

## Pathogen profile

**Wheat leaf rust caused by *Puccinia triticina***MELVIN D. BOLTON<sup>1,†</sup>, JAMES A. KOLMER<sup>2</sup> AND DAVID F. GARVIN<sup>1,\*</sup><sup>1</sup>USDA–ARS, Plant Science Research Unit, 411 Borlaug Hall, University of Minnesota, St. Paul, MN 55108, USA<sup>2</sup>USDA–ARS, Cereal Disease Laboratory, 1551 Lindig St., University of Minnesota, St. Paul, MN 55108, USA**SUMMARY**

Leaf rust, caused by *Puccinia triticina*, is the most common rust disease of wheat. The fungus is an obligate parasite capable of producing infectious urediniospores as long as infected leaf tissue remains alive. Urediniospores can be wind-disseminated and infect host plants hundreds of kilometres from their source plant, which can result in wheat leaf rust epidemics on a continental scale. This review summarizes current knowledge of the *P. triticina*/wheat interaction with emphasis on the infection process, molecular aspects of pathogenicity, rust resistance genes in wheat, genetics of the host parasite interaction, and the population biology of *P. triticina*.

**Taxonomy:** *Puccinia triticina* Eriks.: kingdom Fungi, phylum Basidiomycota, class Urediniomycetes, order Uredinales, family Pucciniaceae, genus *Puccinia*.

**Host range:** Telial/uredinial (primary) hosts: common wheat (*Triticum aestivum* L.), durum wheat (*T. turgidum* L. var. *durum*), cultivated emmer wheat (*T. dicoccon*) and wild emmer wheat (*T. dicoccoides*), *Aegilops speltoides*, goatgrass (*Ae. cylindrica*), and triticale (X Triticosecale). Pycnial/aecial (alternative) hosts: *Thalictrum speciosissimum* (= *T. flavum glaucum*) and *Isopyrum fumaroides*.

**Identification:** Leaf rust is characterized by the uredinial stage. Uredinia are up to 1.5 mm in diameter, erumpent, round to ovoid, with orange to brown uredinia that are scattered on both the upper and the lower leaf surfaces of the primary host. Uredinia produce urediniospores that are sub-globose, average 20 µm in diameter and are orange–brown, with up to eight germ pores scattered in thick, echinulate walls.

**Disease symptoms:** Wheat varieties that are fully susceptible have large uredinia without causing chlorosis or necrosis in the host tissues. Resistant wheat varieties are characterized by various responses from small hypersensitive flecks to small to

moderate size uredinia that may be surrounded by chlorotic and/or necrotic zones.

**Useful website:** USDA Cereal Disease Laboratory: <http://www.ars.usda.gov/mwa/cdl>

**INTRODUCTION**

Leaf rust, caused by *Puccinia triticina* Eriks., is the most common rust disease of wheat (*Triticum aestivum* L.). Leaf rust occurs more regularly and in more world-wide regions than stem rust of wheat (*P. graminis* f. sp. *tritici*) or stripe rust of wheat (*P. striiformis* f. sp. *tritici*). The fungus is heteroecious, and therefore requires a telial/uredinial host (usually wheat) and an alternative (pycnial/aecial) host (*Thalictrum speciosissimum* or *Isopyrum fumaroides*) to complete the full life cycle. It therefore seems likely that the *P. triticina* centre of origin is the Fertile Crescent region of the Middle East, where the natural range of the primary and alternative hosts overlap (D'Oliveira and Samborski, 1966). *Puccinia* spp. have afflicted wheat for thousands of years, as references to rust can be found in the literature of classical Greece and Rome and in the Bible (Chester, 1946). In North America, *P. triticina* was introduced with wheat cultivation in the early 17th century (Chester, 1946), but was often overlooked as an important disease of wheat as it did not appear to affect grain quality as much as other diseases such as stem rust (Leonard and Szabo, 2005) or Fusarium head blight (Goswami and Kistler, 2004). Yield losses in wheat from *P. triticina* infections are usually the result of decreased numbers of kernels per head and lower kernel weights. *Puccinia triticina* is now recognized as an important pathogen in wheat production world-wide, causing significant yield losses over large geographical areas (Kolmer, 2005; Marasas *et al.*, 2004; Roelfs *et al.*, 1992; Saari and Prescott, 1985). In 2007, leaf rust caused a 14% loss in winter wheat yields in Kansas (Kansas Department of Agriculture), the leading wheat-producing state in the US.

Although leaf rust is found almost everywhere that wheat is grown, suitable alternative hosts are rarely present for the fungus

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to complete the sexual cycle. The *Thalictrum* and *Isopyrum* spp. that are native to North America are relatively resistant to basidiospore infection (Jackson and Mains, 1921; Saari *et al.*, 1968). The lack of suitable alternative hosts, together with *P. triticina* molecular genotyping data, suggests that the sexual cycle does not contribute epidemiologically to disease spread and is an insignificant source of genetic variation for *P. triticina* both in North America and in most other wheat production areas of the world (Kolmer, 2005). Nonetheless, over 70 races of the pathogen are detected each year in North America (Kolmer *et al.*, 2007) where the pathogen persists through reproduction from asexual urediniospores.

Genetic resistance is the preferred method to reduce losses from leaf rust, and 60 leaf rust resistance (*Lr*) genes have been designated in wheat (McIntosh *et al.*, 2007). Most *Lr* genes confer race-specific resistance in a gene-for-gene manner. However, wheat varieties relying on race-specific resistance often lose effectiveness within a few years by imposing selection for virulent leaf rust races. In addition, the cultivation of a large acreage of susceptible winter wheat cultivars in the southern US allows a very large leaf rust population to proliferate, creating a reservoir for mutation and selection (Kolmer, 2005). High levels of virulence variation and broad adaptation of the fungus to the diverse climatic conditions where wheat is grown are characteristic of *P. triticina* and contribute to regular losses from leaf rust in global wheat production (Kolmer, 2005; Roelfs *et al.*, 1992).

Here, we provide a concise review of the biology of *P. triticina* and the interaction between *P. triticina* and wheat. We draw upon classic historical literature and combine this with recent state-of-the-art research, to provide an in-depth overview of the pathogen and the *P. triticina*/wheat pathosystem.

## TAXONOMIC HISTORY

Rust fungi belong to the order Uredinales in the Basidiomycetes. The taxonomic classification of the pathogen causing leaf rust of wheat has undergone several revisions. Leaf rust on wheat was initially designated as *Uredo rubigo-vera* (DC) by Augustin de Candolle (1815). Winter (1884) later placed wheat leaf rust in the species complex *P. rubigo-vera*. Eriksson (1899) was the first to describe wheat leaf rust as a single species, *P. triticina*, specific to wheat. Cummins and Caldwell (1956) included wheat leaf rust in the species complex of *P. recondita* that had leaf rusts with overlapping spore morphology and telial hosts of several species of grasses, wild wheat and rye. Based on this nomenclature, *P. recondita* was further subdivided into *formae speciales* based on host range, and wheat leaf rust was placed into *P. recondita* f. sp. *tritici* (Wilson and Henderson, 1966). More recently, Anikster *et al.* (1997) recommended that leaf rusts that have *T. speciosissimum* (family Ranunculaceae) as the alternative host be considered a separate species from leaf rusts with alternative

hosts such as *Anchusa* spp. (family Boraginaceae). Leaf rust of wheat with *T. speciosissimum* as the alternative host is sexually incompatible with leaf rusts that have *Anchusa* as the alternative host (Anikster *et al.*, 1997). Based on sexual incompatibility, the causal agent of wheat leaf rust is now considered a species distinct from leaf rusts on rye and other wheat relatives. The separation of wheat leaf rust from *P. recondita* is supported by phylogenetic ribosomal DNA sequence analyses (Zambino and Szabo, 1993), spore morphology (Savile, 1984) and infection structure morphology (Swertz, 1994). Leaf rust of common wheat (*Triticum aestivum* L.) or durum wheat (*T. turgidum* L. var. *durum*) is currently described as *Puccinia triticina* Eriks.

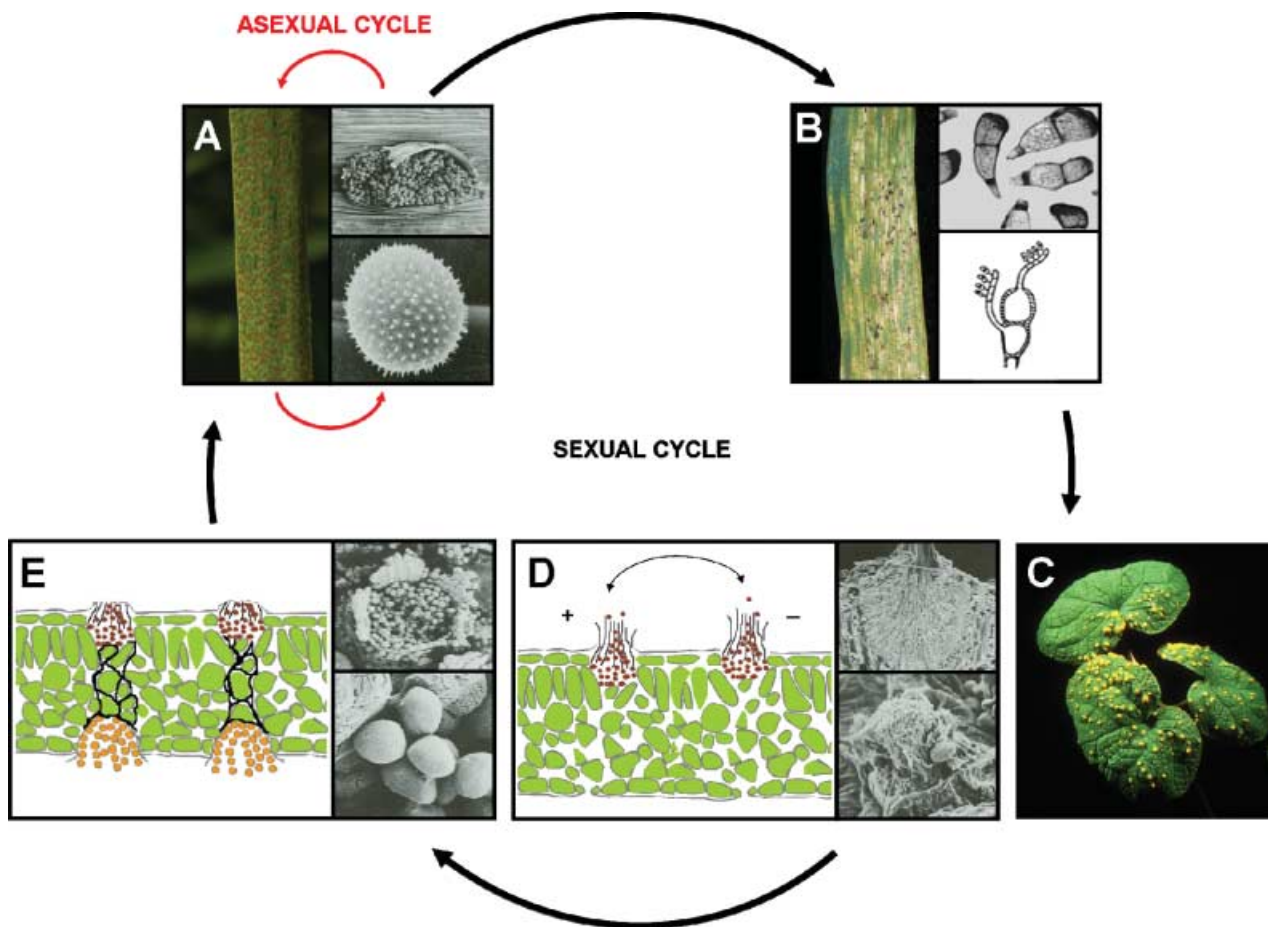
## HOST RANGE

On a world-wide basis, the primary host of *P. triticina* is hexaploid common wheat (Roelfs *et al.*, 1992). *Puccinia triticina* also occurs on tetraploid durum (*T. turgidum* ssp. *durum*), wild emmer (*T. dicoccoides*), domesticated emmer wheat (*T. dicoccon*) and triticale (X Triticosecale). In Israel a form of *P. triticina* that is not found on wheat has been found in limited areas on the diploid species, *Aegilops speltoides* (Yehuda *et al.*, 2004). *Puccinia triticina* is also present on *Ae. cylindrica* (common goatgrass) in the southern Great Plains of the US. Only certain races or virulence phenotypes of *P. triticina* are virulent on a given non-hexaploid wheat host, indicating a high degree of telial host specificity in *P. triticina*. Due to large genetic differences and nearly discrete host ranges (Goyeau *et al.*, 2006; Ordoñez and Kolmer, 2007), isolates of *P. triticina* that occur on durum wheat and *Ae. speltoides* can probably be considered as two different *formae speciales* compared with leaf rust of common wheat. Infections of *P. triticina* have not been noted in natural stands of wild wheat species such as *Ae. sharonensis*, *Ae. tauschii*, *Ae. bicornis*, *Ae. longissima*, *Ae. ovata*, *Ae. variabilis*, *T. timopheevi* or *T. urartu*. However, infections can be obtained on these species when artificially inoculated with *P. triticina* that is virulent to common wheat (Anikster *et al.*, 2005b).

The *P. triticina* sexual stage is rarely found on *Thalictrum* spp. in North America (Levine and Hildreth, 1957). The sexual stage of *P. triticina* has been found on *T. speciosissimum* in southern Europe (Casulli and Siniscalco, 1987; D'Oliveira, 1940; D'Oliveira and Samborski, 1966; Young and D'Oliveira, 1982). Infections on the alternative host *I. fumaroides* appear to be restricted to a region in Siberia (Chester, 1946).

## LIFE CYCLE

*Puccinia triticina* is a macrocyclic and heteroecious rust fungus with five spore stages and two taxonomically unrelated host species (Fig. 1). Urediniospores produced on wheat hosts are dikaryotic, 20 µm in width (Anikster *et al.*, 2005a), and will



**Fig. 1** Life cycle of *Puccinia triticina*. (A) Uredinia on leaf containing single cell dikaryotic urediniospores originating from aeciospores or urediniospores. This asexual uredinal stage may originate either from aeciospores or urediniospores and the uredinal stage is repeated on the wheat host as long as favourable conditions for infection occur. Top inset: surface view of a uredinium ( $\times 100$ ); bottom inset: echinulate surface of a single urediniospore ( $\times 3000$ ). (B) Telia typically form beneath the leaf epidermis near the end of the growing season, are the size of uredinia, and are black and erumpent at maturity. Top inset: teliospores which originate from telia and signal the beginning of the sexual stage of the life cycle; bottom inset: karyogamy and meiosis occur in the mature teliospore. Each teliospore can germinate producing a promycelium which gives rise to four haploid basidiospores, two of each mating type (+ and -). The nuclei undergo mitotic division so that mature basidiospores each have two haploid nuclei (not shown). (C) Pycnia, produced by basidiospores on *Thalictrum*, appear as yellow–orange pustules on upper leaf surfaces. (D) Diagrammatic cross-section of *Thalictrum* with pycnia. Top inset: basidiospores infect *Thalictrum* in which the fungus produces haploid pycnia ( $\times 400$ ); bottom inset: pycniospores and flexuous hyphae are immersed in a liquid ‘nectar’ exudate ( $\times 500$ ). Fertilization occurs when pycniospores and receptive hyphae fuse in compatible pairs of opposite mating type combinations (+/-). (E) Diagrammatic cross-section of *Thalictrum* with both pycnia and aecia. Top inset: following fertilization, a dikaryotic aecium develops ( $\times 200$ ); bottom inset: the aecium produces chains of dikaryotic aeciospores ( $\times 1250$ ). Inset pictures in A, C, and E courtesy of Brown and Brozman (1979), by permission of the University of Missouri Extension Division. Top inset picture in B from Anikster *et al.* (2005a).

re-infect the telial host with free water on the leaf surface and temperatures of 10–25 °C (Fig. 1A). As the host plant matures and the uredinal infections develop, dikaryotic, brown–black, two-celled teliospores, 16  $\mu\text{m}$  in width, with thick, smooth walls are produced in uredinia. In Mediterranean climates, teliospores allow the rust to survive the hot and dry summers to infect the alternative hosts in the autumn. Early in teliospore development, the two haploid nuclei in the teliospore undergo karyogamy to produce a diploid nucleus.

When conditions are suitable, one or both cells in the teliospore produce a hyal protrusion called a promycelium (Anikster,

1986). The diploid nuclei undergo meiosis and four haploid nuclei migrate into the promycelium which become subdivided by septa into four cells, each with one haploid nucleus. A spike-like structure called the sterigma forms on the apical wall of each cell and each haploid nucleus migrates through the sterigma into the newly expanding basidiospore at the apex of the sterigma (Fig. 1B). The nucleus within each basidiospore undergoes mitosis, forming a mature single-cell basidiospore, 6  $\mu\text{m}$  in width, each with two identical haploid nuclei.

Within a few hours after being formed, mature basidiospores are ejected from sterigmata and carried from the telial host by air currents short distances to nearby alternative hosts (Fig. 1C). Light and electron microscopy studies of *P. triticina* infections by basidiospores and subsequent pycnial and aecial development were conducted by Allen (1932) and Gold *et al.* (1979). Basidiospores directly infect epidermal cells resulting in the development of flask-shaped pycnia that develop as yellow–orange pustules on both leaf surfaces. Each pycnium produces haploid pycniospores, 2–3 µm in width, and flexuous (receptive) hyphae that function as male and female gametes, respectively (Fig. 1D). Gold *et al.* (1979) indicated that flexuous hyphae are formed and extend through the pycnia at the upper leaf surface. However, Allen (1932) reported that flexuous hyphae most commonly extend through stomatal guard cells of the lower leaf and that fertilization typically takes place on the lower leaf surface. *Puccinia triticina* is heterothallic and therefore pycniospores and flexuous hyphae originating from the same pycnium are not sexually compatible. Pycniospores emanate from the tip of the pycnium in a liquid ‘nectar’ exudate that attracts insects. The pycniospores are disseminated to different pycnia by insects and by movement in water such as dew and rain splashing. Fertilization occurs when pycniospores and receptive hyphae fuse in compatible pairs of opposite mating type (Fig. 1D). This fusion restores the dikaryotic nuclear condition in the resulting mycelium. The dikaryotic mycelium proliferates through the leaf and culminates on the lower leaf surface as an aecium, typically formed directly below the pycnium (Fig. 1E). Dikaryotic aeciospores, 20 µm in width, are formed in chains within the aecium and are wind-disseminated to the telial/uredinial hosts when the aecia erupt through the leaf epidermis. The life-cycle is complete when aeciospores germinate and penetrate stomata of the telial/uredinial host, resulting in production of asexual urediniospores.

*P. triticina* can cycle indefinitely as uredinial infections on telial hosts such as wheat. In the southern Great Plains of the US, leaf rust infections that over-summer on volunteer wheat can serve as reservoirs of inoculum for the autumn-planted winter wheat. In winter wheat, leaf rust can over-winter as mycelial or uredinial infections in areas with suitable temperature conditions (Roelfs, 1989).

### UREDINIAL INFECTION PROCESS

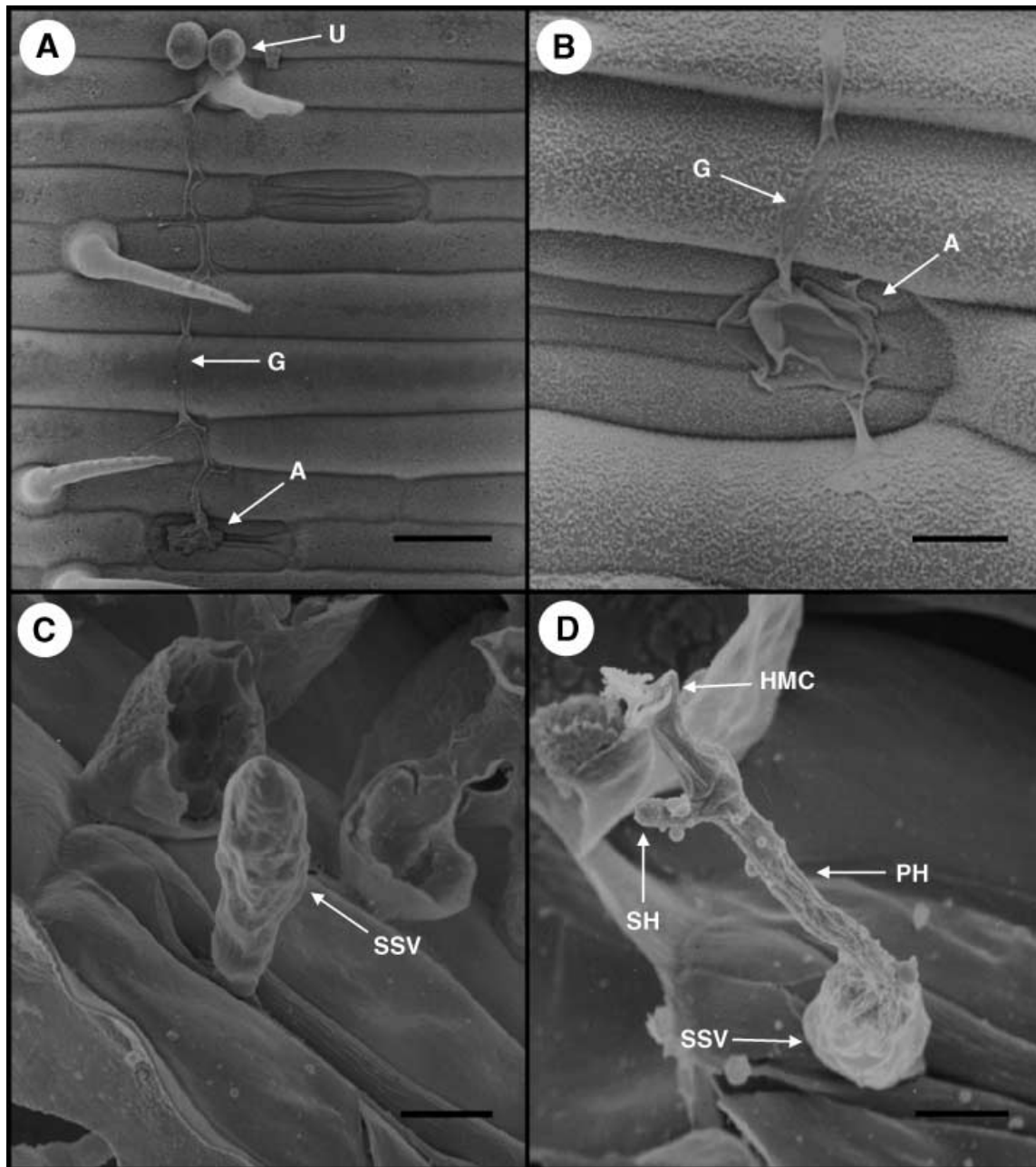
Cytological work by Allen (1926) first described the process of *P. triticina* infection and development in wheat. Urediniospores are deposited by wind or rain on either side of the wheat leaf. Urediniospores imbibe water, swell and develop a germ tube after coming into contact with a film of moisture such as dew or light rain on the leaf surface. Germination occurs after 4–8 h at 20 °C under 100% humidity (Hu and Rijkenberg, 1998; Zhang

and Dickinson, 2001; Zhang *et al.*, 2003), but spores may retain viability 1–3 days after inoculation under field conditions in the absence of an immediate dew period (Allen, 1926). Similar to *P. graminis* and other rust fungi (Collins and Read, 1997; Leonard and Szabo, 2005; Wynn and Staples, 1981), *P. triticina* germ tube growth is controlled by a thigmotropic response to the topography of the leaf surface, with growth orientated perpendicular to the long axis of the epidermal cells (Fig. 2A). Germ tubes continue to grow on the leaf surface until endogenous spore reserves are depleted or until a stoma is encountered (Dickinson, 1969). On artificial membranes that contain surface topographies of plant species other than wheat, germ tubes will often be unable to locate or recognize artificial stomata (Wynn and Staples, 1981). Likewise, germ tubes are also unable to locate or recognize stomata from non-host plants (Wynn and Staples, 1981).

At the stoma, the germ tube stops elongating and protoplasm flows towards the tip to form an appressorium over the stomatal aperture (Fig. 2B). The formation of appressoria occurs within 24 h after inoculation and germ tubes that have not found stomata by this stage do not survive (Zhang *et al.*, 2003). The two nuclei from the urediniospore migrate into the appressorium where they undergo mitosis followed by formation of a septum which isolates the now-empty germ tube from the appressorium. The stomata usually close promptly in response to appressorium formation, and remain closed in the presence of a mature appressorium (Caldwell and Stone, 1936).

A penetration peg originating from the appressorium pushes through the closed stoma to gain entry into intercellular space within the host leaf where the fungus forms a substomatal vesicle and another round of mitosis occurs (Fig. 2C). In contrast to *P. graminis*, *P. triticina* does not require light for entry through stoma and is unaffected by CO<sub>2</sub> concentration (Wynn and Staples, 1981). Subsequently, an infection hypha typically begins to grow inward from the substomatal vesicle towards the mesophyll cells (Allen, 1926), in contrast to intercellular hyphae of *P. graminis* which grow along the inner surface of the epidermis to the mesophyll cells nearest the stoma (Allen, 1923). Cytoplasm and nuclei migrate from the substomatal vesicle into the hypha that grows until the tip comes into contact with a mesophyll cell. After this contact, a septum forms behind the tip to delimit the haustorial mother cell (Fig. 2D). The haustorial mother cell, typically containing three nuclei, forms between 12 and 24 h after appressorium penetration (Hu and Rijkenberg, 1998) and adheres tightly to the host cell wall (Allen, 1926).

Host cell penetration begins with the formation of a penetration peg within an area of contact between the haustorial mother cell and the host cell, followed by the formation of a haustorium within the host cell. The haustorium is a specialized hypha that acts as a feeding structure for the fungus. Although the host cell wall is breached, haustoria are not truly intracellular as they remain separated from host cytoplasm by the extrahaustorial



**Fig. 2** Infection structures of *Puccinia triticina* on wheat leaf surface. (A) Urediniospore (U) germ tube (G) has produced an appressorium (A) over stomatal aperture (bar = 40  $\mu\text{m}$ ). (B) Appressorium contents have moved into substomatal vesicle (SSV) (bar = 12  $\mu\text{m}$ ). (C) Inside of leaf showing SSV orientated perpendicular to leaf surface (bar = 10  $\mu\text{m}$ ). (D) SSV produces primary infection hyphae (PH) and haustorial mother cell (HMC). The HMC produces a haustorium (not shown) within a mesophyll cell. A secondary haustoria (SH) may emerge in the proximity of HMC (bar = 7  $\mu\text{m}$ ). Pictures courtesy of Hu and Rijkenberg (1998), by permission of Elsevier.

membrane, a derivative of the host plasma membrane that tightly envelops the haustorium (Panstruga, 2003; Szabo and Bushnell, 2001). The extrahaustorial membrane is thought to be the interface where nutrient uptake from the host occurs, which is probably the result of fungal manipulation of host cell metabolism to establish and maintain compatibility (Bushnell

and Rowell, 1981; Hahn and Mendgen, 2001; Mendgen *et al.*, 2000; Panstruga, 2003; Szabo and Bushnell, 2001). Although the three nuclei from the haustorial mother cell migrate through the penetration peg and into the haustorium, only one nucleus is found in the mature haustorium due to disintegration of the other two nuclei (Allen, 1926). After haustorial formation, more

infecting hyphae produced from the haustorial mother cell grow and come into contact with additional host cells. These also form haustorial mother cells and haustoria, resulting in a branching network of fungal mycelium. Allen (1926) noted that even up until 6 days after inoculation of a susceptible cultivar, the host cell appeared to be unaltered by the infection. The only visible change seen in host cells during this time was movement of the host nucleus towards the haustorium (Allen, 1926).

At 7–10 days post inoculation, the mycelium growing in susceptible wheat leaf tissue gives rise to uredinia that produce dikaryotic urediniospores. Orange–red urediniospores are released when uredinia rupture the epidermis. The released urediniospores impart the characteristic ‘rusty’ appearance to leaves. In highly susceptible hosts, secondary uredinia form in a small oval around the primary pustule (Schafer, 1987). Even at 16 days post inoculation, less than 1% of host cells have died as a result of infection (Allen, 1926).

### MOLECULAR ASPECTS OF PATHOGENICITY

Currently, very little is known about the molecular and biological basis of *P. triticina* pathogenicity. The dearth of molecular information relates to the inability to culture *P. triticina in vitro*, the relatively large genome size estimated to be 100–124 Mbp (Eilam *et al.*, 1994) and, until recently, the lack of an efficient molecular transformation system. However, recent advances are starting to shed light on the molecular biology of host infection by *P. triticina*.

Webb *et al.* (2006) established stable transgene integration into the *P. triticina* genome using biolistic-mediated transformation. Transformants were selected by observing a change from avirulence to virulence on wheat lines carrying single resistance genes. Several mutants were developed that probably have an insertion into the cognate *Avr* gene, a gene in the *Avr* response pathway or a gene associated with delivery/translocation of the *Avr* protein. Two of the mutants that gained virulence on a wheat line carrying *Lr21* contain transgenes inserted in genes encoding a chitin synthase and a calmodulin-binding protein (Webb *et al.*, 2006). As calmodulin-dependent signalling pathways have been shown to play important roles in virulence and life cycles of several fungi (Kraus and Heitman, 2003), and disruption of fungal chitin synthase genes decreases fungal virulence (Garcerá-Teruel *et al.*, 2004; Soulié *et al.*, 2003), future functional analysis of these potential *Avr* genes will help to illuminate their roles in the *P. triticina* life cycle.

Several strategies have been used to characterize the *P. triticina* transcriptome *in planta*. Thara *et al.* (2003) used suppression subtractive hybridization (SSH) to identify 69 genes induced in the fungus as it developed in plants 4 days after inoculation. Twenty-five of these genes encode fungal ribosomal proteins, presumably indicative of active protein synthesis during the

infection process. In addition, a few genes were induced that have homology to genes encoding virulence factors in other fungi or are induced during infection in other fungal pathogens. Zhang *et al.* (2003) used a complementary DNA-amplified fragment length polymorphism (cDNA-AFLP) approach at several time points after inoculation to identify fungal genes with homologies to sorbitol utilization protein, arabinitol dehydrogenase and chitinase genes. The chitinase gene had homology to a chitinase gene from the bean rust *Uromyces fabae* that is induced in haustoria (Hahn and Mendgen, 1997). The proteome of the susceptible wheat/*P. triticina* interaction was interrogated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) at 9 days post-inoculation (Rampitsch *et al.*, 2006). Using a matrix-assisted laser desorption/ionization (MALDI) source coupled to a tandem quadrupole/time-of-flight (Qq-TOF) tandem mass spectrometer (MS/MS), 22 fungal proteins were characterized. These belonged to several protein classes such as metabolic enzymes, structural proteins and putative virulence-related proteins, the last of which includes heat-shock and 14-3-3-like proteins (Rampitsch *et al.*, 2006).

Recently, an expressed sequence tag (EST) database was developed representing each of several life-cycle stages of *P. triticina* (Hu *et al.*, 2007b). cDNA libraries were constructed from resting urediniospores, germinating urediniospores, an appressorial stage on compatible leaves, a haustorial stage on compatible leaves and an incompatible interaction 24 h after inoculation. Although there was significant overlap in transcripts found between resting and germinated urediniospores, little overlap was found between the other stages, suggesting distinct coordinated gene expression changes at each stage. The presumed fungal cDNAs encoded proteins involved in general metabolism, protein synthesis, and transport-, stress- or virulence-related proteins (e.g. glutathione-S-transferase, catalase, heat shock proteins, chitinases and cytochrome P-450 monooxygenases). One gene generated from this study, *PtMAPK*, encoded a mitogen-activated protein kinase (MAPK), which was functionally analysed by complementation in the corn smut fungus *Ustilago maydis* (Hu *et al.*, 2007a). The results suggest that *PtMAPK* is involved in pathogen development, as are its orthologues in *U. maydis* (Hu *et al.*, 2007a).

### LEAF RUST RESISTANCE GENES IN WHEAT

Genetic studies of leaf rust resistance in wheat have been conducted by wheat researchers world-wide. In the first of these studies, Mains *et al.* (1926) determined that the wheat cultivars Malakof and Webster each had a gene that conditioned leaf rust resistance, later designated as *Lr1* and *Lr2*, respectively (Ausemus *et al.*, 1946). Soliman *et al.* (1964) mapped *Lr* genes by identifying the chromosomes that carried leaf rust resistance genes *Lr1*, *Lr3* and *Lr11*. Dyck and Samborski (1968) demonstrated allelic variation in *Lr* genes when they determined the presence of three alleles at the *Lr2* locus.

Leaf rust resistance genes designated *Lr1* to *Lr60* have been described (McIntosh *et al.*, 2007). These genes have been characterized in common hexaploid wheat, tetraploid durum wheat and many diploid wild wheat species. In hexaploid wheat, leaf rust resistance genes are widely distributed across the genome, being present on nearly every one of the 42 chromosome arms. Four allelic series have also been described. Genes *Lr2a*, *Lr2b* and *Lr2c* were mapped to a locus on chromosome arm 2DS (McIntosh and Baker, 1968), *Lr3a*, *Lr3ka* and *Lr3g* are at a locus on chromosome arm 6BL (Haggag and Dyck, 1973), *Lr17a* and *Lr17b* are at a locus on chromosome arm 2AS (Dyck and Kerber, 1977), and *Lr22a* and *Lr22b* at a locus on chromosome arm 2DS (Rowland and Kerber, 1974). Genes *Lr14a* and *Lr14b* are extremely tightly linked on chromosome 7BL (Dyck and Samborski, 1970) and are considered as alleles for all practical purposes.

Most leaf rust resistance genes condition effective resistance to specific races of *P. triticina*. Race-specific resistance is usually manifested by a hypersensitive response (HR) of rapid cell death that occurs at the interface between fungal haustoria and host cells in the epidermal and mesophyll layers. Different resistance genes condition characteristic resistance phenotypes or infection types (Fig. 3). For instance, the resistance response of wheat lines with *Lr3* is characterized by clearly defined hypersensitive flecks while lines with *Lr2a* have only very light flecks that are difficult to see. Other race-specific resistance responses such as those conditioned by wheat lines with *Lr3ka*, *Lr3bg* and *Lr11* are manifested by small uredinia surrounded by chlorosis, and lines with *Lr16* have small uredinia surrounded by necrosis. Resistance conditioned by these last genes is probably expressed at a later point in the infection and colonization process of *P. triticina*. Race-specific *Lr* genes are effective in seedling plants and remain effective in the adult plant stage. However, the resistance



**Fig. 3** Leaf rust seedling infection types. The lower leaves have resistant infection types that range from hypersensitive flecks to small–moderate size uredinia surrounded by chlorosis and necrosis. The infection type of the top leaf is fully susceptible with large uredinia without surrounding chlorosis or necrosis in the leaf tissue.

conditioned by some genes, such as *Lr12*, *Lr13* and *Lr22a*, is best expressed in adult plants. In wheat lines that have combinations of resistance genes, the gene with greatest resistant infection type is epistatic to genes with less resistant infection types.

To date, three genes that confer race-specific resistance have been cloned: *Lr1* and *Lr10*, originally from common wheat, and *Lr21*, originally from *T. tauschii* (Cloutier *et al.*, 2007; Feuillet *et al.*, 2003; Huang *et al.*, 2003). These genes condition an HR response to isolates of *P. triticina* that carry matching avirulence genes, and encode proteins with nucleotide binding site-leucine rich repeat (NBS-LRR) regions typical of most disease resistance genes in plants.

Not all leaf rust resistance genes condition race-specific resistance. Gene *Lr34* was first described as a modifier of adult plant resistance in the cultivar Frontana (Dyck *et al.*, 1966). This gene was found in a number of wheat lines from around the world (Dyck and Samborski, 1982) and was later mapped to chromosome arm 7DS (Dyck, 1987). The resistance response conditioned by *Lr34* does not involve HR, but instead is characterized by fewer and smaller uredinia with no chlorosis or necrosis on flag leaves of adult plants, often with a decreasing gradient of uredinia density from leaf base to tip. *Lr34* conditions the same resistance response to all isolates of *P. triticina* that have been tested (i.e. non race-specific resistance), and is associated with non-HR resistance to stripe rust and powdery mildew (Spielmeyer *et al.*, 2005). Gene *Lr46* is another adult plant resistance gene originally found in the cultivar Pavon 76. *Lr46* conditions a resistance response of fewer and smaller uredinia, but with varying amounts of chlorosis in adult plants (Singh *et al.*, 1998). Genes that condition non-HR resistance will probably differ both in DNA sequence and protein function compared with race-specific, NBS-LRR resistance genes. Genetic aspects of leaf rust resistance in wheat were previously reviewed by Kolmer (1996).

## GENETICS OF WHEAT–*P. TRITICINA* INTERACTION

Near-isogenic lines of the wheat cultivar Thatcher that differ for single leaf rust resistance genes were developed by P. L. Dyck (McIntosh *et al.*, 1995). These lines have been extremely valuable for conducting analysis of virulence variation in *P. triticina* populations, genetics of leaf rust resistance in wheat and genetics of host–parasite relations between wheat and *P. triticina*.

Samborski and Dyck (1968) showed that single loci in *P. triticina* carry alleles that condition avirulence or virulence to specific corresponding leaf rust resistance genes. In this initial study, four different isolates of *P. triticina* were selfed and the  $S_1$  progeny isolates tested for segregation of virulence on Thatcher isogenic lines. Dominant genes conditioned avirulence to *Lr1* and *Lr11*. Selfed progeny from one isolate segregated for virulence to *Lr3* with avirulence being dominant, while progeny from another

isolate segregated for virulence to *Lr3* with virulence being dominant.

A single dominant gene in the *P. triticina* S<sub>1</sub> progeny segregated that conditioned avirulence to the *Lr2* alleles *Lr2a*, *Lr2b* and *Lr2c* in wheat. Isolates of *P. triticina* that were homozygous or heterozygous for avirulence had very low infection types to all three *Lr2* alleles. Isolates that were homozygous for recessive virulence alleles had high infection to all three *Lr2* alleles. In certain segregating populations, an independent dominant inhibitor gene differentially affected the expression of avirulence to the *Lr2* alleles. Isolates that were heterozygous for both the avirulence and inhibitor genes had intermediate infection types to *Lr2a* and *Lr2b*, and high infection types to *Lr2c*. Dyck and Samborski (1974) confirmed the independent segregation of avirulence and inhibitor genes in a further study with isogenic lines of *Lr2a*, *Lr2b* and *Lr2c* in Thatcher, Prelude and Red Bobs. Single independent genes in *P. triticina* conditioned avirulence/virulence to different resistance alleles at the *Lr3*, *Lr14* and *Lr22* loci in wheat (Bartos *et al.*, 1969; Dyck and Samborski, 1970; Haggag *et al.*, 1973).

Additional genetic studies of avirulence/virulence in *P. triticina* to *Lr* genes in the Thatcher isolines have been conducted (Samborski and Dyck, 1976; Statler, 1977, 1979, 1982, 2000; Statler and Jin, 1991). For a number of resistance genes, the expression (dominant or recessive) and number of genes that conditioned avirulence varied between different segregating populations of *P. triticina*. Either a single gene or two genes segregated in different populations at loci that conditioned avirulence/virulence for *Lr1*, *Lr3*, *Lr10*, *Lr11*, *Lr29* and *Lr30*. Avirulence in *P. triticina* to *Lr16* and *Lr26* was conditioned by two dominant genes, and avirulence to genes *Lr10*, *Lr18* and *Lr23* was conditioned by a single dominant gene in some segregating populations, but recessive genes in other populations. In these genetic studies, almost all *P. triticina* avirulence/virulence loci segregated independently, with close genetic linkage between loci observed only between avirulence to *Lr3ka* and *Lr30* (Kolmer, 1992; Samborski and Dyck, 1976). Loci conditioning avirulence/virulence are apparently widely distributed across the estimated 16–18 chromosomes of *P. triticina* (Leonard and Szabo, 2005).

The effects of gene dosage on expression of avirulence in *P. triticina* have also been noted. An isolate of *P. triticina* that had an intermediate avirulent infection type to the wheat cultivar Transfer with *Lr9* was determined to be heterozygous for a single dominant gene that conditioned avirulence (Samborski, 1963). Selfed progeny isolates that were homozygous for avirulence alleles had lower infection types to wheat lines with *Lr9* compared with heterozygous isolates. Similar effects of gene dosage on avirulent infection types were seen for Thatcher lines with *Lr2a*, *Lr2c*, *Lr3ka*, *Lr3a*, *Lr11*, *Lr17* and *Lr30* (Kolmer and Dyck, 1994). The expression of resistance genes in wheat ranged from completely dominant to recessive, depending on the avirulence

(homozygous or heterozygous) genotype of the *P. triticina* isolate. Similarly, expression of avirulence in *P. triticina* depended on the resistance genotype (homozygous or heterozygous) of the wheat line. Kolmer (1992) noted that isolates of *P. triticina* were often heterozygous for avirulence/virulence genes to resistance genes that were originally derived from hexaploid wheat.

Statler (1985, 1987) conducted mutation studies with isolates of *P. triticina* that were heterozygous for virulence genes to a number of *Lr* genes. Mutant isolates were identified that had gained virulence to a number of genes or had changed from virulent to avirulent. Mutations to virulence could easily be explained by invoking loss of function in the dominant avirulence alleles, leading to non-functional virulence alleles that were not recognized by the host resistance gene. However, mutations from virulence to avirulence implies either that virulence alleles are altered by mutation such that they are recognized by host resistance genes, or that mutations in regulatory loci such as inhibitors of avirulence in *P. triticina* affect expression of avirulence/virulence genes.

These examples reveal that the genetics of the interaction between wheat and *P. triticina* can depart from the classical gene-for-gene relationship first exemplified by Flor in his work with flax rust (Flor, 1971), in which single dominant avirulence genes interact specifically with single dominant host resistance genes to condition incompatible infection types. Although avirulence genes and resistance genes in *P. triticina* and wheat are specific for interaction, these genes are not invariably dominant and do not always interact in a one-for-one relationship. Furthermore, loci that carry alleles that inhibit the expression of avirulence such as at the *Lr2* locus or other types of regulatory loci in *P. triticina* may occur more frequently than has been recognized, thus leading to digenic segregation ratios in genetic analysis.

## POPULATION BIOLOGY OF *P. TRITICINA*

Among plant pathogens, *P. triticina* has a relatively long history of population studies, with nationwide race surveys for this rust beginning in the US in 1926 (Johnston *et al.*, 1968), in Canada in 1931 (Johnson, 1956) and in Australia in 1920 (Waterhouse, 1952). The wheat cultivars Malakof (*Lr1*), Webster (*Lr2a*), Carina (*Lr2b*, *LrB*), Loros (*Lr2c*), Brevit (*Lr2c*, *LrB*), Hussar (*Lr11*), Democrat (*Lr3*) and Mediterranean (*Lr3*) were designated as the International Standard set of leaf rust differentials, and used in the early race identification studies. By 1932, 26 races had been identified in the US (Johnston and Mains, 1932). The International Standard differentials were also used for the initial leaf rust race studies in Europe (Chester, 1946). In Australia, these differentials did not adequately identify leaf rust races (Waterhouse, 1952). The cultivars Gaza (*Lr23*), Thew (*Lr20*) and other cultivars were used to supplement the International Standard differentials in Australian studies (Park, 1996). More recently, isogenic lines of



Thatcher that differ for single leaf rust resistance genes have been used to characterize *P. triticina* populations in Europe and North America. Single gene lines of wheat with *Lr1*, *Lr2a*, *Lr2c* and *Lr3* have been used continuously in the leaf rust race surveys in the US and Canada since their inception. This made it possible to describe changes in virulence to these genes in *P. triticina* in Canada from 1931 to 1986 (Kolmer, 1989, 1991) and from 1987 to 1997 (Kolmer, 1999).

*P. triticina* populations world-wide are highly diverse for virulence phenotypes or races. Up to 70 different leaf rust races are identified on an annual basis in the US on 20 differential lines (Kolmer *et al.*, 2007). In France, 30–50 races are identified annually (Goyeau *et al.*, 2006). In Australia, 10–15 races are detected on an annual basis (Park, 1996). In the US, virulent leaf rust races increase very quickly in response to widespread use of wheat cultivars with race-specific resistance genes. As the leaf rust population is very large, it would be expected that random mutations occur in sufficient numbers, which would lead to the development of virulent races. In Australia, race-specific resistance genes have retained effective resistance for longer periods. This is probably due to the presence of fewer susceptible cultivars, which greatly reduces the population size of *P. triticina* and thus reduces the likelihood of virulent mutations being selected. Cultivars with *Lr24* were first grown in Australia in 1983 (Park *et al.*, 2002), and it was not until 2000 that races with virulence to this gene were selected. In the US, races with virulence to *Lr24* were detected within a few years of cultivars with this gene being released.

Introductions from distant sources of new races with virulence to resistance genes in commonly grown wheat cultivars can also occur. Races with virulence to *Lr17*, *Lr3bg* and *LrB* became common in the southern Great Plains of the US in the mid 1990s. Genetic analysis with AFLP markers indicated that these races were most likely introduced to the Great Plains region from either Mexico or the Pacific Northwest, and were not derived by mutation from the previously existing populations (Kolmer, 2001). In Australia, a new race that was distinct for virulence to genes *Lr16*, *Lr27* and *Lr31* was detected for the first time in 1984, and was probably introduced from another continent (Park *et al.*, 1995). The high degree of virulence and simple sequence repeat (SSR) genotypic similarity between *P. triticina* isolates from durum wheat in Europe, South America, Mexico and California suggested that *P. triticina* populations on durum wheat in these regions originated from a single original founder population (Ordoñez and Kolmer, 2007).

Regional populations of *P. triticina* races can arise within a continent due to use of wheat cultivars with different leaf rust resistance gene composition in different regions. Winter wheat cultivars with *Lr3* and *Lr26* have been grown in central Europe, where races with virulence to *Lr3*, *Lr3bg*, *Lr3ka* and *Lr26* were common. In contrast, *P. triticina* races in western Europe were

generally avirulent to these resistance genes (Park and Felsenstein, 1995). There was also evidence of long-distance movement of *P. triticina* in Europe, as only four races accounted for 64% of tested isolates in this study. In 1998, 105 races were identified in France, Hungary, Italy, Bulgaria and Poland (Mesterhazy *et al.*, 2000), with very few races in common between the countries. Regional populations of *P. triticina* races that differ for virulence to *Lr2a*, *Lr9*, *Lr11* and *Lr16* due to selection by wheat cultivars with these genes have been described in the US (Kolmer *et al.*, 2007).

Molecular markers such as RAPDs and AFLPs have been used to characterize variation in *P. triticina* populations. Different groups of *P. triticina* isolates in Canada and from international collections could be distinguished with RAPD and AFLP markers that also correlated with grouping based on avirulence/virulence to single gene differential lines (Kolmer, 2001; Kolmer and Liu, 2000). In Europe, multiple isolates of the same race from different countries had identical RAPD banding patterns (Park *et al.*, 2000). As *P. triticina* relies upon clonal reproduction nearly everywhere in the world, an effective linkage between virulence and molecular markers is maintained. However, in an experimental greenhouse population of *P. triticina* derived from aeciospores, disequilibrium between individual virulence genes and RAPD markers was often eliminated or reduced (Liu and Kolmer, 1998).

Recently, locus-specific microsatellite or SSR markers have been developed for *P. triticina* (Duan *et al.*, 2003; Szabo and Kolmer, 2007). SSR markers are co-dominant and can distinguish between heterozygote and homozygote genotypes, in contrast to RAPD and AFLP markers that are dominant. *Puccinia triticina* populations studied with SSR markers have several attributes in common, including higher levels of heterozygosity than expected compared with populations in Hardy–Weinberg equilibrium and high levels of linkage disequilibrium, and they are genetically differentiated by continental region (Kolmer and Ordoñez, 2007) or due to selective effects of resistance genes in wheat cultivars (Goyeau *et al.*, 2007). All populations of *P. triticina* that have been examined with SSR markers (Goyeau *et al.*, 2007; Kolmer and Ordoñez, 2007) have genetic characteristics typical of clonal diploid or dikaryotic populations in which high levels of heterozygosity are maintained by sequential mutation in the absence of recombination (Balloux *et al.*, 2003; Halkett *et al.*, 2005). Somatic recombination has been reported in *P. triticina* (Park *et al.*, 1999), although the rates of such variation remain unknown.

## FUTURE PERSPECTIVES

Leaf rust continues to cause losses worldwide in wheat production due to reliance on cultivars with race-specific resistance and the high level of virulence variation in *P. triticina*. The development of wheat cultivars with effective levels of durable resistance will rely on genes that confer non-race-specific resistance. Wheat cultivars

with combinations of *Lr34*, *Lr46* or other non-race-specific resistance genes are highly resistant and are immediately available sources of durable resistance (Singh *et al.*, 2000). Combinations of *Lr34* with seedling resistance genes have also provided high levels of effective resistance that have remained effective for a number of years (Kolmer and Oelke, 2006). The cloning of genes *Lr34* and *Lr46* and analysis of how these genes are expressed may provide a model for understanding the biological basis of non-race-specific resistance.

Many aspects of wheat leaf rust have been well studied, but little is known about the molecular and genetic aspects of *P. triticina* pathogenicity. In rust fungi, little is known about the intrinsic function of Avr proteins in compatible interactions or how Avr proteins interact with resistance gene-encoded proteins to elicit the defence response. Four Avr genes have been cloned from the flax rust pathogen (*Melampsora lini*). Although their function in virulence is not yet known, these genes encode small, secreted proteins that are recognized inside plant cells (Lawrence *et al.*, 2007). Comparable research has yet to be conducted for *P. triticina*.

Research on *P. triticina* would be advanced by genome sequencing. The genome sequences of *P. graminis* and *M. laricina* (poplar rust) have recently been released and will be an important resource for *P. triticina* comparative genomics. Research on the molecular basis of infection and race specificity in *P. triticina* is just beginning, so recent progress in transformation (Webb *et al.*, 2006) and new techniques for functional analysis of *P. triticina* genes (Hu *et al.*, 2007a) will provide new opportunities to study these significant yet poorly understood aspects of this important pathogen.

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