Molecular Plant Breeding 2025, Vol.16, No.6, 349-358
http://genbreedpublisher.com/index.php/mpb

Case Study Open Access

Genome Resequencing-Based Screening and Candidate Gene Mining for Apple Alternaria Leaf Spot Resistance

Wenzhong Huang ✉, Zhongmei Hong
CRO Service Station, Sanya Tihitar SciTech Breeding Service Inc., Sanya, 572025, Hainan, China
✉ Corresponding email: wenzhong.huang@hitar.org
Molecular Plant Breeding, 2025, Vol.16, No.6 doi: 10.5376/mpb.2025.16.0035
Received: 05 Nov., 2025
Accepted: 11 Dec., 2025
Published: 25 Dec., 2025

Preferred citation for this article:
Huang W.Z., and Hong Z.M., 2025, Genome resequencing-based screening and candidate gene mining for apple alternaria leaf spot resistance, Molecular Plant Breeding, 16(6): 349-358 (doi: 10.5376/mpb.2025.16.0035)

Abstract This study mainly introduces how the pathogen of Alternaria leaf spot disease infects apples, explains how the pathogen and apples interact with each other, summarizes some current research progress in apple disease resistance, and also sorts out the methods of developing molecular markers based on resequencing, the identification steps of resistant materials, and the basic strategies of disease-resistant breeding, etc. The research results show that Alt resistance sites, NBS-LRR disease-resistant genes, ROS-related pathways, hormone regulatory modules and some genes related to structural defense are all involved in the disease resistance process of apples together. Combining resequencing with expression data can help identify candidate genes with high credibility more quickly and accurately. This study aims to provide an operational technical framework for future apple disease-resistant breeding and also offer some theoretical references.
Keywords Apple Alternaria leaf spot; Genome resequencing; Candidate gene mining; Genetic basis of resistance; Molecular markers and gene editing

1 Introduction

Alternaria leaf spot is a very serious disease of apple leaves. It is caused by a specialized fungus, *Alternaria alternata* f. sp. *mali*. This disease can be seen in major apple-growing areas such as China, the United States, Japan, South Korea, Russia, Iran, Turkey and Brazil, posing a significant threat to the global apple industry. Pathogenic bacteria can produce some specific toxins (such as AM-toxin) and various secreted proteins. These substances can infect leaves, damage cells, cause brown or black spots on leaves, and eventually lead to early leaf drop, poor fruit quality and reduced yield (Elfar et al., 2023). In China, apple Alternaria leaf spot was first reported in the 1970s and soon became one of the “four major diseases” in the main apple-producing areas, having a significant impact on orchard income (Liu et al., 2022; Cao et al., 2024). The degree of disease susceptibility varies among different apple varieties. For instance, ‘Golden Delicious’ is more prone to disease (Fontaine et al., 2021).

In the past, researchers identified the resistance of apple Alternaria leaf spot mainly through natural field occurrence and artificial inoculation, and then experienced experts would make judgments based on the area of the lesion and the grade of the disease. However, these methods are highly subjective and not very efficient. They are also easily affected by factors such as weather and environment, making it difficult to accurately distinguish the resistance levels of different materials. Although chemical control can suppress diseases in the short term, long-term use will bring problems such as environmental pollution, enhanced pathogen resistance, and pesticide residue risk in fruits (Liu et al., 2022). Therefore, there is now a great need for a faster, more objective and more stable resistance screening method to assist in breeding and precise prevention and control.

In this study, genomic resequencing technology was utilized to screen a large number of apple germplasm resources for resistance to Alternaria leaf spot. Combined with candidate gene mining, the genetic basis of apple resistance was analyzed. By using molecular markers, transcriptome data and functional verification together, genes related to resistance were identified and their regulatory patterns were analyzed. This study aims to provide theoretical support and some available technical references for molecular breeding and green control of disease resistance in apples.

2 Pathogenesis and Genetic Basis of Resistance

2.1 Pathogen biology and infection mechanisms of *Alternaria alternata* apple pathotype

Alternaria alternata apple pathotype (*A. alternata* apple pathotype, AAAP) is a major threat to the global apple industry and is particularly common in East Asia. This pathogen mainly causes disease through a host-specific toxin called AM-toxin. AM-toxin can damage the cellular structure of leaves, causing small brown or black spots on the leaves. As a result, the leaves will fall off prematurely and the fruits will also be affected. AM-toxin not only directly damages chloroplasts and the nuclear membrane of cells, but also causes cells to enter the death process, facilitating the further spread of pathogenic bacteria (Figure 1). In addition, *A. alternata* also secretes various cell wall-degrading enzymes and proteins, such as AltABC, AltAO, AltPDE, etc. Together, they weaken the defense capabilities of apples, making diseases more likely to occur. The AM-toxin toxicity and secreted protein combinations of different strains are different, so their pathogenicity to different varieties will also vary (Cao et al., 2024; Zeng et al., 2024).

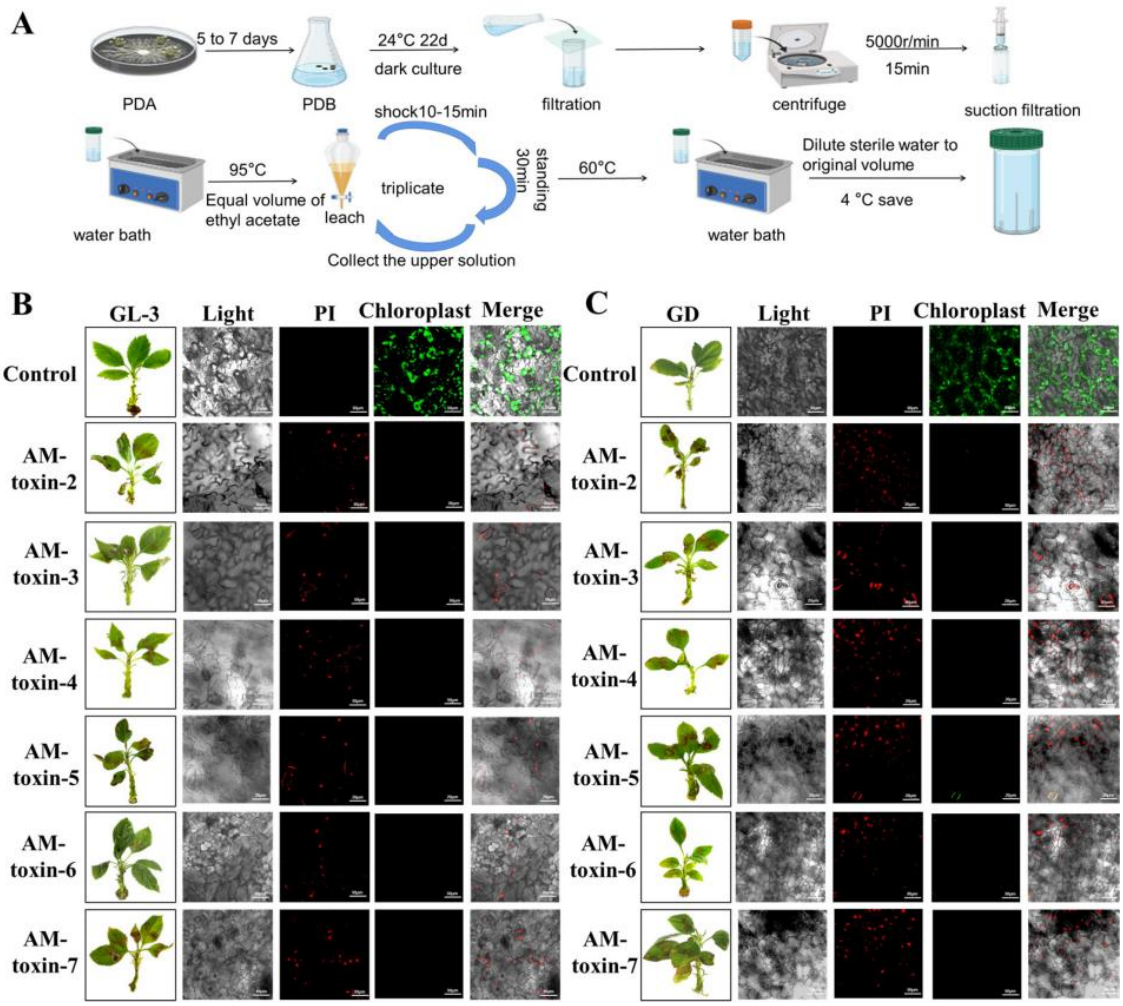

Figure 1 Phenotypes and cellular effects of AM-toxins on apple leaves (Adopted from Cao et al., 2024)

Image caption: (A). Schematic diagram of toxin extraction from *Alternaria alternata* f. sp. *mali* strains ALT2/3/4/5/6/7. (B). AM-toxin-2/3/4/5/6/7-damaged chloroplasts and plasma membranes in GL-3 (Gala-3) leaves. (C). AM-toxin-2/3/4/5/6/7-damaged chloroplasts and plasma membranes in GD (Golden Delicious) leaves. Propidium iodide was used to stain the leaves of control plants and leaves treated with AM-toxin-2/3/4/5/6/7. Confocal microscopy images show red fluorescence at 580-610 nm (representing propidium iodide-stained nuclei) and green fluorescence at 629-670 nm (representing autofluorescence of chloroplasts) (Adopted from Cao et al., 2024)

2.2 Known resistance genes and quantitative trait loci (QTLs)

The resistance of apples to *A. alternata* is not determined by a single factor, but is controlled by major genes and multiple QTLs together. The most important gene known now is the *Alt* gene, which is located at the top of

chromosome 11 and belongs to the CC-NB-LRR class of R genes. The *Alt* gene has different alleles, such as *Alt1* and *Alt2*, which are distributed differently in various varieties. Moreover, *Alt1* is dominant over *Alt2*. Both of these alleles are associated with susceptibility. There is a 12 bp insertion in the *Alt* gene region, which can be used as a molecular marker to distinguish disease-resistant and susceptible materials (Moriya et al., 2019; Moriya et al., 2024). In addition to the *Alt* gene, members of the NS-LRR family (such as *MdNLR16*, *MdRNL2*, and *MdRNL6*) are also important in disease resistance responses. Overexpression of these genes can enhance resistance, and when they are silenced, the plants are more prone to diseases (Meng et al., 2018; Liu et al., 2024). Multiple locations related to resistance have also been identified in QTL studies, among which Alt2 QTL can explain nearly half of the phenotypic differences (Moriya et al., 2024). Some miRNAs (such as miR482, miR827) can also affect the expression of the NBS-LRR gene, thereby influencing the resistance level (Liu et al., 2024; Cao et al., 2025).

2.3 Molecular inheritance patterns of resistance in different apple germplasms

There are significant genetic differences among different apple varieties in their resistance to *A. alternata*. Some varieties, such as 'Jonathan', 'Gala', and 'Hanfu', show strong resistance, while varieties like 'Starking Delicious' and 'Indo' are very susceptible to diseases (Zeng et al., 2024; Chauhan et al., 2025). Resistance is mainly controlled by genotypes such as Alt/alt: Alt/alt often shows susceptibility to diseases, while alt/alt shows resistance to diseases. The resistance performance of different regions and different varieties to the same strain may also vary, which may be related to the toxin level of the strain, the genetic background of the fruit trees themselves, and environmental conditions (Li et al., 2019; Moriya et al., 2019; Moriya et al., 2024). Some transcriptomic and proteomic studies also indicate that resistant varieties will quickly initiate defense responses in the early stage of pathogen infection, such as activating R genes, PR proteins, JA/SA signaling pathways, and genes related to cell wall reinforcement. The responses of susceptible varieties are often relatively slow and may even down-regulate these defense genes (Zhou et al., 2022; Liu et al., 2023; Zhou et al., 2023). In addition, the regulatory effects of some mirnas and epigenetic mechanisms such as histone methylation involving MdSDG26 can also affect the resistance level of apples (Liu et al., 2024; Shen et al., 2024; Cao et al., 2025).

3 Genome Resequencing in Apple Resistance Studies

3.1 Principles and workflow of genome resequencing (WGS and re-seq pipelines)

Whole genome sequencing (WGS) and genome resequencing (re-seq) are both methods that utilize high-throughput sequencing to obtain information on genomic variations. WGS is generally used to establish a reference genome, while resequencing involves comparing many samples based on an existing reference genome to identify variations. Their basic processes are more or less the same, including DNA extraction, library construction, sequencing, data quality control, sequence alignment, as well as variant detection and annotation. The commonly used analysis processes nowadays include BWA-MEM, GATK and DRAGEN, etc. They each have their own characteristics in terms of comparison speed and the accuracy of mutation detection. Therefore, choosing the right process is very important for subsequent analysis (Betschart et al., 2022; Minamikawa et al., 2024; Lu et al., 2025).

3.2 Applications in identifying genomic variation: SNPs, InDels, CNVs, and SVs

Genomic resequencing can simultaneously detect multiple variations, including SNPS, insertions and deletions (InDel), copy number variations (CNV), and structural variations (SV). Among them, SNP and InDel are the most common and are most widely used in population genetics and trait association analysis. CNV is another important variant type, which can affect gene dose, expression level and even phenotype. For instance, in apples, the CNV whole genome map shows that it is involved in biological processes such as defense responses (Xu et al., 2023). SV is also very important. Large fragment insertions, deletions and inversions may all affect gene function. Long-read sequencing and the combined use of different platforms have greatly improved the detection ability of SV (Ebert et al., 2021; Rao et al., 2025). In fruit trees such as apples, the high-density genetic maps and variant data obtained by resequencing provide an important basis for the localization of disease-resistant genes and the screening of candidate genes (Agarwal et al., 2018; Peace et al., 2019).

3.3 Population genetics and genome-wide association analysis (GWAS) for resistance trait mapping

Based on resequencing data, population genetics analysis can help us understand the genetic structure, evolution process, and distribution of resistance genes of apple germplasm. By combining high-density SNP/InDel variations and phenotypic information through GWAS, QTLs and candidate genes related to resistance can be identified across the genome. At present, apple resistance GWAS have identified multiple important QTLs and candidate genes in aspects such as black star disease, fire blight, and Marssonina spot disease. Some overlap with known resistance genes, while others are new discoveries (Noh et al., 2020; Thapa et al., 2021). If combined with transcriptome data and gene function annotation, the accuracy of candidate genes can be further improved, providing a more reliable basis for molecular marker-assisted breeding and resistant variety breeding (Chagné et al., 2019; Peace et al., 2019; Minamikawa et al., 2024).

4 Candidate Gene Mining and Functional Annotation

4.1 Strategies for identifying candidate genes from resistant vs. susceptible genotypes

The screening of candidate genes usually employs both genomic resequencing and multi-omics data simultaneously. Researchers usually compare resistant and disease-susceptible apple varieties together, such as ‘Jonathan’ and ‘Starking Delicious’, or ‘Hanfu’ and ‘Golden Delicious’. By performing whole-genome resequencing and transcriptome sequencing on these materials, genes (DEGs) that are significantly differentially expressed after pathogen infection can be identified (Liu et al., 2023). On this basis, combined with genetic linkage analysis, QTL mapping and high-density SNP typing, some genes related to resistance can be located to specific chromosomal regions, such as Alt sites or Rgls regions (Moriya et al., 2019). In addition, small RNA sequencing can also screen out some disease-resistant genes regulated by miRNA, such as certain NB-LRR R-like genes or WRKY transcription factors (Zhang et al., 2018; Liu et al., 2024). Finally, through population typing, association analysis of genotype and phenotype, and combined with functional verification (such as transient expression, gene silencing, etc.), it is possible to further confirm which are the candidate genes that are truly closely related to resistance (He et al., 2022; Liu et al., 2023).

4.2 Gene function prediction using GO, KEGG, and BLAST annotation

For the candidate genes screened out, functional annotations still need to be made using tools such as GO, KEGG and BLAST. GO analysis revealed that these genes were generally concentrated in biological processes such as defense responses, signal transduction, transcriptional regulation, and REDOX processes. KEGG results often show that they are enriched in pathways such as MAPK signaling pathway - plant, plant hormone signaling pathway, and plant-pathogen interaction (He et al., 2022). BLAST is used to compare the homology and domains of genes, such as typical disease-resistant protein domains like NBS-LRR, TIR, and CC (Moriya et al., 2019; Lv et al., 2022). These annotation results can assist in subsequent functional research and further explain the resistance mechanism.

4.3 Integration with transcriptome data to validate resistance-related expression patterns

After analyzing the candidate genes and transcriptome data together, the expression differences between them in resistance and susceptibility genotypes can be seen more clearly. RNA-seq shows that some genes, such as *MdGRAS53*, *MdERF110*, *MdWRKY75d/e*, and *MdRNL2/6*, are significantly upregulated in resistant varieties after pathogen infection. However, in susceptible varieties, these genes were either expressed at low levels or inhibited by miRNA (He et al., 2022; Liu et al., 2023). Some miRNAs (such as miR482, miR156ab, miR395) can further reduce the resistance of susceptible varieties by inhibiting R genes or transcription factors (Liu et al., 2024). Functional verification, such as transient gene expression and gene silencing experiments, also confirmed the important role of these genes in resistance (He et al., 2022; Liu et al., 2023). Analyzing multi-omics data together can significantly enhance the accuracy of candidate gene screening and also make subsequent annotation and judgment more reliable.

5 Bioinformatics Tools and Data Resources

5.1 Databases for apple genome analysis

The research on the apple genome cannot do without the support of some authoritative databases. GDR (Genome Database for Rosaceae) is currently the most comprehensive Rosaceae database, which integrates genomic

sequences, annotation information, genetic maps, SNPS, QTLs, as well as phenotypic and genotype data of various Rosaceae crops such as apples. It also provides various query tools such as gene function, KEGG pathway, homologous genes, etc., and supports visualization, which greatly facilitates the localization of disease-resistant genes and the screening of candidate genes (Peace et al., 2019; Saud et al., 2024). In addition, NCBI and EnsemblPlants can also provide basic data such as apple genomes, annotations, SNPS and transcriptomes, and are the main sources for many comparative analyses (Peace et al., 2019). AppleMDO integrates multi-omics data such as genomics, transcriptomics and epigenomics, and can conduct co-expression network analysis, functional module analysis and chromatin state studies, which is very helpful for finding resistance-related regulatory relationships (Figure 2) (Da et al., 2019). The ROFT database focuses on transcriptome data during the developmental stage of Rosaceae fruits such as apples, facilitating the comparison of expression patterns among different species and tissues (Li et al., 2023).

5.2 Bioinformatics pipelines for variant calling, annotation, and haplotype analysis

Mutation detection and annotation of high-throughput sequencing data are important steps in mining resistance genes. The general process is to first perform sequence alignment using BWA or DRAGEN, and then use tools such as GATK, Strelka2, Samtools-Varscan2, and DeepVariant to find SNPS and indels. Multiple system evaluations have shown that DRAGEN and DeepVariant are better in terms of accuracy and efficiency and are suitable for large-scale population genome analysis (Chen et al., 2019; Zhao et al., 2020; Betschart et al., 2022; Wong et al., 2025). Due to its high maturity and standardization, GATK is also frequently used for variation detection and haplotype analysis of crops such as apples (Weißbach et al., 2020). Some new tools, such as snpArcher, integrate mutation quality control, visualization and downstream analysis, making the process more stable and more suitable for processing large volumes of data (Mirchandani et al., 2023). Furthermore, the co-expression network analysis tool WGCNA is also frequently used to link haplotypes with phenotypes (Li et al., 2023).

5.3 Functional validation support from protein interaction networks and regulatory pathway mapping

The functional research of candidate genes often requires protein-protein interaction networks and regulatory pathways to assist in the analysis. The AppleMDO database contains protein interaction information, transcription factor families, GO annotations and KEGG pathways of apples, which can help infer gene functions and their regulatory relationships (Da et al., 2019). In addition, algorithms like RSNET can use transcriptome data to infer the regulatory network of apples at specific developmental stages, thereby identifying key regulatory genes (Mochida et al., 2018; Jiang and Zhang, 2022). Tools such as iRegulon analyze the binding sites of cis-elements and transcription factors to infer regulatory relationships in reverse, further helping to confirm the upstream regulation of candidate genes. Protein-protein interaction network and pathway analysis can not only help determine the importance of candidate genes, but also provide theoretical support for subsequent functional verification and mechanism research.

6 Case Study: Genome Resequencing of Resistant Apple Varieties

6.1 Study background: selected resistant and susceptible apple genotypes

This case mainly studies the genomic differences between resistant varieties and susceptible varieties of apple Alternaria leaf spot (*Alternaria alternata*). Common resistant varieties include ‘Hanfu’, ‘Jonathan’, etc. While ‘Golden Delicious’, ‘Starking Delicious’ and ‘NGR196’ are usually used as susceptible controls. These varieties vary greatly in genetic background and disease resistance performance. Therefore, they are highly suitable for mining resistance genes and also for functional validation (Zhang et al., 2018; Zhou et al., 2022; Liu et al., 2023).

6.2 Workflow: resequencing, variant detection, and candidate gene identification

The first step of the research is to conduct whole-genome resequencing on resistant and susceptible varieties to obtain high-quality genomic data. Then compare the sequence with the reference genome to detect variations such as SNPS and indels. Then, by combining population typing, linkage analysis and expression data, the gene regions related to resistance are located. For instance, by comparing the resequencing data of the F1 population with that

of the parents and combining them with phenotypic scores, the loci of resistance or susceptibility such as Alt/Alt2 can be located more precisely (Moriya et al., 2019; Moriya et al., 2024). Meanwhile, RNA-Seq and transcriptome analyses can also identify defense genes and their regulatory networks that are specifically activated in resistant varieties (Zhou et al., 2022; Liu et al., 2023).

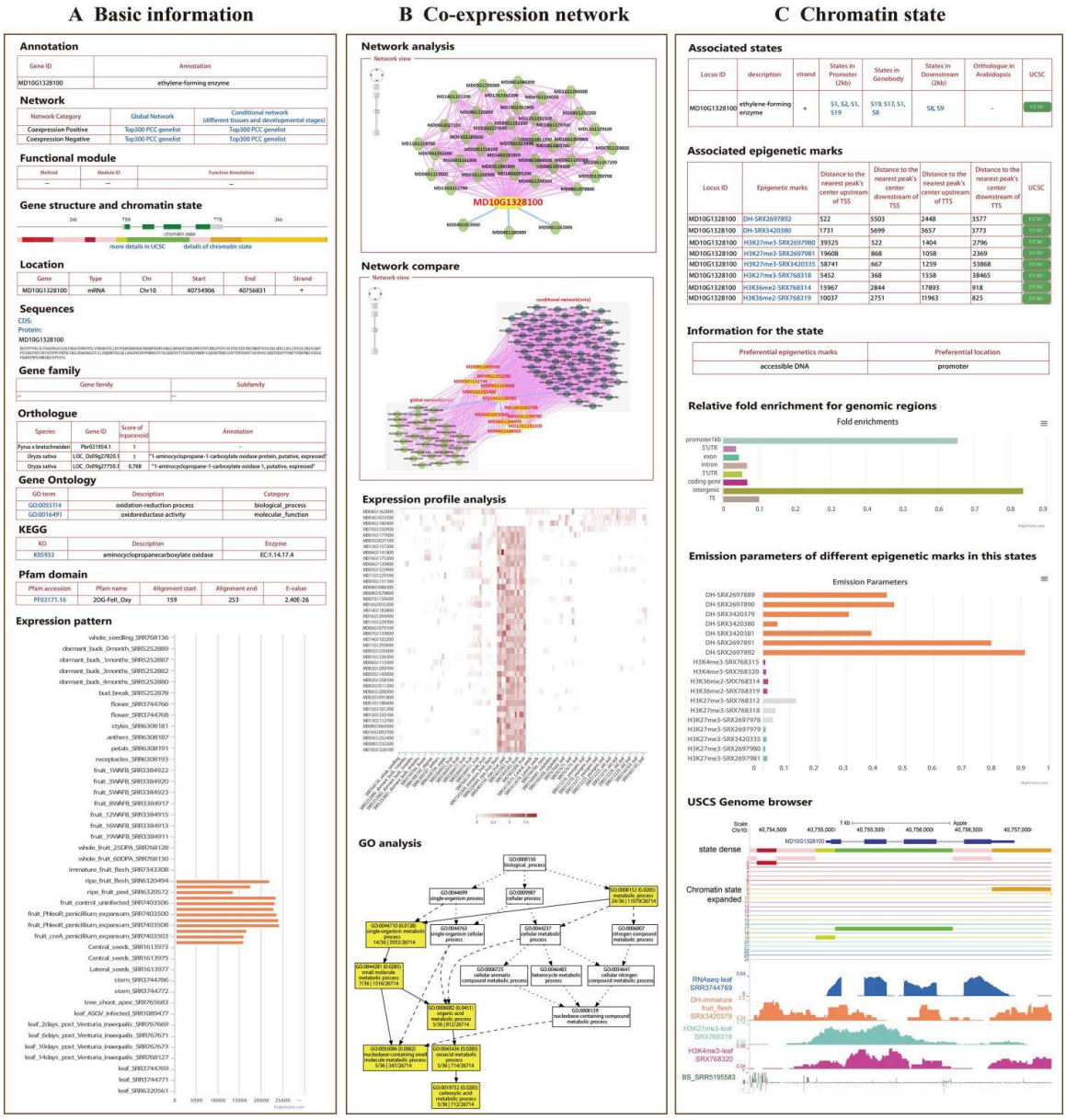

Figure 2 General description of AppleMDO functions (Adopted from Da et al., 2019)

6.3 Key findings: resistance-related alleles, gene clusters, and functional markers

In the fine localization of chromosome 11, the study found that the Alt/Alt2 gene was located near the CC-NB-LRR class of disease-resistant genes, and a 12 bp insertion mutation co-isolated from susceptibility was identified (Moriya et al., 2019; Moriya et al., 2024). In addition, some specific SNPs (such as single nucleotide variations in the promoter region of MdhpRNA277) can also be used to distinguish resistant and susceptible varieties as molecular markers (Zhang et al., 2018). In resistant varieties, gene families such as CNL, TNL, and WRKY are significantly upregulated, and together they form a defense network (Liu et al., 2023). Meanwhile, some miRNAs and epigenetic regulatory modalities (such as m6A modification, SET-domain proteins) have also been demonstrated to be involved in resistance regulation (Liu et al., 2024; Shen et al., 2024; Song et al., 2025). In addition, markers such as SSR and RAPD have also been developed, which can be used to quickly distinguish between resistance and susceptibility genotypes, providing assistance for molecular breeding.

7 Integration with Molecular Breeding and Marker Development

7.1 Marker-assisted selection (MAS) based on SNP and InDel markers

Molecular markers such as SNP and InDel have become very important tools in the breeding of apple Alternaria leaf spot resistance. Studies have shown that some specific SNP loci (such as the SNP in the promoter region of MdhpRNA277) have a close relationship with resistance or susceptibility and can be used to distinguish resistant and susceptible varieties, thereby completing molecular screening at an early stage (Zhang et al., 2018). Furthermore, markers such as SSR (Simple sequence repeat) and RAPD (Random amplification polymorphic DNA) have also been shown to be closely linked to resistance or susceptibility, enabling rapid screening of disease-resistant plants in large-scale breeding populations (Moriya et al., 2019). Through MAS, the breeding speed of disease-resistant varieties was significantly accelerated, and the time and cost of field identification were also reduced (Li et al., 2019).

7.2 Haplotype-based breeding strategies for durable resistance improvement

Haplotype analysis can help study the genetic structure and diversity of resistance genes and also contribute to the breeding of varieties with more persistent resistance. Fine localization and haplotype analysis of major resistance genes such as Alt revealed that some specific insertions or deletions co-occurred with susceptibility. These results laid the foundation for haplotype precision breeding (Moriya et al., 2019). Meanwhile, analyzing the haplotype diversity of resistance gene families such as NB-LRR and CC-NB-LRR can also help integrate superior resistance resources and provide support for the construction of durable resistance varieties (Liu et al., 2023; Liu et al., 2024).

7.3 Prospects of genomic selection and CRISPR-based gene validation

With the continuous advancement of apple genome sequencing and the accumulation of a large number of high-density molecular markers, the prospects of genome selection (GS) in resistance breeding are getting better and better. GS can integrate SNP information from the entire genome to predict resistance phenotypes, thereby enhancing the efficiency of improving multi-gene control traits. Meanwhile, gene editing technologies such as CRISPR/Cas have also provided new means for functional verification and resistance improvement. In the future, it is expected that new disease-resistant varieties can be rapidly obtained through site-specific editing of key resistance genes (Igarashi et al., 2016).

8 Challenges and Future Perspectives

Genome resequencing holds great potential in unearthing resistance genes to apple Alternaria leaf spot, but its effectiveness largely depends on data quality, sequencing depth, and the reliability of the reference genome itself. When the sequencing depth is too low, many SNPS and structural variations may be missed, thereby affecting the localization accuracy of resistance genes. In addition, the genome of apple has a high degree of heterozygosity and a complex structure. The fragmentation of the early reference genome is relatively serious, which also affects the annotation and localization of candidate genes. Although the high-quality T2T reference genomes and pan-genomes introduced in recent years have significantly improved the accuracy of gene mining, there are still structural variations and gene deletions among different varieties. These differences may prevent some resistance genes from being detected. Therefore, enhancing sequencing depth, optimizing the analysis process, and using reference genomes of multiple varieties are the key directions for improving the accuracy of mining.

There are a large number of disease-resistant gene families (such as NBS-LRR, WRKY) in apples, and their functions often overlap. This makes it difficult for a single gene knockout or overexpression to completely change the resistance phenotype. Meanwhile, multilayer regulatory mechanisms such as miRNA also make the resistance regulatory network more complex. The functional verification of candidate genes usually requires transgenic or gene editing techniques. However, the genetic transformation efficiency of apples is not high, the cycle is long, and the phenotype is easily affected by the environment. These factors all increase the difficulty of identifying pathogenic genes. Therefore, integrating multi-omics data and developing a higher-throughput functional verification platform can help break through the bottleneck caused by functional redundancy and more accurately identify key genes.

In the future, the mining of resistance genes will develop from single-omics to multi-omics integration (genome, transcriptome, epigenome, metabolome, etc.), and the resistance regulatory network will be analyzed through systems biology methods, thereby improving the accuracy of candidate gene screening. The establishment of the pan-genome can help to understand the differences in resistance genes among different apple varieties more comprehensively and avoid missing important genes due to relying only on a single reference genome. In addition, the "resistance gene aggregation" strategy of combining multiple resistance genes into the same variety can enable apple varieties to acquire more stable and broad-spectrum resistance . Combining high-density molecular markers and gene editing techniques, the efficiency of disease-resistant breeding of apples in the future will be further enhanced, and it is expected to simultaneously improve resistance and fruit quality.

Acknowledgments

The authors thank Dr. Zhou for his modification suggestions on the manuscript of this study.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Agarwal G., Agarwal G., Agarwal G., Clevenger J., Pandey M., Wang H., Wang H., Shasidhar Y., Chu Y., Fountain J., Fountain J., Choudhary D., Choudhary D., Culbreath A., Liu X., Huang G., Wang X., Deshmukh R., Holbrook C., Bertioli D., Ozias-Akins P., Jackson S., Varshney R., and Guo B., 2018, High-density genetic map using whole‐genome resequencing for fine mapping and candidate gene discovery for disease resistance in peanut, Plant Biotechnology Journal, 16: 1954-1967.
https://doi.org/10.1111/pbi.12930

Betschart R., Thiéry A., Aguilera-Garcia D., Zoche M., Moch H., Twerenbold R., Zeller T., Blankenberg S., and Ziegler A., 2022, Comparison of calling pipelines for whole genome sequencing: an empirical study demonstrating the importance of mapping and alignment, Scientific Reports, 12: 21502.
https://doi.org/10.1038/s41598-022-26181-3

Cao C., Gong S., Li Y., Tang J., Li T., and Zhang Q., 2024, Pathogenic factors and mechanisms of the Alternaria leaf spot pathogen in apple, Horticulturae, 10(3): 212.
https://doi.org/10.3390/horticulturae10030212

Cao L., Luo C., Liu Y., Lan L., Zhou T., Hu K., Wang S., Yu X., and Qu S., 2025, miR827 increases susceptibility to *Alternaria alternata* by shearing the mRNAs of low-affinity phosphate transporters *PHT5-2* and *PHT5-3* in apple, The Plant Journal, 122(3): e70222.
https://doi.org/10.1111/tpj.70222

Chagné D., Vanderzande S., Kirk C., Profitt N., Weskett R., Gardiner S., Peace C., Volz R., and Bassil N., 2019, Validation of SNP markers for fruit quality and disease resistance loci in apple (*Malus* × *domestica* Borkh.) using the OpenArray® platform, Horticulture Research, 6: 30.
https://doi.org/10.1038/s41438-018-0114-2

Chauhan P., Gupta A., Sharma J., and Sharma M., 2025, Assessment of apple germplasm for resistance against Alternaria blotch triggered by *Alternaria alternata* f. sp. *mali* under natural epiphytotic conditions, Discover Plants, 2: 92.
https://doi.org/10.1007/s44372-025-00179-3

Chen J., Li X., Zhong H., Meng Y., and Du H., 2019, Systematic comparison of germline variant calling pipelines cross multiple next-generation sequencers, Scientific Reports, 9: 9345.
https://doi.org/10.1038/s41598-019-45835-3

Da L., Liu Y., Yang J., Tian T., She J., Ma X., Xu W., and Su Z., 2019, AppleMDO: a multi-dimensional omics database for apple co-expression networks and chromatin states, Frontiers in Plant Science, 10: 1333.
https://doi.org/10.3389/fpls.2019.01333

Ebert P., Audano P., Zhu Q., Rodriguez-Martin B., Porubsky D., Bonder M., Sulovari A., Ebler J., Zhou W., Mari R., Yilmaz F., Zhao X., Hsieh P., Lee J., Kumar S., Lin J., Rausch T., Chen Y., Ren J., Santamarina M., Höps W., Ashraf H., Chuang N., Yang X., Munson K., Lewis A., Fairley S., Tallon L., Clarke W., Basile A., Byrska-Bishop M., Corvelo A., Evani U., Lu T., Chaisson M., Chen J., Li C., Brand H., Wenger A., Ghareghani M., Harvey W., Raeder B., Hasenfeld P., Regier A., Abel H., Hall I., Flicek P., Stegle O., Gerstein M., Tubío J., Mu Z., Li Y., Shi X., Hastie A., Ye K., Chong Z., Sanders A., Zody M., Talkowski M., Mills R., Devine S., Lee C., Korbel J., Marschall T., and Eichler E., 2021, Haplotype-resolved diverse human genomes and integrated analysis of structural variation, Science, 372: abf7117.
https://doi.org/10.1126/science.abf7117

Elfar K., Bustamante M., Arreguin M., Nouri M., and Eskalen A., 2023, Identification and pathogenicity of Alternaria species causing leaf blotch and fruit spot of apple in California, Phytopathologia Mediterranea, 62(3): 467-479.

Fontaine K., Fourrier-Jeandel C., Armitage A., Boutigny A., Crépet M., Caffier V., Gnide D., Shiller J., Cam L., Giraud M., Ioos R., and Aguayo J., 2021, Identification and pathogenicity of *Alternaria* species associated with leaf blotch disease and premature defoliation in French apple orchards, PeerJ, 9: e12496.
https://doi.org/10.7717/peerj.12496

He Y., Lan L., Zhao L., Cai B., Qu S., and Wang S., 2022, Comparative transcriptomics analysis reveals *MdGRAS53* contributes to disease resistance against Alternaria blotch of apple, Journal of Plant Physiology, 273: 153697.
https://doi.org/10.1016/j.jplph.2022.153697

Igarashi M., Hatsuyama Y., Harada T., and Fukasawa-Akada T., 2016, Biotechnology and apple breeding in Japan, Breeding Science, 66: 18-33.
https://doi.org/10.1270/jsbbs.66.18

Jiang X., and Zhang X., 2022, RSNET: inferring gene regulatory networks by a redundancy silencing and network enhancement technique, BMC Bioinformatics, 23: 165.
https://doi.org/10.1186/s12859-022-04696-w

Li M., Mount S., and Liu Z., 2023, Rosaceae fruit transcriptome database (ROFT)- a useful genomic resource for comparing fruits of apple, peach, strawberry, and raspberry, Horticulture Research, 10(12): uhad240.
https://doi.org/10.1093/hr/uhad240

Li Y., Hu X., Trigiano R., Aldwinckle H., and Cheng Z., 2019, Evaluation of 110 apple cultivars for resistance to Alternaria blotch caused by *Alternaria alternata* apple pathotype, HortScience, 54(8): 1268-1274.
https://doi.org/10.21273/hortsci13841-18

Liu B., Fan K., Su W., and Peng Y., 2022, Two-stage convolutional neural networks for diagnosing the severity of Alternaria leaf blotch disease of the apple tree, Remote Sens., 14(11): 2519.
https://doi.org/10.3390/rs14112519

Liu F., Tang J., Li T., and Zhang Q., 2024, The microRNA miR482 regulates NBS-LRR genes in response to ALT1 infection in apple, Plant Science, 343: 112078.
https://doi.org/10.1016/j.plantsci.2024.112078

Liu K., Liang Z., Yang A., Yan J., Cong P., Han X., and Zhang C., 2023, Comparative transcriptome analysis of apple cultivars reveals key genes and pathways in response to *Alternaria alternata* apple pathotype infection, Horticultural Plant Journal, 10(3): 641-656.
https://doi.org/10.1016/j.hpj.2023.02.008

Lu Y., Li M., Gao Z., Ma H., Chong Y., Hong J., Wu J., Wu D., Xi D., and Deng W., 2025, Advances in whole genome sequencing: methods, tools, and applications in population genomics, International Journal of Molecular Sciences, 26(1): 372.
https://doi.org/10.3390/ijms26010372

Luo F., Evans K., Norelli J., Zhang Z., and Peace C., 2020, Prospects for achieving durable disease resistance with elite fruit quality in apple breeding, Tree Genetics and Genomes, 16: 21.
https://doi.org/10.1007/s11295-020-1414-x

Lv L., Liu Y., Bai S., Turakulov K., Dong C., and Zhang Y., 2022, A TIR-NBS-LRR gene *MdTNL1* regulates resistance to Glomerella leaf spot in apple, International Journal of Molecular Sciences, 23(11): 6323.
https://doi.org/10.3390/ijms23116323

Meng D., Li C., Park H., González J., Wang J., Dandekar A., Turgeon B., and Cheng L., 2018, Sorbitol modulates resistance to *Alternaria alternata* by regulating the expression of an NLR resistance gene in apple, Plant Cell, 30: 1562-1581.
https://doi.org/10.1105/tpc.18.00231

Minamikawa M., Kunihisa M., Moriya S., Shimizu T., Inamori M., and Iwata H., 2024, Genomic prediction and genome-wide association study using combined genotypic data from different genotyping systems: application to apple fruit quality traits, Horticulture Research, 11(7): uhae131.
https://doi.org/10.1093/hr/uhae131

Mirchandani C., Shultz A., Thomas G., Smith S., Baylis M., Arnold B., Corbett-Detig R., Enbody E., and Sackton T., 2023, A fast, reproducible, high-throughput variant calling workflow for population genomics, Molecular Biology and Evolution, 41(1): msad270.
https://doi.org/10.1093/molbev/msad270

Mochida K., Koda S., Inoue K., and Nishii R., 2018, Statistical and machine learning approaches to predict gene regulatory networks from transcriptome datasets, Frontiers in Plant Science, 9: 1770.
https://doi.org/10.3389/fpls.2018.01770

Moriya S., Abe K., Okada K., Shimizu T., Kunihisa M., Hori A., and Sawamura Y., 2024, Identification of a gene conferring moderate susceptibility to Alternaria blotch (*Alternaria alternata* apple pathotype) in 'Golden Delicious' apples (*Malus* × *domestica* Borkh.), The Horticulture Journal, 93(3): 232-241.

Moriya S., Terakami S., Okada K., Shimizu T., Adachi Y., Katayose Y., Fujisawa H., Wu J., Kanamori H., Yamamoto T., and Abe K., 2019, Identification of candidate genes responsible for the susceptibility of apple (*Malus* × *domestica* Borkh.) to Alternaria blotch, BMC Plant Biology, 19: 132.
https://doi.org/10.1186/s12870-019-1737-7

Noh J., Do Y., Kim G., and Choi C., 2020, A genome-wide association study for the detection of genes related to apple Marssonina Blotch disease resistance in apples, Scientia Horticulturae, 262: 108986.
https://doi.org/10.1016/j.scienta.2019.108986

Peace C., Bianco L., Troggio M., Van De Weg E., Howard N., Cornille A., Durel C., Myles S., Migicovsky Z., Schaffer R., Costes E., Fazio G., Yamane H., Van Nocker S., Gottschalk C., Costa F., Chagné D., Zhang X., Patocchi A., Gardiner S., Hardner C., Kumar S., Laurens F., Bucher E., Main D., Jung S., and Vanderzande S., 2019, Apple whole genome sequences: recent advances and new prospects, Horticulture Research, 6: 59.
https://doi.org/10.1038/s41438-019-0141-7

Rao J., Luo H., An D., Liang X., Peng L., and Chen F., 2025, Performance evaluation of structural variation detection using DNBSEQ whole-genome sequencing, BMC Genomics, 26: 299.
https://doi.org/10.1186/s12864-025-11494-0

Saud S., Atreya P.N., Bhattarai N., Dhakal S., and Bohara A.K., 2024, Assessment of physical and chemical properties of different cultivars of apple (*Malus* spp) in Mustang, Nepal, International Journal of Horticulture, 14(5): 275-282.
https://doi.org/10.5376/ijh.2024.14.0029

Shen W., Zhang D., Zhang Z., He J., Khalil A., Li X., Ma F., Guan Q., and Niu C., 2024, The SET-domain-containing protein MdSDG26 negatively regulates *Alternaria alternata* resistance in apple, Plant, Cell and Environment, 48(1): 323-337.
https://doi.org/10.1111/pce.15136

Song Z., Yang Q., Dong B., Wang S., Xue J., Liu N., Zhou X., Li N., Dandekar A., Cheng L., Meng D., and Fu Y., 2025, Nanopore RNA direct sequencing identifies that m^{6}A modification is essential for sorbitol-controlled resistance to *Alternaria alternata* in apple, Developmental Cell, 60(10): 1439-1453.
https://doi.org/10.1016/j.devcel.2024.12.033

Su Y., Yang X., Wang Y., Li J., Long Q., Cao S., Wang X., Liu Z., Huang S., Chen Z., Peng Y., Zhang F., Xue H., Cao X., Zhang M., Yisilam G., Chu Z., Gao Y., Zhou Y., Liu Z., Xiao H., and Tian X., 2024, Phased telomere-to-telomere reference genome and pangenome reveal an expansion of resistance genes during apple domestication, Plant Physiology, 195(4): 2799-2814.
https://doi.org/10.1093/plphys/kiae258

Thapa R., Singh J., Gutierrez B., Arro J., and Khan A., 2021, Genome-wide association mapping identifies novel loci underlying fire blight resistance in apple, The Plant Genome, 14(2): e20087.
https://doi.org/10.1002/tpg2.20087

Weißbach S., Sys S., Hewel C., Todorov H., Schweiger S., Winter J., Pfenninger M., Torkamani A., Evans D., Burger J., Everschor-Sitte K., May-Simera H., and Gerber S., 2020, Reliability of genomic variants across different next-generation sequencing platforms and bioinformatic processing pipelines, BMC Genomics, 22: 62.
https://doi.org/10.1186/s12864-020-07362-8

Wong M., Liew B., Hum M., Lee N., and Lee A., 2025, Benchmarking of variant calling software for whole-exome sequencing using gold standard datasets, Scientific Reports, 15: 13697.
https://doi.org/10.1038/s41598-025-97047-7

Xu J., Zhang W., Zhang P., Sun W., Han Y., and Li L., 2023, A comprehensive analysis of copy number variations in diverse apple populations, BMC Genomics, 24: 256.
https://doi.org/10.1186/s12864-023-09347-9

Yang Y., Saand M., Huang L., Abdelaal W., Zhang J., Wu Y., Li J., Sirohi M., and Wang F., 2021, Applications of multi-omics technologies for crop improvement, Frontiers in Plant Science, 12: 563953.
https://doi.org/10.3389/fpls.2021.563953

Zeng X., Wu C., Zhang L., Lan L., Fu W., and Wang S., 2024, Molecular mechanism of resistance to *Alternaria alternata* apple pathotype in apple by alternative splicing of transcription factor *MdMYB6-like*, International Journal of Molecular Sciences, 25(8): 4353.
https://doi.org/10.3390/ijms25084353

Zhang Q., Ma C., Zhang Y., Gu Z., Li W., Duan X., Wang S., Hao L., Wang Y., Wang S., and Li T., 2018, A single-nucleotide polymorphism in the promoter of a hairpin RNA contributes to *Alternaria alternata* leaf spot resistance in apple (*Malus* × *domestica*), Plant Cell, 30: 1924-1942.
https://doi.org/10.1105/tpc.18.00042

Zhao S., Agafonov O., Azab A., Stokowy T., and Hovig E., 2020, Accuracy and efficiency of germline variant calling pipelines for human genome data, Scientific Reports, 10: 20222.
https://doi.org/10.1038/s41598-020-77218-4

Zhou T., Cao L., Hu K., Yu X., and Qu S., 2023, miR164-*NAC21/22* module regulates the resistance of *Malus hupehensis* against *Alternaria alternata* by controlling jasmonic acid signaling, Plant Science, 330: 111635.
https://doi.org/10.1016/j.plantsci.2023.111635

Zhou T., He Y., Zeng X., Cai B., Qu S., and Wang S., 2022, Comparative analysis of alternative splicing in two contrasting apple cultivars defense against *Alternaria alternata* apple pathotype infection, International Journal of Molecular Sciences, 23(22): 14202.
https://doi.org/10.3390/ijms232214202