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REVIEW ARTICLE

Elucidating the molecular responses of apple rootstock resistant to ARD pathogens: challenges and opportunities for development of genomics-assisted breeding tools

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Apple replant disease (ARD) is a major limitation to the establishment of economically viable orchards on replant sites due to the buildup and long-term survival of pathogen inoculum. Several soilborne necrotrophic fungi and oomycetes are primarily responsible for ARD, and symptoms range from serious inhibition of growth to the death of young trees. Chemical fumigation has been the primary method used for control of ARD, and manipulating soil microbial ecology to reduce pathogen density and aggressiveness is being investigated. To date, innate resistance of apple rootstocks as a means to control this disease has not been carefully explored, partly due to the complex etiology and the difficulty in phenotyping the disease resistance. Molecular defense responses of plant roots to soilborne necrotrophic pathogens are largely elusive, although considerable progress has been achieved using foliar disease systems. Plant defense responses to necrotrophic pathogens consist of several interacting modules and operate as a network. Upon pathogen detection by plants, cellular signals such as the oscillation of Ca^{2+} concentration, reactive oxygen species (ROS) burst and protein kinase activity, lead to plant hormone biosynthesis and signaling. Jasmonic acid (JA) and ethylene (ET) are known to be fundamental to the induction and regulation of defense mechanisms toward invading necrotrophic pathogens. Complicated hormone crosstalk modulates the fine-tuning of transcriptional reprogramming and metabolic redirection, resulting in production of antimicrobial metabolites, enzyme inhibitors and cell wall refortification to restrict further pathogenesis. Transcriptome profiling of apple roots in response to inoculation with *Pythium ultimum* demonstrated that there is a high degree of conservation regarding the molecular framework of defense responses compared with those observed with foliar tissues. It is conceivable that the timing and intensity of genotype-specific defense responses may lead to different outcomes between rootstocks in response to invasion by necrotrophic pathogens. Elucidation of host defense mechanisms is critical in developing molecular tools for genomics-assisted breeding of resistant apple rootstocks. Due to their perennial nature, use of resistant rootstocks as a component for disease management might offer a durable and cost-effective benefit to tree performance than the standard practice of soil fumigation for control of ARD.

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INTRODUCTION

Apple replant disease (ARD) is caused by a complex of soilborne necrotrophic fungi and oomycetes, and at times can be aggravated by the lesion nematode *Pratylenchus penetrans*.[1–3] When young trees are planted on a site that has a previous history of apple (or closely related species) cultivation, they develop disease symptoms ranging from mildly uneven growth to serious growth inhibition and even death of trees, especially for trees planted in previous orchard rows. In the absence of control, the effects of ARD can exist over the entire lifetime of the orchard in the form of decreased fruit yields. As a result, this disease is a primary limitation to the establishment of an economically viable orchard on replant sites. The principal method for the control of ARD is pre-plant fumigation of orchard soils to eradicate ARD pathogens,[4,5] but fumigation is not feasible after orchard establishment. In addition to the cost, the future availability of currently used fumigants could be restricted due to environmental concerns. Moreover, recent studies have demonstrated that the efficacy of fumigation in terms of plant growth and pathogen pressure in treated soils is short lived.[6] Establishing new plantings on sites where no apple or closely related crops have grown could theoretically be an option, but the availability of such land in the major production regions is limited or non-existent. Fallowing for extended periods as a cultural practice was reported to provide partial control of the peach replant problem,[7] but no detectable benefit to growth and yield of apple tree was observed on replant orchard sites after up to 3 years of fallowing.[8] Measures aimed at managing microbial communities in orchard soil to promote plant heath and minimize pathogen aggressiveness can be effective in many situations, though the satisfactory efficacy of such an approach across the diversity of orchard systems needs further investigation.

Host tolerance/resistance is an economically attractive means of managing diseases in tree fruit production systems. Recent studies suggested that the production of a more fibrous root system contributes to the enhanced performance of certain ARD tolerant rootstocks such as Geneva 210,[9–11] although tolerance to individual components of the ARD pathogen complex has been detected in apple germplasm.[12–14] Even tolerant rootstocks exhibit increased growth in response to soil fumigation indicating incomplete resistance to the causal pathogen complex among the commercially available apple rootstock germplasm. Utilization of innate resistance to ARD pathogens could provide a cost-effective, durable and environment-friendly disease control strategy, yet the molecular basis of apple root resistance responses to ARD pathogens is

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unknown. Due to the hidden nature of the root system and lack of standard phenotyping methods, the molecular characterization of root interactions with soilborne necrotrophic pathogens is currently rare even on model plant species.[15–17] Nevertheless, newly available genomic approaches, accumulated apple genetic resources and the recent progress in the study of molecular plant–necrotroph interactions present the opportunity to elucidate the molecular networks functional in apple root resistance to ARD pathogens. Such a knowledge basis is essential for targeted and efficient introduction of the resistant gene pool by genomics-assisted breeding into future apple rootstock varieties.

A MULTITROPHIC PATHOGEN COMPLEX INCITING ARD

Increasing pathogen densities over time in perennial cropping systems has been well documented[18] and may play a part in reduced productivity over the lifespan of an apple orchard. It also has been shown that this increase in pathogen densities contributes to the general difficulty of replanting of sites with an economically viable crop of the same or similar species. This phenomenon, typically referred to as replant disease or disorder, afflicts the majority of tree fruit and nut crop production systems, including apple,[19,20] in all of the major fruit-growing regions of the world.[21]

Studies employing traditional culture-based methods have yielded the bulk of our knowledge concerning the etiology of apple replant disease, the importance of microbial interactions on disease severity, and the temporal nature of pathogen-complex development over the commercial lifespan of an orchard. Although replant disease has gen erally been attributed to biotic factors, the identity and consistency of the complex inciting this disease have been the subject of much debate. A number of in-depth studies concerning disease etiology are in agreement regarding the cause of replant disease.[1,2,18,20,22,23] Using a multiphasic approach to investigate the etiology of apple replant disease and incorporating a diversity of methods to discern the causal biology, a surprisingly consistent assemblage of pathogens/parasites has been documented as the principal causal agents of replant disease. These elements include, but are not limited to, *Cylindrocarpon*, *Phytophthora*, *Pythium* and *Rhizoctonia* spp., along with the endoparasitic nematode *Pratylenchus penetrans*.[1–3,20,24] While species composition within the fungal/oomycete genera and relative contribution to disease development may vary from orchard to orchard[21,24–26] the complex as a whole has shown consistency across geographic regions.[1,2,20,24] Likewise, non-fumigant approaches, such as soil amendment of brassicaceous seed meals specifically targeting this pathogen complex, have proven effective in controlling the disease highlighting the contribution of this complex to the etiology of apple replant disease.[26] Other components of soil microbial community which influence disease incidence and the manipulation of orchard soil microbial communities as a means to control ARD has been thoroughly reviewed.[27]

ROOTSTOCK RESISTANCE AS A CRUCIAL COMPONENT FOR ARD MANAGEMENT

The utilization of dual genotype plants in perennial tree crops where the root system (rootstock) is of one type and the grafted aerial system (scion) of another, is an ancient technology that has been modernized through breeding and selection of specialized rootstocks.[28,29] The dual nature of the grafted trees has allowed a 'divide and conquer' strategy for the achievement of higher yields by focusing breeding efforts on very different traits in the two constituent parts. Productivity, tolerance to abiotic stresses and resistance to biotic stresses (root diseases and insects) are the target traits of rootstock breeding,[30–35] exploiting one or a combination of many mechanisms, including gene for gene resistance,[36] promotion of a beneficial microbial community,[37] production of antimicrobial substances in the roots[38] and rapid regeneration of root systems.[9,10] It is clear that the implementation of disease tolerant/resistant, high yielding rootstocks has increased per acre productivity of high-quality fruit and gradually decreased labor, fertilizer and antimicrobial compound applications.[39] The complex nature of replant diseases makes breeding for tolerance or resistance very challenging, but some germplasm in the breeding pipeline, such as the progenitor of apple *Malus sieversii*, has been described as possessing resistance to multiple apple diseases.[14,40,41] Evaluations utilized in breeding for resistance have been limited to inoculation with pathogen cocktails[42] and subsequent assessment of seedling death or planting in pathogen-infested fields[34,43] with very little understanding of the mechanisms behind such resistance. Yet breeding strategies for complex diseases like ARD are better served by a reductionist approach that isolates each of the potential culprits and identifies the magnitude of the effects on and responses of plant roots to individual pathogens, with the appreciation that in the field some factors may interact (e.g., root nematodes forming entry wounds for fungal pathogens). The application of robust markers in marker-assisted breeding based on knowledge of how resistance operates in apple roots would also facilitate the development of new resistant cultivars, since most root diseases are difficult to phenotype on single plants.[44,45]

GENOMICS-ASSISTED BREEDING FOR ACCURATE AND EFFICIENT INCORPORATION OF RESISTANT TRAITS

Breeding of rootstock tree crops is a time-consuming and resource-demanding process, with many target traits such as dwarfing, precocity, productivity and resistance to various diseases and insects.[46,47] The detection and exploitation of genetic variation in germplasm collections and breeding populations have always been an integral part of plant breeding, but utilization of DNA-based molecular markers to predict phenotypes can improve the precision and efficiency.[48,49] Genomics-assisted breeding, in general, refers to application of genomic tools in breeding practices for developing superior germplasm with enhanced agronomical traits.[50,51] A range of approaches including genomics, transcriptomics and proteomics can be employed to establish and utilize the relationship between genotype and phenotype, and identify genes or molecular markers associated with traits of interest. The ultimate goal is to use these genomic resources to establish the connection between desirable traits and a tightly linked marker or an allelic form of the gene that is known to contribute significantly towards the target trait. From there forward, the desirable genes can be bred into horticulturally acceptable plant forms from wild germplasm sources with a minimal linkage drag (i.e., the tendency of genes inherited together as they are located proximal to each other on a chromosome). With the increasing availability of abundant markers such as single-nucleotide polymorphism across whole apple genomes, high-throughput genotyping technologies such as whole-genome genotyping array and continually improved statistical software, genomic selection holds promise for the manipulation of complex polygenic traits often controlled by many small effect genes.[52–54] Currently, the specific apple genes or genetic loci associated with resistant responses to ARD pathogens are basically unknown.

ELUSIVE MOLECULAR RESPONSES OF PLANT ROOTS TO SOILBORNE NECROTROPHIC PATHOGENS

Plant pathogens can be classified as biotrophic or necrotrophic based on their mode of attack. Biotrophic pathogens invade and acquire nutrients from living plant cells until the pathogen life cycle is completed, while necrotrophic pathogens kill the plant cell and then utilize nutrients from dead cells.[55] Based on studies using model systems, it is clear that plants use discrete defense mechanisms to deal with these two types of attackers.[56–58] Plant resistance to biotrophic pathogens is based on host induction of localized necrosis to limit pathogen spread. Resistance to necrotrophic pathogens involves production of antimicrobial compounds and cell wall reinforcement to limit pathogen progression and prevent

cell death. While many foliar pathogens are biotrophic, the majority of root pathogens are necrotrophs. Hemibiotrophs may begin the infection as a biotroph and complete infection as a necrotroph, but very likely resistance operates during the initial biotrophic portion of the infection process.[59] All ARD pathogens appear to be necrotrophs; whether or not a brief biotrophic phase exists for some of them during the initial infection stage may require further study. A greater understanding of the mechanisms that underlie rootstock tolerance of root growth influencing groups of fungal endophytes[60] is obtained because recent studies have demonstrated that many soilborne microbial pathogens can establish asymptomatic relationships with the roots of nonhost species.[61] This new insight might account for the persistence of the majority of soilborne pathogens in soil for extended periods of time in the absence of plant hosts. As roots grow in close proximity, certain pathogen propagules may detect root exudates resulting in stimulation of spore germination and mycelial growth toward roots by chemotaxis and chemotropism.[57,62]

As apple root resistance to ARD pathogens is a barely explored and phenotyping-challenged biological process, transcriptomics is a potentially good starting point to uncover the genes, pathways, networks and genetic structure regulating root defense response. Our recent transcriptome profiling of apple root tissue in response to *P. ultimum* infection (as summarized below) revealed that there is substantial similarity to the genes and pathways identified from other plant tissues as they were challenged with necrotrophs. Here we provide an outline of the current understanding of plant–necrotroph interactions as a guideline, with the caveat that most data are derived from studies using non-horticultural species in non-root tissues based on interactions with a few diverse foliar pathogens.

THE MOLECULAR FRAMEWORK OF PLANT DEFENSE RESPONSES TO NECROTROPHIC PATHOGENS

As in animals, plants possess an innate immune system which enables pathogen detection and induction of defense responses. Plant immunity is comprised of distinct signaling sectors interacting in a complex fashion with network properties.[63–65] Plants exploit various strategies to perceive attack and translate the signal into a broad spectrum of inducible defense responses.[63,66,67] Cellular processes during plant defense include accumulation of reactive oxygen species (ROS) and nitric oxide (NO), hormone modulation, biosynthesis of various antimicrobial secondary metabolites and peptides, callose deposition and cell wall modifications.[69,70] Several plant hormones, including SA, jasmonic acid (JA) and ethylene (ET), are central to plant defense mechanisms but the operative mechanisms vary with the pathogen type or mode of attack.[56,58,62,68]

Plant surveillance system, detection of pathogens and early signal transduction

Plants recognize necrotrophic pathogens primarily by the pathogen-associated molecular patterns (PAMP) of structural molecules (or elicitors) through pattern recognition receptors. The necrotrophs produce phytotoxins and cell wall degrading enzymes, and plants in turn activate a wide spectrum of immune responses to counteract these attacks. The cellular activities of plant immediately downstream of elicitor detection are still largely elusive; however, several signaling pathways are correlated with the PTI (PAMP-triggered immunity), including rapid influx of calcium (Ca^{2+}), generation of ROS and NO, and activation of mitogen-activated protein kinases.[71–73]

Calcium concentration. Oscillation of spatial and temporal Ca^{2+} concentration is one of several early signaling events among PAMP-induced defense responses.[74] Several families of proteins, including calmodulins, calmodulin-related proteins and Ca^{2+}-dependent protein kinases function as Ca^{2+} sensors.[75] The molecular connection between Ca^{2+} concentration changes, H_2O_2 production, JA biosynthesis pathway and phytoalexin production has been demonstrated.[76]

Oxidative burst and NO generation. Accumulation of ROS and NO is a commonly observed plant immune response. However, it may possess contrasting defense functions depending on a pathogen's lifestyle. For example, the level of superoxide and hydrogen peroxide generated in plant cells during infection is associated with the relative virulence of *Botrytis cinerea* and *Sclerotinia sclerotiorum*.[77] Pharmacological analyses indicate that there are mutual positive feedback mechanisms between NO generation and JA biosynthesis induction in plant cells under stress conditions.[63] The connection among oxidative burst, cell wall lignification and phytoalexin accumulation is commonly observed during typical PTI responses,[78–80] which can lead to resistant phenotypes.[81]

Kinase. Plant mitogen-activated protein kinase pathways fulfill many functions in plant responses to stress and pathogen infection. MPK6 and MPK3 were shown to phosphorylate ACS (1-aminocyclopropane-1-carboxylic acid synthases) 2 and 6 resulting in increased *B. cinerea*–induced ET biosynthesis.[82–85] Phosphorylation of WRKY (transcription factors containing a conserved WRKYGQR amino acid sequence at their N-terminal ends) 33 by MPK3/MPK6 in response to *B. cinerea* infection is required for camalexin (a pathogen infection induced antimicrobial secondary metabolite) biosynthesis in *Arabidopsis*.[86]

Plant hormone modulation during defense against necrotrophic pathogens

Based on studies using *Arabidopsis* mutants impaired in hormone biosynthesis and perception, as well as pharmacological treatments, it is well established that SA, ET and JA are vital components of plant defense responses,[56,58,87–89] and plants use discrete hormone balances and fine tuning of crosstalk to deal with various attackers. SA-regulated defense mechanisms are activated in response to biotrophic pathogens, whereas JA/ET-mediated signaling pathways are critical to plant defense responses to necrotrophic pathogens.[56,90,91] SA and JA/ET regulated defense pathways are believed to be mutually antagonistic, but examples of synergistic interactions have also been reported.[92–95]

JA. Disruption of genes in JA synthesis and response compromises plant defense to necrotrophs, whereas exogenous application of JA confers resistance to these pathogens.[96–98] Natural variation of sequences for potato allene oxide synthase 2, was shown to contribute to resistance toward two pathogens; *Phytophthora infestans* and *Pectobacterium carotovorum* (previously *Erwinia carotovora*) ssp. *atroseptica*.[99]

ET. Studies of *Arabidopsis* interactions with various necrotrophic pathogens suggest that several components in ET signaling pathways regulate plant defense responses. Over-expression of ERF1 (ethylene response factor 1) enhances resistance against *B. cinerea* and increases susceptibility to the hemibiotroph *Pseudomonas syringae* pv *tomato*.[100,101] Increased susceptibility to necrotrophic fungi such as *Pythium* spp. and *B. cinerea* was linked to defective ethylene signal perception in the *Arabidopsis etr1-1* and *ein2* mutant and ethylene-insensitive transgenic tobacco expressing a defective ethylene receptor ETR1.[102–104]

Other plant hormones. Both tomato and *Arabidopsis* abscisic acid-deficient mutants demonstrated enhanced resistance to necrotrophs, which is attributed to induced transcription of JA/ET-responsive

genes and timely production of hydrogen peroxide.[105] However, in other cases, mutants deficient in abscisic acid biosynthesis or insensitive to abscisic acid are more susceptible to infection by *Altenaria brassicicola, B. cinerea*, and *Pythium irregulare*.[106] Responses to gibberellin can be repressed by DELLA proteins (i.e., contains the conserved amino-acid motifs DELLA), which also promote resistance to necrotrophs by activating JA/ET-dependent defense responses and susceptibility to biotrophs by repressing SA-dependent defense responses. DELLA proteins also promote the expression of genes encoding ROS detoxification enzymes and subsequently regulate the levels of ROS after biotic or abiotic stress.[107,108]

Crosstalk between plant hormones can result in multiple feedback regulations to fine tune gene expression patterns and feed forward regulations to coordinate expression intensity and duration.[68,92,93] The plant transcription factors WRKY, NAC (transcription factor family including three sub groups of NAM, ATAF and CUC), ERF and MYB (myeloblastosis oncogene) families play key roles in plant resistance to necrotrophs under the regulation of plant hormones.[109,110] For example, JA-inducible R2R3-MYB in tobacco protoplast (MYBJS1) is required to activate phenylpropanoid biosynthetic pathway and accumulate phenylpropanoid–polyamine conjugates under stress conditions.[111]

Secondary metabolism as an important component in plant defense

Both preformed antimicrobial compounds (phytoanticipins) and infection induced antimicrobial secondary metabolites (phytoalexins) have long been associated with plant resistance to fungal, oomycete and bacterial pathogens.[112,113] Phytoalexins are small molecules of extreme structural diversity and with effective doses around order of magnitude 10^{-5}–10^{-4} M.[114] In general, closely related plant families use similar secondary metabolites for defense purposes (e.g., isoflavonoids in the Leguminosae and sesquiterpenes in the Solanaceae), although some chemically related defense compounds are shared across taxa (e.g., phenylpropanoid derivatives).[114–117]

Biphenyl and dibenzofuran are the major phytoalexins in rosaceous plants.[118] In a recent study, several biphenyl or dibenzofuran derivatives were reported to accumulate in the transition zone between the infected and healthy shoot segments of apple (*Malus domestica* cv Holsteiner Cox) and pear (*Pyrus communis* cv Conference) in response to inoculation with the fire blight bacterium *Erwinia amylovora*.[119] Functional analysis of biphenyl synthase gene family of apple, which is responsible for the biosynthesis of the biphenyl and dibenzofuran carbon skeleton, suggested that biphenyl synthase 3 is primarily expressed in apple shoot tissue with highest transcript levels in the transition zone in fire blight-infected apple.[120]

Phytoalexins as an integral component of plant defense responses and their roles in disease resistance have been investigated for over half a century, though their roles in resistance phenotypes remain controversial.[121–123] Among other reasons, differences in methods used to quantify phytoalexins may have contributed to the inconsistency regarding its effect. For example, the biosynthesis of camalexin is highly localized surrounding the infection site, but measurement of camalexin may be performed using the whole leaf or whole plant.[124,125] Variations in genotype-specific dynamics of the rate and intensity of phytoalexin accumulation may be important to the outcome of plant–pathogen interactions; moreover, its accumulation at the right place and right time may be more critical in determining the resistant and susceptible phenotypes.[123,126]

GENOMIC APPROACHES TO ELUCIDATE THE DEFENSE NETWORKS IN APPLE ROOT TO ARD PATHOGENS

Although significant progress has been achieved on the molecular dissection of plant–necrotroph interactions in recent years, the vast majority of knowledge has been derived from foliar pathogens interacting with a few model plants. Currently, defense responses in plant root tissues, particularly in perennial tree species such as apple, is far less defined. With progress being made toward deciphering the apple genome and accumulation of germplasm and genetic resources, there is a great opportunity to advance our understanding of apple root responses to soilborne pathogens. Draft genome sequences for apple were released in 2010,[127] and comprehensive apple EST collections now exist (more than 280 000 entries) (Genome Database for Rosaceae; http://www.rosaceae.org/).[128] Available RNA-sequencing (RNA-seq) data were used to develop a comprehensive reference apple transcriptome, which provided improved annotation for apple genome sequences and also revealed many new features of apple transcriptome including novel and antisense transcripts.[129,130] Genome sequences for several founding rootstock genotypes (Ottawa 3, Malling 27, Malling 9, Robusta 5, Geneva 41) are also available (Fazio, unpublished data). Apple genetic maps based on SSRs and single-nucleotide polymorphism marker have also been developed.[52,131,132]

Transcription regulation is a major step in the conversion of genome-encoded information to the agronomic trait.[133–135] Therefore, large-scale transcriptomics is often a primary choice to uncover molecular or genetic bases controlling a less explored biological process. The massive-parallel sequencing technologies, also collectively known as next-generation sequencing, have revolutionized biological research within 10 years.[136–137] RNA-seq, which simultaneously sequences the complementary DNAs of all transcript populations, has become a mainstay of transcriptomic analysis, although the first plant transcriptome analysis using RNA-seq was reported just a few years ago.[138] Compared with the previous microarray technology, the RNA-seq approach offers several obvious advantages. As an open-end platform, RNA-seq is not restricted to only those transcripts deposited on the microarray, but can detect the abundance of all mRNAs in a sample including novel transcripts or alternative splicing variants. RNA-seq, being more sensitive in detecting the dynamic range of gene expression, favors the detection of low-abundance but often function-relevant gene transcripts. RNA-seq can generate more accurate or less biased transcript quantification and distinguish homologous genes and/or alleles at the ultimate resolution of single nucleotide variation.[139,140] With the continuously decreasing cost, RNA-seq methodology can be used to establish the global molecular regulation network underlying the interactions between apple root and ARD.

RNA-seq based large-scale, high-resolution transcriptomic profiling and fast-evolving bioinformatic analysis tools have demonstrated capability for the study of genome-wide sequence polymorphisms on transcriptome variations among intraspecific individuals. One of the recent, large-scale applications of RNA-seq is the detection of expression quantitative trait loci (eQTLs) by sequencing the individual transcriptome in a segregating population. In eQTL analysis, the variation in transcript abundance for each gene is treated as a heritable trait which is subjected to statistical genetic analyses across a population.[141–143] Furthermore, applying a pre-defined network to query the eQTL dataset, or *a priori* network analysis, can be an effective means to link causal gene and resulting phenotype.[144] Therefore, eQTL analysis facilitates the dissection of the molecular basis of complex traits.[144–146] For example, using such approaches, a transcription factor PAP1 in anthocyanin biosynthesis pathway, but not other related transcription factors, was shown to colocate with a phenolic-specific network eQTL.[147–149] Recently, transcriptome profiling for 48 individuals from the 'Ottawa 3' × 'Robusta 5' apple rootstock mapping population identified a small set of thirty genes, physically clustered on the same location of chromosome 12, to be differentially expressed in shoot tips between resistant and susceptible trees to powdery mildew. Similarly, five differentially expressed between trees resistant and susceptible to woolly apple aphid, were clustered on chromosome

17. In each case, the gene clusters were in the vicinity of previously identified a major QTL for the corresponding trait. Several of the differentially expressed genes have been used to develop DNA polymorphism markers linked to powdery mildew disease and woolly apple aphid resistance.[150] Therefore, combined genomic approaches to analyze the various germplasm of apple rootstocks should offer the better opportunity to elucidate the molecular network and identify the genetic components regulating apple root response to ARD pathogens.

PRELIMINARY TRANSCRIPTOMICS ON APPLE ROOT INTERACTING WITH ARD PATHOGEN

With the aim of identifying the transcriptomic changes associated with apple root responses to infection by *Pythium ultimum*, transcriptome profiling using RNA-seq methodology was performed with seven sampling points extending from 0–96 h post-infection (hpi) (Zhu, unpublished data). Comparison of transcriptome changes between mock inoculated and *P. ultimum* inoculated root samples indicated several preliminary findings in terms of molecular defense responses in apple roots: (i) the peak defense response in apple root tissue to *P. ultimum* infection was observed at 48 hpi based on the number of differentially expressed genes; (ii) apple genes functioning in hormone signaling including ET, JA, gibberellin, cytokinin and auxin, and those encoding NAC, WRKY, MYB and ERF transcription factors, which are often associated with defense responses to foliar necrotrophic pathogens, were dynamically regulated; (iii) multiple genes in several families which encode enzymes for the biosynthesis of antimicrobial secondary metabolites and cell wall modification, such as phenylpropanoid and flavonoid biosynthesis pathways, demonstrated consistent upregulation after 24 hpi; (iv) genes encoding defense- and stress-related proteins such as wall-associated receptor kinase (WAK), endochitinase (PR4), thaumatin (PR5)-like protein, laccase, mandelonitrile lyase and cyanogenic beta-glucosidase also showed significant upregulation after 24 hpi; and (v) two cytokinin hydroxylase encoding genes were observed with triple-digit upregulation during the infection process, which may suggest that cytokinin signaling is critical for apple root defense response to *P. ultimum* infection. It appears that there is substantial similarity in term of the molecular defense responses in both foliar and root tissues to necrotrophic pathogens.

CONCLUDING REMARKS AND REMAINING QUESTIONS

Soilborne plant diseases are a devastating and ongoing problem for many agronomically important crops largely due to the persistent and accumulative nature of pathogen inoculum in soil. Although crop rotation can sometimes serve as a viable disease control option in annual crops, it is difficult to apply this practice in perennial tree crop production systems due to limited available orchard sites and long life cycle of a commercial orchard. Chemical fumigation to eradicate ARD pathogens is currently the primary control method, but the effect is short-lived and ARD pathogens are known to recolonize orchard soils rapidly after soil fumigation. Moreover, certain chemicals are facing impending regulatory limitations and fumigation is not feasible after orchard establishment. Exploiting the interactions among microbial communities in orchard soil to promote plant heath and minimize pathogen aggressiveness has been shown to be a promising disease control method and can be effective in many situations. However, the mechanisms and resources of resistance to ARD pathogens have not been carefully investigated. Our recent RNA-seq-based transcriptome profiling on the time course of apple root response to *P. ultimum* infection suggests a conserved molecular framework root defense responses, compared to that identified from leaf tissue of model systems. The molecular characterization of root response to infection by ARD pathogens may be the foundation for subsequent genomics-assisted breeding.

Many questions remain: How do resistance traits in perennial root systems change in relation to tree age? Seedlings are typically the subject of research due to the feasibility of experimental design, but whether or not consistent responses in root systems between seedling and mature tree can be achieved should be investigated. How does the scion genotype influence the performance of root defense responses to pathogen infection? Mutual influence between rootstock and scion genotypes will be an interesting subject. For example, it was shown that rootstock genotypes can affect the performance of scion resistance to fire blight,[151] but the effect of scion genotypes on root resistance to ARD pathogens is unknown. How will the constituent variations of the ARD pathogen complex and other soil microbe communities from orchard to orchard affect performance of resistance traits? How different apple rootstock genotypes alter the soil biota by root exudation and subsequent manipulation of pathogen behavior? Recent study indicated that the previous rootstock genotypes mainly influenced soil bacterial communities and current replanted rootstock genotype affected fungal communities more; pointing to the role of rootstock genotype-specific interactions with soil biota then influencing ARD incidence.[152] Nevertheless, identification of the molecular networks, the genetic loci, the signaling pathways and candidate genes contributing to the resistance to ARD pathogens is an essential first step. Marker assisted selection or genomics-assisted breeding can facilitate incorporating resistant traits more efficiently and accurately to new apple rootstocks. A commercial orchard will stand for several decades, so utilizing resistant rootstocks as an integral component for replant disease management can be more cost-effective and durable.

COMPETING INTERESTS

The authors declare no conflict of interest.

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