Genetic and environmental control of the Verticillium syndrome in Arabidopsis thaliana

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Abstract

Background: Verticillium spp. are major pathogens of dicotyledonous plants such as cotton, tomato, olive or oilseed rape. Verticillium symptoms are often ambiguous and influenced by development and environment. The aim of the present study was to define disease and resistance traits of the complex Verticillium longisporum syndrome in Arabidopsis thaliana (L.) Heynh. A genetic approach was used to determine genetic, developmental and environmental factors controlling specific disease and resistance traits and to study their interrelations.

Results: A segregating F2/F3 population originating from ecotypes ‘Burren’ (Bur) and ‘Landsberg erecta’ (Ler) was established. Plants were root-dip inoculated and tested under greenhouse conditions. The Verticillium syndrome was dissected into components like systemic spread, stunting, development time and axillary branching. Systemic spread of V. longisporum via colonisation of the shoot was extensive in Ler; Bur showed a high degree of resistance against systemic spread. Fungal colonisation of the shoot apex was determined by (a) determining the percentage of plants from which the fungus could be re-isolated and (b) measuring fungal DNA content with quantitative real-time PCR (qPCR). Four quantitative trait loci (QTL) controlling systemic spread were identified for the percentage of plants showing fungal outgrowth, two of these QTL were confirmed with qPCR data. The degree of colonisation by V. longisporum was negatively correlated with development time. QTL controlling development time showed some overlap with QTL for resistance to systemic spread. Stunting depended on host genotype, development time and seasonal effects. Five QTL controlling this trait were identified which did not co-localize with QTL controlling systemic spread. V. longisporum induced increased axillary branching in Bur; two QTL controlling this reaction were found.

Conclusions: Systemic spread of V. longisporum in the host as well as resistance to this major disease trait are described for the first time in natural A. thaliana accessions. This creates the possibility to study a major resistance mechanism against vascular pathogens in this model plant and to clone relevant genes of the involved pathways. Stunting resistance and resistance to systemic spread were controlled by different QTL and should be treated as separate traits. Developmental and environmental effects on pathogenesis and resistance need to be considered when designing and interpreting experiments in research and breeding.

Background

Verticillium spp. are vascular fungal pathogens that induce diverse disease symptoms and severe yield losses on a broad range of dicotyledonous plants [1]. Disease symptoms include wilting, chlorosis, stunting, vascular discoloration, defoliation or premature seed ripening. Infected plants often show unspecific reactions similar to those which occur during senescence or under environmental stress conditions.

In susceptible crops the infection starts in the root and proceeds into the shoot by systemic spread via the transpiration stream in the xylem [2,3]. Certain conditions, for example developmental events, can influence the initiation of the systemic phase [3]. Resistance to systemic spread has been shown to be a major component of Verticillium resistance for many hosts [3-9]. Resistance to systemic spread is regarded as a type of resistance that reduces the rate of epidemic development in the field [10]. Rate-reducing resistance has been
shown to be a major component of field resistance in economically important crops. In soybean for example, yield depends strongly on rate-reducing resistance against Phytophthora root and stem rot [11] and sudden death syndrome caused by *Fusarium* [12]. In both cases a major component of rate-reducing resistance is the host’s ability to restrict fungal colonisation of the plant tissue [12,13].

*V. longisporum* (Stark) Karapapa is a soil-borne fungal pathogen specialised for cruciferous hosts [14]. During the last decades, it has become a serious threat to oilseed rape in Central and Northern Europe [15,16]. Since there are no efficient fungicides available against this pathogen, resistance breeding is the most promising approach to control the disease. To date, only quantitative resistance against *V. longisporum* is known [17]. Quantitative resistance is characterised by being incomplete and conditioned by multiple genes of partial effect [18]. Often, quantitative resistance to pathogens is a pleiotropic effect of genes affecting growth and development [18,19]. The situation in the *V. longisporum-Brassicaeaeae-pathosystem* is further complicated by the diversity of the symptoms induced. Chlorosis and stunting are among the most obvious symptoms in root-dip inoculated *Brassica* plants and are often used to assess disease progression in greenhouse experiments [4,20,21]. Mapping of resistance QTL has mostly been performed on the basis of these symptoms [22]. However, stunting has never been observed in the field. The prevalent symptom on oilseed rape in the field is premature ripening [23], which is accompanied by systemic spread, extensive formation of microsclerotia on shoot tissue, and yield loss [24]. Systemic spread in the host plant has been shown to be a specific component of the *V. longisporum-Brassica-interaction*. Whereas *V. dahliae*-infection is mainly restricted to the roots, *V. longisporum* is capable of invading the shoot system of susceptible *Brassica* genotypes [2,3]. The transition to flowering has been shown to be a crucial developmental stage promoting systemic spread [3], but in extremely susceptible genotypes systemic spread has been observed in even earlier stages [24]. Symptom severity corresponded with the presence of *V. longisporum* in the shoot system for *Brassica* spp. in greenhouse experiments [4,5]. Systemic spread is regarded as a main indicator for disease severity in the field [24]. The genetic basis of resistance to systemic spread in *Brassica* remains unknown.

Recent studies used *A. thaliana* as a model organism to further elucidate the genetic basis of *Verticillium* resistance and disease traits [25-29]. *V. dahliae* and *V. longisporum* are capable of inducing symptoms in *A. thaliana* comparable to those in *Brassica*. Differences in symptom severity have been observed among natural accessions of *A. thaliana* or in specified mutants. A locus on chromosome 4, *Vet1*, was shown to confer resistance to chlorosis and it also delayed flowering after *Verticillium* infection [29]. Two QTL were mapped to different chromosomal positions that were associated with resistance to chlorosis, and a new allele of *rfo1* (resistance to *Fusarium oxysporum*) was described that increased the resistance to fresh weight loss caused by *V. longisporum* [26]. By testing defined *A. thaliana* mutants for their reaction towards *Verticillium* infection, it has been shown that processes as diverse as ethylene signalling [26,27,29], R-gene signalling [26] and post-transcriptional gene-silencing [25] are involved in resistance against *Verticillium*. Disease severity was recorded as stunting, fresh weight loss and chlorosis. In two of these studies the degree of colonisation by *V. longisporum* in the host was recorded. The higher susceptibility of ecotype ‘Col-0’ to *Verticillium*-induced chlorosis was not accompanied by higher numbers of colony-forming units in whole plants when compared to ecotype ‘C24’ [29]. Mutants with impaired endogenous gene silencing showed increased *Verticillium* susceptibility in terms of stunting and chlorosis and also more fungal biomass compared to wild type [25]. Relatively few research studies focus on the natural genetic resources for *V. longisporum*-resistance. *Brassica* as well as *A. thaliana* accessions may still harbour numerous unknown genes capable of influencing the interaction with *V. longisporum* [17,20,26].

The aim of the present study was to localise genomic regions in *A. thaliana* that influence disease and resistance traits in order to identify the associated genes and pathways using a QTL mapping approach. An F2/F3 mapping population was derived from a cross between two ecotypes that displayed striking differences in their interaction with *V. longisporum*. Special emphasis was placed on the definition of disease and resistance traits such as systemic spread and stunting. The degree of systemic fungal spread into the apical parts of the shoot, a trait whose natural variation has not been examined in a genetic study of *A. thaliana* before, was determined with two different methods: Re-isolation from apical shoot segments and qPCR of fungal DNA. To analyse the developmental implications of the disease the branching pattern and the duration of the development were recorded. To study disease traits in closer similarity to the natural situation, a greenhouse testing procedure was used. The dissection of the complex syndrome in a genetic study allowed the detection of QTL controlling different traits in order to identify the associated genes and pathways using a QTL mapping approach. An F2/F3 mapping population was derived from a cross between two ecotypes that displayed striking differences in their interaction with *V. longisporum*. Special emphasis was placed on the definition of disease and resistance traits such as systemic spread and stunting. The degree of systemic fungal spread into the apical parts of the shoot, a trait whose natural variation has not been examined in a genetic study of *A. thaliana* before, was determined with two different methods: Re-isolation from apical shoot segments and qPCR of fungal DNA. To analyse the developmental implications of the disease the branching pattern and the duration of the development were recorded. To study disease traits in closer similarity to the natural situation, a greenhouse testing procedure was used. The dissection of the complex syndrome in a genetic study allowed the detection of QTL controlling different traits, to investigate their relationships and to draw conclusions regarding their role for disease and resistance.

**Methods**

**Material**

*A. thaliana* ecotypes Bur-0 and Ler-0 were originally obtained from the Arabidopsis Information Service (AIS) Frankfurt [30] and maintained in house. The
V. longisporum isolate ‘43’ [31] was used for infestation experiments.

**Fungal culture and preparation of spore suspension**
Fungal stocks with 1-3×10^6 conidia/ml were stored in glycerol-water (1:4) at -75°C. Conidial suspensions for inoculation were produced in liquid Czapek-Dox medium on a shaker at 20°C for 8 days and filtered through sterile gauze. Spore densities were determined using a haemocytometer (Neubauer improved). For inoculation, spore suspensions were diluted to 1×10^6 conidia/ml with sterile tap water.

**Infestation experiments**
For all greenhouse infestation experiments, a root-dip inoculation procedure [31] was applied which was modified for A. thaliana as follows. Seeds were stratified at 8°C for 2 days before sowing and plantlets were grown in a mix of commercial potting soil (Einheitserde Typ P) and sand (3:1 vol. parts) under long-day conditions (16 h light) at 20°C for 19 days. Supplementary lighting was provided using sodium vapour lamps. After uprooting and cutting the root tips plantlets were dipped in a conidial suspension for 1 h. Controls were mock-inoculated in diluted Czapek-Dox medium without spores. Thirty plants were planted per 20×30 cm plastic tray filled with 1.5 l soil/sand mix. Inoculated plants were grown in long-day in the greenhouse at 18-28°C until maturity of the first siliques. Then the height of the aerial parts of the plant (from the hypocotyl to the tip of the longest stalk), their fresh weight, the branching pattern and colonisation of the apical part of the shoot were determined. To test whether infection requires root injury, individual plantlets were raised in pots of 5 cm diameter for 19 days as described above, and then 5 ml of conidial suspension were pipetted into the soil next to the hypocotyls of the seedling. Three F3 infestation experiments were carried out. Experiment 1 (E1) was started in January (winter experiment), experiment 2 (E2) in March and experiment 3 (E3) in April (referred to as spring experiments). Altogether, 108 families were tested. Each experiment comprised 60 F3-families which were partly overlapping between the experiments. The overlap was ten F3-families between E1 and E2, 31 between E2 and E3, and 32 between E1 and E3. Ten selected F3-families, parents and F1 were analysed in every experiment. Of each F3-family, 30 inoculated and 15 mock-inoculated plants were tested. The parental ecotypes Ler and Bur were tested at least eleven times during all seasons of the year with at least 30 replicates per experiment.

**Determination of systemic fungal spread**
Systemic spread of V. longisporum was observed to occur during flowering (see Results) and was therefore determined at the onset of silique maturity. To determine systemic spread by re-isolation, segments of approx. 3 cm length were cut from the apex of the main shoot axis of each plant, surface-sterilised with 0.1% (w/v) sodium hypochlorite solution and subsequently dipped in 70% (v/v) ethanol. After rinsing three times in sterile water, the segments were transferred to Petri dishes containing 9 g/l agar, 10 g/l malt extract and 100 mg/l streptomycin. V. longisporum outgrowth was recorded after 1 and 2 weeks. One shoot segment per plant was plated and the percentage of colonised shoot segments of the total number of shoot segments per F3-family was calculated (degree of colonisation). In experiment E3, apical pieces of the main shoot of parents, F1 and F3-families were also analysed for the amount of fungal DNA in the plant tissue by quantitative real-time PCR. Standards of V. longisporum DNA were prepared as described [32]. Real-time PCR based on internal transcribed sequences of ribosomal RNA genes with SYBR Green fluorescence monitoring was used for the quantification of fungal DNA in plant tissue [2]. The primers were designed to amplify Verticillium spp. but not other organisms potentially infecting A. thaliana. Usually three samples consisting of a mixture of approx. 10 plants were used per family.

**Assessment of the developmental stage**
The following scale was applied: Stage 0 = vegetative; stage 1 = first buds visible; stage 2 = first bud > 1 mm, flowering shoot elongating; stage 3 = 1-3 flowers open; stage 4 = 4-10 flowers open; stage 5 = more than 10 flowers open; stage 6 = 1-3 siliques mature; stage 7 = 4-6 siliques mature; stage 8 = up to 50% of the siliques on main shoot mature; stage 9 = more than 50% of siliques on main shoot mature; stage 10 = all siliques mature, whole plant yellow.

**Generation of the mapping population**
A Bur-0 ♂ × Ler-0 ♀ cross was performed. A single F1-plant was selfed and 243 F2-plants were raised from which leaf material for marker analysis was collected. Each F2-plant was selfed again and F3-seeds were collected from each F2-plant separately. Parents, F1 and F3-families were used for infestation experiments.

**Marker analysis**
The markers used in the present study were identified from different sources. Twenty eight previously published SSR markers [33,34] were used. Eleven sequence-characterised (SCAR) markers were developed for the present study (see Additional File 1) by exploiting length polymorphisms in the sequences of Ler-1 and Bur-0 [35] using the MSQT query tool [36]. The erecta mutation present in Ler [37] was used as a morphological marker.
DNA of shock-frozen rosette leaves was extracted with the CTAB method [38]. The markers were amplified using PCR (94°C for 1 min, 40 cycles of 94°C for 15 s, 50 or 55°C for 15 s, 72°C for 30 s). PCR products were separated on 3.5-4% (w/v) agarose gels (Agarose NEEO Ultra from Roth).

QTL analysis
A linkage map of 40 markers polymorphic for the parental lines was constructed and allele frequencies of each marker were analysed for significant deviations from the 1:2:1 segregation ratio with the program MapDisto [39]. QTL analysis was performed with MapManager QTX 20b [40] using the Kosambi function. Simple interval mapping was performed scanning the genome in 1 cM-steps. In cases where one locus was especially prominent, composite interval mapping using the most significant locus as a background for the calculation of further QTL was performed. MapManager QTX gives the LRS (likelihood ratio statistic) value to assess the probability of a false positive, where $LRS = 4.6 \times \text{LOD}$ [41]. LRS significance threshold values for the 37% (putative), 95% (significant) and 99.9% (highly significant) genome-wide confidence level were determined by permutation tests with 10,000 permutations. The position of a QTL was determined as a peak LRS score exceeding a certain confidence level. Confidence intervals for QTL were determined by bootstrap tests which calculate the QTL position for multiple resampled data sets of the original data set. Only those QTL were considered which were detected either in all three F3 experiments or were detected in at least two experiments and beyond the genome-wide 95%-significance threshold in at least one experiment. For the detection of epistatic interactions the confidence criterion of $p = 10^{-5}$ was set.

Statistical analyses
Frequency distributions of trait values for all F3-families within an infestation experiment were tested for normality with the Kolmogorow-Smirnow-test and the Shapiro-Wilk test. For quantitative assessment of the degree with the Kolmogorow-Smirnow-test and the Shapiro-Wilk test. For quantitative assessment of the degree of interest with the Kolmogorow-Smirnow-test and the Shapiro-Wilk test. For quantitative assessment of the degree of normality with the Kolmogorow-Smirnow-test and the Shapiro-Wilk test. For quantitative assessment of the degree of

Results
Establishment of a linkage map for (Bur×Ler) mapping populations
The A. thaliana ecotypes Landsberg erecta (Ler) and Burren (Bur) were found to differ in their reactions towards challenge with the vascular pathogen V. longisporum in various aspects (see below). To identify chromosomal regions that control the traits, a (Bur×Ler) map based on 28 simple sequence repeat (SSR) markers, 11 sequence-characterised (SCAR) markers and one morphological marker (erecta) was established. The marker locations on the physical map of the Arabidopsis Genome Iniative (AGI) are known for most markers and the marker order of the linkage map was as predicted according to the physical positions. The SCAR markers were developed for the present mapping population from known insertions or deletions in the Bur and Ler genomes and are presented in Additional File 1. In total the map spanned 368.2 cM, with an average marker spacing of 9.4 cM (see Additional File 2). The largest distance between adjacent markers was 24.4 cM. Segregation distortion was only observed at marker EH3-1 on chromosome 3.

Multiple QTL control resistance to systemic spread in A. thaliana ecotype Bur
Significant differences in the extent of systemic spread of V. longisporum in the shoot system were found between the A. thaliana ecotypes Ler and Bur in greenhouse infestation experiments. The degree of systemic colonisation was measured as the percentage of apical shoot segments colonised by the fungus compared to the total number of shoot segments. When explanted on malt agar plates, apical shoot segments of inoculated Ler plants showed fungal outgrowth to a much higher percentage than those of ecotype Bur at the onset of fruit maturation (Figure 1), reflecting a difference in the
probability of viable conidiospores reaching the apical part of the shoot system. To study the progress of infection during plant development, the time course of systemic spread was analysed for both ecotypes. In addition, the amount of fungal DNA in apical shoot segments was measured by qPCR. In *Ler*, shoot colonisation started during the early flowering stages and reached highest values during fruit maturation, whereas the colonisation levels in *Bur* were low throughout all developmental stages (Figure 2). The onset of fruit maturation was chosen as the most appropriate time point to determine the degree of systemic colonisation in all subsequent infestation experiments because by then colonisation was already advanced in susceptible lines and the shoot tissue was still viable enough so that no saprophytic growth could take place. Since *Bur* develops more slowly than *Ler*, and in routine experiments both ecotypes were inoculated at the same time, the possibility had to be considered that the time point of inoculation relative to the onset of flowering was responsible for the difference in systemic spread. To test this hypothesis, both ecotypes were inoculated at specific developmental stages. *Ler* always showed high colonisation rates, whereas *Bur* always showed low colonisation rates (data not shown). Thus the difference in systemic spread between the ecotypes was not caused by differences in the developmental stage during inoculation. It was further tested whether the difference in systemic spread could also be observed when plants were inoculated without prior wounding of the roots. The colonisation rates in this experiment were 0% for *Bur* and 90% for *Ler* and thus very similar to the rates in experiments with dip inoculation of injured roots. For QTL-mapping of loci affecting systemic spread, the percentage of plants showing colonised shoot segments was recorded in F3-families originating from individual F2-plants in three infestation experiments (E1–E3). For E3, data from qPCR quantifying the amount of *Verticillium* DNA in the shoot samples were also available. Means of *V. longisporum*-DNA amounts determined by qPCR were significantly correlated with the colonisation values of the malt agar test in E3 (Figure 3). Broad-sense heritability ($H^2$) of the trait systemic colonisation was moderate to high when data of the malt agar test were used. With the qPCR values available for E3, $H^2$ was much lower (Table 1). Frequency distributions of colonisation data in each experiment were multi-modal and suggest that several QTL are involved in controlling this trait (Additional File 3).

Four QTL that meet the criteria of significance and/or reproducibility applied in the present study were
detected by interval mapping of the trait systemic colonisation as determined by the malt agar test (Figure 4, Table 2). The QTL with the strongest effect (vec1) was detected on chromosome 2 near the morphological marker erecta. Three QTL of lesser effect (vec2, vec3 and vec4) were detected near the markers nga8 and ciw7 on chromosome 4 and near marker nga139 on chromosome 5, respectively. vec2 and vec4 were detected in all three infestation experiments, whereas vec1 and vec3 could be reproduced in two of three experiments. Results from the most representative experiment for each QTL were chosen (see also Additional File 4) and displayed in Figure 4 and Table 2. All vec alleles increasing the degree of colonisation came from ecotype Ler. vec1, vec2 and vec3 were recessive, vec4 showed intermediate inheritance (Table 2). This corresponds with low to moderate colonisation rates in the F1-generation (see Additional File 3). When QTL-mapping was performed with fungal DNA contents in experiment E3, two QTL could be detected whose confidence intervals overlapped with vec1 and vec3, respectively (Figure 4, Table 2). The peak LRS values of vec1 and vec3, however, were lower when qPCR data were applied (Table 2, Additional file 4). vec2 and vec4, which were only detected at the putative level with the re-isolation method in E3, could not be detected with the qPCR values. Epistatic interactions were analysed with MapManager QTX. One epistatic interaction was detected in experiment E3 between marker erecta, which is close to vec1, and marker ciw6 on chromosome 4, which is not within the confidence interval of any of the vec QTL reported here. This interaction was not detected in E1 or E2 or by qPCR.

QTL controlling systemic spread partly overlap with QTL for development time

The degree of colonisation was negatively correlated with the duration of development among F3-families in all three experiments (Table 3). QTL for development time were therefore mapped by recording the number of days from germination to the maturation of the first siliques in mock-inoculated plants. Bur and Ler differed in the duration of their developmental cycle, reflecting the difference in flowering time. The time until the plants reached maturity was always longer for Bur than for Ler, but for both ecotypes it varied considerably with the season in which the experiment was performed. In summer experiments, the fastest development for Ler was 32 days to maturity, for Bur 38 days. In winter, the longest period for both ecotypes was 60 and 72 days to maturity respectively. The segregating F3-population showed transgressive variation in one direction: No F3-family had a

| Table 1 Broad-sense heritability for Verticillium-related traits in the A. thaliana (Bur×Ler) F2/F3 mapping population |
|---------------------------------------------------------------|
| Trait                                      | Exp. | H² | Method |
|---------------------------------------------|------|----|--------|
| Degree of Verticillium colonisation (vec; malt agar test; number of colonised shoot segments/total number of shoot segments in%) | E1   | 0.58 | F3P    |
|                                              | E2   | 0.59 |        |
|                                              | E3   | 0.76 |        |
| Degree of Verticillium colonisation (vec a; real-time qPCR; pg Verticillium DNA/100 mg shoot fresh weight) | E3   | 0.45 | VCA    |
| Development time (dt; days from germination to maturity in mock-inoculated plants) | E1   | 0.46 | VCA    |
|                                              | E2   | 0.49 |        |
|                                              | E3   | 0.62 |        |
| Shoot fresh weight (g)                       | E1   | 0.21 | VCA    |
|                                              | E2   | 0.40 |        |
|                                              | E3   | 0.49 |        |
| Stunting resistance (stre; shoot height of Verticillium-inoculated plants in cm) | E1   | 0.35 | VCA    |
|                                              | E2   | 0.49 |        |
|                                              | E3   | 0.39 |        |
| Verticillium-induced axillary branching (vab; number of plants with a branching score above the overall median of Verticillium-inoculated plants) | E1   | 0.54 | F3P    |
|                                              | E2   | 0.67 |        |
|                                              | E3   | 0.82 |        |

Broad-sense heritability (H²) of traits for each experiment (exp.) was calculated either with variance component analysis (VCA) [42] or with the F3P method [43] as indicated.

Figure 3 Correlation of data from two methods for quantifying systemic spread of V. longisporum. Correlation between the percentage of colonised plants (malt agar test) and the mean V. longisporum DNA content in F3-families tested in experiment E3 (r = 0.59, p < 0.001).
shorter mean development time than Ler, but up to 40% of all F3-families and, in two experiments, also the F1-generation showed a slower development than Bur (Additional File 3). Family means of development time for the controls were normally distributed in all three experiments (Additional File 3). Broad sense heritability of the trait was moderate (Table 1). Development time was significantly affected by genotype and by the experiment, and significant interactions between genotype and experiment were found (Additional File 5). Three QTL controlling development time were found in the top half of chromosome 4 (dt1-dt3), and one QTL was detected near marker nga151 on chromosome 5 (dt4; Figure 4, Table 2). dt1 and dt4 were detected in every experiment, dt2 and dt3 only in two of three F3 experiments (Additional File 4). The QTL with the strongest effect, dt1, was mapped near marker nga8 at the top of chromosome 4 and thus co-localised with a major colonisation QTL, vec2. For the three dt QTL on chromosome 4, the alleles delaying development came from Bur, whereas for dt4, families originating from heterozygous F2-plants flowered significantly later than either maternal or paternal homozygous families. An epistatic interaction could be detected in E1 and E2 between marker EH4-1 and nga151, the latter being within the confidence interval of dt4 on chromosome 5.

V. longisporum-treatment significantly accelerated the development in many F3-families. Acceleration (mean days to maturitycontrols - mean days to maturityinoculated) was closely correlated with the duration of the developmental cycle in controls (Table 3), i.e. slowly developing families were more accelerated after infection than fast families. The correlation was much weaker with the days to maturity of the inoculated variant ($r = 0.39^{**}$ in E1). QTL controlling developmental acceleration, however, could not be detected.

**Stunting depended on development time and season**

Stunting was a particularly striking disease symptom (Figure 5). Stunting resistance was recorded in two different ways in the present study: 1) As the absolute plant height at the onset of maturity of inoculated plants, and 2) as performance ($\text{mean height}_{\text{inoculated}}/\text{mean height}_{\text{control}} \times 100$). The trait performance compensates for the fact that plants carrying a homozygous erecta mutation have a much lower plant height than plants carrying a wild type erecta allele. The performance of Ler and Bur varied between
Table 2: QTL detected for Verticillium-related traits in the A. thaliana (Bur×Ler) F2/F3 mapping population

| Trait                                      | QTL | Exp. | Chr. | Peak pos. (cM) | Nearest marker | LRS   | LOD   | Explained trait variance | Mean ± SD (or median) for F3-families with alleles from: |
|--------------------------------------------|-----|------|------|----------------|----------------|-------|-------|--------------------------|----------------------------------------------------------|
| Degree of Verticillium colonisation (vec; malt agar test; number of colonised shoot segments/total number of shoot segments in%) | vec1 | E1   | 2    | 26 erecta      | 21.2**         | 4.6   | 31%   | Bur                       | 20.4 ± 13.8a                                            |
|                                            | vec2 | E2   | 4    | 10 nga8       | 9.4*           | 2.0   | 11%   | Bur                       | 21.2 ± 17.2b, 26.3 ± 21.8b, 41.8 ± 15.8b               |
|                                            | vec3 | E3   | 4    | 45 ciw7      | 17.0**         | 3.7   | 18%   | Bur                       | 32.0 ± 29.2a, 41.0 ± 20.5a, 65.2 ± 21.5b              |
|                                            | vec4 | E1   | 5    | 11 nga139    | 20.7**         | 4.5   | 31%   | Bur                       | 7.7 ± 6.0a, 23.2 ± 16.5b, 42.0 ± 24.1c                 |
| Degree of Verticillium colonisation (vec a; real-time qPCR; pg Verticillium DNA/100 mg shoot fresh weight) | vec1 a | E3   | 2    | 27 erecta      | 142**         | 3.1   | 20%   | Bur                       | 764 ± 1071a, 1061 ± 1035a, 1994 ± 1588b               |
|                                            | vec3 a | E3   | 4    | 47 ciw7      | 13.4*          | 2.9   | 13%   | Bur                       | 976 ± 1162a, 751 ± 741a, 2015 ± 1615b                 |
| Development time (dt; days from germination to maturity in mock-inoculated plants) | dt1  | E1   | 4    | 5 nga8       | 18.8**         | 4.1   | 29%   | Bur                       | 64.4 ± 7.5a, 58.6 ± 7.4ab, 54.3 ± 3.8b                 |
|                                            | dt2  | E1   | 4    | 16 nga8     | 18.2**         | 4.0   | 28%   | Bur                       | 64.4 ± 7.5a, 58.6 ± 7.4ab, 54.3 ± 3.8b                 |
|                                            | dt3  | E1   | 4    | 33 ciw6    | 17.2**         | 3.7   | 26%   | Bur                       | 63.3 ± 7.2a, 58.0 ± 7.2ab, 54.5 ± 4.7b                 |
|                                            | dt4  | E2   | 5    | 5 nga151   | 17.0**         | 3.7   | 24%   | Bur                       | 50.3 ± 3.1a, 55.0 ± 4.8b, 49.8 ± 5.7a                 |
| Stunting resistance (stre; shoot height of Verticillium-inoculated plants in cm) | stre1 | E1   | 1    | 79 nga111   | 32.5**         | 7.1   | 51%   | Bur                       | 10.9 ± 2.2a, 16.1 ± 3.8b, 20.2 ± 4.3b                 |
|                                            | stre2 | E1   | 1    | 59 nga128  | 22.2**         | 4.8   | 39%   | Bur                       | 11.1 ± 2.3a, 17.4 ± 4.5b, 17.8 ± 3.9b                 |
|                                            | stre3 | E3   | 5    | 31 SO191  | 20.4**         | 4.4   | 39%   | Bur                       | 28.7 ± 3.1ab, 24.7 ± 5.3a, 32.7 ± 3.5b                 |
|                                            | stre4 | E3   | 5    | 39 SO191  | 20.9**         | 4.5   | 40%   | Bur                       | 28.7 ± 3.1ab, 24.7 ± 5.3a, 32.7 ± 3.5b                 |
|                                            | stre5 | E3   | 5    | 53 nga129 | 15.8**         | 3.4   | 32%   | Bur                       | 26.5 ± 3.1a, 25.7 ± 5.5a, 33.5 ± 3.2b                 |
| Verticillium-induced axillary branching (vab; number of plants with a branching score above the overall median of Verticillium-inoculated plants) | vab1 | E1   | 1    | 9 F167-  | 28.1**        | 6.1   | 39%   | Bur                       | 20a, 13b                                                |
|                                            | vab2 | E1   | 1    | 34 EH1-2  | 22.2**        | 4.8   | 33%   | Bur                       | 20a, 12ab, 10.5b                                         |

LRS = likelihood ratio statistic. Asterisks denote genome-wide significance thresholds determined by permutation tests as follows: * 37%, ** 95%, ***99.9%. In each row, values with different letters differ significantly with at least p < 0.05. ANOVA and tukey tests were used for metric values; differences of values that were not normally distributed were confirmed with pairwise Mann-Whitney-U-tests. For axillary branching scores the median is given; values were compared pairwise with U-tests. Results are displayed for the most representative experiment (exp.). For reproducibility see Additional file 4.

Table 3: Correlations between resistance and developmental traits in F3-families

| Traits                                    | Exp. | r     |
|-------------------------------------------|------|-------|
| Degree of Verticillium colonisation vs. days to maturity in mock-inoculated plants | E1   | -0.42** |
|                                            | E2   | -0.73*** |
|                                            | E3   | -0.67*** |
| Verticillium-induced difference in days to maturity vs. days to maturity in mock-inoculated plants | E1   | 0.74*** |
|                                            | E2   | 0.61*** |
|                                            | E3   | 0.68*** |
| Performance ‘Height’ vs. days to maturity in mock-inoculated plants | E1   | -0.67*** |
|                                            | E2   | -0.43*** |
|                                            | E3   | -0.47*** |
| Performance ‘Height’ vs. degree of Verticillium colonisation | E1   | 0.47*** |
|                                            | E2   | 0.26*  |
|                                            | E3   | 0.27*  |

Exp. = experiment, r = Pearson correlation coefficient. Asterisks denote error probability (* α = 5%, ** α = 1%, *** α = 0.1%).
different experiments. Bur showed much more variation across different experiments than Ler (Figure 6). The performance was significantly negatively correlated with the development time that differed between the experiments (Figure 6). While Bur performed better than Ler when maturity was reached early, it showed much more stunting caused by *V. longisporum* when the development was prolonged due to season. The same effect was observed when the performance of F3-families was correlated with their mean development time: Early-flowering families performed better than late-flowering lines (Table 3). The performance showed a weak positive correlation with colonisation rates in all three F3-experiments (Table 2), meaning that severely colonised lines were less stunted than colonisation-resistant lines. This relationship was even more distinct regarding the absolute plant height of inoculated F3-families (*r* = 0.62 in E1; *erecta* plants were excluded). As another method to assess stunting, the fresh weight for each plant was recorded. However, this trait varied to a considerably higher degree than plant height due to differences in branching patterns among plants and small-scale variation in nutrient supply and humidity of the soil. In a two-factorial ANOVA, no significant genotype effect on the fresh weight could be detected, whereas the experiment significantly explained fresh weight variance as well as genotype × experiment-interaction (Additional File 5). A sex-expected trait that is strongly influenced by environmental factors, broad-sense heritability for both plant height and fresh weight was moderate to low (Table 1). No reproducible QTL controlling either the performance or the fresh weight were detected.

As a further approach to identify QTL controlling stunting resistance in the mapping population, we used mean height values of inoculated plants per family after excluding plants with *erecta* phenotype. Significant genotype × experiment-interactions existed for this trait (Additional File 5). In accordance with the strong difference in stunting between seasons, the QTL showed differences in significance level between different infestation experiments. A highly significant locus (*stre1*)

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**Figure 5** *V. longisporum*-induced stunting and axillary branching in *A. thaliana* ecotype Bur. Inoculated plant (right) reached 57% of the height of mock-inoculated plant (left) at the onset of fruit maturation and showed bolting of shoots from the axils of the rosette leaves. Bar = 5 cm.

**Figure 6** Correlation of performance with development time in Ler and Bur. Performance (%): Height inoculated/Height control × 100; Ecotypes Ler (circles) and Bur (squares) are illustrated. Each data point represents a separate infestation experiment. Experiments were performed at different seasons over three years. Ler: *r* = -0.79, *p* = 0.004; Bur: *r* = -0.79, *p* < 0.001.
could be detected near marker nga111 on chromosome 1. A second significant locus was identified on the same chromosome near marker nga128 (stre2). These loci were most significant in the winter experiment E1, but could also be detected in the late spring experiment E3. In E3, three significant QTL controlling the height of *Verticillium*-infected plants (stre3, stre4 and stre5) were detected on chromosome 5 (Figure 4, Table 2). stre3 and stre4 could be reproduced at the putative level in both other experiments and stre5 could be reproduced in experiment E1 (Additional file 4). For stre1 and stre2 on chromosome 1, the alleles increasing the plant height came from Ler, which is in accordance with the finding that Ler plants were more resistant to stunting than Bur plants under winter conditions. Whereas stre1 showed intermediary inheritance (families from F2 plants heterozygous at marker nga111 show an intermediate phenotype), the stre2 allele from Ler was dominant. For stre3 and 4, plants homozygous for the Ler alleles were higher than plants that were homozygous for Bur alleles at these loci, but plants that were heterozygous at these loci showed the most extreme stunting phenotype, indicating a complex inheritance of the trait in this region. For stre5, the increasing allele came from Ler and was recessive (Table 2). All QTL for stunting resistance were absent when mock-inoculated plants were analysed, implying these QTL are not general regulators of plant height but selectively conferred stunting resistance under *V. longisporum* pressure. Apart from erecta, no reproducible locus controlling plant height in the mock-inoculated plants could be identified with the present data set.

Two loci on chromosome 1 control *Verticillium*-induced bolting of axillary buds

*V. longisporum* increased the bolting of basal axillary buds in Bur. In mock-inoculated plants, typically up to two axillary shoots could be observed, whereas in inoculated plants, often more than five shoots emerged from the axillary buds of the rosette leaves (Figure 5). This phenotype could not be observed in Ler. In Figure 7 the branching patterns for both parental ecotypes with or without *V. longisporum*-inoculation are compared. The F3-families showed segregation for this trait. The number of plants with a branching score above the overall median was calculated for each F3-family. QTL mapping resulted in two loci on the upper arm of chromosome 1 (*vab1* and *vab2*) controlling this trait (Figure 4, Table 2). *vab1* was highly significant in experiment E1, significant in E2 and putative in E3. *vab2* was significant in E1 and E3, but absent in E2. Alleles increasing axillary branching originated from Bur and were recessive. Accordingly, this phenotype was never observed in F1 plants.

**Discussion**

By using a mapping approach it was possible to show that the complex *Verticillium* syndrome in *A. thaliana* is controlled by multiple genes which affect different disease and/or resistance traits separately. Stunting resistance did not depend on resistance to the systemic spread of the pathogen, and both traits were controlled by different QTL. *Verticillium* pathogenesis and disease reactions showed a complex cross-talk with host development and were influenced by environmental factors in greenhouse experiments.

Genetic differences in resistance among natural *A. thaliana*-populations to *Verticillium* systemic spread have not yet been reported in the literature. In a previous study, colonisation rates were determined as colony-forming units obtained by plating macerated tissue of whole plants [29], but no differences between the ecotypes Col-0 and C24 were found before the onset of extensive tissue death. These ecotypes however differed only marginally in their colonisation rates in the shoot apex [44]. The present study shows that the ecotypes Bur and Ler differ considerably in the degree to which the apical part of the shoot is colonised by *V. longisporum*. The results were reproducible in independent experiments and allowed the detection of QTL controlling the systemic spread of the pathogen. Four vec QTL detected in the present study accounted for 91% and 86% of the total trait variation in the experiments E1 and E3, where all four vec QTL were detected. The amount of fungal DNA in apical shoot segments correlated significantly with the percentage of colonised shoot segments. The latter reflects the probability of fungal propagules reaching the shoot apex irrespective of the amount of fungal biomass. As expected, the malt...
agar test was more sensitive in detecting low amounts of colonisation, whereas real-time PCR could differentiate between fungal amounts when shoot colonisation rates were high. Factors that affect the amount of fungal biomass in the shoot apex after systemic colonisation can be determined more reliably by quantitative PCR using genetically homogeneous plant material such as RIL. Furthermore, the distribution of fungal biomass in plant tissue is likely to be inhomogeneous. Increasing the amount of material used for the extraction of DNA for real-time PCR is therefore expected to further reduce the variance of fungal biomass estimates [45]. The strongest QTL controlling systemic spread, vec1, explained about 30% of the trait variation and was located on chromosome 2 near the morphological marker erecta. There is substantial evidence in the literature that ERECTA itself, a receptor-like kinase with leucine-rich repeats (EC 2.7.11.30), which is mutated in Ler [37], can play a role in regulating pathogen response in plants [46,47]. In loss-of-function erecta mutants callose formation is impaired at the entry sites of the necrotrophic fungus Plectosphaerella cucumerina [47], a process that also plays a role in protecting xylem vessels against Verticillium infection [48,49]. It is, however, also possible that vec1 represents a gene linked to erecta. vec2 was located near marker nga8 on chromosome 4, in a region where Vet1 had been mapped [29]. Vet1 was reported to confer resistance to Verticillium-induced chlorosis and to act as a negative regulator of flowering. Nothing is known so far about a possible role of Vet1 in inhibiting systemic spread. Recent work focusing on genes differentially regulated after infection by V. longisporum emphasized the role of apoplastic enzymes. In a microarray experiment a high proportion of genes which encode for apoplastic enzymes were identified among the differentially regulated genes in the A. thaliana-V. longisporum-pathosystem [50]. Cell wall modifications could be the basis for the inhibition of systemic spread as well as for the stunting phenotype [50]. Up-regulated genes in the regions of the vec QTL were At4 g23500, a polygalacturonase (EC 3.2.1.15), and At4 g30460, a glycine-rich protein of unknown function, both localised in the region of vec3. Other mechanisms protecting xylem vessels against V. longisporum include apoplastic enzymes like β-1,3-glucanases (EC 3.2.1.6), peroxidases (EC 1.11.1.11) and endochitinases (EC 3.2.1.14) as reported for Brassica [51]. However, none of the A. thaliana genes known to encode wall-localised β-1,3-glucanases [52] nor the A. thaliana homologue of the chitinase up-regulated through V. longisporum in Brassica [51] are close to the positions of any vec QTL. Vascular occlusions through deposition of phenolic substances in hypocotyl vessels of oilseed rape were found to accompany the resistance phenotype in certain Brassica lines [5]. Histological investigations in A. thaliana are needed to clarify whether a similar mechanism is responsible for resistance to systemic spread in Bur and if the vec QTL could correspond to genes involved in the respective metabolism. Resistance to systemic spread has been distinguished as an important resistance component also in other vascular pathosystems, such as Fusarium head blight of wheat [53]. QTL were detected that specifically control resistance to systemic spread of Fusarium [54]. Recently, resistance against northern leaf blight in maize could be dissected into penetration resistance and resistance against the spread of Setosphaeria turcica inside the vascular system. Both types of resistance were controlled by different QTL and differed also for their mode-of-action [55]. Dissecting complex disease and resistance phenotypes in a genetic study allows a more in-depth understanding of the genetic and physiological basis of quantitative resistance.

In the present study, resistance to systemic spread in ecotype Bur was associated with slow development, a correlation that became obvious in the F3-families of the (Bur×Ler) mapping population: Slowly-developing families were less colonised than faster ones. One obvious reason was the overlap of colonisation and developmental QTL. In the present study, the confidence interval of vec2 overlapped with those of dt1 and dt2 (chromosome 4), vec3 with dt3 (chromosome 4) and vec4 with dt4 (chromosome 5). Two interpretations for this phenomenon are possible. Either the QTL influencing development have pleiotropic effects on systemic spread or they are linked to QTL controlling systemic spread. Distinguishing between linkage and pleiotropy is important for breeding because only in the case of linkage it would be possible to implement resistance without co-selection of a certain developmental type. Enhanced resistance to systemic spread in slowly-developing genotypes was also observed for certain accessions of B. oleracea and B. rapa (E. Diederichsen, unpublished results). The localisation of the vec and dt QTL in the present study indicates that the correlation between both traits is due to linkage rather than pleiotropic effects of the same gene(s). Candidate genes located in the region of dt1 are Vet1 [29], and fri, which is known to be impaired in Ler [56]. Further studies are required to determine whether dt1, vec2 and Vet1 are identical. Flowering genes in the region of dt2 include cry1/ky4, a blue-light receptor [57], det1, a suppressor of photomorphogenesis [58] and ted1, an antagonist of det1 [59]. A known flowering gene in the region of dt3 is fca at 9.2 Mb [60,61], which promotes the transition to flowering in the autonomous pathway. dt4 possibly represents two dominant genes delaying development with additive effect originating from different parents. This would
explain why heterozygotes at marker position nga151 developed significantly more slowly than either maternal or paternal homozygotes. A flowering QTL near marker nga151 was also detected in two different RIL populations [34]. *constans* (*co*), *fy* and *flowering locus C* (*flc*) are possible candidates for this QTL. FLC is a potent inhibitor of the transition to flowering integrating the autonomous and the vernalisation pathway [62]. Bur most likely carries a loss-of-function *flc* allele [63], whilst Ler has a weak *flc* allele [64]. The detected epistatic interaction between EH4-1, which is close to *fri*, and nga151 in the vicinity of *flc* may suggest that the pathway nevertheless plays a role in the present mapping population. However, a *fri*-independent late-flowering QTL from Bur on chromosome 5 was described as well which could correspond to *dt4* [63].

Contrary to expectations, high colonisation rates did not coincide with severe disease symptoms such as stunting. Actually a weak positive correlation between colonisation and performance was observed in all three infestation experiments, showing that heavily colonised families were less stunted than sparsely colonised ones. This correlation does not necessarily reflect a causal relationship. Both systemic spread and performance were negatively correlated with development time, a factor that might influence both traits independently. The weaker performance of slowly-developing plants seems to have a physiological basis: In both parental lines Bur and Ler, performance decreased when development was slowed down as a consequence of seasonal changes. An unknown regulatory mechanism might control both stunting severity and developmental velocity differently in interaction with seasonal influences still effective in the greenhouse. Temperature, seasonal changes in light intensity and a higher dosage of blue light in summer are potential factors causing summer/winter differences. Nevertheless, host genetic disposition also plays a role, since Bur shows a much higher difference in performance between summer and winter. In summer experiments with short development times, Bur was consistently less stunted than Ler, whereas the opposite was true in winter experiments. The dependence of quantitative resistance on developmental aspects is a frequently recognised phenomenon [18,19,65,66]. Seasonal differences could also be observed in significance levels of stunting resistance QTL. In E1 (started in January 2007), *stre*1 and *stre*2 were strong, in E3 (started in April 2008) they were present, but only at the putative level. *stre*3 to *stre*5 were strong in E3, while in E1 and E2 they were weak or absent (Additional File 4). The absence of most stunting resistance QTL in E2 (started in March 2007) might have been due to inhomogeneous soil used in this experiment so that nutritional effects masked genetic effects or seasonal impact on development. The finding that the resistant alleles of the *stre* QTL on chromosome 1 were of Ler origin is in good accordance with Ler having been more resistant to stunting in winter. However, the situation for *stre*3 to *stre*5 is more complex. Only for *stre*5, the resistance allele came from Ler. For *stre*3 and 4, heterozygous families were more susceptible than families carrying either of the parental alleles in the homozygous state. A possible explanation would be that *stre*3 and *stre*4 contain an array of susceptibility genes of different origin with additive effects. A gene recently found to be involved in *Verticillium* resistance is *rfo1*, a receptor-like kinase (EC 2.7.11.30) otherwise mediating resistance to Fusarium oxysporum [26]. The *rfo1*-allele from the A. thaliana accession ‘Taynult’ (Ty-0) conferred significant resistance against fresh weight loss caused by *Verticillium* and is located at the bottom of chromosome 1 at 29.9 Mb. Thus it could be a candidate for stunting resistance QTL *stre*1 near marker nga111 at 27.3 Mb, but stunting resistance was not observed for Ler in the respective study [26]. The results of the present study suggest that the symptoms stunting and systemic spread are controlled by different pathways. This underlines the observation that shoot colonisation is not a prerequisite to cause damage to the host in greenhouse or growth chamber assays, which has also been described before for Brassica [2,51]. As stunting can occur without detectable amounts of V. longisporum in the shoot, a translocated signal is likely to be involved in the induction of stunting. *Verticillium* has been shown to interfere with various signalling pathways, like ABA, ethylene, jasmonic acid and salicylic acid [26,27,29,67]. Some components are obviously important for conferring resistance, since mutants in the ethylene or ABA signalling components *ein2*, *ein4*, *ein6* and *aba2* are more susceptible to *Verticillium* than wild type in terms of chlorosis and/or stunting. However, some regulatory mechanisms, especially ethylene production, also seem to be manipulated in a way that enhances disease symptoms in the host plant [27,68]. Plants deficient for the ethylene receptor *etr1* showed enhanced resistance in terms of chlorosis [27,29] and fresh weight loss [26] compared to wild type. Our understanding of the nature and exact role of fungal and plant signals in the context of this pathosystem is only at the beginning [69]. A fungal elicitor protein, *Verticillium dahliae*-necrosis-and-ethylene-inducing factor (VdNEP), was found to be involved in symptom development in cotton [70] and is also present in V. longisporum (Weiberg and Karlovsky, unpublished results), emphasizing the role of ethylene induction in *Verticillium* pathogenesis.

The alteration of the A. thaliana branching pattern after *Verticillium* infection has previously been observed for the A. thaliana ecotypes C24 and ‘Coimbra’ (Co-1) [29].
Scoring the (Bur×Ler) F3-families for basal branch numbers gave clear results for all three experiments. Both loci were on chromosome 1 and together accounted for 72% of the total variation in experiment E1. vab1 had a stronger effect and was located shortly below marker F167-TRB (3.8 Mb, AGI physical map). A promising candidate for vab1 could be supershoot (sp5) at 5.6 Mb. It encodes a P450 cytochrome oxidase (EC 1.9.3.1) that regulates axillary branching by locally modulating cytokinin levels in the leaf axils [71]. Allelic variation of sp5 is significantly associated with basal branching patterns among natural A. thaliana populations [72]. A possible explanation for the differences in the present study would be that Verticillium interferes with cytokinin or other hormonal regulatory pathways, but not every natural sps allele responds to phytohormones in the same way. Other possible candidates for vab1 include bud1 at 6.3 Mb, encoding a MAP kinase kinase (EC 2.7.12.2) involved in auxin transport and systemic acquired resistance [73], and mp5, an auxin-response factor at 6.9 Mb [74]. vab2, the weaker branching QTL, was located between markers nga392 at 9.8 Mb and EH1-2 at 12.8 Mb. A gene in this region influencing branching pattern is axr3 at 11.6 Mb involved in auxin signalling [75]. Whereas ethylene and ABA-signalling have been in the focus of Verticillium researchers [26,27,29], there is no evidence so far for a possible role of cytokinin and auxin. The findings of the present study show that the roles of cytokinin and auxin signalling in Verticillium pathogenesis require further investigation.

The search for genetic resources mediating Verticillium resistance in Brassica has been difficult because qualitative resistance relying on a single gene is not available for this pathosystem. In breeding, quantitative resistance is more difficult to implement as it is mostly controlled by several genes with small effects, and it demands more effort to introgress quantitative resistance. Nevertheless, it is of high interest to breeders since it is often more durable than qualitative resistance. For some pathogens it is the only resistance available [12,18,19]. In greenhouse experiments, A. thaliana has proven to be a good model for genetic studies on quantitative resistance against V. longisporum in crucifers. The complexity of Verticillium resistance in A. thaliana was particularly striking for stunting resistance, which was highly conditional on environmental factors in the present study. A combination of several QTL was necessary to confer good growth performance regardless of different developmental patterns and growing conditions. Fungal spread within the plant was not a prerequisite for increased symptom severity in A. thaliana, but is likely to play a greater role for Brassica crops in the field, as host colonisation enables the fungus to use more host resources for its own proliferation. In Brassica, disease symptoms were indeed correlated with the presence of V. longisporum in shoots [5,24], S. Konietzki, FU Berlin, personal communication]. Breeding for resistance to systemic spread should be a promising strategy to control this disease in Brassica. The present genetic study in A. thaliana provides the basis for the identification of individual resistance genes, their cloning, and the elucidation of the resistance mechanisms involved.

Conclusions
A. thaliana can be used as a model to study typical features of the V. longisporum pathosystem, such as host genotype and developmental effects on pathogenesis and resistance components. Systemic spread of V. longisporum in the host as well as resistance to this major disease trait are described for the first time in natural A. thaliana accessions. This creates the possibility to study a major resistance mechanism against vascular pathogens in this model plant and to clone relevant genes of the involved pathways using the Arabidopsis tool box. Stunting resistance and systemic spread were controlled by different QTL and should be treated as separate traits. Developmental and environmental effects on pathogenesis and resistance need to be considered when designing and interpreting experiments in research and breeding. Further studies will help to determine the exact role of potential candidate genes.

Additional material

Additional file 1: New sequence-characterised (SCAR) markers developed for (Bur×Ler) mapping populations. New markers are listed with name, chromosomal position according to the AGI map, sequences of forward and reverse primers, the annealing temperature for PCR-amplification and the fragment size for Bur and Ler respectively. Length polymorphisms were identified using the MSQT query tool [36].

Additional file 2: Linkage map for the (Bur×Ler) F2 mapping population. The linkage map shows the five A. thaliana chromosomes containing all markers that were analysed. Physical positions of markers according to the AGI map and marker distances in cM as determined in the F2 population are displayed.

Additional file 3: Frequency distributions for trait values of F3-families in individual infestation experiments. Frequency distributions of F3-family values are shown for all three infestation experiments for the following traits: degree of Verticillium colonisation, development time, stunting resistance and Verticillium-induced axillary branching. Parental and F1-values are indicated by boxes.

Additional file 4: QTL information for individual infestation experiments. Peak positions, LRS values and significance levels of QTL in the individual infestation experiments are listed for the following traits: degree of Verticillium colonisation, development time, stunting resistance and Verticillium-induced axillary branching.

Additional file 5: Tests of between-subjects effects in two-factor ANOVA. Two-factor ANOVA was performed to test the influence of genotype and experiment and their interaction on trait values. Results of tests of between-subjects effects are shown for the traits development time, fresh weight of inoculated plants, and the height of inoculated plants (height of plants with and without erecta phenotype were tested separately).
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Authors' contributions

EH planned and carried out all plant experiments, all experiments related to mapping such as marker analysis, mapping and QTL analysis and drafted the manuscript. ED conceived of the study, participated in its design and coordination and helped to draft the manuscript. PK provided the qPCR data on fungal biomass and helped to draft the manuscript. All authors read and approved the final manuscript.

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