Genetic Architecture of Chile Pepper (Capsicum Spp.)
QTLome Revealed Using Meta-QTL Analysis

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Abstract

Chile peppers (Capsicum spp.) are among the most important vegetable crops in the world due to their health-related, economic, and industrial uses. In recent years, quantitative trait loci (QTL) mapping approaches have been widely implemented to identify genomic regions affecting variation for different traits for marker-assisted selection (MAS) in peppers. Meta-QTL analysis for different traits in Capsicum remains lacking, and therefore it would be necessary to re-evaluate identified QTL for a more precise MAS for genetic improvement. We report the first known meta-QTL analysis for diverse traits in the chile pepper QTLome. A literature survey using 29 published linkage mapping studies identified 766 individual QTL from five different trait classes. A total of 311 QTL were projected into a consensus map. Meta-analysis identified 30 meta-QTL regions distributed across the 12 chromosomes of Capsicum. MQTL5.1 and MQTL5.2 related to Phytophthora capsici fruit and root rot resistance were delimited to <1.0 cM confidence intervals in chromosome P5. Candidate gene analysis for the flanking sequences for the P5 meta-QTL revealed biological functions related to DNA repair and transcription regulation. Moreover, epigenetic mechanisms such as histone and RNA methylation and demethylation were predicted, indicating the potential role of epigenetics for P capsici resistance. Allele specific SNP markers for the meta-QTL will be developed and validated using different breeding populations of Capsicum for MAS of P capsici resistant lines. Altogether, results from meta-QTL analysis for chile pepper QTLome rendered further insights into the genetic architecture of different traits for this valuable horticultural crop.

Introduction

The wealth of genomic information available for different crop species and diverse traits has expanded significantly in recent years due to the decreasing costs of high-throughput genotyping and the development of novel and powerful tools to dissect quantitative trait loci (QTL), which are genomic regions affecting variation for quantitative traits\(^1\). A ‘QTLome’ refers to the collection of QTL and their allelic variation shown to affect any quantitative trait for a given trait and species\(^2,3\). The first step to interpret information from QTLome is meta-QTL analysis\(^2,4\). QTL meta-analysis integrates results from multiple QTL studies and could provide more insights into the genetic architecture of traits associated with different genomic regions\(^5\). The QTL identified from a group of different QTL using meta-analysis at a 95% confidence interval are called meta-QTL\(^6\). In meta-QTL analysis, various models can be implemented for identifying the consensus QTL from different studies thereby validating results and refining positions of QTL in the consensus map\(^4,7\). Using information from meta-analysis could therefore allow for a more precise marker-assisted breeding for the genetic improvement of traits across different crops.

Chile peppers, belonging to the genus Capsicum and family Solanaceae, are one of the most important horticultural crops in the world due to their culinary uses, health benefits, and economic impact. Nutrients such as vitamins A, C, and folate are present in varying degrees in peppers\(^8\). Chile peppers have been used to help combat chronic pain, and it was suggested that capsaicin, the compound found predominantly in pungent lines, can induce depletion in nerve sensory terminals, and is commonly found in pain relieving creams\(^9\). In the state of New Mexico in the United States, chile peppers are important cash crops for farmers\(^10\). A present disadvantage of the cultivation of Capsicum species, however, is an inadequate supply due to low yield from farmers. Low yield can be attributed to many factors, including pests and diseases, and undesired agronomic traits such as subpar fruit size or uneven biomass distribution\(^11–13\). In the past, marker-assisted selection through genetic mapping has been implemented in chile peppers to facilitate genetic improvement for different traits including yield and resistance to major diseases.
While many QTL for diverse traits have been identified for chile peppers, there is no known report of meta-study across diverse traits in Capsicum. Most of the meta-QTL studies by far focused on diverse sets of traits across the Solanaceous relatives of chile peppers such as potatoes (Solanum tuberosum)\textsuperscript{14} and tomatoes (S. lycopersicum)\textsuperscript{15}; and in major field crops such as rice (Oryza sativa)\textsuperscript{6,16}, wheat (Triticum aestivum)\textsuperscript{17,18}, barley (Hordeum vulgare)\textsuperscript{19}, maize (Zea mays)\textsuperscript{20,21}, cotton (Gossypium spp.) \textsuperscript{22}, and soybeans (Glycine max)\textsuperscript{23}. In chile peppers, the only known report for meta-QTL to date was a study by Mallard et al.\textsuperscript{24}, who identified meta-genomic regions for \textit{P. capsici} resistance, designated as \textit{MetaPc5.1, MetaPc5.2}, and \textit{MetaPc5.3}, in chromosome P5. Meta-analysis indicated that \textit{MetaPc1} confers resistance against eight isolates, whereas \textit{MetaPc5.2} and \textit{MetaPc5.3} exhibit resistance for three isolates of \textit{P. capsici}.

Given that meta-QTL studies remain lacking in chile peppers, it would be necessary to implement meta-analysis to determine significant genomic regions involved in variation for important traits. The current study aims to perform analysis of meta-QTL to identify regions associated with diverse traits for targeted genetic improvement in chiles. Specifically, our objectives were to (1) develop a consensus genetic map in chile peppers using SNP markers; (2) identify meta-QTL related with different traits such as heat levels (pungency), yield, adaptation, and resistance to diseases in peppers; (3) determine SNP markers linked to meta-QTL; and (4) identify candidate genes present in the meta-QTL regions. We report the first known meta-QTL study of the chile pepper QTLome that could provide insights into the genetic architecture of different traits for this important horticultural crop.

**Results**

**Classification of QTL for diverse traits in** Capsicum **spp.** Overall, 766 individual QTL from 130 unique traits and five different trait classes across the 12 chromosomes of chile pepper were identified. These results were based from 29 linkage mapping studies published within a 10-year period (2010–2020) (Table 1; Fig. 1). Among these studies, majority (90%) of the reported QTL were identified using either a recombinant inbred line (RIL) or an F\textsubscript{2} biparental mapping population. The number of individuals used for QTL mapping ranged between 63 and 440. Marker types used in identifying QTL included amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), and single nucleotide polymorphism (SNP) markers, among others. Across the various linkage-mapping studies surveyed, among the most commonly used parents in creating a biparental mapping population included ‘CM-334’, a \textit{P. capsici} resistant line for identification of QTL linked to chile pepper blight resistance; ‘Bhut Jolokia’, a ‘superhot’ chile pepper for the discovery of QTL associated with capsaicinoid content; and ‘Yolo Wonder’, a bell-pepper type, for linkage mapping of stem and fruit-related traits and disease resistance QTL.
Table 1
Summary of QTL mapping studies used for meta-analysis of diverse traits for the *Capsicum* QTLome. \(^a\) BC-Backcross; \(^b\) DH- Double haploid; \(^b\) RIL- Recombinant inbred line. \(^b\) AFLP- Amplified fragment length polymorphism; \(^b\) CAPS- Cleaved amplified polymorphic sequence; \(^b\) HRM- High resolution melting; \(^b\) InDel- Insertion/Deletion; \(^b\) ISSR- Inter simple sequence repeat; \(^b\) RAPD- Random amplified polymorphic DNA; \(^b\) SCAR- Sequence characterized amplified region; \(^b\) SLAF- Specific locus amped fragment sequence; \(^b\) SNP- Single nucleotide polymorphism; \(^b\) SPP- Single position polymorphism; \(^b\) SSR- Simple sequence repeats. \(^c\) \(F\)_1 progenies of MP crossed with GNM and SM; BC\(_1\) crossed with GNM; For SP, \(F\)_1 and BC\(_1\) crossed with GNM.

| Study No. | Reference | Parents used in crossing | No. of individuals | Mapping population \(^a\) | Trait(s) | Marker type \(^b\) |
|-----------|-----------|--------------------------|-------------------|--------------------------|-----------|-------------------|
| 1         | Alimi et al.\(^55\) | Yolo Wonder x CM334       | 149               | RIL                      | Fruit and stem-related traits | SSR     |
| 2         | Arjun et al.\(^56\) | *C. annuum* FL 201 x *C. galapagoense* TC 07245 | 210               | \(F\)_2                 | Fruit Length | SSR     |
| 3         | Chunthawodtiporn et al.\(^57\) | *C. annuum* Maor x CM334 | 120               | RIL                      | Horticultural traits | SNP     |
| 4         | Du at al.\(^58\) | BVRC 25 x BVRC 1          | 440               | \(F\)_2                 | Bacterial wilt resistance | SNP, InDel |
| 5         | Dwivedi et al.\(^59\) | California Wonder x LCA235 | 74                | RIL                      | Plant height and yield related traits | SSR, SCAR, RAPD |
| 6         | Dwivedi et al.\(^60\) | California Wonder x LCA235 | 74                | RIL                      | Oleoresin content       | SSR, SCAR, RAPD |
| 7         | Eggink et al.\(^61\) | MT x PEN45 (MP); SM x PEN45 (SP); GNM \(^c\) | 250               | BC                       | Agronomic, biochemical, physiological traits | AFLP, SNP |
| 8         | Han et al.\(^41\) | Perennial x Dempsey (PD); TF68 x Habanero (TH) | 56 (PD), 85 (TH)  | RIL                      | Capsaicinoid content     | SNP     |
| 9         | Kim et al.\(^62\) | *C. baccatum* var. Pendulum x Golden-aji | 126               | \(F\)_2                | Anthracnose resistance   | SSR, AFLP, SRAP |
| 10        | Lee et al.\(^63\) | NB1 x Bhut Jolokia         | 175               | \(F\)_2                | Capsaicinoid content     | HRM, SSR, CAPS, Gene-based |
| 11        | Lee et al.\(^64\) | CV4 x Jeju                | 300               | \(F\)_2                | Chili veinal mottle virus | SNP     |
| 12        | Lee et al.\(^65\) | Perennial x Dempsey        | 120               | RIL                      | Fruit-related traits     | SNP     |
| 13        | Li et al.\(^66\) | BJ0747 x XJ0630           | 195               | \(F\)_2                | Cucumber mosaic virus resistance | SLAF-SNP |
| Study No. | Reference          | Parents used in crossing | No. of individuals | Mapping population | Trait(s)                              | Marker type |
|-----------|--------------------|--------------------------|--------------------|--------------------|---------------------------------------|-------------|
| 14        | Liu et al.        | YCM334 x Tean           | 128                | RIL                | *P. capsici* resistance               | SNP         |
| 15        | Lu et al.         | YCM334 x Taean          | 126                | RIL                | Agronomic and morphological traits; *P. capsici* resistance | SNP         |
| 16        | Mahasuk et al.   | Bangchang x PBC932 (BP), PBC80 x CA1316 (PC) | 126 (BP), 146 (PC) | F₂                 | Anthracnose resistance               | SNP         |
| 17        | Mallard et al.   | H3 x Vania (HV), Perennial x Yolo Wonder (PY), YW x CM334 (F5YC) | 101 (HV), 114 (PY), 297 (RIL) | DH (PY, HV), RIL (F5YC) | *P. capsici* resistance               | AFLP, CAPS, RFLP |
| 18        | Naegele et al.   | CM334 x EJ              | 63                 | RIL                | *P. capsici* resistance               | SNP         |
| 19        | Park et al.      | Habanero x Jolokia (HJ), SNU11-001 x Jolokia (SJ) | 87 (HJ), 124 (SJ) | F₂                 | Capsaicinoid content                 | SNP         |
| 20        | Park et al.      | M5 x AG13-3             | 96                 | F₂                 | AGI activity                         | SNP         |
| 21        | Rehrig et al.    | CM334 x EJ              | 66                 | RIL                | *P. capsici* resistance               | SNP         |
| 22        | Siddique et al. | CM334 x ECW30R          | 188                | RIL                | *P. capsici* resistance               | SNP         |
| 23        | Sun et al.       | *C. annuum* 77013 x *C. chinense* PBC932 | 186                | BC                 | Anthracnose resistance               | SSR, InDel, CAPS |
| 24        | Tan et al.       | *C. annuum* BA3 x *C. frutescens* YNXMXML | 154                | F₂                 | Flowering time                       | SSR, InDel  |
| 25        | Wei et al.       | *Capsicum annuum* 007EA x *Capsicum frutescens* P1512 | 120                | F₂                 | Horticultural traits                 | SNP         |
| 26        | Yao et al.       | BJ0747-1-3-1-1 x XJ0630-2-1-2-1-1 | 334                | F₂                 | Cucumber mosaic virus resistance      | SSR, ISSR   |
| 27        | Yarnes et al.    | 2814-6 x NuMex R Naky   | 105                | RIL                | Plant architecture, phenology, fruit quality | SPP         |
| 28        | Zhang et al.     | PM702 x FS871           | 146                | RIL                | First flower node                    | SLAF/SNP    |
| Study No. | Reference | Parents used in crossing | No. of individuals | Mapping population | Trait(s) | Marker type |
|-----------|-----------|--------------------------|--------------------|-------------------|----------|-------------|
| 29        | Zhu et al.⁴⁶ | *C. chinense* 740 x *C. annuum* CA1 | 150                | F₂                | Flower number, flowering time | SLAF       |

Meta-QTL analysis and candidate gene mining. The *Capsicum* consensus map generated from three previously published linkage maps consisted of 14,952 total markers distributed across 12 chromosomes, with an average of 1,246 markers per chromosome (Table 2). Chromosomes P3 (2,231 SNP markers), P7 (1,996), and P6 (1,711) had the greatest number of loci, whereas P8 (431), P4 (707), and P10 (722) had the least. Average length of the consensus map ranged between 94.97 (P6) and 175.09 cM (P8). From the preliminary pool of 766 QTL, 418 remained after further processing and quality control (Supplementary File 1). The consensus map projected a total of 304 QTL, with P1 having the greatest number of projected QTL (61), followed by P5 (45), and P4 (29). Chromosomes P9, P7, and P8 had the least number of QTL projected in the consensus map, with seven, eight, and 11, respectively.

Analysis further identified 30 meta-QTL distributed in the 12 chromosomes of chile pepper, with a mean position of 58.83 cM across all chromosomes (Fig. 2; Table 3). Four meta-QTL were identified for chromosomes P1 and P5, whereas three meta-QTL were detected for P3, P11, and P12. Only a single meta-QTL was identified for chromosome P7. Mean genetic position of individual meta-QTL ranged between 12.19 (MQTL2.1) and 156.87 cM (MQTL5.4). Two meta-QTL in chromosome P5, MQTL5.1 and MQTL5.2, comprised of clusters of QTL for *P. capsici* fruit and root rot resistance had confidence intervals of < 1.0 cM. MQTL1.3 had the greatest number of individual QTL (33), followed by MQTL5.2 (24), and MQTL4.2 (19). Conversely, MQTL5.4, MQTL6.1, MQTL8.2, and MQTL12.2 had only two individual QTL comprising the meta-QTL. QTL related with resistance to *P. capsici* was found in 11 out of the 30 meta-QTL detected (36.6%), whereas loci associated with anthracnose and fruit rot resistance was found in nine (30.0%) and seven (23.3%) of the identified meta-QTL, respectively. Biochemical and physiological traits such as AGI activity and transverse fruit perimeter QTL belong to six different meta-QTL. Akaike information criterion (AIC) values for determining the best QTL models in identifying meta-QTL ranged between 82.13 (P7) and 555.66 (P1), with majority of these models with the lowest AIC value being model 5. Two other chromosomes, P7 and P12, had QTL models 3 and 4 as the best model in identifying meta-QTL, respectively.
Table 2
Length and number of markers for each chromosome of the constructed Capsicum spp. consensus map.

| Chr. | Mean length (cM) | No. of SNP markers |
|------|------------------|--------------------|
| P1   | 134.90           | 986                |
| P2   | 113.95           | 1,442              |
| P3   | 130.42           | 2,231              |
| P4   | 116.43           | 707                |
| P5   | 147.70           | 1,165              |
| P6   | 94.97            | 1,711              |
| P7   | 127.70           | 1,996              |
| P8   | 175.09           | 431                |
| P9   | 97.36            | 878                |
| P10  | 112.47           | 722                |
| P11  | 137.91           | 1,529              |
| P12  | 120.63           | 1,154              |
| Total| 1,509.52         | 14,952             |
| Average| 125.79       | 1,246              |
Table 3

Meta-QTL identified for diverse traits in chile peppers (Capsicum spp.). a Akaike information criterion value for the best QTL model. b Meta-QTL identified in at least two independent linkage mapping studies. c Confidence interval.

| Chr. | No. of QTL | No. of projected QTL | No. of Meta-QTL | QTL model | AIC value a | Meta-QTL b | No. of QTL in the meta-QTL | Mean genetic position (cM) | CI c (cM) |
|------|------------|----------------------|-----------------|-----------|-------------|------------|----------------------------|---------------------------|-----------|
| P1   | 73         | 61                   | 4               | 5         | 555.66      | MQTL1.1    | 15                         | 16.75                     | 2.59      |
|      |            |                      |                 |           |             | MQTL1.2    | 6                          | 40.95                     | 5.27      |
|      |            |                      |                 |           |             | MQTL1.3    | 36                         | 67.62                     | 2.11      |
|      |            |                      |                 |           |             | MQTL1.4    | 4                          | 129.82                    | 4.25      |
| P2   | 64         | 27                   | 2               | 5         | 424.59      | MQTL2.1    | 13                         | 12.19                     | 1.6       |
|      |            |                      |                 |           |             | MQTL2.2    | 14                         | 71.44                     | 2.9       |
| P3   | 43         | 27                   | 3               | 5         | 307.38      | MQTL3.1    | 6                          | 22.33                     | 4.05      |
|      |            |                      |                 |           |             | MQTL3.2    | 6                          | 94.16                     | 5.14      |
|      |            |                      |                 |           |             | MQTL3.3    | 15                         | 146.12                    | 1.65      |
| P4   | 35         | 29                   | 2               | 5         | 295.78      | MQTL4.1    | 10                         | 20.52                     | 3.79      |
|      |            |                      |                 |           |             | MQTL4.2    | 19                         | 84.92                     | 2.02      |
| P5   | 51         | 45                   | 4               | 5         | 316.37      | MQTL5.1    | 13                         | 23.03                     | 0.79      |
|      |            |                      |                 |           |             | MQTL5.2    | 25                         | 29.16                     | 0.55      |
|      |            |                      |                 |           |             | MQTL5.3    | 7                          | 87.33                     | 1.07      |
|      |            |                      |                 |           |             | MQTL5.4    | 2                          | 156.87                    | 12.42     |
| P6   | 27         | 20                   | 2               | 5         | 217.58      | MQTL6.1    | 2                          | 14.7                      | 10.49     |
|      |            |                      |                 |           |             | MQTL6.2    | 19                         | 54.73                     | 1.65      |
| P7   | 11         | 8                    | 1               | 3         | 82.13       | MQTL7.1    | 8                          | 38.99                     | 2.53      |
| P8   | 15         | 14                   | 2               | 5         | 124.72      | MQTL8.1    | 9                          | 22.13                     | 5.61      |
|      |            |                      |                 |           |             | MQTL8.2    | 2                          | 62.24                     | 4.01      |
| P9   | 17         | 10                   | 2               | 5         | 124.95      | MQTL9.1    | 4                          | 43.8                      | 7.87      |
|      |            |                      |                 |           |             | MQTL9.2    | 3                          | 74.89                     | 9.43      |
| P10  | 35         | 26                   | 2               | 5         | 231.24      | MQTL10.1   | 13                         | 22.39                     | 1.86      |
|      |            |                      |                 |           |             | MQTL10.2   | 13                         | 83.87                     | 2.29      |
| P11  | 24         | 23                   | 3               | 5         | 214.53      | MQTL11.1   | 5                          | 19.3                      | 5.72      |
|      |            |                      |                 |           |             | MQTL11.2   | 11                         | 54.56                     | 4.01      |
|      |            |                      |                 |           |             | MQTL11.3   | 7                          | 84.22                     | 3.0       |
| P12  | 24         | 21                   | 3               | 4         | 242.70      | MQTL12.1   | 13                         | 23.51                     | 2.82      |
| Chr. of QTL | No. of QTL | No. of projected QTL | No. of Meta-QTL | QTL model | AIC value | Meta-QTL | No. of QTL in the meta-QTL | Mean genetic position (cM) | CI (cM) |
|------------|------------|----------------------|----------------|-----------|-----------|----------|---------------------------|---------------------------|--------|
| MQTL12.2   | 2          |                      |                |           |           |          | 55.69                     | 10.97                     |        |
| MQTL12.3   | 6          |                      |                |           |           |          | 106.76                    | 14.90                     |        |
| Total      | 419        | 311                  | 30             |           |           |          | 308                       |                           |        |

A total of 346 candidate genes with known biological functions were identified using sequence information for the flanking markers for MQTL5.1 (Scaffold 3155.426970 and Marker1044376) and MQTL5.2 (Cb-HRM288 and Marker635294) in chromosome P5 (Supplementary Table S1). These ranged between five and 318 candidate genes for Scaffold 3155.426970 (MQTL5.1) and Marker635294 (MQTL5.2). A wide range of biological functions for the candidate genes, including those related with DNA replication, repair, transcription regulation, phosphorylation, and glycosylation, among others, for MQTL5.1 and MQTL5.2 has been identified. Moreover, several functions related with epigenetic mechanisms such as RNA, DNA, tRNA, and histone methylation have been predicted. Among the candidate genes identified for MQTL5.2, PHT67052 and PHT65976, have functions related to transcription regulation, histone H3-K36 methylation, and histone lysine methylation; and regulation of gene expression, respectively, in C. annuum. Solyc11g033270.2.1 (MQTL5.2) has functions associated with regulation of defense response to bacterial and fungal infections, as well as activation of protein kinase activity, and stress-activated protein kinase signaling cascade in S. lycopersicum. Gene Solyc11g013370.2.1 has roles in defense response to fungus, leaf morphogenesis, seed dormancy, and positive regulation of cell division in tomatoes. Genes OIS99998 and OIS96773 have roles in methylation in wild tobacco (Nicotiana attenuata), whereas PGSC0003DMT400072321 has functions related to double-strand break repair via homologous recombination; DNA replication, recombination, and repair, and PGSC0003DMT400003724 has functions in tRNA N2-guanine methylation, tRNA processing, and methylation in S. tuberosum.

**Discussion**

The identification and analysis of notable QTL regions associated with different traits has been one of the cornerstones of modern molecular breeding for plant genetic improvement. In Capsicum spp., previous studies focused on identifying QTL linked to diverse traits including but not limited to resistance to major diseases such as chile pepper blight caused by the oomycete P. capsici, yield and yield components, capsaicin (heat) content, and agronomic traits, among others (Table 1). Given this wealth of information from previous QTL studies, it would be necessary to re-evaluate results from linkage mapping using a meta-analysis approach to refine genomic regions associated with important traits resulting in a more efficient implementation of MAS in chile pepper breeding programs. QTL meta-analysis for different traits in Capsicum remains lacking, where a major focus in the past has been the identification of meta-QTL for resistance to P. capsici²⁴. This status quo of meta-studies for chile peppers has thus driven us to explore meta-QTL for the Capsicum QTLome for diverse traits, i.e. not only for those QTL related with disease resistance, but also for those loci linked with other important yield and agronomic characters in chiles. Here, we report the first known meta-analysis of the chile pepper QTLome rendering deeper insights into the genetic architecture of diverse sets of traits for this valuable crop.

We employed a relatively stringent method in declaring a QTL cluster as a meta-QTL: (1) each meta-QTL should be composed of at least two different QTL; and (2) these QTL should come from at least two independent studies.
Accordingly, from an initial set of 39 meta-QTL, only 30 were regarded to be ‘true’ meta-QTL across the 12 chromosomes of chile peppers, with confidence intervals between 0.55 cM (MQTL5.2) and 14.90 cM (MQTL12.3). These criteria were therefore relevant for a more accurate representation of the meta-QTL identified for the chile pepper QTLome. In other crop species, varying numbers of meta-QTL have been identified. Only 11 meta-QTL were detected for seedling stage salinity tolerance in rice, whereas 60 meta-QTL were identified for *Fusarium* head blight resistance in wheat. In another meta-study of QTL in pea plants, 27 meta-QTL were resolved for seed protein content and yield-related traits. Such differences could be a consequence of the genome size, reliability of the consensus map used for meta-analysis, number of QTL regions identified, as well as the intrinsic properties of the reported QTL, such as phenotypic variation explained and LOD scores. As precision in QTL positions are dependent on population size and trait variation explained, re-calculating positions based on the type of mapping populations used for analyses could facilitate a better representation of the genetic positions for each of the QTL evaluated.

One of the objectives of a meta-QTL study is to delimit the region of a QTL using information from multiple linkage-mapping studies. Chromosome P5 represents a major chromosome for *P. capsici* resistance in chile peppers, with large-effect QTL reported in previous studies. In the current study, we reported two meta-QTL regions in chromosome P5, namely MQTL5.1, and MQTL5.2 delimited to < 1.0 cM confidence interval, i.e. 0.79 cM and 0.55 cM, respectively, comprised of QTL mapped for *P. capsici* fruit and root rot resistance. These corresponded to the genomic regions having the most refined genetic distance among all the meta-QTL identified in the present work. Similarly, in peanut (*Arachis hypogea*), a recent meta-analysis of QTL for late leaf spot resistance delimited a region to 0.38 cM and 0.70 cM, whereas in wheat, genomic regions associated with *Fusarium* head blight resistance and root-related traits were narrowed to 0.82 cM and 0.50 cM intervals, respectively. *Capsicum* spp. MQTL5.1 and MQTL5.2 consisted largely of major effect QTL, with percent variation explained ranging between 10 and 52.7% (MQTL5.1) and 8.9 and 67.7% (MQTL5.2) identified from five independent QTL mapping studies, with *N* ranging between 63 and 297 individuals. Notably, these constituent QTL also represent those with the highest phenotypic trait variation explained in the *Capsicum* QTLome evaluated; this could be a reason for a more refined meta-QTL region for disease resistance. Furthermore, MQTL5.2 consisted of 24 QTL, which was next to MQTL1.3 identified to having the highest number of individual QTL. Among the criteria for choosing a meta-QTL for selection are (1) a small confidence interval, (2) a high number individual QTL comprising the meta-QTL, and (3) a high trait variation explained of initial QTL. Considering these factors, MQTL5.1 and MQTL5.2 could serve as potential targets for marker-assisted breeding and selection for improved *P. capsici* resistance in chile peppers. The identification of meta-QTL linked DNA-based markers will help prioritize different QTL for introgression through MAS in plant breeding programs. In this regard, information from the flanking sequences for MQTL5.1 and MQTL5.2 identified in chromosome P5 will be utilized for the development of Kompetitive allele specific (KASP®) SNP assays for marker-assisted breeding. These KASP assays will be further validated using a recombinant inbred line population previously developed at New Mexico State University, and on a diverse population of New Mexican chile peppers to screen for resistance to different races of *P. capsici*.

The power of meta-QTL analysis lies in determining genomic regions that are most frequently involved in phenotypic variation and in delimiting the QTL intervals, therefore enabling candidate gene identification for positional cloning. Also, meta-QTL are potentially genomic regions that are highly rich in genes thereby facilitating pyramiding or stacking of important loci. Putative blight resistant protein homologues and leucine rich repeat (LRR) receptor-like serine/threonine protein kinases have been previously identified as candidate genes for *P. capsici* resistance in chile peppers. In the current study, candidate gene analysis using sequences for markers flanking MQTL5.1 and MQTL5.2 in chromosome P5 identified genes with diverse biological functions related to
disease resistance, including DNA repair, DNA strand renaturation, ion transport, and several epigenetic mechanisms such as DNA, RNA, and histone methylation/demethylation, indicating the possible function of epigenetics in controlling gene expression for disease resistance in chile peppers. Epigenetics and its relationship with conferring disease resistance has been well recognized in other crops such as Arabidopsis\textsuperscript{36}, rice\textsuperscript{37}, and maize\textsuperscript{38}. The denser cytosine methylation profile of the \textit{Capsicum} genome relative to that of the tomato and potato genomes\textsuperscript{39} could indicate the relevance of epigenetics for the expression of different genes in peppers. Accordingly, identifying epialleles near the meta-QTL regions in chromosome P5 could be important in breeding towards improving resistance to \textit{P. capsici} in chile peppers. Nevertheless, while the candidate genes identified here represent promising targets for future breeding, it is not known whether they are the true functional regulators of the detected meta-QTL, as many other genes could be present within the meta-QTL regions\textsuperscript{5}. It would therefore be relevant to perform functional validation of the effects of these candidate genes using different chile pepper germplasm. Overall, meta-QTL analysis confirmed the relevance of chromosome P5 as a major genomic region harboring QTL and different candidate genes for \textit{P. capsici} resistance in \textit{Capsicum}.

Chile peppers are unique among the members of family Solanaceae due to their ability to produce capsaicinoids which render distinct flavors and heat profiles. Previously, gene mapping, allele sequence data, and expression profile analyses collectively identified the pungency gene \textit{Pun1} in chromosome P2 responsible for the biosynthesis of capsaicinoids in chile peppers\textsuperscript{40}. More recently, the quantitative nature of capsaicinoid levels in chile peppers have also been demonstrated through linkage mapping which identified heat level-related QTL on chromosomes P1, P6, and P10\textsuperscript{41–43}. Several meta-QTL (e.g. \textit{MQTL2.1, MQTL2.2}) identified in the present study have pungency-related QTL co-localized with QTL for agronomic and disease resistance traits such as fruit wall (pericarp) thickness, biomass, number of leaves on primary axis, and anthracnose resistance, among others. This demonstrated potential pleiotropy and/or effects of close linkage between the underlying QTL\textsuperscript{44}. Such colocation of QTL related with diverse sets of traits for the identified meta-QTL across different chromosomes of chile pepper indicates the possibility of multi-trait improvement using genomic information from multiple linkage mapping studies.

In the current study, meta-QTL analysis was used to dissect the genetic architecture of diverse traits in \textit{Capsicum}. Genomic regions for disease resistance to \textit{P. capsici} in chile peppers were refined, and the role of chromosome P5 as a major genomic region harboring disease resistance QTL has been confirmed. Two meta-QTL, \textit{MQTL5.1} and \textit{MQTL5.2}, in chromosome P5 have been delimited to < 1.0 cM intervals. Analysis of candidate genes for these meta-QTL revealed biological functions related to DNA repair, response to bacterial and fungal infection, and DNA, RNA, and histone methylation, which demonstrate the potential role of epigenetics on resistance to \textit{P. capsici}. The colocalization of several unrelated QTL on similar chromosomal regions demonstrates potential pleiotropic effects and the effect of linkage due to location. SNP assays will be developed for these meta-QTL and will be used for MAS for resistance to pepper blight. This study by far is the largest reported meta-analysis of different traits and the first known study of the \textit{Capsicum} QTLome. The information presented here could serve as a valuable resource for the genomic improvement of diverse sets of traits in chile peppers.

Materials And Methods

Collection and characterization of QTL for different traits in chile peppers. A comprehensive bibliographic review of 29 QTL mapping studies published between 2010 and 2020 (Table 1) was first conducted to generate a QTL database for \textit{Capsicum}. The evaluated traits in chile peppers were divided into five major categories, namely (1) adaptation; (2) agronomic, quality, and yield; (3) disease resistance; (4) heat profile (pungency); and (5) biochemical
and physiological traits. Each QTL was characterized according to the number of lines used for QTL mapping, type of mapping population (e.g. F₂, RIL, BC, DH), QTL name, trait, chromosome and linkage group designations, LOD, phenotypic variation explained (R²), and chromosome positions (in cM). QTL with LOD scores of < 2.0 and with R² values not reported in the original study were excluded in further analysis. Genomewide association studies were not included in the literature review.

**Projection to a consensus map and analysis of meta-QTL.** Three different genetic maps from four cultivated species of *Capsicum* were used to develop a consensus map for the analysis of meta-QTL in chile peppers. These included an interspecific SNP genetic map derived from the hybridization between *C. annuum* and *C. frutescens*⁴⁵; a SLAF-based SNP array resulting from genotyping an F₂ population of a cross between *C. chinense* and *C. annuum*⁴⁶; and an intraspecific SNP linkage map derived from the hybridization between two *C. baccatum* varieties⁴⁷. Consensus maps for each of the 12 chromosomes of chile pepper were created using the ‘LPmerge’ package⁴⁸ in R⁴⁹. This function implements a linear programming (LP) algorithm to effectively reduce the mean absolute error in combining different genetic or linkage maps.

For QTL projection to the consensus map, the confidence interval (CI) for each QTL were calculated according to Darvasi and Soller⁵⁰ and Guo et al.⁵¹ based on the type of population used for linkage mapping using the following formula: Backcross (BC) and F₂: CI = 530/NR²; Double haploid (DH): CI = 287/NR²; and RIL: CI = 163/NR², where N is the number of lines used in QTL mapping and R² is the phenotypic variation explained by the QTL. QTL were projected to the *Capsicum* consensus map using the QTL projection function in Biomercator v.4.2.3⁵².

Meta-analysis was performed using Biomercator v.4.2.3 which implements a maximum likelihood algorithm developed by Goffinet and Gerber⁴. In this method, an Nnumber of QTL linked to the same trait or set of related traits detected in independent experiments and located in the same genomic regions is determined to be consistent with five different QTL models, namely, 1, 2, 3, 4, and 5-N QTL models. An Akaike information criterion was used to determine the best model in identifying a meta-QTL, or “real” QTL which best represent the original QTL²⁵. Additionally, only those genomic regions where QTL from at least two different genetic mapping studies co-localized to form a meta-QTL were regarded as a meta-QTL⁵. Therefore, no meta-QTL identified in this study consist of only a single QTL (i.e. a singleton). Designations for each identified meta-QTL were based on the corresponding chromosome number and their position relative to the short arm of the chromosome (e.g. “MQTL1.1”, “MQTL2.3”). Pepper chromosomes with the identified meta-QTL and their flanking markers were redrawn using the ‘LinkageMapView’ package⁵³ in R.

**Candidate gene identification for the meta-QTL.** Identification of candidate genes was conducted using the sequences of the flanking markers for the identified meta-QTL that has a < 1.0 cM confidence interval. Flanking sequences were BLASTn searched in EnsemblPlants (https://plants.ensembl.org/index.html)⁵⁴ against the genome of *C. annuum* and annotated genes and their biological functions were listed. Additionally, orthologous genes from Solanaceous plant species, including tomato (*S. lycopersicum*), potato (*S. tuberosum*), and wild tobacco (*N. attenuata*) were identified.

**Declarations**

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Author Contributions

D.N.L. conceived the research and wrote the first draft of the manuscript, L.W. and D.N.L. performed the meta-QTL and candidate gene analysis, A.A. edited the manuscript. All authors reviewed the manuscript.

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