www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 52, No. 2 (2012)

DOI: 10.2478/v10045-012-0040-5

IDENTIFICATION OF PCH1 EYESPOT RESISTANCE GENE IN THE COLLECTION OF WHEAT LINES (*TRITICUM AESTIVUM* L.)

Michał Kwiatek[1]*, *Katarzyna Pankiewicz*[1], *Halina Wiśniewska*[1], *Marek Korbas*[2], *Jakub Danielewicz*[2]

[1] Institute of Plant Genetics Polish Academy of Science
Strzeszyńska 34, 60-479 Poznań, Poland
[2] Institute of Plant Protection – National Research Institute
Władysława Węgorka 20, 60-318 Poznań, Poland

Received: September 16, 2011
Accepted: January 30, 2012

Abstract: Endopeptidase marker *EpD1b* and STS marker *XustSSR2001-7DL* are closely linked to very effective eyespot resistance gene *Pch1*. Because of this, the aim of this study was to compare the results obtained under lab conditions using such markers with the results obtained under field conditions. 134 wheat breeding lines and *Triticum aestivum* L. var. Randevous used as a eyespot resistance control were analized. The combination of three methods allowed to select eight completely resistant or high resistant lines, that could be used in following breeding processes. Results obtained using endopeptidase and STS markers in 100% correlate with the phenotyping scoring.

Key words: eyespot disease, isozyme, marker-assisted selection, resistance gene, *Triticum aestivum*

INTRODUCTION

Eyespot (strawbreaker foot rot), caused by necrotrophic fungal pathogens: *Oculimacula acuformis* (formerly *Tapesia acuformis*) and *O. yallundae* (*T. yallundae*) (Crous *et al*. 2003), is one of most common diseases of cereals in the temperate climate and one of the most dangerous disease between winter and spring. Usually *O. acuformis* and *O. yallundae* simultaneously appears on the field. They have a wide host range among small grain cereals and grasses (Murray 1992). First symptoms appear during the autumn pullulating. Eye-shaped, eliptical lesions on the lower portion of the stem are clearly visible at BBCH 30–32 (Korbas 2004). Crops from infected plants are slighter with reduced quality. Severe infections could result in yield losses of up to 50% (Fitt *et al*. 1988; Janczewska 1991).

Three eyespot resistance genes are reported (*Pch1*, *Pch2*, *Pch3*) (McIntosh 2006). First two are as a main sources of resistance. *Pch1*, *Pch2* are effective at the seedling stage and have been incorporated into cultivated wheat varieties (Chapman *et al*. 2008).

Moderated resistance to eyespot was first recognized in the French wheat cv. Cappelle – Desprez (Vincent *et al*. 1952; Batts and Fiddian 1955; Hollins *et al*. 1998). Most of the resistance of Cappelle Desprez is conferred by resistance gene(s) *Pch*2, located on chromosome 7A with additional effects associated with chromosomes 2B and 5D suggesting the involvement of genes of minor effect (Law *et al*. 1975; Chapman *et al*. 2008).

The *Pch1* resistance gene was identified in *Aegilops ventricosa* (2n = 4x = 28, $D^VD^VM^VM^V$) (Mena *et al*. 1992). First eyespot resist wheat line VPM1 was derived from a cross between an amphiploid (*Ae. ventricosa* x *T. persicum*) and *T. aestivum* cv. Marne (Maia 1967). The translocated sequence with the *Pch1* gene has been mapped in chromosome arm 7DL (Jahier *et al*. 1978; McMillin *et al*. 1986). VPM1 line was a base for breeders to create new resistance cultivars like Randevous (used in presented work as a resistance standard).

An identification of the *Pch1* locus is provided by closely linked endopeptidase gene locus *EpD1b* (Worland *et al*. 1988; Groenewald *et al*. 2003).

Endopeptidase 1 (*EP-1*) in wheat is controlled by 3 loci: *EpA1*, *EpB1* and *EpD1*, located in homoeologous chromosomes 7AL, 7BL and 7DL, respectively (Hart and Langston 1977; McMillin and Tuleen 1977; Koebner *et al*. 1988; Santra *et al*. 2006). *EpD1* has two alleles: *EpD1a* – derived from wheat and *EpD1b* – from *Ae. ventricosa* (Koebner *et al*. 1988). Close localization of *EpD1b* gene locus and *Pch1* gene locus determine utility of endopeptidase markers. Results of researches made by Santra *et al*. (2006) documented that the endopeptidase marker is 100% accurate for predicting strawbreaker foot rot reaction.

Groenewald *et al*. (2003) identified the amplified fragment length polymorphism (AFLP) markers linked to the *EpD1b* and *Pch1* and converse one of them into Polymeraze Chain Reaction (PCR)-based screening system. STS

*Corresponding address:
mkwi@igr.poznan.pl

marker predicted disease reaction with approximately 90% accuracy (Santra *et al*. 2006).

The present paper reports study of identification eyespot resistance of wheat lines using enzymatic and molecular markers and phenotypic evaluation under field control after inoculation.

MATERIALS AND METHODS

Plant materials

The 134 breeding lines of wheat, *Triticum aestivum* L., with random genetic background, were obtained from Polish breeding companies. *T. aestivum* variety Randevous was used in as a resistance control. Seeds from the 134 lines and Randevous variety were planted in the greenhouse and plants were grown at 22°C during 14 days.

Evaluation of resistance under laboratory condition

The endopeptidase assay was made using leaf tissue from two-weeks-old seedlings. The enzyme was extracted by grinding the leaves using a Plexiglas bar in 10 µl of 0.025 M glycylglycine buffer (pH 7.4; SIGMA). To load samples, paper strips were soaked in the enzyme extract for each genotype and inserted in to the gel. The 10% starch (SIGMA) gel was run at 4°C at 200V. After electrophoresis the gel was incubated in the dark at 37°C with 0.5% solution of low melting agarose containing 2.56 mg Fast Black K Salt (SIGMA) and 1.12 mg N-α-Benzoyl-DL-Arginine-LB-Naphthylamide (BANA; SIGMA) in 0.1 M Trizma maleate (SIGMA) – NaOH (pH 5.8; POCH).

The identification of STS marker *XustSSR2001-7DL* (Groenewald *et al*. 2003) was made using the same leaf tissue that have been used for the endopeptidase assay. Total genomic deoxyribonucleic acid (DNA) was extracted from 14-days-old seedlings using heksadecyltrimethylammonium bronhide (CTAB) method according to Doohan *et al*. 1998. The marker was amplified following the protocol of Groenewald *et al*. 2003. PCR profile was modificated with reference to standard protocol and consisted of denaturation at 94°C for 10 min., followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min., followed by final extension for 10 min. at 72°C and a soak temperature of 4°C. The products of amplification were visualized using 3% agarose (SIGMA) gel (1 x TBE buffer, 5h at 100V) stained with ethidium bromide.

Evaluation of resistance under field conditions

Field experiment was conducted at the Kopaczewo Field Station in 2011, where wheat grown in monoculture during 4 years, to increase the risk of the infection. It was carried out in four replication at completely randomized block design. Experiment on the provided all 134 wheat lines and control variety were inoculated by spraying at BBCH 31-32 with a fresh-made conidial-mycelium suspension of *Oculimacula acuformis* and *O. yallundae* (1:1 ratio, 4 x 10^6 spres/ml). Plant material was harvested and than the level of the disease was scored in the Department of Mycology (Institute of Plant Protection, National Research Institute). Only eyespot symptoms were rated. Sample of 50 grains from each replicate of each wheat lines and control variety were evaluated (in a total 200 grains for each genotype). The percent of infected grains was determine and than the grain infection index was calculated (Fig. 1). The level of the grain sample infection scale was measured using I – IV scale (I – no symptoms, II – less than 50% of grain surface infected, III – over 50% of grain surface infected, IV – 100% of grain surface infected, rotten tissue). Results were presented as a mean from each replication.

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

Fig. 1. Grain infection index. I – no symptoms, II – less than 50% of grain surface infected, III – over 50% of grain surface infected, IV – 100% of grain surface infected, rotten tissue noted. *n*- number of evaluated grains

RESULTS

The endopeptidase zymograms analyzed among the 134 wheat breeding lines and cv. Randevous were grouped in to six classes of band patterns (Table 1, Fig. 2). First class was represented by cv. "Randevous" and 8 lines: KBP 06.200, KBP 03 245, KBP 08.3, KBP 0652, NAD 0715, NAD 9721, STH 810, MOB 3982. It had the top and the lower bands for *Ep-D1b*. The second class zymograms had the top band for *Ep-D1b* and two bands for *Ep-D1a.* This class numbers 49 genotypes (lines). Nine lines were classified into third type, having only one (top) band for *Ep-D1b.* Fourth class (47 lines) had the top band for *Ep-D1b* and the lower band for *Ep-D1a*. All four bands (two for *Ep-D1b* and two for *Ep-D1a*) were classified as fifth type (8 lines). Plants from 13 lines had the top band for *Ep-D1b* and the top band for *Ep-D1a*.

The amplification of the SSR locus using *XustSSR2001-7DL* STS marker yielded a product of *240* base pairs (*bp*) in resistant genotypes and *222 bp* product in susceptible lines (Fig. 3). Plants from 8 lines (the same like those in the first class of band patterns in endopeptidase analysis) and *Triticum aestivum* L. var. Randevous, used in as a eyespot resistance standard, had the *240 bp* band. Rest of analyzed lines had *222 bp* band.

The results of the inoculation test of derived wheat lines were divided into 6 groups with regard to infection level. The range of the infection came from 0 to 16,5%. First group of 8 genotypes (STH 810, CHD 3443/07, DED 389/06, KBP 08.14, SMH 8429, HRSM 732, MOB 3989/07, MOB 3975/07) had no symptoms of eyespot infection. Second group of 4 genotypes (KBP 08.03, NAD 0715, MOB 3982/07, MOB 3858/07) had 0.5% of grain infection. Four genotypes (KBP 03.245, KBP 06.52, NAD 06136, STH 9005) had 1% of grain infection and were classified to the third group. Fourth group of two genotypes (KBP 06.200 and NAD 0721) had 1.5 % of grain infection. 2% of infection was observed on plants of 3 genotypes (CHD 382/06, MIB 89/06 and KBP 02.813/2). Rest of used genotypes had 7–16.5% of grain infection.

Table 1. Endopeptidase zymogram types (with band pattern and the number of assigned wheat lines)

		Endopeptidase zymogram types					
		1	2	3	4	5	6
Ep-D1b	top band	–	–	–	–	–	–
	lower band	–				–	
Ep-D1a	top band		–		–	–	
	lower band		–			–	–
Number of lines		8	49	9	47	8	13

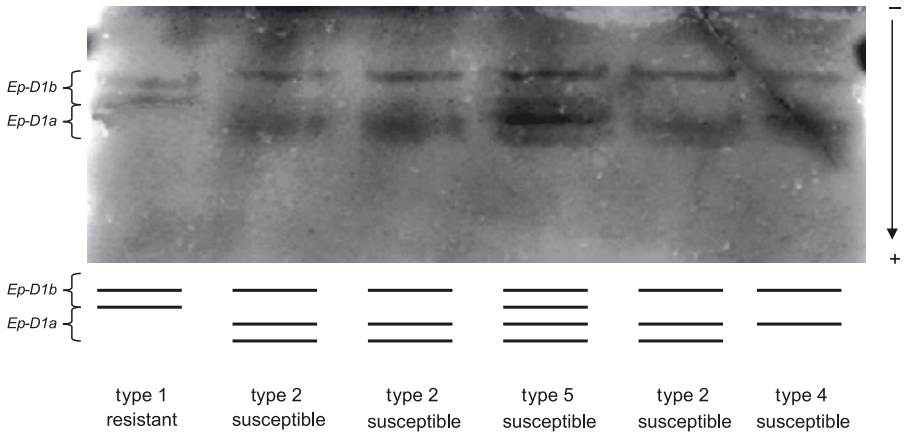

Fig. 2. Endopeptidase zymograms of the given wheat lines and their relationship with the eyespot reaction

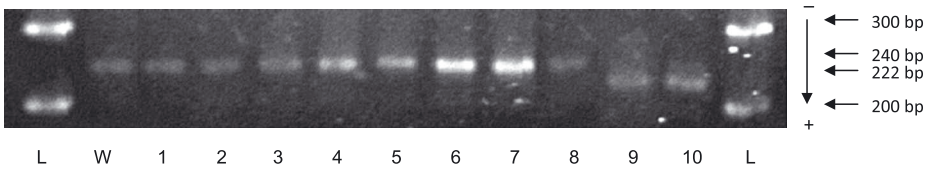

Fig. 3. Amplified products for the STS marker *XustSSR2001-7DL*. Amplified bands 222 bp and 240 bp indicate eyespot susceptibility and resistance specific bands, respectively. (L) DNA size ladder, (W) Rendezvous – resistance standard, (1–8) resistant lines (KBP 06.200, KBP 03 245, KBP 08.3, KBP 0652, NAD 0715, NAD 9721, STH 810, MOB 3982), (9–10) susceptible lines (CHD 974/05-9/06, DED 263/06)

Table 2. Results of the inoculation test of derived wheat lines divided into 6 groups with regard to infection level in comparison to the endopeptidase assay and molelucar marker identification

No.	Line	Infection index K	Average percentage of infected grains	Endopeptidase zymogram types						*XustSSR2001-7DL* bands	
				1 [R]	2 [S]	3 [S]	4 [S]	5 [S]	6 [S]	222 bp [S]	240 bp [R]
1	2	3	4	5	6	7	8	9	10	11	12
C	Rendezvous	0	0	+							+
1	STH 810	0	0	+							+
2	CHD 3443/07	0	0		+					+	
3	DED 389/06	0	0		+					+	
4	KBP 08.14	0	0				+			+	
5	SMH 8429	0	0				+			+	
6	HRSM 732	0	0		+					+	
7	MOB 3989/07	0	0						+	+	
8	MOB 3975/07	0	0						+	+	
9	KBP 08.03	0.06	0.5	+							+

1	2	3	4	5	6	7	8	9	10	11	12
10	NAD 0715	0.06	0.5	+							+
11	MOB 3982/07	0.06	0.5	+							+
12	MOB 3858/07	0.06	0.5		+					+	
13	KBP 03.245	0.13	1	+							+
14	KBP 06.52	0.13	1	+							+
15	NAD 06136	0.13	1		+					+	
16	STH 9005	0.13	1				+			+	
17	NAD 0721	0.19	1.5	+							+
18	KBP 06.200	0.19	1.5	+							+
19	CHD 382/06	0.25	2.00		+					+	
20	MIB 89/06	0.38	2.00		+					+	
21	KBP 02.813/2	0.25	2.00		+					+	
22	LAD 467/06	1.38	7.00		+					+	
…											
134	AND 505/06	3.01	16.50			+				+	

R – resistance; S – susceptible

DISCUSSION

The main goal of this experiment was to compare results obtained using endopeptidase assay and STS marker (*XustSSR2001-7DL*) with the results obtained under field condition after inoculation by *Oculimacula acuformis* and *O. yallundae*. Using enzymatic and molecular markers, eight lines (KBP 06.200, KBP 03 245, KBP 08.3, KBP 0652, NAD 0715, NAD 9721, STH 810, MOB 3982) had been selected and define as resistant. The inoculation test singled out 8 completely resistant lines (0% of infection) and 13 highly resistant lines (0.5–2% of grains infection). The range of the infection was lower in comparison with previous seasons because of the weather conditions, which have not been conductive to proper development of the pathogens. Confrontation of the three, irrespective used, methods let to determine that 8 genotypes: carried *Ep-D1b* locus, that is closely linked with *Pch1* eyespot resistance gene locus and had identified STS marker (*XustSSR2001-7DL*) linked with *Ep-D1b* locus and also were classified into group of completely resistant genotypes and highly resistant genotypes by inoculation tests. The results also documented that the endopeptidase marker and the STS marker is 100% accurate for predicting eyespot reaction in this set of wheat genotypes.

Presented results give some new information about endopeptidase assay of wheat genotypes than these which were reported in previous reports (McMillin *et al*. 1986; Santra *et al*. 2006). Two zymogram band patterns (type 2 and type 5) are novel. In such types of band patterns the second band for *Ep-D1a* was identified. Obtained results also documented that if only one band for *Ep-D1b* is present band (in 9 genotypes – 4–16.5% of grains infection) is not correlated with high or complete eyespot resistance.

Based on the obtained results it is possible to conclude that endopeptidase marker *EpD1b*, STS marker *XustSSR2001-7DL* and phenotypic evaluation under field conditions after inoculation can be used in the breeding programmes to identify new sources of resistance for eyespot of wheat.

ACKNOWLEDGEMENTS

Financial support for this researches came from project of Ministry of Agriculture and Rural Development: „Identification and translocation of eyespot resistance genes to cultivated wheat" (MRiRW:HOR hn-4040dec-26/10).

REFERENCES

Batts C.C.V., Fiddian W.E.H. 1955. Effect of previous cropping on eyespot in four varieties of winter wheat. Plant Pathol. 4: 25–28.

Chapman N.H., Burt C., Dong H., Nicholson P. 2008. The development of PCR-based markers for the selection of eyespot resistance genes *Pch1* and *Pch2*. Theor. Appl. Genet. 117: 425–433.

Crous P.W., Groenewald J.Z.E., Gams W. 2003. Eyespot of cereals revisited Its phylogeny reveals new species relationships. Eur. J. Plant Pathol. 109: 841–850.

Doohan F.M., Parry D.W., Jenkinson P., Nicholson P. 1998. The use of species-specific PCR-based assays to analyse *Fusarium* ear blight of wheat. Plant Pathol. 47: 197–205.

Fitt B.D.L., Goulds A., Pollet R.W. 1988. Eyespot (*Pseudocercosporella herpotrichoides*) epidemiology in relation to prediction of disease severity and yield loss in winter wheat; a review. Plant Pathol. 37: 311–328.

Groenewald J.Z., Marais A.S., Marais G.F. 2003. Amplified fragmentlength polymorphism-derived micro-satellite sequence linked to the Pch1 and *Ep-D1* loci in common wheat. Plant Breed. 122: 83–85.

Hart G.E., Langston P.J. 1977. Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. Heredity 39: 263–277.

Hollins T.W., Lockley K.D., Blackman J.A., Scott P.R., Bingham J. 1988. Field performance of Rendezvous, a wheat cultivar with resistance to eyespot (*Pseudocercosporella herpotrichoides*) derived from *Aegilops ventricosa*. Plant Pathol. 37: 251–260.

Jahier J., Doussinault G., Dosba F., Bourgeois F. 1978. Monosomic analysis of resistance to eyespot in the variety "Roazon". p. 437–440. In: Proc. 5th International Wheat Genet. Symposium (S. Ramamujan, ed.). 23–28, New Dehli, India, February 1978. 510 pp.

Janczewska A. 1991. Yield loss of winter wheat as influenced by the intensity of *Pseudocercosporella herpotrichoides* (Fron.) attack. Phytopathol. Pol. 1 (XIII): 41–44.

Koebner R.M.D., Miller T.E., Snape J.W., Law C.N. 1988. Wheat endopeptidase: genetic control, polymorphism, intrachromosomal gene location, and alien variation. Genome 30: 186–192.

Korbas M. 2004. Choroby i Szkodniki Zbóż. Multum, Poznań, Poland, 88 pp.

Law C.N., Scott P.R., Worland A.J., Hollins T.W. 1975. The inheritance of resistance to eyespot *(Cercosporella herpotrichoides)* in wheat. Genet. Res. 25: 73–79.

Maia N. 1967. Obtention de blés tendres résistants au piétin-verse par croisements interspécifiques blés × *Aegilops*. C.R. Acad. Agric. Fr. 53: 149–154.

McIntosh R.A., Hart G.E., Devos K.M., Gale M.D., Rogers W.J. 2006. Catalogue of Gene Symbols for Wheat.
http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene//supplement2006.pdf

McMillin D.E., Tuleen N.A. 1977. Determination of the linkage relationships and the gene centromere genetic distances for endopeptidase structural genes in hexaploid wheat. Genetics 86 (Suppl.), p. 44.

McMillin D.E., Allan R.E., Roberts D.E. 1986. Association of an isozyme locus and strawbreaker foot rot resistance derived from *Aegilops ventricosa* in wheat. Theor. Appl. Genet. 72: 743–747.

Mena M., Doussinault G., Lopez-Brana I., Aguaded S., Garcia-Olmedo F., Delibes A. 1992. Eyespot resistance gene *Pch-1*. H-93 wheat lines. Evidence of linkage to markers of chromosome group 7 and resolution from the endopeptidase locus *Ep-D1b*. Theor. Appl. Genet. 83: 1044–1047.

Murray T.D. 1992. Pseudocercosporella. p. 149–152. In: "Methods for Research on Soilborne Phytopathogenic Fungi" (L.L. Singleton, J.D. Mihail, C.M. Rush, eds). APS Press, St. Paul, MN, USA, 265 pp.

Santra D.K., Watt C., Little L., Kidwell K.K., Campbell K.G. 2006. Comparison of a modified assay method for the endopeptidase marker *Ep-D1b* with the Sequence Tag Site marker *XustSSR2001–7DL* for strawbreaker foot rot resistance in wheat. Plant Breed. 125: 13–18.

Vincent A., Ponchet J., Koller J. 1952. Recherche de varietes de bles tendres peu sensibles au pietin-verse: resullats preliminaires. [Research on wheat varieties insusceptible to eyespot: Preliminary results]. Ann. de I'Amelioration des Plantes 3: 459–472.

Worland A.J., Law C.N., Hollins T.W., Koebner R.M.D., Giura A. 1988. Location of a gene for resistance to eyespot (*Pseudocercosporella herpotrichoides*) on chromosome 7D of bread wheat. Plant Breed. 101: 43–51.