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Characterization of tetraploid wheat germplasm for resistance to *Pseudocercosporella herpotrichoides*, cause of eyespot disease

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Abstract

Eyespot disease, caused by *Pseudocercosporella herpotrichoides*, can be devastating to winter wheat grown in northern Europe and the northwest USA. Accessions from eight different tetraploid wheat species randomly extracted from core collections were scored for resistance to eyespot disease using a β-glucuronidase (GUS)-transformed strain of *P. herpotrichoides*. The GUS values for the combined population followed a quasi-Gaussian distribution. Three species, *Triticum dicoccoides, T. durum* and *T. turanicum*, showed significant variation ($P < 0.001$) in disease response with *T. dicoccoides* having the lowest disease scores, i.e. highest levels of resistance. All tetraploid accessions were less resistant than resistant diploid *T. tauschii* accessions. Thirteen percent of tetraploid accessions had disease scores that ranged between the average of the resistant accessions of *T. tauschii* and the moderately resistant hexaploid germplasm line Cerco. Eight accessions (three accessions of *T. dicoccoides*, two of *T. turgidum* and three of *T. durum*) with low disease scores (resistance) to infection were selected for further genetic analysis.

Introduction

Resistance to eyespot (syn. foot rot or strawbreaker foot rot) caused by the facultative parasite *Pseudocercosporella herpotrichoides*, (Fron.) Deighton (teleomorph = *Tapesia yallundae* Wallwork & Spooner) has been widely investigated in hexaploid wheats and in some diploid relatives (Vincent et al., 1952; Macer, 1966; Dossinault, 1973; Murray et al., 1995; Yildirim et al., 1995). Tetraploid wheats, wild and cultivated forms, can be crossed easily with hexaploid wheat, but have been not extensively screened as sources of resistance to eyespot.

Eyespot is a limiting factor for wheat farming in the U.S. Pacific Northwest and in Northern Europe (Jones et al., 1995, Koebner & Martin, 1990). The accuracy of the evaluation method for assessing resistance to this pathogen is crucial for efficient plant selection. Several methods of evaluation are available to score eyespot disease reaction such as visual rating, biochemical and molecular techniques (McMillin et al., 1986; Scott, 1971; Lind, 1992; Chao et al., 1989; de la Peña & Murray 1994), and screening in the field. Depending on the method, the evaluation can be performed on either seedlings or adult plants.

Visual scores are affected by subjective errors but can be informative if detected on plants grown to maturity (McMillin et al., 1986). Visual rating based on the host cell epidermal response has high heritability and could be useful to screen breeding lines at the seedling stage (Strausbaugh & Murray, 1989), but is labor intensive. Among biochemical methods, enzyme-linked immunosorbent assay (ELISA) is able to differentiate pre-symptomatic responses at the seedling stage, but consistent differentiation is only achieved from anthesis to plant maturity (Lind, 1992). In contrast, markers linked to gene(s) responsible for resistance have been identified as efficient tools for germplasm screening and selection (McMillin et al., 1986; Chao et al., 1989; Allan et al., 1989, 1990; Koebner & Martin, 1990; de la Peña et al. 1995). The efficiency of marker assisted selection is inversely proportional to the degree of

recombination (linkage) between the marker and the resistance gene (Chao et al., 1989, de la Peña et al., 1995). This method is useful to select resistance genes with large effects (major genes), but it works in a qualitative manner and does not take into account the quantitative component of the disease response due to minor genes (Cox, 1995). Also, a selection system based on disease reaction is necessary to find linked genotypic markers and to quantitatively interpret marker-assisted selection.

To analyze the eyespot disease response of large samples of germplasm within and between species, the evaluation method should be inexpensive, objective, sensitive and applicable at early stages of plant development. A method based on a reporter-gene system was developed by de la Peña & Murray (1994) that allows screening at the seedling stage of a large number of wheat genotypes for resistance to eyespot. This test, which is conducted in a controlled environment chamber, allows a sensitive description of the plant disease response on a quantitative scale.

To date only two genes for resistance to *P. herpotrichoides* are known within the cultivated hexaploid wheat gene pool: *Pch1* transferred from *Aegilops ventricosa* Tausch. (Maia, 1967) located on chromosome 7D of the breeding line VPM-1 (Worland et al., 1988), and *Pch2*, whose origin is not known, located on chromosome 7A of the cultivar Cappelle Desprez (Law et al., 1975; de la Peña et al., 1995). These two lines have been extensively utilized in breeding programs to develop resistant cultivars, but resistance is not complete (Jones et al., 1995). To date no source of resistance has been identified in the B genome.

Resistance gene(s) for eyespot are represented at a high frequency in the wild diploid wheat relatives *T. monococcum* L. (A genome) (Cadle et al., 1997), *T. tauschii* (Coss.) Schmal. (D genome) (Yildirim et al., 1995) and *Dasypyrum villosum* (L.) Candargy (V genome) (Murray et al., 1995). A new resistance gene(s) has been located on chromosome 4V of *D. villosum* (Murray et al., 1994). The use of the resistance identified in these wild species for common wheat breeding is hampered by two constraints: first, the transfer of linked, unwanted characters to cultivated wheats (Law et al., 1988); second, some of these genomes do not pair well with those of *T. aestivum* L. (Lilienfeld & Kihara, 1951; Sears, 1953; Kimber & Feldman, 1987; Dvoràk & McGuire, 1991).

The present work was carried out to explore the extent of variation for eyespot response in tetraploid wheats with the goal of identifying new sources of resistance. The degree of resistance in tetraploid wheat to *P. herpotrichoides* is higher than in common wheat, but still low with respect to *Secale cereale* L., *Avena* spp., *A. ventricosa*, *D. villosum* and some other wild wheat relatives (Sprague, 1936). Germplasm from core collections was utilized to increase the chance of identifying resistant genotypes.

Table 1. Mean GUS activity by species and for the whole sample with relative number of observations standard deviation

Species[a]	Genome	No. of accessions	Mean[b]	Standard deviation
T. durum Desf.	AABB	94	2.7	0.32
T. turgidum. L.	AABB	29	2.6	0.32
T. polonicum L.	AABB	15	2.7	0.27
T. turanicum Jakubz.	AABB	14	2.7	0.26
T. carthlicum Nevski	AABB	10	2.8	0.28
T. dicoccon Schrank	AABB	11	2.8	0.27
T. dicoccoides Koern.	AABB	36	2.8	0.31
T. timopheevii Zhuk.	AAGG	12	2.7	0.38
Whole sample		220	2.7	0.31

[a]Classification adapted from Kuckuck (1970).
[b]$\log_{10}$-transformed GUS scores.

Materials and methods

Germplasm

Two hundred twenty accessions randomly sampled from eight core collections of different tetraploid wheat species were screened for eyespot resistance (Table 1). Each core collection is a 10% random sample of accessions from different countries of origin maintained by the USDA (ARS, National Small Grains Collection, Aberdeen, ID). The cultivated *T. durum* Desf. core-collection includes landrace genotypes only. *T. dicoccoides* Körn. is the only wild tetraploid wheat species available. *T. dicoccon* Schrank and *T. timopheevi* Zhuk. are primitive wheats and *T. turgidum* L., *T. polonicum* L., *T. turanicum* Jakubz., *T. carthlicum* Nevski are landraces. *T. timopheevi* differs from the other tetraploid species tested by having genome AAGG instead of AABB.

Disease reaction

A strain of *P. herpotrichoides* transformed with β-glucuronidase (GUS) reporter gene (Bunkers, 1991)

was used as inoculum at the dose of 1×10^5 conidia/ml. Seedlings were inoculated at the two-leaf stage following the method described by de la Peña and Murray (1994). Seeds were germinated in Petri dishes, placed at 4°C for 3 days to synchronize germination, transferred to pots containing silt loam soil (bottom) and silt loam soil:vermiculite (1:2 w/w, top), and fertilized with N, P, K (14:14:14, w/v). A constant level of moisture was maintained throughout the experiment. After 6 weeks of incubation in a growth chamber (12°C, 10 h/day illumination, 95% RH) the portion of stem inoculated was harvested for GUS analysis. Plant tissues were run through a sap extractor (torq-maxi mod. 4Z522, Ravanel Specialties Company, Seneca, SC) with 2.5 ml of extraction buffer (50 mM $NaHPO_4$, pH 7.0, 5 mM dithiothreitol, 10 mM Na_2EDTA, 0.1% sodium lauryl sarcosine, and 0.1% Triton X-100) (Jefferson et al., 1987). GUS activity was measured fluorometrically using the procedure described by Jefferson et al. (1987) analyzing 50 μl of sample extracted sap instead of ten. This modification was adopted to reduce errors in pipetting.

A split-block experimental design with three blocks was adopted, with "species" as the whole plots and "accession" nested within "species" forming the subplot. Six replicates for each genotype, two per subplot, were evaluated. A second screening was carried out with 65 accessions resulting from three sub-samples of 16 accessions with high, 28 with low and 21 with random GUS values, respectively. The genotypes 'Cerco' (moderately resistant) and 'Chinese Spring' (susceptible) of hexaploid wheat (*T. aestivum* L.), and three resistant accessions (TA 2460, TA 2473 and TA 2524) of diploid wild wheat *T. tauschii* (Yildirim et al., 1995) were included in the second screening. The degree of heterogeneity of GUS response within accession was calculated using a F-test during the second screening. The variance of the GUS response of Cerco (the least variance among the check accessions) was used to test the assumption of equal variances.

Six weeks after inoculation, plants were rated visually for eyespot using a 1–4 scale, where 1 = no visible lesion; 2 = shallow but definite lesion; 3 = lesion involving first and second leaf sheath; 4 = deep lesion visible also at straw level (modification of Scott, 1971). Coleoptile color (1 = green, 1.5 = pink, and 2 = red) and leaf hairiness (1 = absent to 9 = very heavy) were also scored to assess relationships among these morphological traits and the degree of eyespot resistance.

Glutenin analysis

Twenty two random accessions and their single-seed-derived lines for each of the 22 accessions were analyzed for glutenin storage proteins to determine the degree of genetic homogeneity. For each accession and derived-line, nine individuals were tested. Cultivars Langdon and Chinese Spring were used as standards. Glutenin storage protein components were separated by acrylamide electrophoresis using the SDS-PAGE method (Lafiandra & Kasarda, 1985). Components were scored for presence or absence. The degree of heterogeneity was expressed as a percentage of polymorphism (Graner et al., 1990). This value represents the number of informative comparisons among all wheat individuals divided by the total number of comparisons (average pairwise comparisons).

Statistical analysis

Statistical analyses were carried out using SAS 6.10 statistical software (SAS Cary, NC 1993). Analysis of variance for the split-block design was carried out by GLM procedure using the type III sums of squares. Analysis of variance to test "accession" effect was obtained with the above procedure at each level of the factor "species". Fisher's LSD (P = 0.05) test was also calculated. Regression analysis was run with dependent variable "GUS activity" and the independent variables "coleoptile color" and "leaf hairiness". For statistical analysis the GUS activity was transformed in $\log_{10}$ data since the variance of GUS activity increases markedly with the increase of mean value. Chi square test was used to assess the independence of genetic heterogeneity and GUS assay values heterogeneity of the second experiment.

Results

Thirty accessions out of 250 (12%) were excluded from the analysis because of their low germination and/or emergence rate. Lower colonization levels and GUS values have been observed in conjunction with reduced seedling growth rates.

Differences among species were not significant for response to eyespot disease as measured by GUS activity (Table 2). However, significant differences in GUS activity were observed among accessions within the species *T. durum*, *T. dicoccoides*, and *T.turanicum*

Table 2. Analysis of variance of $\log_{10}$ activity of β-glucuronidase enzyme extracted from seedlings inoculated with a GUS-transformed *Pseudocercosporella herpotrichoides* for all the sources of variation in a split block design and within each species

	Source	DF	Mean square	F value
Between whole plots	Blocks	2	4.10	33.59**
	Species	7	0.18	1.45
	Species* Block	14	0.12	
Whole plots	Accession (Species)	212	0.13	2.12**
	Error	739	0.06	
		974		
Triticum durum	Block	2	5.50	86.68**
	Accession	93	0.11	1.80**
	Error	357	0.06	
		452		
Triticum dicoccoides	Block	2	0.65	13.41**
	Accession	35	0.23	4.78**
	Error	125	0.05	
		162		
Triticum turanicum	Block	2	0.67	19.27**
	Accession	12	0.11	3.09**
	Error	44	0.03	
		58		
Triticum carthlicum	Block	2	0.55	10.64**
	Accession	9	0.09	1.65
	Error	41	0.05	
		52		
Triticum dicoccon	Block	2	0.29	7.57**
	Accession	10	0.08	2.05
	Error	20	0.04	
		32		
Triticum polonicum	Block	2	0.82	16.90**
	Accession	14	0.06	1.27
	Error	47	0.05	
		63		
Triticum timopheevi	Block	2	0.28	2.25
	Accession	11	0.14	1.13
	Error	28		
		41		
Triticum turgidum	Block	2	1.18	18.01**
	Accession	28	0.10	1.48
	Error	77		
		107		

** = $P < .001$

Table 3. Percentage of accessions of eight wheat species that show different levels of resistance when inoculated with a GUS-transformed strain of *Pseudocercosporella herpotrichoides.* Underlined values represent the most frequent classes

Species	No.	GUS activity (log_{10})										
		2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2
T. durum	94	2.1	2.1	3.2	11.7	26.6	24.5	18.1	7.5		3.19	1.1
T. turgidum	29	–	–	13.8	10.3	6.9	37.9	20.7	6.9	–	3.5	–
T. polonicum	15	–	–	–	13.3	26.7	40.0	20.0	–	–	–	–
T. turanicum	13	–	7.7	–	7.7	7.7	15.4	38.5	7.7	7.7	7.7	–
T. carthlicum	10	–	–	–	–	20.0	20.0	40.0	10.0	–	–	10.0
T. dicoccon	11	9.1	–	–	–	18.2	18.2	18.2	18.2	9.1	9.1	–
T. dicoccoides	36	8.3	2.8	–	2.8	19.4	8.3	19.4	19.4	16.7	2.8	–
T. timopheevii	12	–	8.3	–	16.7	16.7	25.0	16.7	8.2	–	8.3	–
Whole sample	220	2.7	2.3	3.2	9.1	20.5	23.6	20.9	9.6	4.1	3.6	0.5

Table 4. Pearson correlation coefficients between visual scores and GUS values within experiments and between first and second experiment GUS values

Type of correlation	r	P
Experiment 1	0.20	0.0001
Experiment 2	0.36	0.0001
Experiment 1 vs. 2	0.23	0.094

(Table 2). *T. timopheevii* was the only species with no significant differences among blocks (Table 2).

The distribution of GUS values for the whole population followed an approximately normal distribution with the mean value (log_{10}GUS = 2.69) representing the maximum frequency response, and a standard deviation s = 0.31 (Table 3). Despite the reduced sample size of *T. dicoccoides* in comparison with *T. durum* (36 vs. 94 accessions), *T. dicoccoides* contributed more than each of the other species to determine the lowest values of disease response (resistance) (Table 3). The relative contribution of *T. dicoccoides* to the two lower classes of GUS response was higher than *T. durum* (11.11% vs. 4.26%). *T. dicoccoides* and *T. durum* had the widest range of variation for GUS activity; ranging from 2.16 to 3.12 (*T. dicoccoides*) and to 3.22 (*T. durum*) (Table 3).

The correlation between visual scores and GUS values was low but highly significant (Table 4). Coleoptile color and leaf hairiness were not linearly associated with the variation of the GUS values (R^2 = 0.001). However, heavy leaf hairiness was a species-specific character for *T. timopheevii* and red coleoptile color was scored at high frequency in the wild *T. dicoccoides* and in the primitive wheats *T. dicoccon* and *T. timopheevii*. It was not possible to detect relationships between degree of disease response and accession origin. The germplasm origin reported in the USDA-Germplasm Resources Information Network (GRIN) database in most cases was not the geographic place of origin but the last collecting site or in some cases, the country of the donor scientist.

Seven accessions out of 65 were excluded from the analysis during the second experiment because of their low germination and/or emergence rate. A higher mean value of 0.33 of the GUS activity was measured during the second experiment. A marked variation in the GUS values was observed between experiments (Table 4) and within accessions (Table 5). The Pearson correlation coefficient between the two screenings for GUS values was low (r = 0.23) with a low level of significance (P = 0.09) (Table 4). Thirty five (60.3%) accessions tested during the second screening showed a variance of GUS values significantly higher ($P < 0.01$) than Cerco. Only 6 (10%) accessions showed a variance lower than Cerco and of these, none were significant (Table 5).

All tetraploid accessions had GUS values higher than the resistant *T. tauschii* (more susceptible). About 13% of the GUS values ranged between the moderately resistant hexaploid Cerco and the resistant accessions of *T. tauschii*. The highly susceptible tetraploid wheats gave a reaction similar to Chinese Spring. Seven accessions (3.6%) with low GUS values during the first and second experiment were determined to be resistant. The resistant accessions had GUS values falling within the interval, calculated for both experiments, defined by the lowest GUS value and the value obtained by adding the LSD to the lowest value ($LSD_{.05}$ = 0.37

Table 5. Number and percentage of accessions of the second experiment and for each species having a variance significantly greater than the variance of the check Cerco ($F > 1$ with $^{**} = P < 0.01$) (a), not significantly (ns) greater than Cerco (b), variance lower than Cerco (c)

	(a)		(b)		(c)		Total no.
	F^{**}		F^{ns}		F^{ns}		
Species	No.	%	No.	%	No.	%	
T. durum	8	61.5	5	38.5	–	–	13
T. carthlicum	5	100.0	–	–	–	–	5
T. dicoccoides	5	50.0	3	30.0	2	20.0	10
T. dicoccon	3	75.0	–	–	1	25.0	4
T. polonicum	5	83.3	1	16.7	–	–	6
T. timopheevii	4	80.0	1	20.0	–	–	5
T. turanicum	2	28.6	4	57.1	1	14.3	7
T. turgidum	3	37.5	3	37.5	2	25.0	8
Whole sample	35	60.3	17	29.3	6	10.3	58

Table 6. Tetraloid wheat germplasm resistant to eyespot disease identified by species, accession code, country of origin, reaction to *Pseudocercosporella herpotrichoides* in terms of average GUS activities and visual scores, and average GUS activities and visual scores for the check germplasm

Species	Accession	Origin	GUS activity (nM MU/sample)	Visual score
T. durum Desf.	CI 94705	Israel	637	2.1
	PI 565268	Bolivia	448.1	2.6
	PI 352324	Jordan	550.1	1.7
T. dicoccoides Koern.	PI 428029	Turkey	482.7	1.7
	PI 428134	Lebanon	527.7	1.5
	CI 94689	Armenia	215.7	1.9
T. turgidum L.	PI 352544	Switzerland	398.2	1.8
	PI 374660	Macedonia	259.1	1.0
Check	Cerco		776.4	1.7
	cv. Chinese spring		1453	3.2
	T. tauschii (average)		534.3	1.1

for experiment 1 and $LSD_{.05} = 0.27$ for experiment 2). The resistant accessions were *T. dicoccoides* PI 352324, PI 428029 and PI 428134; *T. durum* CI 94705 and PI 565268; and *T. turgidum* CI 94689, PI 352544 and PI 374660 (Table 6). Five of these accessions with low GUS values also had low visual scores, whereas the two *T. durum* accessions had relatively high visual scores (Table 2).

Eleven out of 22 accessions (50%) tested for glutenin components were polymorphic with the percentage of polymorphism ranging between 22.2% and 97.2% (Table 7). Four of these polymorphic accessions (CI 14434, PI 565268, PI 184543 and PI 435015) were mixtures of genotypes producing high percentages of polymorphism (from 80.5% to 97.2%) with five or seven different electrophoretic patterns out of nine individuals tested. For seven accessions, the single-seed-derived family was not true breeding because of segregation of one or two genotypes out of nine. The hypothesis of independence between genetic heterogeneity of germplasm accessions assessed by glutenin analysis and its heterogeneity for the disease reaction was rejected ($\chi^2 = 4.454 > \chi^2_{1,.05} = 3.840$) (Table 8).

Table 7. Percent polymorphism and number of different patterns for HMW-glutenins of 22 randomly sampled accessions and their single seed derived accessions in relation to the degree of homogeneity of the disease response tested as a ratio of variance of accession GUS value to the variance of the check (Cerco) GUS value during the second experiment

		Glutenins				
		Accession		Single seed derived accession		
Germplasm		Polymorphism (%)	Patterns (No.)	Polymorphism (%)	Patterns (No.)	GUS activity F test[a]
T. durum	CI 14434	83.3	5	55.0	3	**
	PI 192820	0.0	1	0.0	1	ns
	PI 565268	80.5	5	0.0	1	*
T. carthlicum	CI 7665	22.2	2	0.0	1	**
	PI 532476	0.0	1	0.0	1	**
T. dicoccoides	PI 471661	0.0	1	0.0	1	ns
	PI 256029	57.1	2	0.0	1	*
	PI 428029	0.0	1	22.2	2	ns
	PI 428069	0.0	1	0.0	1	ns
	PI 428111	46.4	3	25.0	2	**
T. dicoccon	PI 352347	0.0	1	0.0	1	ns
	PI 532302	0.0	1	0.0	1	**
T. polonicum	CI 3282	22.2	2	0.0	1	**
	PI 352488	22.2	2	0.0	1	ns
T. timopheevii	PI 427381	30.0	2	25.0	2	**
T. turanicum	CI 2431	0.0	1	0.0	1	**
	PI 184543	95.2	5	22.2	2	**
	PI 190973	60.0	2	0.0	1	ns
T. turgidum	PI 372461	41.6	3	22.2	2	**
	PI 435015	97.2	7	22.2	2	ns
	PI 208912	0.0	1	0.0	1	ns
	PI 277125	0.0	1	0.0	1	ns

[a] $F = s^2 \log_{10}$GUS accessions/s^2 $\log_{10}$GUS Cerco.
* = $P < .05$.
** = $P < .001$.
ns = no signif.

Discussion

In wheat, resistance to *P. herpotrichoides* is correlated with low colonization by the fungus and susceptibility with rapid colonization (de la Peña & Murray, 1994). The reporter gene selection method reduces the screening period from one year to two months when compared to other available screening methods. It is more objective and more reliable than other evaluation techniques used at present and, in this study, proved more stringent than the evaluation based on visual scores. Visual scores are also more difficult in genotypes having red pigment in the coleoptile, probably because the contrast between the diseased and the healthy tissues is not as evident. In this experiment, GUS values proved useful for a cross-validation of the visual scores and for more efficient disease response evaluation. However, the screening of genetically diversified germplasm may lead to imprecise estimates of disease ratings at the seedling stage due to variation for morpho-physiological characters within and between species.

In this study, variation for components of storage proteins was used as an estimate of the degree of

Table 8. Observed and expected number of accessions having homogeneous and heterogeneous patterns for glutenins and GUS values respectively

Glutenins	GUS values[a]		Total
	Homogeneous obs. (exp.)	Heterogeneous obs. (exp.)	
Homogeneous	7 (4.5)	3 (5.4)	10
Heterogeneous	3 (5.4)	9 (6.5)	12
Total	10	12	22

[a] $\chi^2_{3,.05} = 4.4$, $\chi^2_{1,.05} = 3.8$.

diversity within an accession. Using these biochemical markers, it was possible to verify that genotypes of landraces and wild wheat germplasm accessions are likely to be heterozygous and can be heterogeneous within an accession. The association between heterogeneity for glutenins and heterogeneity for disease responses ($P = 0.05$) indicates that within an accession there could be a mixture of different individuals, each one having a different disease response. This observation could take into account the variation observed within accessions and between experiments. The high frequency of disease reaction values (65%) captured by the average and its two closest classes (three out of 11 classes of GUS values) indicates that disease response is fairly uniform in this sample of tetraploid wheat accessions. This uniformity associated with the genetic heterogeneity of most accessions may explain the low strength of the linear relationship indicated by low correlation coefficients. Nevertheless, the correlation between GUS values and visual scores, was highly significant. The small number of accessions assayed during the second experiment and their heterogeneity could explain the non-significance of the correlation coefficient between the first and second experiments. Yildirim et al. (1995), using the same method to screen a core collection of the diploid wild wheat *T. tauschii* sampled in different countries of origin, detected a disease response frequency distribution skewed on high and low values. This clear differentiation in two classes of response (resistance and susceptibility) contributed to an unambiguous classification of that germplasm and for a high coefficient of correlation between visual-scores and GUS values.

For genetic analysis it would be useful to derive pure lines from tested wheat accessions to be re-tested for disease resistance. When resistance is rare and accession heterogeneity is frequent, as it is in the case of the tetraploid landraces tested in this experiment, selection for resistance should be continued within an accession using the disease response information from a preliminary screening of a core sample of accessions.

The degree of eyespot disease response seems to be very sensitive to micro- and macro-environmental variation. Significant differences between blocks have been reported by Koebner and Martin (1990), Murray et al. (1994) and between experiments by Lind (1992) and by Murray et al. (1994) regardless of the screening method. An increase of the number of replicates for each accession or line, to test the level of resistance to eyespot would be beneficial for more precise estimates of the disease response and for selection purposes.

Improved strategies for drawing core samples to be used in disease screening studies would be useful to increase the chance of detecting favorable genes. The screening of accessions from a core-collection constituted only by random sampling stratified by origin could explain the uniform disease response observed in this research. A more useful core collection will increase the variance for one and/or more traits of interest over the whole collection (Spagnoletti Zeuli & Qualset, 1995). In fact, we observed that the frequency of the extreme classes is very low and presence of duplicates for the trait of interest reduces its variance. Thus, when core collections are established, genetic diversity should be maximized by using improved sampling strategies. In this study, it was possible to identify the significant contribution for the lowest GUS values due to the wild wheat *T. dicoccoides* after partitioning the variation of the disease response for each species. *T. dicoccoides* could be a source of possible major genes for resistance to *P. herpotrichoides* and it may be useful to examine a larger core sample of *T. dicoccoides*.

The distribution areas of *T. monococcum* (form aegilopoides), where many resistance genes have been identified, are largely overlapping with *T. dicoccoides* and pyramiding of resistance genes might have occurred by spontaneous crosses between these two species (Kimber & Feldman, 1987). Less likely would be to find genetic resistance to *P. herpotrichoides* in the domesticated forms of *T. turgidum* as a result of genetic bottleneck effects during their selection and establishment (Burdon, 1987).

In this study eight accessions with a level of resistance higher than Cerco, but lower than resistant accessions of *T. tauschii* were identified. These accessions will be employed in genetic analyses to identify the genetic basis underlying the low disease response. The goal is to identify major genes for resistance to *P. her-*

potrichoides with special interest for genes located on B genome chromosomes.

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