ITEMS FROM PAKISTAN

AGRONOMIC RESEARCH STATION
Bahawalpur, Pakistan.

Identifying sources of resistance to wheat leaf rust under induced and natural conditions.

Altaf Hussain Tariq, Saeed Ahmad, Muhammad Arshad Hussain, Muhammad Ziaullah,Lal Hussain Akhtar, and Sabir Zameer Siddiqi.

Background. Rust diseases pose a major threat to the productivity of wheat crop when epidemics develop. Leaf rust is world wide in distribution and a most dreaded disease that can spread rapidly and devastate the wheat crop (McIntosh et al. 1997). In Egypt, Abdel Haq et al. (1980) estimated yield losses up to 50 % in wheat. This disease has appeared in epidemic form several times in Pakistan. During 1978, a national loss of 86 x 10^6 USD was estimated (Hussain et al. 1980). Chemical control of rust diseases is not economical. Therefore, cultivation of resistant cultivars is of paramount importance. Breeders need to plan their hybridization program judiciously in order to produce cultivars with different genetic backgrounds for resistance to rusts so that any danger of a disease epidemic can be avoided. The present studies explored new sources for rust resistance in wheat, which will help the breeders in planning future wheat-breeding programs.

Materials and methods. Local Wheat Diseases Screening Nurseries (LWDSN) comprised of 293 and 346 advanced wheat lines were planted at Bahawalpur during 2001 and 2002, respectively. Ten commercial wheat cultivars also were included in the nurseries. The entries, which gave reactions from trace to MRMS at Bahawalpur, and 10 commercial cultivars also were sown at Kaghan. Each entry was planted in a single 2-m row, 30 cm apart, at both the locations. Two rows of susceptible checks (Morocco and Local White) were sown repeatedly after every fifth entry and around the block. The nurseries were inoculated artificially with a spore suspension of leaf rust by injecting, rubbing, and spraying from the first week of February until 10 March at Bahawalpur during both the years. Kaghan is a summer station about 7,000 ft ASL. Natural rust epidemics occur frequently in this area. The planting at Kaghan was made during the first week of June. Observations on rust infections were recorded at 10–15 day intervals throughout the growing period at Bahawalpur and during the end of August at Kaghan. Data were recorded according to the modified Cobb's scale at both locations (Peterson et al. 1948). The observations were compared among years and locations to establish the distribution of rust incidence.

Results and discussion. The observations of leaf rust on the 10 commercial wheats sown at Bahawalpur and Kaghan in 2001 and 2002 indicated that the intensity of rust infection during 2001 was comparatively higher than that in 2002 at both locations. Natural infection at Kaghan was less in 2002 because of less precipitation throughout the country during 2002 and the environmental influence on the host-pathogen interaction at Kaghan where the growing season is shorter (80–90 days) and cooler with a shorter daylength (Table 1). Six cultivars, FSD-85, Inqlab-

Table 1. Reaction to the leaf rust pathogen of commercial wheat cultivars at two different locations in Pakistan during 2001–02. Infection types are listed as TR = trace, R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible.

Cultivar	RARI, Bahawalpur (artificial inoculation)		Kaghan (natural infection)	
	2001	2002	2001	2002
Kohinoor-83	60S	40S	30S	20S
Faisal-85	20R	5R	10MR	5MS
Inqlab-91	TR (< 5 %)	5MR	10MRMS	5MR
Pasban	40S	20S	20S	10S
Rohtas-90	TR	30MRMS	30MRMS	20MRMS
Punjab-96	5MSS	5MS	5MRMS	5MR
Bahawalpur-97	20MRMS	10MRMS	20RMR	10MRMS
MH-97	20MR	20MR	20RMR	5MR
Uqab-2000	10MR	5R	30RMR	20MR
Iqbal-2000	20RMR	30MR	20RMR	5MR

91, Rohtas-90, MH-97, Uqab-2000, and Iqbal-2000, were resistant with < 5–30 % infection during both years at Bahawalpur. At Kaghan, these cultivars exhibited almost the same reaction but with less intensity during 2002. These cultivars have the *Lr10* gene along with *Lr27+Lr31* have been very effective in providing resistance to leaf rust. In field experiments conducted at Faisalabad in Pakistan, Khan et al. (1997) found Pavan, Faisalabad-85, and Inqlab-91 to be slow rusting. Chaudhry et al. (1996) evaluated 14 commercial wheat cultivars in the field and reported Inqlab-91, Parwaz-94, and Chakwal-86 resistant to leaf and yellow rust throughout Punjab and the North Western Frontier Province during 1994 and 1995. Kohinoor-83, Pasban-90, and Punjab-96 remained susceptible to leaf rust at both sites under induced and natural conditions, whereas Bahawalpur-97 maintained its MR–MS level during both the years.

The leaf rust observations at the different locations of new advanced lines during 2001 and 2002 are presented in Table 2. These observations indicate the number of test entries under different categories of rust-infection levels. During 2001, 95 of 293 entries were immune and 135 (46 % of the total) were trace to moderately resistant. Among 346 lines, 77 remained immune, 92 had trace infection, 103 were resistant, and 51 were moderately resistant during 2002. At Kaghan, the number of entries was less compared to Bahawalpur during both years, because they were selected on the basis of disease reactions (traces to resistant and moderately resistant) and yield traits. Generally, the entries that were moderately susceptible under induced conditions at Bahawalpur were mostly resistant to moderately resistant reactions at Kaghan during both years. The inheritance of leaf rust resistance was better in these lines. Rust inoculum is dynamic in nature and changes from year to year and place to place. Virulence in one environment may not necessarily appear in another (Khan et al. 2002). The virulence patterns observed at the two sites confirm this hypothesis.

Table 2. Reaction to the leaf rust pathogen under natural infection and induced conditions in new advanced lines at two different locations in Pakistan during 2001–02. Infection types are listed as I = immune, TR = trace, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, and HS = highly susceptible.

	Number of plants			
	RARI, Bahawalpur (induced epidemic)		Kaghan (natural infection)	
Infection type	2001	2002	2001	2002
I (0)	95	77	17	61
TR (< 5 %)	51	92	45	70
R (5–20 %)	70	103	112	114
MR (21–40 %)	14	51	46	20
MS (41–50 %)	28	10	11	6
S (51–80 %)	35	13	1	4
HS (> 80 %)	—	—	—	—
Total	293	346	232	275

The evolution of new rust races is a permanent feature of the rust pathogen. Whenever new cultivars are deployed in the field, new races of the pathogen develop after several years and the existing cultivars become susceptible. This phenomenon has been reported by number of workers (Ezzahiri 1989; Meshkova 1990; Meena-Kumari et al. 1992). At present, more than 80 % of the area under wheat cultivation is occupied by the single cultivar Inqlab-91, which is fraught with the danger. Under these circumstances, steps to avoid monoculture need to be taken. A number of advanced lines are available from the present studies that were resistant to prevailing rust races to provide sufficient material for developing new, resistant wheat cultivars.

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Performance of advanced wheat genotypes to Helicoverpa armigera Hubner.

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Background. Wheat is the staple diet of the people of Pakistan, contributing 12.1 % to value added in agriculture and 2.9 % to the GDP. Wheat was grown on an area of 6.30 x 10^6 ha with a production of 15.42 x 10^6 tons in 2000–01 in Punjab (Anonymous 2001). The by-products of wheat are used in bakery products and confectionery. For the last few years, Pakistan has become self sufficient in wheat production. Surplus wheat is exported to various countries such as Vietnam, United Arab Emirates, Somalia, Egypt, Ethiopia, Kenya, and Afghanistan. Various rust and smut diseases, aphids, *Helicoverpa armigera*, and termites attack this crop. Ann (2002) observed that aphids can be controlled easily with predators such as Coccinelid beetles and chrysopa and syrphis flies, whereas the reverse is true for *H. armigera,* which is a devastating pest of many crop plants world wide (Patankar et al. 2001). Saleem and Rashid (2000) reported a loss of 13.98 % in grain yield in wheat caused by a single caterpillar of *H. armigera* per tiller. Being the staple diet, the use of chemicals is not feasible for the control of this pest because of residual effects that may be hazardous to human health. The ultimate solution to the problem is the screening of genotypes with built-in resistance to *H. armigera*. Keeping in view the significance of the pest, we screened for genotypes of wheat resistant or tolerant to *H. armigera*.

Materials and methods. To asses wheat losses caused by *H. armigera*, 20 advance strains of wheat including two checks were evaluated for spike and grain damage during the Rabi season 1998–99 at the Regional Agricultural Research Institute, Bahawalpur. The experiment setup was a RCB design with three replications and plot size of 12 m^2. Similar agronomic practices were applied to all genotypes throughout the growing season. Observations of spike damage were recorded at the harvest by counting the total number of spikes and the number of spikes damaged by the pest from three randomly selected spots of 1 ft^2 from each plot. Grain-damage data were recorded by counting the total number of grains and number of grains damaged by the pest from five randomly selected spikes from each plot after harvest. Thus, the percentage of damaged spikes/grains was calculated as follows:

$$\text{Spike/grain damage (\%)} = \frac{\text{No. of damaged spikes / grains}}{\text{No. of total spikes/grains}} \times 100$$

Data were subjected to statistical analysis using a computer package MSTATC. Correlations were computed using the Correlation subprogram of MSTATC. Means were compared by Duncan's New Multiple Range Test (Steel and Torrie 1980).

Results and discussion. Statistical analysis of the data revealed the highly significant differences among the mean values of spike and grain damage ($P < 0.01$) of all the genotypes (Table 3). Spike and grain damage ranged from 19.95 to 80.47 and 3.90 to 22.16 % in the check genotypes, respectively (Table 4). The most susceptible genotypes in terms of spike damage

Table 3. Analysis of variance of data with regard to spike and grain damage of various wheat genotypes after damage by *Helicoverpa armigera*.

Parameter	Damaged spikes (%)	Damaged grains (%)
Means squares	65392	96.35
Probability	0.000	0.000
Coefficient of variation (%)	3.54 %	7.32 %
Cd1 (0.05 %)	2.728	1.558
Cd2 (0.01 %)	3.654	2.086
Standard error	0.953	0.544
Correlation between the two traits (r^2)	0.422	

were D-94654 (80.47 %), PR-68 (76.00 %), WS-94194 (59.53 %), and V-94091 (58.28 %). The genotype 92T001 was found to be the most tolerant with the least spike damage (19.95 %). For grain damage, SD-4 had the maximum damage (22.16 %) and V-8120 had the least (3.89 %), a vast range of damage differences. The present results support the data of Saleem and Rashid (2000) who found that a single caterpillar of *H. armigera* per tiller caused 13.98 % loss in grain yield of wheat. Such information will encourage the wheat breeders to incorporate this character in their breeding program. Efforts are being made to develop the wheat genotypes tolerant to *H. armigera* at our institute.

Table 4. Data for various traits of the wheat genotypes tested for resistance to *Helicoverpa armigera* at the Regional Agricultural Research Institute, Bahawalpur during 1998–99.

Genotype	Damaged spikes	Damaged grains	Yield (kg/ha)
V-95219	41.01	11.48	4,062
94B-3047	39.34	14.19	3,861
WS-94194	59.53	15.18	3,674
V-94105	52.37	9.58	3,861
PR-68	76.00	14.94	3,243
D-94654	80.47	18.09	3,292
SD-4	39.04	22.16	3,049
92T001	19.95	4.09	4,035
V-95153	45.75	20.52	4,021
AUP-9701	34.58	6.12	4,333
V-94091	58.28	11.44	3,597
93B2707	37.85	14.32	3,674
PR-67	46.72	9.01	3,507
V-95069	36.22	12.29	3,604
DN-10	50.48	9.96	2,931
V-8120	24.23	3.90	3,382
91BT010-1	45.33	4.00	3,986
V-94045	52.42	18.24	3,326
INQ-91	52.37	19.78	3,674
Local check	41.31	19.12	3,771

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Manthar – a high-yielding cultivar of wheat released for general cultivation in southern Punjab.

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Background. Wheat is the main staple food of the people of Pakistan and is grown on the largest area covering more than 15 x 10^6 acres in the Punjab. Although Pakistan is a wheat exporter, this situation has been changing for the last 2 years. New steps now are needed to be adopted to progress forward. Agronomic advancement is the utmost need, including the development of genotypes possessing high-yield potential. Wheat breeders are trying to improve the potential at their research stations, resulting in wheat cultivars with acceptable and improved characteristics.

Manthar is selection from CIMMYT material and has been tested at Regional Agricultural Research Institute, Bahawalpur and outstations for 7 years. This strain has the famous CIMMYT line Kauz in its pedigree, which is a more adapted and a high yielder. Genetically, this strain differs from existing commercial cultivars of Punjab. Manthar rated a position among the top five strains in National Uniform Wheat Yield Trial the first year and the first position in late planting and second in 23 sites in Pakistan in its second year. Manthar has improved yield potential and better adaptabil-

ity. Dry and unfavorable conditions in 2001–02 produced a successful wheat crop during a continuous drought. This genotype rated the second position in Pakistan based on drought and heat tolerance. The cultivar is resistant to leaf rust and yellow rust at CDRI, Islamabad. We hope that Manthar will help boost the average wheat yield because of its better economic characteristics while being a general-purpose cultivar.

Materials and methods. Manthar, selected from CIMMYT (Mexico) material, was tested at Regional Agricultural Research Institute, Bahawalpur, and outstations for 7 years (1996–2002) and given the number 97B2210. This line was evaluated for its yield potential in 81 trials at various locations Preliminary Yield and Advanced Yield Trials, the Micro Wheat Yield Trials (2000–01), and the National Uniform Wheat Yield Trial (2000–01). Sowing date and fertilizer trials also were conducted to evaluate its production technology during 2000–01 to 2001–02. The line 97B2210 also was tested for resistance to rusts, loose smut, and Karnal bunt at the Regional Agricultural Research Institute, Bahawalpur; the Wheat Research Institute, Faisalabad, and the Crop Disease Research Institute, NARC, Islamabad during 2000–02 and compared with standard cultivars. The Coordinator Wheat, NARC, Islamabad, also studied the quality characteristics of the line in 2000–01. The Federal Seed Certification and Registration Department, Islamabad, evaluated plant characteristics. The yield data were subjected to ANOVA using the MSTAT statistical program and means were compared using Duncan's Multiple Range Test (Steel and Torrie 1980).

Table 5. Results of the station yield trials at Bahawalpur, Pakistan, for Manthar (97B2210) and the check cultivar Inqlab-91.

Year	Trial	97B2210	Inq-91
1997–98	A1 (N)	5,671 [a]	5,322 [a]
1998–99	B3 (N)	4,750 [a]	4,417 [b]
1999–2000	CI (N)	6,115 [a]	5,693 [b]
Average		5,512	5,144
% increase over check		+ 7.1	

Table 6. Zonal testing of Manthar (97B2210) and the check cultivar Inqlab-91 (Inq-91) at three locations in Pakistan during 1999–2000.

Location	97B2210	Inq-91
CRSS, Haroonabad	5,245	4,936
ORS, Khanpur	4,442	4,393
ARS, Khanewal	4,782	4,630
Average	4,823	4,652
% increase over check	+ 3.7	

Results and discussion.

Yield performance. Station Yield Trials. Manthar was tested in preliminary and advance yield trials at the Regional Agricultural Research Institute, Bahawalpur, between 1996–97 and 2001–02 in late planting and compared with the national checks, Uqab-2000 and Inqlab-91. The performance of Manthar is given in Table 5. Over a 3-year average, the cultivar had a 7.1 % higher yield than Inqlab-91 (Table 5) and also outyielded the check by a margin of 3.7 % in zonal trials conducted at three locations in 1999–2000 (Table 6).

Table 7. Results of the Micro Wheat Yield Trials at various locations in Pakistan in 2000–01. Source: Director Wheat, Faisalabad.

Location	97B2210 (Manthar)	Inqlab-91	Uqab 2000	Iqbal 2000
RARI, Bhawalpur	5,405 [a]	4,826 [a]	5,004 [a]	4,676 [b]
ARF, Rahim Yar khan	5,204 [a]	4,932 [a]	4,721 [b]	4,186 [bc]
CRSS, Haroonabad	6,346 [a]	5,990 [a]	3,741 [c]	4,486 [bc]
WRL, Faisalabad	5,735 [a]	5,920 [a]	5,965 [a]	5,550 [a]
ARF, Vehari	3,290 [ab]	3,660 [a]	3,382 [ab]	3,290 [ab]
PSC, Khanewal	3,799 [b]	4,819 [a]	4,819 [a]	5,097 [a]
Thatta Jawana Jhang	4,263 [a]	3,614 [b]	4,031 [a]	4,031 [a]
Hafizabad Pindi Bhattian	4,170 [a]	4,263 [a]	2,124 [c]	2,965 [b]
ARF, Gujranwala	4,911 [a]	4,726 [a]	4,355 [b]	4,633 [a]
RRI, Kala Shah Kaku	4,720 [a]	4,165 [b]	4,165 [b]	4,165 [b]
Average with PSC	4,784	4,691	4,231	4,307
% increase over check		+ 2	+ 13	+ 11
Average without PSC	4,894	4,677	4,165	4,220
% increase over check		+ 4.66	+ 18	+ 16

Micro Wheat Yield Trial. The Director, Wheat Research Institute, Faisalabad, also evaluated the performance of Manthar under a coded number during 2000–01 at various locations in Punjab in replicated yield trials. The results show yields 2.0, 13, and 11 % higher for Manthar when compared to Inqlab-91, Uqab-2000, and Iqbal-2000, respectively, an average of 10 locations (Table 7).

National Uniform Wheat Yield Trial. The Coordinator Wheat, Islamabad, also evaluated Manthar in a replicated trial called the National Uniform Yield Trial under normal and short conditions throughout Pakistan during 2001–02. The performance of

Manthar in this trial is given is Table 8. Manthar had a 7.1 % higher yield than the local check at the National level on the basis of 12 locations in 24 trials.

Varietal characteristics. Various varietal characteristics recorded by the Federal Seed Certification and Registration Department, Islamabad, in comparison with Inq-91 are given in Table 9.

Agronomic studies. Six trials were conducted at Regional Agricultural Research Institute, Bahawalpur, during 2000–02 to ascertain production technology. Sowing time is 10 November to 10 December at a seeding rate of 125 kg/ha. Fertilizer requirements include 125–100–50 NPK with 4–5 irrigations.

Pathology studies. The response of Manthar to various foliar diseases was studied at Crop Diseases Research Institute, NARC, Islamabad; the Wheat Research Institute, Faisalabad; and the Regional Agricultural Research Institute, Bahawalpur. The data are given in Table 10. The data indicates that Manthar is resistant/tolerant to the yellow rust, leaf rust, loose smut, Fusarium, and Karnal bunt pathogens.

Entomology studies. The response of Manthar to aphid and *Helicoverpa armigera* also was studied at Regional Agricultural Research Institute, Bahawalpur, in 2000–02. Data are given in Table 11 shows the performance of Manthar as compared to commercial checks.

Quality studies. The quality characters were recorded by the National Agricultural Research Centre, Islamabad, and are given in Table 12. The new cultivar is better than the existing checks.

Conclusion. The cultivar Manthar (97B2210) not only is a high-yielder and tolerant/resistant to all diseases but also best suited to a wheat–cotton–wheat rotation. Because of better adaptability, Manthar has the potential of replacing previously approved wheat cultivars, especially in the southern Punjab. This cultivar was approved and released by Variety Evaluation Committee, Islamabad, for general cultivation during 2002.

Table 8. Results of the National Uniform Wheat Yield Trial at various locations in Pakistan in 2001–02. Seeding date is for normal and late dates combined. Source: Anonymous 2002.

Location	97B2210 (Manthar)	Local check
ARF, Rahim Yar khan	3,773	3,348
ORS, Khanpur	4,081	3,619
RARI, Bahawalpur	3,583	3,335
CRSS, Haroonabad,BWN	3,827	3,490
ARF, Vehari	3,852	3,583
PSC, Khanewal	3,919	4,177
WRI, Faisalabad	4,843	4,853
ARF, Layyah Karore	2,977	2,125
Gill Model Farm S.Abad Jhang	3,700	3,382
Hafizabad Pindi Bhattian	4,344	4,567
In service Trg. Sargodha	3,927	3,281
ARF, Sheikhpura	3,813	3,792
Average	3,887	3,629
% increase over check	+ 7.1	

Table 9. Ccharacteristics of Manthar compared to the local check cultivar Inqlab-91.

Characteristic	Manthar	Inqlab-91
Days to handing	98	114
Days to maturity	142	135
Plant height	94 cm	98 cm
Lodging	Resistant	Resistant
Tillers per meter row	145	132
1,000-kernel weight	40–45 g	44.0 g
Protein	12.97 %	10.51 %
Disease reaction	Resistant/tolerant	Resistant
Grain size	Medium	—
Maturity status	Medium	Medium
Growth habit	Erect	Drooping
Yield potential	6,708 kg/ha	6,900 kg/ha

Table 10. Disease response of Manthar and a local check to rust recorded by the Crops Disease Research Institute, Islamabad, during 2000–01.

		ACI		RRI	
Year	Cultivar	leaf rust	yellow rust	leaf rust	yellow rust
2000–01	97B2210	3.4	—	6.7	—
	Local White	56.6	—	—	—
2001–02	97B2210	0.7	0	7.6	8.9
	Local White	45.65	—	—	—

Table 11. Leaf rust reaction of Manthar (97B2210) and a check in the National Wheat Disease Screening Nursery at CDRI, Islamabad, 2001–02.

Cultivar	PRC, SKT	AARI, FSD	RARI, BWP	CCRLD, SBK	NIFA, PWAR	NARC, ISD	CDRI KHI	RRI
97B2210	0	10MR	0	0	0	5MRMS	0	7.6
Morocco	50S	90MS	50MSS	40S	20S	80S	30S	—

Table 12. Resistance to aphids in Manthar compared with the standard commercial check cultivars.

Year	Cultivar	Aphid population	Yield (kg/ha)
2000–01	Manthar	21.4	3,250
	Inq-91	22.3	3,084
2001–02	Manthar	0.50	2,512
	Auquab-2000	0.55	2,392
	Iqbal-2000	0.55	2,332

Table 13. Resistance to *Helicoverpa armigera* in Manthar and some commercial check cultivars in 2000–01

	Aphid population (per tiller)		Yield (kg/ha)	
Cultivar	Normal	Late	Norma	Late
Manthar	0	0.30	4,475	4,175
Inqbal-91	0.33	0.62	4,150	3,880
MH-97	0.34	7.11	4,262	3,925

Table 14. Results of the National Uniform Wheat yield Trial in 2000–01 for Manthar compared to the local check cultivar Inqlab-91.

Characteristic	Manthar	Inqlab-91
1,000-kernel weight	42.3 g	37.0 g
Test weight	79.5 g	74.2 g
PSI (%)	29.0	42.2
Ash (%)	1.55	1.54
Gluten content	MS	MS
Dry gluten (5)	8.20	5.79
Crude protein (%)	12.79	10.06

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Development of 012679, a new wheat strain with special characteristics.

Mushtaq Ahmad, Ghulam Hussain, Muhammad Rafiq, Manzoor Hussain, Lal Hussain Akhtar, and Sabir Zameer Siddiqi.

Wheat not only is the main staple food of Pakistan, but more than 33 % population of world also depend upon it for nourishment. Hybridization efforts are not bearing significant yield improvements. Improvement in grain yield is the ultimate objective of all agronomic and breeding investigations. Genetic yield potential can be improved by increasing the number of grains/unit area and grain weight. Efforts at the Regional Agricultural Research Institute, Bahawalpur, seek to improve grain weight and grain number/unit area and combine them in the same plant with required protein and gluten levels. A new wheat strain was bulked during 2000–01 with number 012679. Strain 012679 is a local cross (Debaria/WL-711) attempted during 1994–95. The F_1 to F_6 were grown from 1995–96 to 2000–01 at RAI, Bahawalpur. The cultivar was evaluated for yield in yield trials during 2001–02 with under the number 012679.

Strain 012679 produced 41.12 % and 61 % more yield than the commercial checks Inqbal-91 and PND-I, respectively (Tables 15 and 16). Further studies are in progress in yield trials during 2002–03 to confirm these results. Strain 012679 differs from the existing cultivars in following characteristics: a thick stem is resistant to lodging; increasing the seeding rate compensates for a lower number of tillers/seed; early maturity fits in a wheat-based cropping pattern; a thick, dense head with 100 % maturity gives more grains/spike; and more grains than commercial standards results in a higher grain yield.

Effect of irrigation and various nitrogen and phosphorus levels on wheat yield.

Muhammad Aslam, Manzoor Hussain, Lal Hussain Akhtar, Mushtaq Ahmad, Ghulam Hussain, Abdul Rashid, Muhammad Safdar, Muhammad Masood Akhtar, Muhammad Arshad, and Sabir Zameer Siddiqi.

Table 15. Yield data for the new cultivar 012679 compared with commercial cultivars in 2001–02 at the Regional Agricultural Research Station, Bahawalpur, Pakistan.

Cultivar	Yield (kg/ha)	Cultivar	Yield (kg/ha)
012672	3,953	012678	4,848
012673	5,222	012679	5,796
012674	4,710	012680	5,219
012675	4,538	Inq-91	4,108
012676	3,810	Punjnad-I	3,600
012677	5,067	Uquab-2000	3,545

Table 16. Yield components of the new cultivar 012679 compared to the local checks in 2001–02 at the Regional Agricultural Research Station, Bahawalpur, Pakistan.

Cultivar	1,000-kernel weight (g)	No. of grain/spike	Spike weight (g)	Yield (kg/ha)
012679	50.05	108	5.24	5,796
Inqbal-91	40.45	55	4.32	4,108
PND-I	38.20	59	4.54	3,600

Background. Wheat, a major food grain of Pakistan, is being adversely affected by shortage of water. During 2001–02, a decline of 2.4 % in cultivated area and yield was found due mainly to dry weather, a shortage of irrigation water, low application of NP fertilizer, and a delayed sowing of the 2001–02 season crop (Sabir et al. 2000; Anonymous 2002). Under these circumstances, the positive role of irrigation and NP levels need to be demonstrated. Similarly, the high use of irrigation water also is being restricted due to shortage of canal water and high prices of subsoil water. The NP fertilizer and irrigation factors play an important role in getting the highest grain yield from the wheat crop. Ibrahim (1999) obtained a high grain yield of 4.6 and 4.8 t/ha using three and four irrigations. Kalita et al. (2000) achieved a high grain yield from three irrigations. Laxminarayana and Thakur (1999) found that grain yield increased with an increase in applied phosphorus up to 90 kg/ha. Sabir et al. (2000) obtained their highest yields with the application of 150:100 kg/ha N:P. Pandey et al. (1999) reported that grain yield increased up to 150:75 N:P levels. Naser et al. (1999) and Maliwal et al. (2000) found that irrigation treatments increase the yield. Therefore, this project was to determine the best NP level with three and five irrigations for obtaining best wheat yield.

Materials and methods. The study was conducted at Regional Agricultural Research Institute, Bahawalpur, during the years, 2000–02. The wheat cultivar Punjnad-1 was sown during both the years on well prepared seed bed with a single-row drill in rows 30 cm apart. Ten treatments involving two irrigation levels (three (at crown root, boot, and milk stages) and five (at crown root, tillering, boot, milk, and grain-formation stages)) with five levels of NP 0–0, 50–50, 100–75, 150–100, and 200–125 kg/ha, were studied. K was kept constant (60 kg/ha) in all treatments. A split-plot design with four replications was used with net plot size of '6 m x 1.8 m'. All phosphorus and potassium was applied as a basal dose at sowing. All nitrogen fertilizer was applied with the first irrigation. Other agronomic practices were kept uniform for all the treatments. Grain yield (kg/ha) was recorded at harvest. The data were analyzed statistically by using Fisher's analysis of variance and differences among the treatments means were compared by Duncan's Multiple Range Test (Steel and Torrie 1980). Table 17 lists the treatments given.

Table 17. Different treatment regimes used in evaluating different nitrogen and phosphorus levels and irrigation levels on wheat yield.

	NP (kg/ha)				
Irrigation	0–0	50–50	100–75	150–100	200–125
Three	T1	T2	T3	T4	T5
Five	T6	T7	T8	T9	T10

Results and discussion. Grain yield significantly increases with interactive effects of irrigation and NP (Table 18). T4 gave the highest grain yield of 3,678 kg/ha, which was more economical than T5 because addition of 50–25 kg/ha more NP in T5 compared to T4 resulted in only 144 kg/ha additional yield which is uneconomical. T7 gave four times more yield (2,360 kg/ha) than T6 (558 kg/ha). Similarly T8 and T9 gave maximum yield of 3,983 and 4,178 kg/ha, respectively. T10 was at par with T9. The present results support the findings of E1-Far and Teama (1999) who reported that

grain yield was the highest when crop was irrigated after every 31 days and lowest when irrigation was applied after every 60 days. Ibrahim (1999) obtained grain yield of 4.6 t/ha and 4.8 t/ha using three and four irrigations, respectively. Kalita et al. (1999) obtained the highest grain yield from three irrigations. Pandey et al. (1999) reported that grain yield increased up to 150:75 kg/ha NP. Sabir et al. (2000) obtained the highest yield with the application of 150–100 NP. Laxminarayana and Thakur (1999) reported that grain yield increased with increase of phosphorus upto 90 kg/ha. Five irrigations were applied at crown root, tillering, boot, milk, and grain-formation stages.

Table 18. Grain yield in Punjand-1 wheat under various treatment regimes varying in level of nitrogen and phosphorus fertilizer and number of irrigations.

	NP (kg/ha)				
Irrigation	0–0	50–50	100–75	150–100	200–125
Three	474 ^{F}	1,482 ^{E}	3,383 ^{C}	3,678 ^{BC}	3,822 ^{ABC}
Five	558 ^{F}	2,360 ^{D}	3,983 ^{A}	4,178 ^{A}	4,082 ^{A}

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Effect of irrigation at different growth stages on the grain yield of wheat.

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Background. Wheat is the most important Rabi cereal crop of Pakistan. Because of deficits in irrigation water in the rivers, the country is facing long-lasting moisture stress. A plan that utilizes our limited sources of irrigation water in such a way that country does not suffer food shortage is needed. Ibrahim (1999) obtained grain yields of 4.3, 4.6, and 4.8 t/ha by applying 2, 3, and 4 irrigations, respectively. Naser et al. (1999) obtained the highest yield with two irrigations applied 30 and 50 days after sowing. Kalita et al. (1999) obtained high grain yields from three irrigations. Similar results have been reported by Lidder et al. (1999), Tripathi et al. (2000), and El-far and Teama (1999). The present study was planned to define the critical stages of the wheat crop using limited number of irrigation water to obtain an optimum yield.

Materials and methods. The study involved 15 treatments laid out in a RCBD with three replications (Table 19). Net plot size was ‘6 m x 1.8 m’. The wheat cultivar Punjnad-I was sown during the first week of December 2000–02.

The recommended fertilizer dose was applied to all the treatments. Punjnad-I was sown during both years on a well-prepared seed bed with a single-row hand drill in rows 30 cm apart. All other agronomic practices were kept

uniform for all treatments. Grain yield/ha was recorded at harvest. The data were analyzed statistically using Fisher's ANOVA and differences among the treatment means were compared by LSD (Steel and Torrie 1980).

Results and discussion. ***One irrigation.*** One irrigation was applied at different four growth stages of wheat crop. Irrigation applied at boot stage gave the maximum yield compared to other stages (Table 20). Similar results were reported by Ibrahim (1999).

Two irrigations. Two irrigations were applied in six of the combinations. Treatment T10 (boot + milk; 2,676 kg/ha) gave the highest yield of these treatments. Ibrahim (1999), Naser et al. (1999), and Lidder et al. (1999) also achieved best results when irrigation was applied at similar stages.

Table 19. Wheat growth stages used to assess the effect of irrigation for optimum yield.

1.	Crown root
2.	Tillering
3.	Boot
4.	Milk
5.	Crown root + tillering
6.	Crown root+ boot
7.	Crown root+ milk
8.	Tillering + boot
9.	Tillering + milk
10.	Boot + milk
11.	Crown root + tillering + boot
12.	Crown root + boot + milk
13.	Tillering + boot + milk
14.	Crown root + tillering + boot + milk
15.	Crown root + tillering + boot + milk + grain formation

Table 20. Grain yield in Punjnad-I wheat with irrigations applied at various growth stages.

Irrigations applied at		Grain yield (kg/ha)
1.	Crown root	1,260 ^{hi}
2.	Tillering	1,433 ^{ghi}
3.	Boot	1,836 ^{fgh}
4.	Milk	1,494 ^{ghi}
5.	Crown root + tillering	2,018 ^{defg}
6.	Crown root + boot	2,620 ^{cde}
7.	Crown root + milk	2,018 ^{efg}
8.	Tillering + boot	2,273 ^{def}
9.	Tillering + milk	2,200 ^{def}
10.	Boot + milk	2,676 ^{cde}
11.	Crown root + tillering + boot	2,776 ^{cd}
12.	Crown root + boot + milk	3,200 ^{c}
13.	Tillering + boot + milk	2,812 ^{cd}
14.	Crown root + tillering + boot + milk	3,987 ^{b}
15.	Crown root + tillering + boot + milk + grain formation	4,139 ^{a}

Cd^{1}=666.7 Cd^{2}=921.21

Three irrigations. Three irrigations were applied in three combinations. Irrigations applied at crown root + boot + milk stages (T12) gave a maximum yield of 3,200 kg/ha. These results are in line with those of Ibrahim (1999), Maliwal et al. (2000), Naser et al. (1999), and Lidder et al. (1999) who studied similar growth stages for irrigation and found that three irrigation applied at above-mentioned stages gave the best yield.

Four and five irrigations. Four and five irrigations were applied according to the tradition in the southern Punjab. Yields of 3,987 and 4,139 kg/ha were recorded for four and five irrigations, respectively. Grain yield declines of 55.6–69.6, 35.4–51.2, 22.7–33.0, and 3.7 % were observed using 1, 2, 3, or 4 irrigations, respectively, compared to five irrigations. The results are supported by the findings of Naser et al. (1999) and Lidder et al. (1999) who studied various numbers of irrigations at various growth stages and found that all irrigation treatments increased yield.

Conclusion. Depending on the amount of irrigation water available, the best growth stage for application of available irrigation water include:

1 irrigation	Boot
2 irrigations	Boot + milk
3 irrigations	Crown root + boot + milk
4 irrigations	Crown root + tillering + boot + milk
5 irrigations	Crown root + tillering + boot + milk + grain formation

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Wheat yield potential—current status and future research strategies in Pakistan.

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Wheat is the staple food for most of the people of Pakistan, and wheat straw is an integral part of the daily rations for livestock. The cultivation of wheat has spread throughout the four provinces of Pakistan. The wheat-growing area and production for the year 1999–2000 were 73 % and 78 %, respectively for the province of Punjab, with smaller amounts in the Sindh (13.5 % and 14.5 %), Northwest Frontier (9.5 % and 5 %), and Baluchistan (4 % and 2.5 %) provinces.

Yield potential. A substantial yield gap has been observed at the experimental stations, progressive growers, and on farmer's fields in each province. Six, high-yielding wheat cultivars were sown at three different locations in D.I. Khan, (Northwest Frontier Province), Pakistan, to explore their yield potential under prevailing climatic conditions. Daman 98 ranked first among all the tested cultivars by producing a grain yield of 12.5 t/ha (Khan et al. 2000). Choudhary and Mehmood (1998) obtained a maximum grain yield of 7 t/ha with Inqlab-91. Sadiq and Khan (1994) also reported 7 t/ha yield from Pak 81 in a study on the effects of intercropping and planting pattern on yield and yield components of wheat. Bajwa et al. (1993) reported the influence of different irrigation regimes on the yield and yield components of the wheat Pak 81, obtained maximum yield of 6.5 t/ha after the application of four irrigations.

Current status. Pakistan's average grain yield ranged between 2,053 to 2,490 kg/ha over the last 5 years, 1996–97 to 2000–01 (Table 21). A huge yield gap lies between the experimental yield and the average yield of the country, so there is great hope for double and even triple the wheat grain yield.

Population and wheat requirements. For 2001, the projected population for Pakistan is estimated to be 140.47×10^6 and wheat production is 19.02×10^6 tons. Domestic consumption requirements have been estimated at 20×10^6 tons. Pakistan became self-sufficient in wheat by producing 21.08×10^6 tons during the year 1999–2000, which was primarily due to 25 % increase in support price of wheat. Wheat growers produced about one million tons of surplus wheat grain, a marginal self sufficiency that can be changed at any time by natural hazards. Therefore, concerted efforts are needed to maintain increased production that meets future requirements.

Table 21. Area, production, and average yield of the wheat crop in the different provinces of Pakistan between 1996–97 and 2000–01. Units are for area (x 10^3 ha), production (x 10^3 tons), and yield (kg/ha). Source: 2002 Pakistan Statistical Year Book, Agricultural Statistics of Pakistan, Government of Pakistan, Islamabad, pp. 114. NWFP is the Northwest Frontier Province.

		Province				
Year		Pakistan	Punjab	Sindh	NWFP	Balochistan
1996–97	Area	8,109.1	5,839.9	1,106.8	842.8	319.6
	Production	16,650.5	12,371.0	2,443.9	1,064.4	771.2
	Yield	2,053.0	2,119.0	2,208.0	1,263.0	2,413.0
1997–98	Area	8,354.6	5,934.6	1,120.2	918.1	381.7
	Production	18,694.0	13,807.0	2,659.4	1,356.0	871.6
	Yield	2,238.0	2,326.0	2,374.0	1,477.0	2,283.0
1998–99	Area	8,229.9	5,934.6	1,123.7	857.6	314.0
	Production	17,857.6	13,212.0	2,675.1	1,221.8	748.7
	Yield	2,169.0	2,227.0	2,381.0	1,425.0	2,384.0
1999–00	Area	8,463.0	6,180.3	1,144.2	806.5	332.0
	Production	21,078.6	16,480.3	3,001.3	1,067.8	529.2
	Yield	2,490.0	2,667.0	2,623.0	1,324.0	1,594.0
2000–01	Area	8,180.8	6,255.5	810.7	790.3	324.3
	Production	19,023.7	15,419.0	2,226.5	164.0	614.2
	Yield	2,325.0	2,465.0	2,476.0	967.0	1,893.0

Yield gap. A substantial yield gap has been observed between yield at the experimental stations and in farmers' fields in each province. This gap is primarily because of the lack of finances on the part of the majority of farmers for implementing modern technology for wheat production. Thus, we hope for improving wheat production and yield in the country.

Constraints to production. Like many developing countries, wheat production is confronted with both biophysical constraints (disease, fertilizer, water, seed, cultivars, cultural practices, and salinity/sodicity) and socioeconomic constraints (credit, knowledge, experience, tradition, and institutions.).

Disease. Although several diseases attack wheat, stripe and leaf rusts, loose and flag smuts, Karnal bunt, powdery mildew, Helminthosporium leaf spots, and foot and root rots are the most important in Pakistan. Other diseases, such as those caused by *Septoria* spp., downy mildew, black point, and black chaff, are of minor importance. Breeding programs try to develop wheat cultivars that are resistant or tolerant to these principal diseases. Measures to minimize their adverse effects on production also are being investigated.

Insect pests. Fortunately, wheat in not attacked by any serious pests, however, infestations of army worm, Hilothus, and green aphids have occurred in localized areas.

Drought. About 21 % of total wheat area in Pakistan is rainfed. The screening of plant material and the testing of new cultivars for drought tolerance are made in rainfed areas or under simulated moisture stress. Some cultivars (Inqlab 91, Punjnad1, and Iqbal 2000) that were developed for irrigated areas also have proven to be very successful under rainfed conditions. The testing of new cultivars under both irrigated and rainfed conditions is encouraged.

Salinity/sodicity. At present, 2.4 x 10^6 ha of land in Pakistan have been rendered saline-sodic. With the continuous use of low-quality water, this menace increases every year. Wheat yield was found to be reduced by 19 % under moderately saline-sodic soils.

Lack of nutrients. Experiments on yield constraints in irrigated and rainfed areas indicate that the proper application of fertilizer is of utmost importance. Yield reductions ranging from 50 to 75 % have been observed without proper fertilizer use and clearly demonstrate that wheat yields can be substantially increased if fertilizer use is properly regulated in the country.

Planting date. More than 50 % of the wheat in Pakistan is planted late, i.e., during the month of December. Planting date experiments have shown that yield is progressively reduced with delayed planting. Yield was found to be reduced by 28.8 and 75.8 % when sowing was delayed from November to December and from November to January, respectively,

Weeds. With the introduction of high-yielding and fertilizer-responsive Mexican wheat cultivars during mid 1960s, weed populations have increased tremendously causing considerable losses in crop yield. No data regarding yield losses due to weeds is available, however, depending upon the degree of infestation, losses yield are estimated to be between 13–42 %. A number of weed species infest the wheat fields; both grasses and broadleaf weeds. Wild oat (*Avena fatua*), canary grass (*Phalaris minor*), *Chenopodium* spp., and *Convolvulis arvensis* has been found to be the major weeds. When weeds were controlled by the herbicides Dicuran M.A., Tribunil, Graminon, and Arelon, yield increases of 41, 22, 22, and 25 %, respectively, over the weedy controls were found (Ahmed et al. 1987).

Future research strategies. Future strategies for the improvement of wheat will involve more emphasis on breeding cultivars that possess wider adaptation and can withstand various types of stress (disease, high temperature, cold and frost, drought, salinity/sodicity, and water logging). Efforts also will be made to develop wheat cultivars with low input requirements. Improving grain characteristics and milling and baking quality of wheat also will receive greater attention.

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ITEMS FROM ROMANIA

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Yield stability and breeding for adaptation in winter wheat.

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Winter wheat provides a substantially larger amount of the world's wheat production than does spring wheat because winter wheat is more productive in those areas where both types can be grown. Thus, winter wheat usually is preferred over spring wheat in the regions where the climate permits production. The limits of winter wheat adaptation are established primarily by winter temperature. Thus, the winter survival temperature determines the northern limit and the winter temperature that is sufficiently low to permit vernalization gives the southern limit of the cropping area. From this point of view, winter wheat cultivars must have a high enough winter hardiness in northern regions and low requirements for vernalization in southern regions to be acceptable to producers.

Improved cultivars substantially contribute to increase wheat production. However, wheat yields in most production regions seem to be no more than one-half of the potential yield of the cultivars and far below the theoretical

maximum yields. This difference reflects powerful production constraints that prevent the true genetic potential for yield to be expressed by the grown cultivars.

Although wheat-breeding programs have some priorities in common, the major objective of increasing the genetic potential of yield for most, if not for all, can be achieved via breeding for higher yield potential or by diminishing or eliminating hazards that reduce yield. Actually, wheat breeding seeks to remove yield constraints by developing cultivars with resistance to disease, insects, lodging, cold, heat, and drought. Other yield constraints can be best dealt with through improved cultural practices and management. Obviously, some yield constraints are fixed by the environment and cannot be manipulated.

As a breeding objective, yield represents an extreme example of a quantitative trait being polygenically inherited and subject to environmental influence to a large extent. Studies have shown that the environmental variation associated with yield often exceeds genotypic variation, which leads to confounding the genotype mean performance with its true value.

Among breeding priorities, stability of performance may be as important as high yield potential. Therefore, 'genotype x environment' interactions are of major importance, because they provide information about the effect of different environments on cultivar performance and have a key role for assessment of performance stability of the breeding materials.

Developing a wheat cultivar generally results from the selection of valuable recombinants found in manageable hybrid populations. During the breeding process, they will be grown in a limited set of environments. Evaluation of breeding material in a wide range of environments seldom is possible, not to mention the multiple environments encountered by new cultivars released for commercial production. Testing over as wide a range of environments generally is essential if widely adapted cultivars are to be identified.

Environments are seldom, if ever, duplicated. Variation in an environment at a single location over years can be as great as those between locations in one year. Therefore, variations in the 'genotype x environment' interaction that are pertinent to wheat breeding problems are those associated with 'cultivar x year', 'cultivar x location', and 'cultivar x year x location'.

We have discussed stability and adaptation of winter wheat. Yield stability has been defined as the ability of a cultivar to produce an expected yield at the level of productivity of a certain environment (i.e., the cultivar that has no 'genotype x environment' interactions). In practice, the wide variation in yield stability are related to the range in adaptation and response to production inputs. Therefore, wheat cultivars must have sufficient potential to maintain competitive yields in various environments and react favorably to conditions or increased production inputs.

That practical wheat breeding can make increases in genetic yield potential without substantial loss in yield stability and adaptation is of question. Some believe that yield potential and yield stability are more or less independent. Others say that yield stability is inversely proportional to the sum of squares for the 'genotype x environment' interaction attributable to that cultivar. The fact that one cultivar has significantly superior mean yields than another over a wide range of environments denotes genetic differences in the behavior of different genotypes. However, high mean yield alone is not necessarily indicative of high stability and wide adaptation.

Finlay and Wilkinson (1963) pointed out that the desired genotype is the one that produces a high mean yield over a range of environments and has average yield stability in comparison with other genotypes in the same conditions. They suggested that each nursery mean yield can be considered as a measure of an environment and, thus, an array of low- to high-yielding environments becomes available from a given set of ecological trials. The response of a particular cultivar to this range of environments can be estimated by the regression of yield of each cultivar on the mean yield of the nursery. The regression coefficient (b) is considered as a parameter of yield stability. So, $b = 1$ denotes cultivars with average stability; $b > 1$ are less stable cultivars, and $b < 1$ denotes very stable cultivars.

Eberhart and Russel (1966) developed this concept of stability and suggested the use of two stability parameters when describing the performance of one cultivar over a range of environments. They proposed that the regression of each cultivar on an environmental index and a function of the squared deviations from regression would provide more useful estimates of yield stability parameters. The environmental index is a coded deviation of each environment from the grand mean over a given range of environments. Environmental index is obtained for each environment by subtract-

ing the grand mean of all cultivars over all environments from the mean of all cultivars in each environment. This forces the regression of the mean of all cultivars on the environmental index to have unit slope (b = 1). Therefore, a stable cultivar can be defined as one that have above average performance in all environments, a unit regression coefficient (b = 1), and a deviation from regression as small as possible ($Sd^2 = 0$).

Evaluating yield stability and adaptation of the ARDS Turda winter wheat cultivars.

We evaluated the data from 22 ecological yield trials to examine the contributions of the ARDS Turda winter wheats to increases in yield and stability of performance in wheat production. The trials are from seven locations and three years (1998–2000) plus one location with one year (1998); a total of 22 trials. These locations are representative of the diverse environmental conditions in Romania. Experimental cultivars in each trial did not exceed 25, including the long-term check Bezostaia 1, used for comparison to newer cultivars and nursery performance over the years. The trials usually were evaluated in a RCB with six replications. Previous crop, seeding date, and fertilization were different at each location and conformed to local practices. Because part of cultivars in the nursery are changed annually and may influence stability parameters, we chose only 11 cultivars that remained in all trials over the experimental years. The 22 trials included 10 Romanian winter wheats plus Bezostaia 1, the check considered to have had a fairly stable yield and satisfactory adaptation. Five of the 10 Romanian cultivars analyzed were released by the ARDS Turda wheat-breeding program.

'Cultivar x year', 'cultivar x location', and 'cultivar x year x location' interactions were significant, indicating that the yield performance of the cultivars varied with the environments.

Stability parameters, computed according to the Eberhart and Russel model, were used to describe the performance of cultivars over environments. According to the model, the environmental index as an independent variable (x) was obtained for each of 22 environments as the mean of those 11 cultivars minus the grand mean (mean of the 11 cultivars in all 22 environments). The mean yield of each cultivar in each environment (y) was than regressed upon the environmental index. The statistical mean yield, regression coefficient (b), and coefficient of determination (r^2) are currently used to evaluate the stability of yield over environments. We prefer coefficient of determination instead of deviation from regression because it directly gives predictability of a cultivar in relation to the environmental index. Although the deviation from regression must be as small as possible (approaching 0), the desired coefficient of determination is one that approaching 1 when considerable confidence can be attributed on one environment's measurement of a cultivar's performance and adaptation.

Stability parameters for the yield of the ARDS Turda winter wheats in the 22 ecological trials and the check Bezostaia 1 are presented in Table 1. According to the statistical model, the mean yields correspond to an environmental index value of 0. Directly evaluating the percentage gain in yield attributed to cultivar improvement is relative to Bezostaia 1. In our case, this value was between 8 % for Transilvania and 17 % for Ariesan. At the same time, in comparison with Bezostaia 1, our cultivars have had regression coefficient of 1 or slightly higher, except for Turda 95, which has a lower value. In addition, coefficient of determination values equal to or higher than Bezostaia 1 show that the cultivar response to environments is predictable to a considerable degree. Turda 95, which has a lower slope of regression (b = 0.87), seems to be well adapted to suboptimal environmental conditions.

Table 1. Stability parameters for yield of five ARDS Turda winter wheat cultivars compared with long-term check Bezostaia 1 and grown in 22 yield trials in northcentral Romania (1998–2000). Percent mean yield is expressed as a percent of Besostaia 1.

	Mean yield		Regression coefficient	Coefficient of determination
Cultivar	q/ha	%	(b)	(r^2)
Transilvania	54.4	108	1.00	0.91
Ariesan	58.7	117	1.07	0.93
Apullum	57.9	115	1.11	0.90
Turda 95	58.2	116	0.87	0.86
Turda 2000	58.2	116	1.07	0.86
Bezostaia 1	50.3	100	0.96	0.86

A higher regression coefficient is desirable for high-yielding cultivars because they must be responsive to favorable conditions or increased cultural input. Above average performance in all types of environments must be

maintained. The regression of the yield of Transilvania (released in 1982) and Turda 2000 (released in 2000) on environmental indexes compared with the Bezostaia 1 check are shown in Fig. 1. Differences in the mean yield of Transilvania and Turda 2000 relative to Bezostaia 1 demonstrate a continuous yield advance achieved by our wheat-breeding program during the last 30 years. The regression lines of the two cultivars are nearly parallel with that of Bezostaia 1, indicating that their superiority is maintained across a wide range of environments. The slope of Bezostaia 1 is b = 0.96, whereas the slope of Transilvania is b = 1 and Turda 2000 is b = 1.07. These cultivars tend to be slightly more favorable to environments. For these two cultivars, breeding progress to improve yield potential was accompanied with improved stability of performance.

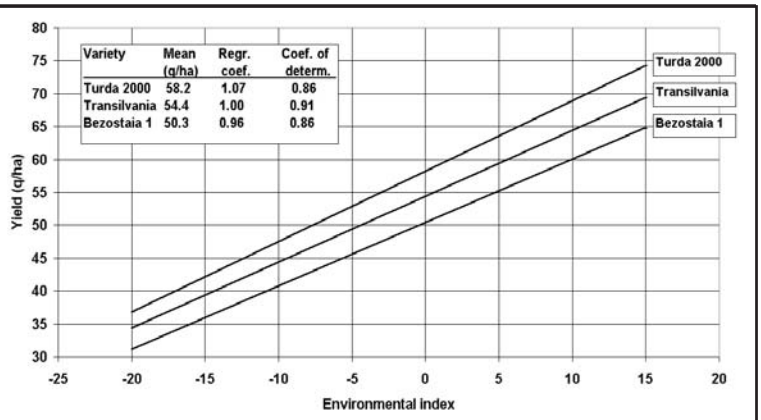

Fig. 1. Regression of the yield of Transilvania and Turda 2000 versus the Besostaia 1 check on environmental indicies in 22 ecological trials.

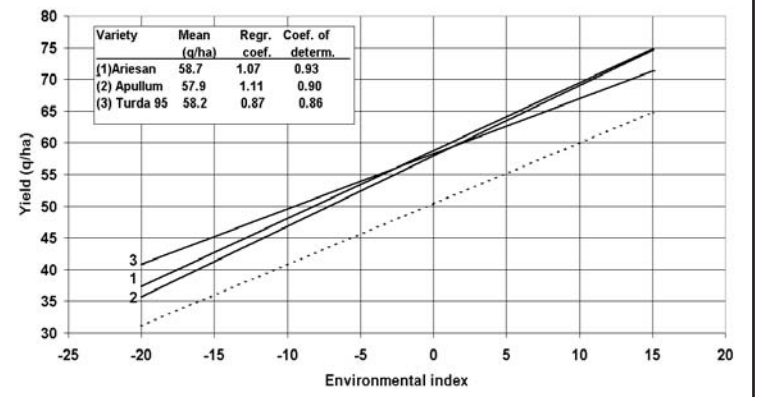

Fig. 2. Regression of the yields of Ariesan, Apullum, and Turda 95 versus the Besostaia 1 (dashed line) check on environmental indicies in 22 ecological trials.

The three other cultivars from our program, Ariesan, Apullum, and Turda 95, had different regressions of yield on environmental indices in the same set of trials; graphically illustrated in Fig. 2. Ariesan, with the largest mean yield and a reasonable regression coefficient (b = 1.07), has the highest coefficient of determination (0.93) denoting a strong, predictable response to changes in environmental conditions. The combination of increased yield potential with good stability of performance may explain the wide acceptance and popularity of Turda-developed wheats like Ariesan. Turda 95, with a larger mean yield (approaching Ariesan) but low regression coefficient (b = 0.87), sharply contrasts with the smaller mean yield and high regression coefficient of Apullum. The coefficients of determination were nearly similar for the two cultivars. The larger mean yield of Turda 95 clearly is associated with its higher yield in the poorer environments, whereas Apullum with a larger regression coefficient seems to be well adapted in favorable environments. The stability parameters in this study do permit comparisons among cultivars for average yields, stability of performance as a degree of response to changing environments, and the predictability of response to specified environments. Such comparisons would be useful for judging the release of cultivars and making recommendations for suitable production conditions and areas of adaptation for different cultivars.

Conclusions and remarks. The final objective of a winter wheat-breeding program is the release of cultivars combining high yield potential and quality with stability of performance and adaptation. Breeding for resistance to diseases and different others biotic and climatic stress promote such stability. The high level of winter hardiness of wheat cultivars is a major requirement for many winter wheat regions. Many genes condition winter hardiness, but the adaptability and stability represent more complex breeding characters that are controlled genetically and encompass a large number of known and unknown morphological, physiological, and biochemical attributes. Therefore, breeding for adaptation must begin with choosing parents for the crosses. They must be well-adapted genotypes that will give valuable hybrid combinations for the desired cultivar. During the generations of selection, the breeding material needs to be grown in the different biotic and climatic conditions with which they will interact to allow the breeder to make sound judgements of chosen material. In addition, testing breeding material in different simulated conditions such as with pathogen inoculations, aluminum toxicity solutions, and sprouting in a mist cabinet, can help achieve the elements of cultivar adaptation. New techniques of selection or manipulation of genetic material also can aid in developing high-yielding and stable cultivars. Previously, we suggested that the pedigree selection method, with only a few reselections, may conserve some heterogeneity in cultivars and buffer against environmental changes resulting in a good stability of performance (Ann

Wheat Newslet **48**:113-115). However, we do not exclude the possibility that homozygous genotypes, like pure lines obtained by double-haploid techniques or other methods, may buffer as any other type of population if selection for increased stability is applied.

Breeders agree that testing over a wide range of environments is essential if stable and widely adapted cultivars are to be identified. However, the extensive trial data required for identification stable cultivars becomes available only in advanced generations, when a cultivar is close to or may be already released. Therefore, the methods for evaluating yield stability proposed in this study have had a little significant impact in the early generations of selection regarding breeding wheat for adaptation. Improved evaluation techniques, applied in early generations, should assist in the early identification of those lines having high yield potential associated with good adaptation in highly variable environments or in alerting breeders to possible deficiencies in adaptation for other lines.

Based on our results presented here, trends in cultivar response to environments in regional performance nurseries indicate that breeders must carefully consider the trade-off between maximum yield potential, stability of performance, and ranges in adaptation during cultivar evaluation. However, convincing breeders to sacrifice high yield for increased stability and wide adaptation is difficult. Nevertheless, assessing the 'genotype x environment' interaction as a factor in determining the yield potential in the different production conditions will remain the most important tool in the breeding wheat for yield.

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Bread-making quality research.

Mss. Rozalia Kadar completed her Ph.D. dissertation in December 2002 under the direction of Prof. Dr. Leon S. Muntean at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The title of Rozalia's thesis was 'Study of the genotype-environment interaction in achieving of bread-making quality in winter wheat'. Her thesis research underscores the fact that most wheat quality characteristics are heritable traits and more or less influenced by environmental conditions and production inputs. The implications of 'genotype x environment' interactions in development of winter wheat cultivars with improved bread-making quality are discussed.

ITEMS FROM THE RUSSIAN FEDERATION

AGRICULTURAL RESEARCH INSTITUTE OF THE CENTRAL REGION OF NON-CHENOZEM ZONE
143026, Nemchinovka-1, Moscow region, Russian Federation.

Genetic linkage between endosperm color and caryopsis size in soft wheat hybrids.

V.G. Kyzlasov.

A method for creating a xenia caryopsis color and its inheritance in soft wheat hybrids has been described previously (Kyzlasov 1998, 2000, 2001). Plants with caryopses of various colors were detected among the progeny with instaminate flowers. These plants arose through the pollination of a spring soft wheat with pollen from spring barley. Instead of stamens, this genotype had formed pistils. The segregation of caryopsis color in the F_1 hybrid plants was 7 light-colored : 9 pigmented (1.284 light : 1.662 dark). The pigment was produced in the caryopses as the result of

complementary interaction of two hypostatic genes determining xenias. In the reciprocal cross, the hybrids did not differ in their segregation pattern (596 light-colored : 819 pigmented ~ 688 light-colored : 843 pigmented).

In the second and subsequent generations of the hybrid populations, the unpigmented caryopses produced light-colored grain progeny only. The progeny of plants with pigmented caryopses segregated 7 light : 9 dark, 1 light : 3 dark or all plants produced pigmented caryopses. Both endosperm and pericarp were colored in the pigmented caryopses. The genes for caryopsis xenia color have an effect on the color of the forming endosperm immediately after fertilization. This phenomenon is known in maize, pea, barley, and rye.

Our investigation revealed that grain xenias can be manifested in other features, e.g., caryopsis size. The dark caryopses obtained from crosses between a light-grained line (female) and a dark-grained line (male) were significantly bigger than those from hybrids of dark-grained line (female) and a light-grained line (male). The ratio the 1,000-kernel weight produced by the dark-grained hybrid to that produced by the light-grained line was (38.8 : 34.8) x 100 = 111.4 % (Table 1).

Table 1. 1,000-kernel weight (g) of different light- and dark-grained wheat lines and their F_1 hybrids. Minimum significant difference (P = 0.05) = 2.5 g

Parental lines	
Light-grained	38.3
Dark-grained	39.2
F_1 hybrids	
Light-grained / light-grained	34.8
Light-grained / dark-grained	38.8

The use of grain xenia-color genes makes it possible to mark and select caryopses within a separate spike that are carriers of the genes determining large grains. Selecting caryopses of different colors within separate spikes of F_1 hybrid plants indicates that dark-colored caryopses are significantly larger than light-colored caryopses (see Table 2).

Table 2. Range in weight of caryopsis after selection according to endosperm color. Values for 1,000-kernel weight are in grams.

Endosperm color	1,000-kernel weight							Average 1,000-kernel weight
	20	25	30	35	40	45	50	
Light	2	13	33	30	19	3	—	33.0 ± 0.6
Dark	—	2	16	36	31	14	1	37.1 ± 0.6

The ratio of the weight of dark to light caryopses was (37.1 : 33.0) x 100 = 112.4 % on average. Linkage between grain color and size was established in other experiments. For example, sweet corn (*Zea mays saccharata*) and garden pea (*Pisum sativum*) also demonstrate linked inheritance of grain size and variety features.

Dark- and light-colored grains taken from the same spike did not differ in their levels of raw protein, K_2O, P_2O_5, and gliadin proteins. Glutenins cause dark-grained wheat. The dark-grained wheat obtained in our experiments is recommended for use when studying the inheritance of grain size in hybrids. The wide distribution of grains of different colors within the same spike indicates that the difference in the size is exclusively a function of genetic factors. The environmental influences are identical for all the grains of a spike. By backcrossing coarse-grain lines can be created that will be analogous to the commercial cultivars with the dark-colored endosperm and pericarp.

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The efficacy of the Ut gene from Saratovskaya 57 (Triticum durum subsp. durum) against loose smut in bread wheat.

A.E. Druzhin.

During two seasons (2001–02), a number of spring bread wheat lines containing chromatin from *T. durum* subsp. *durum* were studied for resistance to loose smut after artificial infection. We selected lines with a high level of resistance to the pathogen. We detected resistance to loose smut in lines with the spring durum wheat cultivar Saratovskaya 57 in their pedigree (see Table 1).

Table 1. Reaction of cultivars and lines of spring bread wheat to race 23 of loose smut during 2001 and 2002.

	% of plants sporulating	
Cultivar/line	2001	2002
L503	58.3	59.5
L504	64.2	59.3
L222	67.5	59.4
Saratovskaya 58 (S58)	70.3	65.2
L504/S57*2//L504/3/S58	5.8	3.1
L503/S57//L503	5.3	4.7
L503/S57//L503/3/L222	0.0	0.0
Saratovskaya 57 (S57)	0.0	0.0

Evaluating resistance to loose smut, bunt, and ergot in spring bread wheat lines containing alien translocations.

A.V. Borozdina.

The Saratov-bred spring bread wheat cultivars and lines containing alien translocations were evaluated under natural infection by pathogen populations of loose smut, bunt, and ergot. Spring bread wheat lines derived from crossings with *S. cereale*; *T. turgidum* subspp. *durum, persicum,* and *dicoccum*; *Ag. elongatum*; *Ag intermedium*; and lines with translocations from these species were most susceptible to ergot. The degree of a susceptibility in the lines containing genes from *T. turgidum* subsp. *durum* + *Ag. elongatum* + *S. cereale* was higher than lines in lacking rye in their pedigree.

Resistance to loose smut was found in lines with translocations *T. turgidum* subsp. *durum* + *T. turgidum* subsp. *dicoccum* + *Ag. elongatum* (L 836-00), *T. turgidum* subsp. *durum* + *Ag. elongatum* (L 2040 and L 164), *T. tudgidum* subsp. *durum* + *Ag. elongatum* + *Ag. intermedium* (L 810-94), *T. turgidum* subsp. *durum* + *T. turgidum* subsp. *persicum* (L 589-94), and *T. turgidum* subsp. *durum* +*Ag. elongatum* + *S. cereale* (L 894-94 and L 255-93).

The study of these lines for resistance to bunt shows that the greatest number of susceptible genotypes in the groups with translocations from *Ag. elongatum, T. turgidum* subsp. *durum*, and their combinations (*T. turgidum* subsp. *durum* + *Ag. elongatum*) and (*T. turgidum* subsp. *durum* + *Ag. elongatum* + *Ag. intermedium*). Lines combining resistance to all three diseases were very rare and observed in lines containing *T. turgidum* subsp. *durum* + *T. turgidum* subsp. *persicum* line L 589-94.

Genetic control for resistance to leaf rust in spring bread wheat lines derived from crosses with tetraploid AB-genome species.

S.N. Sibikeev, S.A. Voronina, and V.A. Krupnov.

In the Department of Genetics at ARISER, spring bread wheat lines resistant to leaf rust were obtained from crosses with *T. turgidum* subspp. *durum*, *dicoccum*, and *dicoccoides*. These lines were produced by the backcross method. In these lines, the following ITs to leaf rust were observed: L164 (pedigree: L504/S57//L504, S57 is a spring durum wheat) – IT = 2–2; L196 (pedigree: S58/*T. turgidum* subsp. *dicoccum**3//S58) – IT = 0;–1; L2870 (pedigree: S55/*T. turgidum* subsp. *dicoccoides**3//S55) – IT = 0;. Genetic analyses indicated that the resistance in L164 was determined by two recessive genes, in L196 by two dominant genes, and in L2870 by one dominant gene. Allelism tests detected that these *Lr* genes are different from *Lr14a* and *Lr23* and from each other.

The evaluation of spring bread wheat cultivars for resistance to stripe rust in 2002.

S.N. Sibikeev and A.E. Druzhin.

Stripe rust of bread wheat in the Saratov district of the Volga Region of Russia occurs seldom and the severity of the epidemics is usually weak. Nevertheless, in the southwestern part of the Saratov district, epidemics of this disease were observed during the last 2 years. A major part of this area is sown with spring bread wheat cultivars L503, L505, Belyanka, and Dobrynya. L503, L505, and Dobrynya had an IT of 0 and that of Belyanka was a 3. L503, L505, and Dobrynaya have resistance gene *Lr19*, and Belyanka has *Lr23+Lr14a*. Resistance to stripe rust in L503, L505, and Dobrynya was surprising, because there is no data regarding the resistance of *Agropyron* translocation with *Lr19* to *P. striiformis tritici*.

The reaction of the bread wheat cultivars and lines to loose smut and bunt.

A.Yu. Buyenkov, A.E. Druzhin, V.A. Krupnov, Yu.V. Lobachev, and M.R. Abdryaev.

We compared the resistance of bread wheat cultivars and lines to loose smut and bunt in an artificial infection. Fourteen cultivars and lines bred at ARISER were infected with spores of loose smut and bunt. The initial inoculum of bunt was collected from the susceptible line L894. Two pathotypes of loose smut were collected from cultivars L505 and Saratovskaya 60, which are susceptible to the named races L 505 and S 60, respectively.

The reactions of bread wheats and lines to bunt and loose smut after artificial inoculation are given in Table 2. Lutescens 62 is resistant to bunt but is moderately susceptible to race L 505 of loose smut and highly susceptible to S 60. L235-01, the line most susceptible to bunt, was resistant to race L 505 and moderately susceptible to S 60. L 2040 was resistant to both races of loose smut but moderately susceptible to bunt (36 %). The majority of bread wheat lines were more susceptible to bunt than resistant to loose smut (Albidum 188, L502-01, L105, and L 108). In most cases, the cultivars and lines were more susceptible when inoculated with race S 60 of loose smut. Approximately similar degrees of susceptibility to bunt and race S 60 of loose smut were observed in L780 and between bunt and races L 505 in L400, L154, and L199. A correlation was detected between the percent susceptible to loose smut and bunt.

Table 2. Percent infection of bread wheat cultivars and lines to bunt and loose smut pathotypes under conditions of artificial infection in 2002.

	% of plants sporulating		
		Loose smut	
Cultivar/line	Bunt	L 505	S 60
Lutescens 62	8.0	47.0	71.0
L503	19.0	29.0	47.0
Yuogo-vostotchnaya 2	22.0	34.0	48.0
Albidum 188	23.0	45.0	48.0
L400	25.0	29.0	59.0
L181 rq	30.0	47.0	51.0
L502-01	33.0	49.0	77.0
L154 Rq	35.0	35.0	55.0
L2040	36.0	4.0	5.0
L780-01	44.0	11.0	47.0
L199-01	46.0	50.0	38.0
L105-01	61.0	19.0	13.0
L108-01	62.0	22.0	36.0
L235-01	68.0	9.0	36.0

The distribution of Puccinia triticina pustules on the flag leaves of Saratov soft spring wheat cultivars.

O.V. Subkova.

In practice, we are selecting soft spring wheat cultivars for general resistance to leaf rust. Quantative estimations, both in the field and greenhouse, include four signs that in total are considered as the final expression of a given interaction. Most methods for estimating pustule distribution contain a number of errors. A wider selection of characteristics is

necessary to increase the accuracy. Finding a new phenotypes (up to two) to help determine the various estimations is desirable.

We visually observed the pustule arrangement on the flag leaves of 12 Saratov soft spring wheat cultivars of the Ugo-Vostok Scientific Research Institute in the 2000–01 season by sketching. The differences between cultivars were assessed from pictures of pustule distribution along the leaf blade. Our hypothesis about pustule distribution includes several points:

in general, in each of the 12 Saratov cultivars, we could determine a typical arrangement from the background pustule arrangement of the flag leaves and defined this as the abstract picture of pustule arrangement (aPDP);

each aPDP of a cultivar consists of a definite design type (from 1 up to 9), one of which can be seen only in single cultivar; others in several; and

a formula describing the pustule arrangement (FDDP) for the resistant and susceptible Saratov wheat cultivars FDDP includes three traits; the number of dominant designs at the bottom, in the middle, and at the tip of the flag leaf.

These data are summarized and expressed by the symbol G, with the index n (1–10). These differences in aPDP are not accidental and are connected with the peculiarities of genotype, plant habit, and the external influences during epidemic pathogen development.

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Determining the genotypes of resistant wheat lines using test pathotypes of the Puccinia triticina.

I.F. Lapochkina and E.D. Kovalenko, and A.I. Zhemchuzhina, T.M. Kolomietz, and D.A. Solomatin (Institute of Phytopathology).

Breeding for resistance to the rust fungus is a great problem for many wheat-cultivating countries such as the Russian Federation, the U.S.A., Canada, Argentina, Brazil, and Australia. One way to solve this problem is to screen for resistance in the world's wheat germ plasm. After testing common, spring-wheat cultivars grown in the territory of the former Soviet Union, only 8–9 leaf rust-resistance genes were identified (Singh et al. 1995). The genetic diversity of winter wheat cultivars is not large; 95 % of the cultivars included in the State Register of Russian Federation in 1998 were the progeny of Bezostaya 1 and Mironovskaya 808 (Martynov and Dobrotvorskaya 2001).

Enriching wheat germ plasm with genes of wild species and establishing new combinations of resistance genes will increase significantly the efficacy of breeding wheat for immunity. The cytogenetic stock collection created at the Agriculture Research Institute of Non-Chernozem Zone contains common wheat genotypes with chromosomes added from *Ae. speltoides* (over 60 genotypes that are grouped into 16 clusters according to disease resistance and morphological traits) (Lapochkina and Volkova 1994; Lapochkina et al. 1998, 2001). The collection also includes hexaploid genotypes with alien material from *Ae. speltoides*, *Ae. triuncialis*, *T. kiharae*, and *S. cereale*. Several stable addition lines of spring wheat obtained by means of wide hybridization with the spring wheat Rodina and the *ph1b* mutant with *Ae. speltoides* and *Ae. triuncialis* species were used in this research and are described in Table 1.

Lines k-62903 and k-62904 are of the *lutescens* type. These lines have a long stem (90–100 cm), a lax multiflowered ear, and anthocyanin-colored anthers. Line k-62905 belongs is a *milturun* type. This line has a short stem, lacks wax on the spike, and has short awn-like sprouts on the ear apex. Line 149/00i is characterized by late ripening, anthocyanin-colored anthers and straw, and waxless spikes. Line 102/00i is *T. aestivum* subsp. *spelta* with a dense spike. This line has anthocyanin-colored anthers and lacks wax. Line 82/00i has a short stem, lax multiflowered spikes with big glumes, and elongated teeth on the lemma. Line 76/00i is characterized by the presence of the morpho-

logical features of wild species; thin, anthocyanin-colored straw and low spike density. Two telomeric SPELT 1 repeats are visualized in the karyotype of this line after FISH (Salina et al. 2000). Line 87/00i has thin straw and is susceptible to powdery mildew. Line 72/00i is characterized by a light-red spike color, low spike density, and narrow, lancet-shaped spike scales. This line is resistant to powdery mildew (10 % infection). Lemmas that adhere to the kernel on the side groove and the existence of a long (over 9 cm) spike are typical of line 99/00i. The line also is susceptible to powdery mildew (40 % infection). Line 85/01i has a low spike density and anthocyanin-colored anthers and straw. Line 97/01i has thin straw and the lemma adheres to the kernel. Line 132/01i is an awned form of *T. aestivum*.

Table 1. Field reaction to powdery mildew and leaf rust and identification of *Lr* genes in wheat–*Aegilops* lines.

Line	Origin	% infection in field		Assumed resistance
		mildew	leaf rust	
k-62903	Rodina/*Ae. speltoides*	0	0	juvenile gene(s)
k-62904	Rodina/*Ae. speltoides*	0	0	juvenile gene(s)
k-62905	Rodina/*Ae. speltoides*	10	20/2	*Lr1 + Lr10*
149/00i	*ph1b*/*Ae. speltoides*	0	0	*Lr10 + Lr26*
102/00i	Rodina/*Ae. speltoides* (10 kR)	40	0	*Lr27 + Lr31 +*
82/00i	Rodina/*Ae. speltoides* (10 kR)	0	0	*Lr10 + Lr26 +*
76/00i	Rodina/*Ae.speltoides* (10 kR)	0	0	adult-plant gene(s)
87/00i	Rodina/*Ae.speltoides* (10 kR)	30	0	juvenile gene(s)
72/00i	Rodina/*Ae. speltoides* (10 kR)	0	0	juvenile gene(s)
99/00i	Rodina/*Ae. speltoides* (10 kR)	5	0	juvenile gene(s)
85/01i	Rodina/*Ae. speltoides* (10 kR)	0	0	juvenile gene(s)
97/01i	Rodina/*Ae. speltoides* (10 kR)	0	0	juvenile gene(s)
132/01i	Rodina/*Ae. triuncialis* (5 kR)	20	0	*Lr28 +*

During the last 5 years, all lines showed a high level of resistance to leaf rust inoculation (genotype of the population 1, 2a, 2b, 2c, 3bg, 3k, 10, 11, 14a, 14b, 16, 17, 18, 20, 21, 23, 25, 26, 27+31, 30) in the field. Fifteen isolates collected from the natural uredopopulations of the pathogen in the Central, Low-Volga, Middle-Volga, North-Caucasian, and West-Siberian regions of the Russian Federation were used as test cultures. Pathotypes of *P. triticina* carried from 12 to 18 virulence genes (Table 2). Disease symptoms were estimated according to the 5-point scale of Mains and Jackson (1926). Infection types 0, 0; 1, 2, and X- mean that a sample possesses resistance genes whereas types 3, 4, and %+ indicate their absence.

Table 2. The genotypes of the *Puccinia triticina* pathotypes used in to identify leaf-rust resistance genes in 13 lines with wheat–alien transfers.

191-7	1,3a,3bg,10,11,14a,14b,15,17,18,21,27+31
238-15	1,2a,2b,2c,3a,3bg,3k,10,11,15,17,18,19,20,21,32,36
242-14	1,2c,3a,3bg,3k,10,11,14a,14b,16,17,18,20,21,25,27+31
245-21	2c, 3a,3bg,3k,10,11,14b,16,17,18,21,27+31
249-6	2b,2c,3a,3bg,3k,10,11,14a,14b,16,17,18,21,26,27+31,32,36
261-7	1,2a,2b,2c,3a,3bg,10,11,14a,14b,15,17,18,20,26
277-23	1,2a,2b,2c,3a,3bg,3k,11,14b,17,18,20,21,25,26,27+31
277-14	1,2a, 2b,2c,3a,3bg,10,11,14a,15,17,18,21,26,27+31
262-6	1,3a,3bg,3k,10,11,14a,14b,16,17,18,20,21,23.25
98-3	1,2a,2b,2c,3a,3bg,3k,11,14a,14b,15,17,18,20,21,26.
270-7	3a,3bg,3k,10,11,14b,15,16,17,18,19,20,21,25,27+31
257-3	1,2a,2b,2c,3a,3bg,3k,11,14a,14b,16,17,18,20,21,26,27+31
277-26	1,2a,2b,2c,3a,3bg,11,14a,14b,17,20,21,26.
258-13	1,2a,2b,2c,3a,3bg,3k,10,11,14a,14b,15,17,18,20,21,27+31,28
269-7	1,2c,3a,3bg,3k,10,11,14b,17,18,20,21.

As a rule, the investigated lines were resistant to the pathogen penetration with ITs of 0, 0; or 1, and 2. A susceptible reaction to some pathotypes suggested that lines k-62905, 149/00i, 82/00i, and 102/00i had a combination of *Lr1+Lr10*, *Lr10+Lr26*, *Lr10+Lr26*, and *Lr27+Lr31* genes, respectively. In addition, lines 82/00i and 102/00i each had one additional, unidentified resistance gene.

Lines k-62903 and k-62904 presumably have new resistance genes from *Ae. speltoides*. The alien translocations and substitutions (T2BL·2SL for k-62903, T1BL·1SS and T5AL·5SL for k-62904, and 7A/7S substitution in k-62905) were identified previously by differential C-banding of chromosomes in k-62903, k-62904 and k-62905

(Lapochkina et al. 1996; Pukhalsky et al. 1999). The presence of resistance genes in these lines probably is related to these translocations.

When lines 87/00i, 99/00i, 85/01i, and 97/01i were inoculated with leaf rust test pathotypes, they showed only the resistant-type reaction (0, 0;); suggesting that the resistance genes from *Ae. speltoides* function in both seedlings and adult plants. Line 76/00i was susceptible to infection by 11 pathotypes and resistant to four. The presence of APR genes in this line possibly are related to the presence of *Ae. speltoides* chromosome 4S in the karyotype. For line 72/00i, the heterogenic type of reaction (x-) was found in the case of three pathotypes, the 12 remaining pathotypes exhibited a resistant reaction (0, 0;). We believe that juvenile resistance genes may be present in this line. *Lr28* and additional unidentified resistance genes from *Ae. triuncialis* were found in line 132/01i.

Conclusions. The testing of 13 wheat–*Aegilops* lines with leaf rust pathotypes with known genotypes showed that most lines had juvenile genes of resistance. Line 76/00i with APR genes was identified. All the lines were classified into three groups: 1) those with unidentified resistance genes from *Ae. speltoides* (k-62903, k-62904, 72/00i, 85/01i , 97/01i , 99/00i, 87/00i, 76/00i); 2) those with known genes of resistance (k-62905 and 149/00i); and 3) those with known resistance genes and an additional unknown resistance gene (82/00i, 102/00i, and 132/01i).

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Technological characteristics of spring wheat cultivars developed in the far-eastern Russian Federation.

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The grain market in the far-eastern part of the Russian Federation is mainly imports from abroad and the central region of the country. The cost is high. Because the far-eastern region has sufficient land and a favorable environment for the production of spring wheat, the area is completely capable of providing the population of the region with bread and

bakery products. To solve this important problem, increasing the wheat yield from 1.0–1.2 t/ha to 1.8–2.0 t/ha, extending the area under cultivation from 200,000 to 450,000–500,000 ha, and having high-quality cultivars is necessary.

In the Russian Federation, *T. aestivum* cultivars are classified into five groups according to their grain-technological characteristics into the categories strong, valuable, medium quality (filler), satisfactory, and weak (Table 1).

The term strong means wheat with high-quality protein content that forms a dough good for intensive mixing and long fermentation, provides for a high volume of bread, and has good mixing quality. The mixing quality is understood to be the capability of strong wheat flour to improve baking quality of a weak wheat flour. The higher the mixing quality of the flour, the less quantity of flour is required as a component of a mixture (from 50–20 %). Valuable and medium (filler) wheats make high-quality bread, but they do not improve the baking quality of weak cultivars. Flour from weak wheat when not combined with a strong wheat flour is not good for bread baking.

Table 1. Classification of *Triticum aestivum* cultivars according to bread-making quality.

Quality indicators	Strong	Valuable	Good filler	Satisfactory	Weak
grain hardness	hard and medium hard		—	—	—
vitreousness, % (not less than)	60	50	50	40	—
protein content in grain (not less than)	14	13	12	11	8
gluten content in grain, % (not less than)	2	~25	24	22	15
gluten content in 70 % flour output, % (not less than)	32	29	27	25	20
dough dilution, pharinograph units, (not more than	30–60	80	120	150	> 150
valorimetric number farinograph units, (not less than)	70–85	55	45	00	< 80
dough deformation, alveograph units, (not less than)	280	260	240	180	< 180
dough elasticity (alveograph), mm (not less than)	80	70	60	50	< 50
bread output from 100 g of flour, ml (not less than)	1,200	1,100	900	800	< 800
baking quality mark (not less than)	4.5	4.0	3.5	3.0	< 3.0

Wheat quality problems are of great economic importance. If 100 g of high baking-quality wheat yields 115 kg of bread, then a low baking-quality wheat will yield only 9l kg (Pumpyansky and Semyonov 1969). Thus, the main importance for obtaining high-quality bread depends on the cultivar.

The far-eastern region has the proper cultivar resources (Shindin 1996; Shindin and Bochkaryov 2001). In 2002, 13 cultivars of soft spring wheat were released for cultivation; 11 are from breeders in the far east (Amurskaya 75, Amurskaya 1495, Amurskaya 90, Dalnevostochnaya 10, Zaryanka, Lyra 98, Monakinka, Primorskaya 14, Primorskaya 21, Primorskaya 39, and Kabarovchanka) and two are from other regions (Krasnofimskaya 90 and Priokskaya). Al-

though no strong wheat cultivar is among these wheats, most are good fillers and are of good quality according to their technological evaluation. All have good agronomic characteristics (high grain yield and resistance to lodging, disease, sprouting, and shattering). The technological and agronomic characteristics of eight far-eastern cultivars follow.

Amurskaya 5. This cultivar was bred at the former Amur Agroexperimental Station (now the Russian Soybean Research Institute, Blagoveshensk), which is situated in the Amur region, a main wheat granary in the far-eastern Russian Federation. The grain is of average size and vitreous (60–78 %). The 1,000-kernel weight is 27–34 g. The 1 L weight is 750–760 g. Baking quality is good. Amurskaya 5 belongs to the valuable class of wheat cultivars. Grain protein content is 14.1–17.8 %, gluten content is 27–40 %, and flour strength is 280–411 units as measured by alveograph. The bread output from l00 g of flour is 620–1,150 ml. Baking quality is 3–4.5. The cultivar is resistant or moderately resistant to lodging, shattering, and *P. graminis*. Grain yield is 2–2.5 t/ha.

Amurskayag 90. This cultivar was bred at the Ear Eastern State Agricultura1 University, Blagoveshensk. The grain is egg-shaped, red, and vitreous with a shallow groove. The 1,000-kernel weight is 32–35 g. According to technological evaluations, Amurskayag 90 is a satisfactory filler, threshes well, and is resistant to *U. tritici* and *P. triticina* but susceptible to *S. nodorum* and *P. gramminis*. Potential yield is 4–4.5 t/ha, with an average yield of 2.5 t/ha.

Dalnevostochnaya 10. This wheat was bred at the Far Eastern Research Institute of Agriculture, Khabarovsk. The egg-shaped grain is red. The 1,000-kernel weight is 30–38 g. Bread-making quality is medium to good and the cultivar is a satisfactory filler. Grain vitreousness is 65 %, and protein content is 28.5–37 %. Flour strength is 230–360 units as measured by alveograph and valorimetric number is 50 units as measured by farinograph. The bread output from 100 g of flour is 650–1,050 ml. Bread-making quality is 2.7–4.1. The cultivar is resistant to lodging and moderately resistant to *P. triticina* and *P. graminis*. Commercial yield is 2–2.5 t/ha with a potential yield of 5 t/ha.

Zaryanka. Bred at the Ear Eastern Research Institute of Agriculture, Khabarovsk, Zaryanka has a grain-protein content of 14–16.7 %, a vitreousness of 60–65 %, gluten of the first-class quality at 28–30 %, and a flour strength of 320–380 units as measured by alveograph. The bread output from l00 g of flour is 960–1,060 ml. The cultivar belongs to the valuable class. Zaryanka is more resistant to *U. tritici*, *F. graminearum*, shattering, and sprouting as compared with the standard and yields between 2.5–3 t/ha.

Lyra 98. Lyra 98 was bred at the Far Eastern Research Institute of Agriculture, Khabarovsk and released for growing in the far-eastern region in 2002. Grain protein content is 16–l7 %, vitreousness 60–70 %, gluten content is 30–38 %, and flour strength is 450–520 units as measured by alveograph. The bread output from l00 g of flour is 1,100–1,200 ml. This cultivar is resistant to lodging, sprouting, and *U. tritici and* moderately resistant to *F. grameniarum.* Potential yield is 4.6–5.0 t/ha.

Primorskaya 14. Bred at the Primorskey Research Institute of Agriculture, Ussuryisk, this cultivar has red, egg-shaped grains with a medium groove and of small to average size. The 1,000-kernel weight is 30–36 g. Baking quality is from satisfactory to good. Vitreousness is 50–78 %, grain protein content is 15–16.8 %, flour gluten is 34.6–39.8 %, and flour strength is 255–343 units as measured by alveograph. The bread output is 620–1,020 ml with a bread-making evaluation of 2.6–3.8 marks. Primorskaya 14 is resistant to lodging except in rainy years, resistant to *P. graminis*, and *U. tritici* and moderately susceptible in rainy years to *P. triticina* and *F. graminearum*. The commercial yield of Primorksaya 14 is 2.5 t/ha with a potential yield of 5 t/ha.

Primorskaya 21. This cultivar was bred at the Primorskey Research Institute of Agriculture, Ussuriysk. The grain is red and oval with a small, narrow groove. The 1,000-kernel weight is 30–42 g. This wheat is of satisfactory baking quality and a good filler. Grain protein content is 14.7–17.6 %, flour gluten content is 37 %, and flour strength is 270–320 units as measure by alveograph. The bread output from 100 g of flour is 800–1,030 ml. Baking evaluation is 3.6–4 marks. Primorskaya 21 is resistant to lodging and moderately susceptible to *P. triticina* and *F. graminearum*. Average yield is 2.5–3 t/ha with a potential yield of 5 t/ha.

Primorskaya 39. The cultivar was bred at the Primorskey Research Institute of Agriculture, Ussuriysk. The grain is red, rounded, and vitreous with a medium groove. The 1,000-kernel weight is 30–34g. Baking quality is good to excellent. Grain protein content is 13–15.9 %, gluten content is 33 %, and flour strength is 440 units as measured by alveograph. The bread output from l00 g of flour is 810 ml. Baking evaluation is 4.6 marks. Primorskaya 39 is resistant

to lodging and moderately susceptible to *P. triticina* and *F. graminearum*. The average yield is 3.5 t/ha with a potential yield between 4.5 and 5 t/ha.

Khabarovchanka. This cultivar was bred at the Far Eastern Research Institute of Agriculture, Khabarovsk. The large, red, egg-shaped grains have a narrow, medium groove. The 1,000-kernel weight is 36–45. Grain protein content is 14–16 %, grain gluten content is 28–31.5 %, and flour gluten is of the first and second quality at 35.7 %, and flour strength is between 280–350 units as measured by alveograph. The bread output from l00 g of flour is 900–1,000 ml. Baking evaluation is 3.6–4.5 marks. Khabarovchanka is highly resistant to lodging, *U. tritici*, *P. graminis*, and *P. triticina*. This cultivar is of the intensive type and has a good responsiveness to improved growing conditions. Commercial yield is 3–4 t/ha with a potential yield of 5 t/ha.

In the future, this list of cultivars will increase as new cultivars with high value and strength are released by breeders from the far-eastern region.

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Chromosome number variations caused by the 2,4-dichlorphenoxyacetic acid in durum wheat calli and regenerant plants cultivated in vitro.

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Introduction. Totipotence is a property of plant cells that makes inheriting information following alterations in the environment and causes the regenerating of plants. The genetic properties of cell populations cultivated on different artificial media and the possibility to introducing hereditable mutations caused by different mutagenic substances are very important (Butenko 1964). Auxins, used for the transformation of isolated plant cells and tissues in culture, were capable of modifying some stages of the mitotic cycle (Gamburg et al. 1990). In addition, the callus tissue itself was shown to be heterogeneous and genetically unstable, being affected by certain environmental compounds such as light, temperature, and nutrition (Shamina 1970). Cytogenetic analysis of durum wheat callus tissues described the ploidy level and revealed a certain genotype and cultivation time (Bennici et al. 1988; Morozova 1991; Yurkova 1989; Yurkova et al. 1985). We studied variations of the chromosome number in durum wheat callus tissue and regenerated plants as they relate to some components of the nutrient medium.

Material and methods. Mature seeds of the durum wheat cultivar Altayskaya Niva were used to obtain callus tissues. The upper part of mature embryos were cut and placed on a nutrient-agar medium. Hamburg medium (B5) (Gamborg et al. 1968) supplemented with 2,4-dichlorphenoxyacetic acid (2,4-D) in concentrations of 2, 4, or 6 mg/l and Murashige-Skoog medium (MS) (Murashige and Skoog 1962) supplemented with 2,4-D at 4 mg/l were used to induce callus growth. Each group consisted of 30–35 explants. Cultivation was in ‘100 x 20-mm’ glass tubes with 10 ml of medium at 24˚C under continuous fluorescent light. After 6–7 weeks, the calli were transferred to another tube containing the same medium, but without the hormone supplement, to induce organogenesis. Cytogenetic analysis of the callus tissues and tissues of the apical root-tip meristem was with temporary squash preparations in acetocarmine. The samples were treated with 0.1 % colchicine at 4˚C and fixed in a solution of alchohol:acetic acid (3:1). The significance of the variation between treatments was determined using the Student’s t test (Lakin 1990).

Results and discussion. After the explants were placed on the agar medium containing 2,4-D, callus formation was observed after 6 weeks of cultivation. Transferring the calli to hormone-free medium caused a range of morphogenic processes such as the continuous proliferation of nondifferentiated cells (nonmorphogenic callus), the appearance of rhyzogenesis zones, shoot induction, and formation of somatic embryoids, the beginning of regenerant plants. The combination and proportion of these processes depended on the auxin concentration in the primary medium (Kuzmina 1997).

The normal chromosome number was 2n = 28 in the regenerant plants (Table 1). Greco et al. (1984) described chromosome numbers and Bennici et al. (1988) identified mosaics in durum wheat plants regenerated from the mesocotyl callus. Reduction in chromosome number depended on 2,4-D concentration and was detected in the roots of rhyzogenic callus. When the calli were grown on a medium supplemented with 4.0 mg/l of 2,4-D, few cells had reduced chromosome numbers, but the proportion of such cells significantly increased with higher concentrations of 2,4-D. The data clearly demonstrated that a high concentration of 2,4-D causes a reduction in chromosome number, at least in root-tip cells, because cells of the nonmorphogenic calli had reduced chromosome number independently of the 2.4-D concentration. This reduction might be explained by mitotic damage and reduction in mitosis rate, particularly due to loss of the final phases. The incapability of the proliferating cells to undergo mitosis might be associated with physiological hyperactivity of the cells maintained in an unusual experimental environment in vitro and with their concurrence for specific regulatory proteins (Gamburg et al. 1990; Shamina 1970; Yurkova et al. 1985).

Table 1. Chromosome numbers of the root and callus cells depending on the 2,4-D concentration in the B5 medium. Numbers are averages with 95 % confidence limits.

Sample origin	2 mg/l 2,4-D	4 mg/l 2,4-D	6 mg/l 2,4-D
Roots of the regenerant plants	28.0 + 0.0	28.0 + 0.0	—
Roots of the rhyzogenic calli	28.0 + 0.0	27.5 + 1.0	26.2 + 1.8
Cells of the non-morphogenic calli	21.2 + 1.0	21.5 + 1.0	20.5 + 1.3

Table 2. Chromosome numbers of the root and callus cells depending on the proportion of NO^{-3} and reduced nitrogen in the nutrition media (MS (Murashigi-Skoog) or B5 supplemented with 2,4-D, 4 mg/l). Numbers are averages with 95 % confidence limits.

Sample origin	MS	B5
Roots of the regenerant plants	28.0 + 0.0	28.0 + 0.0
Roots of the rhyzogenic calli	28.0 + 0.0	27.5 + 1.0
Cells of the non-morphogenic calli	22.7 + 1.8	21.5 + 1.0

The B5 and MS media contained different proportions of a nitrogen salts, NH_4NO_3, KNO_3, and $(NH_4)_2SO_4$. Reduced nitrogen (NH^{+4} and glycine) prevailed in the MS medium, whereas oxygenated nitrogen (NO^{-3}) prevailed in the B5. Because genetic instability might be caused by different components of the nutrition medium (Shamina 1970), we compared results obtained with two nutrition media of different mineral compounds (B5 and MS) supplemented with 2,4-D in equal concentrations of 4.0 mg/l (Table 2). The regenerated plants retained normal chromosome number (2n = 28) independent of the medium used. Roots of the rhyzogenic calli maintained on the MS were diploid, whereas cells of the nonmorphogenic calli had a reduced chromosome number. Only cells of calli maintained on B5 contained reduced chromosome number (2n = 20 or 22). Root-tip cells of the rhyzogenic calli maintained on the B5 contained either normal or reduced chromosome number (2n = 26). Increasing the reduced nitrogen in the MS probably diminished the effect of auxin stress and retracted the chromosome reduction. In one sample, nonmorphogenic calli maintained on MS medium had a single giant cell with 84 chromosomes (not included into the average calculations). Therefore, myxoploidy of the cell populations maintained in vitro might be the result of either reduction or multiplication the chromosome number.

One effect of chromosomal instability on the morphogenetic potency of the cultivated cells is difficult to estimate. Winfield et al. (1995) reported a correlation between the stability of a cell line karyotype and the rate of regeneration of the progeny plants, whereas others detected an absence of morphogenesis in the stable lines. The absence of shoots and regenerant plants in our experiments may be associated with a reduced chromosome number in the cells of nonmorphogenic and rhyzogenic calli. This point needs additional study.

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Aluminium tolerance in spring wheat plants at different levels of potassium and low temperature.

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Aluminum (Al) toxicity is one of the major problems of agriculture worldwide. Some Al-resistant genotypes have been identified, however, conditions that minimize damage from Al are unclear. Breeding material should possess not only specific characters but also a set of positive metabolic responses to environmental stresses typical of particular genotype. On the other hand, we need knowledge of how mineral nutrition and temperature regimes may eliminate the negative effect of Al toxicity. We investigated the Al tolerance of the spring wheat cultivar L-63/1 at different levels of potassium and temperatures.

Materials and methods. Seedlings of spring wheat were grown in a water solution in plastic pots (300 ml, 10 plants/pot). After a 2-day germination (20°C day/18°C night ± 2°C), seedlings were transferred to pots and grown in three nutrition regimes: (1) H_2O + $CaSO_4$ x 10^{-4} M (control), (2) KCl x 10^{-3} M + $CaSO_4$ x 10^{-4} M (low K), and (3) KCl x 10^{-3} M + $CaSO_4$ x 10^{-4} M (high K). After cooling for 2, 3, and 6 h, the solutions were supplemented with Al^{+3} ($AlCl_3$, pH = 5.6) at concentrations of 3 mg/l and 12 mg/l. Two levels of Al^{+3} were studied in each variant; low (3 mg/l) and high (12 mg/l). We examined nine variants in total: (1) control; (2) low K; (3) high K; (4) water + low Al level; (5) water + high Al level; (6) low K + low Al levels; (7) low K + high Al levels; (8) high K + low Al levels; and (9) high K + low Al levels. The root systems of the plants were dipped in solution during the 10 days. Solutions were replaced every 2 d. On the sixth day after germination, some of the plants from each treatment were exposed to cold (+8°C) for 2, 3, and 6 h without light. After a 6-day cold treatment (6 days of reparation), respiration/photosynthesis and linear growth were analyzed in all plants. The experiment was repeated three times.

Table 1. The effect of the low temperature on the length of the root system (cm). LTD_{05} = 2.43.

K^+ level	Length of cold treatment (h)			
	Control	2	3	6
0	48.40	35.4	63.02	53.46
5 x 10^{-3} M	27.27	35.28	38.96	36.30
5 x 10^{-2} M	40.92	41.52	62.08	58.38

Table 2. The effect of Al^{+3} at low and high concentrations on the root length at different levels of K^+ and optimal temperature.

Al level	Water	Low K	High K
0	42.73 ± 1.41	27.80 ± 3.68	43.68 ± 6.60
3 mg/l	22.47 ± 4.20	21.08 ± 4.74	45.18 ± 5.08
12 mg/l	24.41 ± 4.14	18.38 ± 3.82	26.76 ± 4.76

Results. Cold treatment showed a positive effect on root development, increasing the root-system length (RL) (Table 1). The negative effect of Al^{+3} was reduced in K^+-containing solutions. However, the RL at low potassium levels was small. Therefore, the parameter (RL:LL) was the smallest in variants with low K^+.

Minimal RL was formed at low K^+. The ratio of root-system length:seedling length (RL:SL) also was minimal in this variant. The RL in plants grown in water essentially decreased (43–47 %) upon the addition of Al^{+3} ions to solution (Table 2).

The addition of Al^{+3} to the solution changed respiratory metabolism in the plants. In the experimental treatment with water, Al^{+3} decreased respiration intensity by 43-47 % at both doses. At low K^+, respiration intensity decreased by 20 % at low Al^{+3} levels and by 34 % at high Al^{+3} levels. At high K^+, the addition of Al^{+3} to the solution did not change respiration intensity at 3 mg/ml and decreased the intensity by 39 % at 12 mg/ml. We assume that the addition of K^+ to water solutions reduces Al toxicity. Examination of the reparation period after cold treatment has shown that the negative effect of Al^{+3} inhibits growth activation by cold (Table 3). An increase of K^+ decreases the level of Al^{+3} toxicity

The Al^{+3} concentration has a greater effect in the presence of K+ in the nutrition medium. The toxic effects of Al^{+3} ions by suppressing the cold reaction of the root system is manifested by an increase in length. Optimizing K^+ is an essential prerequisite for decreasing Al^{+3} toxicity. In turn, cold treatment is a factor that is capable of adaptating plants to Al^{+3} toxicity, supported by our data from respiration and photosynthesis experiments. In the control plants grown at room temperature, Al^{+3} decreased respiration and photosynthesis activity by 30–68 % after day 6 (Table 4). Respiration in plants kept in the cold either did not change (in the presence of K^+) or decreased by 5–24 %. Finally, we have shown that cooling plants significantly increases the resistance of respiratory metabolism to Al^{+3}.

Table 3. The influence of low temperature on the length of root system (cm) at different K^+ levels and Al^{+3} concentrations.

Cold period (h)	H_2O (control)	H_2O^+ low Al^{+3}	H_2O^+ high Al^{+3}	lowK^+ low Al^{+3}	lowK^+ high Al^{+3}	high K^+ low Al^{+3}	high K^+ high Al^{+3}
0	43.70	22.47	24.41	21.08	18.38	45.18	26.76
2	42.30	26.44	24.02	22.41	21.54	35.59	23.80
3	63.02	23.57	27.10	21.30	17.15	38.10	25.88
6	53.96	22.39	24.28	22.56	20.56	39.38	24.49

Table 4. Variation in respiration and photosynthesis intensity in control plants and after cold treatment at different K^+ and Al^{+3} levels (% relative to control plants without Al^{3+}).

Treatment	Cold-treated plants		Control plants	
	Respiration	Photosynthesis	Respiration	Photosynthesis
H_2O^+, low Al^{+3}	24	5	44	68
H_2O^+, high Al^{+3}	18	18	41	47
Low K^+, low Al^{+3}	—	5	45	57
Low K^+, high Al^{+3}	14	10	45	55
High K^+, low Al^{+3}	—	—	—	—
High K^+, high Al^{+3}	—	—	30	42

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The function of different mitochondrial respiratory-chain pathways in winter wheat mitochondria during short-term cold stress and hardening.

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Plant mitochondria have a branched respiratory chain and, in addition to the main cytochrome pathway, have an alternative pathway that depends on the functioning of alternative cyanide-resistant oxidase (AOX) (Vanlerberghe and McIntosh 1997). Plant mitochondria also are able to oxidize exogenous NAD(P)H because of the presence of additional NAD(P)H dehydrogenases in their structure (Soole et al. 1990; Soole and Menz 1995; Moller and Rasmusson 1998). Recently, a number of proteins that effect mitochondrial activity were found and characterized. Among these are plant uncoupling mitochondrial proteins (plant UCPs) (Ricquier and Bouillaud 2000) and the stress protein CSP 310 (Voinikov et al. 1998), which cause uncoupling of oxidative phosphorylation in mitochondria. AOX (Takumi et al. 2002), *Wh*UCP (Murayama and Handa 2000), and CSP 310 (Kolesnichenko et al. 2000) are present in the mitochondria of winter wheat. Some of these proteins, such as AOX and CSP 310, are induced by cold stress in winter wheat, but others (*Wh*UCP) are not. Although *Wh*UCP is not induced by cold stress in winter wheat, its homologues in other plant species were shown to be induced by cold stress (Laloi et al. 1997; Maia et al. 1998; Ito 1999; Nantes et al. 1999). The main functions of these proteins were established for animals and proposed for plants are thermogenesis, participation in defense against oxidative stress, and regulation of cell metabolism (Sluse and Jarmuszkiewicz 2002). On the other hand, mechanisms that control the different electron-transport pathways in mitochondrial respiration under different stress conditions have not been studied in detail. Using inhibitor analysis that blocks terminal oxidases or respiratory-chain complexes, we studied the role of individual mitochondrial respiratory chain pathways in total mitochondrial respiration to learn how the different electron-transport pathways function in cold-resistant, winter wheat mitochondria during short-term cold stress and hardening.

Materials and methods. Three-day-old etiolated shoots of the winter wheat cultivar Zalarinka were germinated on moist paper at 26°C. Shoots were cold-stressed at –1°C for 1 h or were hardened at 4°C for 7 days. Mitochondria were extracted from seedling shoots by differential centrifugation (Pobezhimova et al. 1996). Isolated mitochondria were resuspended in a medium of 40 mM MOPS-KOH buffer (pH 7.4), 300 mM sucrose, 10 mM KCl, 5 mM EDTA, and 1 mM $MgCl_2$. Mitochondrial activity was recorded polarographically at 27°C using a closed platinum electrode in a 1.4-ml volume cell (Estabrook 1967). The reaction mixture contained 125 mM KCl, 18 mM KH_2PO_4, 1 mM $MgCl_2$, and 5 mM EDTA, pH 7.4. 10 mM malate in the presence of 10 mM glutamate, 8 mM succinate in the presence of 5 mM glutamate and 1 mM NADH were used as oxidation substrates. During succinate and NADH oxidation, 3 mkM rotenone was added to the incubation medium. During NADH oxidation, 0.06 mM $CaCl_2$ was added to incubation medium. The concentrations of inhibitors of the respiratory chain were antimycin A (A-A) (20 mkM), BHAM (1 mM), KCN (0.4 mM), and CSP 310 antiserum (1 mg/mL). Polarograms were used to calculate the rates of phosphorylative respiration (state 3), nonphosphorylative respiration (state 4), respiration control by Chance-Williams (RC), and the ADP:O ratio (Estabrook 1967). The concentration of mitochondrial protein was analyzed according to Lowry et al. (1951). All experiments were performed on 3-6 separate mitochondrial preparations. The data obtained were analyzed statistically and arithmetic means and standard errors were determined.

Results and discussion. We studied the mitochondrial respiratory-chain function of winter wheat during short-term low temperature stress and hardening using different oxidation substrates. When using succinate and NADH as oxidation substrates, rotenone, which blocks electron transfer through complex I of the mitochondrial respiratory chain, was added the mitochondrial-incubation medium. When using malate as oxidation substrate, winter wheat mitochondria isolated from control seedling shoots were well coupled (Table 1). After short-term low-temperature stress, the rates of state-3 and state-4 respiration increased by 19.2 % and 43.8 %, respectively, and the respiratory-control coefficient (RC) decreased (17.3 %) when compared to the control (Table 1). This data shows that these mitochondria were uncoupled. On the other hand, mitochondria isolated from hardened winter wheat seedling shoots had a lower rate of state-3 and state-4 respiration than the control mitochondria and changes in their RC coefficient and ADP:O ratio were to a lesser degree (13 % for state 3 and 11 % for state 4) (Table 1). When succinate was used as an oxidation substrate, we found that neither short-term low-temperature stress nor cold hardening influenced the degree of coupling of isolated mitochon-

Table 1. The energetic activity of winter wheat mitochondria isolated from control (1), stressed (2), and hardened (3) shoots analyzed using different oxidizing substrates. Data are presented as mean ± standard error, n = 6–32.

Substrate	Variant	Rate of oxygen uptake, nMol O_2/min/mg of protein		Respiration control	ADP:O
		State 3	State 4		
10 mM Malate +	1	82.6 ± 1.7	32.0 ± 1.7	2.60 ± 0.15	2.65 ± 0.12
10 mM glutamate	2	98.6 ± 5.6	46.1 ± 2.9	2.15 ± 0.06	2.23 ± 0.05
	3	55.5 ± 5.6	24.7 ± 2.9	2.25 ± 0.06	2.33 ± 0.05
8 mM Succinate +	1	66.9 ± 1.7	45.5 ± 1.8	1.48 ± 0.15	1.80 ± 0.12
5 mM glutamate	2	69.1 ± 5.6	47.5 ± 2.9	1.47 ± 0.06	1.62 ± 0.05
	3	63.5 ± 3.9	44.4 ± 2.7	1.51 ± 0.09	1.56 ± 0.03
1 mM NADH	1	109.5 ± 5.3	96.4 ± 5.6	1.14 ± 0.04	1.05 ± 0.19
	2	105.1 ± 4.2	83.6 ± 6.1	1.27 ± 0.06	1.05 ± 0.06

dria (Table 1). When NADH was the oxidation substrate, results were similar to those of succinate; no significant difference between mitochondria isolated from control, stressed, and hardened shoots (Table 1). Thus, cold stress caused significant changes only in the activity of malate-oxidizing mitochondria but did not influence succinate- and NADH-oxidizing mitochondria. Short-term cold stress caused more pronounced changes in mitochondria energetic activity then cold hardening.

The participation of the main cytochrome and alternative pathways in mitochondrial respiration was studied by adding an oxidation substrate, mitochondria, and ADP to the polarographic cell. When mitochondria were in state-4 respiration, antimycin A, BHAM, and anti-CSP 310 antiserum or KCN were added to the polarographic cell. We found that malate-oxidizing mitochondria isolated from control, stressed, and hardened seedling shoots differed in their reaction to inhibitor addition. Antimycin A in addition to control mitochondria caused ~ 50 % decrease of oxygen consumption. In mitochondria isolated from stressed plants, this treatment caused only ~ 30 % decrease (Fig. 1A). Cold shock caused ~ 20 % increase of antimycin A-resistant mitochondrial respiration. In mitochondria isolated from hardened plants, addition of antimycin A caused ~ 65 % decrease in oxygen consumption. Consequent addition of BHAM to mitochondria isolated from control and hardened plants inhibited oxygen consumption up to 25 % from state-4 respiration but in mitochondria isolated from stressed plants, this treatment inhibited oxygen consumption only up to 33 % (Fig. 1A). Therefore, we can conclude that in control mitochondria about 25 % of the respiration is antimycin A- and BHAM-resistant and that this part of mitochondria respiration increased during short-term low-temperature stress but was at the level of the control plants during cold hardening. The residual mitochondrial oxygen consumption was fully inhibited by consequent addition of anti-CSP 310 antiserum or KCN, so we can conclude that this residual respiration is involved with CSP 310 function.

Adding antimycin A to succinate-oxidizing mitochondria caused ~ 90 % inhibition of oxygen consumption (Fig. 1B). The consequent addition of BHAM to control mitochondria fully inhibited oxygen consumption. Despite the absence of cold-shock influence on total mitochondrial activity (Table 1), this treatment caused an increase of antimycin A-resistant respiration to ~ 20 % of that of state-4 respiration. Consequent addition of BHAM nearly inhibited mitochondrial respiration (Fig. 1B). Cold hardening caused an increase of antimycin A-resistant respiration to ~40 % that of state-4 respiration. This antimycin A-resistant respiration also was nearly inhibited by BHAM addition (Fig. 1B). We conclude that in succinate-oxidizing winter wheat mitochondria only two electron-transport pathways function, the main cytochrome pathway and an alternative antimycin A-resistant oxidase. Both cold shock and especially cold hardening caused an increase in this alternative pathway.

In NADH-oxidizing control winter wheat mitochondria, the addition of antimycin A caused ~ 80 % decrease of oxygen consumption (Fig. 1C). Consequent BHAM addition fully inhibited oxygen consumption in control mitochondria, but this addition and even the consequent addition of anti-CSP 310 antiserum did not fully inhibit oxygen consumption in mitochondria isolated from stressed plants. The residual respiration in this case was about 10 %. Based on these data, we concluded that in succinate- and NADH-oxidizing mitochondria the main part of respiration depends on the functioning of the main cytochrome respiratory chain pathway (77 % and 91 %, accordingly) but only ~ 50 % of respiration depends on this pathway function in malate-oxidizing mitochondria.

Wheat mitochondria have different electron transport pathways. One is an alternative KCN- and antimycin A-resistant oxidase. In addition to this pathway, different types of uncoupling proteins recently were found in plant mitochondria. The plant stress protein CSP 310 is one (Voinikov et al. 1998). Data obtained from inhibitor analyses agree with that about the influence of exogenous CSP 310 on different mitochondrial respiratory-chain complex function (Grabelnych et al. 2001). The effect of CSP 310 addition to isolated plant mitochondria was detected at complex I function but was not detected in the functioning of other respiratory chain complexes. Now, we can show that the main contribution to mitochondrial respiration of the CSP 310-pathway that was inhibited by anti-CSP 310 addition was detected during malate oxidation (25 %).

Because antimycin A addition blocks electron transfer through Q-cycle, i.e., inhibits the main cytochrome respiratory chain pathway, we can conclude that residual mitochondrial respiration depends on the functioning of alternative pathways. Therefore, during malate oxidation, the main cytochrome pathway contributes ~ 50 % to the total mitochondria respiration. The residual 50 % depends on alternative oxidase (25 %) and CSP 310 (25 %) functioning (Table 2). Cold shock caused about a two-fold decrease in the main cytochrome pathway and increased the contribution of alternative pathways. On the other hand, cold hardening caused an increase in the cytochrome pathway contribu-

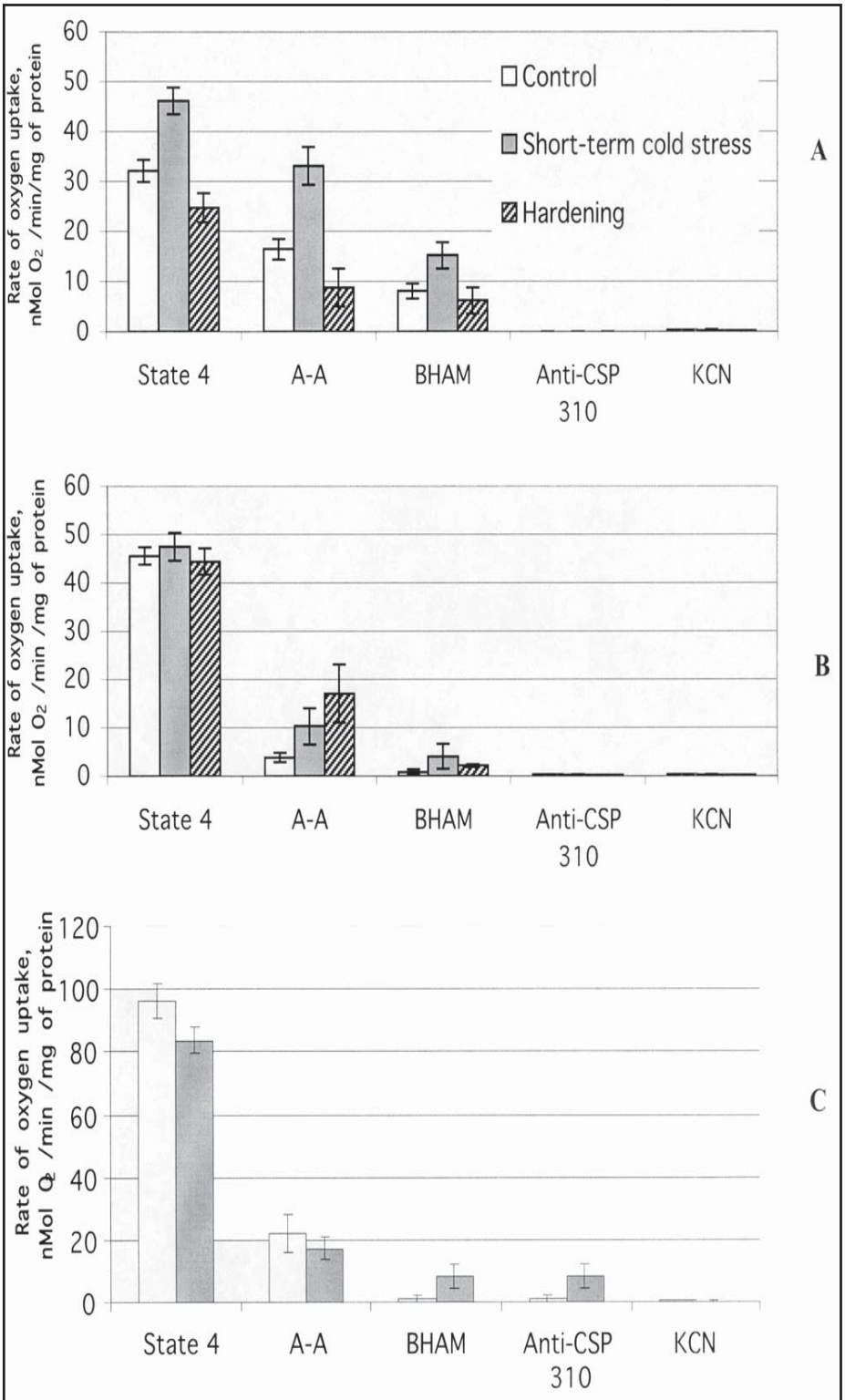

Fig. 1. The effect of different respiratory chain inhibitors on state-4 oxygen uptake by winter wheat mitochondria isolated from control, stressed (short-term cold stress), and hardened (hardening) shoots. The inhibitors were added in the sequence shown reading left to right.
A. 10 mM malate in the presence of 10 mM glutamate used as a substrate of oxidation (M±SE, n = 3–32).
B. 8 mM succinate in the presence of 5 mM glutamate used as a substrate of oxidation (M±SE, n = 3–19).
C. 1 mM NADH used as a substrate of oxidation (M±SE, n = 5–14).

Table 2. The contribution of cytochrome pathway (Cyt) or alternative pathways with cyanide-resistant alternative oxidase (Alt_{AOX}), CSP 310 (Alt_{CSP310}), and outer NADH-dehydrogenase ($NADH_{outer}$) to total respiration of winter wheat mitochondria in control conditions (1), during short-term cold stress (2), and during hardening (3) using different oxidizing substrates. The contribution is expressed as a percent of the respiratory rate in state 4.

	Percent contribution			
Variant	Cyt	Alt_{AOX}	Alt_{CSP310}	$NADH_{outer}$
10 mM malate in the presence of 10 mM glutamate.				
1	48.8	26.0	25.2	0.0
2	28.2	38.8	33.0	0.0
3	64.5	10.4	25.1	0.0
8 mM succinate in the presence of 5 mM glutamate.				
1	91.6	6.6	1.8	0.0
2	78.3	13.1	8.6	0.0
3	61.6	33.4	4.9	0.0
1 mM NADH.				
1	77.2	21.7	0.0	1.1
2	79.4	10.7	0.0	9.9

tion and decreased the contribution of alternative pathways in mitochondrial respiration (Table 2).

During succinate oxidation, the main part of mitochondrial respiration depends on the main cytochrome pathway function (about 90 %). At the same time, during succinate oxidation, short-term low-temperature stress and especially cold hardening caused a significant increase of alternative oxidase function. In NADH-oxidizing winter wheat mitochondria isolated from control plants, the main part of mitochondrial respiration also depends on cytochrome pathway function (about 77 %). Both cold shock and hardening did not significantly influence the contribution of different pathways in NADH-oxidizing mitochondria. Concurrently, we also detected an increase of residual mitochondrial respiration after antimycin A and anti-CSP 310 addition up to 10 % in these conditions (Table 2). In our opinion, this fact could depend on the function of external rotenone-insensitive and antimycin A-insensitive NADH-cytochrome *c* reductase (Soole et al. 1990).

Based on our data, we conclude that the contribution of the different mitochondrial electron-transport pathways significantly depends on the oxidized substrate. Short-term cold stress and cold hardening differ in their influence on the different electron transport pathways in winter wheat mitochondria.

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The use of linoleic acid as an oxidation substrate by winter wheat mitochondria.

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Free fatty acids (FFA) are effective uncouplers of oxidative phosphorylation depending on their protonophoric activity, which causes a significant increase in the conductance of the inner mitochondrial membrane. Some data shows that saturated FFA has less influence on mitochondrial membrane potential then unsaturated FFA (Penzo et al. 2002). In addition, saturated FFA can regulate mitochondrial uncoupling protein activity (Jezek et al. 1997; Jarmuszkiewicz et al. 1998; Costa et al. 1999; Hourton-Cabassa et al. 2002) and even expression of these proteins (Muzzin et al. 1999; Sbrassia et al. 2002).

The major FFA catabolic pathway in the cell is β-oxidation, which results in acetyl-CoA that can be completely oxidized by cell to CO_2 and H_2O via the Kreb's Acid Cycle. Intermediates of this cycle are the main mitochondrial respiration substrate (Schulz 1991). The FFA β-oxidation activity of this pathway significantly increases upon seed germination but dramatically decreases after photosynthesis establishment and during vegetative growth (Masterson and Wood 2000). FFA was used as an oxidation substrate during the very early stages of sunflower and lettuce seed germination (Salon et al. 1988; Raymond et al. 1992) and in potato storage organs (Theologis and Laties 1980). At the same time, data on the capability of wheat-seedling mitochondria to use FFA as an oxidation substrate and about the participation of different mitochondrial electron transport pathways in this process are lacking.

Thus, the aim of this study the function of winter wheat mitochondria during oxidizing of FFA and the participation of different mitochondrial electron-transport pathways in this process.

Materials and methods. Three-day-old, etiolated shoots of winter wheat cultivar Zalarinka were germinated on moist paper at 26°C. Mitochondria were extracted from seedlings shoots by differential centrifugation (Pobezhimova et al. 1996). The isolated mitochondria were resuspended in the following medium: 40 mM MOPS–KOH buffer (pH 7.4), 300 mM sucrose, 10 mM KCl, 5 mM EDTA, and 1 mM $MgCl_2$. Mitochondrial activity was recorded polarographically at 27°C using a closed-type, platinum electrode in a 1.4-ml cell (Estabrook 1967). The reaction mixture contained 125 mM KCl, 18 mM KH_2PO_4, 1 mM $MgCl_2$, and 5 mM EDTA, pH 7.4. Malate (10 mM) in the presence of glutamate (10 mM) and linoleic acid (0.056–750 mkM) were used as oxidation substrates. The concentrations of inhibitors of respiratory chain were rotenone (3 mkM), antimycin A (A–A) (20 mkM), BHAM (1 mM), and CSP 310 antiserum (1 mg/ml). Polarograms were used to calculate the rates of phosphorylative respiration (state 3), nonphosphorylative respiration (state 4), respiration control by Chance-Williams (RC), and the ADP:O ratio (Estabrook 1967). The concentration of mitochondrial protein was analyzed by Lowry method (Lowry et al. 1951). All the experiments were performed on three separate mitochondrial preparations. The data obtained were analyzed statistically and arithmetic means and standard errors determined.

Results and discussion. The amount of total FFA in winter wheat mitochondria is about 15 ng/mg of mitochondrial protein (0.056 mkM) and increases to ~40 ng/mg (0.15 mkM) after short-term cold shock (Vojnikov et al. 1983). In our experiments, we used physiological concentrations of FFA and higher concentrations (1-750 mkM).

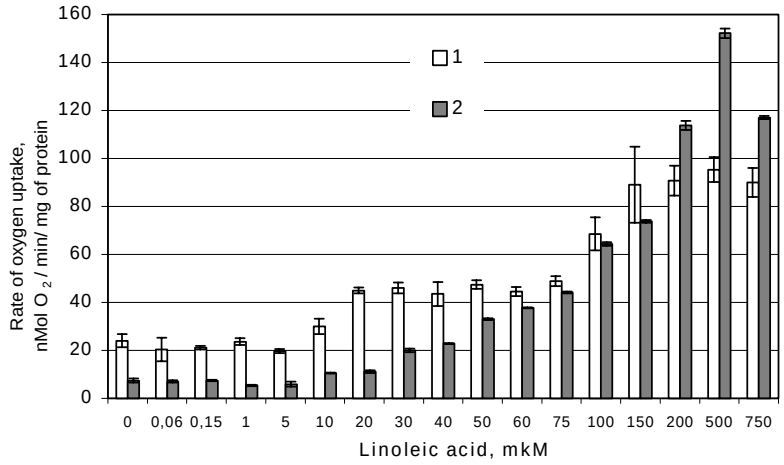

Fig. 2. The influence of linoleic acid on the rate of oxygen uptake in state-4 (1) and linoleic acid-supported oxygen uptake (2) of winter wheat mitochondria. 1. 10 mM malate in the presence of 10 mM glutamate used as a substrate of oxidation (M ± SE, n = 3) and 2. linoleic acid used as a substrate of oxidation (M ± SE, n = 3).

In the first set of experiments, linoleic acid (LA) was added to malate oxidizing mitochondria in state 4 (Fig. 2, 1). We found that LA did not influence mitochondrial oxygen uptake in the range of 0.056–5 mkM. At 10 mkM, LA increased oxygen uptake by 25 %. At 20 mkM, a 87 % increase of oxygen uptake was detected. Further increases in the LA concentration in the mitochondria incubation medium (20–60 mkM) did not cause further increases in state-4 respiration. On the other hand, adding 100 mkM or more LA caused at least a three-fold increase in mitochondrial oxygen uptake with a maximum at 500 mkM. The addition of 100 mkM LA caused an increase in the level of state-4 respiration up to that of state-3 respiration.

Similar results were obtained when the oxidizing of LA was the only oxidation substrate for mitochondria (Fig. 2, 2). Physiological FFA concentrations and concentrations up to 5 mkM did not cause an increase in oxygen uptake by winter wheat mitochondria. At the same time, at a concentration of 10 mkM, mitochondrial oxygen uptake up to 43 % was detected. Higher LA concentrations caused increases in oxygen uptake by mitochondria. The maximum oxygen uptake by winter wheat mitochondria was at LA concentration of 500 mkM. The rate of uncoupled respiration (Fig. 2, 1) and the rate of linoleic acid-supported respiration (Fig. 2, 2) were equal; 50 mkM LA.

Our data show that wheat mitochondria can successfully use linoleic acid as respiration substrate. Therefore, we were interested in determining what mitochondrial electron-transport pathways participate in this process. By looking at the influence of different electron-transport pathway inhibitors on oxygen uptake during 100 mkM LA oxidation, we found that different mitochondrial electron-transport pathways participate in this process. The data indicate that ~31 % of oxygen consumption was inhibited by the addition of antimycin A, ~34 % was inhibited by BHAM addition, ~33 % was inhibited by rotenone addition, and 30 % was inhibited by anti-CSP 310 addition.

During the oxidizing of LA, our data show that electrons can transfer oxygen through all branches of the electron-transport chain. Because rotenone is a complex-I inhibitor, the part of mitochondrial respiration that is not inhibited by its addition could deal with the functioning of complex II and different rotenone-insensitive, internal NADH dehydrogenases (Moller 1997).

Antimycin A addition blocks electron transport through complex III and, after this treatment, only alternative CN-resistant oxidase (Vanlerberghe and McIntosh 1997) and CSP 310 (Kolesnichenko et al. 2002) still function. These results agree with data on the influence of BHAM, which is an inhibitor of alternative CN-resistant oxidase and anti-CSP 310 antiserum, and its addition inhibits oxygen consumption dependent on CSP 310 function. Therefore, the LA-dependent increase in oxygen consumption is involved with the functioning of all branches of mitochondrial electron transport chain, both phosphorylative and nonphosphorylative.

Hermesh et al. (1998) used very high concentrations (0.5–2 mM) of FFA when studing mitochondrial energetic activity and proposed that FFA effects depend on the FFA-dependent uncoupling of oxidative phosphorylation. We have shown that LA concentrations higher than 50 mkM mitochondria change their metabolism to oxidizing LA as an oxida-

tion substrate, because the rate of LA-supported respiration becomes equal to the uncoupled rate after the addition of LA respiration during malate oxidation. The function of the main cytochrome pathway in such conditions could depend on oxidative phosphorylation uncoupling because FFA uncoupling activity causes an increase of oxygen consumption. In addition to this pathway, other alternative electron-transport pathways function during LA oxidation. Based on our data, winter wheat mitochondria can use LA as an oxidation substrate. Linoleic acid oxidation in these conditions depends on the functioning of all electron-transport pathways that exist in plant mitochondria.

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Questioning the possible role of D-amino acids in wheat seedlings.

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The D isomers of different amino acids (alanine, tryptophan, aspartate, glutamate, proline, and other amino acids) and their derivatives have been detected in plants (Bell 1980), but their possible physiological functions are unknown in plants. The presence of nonproteinogenic, D-amino acids in seeds and seedlings is believed to protect plant tissues from pathogens and parasites (Bell 1980).

D-amino acids are actively synthesized by bacteria and low fungi (Davies 1977). Alanine racemase is of great importance to bacteria because it supplies them with D-alanine from available L-alanine. Therefore, alanine racemase may be a key enzyme in the synthesis of the protective peptidoglucan layer of the cell wall. In some cases, the D-amino acids are abundant (Vicario et al. 1987).

Another mechanism by which D-amino acids are formed involves D-amino acid aminotransferase, which produces a diversity of D-amino acids. Perhaps the synergistic action of the two enzymes racemase and D-amino acid transferase accounts for the large amount of different D-amino acids that appear in bacterial cells and plant seedlings.

D-alanine and its dipeptide, D-alanyl-D-alanine, make up a considerable part of the nitrogen pool and probably play a significant part in regulation of nitrogen metabolism in bacteria. D-amino acids are not toxic in plants, perhaps because of neutralization via malonylation, acetylation, and glycosylation followed by compartmentalization in the vacuole. The bonding of D-amino acids with malonyl or acetyl moyeties may be hydrolyzed and reveal amino acids in intact form.

D-alanine and its derivatives in pea seedlings appeared during germination and disappeared on the 8th day of growth (Ogawa et al. 1973). D-alanyl-D-alanine and D-alanylglycine were found in rice seedlings and leaves, respectively (Manabe 1986; Manabe and Ohira 1983). Free and bound D-aspartic and D-glutamic acids were determined in pea seedlings (Ogawa et al. 1977). The N-malonyl-D-tryptophan content increased in leaves of tomato, potato, wheat, and other species during wilting and after drought during the period of recovery after osmotic stress (Rekoslavskaya et al. 1988).

All of these data would seem to indicate that synthesis of D-amino acids and their further conversion have ontogenetic, physiologic, and ecologic significance that is still unknown. As for N-malonyl-D-tryptophan, an acceptable hypothesis is that it functions as a precursor of the plant hormone indoleacetic acid, IAA (Rekoslavskaya et al. 2002). In reality, D-tryptophan has been demonstrated in a number of cases to be as active or even more active than L-tryptophan as an auxin substitute (Rekoslavskaya 1986).

Using D-tryptophan as an IAA precursor illustrates the idea that pools of amino acids for nonprotein synthesis can be created by means of the conversion of L-amino acids to D-amino acids. Direct competition for the amino acid between nonprotein syntheses and protein synthesis occurs in the process of growth and development.

Thus, the appearance of D-amino acids in plants apparently is nonrandom, uncontrolled, and physiological meaningless event, but the physiological significance of D-amino acids remains largely unclear and needs detailed study. We have investigated the content of amino acids in wheat seedlings in relation with some enzyme activities of amino acids metabolism different from protein biosynthesis have been done. The specific activity of racemase, transaminase, and UDPG-transferase were estimated in wheat seedlings during the study.

Materials and methods. The spring wheat cultivar Scala was used in this study. Procedures to determine racemase and transaminase activities were as described by Rekoslavskaya et al. (2002). UDPG-transferase activity was determined according to the modified method primarily described by Kowalczyk and Bandurski (1991). Briefly, 21 g of leaf, 44 g of stem, 5.6 g of young kernel, and 35.1 g of root tissue of green wheat shoots were harvested, ground with mortar and pestle in liquid nitrogen, and extracted with the buffer containing 0.25 M HEPES, 5 mM EDTA Na_2, 0.1 % mercaptoethanol, and 0.025 % Triton X-100, pH 8.5. One mg of phenylmethylsulfonylfluoride was added to the ground material at the time of extraction in order to prevent protease activity. The homogenate was passed through four layers of cheesecloth and centrifuged at 10,000 x g for 20 min at 4^{o}C. The activity of UDPG-transferase was estimated in the

supernatant fraction of each sample. The reaction mixture contained as the substrate 5 mmol of indoleacetic acid (IAA), 5 mmol of UDPG as the cofactor, and in order to prevent the ribosomes activity, 10^{-4} M $CaCl_2$ were added to 1 ml of supernatant. The reaction mixture was then incubated for 16 hours at 37°C. The reaction was stopped by adding of 1 ml of isopropanol. The activity of UDPG-transferase was determined as nmoles of substrate converted during 1 h/mg of protein. The IAA glucose ester content was determined after passing of reaction mixtures through a DEAE-cellulose (acetate form) minicolumn (10 x 20 mm) in 6 ml of eluates of 50 % isopropanol. The Ehrlich reagent was used in order to determine IAA-glucose content with calibration curve made with IAA. A D-amino acids kit was used (Sigma, USA). L-Amino acids were from Reachim (Russian Federation). The content of amino acids were determined on an amino-acid analyzer AAA-1 (Microtechna, Czech Republic).

Results and discussion. The amino-acid content of 7-day-old seedling are presented in Fig. 3. The amino acids Glu, Ala, Val, Pro, Leu, and iLeu had the highest content of > 200 mg/g of fresh weight. The content of Asp was next highest, but the other amino acids were present at levels below 100 nmol/g of fresh weight. Free Try did not contribute any significant content of free amino acids, but the sum of free and bound malonyl D-Try content was nearest to the content of Glu or even greater in seedlings sustaining wilting; 890 nmol/g of fresh weight (Rekoslavskaya et al. 1988).

The appearance of D-amino acids, and especially D-Try, during germination and growth of etiolated seedlings in the dark was shown previously (Rekoslavskaya et al. 2002). The activity of tryptophan racemase was found in the cytosol and etioplast fractions of wheat seedlings. The enzyme was isolated and some biochemical characteristics were studied, but the substrate specificity was broader and racemase used other amino acids as substrates (Table 3).

Table 3. Substrate specificity of the enzymes of amino acid metabolism, % to conversion of tryptophan (Try). Experiments were repeated at least twice.

L- or D-amino acid	D-amino acid oxidase	L-amino acid oxidase	Etioplast racemase	Cytosol racemase	Transaminase
Try	100	100	100	100	100
Ala	135	128	583	112	239
Pro	36	43	350	106	152
Met	104	—	310	106	—
Phe	116	129	101	103	124
Val	83	—	455	102	160
Asp	78	—	197	101	116
Asn	65	128	30	100	0
Thr	50	—	480	98	—
Ser	74	71	449	96	454
Arg	43	52	141	96	—
Cys	45	73	179	95	—
Leu	117	130	331	95	146
His	41	84	66	94	96
Glu	48	58	102	94	125
Tyr	95	85	99	90	—
iLeu	86	91	317	89	—

As shown in Table 3, the chiralic purity of D- or L-amino acids used were estimated with D-amino acid oxidase from hog kidneys (Sigma, USA) or with L-amino acids oxidase from snake venom (Sigma, USA). When D- or L-amino acids were treated with the enzyme preparation from wheat seedlings prepared as described earlier (Rekoslavskaya et al. 2002), we observed higher enzyme activities than in the case of either D- or L-tryptophan. For example, the specificity to Ala, Thr, Val, or Ser was about 5.8 or 4.5 times higher than to Try. The activity of transaminase was higher if Ala, Ser, Val, and some other amino acids were exploited in the study in comparison to Try. Therefore, it might be concluded that there was racemase and transaminase with broad substrate activities in wheat seedlings with some preference to amino acids structurally related to Ala.

About half of the amino acids is in the form of D-enantiomers in etiolated wheat seedlings. The content of D- and L-amino acids in 7-day-old wheat seedlings were 233.4 ± 34.0 and 194.8 ± 9.2 nmol/100 seedlings, respectively. We found two pools of amino acids in growing wheat seedlings and question why half of the amino acids in wheat are in a nonproteinogenic form that is not available for the synthesis of protein.

We tried to explain the appearance of D-Try in wheat seedlings as a creation of nonproteinogenic storage form for the precursor for IAA biosynthesis when the growth was fast during germination. Nevertheless, free Try was essential but not the predominant amino acid in wheat seedlings (Fig. 3). Thus, the role of other D-amino acids still

remained obscure. We searched for other explanations for the possibility of using nonproteinogenic amino acids for wheat seedlings, which they possess in order to survive in ecologically unfavorable conditions.

Amino acids might be used in the formation of plant lectins or phytoagglutenines. Plant lectines may play the role of antibodies against soil bacteria and fungi and participate in the defense response of young seedlings because the localization of lectins was found in embryos and other parts of plant. The binding action of amino acids to a sugar moiety was provided by UDPG-transferase. UDPG-transferases are a widespread and abundant enzyme family with broad substrate specificity. As a model system, we used IAA as a substrate in order to evaluate the activity of UDPG-transferase in wheat shoots, because IAA is a derivative of the amino acid Try and closely related to it in indole and side chain structure (Table 4).

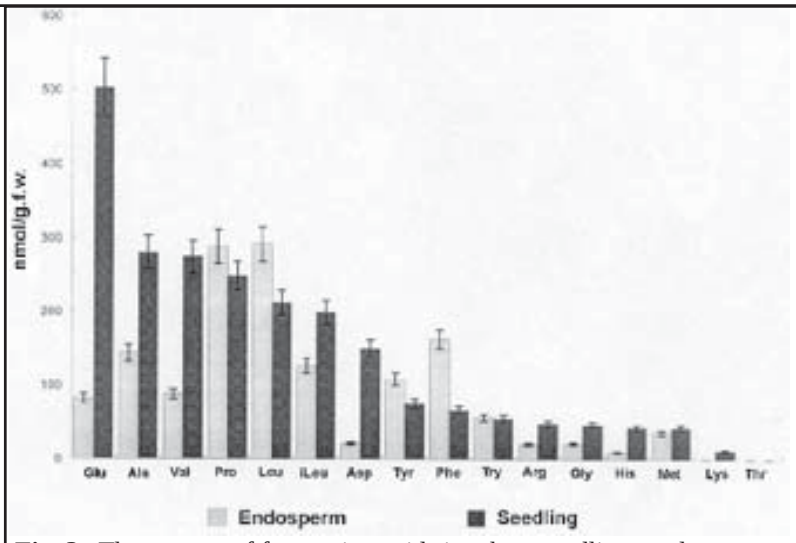

Fig. 3. The content of free amino acids in wheat seedlings and endosperm at 7 days.

Table 4. The specific activity of UDPG-transferase in wheat shoots, nmol of IAA glucosyl ester/mg of protein/h.

Leaves	9.08 ± 0.04
Stems	12.18 ± 0.22
Young kernels	7.92 ± 0.53
Roots	15.43 ± 0.18

The activity of UDPG-transferase was high in all parts of the wheat plant. Therefore, wheat seedlings have a highly active system for balancing the IAA level that was produced by rapid synthesis from D-Try. As a whole, the IAA biosynthesis and its metabolism is sufficiently intense to provide for the fast growth of etiolated seedlings during the heterotrophic period in order to emerge from the soil and initiate photosynthesis. The D-amino acids, which are not involved in protein biosynthesis, might participate in the protection of young seedlings from pathogens, bacteria, and fungi by this very unique manner of joining with glucose or another sugar moiety. This objective will be the subject of following experiments.

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Changes in the aquaporin content in winter wheat membranes after deadaptation.

G.B. Borovskii, A.Yu. Yakovlev, S.V. Vladimirova, and V.K. Voinikov.

In the past decade, we have discovered that water transport in cells is not directly through membranes but through numerous channels in the membranes. These channels are formed by proteins adhering to aquaporins. Aquaporins are found in the plasma and vacuolar membranes in animal and plant cells (Maurel 1997; Connolly et al. 1998). By regulating the degree of aquaporin phosphorylation, the cell controls the permeability of a membrane to water (Maurel et al. 1997; Kjellbom et al. 1999) and changes in the amount of these proteins shift the range of regulation. During adaptation to low temperature, membrane permeability increases and water migrates into the intercellular spaces during freezing (Alberdi and Corcuera 1991). This increase in permeability very likely is associated with an increase of aquaporins in the membranes. We expect the reverse during deadaptation in the spring. To date, changes in the quantity of water-channel proteins during deadaptation of overwintered plants has not been investigated.

Materials and methods. The crowns and leaves of winter wheat plants of the cultivar Irkutskaia ozimaia were used in this study. This genotype is winter hardy and highly productive under the severe climatic conditions of eastern Siberia (Borovskii et al. 2001). Crowns, leaves, and soil monoliths with plants were sampled in the field in January. Crowns and leaves were used for membrane-fraction isolation. The remaining plants in the monoliths were left at room temperature for 1 month under natural illumination for de-adaptation. After 1 month, the crowns and leaves were harvested and the membrane fraction isolated. We identified aquaporins inside the microsomal membrane fraction, because antibodies demonstrated a high degree of specificity (Fig. 4).

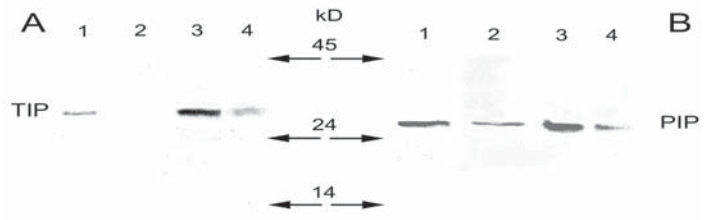

Fig. 4. Specificity using antibodies against two types of aquaporins, TIP (tonoplast-intrinsic protein, A) and PIP (plasmalemma-intrinsic protein, B). Immunoblotting of the proteins obtained from tonoplast (1) and plasmalemma (2). Arrows and numerals between the images indicate molecular weight markers.

Wheat membranes were isolated by centrifugation at 105,000 g for 1 h. Proteins were dissolved in a sample-loading buffer at 65°C. Proteins were separated by SDS–PAGE using a mini-Protean II PAGE cell (Bio-Rad, U.S.A.) according to the manufacturer's instructions. Western blotting and immunodetection were as described by Timmons and Dunbar (1990) using anti-PIP (plasmalemma-intrinsic protein) and anti-TIP (tonoplast-intrinsic protein) primary antibodies (1:1000 dilution), kindly provided by Dr. A. Schaeffner (Institute of Biochemical Plant Pathology, München, Germany) and Dr. C. Maurel (Institut des Sciences Végétales, Gif-sur-Yvette, France), respectively.

Results and discussion. We observed a decrease in aquaporins in both leaves and crowns after deadaptation of winter wheat (Fig. 5). Plasmalemma and tonoplast aquaporins decreased. This data supports the hypothesis that decreases in membrane water permeability occur after spring deadaptation. We assume that the permeability of membrane to water decreases in plants, because permeability is associated closely with freezing resistance. Alternatively, changes in the aquaporin content of the membrane could be connected with the start of the next stage plant development after overwintering plants reinitiate growth.

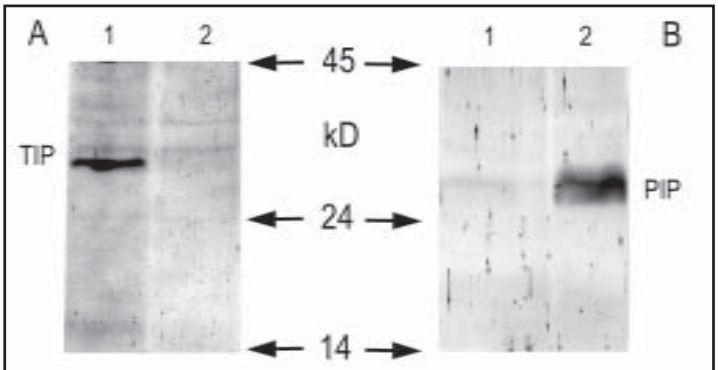

Fig. 5. Changes in aquaporin content in the membrane proteins of winter wheat plants after a 1-month deadaptation. The microsomal fraction was separated from leaves (1, 2) and crowns (3, 4). Plants were taken from field (1, 3) or left for 30 days under ambient conditions (2, 4). Proteins were fractionated on 12 % SDS–PAGE gels. Aquaporins were identified by specific antibodies against TIP (tonoplast-intrinsic protein, A) and PIP (plasmalemma-intrinsic protein, B). Arrows and numerals between the images indicate the molecular size markers.

The aquaporin content culminates after development in the autumn; water exits the cell during freezing. We know that some aquaporins are strongly induced by ABA (Kaldenhoff and Eckert 1999). This fact indirectly confirmed our results, because ABA content is high during winter adaptation and decreases under deadaptation in the spring. Activation of the water channels is useful to expel water and entrance inside under extreme thawing. In our opinion, regulating the action of water channels under the freezing in the external spaces of the cell is the same mechanisms that takes place under the water stress (Kjellbom et al. 1999), by stress-increasing of Ca^{2+} content in the cytoplasm. After winter, a high aquaporin content is dangerous because Ca^{2+} content in the cytoplasm increases under any stress.

Changes in the permeability of cell membranes to water are very important for plant adaptation to freezing. The importance requires a tight control of permeability. Our results suggest that aquaporins are involved in adaptation of wheat to winter and deadaptaion in spring.

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Using urea nitrogen for the nutrition of spring wheat under adverse temperatures.

A.K. Glyanko, N.V. Mironova, and G.G. Vasilieva.

Introduction. Urea is used widely in agriculture and is highly competitive with, and under certain conditions superior to, mineral forms of N fertilizers in its effect on yield and quality. For example, urea contributes to a greater accumulation of protein, gluten, and indispensable amino acids in wheat grain and other cereals during grain formation and maturation (Finney et al. 1957; Pavlov 1967; Schlehuber and Tacker 1967; Slukhai and Zrazhevsky 1971; Mitrofanov et al. 1973; Fox et al. 1986). Urea is taken up rapidly and metabolized by plants (Mokronosov et al. 1966; Pavlov 1967; Andrews et al. 1984). Urea increases the permeability of membranes and tissues and enhances the uptake, transferal, and reutilization of nutrients in plants (Mitrofanov et al. 1973; Turley and Ching 1986).

The mechanisms by which ammonium fertilizer and urea nitrogen affect plant metabolism are different (Tishenko et al. 1991). Thus, the role of urea as a N fertilizer has been studied in relatively sufficient detail, but the influence of adverse environmental factors on plant nutrition and physiology by this form of nitrogen have not. Over the last decade, researchers have had a great interest in studying the physiological response of plants to the nitrate and ammonium forms of N under stress conditions of salinity, low temperature, drought, and inadequate illumination (Chandra et al. 1986; Hubick 1990; Leidi et al. 1991; Gruz et al. 1993; Glyanko 1995).

Our results are derived from studying physiology of nutrition with urea nitrogen when spring wheat plants were exposed to a late spring frost (–6, –7°C) and low soil temperature (> 0°C) to compared to using the mineral forms of nitrogen.

Material and methods. ***Plant material and growth conditions.*** Soft spring wheat plants of the cultivar Skala were grown in containers (eight plants/container) in a growth chamber at the Siberian phytotron (Irkutsk, Russia) at a temperature of 19 ± 1°C/15 ± 1°C (day/night), illuminated by DRL-700 incandescent lamps. The light intensity was 14 ± 0.5 kLx with a 16-hour daylength. Infrared radiation from the lamps was suppressed by a water screen. The plants were

grown using a sand–soil mixture with a small amount of total nitrogen (0.009 %). Macro- and microlements were supplied at half the normal rate into enameled containers filled with dry soil (Grodzinsky and Grodzinsky 1973). Watering was by weight with distilled water up to 70 % of the moisture capacity of the soil. To guard against any nitrification of the ammonium, the nitrification inhibitor 2-chlor-6-trichlormetyl pyridine (N-serve) was introduced into the containers at 1 % of the N dose.

Conditions of the artificial frost. A spring frost condition between –6 and –7°C was produced in a refrigerating chamber of the phytotron once the plants had reached the three-leaf stage. The chamber was not illuminated during the frost period. Temperature in the chamber was controlled automatically under a preset program (Kurets 1974). The program provided for a gradual decrease in temperature within the chamber from the optimum temperature (19 ± 1°C) to 0°C (at the rate of 1°/12 min), followed by a decrease to the minimum subzero temperature (–6 and –7°C) at the rate of 1°/22 min. After a 1.5-hour exposure to temperatures between –6 and –7°C, the temperature was raised to 0°C at the rate of 1°/12 min. The temperature was raised from 0°C to the optimum temperature at the same rate. The total time of exposure of the plants to subzero temperature was 6 hr, of which 1.5 hour corresponds to the minimum subzero temperature. The relative air humidity within the chamber was 85–90 % during the frost. The containers with plants were placed in holes in plastic foam to avoid freezing the soil during the frost. One and one-half hours after the end of the frost (the temperature in the chamber was raised to 19°C), both control and experimental plants were fed through their roots with a mixture of three forms of N, one of which contained labeled ^{15}N. The extra nutrition schemes were variant I, $^{15}NH_4{}^{14}NO_3 + {}^{14}N$ – urea; variant II, $^{14}NH_4{}^{15}NO_3 + {}^{14}N$ – urea; and variant III, $^{14}NH_4{}^{14}NO_3 + {}^{15}N$ – urea.

In variant I, where the label was in the NH_4 group, 25.9 mg ^{15}N were introduced in each container and the enrichment of $^{15}NH_4NO_3$ was 95.31 weight percent of ^{15}N; in variant II, 24.3 mg ^{15}N with an enrichment of $NH_4{}^{15}NO_3$ of 89.66 weight percent of ^{15}N; in variant III, 52.3 mg ^{15}N were introduced with a urea enrichment of 93.84 weight percent of ^{15}N. The total amount of nitrogen that was introduced into the vessels during the extra nutrition was 106.4 mg in the first two variants and 101.3 mg in variant III.

Soil temperature reduction. To reduce the temperature in the root zone, containers with plants were placed in thermal chambers through which water at 5 ± 1°C and 19 ± 1°C was passed, maintaining the required soil temperature (Kurets 1974).

Chemical analyses. Protein in the triturated leaves was precipitated with trichloroacetic acid. Nucleic acids and other soluble compounds were removed from the protein precipitate (Klyachko et al. 1971). The protein was digested in concentrated sulfuric acid with a catalyst, selenium (Se). Protein nitrogen was distilled by the micro-Kjeldale method and determined by the titrimetric method of Ermakov et al. (1987). Samples were analyzed for enrichment of ^{15}N by means of a mass-spectrometer MI-1309. The content of labeled N in samples was determined by a formula for isotopic dilution (Korenkov 1977). The atomic percent of ^{15}N was converted to weight percent of ^{15}N (Korenkov 1977). The activities of glucose-6-phosphate dehydrogenase (G-6-PD) and malate dehydrogenase (MD) were determined using biochemical tests (Boehringer and Soehne GmbH Mannheim, Germany) in cell-free, unpurified root extracts. Urease activity was determined according to Bollard et al. (1968), and the protein in cell-free preparations was quantified according to Lowry et al. (1951). The biological and analytical repeatability of assays was fivefold and threefold, respectively. Results are represented as the arithmetic mean with a standard error. The confidence level of the differences was evaluated by the Student t-test (t_{st}). Least significant difference for comparing treatment means at the 0.95 probability level.

Results and discussion. ***Effect of late spring frost.*** Of 195 plants that underwent frosts, 64 (32 %) had one damaged leaf, eight had two damaged leaves, and three plants died. Thus, 38 % of the plants showed visually observable damages.

The sample for quantifying protein was made from the laminas of two plants having no visible damage. The plants did not show any substantial differences in protein accumulation in their leaves during the first 9 days after the frost, the absolute content in both control and experimental plants increased by a factor of 1.6 to 1.7. Labeled N is incorporated into leaf protein at a different rate depending on the form of N-fertilizer (Table 5). For example, 9 days after the frost, 552.9 μg ^{15}N from urea, 137.0 μg from the ammonium group, and 73.8 μg from the nitrate group were determined in the protein of the control plants. The percentage of labeled N utilization by the plants from fertilizers amounted to 1.06, 0.53, and 0.30, for urea, ammonium, and nitrate, respectively. During frost, this remains regular (Table 5). The difference is that a short exposure to subzero temperature promotes the incorporation into protein of the

label from urea. When compared to control plants, the label incorporation is 115 and 150 % at 3 and 9 days after the frost, respectively (differences at $t_d > t_{st}$).

Table 5. The effect of a late spring frost on ^{15}N uptake by leaf protein during nutrition of wheat from a mixture of different forms of nitrogen. N-protein content is from the dry weight of two plants.

Assay and variant	Within 1 day			Within 3 days			Within 9 days		
	N protein content (mg)	Weight % (excess ^{15}N)	^{15}N content (N protein, g)	N protein content (mg)	Weight % (excess ^{15}N)	^{15}N content (N protein, g)	N protein content (mg)	Weight % (excess ^{15}N)	^{15}N content (N protein, g)
Control	4.19 ± 0.36	0.33	14.5 ± 0.70	3.65 ± 0.20	0.95	36.5 ± 2.41	6.08±0.46	2.14	137.0 ± 5.02
I; frost	3.77 ± 0.05	0.42	16.6 ± 0.73	4.11 ± 0.22	0.67	29.0 ± 1.53	6.17 ±0.15	2.29	148.8 ± 7.41
Control	4.46 ± 0.07	0.02	0.90 ± 0.06	4.23 ± 0.33	0.24	11.3 ± 0.83	7.41 ±0.12	0.89	73.8 ± 8.01
II; frost	4.01 ± 0.22	0.05	2.20 ± 0.17	4.19 ± 0.18	0.32	15.0 ± 1.03	6.88 ± 0.18	1.50	115.6 ± 8.15
Control	4.19 ± 0.09	1.12	50.2 ± 4.31	4.23 ± 0.11	4.32	195.5 ± 8.13	7.62 ± 0.16	6.78	552.9 ± 27.00
II; frost	4.45 ± 0.41	1.09	51.9 ± 3.81	4.22 ± 0.15	4.99	225.3 ± 9.41	7.46 ± 0.04	10.37	827.9 ± 41.30

Label incorporation into protein 1, 3, and 9 days after the frost also is stimulated from the $^{15}NO_3$ group. The confidence level of the differences between the control and the assays are very high ($P > 0.99$). With regard to the effect of frost on the incorporation of the label from the $^{15}NH_4$ group, a reliable decrease in ^{15}N incorporation into protein on day 3 is observed ($P > 0.95$), whereas the differences are unreliable at 1 and 9 days after the frost ($t_d < t_{st}$).

The utilization of labeled N from different forms of N on day 9 after the frost was 1.58, 0.57 and 0.48%, from urea, ammonium, and nitrate, respectively. Thus spring wheat seedlings predominantly utilize urea N in synthesizing the protein. Temperature stress has a stimulating effect on this process. The control and experimental plants did not differ in absolute protein N content in the leaves (Table 5), suggesting that, during increased catabolic processes such as after frost, plants are able to shift the state of decay-synthesis of proteins toward the latter through an intense utilization of urea N.

The predominant utilization of urea from the mixture of three forms of N can probably be explained by a couple of factors. First, the relatively easy uptake of urea by roots. Second, the fast transport of urea (or its products) to aerial organs and subsequent use in metabolism.

In comparison with mineral forms of N (NO_3^- and NH_4^+), the mechanism of urea uptake by plants is not yet understood (Van Beusichem and Neeteson 1982). We anticipate that urea, as a neutral compound, is taken up by root cells with a minimum expenditure of energy and a high proportion is transported to aerial organs in an unchanged form. Urease activity in wheat roots and seedling leaves when the plant roots receive extra nutrition of urea provides some evidence. Activity of urease in leaves increases by a factor of 2.9, whereas enzyme activity in the roots is uncertain.

Chen and Ching (1988) induced leaf urease activity when barley plants are sprayed with urea solution. They detected urease isozymes, which were synthesized only during the period of an abrupt increase in enzyme activity. Our data indicate that spring wheat seedlings contain a sufficiently active constitutive form of urease in their roots and a less active form in leaves (medium without N). Under the influence of extra nutrition of plant roots with urea, urease activity changes little in roots but increases abruptly in leaves. The latter is likely to be associated with the *de novo* synthesis of enzyme.

The reasons for stimulating the uptake of label from urea as an effect of frost are unclear. We determined the urease and nitrate reductase activity in wheat leaves as an effect of the frost (within 1 and 3 days) and found that the activity of both enzymes was enhanced. However, we only can explain the presence in cells of a sufficient number of

NH_4^+ ions needed for the synthesis of amino acids. The mechanism of the effect of low temperature on the transcription-translation apparatus in leaves when plants are fed with different forms of nitrogen remains to be elucidated.

Reduced temperature effect of soil. Urea as fertilizer behaves in a peculiar fashion at low above-freezing temperatures in the root zone. We found that after exposure to low temperature (5 ± 1°C), G-6-PD and MD activity increases in roots by a significantly greater amount when the plants were fed with urea as compared to NO_3^- and NH_4^+. The activity of G-6-PD in the roots by urea is stimulated by 7-fold, as opposed to 3.2- and 3.9-fold for the NO_3^- and NH_4^+ N-sources, respectively. Under normal temperatures, enzyme activity in plants is higher with NO_3 nutrition. The stimulating effect of NO_3 on enzymes of the pentose monophosphate pathway of carbohydrate oxidation has been reported (Givan 1979). The activity of MD at near-freezing temperature increases in roots by 267, 167, and 136 % in variants with urea, NH_4^+, and NO_3^-, respectively. At the optimum temperature in the root zone (19 ± 1°C), however, the activity of these enzymes during urea nutrition of plants is lower when compared to variants with other nitrogen forms. A possible mechanism to explaining the stimulation of the G-6-PD and MD activity under stress could be the dissociation of the multidimensional forms of enzymes into simpler subunits having increased activity. The presence of electrophoretically different forms of enzymes suggests that under different conditions in the medium the relationship of different molecular forms of enzymes can change drastically (Petrova et al. 1985), which is responsible for the increase or decrease in enzyme activity.

We observed a greater stimulating of enzyme activity under low-temperature effect in the presence of urea. In protein chemistry, urea is known as a dissociating agent of proteins (Zolkiewski et al. 1995). At low temperatures, conditions that allow the penetration of urea to places where compartmentalizing of enzymes may be created in cells and the molar concentration suffices to have a dissociating effect on enzymes. An alternative explanation for the activation of the G-6-PD and MD enzymes could be an enhancement, at low temperature, of other processes such as anaplerotic pathways for the assimilation of carbonic acid during the enzymatic decomposition of urea in plant cells. This pathway involves enhancing the carboxylation processes with the participation of root phosphoenolpyruvate carboxylase and other CO_2-fixing enzymes resulting in products that are used in the Krebs cycle.

When urea is used to nourish plants in the root zone at low temperature, root growth is enhanced. According to our data from a water-culture assay, the presence of urea as the only growth source in the nutrient solution causes enhanced growth of plants if the temperature in the root zone was 5 ± 1°C. This effect of urea on root growth was not observed in the root zone at the optimum temperature. This assay was repeated in soil-cultured plants. In this case, nitrogen in the form of different fertilizers was introduced at 42 mg/kg soil (210 mg/container). All other elements were introduced at one-half the normal concentration. Once seedlings appeared, containers with seedlings were placed in different temperature conditions and the plants were grown until the third leaf appeared. At optimum soil temperature, the plants reached the 3-leaf stage within 13–14 days; at low temperature this occurred with in 21–23 days.

Our results showed that at low soil temperature and optimum air temperature (19 ± 1°C), the root dry weight in the variant with urea was higher when compared to plants grown with the other forms of N. The mean length of roots in the variant with urea at both the low and optimum temperatures was greater when compared with the other N-sources (Table 6). The root wet weight during urea nutrition under low temperature conditions in both water and soil culture approaches or exceeds that in the variant without N. Nitrogen deficiency and phosphorus in the medium is known to promote growth of the plant root system (Barber 1979), and the presence of these elements leads to a decrease in

Table 6. Wet weight of roots and of the aerial portion, and mean length of 15 spring wheat seed lings as a function of soil temperature and N-form. Air temperature was the same for all variants, 19 ± 1°C.

	Temperature					
	5 ± 1°C			19 ± 1°C		
Variant	weight of roots (g)	weight of aerial portion (g)	length of roots (cm)	weight of roots (g)	weight of aerial portion (g)	length of roots (cm)
No N (control)	7.3 ± 0.07	2.8 ± 0.17	27 ± 0.6	6.4 ± 0.15	3.2 ± 0.31	29 ±0.5
$Ca(NO_3)_2$	5.6 ± 0.33	4.4 ± 0.39	24 ± 0.4	4.4 ± 0.33	4.7 ± 0.33	19 ± 0.5
$(NH_4)_2SO_4$	6.8 ± 0.12	4.2 ± 0.30	24 ± 0.3	4.1 ± 0.07	5.2 ± 0.10	18 ± 0.7
$(NH_2)_2CO$	8.7 ± 0.08	6.0 ± 0.52	26 ± 0.5	4.6 ± 0.24	5.5 ± 0.41	22 ± 0.1

intensity of growth. In this case, during urea nutrition under low soil temperature conditions, plant roots behave as in the variant without N.

The mechanism responsible for enhancing root growth in the absence of N (or phosphorus) in the medium is unknown. Barber (1979) suggests that a stem-connected feedback mechanism causes an increase in root growth. Such a mechanism could be a hormonal imbalance in wheat roots during nutrition of plants with urea and other forms of N. According to our data, the relation between indoleacetic acid and abscisic acid in root tissues of wheat seedlings varies according to the form of N and soil temperature (Glyanko 1995). Lips (1997) also reported that variation in the balance between abscisic acid and cytokinins in roots during nitrate and ammonium nutrition has an effect on the growth of roots and aerial organs and contributes to adaptation of plants to stress effects (salinization or moisture deficiency). Thus, enhancement of root growth in conditions of near-freezing temperatures is effected under the influence of urea, and activation of urea N in protein molecules as an effect of frost is manifested by the adaptive and reparative changes in wheat plants induced by the form of N.

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Isolation and characterization of antimicrobial peptides from Triticum kiharae.

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All living organisms have evolved mechanisms with which to defend themselves against pathogen attack. This innate immunity involving the production of antimicrobial peptides is one of the most ancient and widespread defense strategies. After defense peptides are produced by transcription and translation of a single gene, they can be delivered rapidly after infection with a limited input of energy and biomass and display differential activity against different types of microorganisms (Thomma et al. 2002). Different families of antimicrobial peptides have been identified, including thionins, defensins, lipid-transfer proteins (LTPs), hevein-type peptides, and knottin-type peptides.

We hoped to identify the antimicrobial peptides in *T. kiharae*, which is highly resistant to most pathogens infecting cultivated wheat. *T. kiharae* has been used in our laboratory in crosses to generate lines resistant to such fungal pathogens as powdery mildew and brown rust.

Materials and methods. The peptide fraction was extracted from *T. kiharae* flour with 10 % acetic acid (flour to solution ratio of 1:10) for 1 h at room temperature. The supernatant was lyophilized and subjected to chromatography. The acid-soluble fraction was separated by gel-exclusion chromatography on a Sephacryl S-100 HR column using 10 % acetonitrile containing 0.1 % TCA as eluent. The chromatographic fractions were tested for the antifungal activity against several fungi (*Helminthosporium sativum, Alternaria consortiale, Rhizoctonia solani, Botritis cinerea,* and *Fusarium culmorum*). The active fraction, which caused inhibition of fungal growth and morphological changes, was separated by reversed-phase high-performance liquid chromatography (RP–HPLC). The HPLC-fractions were tested against fungi and characterized by mass spectrometry (MS) and sequencing.

Results and discussion. Separation of acid-soluble peptides on a Sephacryl column produced six fractions designated from A to G. Only fraction D exhibited antifungal activity against most fungi assayed. This fraction was further separated by RP–HPLC. Several fractions were obtained. Their molecular masses were measured by MS, and N-terminal sequences identified by automatic sequencing. The peptide masses separated by RP–HPLC are in Table 1.

The N-terminal sequences of all fractions were determined. Two fractions were identified: Fr. 4: AXQASQLAVXASAILGGTKPSGE and Fr. 5: KSXXK/RSTL

Table 1. Molecular mass of the RP-HPLC fractions obtained from the fungicidal fraction D. Prevailing masses are indicated in bold.

Fraction number					
1	2	3	4	5	7
1,371.6	1,236.5	1,070.6	1,153.5	1,206.8	3,487.9
	1,425.6	1,344.8	1,644.8	1,405.8	**5,900.9**
	2,734.0	1,535.8	2,189.0	1,574.8	**16,372.1**
	3,451.3	1,829.1	3,484.1	3,021.2	
	7,007.2	2,678.6	**6,972.4**	**3,622.0**	
		3,373.3		4,803.5	
		3,565.6		4,919.6	
		6,983.2			
		7,641.9			

The N-terminal sequence of fraction 4 coincides with that of LTP; however, three substitutions at positions 3, 4, and 5 have been observed (Garcia-Olmedo et al. 1998). Plant LTPs are 90–95 amino acid polypeptides that have been identified (at a protein and/or cDNA levels) in various tissues from a high number of mono- and dicotyledonous species. They were found to be distributed throughout the plant. Antimicrobial activity of LTPs has been reported for all members of the family tested. The relative activities of different LTPs vary between pathogens, suggesting that they have some degree of specificity. The mass of LTP from *T. kiharae* is lower than that described in the literature for other members of this family.

According to the N-terminal sequence, fraction 5 corresponds to α/β purothionins. The toxicity of thionins to plant pathogens is known from investigations into the susceptibility to wheat endosperm thionins of phytopathogenic bacteria in the genera *Pseudomonas, Xanthomonas, Agrobacterium, Erwinia*, and *Corynebacterium*. Purified genetic variants of these thionins differed in activity and showed some degree of specificity. Recent experiments *in planta* also are indicative of a defense role for the thionins.

Other fractions obtained by RP–HPLC of *T. kiharae* peptides were heterogeneous; therefore, their sequencing produced inconclusive results. Some low-molecular peptides were sequenced directly after the separation of the total acetic-acid extract on an RP–HPLC column. The sequences obtained were TRQLSLRG and TRQLSPRG. Homologous proteins were not found in the data banks, so their functions remain unknown.

These results indicate that *T. kiharae* possesses different types of antimicrobial peptides.

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Distribution of hybrid necrosis genes in common wheat cultivars of Australia.

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We studied the necrosis genes in modern cultivars of spring common wheat of Australia. The distribution of hybrid necrosis genes in the old local cultivars was first investigated by Tsunewaki and Hori (1967, 1968), who showed that the *Ne1 ne2* and *ne1 ne2* genotypes prevailed by the end of the 19th and early in the 20th centuries. The available data on the wheat cultivars of Australia and Oceania indicate that 25.4 % are of the *Ne1 ne2* genotype, 18.9 % are *ne1 Ne2*, and 55.7 % are *ne1 ne2* (Pukhalskiy et al. 2000). This genotype distribution resulted from nearly a century of breeding in Australia. We thought it interesting to investigate this parameter at the end of the 20th century.

Materials and methods. The necrotic genotype was analyzed in 48 Australian cultivars of spring common wheat. The spring common wheat cultivars Marquillo (*Ne1^{s}Ne1^s ne2ne2* genotype) and Balaganka (*ne1ne1 Ne2^{s}Ne2^s*) were used as testers. Crosses were conducted under field conditions by standard procedures including emasculation and isolation of spikes. The F_1 and F_2 hybrids were grown in the field. Hybrid necrosis traits were evaluated at different growth stages.

Results and discussion. The distribution of the different necrosis genotypes in Australian wheat cultivars shows that breeding led to complete elimination of the *Ne1ne2* genotype (Table 2). If we estimate the ratios of necrotic genotypes in all 46 cultivars (except for cultivars Beulah and Bt-Schomburgk where the presence of the *Ne2* gene is problematic), the results are as follows: 76.1 % of cultivars possess the *ne1 ne2* genotype and the *ne1 Ne2* genotype is found in 23.9 % of cultivars.

The ratios for wheats at the beginning of the 20th century were different (Tsunewaki et al. 1967). Among 72 cultivars examined, the *ne1 ne2* genotype was found in 57 (79.2 %) of the cultivars, *Ne1 ne2* in 14 cultivars (19.4 %), and *ne1 Ne2* (1.4 %) only in one cultivar. The *ne1 Ne2* genotype was found in the cultivar Atlas (Tsunewaki et al. 1968). The authors did not indicate whether Atlas is a winter or a spring cultivar. In all probability, Atlas was one of the two winter wheat cultivars studied.

We suppose that the observed changes in the distribution of hybrid necrosis genes were due to the Green Revolution and to the wide use of CIMMYT material in the Australian breeding programs.

Pedigree analysis of the Australian wheats using the GRIS 3.5 (Martynov and Dobrotvorskaya 1993) shows the Brazilian landrace Turco as the source of the *Ne2* gene. In addition, this gene could be derived from the Argentinian landrace Barleta or the Japanese cultivar Norin 10, the donor of the short-stem trait, which has the *Ne2* gene from the landrace Mediterranean through the old, American cultivars Lancaster and Fultz.

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Table 2. Genotype of necrosis genes identified in modern Australian cultivars of common spring wheat.

AWCC number	Cultivar	Year of release	Genotype
AUS-25046	Cunningham	1990	*ne1 ne2*
AUS-25139	Lillimur	1990	*ne1 ne2*
AUS-25418	Angas	1991	*ne1 ne2*
AUS-25292	Excalibur	1991	*ne1 ne2*
AUS-25648	Cadoux	1992	*ne1 ne2*
AUS-25468	Katunga	1992	*ne1 ne2*
AUS-27166	Pulsar	1992	*ne1 Ne2*
AUS-25598	Amery	1993	*ne1 ne2*
AUS-25567	Beulah	1993	*ne1?*
AUS-25929	Darter	1993	*ne1 ne2*
AUS-25568	Goroke	1993	*ne1 ne2*
AUS-25868	Houtman	1993	*ne1 Ne2*
AUS-25571	Ouyen	1993	*ne1 ne2*
AUS-25927	Rowan	1993	*ne1 ne2*
AUS-25923	Stiletto	1993	*ne1 ne2*
AUS-25597	Stretton	1993	*ne1 ne2*
AUS-25869	Sunmist	1993	*ne1 Ne2*
AUS-25870	Sunstate	1993	*ne1 Ne2*
AUS-25928	Swift	1993	*ne1 ne2*
AUS-25557	Tasman	1993	*ne1 ne2*
AUS-25924	Trident	1993	*ne1 ne2*
AUS-25925	Vectis	1993	*ne1 ne2*
AUS-25619	Wellstead	1993	*ne1 ne2*
AUS-25600	Bt-Schomburgk	1994	*ne1?*
AUS-25575	Cascades	1994	*ne1 ne2*
AUS-26161	Datatine	1994	*ne1 ne2*
AUS-25931	Sunland	1994	*ne1 ne2*
AUS-26160	Tammin	1994	*ne1 ne2*
AUS-24350	Yarralinka	1994	*ne1 ne2*
AUS-25558	Pelsart	1994	*ne1 ne2*
AUS-26169	Tern	1994	*ne1 ne2*
AUS-26192	Leichhardt	1995	*ne1 Ne2*
AUS-25607	Arnhem	1996	*ne1 Ne2*
AUS-27194	Carnamah	1996	*ne1 ne2*
AUS-27193	Cunderdin	1996	*ne1 Ne2*
AUS-27189	Kalannie	1996	*ne1 ne2*
AUS-27188	Perenjori	1996	*ne1 ne2*
AUS-27191	Petrel	1996	*ne1 ne2*
AUS-27192	Sunlin	1996	*ne1 ne2*
AUS-27199	Yanac	1996	*ne1 ne2*
AUS-27190	Tailorbird	1996	*ne1 Ne2*
AUS-25601	Frame	1997	*ne1 ne2*
AUS-25602	Barunga	1997	*ne1 ne2*
AUS-27203	Krichauff	1998	*ne1 ne2*
AUS-27647	Diamondbird	1997	*ne1 Ne2*
AUS-27694	Baxter	1998	*ne1 Ne2*
AUS-27660	Goldmark	1998	*ne1 Ne2*
AUS-27661	Silverstar	1998	*ne1 ne2*

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Genealogical analysis of Russian and Ukrainian winter wheat resistant to common bunt.

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Common bunt is one of most serious diseases of bread wheat. This disease is distributed in many regions of the Russian Federation including the Northern Caucasus, Central Black Soil region, Volga region, and Non-Black Soil zone. Resistance to common bunt in winter wheat was measured by comparing groups of resistant and susceptible cultivars from the Russian Federation and Ukraine using a genealogical approach.

Data on winter bread wheat cultivars were taken from the database GRIS 3.5 of the Information and Analytical System of Wheat Genetic Resources (Martynov and Dobrotvorskaya 2000). A set of 199 cultivars with known resistance/susceptibility to common bunt and known pedigrees were divided into resistant (Table 1) and susceptible (Table 2) groups.

Tracing expanded pedigrees with the aid of the GRIS program has established the probable donors and sources of resistance to common bunt (Table 1). Except for eight cultivars for which it was impossible to identify the source of resistance, the source of resistance to common bunt 36 cultivars (82 %) was from local sources mainly *A. glaucum* via PPG-599, Crimean, Odessa local cultivar (LV-Odessa) via Zemka, Eliseevskaya rye, and Yaroslav emmer. Other cultivars (18 %) received resistance genes both from local and foreign sources; Florence (*Bt3*) and Oro (*Bt4*, *Bt7*). A number of cultivars have ambiguous estimations of resistance to bunt (marked by an * in Table 1). For example, Bezenchukskaya 380 is considered resistant in the Lower Volga region but susceptible in other areas. Moskovskaya 70 and 642, Moskovskaya nizkostebelnaya, Chaika, and Yantarnaya 50 are classified as resistant, but data from State Varietal Trials indicates susceptibility. Skorospelka 1 and 3, from source data, and Odesskaya 12, from State Varietal Trials, are resistant, but data from the Vavilov Institute identifies them as susceptible. We assume that the conflicting data are a consequence of the different race compositions of local pathogen populations. Krivchenko (1984) has identified 37 different pathogen races. Analyzing the geographical distribution of the pathogen races, we identified two groups appropriate to two conventional regions; north and south of latitude 49°N. Races 1, 9, 15, 17, and 20 comprised the southern group and 2, 14, 16, 25, 31, 34, and 37 were specific to the northern group. Races 6 and 11 were common to both groups. We assume that the sources of resistance differ in southern and northern regions. Therefore, we analyzed groups of resistant and susceptible cultivars divided into southern and northern subgroups (see Tables 1 and 2). Among the cultivars of the southern area, the basic sources of resistance are the Odessa local variety (LV-Odessa) via Zemka, selection from Crimean (CI-1435), and foreign sources via Brevor and CIMMYT cultivars. In the northern subgroup, the number of sources of resistance is more limited; *A. glaucum* via PPG 599 and Eliseevskaya rye.

In a three-way ANOVA of the matrixes of ancestor contribution (Table 3), we investigated the resistance (factor A) with two classes (resistance and susceptibility), the region of origin (factor B) with two classes (south and north), and the original ancestor or hypothetical source of resistance (factor C) with the number of classes (c = 11). The analyzed sample included 52 resistant cultivars (including 23 from the southern and 29 from the northern regions) and 147 susceptible cultivars (including 88 from the southern and 59 from the northern regions). The data were transformed through arcsines. The effects of all investigated factors and interactions, except for interaction (A x B) were highly significant. Highly significant interactions (A x C), (B x C), and (A x B x C) indicate specific differences between the distribution of the contributions of hypothetical sources of resistance in groups of resistant and susceptible cultivars occurring from various regions. Differences in the race composition of regional populations of pathogen explain this fact.

Table 1. Donors of resistance to common bunt in Russian (RUS) and Ukrainian (UKR) winter wheat cultivars. Cultivars marked with an asterisk (*) have conflicting estimates of resistance by different authors.

	Country of origin	Year of release	Donor of resistance	Hypothetical source of resistance
Cultivars bred south of 49°N latitude				
Kooperatorka ***(Bt1)***	UKR	1929	Sel. Crimean	Crimean
Stepnyachka-0496	UKR	1929	Sel. Banatka Kherson	Unknown
Odesskaya-12*	UKR	1947	Zemka	LV-Odessa
Skorospelka-3-B	RUS	1955	Kanred	Crimean (CI 1435)
Deviz	RUS	1978	Skorospelka-3-B, Narodnaya	Crimean (CI 1435), Narodnaya
Odesskaya polukarlikovaya	UKR	1980	Odesskaya-12	LV-Odessa
Stepnyak	UKR	1982	Odesskaya-12	LV-Odessa
Dneprovskaya-39	UKR	1984	Kanred	Crimean (CI 1435)
Prikumskaya-79	RUS	1984	Penjamo-62 (Brevor, Newthatch), Odesskaya-12	Florence, Oro, Yaroslav emmer, LV-Odessa
Dneprovskaya -1029	UKR	1989	Skorospelka -1*	Crimean (CI-1435)
Dneprovskaya -710	UKR	1989	Odesskaya-12, Skorospelka -3B	LV- Odessa, Crimean (CI 1435)
Panna	UKR	1990	Chaika*, Skorospelka -1*	LV- Odessa, Crimean (CI 1435)
Donchanka	UKR	1990	Odesskaya polukarlikovaya, Odesskaya-12, RPG-434-154	LV- Odessa, Eliseevskaya
Donshchina	RUS	1992	Skorospelka -3*, Narodnaya	Crimean (CI 1435), Narodnaya
Odesskaya -133	UKR	1993	Brevor	Florence, Oro
Donchanka 3	UKR	1995	Brevor	Florence, Oro
Delta	RUS	1999	Selkirk	Crimean (CI 1435), Yaroslav emmer
Knyajna	RUS	1999	Selkirk (?)	Crimean (CI 1435), Yaroslav emmer
Prikumskaya 115	RUS	1999	Mida (U.S.), Odesskaya-12	Florence, LV-Odessa
Prima odesskaya	UKR	2000	Odesskaya-12 (?)	LV-Odessa
Zernogradka 10	RUS	2001	RPG-424-154, Skorospelka –3B	Eliseevskaya, Crimean (CI 1435)
Zarnitsa	RUS	2002	Brevor	Florence, Oro
Stanichnaya	RUS	2002	Odesskaya-12, Brevor	LV-Odessa, Florence, Oro

In the northern region, the contributions of *A. glaucum* and Eliseevskaya rye are higher in the group of resistant cultivars. In the southern region, the Odessa local variety prevails among resistant cultivars (Table 4). In the northern region, the contribution of LV-Odessa is higher in the group of susceptible cultivars, confirming the race specificity of this resistance source. Yaroslav emmer, in the northern region, and foreign sources (Oro, Florence, Federation, and *T. timopheevii*), in the south, are effective, although their contribution is not significant when compared with the group of susceptible cultivars.

This analysis shows that number of sources of a vertical resistance to bunt used in the winter wheat-breeding programs in the Russian Federation and Ukraine is not sufficient. The high number of genotypes with identical reaction to bunt causes genetic uniformity in the cultivars. The narrowing of the genetic diversity from a few identical genes can cause a change in the pathogen population and increase susceptibility on homogeneous genetic material.

Efficient horizontal (nonracespecific) resistance, which is shown as incomplete resistance to all races of a pathogen and in varying degrees suppresses its development, also depends on the genetic diversity of the released cultivars. A study of latent genetic diversity in winter wheat cultivars from the Russian Official List has shown that the

Table 1 (continued). Donors of resistance to common bunt in Russian (RUS) and Ukrainian (UKR) winter wheat cultivars. Cultivars marked with an asterisk (*) have conflicting estimates of resistance by different authors.

	Country of origin	Year of release	Donor of resistance	Hypothetical source of resistance
Cultivars bred north of 49°N latitude.				
Milturum-120	UKR	1929	Sel. Krasnokoloska	Krasnokoloska (?)
Moskovskaya-3251	RUS	1929	Sel. Tavtukhi *(T. durum)*	Unknown
RPG-27-36	RUS	1931	Eliseevskaya rye	Eliseevskaya rye
RPG-434-154	RUS	1931	Eliseevskaya rye	Eliseevskaya rye
PPG-599 (*Btz*)	RUS	1948	*Agropyron glaucum*	*A. glaucum*
Lgovskaya-873	RUS	1952	Unknown	Unknown
Belotserkovskaya-198	UKR	1955	Unknown	Unknown
PPG-99 (*Btz*)	RUS	1964	PPG-599	*A. glaucum*
Kalininskaya-27	RUS	1965	*A. glaucum*	*A. glaucum*
Kalininskaya-11 (*Btz*)	RUS	1967	*A. glaucum*	*A. glaucum*
Kharkovskaya-63	UKR	1969	Unknown	Unknown
Zarya (*Btz*)	RUS	1978	PPG-599	*A. glaucum*
Polukarlik-Mytnitskii	UKR	1984	Schlanstedter (?)	Unknown
Bezenchukskaya yubileinaya	RUS	1984	RPG-434-154	Eliseevskaya rye
Yantarnaya-50* (*Btz*)	RUS	1985	Zarya	*A. glaucum*
Moskovskaya nizkostebelnaya*	RUS	1990	Zarya	*A. glaucum*
Inna	RUS	1991	Zarya	*A. glaucum*
Moskovskaya 642*	RUS	1991	Zarya	*A. glaucum*
Moskovskaya 70*	RUS	1991	Zarya	*A. glaucum*
Zvezda*	RUS	1992	*Ag. glaucum*	*A. glaucum*
Nemchinovskaya 25	RUS	1992	Zarya	*A. glaucum*
Pamyati Fedina	RUS	1993	Zarya	*A. glaucum*
Bezenchukskaya 380*	RUS	1994	RPG-434-154	Eliseevskaya rye
Smuglyanka	RUS	1998	PV-18, Brevor, RPG-434-154	Florence, Oro, Yaroslav emmer, Eliseevskaya rye
Povoljskaya 86	RUS	1999	Zarya	*A. glaucum*
Moskovskaya 39	RUS	1999	Yantarnaya -50, Brevor	*A. glaucum*, Florence, Oro
Guberniya	RUS	2000	Unknown	Unknown
Tau	RUS	2001	Selkirk	Crimean (CI 1435), Yaroslav emmer
Omskaya 4	RUS	2001	RPG-434-154	Eliseevskaya rye

overwhelming majority (96 %) of cultivars recommended for cultivation in the Russian Federation are the descendants of Bezostaya 1 and/or Mironovsakaya 808. In the Central Black Soil zone and the Northern Caucasus and Middle and Lower Volga regions, the genetic diversity is acceptable, whereas the Central Non-Black Soil and Volga-Vyatka regions of the Russian Federation are characterized by low genetic diversity. The majority of cultivars recommended for these regions are related at the full- and half-sib level.

A key problem of breeding for resistance to bunt is use of the new sources of resistance. In addition to the 11 known resistance genes (*Bt1–Bt10* and *BtZ*), 11 new genes have now been identified. Ukrainian researchers have identified six new genes; *Bt11* from Sel. M-6623, *Bt12* and *Bt13* from Lutescens 6028, and *Bt14* from Erythrospermum 5221 (Novokharka et al. 1990) and *Bt15* and *Bt16* from Ferrugineum 220/85 (Babayants and Dubinia 1990). CIMMYT researchers have identified five new genes, which, unfortunately, have been given the same gene designations; *Bt11* (from PI-554119), *Bt12* (from PI-119333), *Bt13* (from Thule III), *Bt14* (from Doubbi), and *Bt15* (from Carleton) (Wilcoxson and Saari 1996). In addition, two presumably new genes in lines Erythrospermum 60-89 and Ferrugineum 124-88 were identified (Babayants et al. 1999). Some parental forms of Erythrospermum 5221, Ferrugineum 220/85,

Table 2. Russian (RUS) and Ukrainian (UKR) winter wheat cultivars susceptible to common bunt.

Cultivar	Country of origin	Year of release	Cultivar	Country of origin	Year of release
Cultivars bred south of 49°N latitude.					
Odesskaya 3	UKR	1938	Volgodar	RUS	1990
Pervenets	RUS	1938	Donetskaya 46	UKR	1990
Voroshilovskaya	RUS	1939	Olimpiya 2	RUS	1990
Ferrugineum 622-2	RUS	1939	Khersonskaya 86	UKR	1991
Erythrospermum 161	RUS	1941	Odesskaya 117	UKR	1992
Novoukrainka 83	RUS	1945	Skifyanka	RUS	1992
Krymskaya 1	UKR	1946	Tarasovskaya 87	RUS	1992
Gibrid 481	RUS	1948	Yubileinaya 75	UKR	1992
Osetinskaya 4	RUS	1950	Zernogradka 8	RUS	1993
Osetinskaya G-720	RUS	1950	Kolos Dona	RUS	1993
Gibrid 491	RUS	1951	Odesskaya 120	UKR	1993
Kubanskaya 131	RUS	1951	Soratnitsa	RUS	1993
Kubanskaya 24	RUS	1952	Sfera	RUS	1993
Novoukrainka 84	RUS	1953	Fedorovka	UKR	1993
Odesskaya 16	UKR	1953	Donskaya yubileinaya	RUS	1994
Osetinskaya 3	RUS	1953	Eika	RUS	1994
Yubileinaya Osetii	RUS	1954	Otrada	RUS	1994
Bezostaya 4	RUS	1955	Krasnodarskaya 90	RUS	1995
Lutescens 32	RUS	1965	Leda	RUS	1995
Krasnodarskaya 33	RUS	1967	Nika Kubani	RUS	1995
Skorospelka 35	RUS	1968	Azau	RUS	1997
Donetskaya 5	UKR	1982	Aliza	RUS	1997
Krasnodarskaya 57	RUS	1982	Zimorodok	RUS	1997
Donskaya polukarlikovaya	RUS	1983	Nak	RUS	1997
Prikubanskaya	RUS	1983	Odesskaya 267	UKR	1997
Dar Zaporojya	UKR	1984	Viktoriya Odesskaya	UKR	1998
Donetskaya 38	UKR	1984	Don 95	RUS	1998
Zaporojskaya 60	UKR	1984	Jirovka	RUS	1998
Zirka	UKR	1984	Zernogradka 9	RUS	1998
Olimpiya	RUS	1984	Kroshka	RUS	1998
Brigantina	UKR	1986	Pobeda 50	RUS	1998
Prokofevka	UKR	1986	Uskoryanka	RUS	1998
Stepnaya 7	RUS	1986	Podarok Donu	RUS	1999
Zamena	RUS	1987	Dar Zernograda	RUS	2000
Zimdar 4	RUS	1987	Donskoi mayak	RUS	2000
Peresvet	UKR	1987	Starnad 1	RUS	2000
Prometei	UKR	1987	Tarasovskaya Ostistaya	RUS	2000
Zimdar	RUS	1988	Ermak	RUS	2001
Birlik	RUS	1989	Lira	RUS	2001
Mriya Khersona	UKR	1989	Prestij	RUS	2001
Olviya	UKR	1989	Rosinka Tarasovskaya	RUS	2001
Prikumskaya 98	RUS	1989	Tarasovskaya 97	RUS	2001
Yunnat Odesskii	UKR	1989	Deya	RUS	2002
Albatros Odesskii	UKR	1990	Selyanka	RUS	2002

Erythrospermum 60-89, and Ferrugineum 124-88 are unknown, which does not enable pedigree analysis. We could analyze the pedigree only of Lutescens 6028 and are now able to explain bunt resistance in this line.

Tracing the transmission of *Bt*-genes on the expanded pedigrees the has shown that Lutescens 6028 (Selection 101/Manella//Kavkaz) can have genes *Bt1, Bt3, Bt4, Bt6,* and *Bt7* from Selection 101 (Fig. 1) that has the following

Table 2 (continued). Russian (RUS) and Ukrainian (UKR) winter wheat cultivars susceptible to common bunt.

Cultivar	Country of origin	Year of release	Cultivar	Country of origin	Year of release
Cultivars bred north of 49°N latitude.					
Hostianum 237	RUS	1929	Meshinskaya	RUS	1989
Erythrospermum 20 430	UKR	1934	Mironovskaya 40	UKR	1989
Hostianum 122 76	RUS	1936	Mironovskaya 61	UKR	1989
Kievlyanka 156	UKR	1936	Omskaya ozimaya	RUS	1989
Lesostepka 76	UKR	1937	Kharkovskaya 11	UKR	1989
Svea Pushkinskaya	RUS	1937	Komsomolskaya 56	UKR	1990
Saratovskaya 46 131	RUS	1938	Nemchinovskaya 52	RUS	1990
Erythrospermum 118	RUS	1938	Polesskaya 87	UKR	1990
Lutescens 17	UKR	1940	Lgovskaya 167	RUS	1991
Lesostepka 75	UKR	1945	Nemchinovskaya 86	RUS	1991
Sekisovskaya	RUS	1949	Kharkovskaya 90	UKR	1991
Lutescens 230	RUS	1951	Mironovskaya 27	UKR	1992
PPG 1	RUS	1951	Mironovskaya >AB8AB0O	UKR	1992
PPG 186	RUS	1953	Bazalt	RUS	1993
Veselopodolyanskaya 499	UKR	1954	Lutescens 9	RUS	1993
Mironovskaya 808	UKR	1963	Meshinskaya 2	RUS	1993
Mironovskaya N18;59=0O	UKR	1971	Mironovskaya poluintensivnaya	UKR	1993
Polesskaya 70	UKR	1974	Kharkovskaya 92	UKR	1993
Raduga	RUS	1976	Chernozemka 212	RUS	1993
Akhtyrchanka	UKR	1978	Bagrationovskaya	RUS	1994
Moskovskaya 60	RUS	1979	Veselopodolyanskaya 203	UKR	1995
Mironovskaya 25	UKR	1980	Imeni Rapoporta	RUS	1995
Nemchinovskaya 110	RUS	1980	Saratovskaya 90	RUS	1995
Drujba 2	UKR	1981	Kruiz	RUS	1998
Lgovskaya 77	RUS	1981	Orenburgskaya 105	RUS	1998
Kinelskaya 4	RUS	1985	Orenburgskaya 14	RUS	1998
Mechta 1	UKR	1985	Kharkovskaya 96	UKR	1999
Polesskaya 85	UKR	1985	Malakhit	RUS	2000
Shchedraya Polesya	UKR	1987	Ershovskaya 11	RUS	2002
Volgogradskaya 84	RUS	1989			

cultivars and genes in its pedigree: Rex (*Bt1* and *Bt7*), Rio (*Bt6*), Oro (*Bt4* and *Bt7*), Florence (*Bt3*), Burt (*Bt1, Bt4*, and *Bt6*), and Brevor (*Bt1, Bt3, Bt4*, and *Bt6*). Novokhatka et al. (1990) could not explain the results of segregation of resistance in crosses between 'Lutescens 6028/*Bt4* (monogenic line)' and 'Lutescens 6028/(*Bt6*) Rio'. The first cross segregated 74:26, which corresponds to the theoretical ratio 189:67 ($r^2 = 0.002$) suggesting four genes (one basic and three duplicate-complementary genes (Manjunath and Nadaf 1983). A segregation of 57:58 was found in the second cross, corresponding to a theoretical 121:135 ($r^2 = 0.24$) and

Table 3. Analysis of variance of the contribution of hypothetical sources of resistance to common bunt for Russian and Ukrainian winter wheat cultivars. Factor A is the group of resistant cultivars, factor B is the geographical region of origin, and factor C is the ancestry. * = significance at $P < 0.0001$.

Source	SS	Df	Ms	F
General	71,643.4	2,155		
Factor A	246.6	1	246.6	15.88*
Factor B	402.8	1	402.8	25.93*
Factor C	33,766.3	10	3,376.6	217.45*
Interaction (A x B)	5.4	1	5.4	0.35
Interaction (A x C)	597.8	10	59.8	3.85*
Interaction (B x C)	2,784.3	10	278.4	17.92*
Interaction (A x B x C)	1,029.6	10	102.9	6.63*
Error	32,810.6	2,112	15.5	

suggesting four genes (two basic complementary and two duplicate-complementary genes (Manjunath and Nadaf 1983). Thus, we cannot prove that the resistance genes in Lutescens 6028 are nonallelic and independent from previously described genes *Bt1*, *Bt3*, *Bt4*, *Bt6*, and *Bt7*. The high level of resistance in Lutescens 6028 may come from a combination of all these genes.

Table 4. Average contribution of hypothetical sources of resistance to common bunt for Russian and Ukrainian winter wheat cultivars in groups of resistant and susceptible accessions. Values are followed by letters that indicate significant differences at $P < 0.05$ by Duncan's multiple range test.

		Cultivars bred north of 49°N latitude		Cultivars bred south of 49°N latitude	
Ancestor	Genes	Resistant	Susceptible	Resistant	Susceptible
Agropyron glaucum	*BtZ*	2.11 *b*	0.42 *a*	0.00 *a*	0.02 *a*
Eliseevskaya (rye)		1.95 *b*	0.35 *a*	0.09 *a*	0.32 *ab*
Yaroslav emmer		0.27 *a*	0.17 *a*	0.86 *a*	0.89 *a*
Tr. timopheevii		0.24 *a*	0.28 *a*	0.98 *a*	0.69 *a*
Petkus (rye)		0.00 *a*	0.13 *a*	0.17 *a*	0.09 *a*
LV- Odessa (via Zemka)		0.45 *a*	2.07 *b*	12.05 *d*	7.05 *c*
Oro	*Bt4, 7*	0.22 *a*	0.47 *a*	0.90 *a*	0.59 *a*
Florence	*Bt3*	0.18 *a*	0.38 *a*	1.01 *a*	0.55 *a*
Federation	*Bt7*	0.19 *a*	0.46 *a*	0.82 *a*	0.49 *a*
Hussar	*Bt1, 2, 5*	0.11 *a*	0.11 *a*	0.35 *a*	0.33 *a*

Our analysis was made on the basis from information about resistance or susceptibility of winter wheat received from different authors by different techniques with different combinations of races in local pathogen populations. Therefore, we consider the data on source of resistance and statistical estimations made by comparing samples of resistant and susceptible cultivars as preliminary. Nevertheless, based on genealogical information, the data will be useful in conditions of artificial inoculation with certain races of the pathogen and the use of a standard set of differentials.

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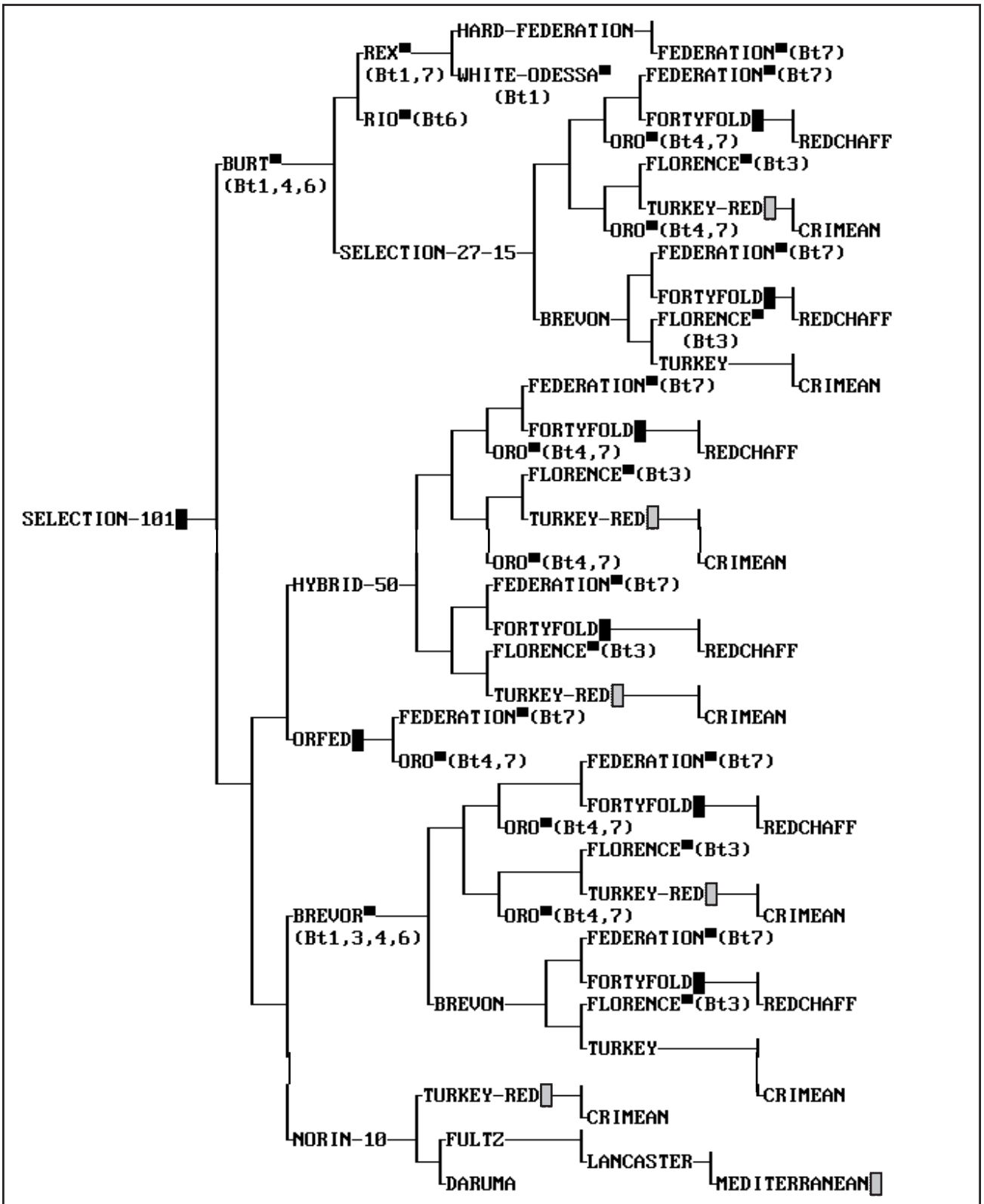

Fig. 1. The tracing of sources of resistance to common bunt for line Selection 101 by GRIS. The resistant cultivars are marked with a symbol ■, susceptible □, and carriers of Bt-genes ■.

ITEMS FROM THE REPUBLIC OF SOUTH AFRICA

SMALL GRAIN INSTITUTE
Private Bag X 29, Bethlehem 9700, South Africa.

Plant Breeding – winter and facultative breeding program.

J.C. Aucamp, D.J. Exley, and H.A. Smit.

During 2002 Small Grain Institute released a new bread wheat cultivar named **Komati**. Komati is a facultative cultivar with moderate vernalization requirements. The lodging resistance of this tall cultivar is good. Komati has a long coleoptile (± 9.3 cm) and excellent resistance to preharvest sprouting, which has been confirmed successfully. Another advantage is the high level of resistance against RWA infestations. Komati is susceptible to stripe rust. Though susceptible, the application of a fungicide is only necessary when conditions are optimal for disease development. The cultivar has no aluminium tolerance and planting on soils with low pH and high levels of acidification is not recommendable. Komati is suitable for planting on low to high potential fields. Yields are stable and competitive with the best cultivars available. Komati has an excellent hectoliter mass and falling number and good protein characteristics and, thus, produces grain of a supreme grade. The cultivar meets with the set standards for flour extraction, protein quality, water absorption, and mixing quality required by the milling and baking industries.

Plant Breeding – spring wheat irrigation program.

W.H.P. Boshoff and H.A. Smit.

The Wheat Technical Committee accepted BSP98/8 for final classification. The line will be marketed as **Olifants**. Olifants yields above average, which appears stable over environments. Important agronomic characteristics of Olifants are a medium growth period, good tillering, and strong resistance to lodging. The cultivar has excellent quality characteristics that comply with requirements of the milling and baking industry. Olifants has high levels of resistance to local foliar diseases including the currently prevailing pathotypes of stripe rust.

Genetic diversity.

T. van A. Bredenkamp and M.V. van Wyk.

The success of a breeding program depends mainly on the genetic diversity available. A constant need exists for the incorporation of new germ plasm to improve locally adapted lines. Activities of the Small Grain Institute Germplasm Collection consist of the conservation of small grain crops, namely wheat, barley, oats, triticale, and rye. This collection is maintained in a cold room facility with a mobile shelving system for medium-term viability at ± 4°C.

International collaboration is of extreme importance because no breeding program can function effectively without sufficient heritable diversity. Over the years a working relationship has been established between South Africa and CIMMYT (Mexico), ICARDA (Turkey), Uruguay, and other countries. Wheat, barley, and triticale nurseries and trials, segregating material, and interspecific crosses are imported annually. These lines are evaluated under quarantine. Microenvironments conductive to disease development are created artificially ensuring high selection pressure. The three quarantine sites are Bethlehem in the Free State, Riviersonderend in the Western Cape, and Vaalharts in the Northern Cape.

Doubled-haploid program.

A.F. Malan and H.A. Smit.

During the 2002 wheat season, several combinations were handled in the DH program. The main purpose of the program is to enhance the development of pure breeding material of promising combinations identified in the different breeding programs. The DH process is involved in the spring, winter, and prebreeding programs and assists in development of lines with good disease resistance, superior quality aspects, and minor gene characteristics.

In the spring wheat-breeding program, cross combinations were made with special emphasis on stripe rust resistance. A potential winter breeding lines' progeny will be tested for agronomic traits, bread-making quality, and disease resistance. Material from the prebreeding program includes combinations for RWA and leaf rust resistance. All these DH lines will be tested in extensive field trials during the 2003 season.

Applying molecular and tissue-culture techniques to problems in disease resistance of wheat with an emphasis on stripe rust.

R. Prins, V.P. Ramburan, and W.H.P. Boshoff (ARC-Small Grain Institute, RSA); L.A. Boyd (Department of Disease and Stress Biology, John Innes Centre, UK); Z.A. Pretorius (Plant Pathology Department, University of Free State, RSA); and J.H. Louw (Genetics Department, University of Stellenbosch, RSA).

Adult-plant resistance to stripe rust in the South African wheat cultivar Kariega was assessed in a DH-mapping population made from the F_1 of a cross between Kariega and the susceptible cultivar Avocet S. A partial linkage map covering all 21 chromosomes was developed with 208 DNA markers and four alternative loci.

Interesting features of the linkage map include the low polymorphism observed in the D genome and a region showing segregation distortion on chromosome 4A. The *Ltn* and *Sr26* genes also were mapped in this study. Two major QTL, together explaining about 55 % of the variation in the trait, were identified on chromosomes 2B and 7D, whereas minor QTL explaining about 14 % of the variation were identified on chromosomes 1A and 4A. The QTL on 7D appears to correspond to *Yr18*, a gene for APR to stripe rust. Markers fairly close to the QTL have been identified and these may be used to detect the presence of these QTL regions in marker-assisted selection. The APR to stripe rust of Kariega appears to be controlled by major QTL, in combination with other minor QTL, which is characteristic of APR in general. The DH population developed and the linkage map constructed are valuable resources for future genetic studies that may include studying APR, plant-pathogen interactions, and the mapping of additional traits polymorphic in this population.

Previous field trials of genetic material derived from Cappelle Desprez (CD) and Palmiet confirmed the effectiveness of *Yr16* (APR) against the South African pathotypes (6E16- and 6E22-). We know that CD also carries a T5BS–7BS translocation that is a complicating factor in studying *Yr16*. Chromosome 2D SSR markers, previously thought to be associated with *Yr16*, were tested on various resistant and susceptible lines. The molecular data suggest that the position of *Yr16* on chromosome 2D needs further verification. Various resistant plants were used in backcrosses to Avocet S and Palmiet and the resulting F_1s were used to produce DHs to simplify future genetic studies. These DH lines will be evaluated for their stripe rust phenotypes in a field trial in 2003.

Preharvest sprouting and falling number.

A. Barnard.

The South African wheat-producing areas, especially the Eastern Free State, are highly subject to the risk of preharvest sprouting because of summer rainfall that occurs just prior to or during harvest. Because preharvest sprouting is closely related to falling number (FN), a substantial amount of research is done on both topics.

Thousands of wheat spikes obtained from various commercial and newly released cultivars are evaluated for preharvest sprouting tolerance with the help of a rain simulator. This information is handed down to the commercial farmer to enable him to make the right decision regarding his cultivar choice for the coming season. Recently, more

attention also was given to breeding programs for sprouting resistance with the help of the rain simulator and protein electrophoresis. This technique is still in developmental and its usefulness still uncertain. Should this technique prove to be useful, direct crosses can be made and the progeny screened for the presence of the necessary electrophoretic bands, ensuring that, as sprouting resistance is a polygenic trade, none of the genes will be lost during the breeding program.

Since the incorporation of the FN method within the grading regulations, attention has been given to the possibility of managing FN within a wheat production system. The effect of early termination of kernel development (early harvest) on the FN of wheat and the effect of fertilizer on FN are being investigated.

Wheat production in the Summer Rainfall Region.

Because of the importance of cultivar choice in the Summer Rainfall Region, an extensive cultivar-evaluation program is followed for each of these areas. Different cultivars are planted in each region and these cultivars are evaluated and characterized in terms of yield reaction and stability in the different areas. Other characteristics that also are evaluated in this program include important quality specifications such as hectoliter mass, protein content, and falling number. These characteristics are used in recommending cultivars best suited for each area in the region.

Dryland production. Almost half of the South African wheat production is in cultivation under dryland conditions in the Summer Rainfall Region. Because of the large variation in climatic conditions and soil types existing in this region, wheat production is very challenging. Not only are good cultivation and management practices essential for successful wheat production, but also the correct cultivar choice. The dryland production area is divided primarily into four homogenous areas where different cultivars, mainly winter and intermediate types, are planted. All cultivar-evaluation trials planted at 18 sites throughout the Western, Central, and Eastern Free State were successful and reported. Eighteen entries were included in the trials, seven were from Small Grain Institute, six from Monsanto, and five from PANNAR.

Production under irrigation. Wheat produced under irrigation amounts to about 20 % of the total wheat production of South Africa and has a stabilizing influence on the total production. Currently, six major irrigation regions exist, although irrigation farming is expanding into new regions.

Mainly spring wheat cultivars are planted in a total of 44 evaluation trials at 23 localities in the different irrigation areas. Entries in these trials originated from Small Grain Institute (7) and from Monsanto (4). A durum cultivar also was included. ANOVA, AMMI analysis, and biplots are used in the interpretation of results and identifying cultivar adaptation and stability in the different production regions. Results from these trials are available in a detailed report.

Wheat production in the Winter Rainfall Region.

There are mainly two wheat producing areas in the Winter Rainfall Region:

- *The Swartland area* stretches from Durbanville in the south to the Sandveld area around Elandsbaai in the north and from Saldana Bay in the west to the mountain ranges in the east.
- *The Rûens or South Coast area*, stretches from Botrivier in the west to the Albertina-district in the east and from Aghullas in the south to the Langeberg mountain range north of Greyton through to Riversdal.

Spring wheat cultivars are grown in these two regions. These cultivars do not require the same amount of cold to break their dormancy as that of the winter wheats grown in the rest of South Africa. Cultivar choice in the Winter Rainfall Region is of extreme importance because of the varied climatic differences between cultivation areas. The yields of available cultivars differ relative to the changing yield-potential conditions that exist in the Winter Rainfall Region. Other important factors that also need consideration are grain quality, hectoliter mass, and disease susceptibility.

In the Winter Rainfall Region, the cultivar-evaluation program is run jointly by The Small Grain Institute and The Directorate of Agriculture of the Western Cape. The program consists of 13 sites in the Swartland and 13 sites in the Rûens, with 11 cultivars included in the trials. Cultivars, from ARC-Small Grain Institute, Monsanto, and PANNAR, are annually tested for yield potential, quality, disease resistance, and adaptability.

Karnal bunt in South Africa.

K. Naudé.

Karnal bunt was identified for the first time in South Africa in December 2000 in the Douglas irrigation area. Karnal bunt is caused by the quarantine organism, *Tilletia indica*, and according to South African regulations, the occurrence thereof should be reported to the National Department of Agriculture (NDA).

To date, the South African wheat industry has been protected against wheat imports from countries where KB already occurs. After the identification of KB in South Africa, a KB Task Team was founded with the objective to compile protocols to limit the spread of the disease in South Africa. These protocols include the testing of all registered seed units and all commercial grain for the presence of teliospores produced by the fungus. Using quarantine regulations and permits for the transportation of grain to intake points and mills also are included.

Karnal bunt occurrence in South Africa. As was the case with the 2001–02 wheat-production season, official surveys were made by the NDA–Directorate Plant Health and Quality (NDA–DPHQ) to test seed and grain for the presence of KB spores and infected kernels. All seed units of the 2001–02 season tested free of spores and infected kernels. Karnal bunt spores and infected kernels, however, were found in grain from the Douglas and Koffiefontein areas. Infection also was found on four farms in the Douglas district, and these farms were placed under quarantine. Results of the 2002–03 season will be available at a later date.

Control of Karnal bunt. At this stage wheat producers are making use of seed free of KB spores. The treatment or nontreatment of seed with chemical fungicidal seed dressing is done at the producer's own discretion. In areas where KB has been identified, spraying twice with Triticonazole is recommended. A first application is done at 25 % ear emergence, followed by a second application 10 days later. This spraying system is used by most wheat producers in the Douglas area with the purpose of limiting KB infection to levels lower than 2 %.

The role of ARC–Small Grain Institute (ARC–SGI). The latest information regarding KB and its control is transferred to producers, agents, and advisors at farmers' days and during courses on a continual basis. ARC–SGI tests all its seed and grain at the KB Laboratory at Bethlehem. All ARC–SGI seed required for planting at the more than 90 localities country wide is washed at the KB Washing Facility according to procedures used by CIMMYT. Fifty lines and cultivars of ARC–SGI were evaluated by CIMMYT in the 2001–02 season for KB resistance. These lines and cultivars will be evaluated by CIMMYT again during the 2002–03 season. Planting of less susceptible or resistant cultivars in the affected areas is regarded as the only sustainable solution for the control of KB.

Research into the utilization of nitrogen by wheat cultivars under irrigation.

W.M. Otto.

The objective of this research was to measure the yield and protein response of irrigated wheat cultivars to nitrogen (N) management options. Furthermore, the effect of split N applications combined with residual soil mineral N on grain yield and quality also is determined. The contributions of soil mineral N, plant uptake of N, and biomass development to N management of the crop also were calculated. The aim is to develop an N management system that the producer can implement to optimize all the relevant production factors.

The yield and grain protein responses to split applications of N applied at planting, late tillering, and flag-leaf stages of six commercially available wheat cultivars (SST 876, SST 822, Kariega, Olifants, Baviaans, and Steenbras) were measured. The trials were planted at Riet River, Vaalharts, Loskop, and Bethlehem.

The tested cultivars differed in response to split applications of applied N, with the magnitude of yield and protein response linked to the adaptability and growth period of the respective cultivar. Measured residual soil mineral N influenced yield response to N. A high level of soil mineral N (205 kg N/ha) decreased the response to applied N, whereas where a low soil mineral N of 60 kg N/ha was measured, significant responses to applied N were found.

Protein percentage of the grain increased with an application of 40 kg N/ha at the flag-leaf stage. A decrease in yield of all the tested cultivars was found when the total N rate was applied at planting. The split application of N, where 80–120 kg N/ha applied at planting was followed by 40–80 kg N/ha at late tillering and 40 kg N/ha at the flag-leaf stage, resulted in the highest yields and protein percentage of the grain. Plant N analysis at the measured growth stages indicated that the split application of N increased plant N concentration to within the optimal N analysis range, showing the potential use of this measurement in N management of the crop.

Wheat Quality Laboratory.

A. Barnard, C.W. Miles, K.B. Majola, M.L.T. Moloi, M.M. Raderbe, N.E.M. Mtjale, C.N. Matla, M.M. Mofokeng, M.L. Dhlamini, and N.M. Mtshali.

One of the main objectives of the Quality Laboratory is to maintain a cost-effective, highly scientific, and objective quality assessment of Small Grain Institute breeding lines, to incorporate contract work for milling and baking industries and private companies, and to provide an objective service to wheat producers. To ensure accurate data to researchers and external parties, the laboratory takes part in quarterly and monthly ring tests. A total of 57,410 analyses were performed during 2002.

Soil Analyses Laboratory.

L. Visser.

Soil analyses form an essential part of a producer's success. The laboratory provides this service and plant and water analyses to external clients and researchers.

During the 2001–02 financial year, the laboratory performed 111,032 tests on 9,329 samples. Fifty-four percent of these samples were received from external clients such as producers, advisors, and representatives of different fertilizer companies. During December 2001, the laboratory bought a new Inductive Coupled Plasma Emission Spectrometer. The instrument is known for accurate, reliable, and fast results. With this instrument the laboratory can handle a larger amount of samples/year and also analyze for elements such as sulphur and boron.

The laboratory is also involved in a research project to evaluate the soil fertility status of resource poor areas where Small Grain Institute operates. The database will help identify trends like increases in soil acidity and also improve the quality of technology transfer in future.

In order to ensure an accurate and reliable service to all clients, the laboratory runs internal control samples and also belongs to Agri-LASA, a national control scheme.

During the past year the external income of the laboratory increased by 10 %. The main objective of the laboratory is to improve on this by rendering an accurate and efficient service to all clients.

Personnel.

Ms. Vicki Tolmay was appointed program manager of Plant Protection replacing Dr. Hugo Smit. Ms. Anri Barnard has resigned to pursue household duties. Labious Masike replaced Godwin Khorommbi as a researcher of Plant Protection. Sanesh Raburam joined Crop Science as a research technician. Willem Otto was transferred to Crop Science to handle the Cultivar Evaluation Programme under irrigation. Pieter Craven and Danie van Niekerk resigned from Small Grain Institute.

Publications.

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G.F. Marais, H.S. Roux, A.S. Marais and W.C. Botes.

Triticale breeding.

Three new triticale cultivars will be available for commercial production in 2003, these are **Bacchus**, a selection from CIMMYTs 28th ITYN-48 (SUPI 3//HARE 7265/YOGUI 1); **Tobie**, a selection from the local cross KIEWIET/4/W.TCL83/HOHI//RHINO 4/3/ARDI 1; and **Ibis**, also derived from a local cross FLORIDA 201/17th ITSN 238 (= DURUM WHEAT/BALBO//BOK"S"). Tobie is a very early maturing triticale with high grain yield and excellent hectoliter mass, whereas Bacchus is a high-yielding, later maturing cultivar. Ibis is a late-maturing, tall straw cultivar selected specifically for the production of fodder. Two cultivars released at Stellenbosch, Tobie and USGEN19 (late maturing), also have been released in Ethiopia under the names Sinan and Maynet, respectively, as part of a collaboration with the Ethiopian Bureau of Agriculture, Amhara Region, and the German G.T.Z. (Technische Zusammenarbeit) program coördinated by Dr. K. Feldner.

Recurrent selection of wheat.

A recurrent-selection procedure for wheat based on genetic male sterility and hydroponic culture of cut tillers was continued and further improvements of the technique were made (Fig. 1). The breeding cycle could be reduced to 4 years (1 year for making crosses and 3 years for inbreeding and field evaluating the F_4 and F_6 male parents). Single-seed descent steps were introduced to advance from the F_1 to the F_6 in the course of 2 years. To accomplish this, two additional plantings (F_2 and F_3) are made during the summer months in an uncooled greenhouse. The F_4 is again planted in

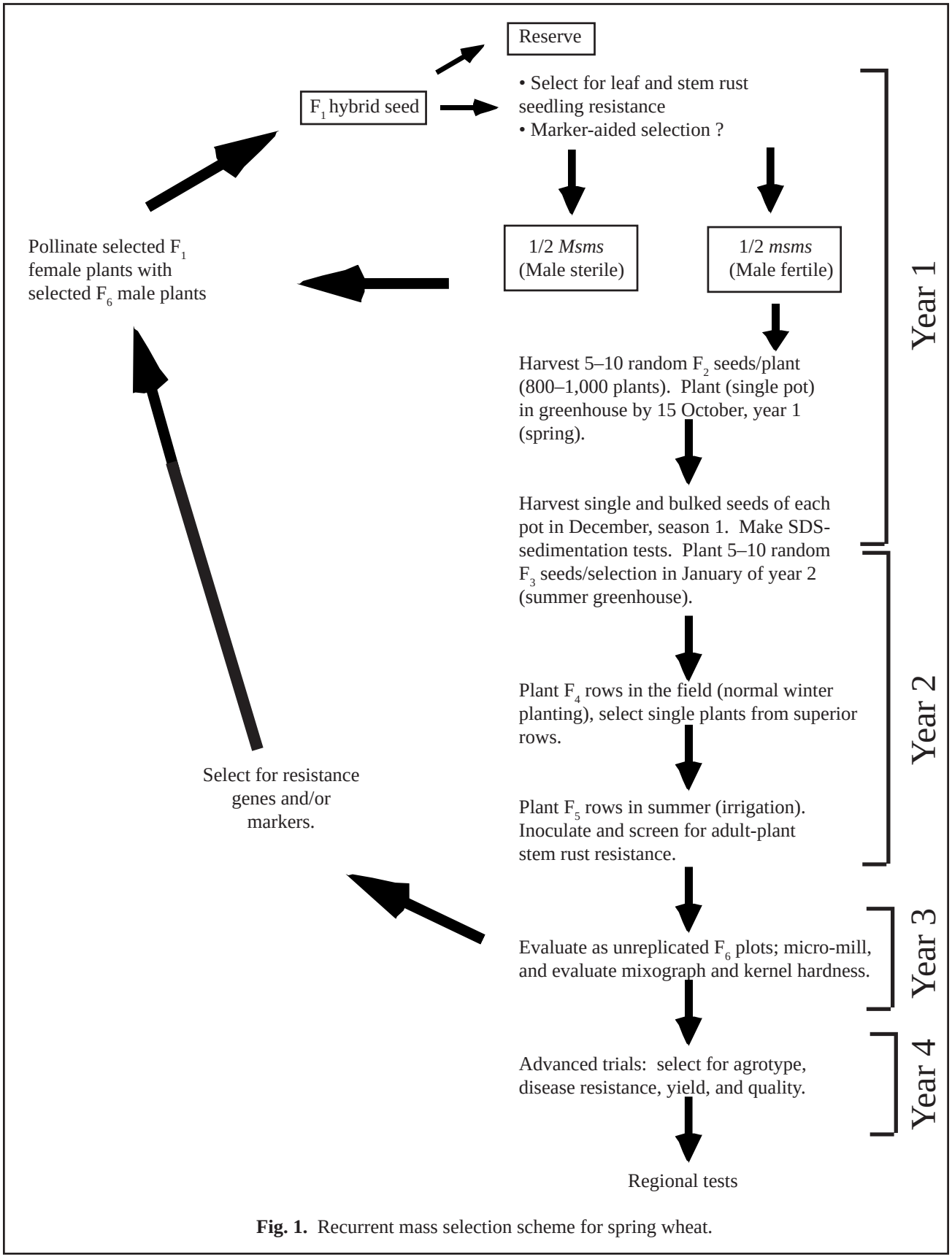

Fig. 1. Recurrent mass selection scheme for spring wheat.

the normal growing season (winter) and the F_5 planted under irrigation during the summer. The F_6 is used for the first yield trials (plots) in the third winter. This modification has several advantages. The shorter breeding cycle allows for a more rapid increase in the frequency of desirable alleles of disease resistance loci. However, properly selecting for agronomic and quality characteristics of lower heritability is still possible. Inbreeding to the F_6 has the same effect as the use of DH technology but can be achieved at considerably reduced costs. When inbred male parents are selected for marker or disease-resistance loci, very rapid shifts in the frequency of desirable alleles occur. Once the frequency of the favorable allele of a number of genes has been raised to 0.70 or higher, a significant proportion of inbreds will have these genes fixed in their genotypes, i.e., the procedure facilitates gene pyramiding. The advantage of pyramiding genes in this manner is that there is no yield ceiling as is the case with backcross-based procedures and numerous diverse genotypes with pyramided genes can be generated over time.

Genetic studies.

In 1993, we initiated a program for transferring leaf rust-resistance genes identified in a collection of wild *Triticum* species. We have developed advanced material of 11 sources that show effective resistance to all known local pathotypes of one or more of the diseases leaf, stem, and stripe rusts. These include a subset of six lines in which resistance (derived from *T. turgidum* subsp. *dicoccoides*, *Ae. sharonensis*, *Ae. speltoides*, *Ae. peregrina*, and *Ae. kotschyi*) appears to occur on wheat chromosomes and five addition lines with added chromosomes from *Ae. peregrina*, *Ae. umbellulata*, *Ae. biuncialis*, and *Ae. neglecta*. In several instances, promising stem rust and/or stripe rust resistance genes were co-transferred with leaf rust resistance. The stripe rust resistance genes (from *T. turgidum*, *Ae. sharonensis*, *Ae. speltoides*, *Ae. peregrina*, *Ae. kotschyi*, and *Ae. biuncialis*) also were effective against four Australian pathotypes and appeared to be novel (evaluations done by Dr. Colin Wellings, University of Sydney). Leaf rust-resistance genes in 10 of the sources showed promising resistance to commonly occurring Western Canadian pathotypes of the disease (evaluated by Dr. Brent McCallum, Cereal Research Centre, Winnipeg, Canada). Stem rust-resistance genes from two *Ae. speltoides* sources were tested with Western Canadian stem rust pathotypes (Dr. Thomas Fetch, Cereal Research Centre, Winnipeg, Canada). One source showed resistance to all pathotypes whereas another was susceptible to one of the pathotypes. All the genes appear to have a wide spectrum of resistance to justify continued introgression into wheat. Preliminary results would suggest that the *Ae. kotschyi*-derived genes (leaf and stripe rust resistance) occur on chromosome 2D, whereas leaf and stripe rust-resistance genes from *Ae. sharonensis* are on 3B. Resistance from *Ae. biuncialis* and *Ae. neglecta* appears to be on group-3 chromosomes of these species. Some of the resistance genes have preferential transmission and the *Ae. speltoides*-derived genes may involve gametocidal effects.

A unique, *Th. distichum*/ 4x rye hybrid (95M1) with genomes $J_1^dJ_2^dRR$ allowed us to identify four *Thinopyrum* chromosomes apparently involved with salt tolerance. When 95M1 was pollinated with diploid rye it yielded F_1 offspring with primarily 21 chromosomes (two complete rye genomes and seven *Thinopyrum* chromosomes). Apparently, the closely related homoeologous chromosomes of the J_1^d and J_2^d genomes regularly formed bivalents during megasporogenesis, and egg cells mostly received a random, yet balanced set of seven *Thinopyrum* chromosomes. F_1 plants were tested for salt tolerance and a set of 15 highly salt-tolerant F_1 plants were selected and maintained as clones for several years. These plants were C-banded and the *Thinopyrum* chromosomes in each line were determined. By comparing segregation patterns, the *Thinopyrum* chromosomes were grouped into seven homoeologous pairs. For each of four homoeologous pairs, one of its members occurred at a higher than expected frequency, implying that these chromosomes are primarily being expressed under salt-stress conditions. The results could be confirmed by backcrossing two of the most tolerant F_1 plants to diploid rye. Although the critical chromosomes can be identified through C-banding, an attempt also was made to find an RFLP marker for each. RFLP probes, diagnostic for the group 2, 3, 4, and 5 homoeologues of wheat, detected polymorphisms on the respective critical *Thinopyrum* chromosomes. However, the preliminary allocation of the critical chromosomes to homoeology groups needs to be confirmed using more and varied markers. An attempt also was initiated to develop triticale plants with disomic additions of the respective critical *Thinopyrum* chromosomes. Disomic addition lines producing the group-3 and 5 RFLPs of two of the target chromosomes have been recovered and are being used in attempts to induce translocations to triticale chromosomes.

ITEMS FROM SPAIN

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Studies in relation to the Hessian fly-resistance gene (H30) transferred from the wild grass Aegilops triuncialis to hexaploid wheat.

The transfer line TR-3531 (42 chromosomes), derived from the cross '*T. turgidum*/*Ae. triuncialis*//*T. aestivum*' and carrying the *H. avenae* resistance gene *Cre*7 (Romero et al. 1998), showed a high level of resistance to the *M. destructor* biotype prevailing in southwestern Spain. A single, dominant gene (*H30*) determines the Hessian fly resistance in this introgression line (Delibes et al. 2001), and its linkage with an isozyme marker (*Acph*-U1) has been studied. A phosphatase marker, resolved into two components, is present in the TR-3531 line, *Ae. triuncialis* (UC), *Ae. umbellulata* (U), and the amphiploid Chinese Spring/*Ae. umbellulata* (ABDU), but is absent in *Ae. caudata* (C). Linkage between *H30* and the *Acph-U1* marker (associated with the U genome) was determined by analyzing 126 individual (TR-3531/H-10-15) F_2 plants. The kernels were cut transversely and the halves without embryos were used to obtain phosphatase zymograms, an enzymatic system associated in wheat with homoeologous group 4 (Delibes et al. 1997a). The linkage in this cross is not very tight, which would be consistent with the recombination expected of the ability of the C genome to suppress the *Ph*-diploidization mechanism of wheat (Romero et al. 1998).

F_2 progeny, derived from crosses between different wheat cultivars from Uniform Hessian Fly Nursery (UHFN and H-93-33 transfer line) and with other sources of resistance, and TR-3531 were tested for resistance in field conditions in order to determine if the new resistance gene was allelic with the *H3*, *H5*, *H6*, *H12*, *H13*, *H18*, *H21*, or *H27* genes. Although the cultivars from UHFN are effective against Hessian fly in the United States, there is no evidence that the selected genes confer resistance to biotype present in Azuaga (southwestern Spain). The results and summarized in Table 1. All UHFN cultivars tested with different genes were resistant to this biotype, except the cultivar Abe with the gene *H5*, which showed a inconsistent reaction. The resistance gene *H30* in line TR-3531 is nonallelic with respect to the genes *H3*, *H6*, *H12*, *H13*, *H18*, and *H21* present in wheat cultivars from UHFN and *H27* in the introgression line H-93-33 (Delibes et al. 1997a and b). Previously, we demonstrated that *H30* in TR-3531 line was not allelic with respect *H9* and *H11* present in cultivars Ella and Kay, respectively (Delibes et al. 2001).

Advanced lines with the *H30* gene were obtained by backcrossing the transfer line and different commercial wheats (cultivars Anza, Betres, Cajeme, Cartaya, Marius, Rinconada, and Osona) as recurrent parents. In all advanced lines, the infestation level was higher, but in the same range, than the donor. Several agronomic characteristics were studied in 16 advanced lines and the results of three of the lines are summarized in the Table 2. The best results were

Table 1. Hessian fly reactions of the parents, F_1, and F_2 populations from crosses between wheats with different resistance genes and the resistant line TR-3531, a carrier of the *H30* gene. Hessian fly reaction of line R-3531 is 25R:0S. R = resistance and S = susceptibility to Hessian fly. UHFN = Uniform Hessian Fly Nursery.

			Hessian fly reaction Crosses between cultivars of the UHFN / TR-3531				
			UHFN line	No. F_1 plants	No. F_2 plants	$X^2_{(1:d.f.)}$ 15:1 ratio	
Cultivar or UHFN line	Gene	Chromo-some	R:S	R:S	R:S	Value	Probability (P)
Howell	*H3*	5A	18:0	7:0	154:8	0.46	0.5
Monon	*H3*	5A	19:0	9:0	187:6	3.25	0.05<P<0.1
Caldwell	*H6*	5A	29:0	8:1	218:14	0.02	0.9
841453							
H15-1-1-2-5-2	*H12*	5A	26:1	2:0	95:1	4.45	0.01<P<0.05
86925 RA1-16	*H13*	6DL	18:2	3:0	140:7	0.55	0.3<P<0.5
Brule	*H18*	—	15:4	7:0	141:2	5.74	0.01<P<0.05
KS86HF012-23-6	*H21*	2BS	19:0	7:0	243:6	6.27	0.01<P<0.05
H-93-33	*H27*	$4M^v$	21:0	7:0	50:5	0.76	0.3<P<0.5

achieved with the Ma-6 line, which displayed good agronomic characteristics, in comparison to the susceptible controls, for the three traits studied. Another fact that increases the importance of this line is that it also carries the CCN resistance gene *Cre*7.

Table 2. Agronomic characteristics of three advanced lines with the *H30* resistance gene in comparison to three bread wheat cultivars.

Cultivar or line	Yield (g/m^2)	Kernels /spikelet	1,000-kernel weight (g)
T. aestivum cv. Osona	1,240.57	2.71	29.49
T. aestivum cv. Astral	897.55	1.64	30.17
T. aestivum cv. Adalid	1,687.51	2.65	33.14
Ma6: TR/OS⊗//OS/3/RN/4/OS/5/RN/6/AZ 3⊗	1,914.12	3.00	43.37
Ma4: TR/BT⊗//AL/3/MA/4/3*BT 2⊗	1,336.08	2.84	27.65
Ma3: TR/3*OS//4*CYÄ/3/CJ 4⊗	1,486.86	2.64	32.50
Least significant difference ($LSD_{P<0.05}$)	409.16	0.47	5.05

Abbreviations used: Ma = advanced lines, TR = TR-353 line, AZ = Anza, BT = Betres, CJ = Cajeme, CY = Cartaya, MA = Marius, OS = Osona, RN = Rinconada, ⊗ = selfing.

Coöperation with other institutions. We are coöperating with Acorex (Cooperative of Extremadura farmers).

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ITEMS FROM TURKEY

CIMMYT AND THE MINISTRY OF AGRICULTURE AND RURAL AFFAIRS P.K. 39 Emek, 06511 Ankara, Turkey.

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The International Winter Wheat Program (IWWIP) is a joint program carried out by the Ministry of Agriculture of Turkey, CIMMYT, and ICARDA. The two main objectives of the program are to develop broadly adapted, disease-resistant, high-yielding winter wheat germ plasm for the winter and facultative wheat-growing areas in Central and West Asia and North Africa (CWANA) and to help facilitate germ plasm exchange among the winter wheat-breeding programs around the world.

Of the 103 million ha of wheat grown in the least-developed countries. Approximately 31 x 10^6 ha are winter and facultative wheat, of which 16.5 x 10^6 ha are sown in CWANA; 13 x 10^6 ha in China; and 1 x 10^6 ha in South America, North Africa, and North Korea. After China, Turkey is the 2nd largest winter wheat grower among the least-developed countries with 6.6 x 10^6 ha; followed by Iran with 4 x 10^6 ha.

News on germ plasm development and cultivar releases.

Since 1980, 27 cultivars from the IWWIP program have been released in CWANA. In 2002 alone, nine cultivars were released in Afghanistan (1), Georgia (1), Turkey (6), and Uzbekistan (1) (Table 1). Three of these cultivars are targeted for rainfed areas and five for irrigated/supplementary irrigation conditions. Thirty-four cultivars are presently included in registration trials in Armenia (6), Georgia (1), Kazakhstan (2), Kyrghyzstan (7), Tajikistan (6), Turkey (5), Turkmenistan (3), and Uzbekistan (4).

The winter wheat program draws heavily on the winter (W)/spring (S) crosses. A major contribution to the winter wheat program is made through the spring wheat lines developed at CIMMYT–Mexico, which are crossed with winter wheats. Many of the most successful CIMMYT spring wheats were derived from W/S crosses. Now, the same is happening for winter wheat. More than 75 % of the IWWIP lines released or in registration trials are selected from crosses between winter and spring wheat lines and three-way crosses (winter/spring//winter). These WSW-derived cultivars are now making their way into registration trials throughout the CWANA region (Fig. 1).

Table 1. International Winter Wheat Program-derived wheat cultivars registered in Central and West Asia and Northern Africa in 2002.

Country	Cultivar	Cross	Type
Afghanistan	Solh02	OK82282//BOW/NKT	WS
Georgia	Mtsjetslaua 1	TAST/SPRW//ZAR	WS
Turkey	Soyer	ATAY/Galvez	WS
Turkey	Yildirim	ID800994.W/VEE	WS
Turkey	Daphan	JUP/4/CLLF/3II14.53/ODIN//CI14431/WA00477	WS
Turkey	Bagco 2002	HN7/Oorfen//BJN8/3/SERI82/4/74CB462/Trapper//Vona	WW
Turkey	Nenehatun	ND/P101/Blueboy	
Turkey	Sakin	PI/FUNO*2//VLD/3/CO723595	WW
Uzbekistan	Dostlik	YMH/TOB//MCD/3/LIRA	WS

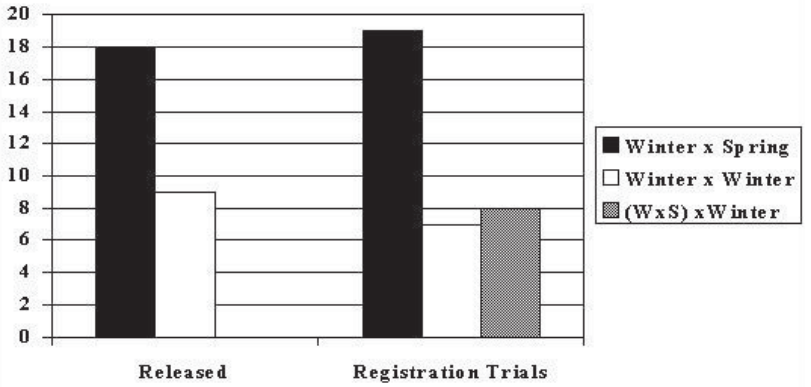

Fig 1. Frequency of winter (W)/spring (S), W/W, and W/S//W crosses in International Winter Wheat Program-derived cultivars registered since 1980 or lines presently in registration trials in Central and West Asia and Northern Africa.

News on germ plasm exchange: the case of yellow rust.

The Facultative and Winter Wheat Observation Nursery (FAWWON) has served as the main vehicle for facilitating germ plasm exchange among winter wheat programs. This nursery consists of lines developed by the IWWIP program and of cultivars submitted by national programs, university programs, or private companies from countries in CWANA, western and eastern Europe, China, South America, and the U.S.A. The 11th FAWWON consisting of 146 entries was distributed for planting in the 2001–02 cropping cycle to around 80 coöperators from more than 40 countries

Yellow rust is one of the most important leaf diseases for the winter wheat areas in west and central Asia. Within the last decade, CWANA countries suffered several major yellow rust epidemics, with losses up to 50 %. Fig. 2 shows the maximum yellow rust score from the evaluation of the 11th FAWWON across 10 locations in Iran (5), Turkey (1), Azerbaijan (1), Tajikistan (1), Syria (1), and China (1). Characteristically, most lines developed by the IWWIP program show good levels of resistance, whereas most other lines are highly susceptible to yellow rust. The fact that many yellow rust-susceptible, but otherwise excellent, lines with highly favorable characteristics will be dismissed by breeders due to yellow rust susceptibility and, therefore, not utilized by breeding programs has forced us to think of ways to restructuring the FAWWON nursery. These changes will be implemented within the coming year.

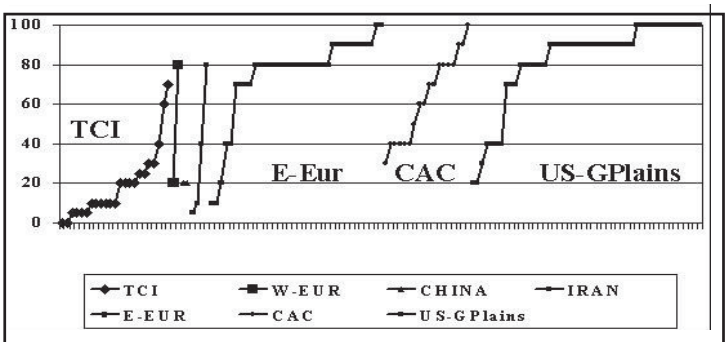

Fig 2. Maximum scores for resistance to yellow rust across 10 locations in six countries in Central and West Asia and North Africa of 146 entries in the 11th Facultative and Winter Wheat Observation Nursery. Entries within each group of origin are sorted by increasing susceptibility.

Research on root rots and nematodes.

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Since 1998, the Ministry of Agriculture and Rural Affairs in Turkey (MARA) in collaboration with CIMMYT staff based in Turkey have initiated two key National/International projects. One of these is on cereal nematodes and the other on cereal root rots. These projects cover a range of research areas including;

– surveys,
– economic importance and population dynamics,
– identifying of sources of resistance, and
– control methods emphasising plant genetic resistance.

Below is a brief summary of some of our findings to date. We very much encourage anyone interested in collaborating with our program to make contact with us.

Preliminary surveys. These surveys have been conducted on the Central Anatolian Plateau, the major winter wheat-growing region of Turkey. The objective was to understand the distribution of two economically important cereal nematodes, cyst (*Heterodera spp.*), and lesion (*Pratylenchus spp*.). Seventy-two percent of the root samples and 83 % of the soil samples contained cysts and in approximately 40 % of soil samples one or both lesion nematodes were found. Cereal cyst nematode was identified to species level using both traditional morphology and a RFLP PCR-based molecular method. None of the samples contained *H. avenae*, the most common cereal cyst nematode documented. Instead, 40 % of the samples contained *H. latipons,* 32 % *H. filipjevi*, and 28 % a mix a both species

A range of Fusarium species have been isolated from cereal crown roots with the most frequently isolated species being *F. culmorum*, *F. nivale*, *F. psuedograminearum*, *F. acuminatum*, and *F. heterosporum*.

The taxonomy of cyst nematode is very time consuming and difficult, and work is underway to optimize the molecular technology to identify the different species of *Heterodera*. We hope in the near future to relate and collate survey data with both classical morphology and molecular methods.

In many cases, several species of nematodes and root rots are present in the same soil, suggesting that we are dealing with a root disease complex and management strategies need to account for this. In addition, zinc deficient soils are widespread and can be considered part of the problem complex (Cakmak et al. 1999).

To understand the economic importance and population dynamics of both nematodes and root rots, multi-location and multi-year yield trials are being conducted. Work with root rots is more advanced than with nematodes. Data from 2-year yield trials with and without root rot inoculation (inoculated as a mix of both *Fusarium* species and *Bipolaris*) indicate that most of the common winter wheat cultivars grown in Turkey are intolerant suffering average yield losses of 37 %. Furthermore, preliminary data of only 1 year suggests strongly that sources of spring wheat resistance identified in Australia and confirmed at CIMMYT–Mexico also are resistant and have high tolerance under field conditions in Turkey. We are in the second year of field testing with nematodes in three locations. Preliminary data from last year clearly show economic grain loss, with most common winter wheat cultivars being intolerant and susceptible (i.e., allow nematode multiplication) to both the lesion and cyst nematodes. Detailed knowledge is available about the population dynamics of the lesion nematodes (*P. thornei* and *P. neglectus*) and at least one of the species of cyst nematode (*H. avenae*). However, little is known about the biology and behavior of other *Heterodera* species that are commonly found in winter wheat areas of the world, namely *H. filipjevi* and *H. latipons*. In addition to monitoring field-population dynamics, we also conduct a range of basic biological experiments to identify factors that affect the hatch of the cysts and to understand the duration of the life cycle and the infection process.

Identification of sources of resistance. A major focus of the work is to screen winter wheat and identify sources of resistance to both the key nematodes and root rots. Again, this work is more advanced with root rots than nematodes on winter wheat. Over 100 crosses/year are being made with sources of both nematode and root rot-resistant germ plasm

(Nicol 2002; Nicol et al. 2001). Spring wheat lines with confirmed root disease resistance also are used in the crossing program.

A large screening program in the south of Turkey near Konya was established where annually around 2,000 accessions are inoculated with a root rot complex. The best accessions form screening nurseries are further tested in replicated screening and yield trials. Resistant lines are confirmed based on field trials and greenhouse work. Screening for nematode resistance concentrates on field screening at present, but work is underway to establish pure nematode cultures in the laboratory and ultimately screen with individual nematodes under controlled laboratory conditions. With cyst nematode, more work is required to understand the biology before such screening can be conducted on a larger scale.

Work within CIMMYT also is utilizing the tools of molecular biology in a MAS strategy. Several markers for known nematode-resistance genes developed in Australia are optimized and are being used routinely on CIMMYT germ plasm. However, given the complexity of the nematode in the region we need to confirm the effectiveness of these known sources of resistance with the range of nematode populations from the region.

Once we have more advanced plant populations (F_4/F_5) where resistance has been incorporated, we will conduct confirmation screening to validate the incorporation of resistances using both traditional and molecular tools where appropriate. As we produce these root disease resistant wheat lines, they will be distributed through the international nurseries.

Cereal nematodes and root rots can be controlled in several ways. The major emphasis in our program is placed on using plant genetic resistance. Because resistance alone is probably not the complete answer (as many resistances are partial), other methods need to be investigated. To control root rots, we will look at the effects of seed treatment with fungicides, application of microelements (including B, Cu, Fe, Mn, S, and Zn), seed-sowing density, and rotation experiments of cereals with other non-cereal crops (such as canola and sugar beet). With nematodes, we will look at the effect of crop rotation and management practices (such a conservation tillage and cultivation) on nematode numbers. As has been proven in the U.S. and Australia, there is no doubt that agronomic practices have a key role to play in the control of these pathogens.

Progress to date on breeding for root rot resistance and tolerance.

Extensive field screening over the last 3 years has assessed the resistance of winter wheat germ plasm against root rots under field conditions. Resistance is defined as a reduction in symptom development of the disease. In Cumra, 40 km south of Konya, field-observation plots were assessed by inoculating seed with a combination of root rot species (*Fusarium* and *Bipolaris*) and comparing symptom development against uninoculated plots. These lines are now entering yield trials to assess tolerance (yield loss) and also have been extensively crossed in the IWWIP program. The best entries after 3-year screening are shown in Table 2. Several of the identified lines are widely grown cultivars such as Gerek 79, Dagdas, and Katia-1.

Training. The IWWIP program is training Turkish scientists and scientists from the region in the field of soil disease cereal research. This includes postgraduate training and special courses such as the one planned for June 2003 in Turkey. Several Australian pathologists will attend this course to provide their expertise and knowledge. The training course is called ‘Soil Borne Pathogens of Cereals’ and will be from 14–28 June under the coördination of the IWWIP. Participants will be trained to work with both nematodes and root rots. We are very grateful to the sponsors, principally led by the ATSE Crawford Fund, CIMMYT, MARA, ICARDA, GRDC, ACIAR, and the Kirkhouse Trust.

Concluding remarks. We believe by conducting this highly focused, complex and difficult research we can clearly define the soilborne constraints in the winter wheat regions of CWANA and ultimately significantly improve wheat production and sustainability of the cropping systems in our region. The key to this will involve a breeding approach to produce high-yielding, quality-adapted germ plasm combined with multiple root disease resistances and microelement efficiencies, complimented with appropriate management practices. This work is large and encompassing, and we welcome any collaboration from interested parties.

Table 2. Germ plasm with field resistance to the root complex after 3 years of field inoculation experiments in Cumra, Turkey. TK = Turkey, TCI = TURKEY/CIMMYT/ICARDA IWWIP.

Cross	Origin
LOV41//LI7/LE2062	Argentina–TCI
Katea-1	Bulgaria–Sadovo
Dachnaya/LAJ3302	TCI
Bilinmiyen96.7	TCI
Burbot-6	TCI
Zargana-2	TCI
Zargana-3	TCI
ECVD12/KAUZ//Unknown	TCI
F12.71/SKA//FKG15/3/F483/4/CTK/VEE	TCI
F130L1.12/Attila	TCI
KRC66/SERI//KINACI79	TCI
KS82W409/SPN//CA8055	TCI
NEMURA/KAUZ//AGRI/NAC	TCI
OK81306//ANB/BUC/3/GRK/7C	TCI
OK81306/SITTA//AGRI/NAC	TCI
Orkinos-1	TCI
Orkinos-3	TCI
PYN//TAM101/AMI/3/KRC66/SERI	TCI
Sultan 95	TCI
TAM200/KAUZ	TCI
BEZ/TVR/5/CFN/BEZ//SUW92/CI13645/3/NA160/4/EMU/6/UNA	TK–Edirne
BEZ/HAWK//ES14	TK–Eskisehir
Cerco/Alondra	TK–Eskisehir
ES 14/Flamura 85	TK–Eskisehir
GEREK79	TK–Eskisehir
BLL2973/Thunderbird	TK–Konya
DAGDAS	TK–Konya
HAWK/AIRI	TK–Konya
PLK70/LIRA”S//30-KZ-1	TK–Konya
TX71A1039-VI*3/AMI(TX81V6603)//MVR16-85	TK–Konya
HARA456/4/61-130/414-44//68111/WARD/3/69T02	TK

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ITEMS FROM THE UKRAINE

INSTITUTE OF PLANT PRODUCTION N.A. V.YA. YURJEV
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The relationship between glume, lemma, and kernel size in polonoid wheats.

R.V. Rozhkov and O.O. Kushchenko.

Polonoid wheats are species that have the traits of *T. turgidum* subsp. *polonicum*, including very long (2.5–4.5 cm or greater) glumes with a straw-like constituency, long lemmas twice the size of the paleas, well-marked knobs on the rachis under the glume that are lacking in other wheat species, and long large kernels. The polonoid wheat group includes the naked species *T. turgidum* subsp. *polonicum*, *T. petropavlovskyi* Udacz. et E.Migusch. (2n = 42), and the hulled wheat *T. ispahanicum* Heslot (2n = 28). The last species does not have knobs on the rachis. Watanabe (2001) determined that the gene for the polonoidy traits are on chromosome 7A in *T. polonicum* and *T. petropavlovskyi* and 7B in *T. ispahanicum*. thus, the polonoidy may have different genetic nature.

Polonoidy may be of practical interest because glumes are one of the photosynthetic organs that are the youngest and nearest to the kernel, therefore, they may be a significant reserve for providing kernels with nutrients and determine kernel size. In 2001 and 2002, we studied the polonoid traits displayed in three polonoid species and their F_1 hybrids with *T. durum* cultivar Kharkivs'ka 19 and *T. aestivum* cultivar Kharkivs'ka 28. The first and second florets of the medial spikelet were analyzed in at least 25 spikes for each accession or hybrid. We measured the lemma and palea - length and width of the glume and the length, width, height, and weight of the grain. Paired correlation coefficients (CC) and degree of dominance (D) were calculated for these traits.

Tables 1 and 2 list the results of the 2-year study. The two tetraploid polonoid species had CCs between dimensions of glume, lemma and palea and grain length and weight of the second floret that exceeded the index for first floret and in most cases were nonsignificant. On the other hand, the CCs between glume, lemma, and palea dimensions for the second floret were less than those for the first. At the same time, almost all the CCs for first floret are greater than those for the second in the hexaploid polonoid and were moderately to highly significant. In the Kharkivs'ka 19 and Kharkivs'ka 28 cultivars, the CCs were low to moderate and differences between the first and second florets were not similar for the different trait pairs.

For the F_1 hybrid '*T. ispahanicum*/Kharkivs'ka 19', the CCs for all trait pairs for the second floret of a spikelet and the most of them for the first floret were high and significant (from 0.71 to 0.94). The CC for one of the most interesting trait pairs, glume length–grain length, is very high, significant for the second floret (0.94) and moderately significant for the first (0.58). The CCs for the another interesting trait pair, glume length–grain weight, is high and significant for the first (0.84) and second (0.74) florets. The CC values indicate heterosis for all trait pairs on the second floret and for the most of the trait pairs on first floret in '*T. ispahanicum*/*T. durum* Kharkivs'ka 19' hybrids in comparison to the parental lines.

In the '*T. polonicum*/Kharkivs'ka 19' F_1 hybrid, the CC for glume length–grain length in the first floret is positive, moderate, and significant. For the other trait pairs including grain length and weight, the CCs were not significant, moderate, or low. The CCs between glume, lemma, and palea dimensions are high and positive. These parents also have low, often negative, CCs, whereas the hybrids have positive moderate, though nonsignificant, CCs as a rule.

In the F_1 hybrid '*T. petropavlovskyi*/Kharkivs'ka 28', nearly all CCs including grain length and weight are nonsignificant for both the first and second florets. The CCs for glume, lemma, and palea are high and significant.

From the three polonoid species, only *T. ispahanicum* displays dominance by glume length (D = 0.2) in hybrids with *T. durum* cultivar Kharkivs'ka 19. In the two other hybrids, '*T. polonicum*/Kaharkivs'ka 19' and '*T. petropavlovskyi*/Kharkivs'ka 28', this trait is inherited as a recessive (D = –0.2 and –0.4 respectively). For grain length

Table 1. Correlation coefficients between the morphological traits of spikelets in the polonoid species, *T. durum*, *T. aestivum*, and their F_1 hybrids, first floret. *Ti* = *Triticum ispahanicum*, *Tdp* = *T. turgidum* subsp. *polonicum*, *Tp* = *T. petropavlovsky*, K19 = *T. durum* subsp. *durum* cultivar Kharkivs'ka 19, and K28 = *T. aestivum* subsp. *aestivum* cultivar Kharkivs'ka 28. Significant correlations are indicated with an asterisk (*).

Trait pairs	Hybrids and parental forms							
	Ti	F_1 *Ti*/K19	K19	*Ttp*	F_1 *Ttp*/K19	*Tp*	F_1 *Tp*/K28	K28
Glume length–grain length	0.16	0.58 *	0.32	–0.40	0.50 *	0.69 *	0.26	0.21
Glume width–grain length	–0.40	0.05	0.08	–0.29	0.38	0.35	–0.02	0.34
Lemma length–grain length	0.35	0.54	0.49 *	–0.21	0.42	0.71 *	0.43 *	0.39
Palea length–grain length	0.32	0.32	0.18	–0.18	0.33	0.65 *	0.23	0.47 *
Glume length–grain weight	-0.11	0.74 *	–0.18	–0.68 *	0.31	0.29	0.20	0.16
Glume width–grain weight	0.13	–0.03	–0.28	–0.72 *	0.30	0.04	–0.26	–0.15
Lemma length–grain weight	0.15	0.68 *	–0.01	–0.53 *	0.20	0.39	0.32	0.01
Palea length–grain weight	0.42	0.67 *	0.07	–0.42	–0.11	0.28	0.09	0.49 *
Glume length–glume width	0.48 *	0.36	0.09	0.81 *	0.85 *	0.52 *	0.73 *	0.21
Glume length–lemma length	0.72 *	0.97 *	0.44 *	0.77 *	0.91 *	0.79 *	0.87 *	0.40
Glume length–palea length	0.62 *	0.83 *	0.53 *	0.66 *	0.70 *	0.39	0.71 *	0.27
Lemma length–palea length	0.55 *	0.83 *	0.41	0.60 *	0.71 *	0.55 *	0.78 *	0.06

Table 2. Correlation coefficients between the morphological traits of spikelets in the polonoid species, *T. durum*, *T. aestivum*, and their F_1 hybrids, second floret.

Glume length–grain length	0.44 *	0.94 *	0.49 *	0.08	–0.03	0.18	0.42	0.38
Glume width–grain length	0.35	0.71 *	0.15	0.13	–0.01	–0.08	0.21	0.28
Lemma length–grain length	0.63 *	0.84 *	–0.03	0.18	0.07	0.37	0.00	0.11
Palea length–grain length	0.39	0.76 *	0.23	0.08	0.18	0.38	–0.12	0.25
Glume length–grain weight	0.04	0.84 *	–0.05	–0.20	0.02	–0.28	0.23	0.40
Glume width–grain weight	0.40	0.84 *	–0.54 *	0.09	–0.00	0.02	–0.06	0.12
Lemma length–grain weight	0.22	0.85 *	–0.20	0.29	0.01	–0.06	–0.05	–0.07
Palea length–grain weight	0.04	0.77 *	0.03	0.17	–0.06	0.05	0.09	0.33
Glume length–glume width	0.41	0.77 *	0.44 *	0.67 *	0.87 *	0.37	0.81 *	0.08
Glume length–lemma length	0.64 *	0.91 *	0.37	0.48 *	0.81 *	0.45	0.78 *	–0.12
Glume length–palea length	0.49 *	0.79 *	0.47 *	0.35	–0.17	0.35	0.43	0.17
Lemma length–palea length	0.34	0.92 *	0.58 *	0.44*	0.02	0.71 *	0.64 *	0.57 *

and weight, dominance of *T. polonicum* (0.7 and 0.9, respectively), overdominance of *T. ispahanicum* (1.4 and 2.6 respectively), and recessiveness of *T. petropavlovskyi* (–0.2 and –0.4, respectively) were observed.

Triticum ispahanicum is dominant over *T. durum* in lemma length (D = 0.7) and is overdominant for palea length (3.7). *Triticum petropavlovskyi* is dominant over *T. aestivum* for lemma and palea length (the both 1.0) and grain height (0.3) and is overdominant for grain width (2.0).

Kharkivs'ka 19 and Kharkivs'ka 28 dominate at various rates for glume width in hybrids with *T. ispahanicum* (D = 0.1) and *T. petropavlovskyi* (0.7). *Triticum durum* is dominant over *T. ispahanicum* for grain width (1.0) and height (0.5), over *T. polonicum* for lemma length (0.6) and grain width and height (the both 1.0), and is overdominant for glume width (2.0) and palea length (3.0). *Triticum aestivum* is dominant over *T. petropavlovskyi* in grain height (0.3) and overdominant for grain width (2.0).

Examining glume length with grain length and weight under the growing conditions at Kharkiv, the '*T. ispahanicum*/Kharkivs'ka 19 durum' F_1 hybrids are early ripening when compared with the durum parent, *T. polonicum*, *T. aestivum*, *T. petropavlovskyi*, and their hybrids. The grains of *T. ispahanicum* have time to fully develop, whereas the other parental lines and hybrids cease grain filling before maturity, which is caused by hot and dry summer temperatures. A clear relationship exists between the glume dimensions and grain length and weight in hybrids with *T. ispahanicum*.

Hence, *T. ispahanicum* seems to by more perspective source of polonoid complex for wheat breeding, than *T. polonicum* and *T. petropavlovskyi*. Moreover, the F_1 hybrids of all the three polonoid species are more adaptive than their parental forms and, as a rule, CCs are more positive and higher in the hybrids than the parents. Improvement may be gained in grain size (and other related traits) in *T. aestivum* and *T. durum* advanced cultivars by means of addition of polonoid complex if early ripening genotypes are used.

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Sowing dates, rates, and phytosanitary state of winter wheat fields.

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These studies were conducted at the Plant Production Institute named after V.Ya. Yuryev (Eastern Forest-Steppe of Ukraine) in 2001–02. The soil was a typical weakly leached, medium humus, black earth soil. The agrotechnique was general use. The three sowing dates were 10–13 September, 20–22 September, 30 September–2 October. The sowing rate was 4.0–5.0 x 10^6 viable seeds/hectare. The relationship between the phytosanitary state of winter wheat, the sowing dates, and the sowing rates was studied by preceding black fallow on a manure background, 30 t/ha along with NPK30 application. Plant damage from disease and cereal flies was studied using conventional methods.

In the experimental years, spread and development of root rots (*Helminthosporium* and *Fusarium*) and the intensity of disease development on winter wheat leaves differed for the sowing dates (Table 3). The later sowing data had a considerable reduction in spread and development of root rots and leaf infection by Septoria in 2001. Leaf rust occurred less at the first sowing date than at the other two. Damage by powdery mildew was low. The least amount of shoot damage by cereal flies was observed at the third sowing date; the highest degree was at the second sowing date. In 2002, the spread and development of root rots did not vary among different sowing dates. The degree of the development of Septoria increased with a delay of the sowing date. Leaf rust and powdery mildew were not observed. Damage to the shoots of winter wheat by cereal flies was not considerable.

Over 2 years, damage by root rots was reduced considerably at later dates of sowing on average. The intensity of Septoria infection on the upper leaves of winter wheat planted at the first sowing date was 1.6 times lower compared with the third date. Leaf rust infection in wheat planted at the second and third dates exceeded that in wheat sown at the first date on all leaves by 2.5–2.6 times and on the upper leaves by 2.2–2.3 times. The lowest degree of shoots damage by cereal flies was recorded at the third date of sowing winter wheat and highest at the first date.

A shift in sowing date led to changes in the phytosanitary state of winter wheat and, as a result, to the changes in yield capacity of the crop. Thus, there was a progressive increase in yield from the first to the third sowing dates in 2001 and, a decreased grain yield with a delay of planting in 2002. On average over 2 years, grain yield increased by 2.6–7.6 c/ha at the third sowing date in comparison with that at the second and first sowing dates.

Table 3. Phytosanitary state of winter wheat plants depending on sowing dates.

Sowing date	Root rots at tillering (%) spread	Root rots at tillering (%) development	Septoria all layers	Septoria upper layer	Powdery mildew all layers	Powdery mildew upper layer	Leaf rust all layers	Leaf rust upper layer	Shoot damage by cereal flies (%)	Grain yield (c/ha)
2001										
I	34.6	13.2	36.6	7.5	0.3	0.2	5.1	6.6	3.0	48.9
II	23.1	8.6	26.0	3.4	1.1	0.4	13.1	15.1	3.7	60.9
III	12.8	4.6	24.0	3.4	1.4	0.1	13.6	14.5	0.7	72.2
LSD_{05}	—	5.3	—	2.6	0.7	—	7.1	7.2	0.8	—
2002										
I	48.7	21.3	46.0	18.2	0.0	0.0	0.0	0.0	2.0	78.6
II	49.7	24.5	52.0	30.6	0.0	0.0	0.0	0.0	0.6	76.7
III	47.0	25.1	53.5	36.7	0.0	0.0	0.0	0.0	0.0	70.6
LSD_{05}	—	6.8	7.8	14.9	—	—	—	—	0.8	—
Mean (2001–02)										
I	41.7	17.3	41.3	12.9	0.2	0.1	2.6	3.3	2.5	63.8
II	36.4	16.6	39.0	17.0	0.6	0.2	6.6	7.6	2.2	68.8
III	29.9	14.9	38.8	20.1	0.7	0.05	6.8	7.3	0.4	71.4

The effect of sowing rates on the phytosanitary state of winter wheat planting is given in Table 4. In 2001, we noted that the sowing rate of 4–5 x 10^6 viable seeds/ha did not differ with respect to the damage by diseases and cereal flies. The 2002 data showed that an increase in sowing rates from 4–5 x 10^6 viable seeds increased damage by root rots and Septoria. Powdery mildew and leaf rust were not factors in 2002.

The spread and development of root rots at the increased sowing rate did not increase considerably on average over 2 years. Septoria infection on all leaves of the winter wheat plants in these variants was approximately the same as that of the upper leaves for planting rates with 5 x 10^6 seeds; exceeding this index compared with a usual rate of 4 x 10^6

Table 4. Phytosanitary state of winter wheat on black fallow depending on a sowing rate ($N_{30}P_{30}K_{30}$).

Sowing date	Root rots at tillering (%) spread	Root rots at tillering (%) development	Septoria all layers	Septoria upper layer	Powdery mildew all layers	Powdery mildew upper layer	Leaf rust all layers	Leaf rust upper layer	Shoot damage by cereal flies (%)	No. of productive tillers (m^2)	Grain yield (c/ha)
2001.											
4.0	34.6	13.2	36.0	7.5	0.2	0.1	3.5	5.5	2.5	601	47.4
5.0	34.9	13.5	36.6	7.5	0.3	0.2	5.1	6.6	3.0	701	48.9
LSD_{05}	—	—	—	—	0.1	—	—	—	—	—	—
2002.											
4.0	48.7	21.3	46.0	18.2	0.0	0.0	0.0	0.0	2.0	664	78.6
5.0	67.6	27.4	53.4	31.8	0.0	0.0	0.0	0.0	1.2	690	84.6
LSD_{05}	—	—	3.4	3.0	—	—	—	—	—	—	—
Mean (2001–02).											
4.0	41.7	17.3	41.0	12.9	0.1	0.05	1.8	2.8	2.3	633	63.0
5.0	51.3	20.5	45.0	19.7	0.2	0.1	2.6	3.3	2.1	696	66.8

seeds. The variants with different sowing rates did not vary greatly in relation to the damage of shoots by cereal flies. In winter wheat sowings at 5 x 10^6 viable seeds/ha, the number of productive tillers increased and, as a result, grain yield was higher by 3.8 c/ha compared to the yield at 4 x 10^6 seeds/ha.

Productivity of spring durum wheats at different seeding rates.

O.V. Golik.

Creating new spring durum wheat cultivars demands a variety of agrotechnics for obtaining the highest yield while maintaining high grain quality. Spring wheat in the Ukraine is grown in risky agricultural conditions. Moisture is the main limiting factor and an optimal seeding rate is the main factor for greatest yield. According to most authors, crops with optimal and dense seeding rates ensure the largest grain yield in any agroecological conditions (Makrushin 1985; Sechnyak et al. 1983). Overgrown plants in thin crop stands have an increased vegetative period and a higher degree of infection by fungal diseases and pests. Thinning crops promotes weed growth. All of these factors decrease the seed-sowing quality (Chulkina et al. 2000). Lelli (1980) stated that the productivity potential of a plant is hereditary and depends on genetically and ecological conditions. Studying the elements of yield structure is a prerequisite of this potential. Thus, we wanted to investigate and determine optimal seeding rates for new cultivars bred for the conditions of the eastern Forest-Steppe Region of the Ukraine.

We analyzed the spring durum wheats Kharkovskaya 15 and Kharkovskaya 23 (standards of the Ukrainian state Service on right protection for plant cultivars), Kharkovskaya 46 (grain quality standard), and Kharkovskaya 19 (lodging-resistance standard) and the new cultivars Kharkovskaya 27, Kharkovskaya 33, and Kharkovskaya 41 under the climatic conditions of the eastern Ukraine (Kharkov) between 1998 and 2000. The characters analyzed included productivity (g/m^2), grain yield (%, the ratio weight of kernels to weight of chaff), plant stand (plant/m^2), productive tillering, and 1,000-kernel weight (g) with seeding rates of 3, 4, 5, 6, and 7 x 10^6 germinating kernels/hectare (MKH). The humidity varied over the years; 1998 and 1999 were severe droughts and 2000 was optimal but irregular in different vegetative phases. The 1-m^2 plots were replicated three times. The cultivars were sown in an experimental field following peas. The results are from a three-factor dispersion analysis.

No differences were observed for durum wheat with different seeding rates. The productivity exceeded the general mean (155 g/m^2) at seeding rates of 3, 5, and 7 MKH (172–175 g/m^2) only in 2000. The least productivity was 146 g/m^2 in 1998. The highest mean productivity (170 g/m^2) was found in Kharkovskaya 33 at 3 MKH (Table 5 represents data for only for best (Kharkovskaya 33) and worst (Kharkovskaya 19) cultivars. The productivity of these cultivars was equal to or higher than the general mean in all

Table 5. The influence of seeding rates on productivity and associated traits in durum wheat cultivars, 1998–2000. * = reliable in comparison with the means by cultivars, ** = reliable in comparison with means by experiment (by trait), and *** = reliable means by cultivar.

		The mean indices of traits				
Cultivar	Seeding rate (x 10^6/ha)	Productivity (g/m^2)	Grain yield (%)	Plant stand plants/m^2	Productive tillering	1,000-kernel weight (g)
Kharkovskaya 33	3	183 *	31.4	172 *	0.99	34.5 *
	4	157	32.2	197 *	1.00 *	34.6 *
	5	184 *	30.6	236	1.00 *	33.5
	6	150	29.8	259 *	0.93	31.4 *
	7	179 *	31.8	292 *	1.01 *	31.0 *
Mean by cultivar		170 **	31.2	231	0.99	33.0 **
Kharkovskaya 19	3	113 *	29.6	146 *	0.91 *	32.7 *
	4	154	29.7	173 *	0.92 *	33.6
	5	135	27.1 *	209 *	0.91 *	32.2 *
	6	128 *	29.2	234	0.89 *	31.2 *
	7	130 *	28.9	264 *	0.89 *	32.1 *
Mean by cultivar		132 **	28.9 **	205 **	0.90 **	32.4 **
Mean by experiment		155	30.8	227	0.96	33.9
Least significant difference 5 %		9.4	1.06	11.9	0.037	0.53

variants. The highest value was 233 g/m^2 at 7 MKH for Kharkovskaya 33 in 2000. Thus, Kharkovskaya 33 was the most productive cultivar grown in the climatic conditions of the Ukraine. The basic group of tested wheats (Kharkovskaya 15, Kharkovskaya 23, Kharkovskaya 27, Kharkovskaya 41, and Kharkovskaya 46) had a productivity equal to the general mean. The optimal seeding rate was 4 MKH. The productivity of Kharkovskaya 19 was minimal (100 g/m^2 in 1999 at 3 MKH). The optimal seeding rate was 5 MKH.

Grain yield depended more on climactic condition. Few cultivars differentiated and did not depend on seeding rates. Those exceeding the general mean of 30.8 % for all cultivars in 1998 (35.7 %) were less in 2000 (25.3 %). The grain yield of Kharkovskaya 23 (33.2 %) and Kharkovskaya 27 (33.5%) reliably exceeded the general mean of Kharkovskaya 19 (28.9 %) and Kharkovskaya 46 (27.8 %) usually less at all seeding rates. This character of other varieties was up-to-date of general mean.

The 1,000-kernel weight also depended more on climatic conditions and less on cultivar differences and seeding rates. This trait always exceeded (34.4–36.5 g) the general mean (33.9 g) in 1998 and 1999 but was lower (30.7 g) in 2000. Kharkovskaya 23 and Kharkovskaya 27 reliably exceeded (35.2–35.3 g) the general mean for all cultivars. Little difference was observed in the 1,000-kernel weight at different seeding rates.

Variability in productive tillering was similar to 1,000-kernel weight but reliable only in some years (1.03 in 1999, 0.89 in 2000, 0.9 for Kharkovskaya 19, and 1.02 for 3 MKH, compared to the general mean of 0.96. Plant stand depended on seeding rate (rates of 3 and 4 MKH reliably exceeded the general mean of 227 plant/m^2) and equaled 160–198 plant/m^2; 5, 6, and 7, MKH were 237–283 plant/m^2. Less reliable were years and cultivar.

Graphics can simplify the dependence estimates of the tested characters by specific factors. For example, productivity in specific ecological conditions by different seeding rates can be represented with the help of a quadratic surface. If the ecological conditions are a mean value of productivity during a suitable year, the maximum of the surface indicates the highest possible display this character (see Figs. 1 and 2).

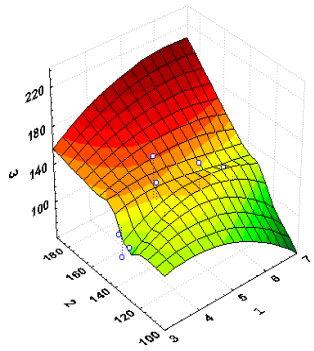

Fig. 1. Productivity of Kharkovskaya 19 for seeding rates and climatic conditions, 1998–2000. Mean productivity of the cultivar in suitable years.

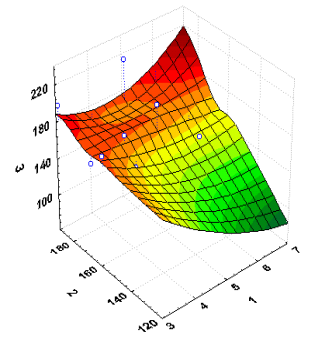

Fig. 2. Productivity of Kharkovskaya 33 for different seeding rates and climatic conditions, 1998–2000. Mean productivity of the cultivar in suitable years.

Thus, the effect of climatic conditions on traits was greatest on 1,000-kernel weight, productive tillering, and grain yield for the variables productivity and plant stand. The lack of difference in productivity and grain yield and the minimal difference in 1,000-kernel weight and productive tillering at different seeding rates indicates the impossibility for estimating the optimal seeding rate for durum wheat. Therefore, this question can be solved only for the concrete cultivar. Kharkovskaya 33 was the most productive with a mean index of other testing traits. The optimal seeding rate was 5 MKH. Kharkovskaya 23 and Kharkovskaya 27 had higher grain yield and 1,000-kernel weight with mean indices of other testing traits. The optimal seeding rate was 4 MKH. Kharkovskaya 41 has mean indices for all traits. The optimal seeding rate was 5 MKH. This cultivar was entered in the Register of Ukraine for 2003. Kharkovskaya 15 and Kharkovskaya 46 have most indices of plant stand, productive tillering, and mean other traits. The optimal seeding rate was 5 MKH. Kharkovskaya 19 had the smallest indices for all tested traits. The optimal seeding rate was 4 MKH.

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Winter wheat gene pool of the CIMMYT international nurseries for improvement of breeding for resistance to fungal diseases and productivity in the eastern Forest-Steppe Region of the Ukraine.

V.V. Sotnikov and T.M. Yevlanova.

We conducted a soil-quarantine control on quarantine pathogens and a primary investigation of 164 bread and 30 samples of hard winter wheat from the CIMMYT International Nurseries at the Introductory Quarantine Nursery of the Plant Production Institute in 2001–02. We looked at the main economical and morphological characters, biological properties, and resistance to nonquarantine, harmful organisms in order to replenish the stocks of the National Centre for Plant Genetic Resources of Ukraine and breeding subdivisions of the Institute with a new foreign germ plasm that is fully free of quarantine pathogens and for the use of highly productive cultivars in breeding. The introductory material was from the 5th Winter Wheat Observation Nursery for Semi-Arid Areas (5th WON-SA, 102 entries), 6th Facultative and Winter Wheat Elite Yield Trial for Rainfed Areas (6th EYTRF, 20 entries), 4th Winter Wheat East-European Regional Yield Trial (4th WWEERYT, 42 entries), and 3rd Winter Durum Wheat East-European Regional Yield Trial (3rd WDEERYT, 30 entries), which contained cultivars from Azerbaijan, Bulgaria, the Czech Republic, Georgia, Hungary, Iran, Kazakstan, Moldova, Romania, the Russian Federation, Syria, Turkey, the Ukraine, and the U.S.A.

The bread wheat cultivar Albatros odeskiy and the hard wheat Kharkivska 32 were used as local checks. The degree of damage was estimated after winter according to a 0–9 scale: 0 = death, 1 = very low, and 9 = very high. The same scale was used to estimate resistance to fungal pathogens in natural conditions where 1 = very high susceptibility, 5 = moderate susceptibility, 6 = moderate resistance, and 9 = very high resistance. Yield was considered very low if it was less than 76 % of the local checks Albatros odeskiy and Kharkivska 32, low = 76–95 %, average = 96–115 %, high = 116–135 %, and very high > 135 %. Thousand-kernel weight was low if less than 39.0 g, average = 39.0–46.9, high = 47.0–54.0, and very high > 54.0 g. Planting in the 5th WON-SA and 6th EYTRF was done with a hand-sower at the planting rate of 60 seeds/m in 0.75-m^2 plots. Planting of the 4th WWEERYT and 3rd WDEERYT was done with a tractor-sower SSFK-7 at a planting rate of 6.0 x 10^6 viable seeds/ha in 8-m^2 plots. Vitavax was used as a seed treatment. Field trials were conducted in arid conditions with a black fallow forecrop. The planting date was very late, 5 October, 2001, or 17 days later than permissible (18.09). Overwintering was satisfactory because of a mild winter. Growth recovery in the spring began much earlier than in comparison with the mean sowing dates of earlier years. Yield level was limited by the late sowing date; an outbreak of fungal diseases including powdery mildew, leaf rust (only on the bread wheats), and Septoria blight; and unfavorable climactic factors including increased daily temperature, hot dry winds, and the lack of soil moisture from the end of tillering to grain filling. Over that period, rainfall was 84 mm (66 % of the mean of many years). During a entire vegetative period, rainfall was approximately 260 mm (105 % of the yearly average). Total rainfall, including winter precipitation, was 408 mm. In spite of these negative effects, the wheats produced a high grain yield. The average yield capacity of the local checks and control cultivars in the 5th WON-SA and in the 6th EYTRF nurseries were Albatros odeskiy, 414 g/m^2; Donetska 48, 534 g/m^2; Kharus, 466 g/m^2; Odeska 267, 485 g/m^2; Kharkivska 96, 627 g/m^2; Tira, 465 g/m^2; Gerek 79, 370 g/m^2; Dagdas 94, 438 g/m^2; Suzen 97, 408 g/m^2; Kirgiz 95, 541 g/m^2; and Gun, 244 g/m^2. In the 4th WWEERYT, average yield capacities were Albatros odeskiy, 277 g/m^2; Donetska 48, 335 g/m^2; Kharus, 415 g/m^2; Myronivska 61, 384 g/m^2; Bezostaya 1, 280 g/m^2; Seri, 199 g/m^2; Jagger, 246 g/m^2; and in the 3rd WDEERYT Kharkivska 32, 314 g/m^2; Aisberg odeskiy, 284 g/m^2; and Kunduru, 177 g/m^2.

A mild winter did not permit evaluation of the entries for winter hardiness. A predominant part of the material had a high degree of wintering (scores of 8–9), with the exception of '494J6.11//TRAP#1/BOW' (wintering score = 4); Unknown 95-3 (3); 'FRTL//AGRI/NAC (2)'; 'Nemura/Kauz//AGRI/NAC (TOP SIEVE95-TOP SIEVE96 TOP SIEVE97)' (5.5); all entries from Turkey; the 5th WON-SA nursery; 'Saulesku #44/TR810200' (5) from Turkey; the 6th EYTRF; 'Brindur/DF 38-86' (5); 'DF900-83/WPB881' (U.S.A) and Altin (4.5); DUT-TA00-22 (1); 'DICLE74/HALKALI058' (5) from Turkey; DYT-CA00-7 (5) from Syria; Turan (1) from Azerbaijan; and the 3rd WDEERYT nursery.

The heading of the bread wheats for the 5th WON-SA compared to the local check Albatros odeskiy (headed 144 days after 1 January) were 55 % similar and 38 % earlier and 41 % and 59 %, respectively, yielded greater than the check. In the 6th EYTRF, 70 % were earlier and 30 % were similar and 86 % and 67 %, respectively, yielded greater than the check. In the 4th WWEERYT, 67 % were similar and 31 % were earlier; the number of entries yielding greater than the check were 82 % and 69 %, respectively. For the hard wheats, when compared to the check Kharkivska 32 (147 days from 1 January) in the 3rd WDEERYT 43 % were similar, 30 % later, and 27 % earlier and those entries with a yield of 96 % of the check were 8 %, 11 %, and 50 %, respectively.

Leaf fungal diseases were caused considerable damage. Susceptibility (1–4 scale) was estimated in the nurseries (Table 6). This natural infection permitted differentiation of the material for the degree of resistance to the major pathogens. A large part of the entries in the 6th EYTRF nursery (83 %) had average or higher grain yields than the check. In the 4th WWEERYT, yield was 76 %, 45 % in the 5th WON-SA, and 21 % in the 3rd WDEERYT compared to the check.

Table 6. Overall susceptibility scores of each of the international nurseries investigated in 2001–02.

	Powdery mildew	Septoria	Leaf rust
5th Winter Wheat Observation Nursery for Semi-Arid Regions	70	62	28
6th Elite Yield Trial for Rainfed Regions	90	80	45
4th Winter Wheat East European Regional Yield Trial	36	98	12
3rd Winter Durum East European Regional Yield Trial	17	97	no damage

We distinguished the following entries that had a combination of useful economic characters and resistance or moderate susceptibility to leaf disease pathogens. These lines are recommended for further study and possible use in breeding highly productive cultivars of bread and hard wheats. Unfortunately, most lines of these group have resistance to two disease pathogens but considerable susceptibility to a third, making their use difficult. The combination of economical characters given include 1 = mean yield, 2 = high yield, 3 = very high yield, 4 = average 1,000-kernel weight, 5 = high 1,000-kernel weight, 6 = very high 1,000-kernel weight, 7 = heading ≥ 2 days earlier compared to the check, and 8 = similar heading date as check.

Moderately susceptible or resistant to powdery mildew and Septoria but highly resistant to leaf rust. CIT90089-0YC-0YC-0YC-7YC-0YC-1SE-0YC-4YC-0YC (4, 7; pedigree: Weston/VEE) and BDKE930161-0YC-0YC-1YC-0YC-3YC-0YC (2, 4, 7; pedigree: Haymana75/4/YMH/TOB//MCD/3/LIRA(BDME 9)). Both these lines were from Turkey and the 5th WON-SA nursery.

Moderately susceptible to powdery mildew and Septoria. MVTD 15-99 (4; Hungary, 3rd WDEERYT nursery,), CIT932314-0SE-0YC-2YE-0YC-2YK-0YK (1, 4, 8; pedigree: RSK/FKG15//CHAM6/3/FDL4; Turkey, 5th WON-SA,), and CIT94072-0SE-1YC-0YC (3, 4, 8; pedigree: PYN//TAM101/AMI/3/KRC66/SERI; Turkey, 6th EYTRF).

Moderately susceptible or resistant to powdery mildew and leaf rust. SG-RU 8069 (3, 4, 8; Czechia), Iveta NTA-92/89-6 (2, 7; Bulgaria); GK Bagoly (1, 4, 8; Hungary), GK Vevecky (2, 4, 8; Hungary), MV Dalma (2, 4, 8; Hungary), GK Forras (3, 8; Hungary), MV 04-96 (3, 5, 8; Hungary), Turda 2000 (2, 4, 8; Romania), Destin (2, 4, 8; Romania), Efekt (1, 4, 8; Romania), Expres (2, 4, 7; Romania), Manypa (3, 8; Moldova), Strumok (3, 4, 8; Ukraine), Erythrospermum 270 (3, 7; Ukraine), Knjazhna (3, 4; Russia), and Akinci-84 (3, 4, 8; Azerbaijan). All entries were from the 4th WWEERYT nursery.

Moderately susceptible to Septoria and resistant or moderately susceptible to leaf rust. CIT922411-0SE-0YC-2YC-0YC-2YC-0YC-3YC-0YC (1, 4, 7; pedigree: CHAM4/TAM200//RSK/FKG15), CIT90089-0YC-0YC-0YC-7YC-0YC-1SE-0YC-3YC-0YC (1, 4, 7; pedigree: Weston/VEE), CIT937256-0SE-0YC-3YE-0YC-1YC-0YC (2, 4, 8; pedigree: PLK70/LIRA//Attila/3/AGRI/NAC), CMSW93WM0071-0AP-0YC-8YE-0YC-3YC-0YC (1, 4, 8; pedigree: FRTL//AGRI/NAC), CIT922229-0SE-0YC-1YC-0YC-7YC-0YC-2YC-0YC (2, 4, 8; pedigree: Necomp1/5/BEZ//TOB/8156/4/ON/3/TH*6/KF//), and MXTK930076-0SE-0YC-12YE-0YC-4YK-0YK (1, 5, 7; pedigree: 1D13.1/MLT/3/LFN/SDY//PVN). All entries are from Turkey and the 5th WON-SA nursery. CIT945175-030SE-0YC-7YE-0YC (3, 4, 7; pedigree: DDZ2141.85.271/ES14//F134.71/NAC) from Turkey and the 6th EYTRF nursery.

Resistant to powdery mildew. ELIDUR (1, 4, 7; Romania) and Perlyna (2, 5, 8; Ukraine) both from the 3rd WDEERYT nursery; CIT935011-0SE-0YC-3YE-0YC-2YC-0YC (1, 4, 7; pedigree: ES14/130L1.12//MNCH) and CIT930082-0SE-0YC-3YE-0YC-2YK-0YK (3, 5, 8; pedigree: KARL/Ariesan) both from Turkey and the 5th WON-SA nursery; ICWH900747-0AP-0YC-0YC-6YC-0YC-9YC-0YC (1, 4, 7; pedigree: Motah-7, Turkey, 6th EYTRF); Demir (2, 5, 7, Turkey, 4th WWEERYT); Kiziltan (5, 8; Turkey, 3rd WDEERYT); and Ankara 98 (6, 8; Turkey), Yilmaz (5, 8;

Turkey), Brindur/DF 38-86 (1, 4, 7; accession #019006; U.S.A.), Brindur/DF 38-86 (2, 4, 7; accession #019007; U.S.A.), UVY162/61.130//HC6654/3/AKB/OVI65/4/WPB881 (5, 8; U.S.A.), WPB881/Rodur (1, 4, 8; U.S.A.), and WPB88/H7092-50B//MI83.84.503 (5; U.S.A.) all entries from the 3rd WDEERYT nursery.

Moderately susceptible to Septoria. CIT922142-0SE-0YC-3YC-0YC-6YC-0YC-1YC-0YC (2, 4, 7; pedigree: JI5418/MARAS), CIT88088T-0SE-1YC-0YC-2YC-0YC-2YC-0YC-8YC-0YC-1YC-0YC (2, 5, 8; pedigree: Zander-34), and CMSW93WM0182-0AP-0YC-5YE-0YC-1YC-0YC (1, 4, 8; pedigree: SW89-3218//ASP/BLT) all from Turkey and the 5th WON-SA nursery; and CMSW94WM00586S-03Y-0B-0SE-1YE-0YC (2, 5, 7; pedigree: Saulesku #44/TR810200) from Turkey and the 6th EYTRF nursery).

Resistant to leaf rust. Capuz (2, 4, 8; Moldova), Kupava (3, 4, 8; Russian Federation), and Kroshka (3, 4, 7; Russian Federation) all from the 4th WWEERYT nursery; 0YA-0YA-5YC-0YC-6YC-0YC (1, 5, 8; pedigree: BUC/5/Naphal/CI13449/4/SEL14.53/3/Lancer//ATL66/CMN), CIT922142-0SE-0YC-3YC-0YC-6YC-0YC-2YC-0YC (3, 4, 7; pedigree: JI5418/Maras), CIT932332-0SE-0YC-7YE-0YC-3YK-0YK (2, 5, 7; pedigree: CHAM6//1D13.1/MLT/3/SHI4414/CROW/4/KVZ/AU//GRK), CIT932282-0SE-0YC-3YE-0YC-3YK-0YK (1, 5, 8; pedigree: Karous-10), CIT935166-0SE-0YC-4YE-0YC-1YK-0YK (3, 4, 7; pedigree: PLK70/LIRA/5/NAI60/3/14-53/ODIN//CI13441/4/GRK/6/MNCH), 0YA-0YA-5YC-0YC-4YC-0YC (3, 5, 7; pedigree: BUC/5/NAPHAL/CI13449/4/SEL14.53/3/L//ATL66/CMN), and CIT935224-0SE-0YC-3YE-0YC-3YC-0YC (3, 5, 8; pedigree: NGDA146/4/YMH/TOB//MCD/3/LIRA/5/F130L1.12) all from Turkey and the 5th WON-SA nursery; F2.96.24-0SE-0YC-1YE (2, 4, 8; pedigree: Bilinmiyen96.24), CIT932332-0SE-0YC-1YE-0YC-2YC-0YC (3, 4, 7; pedigree: CHAM6//1D13.1/MLT/3/SHI4414/CROW/4/KVZ/AU//GRK), and CIT930151-0SE-0YC-9YE-0YC-1YC-0YC (3, 5, 7; pedigree: Jing Dong 1//1D13.1/MLT) all from Turkey and the 6th EYTRF nursery.

Immunological basis for developing initial material resistant to septoriosis for winter and spring bread wheat breeding in Ukraine.

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Among the cereal crops, wheat is the major grain grown in Ukraine. The winter wheat area occupies about 7×10^6 hectares; spring wheats occupy $3–3.5 \times 10^5$ ha. Resistance to disease pathogens for increasing the yield capacity of cereal crops in developed countries has become more significant than other traits. The acuteness of the problem will not decrease in the future as breeding progress for productivity effects a pathogen's development and accelerates adaptation. The breeder, however, must take the lead in order to ensure constant sources and donors of disease-resistance genes. Septoria resistance will play an important role among other diseases. The best results will be achieved only by the use of resistant material in breeding.

Septoria has been one of the most harmful diseases of wheat during the last decade, although it has been known since 1907 (Yachevskiy 1908). At present, this disease is widespread all over the regions where wheat is cultivated, including the Ukraine. The disease symptoms appear as spots, occurring on all above-ground parts of the plant and at all developmental stages. The disease pathogens in the Eastern Forest-Steppe of Ukraine include *S. tritici* and *S. graminum*, which affect the leaves and leaf sheaths of winter and spring wheats (Boublik et al. 1999).

The ascospores are an additional source of infection, but the pycnospores are of great importance in infecting and reinfecting plants. Prolonged moisture, mild, windy weather, and precipitation, especially at the heading and flowering, are favorable to infection. At the Department for the Plant Immunity to Diseases and Pests in the Plant Production Institute named after V. Ya. Yurjev between 1996–2002, we studied some genotypes from a collection of breeding material of winter and spring wheats obtained from the National Centre for Plant Genetic Resources of Ukraine (NC PGRU) and breeding departments of the Institute. Artificial infections for Septoria infection were created according to known procedures (Anonymous 1989a and b). In the Ukraine, a parasite of the Septoria pathogens at the ascospore stage has been found in cereals that belongs to the genus *Leptosphaeria* Ces. et de Not.

The years of the study were characterized by considerable changes in weather conditions, particularly during plant development; 1997 and 2000 were favorable for plant and pathogen development characterized by increased moisture and mild temperatures, and 1996, 1998, and 1999 were years of severe drought during the entire vegetative period of both winter and spring cereals and a reduced level of pathogen development. The weather conditions in 2001

were characterized by increased moisture at the start of plant vegetation and flowering stages and severe drought and heat during grain formation and filling. Epidemics of the disease in 1997 and 2000 did not require creating artificial infections.

Table 7. Maximum infection of cereal crops by Septoria, 1996–2001.

	Year of study					
Crop	1996	1997	1998	1999	2000	2001
Winter wheat	65.0	100.0	60.0	25.0	100.0	65.0
Spring bread wheat	21.0	100.0	65.0	14.0	100.0	65.0
Spring durum wheat	25.0	100.0	25.0	20.0	65.0	40.0

The maximum infection of plants amounted to 65–100 %. In 1996, the pathogen was epidemic among the leaf diseases. In 1998, Septoria in winter wheat was suppressed at the milk stage. In 1999, fungal pycnidia emerged in the first week of May on the bottom leaves of plants, spread to the middle and upper leaves, but symptoms were not found on the flag leaf due to a severe drought (Table 7). In 2001, favorable conditions for disease development in both winter and spring wheats were produced by artificial infections through use of a local population and a population received from the Plant Protection Institute (Kyiv). During 1996–2000, we studied the resistance of 1,542 samples of winter wheat and 1,186 samples of spring wheat. In 2001 with artificial infection, 453 lines of winter wheat and 202 lines of spring wheat were tested.

Table 8. Resistance to Septoria in a collection of winter bread wheats at the Department for Plant Immunity to Diseases and Pests in the Plant Production Institute named after V.Ya. Yurjev between 1996–2001. Scores are on a scale of 1–9 where 0 = susceptible, 5 = medium resistance, and 9 = high resistance; — indicates no test.

		Resistance score by year					
Cultivar	Origin	1996	1997	1998	1999	2000	2001
Myronivska 32	Ukraine	5	5	7–6	7	—	—
Myronivska 33	Ukraine	9	8	8	7	6	6
Myronivska 64	Ukraine	7	5	7–6	7	—	—
Myrich	Ukraine	7	—	7–6	7	6	5
Kyivska 7	Ukraine	6	—	8	6	6	5
Luna 3	Ukraine	8	6	6	6	7	—
Lutescence 20191	Ukraine	—	—	7–6	6	7	6
D 169	Ukraine	—	—	7	7–6	6	5
Atol Odeskiy (*T. durum*)	Ukraine	—	—	—	8	8	6
Plamya	Ukraine	6	—	7–6	7–6	5	5
Don 93	Russia	—	—	6–5	6	5	5
Smouglyanka	Russia	—	—	6	6	6	6
Knyazhna	Russia	—	—	7–6	7	5	5
Arbatka	Russia	—	—	7	6	6	5
Norman	Great Britain	—	—	—	7	8	8–7
Arina	Czech Republic	8	6	6	—	—	—
Ikarus	Austria	7	6–7	7	—	—	—
MV23	Hungary	—	6	7–6	6	—	—
Granada	Germany	8	6	7	—	—	—
Niclas	Germany	8	7	7–8	—	—	—
Olma	Poland	—	—	—	6–7	7	6–7
SMH 2893	Poland	—	6	7–6	7	6	—
Panda	Poland	7	6–7	6–7	—	—	—
N92L228	U.S.A	—	—	7–6	6	6	5
Wakefield	U.S.A	—	—	—	6–7	6	5–7
KS91WGRC11	U.S.A	—	7	—	—	7	6
Charmany	U.S.A	—	6	7–6	—	7	6
TX90V8727	U.S.A	—	6	—	8	—	—

Immune or highly resistant lines were not identified, which shows the low adaptation of resistance genes in the cultivars studied. In the material of the competitive variety trials of the Winter Wheat Breeding Department, two lines were the most resistant (scores 6–7), Lutescens 159-95 and Erythrospermum 224, and 10 lines were of medium resistance (score 5). The rest of the material from the nursery was susceptible or very susceptible (Chernyaeva and Mouraeva 1992; Dolgova et al. 1997; Rabinovich et al. 1999).

Among samples from the world gene pool of winter wheat, we identified genotypes that maintained resistance to the pathogen (scores 6–8) including Granada from Germany and Myronivska 33 and Myrich from the Ukraine. Myrych and Myronivska 33 of Myronivskiy Institute of Wheat n.a. V.M. Remeslo were used as initial material for developing winter wheat resistant hybrids (see Table 8).

Septoria infection in spring wheat was at a lower degree than that of winter wheat. Spring wheats also were infected by leaf rust and powdery mildew fungi. We identified medium-resistant breeding lines (scores 5–8) between 1990–96. In 2002, line 97-171 showed medium resistance.

Among the durum wheats, we identified three lines that maintained resistance during 3 years at a level of 6–7; Leucurum 79, from Kazakhstan, and Hordeifome 1613 and Hordeiforme 1620 from Bulgaria. In 2000, no resistant lines were identified and only two cultivars, Irridur (U.S.A.) and CD 89239 (Mexico), were medium resistant (score 6).

Both spring bread and winter wheats had high levels of Septoria infection. No immune or resistant lines were found after many years. Five genotypes were classified to be of medium resistance (score 5; Largo and Oasis (U.S.A.), and Krasnokutskaya 9, Legenda, and Lutescens 115/85-3 (Russian Federation). In the 2000 epidemic, three lines from the Samariskiy NIISHK scored 6 (medium resistant); Volgouralskaya, Erythrospermum 1508, and Erythrospermum 1509.

Disease dynamics were investigated to analyze resistance in breeding material in an artificial infection. Resistance was determined by the AUDPC. According to the criteria, the best lines were the winter wheats Lutescence 234-99, Erythrospermum 293-99, Lutescence 422-2000, and Lutescence 625-2000 with resistance scores of 6. Infection did not exceed 15 %, with the average indices of resistance at scores 4–5.

Among the NC–PGRU genotypes useful as the sources of resistance were the Ukrainian winter wheats AC-182, Lutescence 20191, TK 121 Line 2, Perlyna Lisostepu, Myronivska 67, and Myronivska 68; cultivars from Great Britain Tara, Brigadier, Hussar, and Norman; the U.S. wheats Wakefield, U 1254, KS91WGRC11, and Charmany; the Russian cultivars Smouglyanka, Douslyk, and Delta; and the Polish cultivar Olma.

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