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Eyespot Resistance Gene *Pch1* and Methods of Study of its Effectiveness in Wheat Cultivars

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Abstract

Dumalasová V., Palicová J., Hanzalová A., Bížová I., Leišová-Svobodová L. (2015): Eyespot resistance gene *Pch1* and methods of study of its effectiveness in wheat cultivars. Czech J. Genet. Plant Breed., 51: 166–173.

The scientific report presents results of our studies on the gene *Pch1*, conferring resistance to eyespot disease in wheat, caused by the fungus *Occulimacula* spp. The presence of the gene *Pch1* in wheat cultivars was analysed using the molecular marker *Xorw1*. In total 106 wheat cultivars registered in the Czech Republic and 54 breeding lines were tested. The gene *Pch1* was found in the cultivars Annie, Beduin, Hermann, Iridium, Manager and Princeps and in three breeding lines. Some of these cultivars were tested for eyespot resistance in field trials and showed a high level of resistance. Cv. Hermann was the most resistant winter wheat cultivar in our experiments in the last two years. The method of artificial infection by *Oculimacula* spp. was optimized.

Keywords: eyespot; molecular markers; *Pch1* gene; resistance; wheat

Eyespot, caused by *Oculimacula yallundae* and *Oculimacula acuformis* (syn. *Pseudocercosporella herpotrichoides* and *Tapesia* spp.; Crous *et al.* 2003), belongs to the stem-base disease complex of wheat, which includes also the pathogens *Rhizoctonia* spp., *Fusarium* spp., *Monographella nivalis*, *Gaeumannomyces graminis*. In the Czech Republic eyespot is a very important disease of cereals, which can cause yield losses up to 40%. The pathogen can survive on plant debris more than 3 years. The occurrence of eyespot varies due to weather conditions (Matusinsky *et al.* 2009). In 2015 eyespot was found in stem-base disease samples of wheat in all regions of the Czech Republic (except northern Moravia).

O. yallundae and *O. acuformis* differ in pathogenicity, occurrence, sensitivity to fungicides etc. but the symptoms on infected plants cannot be distinguished. Moreover, both species very often occur on one stem. Eyespot pathogens have a wide host range among cereals and grasses. Typical symptoms are elliptical eye-shaped spots at the base of the plants, which are visible at the end of stem elongation. However, fungicide treatment should be done at the beginning of stem elongation (BBCH 30–32), otherwise it is not sufficiently effective.

There are several known sources of resistance to eyespot, but only two resistance genes have been used in commercial cultivars so far and neither provides complete control. The most effective and also the most widely used resistance gene in breeding of eyespot-resistant wheat cultivars is the single major gene *Pch1* (*Pch* = *Pseudocercosporella herpotrichoides*), mapped to the distal end of the long arm of chromosome 7D. It was transferred to wheat from *Aegilops ventricosa* in three independent programmes. However, only the gene *Pch1* from the breeding line VPM-1 has been used as a source of resistance in wheat cultivars (Maia 1967). *Pch1* is most effective at the seedling stage and it is highly effective against both species. *Pch1* is linked to deleterious yield limiting traits carried on the *Ae. ventricosa* introgression.

The gene *Pch2* was located on the long arm of chromosome 7AL of the old French cultivar Cappelle-Desprez. Cv. Cappelle-Desprez has been used as a

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source of seedling or young plant resistance since 1953. It was the first commercial wheat cultivar resistant to eyespot. According to some studies it is less effective to *O. yallundae* at young plant stage than against *O. acuformis* (Burt *et al.* 2010).

Additional quantitative resistances are present in cv. Cappelle-Desprez on chromosomes 1A, 2B, 5D and 5A. The single major QTL gene on chromosome 5AL of Capelle-Desprez, designated *Q.Pch.jic-5A,* confers resistance to eyespot at both seedling and adult plant stages. It is effective against both *O. yallundae* and *O. acuformis*. Moreover, genes alongside 5A carry other beneficial traits. QTL controlling time to ear emergence and resistance to Fusarium head blight, which are desirable to be introduced from Capelle-Desprez to other cultivars. The gene *Q.Pch.jic-5A* could be of greater use than *Pch2* (Burt *et al.* 2011).

Another potential source of resistance, the gene *Pch3*, on the distal part of the long arm of chromosome 4V of *Dasypyrum villosum* (Yildirim *et al.* 1998), has not been used in commercial wheat cultivars yet. Multiple resistance genes are present in *D. villosum* on the chromosomes 4V and 5V.

A few other wheat cultivars from Germany and from the Northwest Pacific were reported to show moderate resistance to eyespot apparently not inherited from Cappelle-Desprez. Resistance to eyespot was also identified in *Triticum tauschii*, *T. monococcum*, *T. durum*, *T. dicoccoides*, *T. turanicum*, *Thinopyrum ponticum*, *Th. intermedium*, *Aegilops longissima* and *Ae. kotschyi*.

Development of wheat cultivars with genetic resistance against eyespot is the most cost-effective and environment-friendly strategy. A number of fungicide-resistant strains, environmental concerns and the requirement for additional inputs are the most frequently quoted constraints of the application of fungicides. Breeding for resistance to eyespot is a good example of beneficial use of molecular markers. Resistance evaluations are time-consuming and labour-intensive. Pyramiding is needed to confer a high level of resistance at the adult plant stage. The situation is further complicated by the fact that the symptoms can be masked by other diseases. Also the genotype x environment interaction is high. The same cultivars are sometimes rated moderately resistant and sometimes susceptible.

Pch1 is currently the most effective and extensively used resistance gene in the development of eyespot-resistant wheat cultivars. Various molecular markers linked to *Pch1* were developed (Burt *et al.* 2011; Wei *et al.* 2011). The endopeptidase isozyme marker *Ep-D1b* (McMillin *et al.* 1986) has been widely used. It is effective, codominant and completely linked to *Pch1*. However, it is often difficult to distinguish *Ep-D1b* from certain commonly occurring *Ep-A1* and *Ep-B1* homoeoalleles. Therefore, more user friendly PCR based alternatives have been searched for in multiple studies. Observations putative homoeologous endopeptidases of wheat, rice and maize suggested that *Ep-D1* may encode a serine protease, oligopeptidase B from *E. coli*, which was a starting point for the development of a sequence-tagged-site (STS) marker *Xorw1* (Leonard *et al.* 2008). *Pch1* and *Xorw1* represent the same or tightly linked locus. There is no recombination between *Xorw1*, *Ep-D1* and *Pch1*. We have chosen the *Xorw1* marker for its accuracy on a broad range of wheat cultivars (Meyer *et al.* 2011).

The aim of our study was to observe the expression of resistance in wheat cultivars grown in the Czech Republic and new breeding lines to eyespot using the method of artificial infection. We report on wheat cultivars grown in the Czech Republic (and/or new breeding lines) possessing the resistance gene *Pch1*. We evaluate the influence of the *Pch1* expression on infection by eyespot in field conditions.

MATERIAL AND METHODS

A set of winter wheat cultivars registered in the Czech Republic, selected foreign cultivars, breeding lines and checks carrying *Pch1* and foreign cultivars supposed to carry another translocation from *Ae. ventricosa* (*Lr37*, *Sr38*, *Yr17*) located on the chromosome 2AS were screened with the STS marker *Xorw1* to identify the presence or absence of the *Pch1* gene. The leaf tissue (50 mg) from young plants at the first leaf stage was harvested. DNA extraction for the PCR analysis was performed using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PCR conditions were similar as described by Leonard *et al.* (2008) with minor modifications. A 15 µl reaction volume consisted of 25 ng DNA, 1 U Taq polymerase, 1× PCR buffer, 1.7mM $MgCl_2$ (Qiagen), 0.17mM of each dNTP and 5µM of the primer pair (Generi Biotech, Hradec Králové, Czech Republic). For capillary fragment analysis the forward primer was labelled with a FAM fluorescent dye. The PCR program comprised 95°C for 5 min, 40 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 40 s, and a final extension of 72°C for 5 min. PCR products were preliminarily visualised

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on 4% high resolution agarose gels (Sigma-Aldrich, Saint Louis, USA). The fragment analysis was run on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, USA). The length of fragments was evaluated using GeneScan and Genotyper based on a comparison with a Tamra500 internal lane standard (Applied Biosystems).

The reaction of nine winter wheat cultivars and two breeding lines to artificial infection with *Oculimacula yallundae* and *O. acuformis* was studied in a small plot trial in Prague-Ruzyně. The natural occurrence of the stem-base disease complex in six of these cultivars was assessed under field conditions at Úhřetice (Chrudim district). This experimental field has been sown with wheat for nine years. The set of tested cultivars included cultivars registered in the Czech Republic, four of them possessing the eyespot resistance gene *Pch1* according to the molecular marker *Xorw1* analysis (Annie, Hermann, Manager, Princeps). The inoculum for a small plot trial was prepared from two isolates of *O. yallundae* and one isolate of *O. acuformis*. All the isolates originated from southern Moravia (Kroměříž). The mycelium of pathogens was grown on sterilized barley grains. The inoculum was applied on plots in November and in April (40 g/m^2) (Figure 1). The reaction of the cultivars was rated at the milk growth stage (BBCH 73–77), using a 0 to 5 rating scale (0 – no symptoms, 1 – one small spot, 2 – several spots covering maximally a half of the stem perimeter, 3 – spots covering more than a half of the stem perimeter, 4 – spot covering the whole stem perimeter, 5 – broken stem). Under field conditions (Úhřetice) 25 randomly selected plants with 4 stems were assessed. In inoculated plots (Prague) 14 randomly selected plants with 4 stems were assessed. The methods of inoculation and assessment have been developed at the Crop Research Institute in Prague-Ruzyně for a long time. The first eyespot trials were carried out by RNDr. Eliška Sychrová in the last century. The data were analysed by the UNISTAT 6.5 package (UNISTAT Ltd., London, UK) – Analysis of Variance, Multiple Comparison by Tukey.

Figure 1. Artificially infected plot of winter wheat by barley grains covered by the *Oculimacula* spp. mycelium (photo J. Palicová)

RESULTS AND DISCUSSION

A set of 106 winter wheat cultivars has been analysed for the presence of *Pch1* using the *Xorw1* marker (Table 1). Our results confirmed the presence of *Pch1* in the cultivars Manager and Hermann and its presence in cvs. Annie, Beduin, Iridium and Princeps. The absence of the 183-bp fragment in the remaining 100 cultivars also indicates the absence of *Pch1* in these genotypes. The cultivars Annie, Hermann and Princeps showed low infection levels in field trials with natural infection. Data on low susceptibility of the cultivars Annie, Hermann, Princeps and Manager to eyespot were obtained in our trials with artificial infection as well.

Statistically significant differences were found in the reaction of the tested cultivars at both localities in the years 2014–2015 (Tables 2 and 3). The cultivars with the *Pch1* gene for resistance to eyespot were less infected than those without *Pch1* and the symptoms were mostly different from typical elliptical eye-shaped spots (Figure 2). In 2014 the infection by stem-base diseases (and/or by eyespot) was higher than in 2015. The highest level of resistance to eyespot was detected in the Hermann cultivar in Prague and also at Úhřetice in both years. The typical elliptical eye-shaped spots were found very rarely in cv. Hermann in the field without artificial inoculation by *Oculimacula* spp. at Úhřetice. There were mostly nonspecific necrotic spots on stem bases. The average rating of cv. Hermann to natural infection by stem-base diseases was 1.5 in 2014 and 0.5 in 2015. On the other hand, the typical elliptical eye-shaped spots were found in the plots of cv. Hermann, which were artificially inoculated by *Oculimacula* spp. But

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Table 1. Germplasm analysed for *Xorw1* marker fragments

Genotype	*Pch1* present	Registered since	Genotype	*Pch1* present	Registered since
Annie*	yes	2014	Carroll*	no	2011
Audace*	yes	–	Cimrmanova raná*	no	2012
Balthazar*	yes	–	Citrus	no	2011
Beduin*	yes	2011	Clever*	no	–
Clarus*	yes	–	Corsaire*	no	–
Hermann*	yes	2007	Cubus*	no	–
Iridium*	yes	2009	Dagmar*	no	2012
Madsen*	yes	–	Darwin	no	2004
Manager*	yes	2007	Diadem*	no	–
Piko*	yes	–	Dromos*	no	2006
Princeps*	yes	2012	Dulina*	no	2013
Renan*	yes	–	Ebi	no	1997
Rendezvous*	yes	–	Elan*	no	2012
Roazon*	yes	–	Elly*	no	2010
SG-S 1215-14	yes	–	Etana	no	2013
SG-S 1825-14	yes	–	Etela*	no	2006
SG-S 791-13	yes	–	Eureka*	no	–
Titlis*	yes	–	Eurofit	no	2006
Akteur*	no	2004	Evina*	no	2012
Aladin*	no	2010	F6709*	no	–
Alana*	no	1997	Fabius	no	2013
Altigo*	no	2011	Fakir	no	2013
Anduril	no	2006	Faustina*	no	–
Apache*	no	1999	Federer*	no	2009
Arche*	no	–	Feria*	no	2011
Artist	no	2014	Fermi*	no	2011
Asta	no	1994	Florett	no	2006
Astella	no	1995	Florus	no	2014
Athlon	no	2013	Fortis	no	2009
Avenue	no	2014	Genius	no	2014
Bagou	no	2009	Globus*	no	–
Bakfis*	no	–	Golem	no	2011
Baletka*	no	2008	Gordian	no	2014
Banquet	no	2001	Helmut	no	2008
Barroko	no	2005	Henrik*	no	2010
Barryton*	no	2007	Heroldo*	no	–
Batis*	no	2001	Hewitt*	no	2012
Bill*	no	–	Hussar*	no	–
Biscay*	no	2005	Chevalier*	no	2011
Bodyček*	no	2010	Ilias	no	2003
Bohemia*	no	2007	JB Asano*	no	2012
Brentano*	no	2010	Jindra*	no	2010
Brigadier*	no	–	Julie	no	2014
Brilliant	no	2009	Karolinum	no	2003
Brokat	no	2013	Kerubino	no	2007
Buteo	no	2006	Kodex*	no	2008
Caphorn*	no	–	KWS Ozon*	no	2012

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Table 1 to be continued

Genotype	*Pch1* present	Registered since	Genotype	*Pch1* present	Registered since
Lavantus	no	2013	SG-S 1403-13	no	–
Ludwig	no	2000	SG-S 1503-14	no	–
Magister*	no	2009	SG-S 1670-14	no	–
Matylda*	no	2011	SG-S 1684-13	no	–
Meritto*	no	2003	SG-S 1751-13	no	–
Mladka	no	2002	SG-S 1807-12	no	–
Mona	no	1994	SG-S 2087-14	no	–
Mulan*	no	2007	SG-S 2124-14	no	–
Nikol*	no	2008	SG-S 611-12	no	–
Nordika	no	2014	SG-S 616-14	no	–
Orlando*	no	2008	SG-S 705-14	no	–
Patras	no	2013	SG-S 727-11	no	–
Penalta*	no	–	SG-S 766-11	no	–
Pitbull	no	2008	SG-S 979-10	no	–
Potenzial*	no	2012	SG-U 1104-12	no	–
Raduza*	no	2006	SG-U 1369-13	no	–
Rapsodia*	no	2003	SG-U 2097	no	–
Rheia*	no	2002	SG-U 2138-12	no	–
Rumor	no	2014	SG-U 2513-14	no	–
RW-21106	no	–	SG-U 2712-13	no	–
RW-51121	no	–	SG-U 3165-13	no	–
RW-51210	no	–	SG-U 4101-13	no	–
RW-51313	no	–	SG-U 4107-13	no	–
RW-51320	no	–	SG-U 417-13	no	–
RW-51410	no	–	SG-U 5132-13	no	–
RW-51422	no	–	SG-U 5199-13	no	–
RW-51423	no	–	SG-U 6022-14	no	–
RW-51431	no	–	SG-U 911-12	no	–
RW-51435	no	–	Silueta*	no	–
RW Nadal*	no	2010	Simila	no	2006
S1005-09*	no	–	Sulamit	no	2000
S1272-09*	no	–	Sultan*	no	2008
S1643-08*	no	–	Svitava	no	2001
S269-09*	no	–	SY Passport	no	2013
Sailor	no	2011	Šárka	no	1997
Sakura*	no	2007	Tiguan*	no	2012
Salutos	no	2009	Tilman	no	2014
Samanta*	no	1993	Tobak	no	2013
Saskia	no	1996	Torfrida*	no	–
Secese*	no	2009	Turandot*	no	2012
Seladon*	no	2009	U606810*	no	–
SG-S 1040-13	no	–	U80029*	no	–
SG-S 1145-12	no	–	U907210*	no	–
SG-S 1198-14	no	–	Vanessa*	no	2013
SG-S 1373-14	no	–	Vlasta	no	1996
SG-S 1378-12	no	–	Zeppelin	no	2013

*Dumalasová *et al.* (2013)

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Table 2. The reactions of winter wheat cultivars to natural infection by stem-base diseases in field conditions (Úhřetice) in 2014 and 2015

Cultivar	*Pch1*	2014	2015
Hermann	+	1.53[a*]	0.49[a]
Annie	+	1.57[a]	0.91[b]
Princeps	+	1.65[a]	0.75[b]
Turandot	–	2.06[b]	0.98[bc]
Cubus	–	2.06[b]	1.20[c]
Bohemia	–	2.19[b]	1.46[d]

*used ANOVA – multiple comparisons – Tukey's method (P = 95%); the means in columns followed by the same letter are not significantly different from each other

the average infection by eyespot was very low: 1.7 in 2014 and 1.0 in 2015. The Hermann cultivar shows a resistant reaction to eyespot.

Statistical analyses indicated that all four tested cultivars with the gene *Pch1* were significantly less infected than those without *Pch1* in 2014. In the next year statistical analyses indicated that cv. Hermann was significantly less infected than cvs Princeps and Annie at Úhřetice, although all these cultivars have the gene *Pch1*. The infection of cvs Princeps and Annie was similar to that of cv. Turandot, which does not have *Pch1* because the infection of cv. Turandot was extremely low (1.0) under field conditions in 2015. Cvs. Hermann, Annie and Princeps were significantly less infected than all the cultivars without *Pch1* in inoculated plots in Prague. The reaction of cv. Manager was similar to that of cv. JB Asano (without *Pch1*). The most susceptible reaction to *Oculimacula* spp. was observed in the cultivars Cubus, Turandot and Bohemia on artificially infected plots at Prague-Ruzyně. Cvs. Bohemia and Cubus were most infected by stem-base diseases in the field at Úhřetice.

Several German winter wheat cultivars classified for reaction to eyespot by the German Plant Variety Office (Bundessortenamt 2015) were analysed with markers also in our study. Susceptibility scores from the German "Descriptive Variety List" were rated 2 for the cultivars Manager and Hermann, containing *Pch1*, indicating low susceptibility to eyespot. The cultivars Akteur, Brilliant, Gordian, JB Asano, Mulan, Patras, Potenzial, Rumor, Sailor, Tobak, Zeppelin, Batis, Henrik and Kerubino, without *Pch1*, were scored in the range of 5–6 corresponding to medium susceptibility on a scale 1 (minimum susceptibility) to 9 (maximum susceptibility). The Genius cultivar, also without *Pch1*, had a slightly lower score of 4.

There are at least 6 cultivars (Annie, Beduin, Hermann, Iridium, Manager, Princeps) well adapted to the current conditions of the Czech Republic, that show low susceptibility to eyespot based on the resistance gene *Pch1*. Promising is also the cultivar Rebell, newly registered in Germany. The Rebell cultivar is supposed to carry *Pch1*, however, we have not analysed it with *Xorw1* so far. Another cultivar, very likely containing *Pch1* in its pedigree and showing a disease score of 3 according to the German Plant Variety Office, is Bonanza, newly registered in the Czech Republic and not yet tested in our laboratory. Its parental cultivars Türkis and Hermann were scored 3 and 2 by the German Plant Variety Office. The

Table 3. The reactions of winter wheat cultivars to artificial infection by eyespot (Prague-Ruzyně) in 2014 and 2015

Cultivar	*Pch1*	2014	2015
Hermann	+	1.70[a*]	0.95[a]
Annie	+	2.08[ab]	1.64[ab]
Manager	+	2.27[ab]	2.16[bc]
Princeps	+	2.42[b]	1.86[b]
Potenzial	–	3.38[c]	3.43[de]
JB Asano	–	3.49[c]	2.75[cd]
Bohemia	–	3.51[c]	3.64[e]
Turandot	–	3.58[c]	3.59[e]
Cubus	–	3.73[c]	3.21[de]

*used ANOVA – multiple comparisons – Tukey's method (P = 95%); the means in columns followed by the same letter are not significantly different from each other

Annie JB Asano Turandot

Figure 2. Eyespot symptoms from artificially infected plots in Prague (photo J. Palicová)

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gene *Pch1* was further found in the foreign cultivars or cultivars not registered in the Czech Republic at present: Audace, Balthazar, Clarus, Madsen, Piko, Rendezvous, Renan, Roazon and Titlis.

There are two different translocations from *Ae. ventricosa* in the VPM-1 breeding line, a *Pch1* carrying translocation on 7DL and another translocation with genes *Lr37, Sr38* and *Yr17* on the chromosome 2AS. VPM-1 has been widely used in wheat breeding and the presence of both translocations in the same genotype is relatively common. In our previous study (Dumalasová *et al.* 2013) wheat genotypes known to carry the translocation with genes *Lr37, Sr38* and *Yr17* from *Ae. ventricosa* were tested for the presence of *Pch1* using the molecular marker *Xorw1*. The presence of both translocations in the same genotype was quite frequent in our study. There were thirteen genotypes out of the twenty-eight tested that carried both of them: Audace, Balthazar, Beduin, Clarus, Hermann, Madsen, Manager, Piko, Princeps, Renan, Rendezvous, Roazon and Titlis.

Out of the 54 promising breeding lines we have tested with *Xorw1* for the presence of *Pch1* so far, three were resistant: SG-S 1215-14, SG-S 1825-14 and SG-S 791-13. The breeding lines SG-S 1215-14 and SG-S 1825-14 were included in our plot trials with artificial inoculation and their low level of infection confirms this finding.

Eyespot resistance genes known to date do not generally provide complete control, and environmental effects on its expression can be large. Nevertheless, the difference in the level of infection between the genotypes carrying *Pch1* and the genotypes without *Pch1* was obvious in our trials. The resistance gene *Pch1* appears to provide satisfactory control of eyespot under field conditions with natural infection pressure. Under artificial infection, the level of protection provided through the gene *Pch1* is not 100%, however the lower infection level of cultivars containing the *Pch1* gene is statistically significant. The trials with artificial infection provide results on the reaction of the tested cultivars to a single pathogen, which is not possible under field conditions, where the complex of stem-base pathogens is involved. The methods of eyespot inoculation and assessment were optimized in our experiments at the Crop Research Institute at Prague-Ruzyně and can be used in the future in different trials dealing with e.g. fungicide effectiveness.

Our infection tests proved that even the cultivars resistant to eyespot can be come infected under strong infection pressure. Typical symptoms may then be visible, though not at the high levels causing straw breaking. The visible symptoms typical of eyespot were rare on wheat cultivars with the gene *Pch1* in field trials with natural infection at Úhřetice. Eyespot is only one component of the stem-base disease complex of cereals. The visual diagnosis of eyespot and other diseases is difficult, particularly when more than one component of the complex is present and the symptoms can be masked by other diseases.

Wheat genotypes with resistance genes effective against both forms of the pathogen and effective at both the seedling and adult plant stage are needed. The aim is easier to achieve when suitable molecular markers for resistance genes such as *Xorw1* are available. The paucity of genetic resistances available to plant breeders in hexaploid wheat and the limitations of the existing sources of resistance have led to a renewed interest in novel sources in wheat progenitors and relatives. Cultivars with improved eyespot resistance may be produced through the introgression of novel genes into new cultivars or through pyramiding of several resistance genes with the use of molecular markers.

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