Resistance to *Pyrenopeziza brassicae* (light leaf spot) in *Brassica napus* (oilseed rape)

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Abstract

Light leaf spot (caused by the hemibiotrophic *Pyrenopeziza brassicae*) is one of the most important diseases of winter oilseed rape (*Brassica napus*) in northern Europe, including the UK. In controlled environment and field experiments to study sources of genetic resistance against *P. brassicae*, *R* gene-mediated resistance introduced into *B. napus* slowed growth of *P. brassicae*, prevented asexual sporulation on living tissue, but did not prevent sexual sporulation on senescent tissue. The resistance did not operate in the manner typical of *R* gene-mediated resistance against hemibiotrophs. *P. brassicae* infected the resistant lines but did not elicit an immediate hypersensitive response preventing further fungal growth. Instead, it grew sparsely as sub-cuticular hyphae within green leaves, until a "dark flecking" phenotype associated with collapse of epidermal cells was observed approximately 10 days post inoculation. This resistance may be more durable than that of a typical *R* gene because it reduces secondary infection by splash-dispersed conidia but does not apply selection by preventing the pathogen from completing its life cycle.

Key words: *Brassica napus* (oilseed rape), doubled haploid mapping population, durable resistance, hemibiotroph, *Pyrenopeziza brassicae* (light leaf spot), quantitative PCR, sporulation

Introduction

Plant resistance against pathogens of arable crops makes an important contribution to global food security (Brun *et al*., 2010), especially in areas of the world where subsistence farmers in marginal environments are threatened by devastating epidemics and do not have the option to use fungicides (Flood, 2010; Fitt *et al*., 2011). To exploit such crop resistance effectively, it is important to understand its phenotype in relation to the life cycle of the pathogen. The phenotype of plant *R* gene-mediated resistance to biotrophic pathogens, such as the polycyclic *Blumeria graminis* (cereal powdery mildews) or *Puccinia striiformis* (wheat yellow rust), often involves a hypersensitive response (rapid localised cell death) occurring immediately after invasion of host tissue (Jorgensen, 1994; Bozkurt *et al*., 2010). Hypersensitive resistance responses also occur with hemibiotrophic pathogens such as *Rhynchosporium secalis* (barley leaf blotch) and *Leptosphaeria maculans* (oilseed rape phoma stem canker, leaf spot phase) but much less rapidly, so that the pathogen growth is only slowed, allowing asexual and/or sexual reproduction to continue.

This paper characterises the phenotype of a particular form of resistance against the hemibiotrophic pathogen *Pyrenopeziza brassicae* that has been introgressed into oilseed rape (*Brassica napus*). *P. brassicae* causes light leaf spot, a polycyclic disease initiated in the UK in the autumn by airborne ascospores produced following sexual reproduction of the pathogen on senescent plant debris (Gilles *et al.*, 2000a). After infection by airborne *P. brassicae* ascospores, the fungus grows biotrophically and asymptomatically between the cuticle and the epidermal cells of the leaf, until the first symptoms of asexual sporulation are observed (white *P. brassicae* acervuli breaking through the leaf surface) (Gilles *et al.*, 2000b). The conidia contained in the acervuli are splash-dispersed to cause secondary infections of leaves, stems, meristems and other tissues (Gilles *et al.*, 2001). No clear hypersensitive response following infection of *B. napus* by *P. brassicae* has been reported (Boys *et al.*, 2007). The only report of *R* gene-mediated resistance was by Bradburne *et al.* (1999), who suggested that there were two resistance genes segregating in two mapping populations of doubled haploid (DH) lines produced following introgression of genetic material from *B. rapa* (A genome) and

B. oleracea var. *atlantica* (C genome) into *B. napus* (amphidiploid AC genome) via synthetic lines. These resistance genes were not finely mapped and the resistant phenotypes were not investigated further. This paper reports work to characterise the resistance phenotype as a model system for investigating the operation of plant major gene-mediated resistance to hemibiotrophic pathogens.

Materials and Methods

The susceptible winter oilseed rape cultivar (Apex) and a resistant winter oilseed rape cultivar (Imola) derived from the material studied by Bradburne *et al.* (1999) were used, together with a doubled haploid (DH) mapping population of *B. napus* derived from a cross with this cultivar that segregates for resistance against *P. brassicae*, to characterise the phenotype of resistance observed in *B. napus* against *P. brassicae.* Conidial inoculum of *P. brassicae* was obtained from a selection of winter oilseed rape cultivars grown in field experiments at Rothamsted (Harpenden, UK) and characterised by RAPD-PCR fingerprinting and mating-type PCR. Quantitative PCR was used to measure amounts of *P. brassicae* DNA in leaf samples. Quantitative trait data (symptoms and qPCR) were combined with microsatellite marker mapping of a linkage group corresponding to chromosome A1 of *B. napus*.

Results

The main symptoms of light leaf spot in leaves of the susceptible cv. Apex inoculated with *P. brassicae* in controlled environment conditions were white *P. brassicae* acervuli that erupted through the leaf surface (Fig. 1a) about 16 days post inoculation (dpi). By contrast, a “dark flecking” phenotype (Fig. 1b) was observed on leaves of the resistant cv. Imola from about 10 dpi, most obviously on leaf vein tissue, but also elsewhere on the leaf lamina. Point-inoculation demonstrated that both the resistant (“dark flecking”) and susceptible (asexual sporulation) phenotypes were confined to the area of inoculation and did not induce a visible response elsewhere on the leaf.

Examination of sections of spray-inoculated leaves under a scanning electron microscope showed how the *P. brassicae* hyphae grew within leaf tissue. In the susceptible cv. Apex, the hyphae grew between the cuticle and the upper epidermis, with no evidence that hyphae penetrated host cells, and asexual sporulation was observed at 13 dpi. Sub-cuticular hyphal growth occurred similarly but to a lesser extent in leaves of the resistant cv. Imola. However, no asexual sporulation was observed. The “dark flecking” was shown to be caused by the collapse of epidermal cells. This cell collapse was always associated with the presence of *P. brassicae* hyphae, but the presence of *P. brassicae* hyphae did not always result in cell collapse.

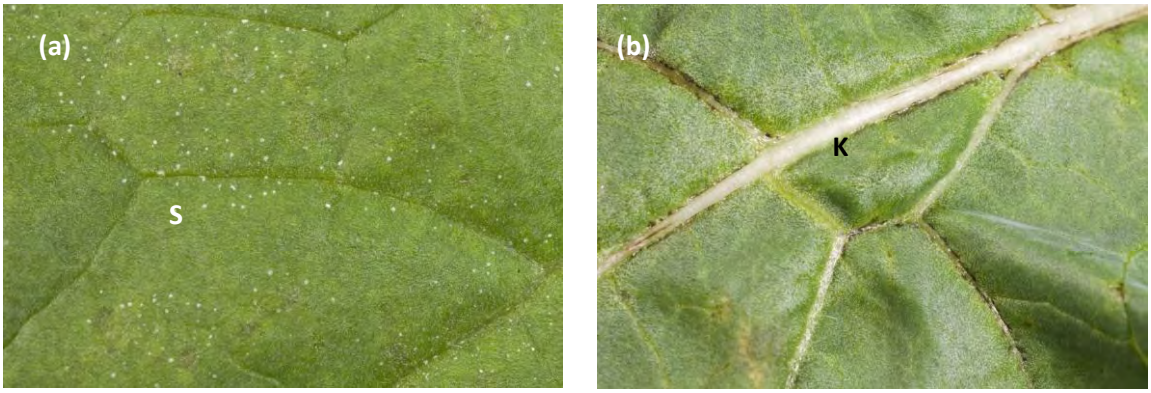

Fig. 1. (a) Light leaf spot symptoms (white *Pyrenopeziza brassicae* acervuli; S) on a leaf of the susceptible *B. napus* cv. Apex at 23 days post spray-inoculation (dpi) with a suspension of conidia from a mixed population of *P. brassicae*; (b) a leaf of the resistant *B. napus* cv. Imola at 23 dpi, showing the “dark flecking” (K) along the midrib.

The amount of *P. brassicae* DNA in leaves of both the susceptible cv. Apex and the resistant cv. Imola increased significantly with time, at the same rate until about 13 dpi. Then the amount of *P. brassicae* DNA in leaves of cv. Imola was significantly less than that in leaves of cv. Apex until about 36 dpi, by which time the leaf tissue was starting to senesce, when the amount in cv. Imola increased until it was the same as in cv. Apex by 45 dpi.

After incubating senescent leaves infected with *P. brassicae*, sexual reproduction was observed in both susceptible and resistant cultivars, in the form of apothecia bearing sexual spores (asci). The apothecia were produced in association with a dark discolouration, spread over the leaf lamina on the susceptible cultivars but confined to the area on and around the leaf veins on the resistant cultivars.

Analysis of resistance or sensitivity in the doubled haploid population showed that the population was segregating for resistance to *P. brassicae* in a ratio of c. 1:1. Of the 125 lines tested in a series of controlled environment experiments, 66 showed a susceptible phenotype with asexual sporulation and no "dark flecking"; of the remaining 59 lines, 57 showed the resistant "dark flecking" phenotype. These results were supported by field experiments. Mapping of both the controlled environment and the field data positioned the resistance locus close to the telomere (end) of the long arm of chromosome A1.

Discussion

These results suggest that a novel form of resistance mediated by a single *R*-gene can limit asexual sporulation but not sexual sporulation of a hemibiotrophic pathogen. This can be compared to the *Leptosphaeria maculans–Brassica napus* pathosystem, where resistance prevents sexual sporulation because it stops the pathogen reaching stem tissues on which sexual sporulation occurs The *R* gene operating against *P. brassicae* may be more durable than the *R* genes operating against *L. maculans*, which have been shown to be rendered ineffective within 4 years (Brun *et al.*, 2000), because *P. brassicae* is able to complete the sexual phase of its life cycle on the senescent debris of resistant lines at the end of the season. It is important to consider the best strategy with which to deploy this *R* gene-mediated resistance in commercial cultivars to maximise its durability (Pink, 2002); for example it has been shown that the durability of an *R* gene may be increased when it is introgressed into a resistant or partially resistant genetic background (Palloix *et al.*, 2009; Brun *et al.*, 2010).

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