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Developing resistance to Fusarium wilt in chickpea: From identifying meta-QTLs to molecular breeding

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Abstract

Fusarium wilt (FW) significantly affects the growth and development of chickpea (*Cicer arietinum* L.), leading to substantial economic losses. FW resistance is a quantitative trait that is controlled by multiple genomic regions. In this study, a meta-analysis was conducted on 32 quantitative trait loci (QTLs) associated with FW resistance, leading to the identification of seven meta-QTL (MQTL) regions distributed across CaLG2, CaLG4, CaLG5, and CaLG6 of the chickpea linkage groups. The integrated analysis revealed several candidate genes potentially important for FW resistance, including genes associated with sensing (e.g., LRR-RLK), signaling (e.g., mitogen-activated protein kinase [MAPK1]), and transcription regulation (e.g., NAC, WRKY, and bZIP). Subsequently, a marker-assisted backcrossing (MABC) trial was executed leveraging the MQTL outcomes to introgress FW resistance from an FW-resistant chickpea cultivar (Ana) into a superior high-yielding Kabuli cultivar (Hashem). The breeding process was extended over 5 years (2018–2023) and resulted in the development of BC_3F_2 genotypes. Consequently, 12 genotypes carrying homozygous resistance alleles were chosen, with three genotypes showing genetic backgrounds matching 90%–96% of the recurrent parent. The findings

Abbreviations: AFLP, amplified fragment length polymorphism; AIC, Akaike information criterion; BC, backcross; BGS, background selection; CAPS, cleaved amplified polymorphic sequences; CIs, confidence intervals; FGS, foreground selection; FW, Fusarium wilt; LG, linkage group; lncRNA, long non-coding RNA; LongSAGE, long serial analysis of gene expression; MABC, marker-assisted backcrossing; MQTL, meta-QTL; PCR, polymerase chain reaction; PVE, proportion of phenotypic variance explained; QTLs, quantitative trait loci; RAPD, random amplified polymorphic DNA; RILs, recombinant inbred lines; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STMS, sequence tagged microsatellite sites.

of this study have significant implications for upcoming programs, encompassing fine-mapping, marker-assisted breeding, and genetic engineering, consequently contributing to the effective control of FW and the improved production of chickpea.

Plain Language Summary

Chickpeas are important crops worldwide but are often threatened by a disease called Fusarium wilt (FW), which causes significant losses. Our study aimed to better understand and improve resistance to FW in chickpeas using genetic approach. By understanding and utilizing specific genetic regions, we can breed chickpeas that are both high-yielding and resistant to disease. We identified several important regions and their associated genes in the chickpea genome that contribute to resistance against FW. Moreover, using specific DNA markers, we successfully bred new chickpea lines that maintain high resistance to the disease. Our research is a significant step toward developing chickpea varieties that can resist FW more effectively. This advancement will help ensure stable chickpea production, benefiting farmers and consumers by providing a more reliable food source.

1 | INTRODUCTION

Chickpea (*Cicer arietinum* L.; $2n = 2x = 16$; genome size of ~750 Mbp) is a prominent grain legume crop that faces production limitations due to the adverse impacts of various biotic and abiotic stresses (Mannur et al., 2019; Suthar et al., 2021). Among biotic stresses, Fusarium wilt (FW), a fungal disease caused by the soilborne pathogen *Fusarium oxysporum* f. sp. *ciceri* (*Foc*) (Jamil & Ashraf, 2021), can lead to complete yield loss in chickpea under favorable conditions (Jendoubi et al., 2017). Although several management approaches, including classical breeding, have been employed to control this pathogen in chickpea (Bhar et al., 2021), their full effectiveness in addressing this challenge remains unproven. Nevertheless, recent genomic and transcriptomic advances have provided valuable insights into potential defense mechanisms (Bhar et al., 2021). To date, eight distinct physiological races (0, 1, 1B/C, 2, 3, 4, 5, and 6) have been identified in this pathogen (Mannur et al., 2019), with varying incidences of these races observed in regions with diverse climates (Pratap et al., 2017).

Several studies have reported various molecular markers associated with FW resistance in chickpea genotypes, which have been successfully utilized for marker-assisted selection (MAS)/introgression of FW resistance trait (Jingade & Ravikumar, 2015; Pratap et al., 2017; Varshney, et al., 2014). Previous research has revealed that FW resistance in chickpea is primarily controlled by a few major genes or quantitative trait loci (QTLs) (Garg et al., 2018) distributed across diverse genomic regions. Furthermore, several QTLs linked to FW resistance have been identified in various chickpea germplasms for specific FW races, including race 0 (Cobos et al., 2005; Halila et al., 2009; Jendoubi et al., 2017; Palomino et al., 2009), race 1 (Garg et al., 2018; Jingade & Ravikumar, 2015; Patil et al., 2014; Raghu, 2016; Sabbavarapu et al., 2013), race 2 (Lakmes et al., 2024), race 3 (Garg et al., 2018), and race 5 (Palomino et al., 2009).

Given the influence of factors such as population size, marker density, and sampling methods on QTL mapping, meta-QTL (MQTL) analysis is increasingly used for identifying consensus genomic regions (Daryani et al., 2022; Loni et al., 2023). MQTL analysis integrates QTL data from various populations across different studies to identify "QTL hotspot" genomic regions with narrow confidence intervals (CIs), serving as a powerful strategy to enhance MAS and genomic selection in plant breeding (Goffinet & Gerber, 2000; Khojasteh et al., 2024). This approach facilitates candidate gene discovery, eventually resulting in the development of more efficient gene-specific molecular markers associated with traits applicable in plant breeding programs (Goffinet & Gerber, 2000; Liu et al., 2020). Although MQTL analysis has been applied to address biotic resistance in various crops, such as Fusarium wilt (*Fusarium oxysporum* f. sp. *vasinfectum*) in cotton (*Gossypium hirsutum* L.), fungal disease resistance in maize (*Zea mays* L.) (Gupta et al., 2023; Zhu et al., 2022), and Fusarium head blight in wheat (*Triticum aestivum* L.) (Liu et al., 2009; Venske et al., 2019; Zheng et al., 2021), there is currently a lack of published studies on MQTL analysis for chickpea.

Utilizing genetically resistant cultivars for fungal disease control is a more environmentally friendly and sustainable strategy compared to other disease management methods

(Suthar et al., 2021). Furthermore, understanding the genetic and molecular mechanisms underlying chickpea–*Foc* interactions is essential for developing tolerant chickpea genotypes (Suthar et al., 2021). The existence of eight known pathogenic *Foc* races, each possessing specific avirulence genes (Jiménez-Díaz et al., 2015), highlights the significance of major disease-resistance (*R*) genes in managing these races in chickpea cultivars (Jendoubi et al., 2016). Suthar et al. (2021) reported that race 1 (described as 1A from an Indian source) is governed by two or three recessive genes, while other studies indicate that races 3 and 5 are regulated by a single gene (Sharma et al., 2005; Tekeoglu et al., 2000). However, classical methods have not reliably identified FW resistance genes. For instance, initial reports suggested that resistance to race 0 is monogenic (Rubio et al., 2003), but subsequent studies revealed it to be polygenic in nature (Choudhary et al., 2023; Yadav et al., 2023)

In addition to QTL mapping and classical genetics approaches, omics technologies, particularly transcriptomics, have been increasingly employed to investigate the mechanisms underlying chickpea–*Foc* interaction and to identify key regulatory genes (Bhar et al., 2021). For instance, Nimbalkar et al. (2006) identified 19 transcript-derived fragments related to gamma-glutamyl-cysteine synthetase, WRKY, and NBS-leucine-rich repeat (LRR)-type transcription factors in chickpea using the cDNA-amplified fragment length polymorphism (AFLP) technique. Furthermore, Gupta et al. (2009) reported interacting pathways of cellular processes of genes involved in sucrose synthase, isoflavonoid biosynthesis, drought stress response, serine-threonine kinases, cystatins, and arginase in chickpea infected by *Foc1*, using the cDNA-AFLP approach. Additionally, several *Foc*-related genes were identified by Gujaria et al. (2011) through cDNA-random amplified polymorphic DNA (RAPD) and semi-quantitative reverse transcriptase-polymerase chain reaction. Furthermore, a study using the suppression subtractive hybridization method reported 162 expressed sequence tags (ESTs) involved in the chickpea–*Foc4* interaction, playing a role in incompatible and compatible interactions, particularly in defense signaling pathways (Saabale et al., 2018). The molecular complexities of chickpea-resistant and susceptible cultivars during *Foc* attacks have been investigated using transcriptome profiling approaches such as long serial analysis of gene expression (LongSAGE) (Upasani et al., 2017), microarray (Ashraf et al., 2018, 2009), and RNA-seq techniques (Gupta et al., 2017; Jain et al., 2015; Kohli et al., 2014). Integrating QTL mapping and omics techniques enables comparisons across various experiments, facilitating the rapid and reliable identification of genes and gene-specific molecular markers linked to disease resistance.

In the present study, a meta-analysis of previously reported QTLs was conducted to identify consensus QTLs, referred to as MQTLs, and integrate them with other relevant data, including FW-responsive genes identified through microarray, SAGE, and RNA-seq techniques. This integration aims to enhance our understanding of FW resistance in chickpea. Subsequently, marker-assisted backcrossing (MABC) was employed to develop superior lines with enhanced FW resistance from a cross between Ana (an FW-resistant cultivar) and Hashem (an enhanced high-yielding cultivar). Evaluating 16 markers on the parents revealed four polymorphic markers located within the MQTL1, MQTL2, and MQTL6 regions. After breeding for five cropping seasons (2018–2023) applying foreground selection (FGS) and background selection (BGS), three highly resistant lines were obtained, which retained 90%–96% of the genetic background of Hashem. These lines are currently undergoing multi-location field trials to assess their yield and agronomic performance, with the potential for future release and cultivation.

Core Ideas

- Finding hotspot genomic regions related to Fusarium wilt (FW) resistance in chickpeas.
- Proposing key candidate genes associated with Fusarium wilt resistance in chickpeas.
- Introducing molecular markers for Fusarium wilt resistance in chickpeas.
- Marker-assisted backcrossing to introgress FW resistance to an improved high-yielding Kabuli cultivar.
- Producing new chickpea breeding lines with high FW resistance.

2 | MATERIALS AND METHODS

2.1 | Collection of initial QTLs associated with FW resistance

A detailed bibliographic review was conducted on 10 distinct published studies focused on QTL mapping for FW resistance in chickpea, encompassing races 0, 1, 3, and 5 from 2005 to 2024. The fundamental details of 32 QTLs extracted from these studies are listed in Table 1. The data collected for each QTL included various aspects, such as the specific resistance race, parents of population, type of population (F_2 and recombinant inbred lines [RILs]), population size (*n*), logarithm of the odds score (LOD), phenotypic variance explained by the QTLs (R^2), and the genotyping method employed (AFLP, cleaved amplified polymorphic sequences [CAPS], dCAPS, inter-simple sequence repeat [ISSR], RAPD, sequence characterized amplified region [SCAR], single nucleotide polymorphism [SNP], simple sequence repeat [SSR], sequence

TABLE 1 Quantitative trait loci (QTLs) information of resistance to some chickpea Fusarium wilt (FW) races used in the meta-analysis.

Race	QTL	Parents	Population type	Population size (*n*)	R^2	CaLG	Genotyping method	LOD score	References
0	R0_1	CA2139 × JG62	RILs ($F_{6:7}$)	79	34.8	5	ISSR, STMS, and RAPD	7.3	(Cobos et al., 2005)
	R0_2	CA2139 × JG62	RILs ($F_{6:7}$)	79	37.8	5	ISSR, STMS, and RAPD	8.2	(Cobos et al., 2005)
	R0_3	CA2139 × JG62	RILs ($F_{6:7}$)	160	26.2	2	STMS	5.4	(Halila et al., 2009)
	R0_4	CA2156 × JG62	RILs ($F_{6:7}$)	160	21.8	5	STMS	8.5	(Halila et al., 2009)
	R0_5	CA2156 × JG62	RILs ($F_{6:8}$)	80	61.2	5	STMS, SSR, SCAR, CAPS, and gene-specific	15.8	(Jendoubi et al., 2016)
	R0_6	CA2156 × JG62	RILs ($F_{6:8}$)	80	88.5	5	STMS, SSR, SCAR, CAPS, and gene-specific	37.1	(Jendoubi et al., 2016)
	R0_7	ILC3279 × JG62	RILs ($F_{6:8}$)	115	82.3	5	STMS, SSR, SCAR, CAPS, and gene-specific	32.0	(Jendoubi et al., 2016)
	R0_8	ILC3279 × JG62	RILs ($F_{6:8}$)	115	75.3	5	STMS, SSR, SCAR, CAPS, and gene-specific	31.0	(Jendoubi et al., 2016)
	R0_9	JG62 × ILC72	RILs ($F_{6:8}$)	102	69.5	5	STMS, SSR, SCAR, CAPS, and gene-specific	20.4	(Jendoubi et al., 2016)
	R0_10	*Ca* × *Cr*	RILs ($F_{6:7}$)	104	20.1	2	CAPS and dCAPS	4.0	(Palomino et al., 2009)
1	R1_1	C214 × WR315	$F_{2:3}$	188	16.4	6	SSR	8.0	(Sabbavarapu et al., 2013)
	R1_2	C214 × WR315	$F_{2:3}$	188	18.8	6	SSR	7.6	(Sabbavarapu et al., 2013)
	R1_3	JG62 × WR315	RILs ($F_{9:10}$)	125	36.0	2	STMS and AFLP	9.2	(Patil et al., 2014)
	R1_4	JG62 × WR315	RILs ($F_{9:10}$)	125	16.0	2	STMS and AFLP	3.5	(Patil et al., 2014)
	R1_5	JG62 × WR315	RILs (F_{12})	125	23.6	2	SSR	6.8	(Raghu, 2016)
	R1_6	JG62 × WR315	RILs (F_{12})	125	25.9	2	SSR	7.9	(Raghu, 2016)
	R1_7	JG62 × WR315	RILs (F_{12})	125	17.3	2	SSR	4.9	(Raghu, 2016)
	R1_8	JG62 × WR315	RILs (F_{12})	125	12.8	6	SSR	3.6	(Raghu, 2016)
	R1_9	JG62 × WR315	RILs (F_{12})	125	8.6	6	SSR	2.5	(Raghu, 2016)
	R1_10	JG62 × WR315	RILs (F_{12})	125	18.6	2	SSR	2.6	(Raghu, 2016)
	R1_11	K850 × WR315	RILs (F_{12})	141	9.5	4	SSR	2.7	(Raghu, 2016)
	R1_12	JG62 × ICCV05530	RILs (F_8)	188	24.6	2	SSR and SNP	18.5	(Garg et al., 2018)
	R1_13	JG62 × ICCV05530	RILs (F_8)	188	7.9	4	SSR and SNP	5.6	(Garg et al., 2018)
	R1_14	JG62 × ICCV05530	RILs (F_8)	188	6.6	6	SSR and SNP	4.4	(Garg et al., 2018)
	R1_15	K850 × WR315	RILs ($F_{10:11}$)	141	69.8	2	SSR	8.9	(Jingade & Ravikumar, 2015)
	R1_16	K850 × WR315	RILs ($F_{10:11}$)	141	60.8	2	SSR	4.4	(Jingade & Ravikumar, 2015)
	R1_17	JG62 × WR315	RILs ($F_{7:8}$)	125	11.89	4	STMS, SSR, SCAR, CAPS, and gene-specific	3.0	(Lal et al., 2022)
	R1_18	JG62 × WR315	RILs ($F_{7:8}$)	125	11.62	2	STMS, SSR, SCAR, CAPS, and gene-specific	3.1	(Lal et al. 2022)
	R1_19	JG62 × WR315	RILs ($F_{7:8}$)	125	26.62	2	STMS, SSR, SCAR, CAPS, and gene-specific	5.2	(Lal et al. 2022)

(Continues)

TABLE 1 (Continued)

Race	QTL	Parents	Population type	Population size (n)	R^2	CaLG	Genotyping method	LOD score	References
3	R3_1	JG62 × ICCV05530	RILs (F_8)	188	17.5	2	SSR and SNP	12.3	(Garg et al., 2018)
	R3_2	JG62 × ICCV05530	RILs (F_8)	188	31.6	4	SSR and SNP	21.0	(Garg et al., 2018)
5	R5_1	*Ca* × *Cr*	RILs ($F_{6:7}$)	104	24.2	2	CAPS and dCAPS	2.9	(Palomino et al., 2009)

Abbreviations: AFLP, amplified fragment length polymorphism; CaLG, chickpea linkage group; CAPS, cleaved amplified polymorphic sequences; ISSR, inter-simple sequence repeat; LOD, logarithm of odds; RAPD, random amplified polymorphic DNA; RILs, recombinant inbred lines; R^2, phenotypic variance explained by QTL; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STMS, sequence tagged microsatellite site.

tagged microsatellite site [STMS], and gene-specific markers) (Table 1).

2.2 | Construction of a consensus genetic map

A novel integrated consensus genetic map was constructed using the *R* package *LPmerge* (Endelman & Plomion, 2014). Initially, a high-density genetic map containing 1328 loci (Hiremath et al., 2012) was obtained from the ICRISAT website (http://cmap.icrisat.ac.in/cmap/). This map was then enriched by merging it with nine additional genetic maps, resulting in a cumulative count of 3914 loci. The supplementary maps comprised Gujaria_300 (Gujaria et al., 2011), Jaganathan_GBS-SNP_1007 (Jaganathan et al., 2015), Mallikarjuna_Consensus_109 (Mallikarjuna et al., 2017), Millan_229 (Millan et al., 2010), Nayak_521 (Nayak et al., 2010), Thudi_1291 (Thudi et al., 2011), Transcript map (Seqclone) (Unknown reference), Varshney_352 (Varshney et al., 2014), and Garg_111 (Garg et al., 2018).

2.3 | QTL projection, meta-analysis, and overview index

After estimating the 95% CI for the initial QTLs on their original genetic map, the QTLs were projected onto the consensus map using BioMercator v4.2 (Table S1). To determine the 95% CI for the initial QTLs, the standard formula proposed by Darvasi and Soller (1997) was applied separately for the F_2 and RILs populations, as follows:

$$95\%\,\mathrm{CI}\left(\mathrm{F}_2\ \mathrm{population}\right) = \frac{530}{N \times R^2} \tag{1}$$

$$95\%\,\mathrm{CI}\ (\mathrm{RILs\ population}) = \frac{163}{N \times R^2} \tag{2}$$

where N is the size of the F_2/RILs population and R^2 is the proportion of variance explained by the QTL.

A meta-analysis was performed on the QTL-harboring linkage groups (LGs) following the standard procedure outlined in BioMercator V4.2 (Arcade et al., 2004; Goffinet & Gerber, 2000). The optimal meta-analysis model was selected based on the Akaike information criterion (AIC) values. Two methods for the QTL meta-analysis were considered, depending on whether the initial number of QTLs was 10 or more. Since the number of original QTLs in a specific region was smaller than 10, the meta-analysis was performed using the Gerber and Goffinet method in BioMercator v4.2. The model with the minimum AIC was chosen to determine the consensus MQTL positions amongst five MQTL models (1, 2, 3, 4, or

N) produced by BioMercator v4.2. Finally, the LGs and QTL positions were visualized using MapChart ver. 2.32 (Voorrips, 2002) and Circos software (Yu et al., 2018), and MQTL-related graphs were drawn using the *R* program (Krzywinski et al., 2009).

Following the meta-analysis, the QTL-overview index was calculated to assess the density distribution of QTLs associated with Fusarium disease traits in chickpea on each LG, based on the equation proposed by Chardon et al. (2004):

$$p\ (x,\ x+0.5) = \frac{\sum_{i=1}^{\text{nbQTL}} \int_{x}^{x+0.5} N\left(p_i, S_i^2\right) d(x)}{\text{nbE}} \quad (3)$$

where nbQTL represents the number of QTLs, *N* denotes the number of 0.5 cM segments on each LG, and Si^2 is the variance position of the individual QTLs on an LG (calculated as $S_i^2 = [\text{CI}/2 \times 1.96]^2$), $d(x)$ is the differential of variable x, and nbE indicates the total number of experiments. The "*pnorm*" function in *R* software was utilized to derive the QTL-overview statistics through a step-by-step calculation of the uniform probability of a 0.5-cM long segment (Daryani et al., 2022).

The average number of QTLs per experiment, represented as nbQTL/nbE, was calculated. This value represents the uniform probability that a segment spanning from x to $x + 0.5$ cM contains a QTL in a specific experiment. To pinpoint genomic regions with a significantly elevated density of QTLs, we utilized the following formulas to compute the $U(x)$ value, which serves as the mean statistic defining the position of "*real QTLs*," and the $H(x)$ value, which establishes the threshold for identifying "*hotspot*" region (Khojasteh et al., 2024):

$$\text{Mean statistic of peak} = U\ (x) = \frac{\text{nbQTL/nbE}}{\text{The total length of map}} \times 0.5 \quad (4)$$

$$\text{Threshold for high values} = H\ (x) = 5 \times U\ (x) \quad (5)$$

2.4 | Identification of FW-responsive genes in chickpea

The differentially expressed genes (DEGs) in response to FW in chickpea were identified based on the previous studies, including one LongSAGE analysis (Upasani et al., 2017), two microarray studies (Ashraf et al., 2018, 2009), and two RNA-seq studies (Gupta et al., 2017; Taxak et al., 2017). For RNA-seq studies, in cases where de novo assembly had been used for analysis, reference-based RNA-seq re-analysis was conducted according to the TopHat/Cufflinks pipeline (Trapnell et al., 2012), utilizing the chickpea reference genome (GCF_000331145.1).

2.5 | Identification of candidate genes

To propose candidate genes associated with chickpea resistance to FW, a series of steps were taken. First, the sequences of the flanking markers of the MQTLs were obtained from the Pulsedb database (https://www.pulsedb.org/find/markers) and subjected to a basic local alignment search tool (BLAST) search in the National Center for Biotechnology Information (NCBI) database to determine their physical locations. Subsequently, the genes located between flanking markers of MQTLs were extracted from the *C. arietinum* genome using the Phytozome13 BioMart tool (https://phytozome-next.jgi.doe.gov/biomart). The identified DEGs were then compared with the genes located within the MQTL regions. Finally, a Venn diagram was generated using InteractiVenn (Heberle et al., 2015).

2.6 | MapMan analysis

MapMan (version 3.5.1; http://mapman.gabipd.org/web/guest) was utilized for pathway analysis of the 98 genes that were both stress-responsive (based on transcriptome studies) and located within MQTL regions, applying a cutoff of p-value ≤ 0.05. Mapping the selected genes onto the pathways of *Medicago truncatula*, a model legume, facilitated the identification of the genes associated with specific pathways (Thimm et al., 2004).

2.7 | Marker-assisted backcrossing to introgress resistance to FW

2.7.1 | Parent materials

The Kabuli chickpea cultivar Ana, which is FW-resistant, was selected as the donor parent, while Hashem, an improved high-yielding Kabuli cultivar, was chosen as the recurrent parent (Figure S1). Despite its high yield potential, the cultivation of Hashem has been limited due to its sensitivity to FW disease.

2.7.2 | Backcross breeding

The breeding process occurred during five cropping seasons (2018–2023). In the first year, Ana (the donor parent) and Hashem (the recurrent parent) were grown in the crossing blocks located in the greenhouse of the Dryland Agricultural Research Institute (DARI) of Iran. The crossing operation was performed between the selected parents (Figure S2), and the F_1 progeny were backcrossed with Hashem to produce the

BC_1F_1 (where BC is backcross) generation. Through three backcrosses and one round of selfing, the BC_3F_2 progeny was obtained.

2.7.3 | Foreground selection

To screen for lines carrying alleles of FW resistance, 16 linked molecular markers previously reported to be associated with FW resistance genes (Table S2) were evaluated in the parents. Four polymorphic markers between the parents (TA59, TA96, TR19, and CaM1402) were selected for genotyping.

2.7.4 | Background selection

Sixteen SSR markers were used to identify the progenies with the greatest similarity to the genetic background of the recurrent parent, Hashem. These markers were selected from a pool of 42 chickpea SSR markers (Halila et al., 2009; Varshney et al., 2014) that displayed the highest levels of polymorphism between the parents.

2.7.5 | DNA extraction and polymerase chain reaction

All the progenies in each generation were cultured. The shoot samples were collected for DNA extraction using the Cetyltrimethylammonium bromide method (Doyle, 1991). PCR was performed in a total volume of 15 μL, and the resulting product was evaluated using 2.5% agarose gel electrophoresis.

2.7.6 | Phenotyping for FW resistance

The selected lines of the BC_3F_2 generation were phenotypically evaluated for FW disease resistance in a growth chamber. Chickpea plants at the 4–6 leaf stage were grown in pasteurized perlite, and the root tips were excised using sterile scissors. The plants were then gently immersed in the FW spore suspension (500,000 spores/mL) for a few seconds. After inoculation, the plants were transferred back to pots and maintained in a growth chamber for 2 months at 25 ± 2°C under a 12-h light/12-h dark photoperiod with a light intensity of 300 μmol/m^2/s. The disease symptoms appeared gradually from the second week after inoculation in susceptible plants. The mortality rate was recorded twice using the approach proposed by Sharma et al. (2005): once in the third and fourth weeks for wilting races, and again in the fifth and sixth weeks for yellowing races post-inoculation.

3 | RESULTS

3.1 | Meta-QTL analysis

3.1.1 | Consensus map construction

A comprehensive consensus genetic map was developed, comprising 3914 markers, including SSR, Diversity Arrays Technology, EST-SSR, RAPD, SNP, CAPS, AFLP, and STMS (Table S1). This consensus map spanned a total length of 811.9 cM, with individual LGs varying in length from 72.1 to 116.6 cM across eight chickpea LGs. The number of markers per LG varied, with CaLG04 containing the highest marker count at 930, while CaLG8 had the lowest, with only 261 markers. The marker density across the LG ranged from 3.2 to 9.9 markers per cM (Figure 1a).

3.1.2 | Major features of initially mapped QTLs

A meta-analysis of 32 initial QTLs within the chickpea LGs revealed an uneven distribution of the compiled QTLs across four out of the eight chickpea LGs. The highest number of QTLs (15 QTLs) was found on CaLG02, followed by CaLG05, CaLG06, and CaLG04, which contained eight, five, and four QTLs, respectively (Figure 1b; Table S3). The majority of the initial QTLs were associated with FW race 1 (19 QTLs), while race 0 accounted for 10 QTLs, race 3 had two QTLs, and race 5 had one QTL (Figure 1b). The initial QTLs were derived from a total of 13 chickpea populations, comprising 12 RIL populations and one F_2 population, with mapping population sizes ranging from 79 to 188 individuals (Table 1). Notably, a significant proportion of these populations were generated using either WR315 (a FW-resistant cultivar) or JG62 (a FW-susceptible cultivar) as one of the parental lines (Table 1).

The 95% CIs of the initial QTLs ranged from 1.66 to 17.19 cM, with an average of ~6.82 cM, whereas six of the 32 QTLs had CIs lower than 2.5 cM (Figure 2a). Moreover, the proportion of phenotypic variance explained (PVE or R^2) by the initial QTLs ranged from 6.63% to 88.5%, while 25 out of these QTLs (~87.5%) exhibiting R^2 values greater than 10% (Figure 2a; Table 1). Furthermore, FW race 0 demonstrated the highest PVE at 88.5%, while FW race 1 displayed the lowest 95% CI (Table 1; Table S3).

3.1.3 | Meta-analysis of QTLs

The projection and meta-analysis of the 32 QTLs on the genetic reference map resulted in the identification of seven MQTLs across four LGs (CaLG02, CaLG04, CaLG05, and

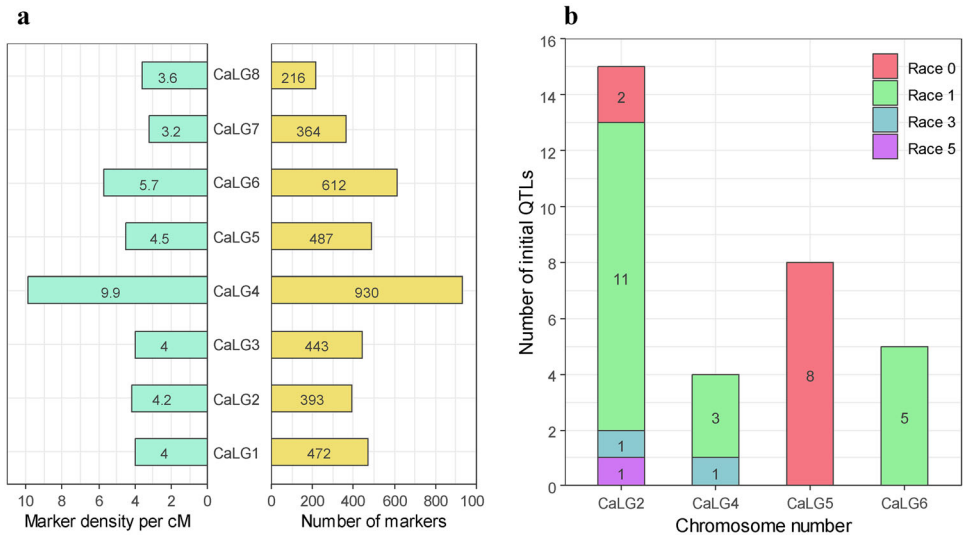

FIGURE 1 Summary of the 32 initial quantitative trait loci (QTLs) associated with Fusarium wilt (FW) resistance used in the QTL meta-analysis: (a) The number of markers and marker density on the chickpea linkage groups (LGs) based on the consensus map used for meta-QTL analysis. (b) Distribution of initial QTLs per LG and their association with each FW race.

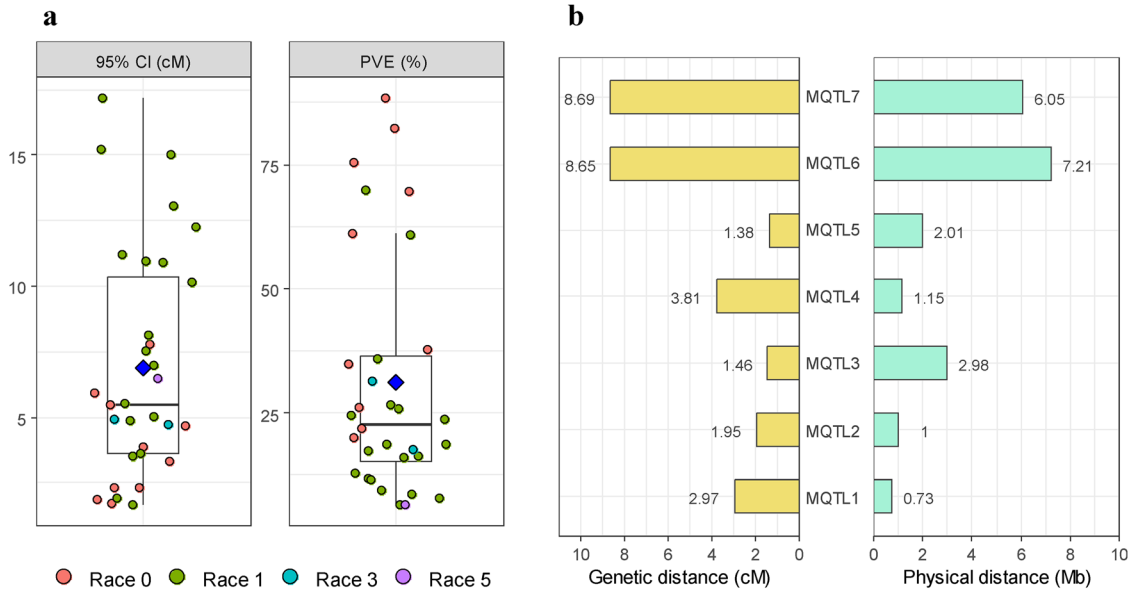

FIGURE 2 Distribution of initial quantitative trait loci (QTLs) and meta-QTLs (MQTLs) associated with Fusarium wilt (FW) resistance in chickpea. (a) Confidence interval (CI (cM);) and the proportion of phenotypic variation explained (PVE (%)) for the 29 surveyed QTLs. (b) The genetic and physical distance of the MQTLs on the chickpea consensus map for FW resistance.

CaLG06) in chickpea (Table 2). CaLG02 contained three MQTLs: MQTL1, MQTL2, and MQTL3, with CIs of 2.87, 1.95, and 1.16 cM, respectively (Figure 2b). In contrast, CaLG04 and CaLG05 each harbored a single MQTL, with CIs of 3.81 and 0.91 cM, respectively. Moreover, two MQTLs, MQTL6 and MQTL7, were identified on CaLG06, both associated with FW race 1 and exhibiting CIs of 8.55 and 8.46 cM, respectively. The initial QTLs linked to MQTL1 were associated with FW races 1 and 3, while the QTLs in MQTL2 were related to races 0, 1, and 5 of FW. MQTL3, MQTL6, and MQTL7 exclusively included QTLs that control resistance to race 1. MQTL4, located on CaLG04, contained QTLs associated with resistance to both races 1 and 3. Additionally, CaLG05, which housed MQTL5, was exclusively composed of QTLs linked to race 0 (Figure 1b; Table 2). The number of initial QTLs varied across the MQTLs, with MQTL1 and MQTL7 containing two QTLs each, while MQTL5 included eight QTLs (Table 2). The PVE by these MQTLs exceeded 10%, ranging from 10.5% in MQTL7 to 58.9% in MQTL5.

TABLE 2 Results of the meta-analysis of quantitative trait loci (QTLs) controlling fusarium resistance in chickpea.

LG	Meta QTL	AIC value	Linked to race(s)	Map position (cM)	No. of initial QTLs	Mean R^2 (%)	L-marker	L-marker position (cM)	R-marker	R-marker position (cM)	No. of studies	Mean CI of initial QTLs	No. of genes
CaLG02	MQTL1	69.71	*Foc1*, *Foc3*	40.01	2	21.0	marker8262	38.52	TA103	41.49	1	4.24	66
	MQTL2		*Foc0*, *Foc1*, *Foc5*	48.02	7	23.0	CAMCTA07	47	TR14	48.95	4	6.58	78
	MQTL3		*Foc1*	53.92	6	36.0	CKaM0759	53.13	CKaM1333	54.59	2	4.77	190
CaLG04	MQTL4	17.55	*Foc1*, *Foc3*	40.73	4	16.3	Pyruvat_Kin_oben	38.91	NCPGR231	42.72	3	9.22	72
CaLG05	MQTL5	19.63	*Foc0*	81.31	8	58.9	CaM0805	81.19	CaM0258	82.57	3	3.45	73
CaLG06	MQTL6	29.36	*Foc1*	47.99	3	11.9	H3A052	43.67	TA80	52.32	2	15.1	394
	MQTL7		*Foc1*	86.11	2	10.5	TOG908356	81.82	TOG899657	90.51	1	12.7	346

Abbreviations: AIC, Akaike information criterion; CaLG, chickpea linkage group; CI, confidence interval; LG, linkage group; MQTL, meta-QTL.

In general, the genetic distances of the identified MQTLs were correlated with their corresponding physical distances in the chickpea genome (Figure 2b). Notably, MQTL5 exhibited the lowest genetic distance at 0.91 cM, whereas MQTL1 had the least physical distance at 0.73 Mb (Figure 2b). As mentioned in the previous section, the lowest CI for the initial QTLs was 1.66 cM. Interestingly, the average CI for the identified MQTLs was significantly lower, approximately 1.63 times less than that of the initial QTLs (Figure 2a,b).

3.1.4 | QTL-overview index

Following the projection of 29 QTLs onto the consensus genetic map, the QTL density, referred to as the "QTL-overview index," was calculated for a designated distance of 0.5 cM on each LG corresponding to the genomic regions related to fusarium resistance traits (Figure 3). Among the obtained peaks of the QTL-overview index, seven peaks exceeded the average index of 0.018 across the genome, suggesting that these QTLs significantly influence FW resistance traits in chickpea. Notably, four peaks exhibited a higher value of 0.090, surpassing the high-value threshold, and therefore can be considered "QTL hotspots" (Figure 3). The number of significant peaks varied, with CaLG04 and CaLG05 each having one peak, while CaLG02 exhibited three peaks. The identified QTL hotspots for FW resistance in chickpea included MQTL1, MQTL2, MQTL3, and MQTL5 (Figure 3):

The distribution of the initial QTLs and MQTLs across the chickpea genome is shown in Figure 4, which was confirmed by the QTL overview index and offers a comprehensive perspective on the genomic regions controlling FW resistance in chickpea. Among the chickpea LGs, CaLG02 displayed the highest number of QTLs and MQTLs, followed by CaLG6, CaLG04, and CaLG05 (Figure 4). Notably, MQTL5 contains eight initial QTLs and is flanked by the CaM0258 and CaM0805 markers (Figure 4).

Figure 5 displays the previously reported molecular markers associated with FW resistance that are specifically linked to the MQTLs. Notably, there was a significant enrichment of linked markers on CaLG02, providing further evidence for the crucial role of this LG in controlling FW resistance. Additionally, the density of the reported markers was found to be significantly higher among the identified MQTLs.

3.2 | Candidate genes associated with FW resistance in chickpea

Candidate genes were detected by comparing the genes located within the MQTL regions (1219 genes; Table S4), with the DEGs identified through the LongSAGE method (767 genes; Table S5), microarray analysis (833 genes; Table S6), and RNA-seq (1877 genes; Table S7) (Figure 6).

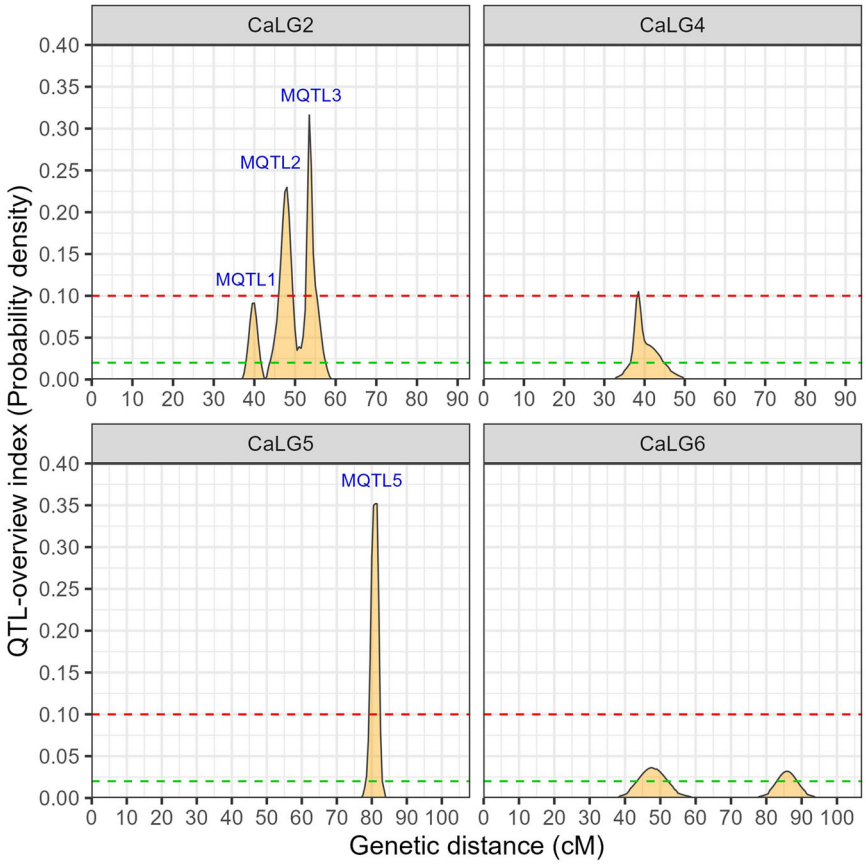

FIGURE 3 The quantitative trait locus (QTL)-overview index for Fusarium resistance trait on the chickpea consensus genetic map. The two dashed lines indicate the average index and high-value threshold, respectively. The labels at the top denote the locations of the identified "QTL hotspot." MQTL: meta-QTL.

Interestingly, 161 genes, including protein-coding genes, long non-coding RNA (lncRNA), and pseudogenes, were found to be both responsive to FW and located within MQTL regions (Table S8; Figure S3). Among these, two genes, *LOC101504229* and *LOC101495836*, located on CaLG02 within MQTL3 and MQTL1, respectively, were consistently recognized as FW-responsive genes across all transcriptome analysis approaches. *LOC101504229* encodes a putative disease resistance protein in *C. arietinum*, and *LOC101495836* is a pathogenesis-related (PR) gene that functions as a transcriptional activator and PTI6-like gene (Table S8).

To further understand the biological function of the 161 common genes, MapMan visualization (Figure S4) was performed to identify FW resistance-related pathways. FW infection in chickpea cells is likely to activate the LRR pathway, which subsequently transmits signals to the nucleus via the auxin signaling pathway and mitogen-activated protein kinase (MAPK) genes. In the nucleus, transcription factors such as WRKYs and NACs regulate the expression of target genes involved in reactive oxygen species production, PR proteins, and lncRNAs (Table S8; Figure 7).

Mapping the common genes onto the stress response pathway overview using MapMan revealed 67 out of the 161 genes which were visually represented on the created graph (Figure S3). The results indicated that chickpea cells respond to FW by regulating genes encoding PR proteins (defense genes), transcription factors such as EFR, WRKY, and bZIP, as well as genes involved in redox reactions, MAPK signaling, secondary metabolite production, proteolysis, and cell wall processes (Figure S4; Table S9).

3.3 | Marker-assisted breeding

3.3.1 | Marker-assisted backcrossing to develop FW-resistant cultivars

To produce the F_1 generation, a cross was made between two parents, Ana (as the donor parent) and Hashem (as the recurrent parent). Hybridization was performed on approximately 120 flowers (Figure S2), resulting in 50 seeds. Among these, around 20 F_1 plants were identified as true hybrids, not derived from self-pollination. These F_1 plants were subsequently backcrossed with Hashem to produce the BC_1F_1 generation. In the first crossing round, 98 seeds were obtained from a total of 210 crosses made in the greenhouse and field, of which 35 progenies were recognized as true hybrids.

To identify the polymorphic markers that differentiate the FW-susceptible and FW-resistant parents, a set of 16 selected markers was examined for both parents (Figure S5a). From this set, four markers (TR19, TA59, TA96, and CaM1402) located in MQTL1, MQTL2, and MQTL6 were selected

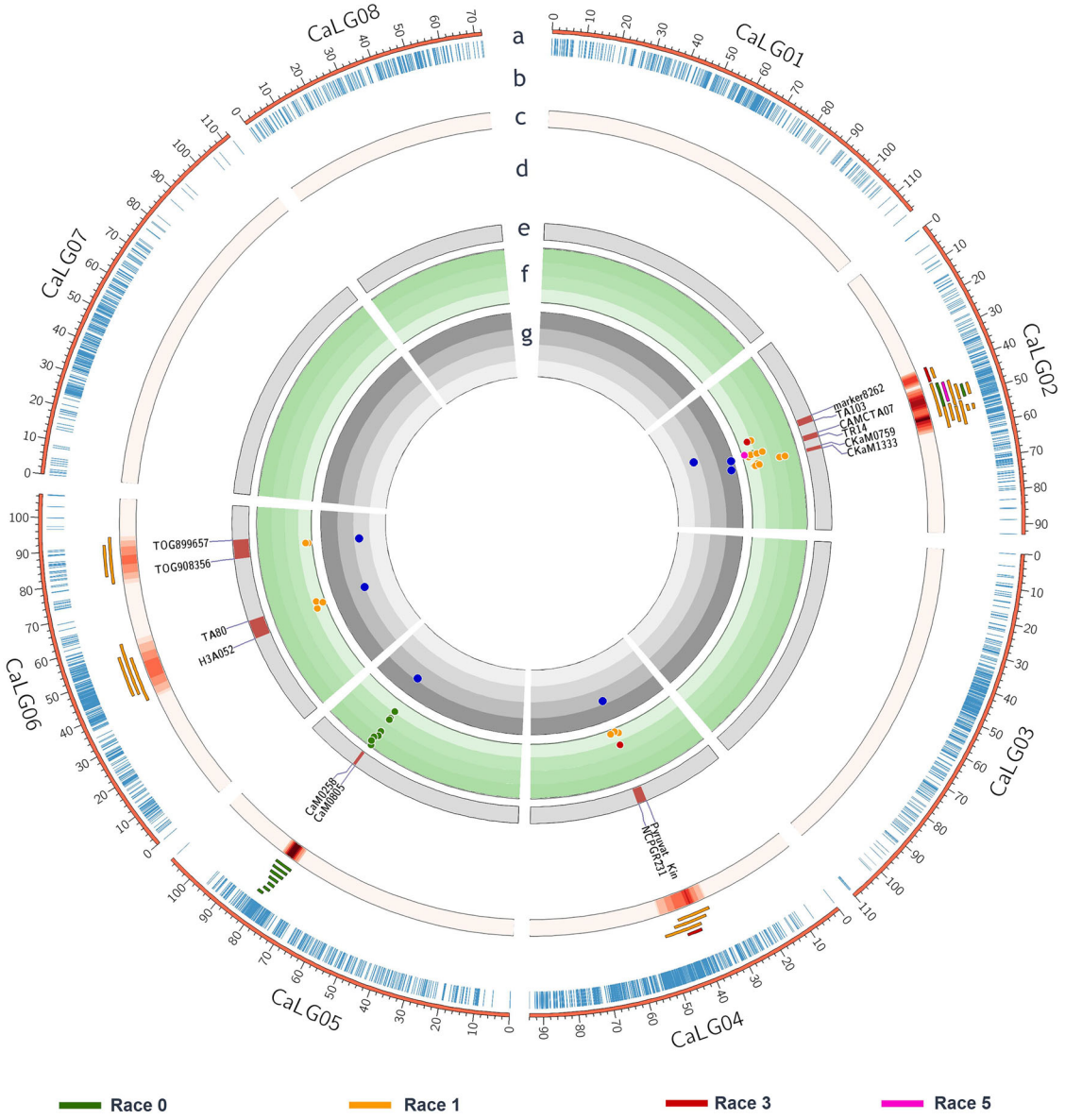

FIGURE 4 Genetic circular plot depicting quantitative trait loci (QTLs) and meta-QTLs (MQTLs) associated with resistance to Fusarium wilt (FW) in chickpea: (a) The eight chickpea linkage groups (CaLGs) are arranged in a clockwise manner and represented as red bars, indicating the molecular marker density on each linkage group (LG). (b) The distribution pattern of QTL across the eight chickpea linkage groups reveals specific resistance against different FW races (race 0, race 1, race 3, and race 5). (c) The QTL-overview index is presented on the consensus genetic map, based on segments measuring 0.5 cM in length. (d) Flanking markers identified within FW-associated meta-QTL regions in chickpea. (e) The genetic positions of MQTLs for FW resistance, with a 95% confidence interval (CI). (f) The proportion of phenotypic variance explained (R^2) by each QTL. (g) The fold reduction in the genetic 95% CI from the mean of the original QTLs to the MQTLs.

(Figure S5b; Figure 5). The CaM1402 marker was located on CaLG06, while the remaining three markers were positioned on CaLG02. Previous reports have indicated their close association with FW resistance genes (Varshney et al., 2014). These markers have been successfully utilized in developing resistant lines against races 1, 2, 3, and 4 (Mannur et al., 2019; Pratap et al., 2017; Varshney et al., 2014). In the present study, FGS of the progenies was conducted using these four markers. Among the BC_1F_1 progenies, six genotypes exhibited all four resistance alleles and were selected to produce the BC_2F_1 generation (Table S10). In the second backcrossing round, a total of 400 crosses were made between the six selected BC_1F_1 genotypes and the recurrent parent (Hashem) under both greenhouse and field conditions, yielding 120 seeds, of which 52 were recognized as true hybrids. Based on the FGS of BC_2F_1 progenies, 12 genotypes with all four resistance alleles were selected. To assess their similarity to the recurrent parent, these genotypes were evaluated using 16 SSR markers (BGS) to determine the recovery percentage of the recurrent parent's genetic background

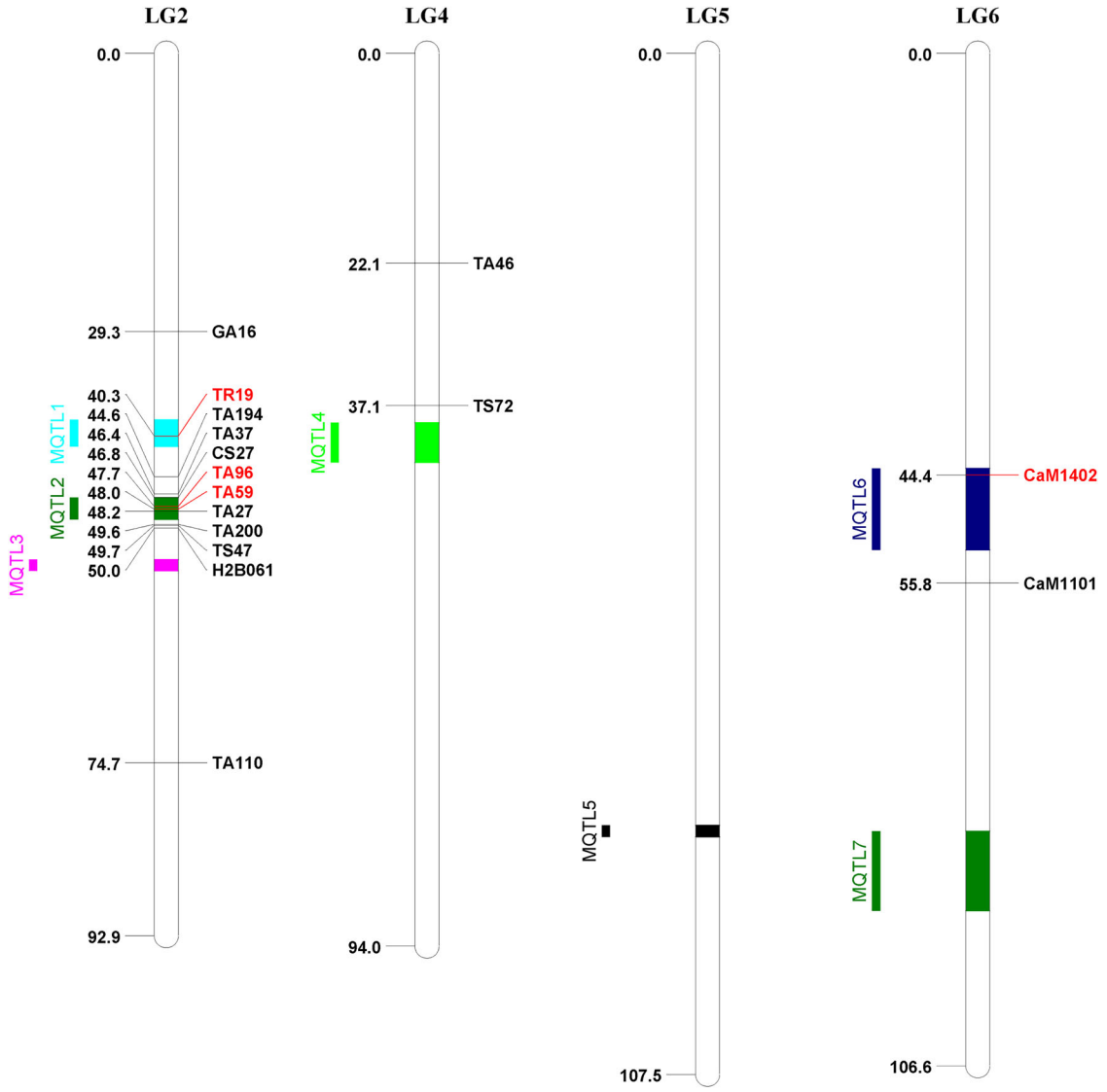

FIGURE 5 Meta-quantitative trait loci (QTLs) for Fusarium wilt resistance, along with the 16 reported linked markers, are shown on the chickpea genetic map. Four markers (TR19, TA59, TA96, and CaM1402) located in the MQTL1, MQTL2, and MQTL6 regions (where MQTL is meta-QTL), which were able to distinguish the FW-susceptible parent from the resistant one, are highlighted in red color. LG, linkage group.

(Figure S6). The results indicated that five BC_2F_1 genotypes exhibited the highest similarity to the recurrent parent, retaining approximately 86%–95% of the genetic background. Subsequently, these progenies were subjected to a third backcrossing round to produce the BC_3F_1 generation with the Hashem cultivar. During this stage, 420 crosses were conducted between five BC_2F_1 genotypes and the recurrent parent under greenhouse and field conditions, resulting in 150 seeds. Among these, 65 seeds were recognized as true hybrids and considered as BC_3F_1 progenies. Further analysis of the FGS and BGS of the BC_3F_1 progenies led to the selection of 11 and 6 genotypes, respectively, which harbored resistant alleles and exhibited the highest genetic similarity to the recurrent parent (with a recovery rate of 89%–97% of the genetic background of the recurrent parent).

The six selected genotypes of the BC_3F_1 generation were self-pollinated to stabilize the genetic background and generate a homozygous population, resulting in the production of 142 seeds. The genotypes of the BC_3F_2 generation were evaluated using FGS and BGS markers, leading to the identification of 12 genotypes with homozygous resistance genes. Among these, three genotypes exhibited a recovery of 90%–96% of the genetic background of the recurrent parent (Table S10).

3.3.2 | Phenotyping to confirm the FW resistance of selected BC_3F_2 lines

To confirm the FW resistance of the selected lines developed through MABC, the resulting lines were evaluated using the

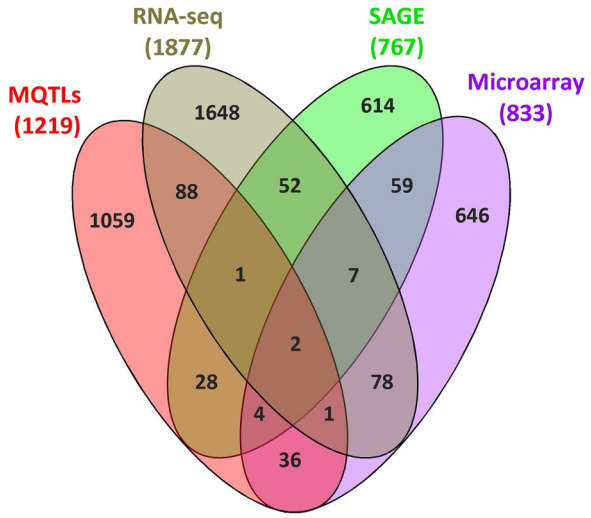

FIGURE 6 Venn diagram representing the common and unique genes identified through meta-QTL (MQTL) analysis and transcriptomic data (SAGE, microarray, and RNA-seq).

method proposed by Sharma et al. (2005). The three selected lines demonstrated resistance to FW, exhibiting minimal disease symptoms with an average disease index of 0%–20% (Table S11; Figure S7).

4 | DISCUSSION

Plants are continuously exposed to biotic stresses, leading to changes in plant metabolism and resulting in physiological damage that ultimately impairs productivity (Gimenez et al., 2018). Resistance to biotic stress in chickpea is a complex trait involving quantitative inheritance and the regulation of multiple genes located at different loci. Among these stressors, FW is a globally recognized threat, causing significant negative impacts on chickpea crop yields and quality. FW is primarily caused by *F. oxysporum*, the most economically damaging species within the Fusarium genus that infects chickpea varieties (Zhou et al., 2021). The genetic control of FW resistance is associated with specific regions of the chickpea genome, and the genetic mechanisms governing this resistance are complex, and characterized by substantial genetic diversity. The use of chickpea lines that exhibit resistance to specific fungal strains, combined with molecular markers closely linked to candidate genes associated with stress resistance, has proven effective in developing new varieties with enhanced resistance (Mannur et al., 2019; Varshney et al. 2013). However, a comprehensive review and detailed assessment are essential to consolidate the wealth of information on candidate genes and clarify the specific genes and mechanisms involved in FW resistance in chickpea (Bhar et al., 2021).

Despite the existence of numerous QTL mapping studies, only a limited number have been implemented in marker-assisted breeding programs (Cobb et al., 2018). This can be attributed to several factors, including (i) the identification of minor QTLs with low proportion of phenotypic variance explained (PVE) in most mapping studies (Bilgrami et al., 2023); (ii) the significant influence of environmental factors on the expression of QTLs (Daryani et al., 2024); and (iii) the potential for differing chromosomal estimations for the position of a single QTL across independent experiments (Goffinet & Gerber, 2000).

4.1 | MQTLs identified for FW in chickpea

Over the last three decades, numerous studies have focused on the mapping of QTLs for FW resistance in chickpea. However, the comparability and transferability of QTL results are hindered by genetic variability, the use of diverse molecular markers, and inconsistencies in genetic linkage maps. Moreover, the large CIs associated with most QTLs create challenges for their effective use in MAS (Loni et al., 2023). MQTL analysis has emerged as a powerful technique to address these limitations. By integrating QTL data from diverse genetic backgrounds and environments, MQTL analysis identifies consistent and reliable QTLs. This method improves the robustness of QTL findings, resolves discrepancies in individual studies, and increases the statistical power to detect significant QTLs. In addition to improving the reliability of QTL mapping, MQTL analysis also offers the potential for identifying QTLs that may have been overlooked or underestimated in individual studies. Through the integration of data from diverse sources, genome-wide association studies, transcriptomics analysis, and microarray data, MQTL analysis offers an enhanced statistical power to identify QTLs that consistently exhibit effects across various genetic backgrounds and environments (Bilgrami et al., 2023). This comprehensive approach enhances our understanding of the genetic basis of complex traits and provides valuable insights for applications such as MAS and crop improvement programs.

To date, various QTL mapping experiments have been conducted to elucidate the genetic mechanisms underlying resistance to FW races in different chickpea populations (Jingade & Ravikumar, 2015; Palomino et al., 2009; Patil et al., 2014; Raghu, 2016; Sabbavarapu et al., 2013). In the present study, QTLs associated with FW resistance were compiled and used for meta-analysis, which resulted in the identification of seven MQTL regions. MQTL1 and MQTL4 were associated with races 1 and 3; MQTL2 was linked to resistance against races 0, 1, and 5 of FW; MQTLs 3, 6, and 7 contained QTLs related to race 1; and MQTL5 was associated with race 0.

This study represents the first report of MQTL identification for FW in chickpea, providing significant advancements

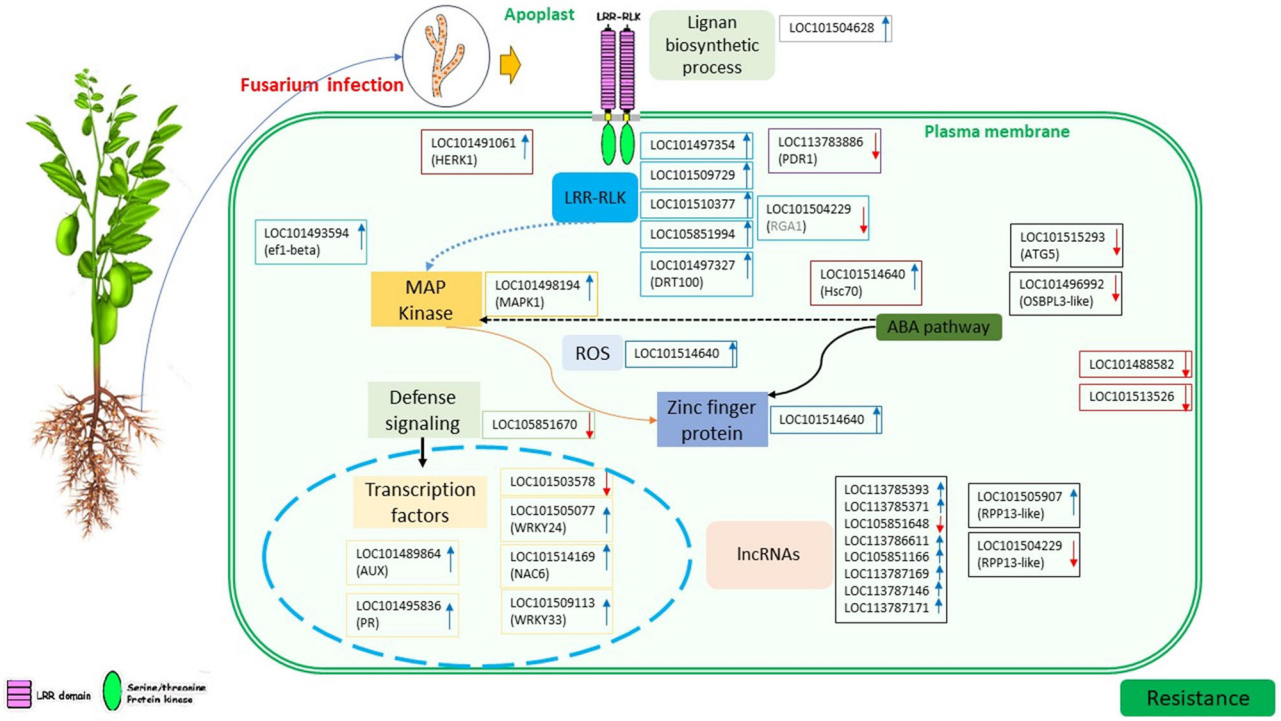

FIGURE 7 A schematic representation of the key Fusarium wilt (FW)-responsive genes in chickpea. lncRNAs, long non-coding RNAs; LRR, leucine-rich repeat; MAP, mitogen-activated protein; ROS, reactive oxygen species.

in understanding this complex trait. Comparatively, 15 MQTLs associated with FW resistance have been reported in upland cotton (Huo et al., 2023), and six MQTLs of FW were identified in Chinese wheat (Cai et al., 2019).

The findings of this study revealed that 32 initial QTLs and seven MQTLs were unevenly distributed across the chromosomes (Figure 4), verifying some previously reported closely linked molecular markers (Jingade & Ravikumar, 2015; Pratap et al., 2017; Varshney et al., 2014). Most of these markers were located on CaLG2, CaLG4, CaLG5, and CaLG6, aligning with the initial QTLs and the MQTLs identified in this study (Figure 4). For instance, Lal et al. (2022) reported that among 42 markers analyzed, 12 were linked to FW resistance, including TA96 and TA59, which were consistently reported in earlier studies (Cobos et al., 2005; Sharma et al., 2005; Winter et al., 2000).

4.2 | Candidate genes for targeted breeding

By integrating the results of MQTL analysis and transcriptomic data, this study identified 161 candidate genes associated with FW resistance (Table S8). Resistance mechanisms involve a cascade of events, including pathogen recognition, signal transduction, transcriptional regulation, and activation of defense responses (Li et al., 2020). In this process, LRR-containing domain genes play a pivotal role in initiating plant defense by detecting pathogens (Ng & Xavier, 2011). Notably, several LRR-RLK genes, such as *LOC101497354*, *LOC101509729*, *LOC101510377*, *LOC105851994*, and *LOC101497327*, were identified within the MQTL regions in this study, and their expression was found to be responsive to FW based on the transcriptomic data.

Furthermore, some signaling genes like MAPK1 (e.g., *LOC101498194*) and defense signaling genes (e.g., *LOC105851670*) emerged as candidates, potentially involved in transmitting signals to the nucleus. Several transcription factor-encoding genes, such as *NAC6* (*LOC101514169*), *WRKY24* (*LOC101505077*), and *WRKY33* (*LOC101509113*), were both upregulated in response to FW and located within the identified MQTL regions (Figure 7). WRKY transcription factors are known for their critical role in plant innate immune signaling, particularly in regulating defense-related genes through binding to their promoters (Rushton et al., 2010). Previous studies have highlighted the exclusive induction of certain WRKY genes in FW-resistant plants upon infection treatment (Chakraborty et al., 2018; Nimbalkar et al., 2006). Similar findings were reported in a previous study (Garg et al., 2013), which identified MAPK, WRKY, and NBS-LRR genes as key contributors to FW resistance in chickpea.

Non-coding RNAs (*LOC113785393*, *LOC113785371*, *LOC105851648*, *LOC113786611*, *LOC105851166*, *LOC113787169*, *LOC113787146*, and *LOC113787171*) were also identified, indicating potential regulatory roles in FW resistance. In addition, among the candidate genes,

a putative disease resistance protein (*LOC101504229* on MQTL1) and a PR protein-encoding gene (*LOC101495836* on MQTL3) were located on high-overview-index MQTLs (MQTL1 and MQTL3, respectively), which may serve as valuable targets for MAS. Previous studies have reported the accumulation of PR proteins in response to *Foc* pathogen exposure in different genotypes of chickpea genotypes (Saabale et al., 2018; Upasani et al., 2017). Consequently, this information can facilitate the identification of closely linked and/or gene-specific molecular markers for use in chickpea breeding programs.

4.3 | Producing BC_3F_1 FW-resistant lines

MAS has proven to be a powerful tool in plant breeding, enabling the identification and validation of molecular markers associated with desirable traits. For FW resistance in chickpea, identifying molecular markers linked to resistance genes is critical for implementing effective breeding programs (Lal et al., 2022). In this study, 16 candidate markers associated with FW resistance genes (Table S2) were evaluated using parental genotypes to identify polymorphic variations that differentiate the FW-resistant genotype (Ana) from the susceptible genotype (Hashem). From this analysis, four markers (TA59, TA96, TR19, and CaM1402) located within MQTL1, MQTL2, and MQTL6 were selected for genotyping.

The introgression of FW genetic resistance into high-yielding but susceptible chickpea cultivars holds great promise for managing this devastating disease. MABC was employed to transfer FW resistance from the highly resistant donor genotype (Ana) into Hashem, an improved high-yielding Kabuli cultivar. This approach allowed for the precise transfer of genomic regions associated with FW resistance while maintaining the favorable agronomic traits of the recurrent parent.

Finally, through the application of MABC, three FW-resistant lines were successfully developed, each retaining a recurrent parent genome ranging from 90% to 96%. These lines represent valuable genetic resources and are well-suited for multi-location trials to assess their yield potential and other critical agronomic traits. The comprehensive evaluation of these lines across diverse agro-climatic conditions will provide crucial insights into their adaptability and stability, supporting the decision-making process for their potential release and widespread cultivation.

5 | CONCLUSION

The findings of this study provide a framework for deeper understanding and improving chickpea resistance to FW by identifying key genomic regions, genes, and molecular markers. The results confirmed that FW resistance is governed by multiple regions of the chickpea genome. MQTL analysis revealed that CaLG02, CaLG04, and CaLG06 are associated with controlling different FW races, while CaLG05 is specifically linked to race 5. The genetic distances of the MQTLs generally aligned with their physical distances on the chickpea genome, with MQTL5 exhibiting the smallest genetic distance and MQTL1 showing the least physical distance. Furthermore, transcriptomic data validated several genes within the identified MQTL regions, suggesting their significant role in conferring FW resistance in chickpea. The study also successfully developed FW-resistant chickpea lines through MABC. Overall, this study enhances our understanding of the genetic mechanisms underlying FW resistance in chickpea and provides valuable resources and insights for breeding programs aimed at developing improved cultivars with enhanced resistance to this devastating disease.

AUTHOR CONTRIBUTIONS

Jahad Soorni: Data curation; formal analysis; investigation; visualization; writing—original draft. **Fatemeh Loni**: Formal analysis; investigation; methodology; validation; writing—original draft. **Parisa Daryani**: Data curation; formal analysis; investigation; methodology; validation. **Nazanin Amirbakhtiar**: Formal analysis; investigation; methodology; validation; writing—original draft. **Leila Pourhang**: Data curation. **Hamid Reza Pouralibaba**: Investigation; methodology; project administration; resources; writing—review and editing. **Hamid Hassaneian Khoshro**: Conceptualization; funding acquisition; project administration; supervision; writing—review and editing. **Hadi Darzi Ramandi**: Formal analysis; methodology; validation; visualization; writing—review and editing. **Zahra-Sadat Shobbar**: Conceptualization; funding acquisition; investigation; project administration; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data supporting the conclusions of this article are provided within the article and in the supplementary material.

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