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Technical Note

Mapping Quantitative Trait Loci onto Chromosome-scale Pseudomolecules in Flax

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Abstract: Quantitative trait loci (QTL) are genomic regions associated with phenotype variation of quantitative traits in a population. To date, a total of 267 QTL for 29 quantitative traits have been reported in 13 studies on flax. Of these, 200 QTL from 12 studies were identified based on genetic maps, scaffold sequences, or pre-released chromosome-scale pseudomolecules. Molecular markers for QTL identification differed across studies but were mainly based on simple sequence repeat (SSR) or single nucleotide polymorphism (SNP) markers. This article provides methods with software tools and database files to uniquely map SSR and SNP markers from different references onto the recently released chromosome-scale pseudomolecules. Using these methods, 195 QTL were successfully sorted onto the 15 flax chromosomes and grouped into 133 co-located QTL clusters. Mapping of QTL from different studies to the same reference enables comparisons and facilitates genome-wide QTL analysis, candidate gene scanning, and breeding applications.

Keywords: flax; association mapping; genome-wide association study (GWAS); simple sequence repeat (SSR); single nucleotide polymorphism (SNP); quantitative trait loci (QTL); chromosome-scale pseudomolecules

1. Introduction

Most traits of importance in plant breeding are quantitative and controlled by polygenes with minor effects on phenotypes. Traditional quantitative genetics can estimate overall genetic effects or variances of polygenes for quantitative traits through dedicated genetic designs [1], providing a theoretical guide for plant breeding. With the development of molecular markers and high-throughput genotyping techniques, individual polygenic loci on chromosomes and their effects can be detected and estimated using statistical genomics approaches. Such polygenic loci on chromosomes are called quantitative trait loci (QTL). They are associated with phenotype variation of quantitative traits and are usually mapped in various populations using molecular markers such as simple sequence repeat (SSR) or single nucleotide polymorphism (SNP) markers. Generally, QTL can be identified by two main approaches: linkage mapping (LM) of bi-parental populations and diverse genetic population-based association mapping (AM) or genome-wide association study (GWAS) [2]. LM uses bi-parental populations, such as F_2, recombinant inbred line (RIL), doubled haploid (DH), and backcross (BC) populations, to identify loci responsible for trait variation between parents based on recombination-based genetic linkage maps [3]. AM relies on linkage disequilibrium (LD) between markers and QTL, using a more diverse genetic panel to overcome the phenotypic diversity limitation of bi-parental populations, such as natural germplasm collections, or, more often, panels including germplasm accessions and breeding lines or, multi-parent populations such as nested association mapping (NAM) [4-6] and multi-parent advanced generation intercross (MAGIC) populations [7-10]. QTL can be exploited for gene cloning, marker-assisted breeding, and genomic selection or prediction.

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Cultivated flax (*Linum usitatissimum* L.) is a self-pollinating annual crop valued for its seed oil or stem fiber. Phenotypic selection remains a major conventional breeding approach to improve traits of agronomic importance in flax. To accelerate the application of molecular breeding, a large number of molecular markers [11-14] and genetic populations [15-18] have been developed to assist QTL identification in the last decade. Using these genetic resources, a total of 267 QTL for 29 traits (11 seed yield and agronomic traits, 11 seed quality traits, four fibre traits, and three disease resistance traits) were reported in 13 studies (Tables 1 and 2). These QTL were identified mainly using SSR or SNP markers (Table 2). Most (200) of the QTL were mapped based on genetic maps [15,18-24], scaffold sequences [17,25,26], or an early (hereafter pre-released) version of chromosome-scale pseudomolecules (PCPs) [27,28]; however, only 67 QTL for pasmo severity (PAS) were mapped on the most recent release of the chromosome-scale pseudomolecules (RCPs) (Table 2) [14,29]. For comparison purposes, coordinates of the QTL based on the PCPs must be converted to the RCPs [29] because the two versions are slightly different. The objective of this technical note is to provide methods and their associated software tools and database files to uniquely map QTL identified in different studies onto the RCPs [29]. Using these methods, 195 out of 200 QTL were successfully mapped onto 15 chromosomes and grouped into 133 co-located QTL clusters. The methods for mapping QTL/markers to the same reference render the QTL identified from different studies comparable, facilitate genome-wide QTL analysis, candidate gene prediction, and breeding applications, present an integrated global view of all QTL identified in flax to date and, provide means to integrate additional QTL in the future.

Table 1. Number of QTL associated with 29 traits in flax.

Category	No	Trait	Abbreviation	Total QTL identified	Total unique QTL	Source
Seed yield and agronomic traits	1	Seed yield	YLD	5	4	[20,22,28]
	2	Thousand seed weight (g)	TSW	24	23	[17,22,26,30]
	3	Seeds per boll	SEB	1	1	[20]
	4	Fruit number	FN	9	8	[17,26]
	5	Branching score	BSC	1	1	[30]
	6	Number of branches	NB	13	13	[26]
	7	Days to flowering	DTF	1	1	[30]
	8	Days to maturity	DTM	3	2	[20,28]
	9	Plant height (cm)	PLH	33	30	[18,22,26,28,30]
	10	Technical length (cm)	TL	17	13	[17,18,22,26]
	11	Lodging	LDG	2	1	[30]
Seed quality	12	Iodine value	IOD	8	7	[19,20,23,28]
	13	Protein content (%)	PRO	2	2	[20,28]
	14	Oil content (%)	OIL	10	10	[20,23,28]
	15	Oleic (%)	OLE	4	4	[20,28]
	16	Palmitic (%)	PAL	7	5	[17,19,20,28]
	17	Stearic (%)	STE	8	7	[17,20,23,28]
	18	Linoleic (%)	LIO	11	9	[17,19,20,23,28]
	19	Linolenic (%)	LIN	12	10	[17,19,20,23,28]
	20	Seed mucilage content	MC	7	7	[27]
	21	Seed hull content	HC	4	4	[27]
	22	Seed color	SC	2	1	[19]
Fibre	23	Straw weight (g)	STW	4	4	[20,22]
	24	Fibre yield (g)	FY	2	2	[22]
	25	Fibre content (%)	FC	4	4	[17,22]
	26	Cell walls (%)	CEW	1	1	[20]

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Category	No	Trait	Abbreviation	Total QTL identified	Total unique QTL	Source
Disease	27	Fusarium wilt rating	FW	2	2	[24]
	28	Powdery mildew rating	PM	3	3	[15]
	29	Pasmo rating	PAS	67	67	[14]

Table 2. QTL identification studies in flax.

Population	Pop size	Markers	Method[1]	Ref[2]	Total QTL	No. of QTL identified/trait[3]	Source
DH	59	8 RFLPs, 213 AFLPs	LM	GM	2	2/FW	[24]
DH	78	113 SSRs, 5 SNPs, 4 genes	LM	GM	9	2/LIO, LIN, IOD; 1/PAL; 2/SC	[19]
F3-F4	300	143 SSRs	LM	GM	3	3/PM	[15]
Core collection	390	464 SSRs	AM	GM	11	5/TSW; 1/DTF; 2/PLH; 1/BSC; 2/LDG	[30]
Core collection	390	460 SSRs	AM	GM	9	1/OIL; 1/STE; 3/LIO; 3/LIN; 1/IOD	[23]
RIL	243	329 SNPs, 362 SSRs	LM	GM	20	1/PAL; 3/STE; 3/OLE;2/LIO; 1/LIN; 2/IOD; 1/OIL; 1/PRO; 1/CEW; 1/STW; 1/TSW; 1/SEB; 1/YLD; 1/DTM	[20]
2 RILs	233	4,497 SNPs	LM	GM	24	14/PLH; 10/TL	[18]
F2	112	2,339 SNPs	LM	GM	12	1/PLH; 1/TL; 3/YLD; 3/STW; 2/FY; 2/FC	[22]
Core collection	224	146,959 SNPs	AM	SS	43	9/PLH; 3/TL; 13/NB; 8/FN; 10/TSW	[26]
Core collection	224	584,987 SNPs	AM	SS	23	2/PLH; 1/FN; 8/TSW; 3/TL; 1/PAL; 2/STE; 1/LIO; 3/LIN; 2/FC	[17]
Core collection	200	771,914 SNPs	AM	PCPs	11	7/MC; 4/HC	[27]
2 RILs and 1 DH	260	17,288 SNPs	AM	PCPs	33	1/YLD; 8/OIL; 5/PLH; 4/PAL; 3/IOD, LIN, LIO, 2/DTM; 2/STE; 1/PRO; 1/OLE	[28]
Core collection	370	258,873 SNPs	AM	RCPs	67	67 PAS	[14]

Pop: population. Ref: reference sequences or linkage maps for QTL identification.

[1] LM: bi-parental population-based QTL mapping; AM: association mapping or genome-wide association study.

[2] GM: genetic map; SS: scaffold based reference sequences [25]; RCPs: recent release of the chromosome-scale pseudomolecules [29]; PCPs: pre-released version of the chromosome-scale pseudomolecules.

[3] See Table 1 for trait name abbreviations.

2. Materials and Methods

2.1. The most Recent Release of the Chromosome-scale Pseudomolecules

Chromosome-scale pseudomolecules for flax were recently released [29]. A total of 622 scaffolds from the flax reference genome [25] were sorted onto 15 chromosomes totaling 316.2 Mb. Thus, SNPs identified based on scaffold reference sequences can be accurately mapped to the pseudomolecules. The 15 pseudomolecule sequences corresponding to 15 chromosomes were downloaded from the National Center for Biotechnology Information (NCBI) database. The accession numbers of the pseudomolecules for the 15 chromosomes are CP027619 (Lu1), CP027626 (Lu2), CP027627 (Lu3), CP027628 (Lu4), CP027629 (Lu5), CP027630 (Lu6), CP027631 (Lu7), CP027632 (Lu8), CP027633 (Lu9),

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CP027620 (Lu10), CP027621 (Lu11), CP027622 (Lu12), CP027623 (Lu13), CP027624 (Lu14), and CP027625 (Lu15). Chromosome sizes are listed in Table S1.

2.2. Marker Infomation of QTL in Flax

All 267 flax QTL identified in different studies are based on three types of markers: amplified fragment length polymorphsims (ALFPs), SSRs, and SNPs. PCR primer sequences of AFLPs and SSRs were retrieved from the literature [15,19-21,23,24]. For SNPs based on the scaffold sequences, scaffold names and coordinates of SNPs on scaffolds were collected directly from the publications [17,26]. For SNPs identified without a reference [18], flanking sequences of the SNP markers were downloaded from the publication [18]. All available primer sequences of SSR markers and flanking sequences of SNP markers for the identified QTL are listed in Tables S2 and S3, respectively.

2.3. Mapping PCR-based Markers to the Most Recent Release of the Chromosome-scale Pseudomolecules

PCR primer sequences of markers were mapped onto the RCPs using the electronic PCR (E-PCR) tool [31]. A pipeline using E-PCR was developed. This pipeline includes two Perl scripts: ProgramS1_prepare_rePCR.pl (Program S1) and ProgramS2_rePCR_pipeline.pl (Program S2). Program S1 is a script that creates a search database of the RCPs, outputting two files for the downstream analysis: *.famap and *.hash. Program 2 is a script that performs electronic PCR to map paired primers onto the RCPs, generating result files with coordinates of the primers on chromosomes and the amplicon sizes. No nucleotide mismatches or gaps are usually allowed. The operations of these programs are described in User guide S1.

PCR primers designed from sequences of different genotypes were not always accurately mapped to the RCPs using the E-PCR approach. In such cases, BLASTN searches were performed to ascertain their map positions.

2.4. Mapping SNPs to the Most Recent Release of the Chromosome-scale Pseudomolecules

If SNPs were identified using the flax scaffold reference sequences [25], then their coordinates were accurately converted to the RCPs' coordinates. The Perl script ProgramS3_convert_scaffold_coordinates_to_pseudochr.pl (Program S3) executes this conversion. A database file for the accurate relationship between scaffolds and the RCPs (Table S4) is required to run this program. This program's implementation is described in User guide S1.

For SNPs identified without a reference sequence [18], SNPs' flanking sequences were searched against the RCPs using BLASTN at an E-value of 10^{-30}. The alignment regions of top hits were used and manually verified.

The coordinates of SNPs based on the PCPs in two publications [27,28] were first retrieved for their scaffold names and corresponding coordinates on the scaffolds because these SNPs were initially identified from scaffold sequences. These SNPs were then converted to the RCPs using Program S3.

2.5. Grouping QTL to Clusters

QTL mapping software tools can detect multiple significant quantitative trait nucleotides (QTNs) that may be grouped into the same QTL or QTN/QTL clusters based on the LD between markers [14]. QTL detected in different populations cannot be grouped based on population-dependent marker LD. To provide a simple solution, we opted to group in a single QTL cluster all QTL located within a 200 kb window covering 100 kb upsteam and downstream of the QTL position.

2.6. Candidate Gene Analysis Based on the Most Recent Release of the Chromosome-scale Pseudomolecules

The RCPs contain 42,277 protein coding genes and 1,327 resistance gene analogs (RGAs) [29]. These genes were mapped to all orthologous genes of the model species *Arabidopsis thaliana* using

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BLASTP of flax protein sequences against *A. thaliana* protein sequences at an E-value of 10^{-10}. A total of 15,323 unique *A. thaliana* genes were mapped. Then the flax genes were searched against the NCBI non-redundant protein database (nr) at an E-value of 10^{-5} and, functional annotations were generated using a custom script that integrates protein annotation information of top hits and the orthologous *A. thaliana* genes. The annotation results were added to the gene lists. The coordinates of all protein coding genes and RGAs with their gene annotations on the released pseudomolecule are listed in Tables S5 and S6, respectively. A genome-wide gene scan along chromosomes for QTL was performed to characterize the underlying genomic regions and identify candidate genes. The genes within a 200 kb window covering upsteam and downstream of the QTL position were scanned. A Perl script ProgramS4_flax_QTL_candidate_gene_scanning.pl was developed (Program S4) to scan potential candidate genes for given QTL based on the gene annotation database files in Table S3 (for all protein coding genes) and Table S4 (for RGAs only). The methods for this program is described in User guide S1.

3. Results and Discussion

3.1. Mapping QTL onto the Most Recent Release of the Chromosome-scale Pseudomolecules

In all 13 publications reporting flax QTL identification, only 67 newly reported pasmo QTL have been mapped on the RCPs [14]. Therefore, mapping of the remaining 200 QTL onto the RCPs was performed. A total of 195 QTL uniquely mapped to the RCPs of 15 chromosomes, including 40 SSRs and 36 SNPs from genetic maps, 75 SNPs from scaffolds, and 44 SNPs from the PCPs (Figure 1 and Table 3). Markers *afB13* and *afXR6* for two powdery mildew QTL were not mapped because their AFLP primer sequences were not available [24]. One QTL for branching score failed to map because its SSR marker *Lu2067a* could not map to any region on the RCPs; this was likely because the marker was designed from a genotype different from the reference genome (cv CDC Bethune). Finally, marker *Lu8_185009* for QTL *uq.C8–2* associated with plant height (PLH) and technical length (TL) [18] mapped to two different chromosomes (Chr 4 and Chr 7).

It is notable that SSR/SNP markers from genetic maps mapped to a genomic region on a pseudomolecule corresponding to a single marker or a pair of flanking markers, while SNP markers from scaffold sequences or the PCPs anchored exclusively to the single nucleotide representing their QTL peak location.

3.2. Identical or Co-located QTL

QTL mapped to the same RCPs are comparable between studies, mapping populations, and traits. Based on our 200 kb upstream and downstream region rule, the 195 QTL/markers for 26 traits mapped to the RCPs were grouped into 133 QTL clusters (Table 3). QTL with the same numbers in the "Co-location" column in Table 3 were deemed to belong to the same QTL clusters, indicating identical or co-located QTL. QTL for 16 of the 29 traits were identified in two or more studies, of which 12 had one or more QTL that located at the same position or in the same QTL cluster (Table 1); thereby supporting the accuracy of the QTL through validation across studies.

Some QTL were validated in several studies that differed in markers (SSRs or SNPs), populations (bi-parental population or diverse genetic panel), or statistical methods used for QTL mapping (Tables 1 and 2). For example, *QTL-195* (*QDTM-Lu4.1*) and *QTL-54* (*QDm.BM.crc-LG4*) on Chr 4 correspond to the same QTL for days to maturity (DTM) identified in two different studies [20,28]. *QTL-187* (*QIOD-Lu7.2*) and *QTL-7* (*QIod.crc-LG7*) on Chr 7 for iodine value (IOD) [19,28], *QTL-190* (*QLIN-Lu7.2*) and *QTL-5* (*QLin.crc-LG7*) on Chr 7 for linolenic acid content (LIN) [19,28], *QTL-6* (*QLin.crc-LG16*) and *QTL-33* (*QLin-LG12.3*) on Chr 12 for LIN [19,23], and *QTL-4* (*QLio.crc-LG16*) and *QTL-30* (*QLio-LG12.3*) on Chr 12 for linoleic acid content (LIO) [19,23] are additional examples of the same QTL identified in different studies. Some QTL or QTNs were grouped into single QTL because their coordinates on chromosomes were close or identical and, historical recombinations may not have been present in the population; for example, *QTL-144* (*scaffold11-96400*) and *QTL-145* (*scaffold11-96569*) on Chr 1 for steric acid content (STE) [17] and, *QTL-155* (*scaffold297-275131*), *QTL-100*

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(*scaffold297_275113*), and *QTL-154* (*scaffold297-275113*) on Chr 1 for technical length (TL) corresponded to unique QTL [17,26].

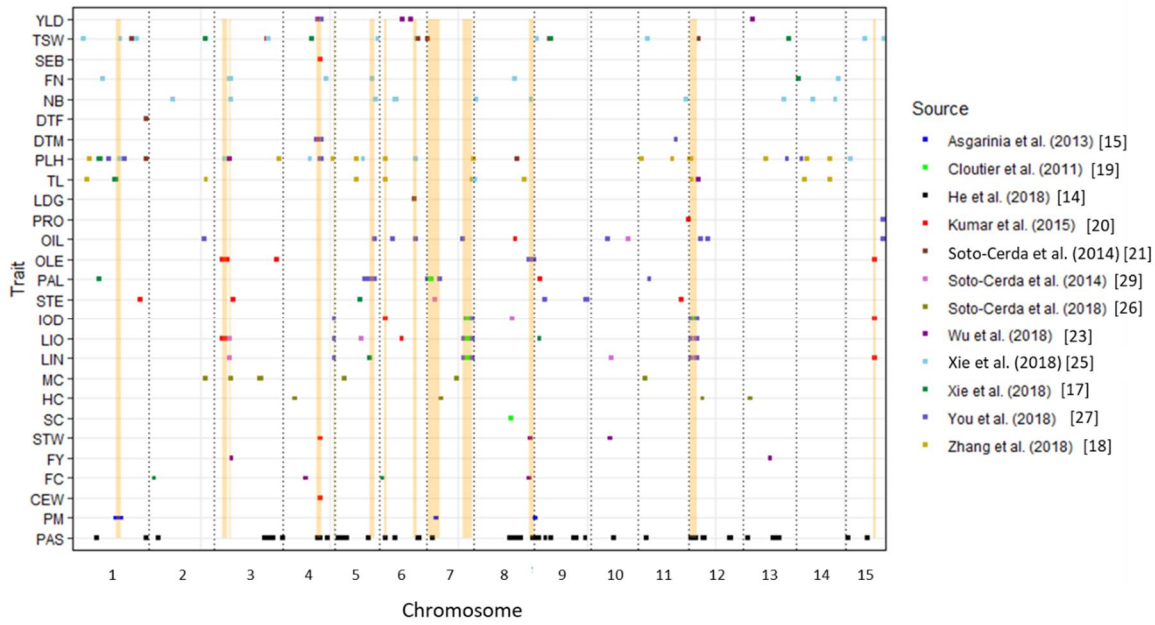

Figure 1. Distribution of 262 QTL associated with 27 traits mapping onto flax chromosomes. Of these QTL, 67 for pasmo resistance have been previously mapped on the most recent release of the flax pseudomolecule. Co-located regions are highlighted in yellow. See Table 1 for the trait name abbreviations.

Some co-located QTL may lead to their pleiotropic effects on multiple traits. Thirteen genomic regions that had at least three identical or co-located QTL were observed (yellow highlights in Figure 1 and Table 3). For example, eight QTL—*QTL-195* (*QDTM-Lu4.1*), *QTL-168* (*QYLD-Lu4.1*), *QTL-179* (*QPLH-Lu4.3*), *QTL-49* (*QCw.BM.crc-LG4*), *QTL-54* (*QDm.BM.crc-LG4*), *QTL-52* (*QSpb.BM.crc-LG4*), *QTL-50* (*QSw.BM.crc-LG4*), and *QTL-53* (*QYld.BM.crc-LG4*)—were co-located between positions 13,170,489 and 15,040,682 bp on Chr 4 and had pleiotropic effects on six traits: DTM, YLD, PLH, cell wall content (%) (CEW), seeds per boll (SEB), and straw weight (STW). This is thus an important genomic region controlling seed yield and related agronomic traits. As noted and discussed previously [19,20,28], *QTL-186* (*QIOD-Lu4.1*), *QTL-189* (*LIN-Lu4.1*), and *QTL-192* (*QLIO-Lu4.1*) were co-located between positions 19,907,982 and 19,907,982 bp on Chr 4; *QTL-193* (*QLIO-Lu7.2*), *QTL-190* (*QLIN-Lu7.2*), *QTL-187* (*QIOD-Lu7.2*), *QTL-7* (*QIod.crc-LG7*), *QTL-5* (*QLin.crc-LG7*), and *QTL-3* (*QLio.crc-LG7*) were between positions 14,540,252 and 17,976,903 bp on Chr 7; *QTL-188* (*QIOD-Lu12.3*), *QTL-191* (*QLIN-Lu12.3*), and *QTL-194* (*QLIO-Lu12.3*) located in the 489,561 and 2,981,562 bp interval on Chr 12; and *QTL-6* (*QLin.crc-LG16*), *QTL-33* (*QLin-LG12.3*), *QTL-4* (*QLio.crc-LG16*), *QTL-30* (*QLio-LG12.3*), and *QTL-8* (*QIod.crc-LG16*) positioned between 2,036,216 and 3,802,807 bp on Chr 12. These four genomic regions contributed greatly to the genetic variation for LIO, LIN, and IOD in flax populations [19,20,28].

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Table 3. QTL mapping to the recently released chromosome-scale pseudomolecules.

QTL No	Trait	*QTL/marker ID*	LG/scaffold	Flanking markers	Chr	Coordinates on chr	Co-location	Source
1	FW	*afB13*	6	*afB13*	NA	NA	NA	[24]
2		*afXR6*	10	*afXR6*	NA	NA	NA	
3	LIO	*QLio.crc-LG7*	7	*FAD3A/Lu44E4*	7	16089395 – 16092602	70	[19]
4		*QLio.crc-LG16*	16	*Lu206-Lu765B*	12	2036216 - 2041030	109	
5	LIN	*QLin.crc-LG7*	7	*FAD3A/Lu44E4*	7	16089395 - 16092602	70	
6		*QLin.crc-LG16*	16	*Lu206-Lu765B*	12	2036216 - 2041030	109	
7	IOD	*QIod.crc-LG7*	7	*FAD3A/Lu44E4*	7	16089395 - 16092602	70	
8		*QIod.crc-LG16*	16	*Lu206-Lu765B*	12	2038322 - 2038517	109	
9	PAL	*QPal.crc-LG9*	9	*Lu741-Lu675*	7	1518897 - 2017169	66	
10	SC	*QL*.crc-LG22*	22	*Colour-Lu178*	8	14838877 - 14839100	75	
11		*Qb*.crc-LG22*	22	*Colour-Lu178*	8	14838877 - 14839100	75	
12	PM	*QPM-crc-LG1*	1	*Lu2698-Lu2712*	1	16920407 - 18739647	11	[15]
13		*QPM-crc-LG7*	7	*Lu2810-Lu2832*	7	3817603 - 3817863	66	
14		*QPM-crc-LG9*	9	*Lu1125a-Lu932*	9	357191 - 357510	83	
15	TSW		3	*Lu2164*	1	22948222 - 22948580	13	[30]
16			6	*Lu2555*	6	14948801 - 14948986	65	
17			7	*Lu2532*	7	661757 - 662020	66	
18			7	*Lu58a*	12	3802629 - 3802807	111	
19			9	*Lu526*	9	5936422 - 5936694	88	
20	DTF		1	*Lu943*	1	28800644 - 28800902	16	
21	PLH		1	*Lu943*	1	28800644 - 28800902	16	
22				*Lu316*	8	17106045 - 17106266	79	
23	BSC		22	*Lu2067a*	NA		NA	
24	LDG		6	*Lu2560*	6	13553559 - 13553779	63	
25			6	*Lu2564*	6	13620999 - 13621234	63	
26	OIL	*QOil-LG9.1*	9	*c31-s67_Lu181*	10	14217309 - 14219605	95	[23]
27	STE	*QSte-LG7.1*	7	*c175-s1216_Lu146*	7	3308199 - 3308517	66	
28	LIO	*QLio-LG3.1*	3	*c729-s156_Lu3262*	3	6080016 - 6080189	24	
29		*QLio-LG5.2*	5	*c30-s11_Lu164*	5	10600927 - 10601125	47	
30		*QLio-LG12.3*	12	*c306-s98_Lu765B*	12	2036216 - 2041030	109	
31	LIN	*QLin-LG3.1*	3	*c729-s156_Lu3262*	3	6080016 - 6080189	24	
32		*QLin-LG5.2*	5	*c202-s39_Lu41*	10	7602629 - 8066018*	94	
33		*QLin-LG12.3*	12	*c306-s98_Lu765B*	12	2036216 - 2041030	109	
34	IOD	*QIod-LG8.1*	8	*c46-s505_Lu2102*	8	15166626 - 15166926	76	

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QTL No	Trait	*QTL/marker ID*	LG/scaffold	Flanking markers	Chr	Coordinates on chr	Co-location	Source
35	PAL	*QPal.BM.crc-LG7*	7	*Lu402/Lu7-1820805*	9	2026186 - 2026487	86	[20]
36	STE	*QSte.BM.crc-LG1*	1	*Lu2183a/Lu1-2670961*	1	26435050 - 26435329	15	
37		*QSte.BM.crc-LG3*	3	*Lu3-8415336/Lu2164*	3	7263087	28	
38		*QSte.BM.crc-LG11*	11	*Lu2128/Lu11-19000928*	11	16797707 - 16797907	102	
39	OLE	*QOle.BM.crc-LG3-1*	3	*Lu3-3979616/Lu3-5950394*	3	3231616 - 4799670	22	
40		*QOle.BM.crc-LG3-2*	3	*Lu658/Lu3150*	3	24238080 - 24238427	33	
41		*QOle.BM.crc-LG5*	5	*Lu5-9728492*	15	11375006	131	
42	LIO	*QLio.BM.crc-LG3*	3	*Lu3-3979616/Lu3-5950394*	3	3231616 - 4799670	22	
43		*QLio.BM.crc-LG6*	6	*Lu2545*	6	8616550 - 8616919	61	
44	LIN	*QLin.BM.crc-LG5*	5	*Lu5-9728492*	15	11375006	131	
45	IOD	*QIod.BM.crc-LG5*	5	*Lu5-9728492*	15	11375006	131	
46		*QIod.BM.crc-LG6*	6	*Lu6-2260313/Lu6-2330258*	6	2018434 - 2088579	57	
47	OIL	*QOil.BM.crc-LG8*	8	*Lu8-22516618/Lu3189*	8	16363106 - 16363334	78	
48	PRO	*QPro.BM.crc-LG11*	11	*Lu11-21716266/Lu52*	11	19594198 - 19594398	105	
49	CEW	*QCw.BM.crc-LG4*	4	*Lu2031*	4	14489225 - 14489333	40	
50	STW	*QSw.BM.crc-LG4*	4	*Lu2031*	4	14489225 - 14489333	40	
51	TSW	*QTsw.BM.crc-LG15*	15	*Lu2010a/Lu2001*	3	20394564 - 20394673	31	
52	SEB	*QSpb.BM.crc-LG4*	4	*Lu2031*	4	14489225 - 14489333	40	
53	YLD	*QYld.BM.crc-LG4*	4	*Lu2031*	4	14489225 - 14489333	40	
54	DTM	*QDm.BM.crc-LG4*	4	*Lu2031*	4	14489225 - 14489333	40	
55	PLH	*uq.C1–1*		*Lu1_396428*	1	6539309 - 6539089	3	[18]
56		*uq.C3–1*		*Lu3_693423*	3	25295008 - 25294801	34	
57		*uq.C4–1*		*Lu4_300701*	4	19453432 - 19453704	42	
58		*uq.C5–1*		*Lu5_8504*	5	8681823 - 8682018	45	
59		*uq.C6–1*		*Lu6_639236*	6	2175711 - 2175911	57	
60		*uq.C8–2*		*Lu8_185009*	7 (4)	6427466 – 6427621 (6238294 - 6238449)		
61		*uq.C8–3*		*Lu8_119488*	8	28706 - 28938	72	
62		*uq.C9–1*		*Lu9_503128*	14	4498680 - 4498955	122	
63		*uq.C11–1*		*Lu11_557617*	11	1276828 - 1277143	96	
64		*uq.C11–1*		*Lu11_447048*	11	13338945 - 13339276	100	
65		*uq.C12–1*		*Lu12_696508*	12	1004697 - 1004929	108	
66		*uq.C12–1*		*Lu12_163596*	12	351979 - 352221	106	
67		*uq.C13–1*		*Lu13_367183*	13	8997700 - 8998007	115	
68		*uq.C14–1*		*Lu14_231853*	14	13485754 - 13486113	126	
69	TL	*uq.C1–1*		*Lu1_695389*	1	5664124 - 5664330	2	

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QTL No	Trait	*QTL/marker ID*	LG/scaffold	Flanking markers	Chr	Coordinates on chr	Co-location	Source
70		*uq.C2–2*		*Lu2_597057*	2	22508975 - 22508683	21	
71		*uq.C5–1*		*Lu5_8504*	5	8681823 - 8682018	45	
72		*uq.C6–1*		*Lu6_639236*	6	2175711 - 2175911	57	
73		*uq.C7–1*		*Lu7_781312*	7	18087445 - 18087733	71	
74		*uq.C8–1*		*Lu8_646184*	8	20045574 - 20045815	80	
75		*uq.C8–2*		*Lu8_185009*	7 (4)	6427466 – 6427621 (6238294 - 6238449)		
76		*uq.C9–2*		*Lu9_618122*	14	3378716 - 3378969	121	
77		*uq.C12–1*		*Lu12_696508*	12	1004697 - 1004929	108	
78		*uq.C14–1*		*Lu14_231853*	14	13485754 - 13486113	126	
79	PLH	*Marker4371*	scaffold156 (LG1)		3	6019156 - 6019499	24	[22]
80	TL	*Marker747228*	scaffold2786 (LG8)		12	3620608 - 3620934	110	
81	YLD	*Marker799956*	scaffold319 (LG10)		13	3856362 - 3856771	114	
82		*Marker770415*	scaffold117 (LG12)		6	11929857 - 11930253	62	
83		*Marker1073071*	scaffold27 (LG12)		6	8701939 - 8702324	61	
84	STW	*Marker326151*	scaffold33 (LG5)		8	22241866 - 22242226	81	
85		*Marker2368217*	scaffold355 (LG15)		10	7140622 - 7140988	92	
86		*Marker614116*	scaffold355 (LG15)		10	7219061 - 7219445	93	
87	FY	*Marker2603286*	scaffold156 (LG1)		3	6573623 - 6574023	27	
88		*Marker1722134*	scaffold127 (LG11)		13	10603161 - 10603485	116	
89	FC	*Marker1051901*	scaffold680 (LG5)		8	21807786 - 21808148	81	
90		*Marker1561746*	scaffold376 (LG11)		4	8748431 - 8748795	36	
91	PLH	*scaffold112_114241*	scaffold112	*scaffold112_114241*	1	18444086	11	[26]
92		*scaffold1491_318496*	scaffold1491	*scaffold1491_318496*	6	14006651	63	
93		*scaffold31_1800846*	scaffold31	*scaffold31_1800846*	3	3929932	22	
94		*scaffold344_309662*	scaffold344	*scaffold344_309662*	1	11008279	6	
95		*scaffold51_1349321*	scaffold51	*scaffold51_1349321*	4	10532424	37	
96		*scaffold59_572553*	scaffold59	*scaffold59_572553*	1	10051709	4	
97		*scaffold156_641874*	scaffold156	*scaffold156_641874*	3	5906791	23	
98		*scaffold147_367986*	scaffold147	*scaffold147_367986*	5	11288517	48	
99		*scaffold859_123972*	scaffold859	*scaffold859_123972*	15	1939372	129	
100	TL	*scaffold297_275113*	scaffold297	*scaffold297_275113*	1	16435852	9	
101		*scaffold361_14957*	scaffold361	*scaffold361_14957*	1	16726904	10	
102		*scaffold273_68457*	scaffold273	*scaffold273_68457*	8	585113	73	
103	NB	*scaffold116_30201*	scaffold116	*scaffold116_30201*	2	9550662	18	
104		*scaffold156_1203677*	scaffold156	*scaffold156_1203677*	3	6468562	26	

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QTL No	Trait	*QTL/marker ID*	LG/scaffold	Flanking markers	Chr	Coordinates on chr	Co-location	Source
105		*scaffold1863_545*	scaffold1863	*scaffold1863_545*	8	1223698	74	
106		*scaffold212_601171*	scaffold212	*scaffold212_601171*	6	6380495	60	
107		*scaffold353_773806*	scaffold353	*scaffold353_773806*	5	16077893	54	
108		*scaffold42_494571*	scaffold42	*scaffold42_494571*	13	15861394	117	
109		*scaffold464_754364*	scaffold464	*scaffold464_754364*	14	15460919	127	
110		*scaffold635_43971*	scaffold635	*scaffold635_43971*	8	22494547	82	
111		*scaffold977_784147*	scaffold977	*scaffold977_784147*	11	18799131	104	
112		*scaffold212_216830*	scaffold212	*scaffold212_216830*	6	5996154	59	
113		*scaffold359_282990*	scaffold359	*scaffold359_282990*	14	6711296	124	
114		*scaffold359_289139*	scaffold359	*scaffold359_289139*	14	6705147	123	
115		*scaffold977_469888*	scaffold977	*scaffold977_469888*	11	18484872	103	
116	FN	*scaffold137_111000*	scaffold137	*scaffold137_111000*	1	11869417	7	
117		*scaffold225_427119*	scaffold225	*scaffold225_427119*	8	15994154	77	
118		*scaffold687_121617*	scaffold687	*scaffold687_121617*	14	16813947	128	
119		*scaffold156_761294*	scaffold156	*scaffold156_761294*	3	6026211	24	
120		*scaffold413_1116527*	scaffold413	*scaffold413_1116527*	4	16914228	41	
121		*scaffold156_1203677*	scaffold156	*scaffold156_1203677*	3	6468562	26	
122		*scaffold413_388319*	scaffold413	*scaffold413_388319*	5	14910709	52	
123		*scaffold687_123666*	scaffold687	*scaffold687_123666*	14	16811898	128	
124	TSW	*scaffold101_354340*	scaffold101	*scaffold101_354340*	3	20942454	32	
125		*scaffold112_184204*	scaffold112	*scaffold112_184204*	1	18514049	11	
126		*scaffold1143_190268*	scaffold1143	*scaffold1143_190268*	1	4375935	1	
127		*scaffold1155_171787*	scaffold1155	*scaffold1155_171787*	15	7690615	130	
128		*scaffold123_1191347*	scaffold123	*scaffold123_1191347*	11	3875819	98	
129		*scaffold1317_154716*	scaffold1317	*scaffold1317_154716*	15	15275145	133	
130		*scaffold132_713877*	scaffold132	*scaffold132_713877*	1	24877317	14	
131		*scaffold1491_58878*	scaffold1491	*scaffold1491_58878*	6	14266269	64	
132		*scaffold15_1207948*	scaffold15	*scaffold15_1207948*	5	16914987	55	
133		*scaffold1519_272169*	scaffold1519	*scaffold1519_272169*	9	1027739	84	
134	FN	*scaffold346-438191*	scaffold346	*scaffold346-438191*	14	1083228	120	[17]
135	TSW	*scaffold43-1111162*	scaffold43	*scaffold43-1111162*	2	21989104	19	
136		*scaffold51-598586*	scaffold51	*scaffold51-598586*	4	11283142	39	
137		*scaffold51-598611*	scaffold51	*scaffold51-598611*	4	11283117	39	
138		*scaffold51-699833*	scaffold51	*scaffold51-699833*	4	11181895	38	
139		*scaffold261-925068*	scaffold261	*scaffold261-925068*	9	6419385	80	
140		*scaffold373-545801*	scaffold373	*scaffold373-545801*	13	17912691	119	

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QTL No	Trait	*QTL/marker ID*	LG/scaffold	Flanking markers	Chr	Coordinates on chr	Co-location	Source
141		*scaffold373-545816*	scaffold373	*scaffold373-545816*	13	17912706	119	
142		*scaffold107-300735*	scaffold107	*scaffold107-300735*	2	22405177	20	
143	PAL	*scaffold59-164258*	scaffold59	*scaffold59-164258*	1	10459958	5	
144	STE	*scaffold11-96400*	scaffold11	*scaffold11-96400*	5	9964973	46	
145		*scaffold11-96569*	scaffold11	*scaffold11-96569*	5	9965142	46	
146	LIO	*scaffold1253-27622*	scaffold1253	*scaffold1253-27622*	9	1922095	85	
147	LIN	*scaffold416-80582*	scaffold416	*scaffold416-80582*	5	13560525	50	
148		*scaffold302-224377*	scaffold302	*scaffold302-224377*	5	13889425	51	
149		*scaffold302-224395*	scaffold302	*scaffold302-224395*	5	13889443	51	
150	FC	*scaffold179-179593*	scaffold179	*scaffold179-179593*	2	2253135	17	
151		*scaffold866-116645*	scaffold866	*scaffold866-116645*	6	1083247	56	
152	PLH	*scaffold344-309662*	scaffold344	*scaffold344-309662*	1	11008279	6	
153		*scaffold59-572553*	scaffold59	*scaffold59-572553*	1	10051709	4	
154	TL	*scaffold297-275113*	scaffold297	*scaffold297-275113*	1	16435852	9	
155		*scaffold297-275131*	scaffold297	*scaffold297-275131*	1	16435834	9	
156		*scaffold361-14957*	scaffold361	*scaffold361-14957*	1	16726904	10	
157	MC	*Lu2-22298066*	2	*Lu2-22298066*	2	22402960	20	[27]
158		*Lu3-25559600*	3	*Lu3-25559600*	3	17645461	29	
159		*Lu3-26033342*	3	*Lu3-26033342*	3	18058033	30	
160		*Lu3-7398487*	3	*Lu3-7398487*	3	6246253	25	
161		*Lu5-3808878*	5	*Lu5-3808878*	5	4087340	44	
162		*Lu7-13225294*	7	*Lu7-13225294*	7	12048040	68	
163		*Lu11-2498303*	11	*Lu11-2498303*	11	2755439	97	
164	HC	*Lu7-6577527*	7	*Lu7-6577527*	7	5834429	67	
165		*Lu10-21552161*	10	*Lu10-21552161*	4	4609469	35	
166		*Lu12-5267706*	12	*Lu12-5267706*	12	5160897	112	
167		*Lu13-2803224*	13	*Lu13-2803224*	13	2764903	113	
168	YLD	*QYLD-Lu4.1*	4	*Lu4-13594936 - Lu4-14968389*	4	13593668 - 14966967	40	[28]
169	OIL	*QOIL-Lu2.1*	2	*Lu2-21913720 - Lu2-21913720*	2	21912675	19	
170		*QOIL-Lu5.2*	5	*Lu5-15704607 - Lu5-15705039*	5	15703416 - 15703848	53	
171		*QOIL-Lu6.3*	6	*Lu6-4879632 - Lu6-4879632*	6	4879493	58	
172		*QOIL-Lu6.4*	6	*Lu6-13799180 - Lu6-13970951*	6	13798861 - 13970632	63	
173		*QOIL-Lu7.4*	7	*Lu7-14209179 - Lu7-14209179*	7	14208772	69	
174		*QOIL-Lu10.5*	10	*Lu10-6517448 - Lu10-6517448*	10	6517339	91	
175		*QOIL-Lu12.6*	12	*Lu12-4591214 - Lu12-7491405*	12	4591134 - 7490902	112	
176		*QOIL-Lu15.7*	15	*Lu15-14665900 - Lu15-15429055*	15	14665228 - 15428383	132	

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QTL No	Trait	*QTL/marker ID*	LG/scaffold	Flanking markers	Chr	Coordinates on chr	Co-location	Source
177	PLH	*QPLH-Lu1.1*	1	*Lu1-13887715 - Lu1-13930292*	1	13887346 - 13929923	8	
178		*QPLH-Lu1.2*	1	*Lu1-20012490 - Lu1-20012490*	1	20011813	12	
179		*QPLH-Lu4.3*	4	*Lu4-14305982 - Lu4-15042104*	4	14304616 - 15040682	40	
180		*QPLH-Lu13.4*	13	*Lu13-17243884 - Lu13-17243884*	13	17242916	118	
181		*QPLH-Lu13.5*	14	*Lu14-2320469 - Lu14-2320469*	14	2320188	121	
182	PAL	*QPAL-Lu5.1*	5	*Lu5-12062376 - Lu5-12182441*	5	12061283 - 12181348	49	
183		*QPAL-Lu5.2*	5	*Lu5-13797851 - Lu5-15668995*	5	13796740 - 15667804	51	
184		*QPAL-Lu7.3*	7	*Lu7-624461 - Lu7-5423691*	7	624439 - 5423600	66	
185		*QPAL-Lu11.4*	11	*Lu11-4417685 - Lu11-4429424*	11	4417306 - 4429045	99	
186	IOD	*QIOD-Lu4.1*	4	*Lu4-19909467 - Lu4-19909467*	4	19907982	43	
187		*QIOD-Lu7.2*	7	*Lu7-15346458 - Lu7-17977459*	7	15346004 - 17976903	70	
188		*QIOD-Lu12.3*	12	*Lu12-489561 - Lu12-2981642*	12	489561 - 2981562	107	
189	LIN	*QLIN-Lu4.1*	4	*Lu4-19909467 - Lu4-19909467*	4	19907982	43	
190		*QLIN-Lu7.2*	7	*Lu7-14540719 - Lu7-17977459*	7	14540265 - 17976903	70	
191		*QLIN-Lu12.3*	12	*Lu12-489561 - Lu12-2981642*	12	489561 - 2981562	107	
192	LIO	*QLIO-Lu4.1*	4	*Lu4-19909467 - Lu4-19909467*	4	19907982	43	
193		*QLIO-Lu7.2*	7	*Lu7-14540706 - Lu7-17977459*	7	14540252 - 17976903	70	
194		*QLIO-Lu12.3*	12	*Lu12-489561 - Lu12-2981642*	12	489561 - 2981562	107	
195	DTM	*QDTM-Lu4.1*	4	*Lu4-13171757 - Lu4-15042104*	4	13170489 - 15040682	40	
196		*QDTM-Lu11.2*	11	*Lu11-14768686 - Lu11-14768686*	11	14767787	101	
197	STE	*QSTE-Lu9.1*	9	*Lu9-4229230 - Lu9-4229230*	9	4229031	87	
198		*QSTE-Lu9.2*	9	*Lu9-20080531 - Lu9-21636823*	9	20079433 - 20654527	90	
199	PRO	*QPRO-Lu15.1*	15	*Lu15-14746288 - Lu15-14746310*	15	14745616 - 14745638	132	
200	OLE	*QOLE-Lu8.1*	8	*Lu8-21782841 - Lu8-23527563*	8	21781910 - 23526575	81	

* Primer sequences did not map to the pseudomolecules; QTL are only mapped to a region corresponding to the whole scaffold. See Table 1 for additional notes.

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3.3. Candidate Genes for QTL

The resolution of current QTL mapping or GWAS technologies is insufficient to pin QTL to accurate locations of genes or genetic features controlling traits. A simple approach for predicting candidate genes is to find related genes on the nearest regions within a QTL, such as a window of 200 kb downstream and upstream of a QTL [14,20]. For example, three QTL for powdery mildew resistance were identified [15] and mapped to chromosomes 1, 7, and 9 (Table 3, Figure 1). Some RGAs were found in the vicinity of the QTL, i.e., within the pre-defined window (Table 4). One nucleotide binding site (NBS) encoding gene (*Lus10026765*), one transmembrane coiled-coil (TM-CC) gene (*Lus10023437*), and several receptor-like protein kinase (RLK) genes co-located with these QTL.

Table 4. Resistant gene analog (RGA) candidates near three QTL for flax powdery mildew resistance.

QTL No.	QTL	Chr	QTL Coordinates (bp)	RGA	Gene location on chr (bp)	Gene annotation
12	*QPM-crc-LG1*	1	16920407 - 18739647	*Lus10026756*	17134471	RLK
				Lus10026761	17159664	RLK
				Lus10026765	17189168	NBS
				Lus10009703	18125241	RLK
13	*QPM-crc-LG7*	7	3817603 – 3817863	*Lus10023437*	3725947	TM-CC
14	*QPM-crc-LG9*	9	357191 – 357510	*Lus10001677*	429431	RLK

NBS: nucleotide binding site; RLK: receptor-like protein kinase; TM-CC: transmembrane coiled-coil.

4. Conclusion

To date, a total of 267 QTL for 29 flax quantitative traits have been reported. However, these QTL were identified based on different references, including genetic maps, scaffold sequences, and chromosome-scale pseudomolecules. This article details the methods, software tools, and database files used to uniquely map these previously identified QTL onto the RCPs. Using these methods, 195 out of 200 QTL that are not based on the RCPs were successfully sorted onto 15 chromosomes and grouped into 133 co-located QTL clusters, showing genomic regions associated with and/or pleiotropic to important agronomic and seed quality traits. Mapping of the QTL identified in different studies to the same reference allows comparisons across QTL and facilitates genome-wide QTL analysis, candidate gene prediction, and breeding applications.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1.
Table S1. Information related to the pseudomolecules of 15 chromosomes in the NCBI database. The downloaded sequences from NCBI are used as input for **Program S1**.
Table S2. Primer sequences of SSR markers for the identified QTL.
Table S3. Flanking sequences of SNP markers for the identified QTL.
Table S4. Coordinates of flax scaffold sequences on the most recent release of the chromosome-scale pseudomolecules. This file is used as input for **Program S2**.
Table S5. Coordinates and annotations of flax protein coding genes on the most recent release of the chromosome-scale pseudomolecules. This file is used as input for **Program S4**.
Table S6. Coordinates and annotations of flax resistance gene analogs on the recently released chromosome-scale pseudomolecules. This file is used as input for **Program S4**.

Program S1. A Perl script to prepare a search database of reference sequences for electronic PCR. Program file name: *ProgramS1_prepare_rePCR.pl*.
Program S2. A Perl script to perform electronic PCR, i.e., map a pair of PCR primer sequences to a reference sequence. Program file name: *ProgramS2_rePCR_pipeline.pl*.
Program S3. A Perl script to convert coordinates of flax scaffold sequences onto the chromosome-scale pseudomolecules. Program file name: *ProgramS3_convert_scaffold_coordinates_to_pseudochr.pl*.

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Program S4. A Perl script to extract all candidate genes and gene annotation information (protein-coding genes or specifically resistance gene analogs) within a genomic region of a QTL or a marker. Program file name: *ProgramS4_flax_QTL_candidate_gene_scanning.pl*

User guide S1. A user guide for executions of **Programs S1**, **S2**, **S3**, and **S4**.

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