

## RESISTANCE OF LITHUANIAN WINTER WHEAT BREEDING MATERIAL TO EYESPOT

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### Abstract

The experiment was conducted at the Lithuanian Institute of Agriculture during 2005–2007 in the winter wheat breeding nurseries with a natural infection. A total of 79 genotypes were evaluated in 2005, 90 in 2006, and 77 in 2007. The present study revealed considerable variation in eyespot resistance within the recently developed Lithuanian winter wheat breeding material. The greatest differences among the lines were recorded in 2005, when the weather conditions in the 2004/2005 season were the most favourable for eyespot occurrence and development. Stems were scored from 1 (healthy stem) to 9 (highest damage level). Eyespot severity on the genotypes tested was estimated by scores from 4.2 to 8.1. The lines in 2006 were characterized by scores from 1.1 to 5.1. The infection scores of lines in 2007 fluctuated from 2.5 to 7.0. The resistance correlation between years was low ( $r = 0.33^*$ ) in the 2005/2006 cycle and medium strong ( $r = 0.63^*$ ) in 2006/2007 cycle with significance level  $p < 0.05$  (\*). The best differentiation between genotypes was achieved in 2005 with medium levels of disease severity. A satisfactory effectiveness of *Pchl* was confirmed. The lines were found to differ significantly in resistance, which mainly depended on the accumulated level of partial resistance.

Key words: winter wheat, *Pseudocercospora herpotrichoides*, partial resistance, resistance genes.

### Introduction

Eyespot disease, caused by facultative parasite *Pseudocercospora herpotrichoides* (Fron) Deighton, can be devastating to winter wheat grown in northern Europe and northwest USA /Jones et al., 1995/. Eyespot is the most severe disease where wheat is grown continuously and the climate is cool and humid. The severity of the disease is greater in winter than in spring wheat. In the years when wheat eyespot is severe, it can directly decrease the yield by up to 50% and indirectly due to severe lodging combined with susceptibility to pre-harvest sprouting by up to 100%. Additional complication is an increasing cost of fungicide application, higher environmental regulations, and the evolution of new strains of the pathogen that are resistant to commonly used fungicides. Exploitation of cultivar resistance to eyespot disease is the most economic and environment friendly way to minimize economic loss of wheat. Therefore, incorporation of genes for eyespot resistance is an important aim of varietal improvement /Murray, 1996; Yanagisawa et al., 2005/.

A number of eyespot resistance genes are known in wheat germplasm. The first and the most effective one is *Pch1*, originally found in *Aegilops ventricosa*. The second source of resistance was found in French cultivar 'Cappelle-Desprez'. A single gene, *Pch2* confers resistance at seedling stages /Pena et al., 1997/. A polygenic component in 'Cappelle-Desprez' confers resistance at adult stage. The third resistance gene *Pch3* was identified in *Dasyphyrum villosum* /Murray et al., 1994/. The fourth source of resistance to eyespot disease was identified in *Triticum tauschii* /Yildirim et al., 1995/. At present, many efforts are described to search and investigate resistance donors. Investigations involved many accessions of *T. monococcum* /Cadle et al., 1997/, *A. kotschy* /Thiele et al., 2002/, *Thinopyrum ponticum*, *T. intermedium*, *T. elongatum*, *T. bessarabicum* /Li et al., 2005/, *Triticum dicoccoides*, *T. durum*, *T. turanicum* /Figliuolo et al., 1998/. High resistance of these accessions offers a promising future. The main problem is distinct relation of these germplasms with grown hexaploid wheats and subsequent complicated and long lasting transfer of resistance genes.

At present, *Pch1* is being actively used both in Europe and the USA because it is more effective in limiting disease development than *Pch2* and is present in several hexaploid wheat varieties. However, neither the *Pch1* nor the *Pch2* confers complete resistance by itself /Cadle et al., 1997/. The breeders can further enhance resistance in wheat adopting different strategies. The first strategy is to pyramid *Pch1* and the *Pch2* into a single variety, but no study about this has been reported so far. The second possible strategy is to transfer into wheat other major alien genes for resistance (for example *Pch3* or the genes from other germplasms) that can be later combined with *Pch1*. For short time strategy resistance breeding can be improved by combining *Pch1* or *Pch2* with resistance polygenes /Lind, 1992; Lind, 1999; Anonymous, 2006/.

The main objective in this study was to screen the eyespot resistance of winter wheat breeding material.

## Materials and Methods

The experiment was conducted at the Lithuanian Institute of Agriculture (LIA) during 2005–2007 in the breeding nurseries with a natural infection. The plot size in the competitive trial nursery was 17 m<sup>2</sup>, replicated four times. A total of 79 breeding lines were evaluated in 2005, 90 in 2006, and 77 in 2007. The plot size in the collection nursery was 2 m<sup>2</sup>. Each cultivar was grown in one replication. Each year, the resistant varieties (with the gene *Pch1*) 'Leiffer', 'Kris', 'Bill' were grown in the collection nursery and used as resistant checks. The soil of the experimental site is loam *Endocalcari-Epihypogleyic Cambisol (sicco)* (CMg-p-w-can) with a clay content of 24–27%, pH 6.5–7.0; organic matter 2.5–2.7%; P<sub>2</sub>O<sub>5</sub> 190–240; K<sub>2</sub>O 185–264 mg kg<sup>-1</sup> soil. N<sub>90</sub>P<sub>60</sub>K<sub>60</sub> was applied annually. Experimental trials were sown with chemically treated seeds. The seeds were treated with Maxim Star at a rate of 11 per 1 t of seed. Phosphorus and potassium fertilisers were applied pre-sowing and nitrogen in spring after resumption of vegetative growth. The plots were sprayed with herbicides in spring when weeds started to grow intensively. Other pesticides and additional fertilisation were not applied.

To analyse adult growth stage resistance in 2005–2007, the disease was assessed post-harvesting. Infestation of 25 stems per replication was evaluated. Each stem was scored from 1 (healthy stem without infestation symptoms) to 9 (stem with strong

symptoms, rotted and snapped of or broken) as described by Thiele et al. /2002/. The average of the scores from 25 stems was taken as mean infestation level.

According to disease severity scores the genotypes were classified as follows: 1.0–3.0 – very resistant (VR), 3.1–5.0 – resistant (R), 5.1–6.0 – medium resistant (MR), 6.1–7.0 – medium susceptible, 7.1–8.0 – susceptible (S), 8.1–9.0 – very susceptible (VS) /Thiele et al., 2002/. The statistical analysis included calculation of mean (Avg.), and its standard deviation (SD); the least significant difference (LSD) and correlation coefficients ( $r$ ) at the significance level  $p < 0.05$  (\*) were calculated using statistical programs ANOVA and STAT-ENG.

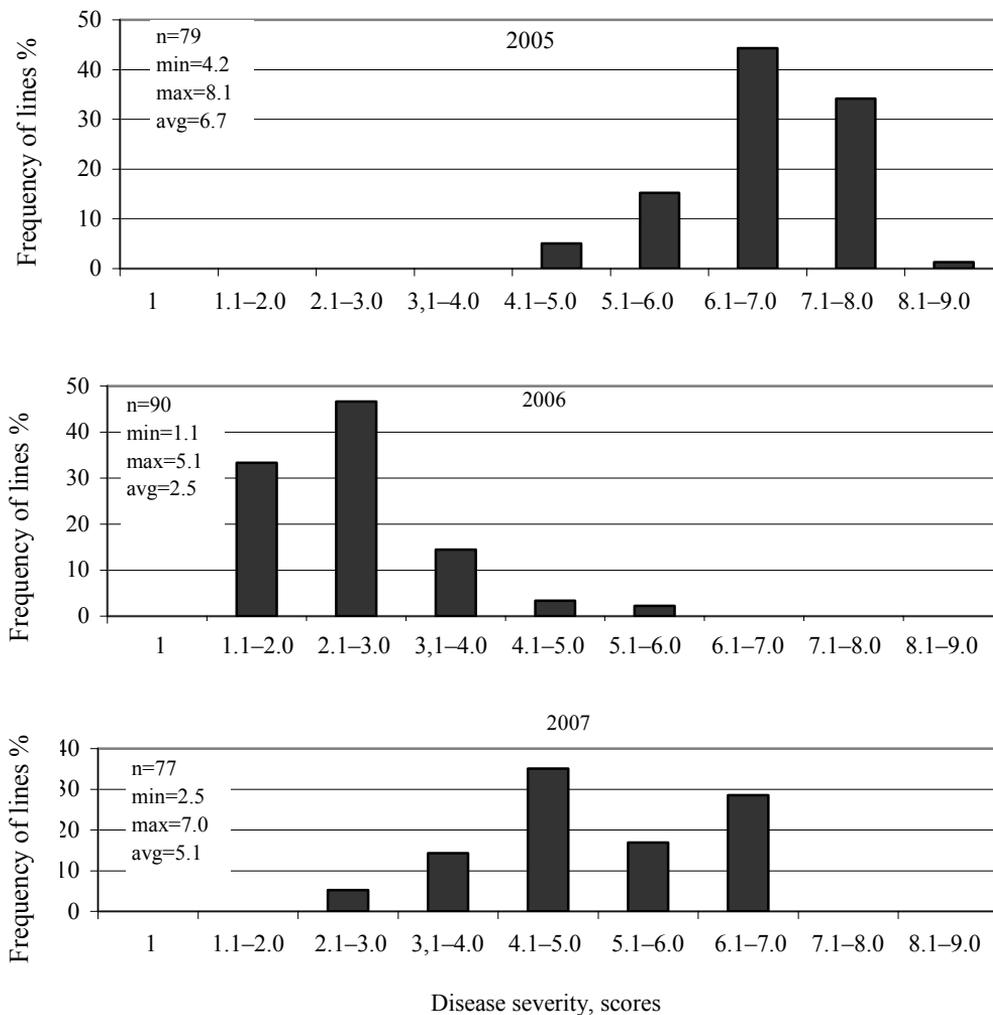
## Results and Discussion

Experimental seasons differed in terms of conduciveness to the development of eyespot. Figure 1 shows the response of winter wheat to eyespot. A total of 79 breeding lines were evaluated in 2005, 90 in 2006, and 77 in 2007. The highest differences among the lines were identified in 2005, when the weather conditions in the 2004/2005 season were the most favourable. Eyespot severity on the genotypes tested was estimated from 4.2 to 8.1 scores. The R and MR genotypes accounted for 5.1% and 15.2% of the lines tested. The most frequent were MS and S genotypes, they accounted for 44.3% and 34.2%, respectively. Only one line was VS and evaluated by 8.1 scores. The lines in 2006 were characterized by scores from 1.1 to 5.1. The lines with the disease level evaluated by 1.1–2.0 (33.3%) and 2.1–3.0 scores (46.7%) dominated. The results of this year did not differentiate the lines by eyespot resistance adequately. However, up to 20% of the lines could be rejected due to the relatively high infection level compared with the rest of the lines. The infection of lines in 2007 was adequate for rejection of susceptible genotypes as lines were infected from 2.5 to 7.0 scores. About a quarter of lines (28.6%) could be rejected by this trait.

The correlation among the resistance scores in contiguous years was low ( $r = 0.33^*$ ) between 2005 and 2006 cycle and medium strong ( $r = 0.63^*$ ) between 2006 and 2007 cycle (Figure 2). The weak correlation between 2005 and 2006 was mainly influenced by the high differences in lines damage by eyespot between years. The second cycle between 2006 and 2007 was characterized by medium correlation as lines were infected more similarly between years. Three check cultivars 'Leiffer', 'Kris', 'Bill' were among the most resistant genotypes.

The breeding lines from competitive trial nursery presented in Table were the most susceptible and the most resistant among the genotypes of the screening years. The differences among the genotypes were significant for response to eyespot if compare susceptible lines with resistant. Among the least damaged lines of 2005 year 'Flair/Lut.9365' was R (score 5.0). The other lines were MR, with scores 5.3–5.9. The most susceptible lines were characterized by scores 7.7–8.1. The difference in eyespot reaction between the most distinct lines was 1.6 times. The most resistant lines of 2006 had scores from 1.1 to 1.8 and the most susceptible by scores 3.8–5.1. The difference in resistance between the most distinct lines was 4.6 times. The most resistant lines of 2007 were evaluated by scores from 2.5 to 3.8 and the most susceptible from 6.5 to 7.0. The lines with cvs 'Dream' and 'Pesma' in the pedigree were the most resistant and were

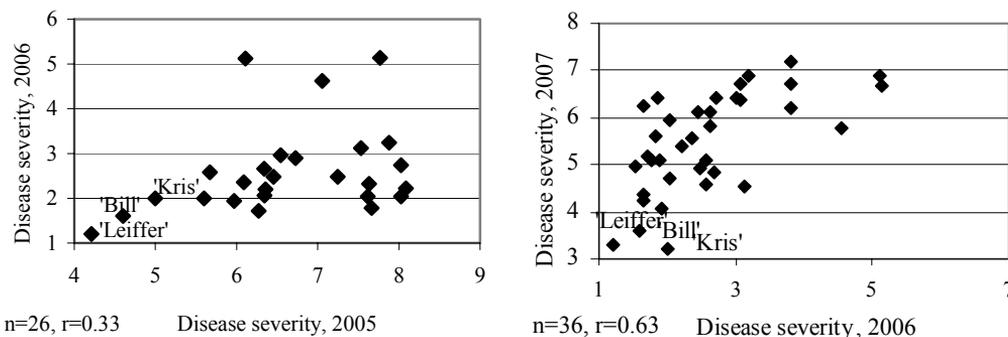
characterized by scores from 2.5 to 3.4. The difference in resistance scores between the most susceptible and the most resistant lines was 2.8 times.



**Figure 1.** Distribution of winter wheat lines from competitive trial nursery by resistance to eyespot in 2005–2007

The present study revealed considerable differences in eyespot resistance among the recently developed Lithuanian winter wheat breeding material. As well as cultivar differences, there were often large differences within a cultivar in different replications. Such differences were probably due to the uneven distribution of soil-borne and air-borne inoculum within the trial. The high level of eyespot in some lines indicated that they had the potential to be heavily infected. Conversely, those lines that had low disease levels in all years can be reasonably claimed to be resistant to this disease, although confirmation in further trials with controllable infection levels would be desirable. Even

unfavourable weather conditions were beneficial for discrimination of quantitative resistance level. It is important to remember that fungal growth is very sensitive to environmental influences and the growth stage of the host. The degree of eyespot disease response seems to be very sensitive to micro- and macro-environmental variation. Significant differences between blocs have been reported by Koebner, Martin /1990/.



**Figure 2.** Correlation of eyespot resistance in winter wheat between years

It shows that under environmental conditions unfavourable for pathogen the present quantitative resistance level is sufficient to control this pathogen. Analysis of genotypes carrying *Pchl* revealed that the genotypic background contributes to the resistance level. Cultivars with *Pchl* were the genotypes with the lowest disease severity. On about 10% of the stems of these cvs. eyespot infection ranged from 7 to 8 scores in 2005. The differences in resistance scores among these cultivars were significant in all years. This situation is similar to that reported by Lind et al. /1994/, where plants also showed remarkably large lesions if the infection conditions were favourable. An increase in the cropping area of wheat cultivars with *Pchl* raises the possibility that the pathogen will adapt due to high infection pressure, and the resistance will break down. Therefore, new resistance sources were investigated in this direction; however distinct relation of wild relatives of wheat makes transfer of new resistance genes very long and complicated /Murray et al., 1994; Yildirim et al., 1995; Cadle et al., 1997; Figliuolo et al., 1998/.

As Lind /1999/ has reported that after transferring *Pchl* into susceptible genetic background, lines were selected that had a relatively low level of resistance; although it was proved that they carried the resistance gene. This suggests that there is sufficient additive gene action for eyespot resistance. Examples for the successful selection of genotypes with improved quantitative resistance in wheat were reported by Lind et al. /1994/. One of the progeny of partial resistance genes in modern wheat is 'Cappelle-Desprez' /Muranty et al., 2002/. The information about other sources of partial resistance is very scarce in scientific literature. However, it is possible to search among the present cultivars for quantitative resistance analyzing their pedigree and selecting cultivars with 'Cappelle-Desprez' in pedigree.

As Thiele et al. /2002/ has reported infection resistance and penetration resistance can be seen at the juvenile growth stage, but stem resistance can only be

analysed at the adult growth stage. In the study the correlation coefficients between juvenile and adult growth stage resistance were low, indicating different resistance mechanisms at juvenile and adult growth stages.

**Table.** The winter wheat breeding lines the least and the most diseased by eyespot Dotnuva, 2005–2007

2005					
The least diseased lines	Avg. *	SD**	The most diseased lines	Avg.	SD
‘Flair/Lut.9365’	5.0	0.61	‘Rufa/Elena’	7.7	0.35
‘Haven/Hamlet’	5.3	0.51	‘Moskovskaya39/A940143’	7.7	0.09
‘Flair/Ukrainka Odesskaya’	5.5	0.88	‘Elena/Lut.956’	7.8	0.54
‘Flair/Lut.3-96’	5.6	0.18	‘Soratnica/Ibis’	7.8	0.42
‘Marabu/Athlet’	5.8	0.57	‘Moskovskaya39/Lut.951’	7.8	0.59
‘Bandit/Tarso’	5.8	0.91	‘Flair/Lut.9329’	8.0	0.14
‘Strumok/Lut.9-365’	5.9	0.42	‘Širvinta1/Batis’	8.0	0.18
‘Rufa/Lut.96-10’	6.1	0.20	‘Flair/Lut.9365’	8.1	0.48
***LSD <sub>05</sub>	0.54		LSD <sub>05</sub>	0.32	
2006					
Rostovchanka/Flair’	1.1	0.09	‘Pegasos/Dream’	3.8	0.25
Rostovchanka/Lars’	1.3	0.21	‘Pegasos/Biscay’	3.8	0.42
Dream/Lut.9329’	1.4	0.15	‘Hussar/Konsul A//Previa’	3.9	0.58
Lut.96-3/Bold’	1.6	0.31	‘Dream/Pesma’	4.2	0.37
Biscay/Dream’	1.7	0.31	Bill/Aspirant’	4.5	0.46
‘Bill/Aspirant’	1.7	0.10	‘Flair/Lut.9329’	4.6	0.48
‘Dream/Pesma’	1.7	0.25	‘Flair/Lut.9365’	5.1	0.31
‘Astron/Tarso//Mobil’	1.8	0.22	‘Elena/Lut.956’	5.1	0.58
LSD <sub>05</sub>	0.23		LSD <sub>05</sub>	0.35	
2007					
‘Dream/Pesma’	2.5	0.43	‘Rostovchanka/Belisar’	6.5	0.58
‘Biscay/Dream’	2.8	0.50	‘Dirigent/Cortez’	6.6	0.45
‘Flair/Pentium’	2.8	0.51	‘Elena/Lut.956’	6.7	0.22
‘Dream/Pesma’	2.9	0.39	‘Dirigent/Cortez’	6.7	0.44
‘Dream/Pesma’	3.3	0.26	‘Pegasos/Lut.96-3’	6.7	0.46
‘Dream/Pesma’	3.4	0.43	Flair/Lut.9365’	6.9	0.45
‘Lone/Inna//Lut.96-6’	3.4	0.41	‘Flair/Ukrainka Odesskaya’	6.9	0.17
‘Lone/Inna//Lut.96-2’	3.8	0.22	‘Flair/Kris’	7.0	0.41
LSD <sub>05</sub>	0.39		LSD <sub>05</sub>	0.40	

\*Avg. – average, \*\*SD – standard deviation, \*\*\*LSD<sub>05</sub> – least significant difference.

In breeding programmes designed to improve the resistance to eyespot, *Pch1* is usually transferred to genotypes with unknown resistance levels but selected for other important traits. Knowledge of the quantitative resistance level of parents is an advantage in planning genetic combinations. Crosses of *Pch1* carriers with quantitative resistant genotypes could yield progenies with the highest resistance levels. Transfer of quantitative resistance requires more expense in breeding, e.g. by selection cycles in segregating generations or by multiple crosses to accumulate resistance genes.

Because yield losses due to eyespot have been demonstrated in many researches to be negligible when disease incidence is below 10%, the most appropriate short time strategy for control of the disease is probably the avoidance of highly susceptible genotypes, especially in early autumn-sown crops amongst which disease incidence is usually greatest. Considering the results of this study, the best differentiation between genotypes was attained in 2005 with medium levels of disease severity. The satisfactory effectiveness of *Pch1* was confirmed. It was determined that lines differed significantly in resistance, which mainly depended on the accumulated level of partial resistance.

### Conclusions

1. Eyespot severity on the genotypes tested in 2005 was estimated by scores from 4.2 to 8.1. The lines in 2006 were characterized by scores from 1.1 to 5.1. The infection scores of lines in 2007 fluctuated from 2.5 to 7.0.

2. The resistance correlation between years was low ( $r = 0.33$ ) in the 2005 vs. 2006 cycle and medium strong ( $r = 0.63$ ) in 2006 vs. 2007 cycle.

3. The best differentiation between genotypes was attained in 2005 with medium levels of disease severity. The satisfactory effectiveness of *Pch1* was confirmed. It was determined that lines differed significantly in resistance, which mainly depended on the accumulated level of partial resistance.

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