Identification of Genomic Regions Associated with Fusarium Wilt Resistance in Cowpea

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Abstract: Fusarium wilt (FW), caused by the soil-borne fungal pathogen Fusarium oxysporum f. sp. Tracheiphilum, is a serious threat to cowpea production worldwide. Understanding the genetic architecture of FW resistance is a prerequisite to combatting this disease and developing FW resistance varieties. In the current study, a genetic diversity panel of 99 cowpea accessions was collected, and they were infected by a single strain, FW-HZ. The disease index (DI) based on the two indicators of leaf damage (LFD) and vascular discoloration (VD) varied highly across the population: most accessions were susceptible, and only seven accessions showed resistant phenotypes by both indicators. Through a genome-wide association study (GWAS), 3 and 7 single nucleotide polymorphisms (SNPs) significantly associated with LFD and VD were detected, respectively, which were distributed on chromosomes 3, 4, 5, 6 and 9, accounting for 0.68–13.92% of phenotypic variation. Based on the cowpea reference genome, 30 putative genes were identified and proposed as the likely candidates, including leucine-rich repeat protein kinase family protein, protein kinase superfamily protein and zinc finger family protein. These results provide novel insights into the genetic architecture of FW resistance and a basis for molecular breeding of FW resistant cultivars in cowpea.

Keywords: cowpea; fusarium wilt; GWAS; candidate genes

1. Introduction

Cowpea (Vigna unguiculata (L.) Walp.) is one of the most important legume crops worldwide. As a multiple-purpose crop, almost all the aboveground parts of the cowpea plant are edible and regularly consumed. The dry grains are used as a staple food in sub-Saharan Africa, and the dry haulms are harvested and sold as fodder for livestock. In addition, the immature pods are usually used as a vegetable, especially in East Asia and Southeast Asia [1]. Being rich in nutrients such as protein, carotene, vitamins and mineral elements in tender pods [2,3], vegetable cowpea is widely cultivated in China, and the pods can be cooked in stew, stir-fried, served cold in a salad or processed into pickled vegetables. China has been the world’s largest producer and consumer of vegetable cowpea, with an annual planting area of more than 533,333 hectares and total yield of more than 20 million tons.
FW is one of the most devastating diseases in cowpea, which is caused by *Fusarium oxysporum* f. sp. *tracheiphilum*. FW is a soil-borne disease. The pathogen invades vascular tissue through the root system, causing browning and necrosis of vascular bundles; blocking the transmission of water and nutrients in plants; and finally, wilting and falling off of leaves, and plant death [4,5]. At the seeding stage, FW often causes destruction in a whole field. At the adult stage, the yield loss caused by FW can reach 70%. At present, there is no effective chemical fungicide to control FW. Therefore, developing disease-resistant varieties is the most economical and effective strategy for management of FW. Recently, with the rapid development of cowpea genome resources, the high-throughput Cowpea iSelect Consortium Array and high-density genetic map made the molecular mapping of FW resistance genes become a reality [6–8]. By infecting a recombinant inbred lines (RIL) population derived from a resistant cultivar California Blackeye27 and a susceptible accession 24-125B-1 using the *Fusarium oxysporum* race 3, Pottorf et al. (2012) [5] mapped a resistance gene *Fot3-1* to LG1, which provides special resistance against race 3. Using a similar method, Pottorf et al. (2014) [9] identified three different RIL populations (IT93K-503-1 × CB46, CB27 × 24-125B-1, CB27 × IT82E-18/Big Buff) and mapped two genes, *Fot4-1* on LG5 and *Fot4-2* on LG3. Both genes provided special resistance against race 4. In addition, Wu et al. (2015) [10] conducted an association mapping analysis for FW resistance in a natural population containing 99 cowpea accessions, and identified 11 SNPs significantly associated with LFD and 7 significantly associated with VD, and transformed the most significant SNP into a PCR-based CAPS (cleaved amplified polymorphic sequence) marker for FW molecular breeding. Since the *Fusarium oxysporum* was first reported in the 1930s [11], several cowpea cultivars with FW resistance have been successfully released; of them, the cowpea varieties California Blackeye 27, California Blackeye 46 and California Blackeye 50 achieved milestone success, significantly improving cowpea production in southern California, USA [12,13]. In China, FW occurs in all cowpea planting areas from northern China to southern China, although some cultivars with moderate resistance, such as Zhijiang 618 and Zhijiang 108, have been released, the research on germplasm identification and FW resistance gene mapping is still relatively weak [14,15].

GWAS is a powerful technology for exploiting genes/quantitative trait loci (QTLs) controlling complex traits. This method does not need to construct segregated populations, thereby largely reducing research time and cost. In addition, more natural alleles for one trait could be detected simultaneously. GWAS has been widely used in crops such as rice, corn, soybean and watermelon [16]. In cowpea, using the GWAS based on the Cowpea iSelect Consortium Array (Illumina, Inc., San Diego, CA, USA) containing 51,128 SNP markers, Xu et al. (2017) [6] identified 72 SNPs associated with pod length, Lo et al. (2018) [17] identified 17 SNPs for grain size and Wu et al. (2021) [18] identified 30 SNPs loci for cowpea drought tolerance. The objective of this study was to screen the FW resistant accessions in Chinese cowpea germplasms and identified the genes/QTLs conferring FW resistance.

### 2. Materials and Methods

#### 2.1. Plant Materials

A total of 99 cowpea accessions collected in China were used in this study.

#### 2.2. Pathogen Isolation and Inoculum Preparation

FW strain for inoculation was isolated from a cowpea plant with FW symptoms in the field in Hangzhou, Zhejiang (29°11' N, 120°30' E). The root section in the junction of healthy and diseased parts was selected and cut into 1 cm² pieces, and then placed on the potato dextrose agar (PDA) medium. After the white mycelium were generated around the root pieces, the mycelium from one piece was picked and transferred onto a new PDA plate. A spore was continuously inoculated in three generations, and it was considered as a single FW isolate. More than 10 FW isolates were selected and identified via the PCR assay using the universal fungal primers ITS1 (TCCGTAAGGT GAACCTGCGG) and ITS4...
(TCCTCGCTTATTGATATGC). An isolate with 99% sequence similarity to the *Fusarium oxysporum* f. sp. *Tracheiphilum* in GeneBank was retained and named FW-HZ. This strain was preserved at −80 °C. Before the greenhouse inoculation experiment, a 1 cm² PDA plug was cut and transferred aseptically to flasks containing 500 mL of potato dextrose broth, then incubated in a shaker at 28 °C and 150 rpm under lighted conditions for 4 days. To eliminate mycelium, the liquid culture was strained through four layers of cheesecloth. The inoculation spore concentration was adjusted to 1.0 × 10⁵ microconidia mL⁻¹ using a hemocytometer.

2.3. Evaluation of FW Resistance

The experiment was carried out in the greenhouse of the Haining Experimental Base of Zhejiang Academy of Agricultural Sciences in the autumns of 2020 and 2021. All the accessions were sown in plastic pots (20 cm in diameter) containing sterile vermiculite and matrix, and 4–5 uniform seedlings for each accession were kept after one week. When the third trifoliate leaves started to emerge, a modified root-dip method was used to inoculate [19]. The test seedlings were gently uprooted and dipped for 1 min in suspended inoculum, and then transferred back to the pots. Then, the plants were covered with a plastic sheet for 2 days to retain moisture. From the third day, the plastic shed was removed and normal management was applied with the greenhouse day temperature set to 28 °C and the night temperature to 16 °C.

Two indicators, LFD and VD, were used to investigate FW symptoms on the 25th day after inoculation. LFD was evaluated on a 0–3 rating scale following Wu et al. (2015) [10], where 0 is no disease symptoms; 1 is slight disease symptoms, such as as necrotic spots on the basis of root and one to two etiolated or exfoliated leaves; 2 is significant disease symptoms, such as more than three etiolated or exfoliated leaves and inhibited plant growth; and 3 is a completely defoliated or dead plant. By uprooting the entire plant, slicing the stem vertically, and based on the Pottorff (2012) [5], the VD was evaluated on a 0–5 scale as follows: 0, no signs of disease; 1, approximately 10% of the plant showing symptoms of disease; 2, approximately 25% of the plant showing symptoms of disease; 3, approximately 50% of the plant showing symptoms; 4, approximately 75% of the plant showing symptoms; and 5, 100% of the plant showing symptoms. The DI was calculated according to the following equation:

\[
\text{DI} = \sum \left( \frac{r \times n_r}{\lambda N_t} \right)
\]

where \( r \) = rating value, \( n_r \) = number of plants with a disease rating of \( r \) in each line, \( N_t \) = total number of plants tested in each line, and \( \lambda \) is the total scale number (3 for LFD and 5 for VD).

Each accession was classified as one of 4 grades according to DI score: highly resistant (HR, scores of 0–0.10); resistant (R, scores of 0.11–0.30); susceptible (S, scores of 0.31–0.50); highly susceptible (HS, scores of 0.51 or higher) [20].

2.4. SNP Genotyping

The genotype data of 99 accessions was retrieved from Xu et al. (2017) [6], which was identified using the Cowpea iSelect Consortium Array (Illumina, Inc.). After filtering the SNPs with minor allele frequency (MAF) ≥ 0.01, missing rate ≤ 20%, and heterozygosity rate ≤ 20%, a total of 29,945 high-quality SNPs were used in this study.

2.5. GWAS Analysis

Population structure was analyzed using principal component analysis (PCA) and unrooted phylogenetic tree analysis from TASSEL 5.0. To detect the genomic regions associated with FW resistance, a GWAS was conducted on LFD and VD using TASSEL 5.0 under the mixed linear model while accounting for PCA and kinship [21,22]. SNPs with the threshold of logarithm of odds (LOD) ≥ 2.5 were defined as significant SNPs. According to
the cowpea linkage disequilibrium (LD) decay distance (350 kb) [18], the two significant SNPs within a same LD decay block were considered to represent the same QTL.

2.6. Comparative Genome Analysis and Candidate Gene Analysis

The known cowpea FW resistance genes were collected by a literature search. The sequences of markers linked to the known genes and the detected significant SNPs in this study were used to blast the cowpea reference genome (IT97K-499-35, https://phytozome-next.jgi.doe.gov) [8] to determine their physical position under an e-value cut-off of $1 \times 10^{-10}$. If two QTLs/genes were located in a LD block, they were considered to represent the same gene.

According to the cowpea LD decay distance, the genes residing in the 350 kb upstream and downstream of each significant SNPs were retrieved as candidate genes according to the genome annotations.

3. Results

3.1. Phenotypic Assessment

According to the LFD and VD performances (Figure 1), it was found that both indicators varied highly across the population, ranging from 0 to 1 and 0.04 to 1 for LFD and VD, respectively. The correlation coefficient between the two traits was 0.823. Of the 99 accessions, 86 accessions were S or HS in LFD (DI: 0.31-1), and 87 accessions were S or HS in VD. Only seven accessions showed R or HR phenotypes for both VD and LFD, indicating that the resistance accessions in Chinese cowpea germplasms were very rare (Table 1). At the population level, the 2020LFD and 2020VD showed near-normal distributions; however, the frequency distributions of 2021LFD and 2021VD deviated from normal, indicating that FW resistance was largely affected by the environment (Figure 2).

![Figure 1. Symptoms for FW. (A) The LFD symptoms of FW, from left to right: grades 0, 1, 2, 3. (B) The VD of FW, from left to right: grades 0, 1, 2, 3, 4 and 5.](image-url)
Table 1. The seven accessions with resistance phenotypes at LFD and VD.

| Number | Variety | 2020 LFD | 2020 VD | 2021 LFD | 2021 VD |
|--------|---------|----------|----------|----------|----------|
| D406   | G136    | 0.05     | 0.25     | 0.15     | /        |
| D501   | X480    | 0.13     | 0.29     | 0.05     | 0.09     |
| D502   | X486    | 0.29     | 0.11     | 0.13     | 0.10     |
| D503   | X487    | 0.04     | 0.17     | 0.07     | 0.11     |
| D506   | X503    | 0.20     | 0.17     | 0.03     | 0.09     |
| D575   | X436    | 0.08     | 0.18     | 0.12     | 0.04     |
| D584   | X450    | 0.25     | 0.20     | 0.00     | 0.08     |

3.2. GWAS for Resistance to FW

In this study, a total of 29,945 high-quality SNPs were used for association analysis. Given a 620 Mb cowpea genome [18], the marker density in the whole genome reached 48/Mb. Based on the 29,945 SNPs genotype data, PCA analysis showed that 99 accessions were divided into two major clusters (Figure 3). The unrooted phylogenetic tree also showed that the population could be classified into two subgroups (Figure 4). These results were consistent with Xu et al. (2017) [6], which analyzed the entire set of a mini-core collection comprising 299 accessions, indicating that the subgroups differentiation was highly correlated with pod length.
Using the 2020 phenotype data, 9 and 15 SNPs significantly associated with LFD and VD were detected, respectively (Figure 5). These SNPs were distributed on chromosomes 3, 4 and 6, explaining 0.20–3.80% of phenotypic variation. Using the 2021 phenotype data, 4 SNPs associated with LFD and 18 SNPs associated with VD were identified, distributed on chromosomes 3, 4, 5 and 9 and accounting for 5.91–13.26% of phenotypic variation. Of the detected SNPs, some formed clusters. For example, nine SNPs significantly associated with 2020LFD were clustered in a 244,223 kb segment from 20,428,139 to 20,672,362 bp on chromosome 4; there are only 825 bp between the SNPs 2_18465 and 2_48662. Similarly, three SNPs significantly associated with 2021LFD were located in an interval of 10.838 kb on chromosome 3. Previous studies showed that the LD decay distance of cowpea is about 350 kb [18], indicating that the SNPs in the same LD block may represent a single locus associated with FW resistance. For clarity, a final set of 3 significant SNPs associated with LFD and 7 SNPs associated with VD was reported after retaining only one representative locus with the highest LOD value in a same LD block (Table 2). Among these loci, no locus was detected simultaneously in the two years experiments, indicating that the FW resistance was greatly affected by the environment. Two significant SNPs, 2_01757 for 2021LFD and 2_19764 for 2021VD, were found to be apart by a distance of 108.477 kb within a same LD block, indicating that they may represent a same QTL or different alleles for the same gene, which also suggests that LFD and VD may be involved in the same pathway for FW responses. All the SNPs showed minor or moderate effects, which were consistent with Wu et al. (2015) [10], indicating that cowpea FW resistance was controlled by multiple minor genes.
Figure 5. Manhattan plot of FW resistance in cowpea. (A) 2020LFD; (B) 2020VD; (C) 2021LFD; (D) 2021VD.

Table 2. Chromosomes, positions, LOD, R² and favorable allelic variation of locus detected by GWAS.

| Loci    | Chromosome | Position (bp) | LOD  | R² (%) | Favored Allele |
|---------|------------|---------------|------|--------|----------------|
| 2020LFD | 2_01757    | Vu03          | 2,213,123 | 2.575  | 13.26          | C               |
|         | 2_28896    | Vu09          | 35,131,104 | 2.680  | 10.63          | C               |
|         | 2020VD     | 2_51816       | 6,580,689 | 2.810  | 1.16           | G               |
|         | 2_13643    | Vu03          | 7,055,960 | 3.401  | 1.66           | G               |
|         | 2_21905    | Vu06          | 22,176,903 | 2.845  | 0.68           | A               |
| 2021LFD | 2_28896    | Vu09          | 35,131,104 | 2.680  | 10.63          | C               |
|         | 2_15091    | Vu05          | 9,255,334 | 2.599  | 7.40           | A               |
| 2021VD  | 2_19764    | Vu03          | 2,104,646 | 3.108  | 10.15          | G               |
|         | 2_30644    | Vu04          | 218,863   | 2.613  | 10.29          | A               |
|         | 2_15091    | Vu05          | 9,255,334 | 2.599  | 7.40           | A               |
|         | 2_37962    | Vu05          | 34,148,948 | 2.654  | 9.65           | T               |

3.3. Candidate Gene Analysis

Based on the annotation information of the reference genome V1.2, a total of 670 candidate genes were searched for the 10 significant loci, and they were classified into multiple gene families. According to their functional annotations, 30 genes were considered as the likely candidates. Of them, 19 genes were annotated as leucine-rich repeat (LRR) family protein kinases, 6 were protein kinase superfamily proteins, 4 were zinc finger structure family proteins and 1 encodes a disease resistance response family protein (Table 3). These candidate genes are clustered in the genome; for example, a cluster of nine LRR genes was located in a 555.672 kb region from 33,944,363 to 34,500,035 bp on Vu05; and another 10 genes, including 5 LRR proteins, 4 protein kinase superfamily genes and 1 zinc finger
structure family gene, formed a cluster in a 247 kb interval on Vu04. Among the 10 loci, 2.37962 had a distance of 2 kb from Vigun05g179900, 2.30644 had a distance of 7 kb from Vigun04g003100 and 2.13643 had a distance of 7.5 kb from Vigun03g084000. 2.21905 and 2.19764 were just located on the genes Vigun06g089000 and Vigun03g027900, respectively. These genes are probably candidates for the corresponding SNPs.

Table 3. The chromosomes, positions and gene annotations of candidate genes.

| LocusName         | Chromosome | Start (bp)        | End (bp)        | Annotation Information                                           |
|-------------------|------------|------------------|----------------|-----------------------------------------------------------------|
| Vigun03g027900    | Vu03       | 2,104,404        | 2,106,257      | Zinc finger C×8C×5C×3H type family protein                     |
| Vigun03g031700    | Vu03       | 2,408,278        | 2,412,054      | LRR protein kinase family protein                               |
| Vigun03g032000    | Vu03       | 2,429,438        | 2,434,956      | Protein kinase superfamily protein                              |
| Vigun03g032400    | Vu03       | 2,497,442        | 2,503,680      | Protein kinase superfamily protein                              |
| Vigun03g083600    | Vu03       | 6,951,058        | 6,955,356      | Lesion simulating disease 1 zinc finger family protein          |
| Vigun03g084000    | Vu03       | 6,976,636        | 6,980,523      | LRR protein kinase family protein                               |
| Vigun03g084100    | Vu03       | 6,982,935        | 6,986,353      | LRR protein kinase family protein                               |
| Vigun03g087200    | Vu03       | 7,235,261        | 7,239,502      | Zinc finger (C2H2 type) family protein                          |
| Vigun04g000300    | Vu04       | 47,941           | 51,995         | Protein kinase superfamily protein                              |
| Vigun04g001000    | Vu04       | 114,870          | 117,801        | LRR protein kinase family protein                               |
| Vigun04g001100    | Vu04       | 119,053          | 122,765        | Protein kinase protein with adenine nucleotide alpha hydrolases-like domain |
| Vigun04g003100    | Vu04       | 225,982          | 229,379        | Protein kinase protein with adenine nucleotide alpha hydrolases-like domain |
| Vigun04g004600    | Vu04       | 294,639          | 295,697        | CCCH-type zinc finger family protein                            |
| Vigun04g005100    | Vu04       | 344,703          | 345,968        | LRR family protein                                              |
| Vigun04g006100    | Vu04       | 426,291          | 434,980        | Protein kinase family protein / WD-40 repeat family protein     |
| Vigun04g007200    | Vu04       | 509,625          | 513,348        | LRR protein kinase family protein                               |
| Vigun04g007300    | Vu04       | 514,759          | 519,028        | LRR protein kinase family protein                               |
| Vigun04g007400    | Vu04       | 526,327          | 529,997        | LRR protein kinase family protein                               |
| Vigun05g093700    | Vu05       | 9,064,360        | 9,065,312      | Disease resistance responsive (dirigent-like protein) family protein |
| Vigun05g180800    | Vu05       | 34,490,099       | 34,500,035     | LRR transmembrane protein kinase                                |
| Vigun05g180700    | Vu05       | 34,379,081       | 34,417,072     | LRR transmembrane protein kinase                                |
| Vigun05g180500    | Vu05       | 34,343,324       | 34,352,845     | LRR transmembrane protein kinase                                |
| Vigun05g180400    | Vu05       | 34,299,036       | 34,314,931     | LRR transmembrane protein kinase                                |
| Vigun05g179900    | Vu05       | 34,170,350       | 34,194,899     | LRR transmembrane protein kinase                                |
| Vigun05g179800    | Vu05       | 34,038,469       | 34,067,363     | LRR transmembrane protein kinase                                |
| Vigun05g179700    | Vu05       | 33,995,083       | 34,002,086     | LRR transmembrane protein kinase                                |
| Vigun05g179600    | Vu05       | 33,968,639       | 33,971,892     | LRR transmembrane protein kinase                                |
| Vigun05g179500    | Vu05       | 33,944,363       | 33,957,091     | LRR transmembrane protein kinase                                |
| Vigun06g089100    | Vu06       | 22,117,622       | 22,122,348     | LRR protein kinase family protein                               |
| Vigun06g089400    | Vu06       | 22,166,131       | 22,178,232     | LRR protein kinase family protein                               |
4. Discussion

Cowpea is an important legume crop in China. It was among the top six of edible legumes and top 10 of vegetables in terms of consumption. FW is one of the three devastating diseases in cowpea production, and the destruction and yield loss caused by FW increased quickly in recent years. Screening the resistant germplasms is the first step for FW resistance breeding. In China, nearly 5000 cowpea accessions had been collected until now; however, only a small number had been precisely identified for FW resistance. In a previous study, Wu et al. (2015) [10] identified the FW resistance of 99 cowpea accessions and obtained 10 resistant accessions. In the current study, another 99 cowpea accessions were evaluated for FW resistance, and seven resistant accessions were obtained. Taken together, these 17 accessions provide a useful resistant resource for cowpea disease resistance breeding.

Through GWAS, 10 SNPs significantly associated with FW resistance were identified in this study. Using similar association mapping analysis, Wu et al. (2015) [10] identified 11 SNPs for LFD and 7 SNPs for VD. However, there were no overlapped SNPs detected in the two studies. This phenomenon may be caused by the FW strain, genotype data and germplasms used in two studies being different. The inoculation strain FW-HZ in this study was isolated from Hangzhou, and the strain used in Wu et al. (2015) was isolated from Lishui. The former was from plain area, and the latter was from mountainous area. The genotype data used in Wu et al. (2015) was the first-generation Illumina cowpea GoldenGate 1536-SNP assay [23], and the genotyping in this study was derived from the second-generation Cowpea iSelect Consortium Array (Illumina, Inc.) with 51,128 SNPs [6]. Moreover, the evaluated germplasms used in the two studies were completely different. Thus, it is not surprising that different results were obtained from these two studies. In addition, Pottorf et al. [5,9] also identified three FW resistance QTLs, Fot3-1, Fot4-1, and Fot4-2, using QTL mapping. Synteny analysis showed that Fot4-2 had a distance of more than 33 Mb from 2_13643 for 2020VD, and Fot3-1 was 4.6 Mb away from 2_21905 for 2020VD, indicating that they were not the same QTL/gene.

FW is a vascular disease caused by *Fusarium oxysporum* [24] which has widely infected many crops, such as cotton, sugarcane, banana, tomato, cucumber and common bean [25]. At present, several FW resistance genes have been cloned in cotton and tomato. *Fov7*, a major FW resistance gene in cotton, encodes a glutamate receptor, and a single nucleotide mutation in this gene leads to the gain or loss of disease resistance [26]. *I*, *I-2*, and *I-7* are the typical *R* genes containing nucleotide binding and leucine-rich repeat domains in tomato, and *I-3* encodes an S-receptor kinase protein [27]. However, no FW resistance genes have been cloned yet in cowpea. In the current study, a total of 670 predicated genes were identified, and 30 genes were defined as the likely candidates for FW resistance. Of them, Vigun05g093700 belongs to the divergent family, and it is known that *PeEG261* [28], one member of this gene family, was considered to have functionality in FW resistance and drought resistance in common bean. Vigun03g027900, Vigun03g087200, Vigun03g083600 and Vigun04g004600 belong to the zinc finger family protein, and the zinc finger proteins play key roles in regulating plant defense gene expression and disease responses [29]. For example, *GhZFP1*, which contains two typical zinc finger motifs (C×8C×5C×3H and C×5C×4C×3H), can enhance the salt stress resistance and fungal resistance of transgenic tobacco, and *GhWRKY106-1*, which contains a typical C2HC zinc finger structure, is thought to play a role in the FW resistance mechanism in cotton [30,31]. Six genes (Vigun03g032000, Vigun03g032400, Vigun04g000300, Vigun04g001100, Vigun04g003100, Vigun04g006100) belong to the protein kinase superfamily, and the *SNF1* of this family shows the ability to reduce the pathogenicity of FW in cabbage and Arabidopsis [32,33]. The other 19 genes are typical *R*-genes, and the known candidate gene of cowpea resistance gene, *Fot3-1* [5], is also an *R* gene. These results provide direction for the cloning of FW resistance genes. Furthermore, it is worth noting that 2_01757 for 2021LFD and 2_19764 for 2021VD are located within the same LD block, and this segment contains a zinc finger family gene, an LRR type family gene and a protein kinase superfamily gene. This means that LFD and VD...
may involve in the same regulation pathway for FW resistance responses, which was not detected in Wu et al. (2015) [10]. To confirm the functional relevance of the candidate genes to FW resistance, more future work, such as fine mapping and mapped-based cloning of these genes, are required.

5. Conclusions

In the current study, a genetic diversity panel of 99 cowpea accessions was evaluated for FW resistance using a single strain, FW-HZ, and seven resistant accessions were screened. GWAS also identified 10 SNPs significantly associated with FW resistance, and 30 predicted genes were proposed as the likely candidates for the FW resistance loci. The screened FW-resistant germplasm in this study enriched the resistance germplasm resources, and the SNPs significant for FW resistance provide a powerful genetic tool for FW breeding in cowpea. Meanwhile, the GWAS results also enlarged the knowledge of FW resistance and will provide a basis for FW resistance gene cloning.

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