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Molecular screening of candidate defense-related genes involved in resistance to Verticillium wilt in luffa (*Luffa aegyptiaca*)

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Abstract

In the current research, primer sets were designed to amplify four defense-related genes (DRGs), including *Ve*, *PRP1*, *RPS2*, and *chi*, which are associated with defense responses against *Verticillium dahliae* in luffa (*Luffa aegyptiaca*), or sponge gourd. Four luffa genotypes, including, Angled, Northern Large, Northern White Seed, and Touri, were evaluated as potential sources of genetic resistance. DNA was extracted from the genotypes, and degenerate primers were designed based on conserved regions of the DRGs using the Primer3 software. The designed primers exhibited high specificity, enabling successful amplification of target gene fragments. The specificity of these primers was validated via PCR, agarose gel electrophoresis, and amplicon sequencing. BLAST of the newly obtained sequences in GenBank showed high similarity to the receptor-like protein, pathogenesis-related protein type 1 (PRP1), resistance to *Pseudomonas syringae* 2 (RPS2) family, and chitinase (*chi*) family, which are encoded by the *Ve*, *PRP1*, *RPS2*, and *chi* genes, respectively. The *Ve* and *PRP1* homologs were found in all genotypes. However, the *RPS2* homologs were not observed in Angled and Touri, and the *chi* homolog was not detected in the Touri genotype. Inoculation of seedlings with *V. dahliae* revealed mild to severe symptoms in genotypes.

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Introduction

Luffa aegyptiaca Mill., also called loofah, sponge gourd, and vegetable sponge, has ancient origins in the tropical and subtropical regions of the Middle East and Eastern Asia (Porterfield, 1955). Plant diseases caused by soil-borne pathogens represent a serious constraint to global agriculture. Soil-borne vascular wilt diseases are among the most devastating plant health problems worldwide. *Verticillium dahliae* Kleb., with a wide host range, persists in soil through microsclerotia for long time, making it extremely difficult to control with crop rotation or chemical treatments. This pathogen causes vascular blockage, chlorosis, wilting, and ultimately plant death, resulting in considerable yield losses in cucurbit crops such as cucumber, pumpkin, and sponge gourd (Liu et al., 2020). Some eco-friendly methods including biological compounds of antagonistic fungi (Farhangniya et al., 2015), powder and liquid formulation of yeast strains (Golzari et al., 2024), green synthesize of nano-particles (Jomeyazdian et al., 2024), micronutrients, biofertilizers and non-chemical compounds (Hamidi et al., 2014; Seyed Mahmoudian et al., 2026), plant extracts (Narouei et al., 2021), direct fungitoxic/fungistatic effects of essential oils (Irandegani et al., 2023), microencapsulated essential oils (Iranmanesh et al., 2022; Rasteh et al., 2024; Ganjali et al., 2024; Namazi et al., 2025, 2026) and microencapsulated oligoDNAs (Delavari et al., 2026; Nouri et al., 2026) are developed for plant diseases management. However, detection of resistance-related genes (R-genes) seems to be reliable method to confirm resistance resources in plants. *L. aegyptiaca* is highly susceptible to *V. dahliae* infection that severely limits its productivity in regions with high pathogen pressure. In recent years, increasing attention has been given to

the identification of resistance sources in wild or underutilized cucurbit species. *L. aegyptiaca* is not only a vegetable crop but also valued for its medicinal and industrial uses (fiber and sponge). Traditional breeding approaches for resistance are time-consuming and often hampered by the polygenic nature of resistance traits. Therefore, molecular marker development and functional characterization of resistance-related genes are critical steps toward improving resistance in *Luffa*. Evaluation of resistance in some *L. aegyptiaca* genotypes against *V. dahliae*, and *Pytium aphanidermatum* (Edson) Fitzp through evaluation of disease severity index (DI) and area under disease progress curve (AUDPC) have been previously documented (Delaramifar et al., 2023; Shirzaei et al., 2025). The results only showed the reaction of genotypes against pathogens and classified them as sensitive/resistant.

Although some accessions show resistance to other soil-borne pathogens like *Fusarium oxysporum* f. sp. *luffae*, little is known about the genetic basis of resistance to *V. dahliae* in luffa (Huang et al., 2023). Given the limited resources for resistance, developing molecular tools is valuable for breeding. Identifying resistance-related sequences in *Luffa* genotypes can facilitate the incorporation of resistance into elite breeding lines. Polymerase chain reaction (PCR)-based techniques provide efficient tools to identify defense-related genes (DRGs), which are conserved sequences resembling known genes in other plant species. DRGs represent a valuable class of molecular markers scattered throughout the genome. DRGs are partial gene sequences encoding domains homologous to known genes and can be used as markers or sources of resistance when functional genes have not been characterized. Such DRGs have been studied in cotton, where many DRGs cluster in the genome and some are responsive to *V. dahliae* infection (Chen et al., 2015). The majority of genes identified across plant species belong to the nucleotide-binding site leucine-rich repeat (NBS-LRR) superfamily, often subdivided into Toll/Interleukin-1 receptor (TIR) and coiled-coil (CC) subfamilies. The above-mentioned genes play an important role in pathogen recognition and the activation of downstream defense pathways, including hypersensitive response and systemic acquired resistance (SAR). Previous studies in cotton, tomato, and cucumber have demonstrated that DRGs can serve as important diagnostic tools for detecting resistance against *V. dahliae*. For example, *GhDSC1*, a TIR-NBS-LRR in cotton, confer resistance through effector-triggered immunity (Li et al., 2019). The conservation of such motifs across plant families suggests that *L. aegyptiaca* may harbor similar sequences that could be exploited in resistance breeding. However, to date, no systematic attempt has been made to design primers for DRGs detection in *Luffa*. Wan et al., (2013) found a few genes encoding NBS-LRR in cucumber that their encoded proteins were different from TIR family in *Arabidopsis thaliana* (L.) Heynh. Seven families of resistance gene analogs (RGAs) were identified in *Mentha longifolia* (L.) L., all of them showed similarities with Toll/Interleukin-1 receptor. Moreover, degenerate primers designed based on the *Ve* gene against Verticillium wilt in tomato will able to amplify *Ve*-like sequences in *M. longifolia*. This finding cleared that *Ve* gene and its homologues are present not only in *Solanaceae* but also in *Lamiaceae* (Vining et al., 2007). RGA belonging to NBS-LRR group which was detected in watermelon is also identified in other cucurbits genera (Harris et al., 2009). A higher level of resistance to *Verticillium* occurred in the co-expression of *Ve1* and *Ve2* RLPs (Kalischuk et al., 2022). Castroverde et al., (2017) reported an increase in transcription/expression of *Ve1/Ve2* in tomato after infection by *Verticillium*. *Ve1* expression is involved in race-specific resistance to *Verticillium* infection (Nazar et al., 2019; Song et al., 2020). Furthermore, *Ve1* developed resistance against Verticillium wilt in *Arabidopsis*, cotton, and tobacco (Fradin et al., 2011). Yang et al., (2018) were identified the *Gbvdr6* gene, which encodes RLPs confers resistance to Verticillium wilt in cotton and *A. thaliana*. *PR3* and *PRP1*/*PRP2* gene are related to the Jasmonic acid/Ethylene, and Salicylic acid signaling pathways respectively, were continuously expressed in transgenic plants received the *Gbvdr6* gene. Major and quantitative resistances have been identified in certain crops, however, in many cucurbit species reliable sources of resistance remain scarce (Vermeulen & van den Berg 2022). Receptor-Like Proteins (RLPs) are also involved in plant disease resistance (Kruijt et al., 2005). RLPs belong to a family of membrane receptors distinguished from receptor-like kinases (RLKs) by lacking a cytoplasmic kinase domain (Sekhwal et al., 2015). RLPs with LRR domains constitute a subgroup of receptors that play an important role in plant immunity. One LRR-RLP in *Nicotiana benthamiana* Domin was responsible for recognizing xyloglucanase of *Phytophthora sojae* Kaufm. & Gerd (Sun et al., 2022).

RLPs are vital sensors in most plant immune and growth processes, sometimes cooperate with RLKs to activate or weaken signal reception. Extracellular signals are received by membrane receptors and respond to stimuli on the cell surface (Jamieson et al., 2018). The *Ve* gene from the RLPs class is responsible for resistance against *Verticillium* species encode the *Ve1*

and *Ve2*, which are exposed to fungal effectors (de Jonge et al., 2012). In tomatoes, resistance to *V. dahliae* is conferred by the *Ve* gene, which encodes a class of cell surface glycoproteins (Kawchuk et al., 2001). Pathogenesis-related proteins (PRPs) are involved in identifying fungal effectors and considered as molecular markers for plant resistance. Nineteen families of PRPs have been identified with different functions that play an important role in plant defense. PRPs act as the first line of defense. The PRP1 with antifungal and antiviral activity is abundantly produced in the apoplastic pathway during the plant-pathogen interaction (dos Santos & Franco, 2023). *PRS2* is a resistance gene of *Arabidopsis thaliana* that confers resistance against *P. syringae* bacteria that express avirulence gene avrRpt2 (Bent et al., 1994). Chitinase (*chi*) which belong to 3, 4, 8, and 11 groups of PRPs, involved in plant defense, played an important role in destroying the cell wall of pathogens through hydrolysis (dos Santos & Franco, 2023). Chitinases confer resistance in plants by destroying chitin in the cell walls of fungi (Kumar et al., 2018). Increase in *chi* activity observed on pistachio roots were colonized simultaneously with *Glomus intraradices* N.C. Schenck & G.S. Sm., and *Armillaria mellea* (Vahl) P. Kumm (Hassani et al., 2021).

The current study aimed to bridge that gap by designing specific primer sets for the detection of DRGs in *L. aegyptiaca* genotypes. Our objectives were to design primers based on conserved DRG motifs and amplification specificity and reproducibility across diverse *Luffa* genotypes. This work provides a foundational step toward molecular breeding and disease management strategies in *L. aegyptiaca* improvement programs.

Materials and Methods

Source of fungal isolates

Verticillium dahliae V117 obtained from the *Verticillium* Laboratory Collection, Department of Plant Pathology, Wageningen University, the Netherlands (Fig. 1). Information on *L. aegyptiaca* genotypes is presented in Table 1.

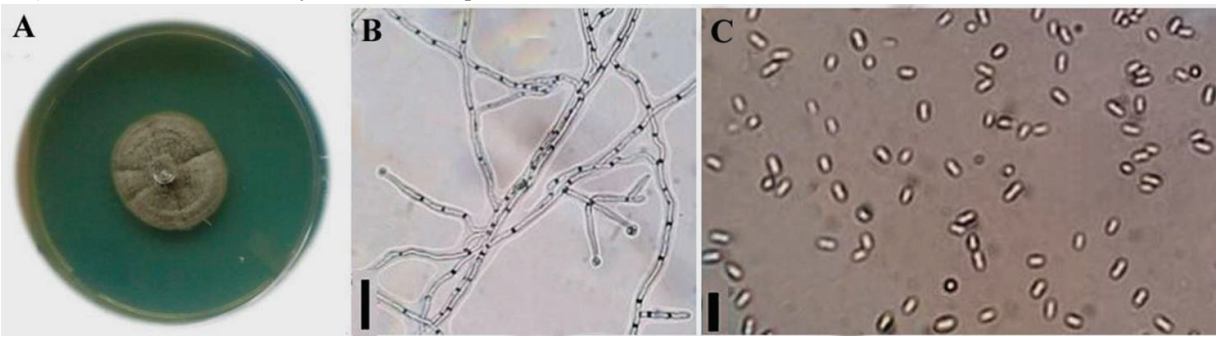

Fig. 1. (A) Colony of *Verticillium dahliae* after 7 days (B) Conidiophores and phialids, scale 10 µm (C) Conidia, scale 20 µm.

Table 1. Name, original region, and symbols of *Luffa aegyptiaca* genotypes used in the text.

Genotype	Original region	Symbol used in the text
Angled	Kerman Province, Iran	-
Northern Large	Mazandaran Province, Iran	NL
Northern White Seed	Mazandaran Province, Iran	NWS
Touri	Sistan & Baluchistan Province, Iran	-

Inoculation of seedlings and monitoring the symptoms

Inoculation was done by root-dipping seedlings in 1×10^6 spore/ml suspension for 5 min. Then, the inoculated seedlings were put in plastic pots containing a mixture of sterile soil and peat moss, incubated in a growth chamber at 25°C with a 12 h photoperiod, and monitored daily. Disease severity index (DI) and the area under disease progress curve (AUDPC) were previously determined by Shirzaei et al., (2025). We referred their results to investigate the presence of DRGs in *L. aegyptiaca* genotypes.

DNA extraction

DNA extraction from leaves was done based on Dellaporta method (Dellaporta et al., 1993). DNA quality and quantity were determined using 1.5% agarose gel and a spectrophotometer (Unico USA UV-2100), respectively.

Design of primer sets

The four candidate genes (*Ve*, *PRP1*, *RPS2*, and *chi*) were selected based on previous reports describing their roles in pathogen recognition or downstream defense responses against fungal pathogens. Complete sequences of these genes from cucurbits (including *Cucumis*, *Cucurbita*) were retrieved from GenBank (https://www.ncbi. nlm.nih.gov/genbank/). The sequences were aligned using the MAFFT algorithm, and the primer sets were designed using the Primer3 software based on conserved regions of genes. Oligonucleotides were synthesized by the Pishgam Biotechnology Company (Tehran, Iran). Details of designed primers are presented in Table 2.

Table 2. Sequence of the primers designed in this study and their features.

Gene target	Primer ID	Sequence (5'-3')	Primer length (bp)	Amplicon size (bp)
Ve	*Ve1*-F	5'-TTTGGAGGTTTTGGACCTTG-3'	20	600-700
Ve	*Ve1*-R	5'-GATGCTCCCAACCAACAGAT-3'	20	
PR1	*PR1*-F	5'-TGGATCTTCTCAAGCTCTCC-3'	20	500-550
PR1	*PR1*-R	5'-ATGGCCTCTGCCCTATGA-3'	18	
RPS2	*RPS2*-F	5'-CGCGACATCGATTCAGTAGA-3'	20	1800
RPS2	*RPS2*-R	5'-TCCCTTCCTTCACTCTTCCA-3'	20	
chi	*Chi*-F	5'-CCATCTCCCTCTTACGAGTAGC-3'	22	350-400
chi	*Chi*-R	5'-CATACGCTTCTAATCTTAGCCTTTGC-3'	26	

PCR, sequencing and sequence analysis

The PCR reaction in a final volume of 25 µL contains template DNA (2 µL), dNTP (10 mM, 1 µL) primers (10-20 µM, 2 µL for each primer), buffer (10X, 2.5 µL), $MgCl_2$ (4 mM, 1 µL), one unit Taq polymerase enzyme (1 U, 0.2 µL) and double distilled water. The PCR program performed in 35 cycles, including initial denaturation (1 min at 94°C), denaturation (5 min at 94°C), annealing (1 min at 56°C), extension (1 min at 72°C), final extension (5 min at 72°C). The PCR products sent to Pishgam Biotechnology Company, Iran, for sequencing. Candidate DRGs related to *Ve*, *PRP1*, *RPS2* and *chi* genes from cucurbits retrieved from GenBank and used for analysis with newly obtained sequences (Table 3). Sequences aligned using ClustalW and analyzed in MEGA v.11 (Tamura et al., 2021). Phylogenetic tree constructed using Neighbor-Joining (NJ) method. A heuristic search was done with Nearest Neighbor Interchange (NNI). Statistical support in nodes was calculated by running a bootstrap analysis of 1000 replicates (Felsenstein, 1985).

Results

Symptoms on inoculated seedlings

The inoculated seedlings were monitored from the day after inoculation to record the progress of disease symptoms. Mild to severe symptoms, along with wilting, were observed in genotypes (Fig. 2).

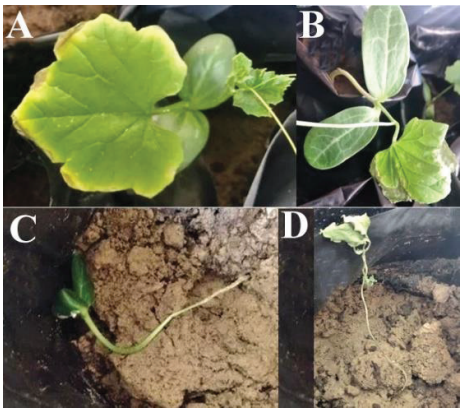

Fig. 2. Reaction *Luffa aegyptiaca* genotypes against *Verticillium dahliae*. (A) Mild blight on the edge of the leaf (Angled), (B) Severe blight on the edge of the leaf (Touri), (C) Wilt symptom (NWS), (D) Wilt symptom (NL).

Table 3. Sequences used in the phylogenetic analysis of the DRGs and their accession numbers.

Target gene	Detected DRGs in GenBank	Host	GenBank accession no.
Ve	Receptor-Like Protein (RLP)	*Luffa aegyptiaca* (Angled)	This study
Ve	Receptor-Like Protein (RLP)	*Luffa aegyptiaca* (NL)	This study
Ve	Receptor-Like Protein (RLP)	*Luffa aegyptiaca* (NWS)	This study
Ve	Receptor-Like Protein (RLP)	*Luffa aegyptiaca* (Touri)	This study
Ve	Receptor-Like Protein (RLP)	*Cucumis melo*	XM_008444165
Ve	Receptor-Like Protein (RLP)	*Cucurbita pepo*	XM_023673411
Ve	Receptor-Like Protein (RLP)	*Cucumis melo*	XM_051084278
Ve	Receptor-Like Protein (RLP)	*Cucumis melo*	XM_008444164
Ve	Receptor-Like Protein (RLP)	*Cucurbita pepo*	XM_023672943
Ve	Receptor-Like Protein (RLP)	*Cucurbita maxima*	XM_023127590
Ve	Receptor-Like Protein (RLP)	*Cucurbita moschata*	XM_023078868
Ve	Receptor-Like Protein (RLP)	*Cucurbita moschata*	XM_023078558
Ve	Receptor-Like Protein (RLP)	*Cucurbita maxima*	XM_023126850
Ve	Receptor-Like Protein (RLP)	*Momordica charantia*	XM_022289915
Ve	Receptor-Like Protein (RLP)	*Benincasa hispida*	XM_039048448
Ve	Receptor-Like Protein (RLP)	*Benincasa hispida*	XM_039048449
Ve	Receptor-Like Protein (RLP)	*Cucumis sativus*	XR_969068
PR1	Pathogenesis-Related Protein (PR1-like)	*Luffa aegyptiaca* (Angled)	This study
PR1	Pathogenesis-Related Protein (PR1-like)	*Luffa aegyptiaca* (NL)	This study
PR1	Pathogenesis-Related Protein (PR1-like)	*Luffa aegyptiaca* (NWS)	This study
PR1	Pathogenesis-Related Protein (PR1-like)	*Luffa aegyptiaca* (Touri)	This study
PR1	Pathogenesis-Related Protein (PR1-like)	*Cucurbita maxima*	XM_023116738
PR1	Pathogenesis-Related Protein (PR1-like)	*Cucurbita pepo*	XM_023698178
PR1	Pathogenesis-Related Protein (PR1-like)	*Cucurbita moschata*	XM_023097100
PR1	Pathogenesis-Related Protein (PR1-like)	*Cucumis melo*	EU556704
PR1	Pathogenesis-Related Protein (PR1-like)	*Cucumis melo*	XM_008456838
PR1	Pathogenesis-Related Protein (PR1-like)	*Cucumis melo*	EU679402
PR1	Pathogenesis-Related Protein (PR1-like)	*Momordica charantia*	XM_022293102
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Luffa aegyptiaca* (NL)	This study
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Luffa aegyptiaca* (NWS)	This study
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucurbita moschata*	XM_023073871
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucurbita ficifolia*	MK618642
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucurbita pepo*	XM_023691176
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucurbita maxima*	XM_023141784
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Benincasa hispida*	XM_039035094
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucumis sativus*	XM_031881483
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucumis melo*	XM_051084404
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucumis melo*	XM_051084403
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucumis melo*	XM_051084402
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucumis melo*	XM_051084401
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucurbita moschata*	EF101667
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucurbita moschata*	EF199759
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucurbita moschata*	EF199760
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucurbita ficifolia*	MG946756
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*C. hystrix* × *C. sativus*	GQ423655
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*C. hystrix* × *C. sativus*	GQ423657
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*C. hystrix* × *C. sativus*	GQ423654
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*C. hystrix* × *C. sativus*	GQ423668

Table 3 (continued)

Target gene	Detected DRGs in GenBank	Host	GenBank accession no.
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*C. hystrix* × *C. sativus*	GQ423670
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucumis sativus*	XM_031881482
RPS2	Resistance to *Pseudomonas syringae* 2 (RPS2-like)	*Cucumis melo*	XM_051084401
chi	chitinase-like/endochitinase-like	*Luffa aegyptiaca* (Angled)	This study
chi	chitinase-like/endochitinase-like	*Luffa aegyptiaca* (NL)	This study
chi	chitinase-like/endochitinase-like	*Luffa aegyptiaca* (NWS)	This study
chi	chitinase-like/endochitinase-like	*Cucurbita pepo*	XM_023689212
chi	chitinase-like/endochitinase-like	*Cucurbita moschata*	XM_023094023
chi	chitinase-like/endochitinase-like	*Benincasa hispida*	XM_039025374
chi	chitinase-like/endochitinase-like	*Panicum virgatum*	XM_039987874
chi	chitinase-like/endochitinase-like	*Quercus robur*	XM_050416720

Designed primer sets were detected *Ve* and *PRP1* homologs in all genotypes (size 600-700 bp and 500-550 bp, respectively). The *RPS2* homologs were found found in NL and NWS genotypes (size 1800 bp). The *chi* homologs were observed in Angled, NL, and NWS genotypes (size 350-400 bp) (Figs. 3 and 4).

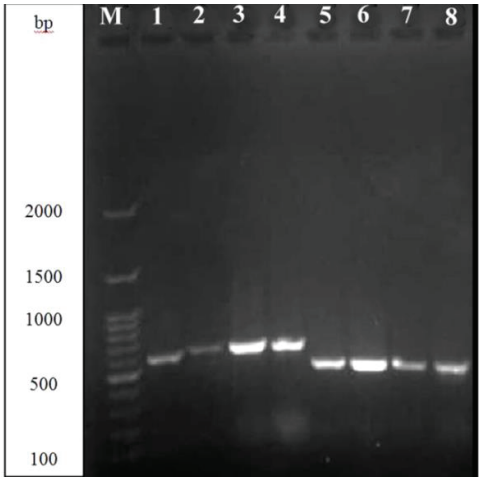

Fig. 3. PCR products related to *Ve* (1-4) and *PRP1* (5-8) homologs in *Luffa aegyptiaca* genotypes. (M) Marker 100bp, lines 1, 5: Angled, lines 2, 6: NL, lines 3, 7: NWS, lines 4, 8: Touri.

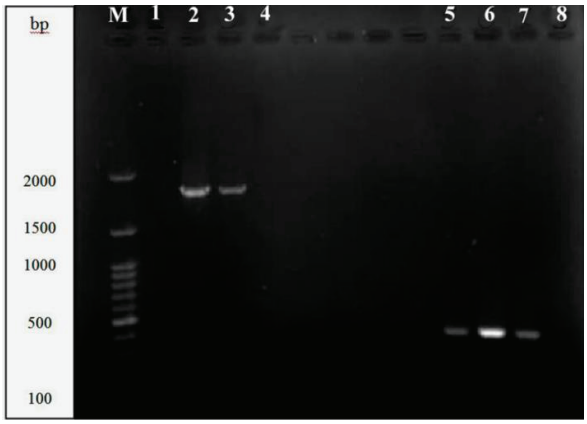

Fig. 4. PCR products related to *RPS2* (1-4) and *chi* (5-8) homologs in *Luffa aegyptiaca* genotypes. (M) Marker 100bp, lines (1, 5) Angled, lines (2, 6) NL, lines (3, 7) NWS, lines (4, 8) Touri.

The DRGs identified in *L. aegyptiaca* genotypes were similar to the sequences deposited in the GenBank from other genera in *Cucurbitaceae* viz. *Benincasa* Savi, *Citrullus* Schrad. ex Eckl. & Zeyh., *Cucumis* L., *Cucurbita* L. and *Momordica* L. (Table 3). Sequences generated in this study and those obtained from GenBank were used for construction of the phylogenetic tree (Fig. 5).

Discussion

V. dahliae is a soil-borne, vascular fungal pathogen causing wilt diseases in many plant species, including important cucurbits, and poses major agronomic challenges due to its persistence in soil and difficulty to control by conventional means. Early and precise detection of DRGs is essential for accelerating breeding programs targeting *V. dahliae* resistance in cucurbit crops. In this study, we designed four primer pairs targeting putative DRGs in *L. aegyptiaca*, evaluated across multiple genotypes that offer tools to identify DRGs in *Luffa* germplasm and facilitate breeding for Verticillium wilt resistance.

The successful design of primer sets targeting DRGs demonstrate the utility of molecular approaches in studying disease resistance in *L. aegyptiaca*, and highlight the potential for identifying DRGs that can be exploited in breeding programs. Since *V. dahliae* resistance is often complex and polygenic, the detection of multiple DRGs provides valuable markers, can serve as diagnostic tools for screening breeding populations and wild germplasm collections. Amplification of four DRGs in *L. aegyptiaca* genotypes demonstrated the feasibility of using molecular tools to investigate resistance against *V. dahliae*. The primer sets designed in this study amplified sequences with high similarity to known defense-related genes from cucurbits, supporting the hypothesis that conserved motifs can be targeted across species to identify potential resistance determinants. Similar results have been reported in cotton and tomato, where variation within NBS-LRR genes influenced recognition specificity and resistance levels against *V. dahliae* (Chen et al., 2015; Li et al., 2019).

Another important implication of our results is the specificity of the designed primers. In cucurbit breeding, one of the major challenges has been the absence of robust molecular markers linked to wilt resistance. The primer sets developed here provide an efficient, low-cost tool for genotyping breeding populations and wild germplasm collections. They can also facilitate association mapping studies by linking sequence of DRGs with phenotypic resistance data under controlled infection assays. Moreover, the integration of these molecular tools with phenotypic screening in field conditions will be crucial to confirm their utility as predictive markers of resistance. Identification of DRGs in cucurbit crops has enabled the discovery of conserved motifs associated with resistance in cucurbits.

Comparison with parallel findings in cotton including the large families of RGAs, and their induction under *V. dahliae* stress underscores that similar mechanisms may be at work in *Luffa* (Chen et al., 2015; Li et al., 2019). Detection of DRGs in *L. aegyptiaca* genotypes offered a practical tool for characterizing genetic resistance against *V. dahliae*. These tools enable screening of germplasm for DRGs and contribute to future molecular breeding strategies aimed at enhancing disease resistance in sponge gourd and related cucurbit crops. The results of present research revealed that Angled was more resistant with mild symptoms, followed by Touri, NWS, and NL genotypes with severe to wilt symptoms (Fig. 2). Our results were in agreement with Shirzaei et al., (2025), who reported that Angled exhibited less sensitivity to *V. dahliae* compared to the other genotypes. The *RPS2* homolog was not detected in the Angled genotype indicates that the *Ve*, *PRP1*, and *chi* homologs contributed to resistance against *V. dahliae*. The *Ve* and *RPP1* homologs were found in the Touri genotype. Moreover, the *RPS2* homologs were found in NL and NWS genotypes, and the *chi* homologs were observed in Angled, NL and NWS genotypes, but both later homologs not detected in Touri. As a result of mild symptoms on Angled and Touri genotypes, it is concluded that the *Ve* and *PRP1* homologs have probably more contributed in resistance against *V. dahliae*. The homologs of four DRGs were detected in NL and NWS genotypes, but the occurrence of wilt symptoms indicates that those homologs were probably not expressed. However, the study did not perform gene expression analysis (qPCR, RNA-seq, or northern blot) to determine whether the DRGs were transcribed.

Consequently, despite designing primers based on published sequences, PCR amplification may detect homologous sequences rather than a single unique locus. Therefore, our PCR data should be interpreted as indicating the presence of gene family-related homologous sequences, rather than definitive proof of a single specific gene.

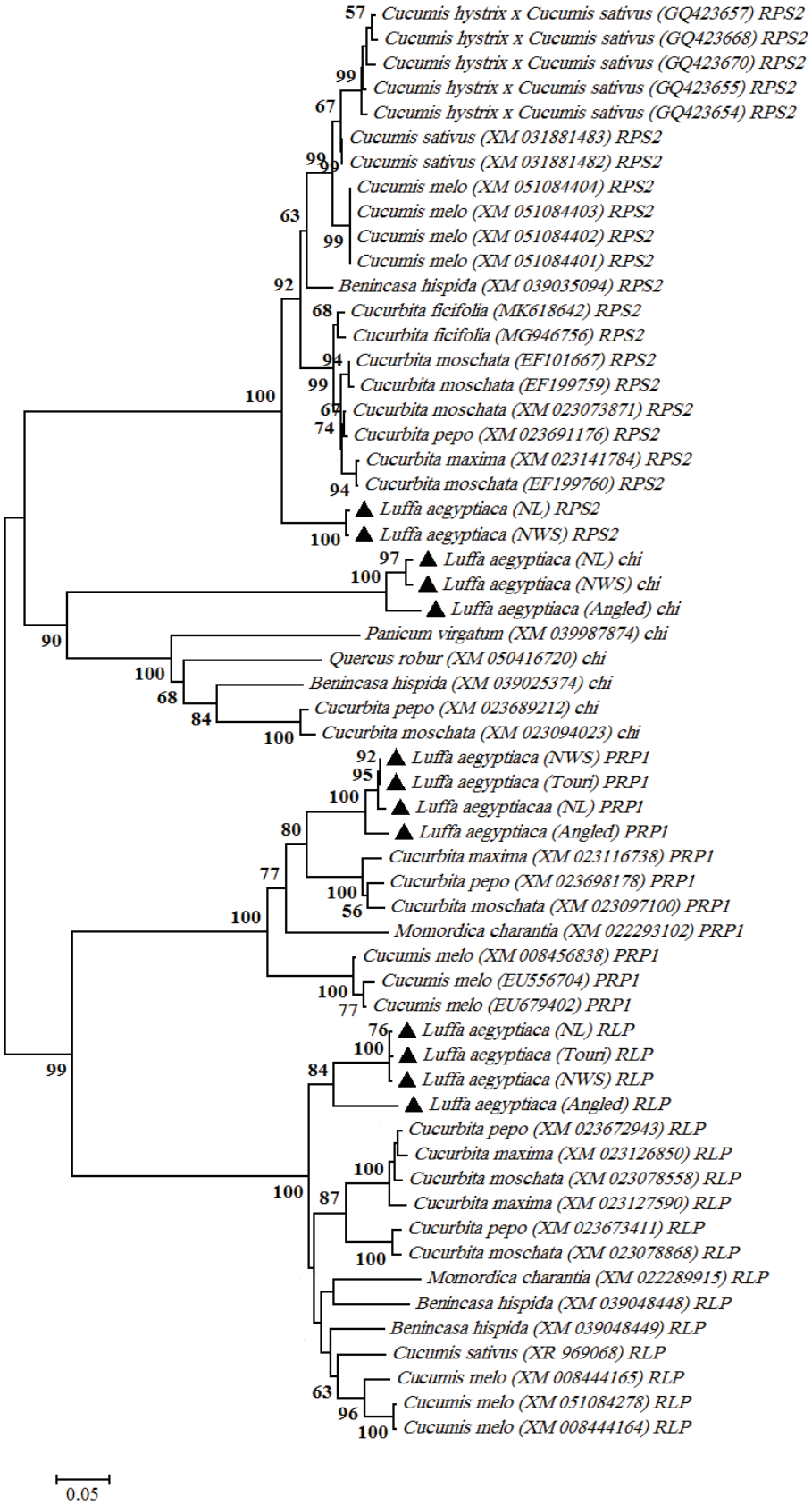

Fig. 5. Neighbor-Joining tree based on the DRGs found in cucurbitaceous plants. Bootstrap values (>50%) with 1000 replicates are indicated above branches. DRGs of *Luffa aegyptiaca* genotypes are marked with black triangles.

Phylogenetic analysis demonstrated that detected DRGs in *L. aegyptiaca* genotypes were similar to previously recognized *Ve*, *PRP1*, *RPS2*, and *chi* genes, indicates that designed primers were able to identify DRGs in *L. aegyptiaca* genotypes. Future work should focus on functional validation of *Ve, PRP1*, *RPS2*, and chitinase-associated loci through gene expression profiling and reverse genetics approaches to determine their direct contribution to resistance against *V. dahliae.*

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Ethical Approval and Consent

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT author statement

M. Miri: Laboratory works. **M. Pirnia:** Conceptualization, Funding acquisition, Investigation, Methodology, Visualization, Validation, Data curation, Formal Analysis, Supervision, Writing – original draft, Writing – review & editing. **M. Keykhasaber:** Conceptualization, Supervision, Methodology, Review & editing, **S. Sarani:** Supervision, Review & editing.

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Data Availability

All datasets generated during this study are available from the corresponding author upon request.

Conflict of interest

The authors declare no conflict of interest.

References

Bent, A. F., Kunkel, B. N., Dahlbeck, D., Brown, K. L., Schmidt, R., Giraudat, J., Leung, J., & Staskawicz, B. J. (1994). RPS2 of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. *Science, 265*(5180), 1856–1860. https://doi.org/10.1126/science.8091210.

Castroverde, C. D. M., Xu, X., Nazar, R. N., & Robb, J. (2017). Biotic factors that induce the tomato *Ve1* R-gene. *Plant Science*, *265*, 61–69. https://doi.org/10.1016/j.plantsci.2017.09.015.

Chen, J. Y., Huang, J. Q., Li, N. Y., Ma, X. F., Wang, J. L., Liu, C., Liu, Y. F., Liang, Y., Bao, Y. M., & Dai, X. F. (2015). Genome-wide comparative analyses of resistance gene analogs in cotton (*Gossypium* spp.) and their response to Verticillium wilt. *BMC Plant Biology*, *15*, 148. https://doi.org/10.1186/s12870-015-0508-3.

Delaramifar, M., Pirnia, M., Keykhasaber, M., Sarani, S., & Khajeh, H. (2023). Reaction of eight luffa genotypes to damping-off disease. *Plant Pathology Science*, *12(2)*, https://doi.org /10.2982/PPS.12.2.76.

Delavari, A., Keykhasaber, M., Miri, M. A., Pirnia, M., & Sarani, S. (2026). Control of *Phytophthora capsici*, which causes root and stem rot, using encapsulated oligonucleotide DNA. *Scientific Reports*, *16*, 4215 https://doi.org/10.1038/s41598-025-34330-7.

Dellaporta, S. L., Wood, J., & Hicks, J. B. (1993). A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter*, *1*, 19–22.

de Jonge, R., van Esse, H. P., Maruthachalam, K., Bolton, M. D., Santhanam, P., Keykhasaber, M., Zhang, Z., Usami, T., Lievens, B., Subbarao, K. V., & Thomma, B. P. H. J. (2012). Tomato immune receptor *Ve1* recognizes effector of multiple fungal pathogens uncovered by genome and RNA sequencing. *Proceedings of the National Academy of Science*, *109(13)*, 5110–5115.

dos Santos, C., & Franco, O. L. (2023). Pathogenesis-related proteins (PRs) with enzyme activity activating plant defense responses. *Plants*, *12*, 2226. https://doi.org/10.3390/ plants12112226.

Farhangniya, S., Naraghi, L., Ommati, F., & Pirnia, M. (2015). Evaluation of the efficacy of the biological compound affected by *Talaromyces flavus* in controlling tomato Fusarium wilt disease in the field condition. *International Journal of Agricultural Science and Research*, *5(2)*, 153-164.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, *39*, 783–791.

Fradin, E. F., Abd-El-Haliem, A., Masini, L., van den Berg, G. C., Joosten, M. H., & Thomma, B. P. (2011). Interfamily transfer of tomato *Ve1* mediates *Verticillium* resistance in *Arabidopsis*. *Plant Physiology*, *156*, 2255–2265. https://doi.org/10.1104/pp.111.180067.

Ganjali, A., Pirnia, M., Miri, M. A., Keykhasaber, M., & Sarani, S. (2024). Microencapsulation of rosemary essential oil by electrospraying: an evaluation of characteristics and antifungal properties. *Research Innovation in Food Science and Technology*, https://doi.org/ 10.22101/jrifst.2024.421841.1530.

Golzari, H., Pirnia, M., Moradi, M., Saberi-Riseh, R., Sabbagh, S. K., & Keykhasaber, M. (2024). Efficacy of some yeast strains for preventing infection of pistachio with *Aspergillus flavus* and aflatoxin. *Journal of Nuts*, *14*. https://doi.org/10.22034/jon.2023.1992574.1241.

Hamidi, S., Moradzadeh-Eskandari, M., Afzali, H., & Pirnia, M. (2014). Effect of various compounds on the control of *Alternaria solani* and *Alternaria alternata* the causal agents of early blight disease on potato. *Applied Research in Plant Protection*, *4(1)*, 57–66.

Harris, K. R., Wechter, W. P., & Levi, A. (2009). Isolation, sequence analysis, and linkage mapping of nucleotide binding site–leucine-rich repeat disease resistance gene analogs in watermelon. *Journal of American Society of Horticultural Science*, *134(6)*, 649–657. https://doi.org/ 10.21273/JASHS.134.6.649.

Hassani, H., Salari, M., Pirnia, M., & Mohammadi, A.H. (2021). The effect of *Glomus intraradices* on chitinase enzyme production in pistachios inoculated with *Armillaria mellea*. *Journal of Biocontrol in Plant Protection*, *9(1)*, 25-33. https://doi.org/10.22092/bcpp. 2021.126377.

Huang, J. H., Namisy, A., Rakha, M., & Chung, W. H. (2023). Resistance to *Fusarium oxysporum* f.sp. *luffae* in *Luffa* germplasm despite hypocotyl colonization. *Plant Disease*, *107(6)*, 1510–1519. https://doi.org/10.1094/PDIS-07-22-1759-RE.

Irandegani, Y., Pirnia, M., Taheri, A., Khaledi, N., Keykhasaber, M., & Sarani, S. (2023). Phylogenetic analyses of *Fusarium oxysporum* species complex on Banana in Iran and evaluation of some essential oils on the growth, sporulation and spore germination of the fungus. *European Journal of Plant Pathology*, *167*, 183–195. https://doi.org/10.1007/s10658-023-02691-2.

Iranmanesh, S., Aran, M., Miri, M. A., & Pirnia, M. (2022). Preparation and characterization of zein electrospun fibers for nano encapsulation of Ajowan essential oil. *Journal of Essential Oil Bearing Plants*, *25*, https://doi.org /10.1080/0972060X.2022.2068970.

Jomeyazdian, A., Pirnia, M., Alaei, H., Taheri, A., & Sarani, S. (2024). Control of Fusarium wilt disease of tomato and improvement of some growth factors through green synthesized zinc oxide nanoparticles. *European Journal of Plant Pathology*, *169*, 333–345. https://doi.org/ 10.1007/s10658-024-02831-2.

Jamieson, P. A., Shan, L., & He, P. (2018). Plant cell surface molecular cypher: receptor-like proteins and their roles in immunity and development. *Plant Science*, *274*, 242–251. https://doi.org/ 10.1016/j.plantsci.2018.05.030.

Kalischuk, M., Müller, B., Fusaro, A.F., Wijekoon, C. P., Watherhouse, P. M., Prüfer, D., & Kawchuk, L. M. (2022). Amplification of cell signaling and disease resistance by an immunity receptor Ve1/Ve2 heterocomplex in plants. *Communications Biology*,

5, 497. https://doi.org /10.1038/s42003-022-03439-0.

Kawchuk, L. M., Hachey, J., Lynch, D. R., Kulcsar, F., van Rooijen, G., Waterer, D. R., Robertson, A., Kokko, E., Byers, R., Howard, R. J., Fischer, R., & Prüfer, D. (2001). *Tomato Ve* disease resistance genes encode cell surface-like receptors. *Proceedings of the National Academy of Science*, *98*, 11 6511–6515. www.pnas.orgycgiydoiy10.1073ypnas.091114198.

Kruijt, M., de Cock, M.J.D., & de Wit, P. J. G. M. (2005). Receptor-like proteins involved in plant disease resistance. *Molecular Plant Pathology*, *6(1)*, 85–97. https://doi.org/ 10.1111/J.1364-3703.2004.00264.X

Kumar, M., Brar, A., Yadav, M., Chawade, A., Vivekanand, V., & Pareek, N. (2018). Chitinases-potential candidates for enhanced plant resistance towards fungal pathogens. *Agriculture*, *8(88)*, https://doi.org/10.3390/agriculture8070088.

Li, T. G., Wang, B. L., Yin, C. M., Zhang, D. D., Wang, D., Song, J., Zhou, L., Kong, Z. Q., Klosterman, S. J., Li, J. J., Adamu, S., Liu, T. L., Subbarao, K. V., Chen, J. Y., & Dai, X. F. (2019). The *Gossypium hirsutum* TIR-NBS-LRR gene GhDSC1 mediates resistance against Verticillium wilt. *Molecular Plant Pathology*, *20(3)*, 310–322. https://doi.org/10.1111/mpp. 12797.

Liu, S., Wang, H., Zhang, B., Li, X., & Sun, J. (2020). Advances in identification and functional analysis of disease resistance genes in cucurbitaceae crops. *Frontiers in Plant Science*, *11*, 593781. https://doi.org/10.3389/fpls.2020.593781.

Namazi, S., Pirnia, M., Miri, M. A., Keykhasaber, M., Sarani, S., & Taliei, F. (2025). Molecular identification of decay fungi *Botrytis deweyae* and *Aspergillus pseudotubingensis* on strawberries, decay control, and fruit durability enhancement by combined thyme-rosemary microencapsulated essential oils. *Journal of Plant Disease and Protection*, *132*, 92 https://doi.org/10.1007/s41348-025-01085-2.

Namazi, S., Pirnia, M., Miri, M. A., Keykhasaber, M., Sarani, S., & Taliei, F. (2026). Molecular identification of decay fungi *Rhizopus arrhizus var. arrhizus* and *Talaromyces oumae-annae* on strawberries, decay control, and fruit durability enhancement by combined garlic-peppermint microencapsulated essential oils. *Journal of Plant Disease and Protection*, *133*, 90. https://doi.org/10.1007/s41348-026-01281-8.

Narouei, A., Pirnia, M., Sarani, S., Abkhoo, J. (2021). Effect of *Hibiscus sabdariffa* extract on growth of *Fusarium graminearum* and the expression of TRI4 and FG08079 genes of the fungus. *International Journal of Basic Science in Medicine*, *6(3)*, 86–90. https://doi.org/ 10.34172/ijbsm.2021.16.

Nazar, R. N., Castroverde, C. D. M., Xu, X., Kurosky, A., & Robb, J. (2019). Wounding induces tomato *Ve1* R-gene expression. *Planta*, *249*,1779–1797. https://doi.org/10.1007/s00425-019-03121-6.

Porterfield, W. M. (1955). Loofah: The sponge gourd. *Economic Botany*, *9*, 211-223. https://doi.org/10.1007/BF02859814.

Nouri, M., Keykhasaber, M., Pirnia, M., Miri, M. A., Sarani, S., & Kamaladini, H. (2026). Antifungal efficacy of microencapsulated miR166 and miR159 oligoDNAs through whey protein concentrate (WPC) as coated protein against *Verticillium dahliae*. *PLOS ONE*, *21(5)*, e0349566. https://doi.org/10.1371/journal.pone.0349566.

Rasteh, I., Pirnia, M., Miri, M. A., & Sarani, S. (2024). Encapsulation of *Zataria multiflora* essential oil in electrosprayed zein microcapsules: Characterization and antimicrobial properties. *Industrial Crops and Products*, *208* https://doi.org/10.1016/j.indcrop.2023.117794.

Sekhwal, M. K., Li, P., Lam, I., Wang, X., Cloutier, S., & You, F. M. (2015). Disease resistance gene analogs (RGAs) in plants. *International Journal of*

Molecular Sciences, *16*, 19248–19290. https://doi.org/10.3390/ijms160819248.

Seyed Mahmoudian, S. M., Pirnia, M., Nasr-Esfahani, M., Sarani, S., & Hasanzadeh-Khankahdani, H. (2026). The effect of some micronutrients, bio-fertilizers and two fungicides on wheat common root rot disease and some growth parameters. *Iranian Journal of Plant Pathology*, (In Press).

Shirzaei, F., Keykhasaber, M., Pirnia, M., Rafiei, V., & Karimi-Jashni, M. (2025). The evaluation of resistance of *Luffa aegytiaca* genotypes to the fungus *Verticillium dahliae*. *Euphytica*, *221* (3). DOI: 10.1007/s10681-025-03472-0.

Song, R., Li, J., Xie, C., Jian, W., & Yang, X. (2020). An overview of the molecular genetics of plant resistance to the Verticillium wilt pathogen *Verticillium dahliae*. *International Journal of Molecular Sciences*, *21*, 1120 https://doi.org/10.3390/ijms21031120.

Sun, Y., Wang, Y., Zhang, X., Chen, Z., Xia, Y., Wang, L., Sun, Y., Zhang, M., Xiao, Y., Han, Z., Wang, Y., & Chai, J. (2022). Plant receptor-like protein activation by a microbial glycoside hydrolase. *Nature*, *610*, 335–342. https://doi.org/10.1038/s41586-022-05214-x.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38(7)*, 3022–3027.

Vermeulen, J., & van den Berg, N. (2022). Scarcity of major resistance genes against *Verticillium dahliae* in cultivated crops. *Plant Breeding*, *141(1)*, 54–66. https://doi.org/10.1111/pbr.13046.

Vining, K. L., Zhang, Q., Smith, C. A., & Davis, T. M. (2007). Identification of resistance gene analogs and Verticillium wilt resistance-like sequences in *Mentha longifolia*. *Journal of American Society of Horticultural Science*, *132(4)*, 541–550. https://doi.org/10.21273/ JASHS.132.4.541.

Wan, H., Yuan, W., Bo, K., Shen, J., Pan, G. X., & Shen, J. (2013). Genome-wide analysis of NBS-encoding disease resistance genes in *Cucumis sativus* and phylogenetic study of NBS-encoding genes in *Cucurbitaceae* crops. *BMC Genomics*, *14*, 109. https://doi.org/ 10.1186/1471-2164-14-109

Yang, Y., Chen, T., Ling, X., & Ma, Z. (2018). Gbvdr6, a Gene encoding a receptor-like protein of cotton (*Gossypium barbadense*), confers resistance to Verticillium wilt in *Arabidopsis* and upland cotton. *Frontiers in Plant Science*, *8*, 2272. https://doi.org/10.3389/fpls.2017. 02272.