Mapping Quantitative Trait Loci for Powdery Mildew Resistance in Flax (Linum usitatissimum L.)

Parvaneh Asgarinia, Sylvie Cloutier,* Scott Duguid, Khalid Rashid, AghaFakhr Mirlohi, Mitali Banik, Ghodratollah Saeidi

ABSTRACT
Powdery mildew (Oidium lini Skoric, [PM]), an obligate biotrophic ascomycete, is a common and widespread foliar disease of flax in most flax-growing areas of the world. In this study, we constructed a linkage map using 143 simple sequence repeat (SSR) markers and an F₂ population of 300 individuals generated from a cross between the susceptible cultivar NorMan and the resistant cultivar Linda. The F₂-derived F₃ families were phenotyped in the field and the F₃-derived F₄ families were phenotyped in a controlled environment growth chamber. The 15 linkage group map covered 1241 cM and was largely collinear with the previously published consensus map. Quantitative trait loci (QTL) analysis was performed and three PM resistance QTL located on LG1, 7, and 9 were identified consistently using phenotyping data from both field and growth chamber studies. These QTL explained 97% of the phenotypic variation exhibiting a mainly dominant gene action. This work represents a first step toward understanding the genetics of PM resistance in flax and map-based cloning of candidate genes underlying the QTL.

Flax (Linum usitatissimum L., 2n = 2x = 30), a historically important and versatile diploid species, is widely grown in Canada, India, and China for its seed oil (linseed) while in Russia, Egypt, Belgium, France, and again China, it is grown primarily for its high-quality cellulose-rich bast fibers (Marchenkov et al., 2003; FAOSTAT, 2013). Flax belongs to the order Malpighiales, the Linaceae family and the genus Linum. Pale flax [L. bienne (L. angustifolium Huds.)] is considered the wild progenitor (Muravenko et al., 2010). The size of the flax genome was originally estimated to be ~675 Mb (Evans et al., 1972; Bennett and Leitch, 2012). However, a recent estimate of the genome size of flax cultivar CDC Bethune was only ~373 Mb (Wang et al., 2012), in agreement with the physical map from Ragupathy et al. (2011).
Powdery mildew (PM), caused by the obligate biotrophic ascomycete *Oidium lini* Skoric, is a common, widespread, and easily recognized foliar disease of flax present in most growing areas worldwide (Gill, 1987; Aly et al., 2012). Early PM infections may cause severe defoliation and reduce both yield and seed quality (Beale, 1991). In Canada, PM in flax was first observed in 1997 (Rashid, 1998; Rashid et al., 1998). Powdery mildew requires living tissue for growth and reproduction and thrives in high humidity and moderate temperature environments. Although the symptoms observed in different environments allude to the presence of different races of the pathogen (Beale, 1991; Saharan and Saharan, 1994), race classification has not yet been reported but studies are underway to establish a system (K. Rashid, personal communication, 2012).

Typical PM infection symptoms start with the appearance of a white powdery mass of mycelia visible as small spots that eventually spread to cover the entire leaf surface. Heavily infected leaves wither, dry up, and die. Genetic resistance is a priority for flax breeders because fungicides can be hazardous, costly, and/or associated with environmental concerns. Classical genetic studies identified several resistant cultivars (Rashid and Duguid, 2005). A single dominant gene designated *pm1* was described in the Canadian cultivars AC Watson, AC McDuff, and AC Emerson, and in the European cultivars Atalante and Linda. Two additional putative dominant genes were also postulated in cultivar Linda (Rashid and Duguid, 2005).

In contrast to the rapidly increasing knowledge on monogenic resistance (Liu et al., 2012; Xue et al., 2012), far less is known about the molecular basis of quantitatively inherited disease resistance, despite its importance in many plant-pathogen interactions. Quantitative trait loci mapping is an effective approach to unravel complex and polygenic traits (Würschum, 2012). In general, QTL analysis is performed on one or more segregating populations derived from diverse parental genotypes providing the high levels of genotypic and phenotypic variation necessary for marker-trait associations. Quantitative trait loci analyses for PM resistance have been performed in grape (Riaz et al., 2011), hop (Henning et al., 2011), wheat (Alam et al., 2011), wild emmer (Liu et al., 2012), barley (Aghnoum et al., 2010), and perennial ryegrass (Schejbel et al., 2008), but not yet in flax.

The development of a comprehensive genetic map and reliable genotyping are paramount to successful QTL identification (Melchinger et al., 2012). Among the different classes of molecular markers, SSRs or microsatellites, have been widely used for the construction of skeletal genetic maps onto which single nucleotide polymorphism (SNP) markers can be added for high saturation or fine mapping (Allen et al., 2011; Yu et al., 2012). Simple sequence repeats, consisting of tandemly repeated short motifs of 2 to 6 nucleotides, are useful for a variety of applications in plant genetics and breeding because they are multi-allelic, highly reproducible, ubiquitous, mostly codominant, and can even be assessed across related species (Powell et al., 1996; Varshney et al., 2005; Kalia et al., 2011; Soto-Cerda et al., 2011b). More than 1400 SSR markers have been developed and analyzed in linseed (Wiesner et al., 2001; Roose-Amsaleg et al., 2006; Cloutier et al., 2009, 2012a; Deng et al., 2010, 2011; Bickel et al., 2011; Rachinskaya et al., 2011; Ragupathy et al., 2011; Soto-Cerda et al., 2011a, 2011b; Kale et al., 2012). To date, QTL mapping in flax remains limited to an amplified fragment length polymorphism (AFLP) map covering approximately 1400 cM that identified two QTL for resistance to Fusarium wilt (Spielmeyer et al., 1998), a restriction fragment length polymorphism (RFLP), and random amplified polymorphic DNA (RAPD) linkage map (Oh et al., 2000), and an expressed sequence tag (EST)-SSR map spanning about 834 cM that detected major QTL for seed coat color and fatty acid composition (Cloutier et al., 2011). Recently, a consensus SSR map was reported using three segregating populations comprising 821 marker loci covering 1551 cM (Cloutier et al., 2012b). A total of 670 markers were anchored to 204 of the 416 fingerprinted contigs of the physical map corresponding to 74% of the estimated flax genome size of 370 Mb (Ragupathy et al., 2011; Cloutier et al., 2012b).

The rapid adoption of high-throughput genotyping technologies enables the construction of multiple genetic maps from the same species that can be combined into a consensus genetic map consolidating the genetic information and offering a wealth of resources for genetic and breeding applications. The consensus genetic map permits the integration of additional markers into the QTL harboring regions (Bohra et al., 2012). Anchoring these markers to contigs or scaffolds of a physical map provides access to specific genomic regions for fine mapping and map-based cloning of candidate genes.

Here, we report on the development of an F2 genetic map derived from a cross between the cultivars Linda and NorMan. The F3 and F4 families were phenotyped for PM resistance in the field and under controlled conditions in growth chambers, respectively. Quantitative trait loci analysis was performed to identify the putative chromosomal segments associated with resistance to PM.

### MATERIALS AND METHODS

#### Plant Materials and DNA Extraction

An F2 population of 300 individuals from a cross between PM susceptible NorMan (Kenaschuk and Hoes, 1986) and PM resistant Linda was generated (Rashid and Duguid, 2005). Linda, introduced from Europe, is not well adapted to flax-growing conditions in Canada and is not registered in Canada, whereas NorMan is a well-adapted Canadian cultivar. The F1 plants were grown in pots in a greenhouse and the F2 seeds were harvested. Genomic DNA was extracted from lyophilized young leaf tissue (100 mg fresh) of the F2 and the parental lines using the DNaseasy...
Genetic Map Construction
Cloutier et al. (2009, 2012a) described the development and analysis of over a thousand polymorphic flax SSR markers. A total of 288 SSR markers distributed on all linkage groups (LGs) were screened on the parental lines for polymorphism assessment. From these, 147 polymorphic SSR markers were assessed on the F2 population. Polymerase chain reactions (PCRs) were performed in 384-well plates as previously described (Huang et al., 2006). The amplification products were resolved on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Output files were imported into Genographer (Benham et al., 1999), as modified by T. Banks for SSR data (http://sourceforge.net/projects/genographer, accessed 5 June 2012), and the three labeled reactions were transformed into independent gel-like images. Fragment sizes were estimated using the GeneScan ROX-500 internal size standard (Applied Biosystems) and were recorded for each individual. References for markers of the linkage map are listed (Supplementary Table S1).

The expected Mendelian segregation ratios of 1:2:1 and 3:1 for codominant and dominant markers, respectively, were tested using the Chi-square goodness-of-fit test. Linkage analysis was performed using JoinMap 4.0 (Van Ooijen, 2006) with logarithmic odds (LOD) of 4.0 and a maximum recombination frequency of 40 cM (Kosambi, 1944). Placement of markers into different LGs was performed as previously described (Cloutier et al., 2012b). Graphical linkage maps were drawn with Mapchart 2.1 (Voorrips, 2002). To assess the amount of colinearity in marker orders between the consensus map of Cloutier et al. (2012b) and the NorMan/Linda F2-derived genetic map, correlation coefficients (r) were calculated and their significance was tested.

Phenotyping
We obtained sufficient seeds from 218 of the greenhouse-grown F1 plants to perform a field evaluation. The parents and 218 F2 families were planted in 2012 at the Morden Research Station, Morden, Manitoba, in single 3m-long row plots (average 75 plants per row) with 0.3 m between rows. No artificial inoculation was used in the field where natural PM infection occurred. Field ratings were performed at the end of flowering and at the middle and late green boll stages by estimating the percent of the leaf area infected (LAI) of all plants in each plot. A scale of 0 to 9 (Rashid and Duguid 2005) was used where 0 meant no sign of infection; 1 meant trace to less than 1% infection; 2, 1 to 5%; 3, 6 to 10%; 4, 11 to 20%; 5, 21 to 30%; 6, 31 to 40%; 7, 41 to 50%; 8, 51 to 60%; and 9, > 60%. The mean disease LAI score for each F3 family was calculated by averaging the scores from the three ratings. Segregation within families was noted and F2 seeds from the F3 families that contained only resistant or susceptible plants were harvested.

A subset of 45 F4 families was grown in a growth chamber on the susceptible variety NorMan at temperatures of 25°C (day) and 20°C (night), with a 16-h photoperiod (high-intensity fluorescent light of 380 μmol m−2 s−1) and > 95% relative humidity. A total of 30 F4 plants from each of the 45 F4 families were grown in duplicate 15 cm pots, i.e., 15 plants × 2 pots per family, using the same conditions as described above. Plants were inoculated with isolate PM97 by uniformly brushing heavily infected flax plants with conidia onto the 20 d-old seedling intended for testing. Additionally, ten pots of heavily infected plants were randomly placed among the F4 pots to provide a continuous supply of inoculum. The inoculated plants were scored for LAI at 45 d post-seeding (pre-flowering stage, 30–40 cm tall) using the 0 to 9 scale described above. Each of the 45 F4 families was tested in three replications using a complete randomized block design. The mean disease score of each line was calculated from the two pots and three replicates. Data from the three replications were subjected to an analysis of variance (ANOVA). Disease scores were converted to percentage of LAI as follows: LAI = Σ [number of infected plants of a given rating × rating value] ×100/[total plant number × highest rating value] (Sabzalian et al., 2012).

QTL Analysis for Powdery Mildew Resistance
Quantitative trait loci (QTL) analysis for PM resistance was performed using the composite interval mapping (CIM) function of WinQTL Cartographer version 2.5 (Wang et al., 2007; Wang et al., 2012). Composite interval mapping analysis was performed applying the Standard Model 6, with a genome scan interval (walk speed) of 1 cM. The “forward-backward stepwise regression” was applied to set the number of marker cofactors to be used as background control. A window size of 10 cM was used to block out signals within 10 cM on either side of the flanking markers. Logarithmic odds thresholds of significance were determined using 1000 permutations and a significance level of 0.05. The estimates of additive and dominance effects, and the coefficients of determination (R2) estimating the percentage of phenotypic variance explained by the genotypes at the putative QTL were obtained using multiple interval mapping (MIM) function of WinQTL Cartographer version 2.5 (Wang et al., 2007).

Identifying Physical Location of the QTL Regions
A physical map of the flax genome (Ragupathy et al., 2011) anchored to the consensus genetic map (Cloutier et al., 2012b) was used to identify genomic sequences (Wang et al., 2012) flanking or spanning the putative QTL identified. BLASTn searches (Altschul et al., 1990) using the bacterial artificial chromosome (BAC) end sequences from which SSR markers Lu2698, Lu2712, Lu2810, and Lu2832 (Cloutier et al., 2012b) and the EST sequences from which SSR markers Lu138, Lu146, Lu151, Lu253, and Lu1125 (Cloutier et al., 2009) were derived, were performed against the flax whole genome shotgun (WGS) sequence assembly (Goodstein et al., 2012). The scaffold hit sequences were downloaded and alignments of the SSR primers were verified.

Identification of SNPs in the QTL Regions
Genomic DNA of parental lines Linda and NorMan was sequenced by the Michael Smith Genome Sciences Centre
of the BC Cancer Agency (Vancouver, BC) on the Illumina HiSeq 2000 (Illumina Inc., San Diego, USA). The equivalent of 1/6 of a HiSeq lane of 100-bp paired end tag sequence was obtained for each of the two genotypes. The sequence output was processed as previously described (Kumar et al., 2012) using the WGS sequence assembly of CDC Bethune as reference (Wang et al., 2012). The two binary alignment/map (BAM) files were combined and alignment of the Linda and NorMan reads against the CDC Bethune reference genome was visualized using Tablet (Milne et al., 2010). Binary alignment map (BAM) files are the binary version of sequence alignment/map (SAM) files, which are tab delimited text files that contain sequence alignment data (Li et al., 2009). Single nucleotide polymorphisms polymorphic between the parental lines were identified by visual assessment of the scaffolds identified with the QTL flanking SSR markers.

RESULTS

Linkage Map and Segregation

The parental lines, NorMan and Linda, were screened for polymorphism using 288 SSR primer pairs, and 147 SSR primer pairs (51%) were chosen on the basis of their polymorphism and amplicon sizes. These primer pairs generated 153 SSR marker loci as detected on the 300 individuals of the F_2 population. The NorMan/Linda F_2 linkage map had 15 LGs comprising 128 codominant and 15 dominant marker loci covering 1241 cM (Fig. 1; Supplementary Table S2). Ten markers were unlinked. The number of markers per linkage group varied from two to 20 with an average distance between markers of 11.4 cM. A total of 38 codominant and three dominant markers deviated significantly (P \leq 0.05) from the expected 1:2:1 and 3:1 ratios, respectively (Supplementary Table S2). Almost half of the distorted markers were located in two linkage groups, namely LG8 and LG12, while the remainder were scattered on eight other LGs. Twenty markers (49%) were skewed toward Linda, 11 (27%) toward NorMan, and 7 (17%) in favor of the heterozygotes (Supplementary Table S2). Three markers had a significant excess of both parental alleles.

Comparison of the NorMan/Linda Genetic Map with the Flax Consensus Map

The colinearity of the NorMan/Linda map and the 770-marker consensus map (Cloutier et al., 2012b) were compared to assess marker order and position and to identify

Figure 1. Linkage map of flax (Linum usitatissimum L.) comprising 143 simple sequence repeat (SSR) loci. Unlinked markers are Lua2, Lu271, Lu1044, Lu1161, Lu1163, Lu2383, Lu2405, Lu2545, Lu2555, and Lu3186. Vertical bars indicate the position of the powdery mildew (PM) quantitative trait loci (QTL). Quantitative trait loci nomenclature is as follows: Q for QTL followed by a trait abbreviation (powdery mildew resistance, PM), originating laboratory (Cereal Research Centre, CRC) and linkage group number (LG).
The disease scores were converted to percent LAI, which was used for QTL mapping. Four major QTL for field resistance to PM, measured as mean LAI, were detected on four chromosomes (Table 1). Due in part to significant dominance effects from the alleles inherited additional markers from the QTL regions. Detailed comparison of the two maps indicated a high degree of conservation in linkage and marker order (Fig. 2; Supplementary Table S2; Supplementary Fig. 1). Only marker Lu2468a was positioned on different LGs between the two maps (Supplementary Fig. 1). Marker positions were in general agreement between the two maps, with a few minor shifts and rearrangements of close markers (Fig. 2).

**Phenotyping and QTL for PM Resistance**

Flax resistance to PM was evaluated in the field on each individual of the F$_3$ families and the two parental lines at three different stages of development using a 0 to 9 scale. Linda had an average score of 1.5 and NorMan, 7 (Figs. 3 and 4a). F$_3$ families with a disease rating between 0 and 1 were considered resistant (R), between 2 and 3 were moderately resistant (MR), between 4 and 5 were moderately susceptible (MS), and between 6 and 9 were susceptible (S). As such, the majority of the F$_3$ families were resistant (Fig. 4a).

The disease scores were converted to percent LAI, which was used for QTL mapping. Four major QTL for field resistance to PM, measured as mean LAI, were detected on four chromosomes (Table 1). Due in part to significant dominance effects from the alleles inherited
from the resistant parent, the genotypes at the three QTL cumulatively explained 95% of the phenotypic variation in PM symptoms. The same four QTL were significant regardless of the rating stage (Supplementary Fig. S2). Using the colinearity information between the consensus and NorMan/Linda maps permitted positioning four and thirteen additional markers into the QTL intervals of LG7 and LG12, respectively (data not shown).

To validate these QTL and to attempt to improve the accuracy of the estimated QTL locations, replicated F4 growth chamber testing of the nonsegregating resistant and susceptible F3 families was performed. Analysis of variance of the F4 families detected significant genotypic differences (P ≤ 0.01) (Supplementary Table S3; Fig. 4b). All resistant and all susceptible F3 families from the field were confirmed resistant and susceptible in the growth chamber evaluations. Quantitative trait loci located at three of the same four locations were also significant (Table 1; Supplementary Fig. S2). Phenotypic variation explained by these QTL ranged from 10 to 48%. The additive and dominance effect estimates ranged from 0.001 to 0.034 and 0.11 to 0.36, respectively.

**Identifying Physical Location of the QTL Regions**

The QTL on LG1 was anchored to two scaffolds and the ones on LG7 and LG9 to a single scaffold each (Supplementary Table S4). Using the sequencing data from NorMan and Linda, 157, 128, and 28 SNPs were identified at the three respective loci.

**DISCUSSION**

**Genetic Linkage Map**

Molecular markers and genetic maps, powerful tools for gaining insight into the inheritance of quantitative characters, are being used to undertake complex trait mapping and the development of marker-assisted breeding strategies in many crop species (Kang and Mian, 2010; Cakir et al., 2011; Zhang et al., 2012). To date, few QTL studies have been conducted in flax (Spielmeyer et al., 1998; Oh et al., 2000; Cloutier et al., 2011) because genetic maps based on user-friendly markers are only now emerging (Cloutier et al., 2012b). This study constitutes the first report identifying QTL associated with PM resistance in flax. The F2 SSR linkage map covered 1241 cM on 15 linkage groups that were mostly collinear with the consensus map (Cloutier et al., 2012b). A few local inconsistencies in marker order like small inversions or local rearrangements were observed, especially for closely linked markers, distorted markers, and markers located at the distal ends of LGs, as previously reported for flax (Cloutier et al., 2012b). This study developed the consensus map published earlier (Cloutier et al., 2012b). Fifty-one percent of the SSR markers were polymorphic in this cross, a relatively high level for flax, where polymorphism between 30 and 40% has been reported (Deng et al., 2010; Cloutier et al., 2011, 2012a; Kale et al., 2012). NorMan, a Canadian variety, and Linda, a European variety, are from two separate gene pools, which are unlikely to contribute to greater polymorphism. LG6 with two markers and LG15 with four markers were the shortest and least saturated LGs because most of the markers on them were monomorphic. LG15 was also the shortest and least saturated linkage group of the consensus map (Cloutier et al., 2012b).

**Marker Segregation Distortion**

Twenty-nine percent of the markers deviated significantly from the expected Mendelian segregation ratios of 1:2:1 and 3:1, similar to previous reports in flax (Spielmeyer et al., 1998, Cloutier et al., 2011) and pigeonpea (Bohra et al., 2012). Segregation distortion, a commonly encountered phenomenon

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**Table 1. Quantitative trait loci (QTL) for resistance to powdery mildew identified in the NorMan/Linda F2 map by phenotyping F3 families in the field and F4 families in growth chambers.**

<table>
<thead>
<tr>
<th>QTL</th>
<th>Linkage group</th>
<th>Flanking markers</th>
<th>Distance between flanking markers (cM)</th>
<th>Nearest marker</th>
<th>Marker position</th>
<th>QTL position</th>
<th>Distance to nearest marker (cM)</th>
<th>LOD† score</th>
<th>Additive effect</th>
<th>Dominant effect</th>
<th>R² or phenotypic variation (%)</th>
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</thead>
<tbody>
<tr>
<td>F3</td>
<td>QPM-crc-LG1</td>
<td>LG1</td>
<td>Lu2698-Lu2712</td>
<td>14.44</td>
<td>Lu2698</td>
<td>94.79</td>
<td>96</td>
<td>1.11</td>
<td>21.1</td>
<td>0.002</td>
<td>0.27</td>
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<td>QPM-crc-LG7</td>
<td>LG7</td>
<td>Lu2810-Lu2832</td>
<td>6.71</td>
<td>Lu2832</td>
<td>101.03</td>
<td>102</td>
<td>0.97</td>
<td>24.0</td>
<td>0.001</td>
<td>0.14</td>
<td>22</td>
</tr>
<tr>
<td>QPM-crc-LG9</td>
<td>LG9</td>
<td>Lu1125a-Lu932</td>
<td>25.21</td>
<td>Lu932</td>
<td>119.81</td>
<td>119</td>
<td>1.21</td>
<td>24.2</td>
<td>0.008</td>
<td>0.40</td>
<td>43</td>
</tr>
<tr>
<td>QPM-crc-LG12</td>
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<td>Lu2779-Lu2485</td>
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<td>F4</td>
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<td>Lu2698-Lu2712</td>
<td>14.44</td>
<td>Lu2698</td>
<td>94.79</td>
<td>97</td>
<td>2.21</td>
<td>4.5</td>
<td>0.001</td>
<td>0.11</td>
</tr>
<tr>
<td>QPM-crc-LG7</td>
<td>LG7</td>
<td>Lu2810-Lu2832</td>
<td>6.71</td>
<td>Lu2832</td>
<td>101.03</td>
<td>101</td>
<td>0.03</td>
<td>7.8</td>
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<td>Lu932</td>
<td>119.81</td>
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<td>7.5</td>
<td>0.034</td>
<td>0.37</td>
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</table>

† LOD, logarithmic odds.
in QTL mapping, is the deviation of observed genetic ratios from the expected Mendelian ratios of a given genotypic class within a segregating population (Lyttle, 1991). A powerful evolutionary force (Taylor and Ingvason, 2003), it has been reported in several crop species (Tai et al., 2000; Kumar et al., 2007; Xu et al., 2008). Segregation distortion is influenced by many factors such as mapping population, residual heterozygosity in parental lines, gametophytic competition, abortion of male or female gametes or zygotes, nonhomologous recombination, transposable elements, chromosome translocation, complementary genes, and experimental techniques (Cloutier et al., 1997; Knox and Ellis, 2002; Zhu et al., 2007; Yamagishi et al., 2010). Similar to the flax consensus map, the majority of the distorted markers clustered onto two LGs, namely LG8 and LG12, suggesting that genetic factors are more likely to have caused the distorted ratios than statistical bias or genotyping or scoring errors (Plomion et al., 1995). The relatively lower correlation between the NorMan/Linda and the consensus maps for LG8 and LG12 may be attributed to the distorted marker clusters. Distorted markers not closely linked to QTL do not significantly impact QTL mapping (Zhang et al., 2010). Also, in small populations, their effect tends to be minor and is generally alleviated in large populations. Distorted markers were not a concern here because they were not present in the vicinity of any of the PM resistance QTL.

QTL Analysis

Quantitative trait loci for PM resistance have been mapped in grape (Riaz et al., 2011), hop (Henning et al., 2011), wheat (Alam et al., 2011), wild emmer (Liu et al., 2012), barley (Aghnoum et al., 2010), and perennial ryegrass (Schejbel et al., 2008). This paper constitutes the first report of PM resistance QTL in flax. There are no known virulence or physiologic races of flax PM, although the symptoms observed in different environments suggest that different morphological variants may exist (Beale, 1991; Saharan and Saharan, 1994). Through conventional pathology analyses, Rashid and Duguid (2005) hypothesized that resistance to the flax PM isolate PM97 is controlled by a single dominant gene, pm1, in the Canadian flax cultivars AC Watson, AC McDuff, and AC Emerson, and by pm1 and possibly two additional dominant genes in Linda. No resistance genes to PM97 were detected in the susceptible cultivars Somme or NorMan. The high level of resistance in Linda (4–5% LAI) was hypothesized to be due to the presence of the three dominant resistance genes while the relatively high level of resistance identified in Atalante (5% LAI), compared with AC Emerson (20% LAI), AC McDuff (14% LAI), and AC Watson (16% LAI), could be attributed to minor resistance genes or the presence of another gene closely linked to pm1. Linda’s PM resistance was consistently observed in both field and growth chamber tests but the disease incidence varied slightly from year to year (1–5% LAI) at both seedling and adult plant stages (Rashid and Duguid, 2005). Our QTL analysis substantiated their results by the identification of three major resistance QTL located on linkage groups 1, 7, and 9 that were identified consistently in the Nor-Man/Linda F2 and F4 families (Supplementary Fig. S2), and explained 95 to 97% of the phenotypic variation. The skewed segregation observed in frequency distribution of the PM resistance in the F3 families (Fig. 4a) is also indicative of the presence of more than one dominant resistance gene and QTL in Linda (Rashid and Duguid, 2005).

The three distinct genotypes of F2 populations permit simultaneous estimation of additive and dominance effects. On the other hand, the additional genetic effects complicate the QTL mapping procedure (Zhang et al., 2008). The additive and dominance effect estimates at the QTL mapped herein indicated that the resistance was predominantly controlled by dominant gene action. Grimmer et al. (2007) reported that three resistance genes exhibited a dominant phenotype in resistance to PM in sugar beet, and that presence of just one of the three resulted in a substantial reduction in infected leaf area per plant. Synteny with the flax consensus map (Cloutier et al., 2012b) located four additional markers to QPM-crc-LG7 that should be useful for genetic to physical map anchoring and to initiate the saturation of the QTL region. Both are important steps towards defining more precisely the QTL regions and eventually facilitating map-based cloning of the candidate genes (Feltus et al., 2006). To date, more than 60 PM resistance genes located at 41 loci (pm1-pm45, pm18 = pm1c, pm22 = pm1e, pm23 = pm4c, pm31 = pm21) (Hsam et al., 1998; Singrün et al., 2003; Hao et al., 2008; Ma et al., 2011; Xie et al., 2011) have been identified and designated in wheat and its wild relatives but only two PM resistance genes have been cloned, including the pm3 allelic series (Yahiaoui et al., 2004; Srichumpa et al., 2005) and pm21 (Cao et al., 2011). Pm3 is localized on the short arm of wheat chromosome 1A (Bougot et al., 2002), and is now known to occur in 15 functional allelic forms (pm3a to pm3g, pm3k to pm3l) which encode CC-NBS-LRR proteins that confer race-specific resistance to different subsets of wheat PM races (Yahiaoui et al., 2004, 2006; Srichumpa et al., 2005). The pm3 locus is conserved in tetraploid wheat, and Yahiaoui et al. (2009) identified 61 pm3 allelic sequences from wild and domesticated tetraploid wheat subspecies. The pm3 sequences formed 24 different haplotypes. They showed low-sequence diversity, differing by only a few polymorphic sequence blocks that were further reshuffled between alleles by gene conversion and recombination (Yahiaoui et al., 2009). While we are a long way in flax from acquiring the extensive knowledge on PM outlined above for wheat, we hope that the research results outlined here constitute an important step towards that goal. Definition of a race system, characterization of host plant resistance, and QTL mapping through biparental populations or association mapping are
key priorities, and, combined with knowledge of the disease from other pathosystems and novel technologies, rapid developments are anticipated.

**FUTURE WORK AND CONCLUSIONS**

In this study, three major PM resistance QTL were identified. As such, the NorMan/Linda linkage map constitutes a first step towards fine mapping of the QTL regions and the identification of the candidate genes. Additional phenotyping of a recombinant inbred line population currently in development, identification of additional recombinant lines in the QTL intervals, and marker saturation are required. Future work will concentrate on identifying additional markers (SNP markers) in an attempt to specify QTL in the chromosomal regions more accurately. The first physical map of the flax genome was constructed (Ragupathy et al., 2011) and incorporated with the SSR consensus map of flax (Cloutier et al., 2012b) to provide the first physical-genetic map integration and the backbone for ordering the whole genome shotgun assembly (Wang et al., 2012). The latter will be aided by the SNPs identified herein between the two parental lines through our genetic and physical map anchoring combined with the next generation sequencing data from NorMan and Linda. Quantitative trait loci validation will be performed using other crosses and isolates to ascertain their resistance spectrum.

Pyramiding resistance genes into the Canadian flax breeding lines with superior agronomic characteristics will enhance the potential for achieving durable resistance to flax PM, reducing or obviating the requirement for chemical control. Strategies such as the pyramidal scheme (Hospital and Charcosset, 1997), QTL complementation selection (Hospital et al., 2000), or genotype building using markers in recurrent selection (Charmet et al., 1999) which could be effective to improve PM resistance in flax, will be tested.

**Supplemental Data Available**

Supplemental data is available with the online version of this manuscript.

Supplemental Table S1. Sources of markers used to construct the linkage map.

Supplemental Table S2. Mapping statistics of the NorMan/Linda F2-derived genetic map including number, type, and location of distorted markers and correlation coefficient (r) between this map and the Cloutier et al. (2012b) consensus map of flax.

Supplemental Table S3. Analysis of variance for powdery mildew resistance of the F4 families.

Supplemental Table S4. Anchoring of the quantitative trait loci (QTL) to the whole genome shotgun sequence of flax and number of single nucleotide polymorphisms (SNPs) identified in each of the powdery mildew QTL interval.

Supplemental Fig. S1. Scatter plots showing the correlations between the consensus and F2 population genetic maps for all 15 linkage groups.

Supplemental Fig. S2. Logarithmic odds (LOD) score and position of four putative quantitative trait loci (QTL) detected for powdery mildew resistance based on the F3 families evaluated in the field at three rating dates (the end of flowering, the middle, and late green boll stages) and overall mean data (mean of all stages), and the simple sequence repeat (SSR) linkage map of linseed, using the composite interval mapping (CIM) approach and the three putative QTL detected using F4 families evaluated in growth chamber tests at 45 d post-seeding (pre-flowering stage, 30–40 cm tall). Means are the averages from three replicates.

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