make wide crosses within the Triticeae and the similarity of the different species led us fairly early in the project to expand our data assembly to include barley, rye, and those wild grasses which can be crossed to wheat. In addition, we have included data from oats and sugarcane. The inclusion of data on many of the small grains suggested to us the name GrainGenes for our database (with an apology to sugarcane).

The database is intended for all researchers working with those crops we cover. While the initial impetus for the project was from consideration of newly accumulating molecular data, we found that there were sources of non-molecular data of great interest to researchers. Much of this additional data was not appropriate for a traditional database, but more suited to browsing or simple searches of large files. To accommodate the different types we are using several different data presentations (gopher,

Acedb, World-Wide-Web, and CD-ROM), but collectively they are referred to as the "GrainGenes" database.

The two main presentations are the GrainGenes gopher and the Acedb GrainGenes. The gopher format is text-based and the Acedb format is a graphical interface with extensive graphic and query capabilities. The gopher has the simplest access. A user must be able to log onto a computer connected to the Internet (the early version of the Information Superhighway we hear so much about). From there the user connects to the GrainGenes gopher and uses basic keyboard strokes to maneuver. From the text-based display the user is able to maneuver to different information such as the following:

- Search the GrainGenes Acedb database for information
- Retrieve files and images to a local computer
- Search or download the Wheat Gene Catalog
- Search the Commercial Wheat Cultivar Catalog
- Browse or download yearly performance and quality evaluations
- Browse the Cereal Rust Bulletin
- Download raw mapping scores
- etc

More complex hardware and software is needed for a graphic connection to the Acedb GrainGenes, whose basic requirements are a direct Internet connection and X-windows software on the user's computer. Acedb has a graphical interface, and is a multi-windowed, mouse-controlled

environment with both graphic and text displays. Among the capabilities of the Acedb format and datasets it contains are:

- Image displays
- Interactive active map displays
- Complex query capability
- 24 maps and 166 linkage groups
- Comparative maps of rice, maize, and wheat
- More than 2500 loci and 600 genes
- More than 2000 probes with nucleotide end sequences of 200
- Information on 10,000 germplasms
- Information on 450 species of plants, plant and insect pathogens
- Results of a QTL study in wheat
- Names, addresses and research interests of 1000 colleagues
- More than 1400 relevant bibliographic citations
- 450 pathology entries, some with digitized images of disease symptoms
- 1000 images of pathogens, morphologies, and southern blots from mapping studies
- HMW-glutenin gene complements for 1800 wheat cultivars

Both presentations have advantages, and a single interface with all data types may eventually develop. Perhaps an early version of this union is *via* the World-Wide-Web (WWW), a graphic interface with the same windowing appearance in all platforms. The user runs resident software of a "Web-browser" on the local computer (PC, Mac, Unix) and interacts *via* nearly identical-looking windows and buttons. The current browser we are using is

Mosaic, but several others, both commercial and free, are becoming available. One endearing characteristic of all the basic software we are currently using is that it is free and can be downloaded over the Internet from a number of source sites. The exception is the X-windowing software which must be purchased from any of a number of vendors (unless your system already has X-window capability).

The final presentation format is via CD-ROM using Mosaic to access the data. The CD-ROM is pressed by the NAL and current plans are to update 2-3 times a year. Distribution is free at the moment, but charging for the cost of the discs will probably eventually be necessary. The second version of the CD-ROM is now being distributed. Several bugs have been fixed from the first prototype. Although there are still some problems to be addressed in future versions, the current CD-ROM is reasonably straightforward to install and includes data not just for GrainGenes, but it also for several other crop databases (maize, rice, solanaceae, soybeans, and trees). The advantage of the CD-ROM is two-fold. First, users without good, or any, network access will be able to use the databases. This can include less-developed countries, users in more remote sites, and users wanting to work at home where no Internet access is yet common. Secondly, the CD-ROM adds a mobile dimension. Besides home use, newer models of laptop computers are beginning to include CD players.

Even though GrainGenes is funded by the United States Department of Agriculture, it is, by necessity, of an international character. Our crops are among the most widely grown in the world, and thus not only are potential users of the database found throughout the world, but many of the important data sources are international (either literally from other countries, or with an international component). Thus we encourage, and are enjoying, successful interactions with scientists throughout the world. Just a few examples include the curator of the Wheat Gene Catalog (Bob McIntosh - Australia), germplasm and trait data from CIMMYT (Mexico City), and maps from Australia, The United Kingdom, Germany, and France.

We are always eager to help users connect to the databases, and we encourage comments and suggestions for improvements. If you are a small grains scientist and, after viewing the GrainGenes databases, wish that data you possess or know about were part of GrainGenes, then you should be in contact with us. Improvements in data presentation and the breadth and depth of data are mainly driven by user interactions with the GrainGenes personnel. Similarly, the collation and maintenance of data is dependent of scientists' contribution to the database project. As an example, we are currently organizing curators for specific areas in pathology. Individuals or small groups will be responsible for curating data in areas of their expertise. Email notices of this effort will be forthcoming as we organize further, and anyone interested in participating is encouraged to contact us.

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GrainGenes Acedb access requires a password from David Matthews.  
Gopher access is via [greengenes.cit.cornell.edu](http://greengenes.cit.cornell.edu), or [probe.nalusda.gov](http://probe.nalusda.gov).  
Mosaic access is via <http://probe.nalusda.gov:8300>.

# Adaptation of Wheat Curl Mite (*Acaroa: Eriophyidae*) to Resistant Wheat

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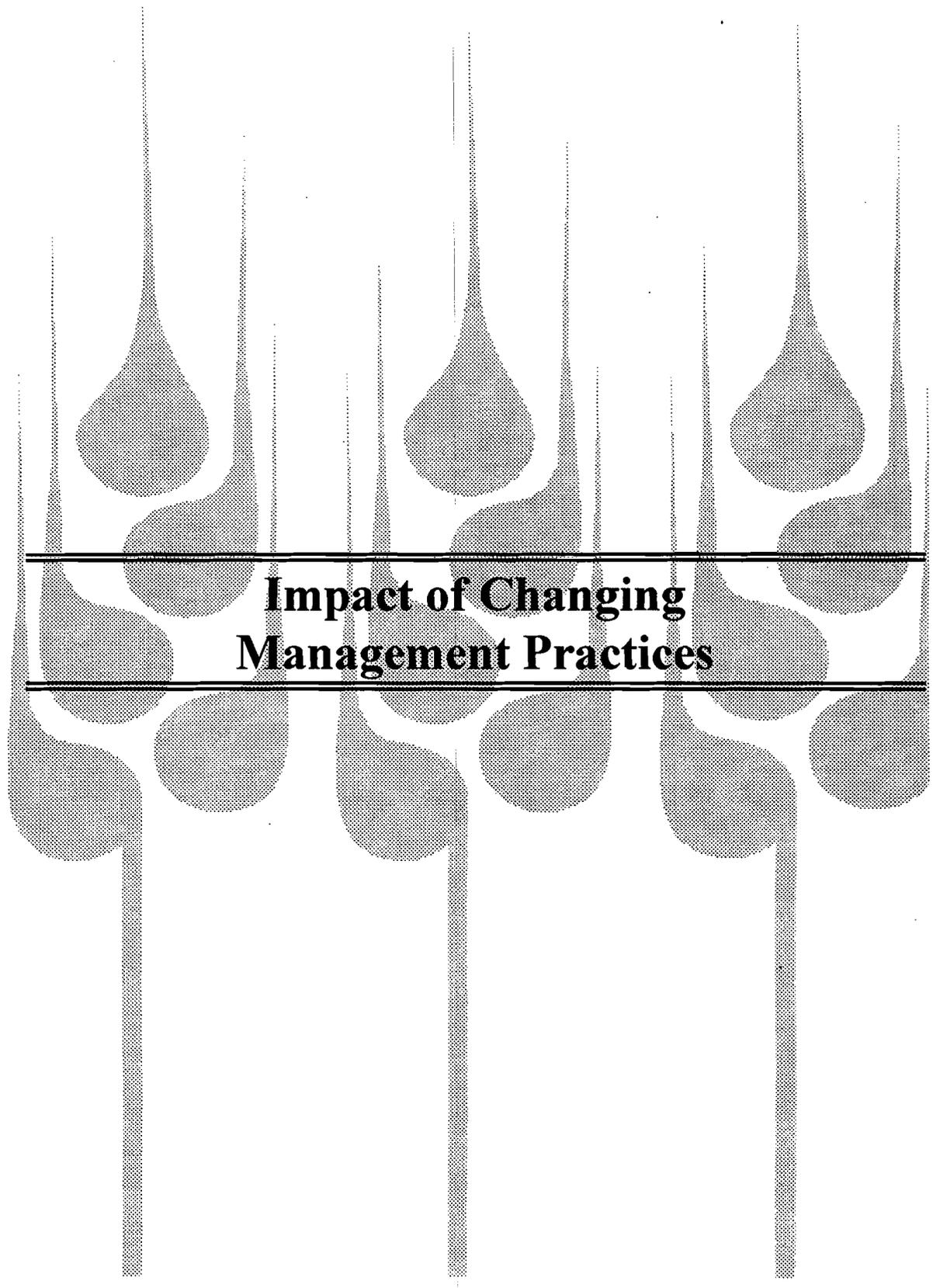
Wheat streak mosaic (WSM) was the most important disease of wheat in Kansas from 1987 to 1991 with an average annual loss of 15.5 million bushels. The incidence of WSM is reduced in the wheat cultivar TAM 107, which has resistance to the wheat curl mite, *Eriophyes tulipae* Keifer, vector of wheat WSM virus. Different physiological strains of the wheat curl mite have previously become adapted to species that were initially poor hosts, so it seemed likely that the mite would become adapted to the resistance of TAM 107, which is the most popular wheat cultivar grown in western Kansas.

Our objectives were to determine whether the wheat curl mite could adapt to or overcome the resistance of TAM 107 in the laboratory and whether resistance-breaking strains or biotypes have developed on TAM 107 in the field

TAM 107 was resistant to each of three field collections of wheat curl mites from susceptible 'Arkan' wheat, made in different years and from different stages of growth; however, each mite collection readily survived and reproduced on TAM 107 after being reared on that cultivar for 2 months. Another field collection from Arkan was largely adapted to TAM 107 after being reared on that cultivar for only 6 weeks.

In 1992 wheat curl mite collections were made from wheat spikes of 'Karl' and TAM 107 from ten Kansas counties. More mites transferred from spikes to wheat seedlings in the greenhouse from Karl than from TAM 107. However, one collection from Graham County differed from the others in that large numbers of mites were collected from both Karl and TAM 107. Further tests verified that the wheat curl mites collected from TAM 107 wheat spikes in Graham County were a resistance-breaking strain that had overcome the resistance of TAM 107.

The numbers of field collections were too small and limited in distribution to provide a reliable estimate of the prevalence and distribution of the resistance-breaking strain in the field. However, resistance is likely to be lost in proportion to the amount of resistant wheat grown; TAM 107 and 'TAM 200' where grown on 49 percent of the western Kansas wheat acreage in 1993. Wheat curl mite populations were generally low in 1993 and 1994, but the effectiveness of the resistance of TAM 107 should be monitored in the future to determine the need to deploy new sources of resistance which may be effective against the strains that have overcome the resistance of TAM 107.



**Impact of Changing  
Management Practices**



# Variety Selection: A Producer's Perspective

Don Oswald, Farmer and Owner  
Super O Seed, Apache, OK

## Farmer -

- Wheat, Cotton, Corn, Alfalfa, Other Hay
- Super O Seed - Certified Wheat Seed, Seed Cleaning
- Custom Farming, Machine Hire

## Cattle -

- Commercial Cow-Calf
- Stockers

Apache, Caddo County, Oklahoma

Southwest Central Oklahoma

## Local Wheat Varieties - currently most popular

- 2180\*
- 2163

## Why?

- \$\$\$\$\$\$\$\$
- Yield Potential!!

## Grain and grazing...dual purpose varieties

- good grazing characteristics
- maximum yield potential
- high end-use quality

## Grazing Very Important!

Value of Forage - \$135-317 million annually. This is from grazing only 1/2 of the wheat planted in Oklahoma. Wheat serves as both the forage base for the beef industry and a

feed grain. Oklahoma's stocker industry has progressively supplied an increasing percentage of the national supply of feeder cattle since 1970.

## Factors for Variety Selection

### Rapid Emergence After Planting

- Coleoptile length -warmer soil temperature along with timely late summer showers in my region helps me to consider other factors.

- Semi-Erect Juvenile Growth Habit
- Prostrate for freeze protection, erect for maximum consumption

- Grazing Potential
- Fall or spring - Does majority of forage production come early in the fall or during spring for graze-out?

- Good Growth Potential

## Grazing Potential

Average	Good	Excellet
#A <1200	1200-1400	>1400
Cimarron	2180	Longhorn
Chisholm	2163	Tomahawk
Karl 92		

### ***Coleoptile Length***

Short	Medium	Long
2180	2163	Longhorn
AGSECO 7853	Karl 92	Thunderbird
Chisholm	Tomahawk	
Cimarron		

### ***Fall Cover Capability***

Slow	Average	Quick
Tomahawk	2180	Longhorn
2163	Chisholm	Thunderbird
Karl 92	Cimarron	
	Custer*	
	Tonkawa*	

\*New OSU variety releases

### ***Grain Production***

Superior wheat varieties and improved management practices, especially in regard to fertilization and pest control have allowed production of hard red winter wheat for grain to dramatically increase on a per acre basis.

### **Factors for Variety Selection**

- Maximum Yields
- Heading Date
- In days after March 31. How early do we want a mature plant to harvest?
- End-Use Quality
- Protein
- Dough Mixing Time
- Mixing Tolerance
- Percent Ash
- H<sub>2</sub>O Absorption
- Crumb Grain
- Loaf Volume Potential

These could be expanded to include nutritional aspects of wheat brand and their whole-wheat flour

- Test Weight
- Another decision by the wheat producer has to be made. In selecting for varieties how important is test weight in relation to higher yields. We want maximum pounds weighing in across the scales regardless of how it gets there.

### ***Yield Potential***

Good	Very Good	Excellent
Chisholm	2180	2163
	Cimarron	Karl 92
	Tomahawk	
	AGSECO 7853	
	Custer	
	Tonkawa	

As we look at these headings in more detail we see my local favorites are still hanging in there! 2180 is tailing off somewhat.

### ***Maturity***

Heading date in days after March 31

Early (20-21)	Med. Early (22-23)	Medium (24-25)
2180	2163	AGSECO 7853
Chisholm	Cimarron	Tomahawk
Karl 92		
Custer		
Tonkawa		

### ***Test Weight***

Light	Average	Above Average
2163	2180	AGSECO 7853
Tomahawk	Chisholm	Karl 92
	Cimarron	Custer
	Tonkawa	

## End Use Quality

Less Desirable	Acceptable	Exceptional
2163	Chisholm	AGSECO 7853
Longhorn	Cimarron	Karl 92
	Tomahawk	
	2180	

## Factors for Variety Selection-Dual Purpose

- pH Tolerance
- Disease Resistance
- Leaf rust
- Soil-borne mosaic virus
- Septoria leaf blotch
- Tan spot
- Powdery mildew
- Wheat streak mosaic virus
- Insect Resistance - main problems
- Greenbugs
- Russian Wheat Aphid
- -lesser problems
- Army worms
- Wheat mites

## Disease Resistance

Most Susceptible	Moderately Susceptible	Moderately Resistant	Most Resistant
<b>Leaf Rust</b>			
Chisholm	Cimarron	AGSECO 7853	Longhorn
Karl 92	Tomahawk		
	2163		
	2180		
<b>Powdery Mildew</b>			
	2180	Chisholm	2163
	Cimarron	Karl 92	
	Longhorn		
	Tomahawk		
	AGSECO 7853		
<b>Septorial Leaf Blotch</b>			
Cimarron	AGSECO 7853	2163	
Karl 92	Chisholm	Longhorn	
2180	Tomahawk		

## Other Factors

Consistency	High pH	Iron Deficiency
Seed Size	Height	
Shattering Reputation	Protein	
Straw Strength	Winterhardness	
Ability to hold dormancy		

## pH Tolerance

Most Sensitive	Average	Above Average	Most Tolerant
Chisholm	Cimarron	AGSECO 7853	2163
Karl 92	2180		
Tomahawk			

They're back. 2180 and 2163. In a continuous wheat cropping system pH tolerance is the single most important factor for me as a producer today. Alternative crops are almost nonexistent. At today's market and target prices for wheat liming is too costly. My pH's range from 4.8 to 7.6. The overall average is 5.8. In two years it has decreased an average of .4. In six years it has decreased an average of almost a full point.

Hard white wheat will be added to my choice dilemma in the years to come. I have talked to our export customers and they all want to know about HWW. I think it will be a significant part of my product mix in the years to come. I am glad to see it on your agenda.

Progress involves risk. As a wheat producer, I am looking forward to the day of being chemically free in a continuous no-till wheat cropping system thanks to your efforts.

# Management Practices and Net Returns in a Wheat-Stocker Enterprise

Gene Krenzer, Small Grains Extension Specialist  
Oklahoma State University

Wheat in the southern Great Plains is unique in that we harvest it for dual purposes. Cattle graze much of the wheat from November through the winter. When the wheat begins to joint, cattle are removed and the wheat is harvested for grain. Because of this dual use, there are additional management factors that are considered by the producer. Today, I would like to share some of what we have been learning about a few of these management factors. The overall objective is to evaluate practices which might increase the net return in a wheat-stocker cattle enterprise.

There are four general ways of increasing the net return in a wheat-stocker cattle enterprise:

- Improve animal performance.
- Increase stocking rate.
- Lengthen the grazing season.
- Increase the grain yield and/or test weight.

In today's discussion, I will concentrate on two of these aspects: increasing the stocking rate and lengthening the grazing season.

## Increasing Stocking Rate

For the last two years, we have conducted trials at our Wheat Pasture Research Unit at Marshall, OK. where we have four varieties each being grazed at four different stocking rates. These trials are conducted in 18 to 24 acre

pastures. Wheat is planted in early September at 90 lb/acre. Enough nitrogen is available at planting to produce 300 lb/acre of beef and 50 bu/acre grain. Cattle begin grazing around November 1 and are removed at the first hollow stem stage of growth. This will be defined later.

As the stocking rate increased, the beef produced per acre has increased (Fig. 1), but the grain yields have decreased. Applying economics to this data, we conclude that the reduction in grain yield has more than compensated for the beef gain differences (Fig. 1). However, we have only conducted this study over two years at one location and desire several years' data before we draw very many conclusions. The preliminary conclusion would be that producers may be trying too hard to take advantage of every bite of forage produced and decreasing their net return per acre by doing so.

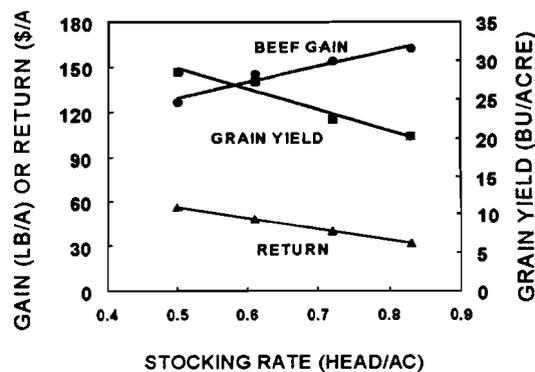


Figure 1. Stocking rate while grazing wheat pasture influences beef gains, grain yield and net return per acre.

## Lengthening the Grazing Season - Start Grazing Earlier

The grazing season can be lengthened by either starting grazing earlier or removing the cattle from wheat pasture later. I will discuss several ways we might be able to influence either end of the grazing season. First we will look at ways we might be able to start grazing earlier.

To start grazing earlier, we have to obtain a minimum amount of forage by an earlier date.

**1. Plant wheat earlier.** Several years ago, there seemed to be a general understanding that wheat should not be planted before the soil temperature was below 85°F in mid-afternoon. Since then, we have learned that one reason for this understanding was because poor stands were obtained when soil temperatures were too high. With shallow planting, no seed deeper than one inch, excellent stands can be obtained even if soil temperatures at mid-afternoon are above 100°F. As long as enough soil moisture and rainfall are available to prevent drought stress, planting date has a major impact on forage yield before first hollow stem (Fig. 2). These data are averages of years with little drought stress in the fall and others like 1993-94 at Lahoma where forage production was severely limited because of drought stress.

It is important to note that the forage yield increases do not come free. For reasons unknown at this time, the earlier planting dates also strongly influence test weight (Fig. 3) and grain yield (Fig. 4). We intend on applying economics to these data in the near

future, but have not gotten them ready yet.

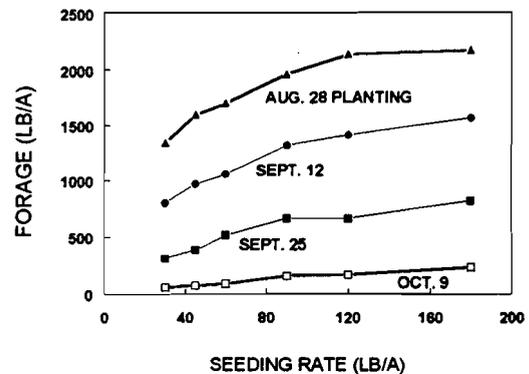


Figure 2. Forage produced by the first hollow stem stage of growth increases as seeding rate increases and wheat is planted earlier.

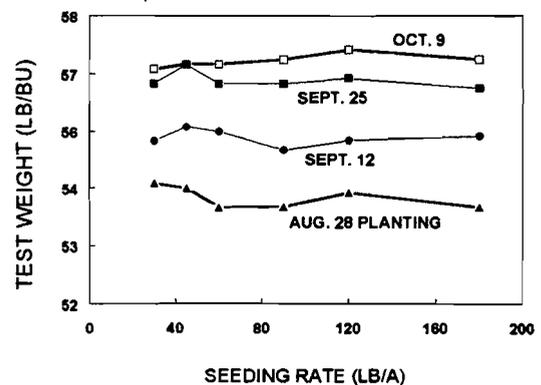


Figure 3. Planting date and seeding rate influences on test weight of wheat from which forage was removed by clipping until the first hollow stem stage.

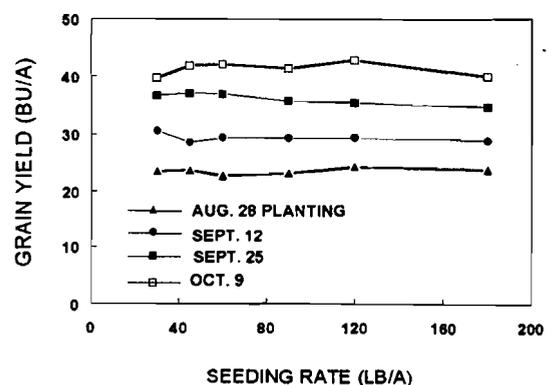


Figure 4. Planting date and seeding rate influences on wheat grain yield of plots clipped to remove forage until the first hollow stem growth stage.

**2. Increase the seeding rate.** Our best guess is that most producers in central Oklahoma involved in the wheat-stocker system are planting approximately 90 lb/acre. We have seen that increasing the seeding rate as high as 180 lb/a has increased the forage produced prior to first hollow stem (Fig. 2). Depending on the price you assign to wheat seed, the return may or may not exceed the increased cost. We have not finished the economics for this data set. The strong point here is that increasing the seeding rate does allow us to produce more forage earlier.

**3. Improve stand establishment.** From Fig. 2, we see how important the number of plants is for increasing forage production. In the fall of 1992 and 1993, we conducted a survey of producer fields to determine how well they were doing in stand establishment. To conduct the survey, we contacted agricultural agents to set a date when many producers were planting in their county. We traveled down a highway where wheat was being grown and stopped every drill we saw planting. Over 100 fields were included in the survey. The average field had 57% of the live seed planted resulting in a plant (Fig. 5).

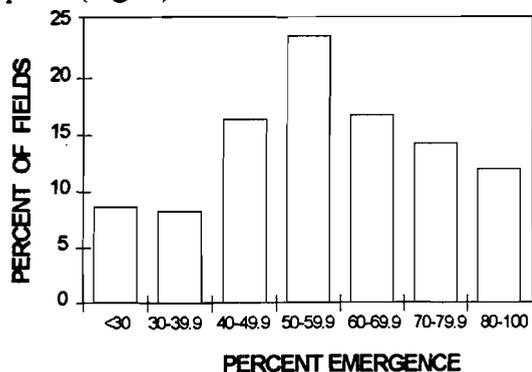


Figure 5. Stand establishment results showing the percent of fields surveyed contained in each percent emergence category. Percent emergence is % of live seed producing plants.

This means that in the average field, two seeds were planted to produce one plant. To me this means the average producer needs to plant twice as many seeds per acre as indicated on the graph in Fig. 2 to obtain the same forage yield. In these research plots, we have never attained less than 80% emergence.

The most frequent reason we were able to identify for poor stands was seed placed so deep the coleoptile could not grow long enough to reach the soil surface. The first true leaf emerged through the coleoptile tip and was trapped below the soil surface. When we removed soil from above the row, the resultant yellow accordion-leaved plant sprung from beneath. Such conditions could be lessened by planting shallower and at a more consistent or uniform depth. One way to reduce this problem is to have a firm seedbed prepared and use very shallow tillage just prior to planting. Another way of reducing the stand establishment difficulties is using varieties with longer coleoptiles. However, under hot soil conditions coleoptile length is reduced with all varieties as seen in the following table.

Variety	Soil Temperatures of Early	
	September	October
Karl	1.6	2.3
2180	1.5	2.0
Chisholm	1.5	2.4
AgriPro Longhorn	2.4	3.5
Scout 66	2.4	3.5

Table 1. Coleoptile length (inches) of selected wheat varieties grown in soil temperatures representing early September and early October planting conditions.

**4. Use starter fertilizer.** For this discussion, a starter fertilizer is one containing both nitrogen and phosphorus and is applied directly in the seed furrow with the seed. Early forage yields have been increased by using a starter fertilizer (Fig. 6). Apparently, the fertilizer in close proximity to the developing root system helps the plant get started faster. No grain yield response has been obtained (Fig. 6).

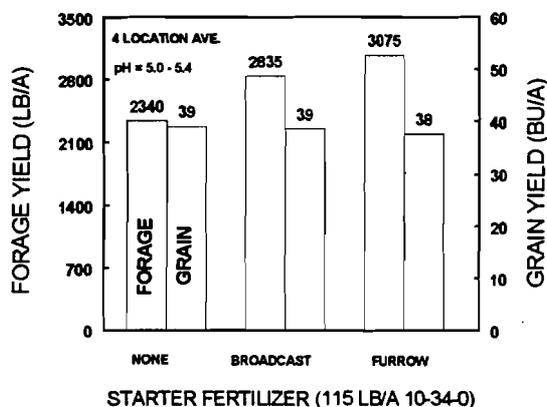


Figure 6. Forage and grain yield differences in a starter fertilizer trial.

**5. Variety selection.** Different wheat varieties produce different quantities of forage prior to first hollow stem. In Fig. 7, we show differences in forage yields of those varieties which were above average in grain yield for that particular year. This shows how big the differences are among varieties that are good grain producers.

We do need to keep in mind that frequently the limiting factor on how early we can graze the wheat pasture is not based on whether we have enough forage or not. Sometimes it is when the cattle are ready for wheat pasture, and other years is when can we get the nodal root system, those roots developing at the crown in contrast to the original roots emerging from the seed, developed.

Producers frequently call these the secondary roots. Normally when the first tillers become visible, we should be able to find nodal roots expanding. If the soil is dry, they will not expand until it is moistened. These roots are important because they anchor the plant in the soil. The seminal root system provides some anchoring, but frequently the internode between the seed and the crown is not strong enough to withstand the force of cattle pulling on the wheat plant as they graze. Therefore, wheat plants are easily pulled up by grazing cattle until the nodal root system develops enough to anchor the plant. Grazing should not be initiated until this has occurred.

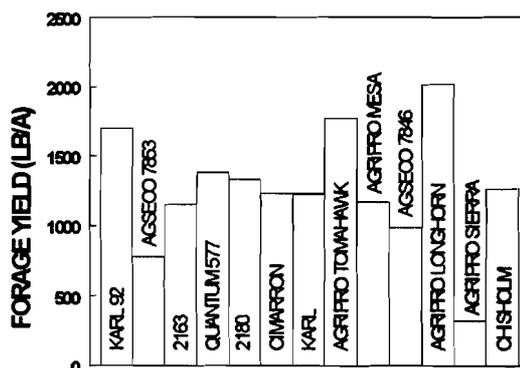


Figure 7. Forage yield prior to the first hollow stem stage of growth of hard red winter wheat varieties which yielded above average in 1992-93 grain yield trials.

### Lengthening the Grazing Season - Terminate Grazing Later

**1. Grazing after first hollow stem.** One of the most frequently asked questions in the late 1980's was "How much does it hurt the grain yield if we graze the cattle a few days or weeks later into the spring?" We have been evaluating this with a trial at Marshall since the 1989-90 wheat year. To accomplish this, we place an enclosure in a wheat field being grazed whenever we want to terminate grazing. An enclosure is erected by placing four 16 foot long

welded wire cattle panels in a square. We are monitoring cattle weights throughout the grazing period so we can calculate cattle weight gains from the pasture. Grain yields are measured inside each enclosure. Combining the economics from grain yields and beef gains, we calculate net return to the system for each grazing termination date.

Stage of wheat development appears to be critical in determining when grain yield begins to decline with continued grazing (Fig. 8).

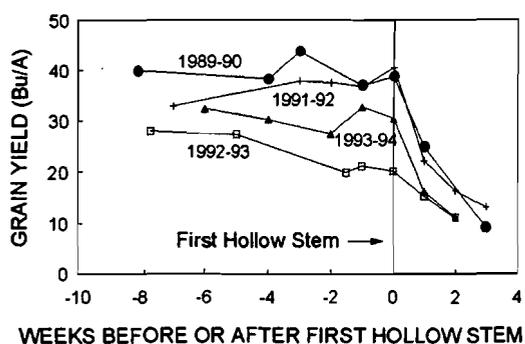


Figure 8. The effects of removing cattle from wheat pasture at different times on grain yield.

Development cannot be predicted by calendar date; therefore, we present the data in terms of time before or after first hollow stem. First hollow stem is defined as the growth stage where hollow stem can first be identified above the crown in larger wheat shoots and occurs before the growing point (head) reaches the soil surface. First hollow stem is the earliest portion of the jointing stage of growth. See "Wheat For Pasture" (FS - 2586) for pictures or the January 1993 "Fine Tuning Wheat Production" (TC 1320) video to see how to determine when first hollow stem occurs. At Marshall, Oklahoma, first hollow stem stage for Karl wheat occurred as early as February 28, 1992 and as late as March 16, 1993.

Temperatures during January and February strongly influence the date first hollow stem occurs.

Removing stocker cattle from wheat pasture one to six weeks prior to first hollow stem had no effect on grain yield (Fig. 8). In Fig. 9, we summarize the grain yield response to show the four-year average. This would also be our best estimate of what will happen next year.

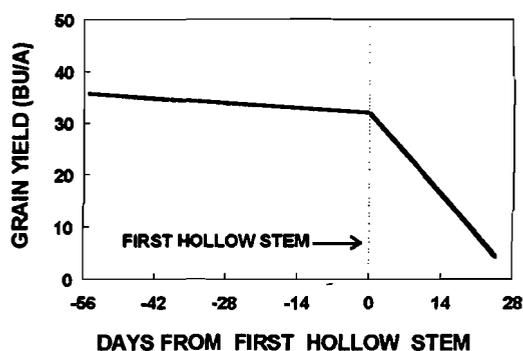


Figure 9. Average effect of removing cattle from wheat pasture at different times on grain yield.

Net return from cattle (Fig. 10) continued to increase with length of grazing season due to continued weight gain per animal. However, net return from wheat grain and total net return from the system decreased as cattle continued to graze beyond first hollow stem. Beef gains after first hollow stem do not compensate for reduced grain yield and rapid decreases in net return occurred when cattle continued to graze just a few days after first hollow stem. To obtain maximum return per acre, producers need to watch closely for the first hollow stem. A few less days of grazing reduced net return far less than grazing a few days too long.

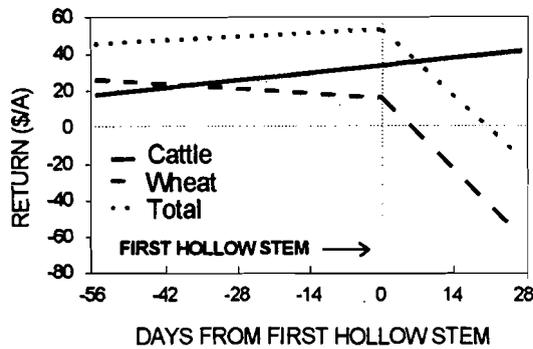


Figure 10. Changing the time of grazing termination effects on return to cattle, wheat, and the combination of cattle and wheat.

Historically, we have been taught that removing cattle from wheat pasture prior to the time they could graze off heads as the wheat stem elongates was critical. Now we learn that stem elongation is delayed by grazing (Fig. 11), and wheat that is not being grazed needs to be monitored to determine when first hollow stem occurs. Figure 11 shows the amount of hollow stem that could be seen in wheat two weeks after the ungrazed wheat reached first hollow stem. No hollow stem could be detected in the continuously grazed wheat, yet grain yield and net return had decreased sharply. Therefore, ungrazed wheat of the same variety, planting date, etc., needs to be checked to determine when first hollow stem occurs.

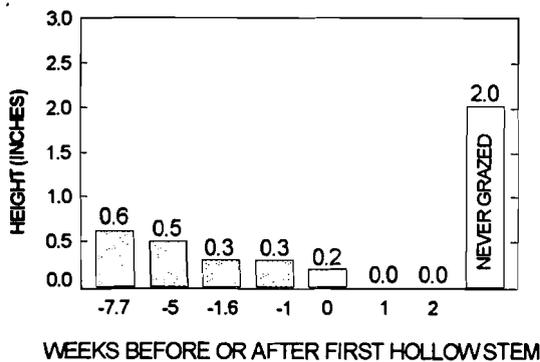


Figure 11. Height of the growing point above the crown two weeks after ungrazed wheat reached the first hollow stem stage of growth.

**2. Variety selection.** Wheat varieties do not all reach the first hollow stem stage at the same time. Producers who would like to extend the grazing season slightly later into the spring could select a variety which reaches first hollow stem at the latest possible time relative to other varieties. We have monitored first hollow stem stage for the variety trial at Marshall the last two years. TAM 202, AgriPro Tomahawk, and 2180 have been the varieties reaching first hollow stem the earliest in both years and AgriPro Ponderosa, Chisholm, Cimarron, Ike and 2163 have been the latest. The difference between these groups was 9 days in 1994 and 18 days in 1995. We had a cold period during this time period each year, but the cold period was much longer in 1995.

Data from Texas indicates there may be a difference in how wheat varieties respond to grazing termination dates. When evaluating grain yield of grazed wheat compared to wheat grown for grain only, the yield of tall wheat varieties was reduced 12 and 25% when they were grazed until Feb. 3 and March 19, respectively, while semidwarf varieties were reduced 36 and 53%. We have included Scout 66 in the trials at Marshall in 1994-95 along with 2180, AGSECO 7853 and AgriPro Longhorn. We will be collecting data on first hollow stem stage as well as grazing termination information as discussed earlier to determine if tall varieties suffer less yield loss when grazed beyond the first hollow stem stage.

**Summary:**

1. Producers seem to want a wheat variety for the wheat-stocker cattle enterprise which has the following characteristics:

Long coleoptile, Late first hollow stem, Produces much forage early  
High total forage, High test weight, High grain yield

2. Planting date information might be summarized as follows:

Very early	> forage	< test weight	< grain yield
Later	< forage	> test weight	> grain yield
Net return	???		

3. Seeding rate between 90 and 150 lb/acre appears ideal depending on your ability to obtain an excellent stand and the cost of seed.
4. Modify practices where possible to obtain an excellent stand or increase seeding rate to compensate.
5. Use a starter fertilizer.
6. Terminate grazing on or before first hollow stem stage of growth in ungrazed wheat.
7. Identify your primary purpose for growing wheat in each field and apply the best production practices for that purpose.

# Possible Effects of Management Practices Associated with Sustainable Agriculture on Diseases

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Department of Plant Pathology  
Kansas State University

The severity of winter wheat diseases is greatly affected by the environment in and around the wheat crop. Any major shift in crop management practices that affects this environment would be expected to influence wheat disease. Widespread adoption of management practices associated with "sustainable agriculture" could have important implications for wheat diseases in the future. Below are four major effects that would occur by grower adoption of more sustainable farming systems.

1. **More crop residues left on or above the soil surface.**
2. **More use of crop rotations.**
3. **Less use of foliar fungicides.**
4. **Less weed control.**

**Surface Residues:** Leaving more crop residues on the soil surface would be expected to increase those diseases whose causal agents survive in surface-borne residue. For winter wheat in the Great Plains, several diseases should be favored by that management practice including; tan spot, take-all, head blight, speckled leaf blotch, glume blotch, and powdery mildew. Although the pathogens that cause head blight, speckled leaf blotch, glume blotch, and powdery mildew can occur in fields with

little or no residue on the soil surface, their severities should increase with reduced-tillage practices. The reason they occur in "clean-till fields" is because they produce a spore phase that is airborne for long distances. However, if more producers leave residue on the soil, this will increase the spore load on a county- or region-wide basis, increasing disease pressure. Finally, wheat streak mosaic would be expected to increase with more crop residues on the soil due to the poorer volunteer wheat control that usually occurs with reduced tillage. The mite vector and virus survive the summer primarily in volunteer wheat and move to seeded wheat in the fall.

One example of a disease that is increased by surface-borne residues would be take-all. In experimental plots infested with the pathogen, inoculum incorporated into the soil at planting caused 58% yield loss compared with the noninoculated control. When the same amount of inoculum was incorporated into the soil 2 months before planting and the soil left bare, there was only 5% yield loss. However, when inoculum was incorporated 2 months before planting and the soil shaded with wheat straw, there was 23% yield loss. The pathogen survived the summer to a higher degree when it was in soil that was shaded

(cooler) compared with when it was under bare-soil conditions.

There are only two diseases of the Great Plains that would be expected to decrease with more crop residues on the soil. These are common root and crown rot (caused by *Cochliobolus sativus*) and dryland foot rot (caused by *Fusarium* spp.). The mature-plant phase of these diseases is favored by drought stress and high temperatures. More water is trapped and held under reduced-tillage conditions. Additionally, there are publications showing that the increased availability of soil water associated with reduced tillage reduces these diseases.

**Crop Rotations:** One of the cornerstones of sustainable agriculture is the use of crop rotations. As a general rule, rotations increase soil fertility, reduce weed populations, and control many diseases. Rotations to nonhost crops would be expected to reduce the following diseases important in the Great Plains: tan spot, take-all, wheat streak mosaic, head blight, speckled leaf blotch, and glume blotch. These are the same diseases that are favored by reduced tillage. Crop rotation would allow the infected host residues to degrade between wheat crops, killing the pathogens.

An example of the effect of rotation on disease would be the control of tan spot with a wheat-sorghum rotation. In this rotation, wheat was grown every other year with grain sorghum grown the other years. To accomplish this, wheat was seeded directly into sorghum residue immediately after sorghum harvest. Additionally, there were three levels of wheat residue management (conventional

till, reduced till, no-till). With continuous wheat, tan spot was very low with conventional tillage (plow), moderately severe with reduced tillage, and very severe with no-till. Under the wheat-sorghum rotation, however, tan spot was controlled in all cases, even where no-till was practiced. The pathogen dies out during the one-year break between susceptible wheat crops.

There are no diseases that would be favored by an increase in the use of crop rotation. However, this would not be the case if the rotation crop was also susceptible to the pathogen. Therefore, wheat in a rotation involving corn would be expected to have head blight because the causal organism (*Fusarium graminearum*) attacks both those crops. The key to a successful rotation is the choice of an alternate crop that is not a host of the pathogens attacking the primary crop.

**Foliar Fungicides:** A shift to more sustainable farming practices would probably involve using less synthetic pesticides. This would result in less acreage treated with foliar fungicides which would affect wheat foliar diseases. Diseases which would be expected to increase with less fungicide would be; leaf rust, tan spot, speckled leaf blotch, glume blotch, and powdery mildew. There are no known diseases that would be reduced with less use of foliar fungicides.

In addition to the increase in foliar diseases, the quantity and quality of wheat seed would be affected by reduced use of foliar fungicides. The target size for seed wheat is to have seeds greater than 6/64 of an inch in diameter. There are numerous reports of advantages of

large seed over smaller seed. In Kansas, a single application of foliar fungicide has consistently provided a 5-10 bushel per acre increase in the amount of large seed produced. This has occurred only on cultivars that are susceptible to one or more important foliar diseases. Increases have not occurred on resistant cultivars. Therefore, reduced use of fungicides may affect the quantity and quality of seed wheat.

**Weed Control:** Adoption of more sustainable farming practices would be expected to result in less weed control. There would probably be less use of herbicides and less mechanical weed control by tillage. Wheat diseases that may increase with less weed control would be wheat streak mosaic, take-all, and tan spot. This would only happen if the weed problems were volunteer wheat or grassy weed hosts of the pathogens; other types of weeds would not greatly affect these diseases.

An example would be the effect of volunteer wheat during the summer on carryover of the take-all pathogen. When inoculum of the fungus was incorporated into soil at planting time, there was 52% yield loss from take-all compared with the noninoculated control. When an equivalent amount of inoculum was incorporated into the soil 2 months before planting, only 12% loss occurred. The fungus had largely died out during the 2-month overwintering period. However, when the same amount of inoculum was incorporated into the soil 2 months before planting and the plots seeded with wheat to simulate volunteer, 26% yield loss occurred in a subsequent wheat crop. The pathogen survived on the volunteer wheat during

the summer and then grew onto the seeded wheat that fall.

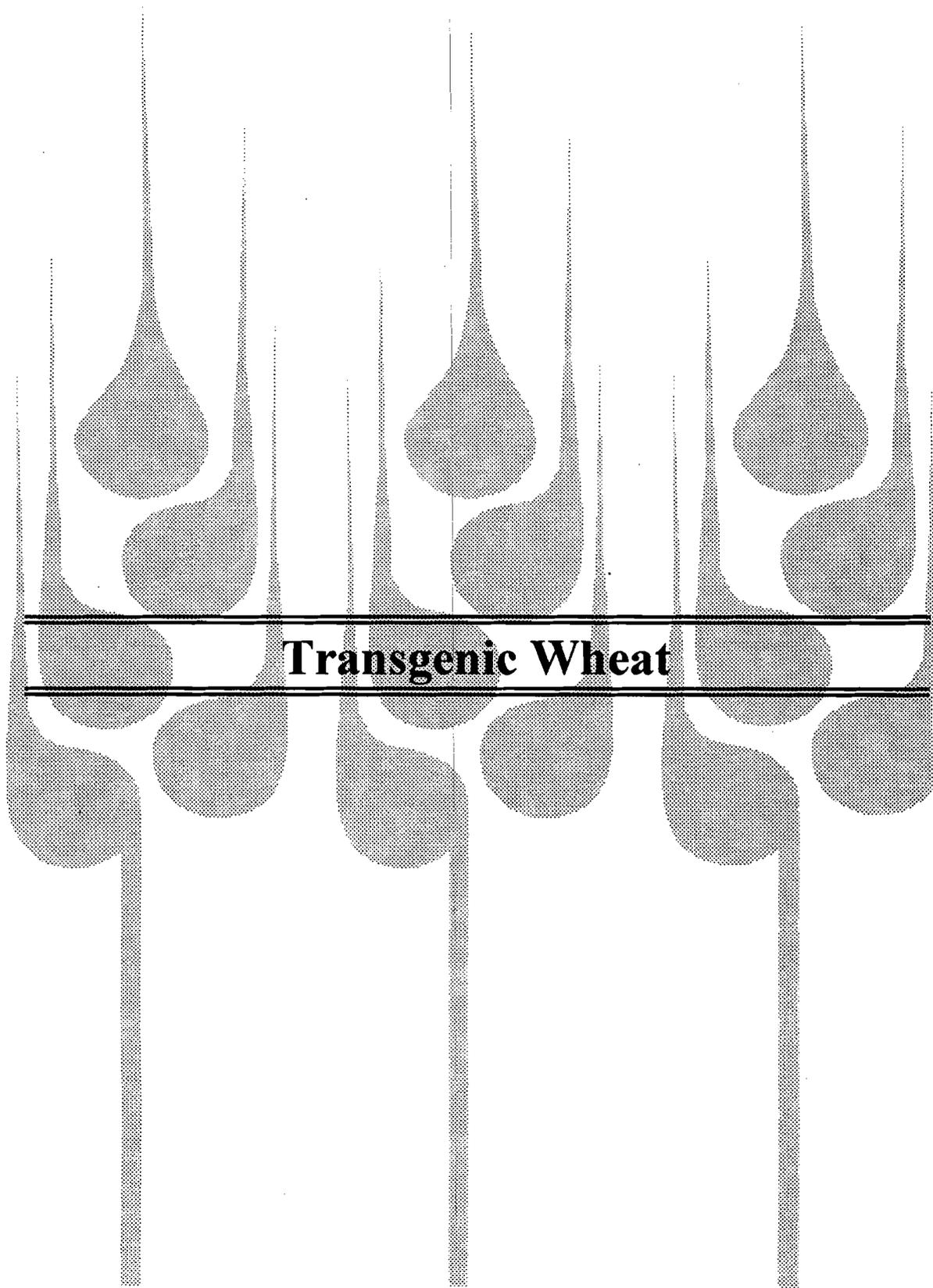
**Summary:** If more sustainable farming practices are adopted, there would be four major effects on the environment of the wheat crop: 1) more residue on the soil; 2) more crop rotations; 3) less foliar fungicide use; and 4) less weed control. These effects could influence the severity of wheat diseases. They will be summarized in reverse order.

Less weed control may increase wheat streak mosaic, take-all, and tan spot. Of these diseases, however, the effect on wheat streak should be of most concern. Because only about 1% of the acreage in Kansas is sprayed with foliar fungicides, less use of fungicides would have minimal impact on the wheat disease picture. Nevertheless, foliar fungicides have great potential to increase the quantity and quality of seed wheat; therefore, less fungicide use may negatively impact the wheat seed industry. Crop rotations are an excellent method to control many diseases; however, in the near future, it is unlikely that there will be widespread increase in the use of rotations in the continuous wheat belt of the Great Plains. The effect listed above with potentially the most impact on the wheat disease picture in the near future would be an increase in the amount of wheat residue on, or above, the soil surface. This should increase several important diseases including; tan spot, take-all, wheat streak mosaic, head blight, speckled leaf blotch, glume blotch, and powdery mildew. There are also wheat diseases that are not currently recognized as problems in the Great Plains that may become severe

such as *Pythium* and *Rhizoctonia* root rots.

Data collected over multiple years for continuous wheat indicate that the more residue producers leave on the soil surface, the less the grain yield (example: Epplin et al. 1994. *J. Soil & Water Cons.*). Continuous, no-till wheat yields about 30% less than continuous, plowed wheat. Several factors result in the reduction including weeds, insects, and diseases. The challenge to wheat breeders/researchers is to solve these

problems. For wheat diseases, this would include incorporation of resistance to those pathogens that become yield limiting under reduced-tillage conditions. Finally, as far as wheat disease control is concerned, crop rotation coupled with reduced tillage would have the advantage of allowing more sustainable farming practices with fewer severe disease problems. For the long term, there should be increased effort to find and implement rotations that would be more attractive to wheat producers in the Great Plains.



**Transgenic Wheat**



# Genetic Engineering of Wheat for Drought Tolerance

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Advances in plant biotechnology will prove to be a novel and powerful tool for plant improvement by the introduction, stable integration and expression of defined foreign genes into most cereal species. In recent years, microprojectile bombardment of tissue derived from immature embryos has become the established procedure for wheat transformation [1,2,3,4]. In our laboratory we were successful in establishing a transformation protocol that yielded multiple transformed wheat lines without excessive effort, was reproducible on a regular basis, and yielded fertile transgenic lines that passed on the genotypes and phenotypes to successive generations.

Research has since been conducted on improving and modifying the existing protocol. These approaches included manipulation of media components, DNA delivery modification, and transformation selection strategies. Vain *et al.* [5] reported that osmotic conditioning of the target cells resulted in a 6.8-fold increase in recovery of stably transformed maize clones. It was suggested that the basis of osmotic enhancement of transient expression and stable transformation resulted from plasmolysis of the cells which may have reduced cell damage by preventing extrusion of the protoplasm from bombarded cells. Our experiments showed that by maintaining scutellar calli on 0.4 M mannitol containing medium four hours pre-bombardment and

sixteen hours post-bombardment would greatly increase the transformation efficiency. The osmotic conditioning treatment has increased our transformation efficiency to greater than one percent.

The selection procedure was another aspect of the protocol that we have improved on. Previous results of ours have shown that both the phosphinothricin acetyl transferase (PAT) and  $\beta$ -glucuronidase (GUS) assays were not strictly indicative of transformation. In addition, the PAT assay involves the use of a radioisotope which makes it inconvenient to use because of the safety and wastes disposal conditions and the GUS assay is destructive, killing the tissue that is sampled.

Also, selection based on herbicide-containing medium can be tedious and time-consuming. Kramer *et al.* [6] have described a method for identifying transformed cells with the inclusion of a pH-indicator in the culture medium. The method allows for the identification of transformed tissue more quickly and efficiently by observing a color change from a red to yellow in the medium. It is also non-destructive to the plant tissue. We have applied this procedure to the selection of putative transformed shoots in our protocol. This method has made it possible to select transformants after two days rather than the usual two weeks. It has also significantly reduced the number of

escapes and cultures that would have to be carried forward.

The modification and optimization of our protocol now makes it feasible to bioengineer agronomically important traits into wheat. One interest in our laboratory is to use the transformation technology to enhance drought resistance in wheat. Drought stress is a major constraint to obtaining maximum wheat yields in semiarid regions. Drought stress limits wheat productivity by reducing tillering, leaf growth, seed weight, and seed number. To increase agricultural productivity, crop plants must be developed to efficiently utilize available moisture.

Tarczynski *et al.* [7] isolated an osmolyte gene (sugar alcohol mannitol) and reported that this gene in vivo protects against high salinity in tobacco and also may enhance other stress tolerances such as water stress. Wheat calli have been bombarded with a osmolyte gene and selected on medium having a low water potential. Callus cells which have been transformed with the osmolyte gene should be able grow in the presence of low water potential and be easily selected. The presence and expression of the osmolyte gene will be critical for selection but also be beneficial to the plant for drought resistance. Wheat plants will be regenerated from calli tolerant to low water potentials. Progress was presented on the development of the osmolyte selection strategy to recover drought resistant wheat plants.

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# Using Transgenic Wheat to Explore Protein Contributions to Bread-Making Quality

Olin D. Anderson, Ann E. Blechl, and J. Troy Weeks

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The past two years has seen reproducible wheat transformation reports from several laboratories (Weeks et al., 1993; Becker et al., 1994; Nehra et al., 1994; Vasil et al., 1994). In addition, several other Triticeae have yielded to similar transformation protocols (rye - Castillo et al., 1994; tritordeum - Barcelo et al., 1994; barley - Yuechun and Lemaux, 1994). Our laboratory has concentrated on the wheat cultivar Bobwhite, a hard white spring wheat which is highly competent to regenerate from immature embryos. Initial experiments were reproducible, but the efficiency was not high enough for routine experimentation. However, the efficiency is now approaching one successfully produced transgenic line per fifty embryos and we are beginning to exploit this new capability.

The next major question facing us is, "What DNAs to integrate?" The first targets for genetic engineering of wheat will be, by necessity, traits controlled or greatly influenced by single genes. The wheat storage proteins have been a long term subject of study in our laboratory because of their known correlation with quality characteristics. Of the several families of seed proteins, the high-molecular-weight glutenin subunits (HMW-GS) have the highest correlation

to quality parameters. This is fortunate since the HMW-GS genes are also the smallest family of wheat storage proteins, with never more than five genes active in a single hexaploid cultivar. Engineering would be more difficult if, for example, the 30-100 member  $\alpha$ -gliadin family was the most important seed protein class for wheat quality. In addition, studies of near-isogenic-lines missing 1, 2, or all 3 of the HMW-GS loci have shown strong additive effects on quality (Lawrence et al., 1988). Thus, there is a high expectation that the simple addition of new HMW-GS loci will have significant effects on parameters such as dough strength.

The creation of new HMW-GS loci requires the prior isolation and characterization of HMW-GS genes. Again, this gene family is a good candidate since all six genes have been isolated and sequenced (2\*, 5, 7, 9, 10 and the silent Ay) from a single cultivar, the hard red winter wheat Cheyenne (Forde et al., 1985; Halford et al., 1987; Anderson and Greene, 1989; Anderson et al., 1989). Clones of these genes are therefore available for molecular manipulation and wheat transformation.

One of the powers of this general approach is the potential to create more genetic diversity for a specific gene than

is possible by traditional breeding. In the present case of the HMW-GS loci, the only previously available strategy has been to screen cultivars and wild wheats for novel alleles which are then crossed into elite lines. The researcher may have no information on the potential usefulness of the new allele until quality testing is carried out after significant time and resources are devoted to moving the new allele into useful wheat lines. Even then, no fundamental information is gained on the basis of the effect of the new allele unless the DNA is cloned and sequenced. In contrast, the molecular biology/transformation approach allows directed gene modifications to be tested in known backgrounds.

For example, while the molecular basis of the functionality of the HMW-GS is not proven, most theories concentrate on the two most prominent characteristics of these polypeptides: the long repeat domain structured from 2 or 3 short peptide motifs that composes most of the subunit; and the presence of the cysteine residues in the non-repetitive N- and C-terminal domains. Almost certainly, most, if not all, of the allelic size heterogeneity in these subunits is caused by variation in the repetitive domain (D'Ovidio et al., 1995a; Anderson et al., in preparation) resulting from duplication/deletion events promoted by the long series of similar, short DNA repeats. The laboratory creation of HMW-GS genes with specific repetitive domain length changes would allow both the creation of new alleles and the determination of the effects on quality of variation in repeat length. As a first step, we have constructed three variants of the Dx5 gene (D'Ovidio et al., 1995b). One contains a repeat domain 20% longer than the original Dx5 gene,

and the others contain repeats 20% and 40% shorter. These constructs have been expressed in a bacterial expression system that produces enough protein for use in physical/chemical studies of repeat structure and in micro-dough-mixing experiments such as in Bekes et al. (1994). Similar constructs can be used to transform wheat to express specific novel HMW-GS in the endosperm.

As a first step in HMW-GS gene transformation into wheat we needed to confirm that the promoters from the cloned genes would function normally in transgenic wheat. This is a major concern since it is not known exactly what portions of the promoter sequence control levels of expression and developmental fidelity. If the transgenes express at too low a level, the amount of new subunit synthesis may be insufficient to confer changes in physical parameters. Since the endogenous HMW-GS genes each contribute about 1% of the wheat endosperm protein, the transgenic loci must express near this level. Lower levels may be sufficient if multiple copies are inserted or if transgenic loci are pyramided. The promoters must also function in a developmentally correct manner. It is undesirable to cause HMW-GS expression in other tissues. While we cannot predict the consequences of such ectopic expression, the result of production of glutenins in anthers or roots is not likely to be favorable. To address both these concerns, we first constructed a gene with a HMW-GS promoter and a coding region from the bacterial gene encoding  $\beta$ -glucuronidase (GUS), a commonly used reporter gene which allows colorimetric detection of the gene product. GUS is an extremely stable enzyme and is considered a sensitive

assay for gene expression. Transgenic wheat lines were produced using this construct, and testing for sites of GUS expression are ongoing. Results in the first several transgenic lines show no GUS activity in any tissue except the endosperm.

The next experiment needed to use a HMW-GS coding sequence to compare endogenous gene expression with the transgenic loci. The only direct assay for the expression of a single HMW-GS is a protein gel of endosperm contents. However, since the cultivar we were using (Bobwhite) contained exactly the same set of HMW-GS genes as those previously isolated from cv Cheyenne, we did not expect to be able to detect increased levels of the same subunit. Our expectation was that levels of expression from the new locus could be lower than from the endogenous loci (we were wrong). We decided to use a HMW-GS subunit that would migrate in SDS-PAGE well separate from any other protein. Unfortunately no such HMW-GS was available, and we decided to construct a novel gene. From our previous work on expression of HMW-GS genes in bacteria we had shown that chimeric subunits made up of segments from different subunits can migrate in different positions from the original subunits (Shani et al., 1992). A construct was selected to encode a subunit which migrated faster than the Dx5 subunit and in a region of the gel devoid of other protein bands. This construct, D10/5, encodes a subunit made up of the N-terminus of the Dy10 subunit and the repetitive and C-terminus of the Dx5 subunit. The promoter was from the Dy10 gene.

Thus far approximately 30 transgenic lines have been produced using this construct. Seventeen lines have been assayed for seed protein expression and 11 of these lines are expressing a new protein band in exactly the correct position for the D10/5 subunit. The intensity of the new band varies from just detectable to as much as five-fold more intense than the band from the natural Dx5 gene of cv Bobwhite. This result has several far-reaching implications. Not only does level of expression of transgenic HMW-GS not appear to be a problem, but the HMW-GS promoters may be the promoters of choice when targeting endosperm molecular engineering. The strength and developmental control evidenced by this promoter, in our preliminary results, make it a logical choice for manipulation of other aspects of grain quality, both to express novel loci and to reduce expression with anti-sense constructions.

As encouraging as are our results there is still one major potential hurdle. Are these transgenes indefinitely stable in their patterns of expression? Abundant evidence from dicot transformations shows that transgenes can vary in their levels of expression or even be completely shut off. This suppression phenomenon is often associated with methylation, both molecular aspects of gene control which are poorly understood. At this stage in technology development it will require following transgenes for many generations to assure stability. Yet the early results with the HMW-GS constructs are sufficiently encouraging to begin crossing the new HMW-GS into other lines, following stability under field and stress conditions, and carrying out quality testing on amplified seed.

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The background of the page features a stylized, halftone-style illustration of wheat stalks. Each stalk has a long, thin stem and a head of grain. The grain heads are composed of several rounded, teardrop-shaped segments. The entire illustration is rendered in a light gray, dotted pattern. A horizontal band with a double-line border is centered across the middle of the page, containing the title text.

**Hard White Wheat Development**



# Improvement of Sprouting Tolerance and Seed Color in Hard White Winter Wheats

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The conversion of the Kansas breeding efforts to the development of hard white winter wheat was accelerated in 1988 as the result of increased support for the effort by the Kansas Wheat Commission, Kansas Crop Improvement Association, and the Kansas Technical Enterprise Corporation. Currently about 50% of the Hays program and about 35% of the Manhattan based program are devoted to the development of hard white wheats.

I would like to address some of the major changes we have had to make in the hard white efforts that were not necessary when we were working exclusively with hard red winter wheat. The two characteristics I want to cover are hard white wheat sprouting tolerance and seed color.

## White Wheat Sprouting Tolerance

There have been a number of white wheat sprouting tolerant sources released as germplasm or cultivars. Table 1 lists the sources used in the Kansas breeding program. The Agripro variety Rio Blanco should also be on that list. Rio Blanco does carry an effective level of preharvest sprouting tolerance.

**Table 1.** Sources of Preharvest sprouting tolerance which have been used by the Kansas breeding program.

Line	Developer	Class
P1520756 to P1520760	Kansas	HWW
Kite	Australia	HWS
Suneca	Australia	HWS
NY6432-18	New York	SWW
NY6708-18	New York	SWW
RL4137	Canada	HRS
Lowsprout	Canada	HWS

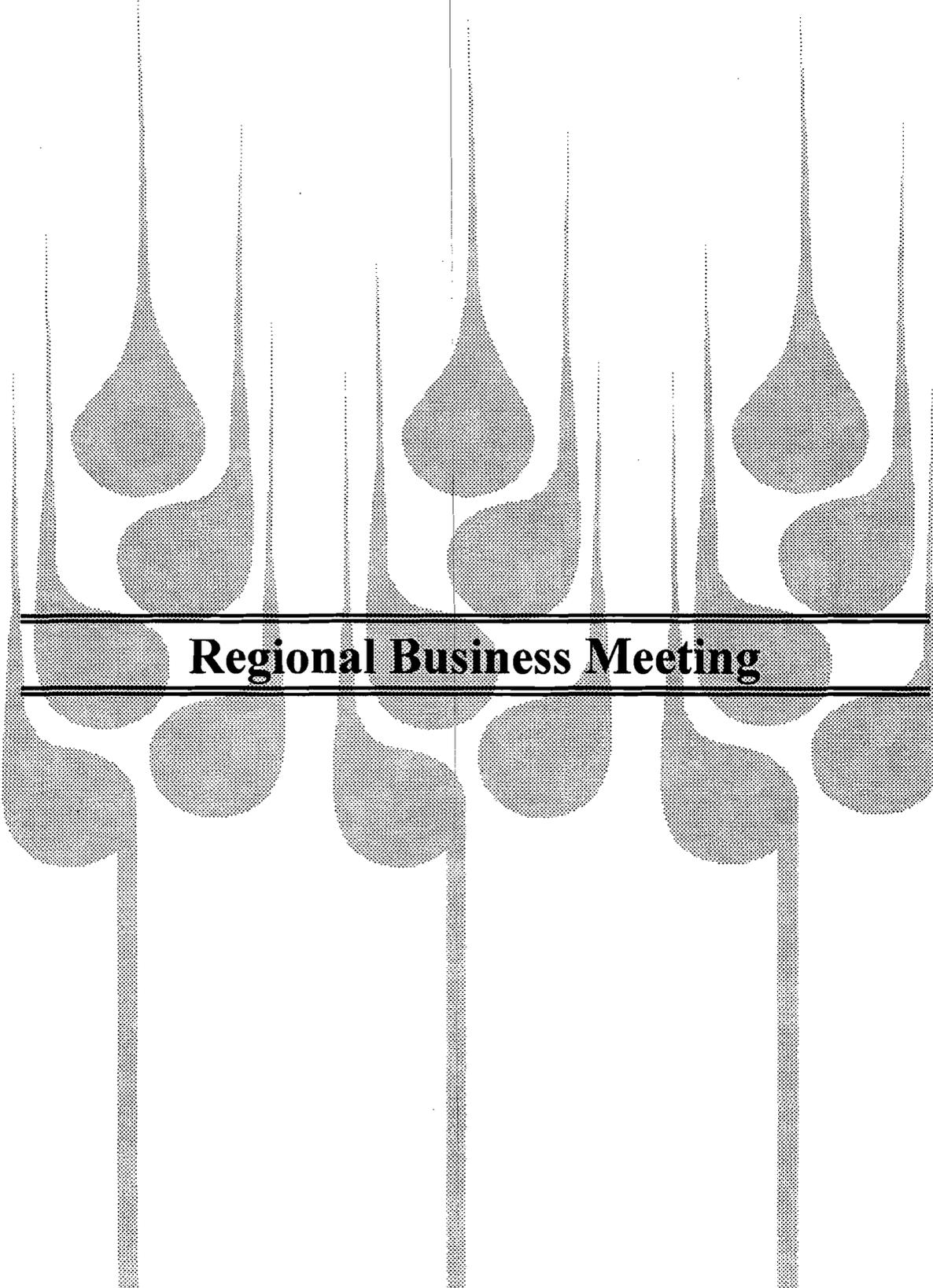
Methods for selecting sprouting tolerance have been described and are readily available in the literature. We have used two methods for sprouting tolerance evaluation. The first is used on intact F<sub>3</sub> head selections that are harvested as they reach physiological maturity (PM). We use clearing of the green from the stem just below the head as an indicator of PM. These heads are then dried in the lab at a moderate temperature for 5 days and then frozen in a deep freeze until the sprouting tests can be conducted. The heads from the F<sub>3</sub> populations and check cultivars Rio Blanco and KS84HW196 are inserted in an upright manner into trays and placed in a mist chamber. We use the same chamber that we have used for leaf rust tests. Normally after about 3 to 4 days

most of the KS84HW196 heads are sprouted and only one or two of the Rio Blanco heads show any sign of sprouting. At this time we sort through the heads discarding any heads that show signs of sprouting. The non-sprouted heads are immediately dried down, threshed and grown as F<sub>4</sub> head rows.

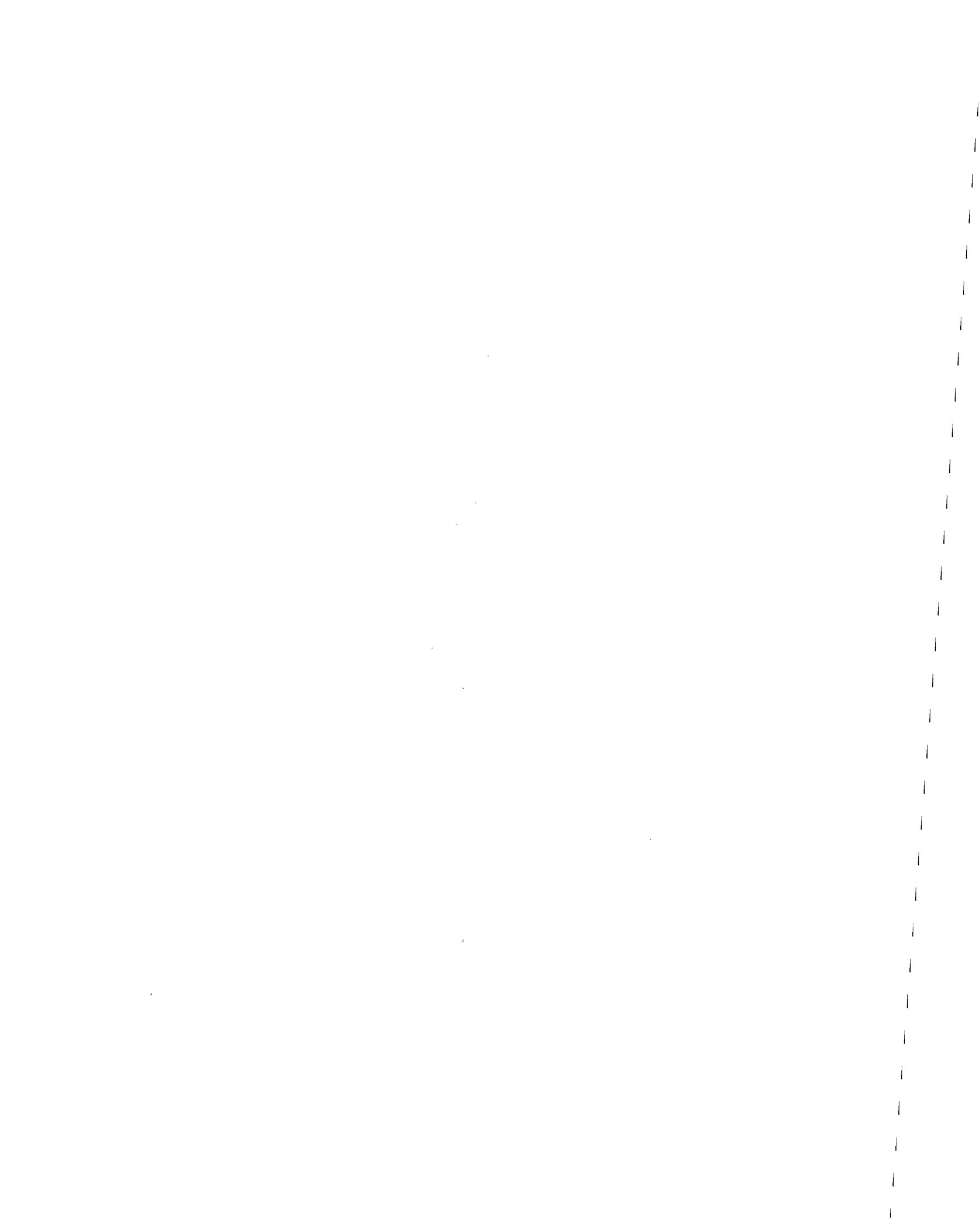
The second test is used to evaluate lines in various performance tests. Ten to fifteen heads from each line are harvested at PM and treated as the F<sub>3</sub> heads for storage. These heads are then threshed by hand and a 100 seed sample is placed on wet filter paper in a petri dish at 65\_F. Normally about 4 to 5 days after the germination test is started we assign a sprouting tolerance score based on total germination at that time. KS84HW196 and Arlin will usually be rated a 9 or 10, for 90 to 100% germination at five days. Rio Blanco will usually rate a 3, for 30% germination.

### **Hard White Wheat Seed Color**

Federal Grain Inspection Service (FGIS) currently has a lot of problems identifying many samples of hard white wheat. Those samples that come in non-weathered in a dark vitreous state are simply not white enough for FGIS to grade as white. Of the three cultivars currently being produced in the great plains, Rio Blanco produces the brightest kernels in an unweathered state, followed closely by Arlin. KS84HW196 will produce the darkest kernels. Using non-weathered seed from the 1992 Hays hard white yield test we got a strong positive correlation between carotene content of the endosperm and seed color. However we need to look at this relationship within a broader range of genotypes to confirm these results. If the relationship does prove to be true, we will need to determine how far we can go, in terms of carotene content, without affect flour color.



**Regional Business Meeting**



# Hard Winter Wheat Improvement Committee

## January 26, 1995

### Oklahoma City, OK

## MINUTES

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Jim Peterson  
USDA-ARS, Lincoln, NE

The meeting was called to order by Chairman Bruns at 1:30 p.m. Jim Peterson read current list of Committee members and Bruns established proper voting procedures and quorum for approving Committee actions. A list of Committee members is included in the minutes.

Members voted to approve minutes of the last meeting held at Lincoln, NE on January 22, 1992, and dispense with reading of the minutes. The minutes are printed in the Proceedings of the Nineteenth Hard Red Winter Wheat Workers Conference, January 21-23, 1992, Lincoln, NE.

#### **Name of Regional Committee**

Peterson proposed to drop the word 'red' from the Committee name and from activities associated with the Regional Nursery program. A motion to that effect was made by Jim Wilson, second by Brett Carver. Motion carried. The Committee will be known as the 'Hard Winter Wheat Improvement Committee' (HWWIC).

#### **Adoption of 'Wheat Worker's Code of Ethics' as policy**

Peterson indicated that the Regional Nursery has operated, essentially informally, under the 1976 'Wheat Worker's Code of Ethics' regarding use and distribution of germplasm through the program. In 1994, The National Wheat Improvement Committee revised the 'Code of Ethics' to account for changes in genetic technologies and plant variety protection laws. Peterson proposed that the 'Wheat Worker's Code of Ethics' be adopted as formal policy for the Regional Nursery program. A motion to that effect was made by Stan Cox, second by Jim Reeder. Motion was passed unanimously. Quick proposed that each institution cooperating in regional testing be informed of the change and ask for each to acknowledge the Code of Ethics as policy for those participating in the Regional Program.

#### **Participation of Private Companies in Regional Nursery Testing**

Private companies participation in Regional Nursery testing has been limited to entering germplasms in the nurseries. They do not receive seed of entries in the performance nurseries, do not grow the nurseries, and do not contribute data to the

final report. If they wish to obtain seed of nursery entries for evaluation, they must contact the originating program directly. The Regional Coordinator can supply seed only to public programs.

Peterson proposed that, with adoption of the 'Code of Ethics' as formal policy, that private companies now be permitted to receive seed, grow nurseries, and contribute data to the regional report. Three requirements were proposed for participation of any company:

- 1) The private company must document they are active in hard winter wheat germplasm development and breeding within the HWW region.
- 2) The company must show evidence that it conducts crossing, manages all segregating generations for evaluation and selection, from F1 through to commercial cultivar or hybrid release.
- 3) The company must sign and document their acceptance of the Wheat Workers Code of Ethics in regard to handling any seed or germplasm received through the Regional Nursery Program.

A motion was made by Jim Quick to allow private companies to receive seed and grow Regional Nurseries based on the company meeting eligibility and participation requirements as proposed. Motion was seconded by Craig Roozeboom and motion carried unanimously.

### **Policies for Approval or Acceptance of New Test Sites**

Participation of private companies in regional testing will result in an increase in number of sites for the Regional Performance Nurseries. Peterson proposed the following be adopted as policy regarding additions or changes in nursery test sites.

- 1) New testing locations, including those proposed by private companies, should complement, not duplicate, those already in place; i.e. contribute data from production areas where nursery information is currently considered inadequate.
- 2) Approval of new test sites, or changes in test sites, will remain the discretion of the Regional Coordinator, with input from members of the HRWWIC as needed.
- 3) The number of replicated test sites allowed will be restricted due to limited quantities of seed available. Smaller amounts of seed may be provided to cooperators for use in unreplicated observation nurseries, as available, in lieu of seed for a replicated test.
- 4) The test site must be accessible for observation by anyone participating in the nursery program during the growing season.

Stephen Baenziger moved to adopt the policy, second by John Moffatt. Motion carried.

## **Regional Germplasm Observation Nursery**

Peterson proposed to replace the Northern and Southern sections of the Uniform Winterhardness Nurseries and Soilborne Mosaic Virus Nursery with a single Regional Germplasm Observation Nursery (RGON). There are several goals: a) Provide a nursery in which materials can be evaluated and exchanged at a generation prior to regional performance testing; b) Obtain information on a larger number of lines for multiple traits; c) Provide a means to exchange and evaluate germplasm that would not normally be included in the performance nurseries; and d) Continue to provide a mechanism for uniform winterhardness testing.

In the Northern and Southern sections of the UWHN, 300 to 350 lines are evaluated per year at 7 northern sites. Single row plots (10 gm/row) are used with 2 replications at each site. A total of 140 grams of seed are required per entry. Using approximately the same amount of seed and resources, germplasm observation nursery could be grown at select, strategic sites from Texas to Montana and substantially enhance information obtained.

The RGON structure was proposed as follows:

- 1) Each breeder/geneticist could contribute up to 30 lines each year for evaluation. Two hundred grams of seed per entry would be required. From three to five check varieties would be included approximately every 25 rows. Current entries in the SRPN, NRPN, and WPRPN

would not normally be included in the RGON, except on request.

- 2) The nurseries would consist of two replications of single row plots (10 gms/row) at each site. Up to 10 nurseries would be grown, strategically located to exploit key environmental or biological constraints within the region.
- 3) Data obtained from the trials would be based on an 'opportunity basis'. That is, notes would be taken at each location based on differential response to disease, insects, or environmental stress that may occur. The trials would not need to be harvested for grain yield data. Evaluation of grain quality, or end-use quality, would also not be a primary program goal. A cooperator may, however, contribute such analyses if they desire. Cooperators could also harvest only those selections of interest, then use select lines for crossing purposes or additional evaluation. Any contributed data would be summarized in the annual nursery report.

The RGON would be a natural outlet for many germplasms and parent stocks that could be of value to the region, but would not normally be entered in the performance nurseries.

Stephen Baenziger moved to adopt the Regional Germplasm Observation Nursery proposal and discontinue Uniform Winterhardness and Soilborne Mosaic

Nurseries. Motion was seconded by Scott Haley and motion carried. Peterson asked for a subcommittee to help identify key test sites, check varieties, and cooperators to grow the nursery. Brett Carver, Scott Haley, Mark Lazar, Stephen Baenziger, and Phil Bruckner indicated their willingness to help organize the nursery.

### **Format of Regional Performance Nurseries**

No changes were proposed to format of Southern, Northern, or Western Plains Regional Performance Nurseries (SRPN, NRPN, and WPRPN, resp.) and each nursery retains a maximum of 45 entries. Check varieties remain Kharkof, Scout 66, and TAM-107 for the SRPN; Kharkof, Roughrider, and Abilene for the NRPN; and Larned, Lamar, Siouxland, and Arapahoe for the WPRPN

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. Peterson indicated the priorities for nursery entries were 1) new and unreleased experimentals; 2) second year entries with promising first year performance; 3) entries from states or companies submitting fewer total number of entries; and 4) released varieties are generally not accepted.

Seed requirements for the regional nurseries are currently 15 lb/entry in the SRPN; 11 lb/entry in NRPN; 2,000 gms in WPRPN; and 200 gms in the RGON. Seed is to be untreated. Seed of check varieties are increased and distributed with new entries each year from Lincoln, NE.

### **Regional Nursery Data on Graingenes**

Peterson indicated that, in the near future, all regional nursery data will be available electronically on the 'Graingenes' gopher. Nursery lists for 1994 and 1995 and yield data are currently available. Access is through 'Graingenes' menus "Wheat Performance Evaluations" and "HWW Regional Performance Nursery Program"

Items that are to be posted, and can be downloaded, include: 1) nursery lists; 2) preliminary yield reports; 3) components of the nursery report as available; 4) final nursery reports - (for last two years); and 6) proceedings of regional meetings. It can also serve as a bulletin board for regional events and include Items from HWWIC members related to the regional nursery program or germplasm evaluations.

### **Quality Analyses for Regional Nursery Samples**

Okky Chung reported on current status of quality testing for SRPN, NRPN, and WPRPN nurseries at the U.S. Grain Marketing Research Laboratory. The 1993 SRPN and NRPN evaluations include individual location analyses for protein, NIR hardness, grain characteristics via the Single Kernel Hardness Tester, and mixograph analyses, in addition to a composite bake evaluation. The individual location analyses were intended to provide measures of stability for key quality traits.

Chung also reported on a new bar-code label system for management of regional nursery quality samples. Brad Seaborn will distribute labels to each nursery cooperator at harvest for attachment to quality samples. The labels, first used in 1994, have been beneficial in sample processing and

management, and have reduced sampling errors and losses.

Bruns reported that the Regional Subcommittee established to advise the Grain Marketing Research Laboratory had been relatively inactive. Scott Haley was appointed as new Chair of the Subcommittee, with the responsibility to work with the GMRL to prioritize regional quality evaluations, propose appropriate analyses, and provide input regarding quality evaluations needed for individual public and private breeding programs.

### **Regional Wheat Research Status Report**

Bruns reported on results of a 1994 survey by the National Wheat Improvement Committee. The survey was conducted to document current wheat research efforts, establish priorities for additional research areas, and identify research areas that will be impacted by retirements in the near future. Survey data was broken out to compare research staffing and priorities on national and regional basis, and compare public vs private responses. Research efforts within the region showed a good balance across 10 discipline areas when compared with other regions and the USDA-ARS. Pathology research was identified as a key research priority that needs additional support. Other priority areas include germplasm breeding, physiology, quality, and molecular biology. There was a lack of confidence in ability to refill many current positions with impending retirements or vacancies. Vulnerability of many core wheat research positions was of great concern. The NWIC and HWWIC will need to communicate with appropriate administrators regarding the strategic importance and future of these research positions. Bruns indicated that the regional research status report will be incorporated

with those from other regions into a national strategic planning guide for the NWIC.

### **Election of Regional Officers**

Joe Martin was elected as Chair of the Hard Winter Wheat Improvement Committee. Brett Carver, Stephen Baenziger, and David Worrall were elected as representatives to the National Wheat Improvement Committee. A resolution of appreciation to Rob Bruns, past chair, and past NWIC representatives Stan Cox, David Worrall, and David Porter will be drafted by Peterson.

### **Site of Next Wheat Breeders Field Day**

The 1995 Regional Breeders Field Day was set for May 31 at Hutchinson, Kansas. Plans are being developed to visit several sites and breeding programs during the day. Based on history of past field days, the 1996 field day is scheduled for Colorado and 1997 field day for Texas.

### **Site of Next Regional Workshop**

An invitation from Jim Quick was accepted to hold the 1998 Regional Wheat Workers Workshop in Colorado; the date and exact location to be determined.

Bruns expressed the Committee's appreciation to the Local Organizing Committee for a very successful 20th Wheat Workers Workshop and a formal resolution of appreciation was approved.

Respectfully submitted,

C. J. Peterson  
Secretary, HWWIC

## Resolutions

The following resolutions were unanimously adopted:

- No. 1. Whereas, Rob Bruns has provided superior and active leadership to the Hard Red Winter Wheat Improvement Committee; and  
Whereas, Dr. Stan Cox, Dr. David Worrall, and Dr. David Porter, along with Rob Bruns, 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 Bruns, Stan Cox, David Worrall, and David Porter for their efforts and superior contributions on behalf of the committee.

- No. 2. Whereas, the 20th Hard Red Winter Wheat Workers Workshop has been an excellent and informative meeting and our hosts have expended much time and effort to ensure the success of the workshop;

Be it therefore resolved, the Hard Red Winter Wheat Workers express their sincere appreciation to Oklahoma State University and USDA-ARS wheat researchers at Stillwater, OK for serving as hosts in this

workshop; to Arron Guenzi for chairing the local arrangements committee; to Gene Krenzer, Bob Hunger, David Porter, Ed Smith, and Brett Carver for local arrangements; to Michelle Kuehn and Debbie Porter for handling registrations; to David Worrall, Stan Cox, David Porter, Bob Hunger, Gene Krenzer, Arron Guenzi, Rob Bruns, and Brett Carver for serving as session chairs; and to regional officers Rob Bruns, Stan Cox, David Worrall, David Porter, and Jim Peterson for contributions to workshop planning.

Be it further resolved, the Hard Winter Wheat Workers express their sincere appreciation for financial support of the workshop from the Oklahoma Wheat Commission; Oklahoma Crop Improvement Association; American White Wheat Producers Association; Johnston Seed Co. and the W.B. Johnston Grain Co.; and Shawnee Mills.

## **WHEAT WORKER'S CODE OF ETHICS**

Adopted by the  
National Wheat Improvement Committee  
November 5, 1994

This seed is being distributed in accordance with the "Wheat Workers' Code of Ethics for Distribution of Germplasm", developed and adopted by the National Wheat Improvement Committee on Nov. 5, 1994. Acceptance of this seed constitutes agreement.

1. The originating breeder, institution, or company has certain rights to the unreleased material. These rights are not waived with the distribution of seeds or plant material but remain with the originator.
2. The recipient of unreleased seeds or plant material shall make no secondary distributions of the germplasm without the permission of the owner/breeder.
3. The owner/breeder in distributing unreleased seeds or other propagating material grants permission for its use in tests under the recipient's control or as a

parent for making crosses from which selections will be made. Uses for which written approval of the owner/breeder is required include:

- (a) Testing in regional or international nurseries;
  - (b) Increase and release as a cultivar;
  - (c) Reselection from within the stock;
  - (d) Use as a parent of a commercial F1 hybrid, synthetic, or multiline cultivar;
  - (e) Use as a recurrent parent in backcrossing;
  - (f) Mutation breeding;
  - (g) Selection of somaclonal variants; or
  - (h) Use as a recipient parent for asexual gene transfer, including gene transfer using molecular genetic techniques.
4. Plant materials of this nature entered in crop cultivar trials shall not be used for seed increase. Reasonable precautions to ensure retention or recovery of plant materials at harvest shall be taken.

# *Hard Red Winter Wheat Improvement Committee*

## **Membership, January 17, 1995**

### Colorado

R. Bruns  
B. Cooper  
J. P. Hill  
J. Moffatt  
S. Perry  
J. S. Quick  
J. Reeder  
J. F. Shanahan

### Idaho

E. Souza

### Kansas

R. Bequette  
B. Bockus  
B. Bowden  
O. K. Chung  
S. Cox  
S. Curran  
J. R. Erickson  
M. Eversmeyer  
D. Fjell  
B. S. Gill  
J. H. Hatchett  
J. Havlin  
W. Heer  
S. L. Kuhr  
R. Lamond  
G. Lookhart  
T. J. Martin  
M. Olewnick  
G. M. Paulsen  
J. Raupp  
C. Roozeboom  
R. G. Sears

D. Seifers  
J. Shroyer  
V. Smail  
G. Wilde  
J. Wilson  
M. Witt

### Montana

P. Bruckner  
D. Mathre

### Nebraska

P. S. Baenziger  
R. C. French  
R. A. Graybosch  
C. J. Peterson  
D. R. Shelton  
J. E. Watkins

### North Dakota

J. Anderson

### Oklahoma

C. Baker  
B. Carver  
A. C. Guenzi  
R. M. Hunger  
D. Porter  
L. Singleton  
E. L. Smith  
J. Webster

### South Dakota

S. Haley

### Texas

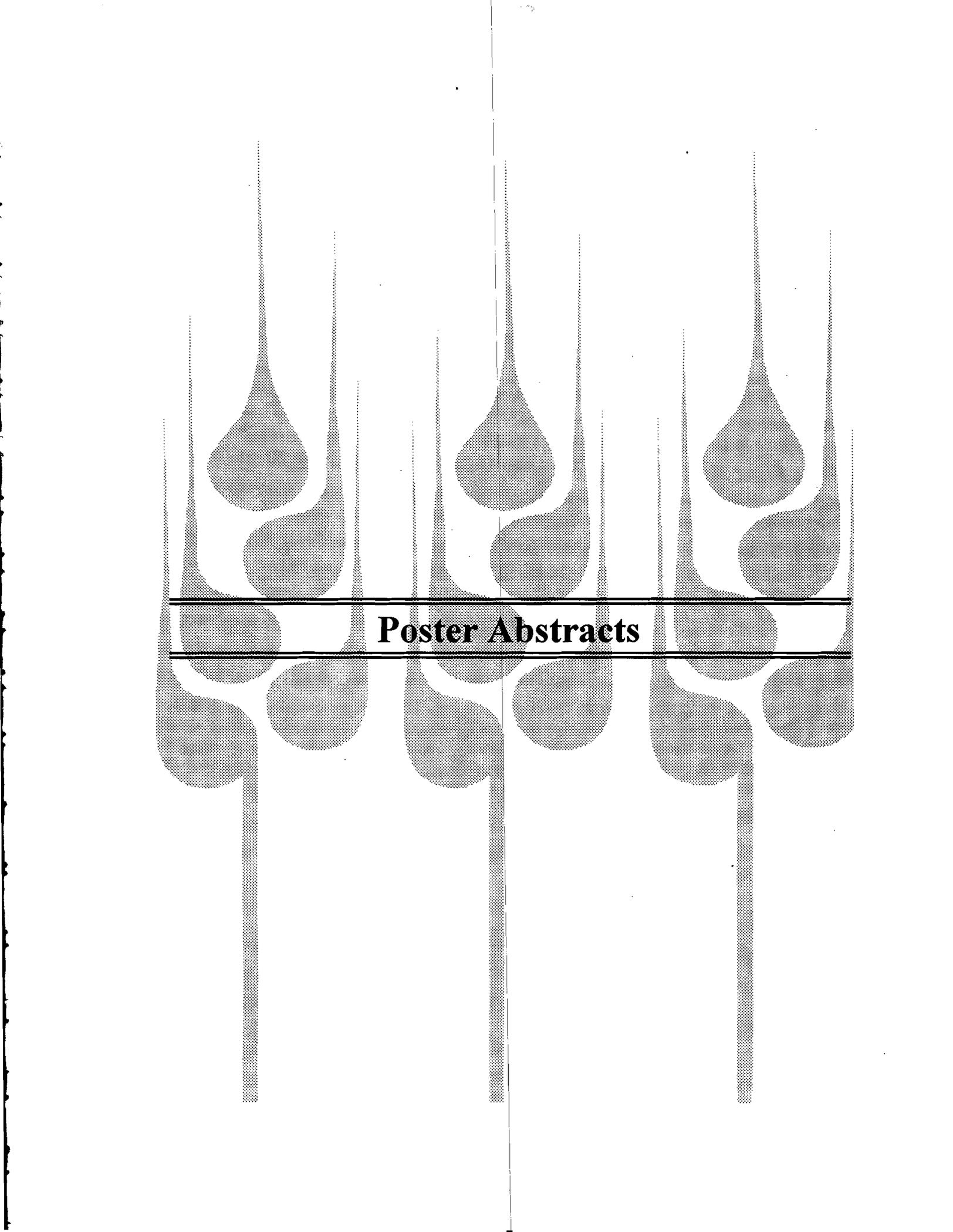
M. Lazar  
D. S. Marshall  
J. Michels  
B. McDonald  
H. Nguyen  
C. Rush  
P. Sebesta  
N. A. Tuleen  
W. D. Worrall

### Washington

E. Donaldson

### Wyoming

J. Krall

The background of the page features a repeating pattern of stylized, teardrop-shaped leaves or petals. Each leaf is filled with a fine, dotted halftone texture. The leaves are arranged in vertical columns, with some overlapping. From the base of each column, a thin, vertical stem extends downwards. The overall effect is a light, textured pattern that frames the central text.

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**Poster Abstracts**

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**TRITICUM TAUSCHII DERIVED LINES AND THEIR EFFECT ON  
BREAD MAKING QUALITY**

M.A. KNACKSTEDT, R.G. SEARS, T.S. COX, R.K. BEQUETTE, and O.K. CHUNG

Agronomy Department - Throckmorton Hall  
Kansas State University  
Manhattan, KS 66506

*Triticum tauschii* is increasingly used as a source for disease and insect resistance genes for common wheat. Detrimental genes, which may inadvertently be introduced into common wheat, need to be monitored. Some previous introductions of resistance genes have resulted in a marked decline in bread making quality. Our research estimates the effect of the use of *T. tauschii* derived lines on bread making quality. Additionally, we identified derived lines carrying novel gliadin protein bands and found, in a least one case, where mixing time was improved while other bread making quality parameters were not adversely affected.

## **HYBRID AND PURELINE RESPONSE TO HIGH TEMPERATURE STRESS AT TWO GROWTH STAGES IN HARD RED WINTER WHEAT**

M.D. ALBRECHT, N.D. VAN MEETEREN, AND R.G. SEARS

Department of Agronomy, Kansas State University, Manhattan, KS 66502

In the hot summer environments of the Great Plains, the ability to maintain both green leaf duration and grain filling at temperatures that often exceed 40°C is necessary to achieve high grain yields. The purpose of this study was to test hybrids, their parents and standard varieties currently in production for tolerance to high temperature stress at Feekes stage 10 (boot) and 10.52 [4 days after anthesis (4DAA)].

Five hybrids, their parents, Karl and Tam 107 were planted in the greenhouse in the spring of 1994. Temperature (25°C-d/20°C-n) and lighting (16 h-d/8h-n) were maintained until initiation of the experiment. At Feekes stage 10 and at Feekes stage 10.52, based on main tiller development, plants were moved into two greenhouse environments: the control environment consisted of temperatures (25°C-d/20°C-n) and the above light regime. The high-temperature treatment consisted of temperatures (35°C-d/30°C-n) with the same light regime. Each environment was arranged as a randomized complete block design with four replications. One plant/pot served as the experimental unit. Plants were grown in one-gallon pots and watered and fertilized to maximize yield potential. Grain yield per plant, main tiller grain weight and total dry matter were measured. Harvest index was then computed from the data. Data are reported as percent reduction from the control environment.

Grain filling days (anthesis to physiological maturity) were shortened approximately 10 days by the high-temperature treatments (27d 'vs' 37 d). Yield reductions at boot (70%) were significantly greater than at 4DAA (67.4%) but both regimes reduced yield substantially compared to the control. At both growth stages, the hybrids collectively had better high-temperature tolerance than their parents, averaging approximately 2% less grain loss under the high temperature treatments. In general, inbreds with better high-temperature tolerance produced hybrids with better high temperature tolerance, but a clear trend was not established with these genotypes. Harvest index reflected similar trends. Reduction of yield in the main tillers was much less than in the whole-plant measurement, averaging 31% at boot and 32% at 4DAA. Percent reduction of main tiller grain weight was significantly, but poorly correlated to plant grain yield ( $r = .38$  boot,  $r = .25$  4DAA). Therefore, genotypes did not all respond alike in regards to temperature stress for their secondary and tertiary tillers. This undoubtedly is a worse-case scenario, as late filling tillers in the field would probably be sloughed off by the plant prior to maturity, in addition to the fact that under field conditions, plants would have fewer tillers to begin with.

Hybrid wheat is currently being introduced and marketed for the higher yielding irrigated regions of the southern plains, primarily southwest Kansas and the panhandles of Oklahoma and Texas. These environments always undergo high-temperature stress during grain filling. Our experiments suggest that hybrids may have better heat tolerance than purelines when exposed to continuous high-temperature stress at either boot or early grain filling.

## HYBRID AND PURELINE RESPONSE TO HIGH TEMPERATURE STRESS UNDER TWO ENVIRONMENTS IN HARD RED WINTER WHEAT

N.D. VAN MEETEREN, T.S. COX, and R.G. SEARS

Throckmorton Hall  
Department of Agronomy  
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Manhattan, KS 66502

High temperature stress of winter wheat in the Great Plains is a frequent occurrence, especially during the grain filling period. Field observations have suggested that hybrid wheat may be more tolerant to high temperature stress than their parents or other pureline varieties. The purpose of this research was to compare the effects of high temperature stress on F1 hybrids, their parents, and pureline varieties grown in both greenhouse and growth chamber environments. In 1994, 7 hybrids, their parents, and 4 varieties were grown in the greenhouse in a randomized complete block design. Four days after anthesis (Feekes 10.52), plants were transferred to either growth chambers or greenhouses set at control (25/20°C), or high temperature stress (35/25°C) 16-h daylength. Data were collected on yield/plant, primary tiller grain weight, seeds/spike, fertile spikelets/spike, tiller number/plant, and total above ground biomass. Direct statistical comparisons of the two environments (growth chambers and greenhouse) was not possible because of heterogeneous variances. However, in the greenhouse experiments, the hybrids overall were less affected by high temperature than their parents. Yield, biomass, and heterosis values were generally lower in the growth chambers. The % reduction due to high-temperature for yield in the greenhouse was 53.2% for hybrids (41.7% - 65.9%). The parents had an average reduction of 57.2% (39.1% -66.9%). In the growth chambers, the hybrids had a 50.5% reduction in yield with a range of 39.5% to 60.3%. The parents had a 50.1% reduction in yield with a range of 38.6% to 52.4%. Hybrids had a significant advantage over their parents of 38.6% to 52.4%. Hybrids had a significant advantage over their parents in the greenhouse for yield, primary tiller grain yield, seeds/spike, and fertile spikelets/spike. There was a significant difference between hybrids and their parents in primary grain tiller yield, and harvest index in the growth chamber. Correlations between main tiller grain yield and total grain yield in the greenhouse were 0.92 and 0.76, for hybrids and parents, respectively. Correlations in the growth chamber were 0.54 and 0.79 for hybrids and parents, respectively. The actual average high parent heterosis for yield in the greenhouse was 1.69% with a high of 13.39%. In the growth chambers, the average high parent heterosis was -5.58% with a high of 17.57%. The hybrids with heat tolerant parents generally had high parent heterosis.

**DEVELOPMENTAL AND REPRODUCTIVE BEHAVIOR OF  
BIOTYPES C AND E GREENBUG RESTRICTED TO WINTER  
WHEAT LINES DIFFERING BY SPECIFIC RESISTANCE GENES**

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The relationship between greenbug (*Schizaphis graminum*) biotypes and wheat (*Triticum aestivum*) resistance genes may provide a good model for understanding biotype development generally, and aid in elucidation of resistance mechanisms. The availability of closely related wheat lines possessing variation at several loci for resistance of susceptibility to both biotypes 'C' and 'E' greenbug enables direct assessment of the effects of the resistance genes per se on greenbug growth and development. Four genotypes were examined in the current study, two cultivars, TAM-105 and TAM-107, and two breeding lines, TXGH12588-273 and TXGH12588-307. TAM-107 is a BC<sub>3</sub> generation derivative of TAM-105, and carries biotype C resistance derived from Amigo. The two breeding lines are BC<sub>4</sub> generation derivatives of a full sib of TAM-107, using the cultivar, Largo, as the nonrecurrent parent, TXGH12588-273 carries biotype E resistance derived from Largo, while TXGH12588-307 is susceptible to biotype E, but resistant to biotype C. TAM-105 is susceptible to both biotypes. Single adult aphids of each biotype were transferred to single plants of TAM-105 for biotype C, or TAM-107 for biotype E. From each plant, first-instar nymphs were removed, and placed on a single plant of each of the four genotypes. The passage of nymphs through developmental stages was then scored on each plant (replicate) by observing molting. As the nymphs passed into adulthood, reproduction of new nymphs was observed each day until the death of the parent. With biotype C, as expected, rate of development and fecundity of aphids was much greater on TAM-105 than on the other three lines, while no significant differences were observed between the three resistant lines. With biotype E, however, significant differences were observed among each of the three susceptible lines for both rate of development and fecundity, as well as between the susceptible lines and the resistant line. Intrinsic rate of increase of aphids, calculated by two methods, on each plant genotype, suggested a similar relationship among the genotypes. The decrease in fecundity observed, without complete toxicity, particularly in TXGH12588-273 infested with biotype E, should be useful in the field as a complement to biological control methods.

## TRAITS RELATED TO DROUGHT SUSCEPTIBILITY VARIATION AMONG CLOSELY RELATED WHEAT LINES

M.D. LAZAR, G. PICCINNI, C.D. SALISBURY, W.D. WORRALL, S.P. CALDWELL,  
Q.W. XUE, and G.L. PETERSON

Texas A & M Research and Extension Center, Amarillo, Texas  
Texas A & M Research and Extension Center, Vernon, Texas

Drought resistance is a complex trait for which systematic improvement has been difficult. One means of simplifying analysis of such a trait is through examination of variability among closely related genotypes. Winter wheat (*Triticum aestivum*) lines derived from a backcrossing program have been found to differ significantly in ratio of dryland to irrigated yield, and in the Fischer-Maurer susceptibility index, S. No significant correlations have been found in this population between yield or S and variables which might be confounded with drought resistance, including earliness, disease or insect susceptibility and lodging. Therefore, it is highly likely that the yield variability observed is related to response to water stress. We have examined seven of these sister lines in a two-year field study at Bushland, Texas, examining yield components in each line in two treatments, fully irrigated and rainfed. Significant variability was observed among the seven lines for dryland yield, tillers per plant, plant dry weight, seed weight and test weight. No significant differences were observed for number of leaves per plant, leaf area per plant or number of seed per head. The only significant, positive correlation between yield rank and any of the above variables involved seed weight, strongly suggesting that the yield variability among the tested lines is mainly related to differences in seed weight. We are continuing to evaluate this variability with a view to specific physiological processes which may underly that variation in seed weight under conditions of water stress.

## VALUE OF STRESS RESISTANCE GENES RELATIVE TO DRY WEIGHT ACCUMULATION IN WHEAT SEEDLINGS

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Amarillo, Texas 79106-1706

Biotic stress on winter wheat (*Triticum aestivum*) seedlings can be extremely damaging. Many genes for resistance to insects and pathogens have been introduced into wheat, however, quantitative estimates regarding the effectiveness of such genes with respect to traits other than yield are generally unavailable. In this study, we have used closely related lines, derived from backcrossing using a single recurrent parent, to assist in estimating genotypic values for four resistance traits. We evaluated *Pm17*, for resistance to powdery mildew, *Gb2*, for resistance to biotype 'C' greenbug, and two sources of resistance to biotype 'E' greenbug, *Gb3* and *Gb6*, each in the TAM-105 genomic background, for dry matter accumulation during a five week period, beginning at the two-leaf stage. For each greenbug resistance gene, two initial infestation rates were examined, 0.5 and 5.0 aphids per plant. Powdery mildew damage to susceptible seedlings developed more slowly than did greenbug damage at either infestation rate. Resistance conferred by *Pm17* was completely effective, however, while greenbug infestation of any of the resistant genotypes, at 5.0 aphids per plant, resulted in significantly reduced dry matter accumulation compared to uninfested control plants, by the end of the study. In TAM-105, which is susceptible to both greenbug biotypes, reduced dry weight occurred earlier when infested with biotype E than with biotype C. Also, while both TAM-105 and TAM-107 are susceptible to biotype E, when infested with that biotype, TAM-105 exhibited reduced dry weight sooner than did TAM-107, which possesses biotype C resistance. These results suggest that biotype E is the more damaging of the two biotypes, but that biotype C resistance may confer some delay in development of symptoms to biotype E. Biotype E resistance from either source is effective, but does not provide immunity.

**ENVIRONMENTAL EFFECTS ON PROTEIN COMPONENTS, CHEMICAL AND  
PHYSICAL PROPERTIES, AND MILLING AND BREAD-MAKING DATA FOR  
KARL WHEATS GROWN AT 6 LOCATIONS IN KANSAS AND HARVESTED IN  
1993**

G.L. LOOKHART, O.K. CHUNG

USDA/ARS, U.S. Grain Marketing Research Laboratory  
1515 College Avenue, Manhattan, Kansas 66502

The effect of environment on the various chemical and physical parameters was determined by examining those parameters in a single cultivar grown in various locations. Six samples of a U.S. hard red winter wheat cultivar, "Karl", were chosen that represented varying protein contents and hardness values. The wheats were all grown in Kansas in 1993; two were grown in irrigated plots and the others on dryland. Crop year 1993 was wet in all Kansas locations. In particular, Reno County received 26 inches of rain in May, making it a nearly irrigated type environment. The parameters tested included: kernel test weight, thousand kernel weight, hardness scores (HS) by near infrared reflectance and single kernel wheat characterization system, and the ash, moisture, and protein contents of wheats and flours. Milling, baking, and protein fractionation characterization were also performed. Irrigated plots of Karl wheat produced grain with consistently higher than average protein contents, a smaller than average percentage of large kernels, and higher than average HS. The bread volumes were higher than average for irrigated samples of Karl. In addition, the ratio of the amount of albumin plus globulin total protein was generally higher in the dryland grown samples which is indicative of smaller amounts of functional proteins (gliadins and glutenins) and of poorer baking quality.

## **RELATIONSHIP BETWEEN SINGLE KERNEL CHARACTERISTICS AND END USE QUALITY. II. SOFT WHEATS**

**O.K. CHUNG, P.L. FINNEY, C.R. MARTIN, J.L. STEELE,  
B.W. SEABOURN, and V.W. SMAIL**

USDA/ARS, U.S. Grain Marketing Research Laboratory,  
1515 College Ave., Manhattan, KS 66502  
USDA/ARS, Soft Wheat Qual. Lab., 1680 Madison Ave., Wooster, OH 44691  
American Institute of Baking, 1213 Baker's Way, Manhattan, KS 66502

The Single Kernel Wheat Characterization System (SKWCS), developed by the U.S. Grain Marketing Research Laboratory (USGMRL), the Engineering Research Unit, was used to study the relationships between the SKWCS parameters and some quality data for the soft wheat samples. Soft wheat samples (140 cleaned and 136 uncleaned) and their quality data were provided by the Soft Wheat Quality Laboratory, Wooster, Ohio. The USGMRL-SKWCS data were used to predict various quality parameters of soft wheats by multiple stepwise regression. Softness Equivalent (S.E.) of both cleaned and uncleaned would be estimated well by SKWCS ( $r = 0.94$ ) for uncleaned vs.  $0.92$  for cleaned set). However, the Adjusted Flour Yields and Milling Scores were significantly better fitted with a prediction equation for and Combined Quality Scores with  $r$  of  $0.91$  and  $0.85$  for the uncleaned set whereas  $r$  of  $0.78$  and  $0.80$  for the cleaned set. Some other parameters such as NIR hardness score, flour protein content, etc. were not greatly affected by cleaning grain prior to testing.

## **RELATIONSHIP BETWEEN SINGLE KERNEL CHARACTERISTICS AND END USE QUALITY. I. HARD WHEATS**

**O.K. CHUNG, J.B. OHM, C.R. MARTIN, J.L. STEELE,  
G.L. LOOKHART, and V.W. SMAIL**

**USDA/ARS, U.S. Grain Marketing Research Laboratory  
Department of Grain Science and Industry, Kansas State University  
American Institute of Baking  
Manhattan, Kansas 66502**

The Single Kernel Wheat Characterization System (SKWCS), developed by the U.S. Grain Marketing Research Laboratory (USGMRL), the Engineering Research Unit, was used to study the relationships between the SKWCS parameters and some quality data. The USGMRL-SKWCS provides the mean values of moisture contents, weights, diameters, and hardness scores of about 300 wheat kernels and their standard deviations. To eliminate environmental effects on end use quality, the set of 12 hard red winter (HRW) and 12 hard red spring (HRS) wheats, grown at the same location (Sacramento Valley, CA) during three crop years (1988-1990) were used. The equations were derived by multiple stepwise or principal component regression analysis to study the relationships between single kernel parameters and quality data. There were significant correlations for experimental micro-milling yields and micro-bread loaf volumes with single kernel parameters. The most significant kernel parameters were kernel sizes for milling yields and kernel weights for loaf volumes. The  $r$  values of mathematical relationships were higher for the HRW wheat classes or HRS wheat classes separately ( $n = 36$  each) than for the combined two wheat classes ( $n = 72$ ). In general, the higher  $r$  values were obtained by the principal component regression analysis than by the multiple stepwise regression analysis.

## **RAPID IDENTIFICATION OF SOME U.S. WHEAT LINES BY NEAR INFRARED DIFFUSE REFLECTANCE SPECTROSCOPY**

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Current methods of wheat varietal identification are the visual, high performance liquid chromatography (HPLC), polyacrylamide gel electrophoresis (PAGE), and the most recent, capillary zone electrophoresis (CZE) determination. While HPLC and PAGE methods are quite accurate, they are not applicable to fast, routine, quality control in the grain industry. The visual method, however, while it is fast, is not as accurate as it used to be due to the increasing number of wheat lines entering the market that represent crosses between distinct varieties. In the present work, the feasibility of rapid varietal identification of some U.S. wheats by near infrared reflectance (NIR) analysis was investigated. Wheat samples of 30 known hard red winter (HRW), hard white winter (HWW), and soft red winter (SRW) commercial wheat lines, which consisted of a total of 1544 samples, were collected over 3 crop years (1992-1994) from 2 sources (Kansas Association of Wheat Growers, and the Kansas Winter Wheat Performance Trials), and their visible and NIR spectra were recorded. Twelve varieties (10 HRW, 1 HWW, and 1 SRW), which consisted of a total of 796 samples, were then selected for multivariate discriminant analysis (MDA). To reduce the effect of nonlinear spectral effects such as granularity, the spectral data were mathematically corrected by removing the linear and quadratic curvature of each spectrum and forcing the standard normal variate of each spectrum to equal 1.0. Principal component analysis and MDA were applied to the corrected data. MDA allowed an overall 81.5% efficiency of identification of the genetic origin of an unknown set of samples based upon the 12 wheat lines selected. A comparison of the MDA results obtained by different spectral math pretreatments was made. Using a coefficient of parentage ( $r$ ), a comparison of efficiency of identification of genetic origin on the basis of between two similar wheat lines (Larned and Scout 66,  $r = 0.98$ ) and two unrelated pedigrees (Cimarron and Scout 66,  $r = 0.20$ ) was also made. Again, MDA allowed an overall 73.3% efficiency of identification of the genetic origin of the 3 wheat lines selected, with the greater error in misclassification going to the two most closely related wheat lines.

## EVALUATION OF A TRITICUM ARARATICUM COLLECTION FOR RESISTANCE TO DISEASE AND INSECT PESTS OF WHEAT

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Wild wheat relatives are regarded as an important source of diverse genes for resistance to disease and insect pests that can be used for wheat improvement. *Triticum timopheevii* var. *araraticum* is a wild tetraploid wheat containing the A<sup>t</sup> and G genomes, which are closely related to the A and B genomes of *T. aestivum* L. and *T. turgidum* L. The collection of *T. araraticum* held at the wheat Genetics Resource Center (WGRC) has been screened for reaction to six foliar diseases of wheat, Hessian fly and wheat curl mite. All *T. araraticum* accessions tested for reaction to *Septoria tritici* leaf blotch were scored as resistant. A very high percentage of tested accessions were resistant to the tan spot fungus and all were resistant to the necrosis-causing toxin produced by *Pyrenophora tritici-repentis*. The frequency of accessions having intermediate and low reaction types to leaf rust was 68% and 36%, respectively. While intermediate levels of resistance to stripe rust and powdery mildew were also common in the collection, the frequency of low infection types was 7% for powdery mildew and 0% and 2% for stripe rust isolates CDL-43 and CDL-45, respectively. Resistance to stem rust was less frequent than resistance to the other rust pathogens with 15% of tested accessions having intermediate reaction types and 6% having low infection types. Ninety-one percent of the accessions tested were scored as resistant or segregating for resistance to Hessian fly biotype D, and 27% of the accessions tested had some level of resistance to wheat curl mite. Eighteen accessions were identified with intermediate to high levels of resistance to at least 5 pests. Pest resistance in the collection was not strictly related to geographic origin. However, accessions from northern Iraq had the highest frequency of resistances, suggesting that it may be a center of diversity of *T. araraticum*. Wheat workers interested in obtaining complete screening data and/or small quantities of seed of individual accessions of *T. araraticum* may do so by contacting the WGRC, Throckmorton Plant Science Center, Kansas State University, Manhattan, Kansas 66506.

## **MECHANICAL MASS SELECTION FOR TEST WEIGHT IN HARD RED SPRING WHEAT**

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The objective of the project was to determine the feasibility of using a gravity table to select for high test weight in a variable population of hard red spring wheat. Eight hundred F<sub>2</sub> and F<sub>3</sub> populations originating from Pioneer Hi-Bred International, Inc. were bulked to form one highly variable population. This population was planted late at Brookings, South Dakota in 1992 so that it matured under considerable foliar disease pressure. After harvest, a gravity table was used to separate the population into fractions differing in mean test weight. After a seed increase in 1993, the fabricated populations were evaluated at 2 locations in 1994. Phenotypic correlations were calculated for the original test weight grouping and agronomic traits. Test weight, kernel weight, and plant height were all positively correlated with test weight group and leaf rust susceptibility was negatively correlated.

## **WHEAT GRAIN YIELD TIMING RELATIONSHIPS**

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Variable timings of the grain-filling-period (GFP) were created with winter wheat by utilizing plantings over a 6 month period. Progressively delayed plantings gradually shortened duration from planting to the flowering period and then later also hastened duration of the GFP. High grain yields showed a very high correlation with early heading dates ( $r = -0.83$ ). Date mature ( $r = -0.63$ ), like heading date, showed a negative reaction to delay. Other factors associated with grain yield showed correlation ( $r$ ) values as follows: 1,000 kernel weight (0.81), test weight (0.72), grain filling days (0.71), kernels per plant (0.70), heads per square foot (0.70), kernels per head (0.55), heads per plant (0.51), GFP average daily maximum temperature (-0.48), mature plant height (0.41), spikelets per head (0.19). Years and planting timings that coincided with wetter and cooler conditions during grain filling typically extended the GFP, produced larger kernels and higher test weights and resulted in higher grain yields.

# VARIATION OF GRAIN FILLING FOR 54 FACULTATIVE AND WINTER WHEATS GROWN ON THE CENTRAL ANATOLIAN PLATEAU OF TURKEY

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On the Central Anatolian Plateau of Turkey (CAP) winter wheat (*Triticum aestivum* L.) is mainly produced in rainfed environments with less than 400 mm annual rainfall. The grain fill (GF) period coincides frequently with periods of drought and high temperatures. Since the market demands wheat cultivars with a high TKW, understanding the factors influencing grain fill rate and TKW is essential for wheat breeding. Duration and GF rate of 54 winter and facultative wheat cultivars from beeding programs in Turkey, Eastern Europe, China, and North America were determined in order to: a) investigate the relationships between grain fill parameters and wheat productivity and b) to identify breeding stocks adapted to the CAP. The experiment was conducted in 91/92 and 92/93 in Eskisehir. Step wise regression was used to find the best polynomial to describe the relationship between kernel weight and accumulated growing degree days (GDD) from anthesis to maturity. Fitted curves were used to estimate duration and rate of GF. Correlaton between kernel weight and GF rate was highly significant ( $r = 0.80$  and  $0.82$ ). GF rate was significant negatively correlated ( $r = -0.76$  and  $-0.51$ ) with GF duration, suggesting that later flowering genotypes have a higher grain fill rate. The absence of a significant correlation between TKW and GF duration indicates that genotypes with high TKW can have variable periods from anthesis to maturity. Yield was not correlated with any of the GF parameters, but a significantly positive relation ( $r = 0.45$ ) was found to tiller density. Grouping of genotypes according to origin showed that, on average, Chinese cultivars had the earliest anthesis date but the longest grain fill duration, while cultivars from Turkey and the Pacific Northwest had the latest anthesis date but the shortest grain fill duration. The long GF duration of Chinese and other early flowering cultivars may have been obtained, since GF duration and rate were estimated from fitted curves, which may not have described the specific grainfill process sufficiently well. Further statistical analyses are needed to answer this question. Grain fill rate was highest for cultivars from Turkey and Mexico. Cultivars with high TKW and high yield were Fundulea 4, RSK/NAC and DOGU 88. These results suggest that grain fill was not limited by high temperatures or terminal drought. Selection for cultivars with high GF rate, high yield, and high TKW without increasing the GF duration should be possible.

## VERNALIZATION (VRN) AND PHOTOPERIOD (PPD) RESPONSE GENES: THEIR ROLE IN WHEAT ADAPTATION TO DIFFERENT ENVIRONMENTS

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Collaborative research project between the Center for Plant Breeding and Reproduction  
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This study is undertaken to assess the role of the dominant genes for vernalization (*Vrn 1, 2, 3*) and photoperiod (*Ppd 1,2*) insensitive responses in the adaptation of wheat to different latitude production environments, and to identify molecular markers to aid specific plant selection in breeding programs. Near isogenic lines (NIL's) are being developed involving different combinations of *Vrn* and *Ppd* genes in the background of the winter daylength sensitive varieties Stephens (SPN) and Mironovskaya 808 (MKO). Development of NIL's are in the F3 generation after four backcrosses to the recurrent parent SPN and in the F3 generation from single crosses in the MKO background. RAPD and RFLP techniques have been implemented to search for molecular markers. Four hundred and twenty primers have been tested using the bulk segregant analysis. Thirty nine primers identified polymorphic DNA differences (9%), which were, however, irrelevant to the genes of interest in this study. Sixty four probes were assayed using the RFLP technique. One identified a loose linkage (20% recombination) with one population segregating for *Vrn2* and *Ppd1*. A PCR technique that requires the enrichment in unique sequences is being implemented to increase the chances of finding meaningful polymorphisms. A preliminary trial of the MKO NIL's will be sent internationally in 1995. Countries in the developing world where yield trials will be conducted include: Pakistan, India, Syria, Algeria, Kenya, Zimbabwe, China, Ukraine, Mexico, Chile, and Argentina. Developed material may reveal the importance of these genes in breeding wheat for adaptation to environments prone to drought, waterlogging, heat, early-late frosts, certain diseases, and insect build-up periods. We aim to study these latter conditions in the near future.

### GENE COMBINATIONS TARGETED<sup>1</sup>

Genes	p	P1	P2
v	vp	vP1	vP2
V1	V1p	V1P1	V1P2
V2	V2p	V2P1	V2P2
V3	V3p	V3P1	V3P2

1. For simplicity only the first letter followed by a number indicates gene name and number e.g.; V1 refers to *Vrn1Vrn1*, while vp refers to a double recessive genotype for all *Vrn* and *Ppd* genes.

**INHERITANCE AND MECHANISMS OF RUSSIAN WHEAT APHID  
(HOMOPTERA: APHIDIDAE) RESISTANCE IN PI 225217**

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The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), is a serious pest of cereal crops in the western United States. Development of Russian wheat aphid-resistant cultivars is a priority in many wheat (*Triticum aestivum* L.) breeding programs. Identification of diverse sources of resistant germplasm, followed by determination of the genetic control of resistance should aid in the timely development of new, genetically diverse cultivars. Determination of the mechanisms of resistance present in different germplasms could influence initial selections of resistant parents to be used in cultivar development. In theory, tolerance may be preferred since it should not promote the development of new insect biotypes and it should also be more compatible with an integrated pest management cropping system.

Studies were conducted to determine the inheritance of Russian wheat aphid resistance in a red winter wheat line selected from PI 225217. Crosses were made between the resistant line and susceptible wheat cultivar 'Chisholm'. F<sub>1</sub>, F<sub>2</sub>, and F<sub>3</sub> progeny were artificially infested with Russian wheat aphid in the greenhouse. F<sub>1</sub> data indicated that resistance is dominant with no evidence of cytoplasmic effects. F<sub>2</sub> and F<sub>3</sub> data indicate that resistance in this line is clearly controlled by a single gene; the F<sub>2</sub> showed a 3R:1S segregation ratio, and F<sub>3</sub> lines segregated in a 1R:2 Seg:1S ratio.

Additional studies were conducted to determine the mechanism(s) of resistance present in this line. Antibiosis and preference tests indicated that the resistant line was not significantly different from the susceptible check beyond the one-leaf stage. The tolerance tests indicated that the resistant line was significantly more tolerant to Russian wheat aphid feeding than the susceptible check for all parameters tested. Therefore, tolerance to Russian wheat aphid appears to be the most important mechanism of resistance present in this line.

# RAPD-PCR TO DETECT GENOMIC POLYMORPHISM AMONG GEOGRAPHICALLY-DISPersed POPULATIONS OF *CEPHUS CINCTUS*

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Wheat stem sawfly, *Cephus cinctus* Norton, is the most destructive chronic insect pest of wheat in the northern Great Plains. However, little is known about the breeding structure and genomic variability of wheat stem sawfly in North America. Over the past 20 years in Montana, wheat stem sawfly has changed from an insect pest exclusive to spring wheat to a pest which causes major economic damage in both spring and winter wheat. Preliminary examination suggests that phenology and maturity of wheat cultivars grown in Montana has not changed enough over this period to account for the difference in insect virulence. Knowledge of the genetic variability within endemic populations of wheat stem sawfly is important for developing management and resistance-breeding strategies.

Our objective in this study was to assay the genomic variability within and among geographically-dispersed collections of wheat stem sawfly from the northern U.S. Great Plains using RAPD-PCR markers. Overwintering sawfly larvae were collected from wheat stubble at eight sites in Montana (Bruckner), six sites in North Dakota (Dr. M.J. Weiss, North Dakota State Univ.), and one site in Wyoming (Dr. G.L. Hein, Univ. of Nebraska). DNA was extracted and evaluated from 10 individual larvae for each Montana collection and from 5 larvae for each North Dakota and Wyoming collections. Sixty-two random decamer primers were screened and 20 of them consistently produced well-amplified and reproducible polymorphic bands. The size of amplified DNA fragments produced by these primers ranged from 200-1900 bp, with individual primers generating from 2-8 bands. Cluster analysis using UPGMA indicated significant variation among individuals within collection sites and among collection sites. All sawfly larvae from Montana collections clustered separately from sawfly larvae collected in North Dakota and Wyoming. Larvae collected from the Wyoming site showed the greatest within site similarity. Whether this genomic diversity observed among sawfly populations at geographically and ecologically diverse collection sites is meaningful in terms of insect virulence and/or biotypic differentiation is not known at this time. If RAPD-PCR molecular markers can be used to identify meaningful differences in wheat stem sawfly virulence, then assessment of insect variability among geographic regions could be used to target resistance screening nurseries to sites where new and/or more virulent biotypes are found. In preliminary research, we also analyzed genomic differences among larval progeny of unmated, caged sawfly females, and between male and female larvae collected from the same sites. Much less variation was found among family members than within the source population and no sex specific differences were detected among male and female individuals collected from the same sites. If funded, future research on sawfly population structure will focus on 1) collection of *Cephus cinctus* populations over an expanded geographical area; 2) development of bioassays for sawfly virulence, morphological, developmental, and/or behavioral diversity to verify potential biotype development; and 3) identification of new and diverse sources of host plant resistance to wheat stem sawfly.

# **A RAPD MARKER FOR PREHARVEST SPROUTING RESISTANCE IN WHITE SEEDED CANADIAN PRAIRIE SPRING WHEAT CROSSES**

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Preharvest sprouting in wheat kernels can occur during wet harvest conditions. This causes harvest losses and reduced end use quality due to high levels of hydrolytic enzymes in the flour. Preharvest sprouting is an especially severe problem in the production of white seeded wheats and is generally attributed to the lack of dormancy. Dormancy is difficult to incorporate into new varieties because heritability is low and the evaluation of dormancy is time consuming and environmentally dependent. Three white kernel sources of dormancy; SC8021V2, SC8019R1, RL4555 are being used in the Canadian Prairie Spring (CPS) wheat breeding programs and all have Kenya 321 in their pedigrees.

Our initial objective was to identify a RAPD marker linked to dormancy in a recombinant inbred (RI) population consisting of 100 individuals from a cross between a red non-dormant CPS line (BiggarBSR) and a white dormant spring line (RL4555). This population was grown in a controlled environment and dormancy was evaluated by germination tests on hand threshed seed. Under these conditions we observed one major gene controlling dormancy which was independent of kernel colour. The RI population was screened for polymorphisms using RAPD primers. The PCR products were visualized by agarose and denaturing gradient gel electrophoresis (DGGE). Out of 50 primers tested, 14 polymorphic loci were identified between the parents. Linkage analysis was performed and one polymorphic band was linked in repulsion to a major dormancy gene and the map distance appeared to be between 15 and 30 cM.

The polymorphic band has been cloned and will be sequenced and converted to a sequence characterised amplified region (SCAR). SCAR markers are more reliable and will allow a more rapid screening of lines from a plant breeding program. The cloned band was used as a probe on genomic DNA and was found to be low copy number DNA, however the polymorphism was not retained. The cloned band will be used as a probe on Chinese Spring nulli-tetra cytogenetic stocks in order to determine the chromosome location of the marker. This will allow us to find closer markers based on the genome map of wheat.

## **RETURNING GRASSLAND TO CROP PRODUCTION IN EASTERN WASHINGTON - SMALL PLOT RESULTS**

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With the probable termination of the Conservation Reserve Program (CRP), management practices to return the grassland to crop production in highly erodible areas are urgently needed. In the fall of 1992, grass breakout using small plots was initiated on the Dryland Research Unit at Lind, Washington. Several factors could effect the successful conversion of grassland into crop production, including; time of breakout, method or tools used, weed and fertilizer management, and the cultivar grown. Many entire farms in the area entered the program in one year (presumably, the entire farm will come out of CRP in one year). Since this is a winter wheat - summer fallow area, a spring crop on half the farm may be advisable to maintain a favorable cash flow.

In the first year, 1992/93, the only variable investigated was fall vs spring breakout and different cultivars. A disc was used for initial tillage. In the second year, this was expanded to include a sweep plow for initial tillage and different rates of nitrogen fertilizer.

Fall breakout was best for spring crop production the first year while spring breakout was best the second year. The first year was above normal in precipitation and had some winter runoff from nontilled plots. The second year was dry with no winter runoff. Yields the first year were as expected for annual recropping, while the yields for the second year were very low and insufficient to produce crop residue to prevent erosion. Winter wheat yields following summer fallow were as expected from continuous cropping with a wheat - summer fallow rotation.

## THE INTERNATIONAL WHEAT INFORMATION SYSTEM AT WORK IN BREEDING

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*"..the scattered bits of information about germplasm are currently beyond the grasp of any one researcher."*

-- a US wheat breeder, 1992.

A revolution in breeding occurred through germplasm exchange. The second revolution will involve the free exchange of information related to germplasm, adding value to germplasm and strengthening bonds between institutions and between scientists. Positive dynamic feedback between genetics, conventional and molecular, and environmental information will provide new insights into crop adaptation.

Costs of generating field and laboratory data are increasing, while the costs of data storage, management and analysis are decreasing rapidly. Conservative estimates indicate that, throughout the world, national agricultural research systems collectively invest more than \$1 million per year in field plot management of the germplasm received from the CIMMYT Wheat Program alone. Now information technology can put the data generated by such major investments to work in crop improvement.

Barriers to data exchange resulted from multiple synonyms for a given cultivar or the use of the same name for different ones. Other barriers reflected fragmentation of research. CIMMYT has developed a research plan based on unique identification of germplasm to remove barriers to association and to facilitate crossing data frontiers. One of many advantages of data integration will be ease of use of data from genebanks and laboratories in the planning of crosses in breeding.

The software being developed at CIMMYT will be transferred to a PC environment for scientists around the world. A "plug and play" CD system featuring annual updates, is scheduled for release in 1996.

Integrating the CIMMYT system for unique identification of germplasm with work of the USDA Plant Genome Research Program and other groups will make it possible for an International Wheat Information System to store, query, and disseminate data on wheat germplasm held by many countries. Such a system could provide a model for other crops.

## **IN VITRO SELECTION OF WINTER WHEAT CULTIVARS FOR FREEZING TOLERANCE**

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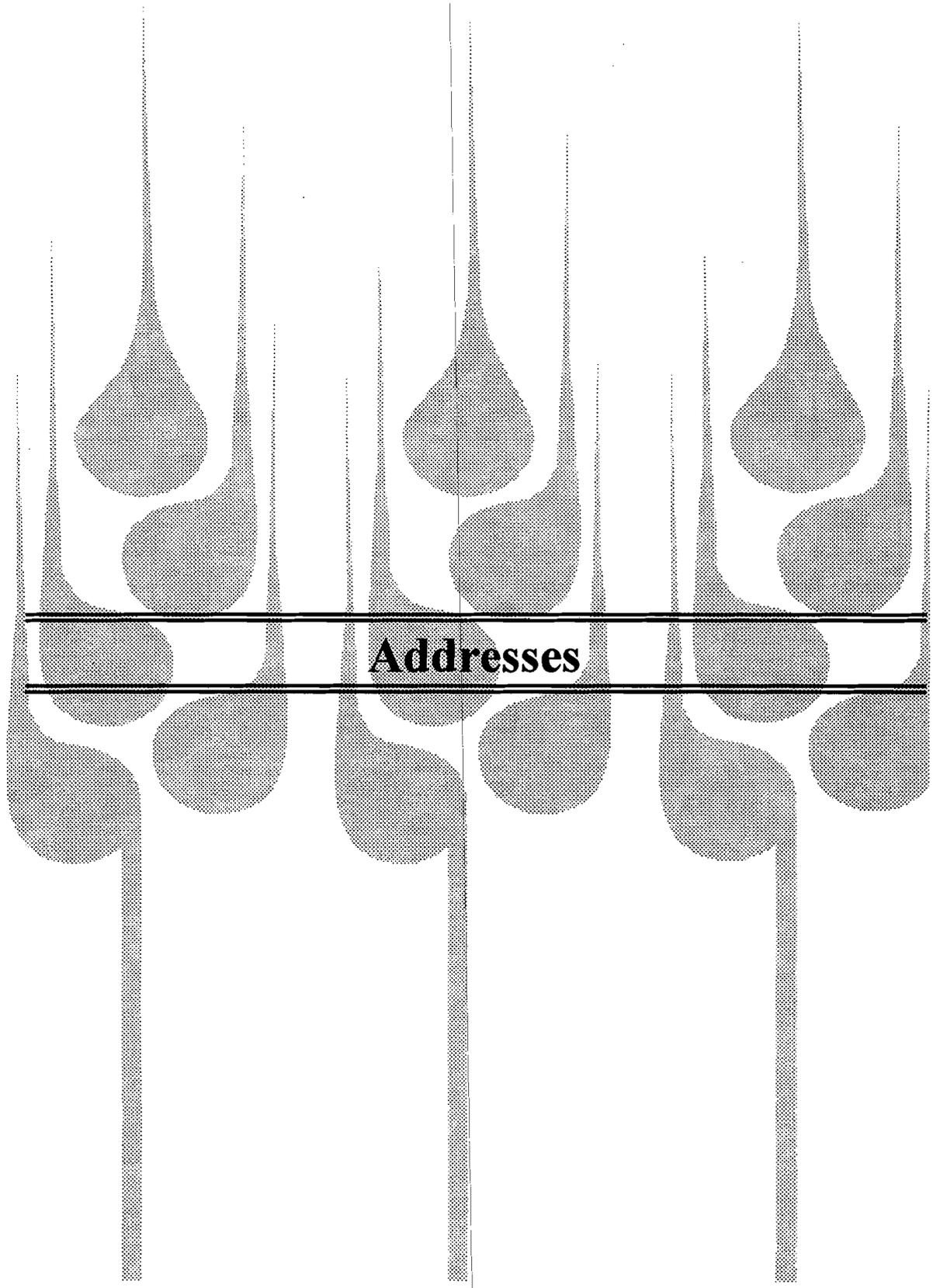
One spring and nine winter wheat (*Triticum aestivum* L. em. Thell.) cultivars were screened for freezing tolerance using a tissue culture procedure. Temperature treatments were applied to immature embryo-derived callus cultures. Regression analysis was done using mean calli weights from 2 to  $-15^{\circ}\text{C}$ . The regression coefficients (b values) of the five most winter-hardy cultivars were negatively correlated with published winter survival under both conventional and no-till conditions. Thus, lower winter hardiness was associated with an increased temperature effect on calli growth. This relationship was not true for the four least hardy winter wheats. The best relationship between calli growth and winter survival was observed at  $-5^{\circ}\text{C}$ . Attempts to regenerate plants were made on all calli. All ten cultivars and progeny from the regenerated plants were evaluated for freezing tolerance at  $-15^{\circ}\text{C}$ . Calli exposed to a moderate freezing temperature of  $-5$  to  $-10^{\circ}\text{C}$  produced the greatest frequency of selected lines. Eighty-one lines exhibited significantly greater freezing tolerance compared to their donor parent. The scheme used in this study appears to be effective for identifying genetic variation for freezing tolerance. Somaclonal variation appears to be responsible for the greater freeze tolerance of selected lines.

## **DEVELOPMENT OF A SMALL-SCALE LABORATORY SHEETED NOODLE DOUGH MIXER**

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A small scale laboratory noodle dough mixer has been developed for making Asian sheeted noodles. The characteristics of noodle doughs made by various mixers were investigated with regard to texture, optimum dough development time, and dough temperature variation during mixing. Noodle dough properties were affected by the length and width of mixing pins. The number of mixing pins and the distance between pins on the mixing shaft influenced noodle dough characteristics. A noodle dough mixer has been developed which provided appropriate noodle doughs for making Asian sheeted noodles. These results may have application to laboratories which analyze small amounts of flour.



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