6-11-2015 12:00 AM

Identification of Putative Plant Defense Genes Using a Novel Hydroponic Co-Cultivation Technique for Studying Plant-Pathogen Interaction

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

IDENTIFICATION OF PUTATIVE PLANT DEFENSE GENES USING A NOVEL HYDROPONIC CO-CULTIVATION TECHNIQUE FOR STUDYING PLANT-PATHOGEN INTERACTION

(Thesis format: Monograph)

By

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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Abstract

Previous work on identifying the molecular mechanisms mediating plant-pathogen interactions and reciprocal host responses have little emphasis on developing models that closely resemble host-microbe interaction *in planta*. This work establishes an amalgamated model of interaction wherein successful pathogens elicit and overcome host defenses activated by microbial signatures and virulence factors. Using a hydroponic co-cultivation model, we assessed the responses of *Arabidopsis thaliana* Col-0 to *Agrobacterium tumefaciens* C58 to ameliorate limitations of previous approaches. Comparisons of differential gene expression between directly and indirectly affected host sites by microarray analysis revealed both reactive and pro-active defense responses, respectively. Selected homozygous single-gene knockouts for proactive defenses show variable *A. tumefaciens* root surface attachment and root secretion profiles. Studying host-microbe responses using hydroponics may improve priming of cash crops against pathogens and in part, may also improve the use of *A. tumefaciens* as a vector for generation of transgenic crops.

Keywords

Pathogen, plant-pathogen interaction, hydroponic co-cultivation, plant defense, virulence, *Arabidopsis thaliana*, *Agrobacterium tumefaciens*, microarray, transgenic

Co-Authorship Statement

Chapter 1:

***Agrobacterium tumefaciens* responses to plant-derived signaling molecules**

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This work has been published in *Frontiers in Plant Science*.

Sujatha Subramoni contributed to this work with writing and editing of this paper.

Naeem Nathoo contributed to this work with writing and editing of this paper.

Eugene Klimov contributed to this work with editing of this paper.

Dr. Ze-Chun Yuan conceptualized the writing and editing of this paper.

Acknowledgments

I would like to take this opportunity to express my thanks of gratitude to my supervisors Dr. Mark A. Bernards and Dr. Ze-Chun Yuan whose patience, guidance and support made the completion of my M.Sc. possible. I would like to especially thank Dr. Yuan for his tireless effort to ensure my experimental execution and writing was completed at a high level of quality.

I would also like to thank my advisor Dr. Greg Thorn, whose guidance and encouragement propelled the completion of my project.

Lastly I would like to thank David Carter for his assistance with processing our microarray data, Dr. Lingrui Zhang for aiding with qPCR troubleshooting, my lab-mate Alexander William Eastman for his help and companionship throughout my project, and the invaluable help and assistance made available by our lab technician Brian Weselowski.

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Chapter 1 – Literature Review

1.1 Background

The rapid rise in the rate of population growth is a major concern, poising those in developing countries on the brink of disaster. At current population growth rates, a billion people are added to the global population approximately every twelve years[1]. However even more troubling is the correlation between population size and the rate of population increase. For years, researchers have attempted to design various models to determine the fate of human beings on planet earth, ultimately verifying the inevitable outcome of an unsustainable growing human population. In the later part of 2011, the United Nations (UN) officially declared the global population had reached seven billion, a third of which were classified as living under a European standard of living[2,3]. At the current rate of resource production, overall consumption would have to be reduced by 50% to sustain our current population at its current rate of growth[3]. Theoretically, cutting consumption in half is a daunting task. Even if the rate of human population growth remained steady, it would not be long until we began running out of food and water. By 2050, our population is estimated to reach approximately 9.6 billion making the battle against poverty, hunger, and resource availability the forefront of global challenges to be faced in the first half of the 22nd century[3]. Despite the seemingly discouraging nature of our population's predicted fate, the future can be approached with cautious optimism.

1.2 Cash Crop Production

Sustaining a growing global population will necessitate greater production and availability of food. Increasing the current production of edible goods invariably requires

the expansion of water irrigation and area for cultivation. Expanding areas for agricultural practice will be particularly challenging as urban development continues to advance, indirectly influencing climate change, and directly reducing available land for irrigation. In 2014, global cash crop production possessed a monetary value of approximately $1.3 trillion USD[4]. However from the point of production to the time produce reaches consumers shelves, a significant proportion of produce is lost. In the pre-harvest stage, biotic factors account for 31% to 42% of cash crop loses and include various challenging pathogens causative of disease, herbivores and weeds[4]. In developing countries, the impacts of disease causing biotic factors tend to be much more severe[5]. Detrimental abiotic factors account for an additional 6% to 20% of crop losses in the pre-harvest stage[4]. Post-harvest, biotic factors further decrease yields by another 6% to 20%[4], resulting in an overall 63% ($806 million USD) loss by the time yields reach their destination even if we considered the most moderate of these ranges of losses. As a means of offsetting the losses caused by abiotic and biotic factors, the amount of land cultivated in developing countries is being expanded to meet increasing demand. By 2050, arable land is expected to decline by 50 million hectares in developed countries and increase by 120 million hectares in developing countries, mainly localized to sub-Saharan Africa and Latin America, by conversion of natural habitats[6]. Much of the available land remaining in other geographic regions is chemically and/or physically restrictive of crop production, disease prone or lacks the infrastructure necessary for crop cultivation. Clearing land for agricultural practice is a possible alternative to meet demands, however this requires significant investment, is destructive to the environment and is subject to expanding urban settlement. Limitations to land expansion will force the development of

alternative procedures to keep pace with the anticipated 60% required increase in agricultural production by 2050[7]. Ideally, these alternative approaches will increase cash crop productivity without necessitating the expansion of land for agricultural use.

1.3 Controlling Biotic Factors for Crop Productivity

Living organisms, both macro- and micro-organisms, capable of influencing vegetation are said to be biotic influencers (factors). Macro-organisms include animals, insects, and plants, where-as micro-organisms refer to fungi, oomycetes, bacteria, and viruses. Together these diverse biotic factors profoundly influence the performance and development of plants by direct and indirect interactions, which may be either detrimental or sometimes beneficial.

Amongst plants, nutrient availability in the rhizosphere and exposure to sunlight are essential requirements for vegetative life. Overcoming competition for limited nutrient resources demands abundant seed production, dispersal and germination[8]. Rate of growth, mode of branching, longevity and surrounding environmental condition requirements are also important factors influencing plant competitiveness [8,9]. Agro-economically, challenges presented by competitive plant species can be controlled effectively by agricultural herbicides. Accordingly in more recent years, many research groups have refocused their effort towards the understanding of associations between herbivorous pests and microbes on plants. Ultimately the goal of such research is the development of sustainable techniques to prevent the detriments associated with herbivores and plant pathogens, while promoting associations of beneficial microbes with crops.

1.4 Herbivorous Pests

Current herbivore pest control primarily includes the application of pesticides that are extremely costly to the economy, human health, ecosystems and biodiversity. According to the Organization for Economic Cooperation and Development (OECD), Canada ranked 22nd out of 29 industrialized countries as one of the worst for pesticide use and 25th for high levels of commercial fertilizer use in 2001[10]. In 1994, Canada reported 29x10^{6} kg of applied pesticides and at the time ranked 18th for pesticide use[10]. This example of an increasingly heavy reliance on applied pesticides is a major contributing factor to the decrease in effectiveness of these chemicals to antagonize pests. The difficulty in producing newer pesticides, which insects are yet to become resistant to, presents additional challenges economically. Production of new pesticides requires 8 to 12 years of development, with investments as high as $50 million USD[11]. Instead, research groups have sought after understanding host responses to insects[12-14], as well as exploring the antagonistic features of plant growth promoting microorganisms[15,16], for exploitation to improve plant resistance to herbivory damage. The introduction of Bt corn is a popular example of such efforts, originally adapted from the successful introduction of Bt tobacco[17,18]. Bt corn is a variant of maize (*Zea mays*) genetically engineered to encode a set of genes introduced from *Bacillus thuringiensis*[18]. *Bacillus thuringiensis*, a soil-dwelling bacterium, produces delta endotoxins (Cyt toxins) that possess an insecticidal action against caterpillars and more than 50 other lepidopteran pest species[19-22]. By introduction of Cyt toxin biosynthesis genes expressed throughout plant tissues, feeding by vulnerable insects ultimately causes the activation of a paralyzing toxin in the digestive tract eventually starving the target[19,23,24]. The challenge in exploiting the use of

naturally occurring solutions against herbivorous pests is identifying the compounds produced either by plants or beneficial microorganisms, and the biochemical mechanisms that result in their production.

1.5 Plant Associative Microbes

Unlike herbivorous pests, the interaction between plants and microorganisms is much more difficult to alter once an association has been established. Whether beneficial, commensal or detrimental in nature, microbial association with a host results in various forms of colonization and in some cases, genetic manipulation of the host. Therefore the promotion of beneficial- and elimination of detrimental-associations necessitates a nuanced understanding of how these interactions are established prior to engineering associations for increased productivity of cash crop production.

Plant associative microbes occupy various regions surrounding their host including the phyllosphere and rhizosphere[25,26]. The phyllosphere includes the aerial portions of the plant host. On the other hand, the rhizosphere refers to the narrow soil region that can be specifically influenced by root systems and secretions. Here, both beneficial and pathogenic microbes compete for space and food, ultimately using their plant target as a host. Phyllospheric microbes include bacteria, yeasts, fungi, and oomycetes. In the phyllosphere, microbes are more predominant on the surfaces of aerial plant structures, although there are a few microbial species that can be isolated from within these plant tissues known as endophytes. Surprisingly, little research has focused on the role microbes play in the phyllosphere, considering such a large amount of plant tissue surface area is exposed to this microbe dense environment. However research suggests this

particular region is considered a hostile environment for microbial communities due to the highly fluctuant nature of environmental conditions. In contrast, the rhizosphere is considered a highly favorable habitat for microbes to proliferate within due to the abundance of nutrients such as amino acids and sugars, and more stable-favorable environmental conditions. The nutrient rich nature of the rhizosphere is reflected by the 10-100 times greater density of bacteria in comparison to surrounding bulk soil[27]. In much the same way as the phyllosphere, microbes occupying the rhizosphere are able to interact directly with plants through adherence to plant root surfaces as well as by colonization of internal root tissues. In addition to these direct associations, microbes in the rhizosphere have the additional benefit of indirectly interacting with plants by two way chemical signaling with a nearby host.

The rhizosphere is populated with a diversity of microorganisms including bacteria, fungi and oomycetes, however bacteria are most predominant, termed rhizobacteria[28]. In contrast to bacteria, the composition and density of fungi and oomycetes cannot be altered or enhanced by the presence of plant root systems. Among bacteria, gram-negative, rod shaped, non-sporulating bacteria, which respond to root exudates, dominate the rhizsophere[29], while gram-positive, rods, cocci and aerobic spore forming genus' are comparatively rare[30]. The rarity of aerobic bacteria is the result of reduced oxygen levels caused by root respiration[30]. Generally speaking, the rhizobacterial population in soil ranges from 10^8 to 10^9 propagules per gram of soil[31], and cover approximately 4-10% of the total root surface area[32]. The high level of attraction to plant root structures is caused by the rhizodeposition of nutrients and growth factors[33-35]. Agronomically, the composition of bacteria in the rhizosphere is particularly important. Plant-associative

rhizobacteria are classified in beneficial, deleterious and neutral subgroups based on their effect on plant growth. The beneficial free-living rhizobacteria are known as plant growth promoting rhizobacteria (PGPR)[36]. PGPRs colonize the rhizosphere, the rhizoplane (root surface) or the radicular tissues within the root itself. A diverse set of genera have been characterized as PGPRs and it is well established these beneficial genera comprise 1 to 2% of the total bacterial population in the rhizosphere[36,37]. PGPRs are known to promote plant growth by two different approaches. First, by direct promotion of plant growth either by supplying the host with compounds synthesized by the bacterium, such as hormones, or by facilitating the uptake of nutrients from the soil[36]. Secondly, PGPRs can also indirectly promote plant growth by antagonizing other detrimental organisms to plant health, as previously described for *B. thuringiensis*[19-22,36]. PGPRs possess various plant growth promoting characteristics that may or may not function independently of one another. For example, PGPRs can act as biocontrol agents[38-42], and independently promote growth by the production of auxin[43], reduce plant ethylene accumulation[44], facilitate the uptake of phosphorus[45,46] and fix nitrogen[47,48] to become more functionally accessible to their host. In addition to these plant growth-promoting processes, a variety of other growth promoting mechanisms have been documented among PGPRs. Some additional mechanisms of plant growth promotion include the production of siderophores to facilitate host uptake of ferric ion[49], production of antifungal antibiotics[36,50,51] and production of bacteriocins[52]. Fundamentally bacteriocins differ from antibiotics by only having toxic effects against a narrow spectrum of microbes that are typically closely related to the producing species[52]. Moreover, non-pathogenic rhizobacteria have also been shown to suppress disease in quite different and unique ways. Non-pathogenic

rhizobacteria, incapable of producing growth promoting and antagonistic compounds, can induce host resistance mechanisms known as "induced systemic resistance" (ISR)[53-55]. ISR is mechanistically very similar to systemic acquired resistance (SAR), which is typically activated upon host perception of a pathogen[56]. The activation of ISR causes host cells to enter a "primed" state against pathogens by induction of plant defense proteins and production of the plant hormones salicylic acid (SA) and jasmonic acid (JA), which are master regulators of host defense processes. By doing so, plant hosts have a defense response proactively mounted prior to pathogen challenge.

Together, these diverse plant growth-promoting properties of PGPRs provide great promise for exploitation in the production of novel, more sustainable forms of agriculture. In stark contrast to PGPRs, plant-associative pathogenic bacteria are causative agents of many serious diseases. The introduction of plant pathogens and their detriment to plant fitness is not only realized in the short term at harvest, but pathogens remaining on the field act as threats to any subsequent cultivation.

1.5.1 Plant Pathogens

Infection of plants with pathogenic microbes is causative of many serious diseases. Pathogenic microbes include various genera of fungi, oomycetes, virus and bacteria. Annually, fungal, oomycete and bacterial pathogens account for 16% of total crop losses[57]. Although pathogenic bacteria cause relatively less damage and economic cost compared to oomycetes and fungal pathogens, they possess the widest host range, which significantly complicates the management of bacterial disease. Traditionally, efforts to inhibit the effects of damaging bacteria was termed plant disease control, but more

recently has become reassigned as plant disease management. In the former, plant disease control is viewed as a reactive treatment including drastic measures such as pesticide application, soil fumigation or burning, however these techniques are progressively being practiced much less frequently. Instead, plant disease management is more proactive in approach; not only managing disease after infection, but also attempting to avoid infection altogether. In plants already infected by phytopathogens, plant disease management utilizes newer techniques to control the spreading of the disease by chemical treatment or heating vegetative areas of plants, which are more susceptible to infection. Ideally, further development of plant disease management in the pre-infection stages would provide the best outcome in terms of increasing cash crop productivity. Pathogenic microbes result in a variety of common plant diseases and disorders including blight, cankers, rots, rusts, wilt, spots and galls. Some very common plant pathogens causative of these diseases include *Phytophthora sojae* (causative of root rot)[58], *Botrytis cinerea* (causative of mold)[59] and *Agrobacterium tumefacines* (causative of crown gal)[60]. Among these pathogens, *A. tumefaciens* has been studied extensively as a model for plant-pathogen interaction and more specifically, as a model of plant pathogenic bacteria. Deepening the understanding of such a pathogen will aid in the development of techniques that are better able to proactively inhibit or slow infection and subsequent disease.

1.5.2 *Agrobacterium tumefaciens*

Agrobacterium tumefaciens is a gram-negative phytopathogenic soil bacterium and is the causal agent of crown gall disease in plants[60]. *Agrobacterium tumefaciens* is infectious of over 391 plant genera, many of which are cash crops[61]. Once a crown gal has formed on a

plant host, this particular disease phenotype is impossible to reverse. However one proactive treatment has been developed in which seeds are coated with non-pathogenic *Agrobacterium radiobacter* K84. Treatment with *Agrobacterium radiobacter* K84 is relatively inexpensive and is effective against *A. tumefaciens* by production of the antibiotic agrocin 84[62]. In addition to agrocin 84, *A. radiobacter* K84 also induces host ISR[53,62], making plant challenge much more difficult for *A. tumefaciens.* Alternatively, soil regions known to be infected with *A. tumefaciens* can be planted with monocotyledonous crops which have been previously shown to be unaffected by *A. tumefaciens*[63,64]. In fact, the immune nature of monocotyledonous crop is beneficial since they are not susceptible to infection; however from a biotechnological perspective, this feature is rather unexciting since monocotyledonous hosts cannot be transformed using *A. tumefaciens* as a vector.

1.5.2.1 *Agrobacterium tumefaciens* Pathogenicity

During the *A. tumefaciens* virulence program, *Agrobacterium* possesses the unique ability to introduce a number of effector (avr) proteins, virulence (vir) proteins and transfer DNA (T-DNA) into a plant host cell without being perceived by plant cell wall localized pathogen recognition receptors. As a direct consequence, targeted plant cells are unable to mount an effective response prior to *A. tumefaciens* infection. There are a variety of *A. tumefaciens* characteristics that aid in explaining how this unique infectious process is possible. First, *Agrobacteria* are the only phytopathogens equipped with a type IV secretion system (T4SS)[65], allowing for avr protein, vir protein and T-DNA injection into the host cell cytoplasm. The T4SS, comprised of *vir*B sub-units 2-11[66] and *vir*D subunits 1-5[65,67-69], is assembled once *vir* gene expression is activated through the bacterial-

detection of rhizodeposited plant phenolic compounds. Phenolic compound recognition by membrane-localized *vir*A causes downstream auto-phosphorylation of *vir*G, inducing transcription of the *vir* operon[70]. *Vir* operon induction is synergistically sensitive to low pH rhizosphere-conditions and monosaccharide components of the plant cell wall[71,72]. Since plants routinely secrete amino acids, carbohydrates and other chemicals that acidify the rhizosphere, infection occurs primarily at the ground level and roots. Root secreted amino acids and acidic carbohydrates are detected by *chromosomal virulence* (*chv*) receptors, *chv*I[73,74] and *chv*E[75,76] respectively. Detection by *chv*E *and chv*I ultimately leads to increased phosphorylation of *vir*G, for increased expression of *vir* operons.

Following *vir* operon induction, T-DNA, a portion of the tumor-inducing (Ti) plasmid, is cleaved and chaperoned by *virD* to the T4SS for transfer to the plant cytosol[65,67-69]. *vir*E*,* transported independently from the T-DNA-*virD* complex into the infected plant cell cytoplasm, later associates with the T-DNA strand in the plant cytosol where it protects the single-stranded DNA (ssDNA) from degradation[77]. The T-DNA complex is transported to the nucleus via *vir*E interaction with plant-encoded and histone-localized *VIP*1[78]. T-DNA encodes the genes necessary for plant regulated auxin (*iaa*M and *iaa*H), cytokinin (*ipt*) and opine biosynthetic production. Once T-DNA is integrated into a target host chromosome, expression of *iaa*H, *iaa*M and *ipt* leads to the synthesis of plant growth regulators causing enhanced host susceptibility to infection, differentiation of cell types with altered morphology and tumor induction (crown gal)[65,79-80], whereas opine is cycled back to *Agrobacteria* to be utilized as a carbon and energy source[81]. In addition to vir protein and T-DNA injection, *Agrobacterium* avr proteins are also introduced. Avr proteins serve to manipulate the host cellular environment to slow the

activation of host defenses and facilitate the successful introduction of T-DNA into the host nuclear genome[82-83]. The evasive properties of *A. tumefaciens* to host perception prior to T-DNA transfer results from mutations in a pathogen-associated molecular pattern (PAMP), that is otherwise common among other plant pathogenic bacteria. *A. tumefaciens* possesses remarkable divergence in the typically conserved N-terminal domain of *flagellin*22 (*flg*22), which is a component of plant pathogenic bacteria perceivable by plant cell wall localized receptors[84-85]. As a result, *A. tumefaciens* avoids the activation of host defenses, until avr proteins are detected in the plant cytoplasm, at which point infection is already eminent and the host can no longer mount a successful response. This unique feature of *A. tumefaciens* pathogenicity has been similarly observed in *P. phaseolicola* with knocked out hypersensitive reaction and pathogenicity (*hrp*) genes[86]. *Agrobacterium tumefaciens* is regarded as a highly virulent plant pathogen and is an important tool in understanding the infectious process of pathogens. Furthermore, *A. tumefaciens* is widely studied for its useful application in dicotyledonous plant transformation technology.

1.5.2.2 *Agrobacterium tumefaciens* Mediated T-DNA Transformation

A. tumefaciens uniquely mobilizes a portion of its Ti-Plasmid, T-DNA, into target plant host cells. The successful introduction of T-DNA into the host's nuclear genome marks a successful infection by *Agrobacterium* and results in the subsequent disease phenotype of gall formation[87]. The Ti-Plasmid is unlimited in size but typically ranges from 180 to 250 Kb, and the T-DNA region is representative of approximately 10%[88]. More frequently, each Ti-Plasmid contains a single T-DNA but multiple T-DNA regions within a single

Ti-Plasmid have also been reported. T-DNA is flanked by border regions, which are recognized by *vir*D for cleavage and subsequent injection into a target cell[65,67-69,89]. The process of T-DNA transfer can be enhanced by "overdrive" sequences flanking T-DNA right borders[90-92], however the mechanism behind this enhancement is very poorly understood.

To take advantage of this mechanism for biotechnological gain, a method had to be developed to eliminate the genes causative of crown gall, while integrating genes of interest to be transferred to a plant cell. At first, many groups attempted to introduce genes of interest into the T-region of the Ti-Plasmid, however this proved to be extremely difficult[93-96]. An alternative technique was developed in which T-DNA and the *vir* genes necessary for T-DNA transformation were integrated on separated replicons[97,98]. The presence of both replicons in the same *Agrobacterium* cell created a system where vir proteins were able to act in trans to initiate the T-DNA mobilization process. In these systems, the replicon containing the *vir* genes (helper plasmid) generally contained a complete or partial deletion of the native T-region containing tumor inducing genes, and many *Agrobacterium* strains have been generated with this two replicon system including LBA4404, GV3101 MP90, AGL0, EHA101, EHA105 and NTI (pKPSF2).

Using this system, many research groups have introduced a number of beneficial genes to create transgenetic plants. Additional advantages of the developed two-replicon system include a number of unique restriction enzyme sites that allow easy cloning of genes into the T-region. A few popular examples of genetically modified crops using the two-replicon T-DNA transfer system include tomato, cotton, potato, soybean, canola and

tobacco to contain various properties including longer shelf life, Bt endotoxin production, and glyphosphate herbicide resistance ("round-up ready" crops).

1.6 Plant-Microbe Interaction

Plants interact with a diverse community of microbes in their immediate environment. Various microbial species inhabit surrounding regions of plants above and below ground, and the composition of these microbes can be influenced by a variety of environmental factors including wind patterns and water flow. In addition, plant root secretions, especially in the rhizosphere, can heavily influence the composition of microbial communities. This feature of plant physiology equips plants with the potential to significantly limit or prevent disease.

Typically most bare soil systems are carbon starved with relatively low soil microbial densities[27]. On the other hand in the rhizophere, the presence of plants and their root systems facilitates rhizodeposition of up to 40% of their photosynthates, increasing microbial population densities, otherwise known as the 'rhizosphere effect'[29,33-35,99]. Despite the high rhizosphere microbial density in comparison to bulk soil, there is far less diversity among the species of microbes present. Dependent on the composition of root secretions, plants can preemptively control the composition of the rhizosphere to facilitate management of herbivorous pests and encourage associations with beneficial microbes[100-102], while altering the physical and chemical properties of the rhizosphere to inhibit growth of plant pathogens and competing plant species[103-105]. Though this phenomenon is widely accepted, the subterranean nature of roots increases the difficulty in elucidating how these chemical signaling processes operate. However in more recent

years, some compounds identified in root exudates have been shown to act as messenger recruitment signals to attract PGPRs. Flavanoids, a class of secondary plant metabolite, recruit *Rhizobium* spp. and subsequently activate genes responsible for the nodulation process[106-108]. The host-facilitated nodule colonization by *Rhizobium* spp. provides a source of nutrients for the rhizobacterium, which in turn provides fixed nitrogen to the host[108]. Other root secreted compounds such as citric acid, succinic acid and malic acid have also been shown to influence the rhizosphere microbiome[109]. Rhizobacterial strains capable of utilizing these organic acids as a sole carbon source is suggestive of their root-colonizing ability. Plant secretions have also been shown to inhibit the growth of specific microbes in the rhizosphere. For example, benzoxazinoids, specifically 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA) produced by maize, contains antimicrobial properties inhibiting the growth of pathogenic rhizosphere microbes[110]. Interestingly while inhibiting the growth of pathogens, DIMBOA is simultaneously tolerated by the plant growth promoting *Pseudomonas putida* KT2440 strain while also acting as a chemoattractant[102]. The diversity of root-secreted rhizodeposits is highly dependent on the plant species, and subsequently, different compositions of microbial communities are attracted and deterred in a host-dependent manner. Plants are also equipped to enhance bacterial growth once a population has been established. Plant-associative bacteria produce diffusible *N*-acyl-homoserine lactones (AHLs) to communicate with other bacteria and regulate their gene expression at a community level[111,112]. This type of cell-to-cell communication is known as ‘quorum sensing’ (QS). Plants can produce compounds that either stimulate or repress QS-regulated responses in bacteria, many of which are phenolic compounds[113-115]. In some cases, the secretion of

phenolic compounds acts as a chemo-attractant for PGPRs, while simultaneously acting as a deterrent for plant pathogens. The unexplored chemo-diversity of root exudates is promising for the identification of novel biologically active compounds for the promotion of crop productivity, however the study of subterranean biochemical signaling remains a challenge.

1.6.1 Plant-Pathogen Interaction

Through recruitment of beneficial microbes and root secretion, plants are well equipped in the prevention of infection. However as sessile organisms, plants have also developed the ability to directly resist challenge by most plant pathogens by activation of intracellular host defense programs. Interactions between plants and associated pathogen require a two-way signaling network in which a plant host must be able to recognize the pathogen and mount a defensive response, while on the other hand the pathogen must be able to detect a compatible host and manipulate its machinery to facilitate infection and colonization. In a co-evolutionary arms race, both plants and pathogens have developed these seemingly complementary mechanistic properties.

1.6.2 Plant Host Defenses

Based on the current understanding of plant responses to invading pathogens, defensive mechanisms are known to be activated at two distinct levels. The first involves the extracellular perception of a pathogen by recognition of pathogen-associated molecular patterns (PAMPs) or alternatively, general microbial-associated molecular patterns (MAMPs)[116,117]. Detection of these compounds initiates the PAMP-triggered immunity (PTI) defense program[118,119]. Pathogens capable of successfully infecting their target host

are able to evade the activation of PTI and in some cases, suppress PTI activation entirely[120]. In the second level, hosts perceive pathogen effectors, also known as avirulence (avr) proteins, which are detected in the host's cytoplasm by resistance (R) proteins[121]. Avirulence proteins are the pathogenic factors responsible for suppressing responses regulated by PTI. Detection of avr proteins mounts the second line of host defense known as effector-triggered immunity (ETI). However, ETI is only a feature of plant-pathogen interaction once the challenging microbe is able to bypass PTI and enter host cells. These pathogens are described as 'virulent' pathogens. Despite mechanistic differences in initiation, ETI is best summarized as a stronger resistive response than that regulated by PTI. Together these responses present a broad range of opportunity to be exploited by researchers for the improvement of plant defenses against pathogens.

1.6.2.1 PTI – First Layer of Host Defense

Extracellular host detection of PAMPs and MAMPs allows the proactive PTI defense program to aid in defense against the initial infection event of a pathogen. PAMPs are highly conserved among plant pathogens, serving to initiate the PTI program that is not specific to an invading pathogen. Among PAMPs, *flg*22, a component of the bacterial mobility protein flagellin, and *elongationfactor*18 (*elf*18), a bacterial elongation factor, are the most commonly host-recognizable molecular patterns of bacteria[122-125]. In the case of fungal pathogens, chitin is the acting PAMP factor, including a variety of others such as fungal xylanase, oomycete heptoglucans, and lipopolysaccharides. Detection of these factors by plant cell wall localized pattern recognition receptors (PRRs) is ultimately the initial point of contact between plant and pathogen[116]. The more common *flg*22 and *elf*18 PAMPs are detected by the host *Flagellinsensing*2 (*FLS*2) and *EF-Tu Receptor* (*EFR*)

respectively[122-125]. Detection of PAMPs by these leucine-rich repeat receptor-like kinases (LRR-RLKs) activates a multi-faceted network cascade of mitogen-activated protein kinases (MAPKs) to activate a reactive oxygen species (ROS) burst by *Respiratory Burst Oxidase Homolog D* (*RBOHD*) producing superoxide (O_2-), later converted to hydrogen peroxide (H_2O_2) by superoxide dismutase[126]. In addition, a variety of transcription factors, including those in the WRKY, basic helix-loop-helix (bHLH) and basic leucine zipper domain (bZIP) families, are activated to increase the expression of defense related proteins[127-129].

As a first layer of activated plant defense, successful pathogens must suppress PTI to establish infection. Accordingly, the inability to overcome PTI is ultimately what allows plants to distinguish virulent pathogens from non-virulent pathogens. Successful pathogenic bacteria, secreting avr proteins inside plant cells, target PAMP receptors and their downstream components to facilitate full microbial virulence. This resistive mechanism of pathogens forced plants to develop another layer of plant defense. Plants developed a second class of cytoplasmic receptive proteins, R proteins, which specifically identify avr proteins or avr protein-targets, to initiate ETI. This seemingly back and forth nature of plant defense and evasion by pathogens is often referred to as the 'zig-zag' model.

1.6.2.2 ETI – Robust Host Defense against Virulent Pathogens

As a secondary layer of plant defense activation, plants are equipped with ETI. To initiate the ETI defense program, host R-proteins must either directly bind to pathogen avr proteins or in some cases bind alternative plant proteins, known as a 'guardee', following modification by pathogen avr proteins[130-132]. The guard hypothesis

was first shown for the *Arabidopsis* encoded RIN4 protein, which is required for resistance against *Pseudomonas syringae* pv tomato DC3000 and mediated by the plant R-genes *RPM*1 and *RPS*2[132]. During the activation of the ETI defense program, Ca^{2+}-dependent protein kinases (CPKs), MAPKs, ROS production and nitric oxide (NO) are induced, in association with the accumulation of various phytohormones including SA and JA[121,133]. Together these processes result in the expression of various defense genes to restrict bacterial growth and programmed cell death (PCD) related proteins. The compensatory mechanisms activated by ETI strengthen the force with which plants are able to prevent successful colonization by a pathogen, however highly virulent pathogens possess the ability to manipulate the host cellular environment effectively such that reproduction and colonization is possible.

Holistically, both PTI and ETI utilize the same intracellular signaling network, albeit with small differences. PTI functionally activates plant defense programs rapidly, however over time, negatively regulates these same processes to fine-tune immune responses such that optimal plant fitness is maintained. In the event of ETI, the suppressive features of PTI in its later stages are inhibited, thus allowing for the continual activation of plant defenses in an effort to produce a longer-lasting immune response.

1.7 Models to Study Plant-Pathogen Interaction

For several decades, many research groups have attempted to uncover the biochemical signaling processes occurring between plants and pathogens. Despite the knowledge generated from this work, little has found any broad-scale application. As we develop better model systems and examine a greater diversity of both plants and pathogens, we

are beginning to understand how variable the molecular interactions mediating each plant-pathogen interaction can be.

1.7.1 *Agrobacterium tumefaciens* as a Model Pathogen

Agrobacterium tumefaciens represent a very unusual yet very useful pathogen. The rare inter-kingdom transfer of DNA mediated by *A. tumefaciens* and the ability to adapt this pre-existing system for use in transformation biotechnology offers an efficient tool to introduce useful exogenous traits into dicotyledonous plants[87,97,98]. In addition, T-DNA transformation has also been utilized to generate mutant plants[134]. The random nature within which T-DNA inserts itself, allows for subsequent identification of the gene interrupted with a known DNA sequence that can be determined by mapping. A vast library of T-DNA insertion mutants has been developed for *Arabidopsis* and was later applied to *Oryze sativa* (rice)[134,135]. T-DNA has directly facilitated the ability to study the effect of eliminating specific genes under specific experimental conditions. This application has been further extended for use in generating T-DNA insertion mutant libraries in yeast and fungi[136,137].

Therefore using *A. tumefaciens* as a model pathogen, we will be able to further develop the understanding of pathogen associated biochemical and molecular processes required to infect a target dicotyledonous plant host, while also improving use as a biotechnological tool. However, the inefficiency in transforming monocotyledonous plants with *A. tumefaciens* remains a challenge. If the transformation efficiency could be improved for monocotyledonous plants, it would represent the ability to transform additional important crops utilizing *A. tumefaciens*.

1.7.2 *Arabidopsis thaliana* as a Model Host

Arabidopsis thaliana is a small weed in the mustard family and has been widely used for a variety of plant genetic approaches. *Arabidopsis thaliana* has a relatively short life cycle, producing a large number of seeds in 16-20 weeks, and encodes one of the smallest genomes among flowering plants. Cloning genes in *A. thaliana* has also been facilitated by the identification of genetic and molecular markers, making co-segregation of a desired phenotype significantly easier. Additionally, *A. thaliana* possess a small rosette structure and develops normal plant root structures when grown in petri dishes, which is a relatively rare feature of petri-dish-grown plants[138]. For these reasons *A. thaliana* serves as an excellent model for dicotyledonous plants.

1.8 Previously developed *A. tumefaciens*-*A.thaliana* model systems

Previous *A. tumefaciens*-*A. thaliana* based studies largely have been limited to two models: inoculation of *A. thaliana* cell suspension cultures with *A. tumefaciens* where virulence has been chemically-induced or artificial site-specific wounding of *A. thaliana* for *A. tumefaciens* inoculation[139-144] (Figure 1). *Arabidopsis thaliana* cell-suspension cultures inoculated with *A. tumefaciens* supplemented with acetosyringone (AS) revealed induction of ETI-related genes including peroxidases, glutathione transferases, pathogenesis-related (PR) proteins and enzymes related to secondary metabolism; common features of plant defense response[139,140]. Similarly, many other groups have utilized *A. thaliana* cell suspension cultures to study host interaction with other plant pathogens including *Xanthomonas campestris* pv. Vesicatoria[145], *Colletotrichum lindmuthianum* stain 172747[146], and *Pseudomonas syringae* pathovar *tomato*[147]. Although

many host responses typical of pathogen challenge were detected, there are significant fundamental issues with the use of plant cell suspension cultures to study host responses. Beginning with plant physiology, plant cell suspension cultures do not contain any of the typical structures and tissues found in whole plants. The absence of syncytial links between cells likely has significant effects on the molecular output of plant cell culture responses to pathogen challenge[148-149]. In addition, without root structures the exudates necessary for activating microbial chemotaxis and virulence are also absent[150-155]. Accordingly, the induction of pathogen virulence requires artificial supplementation with AS. Detecting the responses of plant cell suspension cultures may be reflective of host responses *in planta*, but due to physiological differences, manipulation of host responses based on the data generated in cell suspension techniques is unlikely to have the same affect *in planta*. Moreover, analysis of plant cell suspensions responses is limited in comparison to whole plant studies. Only responses of directly affected cells may be examined, excluding the spatial variation of these responses among both directly and indirectly affected tissues sites.

Significant issues with plant cell suspension techniques are amendable by using the artificial site-specific wounding technique. First, artificial site-specific wounding techniques maintain whole plant structures, establishing the absent syncytial links between cells in plant cell suspensions. In addition, the differential responses of various tissues can be analyzed and whole root systems are maintained. Studies utilizing this technique identified comparable induction of many ETI-related plant defense genes in directly affected tissue, but have also offered insight into a less understood mechanism known as host 'priming' in indirectly affected tissues[156,157]. Priming is a physiological

process in which a plant is proactively prepared to respond to pathogen challenge much more rapidly or aggressively. Directly affected tissue sites are able to signal non-affected (indirectly affected) sites to enter a 'primed state', which causes indirectly affected tissues to heighten the basal expression of plant defenses. This prevents the challenging pathogen from spreading throughout the plant structure and in addition, prevents a secondary infection from occurring[158]. Although this technique has offered newer insight into host responses and resolves many of the challenges associated with plant cell suspensions, there are still a number of problems remaining. By mechanically wounding the host, a much stronger response is generated as a response to the compromised integrity of the plant cell wall. These damaged plant tissues produce damage associated molecular patterns (DAMPs), perceived by nearby cells to initiate a response to the wounding event. In fact, DAMPs are imperative in activating plant defenses associated to herbivorous pests due to the nature in which they consume whole cell contents or whole portions of plant tissue[159-162]. JA primarily regulates the defensive mechanisms activated by plant wounding. Conversely, SA mediates antagonizing responses, since SA is considered the master regulator of host responses to pathogens. Accordingly, since the pathogen is applied following the mechanical wounding event, the host has already mounted a defense response that would not have been activated by typical *A. tumefaciens* infection, and likely has a significant influence on the detectable responses of the host. In addition to activating responses to mechanical wounding, site-specific wounding for inoculation applies the pathogen to aerial regions of the plant structure, which would be an atypical site of infection for *A. tumefaciens*. By applying the pathogen to different tissue sites, it is possible that differences in spatial gene expression patterns could

influence the susceptibility and responses of the host. Finally in similarly to plant cell suspension techniques, the pathogenic virulence must be artificially induced by AS in order to activate *A. tumefaciens*' virulence program for infection.

Together, plant cell suspension and site-specific wounding for inoculation techniques have provided a vast amount of knowledge pertaining to host responses upon pathogen challenge. However, there are a wide number of issues associated with both techniques despite their advantages. In order for the data obtained from such studies to have the greatest impact in the future manipulation of crop plants for improved resistance, experimental models must be developed to mimic conditions in nature as closely as possible. The aim of this project is to develop and make use of such a model. Herein we present the use of hydroponic co-cultivation in order to establish an experimental system that more closely resembles the biochemical and molecular processes that occur between *A. tumefaciens* and *A. thaliana in situ.*

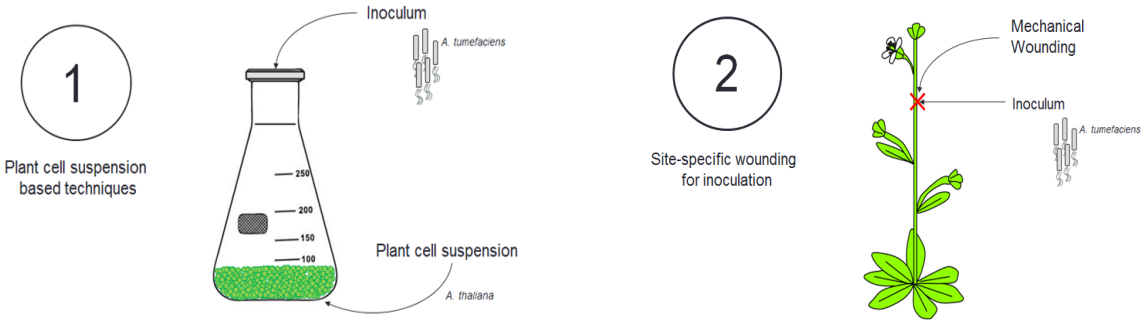


Figure 1. Schematic of conventional model systems to study *A. thaliana-A. tumefaciens* interaction.

1) Plant cell suspension based technique in which *A. thaliana* Col-0 cells are supported in a liquid Murashige and Skoog (MS) media. *A. tumefaciens* is introduced into the plant cell culture in addition to AS to induce virulence. 2) Site-specific wounding based technique where whole *A. thaliana* Col-0 structure is maintained. The stem structure is mechanically wounded for inoculation with *A. tumefaciens* supplemented with AS.

1.9 Hydroponic Co-Cultivation

Hydroponics is a method of growing plants without soil, using mineral nutrients in an aqueous solution. Hydroponic cultivation of plants was documented as early as 1627 and in 1929 was publicly promoted to be used for agricultural crop production purposes. Hydroponics is a subset of soilless culture that does not contain a solid medium for root establishment. There are a wide variety of hydroponic derivatives including static solution culture, continuous-flow solution culture, aeroponics, passive sub-irrigation, flood and drain sub-irrigation, the run to waste system, deep water culture, top-fed deep water culture, fogponics, and rotary hydroponics. Fundamentally, all techniques make use of a liquid culture composed of nutrients necessary to support plant life.

Hydroponic techniques have been widely established for studying optimal nutrient growth conditions and effects of metallic toxicity[163-164]. There are several advantages of utilizing hydroponic models. Hydroponic cultivation has small spatial requirements, maintains whole plant structure facilitating access to various plant tissues, allows tight control of nutrient/environmental conditions, and importantly provides control over the presence or absence of microbes/insects. Hydroponics is also less limiting to plant growth in comparison to agar/phytogel plating techniques. Typically when growing *A. thaliana* using agar/phytogel plating techniques, growth can only be sustained for up to 2-3 weeks before it is limited by petri-dish size. By contrast, growth of *A. thaliana* in a hydroponic system allows growth for up to 4-5 weeks before the shoot structure approaches the lid in an 8 cm tall glass.

In consideration of utilizing hydroponics for the study of plant-pathogen interaction, many of the remaining challenges associated with site-specific wounding techniques are addressed. By maintenance of whole plant structures and suspension of root systems in a liquid medium, the natural root secretion of chemical compounds necessary for microbial virulence induction is facilitated and collected in the liquid culture (Figure 2). The liquid culture essentially mimics the soil in the rhizosphere, becoming richer in root secretions as time progresses. This particular aspect of hydroponic cultivation exemplifies an excellent system mimicking soil grown conditions for inoculation with microbes. Once the microbe is inoculated into the liquid culture, the presence of root-secreted compounds can activate microbial virulence for direct plant-microbe association. Subsequently, the host is also able to perceive the microbe upon attachment and generates a response naturally without supplementation of defense elicitor compounds (Figure 2).

Mechanistically, AS is no longer required to activate microbial virulence in the case of *A. tumefaciens.* In addition, *A. tumefaciens* can act as an opportunistic pathogen by infecting 'naturally' damaged root tips resulting from root elongation, eliminating artificial mechanical wounding. Together the responses of directly affected root tissues can be studied, as well as detecting responses in indirectly affected (shoot) tissues (Figure 3), which have been initiated naturally aside from the initial introduction of the pathogen into the liquid culture. Hydroponic systems can be extremely useful and characteristically are easy to modify as depicted for our system in enabling the use of alternative hosts (Figure 4).

Conversely, hydroponic co-cultivation is one of the few systems available to study microbial responses. Typically when studying the responses of microbes, plant chemical compounds are applied to microbes in concentrations that are reflective of those found *in planta.* This is done in order to induce a level of response that would be typical of microbes experiencing exposure to a plant host. Hydroponic cultivation allows the roots to secrete chemicals that slowly diffuse into the liquid culture. Using this system, microbes can be separated from the liquid culture for study of their responses to true host exposure versus those techniques that apply chemicals to serve as the artificial perception of a host to induce detectable microbial responses (Figure 3). In addition, hydroponic co-cultivation can be utilized to study plant pathogens other than *A. tumefaciens* or can even be utilized to study responses to beneficial microbes.

Therefore, hydroponic co-cultivation may provide a superior system for uncovering a more detailed understanding of *A. thaliana-A. tumefaciens* reciprocal responses, and provides the ability to study responses between a wide variety of hosts and microbes.

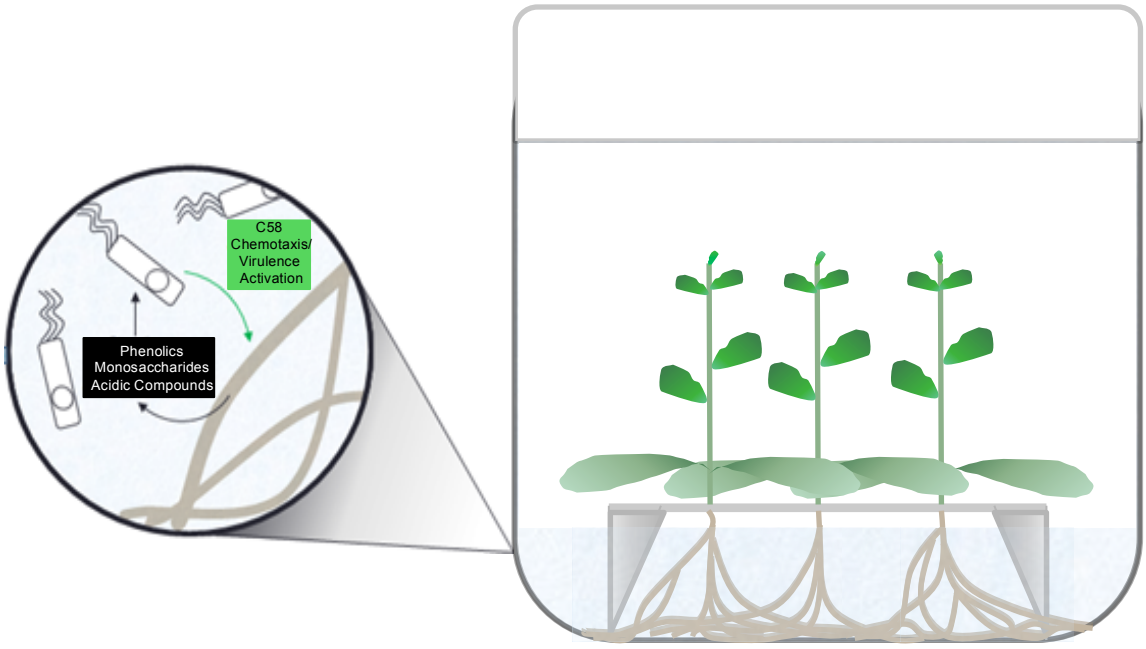


Figure 2. Illustration of hydroponic co-cultivation set-up for visualization of more natural host perception and root attachment.

Accumulation of phenolic, monosaccharide and acidic compounds in liquid culture are perceivable by *A. tumefaciens* C58 for activation of virulence and chemotaxis facilitating subsequent root infection.

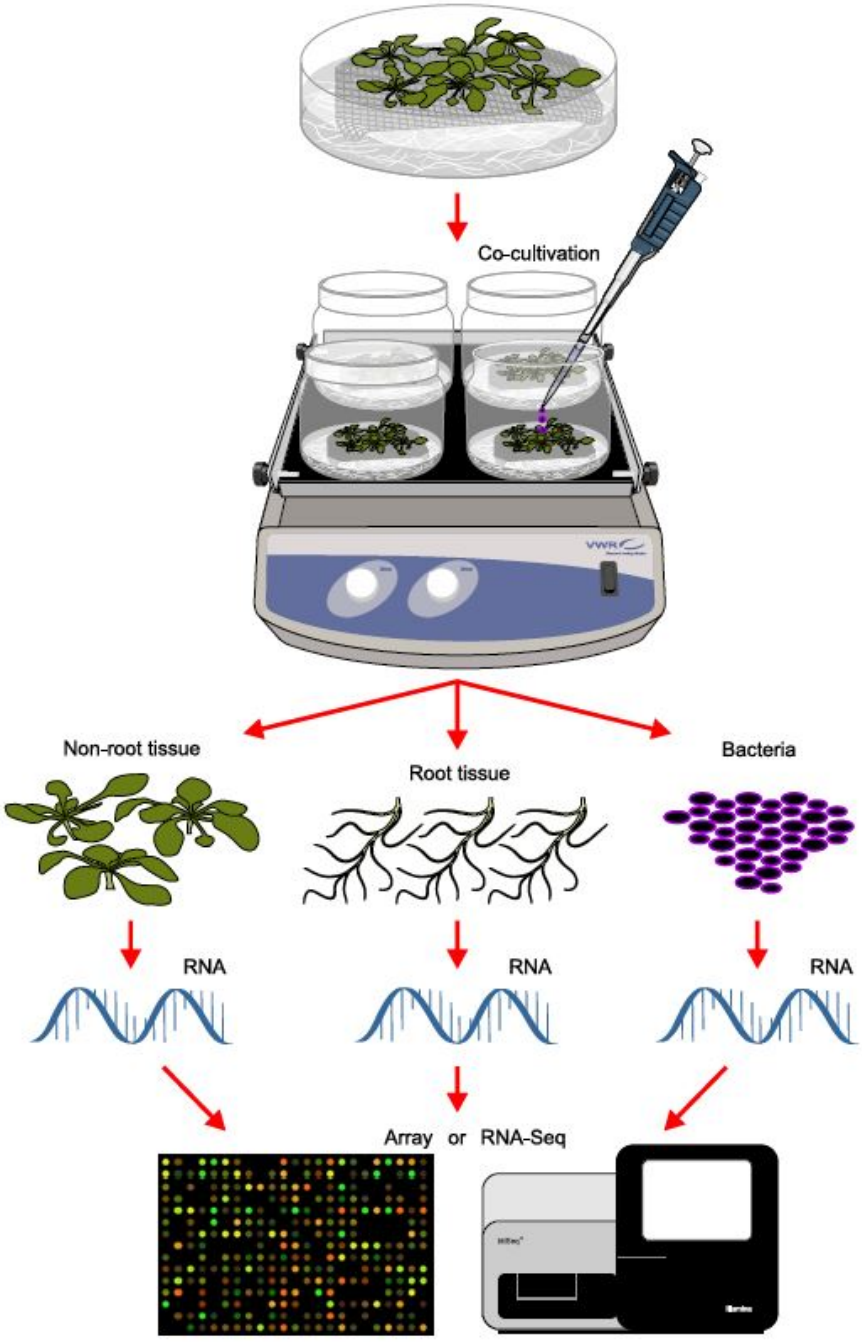


Figure 3. Schematic of tissues/cells that can be analyzed for responses.

Using the hydroponic co-cultivation system, whole plant structures are maintained. Root tissues (directly affected by *A. tumefaciens* C58), shoot tissues (indirectly affected by *A.*

tumefaciens C58) and *A. tumefaciens* C58 responses can be analyzed following separation. Microarray technology was utilized to study root and shoot tissues; *A. tumefaciens* C58 responses were not analyzed in this study.

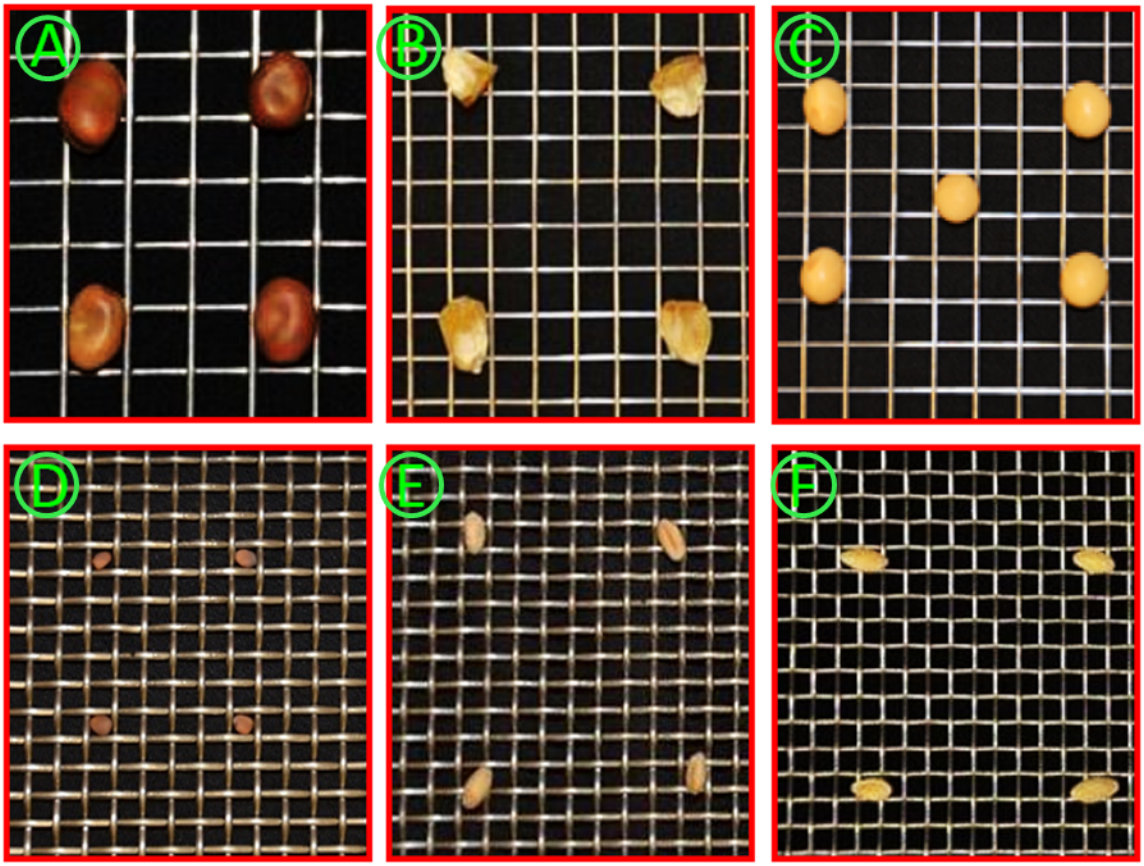


Figure 4. Many hosts can be cultivated in the developed hydroponic system through manipulation of the metal mesh platforms.

Compatibility of a set of metal meshes for hydroponic co-cultivation was tested, and they were shown to be suited to a variety of plant seeds. Stainless steel type 304 weldmesh 3 × 3 mesh × .047" dia wire for *Vickie fava* (A), Stainless steel type 304 weldmesh 4 × 4 mesh × .032" dia wire for *Zeal mays* (B) and *Glycine max* (Soybean) (C), Stainless steel type 304 weldmesh 6 × 6 mesh × .047" dia wire for *Raphanus sativus* (Winter Radish) (D) and *Triticum* spp. (E), and Stainless steel type 304 weldmesh 6 × 6 mesh × .035" dia wire *for Cucumis sativus* (F).

1.10 Hypothesis

Using the hydroponic co-cultivation system to model a host-pathogen interaction between *A. thaliana* Col-0 and *A. tumefaciens* C58, I hypothesize novel gene candidates will be identified with roles in plant defense against phytopathogens. To test this hypothesis, we will detect *A. thaliana* Col-0 total transcriptome changes in the earlier stages of host responses to *A. tumefaciens* C58 in both indirectly affected (shoot) and directly affected (root) tissues using an ATH1 Arabidopsis microarray. Candidate genes, selected from shoot tissue will be tested for roles in *A. thaliana* Col-0 defense against *A. tumefaciens* by analysis of variation in plant root surface attachment and root secretion profile. Candidates were selected from shoot responses since theoretically, these responses are proactively initiated for prevention of infection. Proactive responses may offer more in the reduction of host susceptibility to pathogens versus responses of root tissues that are more reactive in nature. Finally, comparative analysis will be conducted between root and shoot responses, as well as comparison with previously generated data sets analyzing *A. thaliana* Col-0 responses to *A. tumefaciens* C58 using conventional experimental systems.

Chapter 2 – Materials and Methods

2.1 *Arabidopsis* Seed Acquisition

Arabidopsis thaliana Col-0 wild-type and homozygous T-DNA mutant lines were obtained from the ABRC Order Stocks website (www.abrc.osu.edu./order-stocks). Mutant lines were homozygous knock-outs for *A. thaliana* Col-0 encoded *Receptor-like Protein* 32 (*RLP*32; At3G05650; induced in shoot), *Pathogen-Related* 6 proteinase inhibitor (*PR*6 protein; At2G38870; induced in shoot), and *Tempranillo* 1 (*TEM*1; At1G22560; repressed in shoot).

2.2 *Arabidopsis* Seed Surface Sterilization

Approximately 50 seeds of *Arabidopsis thaliana* (Col-0) were suspended in 800 µL of de-deionized RNase/DNase-free (UltraPure) water, vortexed and centrifuged at 10,000 x gravity (x g) for 30 seconds to remove the supernatant. Seeds were re-suspended in 500 µL of 5% NaOCl (bleach) and incubated for 1 minute at room temperature (21^{o}C). The seeds were centrifuged at 10,000 x g for 30 seconds to remove the supernatant. Bleach treated seeds were washed 5 times with UltraPure water, then were re-suspended in 500 µL of 75% ethanol and incubated for 1 minute at room temperature. The sterilized seeds were washed an additional 5 times with UltraPure water and finally re-suspended in 500 µL of UltraPure water. All washing steps were applied to wild-type and mutant *A. thaliana* Col-0 seeds.

2.3 Preparation of Electrocompetent *Agrobacterium tumefaciens* C58 and Electroporation

Wild-type *Agrobacterium tumefaciens* strain C58 (University of Washington) was propagated in a 30 mL culture of LB broth (5 g/L yeast extract, 7.5 g/L tryptone, 5 g/L sucrose, pH 7.0) for 16 hours at 28^{o}C in a shaker (75 rotations per minute). The 30 mL culture of *A. tumefaciens* C58 was added to 300 mL of 1:1 MGL (5.0 g/L mannitol, 1.0 g/L L-glutamic acid, 0.25 g/L potassium phosphate, 0.1 g/L sodium chloride, 0.1 g/L magnesium sulphate, 5 g/L tryptone, 2.5 g/L yeast extract, 1 µg/L biotin, pH 7.0) and NPT (2 g/L nutrient broth, 5 g/L potato dextrose, 6 g/L tryptic soy, pH 7.0). The culture mixture was incubated at 28^{o}C in a shaker until the OD_{600} reached 0.6 (following approximately 3-4 hours of incubation). Once the OD_{600} reached 0.6, the *A. tumefaciens* C58 culture was incubated on ice for 10 minutes with frequent shaking to cool the cells rapidly. The cooled culture was divided into 6 pre-chilled 50 mL falcon tubes and centrifuged at 8000 x g for 10 minutes at 4^{o}C. The supernatant was removed and the *A. tumefaciens* C58 pellet was re-suspended in 5 mL of chilled SG buffer (10 g/L glycerol, 0.5 M Sucrose, 1 mM $MgCl_2$). The re-suspended culture was centrifuged at 8000 x g for 10 minutes at 4^{o}C and the supernatant was removed. The pellet of each tube was re-suspended in 5 mL of SG buffer and the 6 tubes were re-consolidated into 3 tubes. The cultures were centrifuged at 8,000 x g for 10 minutes at 4^{o}C. The supernatant was removed and the pellet was re-suspended in 5 mL of SG buffer. The cultures were centrifuged once more at 8000 x g for 10 minutes at 4^{o}C and the supernatant was removed. The pellet was re-suspended in 1 mL of SG buffer, resulting in 3 mL of culture (3 tubes at 1 mL). The finalized culture was aliquoted into pre-chilled 1.5 Eppendorf tubes and snap frozen with liquid N_2 to be stored at -80^{o}C.[165]

2.4 Fluorescent Labeling of *Agrobacterium tumefaciens* using pCherry

Using M13 forward (M13F) (5' – CGCCAGGGGTTTTCCCAGTCACGAC – 3') and reverse (M13R) (5' – AGCGGATAACAATTTCACACAGGA – 3') primers, pCherry was amplified from the pmp7605 vector under the following PCR conditions: initial melting temperature of 94^{o}C for 30 seconds, 35 cycles of 94^{o}C for 30 seconds, 55^{o}C for 30 seconds and 72^{o}C for 2 minutes, followed by a final extension at 72^{o}C for 5 minutes. In a 50 µL reaction volume: 5 µL of 10x PCR buffer, 4 µL of dNTPs (10 mM), 3 µL of M13F (10 µM), 3 µL of M13R (10 µM), 0.5 µL Genscript Taq Polymerase (200 units/µL), 2 µL of Plasmid DNA (50 ng/µL) and 32.5 µL UltraPure water. The PCR product was purified for restriction enzyme digestion using the Qiagen QIAquick PCR Purification Kit (cat. no. 28106) in accordance to the manufacturer's protocol. Five volumes of Buffer PB were added to 1 volume of the PCR reaction and mixed by slow pipetting. A QIAquick column was placed within a 2 mL collection tube, and the mixture of PCR product and PB buffer was applied. The QIAquick column was centrifuged for 30 seconds at 10,000 x g. The flow-through was discarded and 750 µL of Buffer PE was applied to the column and centrifuged for 30 seconds at 10,000 x g. The flow-through was discarded and the column was centrifuged again for 2 minutes at 15,000 x g to remove residual wash buffer. The QIAquick column was transferred to a new 1.5 mL microcentrifuge tube. To elute the purified PCR product, 50 µL of UltraPure water was applied directly to the center of the column membrane and incubated at room temperature for 2 minutes. The column within the 1.5 mL microcentrifuge tube was centrifuged at 10,000 x g for 1 minute.

The amplified pCherry fragment was digested in a 40 µL reaction mixture containing: 1.5 µL of BamH1 (15 units/µL), 10 µL of the PCR product obtained from M13 (7605; 200 ng/µL), 4 µL of 10x Reaction buffer, and 24.5 µL of UltraPure water at 37^{o}C for 1.5 hours. The BamH1 digested 7605 fragment was ligated into the pJP2 plasmid in a 32 µL reaction mixture containing: 6 µL of pJP2 (10 ng/µL pre-digested by BamH1), 25 µL of mixture from pmp7065 digestion with BamH1, and 1 µL of 5M NaCl. The mixture was mixed by finger flicking and incubated on ice for 10 minutes. Following incubation, 100 µL of 100% ethanol (EtOH) was added, mixed by finger flicking and incubated on ice for an additional 20 minutes. The reaction mixture was centrifuged at 13,000 x g for 10 minutes and the supernatant was removed. The pellet was washed with 500 µL of 100% EtOH and centrifuged at 13000 x g for 10 minutes to separate the supernatant such that the DNA could be air dried completely. The dried pellet was re-suspended in 17 µL of UltraPure water in addition to 2 µL of 10X T4 DNA ligase buffer and 1 µL of T4 DNA ligase (40 units/µL), mixed by finger flicking, and finally incubated at room temperature (21^{o}C) for 2 hours.

From an *Escherichia coli* DH5α culture grown in LB broth to an OD_{600} of 1.0, 1.5 µL was added to the ligation mixture, finger flicked to mix and incubated on ice for 30 minutes. The mixture was heat shocked at 42^{o}C for 1 minute, and put on ice for 2 minutes. Following incubation, 250 µL of LB broth was added to the sample and transferred to a shaker for 1 hour at 28^{o}C. From the reaction mixture, 80 µL was plated onto 0.5% agar LB plates with tetracycline (10 ng/µL) and incubated at 37^{o}C for 24 hours. All plasmid extractions were conducted using the Qiagen QIAPrep Spin Miniprep Kit (cat. no. 27106) using the High Yield Protocol. *E. coli* DH5α retaining the pJP2

plasmid containing pCherry were grown up in a 5 mL culture with 2x YT medium (Qiagen) and incubated for 16 hours at 28^{o}C. The overnight culture was centrifuged at 8000 x g for 3 minutes at 21^{o}C. The supernatant was removed and the pellet was re-suspended in 250 µL of buffer P1. The solution was transferred to a new 1.5 mL microcentrifuge tube and 250 µL of buffer P2 was added and mixed by inverting the 1.5 mL microcentrifuge tube 10-12 times until the mixture turned completely blue, followed by incubation for 5 minutes. Following incubation, 350 µL of buffer N3 was added and mixed immediately and thoroughly by inverting the tube mixture 10-12 additional times until the mixture was colorless. The mixture was centrifuged for 10 minutes at 8000 x g. The supernatant was removed and applied to a QIAprep spin column by pipetting, followed by a quick centrifuge at maximum speed and the flow-through was discarded. The QIAprep spin column was washed with 500 µL of buffer PB, quickly centrifuged at maximum speed and the flow-through was discarded. This step was repeated one additional time. The washed QIAprep column was transferred to a new collection tube and centrifuged at maximum speed for 1 minute to remove residual wash buffer. The dried QIAprep column was transferred to a clean 1.5 microcentrifuge tube and the plasmid DNA was eluted into 60 µL of UltraPure water.

In a 0.1 cm cuvette, 1 µL (10 ng/µL) of purified pJP2 plasmid and 20 µL of electrocompetent *A. tumefaciens* cells were mixed together by gentle tapping and in a single pulse (1.80 kV) *A. tumefaciens* C58 was electroporated with the pJP2 plasmid containing pCherry. Subsequently, 1 mL of SOC medium (20 g/L tyrptone, 5 g/L yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 $MgSO_4$, 20 mM glucose) was immediately added to the cuvette and mixed by slow pipetting. The cell suspension was

transferred to a 17 x 100 mm polypropylene tube and incubated at 28^{o}C for 1 hour with shaking at 75 rpm. Electroporated *A. tumefaciens* C58 cultures were plated on semi-solid LB plates containing 10 µg/mL of Carbenicillin and incubated at 28^{o}C for 48 hours to select successfully electroporated *A. tumefaciens* C58 colonies.

2.5 *Agrobacterium* Propagation

Modified *A. tumefaciens* C58 containing the pJP2 plasmid was propagated in LB for 16 hours in 3 mL cultures. Original cultures were sub-cultured; 1 mL into 3 mL of fresh LB liquid media. Following 8 hours of propagation, all *A. tumefaciens* C58 sub-cultures were combined in Falcon 50 mL conical centrifuge tubes and divided into 1 mL samples contained within 1.5 mL centrifuge tubes. *A. tumefaciens* C58 cultures were centrifuged for 2 minutes at 15,000 x g to remove the supernatant. Spun down *A. tumefaciens* C58 cells were washed twice with 0.85% saline solution (0.85 g/L NaCl) and finally resuspended to an OD_{600} of 1.0 in UltraPure water.

2.6 *Arabidopsis* Seedling Cultivation

To avoid unnecessary damage to seedlings, semi-solid Murashige-Skoog medium (MS; 4 g agar, 2.165 g MS Basal Salts, 10 g sucrose, 0.25 g MES, 59 mL B5 vitamin mix in 1 L de-deionized H_2O, pH 5.75) was used to cultivate *A. thaliana* Col-0 seedlings. MS containing agar was poured in extra-deep petri-dishes (100 mm x 25 mm) and dried for 1 hour in a sterile flow-hood. Stainless steel woven wire mesh (grade: 304; mesh count: 40 × 40; wire DIA: 0.01; clear opening: 0.015; % open area: 36; lbs/sf: 0.279; microns: 381) were remodeled as platforms to be embedded into the surface of the semi-solid MS medium (Figure 5A). The sterilized *Arabidopsis* seeds were spotted onto the metal mesh

and the plates were sealed with 1 inch micropore tape (Figure 5B). The number of seeds sewn per plate varied dependent on the experiment conducted (6 seeds for microarray analysis, 4 seeds for confocal and liquid chromatography). Sealed plates were covered in tinfoil and stratified for 72 hours at 4^{o}C prior to cultivation at 22^{o}C to 24^{o}C with a 16 hour photoperiod for 14 days.

2.7 Hydroponic Co-Cultivation

In a sterilized flow hood, two-week-old seedlings and accompanying stainless steel woven metal mesh platforms were transferred from semi-solid MS plates into a glass tank (100 × 80 mm Pyrex crystalizing dishes with lid; Figure 5C and D). Following transfer, 18 mL of liquid MS (4.33 g MS Basal Salts, 20 g sucrose, 0.5 g MES, 118 mL B5 vitamin mix, pH 5.75) was pipetted into each glass tank and glass lids were sealed with 1 inch micropore tape (Figure 5E). Sealed tanks were placed on trays with lateral shaking at 75 rpm for a 72 hour adjustment period (Figure 5E). Following 72 hours, ~5 × 10^{7} (OD_{600} of 0.01 in 1 mL) of modified *A. tumefaciens* C58 cells were inoculated into the liquid MS solution. *A. thaliana* Col-0 seedlings and *A. tumefaciens* were co-cultivated for the indicated time at the same conditions used for semi-solid cultivation with additional shaking at 75 rpm (Figure 5E).

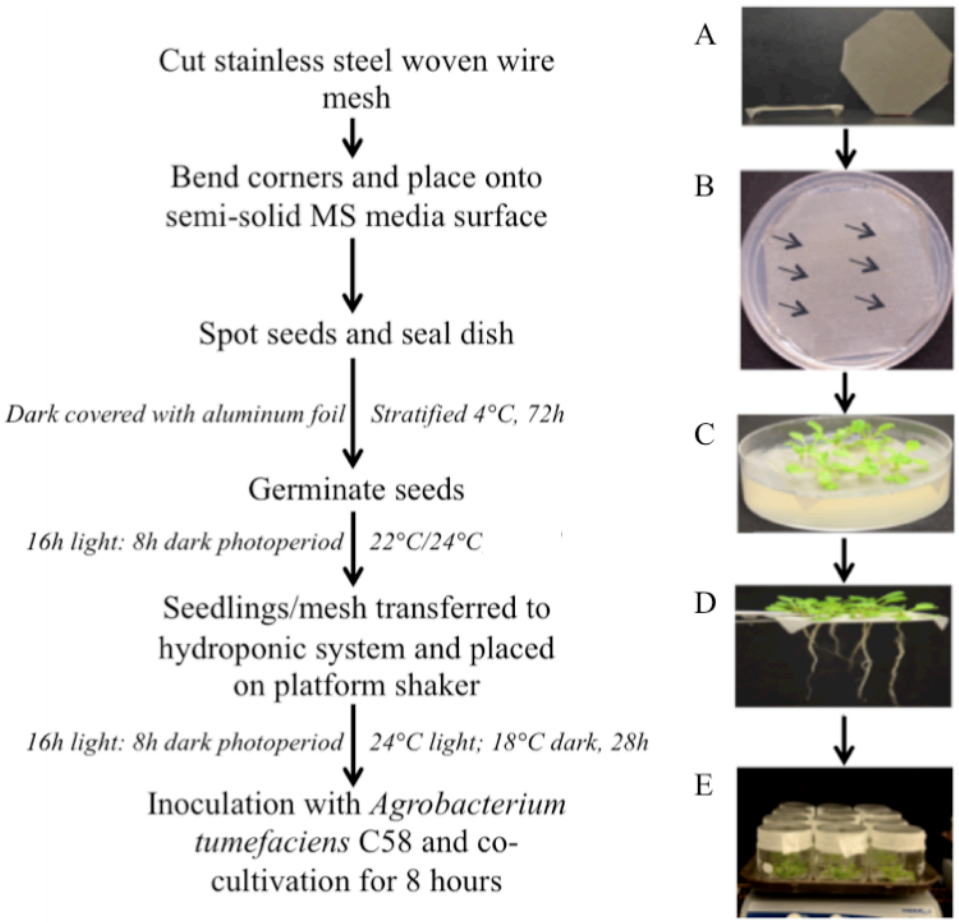


Figure 5. Experimental set-up of hydroponic co-cultivation.

Left panel describes the conditions that accompany each stage of the experimental set-up. A) Stainless steel woven wire mesh is remodeled into platforms. B) Stainless steel mesh is embedded onto the surface of semi-solid MS plates for surface sterilized seeds to be sewn ontop of the platform. C) Two-week old seedlings. D) Separation of metal platform from semi-solid MS plates, with associated 2-week-old seedlings. E) Seedlings and platforms placed in glass tanks for propagation in liquid MS culture.

2.8 Microscopic Observation

Following 48 hours of *A. thaliana* Col-0 hydroponic co-cultivation with pCherry labelled *A. tumefaciens* C58, tanks were unsealed and using sterilized forceps, individual secondary root structures were separated from wild-type and mutant lines. Separated secondary root structures were submerged in 1 mL of UltraPure water and washed twice in order to remove *A. tumefaciens* not directly and irreversibly bound to root structures. Washed roots were placed onto Fisherbrand Colorfrost® microscope slides and submerged in 30 µL of UltraPure water. The root samples were covered with a Fisherfinest Premium Glass cover slip and sealed with nail polish remover to prevent dehydration of the roots. Fluorescent images were obtained using a Leica TCSSP2 confocal microscope under an inverted 63x water lens objective. The mCherry fluorescence was monitored at 590-630 nm with excitation from a He-Ne 543/594 nm laser. Separation and visualization of *A. tumefaciens* C58 attachment to *A. thaliana* Col-0 secondary root structures was done in triplicate for each line.

2.9 Liquid Chromatography

Under sterile conditions, seedlings utilized to separate individual secondary root structures for microscopic observation were transferred into new glass tanks containing 10 mL of fresh liquid MS. Glass tank lids were secured and sealed with 1 inch micropore tape, and placed back onto trays shaking at 75 rpm. Following 72 hours of continued propagation, tanks were unsealed for *A. thaliana* Col-0 and metal mesh removal. In total, 10 mL of the liquid media was separated for each *A. thaliana* Col-0 line (mutant and WT) in triplicate and filter sterilized using a syringe with a sterile nylon 0.2 µm pore filter.

Filter sterilized liquid MS, separated following 72 hours of exposure to inoculated (experimental) and mock-inoculated (control) *A. thaliana* Col-0, were frozen in -80^{o}C freezers overnight in 50 mL Falcon conical tubes. Separation of liquid MS media was done in triplicate for wild-type and mutant *A. thaliana* Col-0 lines. Frozen filter sterilized secretion samples were freeze dried for 36 hours. Freeze dried samples were re-suspended in 5 mL of UltraPure water and the pH of each sample was adjusted to a pH range of 2.0 to 3.0. In a 1:1 ratio, ethyl acetate was utilized to partition the concentrate twice. For each partition, ethyl acetate was added and mixed by inverting 4 times, followed by incubation at room temperature for 5 minutes. The soluble organic compounds from each ethyl acetate-mediated partitioning were pooled and dried under nitrogen gas for approximately 45 minutes. The dried samples are finally re-suspended in 100 µL of 100% methanol before direct injection into the Agilent 1260 HPLC attached to an Agilent 6230 TOF mass spectrometer, equipped with a DualSpray ESI interface. Seperation conditions were as follows: compounds were seperated using a C-18 column (Eclipse Plus C18, 2.1x 50 mm, 1.8 µm; Agilent) at 35 ^{o}C. The flow rate was 0.300 mL/min and the corresponding gradient and solvents utilized are included in Table 1. Post-run, a 6 minute equilibration was done with 100% Solvent A. For processing, data were exported in mzDATA format and imported into MZmine (2.14.1; mzmine.sourceforge.net/) to determine molecular feature identification, alignment and ion abundance across samples. The following parameters were set:

Mass detector → Local Max
    Noise level = 100
Build chromatograms
    Minimum time span (min) = 0.1
    Min Height = 75
    m/z Tolerance = 0.05 m/z or 50 ppm

Alignment → Join
m/z Tolerance = 0.05 m/z or 50 ppm
Weight for m/z = 2
RT Tolerance = 0.5 min
Weight for RT = 0.5
Gap Filling → Same RT and m/z range
m/z Tolerance = 0.05 m/z or 50 ppm

RStudio Script was utilized to generate a heat map. The following commands were

applied:

```
Library("gplots",
lib.loc="/Library/Frameworks/R.framework/Versions/3.1/Resources/library")

# Read in data file
PolarPosAvg <- read.csv("Polar Pos – Averaged Areas Across Reps.csv")

#set first column as a factor, rather than integer value
PolarPosAvg$Compound <- factor(PolarPosAvg$Compound)

#Set up Heat Map parameters according to
#http://sebastianraschka.com/Articles/heatmaps_in_r.html

names <- PolarPosAvg[,1]
PolarPosAvgMatrix <- data.matrix(PolarPosAvg[,2:ncol(PolarPosAvg)]
Rnames <- PolarPosAvg[,1]

#set colours and range
my_palette <- colorRampPalette(c("red", "orange", "yellow"))(n=149)

#establish break points for colow change, and length of each colour break
col_breaks = c(seq(-0,1,length=50),seq(1,3,length=50),seq(3,5,length=50))

#use values from aboveto establish heatmap
heatmap.2(PolarPosAvgMatrix,trace="none",margins=c(12,9),col=my_palette,breaks=co
l_breaks)
```

Table 1. Gradient parameters and solvent percentages for polar metabolite analysis. Solvent A (0.1% formic acid in water); Solvent B (0.1% formic acid in acetonitrile)

Time (min)	Solvent A%	Solvent B%
0	100	0
2	100	0
5.5	5	95
7.5	5	95
8	0	100

2.10 RNA Isolation and Microarray Analysis

Following 8 hours of *A. thaliana* Col-0 hydroponic co-cultivation with wild-type *A. tumefaciens* C58, total RNA was extracted from root and shoot (stem, leaf) tissues using Qiagen RNeasy Plant Mini Kits (cat. no. 74904). The manufacter's protocol was utilized in order to conduct all subsequent RNA extraction procedures. Plant tissue (up to 50 mg) was crushed into a fine power under liquid nitrogen using a pre-chilled mortar and pestle. Tissue power and liquid nitrogen were transferred to 50 mL Falcon tubes and the liquid nitrogen was allowed to evaporate. Following evaporation, 600 µL of lysis buffer (RLT; containing β-mercaptoethanol) was added and vortexed vigorously. The sample was incubated in a heating block at 70^{o}C for 10 minutes with periodic shaking every 2-3 minutes. The lysate was transferred to a QIAShredder spin column that was placed within a 2 mL collection tube and centrifuged for 2 minutes at 15,000 x g. The flow-through was

transferred to a new microcentrifuge tube without disrupting the cell-debris pellet in the collection tube. A half volume of 100% ethanol was added to the lysate and mixed by pipetting 2-3 times. The mixed sample was transferred to an RNeasy spin column placed within a 2 mL collection tube and incubated at room temperature for 2 minutes. Following incubation, the sample was centrifuged for 30 seconds at 12,000 x g. The flow-through was discarded and the RNeasy spin column was transferred into a new 2 mL collection tube. Following transfer, 350 µL of buffer RW1 was added to the column and incubated for 1 minute at room temperature, followed by centrifugation at 15,000 x g for 30 seconds. Subsequently, 4 µL of DNaseI mixture (10 units/µL) was added to the column and incubated at room temperature for 15 minutes. Immediately 2 µL of EDTA (25 mM) was added to the mixture and placed on a heating block at 65°C for 10 minutes to halt the DNaseI reaction. Following incubation, 350 µL of buffer RW1 was added to the column and centrifuged at 15,000 x g for 30 seconds. Flow-through was discarded and an additional 350 µL of buffer RW1 was added to the column and incubated for 2 minutes followed by centrifugation at 15,000 x g for 30 seconds. The flow-through was discarded and 500 µL of buffer RPE was added to the column and incubated for 3 minutes at room temperature. The column was centrifuged at 12,000 x g for 30 seconds; this was repeated twice. Following centrifugation, the RNeasy spin column was placed into a new 2 mL collection tube. The RNeasy spin column was centrifuged at 15,000 x g for 2 minutes to dry the column. With the lid left open, the column was centrifuged again at 15,000 x g for 5 minutes as a final drying step. The RNeasy column was transferred into a new 1.5 microcentrifuge tube and 30 µL of UltraPure water was applied directly to the center of the RNeasy spin column and incubated for 5 minutes at room temperature.

The column was spun at 10,000 x g for 1 minute to elute the RNA from the column. The eluted RNA was re-applied to the column and incubated for an additional 5 minutes before finally being centrifuged at 10,000 x g for 1 minute. The quality of the RNA obtained was analyzed using a NanoDrop to measure OD ratios at 260 nm/280 nm and 260 nm/230 nm.

Prior to microarray analysis, the quality of the RNA obtained was re-assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Palo Alto, CA) and the RNA 6000 Nano kit (Caliper Life Sciences, Mountain View, CA). Only high quality, non-degraded RNA samples proceeded to labeling and hybridization to a GeneChip using a minimum RNA quality indicator (RQI) number of 7. Single stranded amplified RNA (aRNA) was prepared from 500 ng of total RNA as per the Affymetrix 3' IVT Express kit protocol (cat. no. 901228). Pre-hybridization was done with 5 × SSC (0.75 M NaCl and 0.075 M sodium citrate) solution of 3% powered milk at 42°C for 1 hour, then washed in H_2O and propanol, and finally centrifuged dry. 12.5 μg of fragmented end-labeled aRNA was hybridized for 16 hours at 45°C to *Arabidopsis* Genome ATH1 arrays (Alameda, CA, U.S.A.) for five cycles in 2 × SSC and three cycles in 0.1 × SSC.

All liquid handling steps were performed by an Affymetrix GeneChip Fluidics Station 450 and subsequently GeneChips were scanned with a GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA) using Command Console v3.2.4. Initial data quality assessment was performed via Affymetrix's Expression Console. The probe level data (.CEL file) is generated using Affymetrix Command Console v3.2.4 and summarized to gene level data in Partek Genomics Suite v6.6 (Partek, St. Louis, MO) using the RMA algorithm. The fold change and *P*-value for each gene was calculated using Partek's

multi-way ANOVA. Gene lists were created based on a 2-fold change in expression and a p-value cut-off of 0.005. These lists were then analyzed for enriched Gene Ontology terms and KEGG Pathways using a Fisher's Exact test to determine their biological significance. Gene Set Enrichment Analysis (GSEA) and GO-ANOVA was also carried out to determine significance between sets of genes between conditions. All sample labeling and GeneChip processing was performed at the London Regional Genomics Centre (Robarts Research Institute, London, Ontario, Canada). The microarray data generated in this study has been deposited in NCBI's Gene Expression Omnibus [101] and are accessible through GEO series accession number GSE62751 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62751).

2.11 Semi-Quantitative Reverse Transcription PCR

Semi-quantitative reverse transcription PCR (RT-qPCR) was conducted to validate the root and shoot gene expression profiles determined by microarray analysis. Single-stranded cDNA was synthesized using 2.0 µg of RNA with the SuperScriptIII (SSIII) RT Kit (cat. no. 18080051). In an initial mixture volume of 20 µL containing 2 µg of RNA suspended in UltraPure water, 2 µL of oligod(T) (0.5 µg/µL) and 2 µL of dNTPs (10 mM) was added and placed on a heating block at 65°C for 5 minutes. The reaction mixture was placed on ice for a quick chill and the following components were added: 8 µL of 10X First Strand Buffer, 2 µL of DTT (0.1M), 2 µL of RNase Out (40 units/µL). The reaction mixture was incubated at 42°C for 2 minutes to allow oligo d(T) primers to bind mRNA. Finally, 1 µL of SSIII RT (200 units/µL) was added and the reaction mixture was placed onto a heating block under the following conditions to generate cDNA: 25°C for 5

minutes, 50°C for 60 minutes, 70°C for 15 minutes, 80°C for 5 minutes, and a final hold at 4°C.

Quantitative PCR was performed using the SsoFast EvaGreen® Mix Kit (cat. no. 172-5200) on the CFX96 Real-Time PCR Detection System. The quantitative PCR conditions consist of an initial melting point of 95°C (30 s), 40-cycles of 95°C for 10 s and 60°C for 5 s, followed by a final melt curve analysis. Each 20 µL reaction contained: 10 µL of Sso Fast EvaGreen Mix®, 1 µL of forward primer (500 nM final concentration), 1 µL of reverse primer (500 nM final concentration), 0.5 µL of cDNA template and 7.5 µL of UltraPure water. Five root genes of interest were amplified: *HSP*21, *PGIP*1, *ELIP*1, *AGP*1, and *NAS*1 (Table 2). Five shoot genes of interest were amplified: *ATEXT*4, *LTP*3, *LSU*1, *WCOR*413, and *ERF*012 (Table 3). All data represent the mean of four to six independent experiments with SD, using *ACTIN*2 as a reference (Table 2). Note: for reactions amplifying *ATEXT*4, *WCOR*413 and *ERF*012 0.5 µL of $MgCl_2$ (25 mM) was added and accordingly contained 7 µL of UltraPure water. Additionally, reactions amplifying *LTP*3 and *LSU*1 contained 1.5 µL of $MgCl_2$ (25 mM) and accordingly had 6 µL of UltraPure water per reaction.

Table 2. Root RT-qPCR primers.

Gene Amplified	Primer Orientation	Sequence (5'-3')
*HSP*21	Forward	GAAGTCCGCTACACCGTTCT
	Reverse	TCCAACAATCCGAAAGGAGAGA
*PGIP*1	Forward	CAGGAACAAACTTACAGGTTCCAT
	Reverse	TGGAGCTTGTTGCGGGATAAA
*ELIP*1	Forward	GATGGTTGGATTCGTTGCGG
	Reverse	GGACTCAACGCTTATGCCCT
*AGP*1	Forward	ACACCTTCCCCTACCTCCAA
	Reverse	AGGAGGGTAAACTGGTGGGA
*NAS*1	Forward	TGACATCGACTCACACGCAA
	Reverse	TCCAAGTGCTCGATGGCTTT
*ACTIN*II	Forward	CGAGGCTCCTCTTAACCCAAAGG
	Reverse	GACACACCATCACCAGAATCCAGC

Table 3. Shoot RT-qPCR primers

Gene Amplified	Primer Orientation	Sequence (5'-3')
*ATEXT*4	Forward	CTCCTCCTCCGTACACCACT
	Reverse	TCCCGTCAACGATCTTGTGT
*LTP*3	Forward	AGCTTGCAGATGCATCCACT
	Reverse	CGCAAAACGACGACGTAAG
*LSU*1	Forward	TCGAGAGAAGTGGCGGAGAT
	Reverse	GGAAGAGACATGCGATCGT
*WCOR*413	Forward	GCGTCCATCGTTGCAGTTTT
	Reverse	ATTTCCCAACCCCGTTCCTC
*ERF*012	Forward	TCAAAGGCCCTCAAGCCAAT
	Reverse	GACGGCTGATGAAGTAGGGG

2.12 Detection of *Agrobacterium* Virulence Gene Expression

To test if *Agrobacterium* virulence genes are induced in response to hydroponic co-cultivation with *A. thaliana* Col-0, quantitative PCR was conducted on RNA isolated from *A. tumefaciens* C58 cells isolated from the hydroponic liquid MS solution following 8 hours of co-cultivation. Total RNA was extracted using the Qiagen RNAprotect Bacteria Reagent and RNeasy Protect Bacteria kit (cat. no. 74524). A 350 µL *A. tumefaciens* C58 culture volume (OD_{600} of 1.0) was added to 700 µL of RNAprotect Bacteria Reagent and mixed immediately by vortexing for 5 seconds. The mixture was incubated for 5 minutes at room temperature and centrifuged for 10 minutes at 5,000 x g. The supernatant was decanted and residual supernatant was removed by pipetting. 20 µL of Qiagen Proteinase K was added to 200 µL of TE buffer (30 mM Tris·Cl, 1 m M EDTA, 15 mg/mL lysozyme, pH 8.0) and added to the cell pellet. The *A. tumefaciens*

C58 cells were carefully re-suspended by pipetting the mixture several times. The mixture was briefly vortexed for 10 seconds and incubated at room temperature for 10 minutes. Every 2-3 minutes the reaction mixture was briefly vortexed for 10 seconds. Following a 10 minute incubation period, 700 µL of buffer RLT was added and vortexed vigorously. Subsequently 500 µL of 100% ethanol was added and mixed thoroughly by pipetting. Up to 700 µL of the mixture was applied to an RNeasy Mini spin column placed in a 2 mL collection tube. The lid was closed and centrifuged for 30 seconds at 8,000 x g. The flow-through was discarded and 4 µL DNaseI mixture (10 units/µL) was added to the column and incubated at room temperature for 15 minutes. Immediately 2 µL of EDTA (25 mM) was added to the mixture and placed on a heating block at 65°C for 10 minutes to halt the DNaseI reaction. Following incubation, 700 µL of buffer RW1 was applied to the RNeasy spin column, incubated for 1 minute at room temperature, and centrifuged for 30 seconds at 8,000 x g. Supernatant was discarded, 500 µL of buffer RPE was applied to the RNeasy spin column, incubated at room temperature for 5 minutes, and centrifuged at 8,000 x g to discard the supernatant. An additional 500 µL of buffer RPE was applied to the RNeasy spin column and centrifuged at 8,000 x g for 2 minutes. The RNeasy spin column was transferred to a new 1.5 mL collection tube and 30 µL of UltraPure water was applied directly to the spin column membrane, followed by a 2 minute incubation period at room temperature. The RNeasy spin column was centrifuged for 1 minute at 8,000 x g to elute RNA.

In a 20 µL mixture containing 1 µg of RNA suspended in UltraPure water, 4 µL of dNTPs (10 mM) and 1.5 µL of random hexamers (50 ng/µL) were added and incubated for 5 minutes on a heating block at 65°C. Following incubation, the reaction mixture was quick

chilled on ice and the following components were added: 4 µL of 5X First Strand Synthesis buffer, 4 µL of $MgCl_2$ (25 mM), 2 µL of DTT (0.1 mM), 1 µL of RNase out (40 units/µL) and incubated at 25°C for 2 minutes. Finally, 1 µL of SSIII RT (200 units/µL) was added. The reaction mixture was applied to the following amplification cycle to obtain cDNA: 25°C for 5 minutes, 50°C for 60 minutes, 70°C for 15 minutes, 80°C for 5 minutes, and a final hold at 4°C.

Quantitative PCR was performed on the following virulence genes: *chv*G, *vir*D1, *vir*A, *vir*E0, outer membrane protein *rop*B and *vir*H1 (Table 4). Relative gene expression was normalized using *16S* rRNA as a reference (Table 4). Each 20 µL reaction contained: 10 µL of Sso Fast EvaGreen Mix®, 1 µL of forward primer (500 nM final concentration), 1 µL of reverse primer (500 nM final concentration), 0.5 µL of cDNA template and 7.5 µL of UltraPure water. All data represent the mean of four to six independent experiments with SD.

Table 4. *A. tumefaciens* C58 RT-qPCR primers.

Gene Amplified	Primer Orientation	Sequence (5'-3')
*chv*G	Forward	GTGAACCAGTCACCCCAAGT
	Reverse	TGAGTTTCCCGATCAACCCG
*vir*D1	Forward	TCGTCATAGGATGGAGGCCA
	Reverse	CGGACAAAATGGAACGGAGC
*vir*A	Forward	TATACTTGCACGCGAGGGTC
	Reverse	ACCGAAACGGAACCCAAGAA
*vir*E0	Forward	TGATGTTGATCGGACGGCTTT
	Reverse	TTGCCGGAAGCCCACAATC
*rop*B	Forward	GGAAACGATACGCTCCGACA
	Reverse	AACCAGAACACCTGCGTCAA
*vir*H1	Forward	TGGGACGATGTACCGAGAGT
	Reverse	TCTTCTCCCCACGAAGGACT
16S rRNA	Forward	CGAGGCTCCTCTTAACCCAAAGG
	Reverse	GACACACCATCACCAGAATCCAGC

2.13 *Agrobacterium* Growth Curve

Agrobacterium tumefaciens C58 growth in the hydroponic co-cultivation system was determined over a 54-hour period in the presence and absence of an *A. thaliana* Col-0 host. *Agrobacterium tumefaciens* was inoculated into the system at a starting OD_{600} of 0.1 and were analyzed for growth every 4 hours using a SmartSpec Plus Spectrophotometer with 1,000 μL cuvettes.

Chapter 3 – Results

3.1 Microarray

3.1.1 Shoot

To assess the responses of *A. thaliana* Col-0 indirectly affected tissue sites (shoot tissue), a microarray was conducted. RNA isolation was conducted on shoot tissue extracted in triplicate from six plants. Only high quality RNA with 260/280 and 260/230 readings ≥2.0 proceeded for microarray analysis. In shoot tissues, 863 genes were differentially expressed (DE) by ≥2.0 fold. Changes in expression by ≥2.0 fold were considered significant. By applying a 0.005 p-value and excluding hypothetical genes, 401 genes were DE in *A. thaliana* Col-0 shoot tissues in response to hydroponic co-cultivation with *A. tumefaciens* C58 eight hours post-infection (hpi). Of 401 differentially expressed genes (DEGs), 249 were induced (Appendix 1) and 152 were repressed (Appendix 2).

Gene ontology (GO) term analysis was utilized to determine the biological processes most highly influenced by challenging *A. thaliana* Col-0 with *A. tumefaciens* C58. GO analysis revealed stress response, transport and development categories contained the greatest number of DEGs (Figure 6), and these were classified as the most highly affected functional biological processes. Among stress-related process, hyperosmotic stress, reactive oxygen species (ROS), fungal stress, defense activation, bacterial stress, system acquired resistance (SAR), wounding and starvation were most highly affected (Figure 7). Lipid, amino acid, transition metal and nitrate transport were the most highly affected sub-categorized transport processes (Figure 8), in addition to reproductive development as the most highly affected developmental process (Figure 9).

Typically, SA-mediated responses are primarily triggered in response to pathogen infection, and in some cases are even antagonistic to JA-mediated responses[121,133]. Instead, we detected abscisic acid (ABA) responsive genes with the greatest differential expression to phytopathogen challenge, in addition to JA and auxin responsive genes (Figure 10). However further analysis also revealed SA signaling and biosynthesis were also highly influence in shoot tissues (Figure 10). Despite the relatively significant DE of ABA responsive genes, the increased expression of ABA biosynthesis genes was not detected (Figure 10). The detection of ABA, JA and auxin mediated responses suggests that in indirectly affected host sites, SA is not a major player for priming the host in the event of progressive infection.

3.1.2 Selection of Candidate Genes

To investigate the defense responses of shoot tissues, three genes were selected as candidates for roles in mediating *A. thaliana* Col-0 susceptibility to *A. tumefaciens* C58. Of the three genes, two were induced, *RLP32* and a PR6 proteinase inhibitor (Appendix 1), and one repressed, *TEM*1 (Appendix 2). The genes were selected based on a variety of factors including gene family and the biological processes associated with the correspondent gene family.

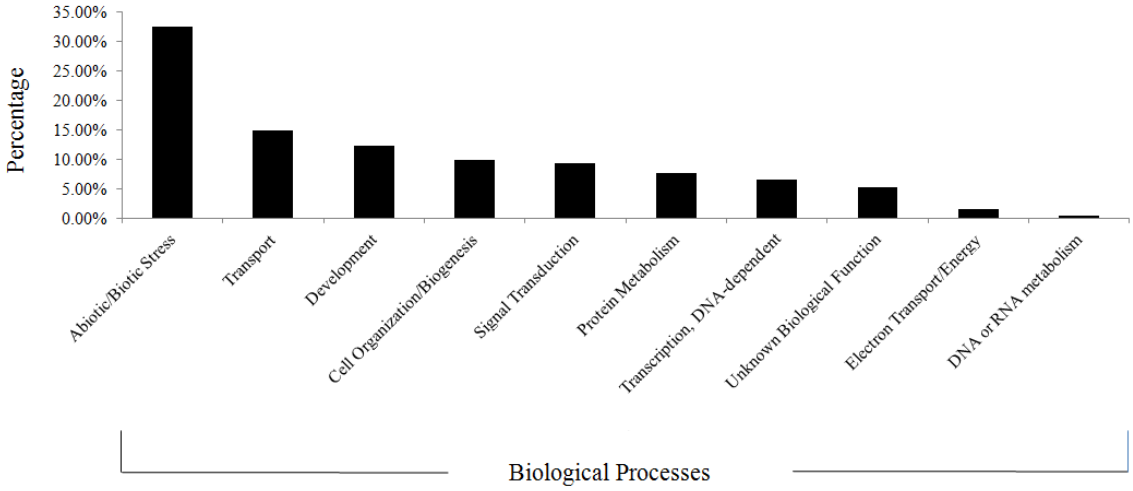


Figure 6. Functional categorization of the biological processes affected by *A. thaliana* Col-0 hydroponic co-cultivation with *A. tumefaciens* C58.

Percentage of genes affected (both induced and repressed) in association with specified biological processes within shoot tissues.

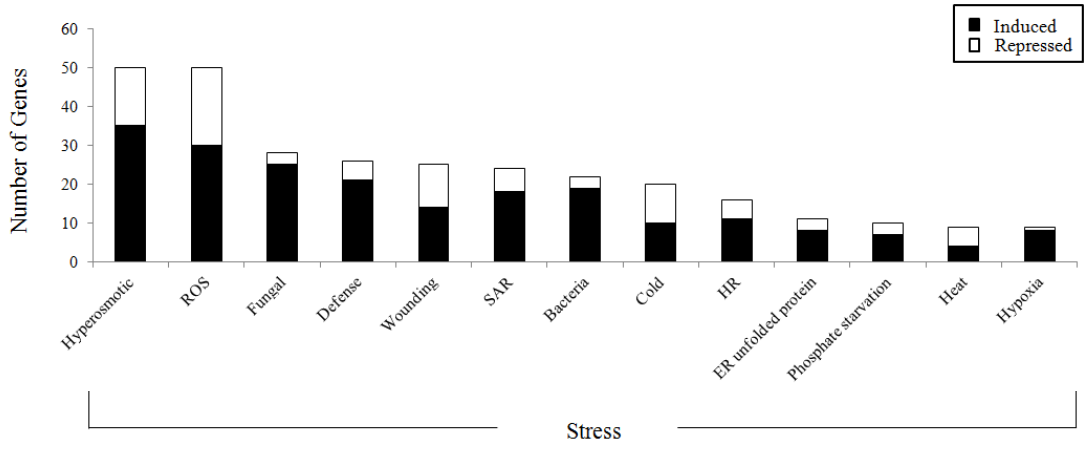


Figure 7. Distribution of genes differentially expressed among various stress responses.

Number of stress-related genes differentially expressed by 2-fold or greater in *A. thaliana* Col-0 shoot tissue in response *A. tumefaciens* C58 infection. All stress responses containing seven differentially expressed genes or less (≤2%) were omitted from the representative data including: flooding, desiccation/drought, freezing, PAMP, viral, insect, DNA repair, misfolded protein, callose deposition, starvation (sugar, nitrogen, sulfur, iron), innate immune and induced systemic responses.

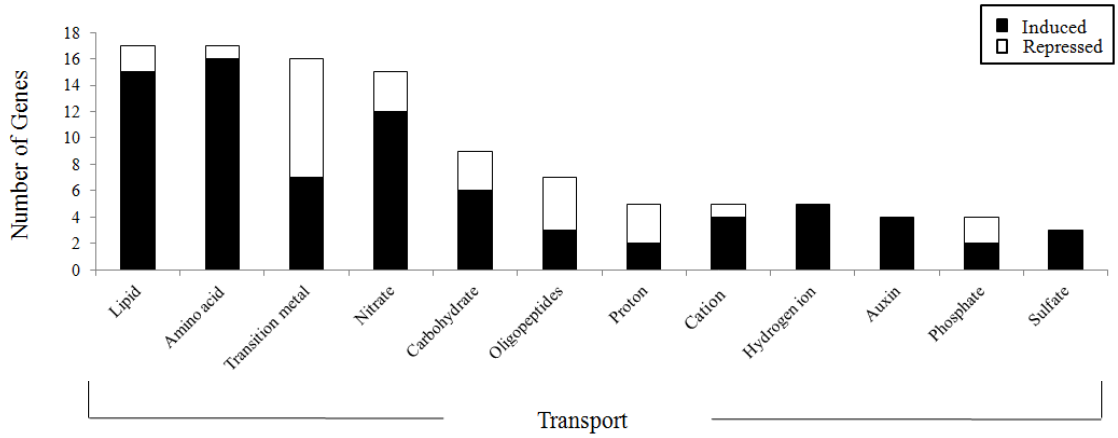


Figure 8. Distribution of genes differentially expressed among various substrate transporters.

Number of transport related genes differentially expressed by 2-fold or greater in *A. thaliana* Col-0 shoot tissues in response to *A. tumefaciens* C58 infection. All substrate specific transporters containing two differentially expressed genes or less were omitted from the representative data including: sorbitol, urea, borate and aluminum transport.

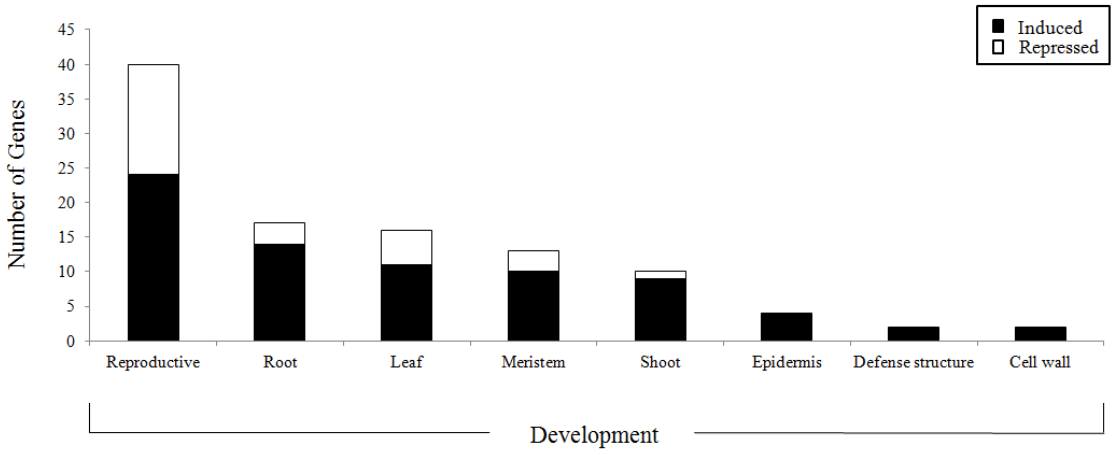


Figure 9. Distribution of differentially expressed genes associated with plant development.

Number of development related genes differentially expressed by 2-fold or greater in *A. thaliana* Col-0 shoot tissue in response to *A. tumefaciens* C58 infection.

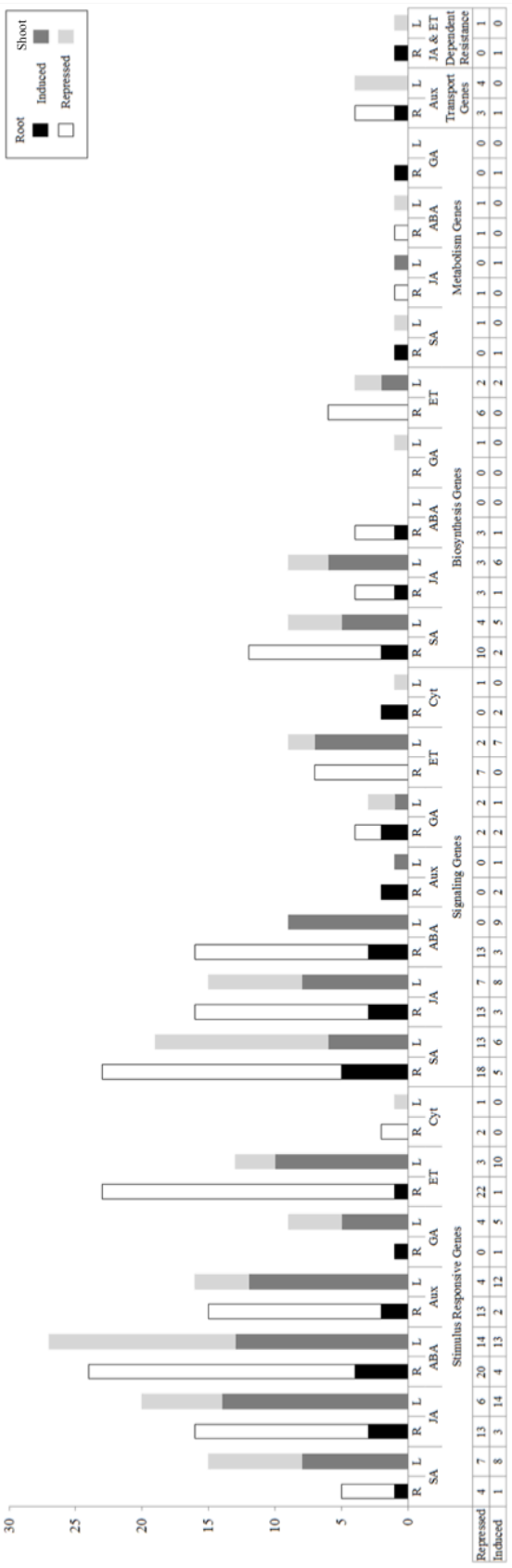


Figure 10. Distribution of differentially expressed genes associated with hormonal

regulation in shoot and root *A. thaliana* Col-0 tissues in response to hydroponic co-cultivation with *A. tumefaciens* C58 for 8 hours.

Black filled bars indicate the number of inducible genes in root tissues; dark grey filled bars indicate the number of inducible genes in shoot tissue. Unfilled bars indicate the number of repressed genes in root tissue; light grey bars indicate the number of repressed genes in shoot tissue. Hormone abbreviations are as follows: SA (salicylic acid); JA (jasmonic acid); ABA (abscisic acid); Aux (auxin); GA (gibberellic acid); ET (ethylene); Cyt (cytokinin).

3.1.2.1 Comparative Analysis with Previously Generated *A. thaliana* Data Sets

For comparative analysis of DEGs detected in *A. thaliana* Col-0 shoot tissue in response to hydroponic co-cultivation with *A. tumefaciens* C58, the obtained dataset was compared with transcriptomic data obtained using conventional systems such as *A. thaliana* cell suspension cultures or site-specific wounding of *A. thaliana* for inoculation. The previously generated transcriptomic data using conventional systems assayed *A. thaliana* Col-0 responses to *A. tumefaciens*[139,156], the fungal pathogen *Fusarium oxyspoarum* (*F. oxysporum*)[166], the plant herbivore *Bemisia tabaci* type B (*B. tabaci*)[167], and the PAMP factor elicitor compound *flg*22[140]. First, our gene list generated from shoot transcriptome data was uploaded to NCBI's Gene Expression Omnibus (GEO) and is accessible through the GEO series accession number GSE62749 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62749). Overlapping comparison of the transcriptomic data sets was completed using a "VLOOKUP" formula generated in Microsoft Excel. Forty-five DEGs detected from our shoot microarray were previously detected in *A. thaliana* responses to *A. tumefaciens*, *F. oxysporum*, *B. tabaci* and *flg*22 (Appendix 3). None of the DEGs detected using hydroponics were identified in the responses of *A. thaliana* cell suspension cultures (Appendix 3). Sixteen DEGs were found to be similarly detected in the *A. thaliana* site-specific wounding based technique (Appendix 3).

Additionally, 358 DEGs in *A. thaliana* Col-0 shoot tissues detected in response to hydroponic co-cultivation with *A. tumefaciens* C58 following 8 hpi have not been previously detected in response to pathogenic bacteria as determined by GO TAIR

annotation (Appendix 4). Of these, three genes that have not been previously characterized for roles in defense against pathogenic bacteria were selected to be further analyzed for roles in *A. thaliana* Col-0 defense. Single-gene knockouts were obtained from the ABRC T-DNA mutagenized *A. thaliana* Col-0 resource for *RLP*32 (At3G05650), PR6 proteinase inhibitor (At2G38870) and *TEM*1 (At1G22560) (See section 3.3 and 3.4)

3.1.3 Root

To assess the responses of *A. thaliana* Col-0 tissue sites directly affected by *A. tumefaciens* C58 inoculation (i.e., root tissue), a microarray was conducted. In root tissues, 827 genes were DE by ≥2.0 fold. Applying a 0.005 p-value and by exclusion of hypothetical genes, 549 genes were DE in *A. thaliana* Col-0 root tissues in response to hydroponic co-cultivation with *A. tumefaciens* C58 eight hpi. From 549 DEGs, 323 were induced (Appendix 5) and 226 genes were repressed (Appendix 6).

To determine the most highly affected biological processes, GO term analysis was also conducted for root tissue microarray expression data. Similar to shoot tissues, stress response, transport and development categories were the most highly affected biological processes (Figure 11). Responses to hyperosmotic stress, ROS, fungal stress, wounding, SAR, bacterial stress, ER unfolded protein, heat stress, iron starvation and light stress were the most highly affected among stress-related processes (Figure 12). Transition metal, nitrate, cation, amino acid and water transport were the most highly affected sub-categorized transport processes (Figure 13). In addition, genes responsible for root development were the most highly affected in response to pathogen challenge (Figure 14).

Interestingly, the distribution of hormone related genes affected in root tissues is similar to that of responses in shoot tissues (Figure 10). ABA responsive genes showed the highest number of DEGs, followed by JA and auxin responsive genes (Figure 10). In addition, SA signaling and biosynthesis genes also showed significant responses to *A. tumefaciens* C58 challenge (Figure 10). Although the pattern of distribution is observationally similar to that of shoot tissues, it is clear that the number of genes induced versus those repressed show opposite patterns of detection. This indicates that although the hormonal processes affected and number of genes affected may be similar between tissue types, the processes that are being activated or inactivated are opposite.

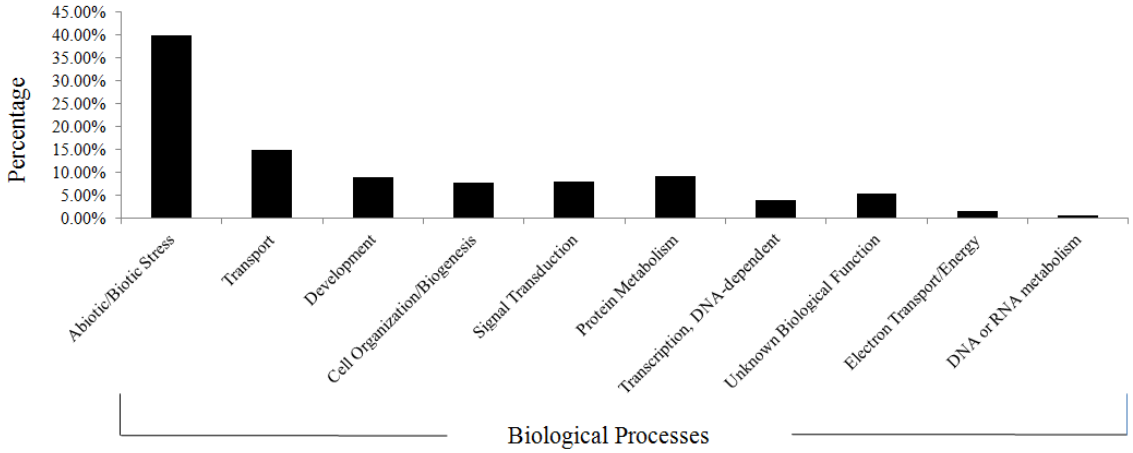


Figure 11. Functional categorization of the biological processes affected by *A. thaliana* Col-0 hydroponic co-cultivation with *A. tumefaciens* C58.

Percentage of genes affected in association with specific biological processes within root tissues.

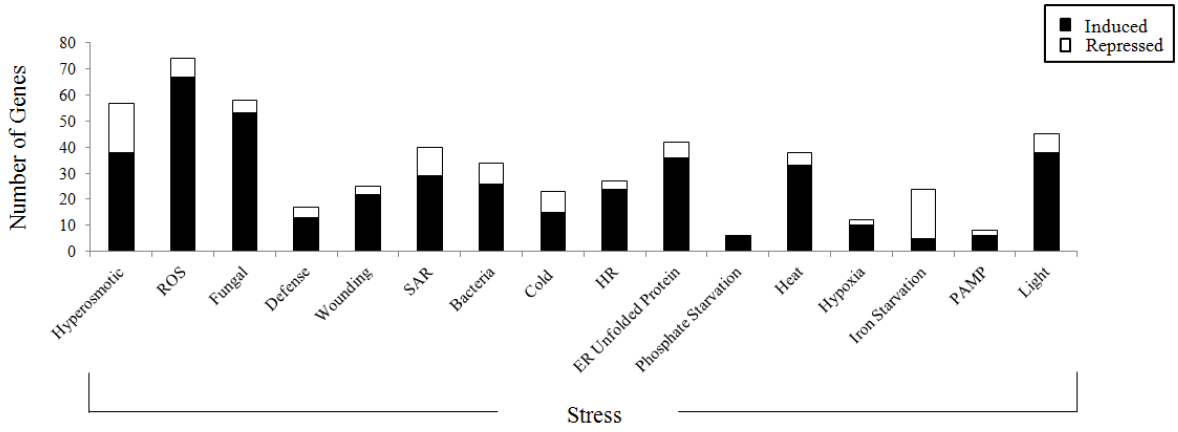


Figure 12. **Distribution of genes differentially expressed among various stress responses.**

Number of corresponding stress-related genes differentially expressed by 2-fold or greater in root *A. thaliana* Col-0 tissue in response *A. tumefaciens* C58 infection. DNA repair, insect, viral, freezing, flooding and anoxia related stress-responses were omitted from the data (≤2%).

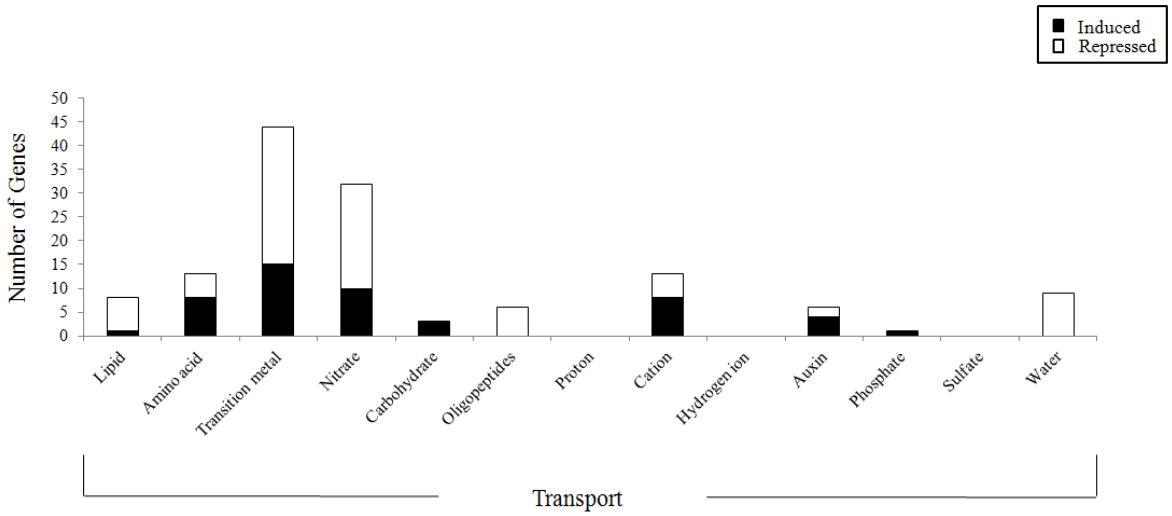


Figure 13. Distribution of genes differentially expressed among various substrate transporters.

Corresponding number of transport related genes differentially expressed by 2-fold or greater in root *A. thaliana* Col-0 tissues in response to *A. tumefaciens* C58 infection. All substrate specific transporters containing two differentially expressed genes or less were omitted from the representative data including: malate, inorganic anion, nucleotide, ammonium, urea, peroxide, carbon dioxide and lithium ion transport. In addition, water transport was highly repressed in *A. thaliana* root tissues.

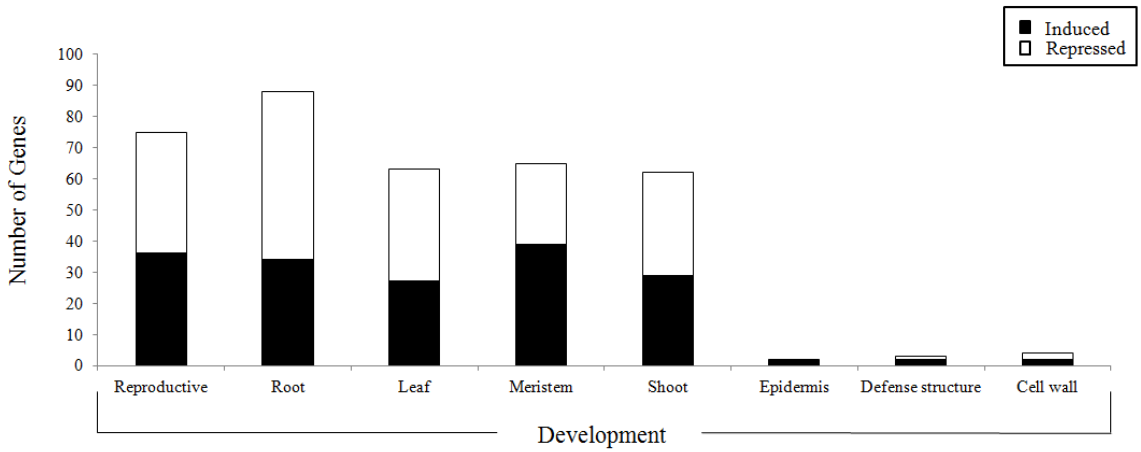


Figure 14. Distribution of differentially expressed genes associated with plant development.

Number of development related genes differentially expressed by 2-fold or greater in root *A. thaliana* Col-0 tissue in response to *A. tumefaciens* C58 infection.

3.1.3.1 Comparative Analysis with Previously Generated *A. thaliana* Data Sets

For comparative analysis of DEGs detected in *A. thaliana* Col-0 root tissue in response to hydroponic co-cultivation with *A. tumefaciens* C58, the obtained dataset was compared with transcriptomic data obtained using *A. thaliana* cell suspension cultures or site-specific wounding of *A. thaliana* for inoculation. The previously generated transcriptomic data using conventional systems assayed *A. thaliana* Col-0 responses to *A. tumefaciens*[139,156], *F. oxysporum*[166], *B. tabaci*[167], and the PAMP factor elicitor compound *flg*22[140]. The transcriptomic data from root tissues was submitted to NCBI's GEO and is accessible through the GEO series accession number GSE62750 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE627590). Overlapping comparison of multiple genes sets was completed using a "VLOOKUP" formula generated in Microsoft Excel. From 549 DEGs, 121 were previously detected in *A. thaliana* responses to *F. oxysporum*, *B. tabaci* and *flg*22. In comparison solely to transcriptomic responses to *A. tumefaciens* C58 challenge, only two DEGS from hydroponic based methods were detected in *A. thaliana* cell suspension cultures (Appendix 7). However, forty-four DEGS were found to be similarly detected in the *A. thaliana* site-specific wounding based technique (Appendix 7). Based on GO TAIR annotation, 527 DEGs detected in *A. thaliana* Col-0 root tissues have not been previously reported in response to pathogenic bacterium (Appendix 8).

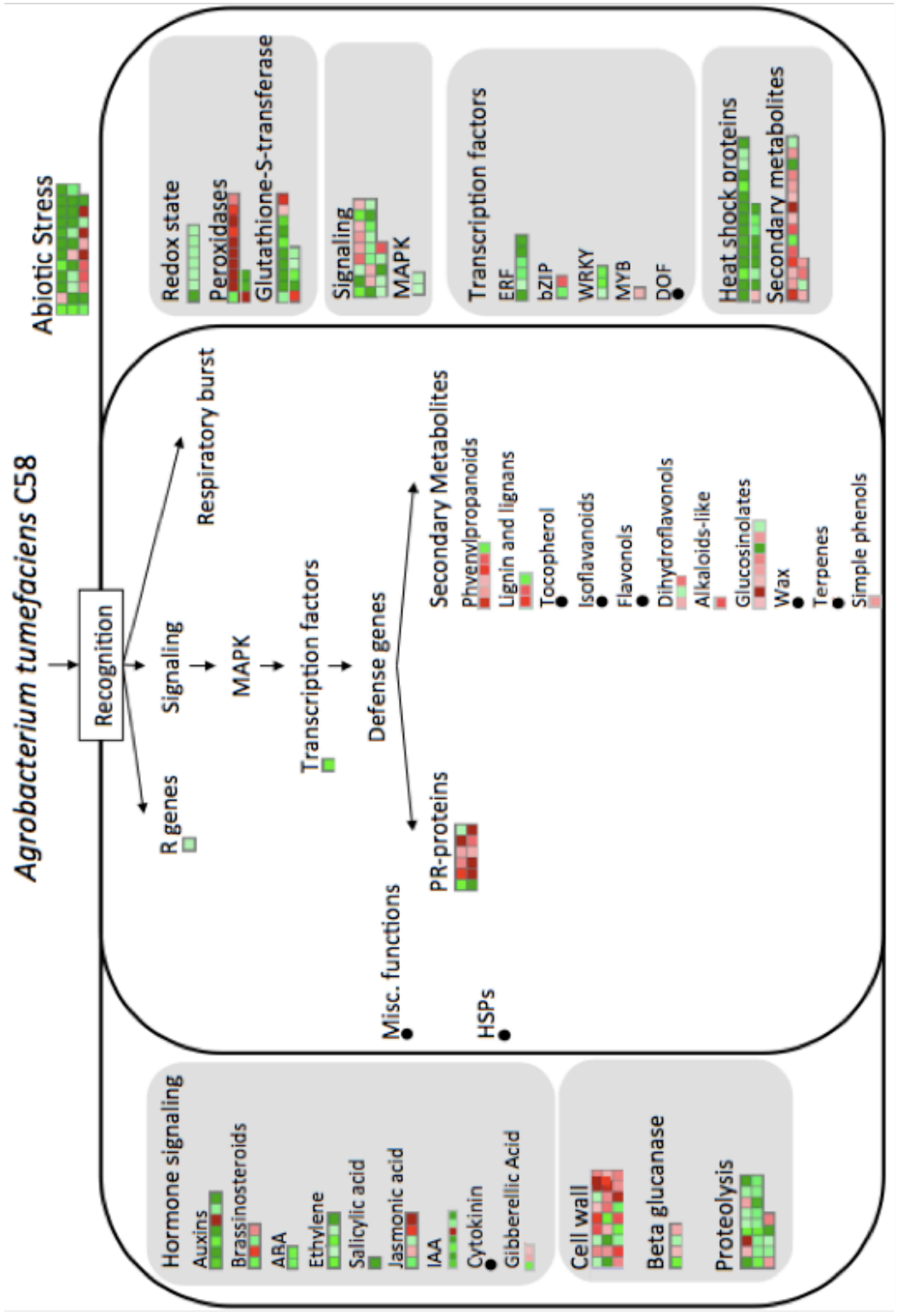
3.1.4 Comparative Analysis between Root and Shoot Microarray Data

Using the previously generated "VLOOKUP" formula for comparative transcriptome analysis with previously generated datasets, 52 DEGs overlapped in both our leaf and root transcriptome profiles (Appendix 9). From these genes, 21 genes had opposite patterns of expression between tissue types (Appendix 9). Three genes were down-regulated in both tissue types and the remainder was induced in both tissue types (Appendix 9).

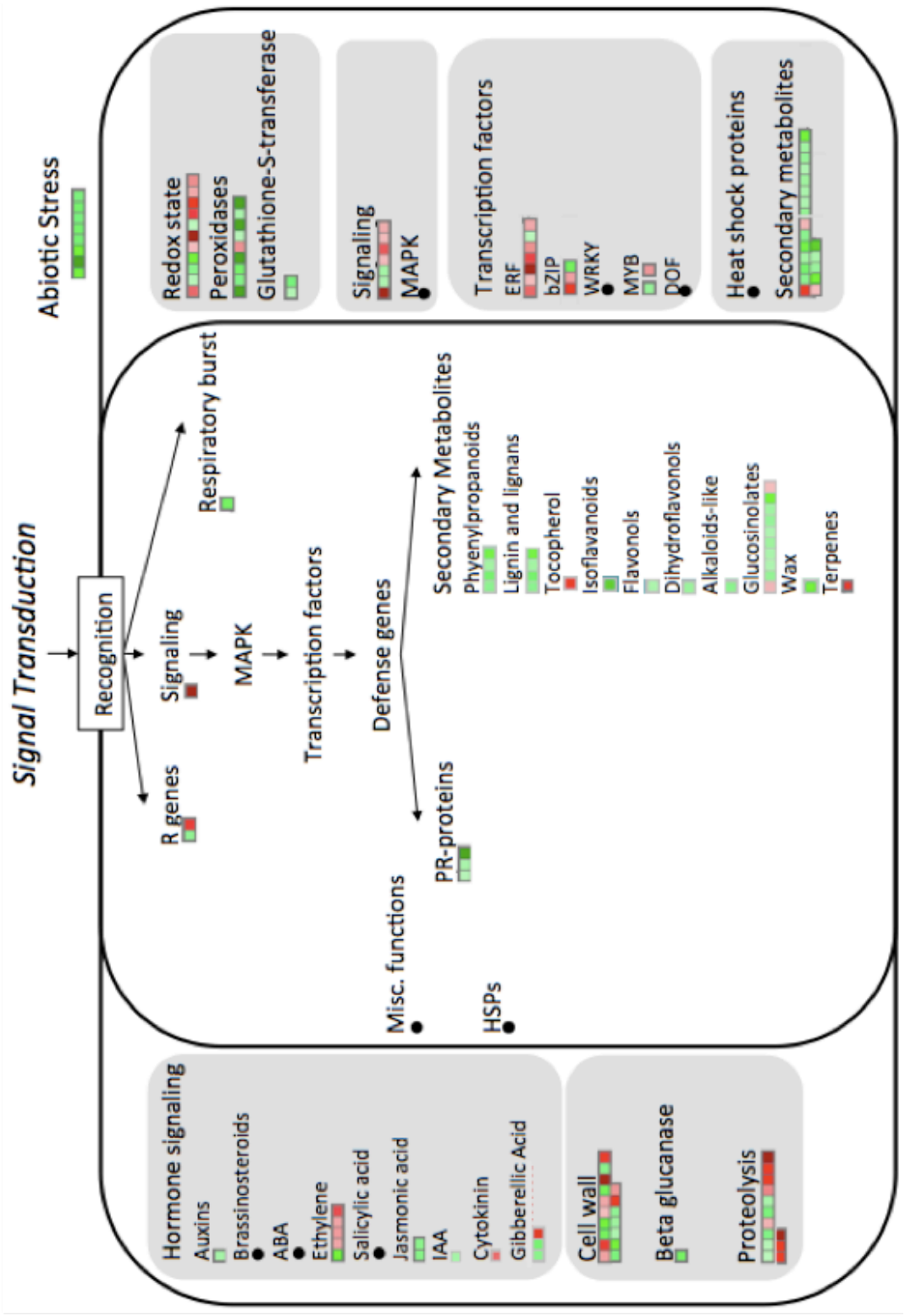
From the schematic generated for comparison of root and shoot cellular responses, several major differences can be identified between tissue types (Figure 15). Beginning with signaling, root cells show a much greater response in the activation of signaling processes including the mitogen-activated protein kinase (MAPK) cascade. In direct association, hormonal signaling involving SA and ABA were strongly induced to serve as mediators in defense activation (Figure 15). Ethylene (ET), auxin and indole-3-acetic acid (IAA) signaling were also highly induced in root cells (Figure 15).

On the other hand, hormonal signaling responses in indirectly affected tissues were opposite to those of directly affected tissues (Figure 10). In shoot tissues, JA and gibberellic acid (GA) signaling processes are activated (Figure 15). JA-mediated responses in shoot sites include cell wall re-organization, re-enforcement, and increased production of waxes and suberin.

Many major families of transcription factors including ethylene responsive factors (ERFs), basic leucine zipper domain (bZIP) containing proteins and WRKY factors are activated in directly affected roots, while primarily repressed in shoot tissues (Figures 15). Down-stream of these regulators in root tissues, heat shock proteins are highly induced in addition to proteolysis proteins and enzymes responsible for maintaining redox state upon *A. tumefaciens* C58 perception (Figure 15). However in shoot cells, we see almost an exact opposite. Instead in systemically affected cells, we see strong induction of secondary metabolite production and cell wall re-organization (Figure 15).


Agrobacterium tumefaciens C58
Abiotic Stress
Recognition
R genes
Signaling
MAPK
Transcription factors
Defense genes
Respiratory burst
Secondary Metabolites
Phenylpropanoids
Lignin and lignans
Tocopherol
Isoflavanoids
Flavonols
Dihydroflavonols
Alkaloids-like
Glucosinolates
Wax
Terpenes
Simple phenols
PR-proteins
Misc. functions
HSPs
Redox state
Peroxidases
Glutathione-S-transferase
Signaling
MAPK
Transcription factors
ERF
bZIP
WRKY
MYB
DOF
Heat shock proteins
Secondary metabolites
Hormone signaling
Auxins
Brassinosteroids
ABA
Ethylene
Salicylic acid
Jasmonic acid
IAA
Cytokinin
Gibberellic Acid
Cell wall
Beta glucanase
Proteolysis

A


Abiotic Stress
Signal Transduction
Recognition
R genes
Signaling
MAPK
Transcription factors
Defense genes
Respiratory burst
Secondary Metabolites
Phyenylpropanoids
Lignin and lignans
Tocopherol
Isoflavanoids
Flavonols
Dihydroflavonols
Alkaloids-like
Glucosinolates
Wax
Terpenes
PR-proteins
Misc. functions
HSPs
Redox state
Peroxidases
Glutathione-S-transferase
Signaling
MAPK
Transcription factors
ERF
bZIP
WRKY
MYB
DOF
Heat shock proteins
Secondary metabolites
Hormone signaling
Auxins
Brassinosteroids
ABA
Ethylene
Salicylic acid
Jasmonic acid
IAA
Cytokinin
Gibberellic Acid
Cell wall
Beta glucanase
Proteolysis

B

Figure 15. Schematic diagram using MapMan of cellular processes activated or repressed in *A. thaliana* Col-0 following 8 hours of hydroponic co-cultivation with *A. tumefaciens* C58.

A) Cellular responses of directly affected *A. thaliana* Col-0 root tissues; B) Cellular responses of systemically affected *A. thaliana* Col-0 shoot tissues. Colored scale indicates the level of induction/repression in response to *A. tumefaciens* C58 challenge. Each colored square signifies at least one transcript is differentially expressed by a factor corresponding to the associated color scale; red indicating transcript repression, green indicated transcript induction (Note: this is not indicative of the number of genes detected at the assigned level of expression). Different expression is shown to a maximum of 5-fold change in expression. Black dots indicate categories either expanded in external borders, within which genes were not differentially expressed or for which data was not shown (misc. functions).

3.2 Validating Microarray Data Sets

To validate the microarray data obtained from *A. thaliana* Col-0 root and shoot tissues, a variety of techniques were applied including principal component analysis (PCA) plotting, MA plotting, and reverse-transcription (RT) semi-quantitative PCR (qPCR).

3.2.1 PCA Plotting

Based on the segregation and grouping of the microarray duplicates conducted, the first principal component can be determined. To determine whether the first principal component is the biological treatment of inoculating versus mock-inoculating *A. thaliana* Col-0 with *A. tumefaciens* C58 in a hydroponic system, plots were generated for both shoot and root tissue microarrays. Co-segregation of treatments assayed in duplicate along the X-axis (first principal component) indicates inoculation with *A. tumefaciens* C58 as the primary factor for differential gene expression. In both corresponding root and shoot PCA plots, each mock-inoculated (red) and inoculated (blue) replicate co-segregates along the X-axis (Figure 16a and 16b respectively). This indicates the DEGs detected in shoot and root microarrays are primarily accounted for by treatment with *A. tumefaciens* C58.

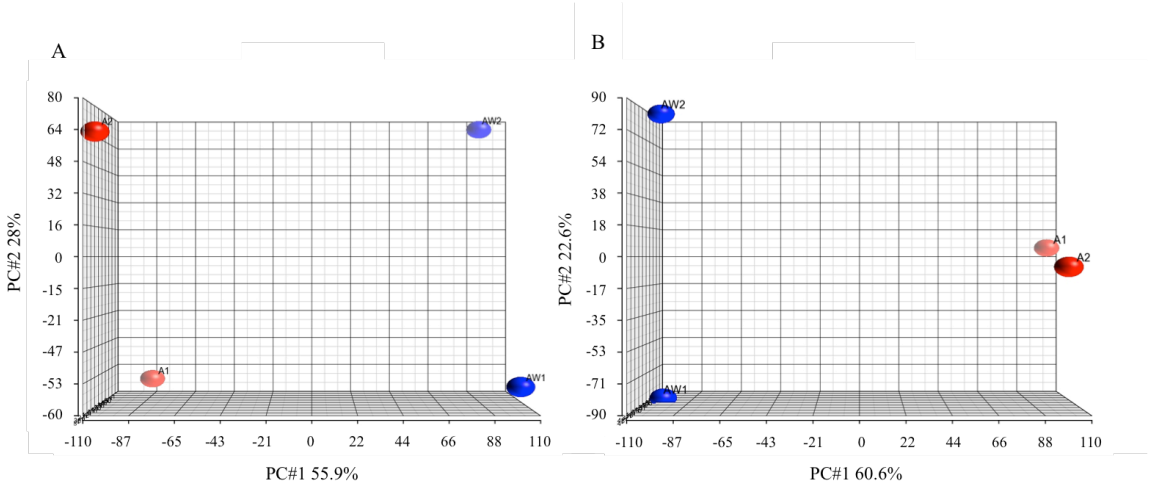


Figure 16. PCA plots for validation of microarray data.

A) PCA plot corresponding to microarray data generated from root RNA samples. B) PCA plot corresponding to microarray data generated from shoot RNA samples. Red indicates RNA samples obtained from mock-inoculated *A. thaliana* Col-0; blue indicates RNA samples obtained from *A. thaliana* Col-0 inoculated with *A. tumefaciens* C58.

3.2.2 MA Plotting

As a second measure of microarray data validation, MA plots were generated. Independently for root and shoot microarray data, MA plots were constructed to compare experimental replicates, compare mock-inoculated replicates, and compare between experimental and mock-inoculated replicates. Accordingly, the variance between experimental replicates was rather low, similar to that of MA plots between mock-inoculated replicates (Figure 17a,b and Figure 18a,b). MA plots assembled between mock-inoculated and experimental replicates, the variance increased by as much as four-fold (Figure 17c-f and Figure 18c-f). Variance within experimental replicates and control replicates is expected to be low since treatment conditions should be identical. However when comparing between experimental and control replicates, the variance is expected to rise due to the biological treatment (*A. tumefaciens* inoculation). Therefore based on the MA plots generated, the DEGs detected for root and shoot tissue were detected in response to *A.tumefaciens* C58 challenge.

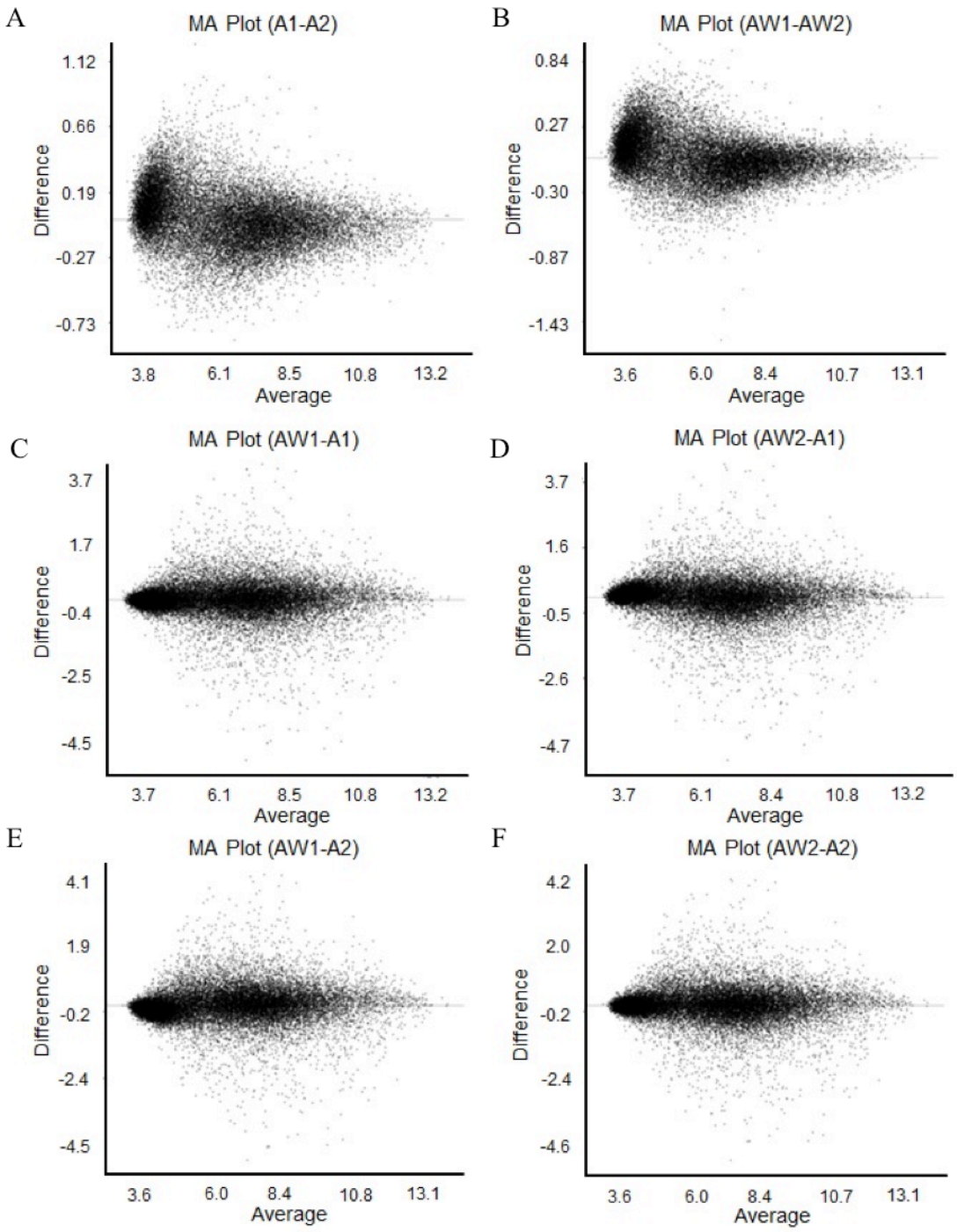


Figure 17. **MA Plots to show transcriptome scale difference in treatment-dependent gene expression for root samples.**

Expression of 22,500 loci in *A. thaliana* Col-0 was tested, of which 549 were differentially expressed. Expression values were plotted on a log scale, with $M = \log_2 R$ -

$\log_2$G differences in expression and A = 1/2 × ($\log_2$R + $\log_2$G) average expression where R = C600 and G = Rob. (A) MA plot of mock-inoculated sample group 1 (A1) and mock-inoculated sample group 2 (A2); (B) MA plot of inoculated sample group 1 (AW1) and inoculated sample group 2 (AW2); (C) MA plot of AW1 and A1; (D) MA plot of AW2 and A1; (E) MA plot of AW1 and A2; (F) MA plot of AW2 and A2. Variance within mock-inoculated microarray replicates is expected to be low since there is no difference in biological treatment. Variance within inoculated microarray replicates is expected to be low since there is no difference in biological treatment. Variance between inoculated and mock-inoculated experiments is expected to be much greater since there are differences in biological treatment. The biological treatment is the primary component responsible for differential gene expression.

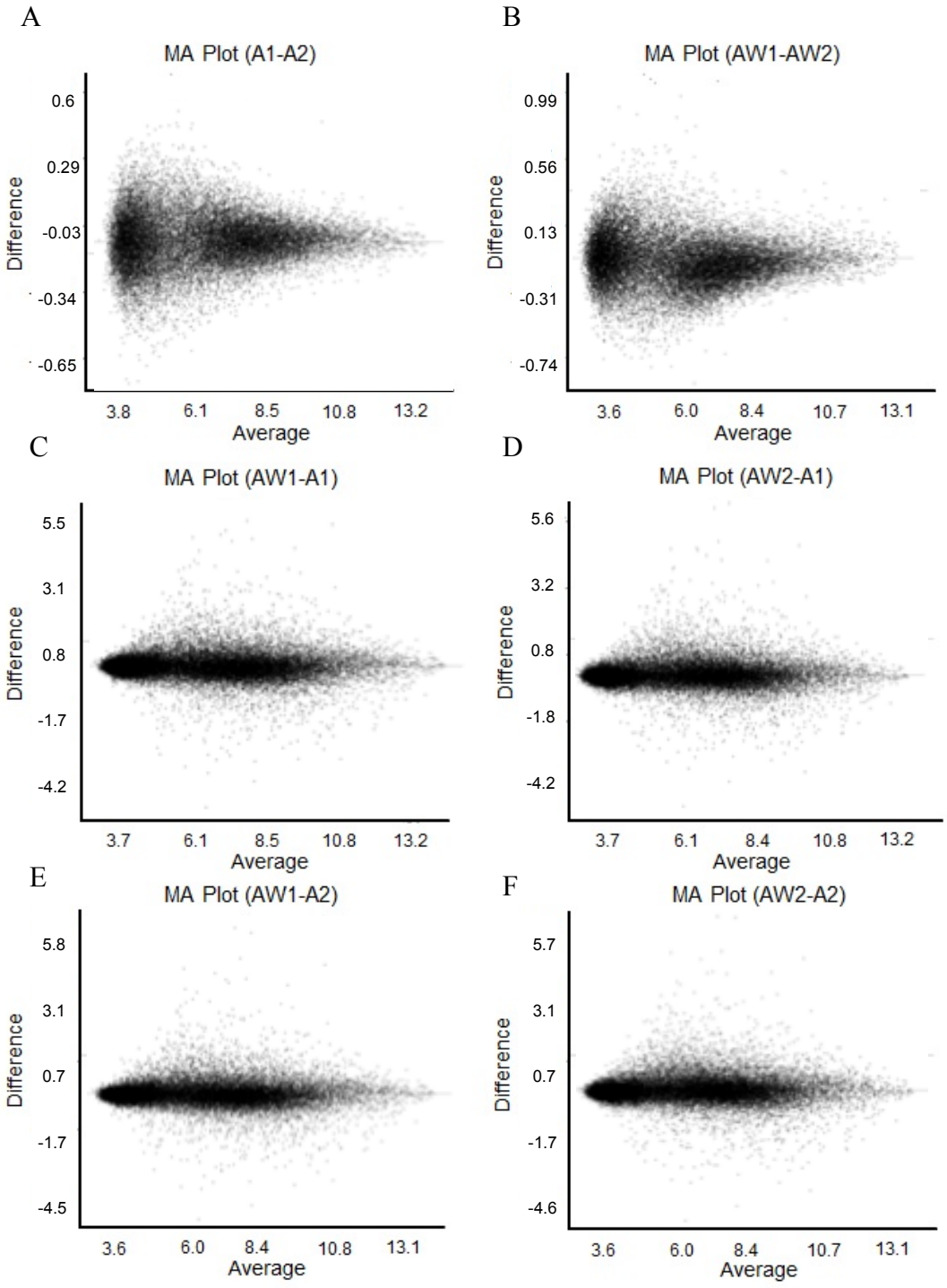


Figure 18. MA Plots to show transcriptome scale difference in treatment-dependent gene expression for shoot samples.

Expression of 22,500 loci in *A. thaliana* Col-0 was tested, of which 549 were differentially expressed. Expression values were plotted on a log scale, with $M = \log_2 R - \log_2 G$ differences in expression and $A = 1/2 \times (\log_2 R + \log_2 G)$ average expression where R = C600 and G = Rob. (A) MA plot of mock-inoculated sample group 1 (A1) and mock-inoculated sample group 2 (A2); (B) MA plot of inoculated sample group 1 (AW1) and inoculated sample group 2 (AW2); (C) MA plot of AW1 and A1; (D) MA plot of AW2 and A1; (E) MA plot of AW1 and A2; (F) MA plot of AW2 and A2. Variance within mock-inoculated microarray replicates is expected to be low since there is no difference in biological treatment. Variance within inoculated microarray replicates is expected to be low since there is no difference in biological treatment. Variance between inoculated and mock-inoculated experiments is expected to be much greater since there are differences in biological treatment. The biological treatment is the primary component responsible for differential gene expression.

3.2.3 Reverse-Transcription Semi-quantitative PCR

3.2.3.1 *A. thaliana* Col-0 Shoot Tissue

Five genes were chosen for RT-qPCR including three induced genes, *ATEXT*4, *LTP*3 and *LSU*1, and two repressed genes, *WCOR*413 and *ERF*012, using *ACTIN*II as a reference (Figure 19). *ATEXT*4, *LTP*3 and *LSU*1 were significantly induced, and *WCOR*413 and *ERF*012 were significantly repressed in response to *A. tumefaciens* C58 as determined by RT-qPCR (Figure 19). Melt curve analysis indicated the specificity of the qPCR target genes (Appendix 10-15).

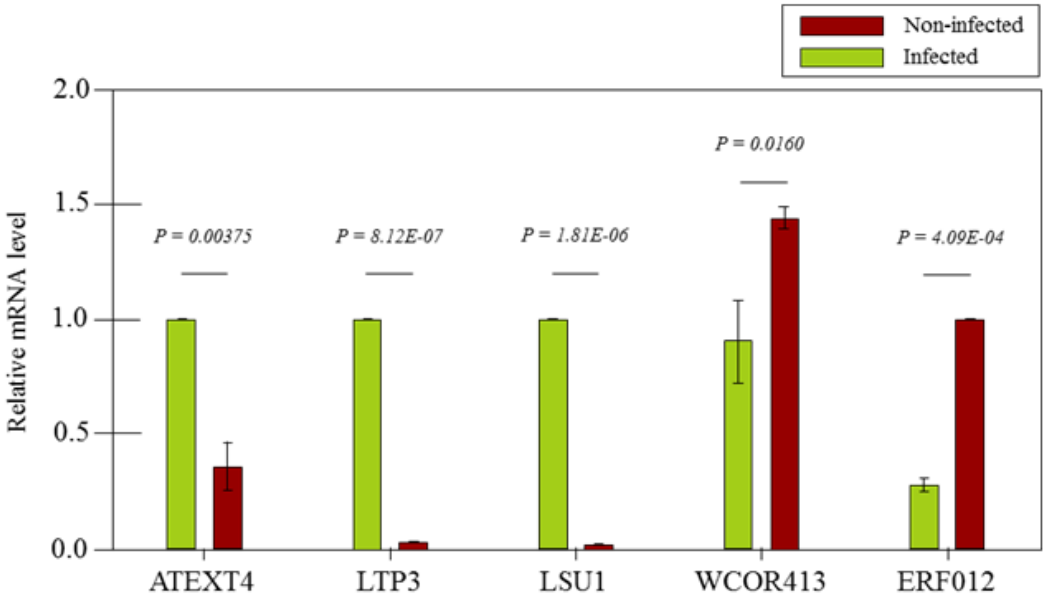


Figure 19. Validation of shoot microarray data by semi-quantitative RT-qPCR.

The mRNA level of two representative down-regulated genes (*WCOR*413 and *ERF*012 respectively) and three representative up-regulated genes (*ATEXT*4, *LTP*3, *LSU*1 respectively) identified from shoot *A. thaliana* Col-0 microarray data was further confirmed by qRT-PCR. The abundance of each mRNA transcript was normalized to that of *ACTIN*II transcripts. The data are representative of mean values of transcript abundance with SD values generated from three biological replicates.

3.2.3.2 *A. thaliana* Col-0 Root Tissue

Five differentially expressed (DE) genes were selected for RT-qPCR analysis. *HSP*21, *PGIP*1 and *ELIP*1 were chosen as representatives of induced genes, where as *AGP*1 and *NAS*1 were chosen from down-regulated genes respectively; *ACTIN*II was utilized as a reference (Figure 20). *HSP*21, *PGIP*1 and *ELIP*1 were significantly induced, and *AGP*1 and *NAS*1 were significantly repressed in response to *A. tumefaciens* C58 as determined by RT-qPCR (Figure 20). Melt curve analysis verified the specificity of the qPCR target genes (Appendix 16-21).

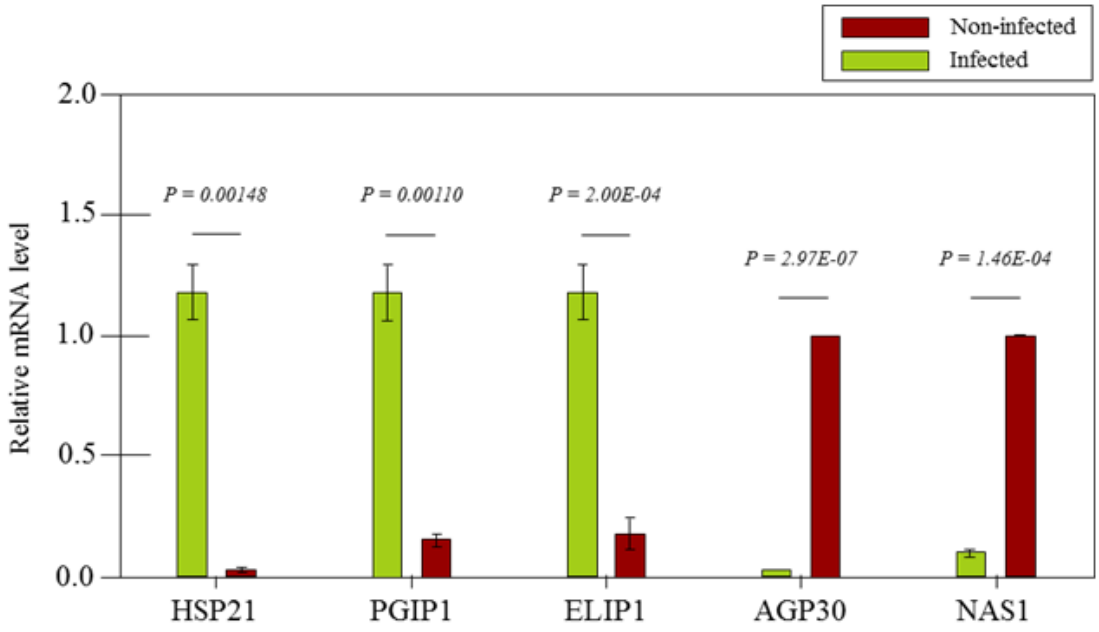


Figure 20. Validation of root microarray data by semi-quantitative RT-qPCR.

The mRNA level of two representative down-regulated genes, *AGP*30 and *NAS*1, and three representative up-regulated genes, *HSP*21, *PGIP*1 and *ELIP*1, found in the root microarray data was further confirmed by qRT-PCR. The abundance of each mRNA transcript was normalized to that of *ACTIN*II transcripts. Data represent means with SD values of three biological replicates.

3.3 *Agrobacterium tumefaciens* Root Attachment

To determine alterations in host susceptibility to irreversible *A. tumefaciens* C58 root attachment, confocal microscopy was utilized to visualize differences in the density of host root attachment. Using WT *A. thaliana* Col-0 as a reference, observational differences were determined for hosts with single-gene knockouts including *RLP*32 (At3G05650), PR6 proteinase inhibitor (At2G38870) and *TEM*1 (At1G22560). *A. tumefaciens* C58 modified to harbor a pJP2 plasmid with a pCherry expression vector were visualized with a helium-neon 543/594 laser and the obtained images were overlayed with white light images. The pJP2 plasmid is a highly stable, low copy number plasmid aiding to ensure plasmid retention to avoid the necessity of antibiotic application to the hydroponic system. Visualization of root attachment was conducted following forty-eight hours of hydroponic co-cultivation with *A. thaliana* Col-0.

A. thaliana Col-0 *rlp*32-/- show little to no observational differences in susceptibility to *A. tumefaciens* C58 root attachment in reference to WT *A. thaliana* Col-0 (Figure 21). Despite the strong induction of PR-6 proteinase inhibitor, *A. thaliana* Col-0 deficient for PR-6 proteinase inhibitor (At2G38870) showed reduced observable levels of *A. tumefaciens* C58 root attachment (Figure 21), contradictory to any suggestive role by microarray analysis in the prevention of phytopathogen infection. Finally, *A. thaliana* Col-0 *tem*1-/- mutants show increased levels of *A. tumefaciens* C58 root attachment (Figure 21).

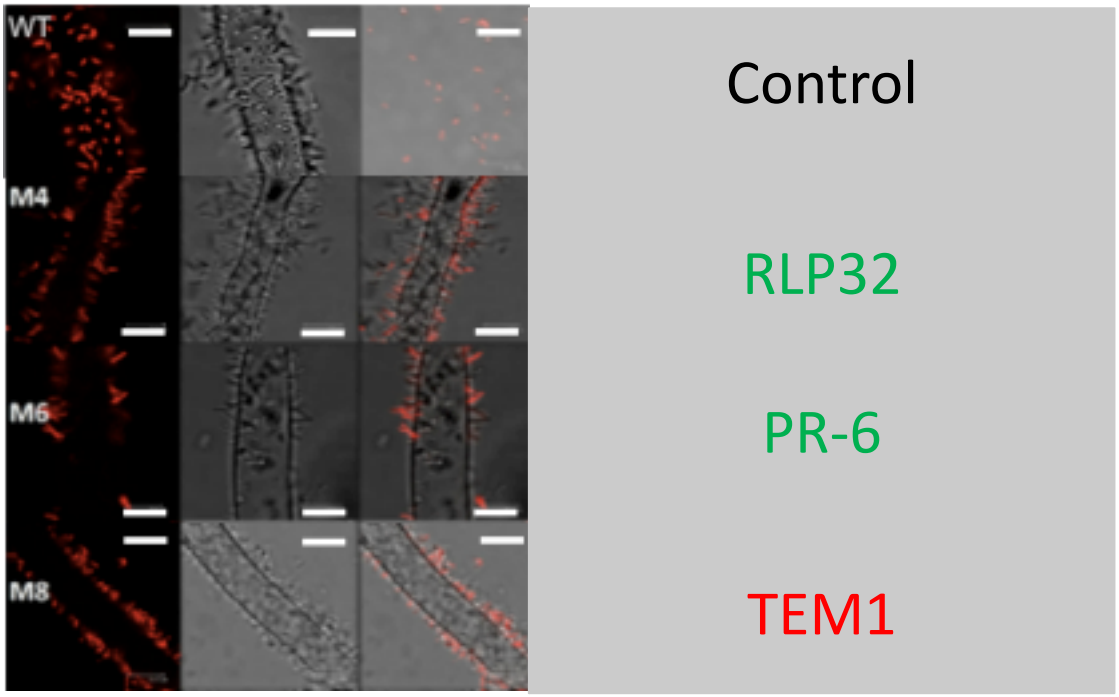


Figure 21. **Compound laser scanning confocal microscopy for visualization of *A. tumefaciens* C58 attachment to *A. thaliana* Col-0 root structures.**

Left panel: fluorescent visualization of *A. tumefaciens* C58 electroporated with a pJP2 plasmid to express mCherry. Middle panel: light microscopy of *A. thaliana* Col-0 root structures and *A. tumefaciens* C58. Right panel: overlay of laser/light microscopic images. All fluorescent images utilized a helium-neon 543/594 laser for mCherry excitation. White solid bars indicate a scale of 5μm.

3.4 Variation in Secretome Profile

To determine whether there are differences in the secretome profile of the various *A. thalaina* Col-0 T lines, liquid chromatography-mass spectrometry (LC-MS) analysis of media components was performed. Following hydroponic co-cultivation of *A. thaliana* Col-0 either mock-inoculated or inoculated with *A. tumefaciens* C58 for confocal microscopy, the liquid Murashige-Skoog (MS) media utilized to sustain the system was isolated and filter sterilized. From inoculated samples, the resultant microbe-free media contained metabolites of *A. tumefaciens*, in addition to plant root released compounds. Mock-inoculated samples do not contain *A. tumefaciens* C58 and therefore do not contain bacterium-associated metabolites. LC-MS analysis inferred differences between each of the *A. thaliana* Col-0 lines (Figure 22). The heat map generated describes 150 molecular features extracted from the LC-MS data using MZmine software. Interestingly despite genetic differences, WT and mutant *A. thaliana* Col-0 line secretome profiles cluster together more closely dependent on challenge with *A. tumefaciens* C58 (Figure 22). There are particular compounds that appear to be completely absent in unchallenged *A. thaliana* Col-0 lines in comparison to challenged lines, shown by strong red coloration (Figure 22). In addition, lower detection of certain compounds (near the top of the heat map) upon pathogen challenge were observed in WT lines, while the same compounds show enhanced detection in mutant lines (Figure 22). Among these same compounds, PR6 mutant lines challenged by *A. tumefaciens* C58 have the greatest enhancement of these compounds. For various molecular features, *TEM*1 lines in particular seems to have many unique features where compound detection is much lower than WT, *RLP*32, and PR6 lines (Figure 22). Finding and identifying compounds that have unique secretion

profiles among *TEM*1 and *PR*6 knockouts may aid in explaining differential levels of root attachment by *A. tumefaciens* C58, as identified by confocal microscopy. Although LC-MS serves as a primary screen for differences in secretome profile, the compounds that are being differentially secreted cannot be determined or quantified without use of further analysis. The data generated is inferred from gene expression data or is a supposition that all of the molecular features measured by LC-MS are secondary metabolites.

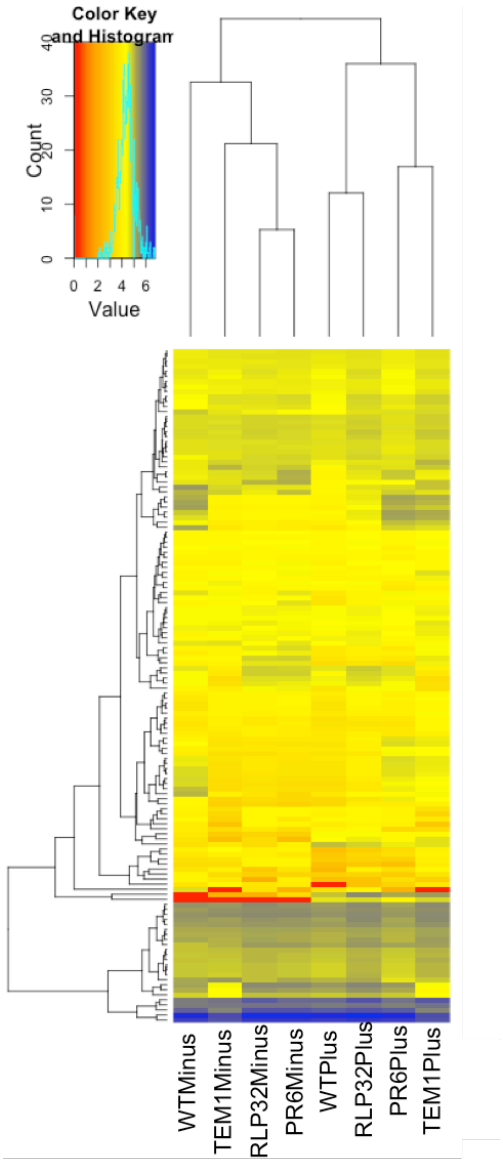


Figure 22. Liquid chromatography analysis of *A. thalaiana* Col-0 secretome profiles.

Mutant *A. thaliana* Col-0 secretome profiles generated by liquid chromatography in reference to wild-type *A. thaliana* Col-0. Secretome profiles were analyzed following 3 days of root secretion collection in a hydroponic system challenged with *A. tumefaciens* C58. All *A. thaliana* Col-0 lines analyzed are 24 days old. Molecular features were extracted from the data using MZmine (2.14), aligned and averaged across replicates. The heat map was generated from log-transformed area values using R. Sample number

corresponds to the following gene knockouts: 4) *RLP*32; 6) PR6 Proteinase Inhibitor; 8) *TEM*1.

3.5 *Agrobacterium tumefaciens* Virulence Induction

To confirm *A. tumefaciens* C58 virulence induction without AS supplementation in the hydroponic system, relative changes in the expression of hallmark virulence genes were tested in the presence and absence of *A. thaliana* Col-0. *Agrobacterium tumefaciens* has been shown to detect compatible hosts via membrane proteins, *vir*A, *chv*I and *chv*E, by perception of plant phenolic, acidic and sugar compounds, respectively[70-76]. Detection of these compounds by *A. tumefaciens* activates a virulence program for plant cell surface attachment and subsequent induction of virulence genes for mobilization of transfer-DNA (T-DNA). By differential detection of *A. tumefaciens* virulence genes, it can be confirmed that no artificial supplementation of virulence inducing compounds is required to establish a plant-pathogen interaction in our co-cultivation system. Differential gene expression of six select virulence genes was confirmed using RT-qPCR (Figure 23). Among the six virulence genes tested, the transcript abundance of all genes were increased in the event *A. tumefaciens* C58 was co-cultivated with *A. thaliana* Col-0 in reference to mock-co-cultivated *A. tumefaciens* C58 (Figure 23). Melt curve analysis verified single products were being amplified (Appendix 22-28). Taken together, this indicated that the virulence program of *A. tumefaciens* C58 is activated in the hydroponic co-cultivation system without additional chemical supplementation.

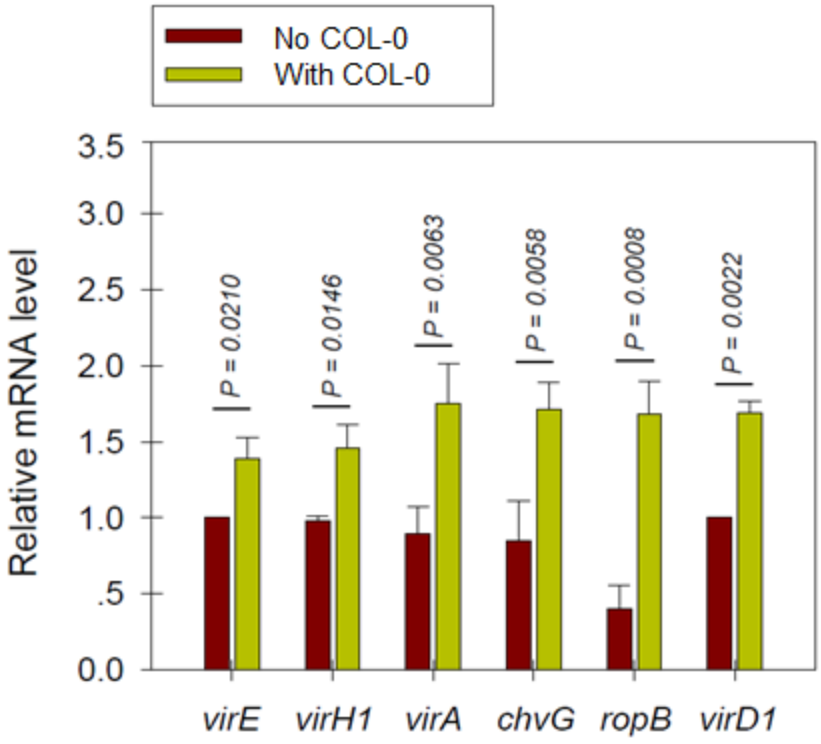


Figure 23. Activation of *Agrobacterium* virulence in the hydroponic co-cultivation system.

The mRNA levels of six *Agrobacterium* virulence factors was quantitatively measured by qRT-PCR in the co-cultivation system containing *A. tumefaciens* C58 and *A. thaliana* Col-0, and in a control in which only *Agrobacterium* was added. The abundance of virulence factor transcripts was normalized to that of 16S rRNA transcripts. Data represents mean values with SD generated from three biological replicates.

3.6 *Agrobacterium tumefaciens* Growth Curve

To determined *A. tumefaciens* C58 growth performance in the hydroponic system containing liquid MS, growth curve analysis was conducted. Using a spectrophotometer adjusted to a cell count factor of $5x10^9$ cells/mL (OD_{600}=1.0) specific for *A. tumefaciens* C58, growth curves were run beginning with an initial OD_{600} value of 0.1 in triplicate. Based on the data generated, the OD_{600} values were measured for up to 48 hours in 4-hour intervals with no detection of a stationary phase (Figure 24). Instead, linear growth was detected up until the final reading. There conceivably may be a challenge in establishing a typical growth curve of *A. tumefaciens* C58 in the co-cultivation system since it is a two-component living system, however it will provide valuable insight into the affects co-cultivation plays in bacterial growth.

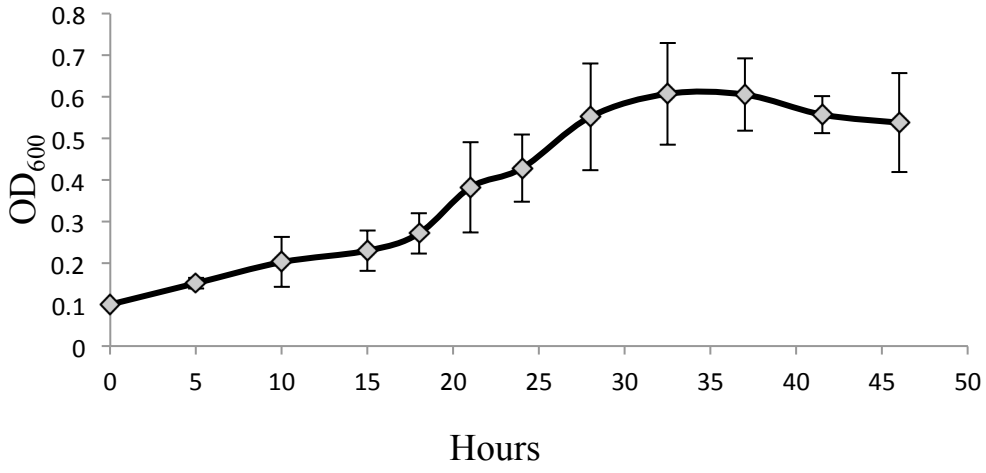


Figure 24. Growth curve analysis for *A. tumefaciens* C58 using hydroponic co-cultivation based techniques.

OD_{600} readings were calculated for 56 hours in 4-hour intervals with a final reading 84 hours post inoculation. OD_{600} values for *A. tumefaciens* C58 without a host with an initial OD_{600} of 0.1.

3.7 Detecting Novel Expression Patterns

While recording the functional annotation and expression patterns of DEGs detected in both root and shoot tissues, a small subset of genes found had not been previously detected in their corresponding tissue sites. From systemically affected tissues sites, three DEGs had not been previously detected in shoot tissues (Appendix 29). On the other hand, 58 DEGs were detected in our root transcriptome that have not been previously identified for expression in root tissues (Appendix 30).

Chapter 4 – Discussion

4.1 Detection of Plant Defenses

Plant responses to pathogen challenge have been studied extensively over the past few decades. Previous reports have uncovered significant information on plant defense mechanisms including the intricate interconnected signaling network of plant phytohormones that activate and mediate the various plant defense programs[168]. Despite these findings, only a small proportion of this research has been translated into application in agriculture. Conceptually, plant cell suspension and site-specific wounding for inoculation techniques, utilized to mimic plant-microbe interaction, lack similarity with conditions *in planta*[139-144]. By comparison of host responses detected using hydroponics versus those of conventional systems, it is clear that the greater the dissimilarity in experimental set-up, the greater the difference in detectable host responses (Appendix 3 and 7). Using conventional systems, not only are there physiological shortfalls, but establishing direct contact at the appropriate host site is also a challenge. Agro-economically, researchers are most highly interested in plant-microbe associations occurring in the soil since the rhizosphere is the most microbe dense interactive interface[169-171]. Accordingly, studying plant-microbe interactions using plant cell suspension or at shoot host sites is inaccurate. Not only are the majority of plant-microbe associations restricted to the root structures, but differences in the spatial expression of host genes also influences the genes detectable in response to pathogen challenge and their abundance. To resolve challenges associated with plant-microbe interactions occurring in the rhizosphere, the roots structure must be the target of the study conducted.

Examination of root responses has been a predominant challenge for subterranean plant-microbe interactions due to limited access to models with natural root structure and tightly controllable experimental conditions. The importance of root-associative studies extends beyond studying the most interactive region of plants, but also opens the possibility to manipulate a more "stable" interface, the rhizosphere, to improve plant productivity[172]. The challenges faced by attempting to study plant-microbe interaction compelled the development of an experimental system more closely mimicking natural plant-microbe interaction for the dissection of complex plant-microbe signaling and responsive pathways.

4.1.1 Plant Defense in Locally Affected Sites

Hydroponic co-cultivation facilitates plant-microbe associations that are tightly controlled at host root structures, serving as the locally or directly affected tissue site. Using a GeneChip Arabidopsis ATH1 Genome array, *A. thaliana* Col-0 transcriptomic changes were detected in directly affected sites following 8 hours of hydroponic co-cultivation with *A. tumefaciens* C58. To organize and better understand the transcriptomic changes occurring in host tissues, MapMan was utilized to generate an illustrative schematic of *A. thaliana* Col-0 responses to *A. tumefaciens* C58.

4.1.1.1 Host Root Perception and Defense

As a successful plant pathogen, *A. tumefaciens* evades host perception by plant cell wall localized receptors[84-85]. Mechanistically, *Agrobacterium* are only perceivable by plants once avr protein, vir protein and T-DNA have been injected into a target host cell,

detected by cytoplasm localized NB-LRR receptor-like proteins[173]. Based on these previous findings, host defenses must rely on the initiation of the more robust ETI.

Salicylic acid (SA) is universally accepted as the major player in regulating host defenses against biotrophic and hemibiotrophic pathogens[174]. However, the mechanism by which SA is able to mediate plant defenses is not yet fully understood. Primarily SA serves as the master regulator of systemic acquired resistance (SAR), conferring resistance to a variety of plant pathogens[174,175]. The association between plant defenses to pathogens and SA was reported by the increased susceptibility of SA defective *A. thaliana sid2-2* mutants[176]. For transcriptional changes to occur in an affected host cell, massive reprogramming is required and is dependent on the SA responsive transcription co-factor *Non-Expressor of Pathogenesis-Related*1 (*NRP*1)[177]. The inducible activity of *NPR*1 by SA not only regulates expression patterns linked to SA accumulation, but also influences a wide variety of other signaling networks regulated by alternate phytohormones. In direct association with SA accumulation in the cell, a redox potential is created facilitating the transport of *NPR*1 into the nucleus to repress the activity of bZIP TGA factors that hinder the expression of PR1 proteins[177-178]. Interestingly, no PR1 genes were induced in response to *A. tumefaciens* challenge in our hydroponic system (Appendix 5). Analyzing *A. thaliana* Col-0 responses 8 hpi may not have provided enough time for all SA-mediated defenses to become fully mounted and hence may explain why no altered expression of PR1 genes was detected.

Signaling cross-talk among various phytohormones ultimately fine tunes host responses best suited to the challenging pathogen. Accordingly, SA does not function independently. The antagonistic nature of SA and JA mediated responses is widely

documented, and *NPR*1 in fact is the modulator of this incompatible nature between defenses regulated by SA and JA[121,133,177,179]. JA has been proposed to have a role in the activation of SAR, as has been shown by the accumulation of JA in response to P. *syringae* pv. *tomato* (*Pst*) and by the application of exogenous JA[180]. However, JA-biosynthesis and –signaling defective mutants have been shown to be intact in SAR[181], and our results corroborate that finding (Figure 15a). Synonymously to PR1, which serves as a hallmark SA-inducible gene, *PDF*1.2 indicates the activity of JA-mediated defenses[182]. Induced expression of *PDF*1.2 was not detected in response to *A. tumefaciens* C58 in root tissues and consequently the downstream pathways associated with JA were not induced including development, secondary metabolite production, senescence, and wound response (Appendix 5; Figure 15a). Wounding response would be manifested in the expression of cell wall-related genes for the restructuring, re-enforcing and repair of damaged cell wall structures, which were also not induced in our transcriptome data (Figure 15a).

Though the induction of SA-mediated defenses and SA-mediated antagonism of JA associated processes is clear in our root transcriptome data, other phytohormonal responses were also affected by pathogen including induction of auxin, ABA and ethylene (ET) –mediated processes. Typically when analyzing plant defense, SA, JA and ET are the first hormones to be considered, however more recently roles for ABA, gibberellins and auxin have also been identified. Similar to JA, ET is typically associated with defenses against necrotrophic pathogens and insects, more commonly working hand-in-hand with JA[183]. However ET has significant roles in the plant-*Agrobacterium* interaction as well. ET is crucial for crown gal development and facilitates crown gal

growth by stimulating vascularization of plant tissue[184]. By contrast, increased levels of ethylene inhibit crown gal development if ethylene (ET) accumulates in the cell prior to T-DNA transfer[185]. By supply of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ET, T-DNA transfer can be inhibited in tomatoes and melons[185]. If ET induction is disrupted in the initial phases of co-cultivation with *A. tumefaciens*, transformation efficiency can be significantly enhanced in melons, cauliflowers, apricots, apple trees and bottle gourds. Together these previous findings suggest that the induction of ET-related processes is a host defensive mechanism to avoid transformation by *A. tumefaciens* C58 challenge (Figure 15a). Auxin also possesses its own place in the SA-JA/ET antagonism model, as a negative regulator of SA-mediated responses and auxin deficiencies resulting in increased susceptibility to necrotrophic fungi[186]. In fact, *A. thaliana* defective in disease resistance gene *RPS*2, responsible for detecting pathogen effectors and accumulating SA in the cell, have increased production of auxin[187]. However when challenged with bacteria, SA and the auxin IAA appear to have a synergistic effect, either working together to facilitate host association with beneficial microbes or deterring incompatible associations. Recent research suggests IAA has a role in plant defense against biotrophic pathogens, however the mechanism of action is yet to be elucidated[188-189]. IAA may have a direct impact of plant defense gene regulation, may play a role in fine-tuning the cross talk between JA/ET-SA pathways, or fine-tuning other phytohormones with secondary roles in plant defense such as ABA. ABA has been extensively shown to have some role in plant defense against biotrophic pathogens, both by increased susceptibility to crown gal development in ABA-insensitive mutants and accumulation resulting from exogenous application of ET[190-191]. The increased

susceptibility to pathogens by accumulation of ABA is contradictory to the induction of ABA-mediated processes in *A. thaliana* in response to *A. tumefaciens* C58 following 8 hpi (Figure 15a). Induction of ABA-mediated processes and the positive correlation between ABA and ET may be linked to their synergistic role in the promotion of crown gall development, which may also be preventative of crown gall prior to T-DNA introduction. Holistically in root tissues, *A. thaliana* Col-0 responses to hydroponic co-cultivation with *A. tumefaciens* C58 reveal a reactive response in which the host is responding directly to the challenging pathogen by induction of proteolysis proteins to degrade avr/vir proteins and increase the expression of SA-mediated responses, while repressing defensive responses associated with necrotrophic or herbivory damage; typical features of plant defense against phytopathogenic bacteria.

4.1.2 Transduced Plant Defense in Distally Affected Sites

In indirectly affected or distally affected sites (shoot tissues) the defense program activated is very different from that initiated in root tissues. Although analysis of GEO functional categories reveals great similarity in the biological processes affected in root and shoot tissues, the genes affected within these biological processes are very different. Only 52 transcripts were detected to be differentially expressed in both root and shoot tissues, suggesting that although similar processes were affected, very different transcriptome profiles were being generated in response to *A. tumefaciens*.

The mechanisms through which directly affected cells are able to perceive pathogen challenge and transduce a downstream signal to distal tissues remains elusive. Although the understanding of cell-to-cell communication *in planta* is still in its infancy, analysis of intracellular communication has revealed significant roles of lipid transport

mechanisms in communication between organelles, and in addition has offered some insight into the roles lipid transport proteins (LTPs) play in intercellular communication[192]. Across a variety of plant species, LTPs have been identified as having roles in signaling reproductive development, pathogen defense and abiotic stress response[193-194]. From our shoot transcriptome data, more than twenty LTPs were differentially expressed, all of which were induced (Appendix 1). The induction of LTPs was not found in root tissues (Appendix 5). The strong induction of these LTPs in systemic tissues corroborates the suggestion that LTPs facilitate a mechanism through which cell-to-cell communication is possible, although many others may be yet to be identified.

Upon perception of potential infection at a distal host site, systemically affected tissues mount defensive responses quite different than locally affected tissues. Beginning with phytohormonal changes, genes associated with JA-mediated responses were most highly induced, followed by auxin and gibberellic acid (GA) mediated responses (Figure 15b). In distally affected tissues, the induced responses mimic those activated by wounding damage[159-162]. JA is the master regulator of the transcriptomic changes occurring in shoot tissues. Generally, JA-mediated signaling pathways are implicated in regulating anti-herbivore defenses to deter insect feeding. Upon host perception of damage associated molecular patterns (DAMPs), a HR-like response is initiated by accumulation of reactive oxygen species (ROS) reaching maximal levels of superoxide production seven minutes after wounding, with four to six hours required for maximal hydrogen peroxide production[195]. In response to increased ROS production, JA is converted into its active form, JA-Ile, to activate the production of a variety of secondary metabolites, up-

regulation of cell wall biosynthesis proteins and induction of PR proteins[196-197]. Similarly, the responses detected in shoot tissues resembles those responses normally detected in tissues perceiving wounding damage. There is strong induction of a variety of genes involved in secondary metabolite biosynthesis, cell wall regulating genes and PR5 proteins (Figure 15b; Appendix 5). The PR proteins activated belong to the PR5 thaumitin-like gene family, which are effective against necrotizing pathogens and herbivorous insects[198]. In addition to PR protein expression, a variety of cell wall biosynthesis, reorganizing and re-enforcing genes were highly induced (Figure 15b, Appendix 5). Although systemically affected cells were not damaged, the induction of cell wall related genes suggests that tissues that are yet to be affected are pre-emptively strengthening their cellular structure to increase the difficulty in compromising their cell wall, while also increasing the expression of PR genes in the event the cellular structure is compromised to rapidly deter additional herbivory or necrotic damage. Among the secondary metabolite production pathways induced based on our transcriptomic data, some act to aid in strengthening cellular structure, while others serve to deter pathogens and insects before a direct point of contact is established as secreted compounds (Figure 15b). For example, waxes are a crucial component of cuticle, which serves as a protective layer of epidermal cells[199], while lignins, poor carbon sources due to their structure complexity, are a difficult component of the plant structure to metabolize and break down, thus increasing the structural complexity of the host[200]. Additionally, increased production of phenylpropoanoids, flavonols and glucosinolates were also highly detected (Figure 15b). These compounds have been previously shown to have significant deterrent properties against a variety of herbivorous insects. Indole glucosinolates (IGs) have been

shown to be extremely successful in deterring feeding and oviposition[201], whereas the reduction of phenylpropanoid and flavonol production has been shown to increase host susceptibility to pathogens[202-203]. Though JA primarily mediates these responses, JA also operates by cross-communication to fine tune host responses.

JA and ABA have been widely documented to mediate wound-activated gene expression[190-191]. However, ABA signaling was shown to be unaffected in shoot tissues. It is possible that JA and ABA work synonymously in the event of wounding damage, however since no wounding is occurring at systemically affected sites, ABA remains unchanged and JA works in isolation to induce wound-related defenses. Interestingly, auxins have been previously identified to have negative effects on wound-induced gene expression. Endogenous IAA accumulation is shown to decrease in wounded tobacco (*Nicotiana tabacum*), and the delay of recovery in IAA accumulation serves as a proposed mechanisms to create a limit to the duration of responses associated with wounding[204]. In shoot tissues, IAA was induced in conjunction with JA (Figure 15b). Although the simultaneous induction of JA and IAA for plant defense has not been previously described, it is clear that secondary phytohormones may be induced or repressed in an activation depend manner to fine-tune plant responses to biotic and abiotic stimuli.

Gibberellic acid (GA) in its active form is a potent regulator of plant growth. GA serves as an effective seed dormancy breaker, increases crop growth and yield, enhances photosynthesis and plant metabolism by production of bigger leaves, and can aid plants suffering nutrient and growth deficiency[205]. The interaction between GA and JA is not understood, however the induction of GA mediated responses is likely to be associated

with accelerated host reproductive growth in response to stress perception. Evolutionarily, plants have developed mechanisms to accelerate reproductive development in the event host fitness is compromised, in order to avoid senescence without the production of offspring[206]. Thus, although the increased production of GA may not play a direct role in plant defense, it is possible that while JA induces plant defense programs, phytohormonal cross-talk also activates GA-mediated responses to accelerate reproductive development in case the inducible plant defense program is ineffective in preventing the spread of necrosis, infection or feeding damage.

In comparison to *A. thaliana* Col-0 root tissues that are directly affected by *A. tumefaciens* C58 challenge, shoot tissues initiate a plant defense program that is proactive to deter direct contact with surrounding microbes and insects. In the event direct contact is established, the plant structure is more difficult to get through and premature expression of cytoplasm-localized plant defense proteins serves to combat feeding and herbivory damage much more rapidly. Therefore these distally affected tissues may offer mechanisms that may benefit in further developing proactive approaches to avoid host susceptibility to pathogens and insects, versus studying responses of directly affected sites which are not only responsive after challenge, but are likely to be fine-tuned dependent on the challenging pathogen or insect.

4.2 Identification of Novel Plant Defense Genes

Three candidate genes were selected from the transcriptomic changes detected in shoot tissues to potentially uncover use as targets to proactively improve host fitness and decrease susceptibility.

4.2.1 *RLP*32

Receptor-like proteins (RLPs), implicated primarily in defense and plant growth regulation, are functionally and structurally similar to receptor-like kinases (RLKs)[207]. RLPs associated with plant growth are under relatively high selective pressure in comparison to those associated with defense, which posses significant sequence divergence among various plant genera[208]. A NCBI sequence BLAST[209] of *RLP*32 revealed significant sequence divergence from that of alternative plant genus'. Based on sequence divergence, a knockout of *RLP*32 may result in a loss of inducible plant defense regulation causing constitutive expression of defense related genes directly linked to *RLP*32 regulation. However, *A. tumefaciens* C58 showed little observational differences in root attachment (Figure 21) on *RLP*32 knockdown plants. Despite detectable differences in secretion profile by LC-MS analysis (Figure 22), the compounds differentially detected may not influence chemotaxis of *A. tumefaicnes* C58 to root structures. *RLP*32 knockout strains did not have any unique detectable molecular features and among those molecular features that were unique to all mutant strains in comparison to the WT strain, *RLP*32 mutants had the most moderate profiles in terms of alterations to compound detection (Figure 22). Although the elimination of *RLP*32 did not manifest in increased *A. thaliana* Col-0 susceptibility to *A. tumefaciens* C58 irreversible root attachment, it is possible that *RLP*32 functions solely within the host cytoplasm to regulate the expression of defense proteins. Consequently, *RLP*32 would not have any influence on secondary metabolite production; a factor likely required in regulating plant-microbe association in the host extracellular environment. To determine if *RLP*32 has

some direct role in intracellular defenses, it would be worth determining if *A. thaliana rpl*32 -/- lines have observational differences in crown gal development.

4.2.2 PR-6 Proteinase Inhibitor

Pathogenesis-related (PR) proteins are inducible by effector-triggered immunity (ETI) and are effective antimicrobial, plant priming, plant structure re-enforcing and virulence inactivating proteins[210-211]. Interestingly, although PR-6 family proteins are proteinase inhibitors that antagonize plant infection by pathogenic fungi[212], they are also strongly induced in response to *A. tumefaciens* C58 eight hpi (Appendix 1) and the elimination of PR-6 expression reduced *A. tumefaciens* C58 attachment (Figure 21). Perhaps the comparably strong induction of a PR-6 proteinase inhibitor may not have been the result of activated defenses specifically targeting pathogenic bacteria, but instead was induced as a result of pathway crosstalk in systemically affected tissues. The activation of PR-6 proteins in systemic tissues by pathogen challenge suggests a preventative measure to decrease susceptibility of aerial regions of the host in the event of secondary infection/infestation. It is unclear why irreversible root attachment of *A. tumefaciens* C58 was reduced, but this result seems to suggest alterations in host susceptibility to pathogen virulence. Though there were detectable differences in secretome profile in PR6 knockouts, it is unclear how the elimination of a PR protein would influence secondary metabolite biosynthesis and secretion. Instead, elimination of a PR6 protein inhibitor is likely to have an effect on influencing intracellular susceptibility. Though based on LC-MS analysis, it is clear that those molecular features unique to all mutant lines in comparison to WT showed the greatest enhancement in PR6 protein knockouts (Figure 22). The reduction in irreversible attachment may be due to the increased production of

compounds that are antagonistic to pathogen challenge. Identifying the compounds differentially secreted in PR6 mutants may offer deeper insight to explain reduced pathogen attachment (Figure 22).

4.2.3 *TEM*1

Tempranillo (*TEM*) gene expression is directly correlated with the expression of genes regulating flowering and biosynthesis of GA[213]. Overexpression of *TEM* genes results in plants resembling GA-deficient mutants, and conversely, down-regulation results in increases in GA content[213]. GA is a plant growth-promoting hormone that plays important roles in diverse aspects of plant growth and development, such as stem elongation[214]. In addition, GA has been shown to crosstalk with JA, mostly documented by the antagonistic affects of GA on JA. Where GA is activated, JA mediated processes are down regulated[215]. By analysis of *TEM1* mutants, GA levels may be increased and subsequently influence JA mediated defenses that have variable roles in plant defense, including production of secondary metabolites for secretion from the root structures. It is possible that there is reduction in root secretable compounds, regulated by JA, possessing antimicrobial properties, and the reduction in secretion is accounting for increased levels of irreversible *A. tumefaciens* C58 root attachment (Figure 21). Based on LC-MS analysis, there were a variety of molecular features that showed reductions in detection in comparison to *RLP*32 and PR6 protein mutant strains upon pathogen challenge (Figure 22). The reduction in the production of these particular compounds may explain why there was such a high level of irreversible *A. tumefaciens* C58 root attachment. The enhanced production of these same compounds and the lower levels of *A. tumefaciens* C58 root attachment to PR6 protein mutants, suggests increased root attachment in *TEM*1

mutants is due to lower levels of production of these compounds (Figure 22). Determining the compounds differentially produced and secreted in *TEM*1 knockouts may offer deeper insight into secreted metabolites that may be interesting to study for affects on *A. tumefaciens* C58 chemotaxis (Figure 22).

4.3 Efficacy of Hydroponics

Though conceptually hydroponic co-cultivation more closely represents conditions *in planta*, more work is required in uncovering the roles these detectable genes have in plant defense and their potential application as candidate targets to corroborate the suggested superior utility of hydroponics to study *Agrobacteria-Arabidopsis* interaction. However by comparing the transcriptomic profiles of shoot and root tissues to previously detected *A. thaliana* transcriptomic changes in response to *A. tumefaciens* using conventional models, the overlap in detectable transcripts is suggestive of the power of using hydroponic co-cultivation. When comparing the responses of root tissues, there is greater overlap with transcriptomic responses detected using site-specific wounding based inoculation techniques (Appendix 3 and 7). This suggests that the absence of typical plant morphology in plant cell suspension cultures creates greater dissimilarity in detectable host responses in comparison to systems that maintain whole plant structure. The lack of greater similarity between hydroponics and site-specific wounding based techniques is likely attributed to mechanically wounding the host that ultimately influences the detectable *A. thaliana* Col-0 transcriptomic output. By evading any artificial introduction of direct contact between *A. thaliana* Col-0 and *A. tumefaciens* C58 post-inoculation into liquid culture, the detection of *A. tumefaciens* C58 virulence is imperative to ensure infection still occurs. By confirming *A. tumefaciens* C58 virulence gene induction (Figure

23), not only are host responses testable using hydroponics, but *A. tumefaciens* C58 responses to host perception can also be analyzed. Over a fifty-six hour period, the growth of *A. tumefaciens* C58 in the hydroponic co-cultivation system was monitored every 4 hours. Interestingly, *A. tumefaciens* growth was stagnant leading to the twenty-hour period where growth sharply declined followed by a sudden spike in growth (Figure 24). The delay in *A. tumefaciens* growth responses indicates an initial lag period, however following the spike, growth oscillates up and down with a gradual increase until the final time point. Though atypical of growth curves, this finding suggests some back and forth communication is occurring due to the presence of *A. thaliana* Col-0. This interesting finding requires more attention in order to determine how this seemingly unique interaction is being established.

4.3.1 Alternative Applications of Co-cultivation in Hydroponics

In addition to the benefit hydroponic co-cultivation offers in the study of *A. thaliana* Col-0 and *A. tumefaciens* C58 interaction, there are a variety of broader applications as well. The versatility of hydroponics for studying plant-stimuli responses extends beyond the *Arabidopsis-Agrobacterium* interaction presented here. Hydroponic co-cultivation can be applied to elucidate plant responses to a wide variety of additional stimuli including other pathogenic or beneficial microbes and chemical signals, as well as enabling the study of microbial responses that closely mimic rhizosphere conditions by avoiding the disruption of natural root secretions. This produces a "real-time" stimulation of microbial virulence, which is more progressive than the sudden artificial induction of virulence resulting from acetosyringone (AS) supplementation. Since the secretion and concentration of these compounds in liquid is time sensitive, time-course studies can be conducted to monitor temporal chemical alterations to the liquid solution and the variations in dynamic plant-microbe interactive responses. In addition to stimuli interchangeability, a variety of plant hosts can be utilized for response analyses (Figure 4). Yet, there are a variety of parameters to consider when cultivating host models, namely mechanical and biological challenges. Identifying plant models that maintain typical plant morphology is of significant importance. Discrepancies in typical root development may impair the host's ability to indirectly communicate with microbes, weakening any possible subsequent direct interaction. In addition, dependent on seed and root size, adjustment in the use of metal mesh and growth container may also be required to ensure root structures fully develop below the mesh surface and shoot tissues proliferate above (Figure 4).

Hydroponics under lab conditions offers additional flexibility in applied pathogens and/or chemical compounds. In the case of bacterial, fungal and herbivory pathogens, the sole requirement is that the liquid media and host can support their survival to facilitate infection/feeding. Insects acting above the root surface can be applied directly to various non-submerged regions of the system (i.e.: mesh platform, plant structure, sides of the hydroponic tank, etc.). Moreover, hydroponics may offer new insight into studying the effects of pre-existing (allelochemicals) and/or applied (synthetic) chemical compounds by introduction into the liquid medium, as well as effects of variation in environmental conditions, as has been previously attempted using other methodologies including root dipping [67,68]. The plasticity in stimuli that can be studied using this system makes hydroponics beneficial for identifying molecular plant responses to a variety of abiotic-biotic stimuli.

4.3.1.1 Gene expression pattern analysis

Developing an innovative system for plant-pathogen interaction creates possibility to detect molecular processes that have not been previously identified. This is not limited to interactive studies between host-microbe, but may also reveal other host properties such as spatial/temporal expression patterns of genes and their associative annotations.

During the process of reviewing gene annotations for differentially expressed transcripts detected in root and shoot tissues, a small subset of genes identified had not been previously detected in their corresponding tissue sites previously. From systemically affected tissue sites, three DEGs had not been previously detected in shoot tissues (Appendix 29). On the other hand, 58 DEGs were detected in our root transcriptome that

have not been previously identified in root tissues (Appendix 30). The relatively smaller amount of DEGs uniquely identified to be expressed in shoot tissue using the hydroponic co-cultivation system is the result of analyzing entirely indirectly affected tissue (leaf, stem, shoot). If the expression patterns were analyzed independently for different indirectly affected sites then it is more likely that many more genes that have not been detected in those specified tissues may have been identified for expression. Together these unintentional findings suggest that by further developing more accurate models for experimental study, we are able to better mimic and uncover host-microbe interactions occurring *in planta*.

4.4 Conclusion

Our current understanding of plant-*Agrobacterium* interaction is extensive in comparison to many other plant-stimuli responses. However, the accuracy of the knowledge generated is impeded due to the models that have been utilized to study these responses. Experimental design mimicking natural conditions of plant-microbe interaction as closely as possible is important for both the dissection of complex signaling pathways and application of results *in planta*. Establishing the efficacy of this system for the study of *A. thaliana-A. tumefaciens* interaction, will also facilitate its use to study a wide range of responses to various organisms and among diverse sets of hosts as well. In addition, hydroponic cultivation is already utilized for the study of optimal nutritional conditions for plant propagation. In the future, hydroponic co-cultivation may also be used to study host responses to chemicals either applied in the field or released as a byproduct of another industrial or economic practices.

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Appendices

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Appendix 1. Reported induced genes in shoot tissues.

Reported 249 up-regulated A. thaliana Col-0 shoot tissue differentially expressed genes with at least 2-fold changes in expression (P-value≤0.005) 8 hours post-inoculation with A. tumefaciens C58. 'AW' indicates A. thaliana Col-0 hydroponic co-cultivation with A. tumefaciens C58; 'A' indicates mock-inoculated A. thaliana Col-0.

Locus Tag	Gene Symbol	Function/Putative Function	Fold-Change
AT5G59320	LTP3	Lipid transport	73.7698
AT4G12500	---	Lipid transport	72.8817
AT5G59310	LTP4	Lipid transport	32.2402
AT4G12490	---	Lipid transport	28.4271
AT3G56980	BHLH039	DNA binding; iron ion homeostasis	25.865
AT3G49580	LSU1	Unknown	24.5827
AT5G48850	ATSDI1	Cellular response to sulfate starvation	21.9284
AT5G24660	LSU2	Regulation of defense response; chloroplast organization	19.7184
AT2G43620	---	Chitinase activity	17.8781
AT1G57750	CYP96A15	Alkane hydroxylase activity	14.8721
AT3G51590	LTP12	Lipid transport	12.8233
AT1G73010	---	Pyrophosphatase activity; galactolipid biosynthetic process	11.34
AT5G26220	ATGGCT2; 1	Response to lead/cadmium ion	11.2679
AT5G64120	PRX71	Peroxidase	10.0666
AT5G37940	---	Oxidoreductase; zinc ion binding	9.72898
AT5G07550	GRP19	Lipid binding/storage	8.86517
AT1G65500	---	Unknown	8.80172
AT5G05340	PRX52	Peroxidase	7.89785
AT4G21990	APR3	Oxidoreductase; adenylyl-sulfate reductase	7.55009
AT4G08770	PRX37	Peroxidase	7.53513
AT4G04610	APR2	Oxidoreductase; adenylyl-sulfate reductase	7.4982
AT1G56600	AtGolS2	Galactose metabolism, inositol 3-alpha-galactosyltransferase activity	7.29814
AT1G56430	NAS4	Nicotianamine synthase	7.14376
AT2G44460	BGLU28	O-glycosyl hydrolase	6.83832
AT2G38870	---	Serine-type endopeptidase activity	6.82034
AT3G12500	ATHCHIB	Chitinase activity; nitrate transport	6.77424
AT1G53070	---	Carbohydrate binding	6.36942
AT1G23730	BCA3	Carbon utilization	6.30956
AT5G04150	BHLH101	Transcription factor responsive to iron	6.13756

		homeostasis	
AT3G54700	ATPT2	Sugar:hydrogen symporter; phosphate ion transmembrane transporter	6.11168
AT1G80130	---	Tetratricopeptide repeat like superfamily protein	5.97983
AT5G62080	---	Lipid transport	5.753
AT2G30750	CYP71A12	Iron ion binding; oxidoreductase activity	5.57792
AT4G01700	---	Chitinase	5.45034
AT2G43590	---	Chitinase activity	5.22968
AT5G42180	PER64	Peroxidase	5.13525
AT3G17790	PAP17	Hydrolase; serine/threonine phosphatase activity	5.1057
AT3G04290	LTL1	Carboxylesterase activity; hydrolase activity	5.07083
AT4G11650	ATOSM34	Response to salt stress, bacterium, fungus	5.0671
AT2G43510	ATTI1	Serine-type endopeptidase inhibitor activity	4.9881
AT2G39510	UMAMIT14	Unknown	4.81965
AT3G12520	SULTR4;2	Secondary active sulfate transmembrane transporter	4.69306
AT1G75280	---	Isoflavone reductase	4.56982
AT5G53450	ORG1	Protein phosphorylation	4.54381
AT4G12480	pEARLI 1	Lipid transport	4.53744
AT4G33070	PDC1	Thiamine pyrophosphate; carboxy-lyase; magnesium ion binding	4.47647
AT1G17745	PGDH	L-serine biosynthetic process	4.39871
AT2G39330	JAL23	Unknown	4.38677
AT5G33370	---	Hydrolase; lipid metabolism	4.33344
AT2G20870	---	Regulation of anthocyanin biosynthetic process	4.32865
AT1G02205	CER1	Production of stem epicuticular wax and pollen fertility	4.19835
AT4G15210	BAM5	Beta-amylase; cation binding	4.17288
AT5G37300	WSD1	Diacylglycerol O-acyltransferase; long-chain-alcohol O-fatty-acyltransferase	4.04475
AT3G15395	ATA20	Unknown	3.94295
AT5G53420	---	Unknown	3.9187
AT5G52390	---	Unknown	3.87268
AT5G48540	---	Response to karrikin	3.83165
AT2G19590	ACO1	Oxidoreductase activity	3.80861
AT5G20150	SPX1	Phosphate ion transport in response to starvation; galactolipid biosynthesis	3.80537
AT4G10270	---	Response to wounding	3.79576
AT1G67360	---	Unknown	3.7411

AT1G26390	---	FAD-binding Berberine family protein; electron carrier	3.72452
AT3G08770	LTP6	Lipid transport	3.70941
AT1G64400	LACS3	Long-chain acyl-CoA synthetase	3.68508
AT3G25290	---	Multicellular organismal development	3.67411
AT4G39510	CYP96A12	Oxidoreductase activity; ferric ion binding	3.64022
AT5G24770	VSP1	Acid phosphatase	3.62034
AT1G36370	SHM7	Glycine hydroxymethyltransferase; glycine/serine metabolism	3.607
AT4G08150	KNAT1	Transcription factor negatively regulating plant development	3.60506
AT4G17030	ATEXLB1	Plant cell wall type loosening; sexual reproduction	3.6023
AT4G33040	---	Disulfide oxidoreductase	3.57721
AT1G54020	---	Lipid hydrolase activity	3.55778
AT1G21310	ATEXT3	Proteasomal ubiquitin-dependent protein catabolism	3.50878
AT5G37950	---	Hexosyl transferase	3.49355
AT3G01420	DOX1	Peroxidase; heme binding	3.49003
AT5G15950	CPuORF10	Adenosylmethionine decarboxylase	3.46736
AT2G22500	UCP5	Oxidative phosphorylation; dicarboxylic acid transporter activity	3.437
AT5G50800	SWEET13	Sugar transmembrane transporter	3.41104
AT4G39330	CAD9	Oxidoreductase; zinc ion binding; nucleotide binding	3.39296
AT5G44400	---	UDP-N-acetylmuramate dehydrogenase; Flavin adenine dinucleotide binding	3.38266
AT1G77120	ADH1	Alcohol dehydrogenase (NAD) activity	3.30117
AT1G26420	---	FAD-binding Berberine family protein; electron carrier	3.29872
AT2G40610	ATEXPA8	Plant cell wall type loosening	3.21671
AT5G07030	---	Aspartic-type endopeptidase	3.20948
AT3G18290	EMB2454	Zinc ion binding	3.19257
AT1G77920	TGA7	Transcription factor responsive to xenobiotic stimulus	3.19128
AT2G42800	AtRLP29	Response to molecule of bacteria origin; stamen development	3.17951
AT4G21650	---	Serine-type endopeptidase	3.15761
AT2G01610	---	Pectinesterase inhibitor activity	3.15583
AT5G22460	---	Plant-type cell wall loosening	3.14655
AT3G47420	ATG3PP1	Sugar:hydrogen symporter	3.14287
AT4G24780	---	Pectate lyase	3.1334
AT1G64170	ATCHX16	Sodium/hydrogen antiporter	3.12744

AT5G47910	RBOHD	NAD(P)H oxidase; oxidoreductase; heme binding; calcium binding	3.12545
AT4G14080	MEE48	O-glycosyl transferase; cation binding	3.10629
AT3G47960	ATNPF2.10	Glucosinolate:hydrogen symporter	3.081
AT5G58390	---	Peroxidase	3.07417
AT4G32940	GAMMA-VPE	Cysteine-type endopeptidase	3.0723
AT5G55450	---	Lipid binding/transport	3.06609
AT5G52310	LTI78	Response to ABA	3.04264
AT1G24020	MLP423	mRNA modification	3.03035
AT1G75040	PR5	Anthocyanin-containing compound biosynthetic process	3.02598
AT3G21240	4CL2	4-coumarate-CoA ligase activity	3.00861
AT1G62180	APR2	Adenosine 5'-phosphosulfate reductase	3.00804
AT5G49630	AAP6	Neutral amino acid transmembrane transporter	3.00356
AT1G66350	RGL1	Transcription factor responsive to salt stress	2.95489
AT2G42530	COR15B	Response to cold	2.95322
AT3G04030	MYR2	Transcription factor	2.94038
AT4G02290	AtGH9B13	O-glycosyl hydrolase	2.93397
AT2G36570	PXC1	ATP binding; protein kinase activity	2.92013
AT5G20740	---	Pectinesterase; enzyme inhibitor activity	2.91867
AT2G36970	---	UDP-glycosyltransferase activity	2.9084
AT1G64390	AtGH9C2	O-glycosyl hydrolase	2.89339
AT1G19670	ATCLH1	Chlorophyllase	2.88342
AT1G74590	GSTU10	Toxic catabolic process; glutathione transferase activity	2.87778
AT5G07560	GRP20	Lipid binding/storage	2.87077
AT1G28290	AGP31	Atypical arabinogalactan	2.84431
AT2G22970	SCPL11	Serine-type carboxypeptidase activity	2.84119
AT4G28250	ATEXPB3	Plant-type cell wall loosening; sexual reproductive development	2.83195
AT4G14400	ACD6	Cell death regulator	2.82462
AT1G13750	---	Hydrolase; serine/threonine phosphatase activity	2.79364
AT5G64080	XYP1	Lipid transport	2.78813
AT4G19810	CHIC	O-glycosyl hydrolase; exochitinase	2.77319
AT2G38010	---	Ceramidase activity	2.76122
AT3G49110	PRXCB	Peroxidase	2.75379
AT5G37600	GSR 1	Copper ion binding; glutamate-ammonia ligase	2.7523
AT2G31980	---	Cysteine-type endopeptidase inhibitor activity	2.74846

AT2G28790	---	Cytokinesis; cell wall organization	2.74263
AT3G45140	LOX2	Lipoxygenase	2.73161
AT1G17860	---	Kunitz family trypsin and protease inhibitor protein	2.71659
AT5G22580	---	Unknown	2.71101
AT3G51895	SULTR3;1	Secondary sulfate transmembrane transporter activity	2.70212
AT5G02890	---	Amino-acyl transferase	2.69743
AT1G18250	ATLP-1	Thaumatin-like protein	2.692
AT4G30290	XTH19	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	2.68697
AT1G76680	OPR1	12-oxophytodienoate reductase activity	2.68603
AT1G15550	GA3OX1	Gibberellic biosynthetic pathway	2.67984
AT2G16060	AHB1	Regulation of hydrogen peroxide metabolic process	2.66724
AT1G26380	---	FAD-binding Berberine family protein; electron carrier	2.66451
AT4G23600	CORI3	Cystathionine beta-lyase; 1-aminocyclopropane-1 carboxylate synthase	2.66202
AT1G75900	---	GDSL-like Lipase/Acylhydrolase superfamily protein	2.65522
AT2G37040	pal1	Phenylalanine ammonia-lyase activity	2.64199
AT4G13195	CLE44	Serine/threonine kinase binding	2.64047
AT4G30110	HMA2	Cadmium-transporting ATPase activity	2.63248
AT1G72260	THI2.1	Toxic receptor binding	2.61724
AT1G48750	---	Lipid transport	2.61522
AT3G29030	EXPA5	Unidimensional cell growth; plant-type cell wall organization/loosening	2.6038
AT1G72230	---	Glucuronoxylan metabolic process; xylan biosynthetic process	2.59051
AT1G55260	LTPG6	Cell wall biogenesis; lipid transport	2.58018
AT1G74210	GDPD5	Phosphoric diester hydrolase activity	2.57559
AT2G47800	ATMRP4	Nucleoside-triphosphatase activity; folic acid transporter activity	2.56533
AT1G18590	SOT17	Desulfoglucosinolate sulfotransferase for cysteine biosynthesis	2.55829
AT1G75780	TUB1	Cytoskeleton organization; gluconeogenesis	2.55432
AT1G43800	FTM1	Fatty acid biosynthesis	2.53724
AT1G06350	ADS4	Oxidoreductase activity	2.53501
AT5G61420	MYB28	Transcription factor response to JA, SA, GA	2.52227
AT2G28080	---	Glycosyl transferase; hexosyl transferase	2.5045
AT1G80760	NIP6;1	Glycerol transmembrane transporter activity	2.48709
AT4G01070	UGT72B1	Hexosyl transferase; UDP-glycosyltransferase	2.47441

AT5G57220	CYP81F2	Oxidoreductase; heme binding response to fungus	2.4498
AT4G30530	GGP1	Peptidase	2.44556
AT3G18830	ATPLT5	Glucose transporter activity	2.4418
AT2G28950	ATEXPA6	Calcium ion transport; cell wall loosening	2.43508
AT4G11190	---	Lignin biosynthetic process	2.42582
AT1G56580	SVB	Cell wall biogenesis	2.42166
AT3G54420	ATEP3	Chitinase	2.41811
AT5G16920	---	Pollen exine formation	2.4167
AT5G45340	CYP707A3	Oxidoreductase; heme binding	2.41642
AT5G10770	---	Aspartic-type endopeptidase	2.41016
AT1G52030	MBP2	Carbohydrate binding; thioglucosidase complex	2.40684
AT2G38530	LTP2	Lipid transport	2.38727
AT3G17820	GLN1.3	Glutamate-ammonia ligase; copper ion binding	2.38489
AT1G05300	ZIP5	Fe (II) transporter isolog family	2.38214
AT1G35720	ANNAT1	Annexin gen family; calcium ion transport	2.38142
AT4G36220	FAH1	Ferulate 5-hydroxylase; monooxygenase; iron binding	2.38057
AT5G23940	EMB3009	Acyl group transfase activity	2.37499
AT2G39420	---	Hydrolase	2.37158
AT1G02360	---	Chitinase	2.3699
AT1G26560	BGLU40	O-glycosyl compound hydrolase	2.36546
AT1G53310	ATPPC1	Phosphoenolpyruvate carboxylase activity; water transport	2.35745
AT2G37710	RLK	Protein serine/threonine kinase activity	2.35243
AT5G13740	ZIF1	Sugar:hydrogen symporter	2.35159
AT5G16570	GLN1;4	Glutamate-ammonia ligase; copper ion binding	2.34999
AT3G07390	AIR12	Extracellular matrix structural constituent	2.34217
AT2G21590	APL4	Nucleotidyltransferase; glucose-1-phosphate adenylyltransferase	2.33708
AT3G46900	COPT2	High affinity copper ion transmembrane transporter	2.31768
AT5G08260	scpl35	Serine-type carboxypeptidase activity	2.31512
AT4G36430	---	Heme binding; peroxidase	2.31202
AT3G20470	GRP5	Structural constituent of cell wall	2.29141
AT1G67520	---	Protein tyrosine kinase activity	2.28749
AT2G26020	PDF1.2b	Defense response protein	2.2872
AT1G58430	RXF26	Lipid hydrolase activity	2.28594
AT5G45670	---	Hydrolase activity; lipid metabolism	2.28445
AT1G62500	---	Lipid transport	2.28407

AT4G39950	CYP79B2	Oxidoreductase; heme binding response	2.27969
AT3G48740	SWEET11	Sucrose transport	2.27116
AT1G30530	UGT78D1	UDP-glycosyltransferase; flavonol biosynthetic process	2.2636
AT5G18060	SAUR23	Response to auxin	2.22791
AT1G69530	ATEXPA1	Plant cell wall type loosening	2.22301
AT4G31500	CYP83B1	Oxidoreductase activity; ferric ion binding	2.2216
AT1G62560	FMO GS-OX3	Glucosinolate biosynthetic process; flavin adenine dinucleotide binding	2.21816
AT5G19240	---	Indoleacetic acid biosynthesis	2.21617
AT1G04680	---	Pectin lyase-like superfamily protein	2.20613
AT3G27060	TSO2	Ribonucleoside-diphosphate reductase; transition metal ion binding	2.20155
AT3G16530	---	Carbohydrate binding; response to fungus	2.20079
AT3G54340	AP3	Transcription factor; post-embryonic development/plant-type cell wall	2.19809
AT2G42690	---	Phosphatidylcholine; triglyceride lipase activity	2.19356
AT5G01820	ATSR1	Serine/threonine kinase binding	2.1884
AT1G61070	LCR66	Defense response protein	2.18242
AT3G13790	ATBFRUCT1	Beta-fructofuranosidase; O-glycosyl hydrolase	2.18089
AT2G29350	SAG13	Nucleotide binding; oxireductase activity	2.17827
AT1G69080	---	Adenine nucleotide alpha hydrolases-like superfamily protein	2.17456
AT1G51060	HTA10	Nucleosome assembly	2.16953
AT4G14680	APS3	Sulfate adenylyltransferase	2.16862
AT1G49430	LACS2	Cutin biosynthesis; fatty acid metabolism	2.16801
AT1G08310	---	Alpha/Beta-hydrolase superfamily	2.15828
AT5G17330	GAD	Glutamate decarboxylase; pyridoxal phosphate binding; calmodulin binding	2.15777
AT3G18080	BGLU44	O-glycosyl hydrolase; amygdalin beta-glucosidase	2.15773
AT1G12110	NRT1.1	Nitrate transporter	2.14542
AT1G02930	ATGSTF6	Glutathione transferase	2.14314
AT2G29980	FAD3	omega-3 fatty acid desaturase activity; oxireductase activity	2.1427
AT1G70370	PG2	Auxin polar transport; polgalacturonase activity	2.14205
AT1G62800	ASP4	Asparagine catabolic transamidation (catabolism)	2.13955
AT4G39940	AKN2	Adenylylsulfate kinase activity	2.12241
AT2G22240	MIPS2	Inositol-3-phosphate synthase activity	2.10953
AT1G29050	---	Unknown	2.10813

AT4G08780	---	Peroxidase; heme binding	2.10243
AT2G04160	AIR3	Serine-type endopeptidase activity; glucosinolate biosynthetic process	2.10194
AT3G05650	AtRLP32	Kinase activity	2.09216
AT5G53120	SPDS3	Spermidine synthase	2.08694
AT2G25060	---	Electron carrier activity; copper ion binding	2.08061
AT3G04210	---	ADP binding	2.07626
AT1G11840	ATGLX1	Glyoxalase	2.06972
AT3G49220	---	Pectinesterase; enzyme inhibitor activity	2.06256
AT1G32900	GBSS1	UDP-glycosyltransferase; galactolipid biosynthetic process	2.05675
AT5G51750	ATSBT1.3	Serine-type endopeptidase	2.04921
AT4G14440	HCD1	Dodecenoyl-CoA delta-isomerase; carnitine racemase	2.04647
AT2G14750	APK	Adenylylsulfate kinase activity	2.04103
AT4G38740	ROC1	Peptidyl-prolyl cis-trans	2.04035
AT4G25900	---	Carbohydrate binding; aldose 1-epimerase	2.03623
AT3G12610	DRT100	Nucleotide binding response to drug	2.02264
AT1G13110	CYP71B7	Cellular cation homeostasis; tryptophan catabolic process	2.02208
AT5G63180	---	Pectate lyase activity	2.02119
AT4G31840	ATENODL 15	Copper ion binding	2.00845
AT4G30960	SIP3	Protein kinase; calcium mediated signaling; development	2.0055
AT5G11930	---	Disulfide oxidoreductase	2.00526
AT4G25480	DREB1A	Transcription factor in response to cold	2.00518
AT3G18490	ASPG1	Aspartic-type endopeptidase	2.00277
AT2G39210	---	Amino acid transport	2.00025

Appendix 2. Reported repressed genes in shoot tissues.

Reported 152 down-regulated A. thaliana Col-0 shoot tissue differentially expressed genes with at least 2-fold changes in expression (P-value≤0.005) 8 hours post-inoculation with A. tumefaciens C58. 'AW' indicates A. thaliana Col-0 hydroponic co-cultivation with A. tumefaciens C58; 'A' indicates mock-inoculated A. thaliana Col-0.

Locus Tag	Gene Symbol	Function/Putative Function	Fold-Change
AT2G24850	TAT3	1-aminocyclopropane-1-carboxylate synthase activity	-2.00585
AT3G52840	BGAL2	O-glycosyl hydrolase; cation binding	-2.00589
AT1G77060	---	Phosphoenolpyruvate carboxylase family protein	-2.00738
AT3G16450	JAL33	Response to zinc; cold response	-2.01007
AT5G13330	Rap2.6L	Transcription factor responsive to ABA/JA/SA	-2.01617
AT1G10060	ATBCAT-1	Aminotransferase	-2.03159
AT3G50770	CML41	Calcium ion binding	-2.03501
AT3G15060	AtRABA1g	GTP binding; small GTPase mediated signal transduction	-2.03644
AT2G47700	RFI2	Ubiquitin-protein ligase activity	-2.03664
AT1G22570	---	Oligopeptide transporter	-2.03729
AT5G18600	---	Arsenate reductase; oxidoreductase	-2.03827
AT1G05810	RABA5E	Small GTPase mediated signal transduction	-2.03883
AT3G16690	SWEET16	Sugar transmembrane transport	-2.04331
AT4G35770	SEN1	Response to wounding, sucrose, light for senescence	-2.05695
AT2G48030	---	Hydrolase	-2.06063
AT1G79440	ALDH5F1	Gamma-aminobutyric acid catabolic process	-2.08417
AT3G59900	ARGOS	Positive regulation of organ growth	-2.10171
AT3G60940	---	Unknown	-2.10751
AT1G58180	BCA6	Carbonate dehydratase activity; zinc ion binding	-2.11718
AT5G23010	MAM1	2-(2'-methylthio) ethylmalate synthase; acyl transferase	-2.11859
AT4G35250	HCF244	Translation initiation factor	-2.13639
AT5G24210	---	Tirglyceride lipase	-2.14302
AT4G16690	MES16	Methyl jasmonate esterase; methyl indole-3-acetate esterase; hydrolase	-2.15167
AT4G10030	---	Hydrolase activity	-2.17491
AT3G22640	PAP85	Nutrient reservoir activity	-2.18594
AT3G50560	---	Oxidoreductase	-2.19535

AT1G14290	SBH2	One of two sub-unity sphingoid base hydroxylases	-2.20688
AT4G19530	---	ADP binding; regulation of proton transport	-2.20905
AT5G15410	DND1	Intracellular cAMP activated cation channel	-2.26194
AT1G60140	ATTPS10	Trehalose biosynthetic process	-2.27143
AT1G20630	CAT1	Hydrogen peroxide reductase	-2.28982
AT4G34950	---	glucosinolate biosynthetic process	-2.29455
AT5G21170	AKINBETA 1	AMP-activated protein kinase	-2.3008
AT2G29670	---	Unknown	-2.3036
AT5G01740	---	Unknown	-2.31215
AT4G39780	---	Transcription factor responsive to cold	-2.31864
AT2G40300	ATFER4	Oxidoreductase activity; ferric ion binding	-2.32208
AT4G30270	MERI5B	Xyloglucan:xyloglucosyl transferase	-2.32734
AT5G44210	ERF9	Transcription factor responsive to ethylene for oligopeptide transport	-2.33614
AT5G01600	FER1	Oxidoreductase activity; ferric ion binding	-2.34119
AT1G77760	NIA1	Flavin adenine dinucleotide binding; oxidoreductase activity	-2.34451
AT1G72430	SAUR78	Sterol biosynthetic process	-2.35524
AT5G47220	ERF2	Transcription factor responsive to ET, fungus	-2.36242
AT2G34600	JAZ7	Response to JA; fungal attack	-2.36293
AT4G26530	ATFBA5	Fructose-bisphosphate aldolase	-2.36574
AT1G23870	ATTPS9	Putatively involved in trehalose biosynthesis	-2.36958
AT2G34500	CYP710A1	Heme binding; oxidoreductase activity	-2.37447
AT5G65660	---	Unknown	-2.39843
AT1G12780	UGE1	UDP-glucose epimerase	-2.39909
AT1G77210	ATSTP14	Galactose transmembrane transporter activity	-2.42279
AT2G34770	FAH1	Iron ion binding; oxidoreductase activity	-2.44485
AT1G04310	ERS2	Ethylene receptor related to bacterial two-component histidine kinases	-2.48314
AT5G05440	ATPYL5	ABA binding	-2.49072
AT2G44080	ARL	Response to brassinosteroid	-2.49194
AT2G38240	---	Oxidoreductase activity	-2.5118
AT1G71030	MYBL2	Transcription factor responsive to salt stress	-2.52476
AT1G32170	XTR4	Glycosyl hydrolase; xyloglucosyl transferase	-2.54093
AT3G15850	FAD5	Oxidoreductase activity	-2.54529

AT2G04450	ATNUDT6	ADP-ribose diphosphatase activity; NADH pyrophosphatase activity	-2.55374
AT4G33420	---	Peroxidase; heme binding	-2.56857
AT3G60420	---	Negative regulation of defense response	-2.57699
AT5G05600	---	Oxidoreductase activity	-2.58591
AT4G15530	PPDK	Ligase; phosphate dikinase activity	-2.60018
AT5G67480	BT4	Transcription cofactor activity; zinc ion binding; response to auxin, JA, GA	-2.61245
AT1G79700	WRI4	Transcription factor	-2.61665
AT1G80380	---	Glycerate kinase activity	-2.61802
AT1G11260	STP1	H+/hexose transporter	-2.63653
AT2G39705	RTFL8	Fatty acid catabolic process	-2.6588
AT2G42280	AKS3	Transcription factor responsive to arsenic-containing substance	-2.66119
AT4G17245	---	Zinc ion binding	-2.66733
AT1G43670	ATCFBP	Glycolysis fructose 1,6-bisphosphate 1-phosphate activity	-2.67143
AT5G47240	atnudt8	Hydrolase responsive to wounding	-2.67326
AT2G40000	HSPRO2	Protein binding; response to chitin	-2.67947
AT1G18270	---	Ketose-bisphosphate aldolase class-II family protein for glycolysis	-2.70683
AT2G01860	EMB975	Unknown	-2.7301
AT1G02660	---	Lipid metabolism	-2.75754
AT1G78600	LZF1	Anthocyanin-containing compound biosynthetic process	-2.75789
AT5G01210	---	Amino-acyl transferase	-2.76084
AT5G52250	EFO1	Nucleotide binding responsive to light; flavonoid biosynthesis	-2.79404
AT2G14170	ALDH6B2	Methylmalonate-semialdehyde dehydrogenase (acylation) activity	-2.82319
AT4G34030	MCCB	Ligase; cobalt ion binding; biotic carboxylase	-2.8882
AT5G55970	---	Zinc ion binding	-2.9027
AT2G33830	---	Unknown	-2.94985
AT4G27410	RD26	Transcription factor responsive to JA, ABA	-2.98244
AT1G22400	UGT85A1	Jasmonic acid signaling	-3.00275
AT5G53980	ATHB52	Transcription factor response to light	-3.00339
AT5G06690	WCRKC1	Electron carrier activity; disulfide oxireductase activity	-3.03192
AT1G25560	TEM1	Cellular cation homeostasis; divalent metal ion transport	-3.0516
AT1G22740	RABG3B	Autophagic cell death	-3.19256
AT5G14120	---	Nitrate transport; cysteine biosynthesis	-3.257

AT5G56100	---	Lipid storage	-3.28945
AT3G60530	GATA4	Transcription factor response to light	-3.34799
AT1G43160	RAP2.6	Transcription factor responsive to wounding	-3.36476
AT1G12520	ATCCS	Copper-zinc superoxide dismutase copper chaperone	-3.39085
AT4G15550	IAGLU	Hexosyl transferase; UDP-glycosyltransferase	-3.39596
AT3G06850	BCE2	Zinc ion binding; dihydrolipoamid branched chain acyltransferase activity	-3.39995
AT5G20230	ATBCB	Copper ion	-3.42022
AT3G49620	DIN11	Oxidoreductase	-3.42316
AT5G20250	DIN10	O-glycosyl transferase	-3.42522
AT3G13450	DIN4	3-methyl-2-oxobutanoate dehydrogenase	-3.4725
AT5G36910	THI2.2	Toxin receptor	-3.51124
AT5G44130	FLA13	Unknown	-3.51764
AT4G33150	LKR	Oxidoreductase; nucleotide binding	-3.52191
AT2G22860	ATPSK2	Growth factor activity	-3.60613
AT4G14130	XTR7	O-glycosyltransferase; xyloglucan:xyloglucosyl transferase	-3.61474
AT1G06570	PDS1	p-hydroxyphenylpyruvate dioxygenase (HPPDase)	-3.65612
AT3G13750	BGAL1	Beta-galactosidase activity; O-glycosyl hydrolase	-3.69984
AT2G37030	SAUR46	Response to auxin	-3.74841
AT4G03510	RMA1	Ubiquitin-protein ligase activity	-3.75164
AT1G49210	---	Ubiquitin-protein ligase activity	-3.75706
AT2G43400	ETFQO	Electron transferring flavoprotein dehydrogenase activity	-3.7911
AT4G36410	UBC17	Ubiquitin-protein ligase activity	-3.81204
AT4G18340	---	O-glycosyl hydrolase; cation binding	-3.95426
AT5G56870	BGAL4	O-glycosyl hydrolase; cation binding	-4.04649
AT4G26260	MIOX4	Inositol oxygenase activity; iron ion binding	-4.07571
AT5G57560	TCH4	Glcosyl hydrolase; xyloglucan:xyloglucosyl transferase	-4.09767
AT4G37610	BT5	Transcription co-factor; histone acetyltransferase; calmodulin binding	-4.14992
AT3G21870	CYCP2;1	Cyclin-dependent protein kinase	-4.1576
AT5G42900	COR27	Response to cold	-4.16759
AT5G44260	ATTZF5	Zinc ion binding transcription f actor	-4.25605
AT5G49360	ATBXL1	O-glycosyl hydrolase; alpha-N-arabinofuranosidase	-4.26427
AT2G39980	---	Acyl group transfase activity	-4.29061

AT2G30600	---	Cell adhesion; proximal/distal pattern formation	-4.31087
AT3G13700	---	Nucleic acid binding, RNA	-4.36347
AT1G75750	GASA1	Cell tip growth	-4.36588
AT2G05540	---	Unknown	-4.4362
AT5G39610	ATNAC6	Transcription factor responding to JA,ET,ROS,ABA	-4.4532
AT2G23030	SNRK2.9	Protein serine/threonine kinase	-4.69852
AT3G45300	IVD	Isovaleryl-CoA dehydrogenase;oxidoreductase	-5.23933
AT4G28040	UMAMIT33	Unknown	-5.37134
AT5G45820	CIPK20	Serine/threonine kinase binding	-5.46179
AT3G57520	AtSIP2	O-glycosyl hydrolase; raffinose alpha-galactosidasae	-5.62147
AT5G57550	XTR3	O-glcosyl hydrolase; xyloglucan:xyloglucosyl transferase	-5.66993
AT1G03090	MCCA	Leucine degradation	-5.68113
AT3G48360	BT2	Transcription cofactor activity; calmodulin binding; response to JA	-5.7919
AT3G15500	ANAC055	DNA binding; response to fungus	-5.96384
AT1G33960	AIG1	Endocytosis	-6.09571
AT1G35140	PHI-1	Carbon starvation	-6.48827
AT2G17880	DJC24	Heat shock protein binding	-6.55334
AT2G19800	MIOX2	Inositol oxygenase activity; iron ion binding	-6.59306
AT3G30775	ERD5	Proline dehydrogenase	-6.83576
AT1G21910	DREB26	Transcription factor responsive to temperature	-6.91889
AT1G10070	ATBCAT-2	Aminotransferase	-7.1259
AT3G19390	---	Cysteine-type peptidase activity	-7.56505
AT1G76410	ATL8	Determination of bilateral symmetry	-8.93178
AT5G41080	ATGDPD2	Phosphoric diester hydrolase; glycerophosphodiester phosphodiesterase	-9.15144
AT1G62510	---	Lipid transport	-9.66584
AT3G59930	---	Unknown	-10.1924
AT3G47340	ASN1	Asparagine synthase	-10.6728
AT4G37220	---	Response to glucose, sucrose	-11.3582
AT1G08630	THA1	Threonine aldolase to degrade glycine	-16.6906
AT3G62950	---	Disulfide oxidoreductase	-27.202

Appendix 3. Shoot microarray data comparative analysis with previously produced data sets.

From 401 DEGs detected in shoot tissue, 45 had been previously identified in at least one of five other representative transcriptome response microarrays to biotic stresses.

Wounding	Suspension	Insect	Fungal	FLG22	Function
+		+	+		Chitinase family protein
+					Myristoylation (response to fungal stress)
+					Peroxidase Superfamily Protein
+			+		Peroxidase Prx37
+					Basic Chitinase
+					Extensin (ATEXT1)
+					Pectin lyase-like superfamily protein
+					ACC Oxidase 1
+					Cell fate commitment (KNAT1)
+					Plant Cell Wall Organization (ATEXT3)
+					Pectin lyase-like superfamily protein
+		+			Oligopeptide transport
+		+			Pectin biosynthetic process
		+	+		Induced Systemic Response (CYP71A12)
		+	+		L-serine biosynthetic process (PGDH)
		+	+		Carbohydrate metabolic process
		+	+		Plant-type cell wall loosening (EXPANSIN LIKE)
		+	+		bZIP transcription factor (defense response to bacterium)
		+	+		Lignin Biosynthesis (defense response)
		+	+		Alpha/Beta-hydrolase superfamily protein
		+			Glucosinolate metabolic process
		+			Glutathione S-transferase
		+			Positive regulation of organ growth (ARGOS)
		+			Thioredoxin superfamily protein
		+			Carbohydrate metabolic process
		+			Abscisic acid mediated signaling pathway
		+			Negative regulation of ethylene signaling pathway (EBF2)
		+			L-lysine catabolic process

		+			Glycine-rich protein family
		+	+		Chitinase Family Protein
		+	+		Oligopeptide transport
			+	+	Defense response to fungus, proline transport
			+		Beta carbonic anhydrase 3, carbon utilization
			+		Cytoskeleton organization
				+	Amino Acid Transport
				+	Disease Resistance-responsive amino acid biosynthetic process
				+	Carbohydrate homeostasis
				+	Cell death protein
				+	Nitrate transport
				+	Ethylene mediated signaling pathway
+				+	Jasmoninc acid and ethylene-dependent response to ethylene
+				+	MAPK cascade
		+	+	+	Disease resistance-responsive, lignin biosynthesis
+					Amino acid metabolism
16	0	22	15	10	

Appendix 4. Shoot transcripts not previously annotated in response to *A. tumefaciens* C58.

A. thaliana Col-0 shoot genes not previously detected for differential expression in response to A. tumefaciens C58.

Locus Tag	Gene Symbol	Function/Putative Function	Fold-Change
AT5G59320	LTP3	Lipid transport	73.7698
AT4G12500	---	Lipid transport	72.8817
AT5G59310	LTP4	Lipid transport	32.2402
AT4G12490	---	Lipid transport	28.4271
AT3G56980	BHLH039	DNA binding; iron ion homeostasis	25.865
AT3G49580	LSU1	Unknown	24.5827
AT5G48850	ATSDI1	Cellular response to sulfate starvation	21.9284
AT5G24660	LSU2	Regulation of defense response; chloroplast organization	19.7184
AT2G43620	---	Chitinase activity	17.8781
AT1G57750	CYP96A15	Alkane hydroxylase activity	14.8721
AT3G51590	LTP12	Lipid transport	12.8233
AT1G73010	---	Pyrophosphatase activity; galactolipid biosynthetic process	11.34
AT5G26220	ATGGCT2; 1	Response to lead/cadmium ion	11.2679
AT5G64120	PRX71	Peroxidase	10.0666
AT5G37940	---	Oxidoreductase; zinc ion bingin	9.72898
AT5G07550	GRP19	Lipid binding/storage	8.86517
AT1G65500	---	Unknown	8.80172
AT5G05340	PRX52	Peroxidase	7.89785
AT4G21990	APR3	Oxidoreductase; adenylyl-sulfate reductase	7.55009
AT4G04610	APR2	Oxidoreductase; adenylyl-sulfate reductase	7.4982
AT1G56600	AtGolS2	Galactose metabolism, inositol 3-alpha-galactosyltransferase activity	7.29814
AT1G56430	NAS4	Nicotianamine synthase	7.14376
AT2G44460	BGLU28	O-glycosyl hyrdrolase	6.83832
AT2G38870	---	Serine-type endopeptidase activity	6.82034
AT3G12500	ATHCHIB	Chitinase activity; nitrate transport	6.77424
AT1G53070	---	Carbohydrate binding	6.36942
AT1G23730	BCA3	Carbon utilization	6.30956
AT5G04150	BHLH101	Transcription factor responsive to iron homeostasis	6.13756
AT3G54700	ATPT2	Sugar:hydrogen symporter; phosphate ion transmembrane transporter	6.11168

AT1G80130	---	Tetratricopeptide repeat like superfamily protein	5.97983
AT5G62080	---	Lipid transport	5.753
AT4G01700	---	Chitinase	5.45034
AT2G43590	---	Chitinase activity	5.22968
AT5G42180	PER64	Peroxidase	5.13525
AT3G17790	PAP17	Hydrolase; serine/threonine phosphatase activity	5.1057
AT3G04290	LTL1	Carboxylesterase activity; hydrolase activity	5.07083
AT4G11650	ATOSM34	Response to salt stress, bacterium, fungus	5.0671
AT2G43510	ATTI1	Serine-type endopeptidase inhibitor activity	4.9881
AT2G39510	UMAMIT14	Unknown	4.81965
AT3G12520	SULTR4;2	Secondary active sulfate transmembrane transporter	4.69306
AT1G75280	---	Isoflavone reductase	4.56982
AT5G53450	ORG1	Protein phosphorylation	4.54381
AT4G33070	PDC1	Thiamine pyrophosphate; carboxy-lyase; magnesium ion binding	4.47647
AT2G39330	JAL23	Unknown	4.38677
AT5G33370	---	Hydrolase; lipid metabolism	4.33344
AT2G20870	---	Regulation of anthocyanin biosynthetic process	4.32865
AT1G02205	CER1	Production of stem epicuticular wax and pollen fertility	4.19835
AT4G15210	BAM5	Beta-amylase; cation binding	4.17288
AT5G37300	WSD1	Diacylglycerol O-acyltransferase; long-chain-alcohol O-fatty-acyltransferase	4.04475
AT3G15395	ATA20	Unknown	3.94295
AT5G53420	---	Unknown	3.9187
AT5G52390	---	Unknown	3.87268
AT5G48540	---	Response to karrikin	3.83165
AT2G19590	ACO1	Oxidoreductase activity	3.80861
AT5G20150	SPX1	Phosphate ion transport in response to starvation; galactolipid biosynthesis	3.80537
AT4G10270	---	Response to wounding	3.79576
AT1G67360	---	Unknown	3.7411
AT1G26390	---	FAD-binding Berberine family protein; electron carrier	3.72452
AT3G08770	LTP6	Lipid transport	3.70941
AT1G64400	LACS3	Long-chain acyl-CoA synthetase	3.68508
AT3G25290	---	Multicellular organismal development	3.67411
AT4G39510	CYP96A12	Oxidoreductase activity; ferric ion binding	3.64022

AT5G24770	VSP1	Acid phosphatase	3.62034
AT1G36370	SHM7	Glycine hydroxymethyltransferase; glycine/serine metabolism	3.607
AT4G08150	KNAT1	Transcription factor negatively regulating plant development	3.60506
AT4G17030	ATEXLB1	Plant cell wall type loosening; sexual reproduction	3.6023
AT4G33040	---	Disulfied oxidoreductase	3.57721
AT1G54020	---	Lipid hydrolase activity	3.55778
AT1G21310	ATEXT3	Proteasomal ubiquitin-dependent protein catabolism	3.50878
AT5G37950	---	Hexosyl transferase	3.49355
AT5G15950	CPuORF10	Adenosylmethionine decarboxylase	3.46736
AT2G22500	UCP5	Oxidative phosphorylation uncoupler transporter	3.437
AT5G50800	SWEET13	Sugar transmembrane transporter	3.41104
AT4G39330	CAD9	Oxidoreductase; zinc ion binding; nucleotide binding	3.39296
AT5G44400	---	UDP-N-acetylmuramate dehydrogenase; flavin adenine dinucleotide binding	3.38266
AT1G26420	---	FAD-binding Berberine family protein; electron carrier	3.29872
AT2G40610	ATEXPA8	Plant cell wall type loosening	3.21671
AT5G07030	---	Aspartic-type endopeptidase	3.20948
AT3G18290	EMB2454	Zinc ion binding	3.19257
AT2G42800	AtRLP29	Response to molecule of bacteria origin; stamen development	3.17951
AT4G21650	---	Serine-type endopeptidase	3.15761
AT2G01610	---	Pectinesterase inhibitor activity	3.15583
AT5G22460	---	Plant-type cell wall loosening	3.14655
AT3G47420	ATG3PP1	Sugar:hydrogen symporter	3.14287
AT4G24780	---	Pectate lyase	3.1334
AT1G64170	ATCHX16	Sodium/hydrogen antiporter	3.12744
AT5G47910	RBOHD	NAD(P)H oxidase; oxidoreductase; heme binding; calcium binding	3.12545
AT4G14080	MEE48	O-glycosyl transferase; cation binding	3.10629
AT3G47960	ATNPF2.10	Glucosinolate:hydrogen symporter	3.081
AT5G58390	---	Peroxidase	3.07417
AT4G32940	GAMMA-VPE	Cysteine-type endopeptidase	3.0723
AT5G52310	LTI78	Response to ABA	3.04264
AT1G75040	PR5	Anthocyanin-containing compound biosynthetic process	3.02598
AT3G21240	4CL2	4-coumarate-CoA ligase activity	3.00861

AT1G62180	APR2	Adenosine 5'-phosphosulfate reductase	3.00804
AT5G49630	AAP6	Neutral amino acid transmembrane transporter	3.00356
AT1G66350	RGL1	Transcription factor responsive to salt stress	2.95489
AT2G42530	COR15B	Response to cold	2.95322
AT3G04030	MYR2	Transcription factor	2.94038
AT4G02290	AtGH9B13	O-glycosyl hydrolase	2.93397
AT2G36570	PXC1	ATP binding; protein kinase activity	2.92013
AT5G20740	---	Pectinesterase; enzyme inhibitor activity	2.91867
AT2G36970	---	UDP-glycosyltransferase activity	2.9084
AT1G64390	AtGH9C2	O-glycosyl hyrdrolase	2.89339
AT1G74590	GSTU10	Toxic catabolic process; glutathione transferase activity	2.87778
AT5G07560	GRP20	Lipid binding/storage	2.87077
AT1G28290	AGP31	Atypical arabinogalactan	2.84431
AT2G22970	SCPL11	Serine-type carboxypeptidase activity	2.84119
AT4G28250	ATEXPB3	Plant-type cell wall loosening; sexual reproductive development	2.83195
AT4G14400	ACD6	Cell death regulator	2.82462
AT5G64080	XYP1	Lipid transport	2.78813
AT4G19810	CHIC	O-glycosyl hydrolase; exochitinase	2.77319
AT2G38010	---	Ceramidase activity	2.76122
AT5G37600	GSR 1	Copper ion binding; glutamate-ammonia ligase	2.7523
AT2G31980	---	Cysteine-type endopeptidase inhibitor activity	2.74846
AT2G28790	---	Cytokinesis; cell wall organization	2.74263
AT1G17860	---	Kunitz family trypsin and protease inhibitor protein	2.71659
AT5G22580	---	Unknown	2.71101
AT3G51895	SULTR3;1	Secondary sulfate transmembrane transporter activity	2.70212
AT5G02890	---	Amino-acyl transferase	2.69743
AT4G30290	XTH19	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	2.68697
AT1G76680	OPR1	12-oxophytodienoate reductase activity	2.68603
AT1G15550	GA3OX1	Gibberellic biosynthetic pathway	2.67984
AT2G16060	AHB1	Regulation of hydrogen peroxide metabolic process	2.66724
AT1G26380	---	FAD-binding Berberine family protein; electron carrier	2.66451
AT4G23600	CORI3	Cystathionine beta-lyase; 1-aminocyclopropane-1 carboxylate synthase	2.66202

AT1G75900	---	GDSL-like Lipase/Acylhydrolase superfamily protein	2.65522
AT2G37040	pal1	Phenylalanine ammonia-lyase activity	2.64199
AT4G13195	CLE44	Serine/threonine kinase binding	2.64047
AT4G30110	HMA2	Cadmium-transporting ATPase activity	2.63248
AT1G48750	---	Lipid transport	2.61522
AT3G29030	EXPA5	Unidimensional cell growth; plant-type cell wall organization/loosening	2.6038
AT1G72230	---	Glucuronoxylan metabolic process; xylan biosynthetic process	2.59051
AT1G55260	LTPG6	Cell wall biogenesis; lipid transport	2.58018
AT1G74210	GDPD5	Phosphoric diester hydrolase activity	2.57559
AT2G47800	ATMRP4	Nucleoside-triphosphatase activity; folic acid transporter activity	2.56533
AT1G75780	TUB1	Cytoskeleton organization; gluconeogenesis	2.55432
AT1G06350	ADS4	Oxidoreductase activity	2.53501
AT2G28080	---	Glycosyl transferase; hexosyl transferase	2.5045
AT1G80760	NIP6;1	Glycerol transmembrane transporter activity	2.48709
AT4G01070	UGT72B1	Hexosyl transferase; UDP-glycosyltransferase	2.47441
AT4G30530	GGP1	Peptidase	2.44556
AT3G18830	ATPLT5	Glucose transporter activity	2.4418
AT2G28950	ATEXPA6	Calcium ion transport; cell wall loosening	2.43508
AT4G11190	---	Lignin biosynthetic process	2.42582
AT1G56580	SVB	Cell wall biogenesis	2.42166
AT3G54420	ATEP3	Chitinase	2.41811
AT5G16920	---	Pollen exine formation	2.4167
AT5G45340	CYP707A3	Oxidoreductase; heme binding	2.41642
AT5G10770	---	Aspartic-type endopeptidase	2.41016
AT1G52030	MBP2	Carbohydrate binding; thioglucosidase complex	2.40684
AT2G38530	LTP2	Lipid transport	2.38727
AT3G17820	GLN1.3	Glutamate-ammonia ligase; copper ion binding	2.38489
AT1G35720	ANNAT1	Annexin gen family; calcium ion transport	2.38142
AT4G36220	FAH1	Ferulate 5-hydroxylase; monooxygenase; iron binding	2.38057
AT5G23940	EMB3009	Acyl group transfase activity	2.37499
AT2G39420	---	Hydrolase	2.37158
AT1G02360	---	Chitinase	2.3699
AT1G26560	BGLU40	O-glycosyl compound hydrolase	2.36546

AT1G53310	ATPPC1	Phosphoenolpyruvate carboxylase activity; water transport	2.35745
AT5G13740	ZIF1	Sugar:hydrogen symporter	2.35159
AT5G16570	GLN1;4	Glutamate-ammonia ligase; copper ion binding	2.34999
AT3G07390	AIR12	Extracellular matrix structural constituent	2.34217
AT2G21590	APL4	Nucleotidyltransferase ; glucose-1-phosphate adenylyltransferase	2.33708
AT3G46900	COPT2	High affinity copper ion transmembrane transporter	2.31768
AT5G08260	scpl35	Serine-type carboxypeptidase activity	2.31512
AT4G36430	---	Heme binding; peroxidase	2.31202
AT3G20470	GRP5	Structural constituent of cell wall	2.29141
AT1G67520	---	Protein tyrosine kinase activity	2.28749
AT1G58430	RXF26	Lipid hydrolase activity	2.28594
AT5G45670	---	Hydrolase activity; lipid metabolism	2.28445
AT1G62500	---	Lipid transport	2.28407
AT3G48740	SWEET11	Sucrose transport	2.27116
AT1G30530	UGT78D1	UDP-glycosyltransferase; flavonol biosynthetic process	2.2636
AT5G18060	SAUR23	Response to auxin	2.22791
AT1G69530	ATEXPA1	Plant cell wall type loosening	2.22301
AT1G62560	FMO GS-OX3	Glucosinolate biosynthetic process; flavin adenine dinucleotide binding	2.21816
AT5G19240	---	Indoleacetic acid biosynthesis	2.21617
AT1G04680	---	Pectin lyase-like superfamily protein	2.20613
AT3G27060	TSO2	Ribonucleoside-diphosphate reductase; transition metal ion binding	2.20155
AT3G16530	---	Carbohydrate binding; response to fungus	2.20079
AT3G54340	AP3	Transcription factor; post-embryonic development/plant-type cell wall	2.19809
AT2G42690	---	Phosphatidylcholine; triglyceride lipase activity	2.19356
AT5G01820	ATSR1	Serine/threonine kinase binding	2.1884
AT3G13790	ATBFRUCT1	Beta-fructofuranosidase; O-glycosyl hydrolase	2.18089
AT2G29350	SAG13	Nucleotide binding; oxireductase activity	2.17827
AT1G69080	---	Adenine nucleotide alpha hydrolases-like superfamily protein	2.17456
AT1G51060	HTA10	Nucleosome assembly	2.16953
AT4G14680	APS3	Sulfate adenylyltransferase	2.16862
AT1G49430	LACS2	Cutin biosynthesis; fatty acid metabolism	2.16801
AT1G08310	---	Alpha/Beta-hydrolase superfamily	2.15828

AT5G17330	GAD	Glutamate decarboxylase; pyridoxal phosphate binding; calmodulin binding	2.15777
AT3G18080	BGLU44	O-glycosyl hyrdrolase; amygdalin beta-glucosidase	2.15773
AT1G12110	NRT1.1	Nitrate transporter	2.14542
AT2G29980	FAD3	omega-3 fatty acid desaturase activity; oxireductase activity	2.1427
AT1G70370	PG2	Auxin polar transport; polgalacturonase activity	2.14205
AT1G62800	ASP4	Asparagine catabolic transamidation (catabolism)	2.13955
AT1G29050	---	Unknown	2.10813
AT4G08780	---	Peroxidase; heme binding	2.10243
AT2G04160	AIR3	Serine-type endopeptidase activity; glucosinolate biosynthetic process	2.10194
AT5G53120	SPDS3	Spermidine synthase	2.08694
AT2G25060	---	Electron carrier activity; copper ion binding	2.08061
AT1G11840	ATGLX1	Glyoxalase	2.06972
AT3G49220	---	Pectinesterase; enzyme inhibitor activity	2.06256
AT1G32900	GBSS1	UDP-glycosyltransferase; galactolipid biosynthetic process	2.05675
AT5G51750	ATSBT1.3	Serine-type endopeptidase	2.04921
AT2G14750	APK	Adenylylsulfate kinase activity	2.04103
AT4G38740	ROC1	Peptidyl-prolyl cis-trans	2.04035
AT4G25900	---	Carbohydrate binding; aldose 1-epimerase	2.03623
AT3G12610	DRT100	Nucleotide binding response to drug	2.02264
AT1G13110	CYP71B7	Cellular cation homeostasis; tryptophan catabolic process	2.02208
AT5G63180	---	Pectate lyase activity	2.02119
AT4G31840	ATENODL 15	Copper ion binding	2.00845
AT4G30960	SIP3	Protein kinase; calcium mediated signalling; development	2.0055
AT5G11930	---	Disulfied oxidoreductase	2.00526
AT4G25480	DREB1A	Transcription factor in response to cold	2.00518
AT3G18490	ASPG1	Aspartic-type endopeptidase	2.00277
AT3G52840	BGAL2	O-glycosyl hydrolase; cation binding	-2.00589
AT1G77060	---	Phosphoenolpyruvate carboxylase family protein	-2.00738
AT3G16450	JAL33	Response to zinc; cold response	-2.01007
AT5G13330	Rap2.6L	Transcription factor responsive to ABA/JA/SA	-2.01617
AT1G10060	ATBCAT-1	Aminotransferase	-2.03159
AT3G50770	CML41	Calcium ion binding	-2.03501

AT3G15060	AtRABA1g	GTP binding; small GTPase mediated signal transduction	-2.03644
AT2G47700	RFI2	Ubiquitin-protein ligase activity	-2.03664
AT1G22570	---	Oligopeptide transporter	-2.03729
AT5G18600	---	Arsenate reductase; oxidoreductase	-2.03827
AT1G05810	RABA5E	Small GTPase mediated signal transduction	-2.03883
AT3G16690	SWEET16	Sugar transmembrane transport	-2.04331
AT4G35770	SEN1	Response to wounding, sucrose, light for senscence	-2.05695
AT2G48030	---	Hydrolase	-2.06063
AT1G79440	ALDH5F1	Gamma-aminobutyric acid catabolic process	-2.08417
AT3G59900	ARGOS	Positive regulation of organ growth	-2.10171
AT3G60940	---	Unknown	-2.10751
AT1G58180	BCA6	Carbonate dehydratase activity; zinc ion binding	-2.11718
AT5G23010	MAM1	2-(2'-methylthio) ethylmalate synthase; acyl transferase	-2.11859
AT4G35250	HCF244	Translation initiation factor	-2.13639
AT4G16690	MES16	Methyl jasmonate esterase; methyl indole-3-acetate esterase; hydrolase	-2.15167
AT4G10030	---	Hydrolase activity	-2.17491
AT3G22640	PAP85	Nutrient reservoir activity	-2.18594
AT3G50560	---	Oxidoreductase	-2.19535
AT1G14290	SBH2	One of two sub-unity sphingoid base hydroxylases	-2.20688
AT4G19530	---	ADP binding; regulation of proton transport	-2.20905
AT5G15410	DND1	Intracellular cAMP activated cation channel	-2.26194
AT1G60140	ATTPS10	Trehalose biosynthetic process	-2.27143
AT1G20630	CAT1	Hydrogen peroxide reductase	-2.28982
AT4G34950	---	glucosinolate biosynthetic process	-2.29455
AT5G21170	AKINBETA1	AMP-activated protein kinase	-2.3008
AT2G29670	---	Unknown	-2.3036
AT5G01740	---	Unknown	-2.31215
AT4G39780	---	Transcription factor responsive to cold	-2.31864
AT2G40300	ATFER4	Oxidoreductase activity; ferric ion binding	-2.32208
AT4G30270	MERI5B	Xyloglucan:xyloglucosyl transferase	-2.32734
AT5G44210	ERF9	Transcription factor responsive to ethylene for oligopeptide transport	-2.33614
AT1G77760	NIA1	Flavin adenine dinucleotide binding; oxidoreductase	-2.34451

AT1G72430	SAUR78	Sterol biosynthetic process	-2.35524
AT5G47220	ERF2	Transcription factor responsive to ET, fungus	-2.36242
AT2G34600	JAZ7	Response to JA; fungal attack	-2.36293
AT4G26530	ATFBA5	Fructose-bisphosphate aldolase	-2.36574
AT1G23870	ATTPS9	Putatively involved in trehalose biosynthesis	-2.36958
AT2G34500	CYP710A1	Heme binding; oxidoreductase activity	-2.37447
AT5G65660	---	Unknown	-2.39843
AT1G12780	UGE1	UDP-glucose epimerase	-2.39909
AT1G77210	ATSTP14	Galactose transmembrane transporter activity	-2.42279
AT2G34770	FAH1	Iron ion binding; oxidoreductase activity	-2.44485
AT5G05440	ATPYL5	ABA binding	-2.49072
AT2G44080	ARL	Response to brassinosteroid	-2.49194
AT2G38240	---	Oxidoreductase activity	-2.5118
AT1G71030	MYBL2	Transcription factor responsive to salt stress	-2.52476
AT1G32170	XTR4	Glycosyl hydrolase; xyloglucosyl transferase	-2.54093
AT3G15850	FAD5	Oxidoreductase activity	-2.54529
AT4G33420	---	Peroxidase; heme binding	-2.56857
AT5G05600	---	Oxidoreductase activity	-2.58591
AT4G15530	PPDK	Ligase; phosphate dikinase activity	-2.60018
AT5G67480	BT4	Transcription cofactor; zinc ion binding; response to auxin, JA, gibberellin	-2.61245
AT1G79700	WRI4	Transcription factor	-2.61665
AT1G80380	---	Glycerate kinase activity	-2.61802
AT2G39705	RTFL8	Fatty acid catabolic process	-2.6588
AT2G42280	AKS3	Transcription factor responsive to arsenic-containing substance	-2.66119
AT4G17245	---	Zinc ion binding	-2.66733
AT1G43670	ATCFBP	Glycolysis fructose 1,6-bisphosphate 1-phosphate activity	-2.67143
AT5G47240	atnudt8	Hydrolase responsive to wounding	-2.67326
AT1G18270	---	Ketose-bisphosphate aldolase class-II family protein for glycolysis	-2.70683
AT2G01860	EMB975	Unknown	-2.7301
AT5G01210	---	Amino-acyl transferase	-2.76084
AT5G52250	EFO1	Nucleotide binding responsive to light; flavonoid biosynthesis	-2.79404
AT2G14170	ALDH6B2	Methylmalonate-semialdehyde dehydrogenase (acylating) activity	-2.82319
AT4G34030	MCCB	Ligase; cobalt ion binding; biotic	-2.8882

		carboxylase	
AT5G55970	---	Zinc ion binding	-2.9027
AT2G33830	---	Unknown	-2.94985
AT4G27410	RD26	Transcription factor responsive to JA, ABA	-2.98244
AT1G22400	UGT85A1	Jasmonic acid signalling	-3.00275
AT5G53980	ATHB52	Transcription factor response to light	-3.00339
AT5G06690	WCRKC1	Electron carrier activity; disulfide oxireductase activity	-3.03192
AT1G25560	TEM1	Cellular cation homeostasis; divalent metal ion transport	-3.0516
AT1G22740	RABG3B	Autophagic cell death	-3.19256
AT5G14120	---	Nitrate transport; cysteine biosynthesis	-3.257
AT5G56100	---	Lipid storage	-3.28945
AT3G60530	GATA4	Transcription factor response to light	-3.34799
AT1G43160	RAP2.6	Transcription factor responsive to wounding	-3.36476
AT1G12520	ATCCS	Copper-zinc superoxide dismutase copper chaperone	-3.39085
AT4G15550	IAGLU	Hexosyl transferase; UDP-glycosyltransferase	-3.39596
AT3G06850	BCE2	Zinc ion binding; dihydrolipoamid branched chain acyltransferase activity	-3.39995
AT5G20230	ATBCB	Copper ion	-3.42022
AT3G49620	DIN11	Oxidoreductase	-3.42316
AT5G20250	DIN10	O-glycosyl transferase	-3.42522
AT3G13450	DIN4	3-methyl-2-oxobutanoate dehydrogenase	-3.4725
AT5G36910	THI2.2	Toxin receptor	-3.51124
AT5G44130	FLA13	Unknown	-3.51764
AT4G33150	LKR	Oxidoreductase; nucleotide binding	-3.52191
AT2G22860	ATPSK2	Growth factor activity	-3.60613
AT4G14130	XTR7	O-glycosyltransferase; xyloglucan:xyloglucosyl transferase	-3.61474
AT1G06570	PDS1	p-hydroxyphenylpyruvate dioxygenase (HPPDase)	-3.65612
AT3G13750	BGAL1	Beta-galactosidase activity; O-glycosyl hydrolase	-3.69984
AT2G37030	SAUR46	Response to auxin	-3.74841
AT4G03510	RMA1	Ubiquitin-protein ligase activity	-3.75164
AT1G49210	---	Ubiquitin-protein ligase activity	-3.75706
AT2G43400	ETFQO	Electron transferring flavoprotein dehydrogenase activity	-3.7911
AT4G36410	UBC17	Ubiquitin-protein ligase activity	-3.81204
AT4G18340	---	O-glycosyl hydrolase; cation binding	-3.95426

AT5G56870	BGAL4	O-glycosyl hydrolase; cation binding	-4.04649
AT4G26260	MIOX4	Inositol oxygenase activity; iron ion binding	-4.07571
AT5G57560	TCH4	Glcosyl hydrolase; xyloglucan:xyloglucosyl transferase	-4.09767
AT4G37610	BT5	Transcription co-factor; histone acetyltransferase; calmodulin binding	-4.14992
AT3G21870	CYCP2;1	Cyclin-dependent protein kinase	-4.1576
AT5G42900	COR27	Response to cold	-4.16759
AT5G44260	ATTZF5	Zino ion binding transcription f actor	-4.25605
AT5G49360	ATBXL1	O-glycosyl hydrolase; alpha-N-arabinofuranosidase	-4.26427
AT2G39980	---	Acyl group transfase activity	-4.29061
AT2G30600	---	Cell adhesion; proximal/distal pattern formation	-4.31087
AT3G13700	---	Nucleic acid binding, RNA	-4.36347
AT1G75750	GASA1	Cell tip growth	-4.36588
AT2G05540	---	Unknown	-4.4362
AT5G39610	ATNAC6	Transcription factor responding to JA,ET,ROS,ABA	-4.4532
AT2G23030	SNRK2.9	Protein serine/threonine kinase	-4.69852
AT3G45300	IVD	Isovaleryl-CoA dehydrogenase;oxidoreductase	-5.23933
AT4G28040	UMAMIT33	Unknown	-5.37134
AT5G45820	CIPK20	Serine/threonine kinase binding	-5.46179
AT3G57520	AtSIP2	O-glycosyl hydrolase; raffinose alpha-galactosidasae	-5.62147
AT5G57550	XTR3	O-glcosyl hydrolase; xyloglucan:xyloglucosyl transferase	-5.66993
AT1G03090	MCCA	Leucine degradation	-5.68113
AT3G48360	BT2	Transcription cofactor activity; calmodulin binding; response to JA	-5.7919
AT3G15500	ANAC055	DNA binding; response to fungus	-5.96384
AT1G35140	PHI-1	Carbon starvation	-6.48827
AT2G17880	DJC24	Heat shock protein binding	-6.55334
AT2G19800	MIOX2	Inositol oxygenase activity; iron ion binding	-6.59306
AT3G30775	ERD5	Proline dehydrogenase	-6.83576
AT1G21910	DREB26	Transcription factor responsive to temperature	-6.91889
AT1G10070	ATBCAT-2	Aminotransferase	-7.1259
AT3G19390	---	Cysteine-type peptidase activity	-7.56505
AT1G76410	ATL8	Determination of bilateral symmetry	-8.93178
AT5G41080	ATGDPD2	Phosphoric diester hydrolase;	-9.15144

		glycerophosphodiester phosphodiesterase	
AT1G62510	---	Lipid transport	-9.66584
AT3G59930	---	Unknown	-10.1924
AT3G47340	ASN1	Asparagine synthase	-10.6728
AT4G37220	---	Response to glucose, sucrose	-11.3582
AT1G08630	THA1	Threonine aldolase to degrade glycine	-16.6906
AT3G62950	---	Disulfied oxidoreductase	-27.202

Appendix 5. Reported induced genes in root tissues.

Reported 323 up-regulated A. thaliana Col-0 root tissue differentially expressed genes with at least 2-fold changes in expression (P-value≤0.005) 8 hours post-inoculation with A. tumefaciens C58. 'AW' indicates A. thaliana Col-0 hydroponic co-cultivation with A. tumefaciens C58; 'A' indicates mock-inoculated A. thaliana Col-0.

Locus Tag	Gene Symbol	Function/Putative Function	Fold-Change
AT4G27670	HSP21	Protein folding responsive to light/ROS/heat	35.8896
AT2G43590	---	Chitinase activity	24.1334
AT1G16030	Hsp70b	ATP binding; response to heat/light/virus/ROS	23.9217
AT1G52560	---	Protein folding in response to heat/light/ROS	23.3771
AT1G07400	---	Response to heat/oxidative stress	21.7988
AT2G41380	---	methyltransferase	19.3001
AT3G24500	MBF1C	Selenium binding	18.2832
AT3G28210	PMZ	Zinc ion binding	17.9177
AT5G37670	---	Protein folding responsive to light/heat/ROS	16.7789
AT1G17170	ATGSTU24	Glutathione transferase/binding	15.9119
AT4G37370	CYP81D8	Oxidreductase; iron ion binding	15.5508
AT2G23170	GH3.3	Indole-3-acetic acid amido synthetase	15.2514
AT1G71000	---	HSP binding in response to light/heat	14.5845
AT5G06860	PGIP1	Polygalacturonase inhibitor	12.5274
AT3G09640	APX2	Peroxidase; heme binding	12.3951
AT2G35980	YLS9	Regulation of ROS metabolism; negative regulation of defense	11.8331
AT1G05680	UGT74E2	UDP-glycosyltransferase; hexosyl transferase	11.3811
AT3G22840	ELIP1	Chlorophyll binding	10.8473
AT2G46240	BAG6	Chaperone binding; calmodulin binding	10.7276
AT1G54050	---	Protein folding in response to heat/light/ROS	10.0314
AT3G54150	---	methyltransferase	9.57213
AT3G09350	Fes1A	HSP70 protein binding	9.55315
AT1G26380	---	FAD binding; UDP-N-acetylmuramate dehydrogenase	9.29824
AT2G26150	ATHSFA2	Positive regulation of transcription in response to light/heat/ROS	8.98188
AT5G64250	---	Nitronate monooxygenase; IMP dehydrogenase	8.91544
AT1G76520	PILS3	Auxin:hydrogen symporter	8.8438
AT1G14540	PER4	Peroxidase; heme binding	8.74663
AT5G12030	AT-HSP17.6A	Responsive to unfolded protein due to heat/light	8.49355

AT1G17180	ATGSTU25	Glutathione transferase	8.33537
AT1G72900	---	ADP binding; responsive to fungus and preventative of cell death	8.18787
AT5G05410	DREB2A	Transcription factor responsive to light/heat/ROS/water deprivation	8.08615
AT1G74310	ATHSP101	Nucleoside-triphosphate activity in response to heat/light/ROS	7.93301
AT3G21720	ICL	Isocitrate lyase	7.8989
AT4G02520	ATGSTF2	Glutathione transferase activity	7.69324
AT2G15490	UGT73B4	Hyxosyl/UDP-glucosyl transferase; quercetin 3-O-glucosyltransferase	7.5953
AT5G59820	RHL41	Transcription factor responsive to wounding/ROS/light/salinity/cold	7.58731
AT5G64120	PRX71	Peroxidase; heme binding	7.57792
AT1G77450	anac032	Transcription regulation of toxic catabolism	7.46134
AT4G30270	MERI5B	Xyloglucan:xyloglucosyl transferase; O-glycosyl transferase	7.3239
AT4G34131	UGT73B2	UDP-glycosyltransferase; hexosyl transferase; quercetin 3-O-glycosyltransferase	7.25787
AT5G07570	---	Unknown	7.10518
AT1G01720	ATAF1	Transcription factor responsive to JA/ABA	7.02467
AT1G53540	---	Protein folding in response to heat/light/ROS	6.93922
AT5G64260	EXL2	Response to arsenic-containing substance	6.83995
AT3G28740	CYP81D1	Oxygen binding; oxidoreductase	6.69623
AT4G20070	ATAAH	Metallopeptidase; allantoate deiminase; carbon-nitrogen hydrolase	6.65251
AT1G67810	SUFE2	Enzyme activator for sulfur metabolism	6.62926
AT2G32120	HSP70T-2	ATP binding response to heat/light	6.61627
AT3G55090	ABCG16	ATPase activity; nucleotide binding	6.61187
AT1G55530	---	Zinc ion binding	6.61073
AT3G22370	AOX1A	Alternate oxidase	6.56036
AT1G59500	GH3.4	Indole-3-acetic acid amido synthetase	6.54002
AT2G26560	PLA2A	Nutrient reservoir activity; lipase activity	6.45512
AT1G57980	ATPUP18	Purine nucleobase transmembrane transporter activity	6.39702
AT1G69920	ATGSTU12	Glutathione transferase	6.37763
AT3G22910	---	Metal ion binding; calcium-transporting ATPase activity	6.34193
AT1G64590	---	Oxidoreductase	6.26659
AT1G60730	---	Aldo-keto reductase (NADP)	6.02789
AT3G46230	HSP17.4	Calcium ion binding	5.95804
AT2G40340	ATERF48	Transcription factor responsive to ABA	5.71755
AT1G02930	ATGSTF6	Copper/camalexin/cobalt ion binding	5.6337

AT1G26390	---	UDP-N-acetylmuramate dehydrogenase; FAD binding	5.57189
AT1G18970	GLP4	Nutrient reservoir activity; transition metal binding	5.29893
AT4G01870	---	Toxin catabolism; proteolysis in response to ABA/ET	5.27926
AT2G20560	---	HSP binding; unfolded protein in response to light/heat/ROS	5.24252
AT1G06430	FTSH8	ATP binding; ATPase activity; zinc binding; metalloendopeptidase	5.24027
AT5G57220	CYP81F2	Iron ion binding; oxidoreductase	5.20586
AT3G53230	ATCDC48B	Positive regulation of protein catabolism	5.12803
AT2G37540	---	Oxidoreductase	5.10052
AT1G33110	---	Drug antiporter	5.03114
AT5G26920	CBP60G	Negative regulator of defense response; SA biosynthesis	4.97215
AT3G61630	CRF6	Transcription factor for leaf/cotyledon development	4.94897
AT2G43000	anac042	Negative regulator of leaf senescence	4.91828
AT5G39580	---	Peroxidase; heme binding	4.91344
AT1G14200	---	Zinc ion binding; protein folding	4.90207
AT5G12020	HSP17.6II	Protein folding responsive to heat/light	4.89005
AT3G26830	PAD3	Monooxygenase;oxidoreductase; dihydrocamalexic acid decarboxylase	4.80184
AT5G58070	TIL	Transporter responsive to cold/heat/light	4.76754
AT1G08430	ALMT1	Malate transmembrane transporter	4.68606
AT5G14760	FIN4	Oxidoreductase; L-aspartate oxidase	4.63431
AT5G50760	SAUR55	Nitrate transport in response to auxin	4.60409
AT5G02780	GSTL1	Protein glutathionylation	4.53553
AT1G03070	ATLFG4	Glutamate binding	4.43321
AT4G13420	HAK5	Potassium ion transport; potassium:sodium symporter	4.41267
AT4G25380	SAP10	DNA binding responsive to nickel/col/heat/ROS/zinc/manganese	4.36616
AT2G30140	UGT87A2	Hexosyl transferase; glycosyl transferase;	4.35234
AT3G28580	---	Nucleoside-triphosphate activity; nucleotide binding	4.31856
AT3G47780	ATH6	Nucleotide-triphosphate activity; ATPase activity	4.30135
AT1G32940	SBT3.5	Serine-type endopeptidase	4.25176
AT1G69930	ATGSTU11	Galactolipid biosynthesis; glutathione tranferase	4.24264
AT5G08250	---	Oxidoreductase; heme binding	4.23089
AT1G72060	---	Serine-type endopeptidase	4.20207
AT5G43450	---	Oxidoreductase; 1-aminocyclopropane-1-	4.19297

		carboxylate oxidase	
AT3G62260	---	Serine/threonine phosphatase	4.18823
AT4G31140	---	O-glycosyl hydrolase	4.16275
AT1G64950	CYP89A5	Heme binding; oxidoreductase	4.16061
AT3G14200	---	Heat shock protein binding	4.13868
AT4G13180	---	Oxireductase; nucleotide binding	4.11315
AT5G63790	ANAC102	Transcription factor responsive to unfolded protein response	4.10657
AT4G38420	sks9	Oxidoreductase; copper ion binding	4.08849
AT3G60420	---	Negative regulation of defense response	4.08233
AT3G50260	CEJ1	Transcription factor responsive to salinity/wounding/temperature	4.04328
AT3G01420	DOX1	Lipoxygenase; peroxidase	4.02665
AT5G64900	PROPEP1	Cell surface protein binding responsive to ET/JA/wounding	4.0144
AT3G44190	---	Xyloglucan-specific endo-beta-1,4-glucanase; glycosyl hydrolase	3.97832
AT1G51340	---	Drug antiporter	3.9677
AT5G53970	TAT7	L-tyrosine:2-oxoglutarate aminotransferase	3.91818
AT3G08970	ATERDJ3A	HSP binding; unfolded protein in response to light/heat/ROS	3.89602
AT4G20830	---	FAD binding; UDP-N-acetylmuramate dehydrogenase; oxidoreductase	3.88336
AT5G17860	CAX7	Cation:sodium ion transporter	3.87806
AT5G18470	---	Carbohydrate binding responsive to karrikin	3.87106
AT1G57630	---	Amino acid import; SA biosynthesis/signaling; JA signaling	3.86487
AT1G07350	SR45A	RNA splicing in response to light	3.82562
AT2G30250	WRKY25	Transcription factor responsive to heat/cold/insect	3.75205
AT5G20230	ATBCB	Copper ion binding responsive to wounding/ROS/light	3.74197
AT2G18660	EXLB3	Alternative respiration; SAR	3.70956
AT3G47480	---	Chitinase	3.6709
AT4G08950	EXO	Plant-type cell wall; sterol biosynthesis	3.66151
AT1G30040	ATGA2OX2	Iron ion binding; oxidoreductase	3.64528
AT3G16530	---	Carbohydrate binding	3.64498
AT3G15450	---	Response to sucrose; unknown	3.62338
AT2G47890	---	Transcription factor responsive to wounding; flavonoid biosynthesis	3.59955
AT1G55920	ATSERAT2;1	Serine O-acetyltransferase	3.59313
AT1G10170	ATNFXL1	Transcription factor responsive to salt/light; zinc binding	3.54657
AT5G01600	FER1	Oxidoreductase; ferric iron binding	3.53523

AT1G15380	---	Lactoylglutathione lyase	3.52913
AT4G20000	---	Unknown	3.52022
AT1G02850	BGLU11	O-glycosyl hydrolase; cation binding	3.51692
AT5G57560	TCH4	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	3.45149
AT2G29460	ATGSTU4	Glutathione transferase; toxin catabolism	3.44004
AT2G19310	---	Response to heat/ROS/light; unknown	3.41635
AT5G08350	---	Unknown	3.40573
AT4G27940	MTM1	Mitochondrial transport; unknown	3.38989
AT1G70170	MMP	Metallopeptidase; zinc ion binding	3.38691
AT1G72680	CAD1	Oxidoreductase; cofactor in nucleotide binding	3.38082
AT2G37970	SOUL-1	Flavonoid biosynthesis in response to light	3.37229
AT2G23150	NRAMP3	Manganese ion transporter/inorganic anion transporter	3.34673
AT1G62300	WRKY6	Transcription factor responsive to fungal; toxin metabolism	3.34365
AT5G16010	---	3-oxo-5-alpha-steroid 4-dehydrogenase; oxidoreductase	3.30977
AT5G49130	---	Drug antiporter	3.30738
AT3G63310	BIL4	Glutamate binding	3.29636
AT2G32020	---	N-acetyltransferase	3.28629
AT3G09440	---	ATP binding response to heat/light	3.24158
AT1G68850	---	Peroxidase; heme binding	3.22903
AT4G21990	APR3	Adenylyl-sulfate reductase; oxidoreductase	3.22695
AT5G13900	---	Lipid transport/binding	3.21673
AT5G47560	TDT	Malate transporter; sodium:dicarboxylate symporter	3.20717
AT5G18170	GDH1	Glutamate dehydrogenase [NAD(P)+]; oxidoreductase	3.20449
AT5G20910	AIP2	Ubiquitin-protein ligase; zinc ion binding	3.20099
AT3G14990	ATDJ1A	Thiamine biosynthesis; catalytic activity	3.18019
AT2G46600	---	Calcium ion binding; protein binding	3.17855
AT4G09150	---	Phosphopantetheine binding	3.17123
AT3G49580	LSU1	Unknown	3.16241
AT2G22240	MIPS2	Inositol-3-phosphate synthase	3.1608
AT5G38900	---	Disulfide oxidoreductase	3.1509
AT3G62150	PGP21	Nucleoside triphosphate activity; ATPase activity	3.14772
AT4G25810	XTR6	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	3.13915
AT4G32940	GAMMA-VPE	Cysteine-type endopeptidase	3.09321
AT5G11260	HY5	Transcription factor responsive to light	3.08535

		regulation of cell proliferation	
AT3G25190	ATVTL5	Response to iron ion, nitric oxide, ethylene	3.06649
AT4G21580	---	Oxidoreductase; zinc ion binding responsive to fungus	3.06166
AT5G51830	---	Ribokinase; D-ribose metabolism	3.06061
AT2G47180	AtGolS1	Inositol 3-alpha-galactosyltransferase; hexosyl transferase	3.04968
AT4G34710	ADC2	Arginine decarboxylase	3.03866
AT1G55020	LOX1	Lipoxygenase	3.03724
AT4G28390	AAC3	ATP:ADP antiporter	3.03465
AT4G00080	UNE11	Pectinesterase; enzyme inhibitor	3.02248
AT5G38895	---	Zinc ion binding responsive to auxin/ROS/ABA	3.01654
AT5G06320	NHL3	Unknown	2.98715
AT4G22530	---	Methyltransferase	2.96842
AT3G19580	AZF2	Zinc ion binding; transcription factor responsive to wounding	2.96717
AT2G47520	HRE2	Transcription factor responsive to anoxia	2.9521
AT1G22985	CRF7	Transcription regulation responsive to ethylene	2.93195
AT1G11330	---	Serine/threonine kinase; carbohydrate/zinc binding	2.91625
AT1G32870	ANAC13	Transcription factor responsive to light; galactolipid biosynthesis	2.89459
AT5G47070	---	Serine/threonine kinase	2.881
AT3G12580	HSP70	Ubiquitin-protein ligase	2.87352
AT3G11430	GPAT5	Organic anion transporter; acyl transferase	2.86817
AT4G21980	APG8A	APG8 ligase; APG8-specific protease/activating enzyme	2.86793
AT1G63800	UBC5	Acid-amino acid ligase; ubiquitin-protein ligase activity	2.85324
AT1G72660	---	GTP binding in response to light/heat	2.85115
AT3G13610	F6'H1	Oxidoreductase in response to wounding	2.83694
AT4G15550	IAGLU	Hexosyl transferase; UDP-glycosyltransferase	2.83282
AT3G47540	---	ATP binding; transporter activity; toxic catabolism	2.82711
AT2G29990	NDA2	FAD binding; oxidoreductase; NADH dehydrogenase	2.82638
AT3G18250	---	Amino acid import	2.81123
AT3G07700	ATSIA1	Phosphorus transfer	2.78857
AT4G29070	---	Unknown	2.78491
AT5G64870	---	Response to chitin mediated by ET	2.75696
AT1G17960	---	Threonine-tRNA ligase; aminoacyl-tRNA ligase; ATP binding	2.7521

AT3G63380	ACA12	Calmodulin binding; calcium ion transport	2.74001
AT3G07370	CHIP	Ubiquitin-protein ligase	2.72502
AT2G03760	ST	Brassinosteroid sulfotransferase	2.70909
AT4G03320	tic20-IV	Toxin catabolism	2.67967
AT5G48850	ATSDI1	Regulation of sulfur utilization in response to starvation	2.6725
AT1G50250	FTSH1	Nucleoside-triphosphate activity; metalloendopeptidase	2.66234
AT3G50930	BCS1	ATP binding; ATPase activity	2.66102
AT5G53588	CPuORF50	Unknown	2.61441
AT3G55840	---	Ethylene biosynthesis	2.60835
AT1G67360	---	Unknown	2.59821
AT5G03240	UBQ3	Ubiquitin dependent protein catabolic process	2.59535
AT5G49350	---	Unknown	2.59489
AT2G32150	---	Hydrolase	2.59171
AT2G24600	---	Unknown	2.58749
AT5G39670	---	Calcium ion binding	2.58145
AT3G01290	ATHIR2	Negative regulator of programmed cell death	2.56092
AT1G60610	---	Zinc ion binding	2.55819
AT4G10955	UGE5	Triglyceride lipase	2.55185
AT4G15120	---	Unknown	2.5455
AT2G15480	UGT73B5	Hyxosyl/UDP-glucosyl transferase; quercetin 3-O-glucosyltransferase	2.52889
AT2G29420	ATGSTU7	Glutathione transferase; toxin catabolism	2.52222
AT2G24180	CYP71B6	Oxidoreductase; heme binding	2.50678
AT4G30280	XTH18	Xyloglucan:xyloglucosyl transferase; O-glycosyl transferase	2.50609
AT5G01990	PILS6	Auxin:hydrogen symporter	2.48552
AT5G11520	ASP3	Pyridoxal phosphate binding; L-aspartate:2-oxoglutarate aminotransferase	2.48266
AT2G34660	ATMRP2	Nucleoside-triphosphatase activity; ATPase activity	2.46156
AT4G25200	ATHSP23.6-MITO	Protein folding responsive to light/ROS/cadmium/heat	2.4611
AT2G36670	---	Aspartic-type endopeptidase	2.45495
AT3G55470	---	Positive regulation of flavonoid biosynthesis	2.45263
AT2G26480	UGT76D1	quercetin 7-O-glucosyltransferase; UDP glycosyl transferase	2.44041
AT1G03850	ATGRXS13	Disulfide oxidoreductase	2.43441
AT4G22340	CDS2	Phosphatidate cytidylyltransferase; phosphorus transferase activity	2.43434
AT5G64060	anac103	Transcription factor responsible for organismal development	2.43108

AT2G34770	FAH1	Fatty acid alpha-hydroxylase; oxidoreductase; iron ion binding	2.43008
AT5G54080	HGO	Homogentisate 1,2-dioxygenase	2.42041
AT5G62480	ATGSTU9	Glutathione transferase; toxin catabolism	2.39403
AT1G74460	---	Hydrolase; carboxylesterase	2.39027
AT3G48450	---	Response to nitrate	2.38106
AT5G52450	---	Drug antiporter	2.38001
AT3G57520	AtSIP2	O-glycosyl hydrolase; raffinose alpha-galactosidase	2.37886
AT5G47635	---	Unknown	2.37535
AT5G51440	---	Protein folding responsive to light/heat/ROS	2.37408
AT4G27830	BGLU10	O-glycosyl hydrolase; cation binding	2.35158
AT5G14780	FDH	Co-factor binding; oxidoreductase	2.35005
AT1G14040	PHO1;H3	Phosphate ion transport; phosphate starvation	2.34788
AT2G16060	AHB1	Heme binding; oxidoreductase	2.34737
AT4G38540	---	Monooxygenase; oxidoreductase; toxin catabolism	2.34717
AT4G33040	---	Disulfide oxidoreductase activity	2.33645
AT3G57380	---	Glycosyl transferase	2.33509
AT5G19440	---	Alcohol dehydrogenase (NAD) activity	2.33281
AT5G64220	CAMTA2	Transcription regulation by calmodulin binding	2.3245
AT5G47990	CYP705A5	Thalian-diol desaturase; iron ion binding	2.31955
AT2G17570	CPT1	Alkyl/aryl transferase	2.31859
AT5G27760	---	Response to hypoxia; unknown	2.31462
AT4G24020	NLP7	Transcription factor responsive to nitrate/water deprivation	2.31173
AT2G41100	TCH3	Calcium binding	2.31029
AT1G15170	---	Drug antiporter	2.30877
AT2G44080	ARL	Cell growth responsive to brassinosteroid	2.29985
AT3G55430	---	O-glycosyl hydrolase	2.29793
AT1G59870	PEN3	Nucleoside-triphosphate activity; cadmium ion transporter	2.29703
AT3G08990	---	Unknown	2.28913
AT4G13010	---	Oxireductase; zinc ion/nucleotide binding	2.28653
AT1G30620	MUR4	UPD-glucose 4-epimerase; UPD-arabinose 4 epimerase; coenzyme	2.27611
AT3G57070	---	Disulfide oxidoreductase activity	2.276
AT4G05020	NDB2	FAD binding; disulfide oxidoreductase activity	2.27291
AT4G14690	ELIP2	Flavonoid biosynthesis; response to light/sucrose	2.27216
AT3G19390	---	Cysteine-type peptidase	2.27116

AT5G25450	---	Ubiquinol-cytochrome-c reductase	2.26041
AT3G22160	JAV1	Protein targeting; response to ABA/SA	2.24631
AT1G30660	---	Nucleotide binding	2.24557
AT3G10500	anac053	Transcription factor responsive for drought recovery; toxin catabolism	2.24423
AT5G48180	NSP5	Ubiquitin-dependent protein catabolic process; toxin catabolism	2.2386
AT4G11600	ATGPX6	Glutathione peroxidase	2.23223
AT1G32350	AOX1D	Alternate oxidase	2.22872
AT2G31570	ATGPX2	Glutathione peroxidase	2.2286
AT2G40000	HSPRO2	Protein binding in response to light,SA,chitin,ROS,bacteria	2.2263
AT1G51950	IAA18	Transcription regulation in response to JA/auxin/brassinosteroid	2.21902
AT3G04640	---	Protein acetylation in response to ET, wounding, chitin	2.21614
AT3G29810	COBL2	Glutathione transferase activity	2.21075
AT4G35420	DRL1	Catalytic activity; seed/pollen exine formation	2.20874
AT4G02940	---	Oxidoreductase	2.20234
AT5G54840	SGP1	GTP binding; small GTPase mediated signal transduction	2.19847
AT4G26470	---	Calcium ion binding; toxin catabolism	2.19723
AT1G22930	---	Unknown	2.19477
AT1G11100	FRG5	Helicase; zinc ion binding	2.19347
AT1G35230	AGP5	Unknown	2.18414
AT2G31260	APG9	Toxin catabolism; ubiquitin-dependent protein catabolism	2.179
AT2G20142	---	Signal transduction; unknown	2.17859
AT1G33590	---	Negative regulator of defense; transcription regulation of cell wall	2.17153
AT3G53510	ABCG20	ATPase activity; nucleotide binding	2.15957
AT1G78820	---	Carbohydrate binding	2.15942
AT4G36610	---	Hydrolase	2.15805
AT4G18360	GOX3	glycolate oxidase; oxidoreductase	2.15753
AT3G26470	---	Toxin catabolism	2.15718
AT4G24690	NBR1	Ubiquitin binding	2.15704
AT4G33940	---	Intracellular signal transduction to ABA/auxin/chitin/ROS/carbohydrate	2.15631
AT5G03030	---	Heat shock protein binding	2.14346
AT1G10050	---	O-glycosyl hydrolase; cation binding; endo-1,4-beta-xylanase	2.14335
AT1G53670	MSRB1	Peptide-methionine (S)-S-oxide reductase activity	2.13502
AT3G17611	ATRBL14	Zinc ion binding; serine-type endopeptidase	2.13237

AT5G40850	UPM1	Precorrin-2 dehydrogenase; uroporyphyrin-III C-methyltransferase	2.12892
AT3G23150	ETR2	2-component response regulator; ethylene responsive receptor	2.12825
AT5G11670	ATNADP-ME2	Oxidoreductase; malate dehydrogenase; malic enzyme activity	2.12243
AT1G29400	AML5	RNA binding	2.11972
AT3G22200	POP2	4-aminobutyrate; transaminase	2.11553
AT3G03270	---	Response to molecule of fungal origin	2.11233
AT3G17690	CNGC19	Ion channel activity; cyclic nucleotide binding; calmodulin binding	2.0979
AT3G54420	ATEP3	Chitinase	2.08912
AT4G19030	NLM1	Arsenite/water transport	2.08487
AT1G73500	MKK9	Protein kinase activator; serine/threonine kinase	2.07805
AT5G20380	PHT4;5	Nitrate transport	2.07315
AT2G16900	---	Callose deposition in response to wounding	2.07286
AT1G78780	---	Unknown	2.07092
AT1G69490	NAP	Transcription factor for floral development/senescence	2.06991
AT1G06840	---	Tyrosine kinase; serine/threonine kinase	2.06913
AT3G09020	---	Glycosyl transferase; galactosyltransferase	2.05995
AT1G65610	KOR2	O-glycosyl hydrolase	2.05969
AT2G23320	WRKY15	Transcription factor regulating ET biosynthesis/chitin response/ROS	2.05196
AT1G67820	---	Serine/threonine phosphatase	2.04962
AT3G50400	---	Hydrolase acting on ester bonds	2.0471
AT3G11020	DREB2B	Transcription factor responsive to water deprivation	2.04366
AT4G26270	PFK3	6-phosphofructokinase; ATP binding	2.0371
AT1G01560	ATMPK11	Serine/threonine kinase	2.03517
AT1G54100	ALDH7B4	Oxidoreductase activity; 3-chloroallyl aldehyde dehydrogenase	2.03202
AT4G15230	PDR2	ATPase; nucleoside-triphosphate activity	2.03032
AT1G55510	BCDH BETA1	3-methyl-2-oxobutanoate dehydrogenase	2.0275
AT5G37690	---	Lipid hydrolase	2.0262
AT2G44100	ATGDI1	RAB GDP-dissociation inhibitor activity	2.02576
AT3G07560	PEX13	Lipid transport	2.02333
AT5G47880	ERF1-1	Translation release factor	2.01512
AT4G11280	ACS6	1-aminocyclopropane-1-carboxylate synthase; pyridoxal phosphate binding	2.00714
AT2G02390	ATGSTZ1	Glutathione transferase	2.00658
AT4G35985	---	Unknown	2.00201

Appendix 6. Reported repressed transcripts in root tissues.

Reported 226 down-regulated A. thaliana Col-0 root tissue differentially expressed genes with at least 2-fold changes in expression (P-value≤0.005) 8 hours post-inoculation with A. tumefaciens C58. 'AW' indicates A. thaliana Col-0 hydroponic co-cultivation with A. tumefaciens C58; 'A' indicates mock-inoculated A. thaliana Col-0.

Locus Tag	Gene Symbol	Function/Putative Function	Fold-Change
AT3G43800	ATGSTU27	Oxidoreductase; FAD binding	-2.00645
AT1G03870	FLA9	Unknown	-2.01227
AT2G41660	MIZ1	Chloroplast organization	-2.02175
AT3G62390	TBL6	Unknown	-2.02489
AT1G50560	CYP705A25	Iron ion binding; oxidoreductase	-2.02567
AT5G23010	MAM1	2-(2'metyhlthio)ethylmalate synthase; acyl transferase	-2.0295
AT4G01240	---	methyltransferase	-2.04259
AT3G02885	GASA5	Response to SA/GA for heat acclimation	-2.05244
AT5G05880	---	UDP-glycosyltransferase; hexosyl transferase	-2.06253
AT3G62600	ATERDJ3B	HSP binding; unfolded protein binding	-2.06528
AT2G43100	IPMI2	Hydrolase	-2.06605
AT4G25010	SWEET14	Sucrose transport	-2.07828
AT1G13830	---	Unknown	-2.08866
AT1G70690	HWI1	Autophagy in response to bacterium	-2.10187
AT4G21830	ATMSRB7	Peptide-methionine (S)-S-oxide reductase activity	-2.10272
AT2G41560	ACA4	Calcium-transporting ATPase	-2.10795
AT1G18250	ATLP-1	Cell proliferation (anaphase)	-2.10971
AT4G21600	ENDO5	Ester hydrolase; T/G mismatch-spec. endonuclease; nitrate/iron trans.	-2.11444
AT3G55700	---	UDP-glycosyltransferase; hexosyl transferase	-2.11943
AT3G15950	NAI2	Peroxisome	-2.12079
AT3G06990	---	Disulfide reductase activity; zinc ion binding	-2.12239
AT2G37440	---	Hydrolase	-2.1239
AT2G35210	RPA	ARF GTPase activator; zinc ion binding	-2.12787
AT4G36360	BGAL3	Beta-galactosidase; lactose catabolism; O-glycosyl transferase	-2.13146
AT1G66440	---	Disulfide reductase activity	-2.144
AT1G11540	---	Nite transport	-2.14907
AT2G21830	---	Disulfide reductase activity; zinc ion binding	-2.15003
AT4G12420	SKU5	Oxireductase for development; anthocyanin	-2.15108

		accumulation	
AT5G26660	ATMYB86	Negative regulation of transcription; Unknown	-2.15212
AT1G09210	CRT1b	Calcium ion binding; unfolded protein binding	-2.15987
AT3G52500	---	Aspartic-type endopeptidase	-2.16236
AT2G40230	---	Amino-acyl transferase	-2.16855
AT2G36870	XTH32	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	-2.1701
AT4G32830	AtAUR1	Histidine phosphorylase	-2.17737
AT2G44380	---	Disulfide reductase activity	-2.18106
AT2G40460	---	Oligopeptide transport	-2.18789
AT5G61790	CNX1	Calcium ion binding; unfolded protein binding	-2.1947
AT2G03090	ATEXPA15	Plant-type cell wall loosening	-2.196
AT3G12500	ATHCHIB	Chitinase; nitrate transport	-2.19803
AT3G16450	JAL33	Response to zinc ion	-2.1985
AT5G67100	ICU2	Nucleoside binding; DNA-directed DNA polymerase activity	-2.20666
AT1G23205	---	Pectinesterase inhibitor	-2.22551
AT2G39220	PLP6	Nutrient reservoir; lipid metabolism	-2.2289
AT2G22930	---	Hexosyl transferase; glycosyl transferase	-2.23095
AT1G44575	NPQ4	Xanthophyll/chlorophyll binding	-2.23243
AT1G23040	---	Nutrient reservoir activity	-2.24248
AT2G42570	TBL39	Unknown	-2.24312
AT1G70890	MLP43	Divalent metal ion transport; cation homeostasis	-2.25639
AT2G43530	---	Ion channel inhibitor	-2.25837
AT1G49320	USPL1	Seed development	-2.26009
AT4G25050	ACP4	ACP Phosphopantetheine binding for fatty acid biosynthesis	-2.26646
AT5G19240	---	Tryptophase catabolism; indoleacetic acid biosynthesis	-2.27084
AT4G10380	NIP5;1	Water/borate transport	-2.27468
AT2G43820	UGT74F2	Glucosyltransferase	-2.28101
AT5G42100	BG_PPAP	O-glycosly hydrolase; glucan endo-1,3-beta-D-glucosidase	-2.28158
AT4G12880	ENODL19	Copper ion binding	-2.29046
AT1G24530	---	Nucleotide binding; oligopeptide transport	-2.29354
AT1G74500	BS1	Transcription factor for brassinosteroid signaling	-2.29484
AT3G54220	SCR	Transcription factor responsive to gravitropism	-2.30237
AT5G23840	---	Unknown	-2.30471

AT1G08560	SYP111	SNAP receptor activity	-2.30721
AT1G05210	---	Unknown	-2.31135
AT3G54560	HTA11	DNA binding, regulation of plant development	-2.31157
AT1G22550	---	Oligopeptide transport	-2.32805
AT4G38970	FBA2	fructose-bisphosphate aldolase	-2.33269
AT3G51280	---	Cell cycle regulation	-2.34215
AT5G56040	SKM2	Tyrosine kinase; serine/threonine kinase	-2.35046
AT5G15230	GASA4	Cell redox homeostasis responsive to GA	-2.35121
AT4G11210	---	lignin biosynthesis; root hair cell differentiation	-2.35183
AT5G14060	AK3	Amino acid binding; aspartate kinase	-2.36095
AT1G20020	FNR2	NADP dehydrogenase/flavin adenine dinucleotide reductase	-2.37195
AT4G04830	ATMSRB5	Peptide-methionine (S)-S-oxide reductase activity	-2.37282
AT3G20015	---	Aspartic-type endopeptidase	-2.3767
AT3G20820	---	Defense response signal transduction	-2.39751
AT2G31790	---	Hexosyl transferase; glycosyl transferase; UDP-glycosyl transferase	-2.40622
AT2G40900	UMAMIT11	Unknown	-2.42185
AT3G50300	---	Acyl transferase	-2.43695
AT4G33260	CDC20.2	Signal transduction for mitotic processes; heterotrimeric G-protein	-2.45548
AT2G28160	FRU	Transcription factor responsive to ET, Cyt,ROS	-2.46432
AT1G72200	---	Zinc ion binding	-2.48333
AT1G21440	---	Isocitrate lyase; glucosinolate biosynthesis	-2.4876
AT1G80050	APT2	Phosphate ion transport; adenine phosphoribosyltransferase	-2.48933
AT2G30210	LAC3	Oxidoreductase; hydroquinone:oxygen; copper ion	-2.49032
AT1G80830	NRAMP1	Cadmium/manganese ion transport	-2.49312
AT3G48740	SWEET11	Sugar transport	-2.52271
AT5G60890	MYB34	Transcription factor responsive to JA/sulfur/insect	-2.53316
AT1G03410	2A6	Oxidoreductase	-2.53446
AT1G48500	JAZ4	Protein binding in response to wounding/JA	-2.53735
AT3G11520	CYCB1;3	Protein kinase	-2.54229
AT3G02610	---	Acyl-[acyl-carrier-protein] desaturase; transition metal ion binding	-2.56804
AT5G48290	---	Metal ion transport	-2.57244
AT1G70370	PG2	Polygalacturonase for cellular biogenesis	-2.6034
AT1G12090	ELP	Lipid transport	-2.61274

AT5G17700	---	Drug antiporter	-2.61326
AT1G23750	---	Unknown	-2.61647
AT1G56010	NAC1	Transcription factor regulating organ development	-2.62516
AT3G27020	YSL6	Oligopeptide transport	-2.63894
AT3G54770	ARP1	Nucleic acid binding for mRNA splicing	-2.64275
AT2G01610	---	Pectinesterase inhibitor	-2.6447
AT1G56430	NAS4	Nicotianamine synthase	-2.6564
AT2G45960	PIP1B	Water transporter activity responsive to hyperosmotic stress	-2.65687
AT5G47500	PME5	Pectinesterase	-2.6571
AT3G26520	TIP2	Water transport	-2.6597
AT5G50375	CPI1	Cycloeucalenol cycloisomerase	-2.68743
AT1G11545	XTH8	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	-2.70426
AT5G44480	DUR	Nucleotide binding; UDP-glucose 4-epimerase	-2.71447
AT2G20750	ATEXPB1	Plant-type cell wall loosening	-2.7186
AT5G24410	PGL4	6-phosphogluconolactonase	-2.72536
AT4G25260	---	Pectinesterase inhibitor activity	-2.74463
AT1G62560	FMO GS-OX3	FAD binding; 3/4/5/6/8-methylthiopropyl glucosinolate S-oxygenase	-2.74766
AT2G28670	ESB1	Suberin biosynthesis	-2.75943
AT4G23400	PIP1;5	Water transport	-2.76338
AT5G20950	---	O-glycosyl transferase	-2.76458
AT2G15970	COR413-PM1	Response to water/cold/ABA/salt stress	-2.76707
AT5G17820	---	Peroxidase; heme binding	-2.78182
AT5G04970	---	Pectinesterase activity	-2.79418
AT1G15670	KFB01	Phenylpropanoid metabolic process	-2.80584
AT3G54820	PIP2;5	Water transport	-2.82549
AT3G51600	LTP5	Lipid transport	-2.86886
AT2G19990	PR-1-LIKE	Unknown	-2.901
AT5G07990	TT7	Oxidoreductase; flavonoid 3'-monooxygenase; iron ion binding	-2.91185
AT3G10040	HRA1	Transcription factor for organ development	-2.91826
AT4G12470	AZI1	Lipid transport; nitrate transport; amino acid transport	-2.92812
AT5G50740	---	Metal ion binding/transport	-2.94595
AT4G30460	---	Unknown	-2.96575
AT5G57540	XTH13	Xyloglucan:xyloglucosyl transferase; O-glycosyl transferase	-2.96671
AT4G12480	pEARLI 1	Lipid transport; response to salt stress/fungus/col	-2.98839

AT5G45340	CYP707A3	Oxireductase; (+)-abscisic acid 8'-hydroxylase; iron ion binding	-2.99992
AT4G22212	---	Transition metal ion transport for defense response	-3.00065
AT1G08590	PXL1	Serine/threonine kinase for stamen development	-3.01082
AT1G30370	DLAH	Phosphatidylcholine 1-acylhydrolase; triglyceride lipase	-3.03207
AT4G23496	SP1L5	Unknown	-3.09356
AT4G29690	---	Hydrolase	-3.11803
AT5G66690	UGT72E2	Hexosyl transferase; UDP-glycosyltransferase	-3.12958
AT1G05260	RCI3	Peroxidase; heme binding	-3.1354
AT3G58120	BZIP61	Transcription factor responsive to xenobiotics	-3.15428
AT4G14980	---	Disulfide reductase	-3.17203
AT5G52310	LTI78	Leaf senescence responsive to cold/desiccation/salinity	-3.19218
AT5G23660	MTN3	Sucrose transporter	-3.20135
AT3G14680	CYP72A14	Oxidoreductase; transition metal ion transport	-3.21134
AT2G23050	NPY4	Gravitropism/light signal transducer	-3.21239
AT2G41290	SSL2	Strictosidine synthase; myo-inositol hexakisphosphate biosynthesis	-3.21281
AT4G02850	---	Circadian rhythm	-3.24531
AT2G18800	XTH21	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	-3.26648
AT4G16980	---	Water transport responsive to salt/fructose/light	-3.28309
AT5G60530	---	Transition metal ion transport	-3.28968
AT1G64780	ATAMT1;2	Ammonium transporter	-3.29606
AT1G67110	CYP735A2	Oxidoreductase; iron ion binding	-3.31512
AT3G23800	SBP3	Nitrate transport; iron ion transport	-3.3689
AT2G26690	---	Oligopeptide transport	-3.38995
AT4G31470	---	Unknown	-3.40173
AT2G24762	AtGDU4	Regulation of amino acid export	-3.41355
AT1G12080	---	Acetyl-CoA metabolism	-3.42785
AT1G78090	ATTPPB	Trehalose-phosphate	-3.43265
AT3G55150	ATEXO70H1	Vesicle docking involved in exocytosis	-3.4342
AT3G23190	---	2-component sensor; ethylene binding; histidine kinase	-3.48944
AT4G28250	ATEXPB3	Plant-type cell wall loosening for sexual reproduction	-3.49362
AT4G29340	PRF4	Actin binding for cytoskeleton organization	-3.51093

AT3G50740	UGT72E1	Glycosyl transferase; hexosyl transferase; UDP-glycosyltransferase	-3.6204
AT2G36830	GAMMA-TIP	Water transporter activity responsive to ABA	-3.71441
AT2G36100	CASP1	Protein homodimerization; cell wall modification	-3.73427
AT5G56540	AGP14	Protein binding for root hair elongation	-3.75152
AT5G48430	---	Oligopeptide transport	-3.77541
AT1G78370	ATGSTU20	Glutathione transferase	-3.80306
AT5G25830	GATA12	Transcription factor responsive to light for circadian rhythm regulation	-3.84851
AT4G30170	---	Peroxidase; heme binding	-3.92224
AT1G52060	---	Unknown	-3.93465
AT2G37170	PIP2;2	Water transporter activity responsive to ABA	-3.9988
AT3G50660	DWF4	Oxidoreductase; heme binding	-4.08634
AT3G44990	XTR8	Response to heat	-4.11898
AT4G13580	---	Unknown	-4.23076
AT4G37540	LBD39	Sterol biosynthesis	-4.24966
AT2G37130	---	Peroxidase; heme binding	-4.25735
AT4G35030	---	Serine/threonine kinase	-4.29286
AT1G75780	TUB1	GTP binding in organ development	-4.30379
AT3G62040	---	Hydrolase activity; nitrate transport	-4.32248
AT1G49860	ATGSTF14	Glutathione transferase	-4.51206
AT4G31910	BAT1	Acyl-CoA ligase; iron ion/nitrate transport	-4.56468
AT4G15390	---	Acyl transferase; transition metal ion transport	-4.6263
AT1G48930	AtGH9C1	O-glycosyl hydrolase; carbohydrate binding	-4.6541
AT3G54700	PGT1;7	Inorganic phosphate transporter; sugar:hydrogen symporter	-4.65562
AT1G62980	ATEXPA18	Trichoblast/root hair differentiation; plant type cell wall loosening	-4.6588
AT5G04960	---	Pectinesterase activity	-4.86267
AT4G25250	---	Pectinesterase inhibitor activity	-5.03158
AT2G45180	---	Lipid transport	-5.07169
AT3G59930	---	Unknown	-5.17541
AT4G26010	---	Peroxidase; heme binding	-5.19962
AT4G22010	sks4	Copper ion binding; oxidoreductase	-5.31254
AT5G66460	ATMAN7	O-glycosyl hydrolase; cation binding	-5.34858
AT3G49960	---	Sugar transport	-5.64543
AT1G52050	---	Unknown	-5.66864
AT3G62680	PRP3	Root and trichoblast development	-5.842
AT5G44020	---	Acid phosphatase	-5.87456
AT5G67400	RHS19	Peroxidase; heme binding	-6.03164

AT4G02270	RHS13	Nitrate transport; trichoblast differentiation	-6.08117
AT3G56980	BHLH039	Transcription factor responsive to iron homeostasis	-6.12045
AT2G32300	UCC1	Copper ion binding; root hair elongation	-6.17691
AT1G73620	---	Unknown	-6.23959
AT1G74770	---	Zinc ion binding; nitrate transport	-6.41425
AT5G54370	---	Unknown	-6.49389
AT2G32270	ZIP3	Metal ion transporter; root hair elongation	-6.57192
AT5G47450	TIP2;3	Ammonia transporter; water transport	-6.61023
AT1G30870	---	Peroxidase; heme binding	-6.73989
AT3G61430	PIP1A	Water transport	-6.75586
AT2G39430	---	lignin biosynthesis; root hair cell differentiation	-6.78424
AT4G00680	ADF8	Actin binding	-6.91577
AT1G32450	NRT1.5	Oligopeptide transport; nitrate transporter	-7.03232
AT1G20160	ATSBT5.2	Serine-type endopeptidase	-7.41482
AT4G31320	SAUR37	Calmodulin binding responsive to auxin	-7.49889
AT3G58990	IPMI1	Hydro-lyase; 3-isopropylmalate dehydratase	-7.67531
AT2G43600	---	Chitinase activity	-7.82159
AT3G04320	---	Endopeptidase inhibitor	-8.68627
AT5G42180	PER64	Peroxidase; heme binding	-9.44853
AT1G05240	---	Peroxidase; heme binding	-9.70595
AT5G50200	WR3	Nitrate/nucleotide/ammonium transport responsive to wounding	-10.2082
AT5G62340	---	Pectinesterase inhibitor	-10.5598
AT5G04950	NAS1	Nicotianamine synthase	-11.3602
AT5G10130	---	Unknown	-11.5431
AT4G37160	sks15	Oxidoreductase; copper ion binding	-13.3488
AT5G46890	---	Lipid transport/binding	-16.032
AT4G12510	---	Lipid transport	-16.4018
AT5G60520	---	Unknown	-17.0322
AT4G17340	TIP2;2	Water transport responsive to nitrate	-17.5737
AT3G01190	---	Peroxidase; heme binding	-18.1944
AT3G19430	---	Unknown	-18.499
AT2G33790	AGP30	Unknown	-22.0791

Appendix 7. Root microarray data comparative analysis with previously produced data sets.

From 549 DEGs detected in root tissue, 121 had been previously identified in at least one of five other representative transcriptome response microarrays to biotic stresses.

Wounding	Suspension	Insect	Fungal	FLG22	Function
+				+	Chitinase family protein
		+			Chlorophyll binding
			+		S-adenosyl-L-methionine-dependent methyltransferase-like protein
			+		FAD-binding and BBE domain-containing protein
			+		Peroxidase
				+	Toll-interleukin-resistance domain-containing protein
			+		Isocitrate lyase
+					Glutathione transferase
			+		Glycosyltransferase
+	+	+			Peroxidase
+					Glycosylhydrolase
		+			Exordium-like 2
+					Alternative oxidase
+					Indole-3-acetic acid amido synthetase
		+			Lipase
			+		Glutathione transferase
		+			Calcium transporter
+	+				Aldo-keto reductase
+		+	+		Copper/glutathione binding
		+	+		FAD-binding and BBE domain-containing protein
			+		Manganese ion binding
		+			Calmodulin binding
			+		Transcription factor
+			+		Peroxidase
		+	+		Dihydrocamalexic acid decarboxylase
+					Glutathione transferase
			+		ATPase family protein
		+			ATPase family protein
		+			Serine-type endopeptidase
+					Glutathione transferase

			+	Elicitor peptide
		+		Mannose-binding lectin protein
			+	Toll-interleukin-resistance domain-containing protein
		+		Copper ion binding
		+		Calcium binding
+				Aluminum induced protein
+				Zing finger protein
+				xyloglucosyl transferase
+			+	Glutathione transferase
		+		Cinnamyl-alcohol dehydrogenase
+				Transcription factor
			+	Acyltransferase superfamily protein
+				Peroxidase
		+		Calcium binding
			+	Thioredoxin superfamily protein
+				Hydrolase
		+		Carbohydrate kinase family
		+		Arginine decarboxylase
+				Lipoxygenase
		+		ATP:ADP antiporter
			+	Plant defense
		+		Ubiquitin-protein ligase
+		+	+	Hydroxylase
			+	Chitinase
		+	+	Lipoprotein
		+		Kinase
		+	+	Calcium transporter
		+		Translocon at the inner envelope membrane of chloroplasts
		+		Sulphur deficiency responsive
		+		ATPase family protein
		+		Glutaredoxin
			+	Transcription factor
			+	Heat shock protein 23.5
		+		Hydrolase
		+		Oxygen transporter
		+		Alcohol dehydrogenase-like protein
		+		Cell expansion regulator
		+		ATPase family protein

+			Epimerase
	+		Transcription factor
+			Glutathione peroxidase
+			Cobra-like protein 2 precursor
	+		Chaperone protein
		+	Toll-interleukin-resistance domain-containing protein
+			Leucine rich repeat protein
	+		Autophagy substrate
+			Oxireductase
+			Ethylene binding
	+		RNA binding
		+	Chitinase
+		+	Pathogenesis related
	+		Glycosyltransferase protein
+			Transcription factor
+			Transcription factor
	+		Guanosine nucleotide diphosphate
+			1-aminocyclopropane-1-carboxylic acid (ACC) synthase 6
	+		Glutathione S-transferase
	+		Trichome localized protein
	+		Acetylornithine transaminase
	+		Methionine sulfoxide reductase
+		+	Thaumatin-like protein
+			Endoplasmic reticulum body formation
+			Endotransglucosylase
+		+	Chitinase
+			Defense protein
	+		Transducin
	+		Receptor protein
+			Transcription factor
+			Nitrate transporter
+			Glycosyltransferase
	+		Transcription factor
+			Epimerase
	+		Methyltransferase inhibitor
		+	Suberin production
	+		Kelch-repeat protein
+		+	Pathogenesis related

			+		Transcription factor
+					Defense protein
			+		Acylhydrolase
				+	Pathogenesis related
				+	Lesion-inducing like protein
+					Glutathione transferase
		+			Hydroxylase
				+	Dirigent domain-containing
		+			LOB domain-containing
+		+			Peroxidase
			+		Pectinesterase inhibitor
+					Endo-1,4-beta-mannosidase
				+	Thaumatin-like protein
			+		Ammonia transporter
				+	Chitinase protein
44	2	51	26	18	

Appendix 8. Root transcripts not previously annotated in response to *A. tumefaciens* C58.

Root A. thaliana Col-0 genes not previously detected for differential expression in response to A. tumefaciens C58.

Locus Tag	Gene Symbol	Function/Putative Function	Fold-Change
AT4G27670	HSP21	Protein folding responsive to light/ROS/heat	35.8896
AT2G43590	---	Chitinase activity	24.1334
AT1G16030	Hsp70b	ATP binding; response to heat/light/virus/ROS	23.9217
AT1G52560	---	Protein folding in response to heat/light/ROS	23.3771
AT1G07400	---	Response to heat/oxidative stress	21.7988
AT2G41380	---	methyltransferase	19.3001
AT3G24500	MBF1C	Selenium binding	18.2832
AT3G28210	PMZ	Zinc ion binding	17.9177
AT5G37670	---	Protein folding responsive to light/heat/ROS	16.7789
AT1G17170	ATGSTU24	Glutathione transferase/binding	15.9119
AT4G37370	CYP81D8	Oxidreductase; iron ion binding	15.5508
AT2G23170	GH3.3	Indole-3-acetic acid amido synthetase	15.2514
AT1G71000	---	HSP binding in response to light/heat	14.5845
AT5G06860	PGIP1	Polygalacturonase inhibitor	12.5274
AT3G09640	APX2	Peroxidase; heme binding	12.3951
AT2G35980	YLS9	Regulation of ROS metabolism; negative regulation of defense	11.8331
AT1G05680	UGT74E2	UDP-glycosyltransferase; hexosyl transferase	11.3811
AT3G22840	ELIP1	Chlorophyll binding	10.8473
AT2G46240	BAG6	Chaperone binding; calmodulin binding	10.7276
AT1G54050	---	Protein folding in response to heat/light/ROS	10.0314
AT3G54150	---	methyltransferase	9.57213
AT3G09350	Fes1A	HSP70 protein binding	9.55315
AT1G26380	---	FAD binding; UDP-N-acetylmuramate dehydrogenase	9.29824
AT2G26150	ATHSFA2	Positive regulation of transcription in response to light/heat/ROS	8.98188
AT5G64250	---	Nitronate monooxygenase; IMP dehydrogenase	8.91544
AT1G76520	PILS3	Auxin:hydrogen symporter	8.8438
AT1G14540	PER4	Peroxidase; heme binding	8.74663

AT5G12030	AT-HSP17.6A	Responsive to unfolded protein due to heat/light	8.49355
AT1G17180	ATGSTU25	Glutathione transferase	8.33537
AT1G72900	---	ADP binding; responsive to fungus and preventative of cell death	8.18787
AT5G05410	DREB2A	Transcription factor responsive to light/heat/ROS/water deprivation	8.08615
AT1G74310	ATHSP101	Nucleoside-triphosphate activity in response to heat/light/ROS	7.93301
AT3G21720	ICL	Isocitrate lyase	7.8989
AT4G02520	ATGSTF2	Glutathione transferase activity	7.69324
AT2G15490	UGT73B4	Hyxosyl/UDP-glucosyl transferase; quercetin 3-O-glucosyltransferase	7.5953
AT5G59820	RHL41	Transcription factor responsive to wounding/ROS/light/salinity/cold	7.58731
AT5G64120	PRX71	Peroxidase; heme binding	7.57792
AT1G77450	anac032	Transcription regulation of toxic catabolism	7.46134
AT4G30270	MERI5B	Xyloglucan:xyloglucosyl transferase; O-glycosyl transferase	7.3239
AT4G34131	UGT73B2	UDP-glycosyltransferase; hexosyl transferase; quercetin 3-O-glycosyltransferase	7.25787
AT5G07570	---	Unknown	7.10518
AT1G01720	ATAF1	Transcription factor responsive to JA/ABA	7.02467
AT1G53540	---	Protein folding in response to heat/light/ROS	6.93922
AT5G64260	EXL2	Response to arsenic-containing substance	6.83995
AT3G28740	CYP81D1	Oxygen binding; oxidoreductase	6.69623
AT4G20070	ATAAH	Metallopeptidase; allantoate deiminase; carbon-nitrogen hydrolase	6.65251
AT1G67810	SUFE2	Enzyme activator for sulfter metabolism	6.62926
AT2G32120	HSP70T-2	ATP binding response to heat/light	6.61627
AT3G55090	ABCG16	ATPase activity; nucleotide binding	6.61187
AT1G55530	---	Zinc ion binding	6.61073
AT3G22370	AOX1A	Alternate oxidase	6.56036
AT1G59500	GH3.4	Indole-3-acetic acid amido synthetase	6.54002
AT1G57980	ATPUP18	Purine nucleobase transmembrane transporter activity	6.39702
AT1G69920	ATGSTU12	Glutathione transferase	6.37763
AT3G22910	---	Metal ion binding; calcium-transporting ATPase activity	6.34193
AT1G64590	---	Oxidoreductase	6.26659
AT1G60730	---	Aldo-keto reductase (NADP)	6.02789
AT3G46230	HSP17.4	Calcium ion binding	5.95804

AT2G40340	ATERF48	Transcription factor responsive to ABA	5.71755
AT1G26390	---	UDP-N-acetylmuramate dehydrogenase; FAD binding	5.57189
AT1G18970	GLP4	Nutrient reservoir activity; transition metal binding	5.29893
AT4G01870	---	Toxin catabolism; proteolysis in response to ABA/ET	5.27926
AT2G20560	---	HSP binding; unfolded protein in response to light/heat/ROS	5.24252
AT1G06430	FTSH8	ATP binding; ATPase activity; zinc binding; metalloendopeptidase	5.24027
AT3G53230	ATCDC48 B	Positive regulation of protein catabolism	5.12803
AT2G37540	---	Oxidoreductase	5.10052
AT1G33110	---	Drug antiporter	5.03114
AT3G61630	CRF6	Transcription factor for leaf/cotyledon development	4.94897
AT2G43000	anac042	Negative regulator of leaf senescence	4.91828
AT5G39580	---	Peroxidase; heme binding	4.91344
AT1G14200	---	Zinc ion binding; protein folding	4.90207
AT5G12020	HSP17.6II	Protein folding responsive to heat/light	4.89005
AT3G26830	PAD3	Monooxygenase;oxidoreductase; dihydrocamalexic acid decarboxylase	4.80184
AT5G58070	TIL	Transporter responsive to cold/heat/light	4.76754
AT1G08430	ALMT1	Malate transmembrane transporter	4.68606
AT5G14760	FIN4	Oxidoreductase; L-aspartate oxidase	4.63431
AT5G50760	SAUR55	Nitrate transport in response to auxin	4.60409
AT5G02780	GSTL1	Protein glutathionylation	4.53553
AT1G03070	ATLFG4	Glutamate binding	4.43321
AT4G13420	HAK5	Potassium ion transport; potassium:sodium symporter	4.41267
AT4G25380	SAP10	DNA binding responsive to nickel/col/heat/ROS/zinc/manganese	4.36616
AT2G30140	UGT87A2	Hexosyl transferase; glycosyl transferase;	4.35234
AT3G28580	---	Nucleoside-triphosphate activity; nucleotide binding	4.31856
AT3G47780	ATH6	Nucleotide-triphosphate activity; ATPase activity	4.30135
AT1G32940	SBT3.5	Serine-type endopeptidase	4.25176
AT1G69930	ATGSTU11	Galactolipid biosynthesis; glutathione tranferase	4.24264
AT5G08250	---	Oxidoreductase; heme binding	4.23089
AT1G72060	---	Serine-type endopeptidase	4.20207
AT5G43450	---	Oxidoreductase; 1-aminocyclopropane-1-carboxylate oxidase	4.19297

AT3G62260	---	Serine/threonine phosphatase	4.18823
AT4G31140	---	O-glycosyl hydrolase	4.16275
AT1G64950	CYP89A5	Heme binding; oxidoreductase	4.16061
AT3G14200	---	Heat shock protein binding	4.13868
AT4G13180	---	Oxireductase; nucleotide binding	4.11315
AT5G63790	ANAC102	Transcription factor responsive to unfolded protein response	4.10657
AT4G38420	sks9	Oxidoreductase; copper ion binding	4.08849
AT5G64900	PROPEP1	Cell surface protein binding responsive to ET/JA/wounding	4.0144
AT3G44190	---	Xyloglucan-specific endo-beta-1,4-glucanase; glycosyl hydrolase	3.97832
AT1G51340	---	Drug antiporter	3.9677
AT5G53970	TAT7	L-tyrosine:2-oxoglutarate aminotransferase	3.91818
AT3G08970	ATERDJ3A	HSP binding; unfolded protein in response to light/heat/ROS	3.89602
AT4G20830	---	FAD binding; UDP-N-acetylmuramate dehydrogenase; oxidoreductase	3.88336
AT5G17860	CAX7	Cation:sodium ion transporter	3.87806
AT5G18470	---	Carbohydrate binding responsive to karrikin	3.87106
AT1G07350	SR45A	RNA splicing in response to light	3.82562
AT5G20230	ATBCB	Copper ion binding responsive to wounding/ROS/light	3.74197
AT2G18660	EXLB3	Alternative respiration; SAR	3.70956
AT3G47480	---	Chitinase	3.6709
AT4G08950	EXO	Plant-type cell wall; sterol biosynthesis	3.66151
AT1G30040	ATGA2OX 2	Iron ion binding; oxidoreductase	3.64528
AT3G16530	---	Carbohydrate binding	3.64498
AT3G15450	---	Response to sucrose; unknown	3.62338
AT2G47890	---	Transcription factor responsive to wounding; flavonoid biosynthesis	3.59955
AT1G55920	ATSERAT2 ;1	Serine O-acetyltransferase	3.59313
AT5G01600	FER1	Oxidoreductase; ferric iron binding	3.53523
AT1G15380	---	Lactoylglutathione lyase	3.52913
AT4G20000	---	Unknown	3.52022
AT1G02850	BGLU11	O-glycosyl hydrolase; cation binding	3.51692
AT5G57560	TCH4	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	3.45149
AT2G29460	ATGSTU4	Glutathione transferase; toxin catabolism	3.44004
AT2G19310	---	Response to heat/ROS/light; unknown	3.41635
AT5G08350	---	Unknown	3.40573

AT4G27940	MTM1	Mitochondrial transport; unknown	3.38989
AT1G70170	MMP	Metallopeptidase; zinc ion binding	3.38691
AT1G72680	CAD1	Oxidoreductase; cofactor in nucleotide binding	3.38082
AT2G37970	SOUL-1	Flavonoid biosynthesis in response to light	3.37229
AT2G23150	NRAMP3	Manganese ion transporter/inorganic anion transporter	3.34673
AT1G62300	WRKY6	Transcription factor responsive to fungal; toxin metabolism	3.34365
AT5G16010	---	3-oxo-5-alpha-steroid 4-dehydrogenase; oxidoreductase	3.30977
AT5G49130	---	Drug antiporter	3.30738
AT3G63310	BIL4	Glutamate binding	3.29636
AT2G32020	---	N-acetyltransferase	3.28629
AT3G09440	---	ATP binding response to heat/light	3.24158
AT1G68850	---	Peroxidase; heme binding	3.22903
AT4G21990	APR3	Adenylyl-sulfate reductase; oxidoreductase	3.22695
AT5G13900	---	Lipid transport/binding	3.21673
AT5G47560	TDT	Malate transporter; sodium:dicarboxylate symporter	3.20717
AT5G18170	GDH1	Glutamate dehydrogenase [NAD(P)+]; oxidoreductase	3.20449
AT5G20910	AIP2	Ubiquitin-protein ligase; zinc ion binding	3.20099
AT3G14990	ATDJ1A	Thiamine biosynthesis; catalytic activity	3.18019
AT2G46600	---	Calcium ion binding; protein binding	3.17855
AT4G09150	---	Phosphopantetheine binding	3.17123
AT3G49580	LSU1	Unknown	3.16241
AT2G22240	MIPS2	Inositol-3-phosphate synthase	3.1608
AT5G38900	---	Disulfide oxidoreductase	3.1509
AT3G62150	PGP21	Nucleoside triphosphate activity; ATPase activity	3.14772
AT4G25810	XTR6	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	3.13915
AT4G32940	GAMMA-VPE	Cysteine-type endopeptidase	3.09321
AT5G11260	HY5	Transcription factor responsive to light regulation of cell proliferation	3.08535
AT3G25190	ATVTL5	Response to iron ion, nitric oxide, ethylene	3.06649
AT4G21580	---	Oxidoreductase; zinc ion binding responsive to fungus	3.06166
AT5G51830	---	Ribokinase; D-ribose metabolism	3.06061
AT2G47180	AtGolS1	Inositol 3-alpha-galactosyltransferase; hexosyl transferase	3.04968
AT4G34710	ADC2	Arginine decarboxylase	3.03866
AT4G28390	AAC3	ATP:ADP antiporter	3.03465

AT4G00080	UNE11	Pectinesterase; enzyme inhibitor	3.02248
AT5G38895	---	Zinc ion binding responsive to auxin/ROS/ABA	3.01654
AT4G22530	---	Methyltransferase	2.96842
AT3G19580	AZF2	Zinc ion binding; transcription factor responsive to wounding	2.96717
AT2G47520	HRE2	Transcription factor responsive to anoxia	2.9521
AT1G22985	CRF7	Transcription regulation responsive to ethylene	2.93195
AT1G11330	---	Serine/threonine kinase; carbohydrate/zinc binding	2.91625
AT1G32870	ANAC13	Transcription factor responsive to light; galactolipid biosynthesis	2.89459
AT5G47070	---	Serine/threonine kinase	2.881
AT3G12580	HSP70	Ubiquitin-protein ligase	2.87352
AT3G11430	GPAT5	Organic anion transporter; acyl transferase	2.86817
AT4G21980	APG8A	APG8 ligase; APG8-specific protease/activating enzyme	2.86793
AT1G63800	UBC5	Acid-amino acid ligase; ubiquitin-protein ligase activity	2.85324
AT1G72660	---	GTP binding in response to light/heat	2.85115
AT3G13610	F6'H1	Oxidoreductase in response to wounding	2.83694
AT4G15550	IAGLU	Hexosyl transferase; UDP-glycosyltransferase	2.83282
AT3G47540	---	ATP binding; transporter activity; toxic catabolism	2.82711
AT2G29990	NDA2	FAD binding; oxidoreductase; NADH dehydrogenase	2.82638
AT3G18250	---	Amino acid import	2.81123
AT3G07700	ATSIA1	Phosphorus transfer	2.78857
AT4G29070	---	Unknown	2.78491
AT5G64870	---	Response to chitin mediated by ET	2.75696
AT1G17960	---	Threonine-tRNA ligase; aminoacyl-tRNA ligase; ATP binding	2.7521
AT3G63380	ACA12	Calmodulin binding; calcium ion transport	2.74001
AT3G07370	CHIP	Ubiquitin-protein ligase	2.72502
AT4G03320	tic20-IV	Toxin catabolism	2.67967
AT5G48850	ATSDI1	Regulation of sulfur utilization in response to starvation	2.6725
AT1G50250	FTSH1	Nucleoside-triphosphate activity; metalloendopeptidase	2.66234
AT3G50930	BCS1	ATP binding; ATPase activity	2.66102
AT5G53588	CPuORF50	Unknown	2.61441
AT3G55840	---	Ethylene biosynthesis	2.60835
AT1G67360	---	Unknown	2.59821

AT5G03240	UBQ3	Ubiquitin dependent protein catabolic process	2.59535
AT5G49350	---	Unknown	2.59489
AT2G32150	---	Hydrolase	2.59171
AT2G24600	---	Unknown	2.58749
AT5G39670	---	Calcium ion binding	2.58145
AT3G01290	ATHIR2	Negative regulator of programmed cell death	2.56092
AT1G60610	---	Zinc ion binding	2.55819
AT4G10955	UGE5	Triglyceride lipase	2.55185
AT4G15120	---	Unknown	2.5455
AT2G15480	UGT73B5	Hyxosyl/UDP-glucosyl transferase; quercetin 3-O-glucosyltransferase	2.52889
AT2G29420	ATGSTU7	Glutathione transferase; toxin catabolism	2.52222
AT2G24180	CYP71B6	Oxidoreductase; heme binding	2.50678
AT4G30280	XTH18	Xyloglucan:xyloglucosyl transferase; O-glycosyl transferase	2.50609
AT5G01990	PILS6	Auxin:hydrogen symporter	2.48552
AT5G11520	ASP3	Pyridoxal phosphate binding; L-aspartate:2-oxoglutarate aminotransferase	2.48266
AT2G34660	ATMRP2	Nucleoside-triphosphatase activity; ATPase activity	2.46156
AT4G25200	ATHSP23.6 -MITO	Protein folding responsive to light/ROS/cadmium/heat	2.4611
AT2G36670	---	Aspartic-type endopeptidase	2.45495
AT3G55470	---	Positive regulation of flavonoid biosynthesis	2.45263
AT2G26480	UGT76D1	quercetin 7-O-glucosyltransferase; UDP glycosyl transferase	2.44041
AT1G03850	ATGRXS13	Disulfide oxidoreductase	2.43441
AT4G22340	CDS2	Phosphatidate cytidylyltransferase; phosphorus transferase activity	2.43434
AT5G64060	anac103	Transcription factor responsible for organismal development	2.43108
AT2G34770	FAH1	Fatty acid alpha-hydroxylase; oxidoreductase; iron ion binding	2.43008
AT5G54080	HGO	Homogentisate 1,2-dioxygenase	2.42041
AT5G62480	ATGSTU9	Glutathione transferase; toxin catabolism	2.39403
AT1G74460	---	Hydrolase; carboxylesterase	2.39027
AT3G48450	---	Response to nitrate	2.38106
AT5G52450	---	Drug antiporter	2.38001
AT3G57520	AtSIP2	O-glycosyl hydrolase; raffinose alpha-galactosidase	2.37886
AT5G47635	---	Unknown	2.37535
AT5G51440	---	Protein folding responsive to	2.37408

		light/heat/ROS	
AT4G27830	BGLU10	O-glycosyl hydrolase; cation binding	2.35158
AT5G14780	FDH	Co-factor binding; oxidoreductase	2.35005
AT1G14040	PHO1;H3	Phosphate ion transport; phosphate starvation	2.34788
AT2G16060	AHB1	Heme binding; oxidoreductase	2.34737
AT4G38540	---	Monooxygenase; oxidoreductase; toxin catabolism	2.34717
AT4G33040	---	Disulfide oxidoreductase activity	2.33645
AT3G57380	---	Glycosyl transferase	2.33509
AT5G19440	---	Alcohol dehydrogenase (NAD) activity	2.33281
AT5G64220	CAMTA2	Transcription regulation by calmodulin binding	2.3245
AT5G47990	CYP705A5	Thalian-diol desaturase; iron ion binding	2.31955
AT2G17570	CPT1	Alkyl/aryl transferase	2.31859
AT5G27760	---	Response to hypoxia; unknown	2.31462
AT4G24020	NLP7	Transcription factor responsive to nitrate/water deprivation	2.31173
AT2G41100	TCH3	Calcium binding	2.31029
AT1G15170	---	Drug antiporter	2.30877
AT2G44080	ARL	Cell growth responsive to brassinosteroid	2.29985
AT3G55430	---	O-glycosyl hydrolase	2.29793
AT1G59870	PEN3	Nucleoside-triphosphate activity; cadmium ion transporter	2.29703
AT3G08990	---	Unknown	2.28913
AT4G13010	---	Oxireductase; zinc ion/nucleotide binding	2.28653
AT1G30620	MUR4	UPD-glucose 4-epimerase; UPD-arabinose 4 epimerase; coenzyme	2.27611
AT3G57070	---	Disulfide oxidoreductase activity	2.276
AT4G05020	NDB2	FAD binding; disulfide oxidoreductase activity	2.27291
AT4G14690	ELIP2	Flavonoid biosynthesis; response to light/sucrose	2.27216
AT3G19390	---	Cysteine-type peptidase	2.27116
AT5G25450	---	Ubiquinol-cytochrome-c reductase	2.26041
AT3G22160	JAV1	Protein targeting; response to ABA/SA	2.24631
AT1G30660	---	Nucleotide binding	2.24557
AT3G10500	anac053	Transcription factor responsive for drought recovery; toxin catabolism	2.24423
AT5G48180	NSP5	Ubiquitin-dependent protein catabolic process; toxin catabolism	2.2386
AT4G11600	ATGPX6	Glutathione peroxidase	2.23223
AT1G32350	AOX1D	Alternate oxidase	2.22872
AT2G31570	ATGPX2	Glutathione peroxidase	2.2286

AT1G51950	IAA18	Transcription regulation in response to JA/auxin/brassinosteroid	2.21902
AT3G04640	---	Protein acetylation in response to ET, wounding, chitin	2.21614
AT3G29810	COBL2	Glutathione transferase activity	2.21075
AT4G35420	DRL1	Catalytic activity; seed/pollen exine formation	2.20874
AT4G02940	---	Oxidoreductase	2.20234
AT5G54840	SGP1	GTP binding; small GTPase mediated signal transduction	2.19847
AT4G26470	---	Calcium ion binding; toxin catabolism	2.19723
AT1G22930	---	Unknown	2.19477
AT1G11100	FRG5	Helicase; zinc ion binding	2.19347
AT2G31260	APG9	Toxin catabolism; ubiquitin-dependent protein catabolism	2.179
AT2G20142	---	Signal transduction; unknown	2.17859
AT1G33590	---	Negative regulator of defense; transcription regulation of cell wall	2.17153
AT3G53510	ABCG20	ATPase activity; nucleotide binding	2.15957
AT1G78820	---	Carbohydrate binding	2.15942
AT4G36610	---	Hydrolase	2.15805
AT4G18360	GOX3	glycolate oxidase; oxidoreductase	2.15753
AT3G26470	---	Toxin catabolism	2.15718
AT4G24690	NBR1	Ubiquitin binding	2.15704
AT4G33940	---	Intracellular signal transduction to ABA/auxin/chitin/ROS/carbohydrate	2.15631
AT5G03030	---	Heat shock protein binding	2.14346
AT1G10050	---	O-glycosyl hydrolase; cation binding; endo-1,4-beta-xylanase	2.14335
AT1G53670	MSRB1	Peptide-methionine (S)-S-oxide reductase activity	2.13502
AT3G17611	ATRBL14	Zinc ion binding; serine-type endopeptidase	2.13237
AT5G40850	UPM1	Precorrin-2 dehydrogenase; uroporphyrin-III C-methyltransferase	2.12892
AT3G23150	ETR2	2-component response regulator; ethylene responsive receptor	2.12825
AT5G11670	ATNADP-ME2	Oxidoreductase; malate dehydrogenase; malic enzyme activity	2.12243
AT1G29400	AML5	RNA binding	2.11972
AT3G22200	POP2	4-aminobutyrate; transaminase	2.11553
AT3G03270	---	Response to molecule of fungal origin	2.11233
AT3G17690	CNGC19	Ion channel activity; cyclic nucleotide binding; calmodulin binding	2.0979
AT3G54420	ATEP3	Chitinase	2.08912

AT4G19030	NLM1	Arsenite/water transport	2.08487
AT1G73500	MKK9	Protein kinase activator; serine/threonine kinase	2.07805
AT5G20380	PHT4;5	Nitrate transport	2.07315
AT2G16900	---	Callose deposition in response to wounding	2.07286
AT1G78780	---	Unknown	2.07092
AT1G06840	---	Tyrosine kinase; serine/threonine kinase	2.06913
AT3G09020	---	Glycosyl transferase; galactosyltransferase	2.05995
AT1G65610	KOR2	O-glycosyl hydrolase	2.05969
AT2G23320	WRKY15	Transcription factor regulating ET biosynthesis/chitin response/ROS	2.05196
AT1G67820	---	Serine/threonine phosphatase	2.04962
AT3G50400	---	Hydrolase acting on ester bonds	2.0471
AT3G11020	DREB2B	Transcription factor responsive to water deprivation	2.04366
AT4G26270	PFK3	6-phosphofructokinase; ATP binding	2.0371
AT1G01560	ATMPK11	Serine/threonine kinase	2.03517
AT1G54100	ALDH7B4	Oxidoreductase activity; 3-chloroallyl aldehyde dehydrogenase	2.03202
AT4G15230	PDR2	ATPase; nucleoside-triphosphate activity	2.03032
AT1G55510	BCDH BETA1	3-methyl-2-oxobutanoate dehydrogenase	2.0275
AT5G37690	---	Lipid hydrolase	2.0262
AT2G44100	ATGDI1	RAB GDP-dissociation inhibitor activity	2.02576
AT3G07560	PEX13	Lipid transport	2.02333
AT5G47880	ERF1-1	Translation release factor	2.01512
AT4G11280	ACS6	1-aminocyclopropane-1-carboxylate synthase; pyridoxal phosphate binding	2.00714
AT2G02390	ATGSTZ1	Glutathione transferase	2.00658
AT4G35985	---	Unknown	2.00201
AT3G43800	ATGSTU27	Oxidoreductase; FAD binding	-2.00645
AT1G03870	FLA9	Unknown	-2.01227
AT2G41660	MIZ1	Chloroplast organization	-2.02175
AT3G62390	TBL6	Unknown	-2.02489
AT1G50560	CYP705A2 5	Iron ion binding; oxidoreductase	-2.02567
AT5G23010	MAM1	2-(2'metyhlthio)ethylmalate synthase; acyl transferase	-2.0295
AT4G01240	---	methyltransferase	-2.04259
AT3G02885	GASA5	Response to SA/GA for heat acclimation	-2.05244
AT5G05880	---	UDP-glycosyltransferase; hexosyl transferase	-2.06253
AT3G62600	ATERDJ3B	HSP binding; unfolded protein binding	-2.06528

AT2G43100	IPMI2	Hydrolase	-2.06605
AT4G25010	SWEET14	Sucrose transport	-2.07828
AT1G13830	---	Unknown	-2.08866
AT4G21830	ATMSRB7	Peptide-methionine (S)-S-oxide reductase activity	-2.10272
AT2G41560	ACA4	Calcium-transporting ATPase	-2.10795
AT1G18250	ATLP-1	Cell proliferation (anaphase)	-2.10971
AT4G21600	ENDO5	Ester hydrolase; T/G mismatch-spec. endonuclease; nitrate/iron trans.	-2.11444
AT3G55700	---	UDP-glycosyltransferase; hexosyl transferase	-2.11943
AT3G15950	NAI2	Peroxisome	-2.12079
AT3G06990	---	Disulfide reductase activity; zinc ion binding	-2.12239
AT2G37440	---	Hydrolase	-2.1239
AT2G35210	RPA	ARF GTPase activator; zinc ion binding	-2.12787
AT4G36360	BGAL3	Beta-galactosidase; lactose catabolism; O-glycosyl transferase	-2.13146
AT1G66440	---	Disulfide reductase activity	-2.144
AT1G11540	---	Nite transport	-2.14907
AT2G21830	---	Disulfide reductase activity; zinc ion binding	-2.15003
AT4G12420	SKU5	Oxireductase for development; anthocyanin accumulation	-2.15108
AT5G26660	ATMYB86	Negative regulation of transcription; Unknown	-2.15212
AT1G09210	CRT1b	Calcium ion binding; unfolded protein binding	-2.15987
AT3G52500	---	Aspartic-type endopeptidase	-2.16236
AT2G40230	---	Amino-acyl transferase	-2.16855
AT2G36870	XTH32	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	-2.1701
AT4G32830	AtAUR1	Histidine phosphorylase	-2.17737
AT2G44380	---	Disulfide reductase activity	-2.18106
AT2G40460	---	Oligopeptide transport	-2.18789
AT5G61790	CNX1	Calcium ion binding; unfolded protein binding	-2.1947
AT2G03090	ATEXPA15	Plant-type cell wall loosening	-2.196
AT3G12500	ATHCHIB	Chitinase; nitrate transport	-2.19803
AT3G16450	JAL33	Response to zinc ion	-2.1985
AT5G67100	ICU2	Nucleoside binding; DNA-directed DNA polymerase activity	-2.20666
AT1G23205	---	Pectinesterase inhibitor	-2.22551
AT2G39220	PLP6	Nutrient reservoir; lipid metabolism	-2.2289
AT2G22930	---	Hexosyl transferase; glycosyl transferase	-2.23095

AT1G44575	NPQ4	Xanthophyll/chlorophyll binding	-2.23243
AT1G23040	---	Nutrient reservoir activity	-2.24248
AT2G42570	TBL39	Unknown	-2.24312
AT1G70890	MLP43	Divalent metal ion transport; cation homeostasis	-2.25639
AT2G43530	---	Ion channel inhibitor	-2.25837
AT1G49320	USPL1	Seed development	-2.26009
AT4G25050	ACP4	ACP Phosphopantetheine binding for fatty acid biosynthesis	-2.26646
AT5G19240	---	Tryptophase catabolism; indoleacetic acid biosynthesis	-2.27084
AT4G10380	NIP5;1	Water/borate transport	-2.27468
AT2G43820	UGT74F2	Glucosyltransferase	-2.28101
AT5G42100	BG_PPAP	O-glycosly hydrolase; glucan endo-1,3-beta-D-glucosidase	-2.28158
AT4G12880	ENODL19	Copper ion binding	-2.29046
AT1G24530	---	Nucleotide binding; oligopeptide transport	-2.29354
AT1G74500	BS1	Transcription factor for brassinosteroid signalling	-2.29484
AT3G54220	SCR	Transcription factor responsive to gravitropism	-2.30237
AT5G23840	---	Unknown	-2.30471
AT1G08560	SYP111	SNAP receptor activity	-2.30721
AT1G05210	---	Unknown	-2.31135
AT1G22550	---	Oligopeptide transport	-2.32805
AT4G38970	FBA2	fructose-bisphosphate aldolase	-2.33269
AT3G51280	---	Cell cycle regulation	-2.34215
AT5G15230	GASA4	Cell redox homeostasis responsive to GA	-2.35121
AT4G11210	---	lignin biosynthesis; root hair cell differentiation	-2.35183
AT5G14060	AK3	Amino acid binding; aspartate kinase	-2.36095
AT4G04830	ATMSRB5	Peptide-methionine (S)-S-oxide reductase activity	-2.37282
AT3G20015	---	Aspartic-type endopeptidase	-2.3767
AT3G20820	---	Defense response signal transduction	-2.39751
AT2G31790	---	Hexosyl transferase; glycosyl transferase; UDP-glycosyl transferase	-2.40622
AT2G40900	UMAMIT11	Unknown	-2.42185
AT3G50300	---	Acyl transferase	-2.43695
AT4G33260	CDC20.2	Signal transduction for mitotic processes; heterotrimeric G-protein	-2.45548
AT2G28160	FRU	Transcription factor responsive to ET, Cyt,ROS	-2.46432
AT1G72200	---	Zinc ion binding	-2.48333

AT1G21440	---	Isocitrate lyase; glucosinolate biosynthesis	-2.4876
AT1G80050	APT2	Phosphate ion transport; adenine phosphoribosyltransferase	-2.48933
AT2G30210	LAC3	Oxidoreductase; hydroquinone:oxygen; copper ion	-2.49032
AT1G80830	NRAMP1	Cadmium/manganese ion transport	-2.49312
AT3G48740	SWEET11	Sugar transport	-2.52271
AT5G60890	MYB34	Transcription factor responsive to JA/sulfur/insect	-2.53316
AT1G03410	2A6	Oxidoreductase	-2.53446
AT1G48500	JAZ4	Protein binding in response to wounding/JA	-2.53735
AT3G11520	CYCB1;3	Protein kinase	-2.54229
AT3G02610	---	Acyl-[acyl-carrier-protein] desaturase; transition metal ion binding	-2.56804
AT5G48290	---	Metal ion transport	-2.57244
AT1G70370	PG2	Polygalacturonase for cellular biogenesis	-2.6034
AT1G12090	ELP	Lipid transport	-2.61274
AT5G17700	---	Drug antiporter	-2.61326
AT1G23750	---	Unknown	-2.61647
AT1G56010	NAC1	Transcription factor regulating organ development	-2.62516
AT3G27020	YSL6	Oligopeptide transport	-2.63894
AT3G54770	ARP1	Nucleic acid binding for mRNA splicing	-2.64275
AT2G01610	---	Pectinesterase inhibitor	-2.6447
AT1G56430	NAS4	Nicotianamine synthase	-2.6564
AT2G45960	PIP1B	Water transporter activity responsive to hyperosmotic stress	-2.65687
AT5G47500	PME5	Pectinesterase	-2.6571
AT3G26520	TIP2	Water transport	-2.6597
AT5G50375	CPI1	Cycloeucalenol cycloisomerase	-2.68743
AT1G11545	XTH8	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	-2.70426
AT5G44480	DUR	Nucleotide binding; UDP-glucose 4-epimerase	-2.71447
AT2G20750	ATEXPB1	Plant-type cell wall loosening	-2.7186
AT5G24410	PGL4	6-phosphogluconolactonase	-2.72536
AT4G25260	---	Pectinesterase inhibitor activity	-2.74463
AT1G62560	FMO GS-OX3	FAD binding; 3/4/5/6/8-methylthiopropyl glucosinolate S-oxygenase	-2.74766
AT2G28670	ESB1	Suberin biosynthesis	-2.75943
AT4G23400	PIP1;5	Water transport	-2.76338
AT5G20950	---	O-glycosyl transferase	-2.76458
AT2G15970	COR413-	Response to water/cold/ABA/salt stress	-2.76707

	PM1		
AT5G17820	---	Peroxidase; heme binding	-2.78182
AT5G04970	---	Pectinesterase activity	-2.79418
AT1G15670	KFB01	Phenylpropanoid metabolic process	-2.80584
AT3G54820	PIP2;5	Water transport	-2.82549
AT3G51600	LTP5	Lipid transport	-2.86886
AT5G07990	TT7	Oxidoreductase; flavonoid 3'-monooxygenase; iron ion binding	-2.91185
AT3G10040	HRA1	Transcription factor for organ development	-2.91826
AT4G12470	AZI1	Lipid transport; nitrate transport; amino acid transport	-2.92812
AT5G50740	---	Metal ion binding/transport	-2.94595
AT4G30460	---	Unknown	-2.96575
AT5G57540	XTH13	Xyloglucan:xyloglucosyl transferase; O-glycosyl transferase	-2.96671
AT4G12480	pEARLI 1	Lipid transport; response to salt stress/fungus/col	-2.98839
AT5G45340	CYP707A3	Oxireductase; (+)-abscisic acid 8'-hydroxylase; iron ion binding	-2.99992
AT4G22212	---	Transition metal ion transport for defense response	-3.00065
AT1G08590	PXL1	Serine/threonine kinase for stamen development	-3.01082
AT1G30370	DLAH	Phosphatidylcholine 1-acylhydrolase; triglyceride lipase	-3.03207
AT4G23496	SP1L5	Unknown	-3.09356
AT4G29690	---	Hydrolase	-3.11803
AT5G66690	UGT72E2	Hexosyl transferase; UDP-glycosyltransferase	-3.12958
AT1G05260	RCI3	Peroxidase; heme binding	-3.1354
AT3G58120	BZIP61	Transcription factor responsive to xenobiotics	-3.15428
AT4G14980	---	Disulfide reductase	-3.17203
AT5G52310	LTI78	Leaf senscence responsive to cold/desiccation/salinity	-3.19218
AT5G23660	MTN3	Sucrose transporter	-3.20135
AT3G14680	CYP72A14	Oxidoreductase; transition metal ion transport	-3.21134
AT2G23050	NPY4	Gravitropism/light signal transducer	-3.21239
AT2G41290	SSL2	Strictosidine synthase; myo-inositol hexakisphosphate biosynthesis	-3.21281
AT4G02850	---	Circadian rhythm	-3.24531
AT2G18800	XTH21	O-glycosyl hydrolase; xyloglucan:xyloglucosyl transferase	-3.26648
AT4G16980	---	Water transport responsive to	-3.28309

		salt/fructose/light	
AT5G60530	---	Transition metal ion transport	-3.28968
AT1G64780	ATAMT1;2	Ammonium transporter	-3.29606
AT1G67110	CYP735A2	Oxidoreductase; iron ion binding	-3.31512
AT3G23800	SBP3	Nitrate transport; iron ion transport	-3.3689
AT2G26690	---	Oligopeptide transport	-3.38995
AT4G31470	---	Unknown	-3.40173
AT2G24762	AtGDU4	Regulation of amino acid export	-3.41355
AT1G12080	---	Acetyl-CoA metabolism	-3.42785
AT1G78090	ATTPPB	Trehalose-phosphate	-3.43265
AT3G55150	ATEXO70 H1	Vesicle docking involved in exocytosis	-3.4342
AT3G23190	---	2-component sensor; ethylene binding; histidine kinase	-3.48944
AT4G28250	ATEXPB3	Plant-type cell wall loosening for sexual reproduction	-3.49362
AT4G29340	PRF4	Actin binding for cytoskeleton organization	-3.51093
AT3G50740	UGT72E1	Glycosyl transferase; hexosyl transferase; UDP-glycosyltransferase	-3.6204
AT2G36830	GAMMA-TIP	Water transporter activity responsive to ABA	-3.71441
AT2G36100	CASP1	Protein homodimerization; cell wall modification	-3.73427
AT5G56540	AGP14	Protein binding for root hair elongation	-3.75152
AT5G48430	---	Oligopeptide transport	-3.77541
AT1G78370	ATGSTU20	Glutathione transferase	-3.80306
AT5G25830	GATA12	Transcription factor responsive to light for circadian rhythm regulation	-3.84851
AT4G30170	---	Peroxidase; heme binding	-3.92224
AT1G52060	---	Unknown	-3.93465
AT2G37170	PIP2;2	Water transporter activity responsive to ABA	-3.9988
AT3G50660	DWF4	Oxidoreductase; heme binding	-4.08634
AT3G44990	XTR8	Response to heat	-4.11898
AT4G13580	---	Unknown	-4.23076
AT4G37540	LBD39	Sterol biosynthesis	-4.24966
AT2G37130	---	Peroxidase; heme binding	-4.25735
AT4G35030	---	Serine/threonine kinase	-4.29286
AT1G75780	TUB1	GTP binding in organ development	-4.30379
AT3G62040	---	Hydrolase activity; nitrate transport	-4.32248
AT1G49860	ATGSTF14	Glutathione transferase	-4.51206
AT4G31910	BAT1	Acyl-CoA ligase; iron ion/nitrate transport	-4.56468
AT4G15390	---	Acyl transferase; transition metal ion	-4.6263

		transport	
AT1G48930	AtGH9C1	O-glycosyl hydrolase; carbohydrate binding	-4.6541
AT3G54700	PGT1;7	Inorganic phosphate transporter; sugar:hydrogen symporter	-4.65562
AT1G62980	ATEXPA18	Tichoblast/root hair differentiation; plant type cell wall loosening	-4.6588
AT5G04960	---	Pectinesterase activity	-4.86267
AT4G25250	---	Pectinesterase inhibitor activity	-5.03158
AT2G45180	---	Lipid transport	-5.07169
AT3G59930	---	Unknown	-5.17541
AT4G26010	---	Peroxidase; heme binding	-5.19962
AT4G22010	sks4	Copper ion binding; oxidoreductase	-5.31254
AT5G66460	ATMAN7	O-glycosyl hydrolase; cation binding	-5.34858
AT3G49960	---	Sugar transport	-5.64543
AT1G52050	---	Unknown	-5.66864
AT3G62680	PRP3	Root and trichoblast development	-5.842
AT5G44020	---	Acid phosphatase	-5.87456
AT5G67400	RHS19	Peroxidase; heme binding	-6.03164
AT4G02270	RHS13	Nitrate transport; trichoblast differentiation	-6.08117
AT3G56980	BHLH039	Transcription factor responsive to iron homeostasis	-6.12045
AT2G32300	UCC1	Copper ion binding; root hair elongation	-6.17691
AT1G74770	---	Zinc ion binding; nitrate transport	-6.41425
AT5G54370	---	Unknown	-6.49389
AT2G32270	ZIP3	Metal ion transporter; root hair elongation	-6.57192
AT5G47450	TIP2;3	Ammonia transporter; water transport	-6.61023
AT1G30870	---	Peroxidase; heme binding	-6.73989
AT3G61430	PIP1A	Water transport	-6.75586
AT2G39430	---	lignin biosynthesis; root hair cell differentiation	-6.78424
AT4G00680	ADF8	Actin binding	-6.91577
AT1G32450	NRT1.5	Oligopeptide transport; nitrate transporter	-7.03232
AT1G20160	ATSBT5.2	Serine-type endopeptidase	-7.41482
AT4G31320	SAUR37	Calmodulin binding responsive to auxin	-7.49889
AT3G58990	IPMI1	Hydro-lyase; 3-isopropylmalate dehydratase	-7.67531
AT2G43600	---	Chitinase activity	-7.82159
AT3G04320	---	Endopeptidase inhibitor	-8.68627
AT5G42180	PER64	Peroxidase; heme binding	-9.44853
AT1G05240	---	Peroxidase; heme binding	-9.70595
AT5G50200	WR3	Nitrate/nucleotide/ammonium transport responsive to wounding	-10.2082
AT5G62340	---	Pectinesterase inhibitor	-10.5598

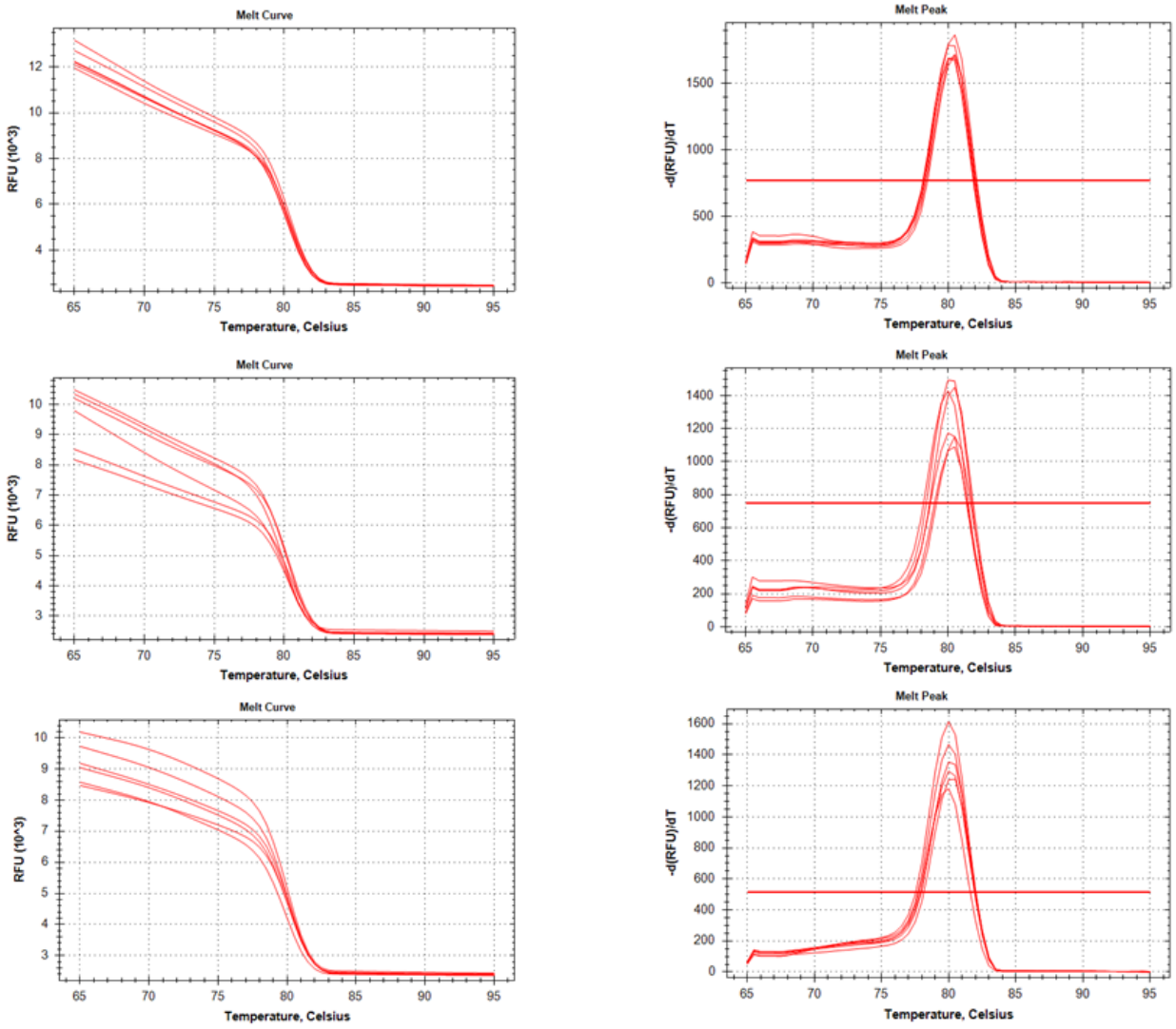
AT5G04950	NAS1	Nicotianamine synthase	-11.3602
AT5G10130	---	Unknown	-11.5431
AT4G37160	sks15	Oxidoreductase; copper ion binding	-13.3488
AT5G46890	---	Lipid transport/binding	-16.032
AT4G12510	---	Lipid transport	-16.4018
AT5G60520	---	Unknown	-17.0322
AT4G17340	TIP2;2	Water transport responsive to nitrate	-17.5737
AT3G01190	---	Peroxidase; heme binding	-18.1944
AT3G19430	---	Unknown	-18.499
AT2G33790	AGP30	Unknown	-22.0791

Appendix 9. Transcripts detected in root and shoot tissues in response to *A. tumefaciens* C58.

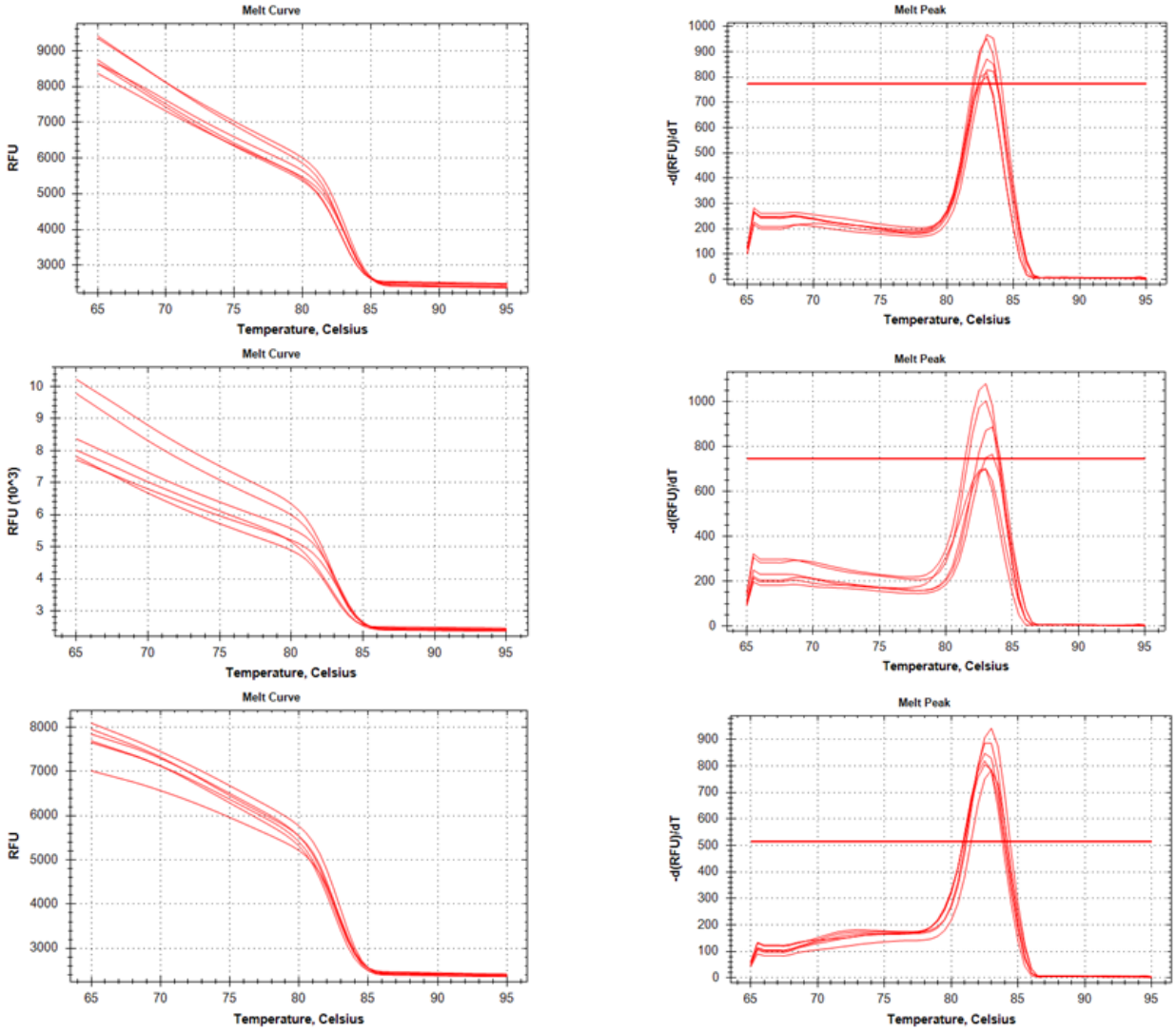
A. thaliana Col-0 DEGs detected in both root and shoot tissue in responses to *A.tumefaciens* C58 hydroponic co-cultivation 8hpi.

Gene Name	Locus Tag	Leaf	Root
AHB1	AT2G16060	2.66724	2.34737
APR3	AT4G21990	7.55009	3.22695
ARL	AT4G30960	2.0055	2.29985
ATBCB	AT1G19670	2.88342	3.74197
ATEP3	AT3G54420	2.41811	2.08912
ATEXPB3	AT4G28250	2.83195	-3.49362
ATFER1	AT2G38010	2.76122	3.53523
ATGSTF6	AT4G10270	3.79576	5.6337
ATHCHIB	AT3G12500	6.77424	-2.19803
ATLP-1	AT1G18250	2.692	-2.10971
ATPT2	AT3G54700	6.11168	-4.65562
ATSDI1	AT5G48850	21.9284	2.6725
AtSIP2	AT2G25060	2.08061	2.37886
BHLH039	AT3G56980	25.865	-6.12045
SWEET11	AT3G48740	2.27116	-2.52271
Chitinase family protein	AT2G43590	5.22968	24.1334
CYP707A3	AT5G45340	2.41642	-2.99992
CYP81F2	AT4G39510	3.64022	5.20586
defensin-like protein 206	AT3G59930	-10.192	-5.17541
DOX1	AT3G01420	3.49003	4.02665
FAD-binding and BBE domain-containing protein	AT1G56430	7.29814	9.29824
FAD-binding Berberine family protein	AT1G67360	3.7411	5.57189
FAH1	AT1G29050	2.10813	2.43008
FMO GS-OX3	AT1G62560	2.21816	-2.74766
GAMMA-VPE	AT4G32940	3.0723	3.09321
Glutaredoxin-C6	AT4G33040	3.57721	2.33645
Glycerophosphoryl diester phosphodiesterase family protein	AT1G02360	2.3699	2.85646
GPI-anchored glycoprotein membrane precursor	AT5G19240	2.21617	-2.27084
Granulin repeat cysteine protease family protein	AT5G13330	-2.0161	2.27116
HSPRO2	AT3G16690	-2.0433	2.2263
IAGLU	AT5G16570	2.34999	2.83282
Invertase/pectin methylesterase inhibitor-like protein	AT2G01610	3.15583	-2.6447
Jacalin-related lectin	AT3G16450	-2.0100	-2.1985
Legume lectin-like protein	AT4G28250	2.83195	3.64498

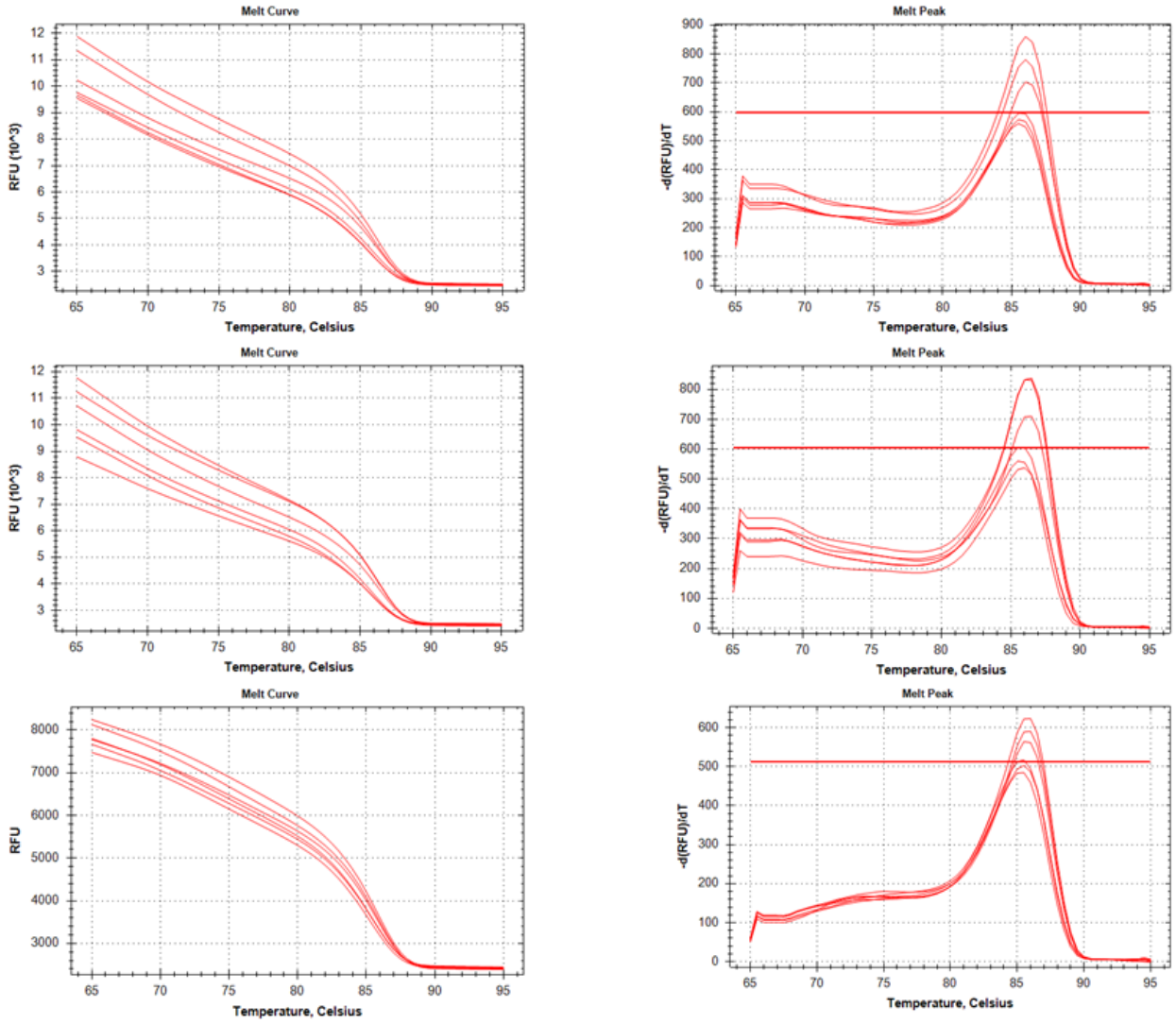
LSU1	AT3G49580	24.5827	3.16241
LTI78	AT5G52310	3.04264	-3.19218
LZF1	AT2G34600	-2.3629	2.08516
MAM1	AT5G23010	-2.1185	-2.0295
MERI5B	AT4G11650	5.0671	7.3239
MIPS2	AT1G74210	2.57559	3.1608
NAS4	AT1G56430	7.14376	-2.6564
NIA1	AT5G19240	2.21617	2.60561
pEARLI 1	AT4G12480	4.53744	-2.98839
Peroxidase	AT5G42180	5.13525	-9.44853
Peroxidase 71	AT5G64120	10.0666	7.57792
Phosphoglycerate mutase family protein	AT1G24020	3.03035	4.08233
Polygalacturonase 2	AT1G70370	2.14205	-2.6034
Protein aspartic protease in guard cell 1	AT3G18490	2.00277	-2.02896
Rubber elongation factor protein	AT1G67360	3.7411	2.59821
Serine carboxypeptidase S10 family protein	AT2G22970	2.84119	-2.12205
TCH4	AT2G28790	2.74263	3.45149
TUB1	AT1G75780	2.55432	-4.30379



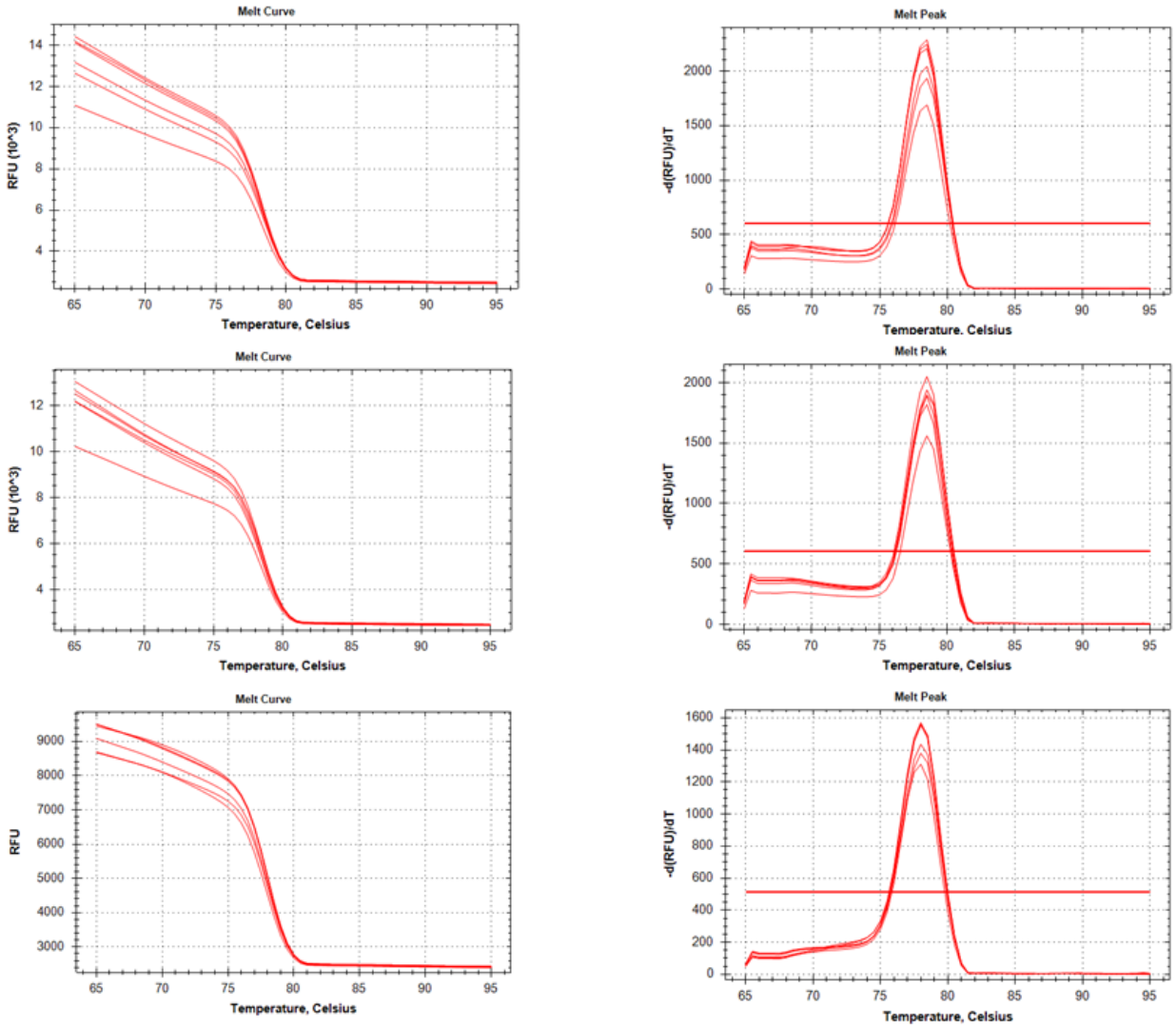
Appendix 10. Melt curve analysis for ATEXT4.



Appendix 11. Melt curve analysis for LTP3.



Appendix 12. Melt curve analysis for LSU1.



Appendix 13. Melt curve analysis for WCOR413.

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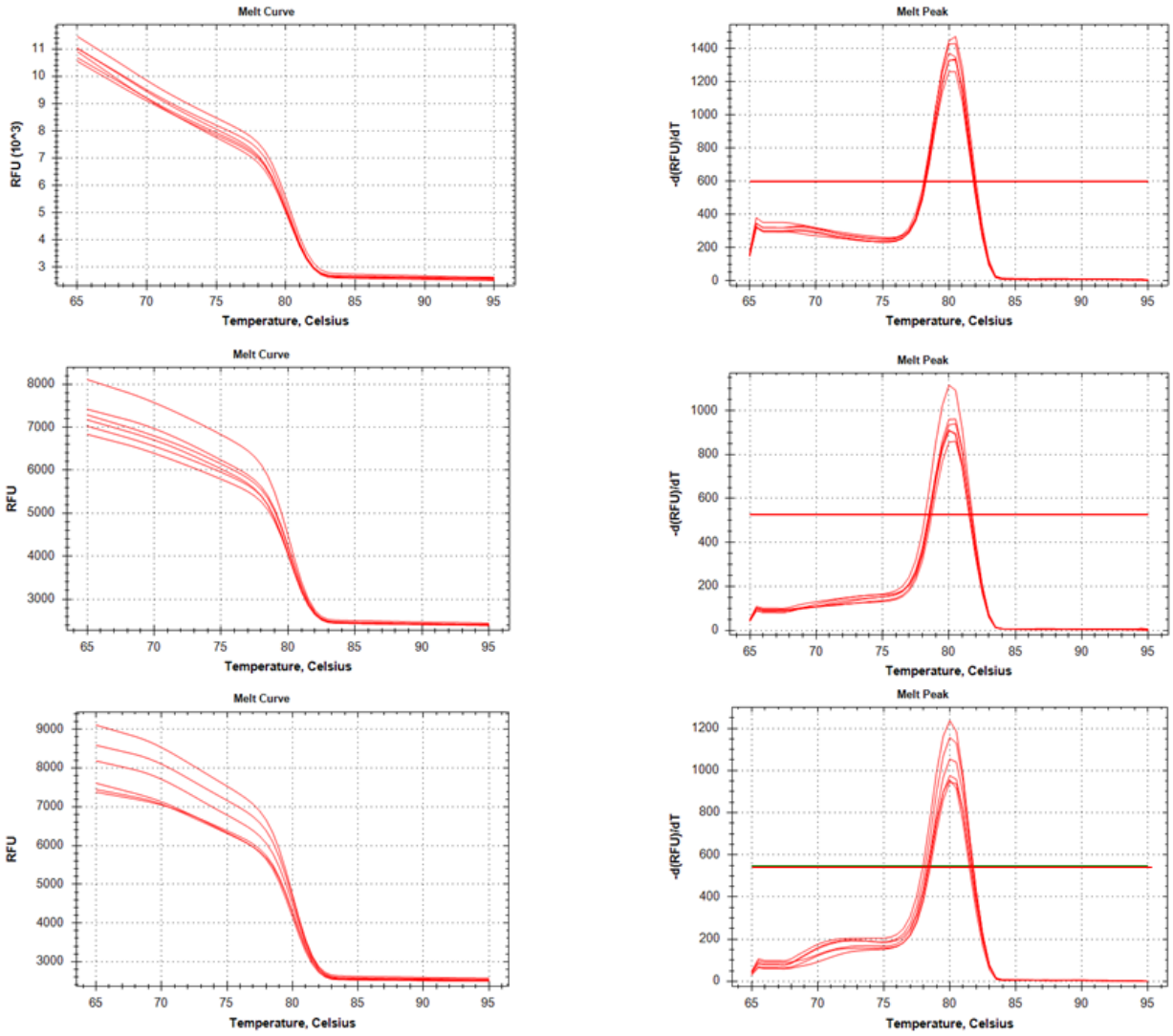
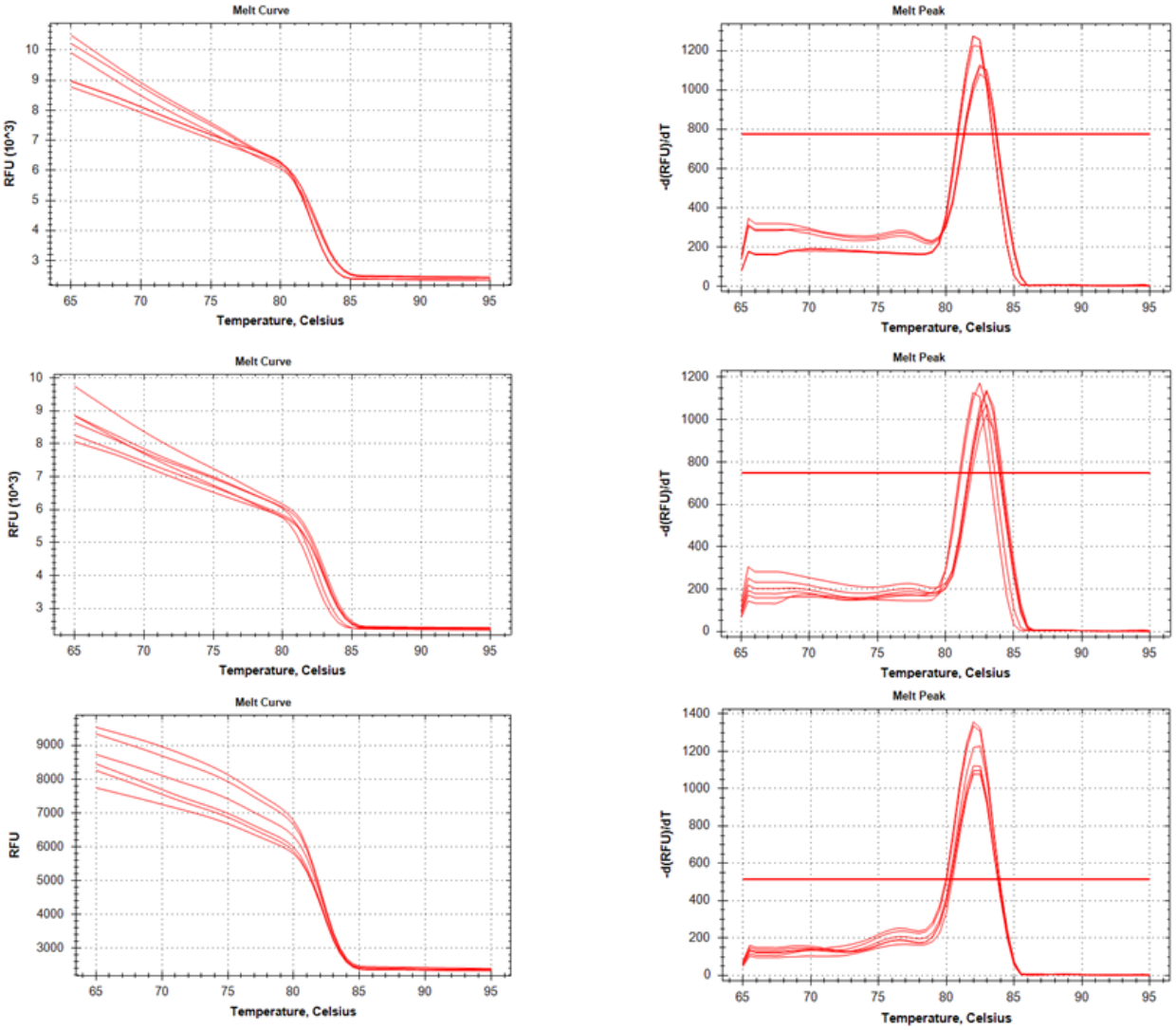
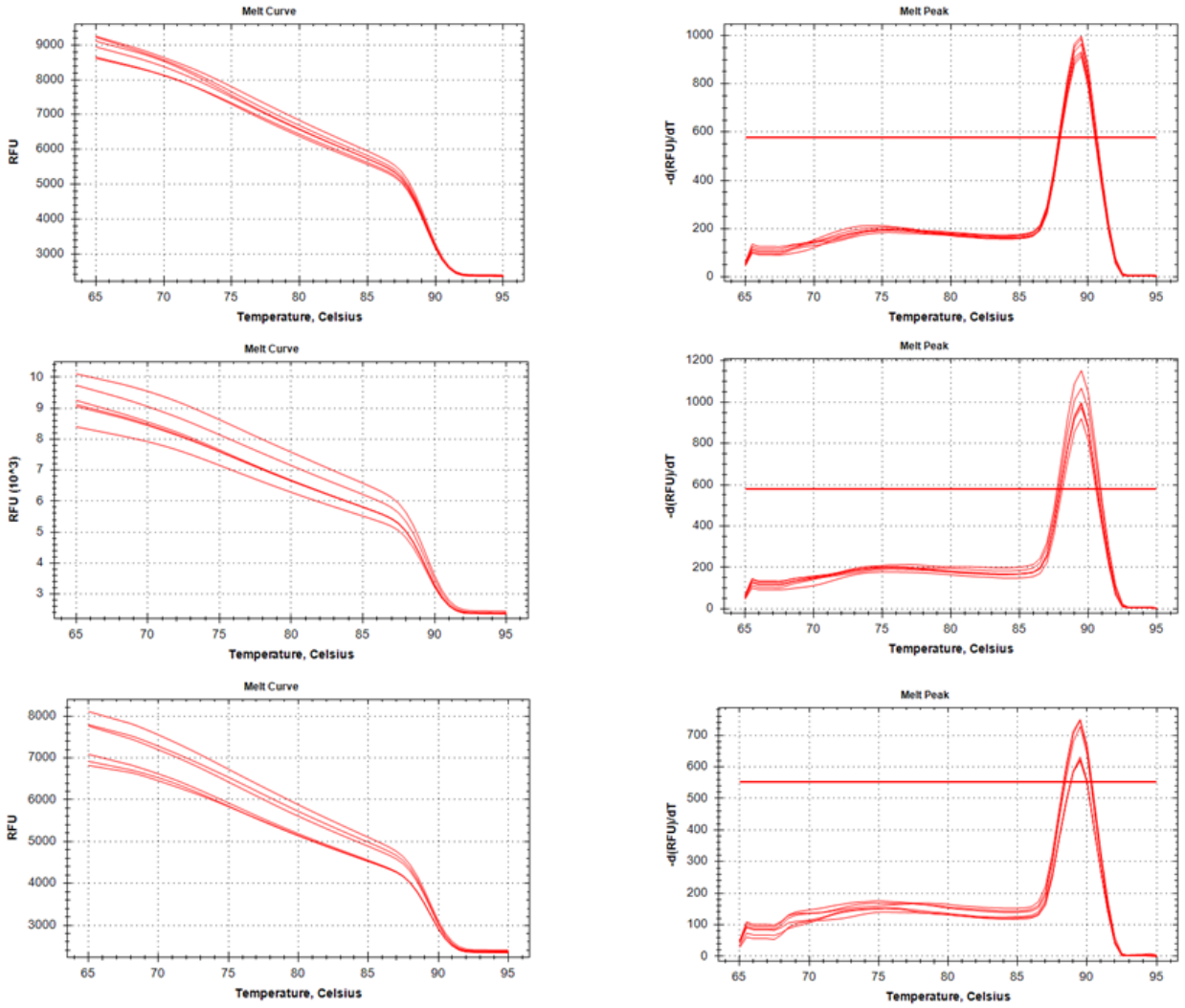


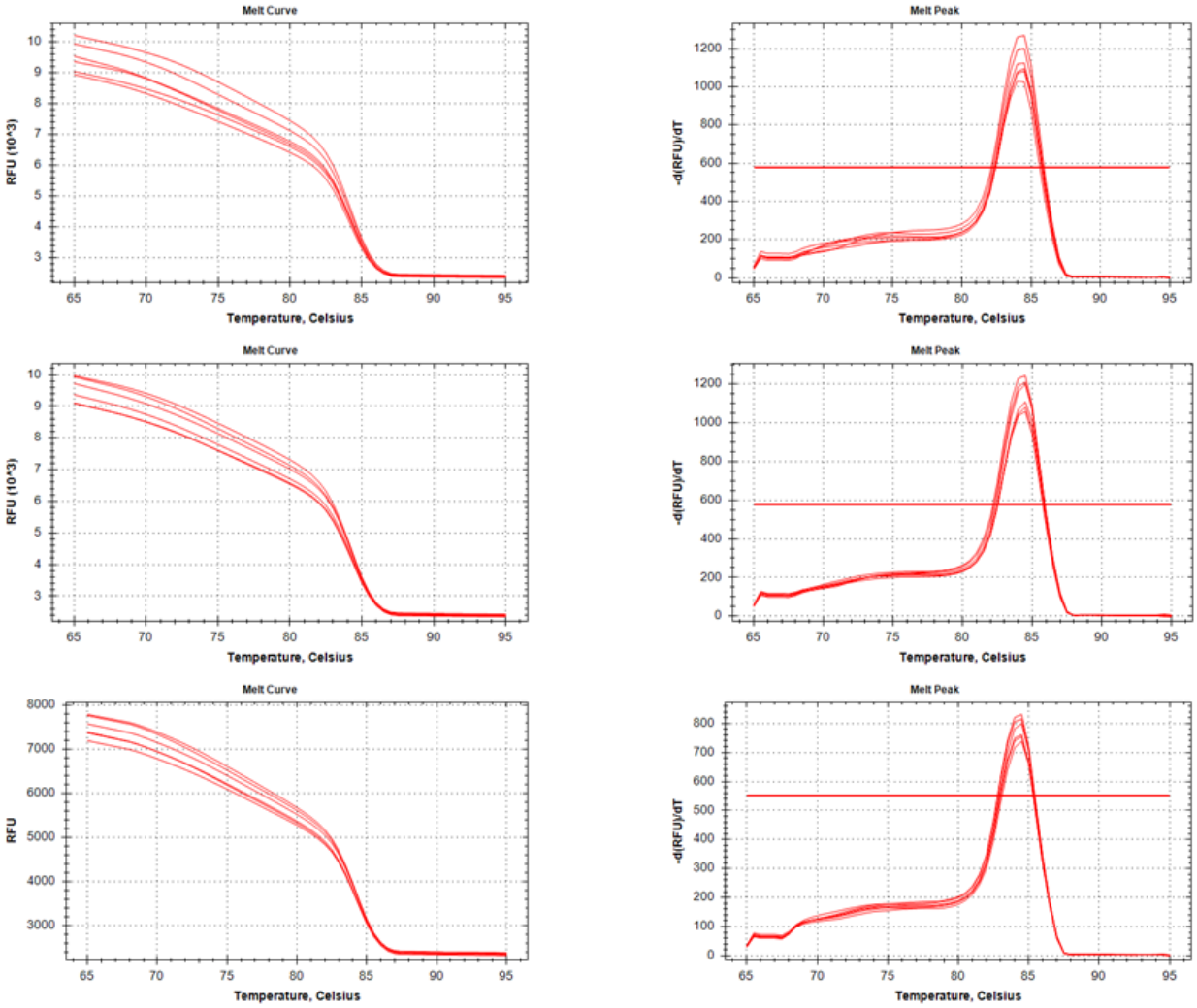
Appendix 14. Melt curve analysis for ERF012.



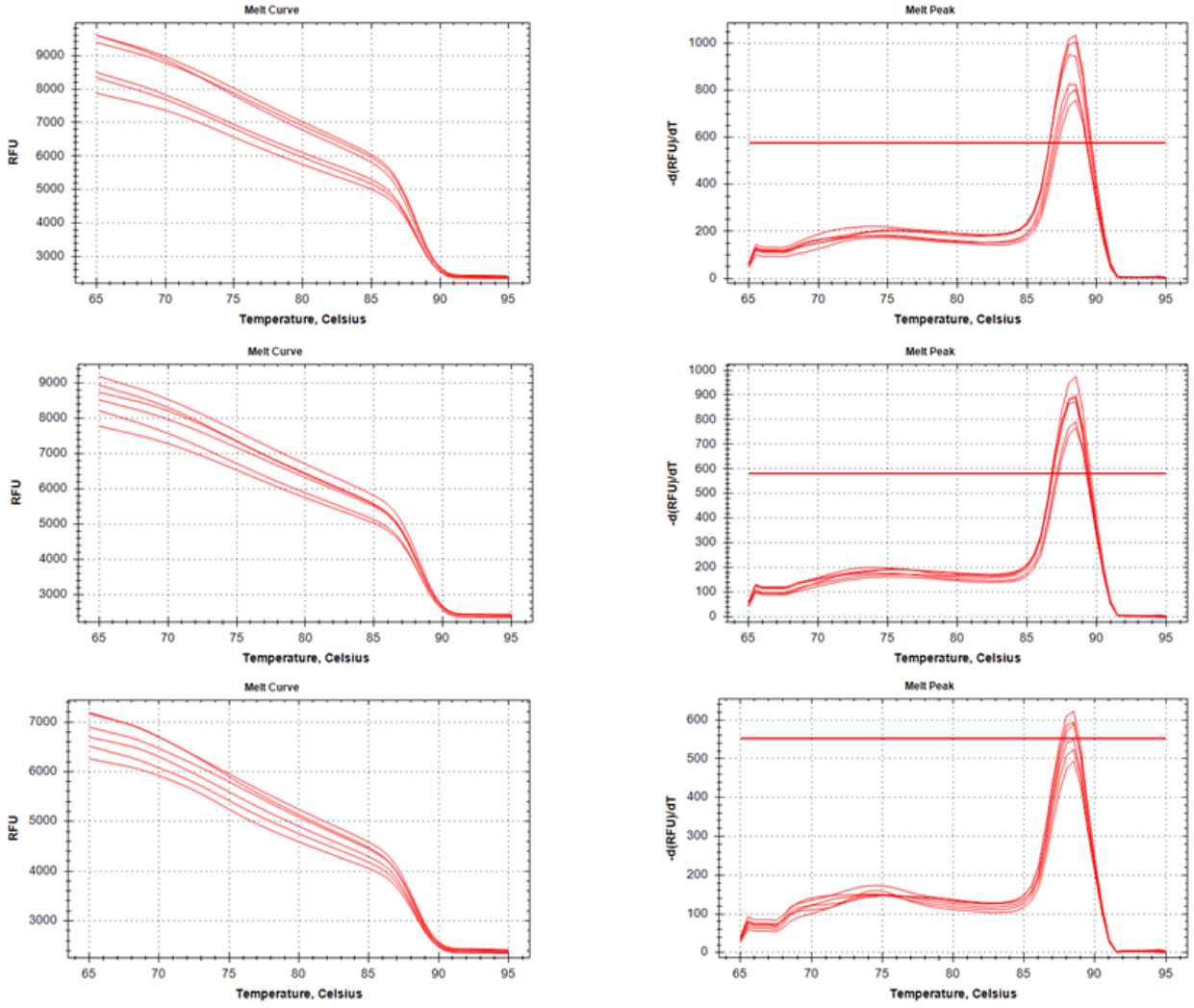
Appendix 15. Melt curve analysis for ACTINII.



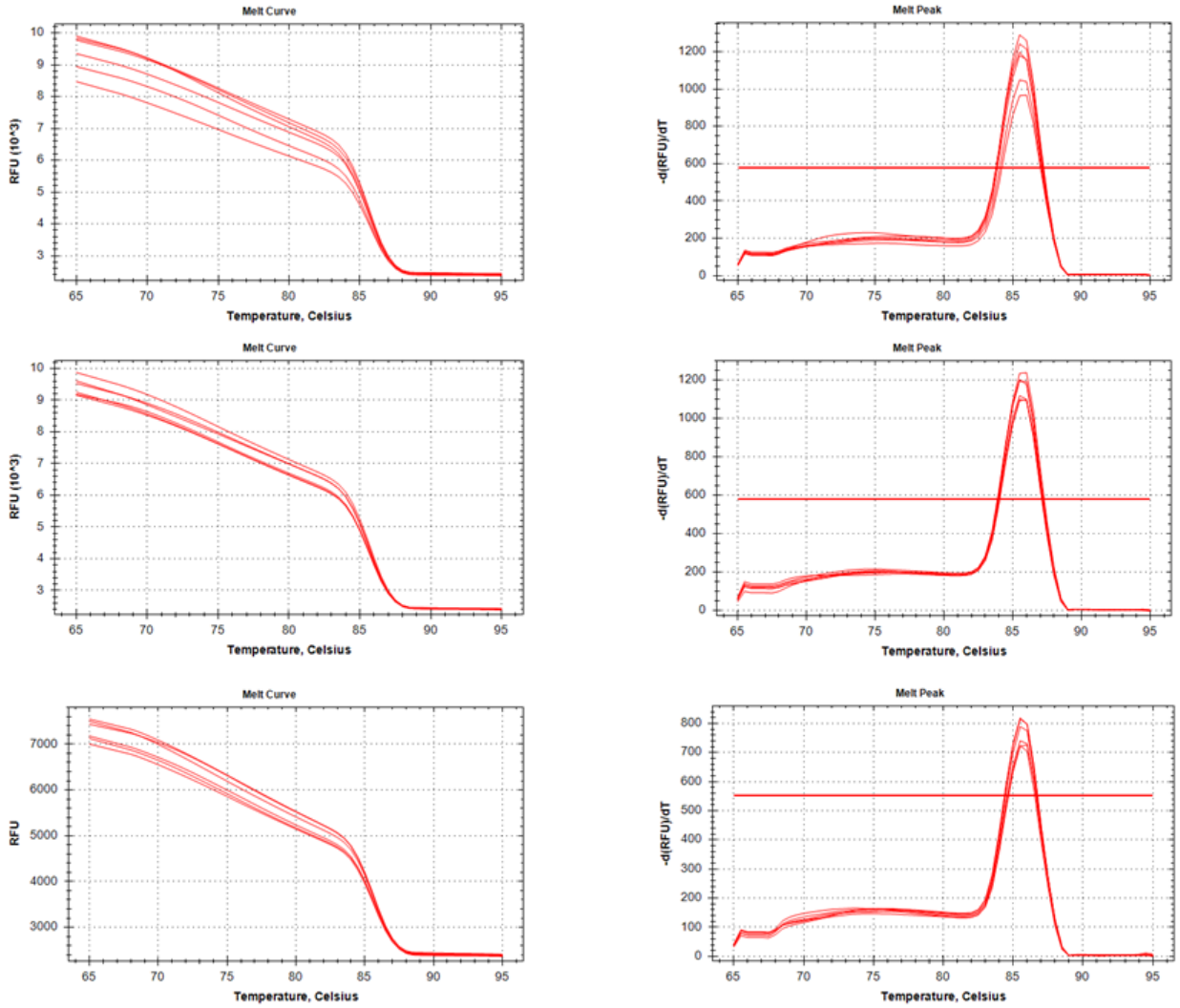
Appendix 16. Melt curve analysis for HSP21.



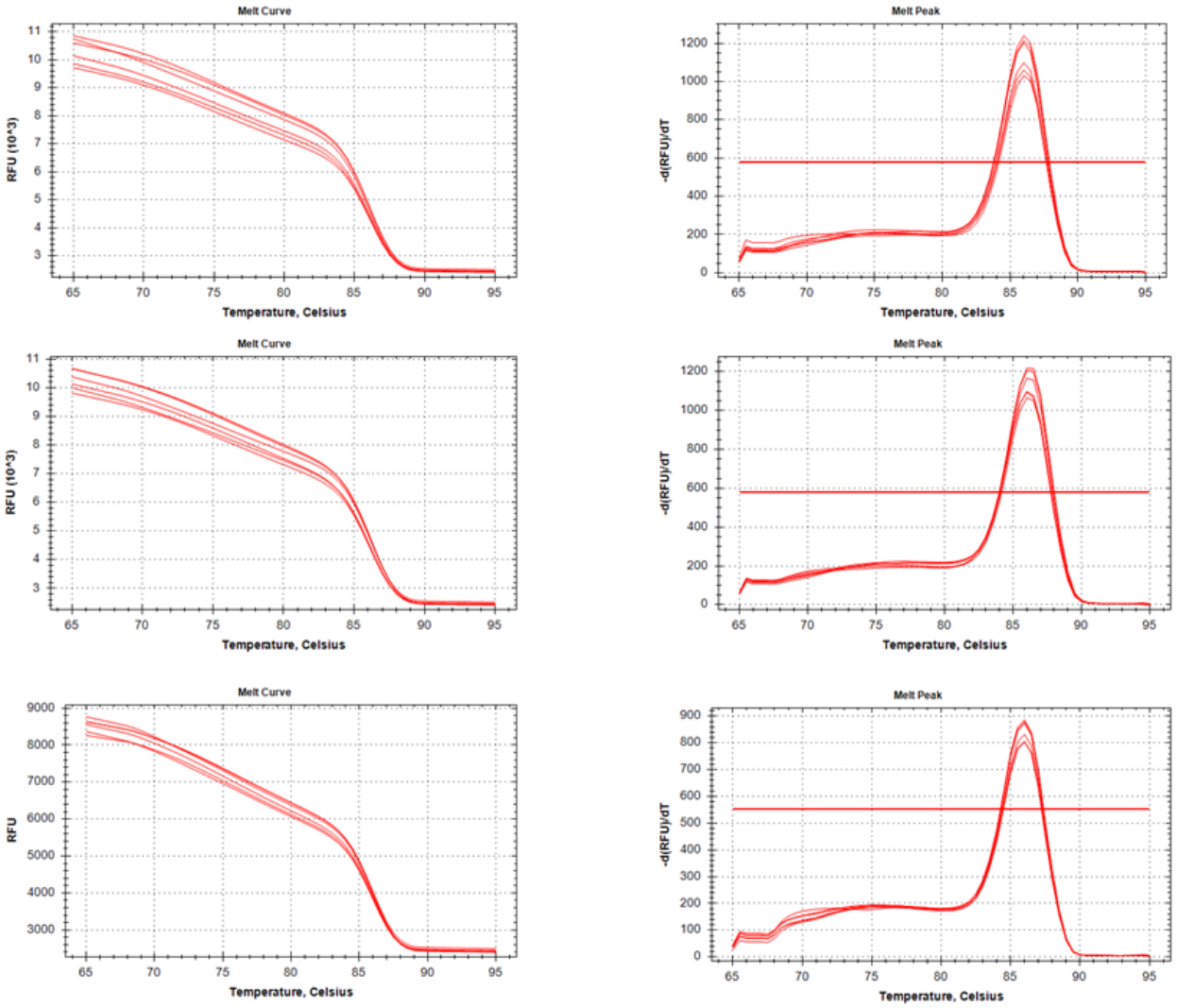
Appendix 17. Melt curve analysis for PGIP1.



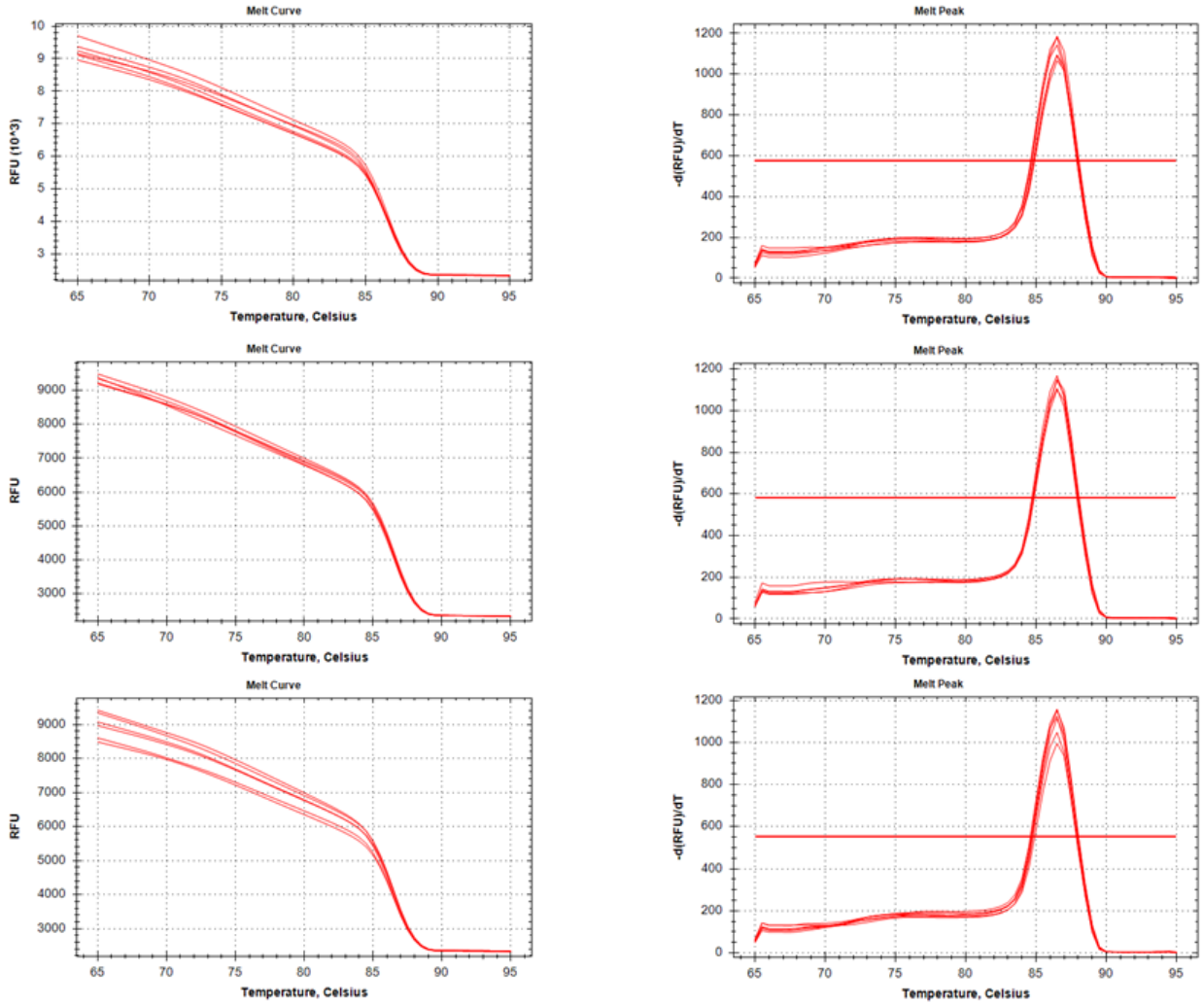
Appendix 18. Melt curve analysis for ELIP1.



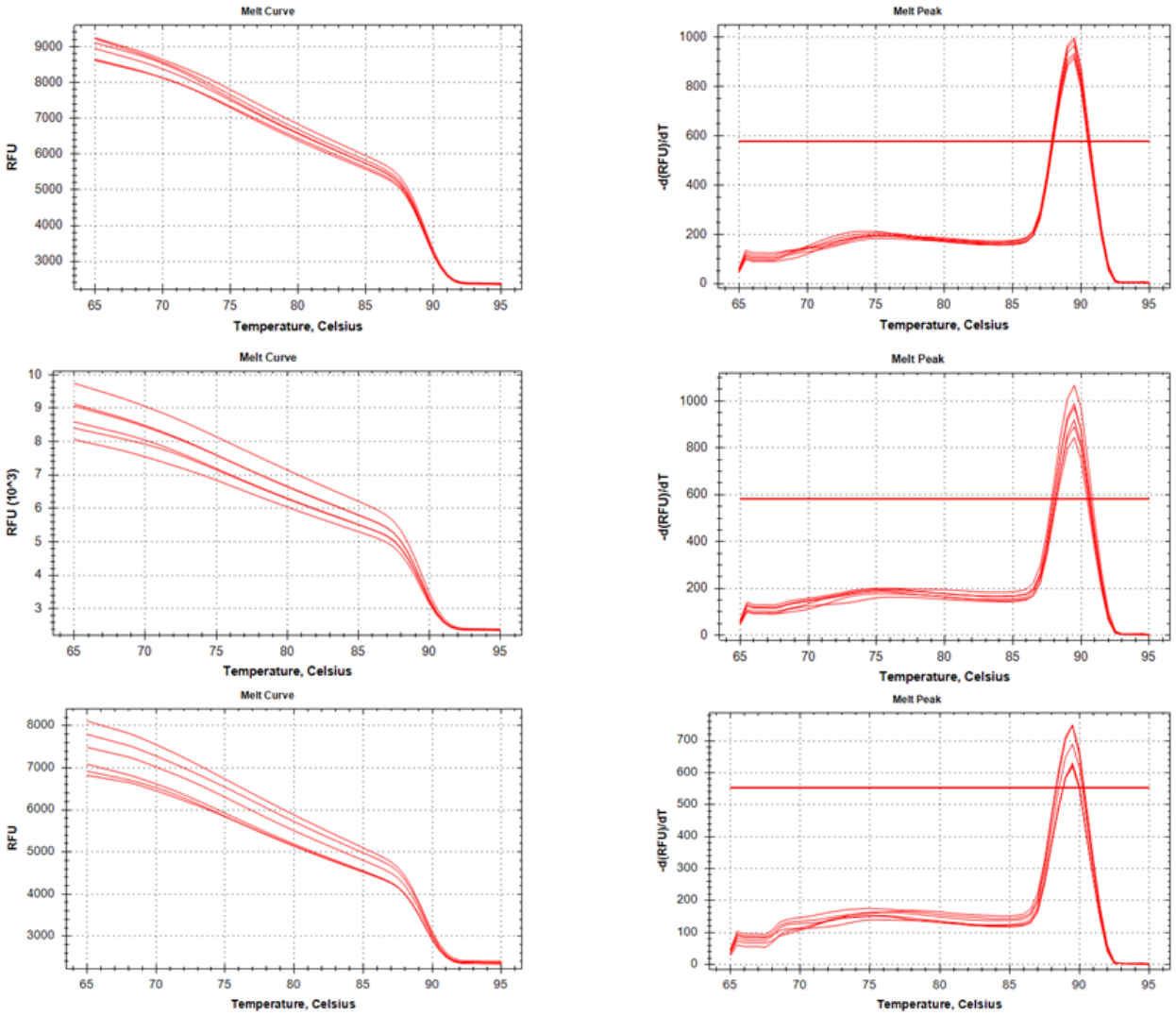
Appendix 19. Melt curve analysis for AGP30.



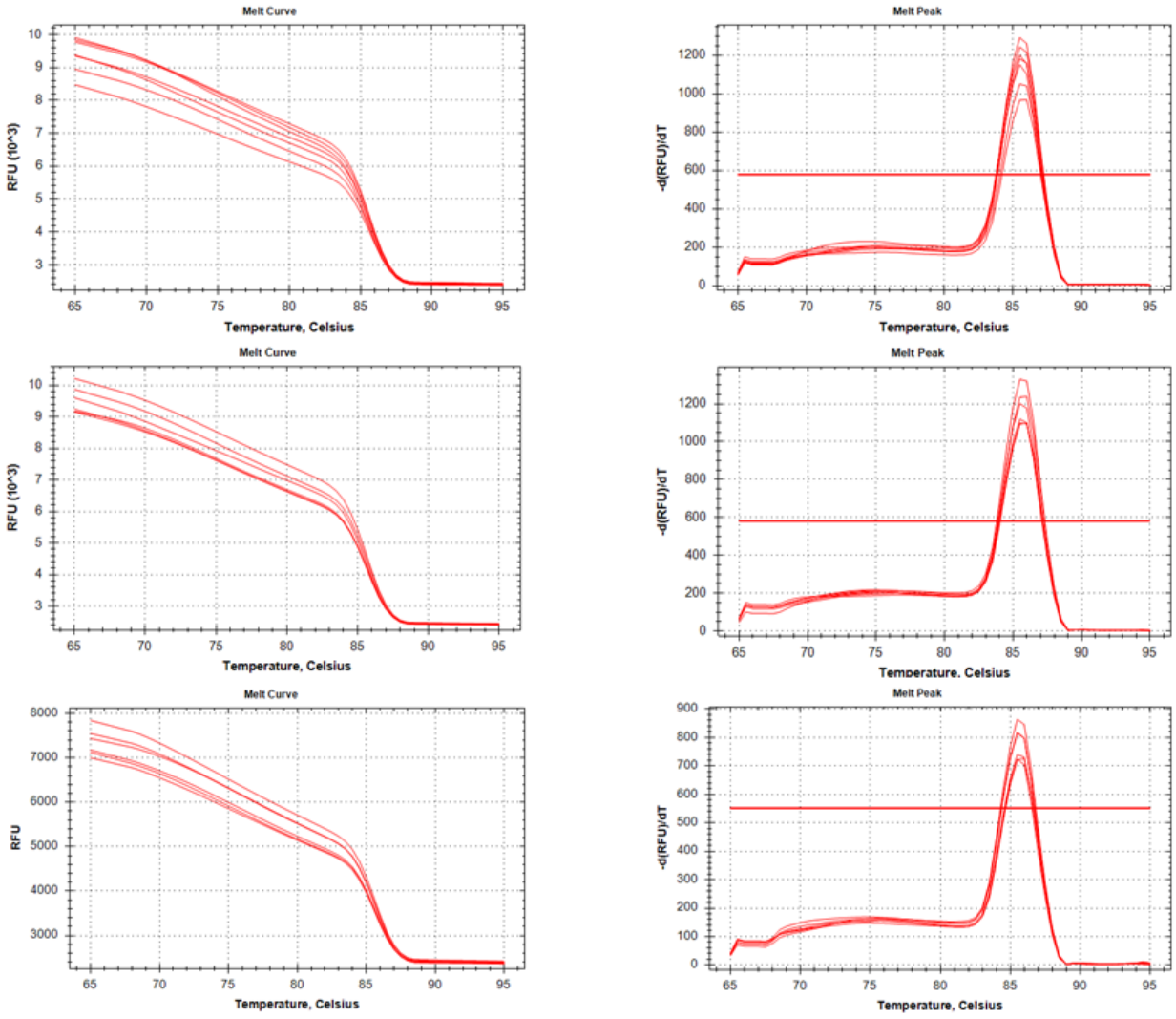
Appendix 20. Melt curve analysis for NAS1.



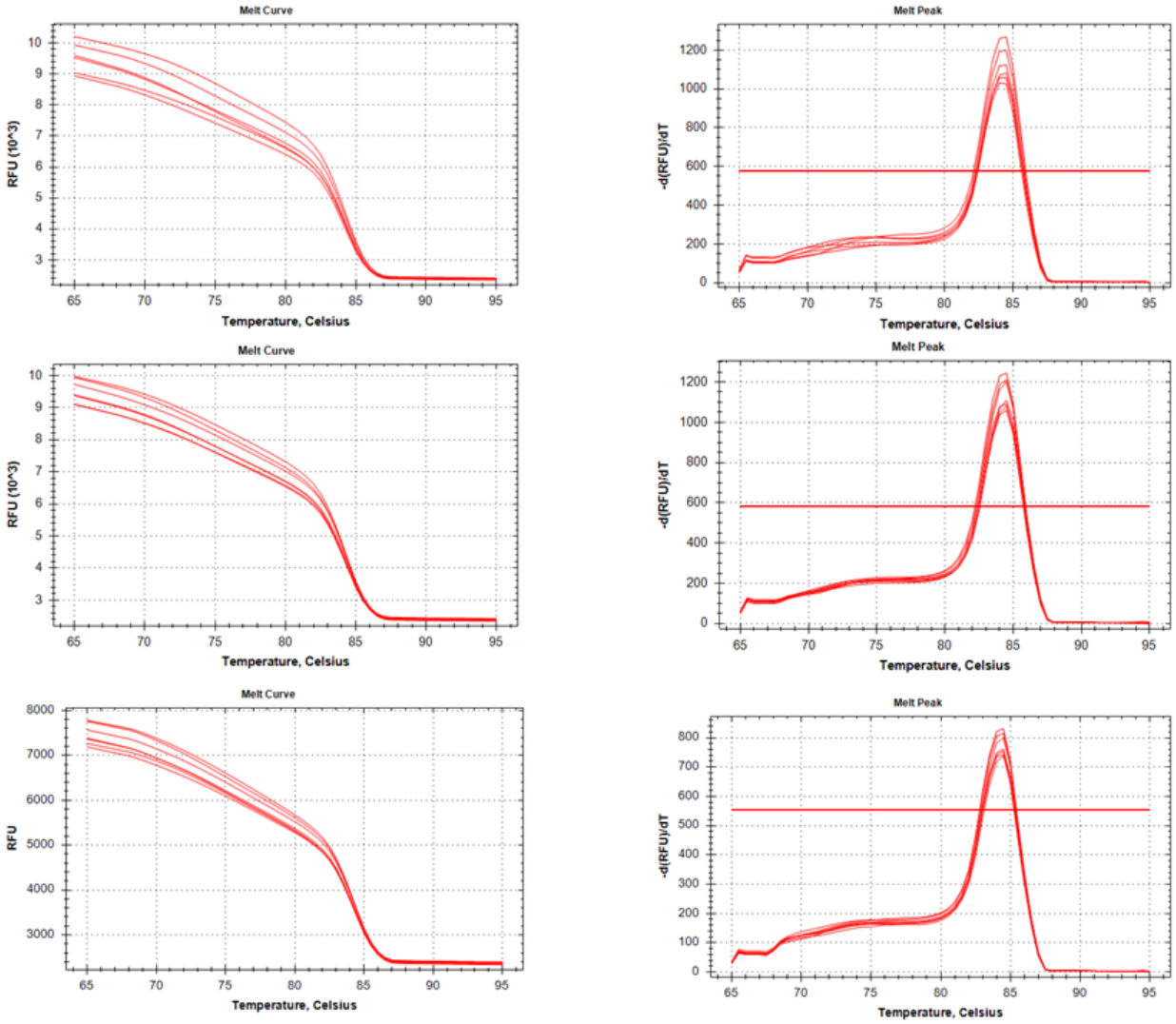
Appendix 21. Melt curve analysis for ACTINII.



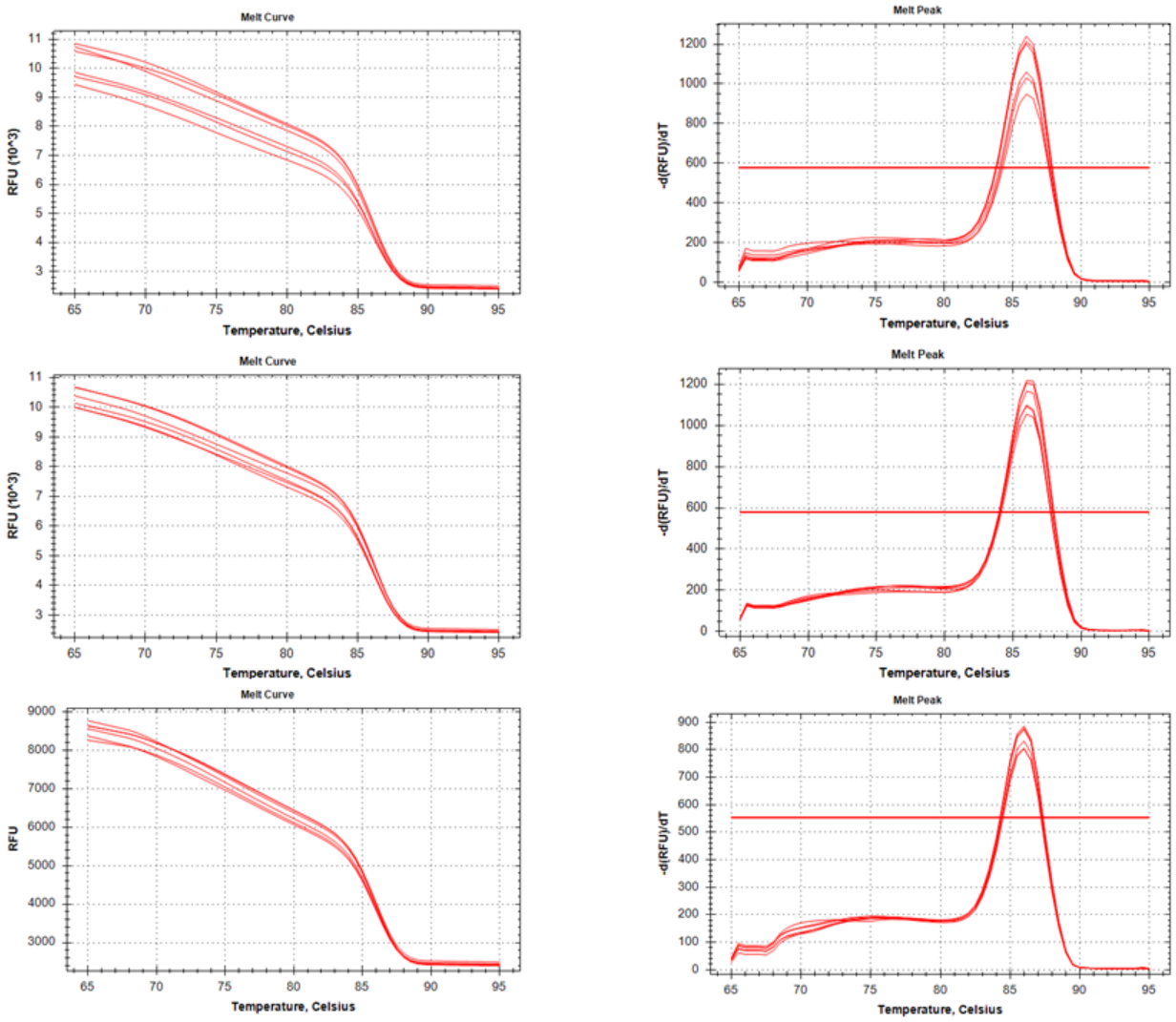
Appendix 22. Melt curve analysis for chvG.



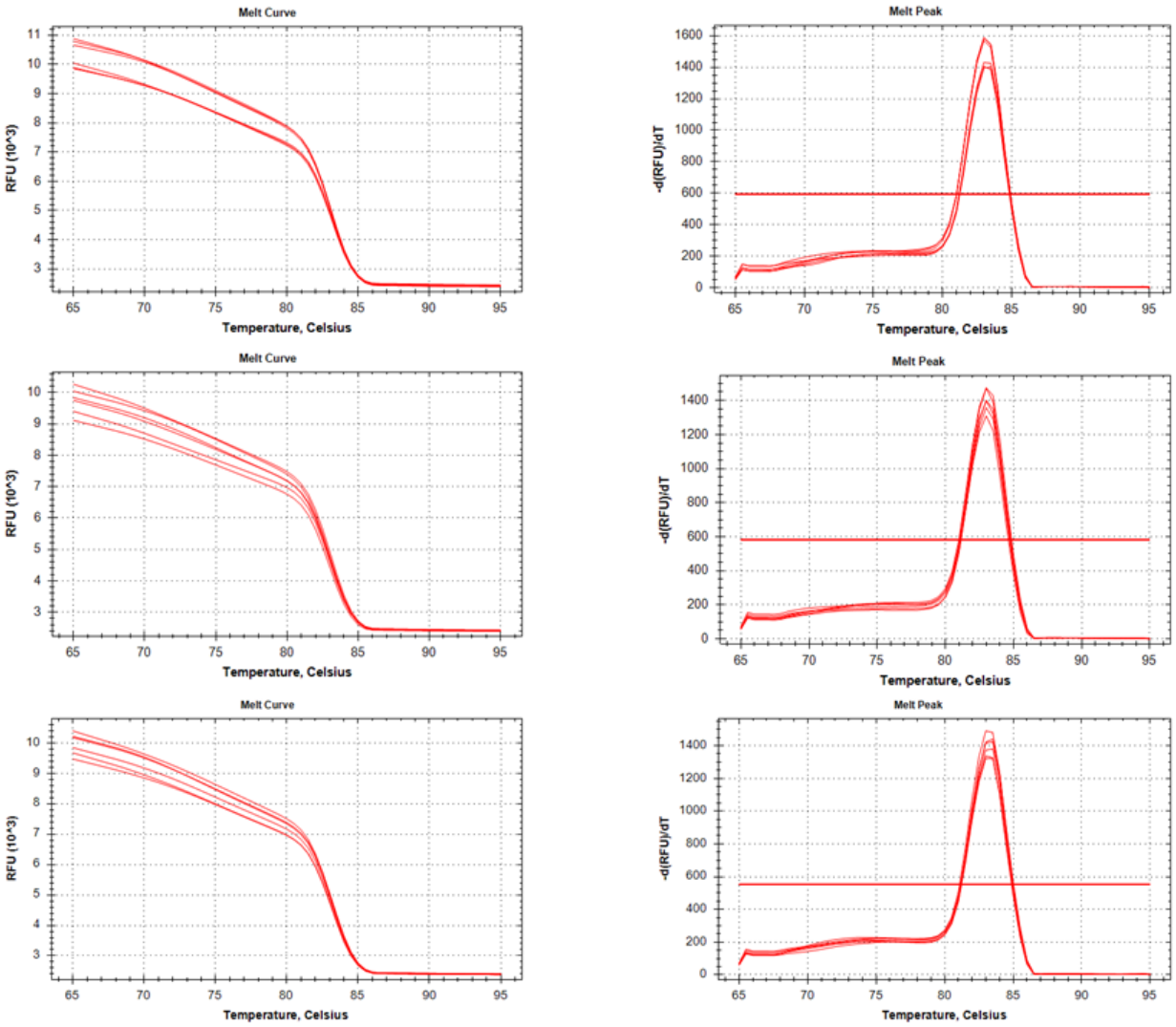
Appendix 23. Melt curve analysis for virA.



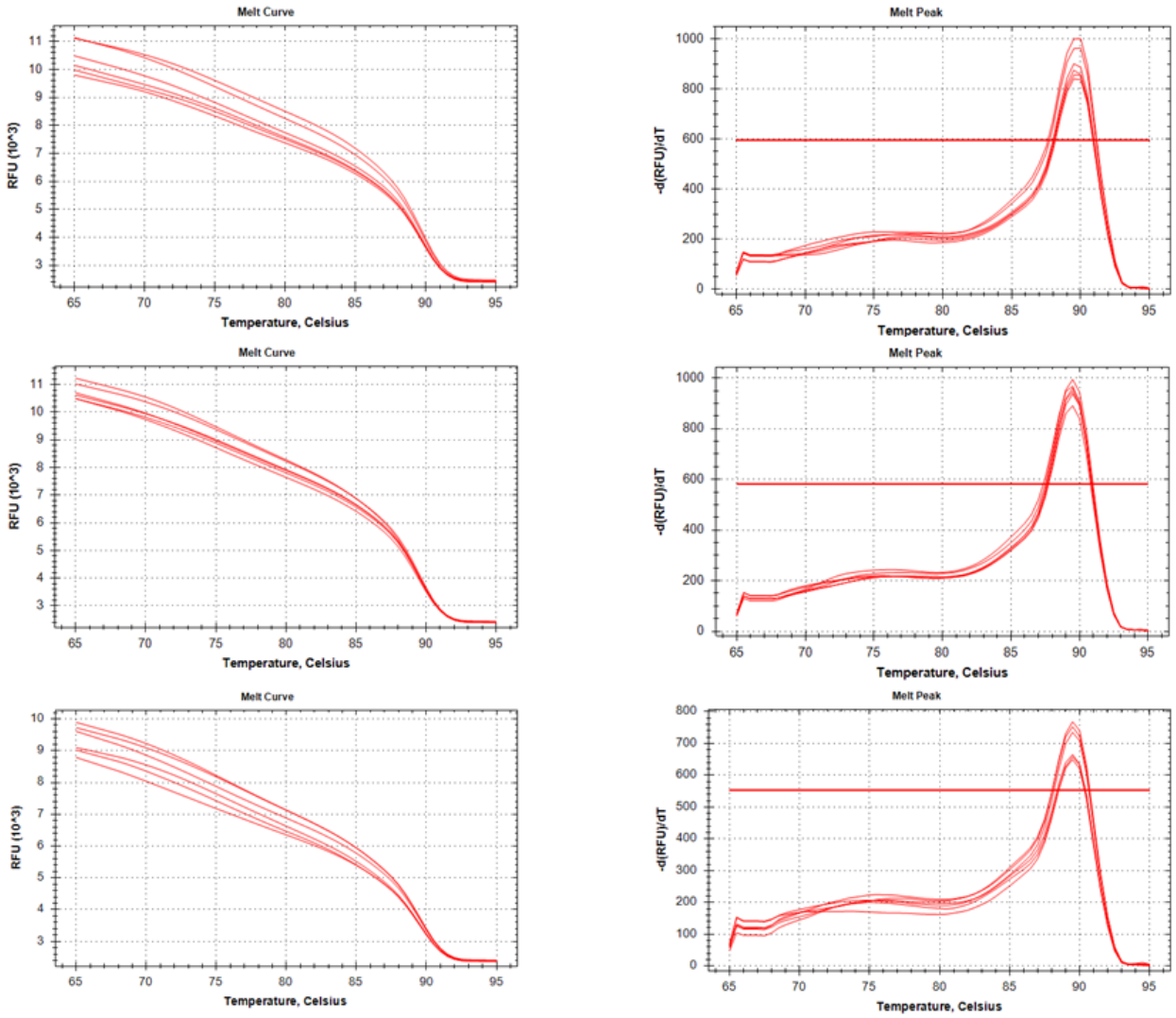
Appendix 24. Melt curve analysis for virD1.



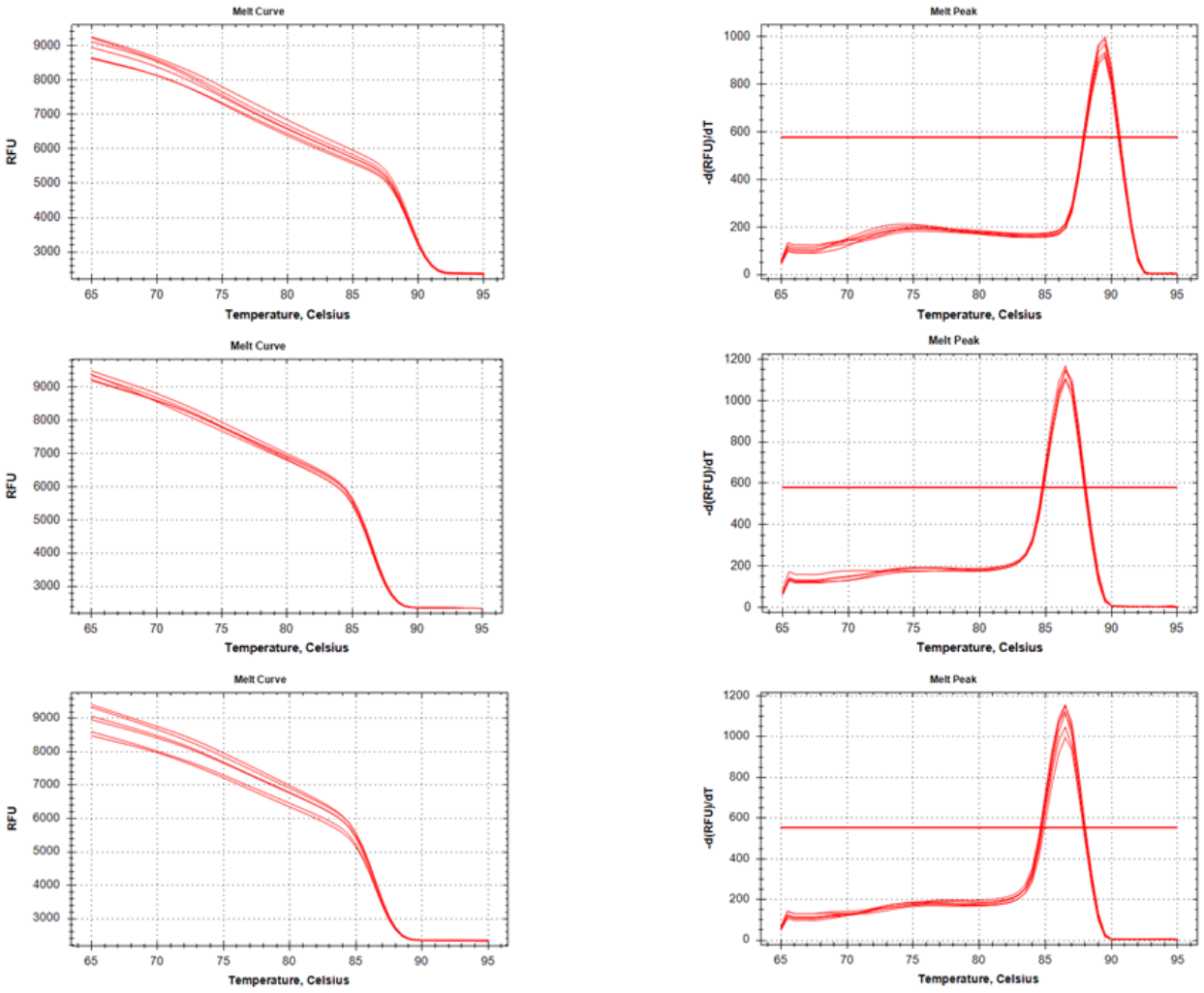
Appendix 25. Melt curve analysis for virE0.



Appendix 26. Melt curve analysis for virH1.



Appendix 27. Melt curve analysis for rpoB.



Appendix 28. Melt curve analysis for 16S rRNA.

Appendix 29. Shoot DEGs that have not been previously detected in shoot tissues.

Locus Tag	Gene Symbol	Function/Putative Function	Fold-Change
AT5G37950	---	Hexosyl transferase	3.49355
AT3G60940	---	Unknown	-2.10751
AT3G59930	---	Unknown	-10.1924

Appendix 30. Root DEGs that have not been previously detected in root tissue.

Locus Tag	Gene Symbol	Function/Putative Function	Fold-Change
AT4G27670	HSP21	Protein folding responsive to light/ROS/heat	35.8896
AT1G07400	---	Response to heat/oxidative stress	21.7988
AT2G23170	GH3.3	Indole-3-acetic acid amido synthetase	15.2514
AT3G09640	APX2	Peroxidase; heme binding	12.3951
AT1G05680	UGT74E2	UDP-glycosyltransferase; hexosyl transferase	11.3811
AT3G22840	ELIP1	Chlorophyll binding	10.8473
AT1G26380	---	FAD binding; UDP-N-acetylmuramate dehydrogenase	9.29824
AT3G21720	ICL	Isocitrate lyase	7.8989
AT4G34131	UGT73B2	UDP-glycosyltransferase; hexosyl transferase; quercetin 3-O-glycosyltransferase	7.25787
AT5G07570	---	Unknown	7.10518
AT1G59500	GH3.4	Indole-3-acetic acid amido synthetase	6.54002
AT1G57980	ATPUP18	Purine nucleobase transmembrane transporter activity	6.39702
AT1G69920	ATGSTU12	Glutathione transferase	6.37763
AT1G60730	---	Aldo-keto reductase (NADP)	6.02789
AT2G40340	ATERF48	Transcription factor responsive to ABA	5.71755
AT1G06430	FTSH8	ATP binding; ATPase activity; zinc binding; metalloendopeptidase	5.24027
AT2G43000	anac042	Negative regulator of leaf senescence	4.91828
AT1G32940	SBT3.5	Serine-type endopeptidase	4.25176
AT5G08250	---	Oxidoreductase; heme binding	4.23089
AT5G43450	---	Oxidoreductase; 1-aminocyclopropane-1-carboxylate oxidase	4.19297
AT1G64950	CYP89A5	Heme binding; oxidoreductase	4.16061
AT4G38420	sks9	Oxidoreductase; copper ion binding	4.08849

0			
AT3G6042			
0	---	Negative regulation of defense response	4.08233
AT5G5397			
0	TAT7	L-tyrosine:2-oxoglutarate aminotransferase	3.91818
AT5G1786			
0	CAX7	Cation:sodium ion transporter	3.87806
AT1G5763		Amino acid import; SA biosynthesis/signalling;	
0	---	JA signalling	3.86487
AT2G1866			
0	EXLB3	Alternative respiration; SAR	3.70956
AT3G4748			
0	---	Chitinase	3.6709
AT1G3004	ATGA2O		
0	X2	Iron ion binding; oxidoreductase	3.64528
AT2G2946	ATGSTU		
0	4	Glutathione transferase; toxin catabolism	3.44004
AT2G3797			
0	SOUL-1	Flavonoid biosynthesis in response to light	3.37229
AT5G4913			
0	---	Drug antiporter	3.30738
AT3G4958			
0	LSU1	Unknown	3.16241
AT2G4752			
0	HRE2	Transcription factor responsive to anoxia	2.9521
AT3G1825			
0	---	Amino acid import	2.81123
AT1G5025		Nucleoside-triphosphate activity;	
0	FTSH1	metalloendopeptidase	2.66234
AT4G1095			
5	UGE5	Triglyceride lipase	2.55185
AT5G6406		Transcription factor responsible for organismal	
0	anac103	development	2.43108
AT3G5738			
0	---	Glycosyl transferase	2.33509
AT3G2216			
0	JAV1	Protein targeting; response to ABA/SA	2.24631
AT1G3235			
0	AOX1D	Alternate oxidase	2.22872
AT4G3542			
0	DRL1	Catalytic activity; seed/pollen exine formation	2.20874
AT1G3523			
0	AGP5	Unknown	2.18414
AT4G2501	SWEET1		
0	4	Sucrose transport	-2.07828
AT1G4457	NPQ4	Xanthophyll/chlorophyll binding	-2.23243

5			
AT4G25050	ACP4	ACP Phosphopantetheine binding for fatty acid biosynthesis	-2.26646
AT1G20020	FNR2	NADP dehydrogenase/flavin adenine dinucleotide reductase	-2.37195
AT4G33260	CDC20.2	Signal transduction for mitotic processes; heterotrimeric G-protein	-2.45548
AT1G48500	JAZ4	Protein binding in response to wounding/JA	-2.53735
AT3G02610	---	Acyl-[acyl-carrier-protein] desaturase; transition metal ion binding	-2.56804
AT4G12480	pEARLI 1	Lipid transport; response to salt stress/fungus/col	-2.98839
AT4G29340	PRF4	Actin binding for cytoskeleton organization	-3.51093
AT3G54700	PGT1;7	Inorganic phosphate transporter; sugar:hydrogen symporter	-4.65562
AT3G59930	---	Unknown	-5.17541
AT3G04320	---	Endopeptidase inhibitor	-8.68627
AT1G05240	---	Peroxidase; heme binding	-9.70595
AT5G46890	---	Lipid transport/binding	-16.032
AT4G12510	---	Lipid transport	-16.4018

Curriculum Vitae

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Eastman, A. W., Weselowski, B., Nathoo, N. & Yuan, Z. C. (2014). **Complete Genome Sequence of *Paenibacillus polymyxa* CR1, a Plant Growth-Promoting Bacterium Isolated from Corn Rhizosphere Exhibiting Potential for Biocontrol, Biomass Degredation, and Biofuel Production.** *Genome Announc* **2,** pii: e01218-13.