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Review Article

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Advances and Prospects in Wheat Eyespot Research: Contributions from Genetics and Molecular Tools

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Abstract

Eyespot disease caused by the soil-borne facultative fungi *Oculimacula yallundae* and *O. acuformis* is the major component of the stem-base disease complex of wheat in temperate regions of the world with a cool and wet climate. In this review, we focus on results of genetic studies concerning both partners of the host–pathogen interaction. This comprises analyses of genetic diversity of the pathogen and identification of particular genes within it, evaluation and screening methods for host resistance, resistance sources and genetics of these resistances, breeding of resistant cultivars in wheat, and application of genetic markers in tagging and tracking of eyespot resistance genes. We also attempt to foresee some of the key issues and developments that may occur in future. The identification of markers tightly linked to eyespot resistance genes is the important research focus opening the door to marker-assisted selection of resistant varieties.

Introduction

Wheat production is limited by various abiotic and biotic factors. It is influenced by a number of diseases, mainly of pathogenic fungal origin. These can reduce kernel yield and quality and cause dramatic yield losses (King 1977; Fitt and Goulds 1988; Griffey et al. 1994; Marshall and Sutton 1995). Eyespot, caused by the soil-borne facultative fungi *Oculimacula yallundae* (Wallwork and Spooner) Crous and W. Gams [formerly *Tapesia yallundae*, anamorph *Helgardia* (formerly *Pseudocercosporella*) *herpotrichoides*] and *O. acuformis* (Boerema, R. Pieters and Hamer) Crous and W. Gams [formerly *Tapesia acuformis*, anamorph *Helgardia* (*Pseudocercosporella*) *acuformis*], is a component of the stem-base disease complex of wheat (Lucas et al. 2000; Crous et al. 2003). Eyespot forms lesions on the leaf sheaths and culms near the soil level, and their elliptical shape gives rise to the name of the disease which is also known as strawbreaker or foot rot. Eyespot pathogens have a wide host range among cereals and grass species (Lucas et al. 2000). Wheat, barley, rye, oats and other related grasses can be affected, with wheat being the most susceptible (Murray et al. 1994; Chapman et al. 2008). Sexual reproduction of *O. yallundae* can occur on some wild grasses [e.g. *Bromus diandrus*, *Hordeum leporinum* (Wallwork 1987) or *Holcus lanatus* (Dyer and Bradshaw 2002)], which may provide a reservoir of primary inoculum to infect cultivated fields. Significant damages due to eyespot are observed on winter wheat and fall-sown spring wheat in temperate regions of the world with cool and wet climates where fall-sown cereals predominate (Lucas et al. 2000). Measures to control eyespot include cultural practices (sowing date and density, tillage practice, crop rotation), chemicals (fungicides) and biological control. The development of wheat cultivars with genetic resistance is recognized as the most effective, economic, environmental-friendly and sustainable strategy to control the disease. Previous reviews mainly concerned epidemiology and management of eyespot (Fitt and Goulds 1988; Fitt et al. 1990) or the biology and genetics of *Oculimacula* species (Lucas et al. 2000). Furthermore, eyespot was one of the examples in reviews concerning sensory biology of wheat pathogens (Lucas 2004), molecular mechanisms of fungicide resistance (Ma and Michailides 2005) or coexistence of sister pathogen species in arable crops (Fitt et al. 2006). The current review focuses on molecular genetic studies on the pathogen on one

hand and on resistance to eyespot in host plants and practical implications for wheat breeders on the other.

Molecular Genetic Studies of the Pathogens

As mentioned by Lucas et al. (2000), molecular genetic studies of eyespot pathogens are practicable: they can be cultivated on artificial media, they can be transformed (Blakemore et al. 1989) to study complementation, to insert reporter genes (Bunkers 1991; Bowyer et al. 2000; Eckert et al. 2005) or to obtain mutants, and techniques allowing *in vitro* sexual crosses between compatible strains were developed (Dyer et al. 1993). However, few genes of *Oculimacula* sp. have been identified so far, with only 27 sequences in the NCBI nucleotide database and nine in the protein database (15 July 2010).

During the 1990s, several genetic marker systems were used to study genetic diversity in eyespot pathogens. These were isozymes (Julian and Lucas 1990; Priestley et al. 1992), restriction fragment length polymorphisms (RFLP) and other DNA-hybridization-based marker systems (Nicholson et al. 1991, 1993; Thomas et al. 1992; Frei and Wenzel 1993; Poupard et al. 1995; Takeuchi and Kuninaga 1996), and random amplified polymorphic DNAs [RAPDs, (Nicholson and Rezanoor 1994; Nicholson et al. 1994; Papaikonomou and Lucas 1994; Vanova et al. 2000)]. These studies mainly targeted identification of molecular fragments enabling discrimination of the two *Oculimacula* species. They also showed that *O. yallundae* had a higher degree of polymorphism than *O. acuformis*. The competitive PCR assay developed by Nicholson et al. (1997) on the basis of specific RAPD fragments was later used in several studies that evaluated the effects of various factors, e.g. fungicide treatments, cultivars with different eyespot susceptibilities, soil management, farming practices or the preceding crop, on the incidence and severity of stem-base diseases of wheat (Bateman et al. 2000; Turner et al. 2001; Nicholson et al. 2002; Matusinsky et al. 2008a,b, 2009).

The rDNA genes were used to design PCR-based tests to rapidly differentiate the two types of *Oculimacula* isolates (Poupard et al. 1993; Gac et al. 1996) that were not definitively known as separate species at that time. These tests were used to study the development of *O. yallundae* and *O. acuformis* in the field and to evaluate the effect of a fungicide seed treatment (Gac et al. 1999). Ray et al. (2004) used competitive PCR assays designed on rDNA genes that enabled quantification of *O. yallundae*, *O. acuformis* and other pathogens involved in stem-base diseases of wheat to study the effectiveness of various fungicide treatments on disease index and yield. Similar assays were also used to evaluate the effects of eyespot on stem strength, lodging resistance and yield (Ray et al. 2006). The rDNA genes of four isolates were sequenced by Stewart et al. (1999), and these results lead Crous et al. (2003) to define the specific genus name *Oculimacula* for the fungi associated with eyespot in cereals. These sequences were used to design a real-time PCR assay to discriminate *O. yallundae* and *O. acuformis* from other fungal species found on wheat and to quantify the pathogens in wheat plants (Walsh et al. 2005). The rDNA sequence of *Helgardia anguioides* obtained by Stewart et al. (1999) enabled the identification of this fungus within the wheat root microbial community (Kwasna et al. 2010).

The two mating-type alleles of *O. yallundae* were isolated by Singh et al. (1999). With these data, Dyer et al. (2001) designed a multiplex PCR test for determining mating type in both *O. yallundae* and *O. acuformis*. Douhan et al. (2002a) used this test to analyse *Oculimacula* populations found in the US Pacific Northwest. These results and a complementary AFLP analysis indicated that both species were represented by random mating populations undergoing partial asexual reproduction at the scale studied (Douhan et al. 2002b, 2003).

The ornithine decarboxylase (ODC) gene was studied by Mueller et al. (2001) as a candidate gene involved in polyamine metabolism, and that could be critical during cell proliferation observed in the first steps of the infection process. The ODC knockout mutants obtained in this study were unable to differentiate infection plaques *in vitro* but were not reduced in virulence towards wheat when compared to a normal strain. Consequently, the ODC gene was considered not to be a suitable target for fungicides.

Two genes involved in fungicide resistance were also studied. Mutations in the β-tubulin gene were associated with different phenotypes of benzimidazole resistance (Albertini et al. 1999), whereas contradictory results were obtained concerning the association of mutations in the 14a-demethylase gene (CYP51) with resistance to DMI (sterol 14α-demethylase inhibitor) fungicides like prochloraz (Wood et al. 2001; Albertini et al. 2003). Genetic analyses in sexual crosses between isolates of *O. yallundae* with varying levels of prochloraz resistance indicated that resistance is controlled by a major gene and several minor genes (Dyer et al. 2000).

Methods for the Assessment of Eyespot Resistance

Various techniques for evaluating eyespot resistance in wheat genotypes have been used by breeders. It was early realized that controlled inoculations with the pathogen would give a more reliable assessment of eyespot resistance than natural infection because artificial inoculation is more uniform than the natural one. Techniques were described for the production of inoculum and for inoculation in the field (Bruehl and Nelson 1964) or in growth chambers (Macer 1966). The advantage of the growth chamber test was that it was more rapid (2–3 months) than a field test, which requires a quite complete growing season. The growth chamber test measures mainly resistance to penetration of the leaf sheaths, whereas the field test measures mainly resistance of the stem to invasion by the fungus. Because the two kind of resistance are not

completely correlated, it should be better to measure both (Doussinault 1973). In both tests, ratings are based on an index: several plants (10–20 in growth chamber tests and approximately 50 tillers in field tests) are scored for number of penetrated leaf sheaths (growth chamber tests) or the portion of the stem attacked by the fungus (field tests), and their scores are averaged to obtain the index. With such index techniques, resistance evaluations are time-consuming and labour-intensive (Johnson 1992b). They are also difficult to apply to heterogeneous material such as segregating families in early generations after crossing. For heterogeneous populations, it was preferred to select for yield or seed size in a naturally infested environment or in inoculated trials (Doussinault 1973; Roberts and Allan 1990). However, it soon appeared that if the population was also heterogeneous for plant height or earliness, this procedure tended to select tall and vigorous plants and that the selection pressure on eyespot resistance was not sufficient. Consequently, it was proposed that selection for yield in inoculated trials should be delayed until attainment of near-homozygosity by single-seed descent (Roberts and Allan 1990) or should be applied after classifying plants on height (Doussinault 1973).

Several variations of the growth chamber test were proposed to reduce its duration. For example, Murray and Ye (1986) observed that papillae formation, hypersensitive reaction at papillae sites and number of successful penetrations were correlated with host resistance, and Strausbaugh and Murray (1989) used the percentage of successful penetrations in the first leaf sheath at 50 infection sites to study the inheritance of eyespot resistance in segregating F_2 and backcross populations. This method allowed assessment 4 weeks after inoculation but remained time-consuming and quite subjective.

An improved method using a β-glucuronidase (GUS)-transformed strain of the pathogen was developed to measure differences in disease development on 4- to 8-week-old wheat seedlings (de la Peña and Murray 1994). This method differentiated highly resistant, resistant and susceptible genotypes (Jones et al. 1995). After inoculation with the GUS-transformed strain, production of the GUS enzyme is highly correlated with the amount of fungal growth in the plant. Thus, disease severity or differences in resistance to eyespot are directly related to the amount or differences in GUS activity in seedling tissues of wheat genotypes. The results of this seedling test are highly correlated with visual ratings made on 6- to 8-week-old young plants and were considered to be sufficient to predict adult plant resistance under Pacific Northwest conditions. Using this method, evaluation of resistance could be reduced from approximately 11 to 2 months (Jones et al. 1995). The GUS seedling test has been used to identify new sources of eyespot resistance in wild relatives of wheat, to determine the genetic control of resistance and to facilitate mapping and tagging of eyespot resistance genes (Murray et al. 1994; Yildirim et al. 1995, 1998; de la Peña et al. 1996, 1997; Cadle et al. 1997; Figliuolo et al. 1998; Lucas et al. 2000; Li et al. 2004, 2005). With only one GUS-transformed strain used for resistance testing, there was a risk of selecting specific resistance genes conferring resistance to only a limited portion of the pathogen populations. That is probably why Li et al. (2004) used a mixture of four GUS-transformed strains in their study.

Lind (1992) developed a method based on an enzyme-linked immunosorbent assay (ELISA) to measure quantitative differences in eyespot resistance between wheat cultivars. This method detected the fungus in presymptomatic wheat plants and could be applied at different growth stages, but only measurements taken at or after anthesis correlated well with response at the adult stage (Lind 1992), whereas measurements taken at younger growth stages, particularly around tillering, were not able to discriminate resistant from highly resistant genotypes (de la Peña and Murray 1994). The method was applied to study the stability of eyespot response measured by ELISA in 20 wheat cultivars studied in six environments (Lind et al. 1994), relative rates of *O. yallundae* and *O. acuformis* development in wheat (Poupard et al. 1994), variation in eyespot response in *Pch1*-carrying genotypes (Lind 1999) and quantitative inheritance of eyespot resistance in diallel crosses (Lind 2000).

The competitive PCR assay developed by Nicholson et al. (1997) was rarely used by wheat breeders or geneticists, probably for cost reasons and because it is time-consuming. Uslu et al. (1998) observed that *Oculimacula* DNA quantification by competitive PCR did not correlate well with visual ratings and was not powerful in revealing small differences between genotypes, probably because the method evaluates the level of colonization rather than penetration and had to be applied on bulks of individual plants that had different visual disease scores. More recently, real-time PCR was demonstrated to be useful in discriminating and quantifying *O. yallundae* and *O. acuformis* in plants (Walsh et al. 2005). In the context of wheat genotype evaluation, assays based on this technique were developed by Meyer et al. (2006, 2008) and by Gedye and Murray [unpublished work cited by Li et al. (2008)].

None of these tests is simple and highly effective; all still need inoculation with pathogens, and replicated testing is necessary owing to environmental variation and genotype-by-environment interaction.

Sources of Eyespot Resistance and Resistance Genes Found in Relative Species of Wheat

There are several known sources of resistance to eyespot, but only three resistance genes have been described. The French cultivar 'Cappelle-Desprez' reported by Vincent et al. (1952) was the first commercial wheat cultivar resistant to eyespot. Most of its resistance is conferred by *Pch2* which is located at the distal end of the long arm of chromosome 7A and acts at the seedling or young plant stage (Law et al. 1975; Koebner and Martin 1990; de la Peña et al. 1996,

1997; Chapman et al. 2008). Other genes on chromosomes 1A, 2B and 5D of 'Cappelle-Desprez' modify the levels of infection at the young plant stage (Law et al. 1975). *Pch2* can also be analysed as a quantitative trait when its effect does not permit plants to be clearly classified into resistant and susceptible types (Hollins et al. 1988; Chapman et al. 2008). Recently, *Pch2* was found to confer a significantly less effective resistance against *O. yallundae* than against *O. acuformis* at the young plant stage (Burt et al. 2010). In another study, chromosomes 5A, 1A and 2B were shown to carry genes for resistance against *O. yallundae* at the adult stage, whereas *Pch2* did not have an effect (Muranty et al. 2002). Although the pedigree of 'Cappelle-Desprez' is known, the origin of *Pch2* and other eyespot resistance genes in 'Cappelle-Desprez' is not known (Murray et al. 1994). The origin of *Pch2* could be in an A-genome species like *Triticum monococcum*, as suggested by Cadle et al. (1997). 'Cappelle-Desprez' was selected in an environment with a long history of exposure to the disease where selection for resistance was probably performed without much effort (Law et al. 1975). In the UK during the 1960s and 1970s, selection for the resistance carried by 'Cappelle-Desprez' was usually achieved by breeding within a pool of varieties derived from 'Cappelle-Desprez' and thought to be homozygous for resistance (Law et al. 1988).

A few other wheat cultivars were reported to show moderate resistance to eyespot apparently not inherited from 'Cappelle-Desprez'. These are for example 'Kanzler', 'Florida' and 'Kraka' from Germany (Lind et al. 1994), 'Cerco' (Peterson et al. 1974), and 'Edwin' (Jones et al. 2000) developed in the Pacific Northwest. The genetic control of eyespot resistance in these cultivars has not been studied.

Some cultivars are also sometimes rated moderately resistant and sometimes susceptible: for example, 'Stephens' (Kronstad et al. 1978) and 'Viking' [mentioned as moderately resistant by Murray and Ye (1986) and Murray and Bruehl (1986) and as susceptible by Strausbaugh and Murray (1989) and Murray and Bruehl (1983)]. This illustrates the high genotype × environment interaction that confuses phenotypic test results or possibly unreliability of the assays.

The most effective resistance is due to the single major gene *Pch1*, which was transferred to wheat from *Aegilops ventricosa* in three independent programmes. *Pch1* was transferred to hexaploid wheat by first crossing *Ae. ventricosa* ($2n = 4\times = 28$, genome $D^VD^VM^VM^V$) with an accession of the tetraploid species *Triticum persicum* ($2n = 4\times = 28$, genome AABB) to obtain a fertile amphidiploid and subsequently backcrossing with the hexaploid wheat ($2n = 6\times = 42$, genomes AABBDD) variety 'Marne-Desprez' for three generations to develop Ventricosa × Persicum × Marne (=VPM-1) (Simonet 1957; Maia 1967). *Pch1* was transferred to the distal part of chromosome arm 7DL of wheat via recombination between the $7D^v$ chromosome of *Ae. ventricosa* and the 7D chromosome of a susceptible wheat (Gale et al. 1984; Chao et al. 1989). VPM-1 became a ready source of the eyespot resistance gene *Pch1* for wheat breeders. Genetic material from *Ae. ventricosa* was also transferred to hexaploid wheat via an intermediate male-sterile hybrid between *T. turgidum* ($2n = 4\times = 28$, genomes AABB) and *Ae. ventricosa* that was backcrossed as female parent with hexaploid wheat. The progeny were then repeatedly selfed to obtain stable wheat lines with 42 chromosomes, designated H-93 lines (Doussinault et al. 1983a). The resistance factor in line H-93-70 was confirmed to be allelic to the *Pch1* gene of VPM-1 and to be transferred from chromosome $7D^V$ of *Ae. ventricosa* to chromosome 7D (Delibes et al. 1988; Worland et al. 1988; Mena et al. 1992). In the third programme, eyespot resistance from *Ae. ventricosa* was transferred to bread wheat by a direct cross. A hybrid between *T. aestivum* 'Moisson' and *Ae. ventricosa* was open-pollinated with an unidentified *T. aestivum* plant, and a resulting hybrid was backcrossed with *T. aestivum* 'Courtot', followed by a further cross with *T. aestivum* 'Moisson'. The resulting plants were selfed to establish pure lines (Doussinault et al. 1988). These lines, known as F-210, were shown to have a very high resistance level (Lind 1999).

Eyespot-resistant accessions were identified with a GUS-transformed strain of *O. yallundae* in *Triticum tauschii* (Yildirim et al. 1995), *T. monococcum* (Cadle et al. 1997), *T. durum*, *T. dicoccoides* and *T. turanicum* (Figliuolo et al. 1998), *Dasypyrum villosum* (Yildirim et al. 2000), *Thinopyrum ponticum* and *Th. intermedium* (Li et al. 2004, 2005), and *Aegilops longissima* (Sheng and Murray 2009). Immune and highly resistant accessions were also identified in *T. tauschii* with a non-transformed strain (Assefa and Fehrmann 1998). Hundreds of accessions of *T. tauschii* were screened and shown to carry a high frequency of resistance (Jones et al. 1995; Yildirim et al. 1995). Crosses between resistant and susceptible *T. tauschii* accessions indicated that a single gene controlled eyespot resistance in each cross, and tests with molecular markers showed that this gene is probably not allelic to *Pch1* (Cadle et al. 1998), but the chromosome location of a resistance gene in *T. tauschii* is still unknown. Sheng and Murray (2009) identified eyespot-resistant *A. longissima* accessions and initiated genetic analyses of eyespot resistance with molecular markers in crosses between resistant and susceptible accessions. Among 22 *T. monococcum* accessions, a dozen were shown to have an intermediate to high level of eyespot resistance, and four of these had significantly different responses to *O. yallundae* and *O. acuformis* (Burt et al. 2010).

A part of the Gatersleben genetic resource collection was tested for eyespot resistance, mainly under natural disease pressure but also with artificial inoculations (Boerner et al. 2006). A quite high number of accessions of the *Triticum* genus (46 at the seedling stage, 412 at the adult stage) were scored with no visible infection under natural infection, and more than half

the *Aegilops* accessions had no visible infection under natural infection. These putatively highly eyespot-resistant accessions deserve further investigation using artificial inoculations.

High levels of resistance reported by Sprague (1936) in *D. villosum* (L.) Candargy ($2n = 14$, genome VV), a distant relative of wheat, were confirmed by Murray et al. (1994) who also showed that a chromosome 4V addition line in a 'Chinese Spring' background was as resistant as VPM-1. In a cross between the susceptible 'Yangmai-5' (4V(4D)) substitution line and the resistant 4V disomic addition line, the resistance gene *PchDv* (*Pch3*) from *D. villosum* was located on the distal part of the long arm of chromosome 4V (Yildirim et al. 1998). Although five tested accessions of *D. villosum* were resistant to both *O. yallundae* and *O. acuformis*, analysis of single chromosome addition lines in a 'Chinese Spring' background showed that resistance to the two eyespot pathogens may be conferred by different genes (Uslu et al. 1998).

The wheatgrasses *Th. ponticum* and *Th. intermedium* were reported as potential sources of resistance to eyespot that could be used in perennial wheat (= *Triticum* spp. × *Th. ponticum* or *Th. elongatum*) breeding (Cox et al. 2002), whereas an eyespot tolerant germplasm line with *Th. ponticum* in its pedigree was registered by Allan et al. (1993). Genetic analyses of a chromosome substitution line in which chromosome 4D was replaced by chromosome 4J of *Th. ponticum* indicated that eyespot resistance in offspring of this line was associated with the 4J chromosome (Li et al. 2004). A related study with chromosome substitution or translocation lines incorporating *Th. intermedium* chromosomes or chromosome arms indicated that eyespot resistance is associated with the short arm of chromosome 4Ai#2 (= $4J^S$) (Li et al. 2005). These resistance sources require further chromosomal engineering to remove deleterious factors introduced with the alien chromatin (Li et al. 2008).

Aegilops kotschyi ($2n = 28$, genome UUS^vS^v) was described as a further source of resistance to eyespot pathogens [Bang (1986), cited in Lind (2000)] and was used to develop introgression lines in a wheat background by crossing and twice backcrossing with three German wheat varieties, followed by selfing for line development (Thiele et al. 2002). Among these lines, several were as resistant as 'Cappelle-Desprez' but none were as resistant as *Pch1*-carrying controls. The genetic basis of this resistance was studied by Meyer et al. (2008) in a doubled haploid population, and it seemed to be of more minor effect than previously thought and due to several minor genes.

Development of Eyespot-Resistant Cultivars

Variable emphasis has been placed on eyespot resistance in different breeding programmes around the world during the last 60 years. When fungicides were not used, selection of wheat in Western Europe occurred in environments largely attacked by the disease and selection for resistance went along with selection for yield (Law et al. 1975). When breeders began to use fungicides in yield trials, nowadays current practice, they had to perform separate tests to select for eyespot resistance.

The cultivar 'Cappelle-Desprez' and the gene *Pch1* from the breeding line 'VPM-1' are the most widely used sources of resistance to eyespot. Examples of cultivars that are thought to have inherited 'Cappelle-Desprez' resistance are 'Hobbit sib' (Worland et al. 1988), 'Avalon', 'Longbow', 'Norman', 'Virtue' (Hollins et al. 1988), 'Maris Huntsman' (Johnson 1992b), 'Apollo', 'Sperber', 'Boxer', 'Sorbas', 'Rektor' (Lind et al. 1994), 'Joss', 'Maris Beacon', 'Xanthos' and 'Adular' (Lind 2000). The presence of *Pch2* was confirmed or inferred from molecular genotyping results in 'Hobbit sib', 'Lynx', 'Rendezvous' and 'Riband' (Burt et al. 2010).

Of the three described resistance genes, *Pch1* is the most extensively used in the development of eyespot-resistant wheat cultivars due to its tight linkage with an isozyme marker (McMillin et al. 1986). However, as early as the 1970s, Doussinault et al. (1974) started to breed new wheat lines with a VPM parent, selecting for eyespot resistance at the young plant and adult stages and for other agronomic traits like earliness, height, yield and quality. These efforts led to the registration of the French cultivar 'Roazon', which was the first commercial wheat to contain the eyespot resistance gene *Pch1* of VPM-1. The two other sources where *Pch1* was introduced, i.e. the H-93 lines (Delibes and Garcia-Olmedo 1973; Mena et al. 1992) and the F-210 lines (Doussinault et al. 1988; Lind 1999), have not been reported as sources of eyespot resistance in wheat cultivars. This is perhaps due to the fact that the *Ae. ventricosa* accession used to develop these lines was different from the accession used to obtain VPM, and the associated allele at the linked isozyme locus is a null allele in these sources (Huguet-Robert et al. 2001; J. Jahier, personal communication). The French cultivar 'Roazon' was never widely grown (Jones et al. 1995).

The USDA-ARS winter wheat breeding programme at Washington State University started to work with *Pch1*-carrying lines in 1974. The first lines combining high yield potential, adequate cold hardiness, good milling quality and eyespot resistance conferred by *Pch1* were released as 'Madsen' (Allan et al. 1989) and 'Hyak' (Allan et al. 1990) and were derived from VPM/Moisson selections. Further varieties with *Pch1* were developed for the Pacific Northwest and several other regions around the world (Table 1).

Genetic Markers of Eyespot Resistance Genes

It has long been recognized that markers that could be used to indirectly select resistant individuals and manipulate eyespot resistance genes would greatly facilitate breeding. Several markers linked with the three described eyespot-resistant genes (*Pch1*, *Pch2* and *Pch3*) have been published, and these results are summarized in Table 2.

Table 1
Wheat germplasm containing *Pch1* resistance against eyespot

Name of line	Registration year	Pedigree
Alcazar[a]	2004	
Allister[a]	2003	
Andante[b]	1992	Moulin/D-172-6-4
Arbon[a]	1990	Maris Huntsman/US-363
Astuce[a]	2004	
Attlass[a]	2005	
Audace[a]	1995	(VM1347 × VM480.18) × VM480.4
Azimut[a]	2004	
Balthazar[a]	1995	Faucon/Rendezvous
Beamer[c]		
Bill[d]	1998	
Brandt[e]		
Cara[f]	2006	WA7752//WA6581/WA7217
Cardos[a,g]	2000	Cappelle-Desprez//Taras/Hadmerslebener-230-60
Certo[h,g]		
Cetus[g,i]	2005	
Chukar[j,k]	2003	WA7665/Rulo
Coda[j,l]		Tres//Madsen/Tres
Eclipse[a]	1999	
Ecu[a]	1988	L-1035/L-1474//Moisson
Farandole[a]	1999	VM713/CF1851//CF1616/Renan
Finch[j]	2003	Dusty//Wa7164/Dusty
Flèchedor[a]	1992	VPM/Moisson//US-60-43/3/Prieur-61/4/Fidel VPM/Moisson//US-60-43/3/Prieur/4/Fidel
Format[g]	2007	
FR-50[j]		VPM-1/McCall
Grisby[a]	2002	Wild emmer/Obelisk/Taurus
Hermann[g,m]	2004	
Hyak[n]	1989	VPM-1/Moisson 421//2*Tyee
Hybnos-1[h]	1999	
Intense[a]	2001	
Kris[d]	1997	
Leiffer[d,g]	2004	
Limes[g]	2002	
Lone[e]	1992	
Lynx[e]	1992	Sleipner/Rendezvous
Madsen[n]	1988	VPM-1/Moisson 951/2*Hill 81
Manager[g]	2006	
Mitchel[a]	2001	
Mobil[o]	1991	Kronjuwel/Roazon
Mohler[c]		
Oratorio[a]	1995	H-84290/Genial
Osmin[h]	2004	
Pactole[a]	1986	Top/VPM-71
Piko[g,h]	1994	CWW-3319.5/3/Kraka//Maris Huntsman/Fruhgold
PR22R28[a]	2001	
Ralf[a]	1997	Cario/Tadorna//Ibis/Ferto/3/Burma/4/Rendezvous
RE8714[a]		(*Aegilops squarrosa* no. 33/ *Triticum dicoccum* no. 119)//(VPM/Moisson)/Beauchamp
RE9001[a]		(80MH3/R3.7)//(R3.7/74RHD8.4)
Regain[a]	1995	R-3-7/Bounty//Adam/3/R-3-7/Bounty//Darius
Renan[a]	1989	(Mironovskaia × Maris Huntsman) × [(VPM × Moisson) × Courtot]
Rendezvous[a]		(VPM × Hobbit) × Virtue
Ressor[a]	2004	RE9001/82RmultiHD10
Roazon[a]	1978	VPM-1-1-1-2-R-4/Moisson
Rulo[j]	1994	Tyee//Roason/Tres
Rumba[a]	2000	(Fresco/R3-7)//(CWW3547/46/Florin)
Sankara[a]	2004	
Simon[j]		Haven/Lambert//Madsen
Sinope[a]	2003	
Striker[g]	2004	
Temple[j]	1997	Tres/VPM-1
Titlis[a]	2005	
Tubbs[j]	2004	Madsen/Malcolm
Türkis[g,h]	2004	
Virtuose[a]	1998	VM713/CF1851//CF1616/Renan
Voltige[a]	2002	Soissons/VM802//C1723/C7128
VPM-1[a]		(*Ae. ventricosa* 10 × *T. persicum*) × Marne[3]
VPM-1/Moisson 421[n]	1974	VPM-1/Moisson
VPM-1/Moisson 951[n]	1974	VPM-1/Moisson

Table 1
Continued

Name of line	Registration year	Pedigree
WA 7217[j,p]		VPM/Moisson-951//2*Barbee
WA 7621[j]		VPM/Moisson 421/2/VH 66354/WA 5827/WA 6241/3/Tres
WA 7625[j]		VPM-1/Moisson 951//2*Hill 81
WA 7666[j,p]		VPM/Moisson 951//CI 13438
WA 7671[j]		VPM-1/Moisson 421//VH-66354/WA 5827/WA 6241/3/2*Hill 81
WA 7690[j]		VPM-1/Moisson 951//Yamhill/Hyslop/Hill 81/3/WA 6910
Weatherford[j]	2001	Malcolm/3/VPM/Moisson 951//Hill/4/VPM/Moisson 951//2*Hill
Zobel[g]	2006	

[a]Accession lists from INRA, France (H. Muranty, unpublished data).
[b]Burt et al. (2010).
[c]Accession list in Santra et al. (2006).
[d]Liatukas and Ruzgas (2008).
[e]Borum (2001).
[f]http://www.ars-grin.gov/npgs/acc/acc_queries.html.
[g]Meyer et al. (2010).
[h]Thiele et al. (2002).
[i]Wheat Pedigree and identified alleles of genes on line http://genbank.vurv.cz/wheat/pedigree/default.htm.
[j]Accession lists from the US department of agriculture (Leonard et al. 2008).
[k]Campbell et al. (2005).
[l]Allan et al. (2000).
[m]Meyer et al. (2008).
[n]Accession lists from Jones et al. (1995).
[o]Accession list in Lind (1999).
[p]Allan et al. (1993).

The endopeptidase allele *Ep-D1b* derived from the long arm of chromosome $7D^V$ of *Ae. ventricosa* like *Pch1* provides a particularly efficient marker for the presence of *Pch1* and is widely used for classification and selection of resistant breeding lines (McMillin et al. 1986; Vahl et al. 1987; Koebner et al. 1988; Law et al. 1988; Summers et al. 1988; Worland et al. 1988; Vahl and Müller 1991; Mena et al. 1992; Santra et al. 2006). The other tightly linked markers are a RFLP marker *Xpsr121* (Chao et al. 1989), whose probe was found to encode a beta-glucanase, the dominant simple sequence repeat (SSR) markers *Xbarc97*, *Xwmc14* and *Xcfd175*, failing to amplify an *Ae. ventricosa* allele, which are suitable only for screening homozygous materials and cannot detect failed reactions (Chapman et al. 2008), a dominant sequence-tagged site (STS) marker *XB-glu7D* derived from a 7D beta-glucanase expressed sequence tag (EST) (Chapman et al. 2008), and three STS markers *Xorw1*, *Xorw5* and *Xorw6* (Leonard et al. 2008).

Several markers loosely linked to *Pch2* were identified in the 1990s: these were an isozyme marker *Ep-A1* (Koebner and Martin 1990) and the RFLP markers *Xpsr121* (de la Peña et al. 1996), *Xcdo347* and *Xwg380* (de la Peña et al. 1997). More recently, the first association of *Pch2* with PCR-based markers was reported (Chapman et al. 2008). In this study, *Pch2* was shown to be associated with three SSR markers and to map close to *Xwmc525* within a 7-cM interval flanked by *Xwmc346* and *Xcfa2040* (Chapman et al. 2008). Markers for *Pch2* were also developed from cDNA-AFLP fragments differentially expressed between 'Chinese Spring' and 'Chinese Spring (Cappelle-Desprez 7A)': two markers, *X4CD7A8* and *X33CD7A8*, were mapped in the same terminal deletion bin of chromosome arm 7AL (7AL15-0.99-1.00) as *Xcfa2040* but could not be mapped in the 'Chinese Spring (Cappelle-Desprez 7A)' × 'Chinese Spring' population due to a lack of polymorphism (Chapman et al. 2009). Finally, five AFLP markers were significantly associated with eyespot resistance in a double haploid (DH) population segregating for *Pch2* (Meyer et al. 2008).

Restriction fragment length polymorphisms markers *Xcdo949* and *Xbcd588* bracket the gene *Pch3* on chromosome 4V in a wheat background in a 33-cM interval, and simultaneous selection for both flanking markers would theoretically select 96.7% of the genotypes having *Pch3* (Yildirim et al. 1998).

Durability of Eyespot Resistance Genes

The possibility of pathogenic specialization in *O. yallundae* and *O. acuformis* is a question that has not been studied in depth. Old studies on this subject were all published before the identification of the two *Oculimacula* species, and host-specific pathogenicity was often analysed in relation to different species at the same time as in relation to different cultivars. Evaluation of response specificity demands tests of several genotypes with several isolates and assessments of the statistical interaction between isolates and genotypes while avoiding the confounding direct and interacting effects of environment. Scott and Hollins (1977) reported such a study and concluded that genotype × isolate × environment was much larger than genotype × isolate interaction, which means that differential responses of cultivars to isolates were not repeatable over experiments.

Resistant wheat cultivars derived from the French cultivar 'Cappelle-Desprez' dominated European wheat

Table 2
Genes for resistance to eyespot mapped using genetic markers

Linked genes	Marker name	Marker type	Marker interval	Population structure	References
Pch1	*Ep-Db1*	Isozyme	Tight linkage	30 F_5 lines 'VPM/Moisson 421'//'Selection 101'	McMillin et al. (1986)
				689 resistant lines	Summers et al. (1988)
	Xpsr121	RFLP	Tight linkage	68 RSL HS(VPM-7D)/HS	Chao et al. (1989)
	Xust2001-7DL	SSR	3 cM away from *Pch1*	38 breeding lines	Santra et al. (2006)
	XW7Dest	SSR	6 cM away from *Pch1*	90 BC_5 lines HS(VPM-7D)/HS	Chapman et al. (2008)
	Xgwm428	SSR	8 cM away from *Xwmc14*		
	XB-glu7D	EST	Tight linkage		
	Xwmc273	SSR	Loose linkage	254 RIL_6 (Coda×Brundage),	Leonard et al. (2008)
	Xcfa2040	SSR	(between 9.4 and	germplasm survey of 44 lines	
	Xwmc634	SSR	7 cM from *Pch1*)	94 RILs of ITMI population	Chapman et al. (2008)
	Xgwm37	SSR		(W7984 × Opata85)	
	Xwmc14	SSR	Tight linkage		
	Xbarc97	SSR			
	Xcfd175	SSR			
	Xorw5	STS	Tight linkage	254 RIL_6 (Coda × Brundage),	Leonard et al. (2008)
	Xorw1	STS		germplasm survey of 44 lines;	
	Xorw6	STS		23 DH lines '293' (*Pch1*) × 'St906' (susceptible), 24 DH lines '359' (*Pch1*) × 'St906' (susceptible) and 80 DH lines 'Chevalier' (susceptible) × WW3640 (*Pch1*)	Meyer et al. (2010)
	K110	AFLP	Loose linkage	DH populations segregating	Meyer et al. (2008)
	SSR03	SSR	(2.1 and 4.3 cM	for *Pch*	
			from *Pch1*)		
	K210	AFLP	Tight linkage		
	Xust2001-7DL	SSR			
	SSR01	SSR			
	SSR02	SSR			
	SSR08	SSR			
	Xorw6	STS			
	Xorw5	STS			
Pch2	*Ep-A1b*	Isozyme	15% recombined with *Pch2*	80 RSL CS(CD7A)/CS	de la Peña et al. (1996)
	Xpsr121	RFLP	3.8% recombined with *Ep-A1b*		
	Xcdo347	RFLP	11 cM distal to *Pch2*	102 RSL CS(CD7A)/CS	de la Peña et al. (1997)
	Xwg380	RFLP	18.8 cM proximal to *Pch2*		
	Xwmc525	SSR	*Xwmc525* Linked with *Pch2* in	192 F_2 CS(CD7A)/CS	Chapman et al. (2008)
	Xwmc346	SSR	7 cM interval, flanked by		
	Xcfa2040	SSR	*Xwmc346* and *Xcfa2040*		
	Five fragments	AFLP	Unknown	DH population segregating for *Pch2*	Meyer et al. (2008)
Pch3	*Xcdo949*	RFLP	The two markers bracket	82 F2 [Yangmai-5(4V(4D)) ×	Yildirim et al. (1998)
	Xbcd588	RFLP	the *Pch3* in a 33 cM interval	a disomic addition line (CS + 4V)]	

RSL, single chromosome recombinant lines; RIL, recombinant inbred lines; RFLP, restriction fragment length polymorphisms; DH, double haploid; SSR, simple sequence repeat; STS, sequence-tagged site.

markets for two decades from 1953 (Hollins et al. 1988). Their resistance remained effective during prolonged and widespread use and can be recorded as durable (Gale et al. 1984; Law et al. 1988; Johnson 1992a).

Few and contradictory results were reported regarding durability of resistance conferred by *Pch1*: increases in yield loss of 'Madsen' relative to susceptible cultivars were observed over a 12-year period in eyespot field evaluation tests in the Pacific Northwest, where two cultivars carrying *Pch1*, 'Madsen' and 'Hyak', were grown over 500 000 ha (Jones et al. 1995), whereas no isolate of the pathogen obtained from France was found virulent on *Pch1*-carrying genotypes (Saur and Cavelier 1995; J. Jahier, personal communication), but *Pch1*-carrying cultivars were not widely grown in France until recently.

Differential response to the two *Oculimacula* species is a related subject more often discussed in recent publications. Poupard et al. (1994) showed that cultivars with *Pch1* ('Roazon' and 'Rendezvous') carried much less *O. acuformis* material as measured by ELISA than *O. yallundae* material, in the same experimental field and the same environmental conditions where plants had been inoculated separately with isolates of the two species. In this experiment, susceptible and moderately resistant cultivars had similar ELISA values with the two species. On the contrary, *Pch1* was found to be highly effective against both species, whereas *Pch2* was significantly less effective against *O. yallundae* than *O. acuformis* (Burt et al. 2010). While studying the eyespot resistance found in *D. villosum*, Uslu et al. (1998) observed that resistance to the two pathogen species could be conferred by different genes. Similarly, Sheng

and Murray (2009) observed that 20% of the *A. longissima* lines they tested responded differently to the two species, and Burt et al. (2010) identified four *T. monococcum* lines that responded differently to the two species.

Pathogenic specialization seems to exist in interactions between *Oculimacula* and wheat relatives. In *T. tauschii*, a differential response was repeatedly observed with two lines and several isolates in three European laboratories (Scott et al. 1976). Similarly, a differential response was observed in triticale (× *Tricosecale*) when challenged with *O. acuformis* isolates (M. Trottet, personal communication).

Problems and Future Prospects

Quite a few problems and significant new perspectives on eyespot of wheat have been provided by the research findings over many years. The fact that eyespot can be caused by two different species, *O. yallundae* and *O. acuformis* (Lucas et al. 2000; Crous et al. 2003), and that both have the ability to adapt to selection pressures (King and Griffin 1985; Murray 1996; Leroux and Gredt 1997) has important implications for disease management. Disease monitoring, chemical control and plant breeding will depend on a clear understanding of pathogenic specialization, genetics and population biology of the pathogen species (Dyer and Lucas 1995; Dyer et al. 2000; Bateman and Jenkyn 2001).

Alternative cultural practices, such as cultivar mixtures, induced resistance and biocontrol, deserve further research in regard eyespot management. Mixtures of resistant and susceptible cultivars seem to be able to reduce lodging significantly under severe eyespot attacks, even if symptoms are not reduced (Mundt 2002). This effect is likely due to resistant cultivars physically supporting susceptible cultivars. The endophyte *Piriformospora indica*, a Basidiomycota originating from the Thar desert of Rajasthan, India, was shown to colonize wheat roots and to have a significant reducing effect on eyespot symptoms (Serfling et al. 2007). It is not clear whether this effect is a result of host defence induction (systemic acquired resistance or priming) or an increased plant growth rate that helps the host to produce a leaf sheath faster than the pathogen goes through them. However, *P. indica* was apparently not able to restrict leaf pathogens in the field, raising doubts about its applicability for wheat production. Finally, the resistance inducer Benzo (1,2,3) thiadiazole-7-carbothioic acid *S*-methylester had no effect against eyespot disease in a field experiment that aimed primarily to evaluate its effects on foliar diseases and grain yield of winter wheat (Stadnik and Buchenauer 1999).

Molecular genetic studies of the eyespot pathogens are practicable and could be applied to identify factors determining fungicide resistance, pathogenicity and host specificity, to understand pathogen reproduction and to reveal the mechanism of the infection process. Such studies could ultimately enable the design of novel chemical compounds to interfere with key steps in the infection processes or spore production and hence prevent pathogen dispersal. However, very few results in these research areas were published in the last 10 years, and genomic studies do not seem to be underway for the eyespot pathogens.

Field evaluations and seedling tests in greenhouses or growth chambers based on visual scores are time- and resource-consuming, labour-intensive and sometimes inaccurate, because the pathogen grows slowly *in planta* and damage to plants is difficult to assess because it is not restricted to the plant surface. Additionally, the test requires substantial replication to obtain reliable results, owing to a significant degree of non-genetic interference and is seldom effective when applied to single plant selection (Koebner and Summers 2003). Moreover, young plant and adult plant responses to eyespot are only partially correlated. Finally, field tests are quite slow, taking up to 11 months. As a consequence, if phenotypic tests are useful to discover and map resistance genes, breeders need genetic markers for these resistance genes in order to manipulate them efficiently in breeding.

The introgressed segment surrounding *Pch1* in VPM-1 seems to have unfavourable effects for optimal yield. A yield penalty is associated with *Pch1* in the absence of the pathogen (Worland et al. 1988). Substitution of chromosome 7D of VPM-1 into several adapted UK wheat varieties depressed yield by approximately 6% (Law et al. 1988). Yield potential of VPM-1 was 30% lower than that of the long-term check 'Nugains' on the basis of 16 site-years of tests in Washington State (Jones et al. 1995). VPM-1 and some of its derivatives possess a large segment of chromosome from the D^V genome of *Ae. ventricosa*, and yield-depressing genes are probably carried together with eyespot resistance on this segment. Chao et al. (1989) considered that the $7D^v$ segment in VPM-1 represented most of the 7D chromosome because VPM-1 was different from the reference *Ae. ventricosa* accession they used at only one RFLP locus (*Xpsr129*) and one isozyme locus (*alpha-Amy-D2*), whereas it had the *Ae. ventricosa* allele at 12 RFLP loci, including the most distal at both ends of the chromosome and the most proximal ones near the centromere, and at three other loci (*Pch1*, *Ep-D1* and *Rc3*). Similarly, according to C-banding analysis, chromosome 7D appeared entirely substituted by chromosome $7D^V$ in four VPM lines (Badaeva et al. 2008). Quite long ago, it was shown that the deleterious linkages between low yield and *Pch1* can be broken (Worland and Law 1986; Law et al. 1988). More recently, molecular results showed that some cultivars carrying *Pch1*, for example 'Coda', have a much shorter $7D^v$ segment than VPM-1 (Leonard et al. 2008).

The codominant endopeptidase marker *Ep-D1* was long considered useful to monitor the introgression of *Pch1* from VPM-1 to elite lines. However, the endopeptidase test is destructive for single seed and generally not sufficiently accurate when applied on an

embryo-less half-grain basis. It is therefore usually applied as a bulked progeny test, delaying selection by one generation (Koebner and Summers 2003). An improved method of assaying for the *Ep-D1b* marker using roots from a single seedling was shown to be accurate in predicting resistance (Santra et al. 2006). This isoelectric focusing assay is not highly robust; it is a technically demanding procedure and produces toxic chemical compounds, and it is often difficult to distinguish the *Ep-D1b* allele from certain orthologous *Ep-A1* and *Ep-B1* alleles (Koebner et al. 1988). A DNA-based marker for the presence of the eyespot resistance gene is thus desirable for routine use in selection programmes, because it would provide a simple, rapid and accurate assay for resistance at all stages of plant growth and could be multiplexed with DNA markers for other traits. Fortunately, several SSR and STS markers have been identified that can be used in this context.

Yield losses due to eyespot can still occur in cultivars with the resistance of 'Cappelle-Desprez' (Hollins et al. 1988). A significant loss in grain yield due to eyespot was observed with VPM-1 once in 4 years under favourable disease conditions (Murray and Bruehl 1986). 'Madsen' sustained significant yield losses (average 15%) in five out 13 tests when inoculated and non-inoculated plots were compared (Jones et al. 1995), and the level of eyespot resistance varied among material with *Pch1* resistance gene (Lind 1999). On the contrary, additional fungicide treatment for eyespot control was predicted to be no longer routinely required in 'Rendezvous' that combines at least *Pch1* and *Pch2* (Hollins et al. 1988; Law et al. 1988; Burt et al. 2010). Similarly, Doussinault and Douaire (1978) observed that F_2 families obtained by crossing VPM and Cappelle-Desprez were slightly more eyespot resistant than VPM itself at the adult stage and obtained transgressive progenies in the F_4 generation. Two lines derived from these crosses showed significantly higher resistance than VPM-1 a few years later (Doussinault et al. 1983b). Allan and Roberts (1991) also identified transgressive progenies for eyespot resistance in a cross between VPM-1/Moisson 951 (resistant with *Pch1*) and 'Cerco', with resistance at the level of 'Cappelle-Desprez'. Through the use of SSR or STS markers flanking *Pch2* in combination with *Pch1*-linked loci like *Xorw1*, *Xorw5* and *Xorw6,* marker-assisted selection of genotypes carrying *Pch1* and *Pch2* could be successful and much easier than with phenotypic screens. This should provide farmers with cultivars having adequate eyespot resistance in the majority of the years.

It has often been suggested that the combination of *Pch1* and the resistance of 'Cappelle-Desprez' should also sustain *Pch1* durability, largely because of 'Cappelle-Desprez' resistance durability. Whether the combination of *Pch1* and *Pch2* will be durable remains an unanswered question, and for this reason, further sources of resistance should be identified at the genetic level.

Eyespot resistance is generally not complete, and environmental effects on its expression can be large. For these reasons, potentially valuable genotypes can be lost if breeders are not able to identify resistant genotypes. For example, *Pch3* was mapped with an assay performed with the GUS-transformed strain, and this would not have been possible with visual disease ratings (Jones et al. 1995).

In the event of *Pch1* resistance breakdown, new sources of eyespot resistance genes will be needed (Thiele et al. 2002) together with suitable genetic markers. Cultivars with improved eyespot resistance will then be produced through introgression of the new genes into new cultivars or through pyramiding of several resistance genes. This probably will be possible only with closely linked molecular markers for the various eyespot resistance genes. Various accessions of *T. tauschii*, *T. monococcum*, *T. durum*, *T. dicoccoides* and *T. turgidum* were identified in the 1990s as potential sources of eyespot resistance, but transfer of these putatively new source genes into hexaploid wheat, identification and mapping of the major genes represent a huge amount of work that is still incomplete. Association mapping with DArT (Semagn et al. 2006; Crossa et al. 2007), SNP markers when available in sufficient density or other marker techniques could help in localizing genes of interest on the basis of currently available phenotypic results, and identified markers could help in incorporating, pyramiding and stacking of resistance genes in commercial wheat cultivars. Wheat is well served in the development of genomic tools, which offers the promise of improved genetic control of eyespot as well as a broader genetic base to exploit in variety improvement.

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