Short title: ***Genetics for Downy mildew resistance in Brassica oleracea***

Genetics of resistance to downy mildew in *Brassica oleracea* and breeding towards durable disease control for UK vegetable production

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ABSTRACT

Downy mildew resistance was previously identified from screening a *Brassica oleracea* collection against two standard UK isolates of *Hyaloperonospora parasitica*. Sources of resistance were chosen from this material and developed further in this study by generating doubled haploid (DH) and inbred lines. Seedlings from the new lines were tested for resistance to a larger collection of *H. parasitica* isolates collected in 2001-02 and 2007-08 from the main broccoli and cauliflower production regions of the UK. Three lines (derived from borecole or summer cabbage) were broadly resistant to the pathogen isolates. Three of the remaining lines exhibited strong isolate-specific resistance; several examples of weak or basal level of resistance to some isolates were observed. A new *H. parasitica* variant collected in 2008 was virulent in the broadly resistant lines, but was avirulent in a line with narrow specificity of resistance. The F_2 and BC_1 seedlings derived from outcrossing each of the three broadly resistant lines to susceptible broccoli and cauliflower lines segregated in a manner indicating that the resistance is controlled by a single dominant gene. No susceptibility was observed amongst F_2 seedlings derived from intercrossing the three resistant lines, indicating that they all share the same or closely linked broad spectrum resistance gene(s). DH lines were produced from F_1 plants and resistant plants were further back-crossed to produce broccoli and cauliflower-like lines that could be useful pre-breeding material. A combination of resistance from lines with broad and narrow specificity is recommended for controlling downy mildew in UK brassica production.

Introduction

Downy mildew caused by the oomycete *Hyaloperonospora parasitica* (Pers. ex Fr.) Constant. (syn. *Peronospora parasitica* Constantinescu & Fatchi, 2002) is an economically important foliar disease of *Brassica oleracea* crops (cabbage, broccoli, cauliflower, kale and Brussel sprouts). The disease is distributed worldwide wherever brassica crops are grown and is favoured by cool humid weather that is frequent in spring or autumn (Channon, 1981). Downy mildew can affect plants at all growth stages, but the most damaging effects are generally restricted to young seedlings, causing heavy losses in plant nurseries, and to particular organs like cauliflower curds (Channon, 1981). Repeated application of fungicides is generally used for control, but the effectiveness of this approach has been limited because there is a restricted range of active ingredients effective against oomycete pathogens and frequent prophylactic application of fungicides has led to the selection and widespread distribution of insensitive variants of the pathogen (Brophy & Laing, 1992).

Downy mildew resistant cultivars would offer a practical, environmentally acceptable alternative method to control downy mildew. Several sources of seedling resistance to *H. parasitica* in *B. oleracea* have been reported previously, and heritability of the resistance has been characterised in many cases including: single dominant genes in broccoli and cauliflower (Natti *et al.*, 1967; Jensen *et al.*, 1999a; Farnham *et al.*, 2002), a single recessive gene in cauliflower (Hoser-Krauze *et al.*, 1984), recessive gene(s) in kale, Savoy cabbage and Brussels sprouts (Carlsson *et al.*, 2004) and multiple (additive) genes in cauliflower, broccoli and cabbage (Moss *et al.*, 1988; Hoser-Krauze *et al.*, 1995; Jensen *et al.*, 1999b). The breadth of resistance provided against a collection of *H. parasitica* isolates was not investigated in these previous reports, so the potential durability of each resistance is unknown.

The development and deployment of cultivars with single major disease resistance genes has frequently failed to provide durable disease control due to the quick evolution of pathotypes virulent on resistant cultivars, so in cases like downy mildew (*Bremia lactucae*) of lettuce, blackleg (*Leptosphaeria maculans*) of oilseed rape, northern leaf blight (*Setosphaeria turcica*) of maize, leaf and stripe rust (*Puccinia triticina* and *P. striiformis*) of wheat and potato late blight (*Phytophthora infestans*) there has been a continuous effort to find sources of broad-spectrum resistance and to devise strategies to deploy the resistant cultivars (Crute & Norwood, 1981; Sivasithamparam *et al.*, 2005; Stuthman *et al.*, 2007; Zhang *et al.*, 2009).

In the current study, we have followed a systematic approach pioneered more than four decades ago at the then National Vegetable Research Station (NVRS), Wellesbourne, UK, for predictive breeding of durable resistance to downy mildew in lettuce, and subsequently applied to other major vegetable diseases (Crute, 1992; Taylor *et al.*, 1996; Taylor *et al.*, 2002). The approach began in each case with an initial characterisation of differential reactions amongst standard vegetable cultivars for resistance to a small sample of pathogen isolates. A broadly virulent isolate (one which could cause typical disease symptoms in the initial subset of cultivars) could then be chosen to screen a much larger host diversity collection to identify new sources of disease resistance. Inbreeding and selection of true-breeding resistant lines is often required at this stage, since plant genetic resources (e.g. older open pollinated cultivars and landraces or wild crop relatives) may comprise heterogeneous mixtures of resistant and susceptible genotypes. Genetically uniform resistant lines can then be screened against a more extensive sample of pathogen isolates, collected widely from vegetable production regions, to identify examples of broad spectrum disease resistance against the entire pathogen collection. The NVRS approach was used successfully to identify race non-specific resistance to lettuce downy mildew and bacterial diseases of

common bean and pea (Crute & Norwood, 1981; Schmit *et al.*, 1993; Taylor *et al.*, 1996). The same approach was subsequently applied to *Brassica oleracea* in a coordinated effort to identify sources of resistance to black rot (*Xanthomonas campestris* pv. *campestris*), downy mildew (*Hyaloperonospora parasitica*), white blister rust (*Albugo candida*) and cabbage aphid (*Brevicoryne brassicae*) in a core diversity collection of more than 400 *B. oleracea* accessions (Leckie *et al.*, 1996; Ellis *et al.*, 1998; Taylor *et al.*, 2002).

The objective of our current study was to complete the assessment of the downy mildew resistant sources selected in previous work in order to determine if they could provide potentially durable downy mildew control for UK vegetable production. To achieve this, we assessed the spectrum of resistance of selected lines against UK isolates of *H. parasitica*, studied the inheritance of resistance in some of the resistant lines and initiated breeding for the development of broccoli and cauliflower lines possessing the resistance identified in selected lines.

Materials and methods

Plant materials

The experimental lines used in this study are listed in Table 1. Nine lines of *B. oleracea* were derived from accessions identified by Leckie *et al.* (1996), which were selected for resistance to an isolate of either *H. parasitica* (P005 or P006) or *A. candida* (A001). Resistant plants were crossed with rapid cycling plants (line CrGC3.4) and double haploids were produced via microspore culture from resistant F_3 plants. Six additional *B. oleracea* lines and three *B. napus* lines were also included in experiments as controls. Seeds were sown in Levington F2 compost (The Scotts Company, Ipswich, UK) in P286 plug trays (Plant Pak, Cookson Ltd.) cut to 9 x 15 array and placed in seed trays. Seeds were covered with medium grade

vermiculite and watered. The trays were covered with propagator lids and placed in a growth room for 9 days at 20 ±2°C with a day length of 10 hours prior to inoculation.

The downy mildew susceptible kale cultivar Maris Kestrel (Kings Seeds, UK) was used for maintaining cultures and multiplying sporangial inoculum of *H. parasitica* isolates. Each week, approximately 40 seeds were sown in four 4 cm square modules filled with Levington F2 compost and soaked in water. The seeds were covered with a layer of vermiculite. The modules were placed in a covered propagator in a growth room at 20 ±2°C with 10h days until inoculation.

For genetic experiments, plants were raised from seed sown initially in module trays. One-month-old plants were transplanted into 9-cm pots filled with Levington M2 compost. Plants were kept in a glasshouse with a minimum temperature of 17/14°C (day/night), venting at 19/16°C (day/night) with supplementary lighting from October to March to give 16 h days. Hybridisations were performed using bud pollination. Self-compatible plants were naturally self-pollinated and self-incompatible plants were self pollinated by bud pollination. All plants were covered with perforated polyethylene bags to prevent contamination with other pollen.

Hyaloperonospora parasitica **isolates**

The pathogen isolates used in this study are listed in Table 2. Two standard isolates (P005 and P006) were included, which were previously used for genetic studies and for selection of the original downy mildew accessions (Leckie *et al.*, 1996). Twenty-five single-spore isolates were included, which were derived from infected tissue samples that were collected from transplant nurseries or commercial farms in northern and southern England during the 2001 and 2002 growing seasons. Fifteen additional isolates were collected from plant nurseries in 2007 and 2008.

For maintenance of cultures and inoculum production, infected leaf tissue with downy mildew sporulation was washed with 2-3ml of distilled water, and the resulting suspension was drop inoculated onto 9-day-old kale (cv. Maris Kestrel) seedlings. The inoculated seedlings were then incubated for 7 to 14 days in a growth room at 15±2ºC with a day length of 12 hours. Infected cotyledons with sporulation were harvested and washed and the spore suspension was inoculated again onto kale seedlings. After 7 days incubation, cotyledons displaying heavy sporulation were used to prepare a suspension for seedling experiments. Surplus seedlings with heavy sporulation were harvested, placed in small, sealed pots and frozen at –80^{o}C for long-term storage.

Phenotypic assessment

For assessment of the phenotypic interaction of different combinations of *B. oleracea* line and *H. parasitica* isolate, seedlings were grown in plug trays (described above) and inoculated at 9-days-old with droplets of sporangiospore suspension. The inoculum was prepared by placing heavily sporulating seedlings of Maris Kestrel in sterile distilled water, and agitating gently to dislodge the sporangia. The inoculum concentration was measured using a haemocytometer, and adjusted to 4 x 10^4 spores/ml. Two 10 µl droplets were placed on each cotyledon of the experimental lines using a repetitive pipette. Trays of inoculated seedlings were covered with a transparent propagator lid, with the vents closed and sealed with tape, and transferred to a growth room set at 15±2ºC with a day length of 12 hours.

The interaction phenotype (IP) of each seedling was assessed seven and fourteen days after inoculation, by rating the degree of sporulation and host response using a scale adapted from Leckie *et al.* (1996), and converting scores to a numerical IP value (Table 3). The highest score of each seedling was used to calculate a mean IP value per line. A mean IP lower than 2.5 was considered as fully resistant, a mean IP higher than 4.5 was considered as

fully susceptible, and an intermediate value indicated a weak level of resistance with restricted sporulation (possible examples of basal or rate-reducing resistance).

Production of doubled haploid lines

The microspore culture protocol described by Takahata and Keller (1991) was modified for this study. Plants selected for microspore culture were transferred to an air-conditioned glasshouse at 15°C with supplementary lighting from October until March to give 16 hours day length to produce buds for microspore culture. Buds 3.5 - 5.5mm in length were collected, sterilised in Fichlor solution (1.7% w/v) containing a small volume of wetting agent Nonidet (5%), and washed three times with sterile RO water. The buds were then mashed in 5ml of cold (4°C) liquid medium NLN to release immature pollen into suspension. The mashed bud suspension was filtered through a 45 micron nylon filter cloth (Cadish Precision Meshes Ltd.) and the microspore suspension collected and made up to 40ml with cold NLN medium. The microspores were washed three times by centrifugation at 1000rpm for 3.5min, with cold NLN medium added after each spin. After the final wash the microspores were re-suspended in 60ml of cold NLN medium and 5-6ml were dispensed into 5cm deep form Petri plates. The plates were incubated in the dark at 32^{o}C for 24 hours and followed by incubation at 25^{o}C. After two weeks the plates were transferred to a shaker at 25^{o}C in the dark. Embryos became apparent to the naked eye after a further week.

Large embryos were transferred to Gamborg's B5 solid basal medium in 5cm Petri dishes and incubated at 22±2^{o}C with a day length of 16 hours at a photon flux density of 80-180 µmolm^{-2} s^{-1} for 2-3 weeks. Every 2-3 weeks, shoots and differentiating tissues were transferred onto fresh basal medium. When shoots had grown sufficiently, they were transferred back onto basal medium in small pots (Sterilin) and grown until large enough to be transferred to compost in 4cm pots (P24, Plantpak). After 3-4 weeks, the plants had grown sufficiently in soil to be transferred to glasshouse conditions. Leaf samples originating from

different embryos were analysed for ploidy number by flow cytometry (Plant Cytometry Services, The Netherlands) to identify the doubled haploid lines. When inflorescences developed they were bagged to prevent cross-pollination. Plants confirmed as diploid lines were grown to produce sufficient seed for pathogen screening in subsequent experiments.

Results

Specificity of downy mildew resistance

Fifteen *Brassica oleracea* and three *B. napus* lines were inoculated with the full collection of 42 *H. parasitica* isolates (Figure 1). Three lines (EBH525, EBH544 and EBH550) were resistant to all of the isolates collected in 2001, 2002 and 2007. However, one of the eleven isolates collected in 2008 (Hp806) was virulent on these lines, indicating that rare variants occur in the UK pathogen population which could overcome this broad spectrum resistance (Figure 1; Table 4).

Three lines exhibited differential resistance including: EBH502 which was fully resistant to five *H. parasitica* isolates (Hp006, 710, 735, 807, 815) and weakly resistant to 19 isolates; EBH508 which was fully resistant to only two isolates (Hp006, Hp704) and weakly resistant to eight isolates; and EBH527 which was also fully resistant to only two isolates (Hp702, Hp801) and weakly resistant to 27 isolates (Figure 1; Table 4). Three white rust resistant lines were included (EBH516, EBH535 and EBH553) which were susceptible to most of *H. parasitica* isolates, but exhibited weak resistance to 3, 8 and 6 isolates, respectively. Similarly, three lines (GDDH33, Lateman, A12DHd) were susceptible to most isolates, but exhibited weak resistance to 2, 3 and 15 isolates, respectively. Three other control lines (Cal18b, Surfrider and Senna) were susceptible to all isolates in the collection.

Importantly, this combined information of variable resistance specificity indicates a high degree of pathotypic variability amongst the UK *H. parasitica* isolates used in this study.

Similarly, the *B. napus* lines included in this study also differed in responses to the *H. parasitica* collection (Figure 1). The line N-o-9 was fully resistant to all of the isolates, whereas COB76 was resistant to all of the isolates apart from a weak level of resistance to three isolates (Hp717, Hp801 and Hp811). N-o-1 was the only *B. napus* line, which exhibited susceptibility, in this case to one isolate (Hp727).

Inheritance of resistance and allelism tests

The F_2 and back-crosses derived from six crosses between the three sources of broad specificity resistance (EBH525, EBH544, EBH550) and susceptible broccoli or cauliflower lines were tested with a single *H. parasitica* isolate (Hp717). In all three cases, the F_2 and the BC_1 generation segregated in agreement with a 3:1 and 1:1 ratio respectively (Table 5) (χ^2 probabilities higher than 0.1), which indicates that the resistance in each line is controlled by a single dominant gene.

The F_2 lines derived from inter-crosses between the three sources of broad spectrum resistance were also tested with the *H. parasitica* isolate Hp717 (Table 5). Over 3200 F_2 seedlings were tested (minimum of 500 seedlings per cross) together with controls including F_1 seedlings, parental lines and a susceptible control (Senna). The F_1 seedlings derived from the crosses between lines EBH525 and EBH544, and EBH525 and EBH550 were fully resistant (IP of 1); whereas F_1 seedlings derived from the cross of EBH544 and EBH550 exhibited IP values ranging from 1 to 3 (with some restricted sporulation). Most F_2 plants exhibited a resistant phenotype (IP 1 or 2) with a small proportion of plants showing restricted sporulation (IP 3). No fully susceptible F_2 plants were found, indicating that the genetic basis of the three different sources of downy mildew resistance is most likely

determined by the same genetic locus, conferring resistance via a single major R-gene or a combination of tightly linked genes.

Doubled-haploid lines derived from crosses with broccoli and back-crosses to broccoli and cauliflower

The lines EBH525 and EBH550 were used to initiate introgression of the broad spectrum resistant R-gene into a broccoli background. Forty-five doubled haploid plants were produced from F_1 plants from a cross between each resistant line to the susceptible broccoli line Cal18b. Five of these doubled haploid were self-incompatible, however, seed was produced from all plants by bud pollination. Forty lines were tested against Hp717, and approximately half (21 lines) were fully resistant, five exhibited weak resistance, and the remainder were susceptible (Table 6). The lines displayed variable levels of self-fertility and variable morphology.

The plants derived from crosses between the sources of resistance and the susceptible broccoli Cal18b and cauliflower cv. Lateman were back-crossed twice to the susceptible parent. Plants of the BC_1 and BC_2 generations segregated for downy mildew resistance as expected (in agreement with 1:1 ratio) and two plants obtained from self-pollination of BC_1 plants, segregated in agreement with a 3:1 ratio (results not shown). Generally the morphology of BC_2 plants resembled more the broccoli and cauliflower parent than the resistant line. These plants formed tighter heads and had smaller buds than the F_1 and BC_1 plants although generally their heads were still not as large and tight as the susceptible broccoli (Cal18b) and cauliflower (Lateman). Plants from the BC_1 generation were also used to produce further doubled-haploid lines via microspore culture.

Discussion

The current study follows an applied genetics approach that was pioneered at the then National Vegetable Research Station (NVRS, Wellesbourne, UK) in the 1970-80s, which enabled the successful identification of sources of race non-specific and potentially durable disease resistance for use in vegetable breeding programmes (Crute, 1992; Schmit *et al.*, 1993; Taylor *et al.*, 1996; Taylor *et al.*, 2002). Lettuce downy mildew provided the first example, and polygenic resistance that protects adult leaf tissue from severe downy mildew under field conditions in a pathotype nonspecific manner was eventually characterised (Crute & Norwood, 1981; Norwood *et al.*, 1983). Although this 'field resistance' has thus far been difficult to incorporate routinely into lettuce cultivars, it demonstrates an example for development of molecular marker assisted selection in breeding programmes (Zhang *et al.*, 2009; Kou & Wang, 2010). In contrast, a single recessive gene conferring nonspecific resistance to halo blight in common bean (*Phaseolus vulgaris*) has been used effectively for at least two decades to control the disease in crop production across the globe, and the subsequent characterisation of at least two alternative genes provides the potential to 'back up' this resistance if there is a shift in virulence within the pathogen population (Taylor *et al.*, 1996); (Taylor *pers. comm.*). Similarly, this approach was used to identify a single major QTL gene from the wild brassica relative, *Arabidopsis thaliana*, which confers nonspecific resistance to at least four physiological races of *Albugo candida,* and is transferable for use in transgenic oilseed brassicas (Borhan *et al.*, 2008; Borhan *et al.*, 2010).

Two key elements of the previous research effort were the availability of crop diversity collections from genebanks and a concerted effort to assemble extensive collections of pathogen isolates primarily from major crop production regions in order to sample the diversity within the pathogen population. Both elements were included in the current study, in our attempt to identify useful sources of downy mildew resistance for vegetable brassica

production in the UK. A core diversity collection of more than 400 *B. oleracea* accessions had previously been screened for downy mildew resistance using two standard isolates of *H. parasitica* (Leckie *et al.*, 1996). The potential sources of resistance were heterozygous; an additional component of the current research was to 'fix' resistance in genetically uniform experimental lines to allow robust phenotyping to be carried out. Resistant plants were crossed with rapid cycling plants and doubled haploid lines generated from F_3 resistant plants as the first step in the current work. We also generated doubled haploid lines from white rust resistant plants selected in the earlier research effort. These were included in the current study because co-segregation of downy mildew resistance was observed following selection for white rust resistance in a rapid cycling population of *B. rapa* (Mitchell-Olds *et al.*, 1995). Indeed, one of the recommended downy mildew resistant lines (EBH502, described below) was originally selected for white rust resistance in *B. oleracea*.

Three lines (EBH525, EBH544, EBH550) were promising as examples that exhibited broad seedling resistance to the sample of UK *H. parasitica* isolates collected in 2001-02. An additional sample of *H. parasitica* isolates from UK production sites in 2007-08 revealed that isolate Hp806 (one out of 15) was capable of heavy sporulation following inoculation of EBH525, EBH544, EBH550 seedlings. This resistance can also be broken or partially broken by some isolates from Portugal and France (Coelho, P., *pers. com.*). Nevertheless, these three lines represent the best candidates of selection for downy mildew resistance from the core diversity collection of *B. oleracea.* Thus, our results indicate that no pathotype nonspecific seedling resistance occurs in the core diversity collection. Similarly, dominant resistance reported by Jensen *et al.* (1999a) which appears to be effective against pathogen isolates from Denmark, is not effective against all UK isolates. Allelism tests indicated that all three lines share at least one downy mildew resistance gene. Testing of F_1, F_2 and back-cross generations from outcrossing to susceptible broccoli and cauliflower lines indicated that the resistance is

conferred by a single major (fully dominant) resistance gene. We did not determine whether this resistance gene is allelic to sources of downy mildew resistance that have been reported previously (Natti *et al.*, 1967; Jensen *et al.*, 1999a).

Three doubled haploid lines (EBH502, EBH508 and EBH527) exhibited pathotype-specific resistance. Two lines, EBH502 and EBH508, have differential reactions that can be used to differentiate downy mildew isolates and to divide them into pathotypes or physiological races (Table 4). The line EBH527 has partial resistance to over half of the isolates tested indicating that this resistance can also be valuable for controlling the disease. This line can also differentiate some isolates but, it appears to be less useful to differentiate isolates because it frequently showed intermediate phenotypes. The groups of isolates defined in table 4 are not related in their origin, as groups contain isolates from up to five different UK counties and also from organic and conventional production systems. Gene-for-gene relationships can be used to explain the interaction between *H. parasitica* isolates with different avirulence genes and *B. oleracea* lines with different resistance genes. It is possible that the isolates can be further divided using other plant lines, but the current differentiation based on three lines can be a starting point for the selection of isolates for further studies.

Preliminary genetic analyses (data not shown) indicate that resistance of EBH508 to *H. parasitica* isolate Hp717 is conferred by a single dominant resistance factor, whereas the resistance in lines EBH502 and EBH527 appears to be conferred by a single recessive (or co-dominant) factor. Importantly, EBH502 is resistant to *H. parasitica* isolate Hp806, suggesting that this resistance would complement the broader resistance in lines EBH525, EBH544 and EBH550 and a combination of the two would provide control against the virulences sampled in the collection of *H. parasitica.*

We have started to transfer the resistance from the lines EBH525, EBH544, EBH550 to broccoli and cauliflower through a back-cross programme. The doubled-haploid lines

produced from the F_1 plants, have segregated in agreement with a 1:1 ratio as expected for resistance controlled by a single gene. Five doubled haploid lines obtained from one of the crosses showed an intermediate phenotype with restricted sporulation that indicated that there might be additional genes which determine a partial resistance phenotype. Some of the doubled haploid lines produced from F_1 plants have very strong resistance and should be a good starting material for further breeding. Plants of the back-cross generation are morphologically more similar to the parental broccoli or cauliflower lines and some doubled haploid lines are currently being produced from these plants.

Nonspecific downy mildew resistance may occur as adult plant resistance as demonstrated by Crute and Norwood's (1981) investigation of field resistance in lettuce. *B. oleracea* can exhibit resistance to downy mildew in adult leaf tissue which, in some cases, can be independent of seedling resistance (Coelho & Monteiro, 2003). In contrast, the resistance identified at the seedling stage in cauliflower by Jensen et al. (1999a) is also expressed during the adult plant stage. It is still unknown if the resistance studied here at seedling stage is effective in adult plants and therefore, it will be important to test the lines at a later developmental stage.

Race-nonspecific downy mildew resistance genes may occur in other brassica species or wild relatives of *B. oleracea*. Sheriff and Lucas (1990) had previously reported a high degree of host preference amongst physiological races of *H. parasitica* that were derived from different brassica species. The pathogen population of *B. oleracea* is probably relatively narrow as co-evolution on a restricted range of host genotypes might have taken place. For example, isolates collected from *B. oleracea* are typically most virulent in *B. oleracea*, however, weak sporulation by some isolates in oilseed *B. napus* cultivars was observed (Sherriff & Lucas, 1990). Similarly, we included three *B. napus* accessions in experiments to assess the pathogenic variation amongst the *H. parasitica* isolates collected from *B. oleracea*.

Two *B. napus* accessions were mostly resistant to the collection of *H. parasitica* isolates, whereas the double haploid line N-o-1 exhibited intermediate phenotypes to over half of the isolates. The resistance in *B. napus* can possibly be mapped using available mapping populations and recent integrated linkage map information (Wang *et al.*, 2011).

The 'broccoli and cauliflower-like' lines developed in this study possess resistance to a wide range of *H. parasitica* isolates in the UK and therefore can be used to develop new cultivars in these two crops. The lines can also be used for molecular studies aimed at mapping and cloning the resistance genes involved. It would then be possible to use plant transformation to speed the introduction of useful genes into new cultivars, but currently it is unlikely that these cultivars would be accepted in the UK. Alternatively, the double haploid lines are ideal material for construction of genetic linkage maps and genetic analysis of traits (Pink *et al.*, 2008). The resistance can then be introduced in different cultivars through classical breeding that can be aided by marker assisted selection. Combining or 'pyramiding' pathotype specific resistance genes in the same cultivar has long been recognised as an effective means of achieving disease control in plants (McIntosh & Brown, 1997; Pink & Puddephat, 1999; Pink, 2002). The use of single gene-based resistance might not provide durable disease resistance (Pink, 2002; Sivasithamparam *et al.*, 2005; Stuthman *et al.*, 2007), but the combination of isolate specific genes and genes that confer broad spectrum resistance together with an appropriate strategy for deployment of cultivars, can contribute to the management of the disease. Monitoring pathogen variability is critical for sustained use of gene combinations. Based on current information of the virulence of the UK *H. parasitica* population, the combined resistance available in EBH525/544/550 and EBH502 should provide effective control of downy mildew in UK production of *B. oleracea* crops.

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Table 1. Summary of *Brassica oleracea* accessions including doubled haploid lines that were generated for this study from different sources of downy mildew resistance, and additional susceptible or resistant control accessions.

Accession	Crop source (accession/cultivar)[c]	Seed stock	Origin or Reference	Seedling resistance[d]
EBH502	Cabbage (OL21260)	Doubled haploid	This study	Narrow isolate specific
EBH508 [a]	Calabrese (OL21250)	Doubled haploid	This study	Narrow isolate specific
EBH516 [a]	Cabbage (OL21410)	Doubled haploid	This study	None or weak (basal)
EBH525 [b]	Borecole (OL21264)	Inbred	This study	Broad specificity
EBH527 [a]	Cauliflower (OL21278)	Doubled haploid	This study	Narrow isolate specific
EBH535 [a]	Cauliflower (OL21311)	Doubled haploid	This study	None or weak (basal)
EBH544	Cabbage (OL21287)	Doubled haploid	This study	Broad specificity
EBH550	Borecole (OL21396)	Doubled haploid	This study	Broad specificity
EBH553 [a]	Kale (OL21361)	Doubled haploid	This study	None or weak (basal)
Cal18b	Broccoli	Doubled haploid	WHRI, unpublished	None
Lateman	Mini-cauliflower	Open pollinated	Mr Fothergill's, Kentford, UK	None
Surfrider	Cauliflower	Doubled haploid	WHRI, unpublished	None
A12DHd	Chinese kale	Doubled haploid	Bohuon *et al.* (1996)	None or weak (basal)
GDDH33	Calabrese (Green Duke)	Doubled haploid	Bohuon *et al.* (1996)	None
Senna	Rapid cycling (OL2050)	Doubled haploid	WHRI, unpublished	None
N-o-1[e]	*B. napus* spring rape	Doubled haploid	Sharpe *et al.* (1995)	Narrow isolate specific
N-o-9	*B. napus* winter rape	Doubled haploid	Sharpe *et al.* (1995)	Broad specificity
COB76	*B. napus* (Cobra)	Doubled haploid	WHRI, unpublished	Broad specificity

[a] These doubled haploids were originally developed as different sources of white blister rust resistance in a parallel project, which in three cases (EBH516, EBH527 and EBH535) exhibited broad spectrum resistance to 35 UK isolates of the oomycete *Albugo candida* race 9, and in two cases (EBH508 and EBH553) exhibited narrow isolate specific resistance (Holub & Gunn, unpublished).

[b] EBH525 segregates for white or yellow flower colour, but is uniformly resistant to *H. parasitica.*

[c] OL prefix indicates an accession which is included in the core diversity collection maintained for distribution by the Genetic Resource Unit, Warwick Crop Centre, Wellesbourne Campus, Warwick CV35 9EF, UK.

[d] Based on inoculations with UK isolates of *H. parasitica* described in this study.

[e] This accession is derived from cv. Westar; a *B. napus* mapping population (N-o-72-8) and linkage map have been generated from a N-o-1 x N-o-9 cross (Sharpe *et al.*, 1995).

Table 2. Sample year and origin of *Hyaloperonospora parasitica* isolates used in this study.

Year	Isolate No.	County	Location	Production System	Host crop type (cultivar)
1977	P005	Nottinghamshire	Holystone	Conventional	Cauliflower (Barrier Reef)
1983	P006	Lincolnshire	not recorded	Conventional	Cauliflower
2001	Hp701	Cambridgeshire	Doddington Nursery	Organic	Cabbage (Autoro)
	Hp702	Cambridgeshire	Doddington Nursery	Organic	Calabrese (Marathon)
	Hp703	Cambridgeshire	Doddington Nursery	Organic	Savoy Cabbage (Tarvoy)
	Hp704	Lancashire	Holmeswood	Organic	not recorded
	Hp705	Lincolnshire	Old Leake Nursery	Conventional	not recorded
	Hp706	Lancashire	NIAB (Holmeswood)	Organic	not recorded
	Hp710	Lincolnshire	Low Fulney	Conventional	Cauliflower (Optimist)
	Hp711	Lincolnshire	Wainfleet	Conventional	White cabbage
	Hp713	Lincolnshire	Wainfleet	Conventional	Cauliflower
	Hp715	Lincolnshire	Wainfleet	Conventional	White cabbage
	Hp717	Lincolnshire	Wainfleet	Conventional	Calabrese
	Hp718	Lincolnshire	Wainfleet	Conventional	Calabrese
	Hp 724	Cambridgeshire	Doddington Nursery	Organic	Cabbage (Duncan)
	Hp726	Lincolnshire	Wainfleet	Conventional	Calabrese
	Hp727	Lancashire	Holmeswood	Organic	not recorded
	Hp 728	Lincolnshire	Kirton	Organic	not recorded
	Hp729	Cornwall	Camborne	Organic	Spring Greens
	Hp730	Cornwall	Camborne	Organic	Savoy Cabbage
	Hp731	Cornwall	Bristol	Conventional	Cabbage (Duncan)
2002	Hp733	Gloucestershire	Penzance	Organic	Cauliflower
	Hp735	Cornwall	Mount Hawke	Organic	Cauliflower (Castle Grand)
	Hp736	Cornwall	Mount Hawke	Organic	Calabrese (Marathon)
	Hp738	Yorkshire	Epworth	Organic	Broccoli
	Hp739	Nottinghamshire	Bildworth	Organic	Cauliflower
	Hp741	Warwickshire	Wellesbourne	Conventional	Broccoli
2007	Hp801	Somerset	Wessex Nursery	Conventional	Cauliflower (Pavilion)
	Hp802	Lincolnshire	Westhorpe Nursery	Organic	Cabbage (Duchy)
	Hp803	Lincolnshire	Westhorpe Nursery	Organic	(Tinman)
	Hp804	Lincolnshire	Westhorpe Nursery	Organic	Calabrese (Marathon)
2008	Hp805	Lancashire	Farringtons Nursery	Conventional	(Boris)
	Hp806	Lancashire	Farringtons Nursery	Conventional	(Helsinki)
	Hp807	Lancashire	Farringtons Nursery	Conventional	(Trent)
	Hp808	Lincolnshire	Sheepgate Nursery	Conventional	Cauliflower (Pavilion)
	Hp809	Lincolnshire	Sheepgate Nursery	Conventional	Cauliflower (Concept)
	Hp810	Lincolnshire	Sheepgate Nursery	Conventional	(Valtos)
	Hp811	Lincolnshire	Sheepgate Nursery	Conventional	(Kronas)
	Hp812	Lincolnshire	Sheepgate Nursery	Conventional	(Valtos)
	Hp813	Lincolnshire	Sheepgate Nursery	Conventional	Cauliflower (Concept)
	Hp814	Lancashire	Farringtons Nursery	Conventional	Calabrese
	Hp815	Lancashire	Farringtons Nursery	Conventional	Calabrese

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Table 3. Interaction phenotypes between *Hyaloperonospora parasitica* and brassicas.

Class	Pathogen sporulation	Host response
0	None	None visible
1	None	Light to heavy necrotic flecking, confined to inoculation tissue
2	None	Heavy necrotic flecking spreading on cotyledon
3	Sparse, confined to inoculated patch of tissue	Flecking or diffuse necrosis of sporulating tissue
4	Moderate to heavy, confined to inoculated tissue	Flecking or diffuse necrosis of sporulating tissue
5	Moderate to heavy, spreading across the cotyledon	Flecking or diffuse necrosis
6	Heavy sporulation, across entire cotyledon	Flaccid cotyledon without necrosis

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Table 4. Seedling phenotypes of five downy mildew *Brassica oleracea* accessions following inoculation with 42 *Hyaloperonospora parasitica* isolates that were collected from UK brassica crops.

Year Collected	*H. parasitica* isolate	*B. oleracea* line [b]		
		EBH525, EBH544, EBH550	EBH502	EBH508
1977	005	R	S	S
1983	006[a]	R	R	R
2001-02	704	R	I	R
	718	R	I	I
	710, 735	R	R	S
	705, 706, 713, 730, 731, 733, 739	R	I	S
	711, 726, 728, 738, 741	R	S	I
	701, 702, 703, 715, 717, 724, 727, 729, 736	R	S	S
2007-08	802	R	I	I
	807, 815	R	R	S
	808, 809, 810, 811, 812, 813	R	I	S
	805, 814	I	I	S
	804	R	S	I
	801, 803	R	S	S
	806	S	I	S

[a] Indicates the *H. parasitica* isolate that was used to select plants from the original source of downy mildew resistance for EBH525, EBH544 and EBH550.

[b] R= resistant host with mean interaction phenotype score < 2.5; S= susceptible host with mean score ≥4.5; and I= partially resistant host with intermediate mean score.

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Table 5. Segregation of downy mildew resistance to *Hyaloperonospora parasitica* in the F_1, F_2 and BC_1 generations derived from crosses between the downy mildew resistant accessions EBH525, EBH544, EBH550 and susceptible cultivars of broccoli (Cal18b) or cauliflower (Lateman). Additional intercrosses in all pairwise combinations of the resistant accessions were also tested.

Plant material	Observed ratio (R : S)*	Predicted ratio	χ^2 probability	Conclusion
F_1 Cal18b x EBH525	22 : 0	1 : 0		
F_2 Cal18b x EBH525	133 : 45	3 : 1	0.93	Dominant
BC_1 (Cal18b x F_1)	50 : 37	1 : 1	0.16	Single gene
EBH525	9 : 0	1 : 0		
Cal18b	0 : 8	0 : 1		
F_1 EBH525 x Lateman	11 : 0	1 : 0		
F_2 EBH525 x Lateman	136 : 43	3 : 1	0.76	Dominant
BC_1 (F_1 x Lateman)	45 : 38	1 : 1	0.44	Single gene
EBH525	7 : 0	1 : 0		
Lateman	0 : 10	0 : 1		
F_1 Cal18b x EBH544	20 : 0	1 : 0		
F_2 Cal18b x EBH544	136 : 34	3 : 1	0.13	Dominant
BC_1 (Cal18b x F_1)	40 : 38	1 : 1	0.82	Single gene
EBH544	8 : 0	1 : 0		
Cal18b	0 : 10	0 : 1		
F_1 EBH544 x Lateman	22 : 0	1 : 0		
F_2 EBH544 x Lateman	141 : 48	3 : 1	0.90	Dominant
BC_1 (F_1 x Lateman)	34 : 43	1 : 1	0.30	Single gene
EBH544	8 : 0	1 : 0		
Lateman	0 : 11	0 : 1		
F_1 Cal18b x EBH550	10 : 0	1 : 0		
F_2 Cal18b x EBH550	119 : 50	3 : 1	0.17	Dominant
BC_1 (Cal18b x F_1)	43 : 35	1 : 1	0.36	Single gene
EBH550	10 : 0	1 : 0		
Cal18b	0 : 9	0 : 1		
F_1 EBH550 x Lateman	24 : 0	1 : 0		
F_2 EBH550 x Lateman	127 : 52	3 : 1	0.21	Dominant
BC_1 (F_1 x Lateman)	47 : 42	1 : 1	0.60	Single gene
EBH550	11 : 0	1 : 0		
Lateman	0 : 9	0 : 1		
F_2 EBH525 x EBH544	523 : 0	1 : 0		Allelic
F_2 EBH544 x EBH525	592 : 0	1 : 0		Allelic
F_2 EBH550 x EBH544	502 : 0	1 : 0		Allelic
F_2 EBH544 x EBH550	520 : 0	1 : 0		Allelic
F_2 EBH525 x EBH550	531 : 0	1 : 0		Allelic
F_2 EBH550 x EBH525	540 : 0	1 : 0		Allelic

* R= resistant; S= susceptible

Table 6. Doubled haploid (DH) plants from F_1 plants that were derived from outcrosses of a downy mildew susceptible broccoli line (Cal18b) and two downy mildew resistant lines (EBH525 and EBH550). The *Hyaloperonospora parasitica* isolate Hp717 was used to assess the interaction phenotype of each DH line.

F_1 outcross	No. DH lines tested	DH phenotype based on progeny test (resistant : intermediate : susceptible)
Cal18b x EBH525	36	20 : 5 : 11
Cal18b x EBH550	4	1 : 0 : 3
Total	40	21 : 5 : 14