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Rapid communication

How do eyespot resistance genes transferred into winter wheat breeding lines affect their yield?

Michał Kwiatek[1*†], *Halina Wiśniewska*[1†], *Marek Korbas*[2], *Magdalena Gawłowska*[1], *Jolanta Belter*[1], *Maciej Majka*[1], *Jakub Danielewicz*[2]

[1] Institute of Plant Genetics of the Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland
[2] Institute of Plant Protection – National Research Institute, Władysława Węgorka 20, 60-318 Poznań, Poland
†These authors participated equally in the research

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Abstract: Eyespot can reduce yields, even up to 50%. There are four genetically characterized resistances in wheat varieties, controlled by: (1) the *Pch1* gene, transferred from *Aegilops ventricosa*; (2) the *Pch2* gene, originating from wheat variety Capelle Desprez; (3) the *Pch3* gene, originating from *Dasypyrum villosum*; and (4) the *Q.Pch.jic-5A* gene, a quantitative trait locus (QTL) located on chromosome 5A of Capelle Desprez. However, those loci have drawbacks, such as linkage of *Pch1* with deleterious traits and limited effectiveness of *Pch2* against the disease. Here we present an initial study which aims to characterize wheat pre-registration breeding lines carrying 12 eyespot resistance genes, consider their resistance expression in inoculation tests and the influence of resistance genotypes on the yield. We selected four groups of breeding lines, carrying: (1) the *Pch1* gene alone: one line; (2) the *Pch2* gene alone: four lines; (3) the *Q.Pch.jic-5A* gene alone: one line; and (4) *Pch1* + *Q.Pch.jic-5A*: three lines. For the first time, the effect of the combination of *Pch1* and *Q.Pch.jic-5A* genes was compared with resistance conferred by *Pch1* or *Q.Pch.jic-5A* alone. We found significant differences between infection scores evaluated in resistant lines carrying *Pch1* and *Q.Pch.jic-5A* alone, while no differences in terms of the level of resistance expression were detected between *Pch1* alone and *Pch1* + *Q.Pch.jic-5A*, and between wheat lines carrying *Pch1* and *Pch2* alone. Moreover, we demonstrated that the *Pch1* gene, together with an *Ae. ventricosa* segment, caused statistically significant yield losses, both as a single eyespot resistance source or in a combination with *Q.Pch.jic-5A*. Yield scores showed that wheat lines with *Q.Pch.jic-5A* had the highest yields, similar to the yielding potential of *Pch2*-bearing lines and control varieties.

Key words: eyespot, inoculation tests, isozyme, molecular markers, resistance, *Triticum aestivum*, wheat, yield

Eyespot is a stem base disease of winter wheat (*Triticum aestivum* L.) caused by two closely related fungal species: *Oculimacula yallundae* (formerly *Tapesia yallundae*) and *Oculimacula acuformis* (formerly *Tapesia acuformis*; Crous *et al*. 2003), which often coexist in the same field. Eyespot causes the reduction of nutrient transport at the stem base and lodging, which consequently, can lead to a significant yield reduction (Lucas *et al*. 2000), even up to 50% (Murray 2010). Yield losses are due to the reduced number of tillers, premature wilting or death of stems, and smaller kernels (Scott and Hollins 1974; Murray and Bruehl 1986).

There are four sources of eyespot resistance identified in commercial wheat varieties: *Pch1* (Worland *et al*. 1988), *Pch2* (de la Peña *et al*. 1996), *Pch3* (Murray *et al*. 1994) and *Q.Pch.jic-5A*, a quantitative trait locus (QTL) on chromosome 5A (Burt *et al*. 2010). *Pch1* is the most effective eyespot resistance gene. It was introduced into the VPM-1 wheat line (VPM = Ventricosa × Persicum × Marne; Maia 1967) from the wild grass *Aegilops ventricosa* (Zhuk.) Chennav. (Maia 1967) and is located on the distal end of chromosome 7D (Doussinault *et al*. 1983; Worland *et al*. 1988). According to McMillin *et al*. (1986), there is a close association between the *Pch1* resistance gene and *EP-D1b*, an endopeptidase isozyme allele from *Ae. ventricosa* chromosome 7DL. This loci is linked to simple sequence repeat (SSR) markers *XustSSR2001-7DL* as well as *Xbarc97* (Groenewald *et al*. 2003; Chapman *et al*. 2008). However, Santra *et al*. (2006) showed that *EP-D1b* is more effective than *XustSSR2001-7DL*. VPM-1 is the most popular breeding line and has been used extensively as a source of *Pch1* in breeding programs (Doussinault *et al*. 1983). The second eyespot resistance gene, *Pch2*, is present in the variety Cappelle Desprez (Hollins *et al*. 1988). Vincent *et al*. (1952) found that Cappelle Desprez was less infected by eyespot than other cultivars in field trials. However, Johnson (1992) reported that *Pch2* is less effective than *Pch1*. The *Pch2* gene was identified in Cappelle Desprez wheat using three closely linked SSR markers (Table 1) (Chapman *et al*. 2008). Muranty *et al*. (2002) showed that *Pch2* caused resistance to eyespot at the seedling stage and an

*Corresponding address:
mkwi@igr.poznan.pl

Table 1. Markers and their products linked with eyespot resistance expression identified in presented breeding lines of wheat

Resistance gene	Marker	Product		References
		resistant plants	susceptible plants	
Pch1	*EpD1*	*EpD1b*	*EpD1a*	McMillin *et al*. 1986
	XustSSR2001-7DL	240 bp	220 bp	Groenewald *et al*. 2003
	Xbarc97	0 bp	260 bp	Chapman *et al*. 2008
Pch2	*Xwmc346*	205 bp	210 bp	Chapman *et al*. 2008
	Xwmc525	205 bp	210 bp	Chapman *et al*. 2008
	Xcfa2040	320 bp	300 bp	Chapman *et al*. 2008
Q.Pch.jic-5A	*Xgwm639*	140 bp	165 bp	Burt *et al*. 2011
	Xbarc197	185 bp	180 bp	Burt *et al*. 2011

bp – base pairs

additional gene on chromosome 5A was responsible for eyespot resistance at the adult stage. Furthermore, Burt *et al*. (2011) described a QTL on chromosome 5A, conferring effective resistance to *O. acuformis* and *O. yallundae* at both seedling and adult stages.

The main aims of this study were (1) to screen the collection of 150 winter wheat breeding lines in order to identify genotypes with *Pch1*, *Pch2* and *Q.Pch.jic-5A* loci, (2) to analyze the resistance expression in those lines using inoculation tests at BBCH31-32 growth stage and (3) to compare the yield of these genotypes with positive and negative control varieties.

Marker analyses (Table 1) were made on 10 randomly chosen plants of each pre-registration breeding line and both positive (Rendezvous with *Pch1*, *Pch2* and *Q.Pch.jic-5A* loci) and negative (Ozon and Patras, eyespot susceptible) control varieties. *Pch1*, *Pch2* and *Q.Pch.jic-5A* loci identification was made using eight verified markers (Table 1) according to McMillin *et al*. (1986); Groenewald *et al*. (2003); Chapman *et al*. (2008) and Burt *et al*. (2011). Based on the marker analysis, we selected five groups of breeding lines, carrying: (1) the *Pch1* gene alone: one line; (2) the *Pch2* gene alone: four lines; (3) the *Q.Pch.jic-5A* alone: one line; and (4) *Pch1* + *Q.Pch.jic-5A*: three lines. The fifth group consisted of two cultivars (Ozon and Patras) considered as negative controls.

Resistance expression was evaluated using inoculation tests made on 100 randomly chosen plants from each of four replications by calculating the infection index (*K*) and the percentage of infected stems according to Kwiatek *et al*. (2012; 2015). A sample of 50 plants from each replicate of each wheat genotype and the control varieties were evaluated (a total of 200 stems for each genotype). The percentage of infected stems was determined and the leaf sheath infection index was calculated using the *K*-index formula:

$$K = \frac{[n(\mathrm{II}) \times 0.25] + [n(\mathrm{III}) \times 0.75] + n(\mathrm{IV})}{n(\mathrm{I} + \mathrm{II} + \mathrm{III})},$$

where: I – no symptoms; II – less than 50% of stems surface infected; III – over 50% of stems surface infected; IV – 100% of stems surface infected, rotten tissue; *n* – number of stems.

All hypotheses about the equality of genotype groups were tested at p = 0.05 and p = 0.01 significance levels by using analysis of variance (ANOVA). After the rejection of the null hypothesis of no differences between groups, Tukey's tests ($HSD_{0.05}$ and $HSD_{0.01}$) for unequal replications were used for the planned pair comparisons (Gomez and Gomez 1984).

Analysis of variance (ANOVA) showed that there were no significant differences at p = 0.05 and p = 0.01 between lines and their replications within the groups considering the *K*-index and the percentage of infected leaf sheaths. Hence, the scores within the groups of resistance types were pooled. For the first time, the effect of the combination of *Pch1* and *Q.Pch.jic-5A* genes was compared with resistance conferred by *Pch1* or *Q.Pch.jic-5A* genes alone. Significant differences between infection scores evaluated in resistant lines carrying *Pch1* and *Q.Pch.jic-5A* were observed, while no significant differences were detected between *Pch1* and *Pch1* + *Q.Pch.jic-5A* wheat lines (Table 2). In contrast, the resistance provided by *Q.Pch.jic-5A* is similar to that conferred by *Pch1* + *Q.Pch.jic-5A*. Moreover, there were no significant differences between resistance conferred by *Pch1* and *Pch2*. Previously, Burt *et al*. (2010) reported no reduction in resistance to eyespot in lines combining *Pch1* and *Pch2*, suggesting that the potent effect of *Pch1* is sufficient to mask the differential resistance conferred by *Pch2*. All four groups of resistant breeding lines were less infected in comparison to cultivars Ozon and Patras (negative controls), which was confirmed by Tukey's test: $HSD_{0.05} = 0.48$ and $HSD_{0.01} = 0.58$ for *K*-index scores and $HSD_{0.05} = 3.25$ and $HSD_{0.01} = 3.94$ for percentage of infected sheaths (Table 2).

Finally, the yield level (dt · ha^{-1}) of breeding lines was measured in two replications in each of four locations in Poland (Kobierzyce, Nagradowice, Smolice and Strzelce). During the experiment natural infections caused by *O. yallundae* and *O. acuformis* were not present, so the results of yield potential evaluation were not interfered with and show the real differences between resistant and non-resistant wheat genotypes considering the yield level. The results indicate that the yield level of the compared breeding lines is connected with their diversified eyespot resistance traits (Table 3). The lowest yield was measured in plants carrying the *Pch1* gene (mean = 132.25 dt · ha^{-1}). This agrees with previous reports indicating a linkage between *Pch1* and yield limitations (Johnson 1992). Furthermore, the breeding lines with resistance conferred by

Table 2. Summary of variance components and Tukey's test for *K*-index and percentage of infected stems

No.	Sources of resistance	*K*-index							Infected stems [%]						
		N	Σx	mean	Σx^2	variance	SD	SE	N	Σx	mean	Σx^2	variance	SD	SE
1	*Pch1*	4	2.00	0.5	1.13	0.04	0.20	0.10	4	12	3.00	46	3.33	1.83	0.91
2	*Pch2*	16	11.84	0.74	9.38	0.04	0.20	0.05	16	66	4.13	332	3.98	2.00	0.5
3	*Q.Pch.jic-5A*	4	4.03	1.01	4.12	0.02	0.14	0.07	4	28	7.00	214	6.00	2.45	1.22
4	*Pch1* + *Q.Pch.jic-5A*	12	7.41	0.62	5.06	0.04	0.21	0.06	12	51	4.25	281	5.84	2.42	0.70
5	Non-resistant controls	24	42.74	1.78	80.59	0.19	0.44	0.09	24	263	10.96	2995	4.91	2.22	0.45

Tukey's Honest Significant Difference (HSD) test												
HSD level	$HSD_{0.05}$	$HSD_{0.01}$	1vs2	1vs3	1vs4	1vs5	2vs3	2vs4	2vs5	3vs4	3vs5	4vs5
K-index	0.48	0.58	ns	$p < 0.05$	ns	$p < 0.01$	ns	ns	$p < 0.01$	ns	$p < 0.01$	$p < 0.01$
Infected leaf sheaths [%]	3.25	3.94	ns	$p < 0.01$	ns	$p < 0.01$	ns	ns	$p < 0.01$	ns	$p < 0.01$	$p < 0.01$

N – number of replications; Σx – sum of scores; Σx^2 – sum of square scores; SD – standard deviation; SE – standard error; ns – not significant

Table 3. Summary of variance components and Tukey's test for yield potential

No.	Sources of resistance	Yield						
		N	Σx	mean	Σx^2	variance	SD	SE
1	*Pch1*	8	1,058.00	132.25	139,922	0.23	0.48	0.17
2	*Pch2*	32	4,623.52	144.49	668,622	19.11	4.37	0.77
3	*Q.Pch.jic-5A*	8	1,205.20	150.65	181,566	0.35	0.59	0.21
4	*Pch1* + *Q.Pch.jic-5A*	24	3,169.60	132.07	418,960	15.72	3.96	0.81
5	Non-resistant controls	48	7,084.00	147.58	1,045,697	4.60	2.15	0.31

Tukey's Honest Significant Difference (HSD) test												
HSD level	$HSD_{0.05}$	$HSD_{0.01}$	1vs2	1vs3	1vs4	1vs5	2vs3	2vs4	2vs5	3vs4	3vs5	4vs5
Yield	3.29	3.95	$p < 0.01$	$p < 0.01$	ns	$p < 0.01$	$p < 0.01$	$p < 0.01$	ns	$p < 0.01$	ns	$p < 0.01$

N – number of replications; Σx – sum of scores; Σx^2 – sum of square scores; SD – standard deviation; SE – standard error; ns – not significant

Pch1 + *Q.Pch.jic-5A* also had a reduced yield level (mean = = 132.06 dt · ha^{-1}), which was comparable with that of *Pch1*-bearing plants. This shows that despite the great impact in limiting *Oculimacula* spp. infection, the reduction of yield is substantial, thereby creating a need to increase the recombination of the translocated *Ae. ventricosa* segment. So far, several *Pch1*-bearing wheat varieties with a satisfying yield potential have been released (HGCA 2010; Burt 2010). Nevertheless, the reason of their high yields is unclear and it can only be speculated that it is caused by a reduced *Ae. ventricosa* segment in which the linkage has been broken, or to another genetic source that promotes yield potential. Previous reports (Hollins *et al*. 1988; Lind 1999) have shown that cultivars carrying *Pch1* with Cappelle Desprez (*Pch2* and *Q.Pch.jic-5A*) in their pedigrees, such as Rendezvous, have enhanced adult plant resistance. However, those varieties are not widely used in breeding as a result of their relatively low yield in spite of the absence of the disease. Furthermore, yield scores show that wheat lines with *Q.Pch.jic-5A* had the highest yield (mean = 150.65 dt · ha^{-1}), which is similar to the yielding potential of *Pch2*-bearing lines and positive control varieties (Table 3). This single major QTL, on the long arm of chromosome 5A, confers resistance to both *O. yallundae* and *O. acuformis* at both the seedling and adult plant stages (Burt *et al*. 2011).

In conclusion, we found that the *Pch1* gene, together with an *Ae. ventricosa* segment, caused statistically significant yield losses, either as a single eyespot resistance source or in a combination with the QTL located on chromosome 5A, responsible for eyespot tolerance. However, there are no significant differences in the resistance effects of *Q.Pch.jic-5A* when compared to combined *Pch1* + + *Q.Pch.jic-5A* genes or *Pch2* gene. Together with the high yield potential this could be interesting material for production of eyespot-tolerant wheat varieties.

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