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Nucleotide variations of *WRKY70* gene sequence related to Huanglongbing resistance in citrus

Kristianto Nugroho[1,3], Agus Purwito[2*], Dewi Sukma[2], Mia Kosmiatin[3], Tri Joko Santoso[3], Reflinur[4], Mastur[5]

[1]Graduate Program of Plant Breeding and Biotechnology, Faculty of Agriculture, IPB University, Jalan Meranti IPB Dramaga Campus, Bogor 16680, West Java, Indonesia

[2]Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jalan Meranti IPB Dramaga Campus, Bogor 16680, West Java, Indonesia

[3]Research Center for Horticulture, Research Organization for Agriculture and Food, National Research and Innovation Agency, Jalan Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia

[4]Research Center for Applied Botany, Research Organization for Life Sciences and Environment, National Research and Innovation Agency, Jalan Raya Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia

[5]Politeknik Pembangunan Pertanian Yogyakarta-Magelang, Ministry of Agriculture Republic of Indonesia, Jalan Kusumanegara 2, Umbulharjo 55167, Yogyakarta, Indonesia

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*Corresponding Author:
Agus Purwito,
Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Jalan Meranti IPB Dramaga Campus, Bogor, West Java, Indonesia
Email: apurwito@apps.ipb.ac.id

Abstract: Huanglongbing, inflicted by *Candidatus* Liberibacter asiaticus in Asia region, is a destructive disease affecting citrus productions worlwide. Several studies have identified resistance genes that play essential roles in the citrus defense system against this pathogen. The goals of this study were to design the specific gene primers from the *WRKY70* gene sequence and analyze the nucleotide variations and genetic diversity among several citrus genotypes. Genomic DNA from nine citrus genotypes were amplified using *WRKY70*-specific gene primers and the products of PCR were sent to Sanger sequencing, while the sequences of the other 12 genotypes were collected from Citrus Genome Database. The results revealed a total of 282 nucleotide variations which consisted of 157 SNPs, 28 insertions, and 97 deletions, were identified in the *WRKY70* gene fragment sequence. There were three notable SNPs detected, with only one SNP [C/T] in first intron area at the position of 524 bp downstream from START codon that showed its ability to distinguish between susceptible and tolerant/resistant citrus genotypes. The phylogenetic analysis also revealed the clearly separation among citrus genotypes in two main clusters. The discovery of this SNP is useful for designing a functional marker as a screening tool in citrus breeding program in the future.

Keywords: *Candidatus* Liberibacter asiaticus, Citrus Genome Database, resistance gene, Sanger sequencing, SNP

Introduction

Huanglongbing (HLB), a destructive disease to citrus plantations worldwide, in Asia region is caused by a heat tolerant, uncultured, Gram-negative bacterium namely *Candidatus* Liberibacter asiaticus which transmitted by Asian citrus psyllid (*Diaphorina citri*) (Jagoueix *et al*., 1994; Bové, 2006; Gottwald *et al.*, 2007; Grafton-Cardwell *et al.*, 2013). The bacteria are infected the citrus phloem tissue that induced the plant defense system to accumulate a starch namely callose (β-1,3-glucan) in phloem sieve tube to restrict the movement of the bacteria, but on the contrary it is inhibited the photosynthate flow to other plant organs and leads the phloem damaged (Etxeberria & Narciso, 2015; Welker *et al*., 2022; Bernardini *et al*., 2024). The symptoms of this disease are varied including yellow shoots, blotchy-mottled leaf with corky vein, small fruit size with dull color, abortus fruit, and stunted growth that occassionally misinterpreted as nutrient deficiency

symptoms (Lee *et al.,* 2015; Li *et al*., 2019; Cervantes-Santos *et al.,* 2025). The economic losses caused by this disease are ranging from 30 to 100% (Iftikhar *et al.*, 2016).

The commercial citrus cultivars that preferred by the consumers are vulnerable to HLB disease (Pandey *et al.*, 2022). Therefore, the breeding program to improve the HLB-resistant commercial cultivars are challenging due to the unavailability of citrus germplasms which are particularly resistant or tolerant to HLB (Bassanezi *et al.*, 2020). However, a number of studies have reported the existance of several citrus species and their relatives that exhibited their resistance or tolerance to the disease, such as rough lemon (*Citrus jambhiri*) (Fan *et al*., 2012); Australian finger lime (*Citrus australasica*) (Weber *et al,*. 2022); trifoliate citrus (*Poncirus trifoliata*) (Hall *et al*., 2017); Chinese-box orange (*Severinia buxifolia*) (Miles *et al*., 2017); and orange jasmine (*Murraya paniculata*) (Cifuentes-Arenas *et al*., 2019). Several pomelo (*Citrus maxima*) cultivars such as Pangkajene Merah, Pangkajene Putih, Raja, and Magetan also showed their resistance to HLB disease according to Prasetyaningrum *et al.* (2012).

Evaluation of citrus resistance to HLB should be adressed continuously to obtain more cultivars or species with highly resistance or tolerance to the disease, as the genetic material sources in breeding programs. All this time, the evaluation of citrus resistance to HLB is challenging due to the inability of the bacteria to be cultured in artificial medium. Previous study from Sechler *et al.* (2009) has been successfully formulated Liber A medium for HLB bacteria culture. Additionally, Parker *et al.* (2014) reported the successful of HLB bacteria culture using one third consentration of King's B medium supplemented with citrus commercial juice.

The discovery of other evaluation methods should be performed, specifically using molecular marker. The utilization of molecular marker to analyze the citrus resistance to HLB is promote several benefits such as early detection in seedling phase, unaffected by enviromental factors, high reproducibility, and high polymorphisms to detect variation in nucleotide level (Nadeem *et al.*, 2018; Garrido-Cardenas *et al*., 2018). Previous study from Nugroho *et al*. (2025) could identify a Single Nucleotide Polymorphism (SNP) that located in 200 bp downstream from START codon in *callose synthase 7* gene, which could distinguish between resistant/tolerant and susceptible citrus genotypes. However the other genes that play significant role in HLB resistance should be explore to find the other SNPs that could be utillized as selection marker.

Study from Mafra *et al.* (2012) demostrated a gene-encoded transcription factor, namely *WRKY70*, which showed an essential role in response to HLB infection. Wu *et al.* (2021) reported a gene, named *NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1* (*CsNPR1*) in citrus that played an important role in Huanglongbing pathogen's plant defense. Dong *et al.* (2024) also reported a gene, namely *salicylic acid binding protein 2* (*CaSABP2*), that responsible for methyl salicylate conversion to salicylic acid and is essentially functional in the systemic acquired resistance (SAR) mechanism in citrus plants. The goals of this study were to design the specific gene primers from the *WRKY70* gene sequence and analyze the nucleotide variations and genetic diversity among several citrus genotypes using *in silico* method. The discovery of the nucleotide variations in this study, specifically SNPs, that could distinguish between resistant/tolerant and susceptible citrus genotypes, is expected could be useful for developing functional marker as a screening tool in citrus breeding activities in the future.

Materials and Methods

Plant Genetic Materials

This research was conducted from June to August 2025 in Plant DNA Experiment Laboratory, PP4 Building, BRIN, Cibinong Bogor. A total of 21 citrus genotypes were analyzed in this study (Table 1). As many as nine genotypes were utilized in Sanger sequencing analysis. Of these nine genotypes, as many as seven genotypes i.e. *Citrus nobilis* cv. Pontianak, *C. nobilis* cv. Madu, *C. reticulata* cv. Terigas, *C. reticulata* cv. Batu55, rough lemon (*C. jambhiri*), *C. maxima* cv. Pangkajene Putih, and *C. maxima* cv. Magetan were collected from the Agency for

Agricultural Assemblies and Modernization for Citrus and Subtropical Fruits (BRMP Jestro) in Malang, East Java, Indonesia, while orange jasmine (*Murraya paniculata*) and Chinese-box orange (*Severinia buxifolia*) were collected from local farmers. The other 12 citrus genotypes data were collected *in silico* from Citrus Genome Database (https://www.citrusgenomedb.org/blast/nucleotide/nucleotide).

Table 1. List of citrus genotypes used in this study

No	Citrus Genotypes	Collection Origin
1.	*Citrus nobilis* cv. Pontianak	BRMP Jestro
2.	*C. nobilis* cv. Madu	BRMP Jestro
3.	*C. reticulata* cv. Terigas	BRMP Jestro
4.	*C. reticulata* cv. Batu55	BRMP Jestro
5.	*C. jambhiri*	BRMP Jestro
6.	*C. maxima* cv. Pangkajene Putih	BRMP Jestro
7.	*C. maxima* cv. Magetan	BRMP Jestro
8.	*C. ichangensis* cv. ZGYCC	Citrus Genome Database
9.	*C. changshanensis cv.* Huyou	Citrus Genome Database
10.	*C. garrawayi* UQ	Citrus Genome Database
11.	*C. glauca* CRC3463	Citrus Genome Database
12.	*C. hongheensis* cv.HH	Citrus Genome Database
13.	*C. inodora* CRC3784	Citrus Genome Database
14.	*C. linwuensis* cv. LW	Citrus Genome Database
15.	*C. mangshanensis* cv. MSYG	Citrus Genome Database
16.	*C. australasica* cv. Rainbow	Citrus Genome Database
17.	*Severinia buxifolia*	Local farmer in Bandung, West Java
18.	*Citropsis gilletiana* cv. CGI	Citrus Genome Database
19.	*Fortunella hindsii* S3y-45	Citrus Genome Database
20.	*Poncirus trifoliata* DPI 50-7	Citrus Genome Database
21.	*Murraya paniculata*	Local farmer in Citayam, West Java

WRKY70 Gene Sequence Searching and Specific Gene Primers Designing

The searching of *WRKY70* gene sequence was performed utilizing the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). The mRNA sequence collected from NCBI was then compared with *Citrus sinensis* v1.0 genome (JGI) CDS in the Citrus Genome Database (https://www.citrusgenomedb.org/blast/nucleotide/nucleotide), using the Basic Local Alignment Search Tool nucleotide (BLASTn) program (Altschul *et al*., 1997). The sequence obtained from BLASTn result was subsequently employed for primer design using Primer3Plus (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (Untergasser *et al.*, 2007). The sequences of primer acquired from Primer3Plus were then rechecked using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi) (Ye *et al*., 2012) to validate that the primers only have a single annealing site. The sequences of the primers are displayed in Table 2.

Table 2. The sequence of specific gene primers designed in this study

No	Primer's name	Sequences (5'-3')	Tm (°C)	Target size (bp)
1.	CsWRKY70-F	GAAAATGGAGGCCGGTCAG	62,9	1380
2.	CsWRKY70-R	ATCATCAAAGTTAACAGACATATCCA	58,1	

Genomic DNA Extraction, DNA Amplification, and Sanger Sequencing

Genomic DNA from nine citrus genotypes, i.e. *Citrus nobilis* cv. Pontianak, *C. nobilis* cv. Madu, *C. reticulata* cv. Terigas, *C. reticulata* cv. Batu55, rough lemon (*C. jambhiri*), *C. maxima* cv. Pangkajene Putih, and *C. maxima* cv. Magetan, *M. paniculata*, and *S. buxifolia*, were extracted from leaves, using modified Doyle & Doyle (1990) method, by freshly added 0.38%

(w/v) sodium bisulfite and 2% (w/v) polyvinylpyrrolidone (PVP) to the extraction buffer. The DNA stock solutions were then diluted to a final concentration of 20 ng/µL and used as the DNA templates. The PCR cocktail for DNA amplification for each sample was consisted of 20 ng/µL DNA template at the volume of 2 µL, 2× MyTaq™ HS Red Mix (Bioline, UK) at the volume of 20 µL, 10 µM forward and reverse primers, each at the volume of 2 µL, and sterile ddH_2O adjusted to a final volume of 40 µL. The PCR process was performed employing T100 Thermal Cycler (Bio-Rad, USA) with the following profiles, i.e.: first denaturation at 95 °C as long as 5 minutes, continued by 35 cycles of denaturation at 94 °C as long as 30 seconds, annealing at 55 °C as long as 1 minute, and first extension at 72 °C as long as 1 minute. The PCR reaction was finished with the final extension at 60 °C for 15 minutes.

The products of PCR were then validated using 1% (w/v) agarose gel electrophoresis. The gel was then stained using RedSafe™ (iNtRON Biotechnology, South Korea) and envisioned using a UV transilluminator. The PCR products of nine citrus genotypes with clearly visible amplicon bands were then sent to PT Genetika Science Indonesia for Sanger sequencing. Additionally, the Sanger sequencing of the other twelve citrus genotypes were not employed in this study due to the limitation of plant genetic materials in our study. Therefore the *WRKY70* fragment gene sequence of those twelve citrus genotypes were collected *in silico* via Citrus Genome Database.

Data Analysis

The *WRKY70* fragment gene sequence, both collected from Sanger sequencing results and Citrus Genome Database collection, were further analyzed using Clustal Omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo) (Sievers & Higgins, 2014) to identifiy the nucleotide variations, specifically single-nucleotide polymorphisms (SNPs), insertions, and deletions. The phylogenetic tree was constructed from all of the citrus genotypes sequences using the Unweighted Pair Group Method with Arithmetic (UPGMA) program and the Tamura-Nei model, utilizing 1000 times bootstrap replication in Mega X (Kumar *et al*., 2018).

Results and Discussion

Searching and Designing of *WRKY70* Specific Gene Primers

The searching for *WRKY70* gene sequence in the NCBI database resulted the accession number of XM_006481140.4 that represented the PREDICTED: *Citrus sinensis* probable WRKY transcription factor 70 (LOC102630555). This sequence showed 98.78% homology to a gene located at the orange1.1g022398m locus in the Citrus Genome Database. The length of gene sequence was 1590 bp, with the length of coding sequence was 984 bp. The gene annotation revealed its relation to WRKY domain superfamily with the function as DNA-binding transcription factor. The coding sequence of the gene consisted of three exons interspersed with two introns. The forward primer in this study was designed in the first exon, while the reverse primer was designed in the third exon, as presented in Figure 1.

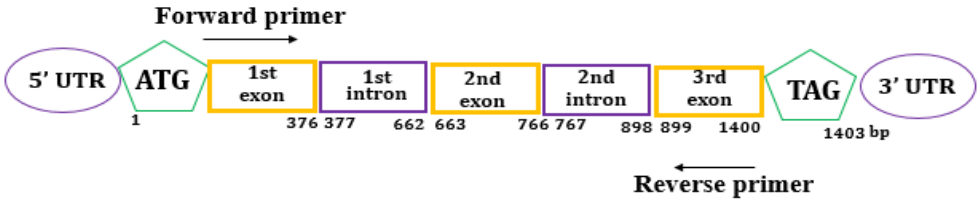

Figure 1. The arrangement of specific gene primers designed based on citrus *WRKY70* gene sequence. Information: ATG: START codon, TAG: STOP codon, 5' and 3'-UTR: untranslated region

The sequence collected from orange1.1g022398m locus was also used as the reference to obtain the *WRKY70* fragment gene sequence from the other twelve citrus genotypes that were not analyzed using Sanger sequencing. The sequence was used as the input in BLASTn

program compared to the twelve citrus genomes data that were available in Citrus Genome Database. The homology analysis results revealed high similarities percentage that ranging from 92.55 to 98.62% , as presented in Table 3.

The result of 1% (w/v) agarose gel electrophoresis revealed the clearly and visible amplicon bands from all of the nine citrus genotypes DNA. Each genotypes produced amplicon band with the size as targeted at 1380 bp (Figure 2), with no unspecific band appeared. In this study, there was no polymorphism detected among the nine citrus genotypes based on *WRKY70* specific gene primers, in contrast to previous study from Nugroho *et al*. (2025), which showed non-specific amplicon band from *M. paniculata* genotypes, using *CalS7* specific gene primers.

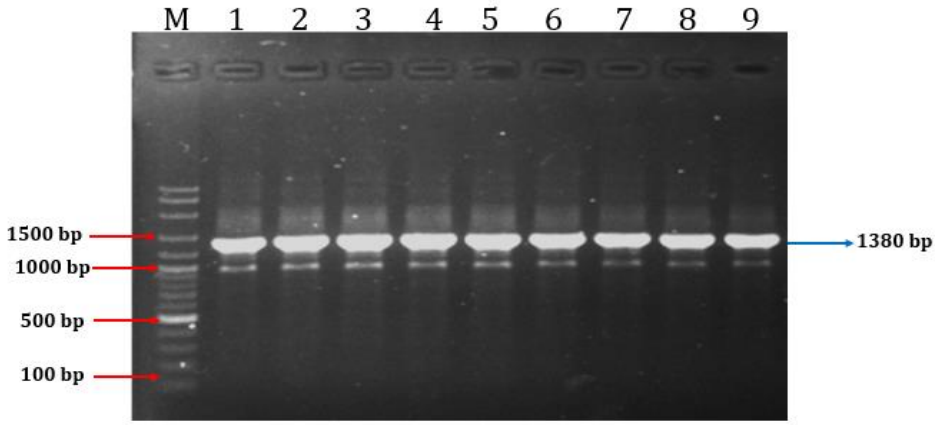

Figure 2. Visualization of PCR products from nine citrus genotypes that were amplified using *WRKY70*-specific gene primers using gel electrophoresis of 1% (w/v) agarose. Information: M: DNA ladder of 100 bp plus (Vivantis Technologies), 1: *C. nobilis* cv. Pontianak, 2: *C. nobilis* cv. Madu, 3: *C. reticulata* cv. Terigas, 4: *C. reticulata* cv. Batu55, 5: *C. jambhiri*, 6: *C. maxima* cv. Pangkajene Putih, 7: *C. maxima* cv. Magetan, 8: *M. paniculata*, and 9: *S. buxifolia*

Table 3. Homology analysis results of *WRKY70* gene sequence reference with the twelve citrus genomes data available in Citrus Genome Database

No	Reference Locus	Genome Target	Homolog Locus	Similarity Percentage (%)
1.	orange1.1g022398m scaffold00036:115495.117084- (*Citrus sinensis*)	*Citrus ichangensis* cv. ZGYCC v2.0 genome	contig442 (7960844..7962427)	98.05
2.		*C. changshanensis* cv. Huyou v1.0 genome	chr6 (17050673.17049093)	97.86
3.		*C. garrawayi UQ v1.0 genome*	hap2-C.garr_06 (7680379. 7681951)	96.86
4.		*C. glauca* CRC3463 v1.0 genome	Pri_Scaffold_6 (23607160.23605572)	97.11
5.		*C. hongheensis* cv. HH v1.0 genome	Contig563 (2702232.2700668)	96.60
6.		*C. inodora* CRC3784 v1.0 genome	Alt_Scaffold_6 (19250761.19249172)	98.18
7.		*C. linwuensis* cv. LW v1.0 genome	contig375 (6106376.6104778)	97.75
8.		*C. mangshanensis* cv. MSYG v1.0 genome	contig352 (3128989..3127401)	97.49
9.		*C. australasica* cv. Rainbow v1.0 genome	Chr06.H1 (7796474. 7798062)	97.93

No	Reference Locus	Genome Target	Homolog Locus	Similarity Percentage (%)
10.		*Poncirus trifoliata* DPI 50-7 v1.3.1 genome CDS	Ptrif.0006s1042.1.v1.3.1 (14970466.14972132-)	96.88
11.		*Citropsis gilletiana* cv. CGI v1.0 genome	ctg000159 (5235000..5233430)	92.55
12.		*Fortunella hindsii* S3y-45 v1.0 genome CDS	sjg209700.1 (5020096.5023338-)	98.62

Variations of Nucleotide among Citrus Genotypes

In this study, as many as 282 nucleotide variations which consisted of 157 (55.7%) Single Nucleotide Polymorphisms (SNPs), 28 (9.9%) insertions, and 97 (34.4%) deletions,were identified in the *WRKY70* gene fragment sequence, from 21 citrus genotypes used in this study. The majority of the nucleotide variations were categorized as SNPs, followed by deletions and insertions, similar to previous study from Nugroho *et al.* (2025). According to Tatarinova *et al.* (2016), the genetic variations in organisms mostly identified as SNPs and the small portion of insertions and deletions, with their functions in genetic diversity and evolutionary process for conservation of the original state or development new varieties with desired characters.

There were three notable SNPs that were identified in this study, but only one SNP that significantly could distinguish between the susceptible and resistant/tolerant citrus genotypes used in this study (Table 4). The first SNP [G/C] is located at 108 bp downstream from START codon. However the SNP was not only possessed by the susceptible genotypes, but also possessed by the resistant genotypes such as *Murraya paniculata*, *Poncirus trifoliata*, and *Severinia buxifolia*. Therefore this SNP can not be utilized as selectable marker. The second SNP [C/T] is located at 237 bp downstream from START codon. Actually the SNP is almost possessed by all of the HLB resistant/tolerant citrus genotypes, except *S. buxifolia* and *Citropsis gilletiana*. However the C allele, which possessed by the resistant/tolerant genotypes, is also possessed by the reference sequence obtained from *C. sinensis* (sweet orange), which is categorized as susceptible to HLB disease. Hence, this SNP also can not be used as selectable marker.

The third SNP [C/T] is located at 524 bp downstream from START codon. This SNP could distinguish clearly between the susceptible and resistant/tolerant citrus genotypes used in this study. Several mandarin citrus, such as *Citrus nobilis* cv. Pontianak, *C. nobilis* cv. Madu, *C. reticulata* cv. Terigas, *C. reticulata* cv. Batu55, including reference sequence obtained from *C. sinensis*, were possessed the C allele, while the other genotypes possessed the T allele. Mandarin and sweet orange are comercial citrus genotypes that categorized as susceptible to HLB disease. Interestingly, *Citrus jambhiri* or rough lemon genotype was also possessed the C allele, similar to mandarin and sweet orange. Fan *et al*. (2012) previously reported that this genotype is categorized as resistant to HLB infection. On the other side, Ramadugu *et al*. (2016) demonstrated that rough lemon is susceptible to HLB infection according to field evaluations. Previous study from Nugroho *et al*. (2025) also revealed the existance of a SNP in rough lemon which also possessed by the susceptible citrus genotypes such as mandarin and sweet orange, based on *CalS7* specific gene primers. Phylogenetic analysis also revealed the clustering of rough lemon together with the other susceptible genotypes such as mandarin and sweet orange (Figure 4), strengthen about the susceptability of this genotype.

Table 4. List of notable SNPs identified in this study

SNP position from START codon in reference sequence (bp)	**108**	**237**	**524**
WRKY70 reference sequence (*C. sinensis*)	G	C	C
Citrus nobilis cv. Pontianak	C	T	C
C. nobilis cv. Madu	C	T	C
C. reticulata cv. Terigas	C	T	C
C. reticulata cv. Batu55	C	T	C
C. jambhiri	C	T	C
C. maxima cv. Pangkajene Putih	G	C	T
C. maxima cv. Magetan	G	C	T
C. ichangensis cv. ZGYCC	G	C	T
C. changshanensis cv. Huyou	G	C	T
C. garrawayi UQ	G	C	T
C. glauca CRC3463	G	C	T
C. hongheensis cv.HH	C	C	T
C. inodora CRC3784	G	C	T
C. linwuensis cv. LW	G	C	T
C. mangshanensis cv. MSYG	C	C	T
C. australasica cv. Rainbow	G	C	T
Severinia buxifolia	C	T	T
Citropsis gilletiana cv. CGI	C	T	T
Fortunella hindsii S3y-45	G	C	T
Poncirus trifoliata DPI 50-7	C	C	T
Murraya paniculata	C	C	T
SNPs posiition region	First exon	First exon	First intron

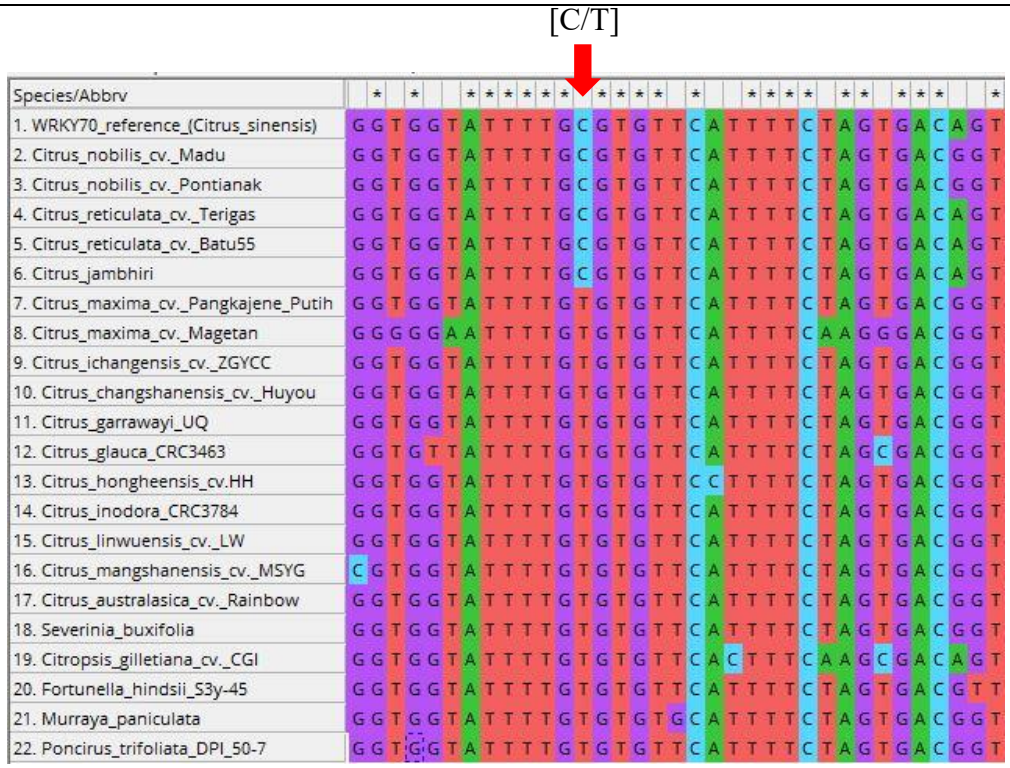

Figure 3. The SNP [C/T], highlighted by red arrow, at the position of 524 bp downstream from the START codon at the reference sequence that could distinguish the susceptible and resistant citrus genotypes to HLB disease

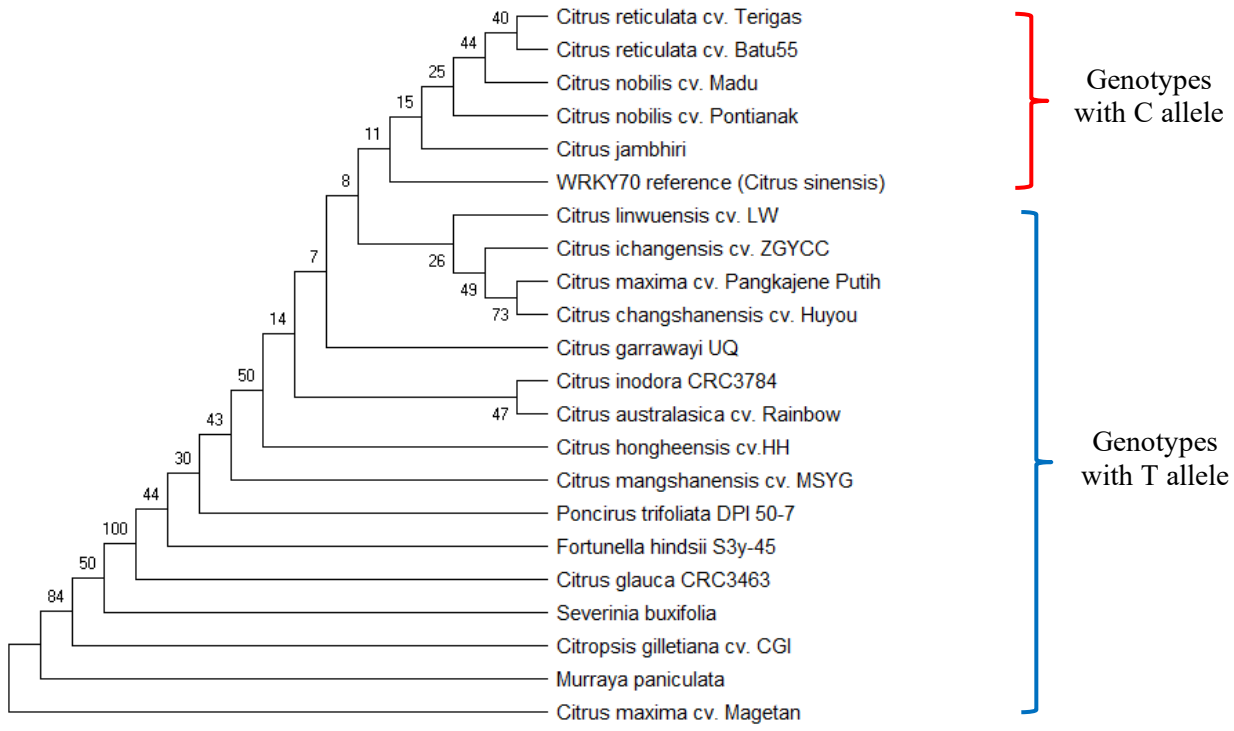

Figure 4. Phylogenetic tree of 21 citrus genotypes used in this study, based on variations of nucleotide in *WRKY70* gene fragment sequence, constructed using the UPGMA method and Tamura-Nei model

On the other side, the T allele was detected in 16 citrus genotypes that mostly known as resistant/tolerant genotypes. All of these genotypes were grouped together in similar cluster, separated from mandarin, sweet orange, and rough lemon genotypes (Figure 4). According to Tsai *et al.* (2008), mostly commercial citrus cultvars before 1970's were susceptible to HLB infection, except pomelo (*C. maxima*). In 1970's, pomelo is started to be infected by new HLB strain in Taiwan and Southeast Asia region (Tsai *et al*., 2008). However, Prasetyaningrum *et al.* (2012) reported the resistance of Indonesia's local pomelo cultivars namely Pangkajene Putih and Magetan cultivars. Australian finger lime (*C. australasica*) (Weber *et al*,. 2022); trifoliate citrus (*Poncirus trifoliata*) (Hall *et al*., 2017); Chinese-box orange (*Severinia buxifolia*) (Miles *et al*., 2017); and orange jasmine (*Murraya paniculata*) (Cifuentes-Arenas *et al*., 2019) were also showed the resistance to the disease.

Study from Zhao *et al.* (2017) revealed the tolerance of *Citrus mangshanensis* to HLB due to its different protein degradation pathway compared to sweet orange. *Microcitrus inodora* or *Citrus inodora* was categorized as tolerant genotype with different disease responses due to seedling variations, according to Ramadugu *et al.* (2016) study. However, Alves *et al.* (2021) was categorized *C. inodora* and *Citropsis gilletiana* as partial resistant due to the existance of several scions with positive HLB when they used as the rootstocks, in grafting inoculation. *Eremocitrus glauca* or also known as *Citrus glauca* is categorized as HLB resistant according to previous studies (Ramadugu *et al*. 2016; Alves *et al.* 2021). The tolerance of *Citrus ichangensis* to HLB disease has also been reported in previous study by and Wu *et al.* (2020). Kumquat (*Fortunella margarita*) was initially resistant to HLB disease, however this genotypes also became infected and showed HLB symptoms based on Tsai *et al*. (2006). On the other side, the resistance information of Hong Kong kumquat (*Fortunella hindsii*) that was utilized in this study is still unclear due to limited informations (Ramadugu *et al*., 2016),

In this study, there are also several citrus genotypes with lack of HLB resistance information such as *Citrus linwuensis*, *Citrus changshanensis*, *Citrus garrawayi*, and *Citrus hongheensis*. However all of this genotypes were possessed T allele, similar to the resitant /tolerant genotypes. They were also grouped together in the similar cluster with resitant /tolerant

genotypes in the phylogenetic tree (Figure 4). Thus, further studies should be adressed to validate the HLB resistance character of these citrus genotypes to validate our study results.

WRKY70 is a gene that belongs to *WRKY* family gene, which possessed crucial roles in plant resistance to biotic and abiotic stress, nutrient deficiencies, plant development and senescence, as well as hormone-controlled processes (Bakshi & Oelmüller, 2014),. Previous study demostrated the essential role of *WRKY70* gene in citrus defense against HLB infection by working as the salysilic acid-dependent genes activator and jasmonic acid regulated genes repressor (Mafra *et al.* 2012). The activation of *WRKY70* gene is partly triggered by NPR1-dependent mechanisms and related to the salysilic acid signaling pathway and simultaneously repressed the jasmonic acid pathway (Qiu *et al*., 2021). Another study reported the role of *NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1* (*NPR1*) gene in citrus that played an important role in Huanglongbing pathogen's plant defense (Wu *et al.* 2021). Additionally, Deng *et al.* (2020) also revealed that the *WRKY70* gene is actively contributed in the salysilic acid-induced immune responses of the citrus fruit against *Penicillium digitatum* infection.

The SNP [C/T] that identified in this study is located in the intron area of *WRKY70* gene sequence and eventhough it is promoted the amino acid changes from alanine to valine, but the intron area will be spliced and will not affect the gene expression. The existance of the SNP that is located in the intron area has also been reported in previous studies (Koeda *et al*. 2021; Nugroho *et al*. 2025). However the SNP still could be converted to a functional marker such as Single Nucleotide Amplified Polymorphism (SNAP) and can be used as a screening tool in citrus breeding program in the future.

Conclusion

The study of *WRKY70* gene sequence related to HLB resistance revealed the nucleotide variations among 21 citrus genotypes. Most of the nucleotide variations identified were SNPs, followed by deletions and insertions. There were three notable SNPs detected in this study, with only one SNP [C/T] in first intron area at the position of 524 bp downstream from START codon showed its ability to distinguish between susceptible and tolerant/resistant citrus genotypes. The phylogenetic analysis also showed the clearly separation among citrus genotypes in two main clusters. The discovery of this SNP is provides new information about citrus resistance to HLB disease that could be explored continously in future studies

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