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# Genetic analysis of phenylpropanoid metabolites associated with resistance against *Verticillium longisporum* in *Brassica napus*

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**Abstract** *Verticillium longisporum* is a major threat to production of oilseed rape (*Brassica napus*) in Europe. The aim of the study was to develop new markers and obtain insights into putative mechanisms and pathways involved in the resistance reaction. A genetic approach was used to identify quantitative trait loci (QTL) for *V. longisporum* resistance and metabolic traits potentially influencing resistance in a *B. napus* mapping population. Resistance to *V. longisporum* was mapped in a doubled haploid (DH) population from a cross between the partially resistant winter oilseed rape variety Express 617 and a resistant resynthesized *B. napus* line, R53. One major resistance QTL contributed by R53 was identified on chromosome C5, while a further, minor QTL contributed by Express 617 was detected on chromosome C1. Markers flanking the QTL also significantly correlated with *V. longisporum* resistance in four further DH populations derived from crosses between elite oilseed rape cultivars and other resynthesized *B. napus* lines originating from genetically and geographically diverse brassica A and C genome donors. The tightly-linked markers developed enable the combination of favorable alleles for novel resistance loci from resynthesized *B. napus* materials with existing resistance loci from commercial breeding lines. HPLC analysis of hypocotyls from infected DH lines revealed that concentrations of a number of phenylpropanoids were correlated with *V. longisporum* resistance. QTL for some of these phenylpropanoids were also found to co-localize with the QTL for *V. longisporum* resistance. Genes from the phenylpropanoid pathway are suggested as candidates for *V. longisporum* resistance.

**Keywords** *Verticillium longisporum* · Resistance · Quantitative trait loci (QTL) · *Brassica napus* · Metabolic profiling · Marker-assisted breeding

## Introduction

Oilseed rape (*Brassica napus*) is an amphidiploid species ($2n = 38$, genome AACC) derived from a more recent, medieval hybridization of the two closely related diploid species *B. rapa* ($2n = 20$, genome AA)

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and *B. oleracea* ($2n = 18$, genome CC) (U 1935). Oilseed rape is the second most important source of vegetable seed oil in the world, after soybean. It is the most important oilseed crop in China, Canada, Europe and Australia, where it delivers a high-quality, unsaturated nutritional oil that in Europe is also a major source of biodiesel. Since the 1970s the worldwide production area of oilseed rape has increased continuously to the current estimated level of about 30 million hectares in 2009/2010. The European Union produced rapeseed oil from a harvested area of 6.4 million hectares in 2009/2010, an increase of 32 % in comparison with 2005/2006 (Mielke 2010). This sharp increase was accompanied by a shortening of crop rotation cycles, sometimes down to 2 years in Northern Europe. One consequence of this has been an increase of *Verticillium longisporum* pathogen infection threatening oilseed rape production in Europe (Dunker et al. 2008).

In Europe *Verticillium* disease of oilseed rape became economically important in Sweden in the 1970s (Johansson et al. 2006a) and has increased strongly in Germany, France, Poland, England, Russia and the Ukraine since the 1980s (Karapapa et al. 1997). Although the yield-damaging potential of the disease is high, with up to 80 % reduction of single plant yield, the overall yield losses can vary considerably between years depending on climatic conditions (Dunker et al. 2008). Control of *Verticillium* diseases and other soil-borne and vascular diseases by fungicide treatment is not effective (Klosterman et al. 2009), hence the most promising long-term measure to control the disease is breeding of resistant cultivars.

The soil-borne phytopathogenic ascomycete *V. longisporum* can survive in soils for many years through the production of microsclerotia (Karapapa et al. 1997; Heale and Karapapa 1999; Steventon et al. 2002; Fahleson et al. 2003, 2004). After germination of microsclerotia in the soil, the fungal hyphae penetrate the epidermal cells of the roots and eventually enter the xylem elements. The spread and blocking of xylem vessels by fungal structures has been suggested to cause restricted water and nutrient transportation, leading to chlorosis and leaf yellowing, premature ripening and senescence as typical symptoms of *Verticillium* disease in oilseed rape (Eynck et al. 2007). Clear identification of symptoms on winter oilseed rape plants infected by *V. longisporum* is difficult until late in the growing season after flowering and during ripening (Supplementary Figure S1). In contrast, in greenhouse infection experiments applied in resistance screening programmes, when plants are infected by root-dip inoculation with high densities of fungal spores early in their development, rapid systemic spread of *V. longisporum* occurs in susceptible oilseed rape cultivars. This results in typical leaf yellowing symptoms and severe stunting of plants (Supplementary Figure S2) (Happstadius et al. 2003; Rygulla et al. 2007a, b, 2008; Eynck et al. 2009a).

Oilseed rape has a comparatively narrow gene pool which originated in a limited geographic region of southern Europe through spontaneous hybridizations between a restricted number of turnip rape (*B. rapa*) and cabbage (*B. oleracea*) genotypes (Allender and King 2010). Genetic diversity in elite breeding material has been further eroded by continuous selection for seed quality traits since the 1970s, in particular through introgression of double-low (00) seed quality (zero erucic acid, low glucosinolate content) essentially from two varieties as donors of superior quality (Allender and King 2010). As a consequence, 00-quality winter oilseed rape has a relatively low genetic diversity and lacks a broad spectrum of disease resistances. Extensive screening of diverse *B. napus* germplasm for resistance to *V. longisporum* under controlled greenhouse conditions revealed no resistance sources (Happstadius et al. 2003), and current European winter oilseed rape cultivars exhibit only low levels of tolerance against *V. longisporum*. On the other hand, *V. longisporum* resistance has been successfully transferred from the two progenitor species into resynthesized *B. napus* lines via the embryo rescue technique (Happstadius et al. 2003; Rygulla et al. 2007a, b). The quantitative resistance in these lines is predominantly derived from the *B. oleracea* C genome donor.

The genetic basis of resistance to the systemic spread of *V. longisporum* in *B. napus* and other *Brassica* species is unknown. A proteome expression study identified proteins up-regulated upon *V. longisporum* infection in roots, hypocotyls and leaves, including the typical pathogen defense-related enzymes $\beta$-1,3-glucanase, peroxidase, PR4 and endochitinase (Floerl et al. 2008). Histochemical analysis of *B. napus* and *B. oleracea* genotypes with different levels of resistance revealed that the resistance response to systemic *V. longisporum* spread is

localized internally within the hypocotyls, and that the build-up of physical barriers, deposition of cell wall-bound phenolic compounds and lignin modification within the vascular system play a crucial role in the defense reaction (Eynck et al. 2009b). Similarly, various structural barriers that restrict invading hyphae of *V. dahliae* and other *Verticillium* species have been described in roots and stems of cotton, potato and tomato (Klosterman et al. 2009; Xu et al. 2011).

A number of studies characterising the interaction of *Verticillium* species have been performed in the model crucifer *Arabidopsis thaliana*, a close relative of *Brassica* crops. In contrast, there are only limited reports on the genetic basis of resistance to *Verticillium* ssp. in crop species (Klosterman et al. 2009). For example, the gene *Ve*1 conferring resistance to *V. dahliae* and *V. albo-atrum* race 1 has been identified in tomato (*Solanum lycopersicum*). *Ve*1 encodes a cell surface receptor that belongs to the extracellular leucine-rich repeat (LRR) class of receptor-like proteins. *Arabidopsis* plants transformed with tomato *Ve*1 showed resistance to *V. dahliae* and *V. albo-atrum* race 1, but not to *V. longisporum* (Fradin et al. 2011). In *A. thaliana* a single dominant locus on chromosome IV, *VET*1, was found to confer resistance to *Verticillium* infection (Veronese et al. 2003). Häffner et al. (2010) found that genes involved in flowering control on *A. thaliana* chromosome IV are localized within quantitative trait loci (QTL) for resistance to systemic spread, although further studies are required to determine if these regions coincide with *VET*1. Also nearby are nucleotide-binding LRR protein genes of the RPP5 super-family, with Toll/interleukin-1 receptor domains controlling resistance to the fungal pathogen *Peronospora parasitica* (Häffner et al. 2010).

In different *A. thaliana* ecotypes, high infection rates are not always correlated with severe disease symptoms such as stunting which can be observed in *B. napus* after greenhouse infections (Floerl et al. 2010; Veronese et al. 2003; Häffner et al. 2010; Steventon et al. 2001). Analyses of mutants deficient in hormone signaling suggest that the jasmonic acid and ethylene signaling pathways, but not salicylic acid signaling, are involved in resistance activation in *A. thaliana* (Fradin et al. 2011; Johansson et al. 2006b; Pantelides et al. 2010). In contrast, however, salicylic acid and its glucoside were detected in xylem sap from *B. napus* roots and hypocotyls, and increased in the shoots above the hypocotyls after infection by *V. longisporum*, whereas jasmonic acid and abscisic acid levels remained unchanged (Ratzinger et al. 2009). Since *A. thaliana* is not naturally infected by *V. longisporum*, however, the *A. thaliana*–*V. longisporum* pathosystem has limitations for simplistic transfer of information on putative resistance mechanisms to *B. napus*.

It is not known whether different races or pathotypes of *V. longisporum* exist. Nevertheless, genetic mapping in a doubled haploid (DH) population, with resistance derived from a resynthesized *B. napus* line originating from a cross between white cabbage (*B. oleracea* ssp. *oleracea* convar. *capitata*) and a winter turnip rape (*B. rapa* ssp. *oleifera*) as genome donors, revealed two major QTL for *V. longisporum* resistance on chromosomes C4 and C5. After infection with either a mixture of five Swedish *V. longisporum* isolates or a single-spore isolate, respectively, these two QTL consistently exhibited significant effects on resistance in multiple greenhouse environments (Rygulla et al. 2008).

The objective of the present study was to identify genomic regions involved in *V. longispourm* disease development and resistance in different *B. napus* genetic backgrounds. To achieve this, a QTL mapping approach was applied to identify QTL that are stable across environments and breeding populations, and to develop markers to pyramid complementary resistance loci from resynthesized *B. napus* and commercial oilseed rape lines. Furthermore, QTL analyses of phenolic compounds in hypocotyl tissues were performed to determine their relevance in the *V. longisporum* disease reaction and resistance in *B. napus*.

## Materials and methods

### Resistance donors and mapping populations

A total of 214 homozygous DH lines of the population ExR53-DH were used for genetic mapping. This DH population was produced by Saaten Union BioTec GmbH (Leopoldshöhe, Germany) (Radoev et al. 2008; Basunanda et al. 2010) from a cross between the inbred line Express 617, derived from the partially *V. longisporum*-resistant German winter oilseed rape cv. Express, and R53, a resistant, resynthesized

rapeseed line. R53 originates from an interspecific hybrid between *B. oleracea* ssp. *oleracea* convar. *acephela* (kale) and *B. rapa* ssp. *pekinensis* (Chinese cabbage). Four other DH populations used for marker validation, SW08-190001, SW08-190002, DSV-1575 and DSV-1605, were produced by the plant breeding companies Lantmännen SW Seed AB (Svalöv, Sweden) and Deutsche Saatveredelung AG (Lippstadt, Germany) using genetically divergent resynthesized *B. napus* accessions as resistance donors. Details of the pedigree and resistance donors of all populations are listed in Supplementary Table S1.

## Resistance screening

Seedlings of the DH lines and parents were inoculated with a *V. longisporum* isolate mixture in four separate greenhouse experiments during 2009 and 2010 at Göttingen University. A mixture of the German *V. longisporum* isolates VL40 and VL43 (Zeise and von Tiedemann 2002) in equal densities of $1 \times 10^6$ spores/ml plus mycelium fragments each was used for the inoculations. Each experiment included a total of 20–24 inoculated and 10–24 non-inoculated plants for each of the DH lines. Furthermore, 20–24 inoculated and 10–24 non-inoculated plants each of the susceptible *B. napus* cultivars Falcon and Laser and the partially resistant cultivars Express and Lion were included in each experiment as controls. In the four experiments a total of 3,146, 1,212, 2,591 and 4,895 plants were monitored, respectively. The plants were grown in individual pots with the genotypes arranged in a completely random design. In experiments 1–4 a total number of 100, 32, 82 and 98 DH lines were tested, respectively. In experiment 4 the two parents were also included. A total number of 214 different DH lines were tested in these four experiments. In experiment 4, randomly selected DH lines were retested: 52 from experiment 1, 15 from experiment 2 and 31 from experiment 3. The inoculations were carried out using a root-dip inoculation method described by Eynck et al. (2009a). The seedlings were grown at 22 °C in a greenhouse with a light regime of 14 h per day. Disease scoring was carried out weekly for disease symptoms over a time period of 4 weeks using a 9-scale assessment key (Eynck et al. 2009a). Area under the disease progress curve (AUDPC) values were calculated from the disease severity values. Growth reduction was calculated 28 days post-inoculation from the difference in the means of the height (in cm) from 20–24 non-inoculated and 20–24 inoculated plants.

## Simple sequence repeat (SSR) marker analysis

Genomic DNA samples from the DH lines were isolated from fresh or freeze-dried leaf material according to Doyle and Doyle (1990). For all SSR analyses the M13-tailing procedure described by Berg and Olaisen (1994) was used. In this method the fluorescently labeled universal M13 primer 5′-AGGGTTTTCCCAGTCACGACGTT-3′ is added to the PCR reaction, and the forward primer of each SSR is appended with the sequence 5′-TTTCCCAGTCACGACGTT-3′. After the first cycle of amplification, the PCR fragments are subsequently amplified by the labeled universal primer. All SSR amplification products were separated using a LI-COR 4200 DNA Analyzer (LI-COR Biosciences) and scored visually. The SSR markers used in the present study derived from numerous published *B. napus* maps (Lowe et al. 2004; Suwabe et al. 2004; Piquemal et al. 2005; Qiu et al. 2006; Basunanda et al. 2007, 2010 Radoev 2007, 2008; Rygulla et al. 2008; Iniguez-Luy et al. 2009; Cheng et al. 2009; Nagaoka et al. 2010). See Supplementary Table S2 for detailed information on the SSR markers successfully mapped to the chromosomes of interest.

## Map construction

Genetic mapping used a framework map published by Radoev et al. (2008) as a basis, with 191 markers mapped in 275 ExR53-DH lines. In addition, 15 polymorphic SSR markers mapping to chromosomes C1 and C5 were genotyped in 214 DH lines and added to the framework map using the program JoinMap 3.0 (Stam 1993). Of these 15 polymorphic SSR markers, 10 are shown in the genetic maps in Figs. 2 and 3 which are at least 1 cM distant from previously mapped SSR markers. Map distances measured in cM between markers were derived from Haldane's function.

## Statistical analyses and QTL mapping

QTL positions were localized using the software QGene, version 4.3.9 (Joehanes and Nelson 2008), by

composite interval mapping with automatic forward cofactor selection allowing QTL cofactors instead of markers. The map was scanned at 2 cM intervals. A value of $p \leq 0.005$ for Type I errors and a $\log_{10}$-likelihood ratio (LOD) value of 2.5 were used as criteria to indicate putative QTL positions. Estimates of the additive effect and phenotypic variation ($R^2$) explained by each QTL were computed. For each QTL, confidence intervals were calculated based on the 1-LOD drop-off method according to Lander and Botstein (1989). QTL were confirmed by re-analysis using the alternative software packages PlabQTL version 1.2 (Utz and Melchinger 1996), WindowsQTL Cartographer version 2.5 (Wang et al. 2011) and R/qtl (Broman et al. 2003). Calculation of broad-sense heritability was also performed using PlabQTL, while all other statistical analyses were carried out with SAS version 9.2 (SAS Institute Inc.).

### RP-HPLC analysis

Phenolic compounds from hypocotyls were extracted with 80 % methanol using a method which was shown to provide the highest phenolic acid extraction efficiency from plant samples (Padda and Picha 2007). Extraction was performed from pooled samples of 24 plants according to Eynck et al. (2009b) from hypocotyls sampled 28 days after inoculation (38 days after sowing). Reverse phase–high performance liquid chromatography was performed using a Merck/Hitachi machine with a Nucleodur Sphinx RP column (Macherey–Nagel) and UV detection at 280 nm. The mobile phase consisted of water with 4.5 % formic acid (solvent A) and acetonitrile (solvent B). The elution was performed for 20 min with a 95/5, 10 min with a 75/25, followed by 20 min with a 2/98 mix of solvents A and B and a 1 ml/min flow rate, according to Lipsa et al. (2012). Peaks putatively corresponding to *p*-coumaric acid, caffeic acid, ferulic acid and sinapic acid were identified by comparison of the retention time with HPLC quality external standards obtained from Sigma-Aldrich and further confirmed by co-injection with standards. Further fractionation of the phenolic compounds from the 80 % methanolic extract of the hypocotyl from selected samples, via solid phase extraction C18 mini-columns (Alltech) into fractions representing phenolic acids, monomeric and oligomeric proanthocyanidines and polymeric proanthocyanidine/anthocyanidines (Lipsa et al. 2012), revealed that the majority of peaks were in the phenolic acid fraction and only one of approximately 45 peaks was also present in the monomeric and oligomeric proanthocyanidine fractions.

## Results

### Phenotypic evaluation of *V. longisporum* resistance

Mean values for the area under the disease progress curve (AUDPC, $n = 20$–24) ranged from 21.5 to 33.3 for the partially resistant reference cultivars Express and Lion, and from 27.8 to 69.6 for the highly susceptible reference cultivars Falcon and Laser in four different experiments. For these repeated measures for the four reference cultivars, multiple pairwise comparison showed significant differences between mean AUDPC values for some of the four experiments [Fig. 1A, $p \leq 0.05$, least squares difference (LSD) test].

Based on the reaction of the reference cultivars, the disease pressure in experiment 2 was lower than in the other three experiments, resulting in up to 60 % lower mean AUDPC values (white bars in Fig. 1A). Similar results were also found for the mean growth reduction values between these four experiments (Fig. 1B). Differences between experiments for the mean AUDPC and growth reduction values were also significant when including all genotypes in the analysis ($p \leq 0.001$, LSD test).

This could be due to differences in the accuracy of visual disease assessment, or to environmental effects (e.g. temperature and humidity fluctuations between greenhouse experiments). Because all visual resistance scoring was done by the same person using a standardized scheme, the observed variations between the four experiments are considered to be due to differences in disease pressure rather than inaccuracies in disease scoring. The interaction between varieties and experiments for AUDPC and growth reduction was significant ($p \leq 0.001$). Because a standardized inoculum and a standardized scoring scheme were used in all four experiments, these differences between experiments are most likely due to environment differences. Accordingly, the four greenhouse screening experiments were treated as

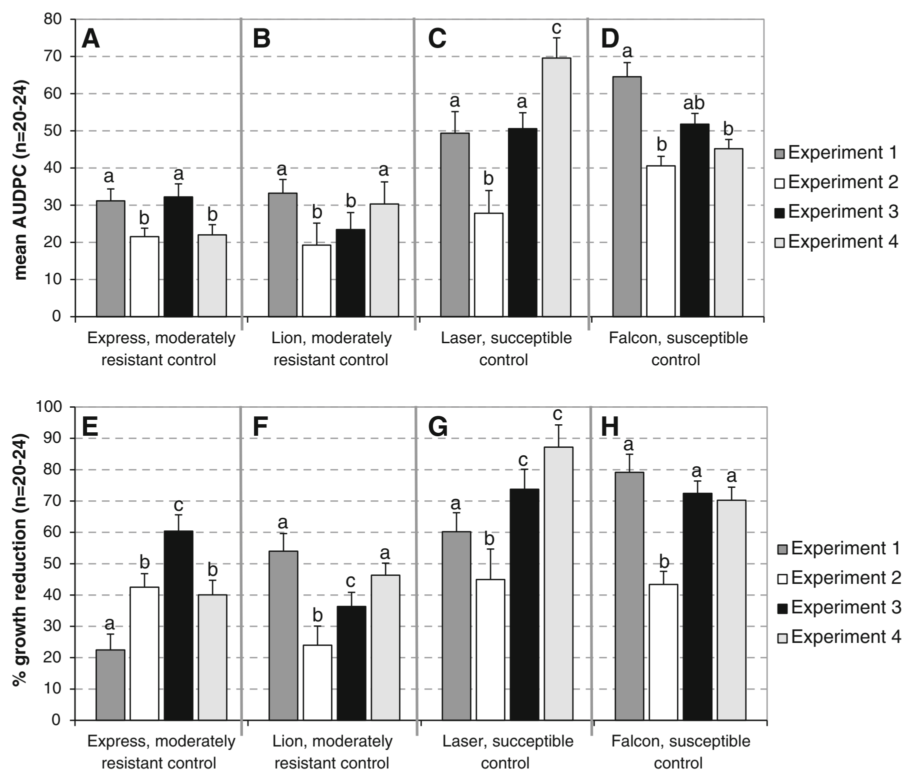


**Fig. 1** Mean area under the disease progress curve (AUDPC) and mean growth reduction values for four reference cultivars inoculated with *V. longisporum* in four greenhouse screening experiments. *Bars* show the standard error of the mean calculated from 20 to 24 single plants. Alphabetic annotations show significant differences based on pairwise comparison for each cultivar (**A**–**H**, LSD post hoc test, $p \leq 0.05$)

different environments and used for four independent QTL analyses instead of one combined QTL analysis.

Phenotypic variation in the AUDPC and the degree of disease-induced growth reduction in the DH mapping population Express 617 × R53 (ExR53-DH) revealed similar, continuous frequency distributions in the four resistance screening experiments with different subsets of the population. In experiment 4, randomly selected DH lines from experiments 1 to 3 were retested. In all four experiments the distributions for both normalized AUDPC (Supplementary Figure S3) and growth reduction (data not shown) were slightly skewed towards high susceptibility.

In greenhouse experiment 4, 52 DH lines (53 %) showed a lower mean AUDPC than the resistant parent R53, while eight DH lines (8 %) had a higher mean AUDPC than the parent Express 617 (Supplementary Figure S3). Estimates of broad-sense heritability ($h^2$) were high, ranging from 0.73 to 0.87 for both resistance-related traits in the different subsets of DH lines retested in experiment 4 and calculated from the four experiments. This high heritability was confirmed by significant Pearson correlation coefficients ($R = 0.59$–$0.82$) between the results of the independent greenhouse phenotyping experiments for different populations or subpopulations.

High correlations ($R = 0.87$, 0.67, 0.89 and 0.81; significant at $p \leq 0.01$) were observed between leaf symptoms and the degree of growth reduction in infected plants from experiments 1 to 4, respectively.

## QTL for *V. longisporum* resistance

In the three independent resistance tests 1, 3 and 4, significant and stable major QTL with LOD scores larger than 2.5 for both resistance-related traits, AUDPC and growth reduction, were found at the same position on chromosome C5 (Fig. 2). Resistance tests 1 and 3 each comprised different subsets of the ExR53-DH population; in resistance test 4 some randomly selected ones were retested. When analyzed using composite interval mapping (CIM) with forward cofactor selection in the software QGENE, this QTL explained between 16 and 34 % of the phenotypic variance for the different traits and datasets (Supplementary Table S3). Similar results were obtained using different CIM approaches implemented in the software packages PlabQTL, R/qtl and Windows QTL Cartographer (data not shown).

Besides the major QTL on chromosome C5, an additional minor QTL was identified on chromosome C1 for AUDPC values in experiments 1 and 3, and for growth reduction in experiment 3 (Fig. 3, Supplementary Table S3). No significant resistance-related QTL were detected in experiment 2, presumably due to the low number of DH lines tested ($n = 32$). No clear evidence for epistatic interactions between digenic loci or main effect QTL was found using PlabQTL or R/qtl.

Rygulla et al. (2008) detected two major QTL for *V. longisporum* resistance on C4 and C5 in the mapping population SW99-307. Mapping of the SSR markers Ra2F11_b and BRMS030, which flank one of the major QTL on C5 in the SW99-307 DH population, revealed that they also flank the major QTL on C5 in ExR53-DH (Fig. 2; here called Ra2F11_230 and BRMS030_210 according to the allele sizes in bp).

## Combined effect of resistance alleles from two QTL and both parents

The allele contributing resistance at the detected major QTL on C5 was derived from the C genome donor of the resistant resynthesized rapeseed parent R53, whereas the allele contributing resistance at the minor QTL on C1 originates from the inbred line Express 617 (Supplementary Table S3). Express 617 was derived by repeated self-pollination from the partially resistant cv. Express, which is considered to exhibit a medium field resistance towards *V. longisporum* infection. The mean normalized AUDPC values of *V. longisporum*-infected DH lines drop about 40 % from an average of 0.98 for lines containing no favorable allele to 0.58 for lines containing two favorable alleles at the two QTL regions on C1 and C5 from both parents (Table 1). Similarly, the mean normalized growth reduction upon *V. longisporum* infection compared to mock-inoculated DH lines is about 33 % in DH lines with both favorable alleles in these QTL regions, compared to about 50 % without the two favorable alleles from the respective parents.

## QTL validation

From 55 publicly available SSR markers mapped on chromosomes C1 and C5 in 18 other mapping populations, 13 markers are located 10 cM either side

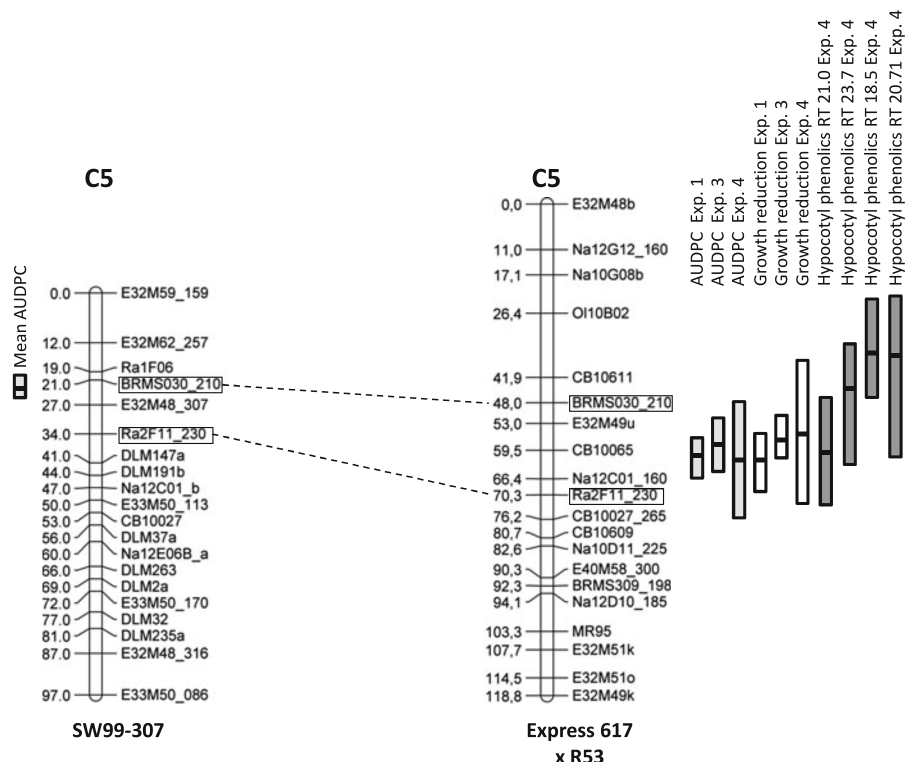


**Fig. 2** Comparison of quantitative trait loci for *V. longisporum* resistance-related traits and soluble phenolic metabolites in the hypocotyl localized on chromosome C5. *Blocks* indicate confidence intervals of the QTL. Data for the population SW99-307 are from Rygulla et al. (2008). Marker alleles BRMS030_210 and Ra2F11_230 are designated BRMS030 and Ra2F11_b, respectively, in Rygulla et al. (2008). *Exp* experiment

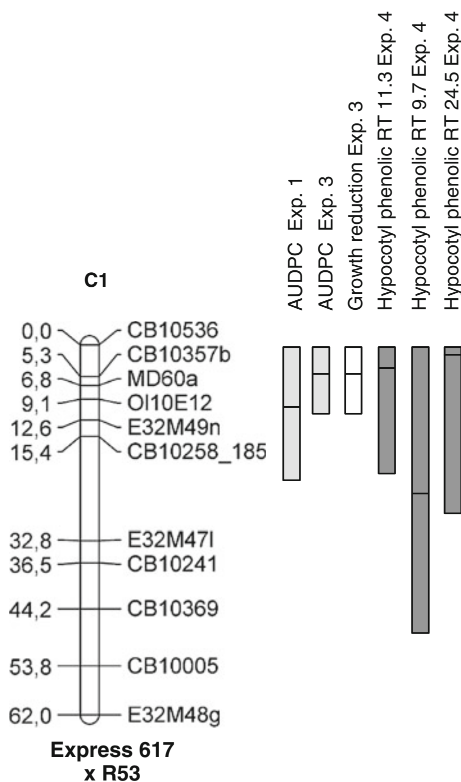


**Fig. 3** Comparison of quantitative trait loci for *V. longisporum* resistance and soluble phenolic metabolites in the hypocotyl localized on chromosome C1. *Blocks* indicate confidence intervals of the QTL. *Exp* experiment

of the QTL peaks in ExR53-DH. The percentages of phenotypic variation explained by three selected marker alleles derived from the QTL regions on C1 and C5 with mean AUDPC values in the two mapping populations and in four genetically diverse DH populations are shown in Table 2.

Two of the DH populations used for QTL validation tested together in one resistance screening are genetically similar half-sister populations, SW08-190001 and SW08-190002, which only differ in the C genome resistance donor (BRA 1008 or HRI 8207) of their respective resynthesized rapeseed parent (Supplementary Table S1). Although on average these DH populations should be about 75 % genetically identical, their normalized AUDPC values in fact show contrasting associations with different marker alleles (Table 2). Whereas SW08-190001 significantly associates only with marker Ol10E12 derived from the minor QTL on C1 in one of the two experiments, SW08-190002 shows a significant association only with the marker CB10065 derived from the major QTL region on C5 in one of the two experiments. This result suggests that the markers allow differentiation between different C genome resistance donors and the resistance QTL on C1 and C5. Comparison of the marker–AUDPC association of SW08-190002 with population DSV-1575, which is genetically diverse but shares the same C genome resistance donor HRI 8207, confirms that the marker CB10065 (derived from the C5 major QTL) is also significantly related to resistance. This concurs with the pedigree information in Supplementary Table S1 and confirms that CB10065 can be effectively used to select for this resistance QTL in different genetic backgrounds.

## Co-localization of QTL for *V. longisporum* resistance and metabolites from the phenylpropanoid pathway

The total soluble phenolics concentration in the hypocotyl of 98 DH lines from the mapping population measured by RP-HPLC increased for every single DH line 1.2-fold up to 5-fold in experiment 4 upon infection with *V. longisporum*. Supplementary

**Table 1** Disease symptoms (AUDPC, growth reduction) in ExR53-DH lines with different combinations of resistance alleles from the two *V. longisporum* resistance QTL on chromosomes C1 and C5 from Express 617 and R53, respectively

| Genotypes | Number of QTL-derived resistance alleles | | Number of DH lines | Mean normalized AUDPC | Mean normalized growth reduction |
|---|---|---|---|---|---|
| | C1 | C5 | | | |
| 'Express 617' × 'R53' | 0 | 0 | 66 | 0.98 | 0.50 |
| | 1 | 0 | 46 | 0.87 | 0.45 |
| | 0 | 1 | 31 | 0.74 | 0.38 |
| | 1 | 1 | 46 | 0.58 | 0.33 |
| 'Express 617' | 1 | 0 | – | 1.51 | 0.68 |
| 'R53' | 0 | 1 | – | 0.79 | 0.41 |

**Table 2** The percentage of variation explained based on simple linear regression of marker alleles from *V. longisporum* resistance QTL regions and disease scores in genetically diverse DH populations

| Population used for | Name of population | *n* | Ol10E12_285 QTL on C1 | BRMS030_210 QTL on C5 | CB10065_198 QTL on C5 |
|---|---|---|---|---|---|
| QTL mapping | ExR53 | Exp. 1: 100 | 11.8** | 15.3** | 29.5** |
| | | Exp. 2: 32 | ns | ns | ns |
| | | Exp. 3: 82 | 10.4** | 10.9** | 16.3** |
| | | Exp. 4: 98 | ns | 16.9** | 17.8** |
| | SW99-307 | Exp. 1: 163 | ns | 6.0** | mm |
| | | Exp. 2: 163 | ns | 7.6** | |
| | | Exp. 3: 163 | ns | 6.4** | |
| QTL validation | SW08-190001 | Exp. 1: 25 | 17.9* | ns | |
| | | Exp. 2: 19 | ns | ns | ns |
| | SW08-190002 | Exp. 1: 25 | ns | ns | ns |
| | | Exp. 2: 12 | ns | mm | 54.7 |
| | DSV-1575 | Exp. 1: 37 | ns | 10.2* | 13.9* |
| | | Exp. 2: 26 | ns | 18.6** | 13.4* |
| | DSV-1605 | Exp. 1: 39 | ns | 21.7** | 12.2** |
| | | Exp. 2: 29 | ns | ns | ns |

Marker bands (size in bp indicated in marker name after underscore) were scored in a 1–0 binary format and used in regression analysis with disease scores calculated as AUDPC values in DH populations representing diverse resistance donors. For details on the population pedigrees see Supplementary Table S1. Mapping data for SW99-307 was obtained from Rygulla et al. (2008). *Exp.* experiment, *mm* monomorphic marker, *ns* not significant, * Significant at 0.1 level, ** Significant at 0.05 level

Figure S4 shows the frequency distributions for the total soluble phenolics concentration in the hypocotyls of 98 DH lines for the *V. longisporum*-inoculated compared to the mock-inoculated set in experiment 4.

Significant but weak correlation ($R^2 = 4\ \%$) was observed between total phenolics concentration and AUDPC in the *V. longisporum*-inoculated DH line set ($n = 98$), but not in the mock-inoculated DH line set. For the individual phenolic acids, 34 QTL with a LOD score threshold of 2.5 were detected for a total number of 43 RP-HPLC peaks from mock-inoculated DH lines, and 37 QTL for 49 peaks from *V. longisporum*-inoculated lines. Four major regions were identified in the DH line set for the mock-inoculation treatment on chromosomes A9, C1, C6 and C8 showing overlapping confidence intervals for at least three QTL involved in the concentrations of individual phenolic acids in the hypocotyl. In the DH line set for the *V. longisporum*-inoculation treatment, two major regions were identified on chromosomes C1 and C5 (for details see Supplementary Table S4).

Further analysis focused on the phenylpropanoid compounds that exhibited a significant correlation with AUDPC and explained at least 5 % of the phenotypic variation in AUDPC. These were 13 compounds from the *V. longisporum*-inoculated DH line data set and five compounds from the mock-inoculated DH line data set (see Supplementary Table S4). Of these 18 phenylpropanoid compounds significantly correlated with AUDPC, seven produced QTL with a LOD score of 2.5 which showed at least one QTL co-localizing with the QTL for AUDPC on chromosome C1 or C5 (summarized in Supplementary Table S3, detailed data in Supplementary Table S4, co-localizing QTL labelled in green). Six of these soluble phenylpropanoid compounds where QTL also co-localized with the QTL for AUDPC on C1 or C5 (Figs. 2, 3) were detected only in the *V. longisporum*-inoculated data set (Table 3). These six compounds explained 7–37 % of the phenotypic variation in AUDPC. In contrast, only one phenylpropanoid compound identified as caffeic acid, where a QTL for this compound also co-localized with the QTL for AUDPC on C1 (Table 3, Supplementary Table S3), was detected in the mock-inoculated data set. In this case, 15 % of the phenotypic variation of AUDPC was explained by the variation in caffeic acid concentration in the mock-inoculated data set (Table 3).

Correlations of constitutive or induced concentrations of the upstream lignin biosynthesis precursors *p*-coumaric acid, ferulic acid and sinapic acid with AUDPC were not observed. In contrast, the constitutive concentration of the lignin precursor caffeic acid significantly correlated with AUDPC, and a QTL for the constitutive expression of caffeic acid in the hypocotyl co-localized with the minor QTL for *V. longisporum* resistance on C1 (retention time 11.3 min; Fig. 3; Table 3). Another two, as yet unidentified, induced soluble phenolic compounds correlated with AUDPC and co-localized with the *V. longisporum* QTL on C1 (retention times 9.7 and 24.5 min; Fig. 3; Table 3). Furthermore, five other soluble phenolic compounds that were induced upon *V. longisporum* infection also correlated with resistance and co-localized with the major resistance QTL on C5 (Fig. 2; Table 3).

These results provide preliminary evidence for regulatory changes in the phenylpropanoid biosynthesis pathway as a potential component of resistance to *V. longisporum* infection.

## Discussion

*Verticillium* disease is an increasing threat to oilseed rape production in Europe. Resistance to *V. longisporum* infection in *B. napus* has been described as being polygenic or multifactorial (Happstadius et al. 2003; Rygulla et al. 2007a, b, 2008; Eynck et al. 2009a). In an earlier study we identified major QTL for resistance on chromosomes C4 and C5 and minor QTL on A6 and C8 in a DH mapping population, SW99-307, produced from resynthesized rapeseed (Rygulla et al. 2008). In the current study a different DH population, ExR53-DH, was used for resistance mapping to dissect the genetic basis of *V. longisporum* resistance present in commercial and resynthesized rapeseed material. This was approached by genetic analysis using a cross of an inbred line from a commercial cultivar exhibiting partial resistance, Express 617, and a resynthesized rapeseed line, R53, of entirely different origin exhibiting pronounced resistance.

High estimates of broad-sense heritability ($h^2$) for both major resistance-related traits, AUDPC and growth reduction, showed that the applied greenhouse test procedure is useful for confidently selecting resistant progenies in breeding, and for accurate genetic mapping. Screening for resistance against *V. longisporum* is extremely time-consuming and costly, however, making it impracticable to implement in a breeding program. On the other hand, phenotypic correlations between the AUDPC values normalized based on the reaction of reference cultivars from different greenhouse experiments, and between greenhouse and field experiments (data not shown), suggest that QTL influencing resistance to artificial inoculation in the greenhouse are highly relevant for field resistance against *V. longisporum*. Effective molecular markers derived from resistance QTL are therefore an extremely valuable resource for breeding of new oilseed rape cultivars with effective field resistance against this important disease.

High correlations between leaf symptoms and degree of growth reduction in infected plants confirm previous observations from greenhouse resistance tests, where a high correlation has been documented

**Table 3** QTL for soluble phenolic compounds in the hypocotyls of the mock- and *V. longisporum*-inoculated mapping population ExR53-DH

| Retention time (min) | Treatment | Correlation with AUDPC, *R* | $R^2$ | Chromosome | QTL peak, position, cM |
|---|---|---|---|---|---|
| 21.0 | VL | 0.61 | 0.37 | C5 | 60 |
| 11.3 (caffeic acid) | Mock | 0.39 | 0.15 | C1 | 4 |
| 9.7 | VL | −0.34 | 0.12 | C1 | 24 |
| 23.7 | VL | 0.33 | 0.11 | C5 | 42 |
| 18.5 | VL | 0.32 | 0.10 | C5 | 34 |
| 20.1 | VL | 0.26 | 0.07 | C5 | 36 |
| 24.5 | VL | −0.26 | 0.07 | C1 | 0 |

Data for experiment 4. Only compounds that co-localize with resistance QTL on C1 and C5, are significantly correlated with AUDPC, and where the QTL explains at least 5 % of the phenotypic variation are shown. *VL V. longisporum*-inoculated

between AUDPC and stunted growth for *V. longisporum*-infected *B. napus* accessions (Eynck et al. 2009a). On the other hand, *Verticillium*-induced stunting in *A. thaliana* under greenhouse conditions appears to be genotype-dependent (Floerl et al. 2010; Veronese et al. 2003; Häffner et al. 2010; Steventon et al. 2001), while *B. rapa* accessions seem to show a weaker correlation between AUDPC and stunting than *B. napus* (Eynck et al. 2009a). Furthermore, *V. longisporum*-induced stunting has never been observed in *B. napus* infested in the fields excluding relative growth reduction as a suitable parameter for scoring of field resistance.

The continuous frequency distributions in four resistance screening experiments with different subsets of the ExR53-DH population confirm the quantitative inheritance of *V. longisporum* resistance. On the other hand, this population exhibited a transgressive segregation for resistance that was demonstrated to be due to recombination of additive alleles contributed by both parents, the inbred line Express 617 and the resynthesized rapeseed line R53, without clear evidence for epistatic interaction. This underlines the value of effective genetic markers for combination of different resistance QTL from different genetic backgrounds, including less resistant genotypes.

In an earlier QTL analysis using the mapping population SW99-307, the resistance was derived from a white cabbage (*B. oleracea* ssp. *oleracea* convar. *capitata*), which had been crossed with a low erucic acid winter turnip rape (*B. rapa* ssp. *oleifera*) to generate a synthetic *B. napus* in Sweden in 1976 (Rygulla et al. 2008). In contrast, the resistance donor R53 was produced at Göttingen University from a cross between a kale (*B. oleracea* ssp. *oleracea* convar. *acephela*) and a chinese cabbage (*B. rapa* ssp. *pekinensis*) (Radoev et al. 2008). Markers derived from R53 that flank the major QTL for *V. longisporum* resistance on C5 also flank the major QTL for the same trait found on C5 in the former study. This chromosome region therefore appears to play an important role in the expression of *V. longisporum* resistance from very different C genome genetic backgrounds, so that molecular markers linked to resistance alleles in this region might be broadly applicable in marker-assisted breeding for *V. longisporum* resistance. Combination with markers linked to the minor resistance QTL on chromosome C1, which represents yet another genetic origin for resistance against *V. longisporum*, underlines the potential for a marker-assisted pyramiding strategy to assist the effective combination of *V. longisporum* resistance from resynthesized rapeseed donors with existing resistance in elite breeding lines.

On the other hand, QTL and associated markers identified in biparental mapping populations are rarely directly useful in marker-assisted selection, requiring verification that they are effective in different genetic backgrounds. Due to limited polymorphism of individual QTL-linked markers in different genotypes, a suite of additional markers within a narrow window spanning the QTL are often required for QTL validation and establishment of a broadly applicable marker-assisted selection strategy (Akhtar et al. 2010). In the present study a QTL validation experiment showed that a number of selected QTL-derived markers were polymorphic in numerous, genetically diverse breeding populations. These markers now allow breeders to distinguish the common resistance QTL on chromosomes C1 and C5 and combine them into new oilseed rape breeding lines and cultivars with increased quantitative resistance to *V. longisporum*.

The resistance mechanism active against *V. longisporum* in rapeseed is largely unknown. However, histochemical analysis of a susceptible and a resistant *B. napus* genotype revealed that the phenol metabolism plays a crucial role in defence against *V. longisporum* and that these defence responses are located in the vascular tissues of plant hypocotyls, leading to the accumulation of soluble phenolics and deposition of cell wall-bound phenolics and lignin (Eynck et al. 2009b). Therefore, resistance to *V. longisporum* was not only studied by measuring AUDPC, but also by quantifying the concentration of soluble phenolic compounds in the hypocotyl of the mapping population in the mock- and *V. longisporum*-inoculated treatment.

By correlation analysis, genetic mapping and QTL analysis, the empiric observations for the total phenolic concentrations in the hypocotyls of resistant and susceptible lines published by Eynck et al. (2009b) were confirmed. In addition, it was shown in more detail that some individual phenylpropanoid compounds expressed in the hypocotyl of the mapping population and measured by HPLC exhibited a weak to medium correlation with *V. longisporum* resistance in *B. napus*. Some of the detected QTL regions for these phenylpropanoid concentrations also

co-localized in genomic regions together with the major and minor resistance QTL.

Over 30 QTL were detected for soluble phenolic compounds in the hypocotyl of the mapping population in the mock- as well as in the *V. longisporum*-inoculated data set. In only about half of the cases, the QTL were located in the same genomic region, suggesting a strong *V. longisporum*-induced activation of different genomic regions for synthesis or modification of soluble phenolic compounds. There were four major regions (on A9, C1, C6 and C8) involved in constitutive soluble phenolic compound concentrations and two major regions (on C1 and C5) involved in induced soluble phenolic compound concentrations. One major region on C1 was detected in the mock- as well as in the *V. longisporum*-inoculated data set. Data on the genetic mapping of phenolic acids in rapeseed is limited. However, a QTL for acid detergent lignin (ADL) concentration in seeds within the same major region on C5 was mapped in the sister DH population Express 617 × 1012-98 (data not shown). Also, two QTL for phenolic compounds, sinapic acid and sinapoyl-glucose, have been mapped in seeds within the major region on C1 (data not shown) and the gene UDP-glucose:sinapate glucosyltransferase has been mapped in the same region (Mittasch et al. 2010), also supporting that these regions on C1 and C5 are major genomic regions involved in phenylpropanoid synthesis or modification in rapeseed.

Of a total of 71 QTL for phenylpropanoid compounds, 18 co-localized with the major and minor QTL for *V. longisporum* resistance on C1 or C5. Less than half of these phenylpropanoid compounds (seven out of 18) also showed significant correlations of their concentrations with AUDPC, suggesting that they do not co-localize by chance, but might be associated with resistance. Most of these co-localizing QTL where in addition the concentrations of the respective phenylpropanoid compounds are significantly correlated with resistance resulting in an $R^2$ of more than 5 % were detected in the *V. longisporum*-inoculated data set (six out of seven). The variation in concentration of these six phenylpropanoid compounds explained 7–37 % of the phenotypic variation in AUDPC. This indicates that, for most of these phenylpropanoid compounds, concentrations are changed in the DH lines upon *V. longisporum* infection. Of these six phenylpropanoid metabolites, the concentrations of two were negatively correlated with AUDPC, meaning higher concentrations in resistant lines upon infection. Concentrations of the four other compounds were positively correlated with AUDPC, meaning higher concentrations in susceptible lines upon infection, including one compound which explained 37 % of the phenotypic variation in AUDPC. These compounds might not be directly involved in a resistance reaction, but rather might be precursors of compounds involved in resistance. However, the phenolic compound which reveals the second largest $R^2$ value, explaining 15 % of the phenotypic variation in AUDPC values, is constitutively expressed in the hypocotyl. This compound was identified by co-migration with an external standard as caffeic acid (RT 11.3). The QTL for the constitutive caffeic acid concentration in the hypocotyls co-localized with the minor resistance QTL on C1. Caffeic acid is a key component of the phenylpropanoid pathway and major lignin precursor (e.g. Dixon and Reddy 2003). Resistant lines showed a lower constitutive concentration of caffeic acid than susceptible lines. This might suggest that caffeic acid was more effectively incorporated in resistant lines into other phenolic compounds or cellular structures which are part of preformed physical or physiological barriers to infection, e.g. in lignin of cell walls within the vascular system. This is in agreement with biochemical and microscopical investigations of a susceptible and resistant *B. napus* line by Eynck et al. (2009b), who described that at earlier time points of infection preformed soluble and cell wall-bound phenolics appear to limit the extent of infection and colonization by the fungal pathogen, whereas de novo formation of lignin and lignin-like polymers becomes more important at later stages of infection.

Resistance of plants to plant pathogens is often a multifactorial process. The accumulation of low-molecular-weight phenols leading to the formation of biopolymers that restrict the spread of the pathogens (e.g. lignin and callose) is only one part of the diverse layers of plant responses to pathogen infection. Low-molecular-weight phenols accumulate in many host–pathogen systems in both resistant and susceptible interactions early after infection. These phenolic compounds, including caffeoyl glucose and caffeoyl esters, might ultimately serve as precursors for compounds essential to expression of resistance. These compounds might also be associated with cellular browning and tissue necrosis that may result

from oxidation and polymerization of *O*-diphenols (Nicholson and Hammerschmidt 1992). However, enhanced lignification and differences in lignin composition have been described as being the main resistance pathway to fungal invasion in a number of different host–pathogen interactions (reviewed by Nicholson and Hammerschmidt 1992; Dixon and Paiva 1995; Vance et al. 1980). Lignification is not only an induced mechanism in the resistance of plants to fungal pathogens, but also plays an important role as a constitutive resistance component (e.g. Eynck et al. 2012, resistance of *C. sativa* to S. s*clerotiorum*). Phenolic acids and phenylpropanoid metabolites have also been assumed to be implicated in the interaction of *V. longisporum* and *A. thaliana*. In a study by Götze et al. (2011) it was shown that leaves of *A. thaliana* accumulate phenylpropanoids and lignans soon after *V. longisporum* root infection. An activation of phenylpropanoid metabolism-related genes and lignin deposition in the hypocotyl after inoculation of cotton with *V. dahliae* was reported from a study based on the global gene regulation analysis of phenylpropanoid metabolism-related genes using RNA-Seq, in combination with histochemical analyses of a resistant and susceptible cotton genotype (Xu et al. 2011).

The *V. longisporum*-induced regulation of individual soluble phenolic acids in the hypocotyl of resistant genotypes, and their correlation and co-localization with QTL for resistance-related traits, described in this study indicates that genes from the phenylpropanoid pathway should be considered as promising candidate genes for expression of *V. longisporum* resistance in oilseed rape. Identification of the as yet unidentified HPLC peaks correlating with *V. longisporum* resistance and co-localizing with resistance QTL by mass spectroscopy will allow the identification of further putative genes and the evaluation of which role they play in this host–pathogen interaction.

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## References

Akhtar S, Bhat MA, Wani SA, Bhat KA, Chalkoo S, Mir MR, Wani SA (2010) Marker assisted selection in rice. J Phytol 2:66–81

Allender CJ, King GJ (2010) Origins of the amphiploid species *Brassica napus* L. investigated by chloroplast and nuclear molecular markers. BMC Plant Biol 10:54

Basunanda P, Spiller TH, Hasan M, Gehringer A, Schondelmaier J, Lühs W, Friedt W, Snowdon RJ (2007) Marker-assisted increase of genetic diversity in a double-low seed quality winter oilseed rape genetic background. Plant Breed 126:581–587

Basunanda P, Radoev M, Ecke W, Friedt H, Becker HC, Snowdon RJ (2010) Comparative mapping of quantitative trait loci involved in heterosis for seedling and yield traits in oilseed rape (*Brassica napus* L.). Theor Appl Genet 120:271–281

Berg ES, Olaisen B (1994) Hybrid PCR sequencing—sequencing of PCR products using a universal primer. Biotechniques 17:896–901

Broman KW, Wu H, Sen Ś, Churchill G (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890

Cheng X, Xu J, Xia S, Gu J, Yang Y, Fu J, Qian X, Zhang S, Wu J, Liu K (2009) Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor Appl Genet 118:1121–1131

Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097

Dixon RA, Reddy MSS (2003) Biosynthesis of monolignols. Genomic and reverse genetic approaches. Phytochem Rev 2:289–306

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Dunker S, Keunecke H, Steinbach P, von Tiedemann A (2008) Impact of *Verticillium longisporum* on yield and morphology of winter oilseed rape (*Brassica napus*) in relation to systemic spread in the plant. J Phytopathol 156:698–707

Eynck C, Koopmann B, Grunewaldt-Stoecker G, Karlovsky P, von Tiedemann A (2007) Differential interactions of *Verticillium longisporum* and *V. dahliae* with *Brassica napus* detected with molecular and histological techniques. Eur J Plant Pathol 118:259–274

Eynck C, Koopmann B, von Tiedemann A (2009a) Identification of *Brassica* accessions with enhanced resistance to *Verticillium longisporum* under controlled and field conditions. J Plant Dis Protect 116:63–72

Eynck C, Koopmann B, Karlovsky P, von Tiedemann A (2009b) Internal resistance in winter oilseed rape inhibits systemic spread of the vascular pathogen *Verticillium longisporum*. Phytopathology 99:802–811

Eynck C, Séguin-Swartz G, Clarke WE, Parkin IAP (2012) Monolignol biosynthesis is associated with resistance to *Sclerotinia sclerotiorum* in *Camelina sativa*. Mol Plant Pathol 18:887–899. doi:10.1111/J.1364-3703.2012.00798.X

Fahleson J, Lagercrantz U, Hu Q, Steventon A, Dixelius C (2003) Estimation of genetic variation among *Verticillium*

isolates using AFLP analysis. Eur J Plant Pathol 109:361–371

Fahleson J, Hu Q, Dixelius C (2004) Phylogenetic analysis of *Verticillium* species based on nuclear and mitochondrial sequences. Arch Microbiol 181:435–442

Floerl S, Druebert C, Majcherczyk A, Karlovsky P, Kües U, Polle A (2008) Defense reactions in the apoplastic proteome of oilseed rape (*Brassica napus* var. *napus*) attenuate *Verticillium longisporum* growth but not disease symptoms. BMC Plant Biol 8:129

Floerl S, Druebert C, Arroud HI, Karlovsky P, Polle A (2010) Disease symptoms and mineral nutrition in *Arabidopsis thaliana* in response to *Verticillium longisporum* VL43 infection. J Plant Pathol 92:695–702

Fradin EF, Abd-El-Haliem A, Masini L, van den Berg GCM, Joosten MHAJ, Thomma BPHJ (2011) Interfamily transfer of tomato *Ve*1 mediates *Verticillium* resistance in *Arabidopsis*. Plant Physiol 156:2255–2265

Götze S, Feussner K, Kaever A, Landesfeind M, Drübert C, Herrfurth C, Polle A, Karlovsky P, Meinicke P, Feussner I (2011) Phenylpropanoids in the interaction of *Verticillium longisporum* and *Arabidopsis thaliana*. In: Gatz C (ed) From foliar to root-interacting pathogens and symbionts, 16–18 Feb 2011, Georg August University Göttingen: poster presentations: abstract 33. http://wwwuser.gwdg.de/~rootmicr/. Accessed 1 March 2012

Häffner E, Karlovsky P, Diederichsen E (2010) Genetic and environmental control of the *Verticillium* syndrome in *Arabidopsis thaliana*. BMC Plant Biol 10:235

Happstadius I, Ljungberg A, Kristiansson B, Dixelius C (2003) Identification of *Brassica oleracea* germplasm with improved resistance to Verticillium wilt. Plant Breed 122:30–34

Heale JB, Karapapa VK (1999) The *Verticillium* threat to Canada's major oilseed crop: Canola. Can J Plant Pathol 21:1–7

Iniguez-Luy LF, Lukens L, Farnham MW, Amasino RM, Osborn TC (2009) Development of public immortal mapping populations, molecular markers and linkage maps for rapid cycling *Brassica rapa* and *B. oleracea*. Theor Appl Genet 120:31–43

Joehanes R, Nelson JC (2008) QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788–2789

Johansson A, Goud JKC, Dixelius C (2006a) Plant host range of *Verticillium longisporum* and microsclerotia density in Swedish soils. Eur J Plant Pathol 114:139–149

Johansson A, Staal J, Dixelius C (2006b) Early responses in the *Arabidopsis-Verticillium longisporum* pathosystem are dependent on NDR1, JA- and ET-associated signals via cytosolic NPR1 and RFO1. Mol Plant Microbe Interact 19:958–969

Karapapa VK, Bainbridge BW, Heale JB (1997) Morphological and molecular characterization of *Verticillium longisporum* comb. nov., pathogenic to oilseed rape. Mycol Res 101:1281–1294

Klosterman SJ, Atallah ZK, Vallad GE, Subbarao KV (2009) Diversity, pathogenicity, and management of *Verticillium* species. Annu Rev Phytopathol 47:39–62

Lander ES, Botstein DB (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lipsa FD, Snowdon R, Friedt W (2012) Quantitative genetic analysis of condensed tannins in oilseed rape meal. Euphytica 184:195–205

Lowe AJ, Moule C, Trick M, Edwards KJ (2004) Efficient large-scale development of microsatellites for marker and mapping applications in *Brassica* crop species. Theor Appl Genet 108:1103–1112

Mielke T (2010) Global analysis. All major oilseeds, oils & oilmeals. Supply, demand and prices. Oil World Annual 2. ISTA Mielke GmbH. http://www.oilworld.de

Mittasch J, Mikolajewski S, Breuer F, Strack D, Milkowski C (2010) Genomic microstructure and differential expression of the genes encoding UDP-glucose:sinapate glucosyltransferase (UGT84A9) in oilseed rape (*Brassica napus*). Theor Appl Genet 120:485–500

Nagaoka T, Doullah MAU, Matsumoto S, Kawasaki S, Ishikawa T, Hori H, Okazaki K (2010) Identification of QTLs that control clubroot resistance in *Brassica oleracea* and comparative analysis of clubroot resistance genes between *B. rapa* and *B. oleracea*. Theor Appl Genet 120:1335–1346

Nicholson RL, Hammerschmidt R (1992) Phenolic compounds and their role in disease resistance. Annu Rev Phytopathol 30:369–389

Padda MS, Picha DH (2007) Methology optimization for quantification of total phenolics and individual phenolic acids in sweetpotato (*Ipomoea batatas* L.) roots. J Food Sci 72:412–415

Pantelides IS, Fjamos SE, Paplomatas EJ (2010) Ethylen perception via ETR1 is required in *Arabidopsis* infection by *Verticillium dahliae*. Mol Plant Pathol 11:191–202

Piquemal J, Cinquin E, Couton F, Rondeau C, Seignoret E, Doucet I, Perret D, Villeger M-J, Vincourt P, Blanchard P (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor Appl Genet 111:1514–1523

Qiu D, Morgan C, Shi J, Long Y, Liu J, Li R, Zhuang X, Wang Y, Tan X, Dietrich E, Weihmann T, Everett C, Vanstraelen S, Beckett P, Fraser F, Trick M, Barnes S, Wilmer J, Schmidt R, Li J, Li D, Meng J, Bancroft I (2006) A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. Theor Appl Genet 114:67–80

Radoev M (2007) Genetic Analysis of Heterosis in Rapeseed (*B. napus* L.) by QTL Mapping. Dissertation, Georg August University Göttingen, Germany. http://webdoc.sub.gwdg.de/diss/2007/radoev. Accessed 1 March 2012

Radoev M, Becker HC, Ecke W (2008) Genetic analysis of heterosis for yield and yield components in rapeseed (*Brassica napus* L.) by QTL mapping. Genetics 179:1547–1558

Ratzinger A, Riediger N, von Tiedemann A, Karlovsky P (2009) Salicylic acid and salicylic acid glucoside in xylem sap of *Brassica napus* infected with *Verticillium longisporum*. J Plant Res 122:571–579

Rygulla W, Seyis F, Lühs W, Eynck C, von Tiedemann A, Friedt W, Snowdon RJ (2007a) Combination of resistance to *Verticillium longisporum* from zero erucic acid *Brassica oleracea* and oilseed *Brassica rapa* genotypes in resynthesised rapeseed (*Brassica napus*) lines. Plant Breed 126:596–602

Rygulla W, Snowdon RJ, Eynck C, Koopmann B, von Tiedemann A, Lühs W, Friedt W (2007b) Broadening the genetic basis of *Verticillium longisporum* resistance in *Brassica napus* by interspecific hybridization. Phytopathology 97:1391–1396

Rygulla W, Snowdon RJ, Friedt W, Happstadius I, Cheung WY, Chen D (2008) Identification of quantitative trait loci for resistance against *Verticillium longisporum* in oilseed rape (*Brassica napus* L.). Phytopathology 98:215–221

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Steventon LA, Okori P, Dixelius C (2001) An investigation of the susceptibility of *Arabidopsis thaliana* to isolates of two species of *Verticillium*. J Phytopathol 149:395–401

Steventon LA, Fahleson J, Hu Q, Dixelius C (2002) Identification of the causal agent of Verticillium wilt of winter oilseed rape in Sweden, *V. longisporum*. Mycol Res 106:570–578

Suwabe K, Iketani H, Nunome T, Ohyama A, Hirai M, Fukuoka H (2004) Characteristics of microsatellites in *Brassica rapa* genome and their potential utilization for comparative genomics in *Cruciferae*. Breed Sci 54:85–90

U N (1935) Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot 7:389–452

Utz HF, Melchinger AE (1996) PLABQTL: A program for composite interval mapping of QTL. J Agr Genomics 2:1-5. http://wheat.pw.usda.gov/jag/papers96/paper196/indexp196.html. Accessed 1 March 2012

Vance CP, Kirk TK, Sherwood RT (1980) Lignification as a mechanism of disease resistance. Annu Rev Phytopathol 18:259–288

Veronese P, Narasimhan ML, Stevenson RA, Zhu JK, Weller SC, Subbarao KV, Bresan RA (2003) Identification of a locus controlling Verticillium disease symptom response in *Arabidopsis thaliana*. Plant J 35:574–587

Wang S, Basten CJ, Zeng Z-B (2011) Windows QTL Cartographer 2.5, Department of Statistics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm. Accessed 1 March 2012

Xu L, Zhu L, Tu L, Liu L, Yuan D, Jin L, Long L, Zhang X (2011) Lignin metabolism has a central role in the resistance of cotton to the wilt fungus *Verticillium dahliae* as revealed by RNA-Seq-dependent transcriptional analysis and histochemistry. J Exp Bot 62:5607–5621

Zeise K, von Tiedemann A (2002) Host specialization among vegetative compatibility groups of *Verticillium dahliae* in relation to *Verticillium longisporum*. J Phytopathol 150:112–119