PLANT HORMONE PATHWAYS PLAY A CRUCIAL ROLE IN SOLANUM SPP. INTERACTIONS WITH THE SOIL ENVIRONMENT

by

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Dedicated to my Grandpa Pedersen, for giving me my love of all things green and beautiful.

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LIST OF ABBREVIATIONS

TERMS

ACC	1-aminocyclopropane-1-carboxylic acid
ASV	Amplicon sequence variant
BC	Biochar
BW	Bacterial wilt
ddH_2O	Double distilled water
Dpi	Days post inoculation
ET	Ethylene
Hpi	Hours post inoculation
JA	Jasmonic acid
SA	Salicylic acid
RS	*Ralstonia solanacearum*
RNA-seq	RNA sequencing

TOMATO GENOTYPES

H7996	*Solanum lycopersicum* cv. Hawaii7996
M82	*S. lycopersicum* cv. M82
WV	*S. pimpinellifolium* accession West Virginia700
SP	*S. pennellii* accession LA0716
LA2093	*S. pimpinellifolium* accession LA2093
ACD	ACC-deaminase transgenic mutant, background UC82B
UC82B	*S. lycopersicum* cv. UC82B
Nr	*Never ripe* mutant, background Pearson
Pearson	*S. lycopersicum* cv. Pearson
def1	*defenseless1* mutant, backround CMII
CMII	*S. lycopersicum* cv. Castlemart II
NahG	salicylate hydroxylase transgenic mutant, background MM
MM	*S. lycopersicum* cv. Money Maker

ABSTRACT

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Plants regulate responses to their environment through complex hormone signaling; these hormones can be categorized broadly into two categories: growth and defense, though many have roles in both. Much remains to be understood about the complexity of hormone signaling in relation to environmental responses, especially species- and genotype-specific differences. Unraveling this complexity of hormone signaling will lead to the development of resilient crops that are able to respond appropriately to their environment. In this dissertation, I hypothesize novel roles for growth and defense hormones in *Solanum* spp. responses to 1) biochar, a black carbon soil amendment (Chapter 2), 2) infection with *Ralstonia solanacearum*, an economically important soilborne pathogen causing bacterial wilt (Chapter 3), and 3) endophytic colonization by the soil bacterial community (Chapter 4). In Chapter 2, I showed that biochar upregulates GA signaling and affects GA-related traits in a species- and cultivar-specific manner. Biochar amendment also downregulates defense signaling. In Chapter 3, I demonstrated a novel role for auxin in resistance against *R. solanacearum*, including differential expression of auxin signaling genes in resistant genotype H7996 compared to susceptible WV in response to *R. solanacearum* infection. In addition, I observed stronger and faster upregulation of defense hormone marker genes for SA and ET in H7996 compared to WV. In Chapter 4, I showed that SA and ET are required for normal tomato root microbial community assembly, affecting the colonization of a few key taxa in order to promote alpha diversity. H7996 and WV root communities differ in alpha diversity, and a panel of H7996 x WV RILs showed quantitative variation in alpha diversity that correlated negatively with the abundance of these key taxa. In conclusion, I elucidated novel roles for hormones in responses to the soil environment, pathogen infection, and root community colonization. These findings are important for developing resilient, sustainable crops.

CHAPTER 1: INTRODUCTION

Hormone pathways involved in plant responses to the environment

Plant hormones are essential for proper plant growth and development as well as defense against pests such as herbivores, bacterial and fungal pathogens. They play an integral role in responding to soil environmental cues, like nutrient levels, pH, obstructions, and other organisms including other plants, insects, microbes, both pathogenic and non-pathogenic. In the following section, I will briefly introduce each hormone pathway, give an overview of its known major functions, and known responses to soil environmental conditions in tomato. In the following sections giving background specific to each project, I will give more detail on the known roles of each hormone in 1) modulating plant responses to the soil amendment biochar, 2) defending against bacterial pathogen *Ralstonia solanacearum*, and 3) shaping the microbial community in and around the roots. For the purposes of this literature review, I will focus on auxin, gibberellins (GA), salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), though abscisic acid, brassinosteroids, cytokinins, and strigolactones are also important modulators of plant environmental responses.

Tomato as a model system

Tomato is the second most important vegetable crop in the world (FAO) after potatoes. Moreover, as it is a model vegetable crop for molecular studies, it has excellent genomic resources, including a well-annotated genome sequence. Tomato is amenable to transformation, and many mutants for a variety of traits are available. In particular, several mutants for the growth/defense hormones auxin, JA, ET, SA are available, making it an excellent model to interrogate the roles of these hormones in tomato interactions with the soil environment. Tomato is also susceptible to a variety of important pathogens, making it an ideal model vegetable crop for molecular plant-pathogen interactions.

Auxin

Auxin is a major plant growth hormone derived from the amino-acid tryptophan (Jones et al. 2012). Auxin is involved in basically all aspects of plant growth, including light responses,

root gravitropism, apical dominance in shoots, lateral root branching, and integration of growth signaling pathways (Jones et al. 2012). Auxin also plays roles in disease response and nodule formation (Etemadi et al. 2014; Naseem et al. 2015). Gradients of auxin concentration regulate developmental patterning in all parts of the plant (Jones et al. 2012). In roots, local auxin maxima determine lateral root initiation sites (Dubrovsky et al. 2008). High concentrations of auxin inhibit root elongation and mediate gravitropic responses (Jones et al. 2012). Plants are able to fine-tune responses to environmental cues through auxin signaling due to the vast diversity of auxin-dependent signaling proteins that function together in a multitude of combinations to produce specific outcomes (Jones et al. 2012; Bouzroud et al. 2018).

Auxin's role in plant defense has been less well studied, but can result in both positive and negative outcomes depending on pathogen trophic lifestyles (Kazan and Manners 2009; Fu and Wang 2011; Ludwig-Müller 2015). Auxin signaling is required for resistance to some necrotrophs, like *Botrytis cinerea* and *Plectosphaerella cucumerina* (Llorente et al. 2008), *Alternaria brassicicola* (Qi et al. 2012) and *Pythium irregulare* (Tiryaki 2002). Necrotrophic pathogens consume nutrients from dead cells; thus, they invade and kill plant tissues quickly, colonizing the dead tissue. On the other hand, increased auxin signaling has been shown to increase susceptibility in several biotrophic pathogen-plant interactions like *Pseudomonas syringae* and Arabidopsis (Chen et al. 2007; Navarro et al. 2016) and *Xanthomonas oryzae* pv. *oryzae* (Ding et al. 2008), *X. oryzae* pv. *oryzicola*, and *Magnaporthe grisea* (Fu and Wang 2011) with rice. Biotrophs derive nutrients from living cells, so they grow on living tissue and kill plants more slowly. Many plant pathogens are able to produce auxin (Spaepen et al. 2007; Ludwig-Müller 2015), and thus may be able to exploit this relationship between auxin signaling and susceptibility. Conversely, the ability to suppress auxin pathways may be important for plant resistance to biotrophic and hemibiotrophic pathogens, especially vascular wilt pathogens (Fu and Wang 2011; Kidd et al. 2011; Denancé et al. 2012; Ludwig-Müller 2015). Arabidopsis mutants for auxin transport were less susceptible to *Fusarium oxysporum*, causal agent of Fusarium wilt (Kidd et al. 2011), and the *wat1* auxin transport mutant is less susceptible to bacterial wilt caused by *R. solanacearum* but not non-vascular pathogen *Pseudomonas syringae* pv. *tomato* (Denancé et al. 2012). Arabidopsis mutants for auxin influx had fewer and smaller galls from root-knot nematode infection (Kyndt et al. 2016). In a compatible interaction, nematodes redirected auxin flow to galls, promoting gall growth and development (Kyndt et al.

2016). Together, these studies show that auxin plays important roles in plant defense, dependent on pathogen trophism.

In tomato, auxin plays roles in responding to both biotic and abiotic stresses in the soil environment (Bouzroud et al. 2018), including fungal pathogens (Zhang et al. 2018). Bouzroud et al (2018) used an in silico approach to investigate differential expression of auxin response factors (ARFs) in response to various pathogens, mined from public datasets. They then used quantitative PCR (qPCR) to examine these ARFs' expression in response to abiotic stress – salt, drought, and flooding – and found differential expression of many ARFs due to stresses, indicating a role for auxin in biotic and abiotic stress response. Auxin also plays a role in beneficial interactions with microbes, as it is important for arbuscule formation in mycorrhizal associations with tomato roots (Etemadi et al. 2014).

Gibberellins

Gibberellins (GA) are a family of related ditepernoid secondary metabolites (Hedden and Sponsel 2015). GAs are involved in many aspects of plant development, including shoot growth, germination, flowering, fruiting, as well as responses to flooding stress (Colebrook et al. 2014; Hedden and Sponsel 2015). Activation of the GA pathway in stems results in cell wall expansion and cell division, resulting in shoot growth (Hedden and Sponsel 2015). In seeds, GA activation results in initiation of germination (Hedden and Sponsel 2015). In response to flooding, some plants are able to repress GA signaling in order to stop shoot elongation and preserve energy until water recedes (Colebrook et al. 2014). GA signaling is highly controlled in time and space with tissue and cell-type specific inactivation pathways (Hedden and Sponsel 2015).

Few studies have examined the role of GAs in tomato root responses to the environment. Martin-Rodriquez et al. (2015) found a link between levels of active GA and mycorrhization, with higher levels of GA associated with lower levels of colonization with mycorrhizal fungi, indicating a role for GA in restricting endophytic fungal colonization. Another study observed a role for GA in drought stress (Gaion et al. 2018). Grafting the wild type background Micro-Tom with the *procera* (*pro*) mutant, characterized by constitutive GA signaling in any root-shoot combination lacked the growth inhibition observed in WT tomato under drought stress, suggesting a role for GA in restricting growth under drought conditions.

Salicylic acid

Salicylic acid (SA) is a secondary metabolite derived from the isochorismate and phenylpropanoid pathways (Pieterse et al. 2012). SA plays a major role in defense against biotrophic pathogens (Pieterse et al. 2012). Local activation of the SA pathway upon pathogen recognition leads to the hypersensitive response, characterized by production of reactive oxygen species (ROS), callose deposition, induction of pathogenesis-related (PR) genes, and eventually cell death at the site of infection (Jones et al. 2012). This process is thought to contain biotrophs to the initial infection site. Local induction of the SA pathway can also lead to systemic acquired resistance (SAR), where plant tissues distant from the initial site of infection become primed for defense to future attacks and is characterized by activation of specific PR genes (Pieterse et al. 2014).

In tomato, SA has been implicated in tolerance to both abiotic and biotic stresses. Various studies have shown that exogenous application of SA results in increased tolerance to salinity (Stevens et al. 2006; Szepesi et al. 2009; Gharbi et al. 2018), heat, drought and cold (Senaratna, T., Touchell, D., Bunn, E., and Dixon 2000). Exogenous SA has also been shown to increase resistance to *Fusarium lycopersicum* (Mandal et al. 2009) when applied to either roots or leaves and to *Alternaria solani* (Spletzer and Enyedi 1999) when applied to roots of hydroponically grown tomatoes. Using mutant analysis, Lopez-Gresa (2016) demonstrated that SA biosynthesis is required for basal resistance to two viruses affecting tomato, Citrus Exocortis Viroid and Tomato Spotted Wilt Virus. Together, these studies show that SA is important for tolerance to soil environmental stresses in tomato.

Jasmonic acid

Jasmonates (JA) are a family of oxylipin hormones derived from free-fatty acids (Pieterse et al. 2012). JA is integral in defense against necrotrophic pathogens and herbivores such as insects (Pieterse et al. 2012). JAs accumulate in response to wounding, and pathogen, and herbivore damage to plant tissues and lead to production of phytoalexins, which are chemically diverse, broad-spectrum antimicrobial compounds, as well as antimicrobial peptides and proteins (Nojiri et al. 1996; Jones et al. 2012; Pieterse et al. 2012). JAs also play an important role in the establishment of symbiotic interactions with nodulating rhizobia, which fix nitrogen into plant-

available forms, and mycorrhizal fungi, which provide mineral nutrients to plant roots in exchange for fixed carbon (Herrera-Medina et al. 2008; Suzuki et al. 2011; Carvalhais et al. 2017).

In tomato, the JA pathway has been found to be important for disease resistance mediated by soil amendments like silicon (Ghareeb et al. 2011) and biocontrol microbes (Hase et al. 2008; Nair et al. 2015a, b; Jogaiah et al. 2018). For example, Nair et al. (2015) observed that mycorrhizal induced resistance to *Alternaria alternata* was due to increased JA biosynthesis and signaling, and induced resistance was lost when a JA inhibitor was applied. Interestingly, JA has been found to have positive and negative effects on mycorrhizal colonization with one study showing that application of JA restricts mycorrhizal colonization (Herrera-Medina et al. 2008), while another showed that a JA biosynthesis mutant had reduced colonization levels (Tejeda-Sartorius et al. 2008), suggesting that JA plays roles in both allowing and maintaining appropriate levels of mycorrhizal colonization.

JA has also been shown to have direct roles in disease resistance in tomato. JA signaling mutant *jai1* showed reduced susceptibility to root knot nematode in a susceptible tomato cultivar, but biosynthesis mutant *def1* had no effect (Bhattarai et al. 2008). Exogenous JA treatment was shown to increase resistance to insect herbivores in the field (Thaler 1999), fitting the standard paradigm of JA-mediated defenses against herbivorous pests.

Ethylene

Ethylene is a small volatile organic compound involved in plant senescence, organ expansion (especially leaves and roots), reproductive development, and responses to pathogens and abiotic stresses like flooding (Van de Poel et al. 2015). ET is regulated by a complex signaling pathway normally maintained in a repressed state by the fast turnover of the key biosynthesis enzyme, ACC synthase (ACS) (Merchante et al. 2013; Berens et al. 2017). When ACS is stabilized by phosphorylation in response to wounding, pathogen attack, or developmental cues, ET accumulates, resulting in downstream responses (Jones et al. 2012; Berens et al. 2017). Ethylene generally works synergistically with JA to combat necrotrophic pathogens (Pieterse et al. 2012).

In tomato, ethylene is important for interactions with soilborne, growth-promoting rhizobacteria (PGPRs) (Yan et al. 2002; Ribaudo et al. 2006; Ibort et al. 2017, 2018),

mycorrhizal association (Fracetto et al. 2017), and interactions with pathogens (Lund et al. 1998). Both ET and JA pathways were required for induction of ISR against tomato late blight by soil-applied PGPRs (Yan et al. 2002). The ethylene overproducing mutant *epinastic* was shown to have decreased mycorrhizal colonization that was rescued with the application of an ethylene inhibitor. In complimentary studies, Ibort et al. (2017, 2018) found that ethylene perception is required for tomato growth promotion by a strain of *Bacillus megaterium*, but not an *Enterobacter C7* strain. The *Nr* ethylene perception mutant also showed reduced susceptibility to two aboveground bacterial pathogens *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*, as well as one soilborne fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* (Lund et al. 1998). Together these studies suggest a role for ethylene both in interactions with plant-growth promoting microbes and pathogens.

Ethylene is also important for tomato responses to abiotic stresses like flooding (Vidoz et al. 2010) and salt stress (Poór et al. 2015). Using mutant analysis, Vidoz et al (2010) demonstrated a role for both ET biosynthesis and perception in promoting adventitious root formation in response to flooding. Additionally, the *Nr* ET perception mutant has also been shown to be more sensitive to salt stress (Poór et al. 2015), indicating that ethylene signaling is required for responses to environmental stress.

Hormone crosstalk for growth and defense

Plant hormones do not act alone to modulate plant responses to the soil environment. Instead, these hormones work together in complex networks to fine-tune responses to environmental conditions. Generally speaking, SA and JA/ET act antagonistically with activation of one leading to repression of the other (Robert-Seilaniantz et al. 2011). GA also plays a role in this antagonism, activating SA biosynthesis and attenuating JA signaling (Robert-Seilaniantz et al. 2011). Auxin and SA also act antagonistically, with auxin signaling activation repressing SA pathways, and SA stabilizing repressors of auxin signaling (Robert-Seilaniantz et al. 2011). Auxin generally works synergistically with GA to promote plant growth and organ expansion, while ethylene often negatively regulates GA signaling (Jones et al. 2012). JA and ET may act either synergistically or antagonistically in order to confer specificity to defense or wounding responses, resulting in differential activation of downstream genes. JA and ET also work synergistically for induced systemic resistance (ISR) signaling.

Hormone crosstalk has been shown to be important for various soil environmental interactions in tomato. Negi et al. (2010) observed ethylene modulation of auxin transport and content in roots, affecting lateral and adventitious root formation, which are important for plastic root responses to soil environment. In tomato responses to *Fusarium oxysporum* f. sp. *lycopersici*, SA is a negative regulator and ET is a positive regulator of susceptibility. However, both pathways are required for defense gene induction in wild type plants (Di et al. 2017). When looking at *B. thuringiensis* induced defense against *R. solanacearum*, Takahashi et al. (2014) found transcriptional profiles consistent with activation of SA and ET pathways, with concurrent downregulation of JA pathways, suggesting SA-JA antagonism in induced resistance to bacterial wilt. Crosstalk of hormone pathways is essential for modulating specific root-soil environment interactions.

Biochar: Introduction

Biochar is a black carbon soil amendment patterned after Terra Preta anthropogenic soils found in the Amazon (Woods and McCann 1999; Marris 2006). Terra Preta soils remain more fertile than surrounding soils even today likely due to "slash and char" agricultural practices by native inhabitants thousands of years ago (Woods and McCann 1999). Modern biochars are produced through pyrolysis of organic material – burning under low oxygen conditions – to produce highly recalcitrant black carbon with trace amounts of mineral ash (Laird et al. 2009). Biochar has been shown to affect yield (Jeffery et al. 2011; Spokas et al. 2012; Biederman and Harpole 2013), biomass partitioning (Biederman and Harpole 2013), germination (Solaiman et al. 2012; Bargmann et al. 2013; Free et al. 2017; Gascó et al. 2017), root architecture (Xiang et al. 2017), and response to pathogens and pests (Elad et al. 2010; Elmer and Pignatello 2011; Elmer 2012; Meller Harel et al. 2012; Jaiswal et al. 2014; Graber et al. 2014; Copley et al. 2015, 2017; Mehari et al. 2015; Eizenberg et al. 2017), though its effects on these traits are often inconsistent and depend on a complex interaction of factors such as biochar type, amendment rate, environment, plant species, and the soil microbiome. Understanding how plants respond to biochar on a molecular level may improve our understanding of these inconsistencies in plant responses to biochar.

Understanding underlying molecular plant responses to biochar will improve our ability to optimize biochar use in agriculture, potentially expanding its use to complement existing

horticultural practices. Therefore, the aim of this introduction is to discuss recent advances in our understanding of the mechanisms behind plant growth and disease responses to biochar as well as discuss the potential factors behind the inconsistency in biochar-plant fitness outcomes.

Mechanisms of biochar-mediated plant growth promotion

Biochar can improve plant growth through a variety of mechanisms, though our understanding of each varies: 1) effects on soil conditions, 2) bioactive molecules present in biochar, and 3) effects on the soil microbiome.

1) Mechanisms of growth promotion: effects on soil conditions

Soil amendment with biochar is thought to promote plant growth through its positive effects on the soil environment, like alterations in pH, nutrient availability, CEC, bulk density, and water holding capacity (Jeffery et al. 2011; Biederman and Harpole 2013). These effects are likely to promote growth under otherwise poor soil conditions (Spokas et al. 2012; Biederman and Harpole 2013; Jeffery et al. 2017b, a) and have been well-reviewed elsewhere (Jeffery et al. 2011; Spokas et al. 2012; Biederman and Harpole 2013).

2) Mechanisms of growth promotion: bioactive molecules present in biochar

Additionally, many biochars contain small molecules and volatiles that may directly impact plant growth and defense, such as hormone or hormone-like compounds (Spokas et al. 2010; Lin et al. 2012; Jamieson et al. 2014; Graber et al. 2015; Kochanek et al. 2016), phenolics (Wang et al. 2015a), and other volatile organic compounds (Spokas et al. 2011). In a survey of biochars produced under different production methods and conditions, Spokas et al. (2011) found a high diversity of VOCs present in biochar, with significant differences in abundance and type of VOC dependent on pyrolysis conditions. Interestingly, certain biochars have been shown to produce ethylene (Spokas et al. 2010), which impacts plant root architecture (Jung and McCouch 2013) as well as defense (Pieterse et al. 2012). Recently, Kochanek et al. (2016) showed that biochar produced from certain feedstocks under certain conditions can produce high levels of karrikins, a plant hormone present in smoke that induces germination in some species after fires (Nelson et al. 2012). Additionally, they showed that biochar containing karrikins was able to improve germination of a karrikin-requiring species. Biochars may also contain humic

substances products (HSPs), which have been shown to have hormone-like effects on plant growth under controlled conditions (Lin et al. 2012; Jamieson et al. 2014; Graber et al. 2015). Graber et al. (2015) observed a reduction in root hair density in Arabidopsis seedlings treated with biochar-derived HSPs, under phosphorus (P) sufficiency and starvation, suggesting a role for these compounds in reducing P stress. Lastly, phenolic compounds found in biochar decreased diversity and abundance of ammonia-oxidizing bacterial community, thus potentially affecting soil nitrogen cycling (Wang et al. 2015b).

3) Mechanisms of growth promotion: effects on microbial community

Biochar is known to alter the soil microbial community, affecting both bacterial and fungal members (Matsubara et al. 2002; Elmer and Pignatello 2011; Kolton et al. 2011, 2016; Dai et al. 2016; Jenkins et al. 2016; Jaiswal et al. 2017), often increasing overall diversity (Kolton et al. 2016; Zhang et al. 2016; Jaiswal et al. 2017) and promoting community members known to have plant-growth promoting or induced systemic resistance (ISR)-inducing properties (Matsubara et al. 2002; Kolton et al. 2011; De Tender et al. 2016a; Zhang et al. 2016; Jaiswal et al. 2017). Biochar amendment is associated with increased nodulation in leguminous crops and increased root length colonized by mycorrhizal fungi (Xiang et al. 2017). Biochar has also been shown to affect functional diversity and metabolic potential of the microbiome, affecting substrate utilization (Kolton et al. 2016) and nitrogen cycling (Wang et al. 2015b; Harter et al. 2016; Li et al. 2016). These factors may contribute to biochar's ability to improve plant performance; however, to our knowledge no causal links between biochar-mediated growth promotion and the microbial community have been established.

Molecular basis of biochar-mediated plant growth promotion: what do molecular studies tell us?

Currently, relatively little is known about the molecular basis for plant growth outcomes with biochar amendment, though altered transcription in several transcriptional pathways involved in plant growth have been linked to biochar application (Viger et al. 2014; Copley et al. 2015). A microarray study comparing global gene expression between biochar-grown and control Arabidopsis plants observed upregulation of genes involved in cell wall expansion and modification, water and nutrient transport (Viger et al. 2014). Soybean plants grown in 5%

biochar exhibited upregulated transcription of β-1,4-glucanase, also involved in cell wall expansion (Copley et al. 2015). These results together suggest that biochar induces shoot growth through increases in cell wall growth and modifications of water and nutrient transport within the plant. Viger et al. (2014) also observed upregulation of auxin and brassinosteroid (BR) signaling and biosynthesis genes, two major hormones involved in growth and development. Further insight into the molecular basis for biochar-mediated plant growth promotion is important for understanding how the plant perceives and responds to biochar's broad abiotic and biotic effects on the soil environment. This information will help us to better predict plant response outcomes to biochar amendment in order to optimize agricultural uses for biochar.

Mechanisms of biochar-mediated disease suppression

Various studies have examined the effects of biochar on disease outcomes from the major groups of plant-pathogenic microbes and fauna – fungi, bacteria, oomycetes, and nematodes (Elad et al. 2010; Zwart and Kim 2012; Meller Harel et al. 2012; Huang et al. 2015; Jaiswal et al. 2015; Mehari et al. 2015; Ebrahimi et al. 2016; George et al. 2016). Similar to results from studies on biochar-mediated plant growth promotion, results vary significantly from positive to neutral to negative. We will focus first on the potential mechanisms for plant disease suppression and evidence for those, followed by a discussion of the factors affecting plant response outcomes to biochar amendment for both growth and disease.

Four potential mechanisms for biochar-mediated disease suppression have been proposed (Graber et al. 2014): 1) increase in plant vigor reducing disease susceptibility, 2) direct inhibition of pathogen growth or infection capability, 3) direct stimulation of plant defenses by biochar compounds, or 4) alterations in the soil microbiome resulting in stimulation of plant defenses, an unfavorable community structure for pathogen infection, or a combination of the two.

1) Mechanisms of disease suppression: Increase in plant vigor

The first potential mechanism is that biochar improves plant growth, thus reducing susceptibility to disease. This has been largely accounted for in greenhouse experiments where plants were given high adequate levels of fertilizer to "wash out" biochar fertilizing effects or biochar had no effect on plant tissue nutrient contents (Elad et al. 2010; Jaiswal et al. 2017). This mechanism may be more important in the field where conditions are more variable or soil quality

is poor, though few studies have been performed in the field to look at biochar's effects on disease resistance. For example, biochar largely increases plant-available potassium (Biederman and Harpole 2013), which has been linked to reduced susceptibility to some diseases such as Verticillium wilt in the field (Hafez et al. 1975).

2) Mechanisms of disease suppression: Direct inhibition of pathogen growth or infection capability

The second hypothesis regarding biochar inhibition of plant disease through direct interactions with pathogens has been examined in a variety of pathosystems, with conflicting results. Biochar or biochar water extracts had no direct toxicity effects on *Pythium ultimum* (Gravel et al. 2013) or *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) (Jaiswal et al. 2017) through *in vitro* assays, though in the case of FORL, a reduction in both growth and infectivity was observed in biochar-amended soil, suggesting an alternate mechanism for disease suppression. However, in a previous study using the same types of biochar, Jaiswal et al. (2015) demonstrated an *in vitro* inhibitory effect on mycelial growth of *Rhizoctonia solani* that increased with biochar concentration. Increasing concentrations of biochar in the potting mix led to a decrease in biochar's effectiveness at disease suppression, despite its increase in effectiveness at inhibiting mycelial growth in vitro suggesting that *in vitro* inhibition may not be an ideal indicator for the level of protection provided by these biochars (Jaiswal et al. 2015, 2017). In contrast, Copley et al. (2015) observed a positive effect of maple bark biochar on *R. solani* growth *in vitro*.

When examining the bacterial pathogen *Ralstonia solanacearum*, Gu et al., (2016) observed direct impacts of biochar on *R. solanacearum* chemotaxis and swarming motility. Biochar decreased *R. solanacearum's* swarming motility and rhizosphere competence and was directly able to adsorb *R. solanacearum* cells onto its surface. Their data also suggested that biochar was able to adsorb root exudates, thus they proposed a model where biochar was able to reduce bacterial wilt through either direct adsorption of *R. solanacearum* cells or adsorption of root exudates which attracted the bacteria to the biochar particles rather than to the tomato root rhizosphere (Gu et al. 2016). Interestingly, they only observed this effect when using finely ground biochar and not coarsely ground, suggesting that surface area or surface chemical groups play a role in biochar's effects on *R. solanacearum*. Together these studies indicate that direct

toxicity effects of biochar do not play a large role in suppressing disease, though some evidence suggests that biochar may directly adsorb bacterial pathogens or affect their attraction to their host rhizospheres, decreasing their ability to infect plants. Further work is required to confirm this in more pathosystems and in the field.

3) Mechanisms of disease suppression: Direct stimulation of plant defenses by biochar compounds

While compounds contained in biochar may not be directly toxic to pathogens, evidence exists for a direct effect of these compounds on the plants themselves. Zwart and Kim (2012) showed that low biochar additions (5% by volume) reduced the size of stem lesions caused by *Phytophthora* spp., but larger additions resulted in neutral to increase in lesion size compared to the controls. Addition of two types of biochar at different rates to soil infested with *Rhizoctonia solani* revealed an interesting phenomenon in damping-off of common bean: for greenhouse waste biochar, disease control peaked with the lowest level of biochar addition (0.5%) and decreased with increasing amendment levels (Jaiswal et al. 2015). At 3% addition, disease incidence was not significantly different from the 0% biochar amended controls. However, when eucalyptus biochar was added instead, disease control peaked at 1% amendment, with both 0.5% and 3% amendment levels offering lower levels of control, though still greater than the 0% control. Likewise, when the same biochars were applied at these rates to cucumber plants, similar results were observed for damping-off severity (Jaiswal et al. 2014). On the other hand, a linear decrease in FORL symptoms in tomato was observed when the same biochar was applied (Jaiswal et al. 2017).

These observations have been labeled the “u-shaped curve” response to biochar additions and point to a hormesis effect where low levels of chemicals present in biochar may stimulate defense, but high levels increase susceptibility. The specific compounds responsible for these effects have not yet been confirmed in biochar studies, but many organic compounds found in biochar like ethylene, phenols, carboxylic acids, furans, and ketones can produce hormetic effects on plant growth and disease outcomes (Calabrese and Blain 2009; Spokas et al. 2011). It is important to note that in these studies (Jaiswal et al. 2014, 2015), two different pyrolysis temperatures were examined for each feedstock, but temperature was not found to affect disease severity indicating that feedstock was a more important factor for disease resistance properties.

Interestingly, the temperature at which a biochar is produced plays a key role in what organic compounds are produced in the pyrolysis process. Low temperature biochars (<350 °C) generally contain furans, ketones, and short carbon chain aldehydes, while higher temperature biochars (> 350 °C) produce biochars with aromatic compounds and longer chain hydrocarbons (Spokas et al. 2011). Evidence against this hypothesis comes from a study that compared the effects of biochar and "stripped" biochar, from which all labile components had been removed, on tomato resistance to *Botrytis cinerea* and showed similar disease-suppressive effects of both compared to a control with no biochar. Together these data suggest that organic compounds present in biochar may play a role in disease resistance, but other factors are also important.

4) Mechanisms of disease suppression: Alterations in the soil microbiome

A major reason for why the biochar-plant-disease outcomes mechanism has remained elusive lies in the difficulty in experimentally teasing apart different potential mechanisms. A large body of evidence exists to support either of the third or fourth potential mechanisms as the difficulty in distinguishing between direct effects of biochar on plant fitness and indirect effects on the soil microbiome that then act on the plant in natural systems is quite high. In pathosystems where biochar promotes disease resistance, a role for induced systemic resistance (ISR) has been discovered (Elad et al. 2010; Meller Harel et al. 2012; Mehari et al. 2015). Biochar application increased resistance to foliar fungal pathogens, despite spatial separation (Elad et al. 2010; Meller Harel et al. 2012; Mehari et al. 2015; De Tender et al. 2016a, b). Investigating expression of marker genes for systemic resistance revealed 'priming' of three strawberry genes: *OLP2*, *LOX*, and *WRKY1*, which increased expression after inoculation with *B. cinerea* more strongly when biochar was applied (Meller Harel et al. 2012). However, when a similar study was done in the tomato-*B. cinerea* pathosystem using quantitative RT-PCR for 12 tomato defense marker genes across 7 genotypes, only a few showed this 'priming' effect of biochar application in a genotype-specific manner (Mehari et al. 2015). Despite the lack of marker gene expression evidence, they demonstrated that an intact jasmonic acid (JA) pathway was required for biochar-mediated resistance to *B. cinerea* through mutant analysis, which supports the ISR-induction hypothesis. However, whether this induced resistance is due to a direct influence of biochar-borne organic compounds on the plant or to biochar's effects on the soil microbiome is unclear.

The current body of evidence offers only a few clues as to whether one or both mechanisms are acting in biochar-plant disease interactions. Evidence for a role of the microbiome in biochar-mediated disease suppression is primarily correlational, as biochar has been shown to increase the abundance of bacterial community members known to induce ISR (Kolton et al. 2011). In multiple studies, increased microbial abundance and diversity in the rhizosphere was associated with decreased incidence and severity of disease (Zhang et al. 2016; Jaiswal et al. 2017). However, these effects could not be separated from improved plant growth parameters (Jaiswal et al. 2017) and soil physicochemical properties (Zhang et al. 2016), which may have also contributed to reductions in disease. In a study examining the interactive effects of biochar and arbuscular mycorrhizal (AM) fungi colonization on fusarium root rot in Asparagus, Matsubara et al. (2002) found that biochar and AM inoculation together reduced root rot symptoms, more than either biochar or AM inoculation alone. Together, these results suggest a role for the microbiome in biochar-mediated disease suppression, though a definitive link has not yet been established.

Factors affecting biochar outcomes

As mentioned above, studies looking at biochar-mediated growth promotion and disease suppression are far from consistent. Many factors may contribute to differences in plant response outcomes, including soil type and environment, biochar type and amendment levels, phytotoxicity, species-specific responses, and trait-specific effects, including growth and defense tradeoffs.

1) Factors affecting outcomes: Soil type and environment

Multiple meta-analysis studies have uncovered a greater positive effect for biochar in acidic vs. alkaline soils and in tropical over temperate regions (Jeffery et al. 2011, 2017a; Biederman and Harpole 2013; Xiang et al. 2017). Additionally, Jeffery et al. (2011) showed that biochar has a greater effect in coarse or medium textured soils, which is corroborated by Xiang et al. (2017) who found that root biomass was increased more in sandy than loamy soils when treated with biochar. These authors claim that these results are likely because acidic soils in tropical regions tend to be poorer quality soils, and biochar has a more obvious positive effect when soils are poor and biochar addition improves their physicochemical properties for plant

growth. This is exemplified in a study by Laird et al. (2017) who surveyed the effects of biochar addition at six field sites across the United States and only observed a positive effect of biochar on plant growth in one location – which had a poor quality, sandy soil, unlike the rest of the sites.

Little is known about soil/environmental-biochar-disease interactions, as most biochar-disease studies have been performed in soilless substrates under greenhouse conditions. Shoaf et al. (2016) showed soil-type specific effects of biochar on *Phytophtora capsici* infection in pepper under greenhouse conditions. A study examining the effects of biochar on asparagus root rot showed reductions in asparagus size, suggesting higher root rot, during a season where rains were heavy (Elmer and Pignatello 2011). The authors proposed that this may have been due to improved conditions for disease with the increased water-holding capacity of the biochar-treated soil (Elmer and Pignatello 2011). Further studies are needed to confirm the role of soil type and environment in biochar-disease interactions.

2) Factors affecting outcomes: Biochar type and amendment level

Biochar is a complex agricultural amendment as it can be made from many different feedstocks under a variety of production conditions, all of which affect the composition of the final product. Biochar characteristics that may influence its effect on plant responses include pH, amount of ash (labile nutrients), surface charge, porosity, carbon recalcitrance, levels and composition of VOCs and other bioactive compounds (Spokas et al. 2011; Harvey et al. 2012; Kochanek et al. 2016). Meta-analyses have shown that for overall crop productivity, biochar feedstock plays an important role (Jeffery et al. 2011; Biederman and Harpole 2013), but for root traits biochar production conditions were more important than feedstock (Xiang et al. 2017). Multiple studies have shown a role for biochar type in disease suppression (Jaiswal et al. 2014; George et al. 2016; Shoaf et al. 2016).

Amendment level is also an important factor to consider for biochar use in agriculture. Many biochar/cropping systems have been reported to exhibit “inverted U-shaped” biochar dose/plant growth relationships (Rondon et al. 2007; Baronti et al. 2010; Gaskin et al. 2010; van Zwieten et al. 2010; Elmer 2012; Rajkovich et al. 2012; Spokas et al. 2012), with optimum growth or disease resistance at a low to intermediate biochar concentration and diminishing returns or even negative effects at higher temperatures. In two similar studies, low levels of biochar decreased disease severity, but higher levels of biochar resulted in a disease severity

equal to or greater than the control (Jaiswal et al. 2014, 2015). When measuring the effects of the same biochar rates on plant growth, a higher level of biochar amendment was required to see the maximum effects of biochar on plant growth.

While some disease resistance studies show this "U-curve effect" (Jaiswal et al. 2014, 2015), others have shown an increasingly positive (Elmer 2012) or negative effect (Copley et al. 2015) of biochar with increasing additions. Interestingly, Elmer (2012) observed a "u-shaped curve" for root growth in the absence of pathogen, but linear increases in root weight with increasing biochar addition in Fusarium-infested soil. Together these studies suggest that biochar-pathogen interactions are complex and point to the need for considering multiple factors when applying biochar, including biochar type, amendment level, and potential biotic stresses.

Amendment level: Growth and defense tradeoffs.

Together, the literature suggests that for some types of biochar at relatively high amendment levels, biochar promotes growth at the expense of defense, while at low concentrations biochar promotes disease resistance. Viger et al. (2014) and Copley et al. (2017) both showed that biochar addition promoted gene expression of genes involved in plant growth and cell expansion, while simultaneously downregulating genes involved in disease resistance. The idea of growth-defense tradeoffs has been well-studied (reviewed in Huot et al. 2014). While the full mechanism has not yet been elucidated, the role of hormone crosstalk is central to growth-defense tradeoffs. Viger et al. (2014) observed upregulation of auxin and brassinosteroid related genes with concomitant downregulation of jasmonic acid (JA) and salicylic acid (SA)-related genes, which they suggest revealed a tradeoff between growth and defense in biochar-treated plants.

Consistent with this, Copley et al. (2017) showed downregulation of several defense genes prior to defense. However, no effect of biochar on growth was observed in this study, though plants were only grown to unifoliate stage. Copley et al. (2017) confirmed the biological significance of the downregulated defense genes by challenging the soybean plants with *Rhizoctonia solani* and confirmed that biochar addition, in agreement with the gene expression data, resulted in higher foliar blight severity. This evidence suggests that biochar addition, at least at relatively high rates (5% w/w) may result in a growth-defense tradeoff.

Conversely, biochar may have a negative effect on disease, but a positive effect on growth. While maple bark biochar had a negative effect on control of *R. solani* across all eleven species examined, it had mixed effects on seedling length depending on plant species (Copley et al. 2015). Soybean seedling length increased with increasing amendment, while pea, alfalfa, cucumber, tomato, leek, carrot, and sugar beet seedling lengths did not change significantly, except with 5% amendment, where lengths were negatively affected. Biochar effects on pepper and lettuce were positive at low amendment rates, but neutral to negative at the 5% level. Further research is needed to dissect the potential tradeoffs between growth and defense in biochar-grown plants, as well as biochar's effects on hormone cross-talk and subsequent effects on plant fitness outcomes.

3) Factors affecting outcomes: Species

As exemplified in the study above, several lines of evidence have demonstrated that a given biochar may act differently on different species of plants. In their meta-analysis of 371 independent studies, Biederman and Harpole (2013) found that overall, biochar's effect on total plant biomass differs between annual and perennial plants, having a positive effect on annual biomass and a neutral effect on perennial biomass. Similar effects were observed for root traits (Xiang et al. 2017), and additionally biochar promoted greater root biomass in legumes over non-leguminous plants.

When five different plant species were grown in potting soil amended 1:1 (v/v) with a commercial softwood biochar, shoot biomasses of sweet pepper, geranium and basil were unaffected, while coriander biomass increased by 45% and lettuce biomass decreased by 44% (Gravel et al. 2013). To further understand biochar's species-specific effects, germination tests in potting mix showed a negative effect on lettuce and basil and neutral effects on the other three species. However, when water extracts of biochar were used to germinate the seeds, no effect was observed on lettuce and a negative effect was observed on coriander.

The reason for this species-dependent response is poorly understood. One group suggested that lettuce's poor initial response to biochar was due to its high salt content (Artiola et al. 2012). Artiola et al. (2012) showed that lettuce growth was decreased by a 4% addition of pine waste biochar. However, when the same soil was used to grow a second crop of lettuce, biochar had a positive effect, which the authors suggested was the result of salts in the biochar

reducing lettuce growth in the first trial but having leached away by the second trial. Though lettuce may be more sensitive to biochar's salt content, lettuce has also been reported to have a positive response to biochar amendment (Viger et al. 2014), so the effect of biochar on lettuce growth is biochar-type dependent.

These species-specific effects are not limited to growth responses. When comparing *Pythium* colonization between five plant species grown in potting mix with or without softwood biochar, biochar had a positive effect on colonization in basil, geranium, and sweet pepper, and a neutral effect on colonization in coriander and lettuce (Gravel et al. 2013). De Tender et al. (2016) showed that biochar had a positive effect on strawberry growth and resistance to *Rhizoctonia solani*, but a neutral effect on lettuce, though this may be confounded by the fact that different soil mixes were used for each species.

No studies thus far have examined differences in closely related species or genotypes within species, though this is important for agricultural growers who may observe differing effects if biochar promotes growth or disease resistance in some genotypes but not others.

4) Factors affecting outcomes: Phytotoxicity

Some biochars contain compounds that may produce detrimental effects on plant growth, which may contribute to the number of studies where negative effects were observed with biochar addition. Xiao et al. (2016) observed short-term negative effects of biochar on root growth, but later saw overall positive effects on root growth and yield, which suggests that the biochar applied may have contained water-soluble phytotoxic compounds that leached away over the course of the experiment. Consistent with this finding, Gale et al. (2016) observed that the phytotoxicity of phenolic compounds present in biochar was relieved when biochars were first leached or thermally treated. Application of the leachate to plants resulted in toxicity, confirming the role of these compounds in negatively affecting plant growth. Some biochar may also contain high levels of free radicals as a result of the pyrolysis process. Biochars containing high levels of free radicals can inhibit germination, root and shoot growth and damage seedling plasma membranes (Liao et al. 2014).

Li et al. (2015) detected a phytotoxic effect of water-extractable components of a corn stover biochar on tomato seedling germination and growth at high doses, but growth promotion at low doses, consistent with the "U-curve" observed in other studies. This observation from

multiple studies may be explained by the phenomenon of hormesis, where some chemical compounds may promote plant growth at low doses while being toxic at higher doses (Belz and Cedergreen 2010; Graber et al. 2014). This effect could also explain how biochars become more susceptible to disease with increasing addition. Compounds present in the biochar may damage plant roots, thus increasing their susceptibility pathogen attack.

On the other hand, biochar may also adsorb detrimental chemicals already present in the soil. For example, addition of pine charcoal partially rescued Scots pine germination inhibited in control soils by a natural phytotoxin (Hille and den Ouden 2005). Biochar addition was also shown to mitigate the effects of allelopathic chemicals present in asparagus replant soils on AM fungi colonization (Elmer and Pignatello 2011), which suggests adsorption of the allelopathic compounds.

5) Factors affecting outcomes: Trait-specific effects

As mentioned in some of the above studies, it is important to note that a given biochar may affect different traits differently (i.e. positive effect on growth but a negative effect on disease resistance; negative effect on germination, but a positive effect on growth) (Solaiman et al. 2012; Copley et al. 2015; Gascó et al. 2017). Solaiman et al. (2012) offers an excellent example of these trait specific effects. They observed a neutral to negative effect of their five biochars on germination in three plant species (wheat, mung bean, and clover) but a range of effects on early seedling growth from positive to negative. These effects depended on biochar type, amendment and species.

Summary and study focus

Therefore, given the current body of biochar literature, it is essential to consider the complexity of plant fitness outcomes in response to biochar addition, as they relate to soil type and environment, biochar type, amendment level, potential positive or negative effects of compounds present in biochar, and trait-specific effects. Further, gaps exist in our current knowledge base that will promote the effective use of biochar in agriculture. First, biochar has demonstrated differences in its effects on different species, but no studies have examined its effects on closely related species or on different genotypes within a species. Second, the molecular basis for biochar-mediated plant growth promotion and interaction with pathogens is

poorly understood, especially in crop plants. My research seeks to fill these gaps in order to improve our understanding of how biochar works to affect plant growth and to better optimize its use in agriculture. In the study described in Chapter 2, I used global gene expression to examine the molecular basis of biochar-induced growth promotion and interactions with disease in tomato.

Ralstonia solanacearum: Introduction

Ralstonia solanacearum (RS) is a soilborne, gram-negative plant pathogen from the class Betaproteobacteria, causing Bacterial Wilt (BW) (Genin and Denny 2012). It has a broad host range, infecting more than 200 species in over 50 families, and is ranked as one of the overall most destructive and economically important bacterial plant pathogens (Genin and Denny 2012; Mansfield et al. 2012). RS is particularly devastating to solanaceous species, including tomato, and significantly affects crop production in hot, humid regions such as Central and South America and the South-east United States, where yield losses in infested fields can reach up to 90% (Denny 2006). RS is a highly diverse species complex divided into four phylotypes that roughly correspond to geographic location: phylotype I in Asia, II in the Americas, III in Africa and IV in the Australia-Indonesia region (Genin and Denny 2012).

Infection process and symptoms

RS infects its hosts through wounds in the root, either at lateral root emergence sites or in the elongation zone (Genin 2010). Invading bacteria then colonize the cortex, eventually moving into the xylem vascular tissue (Genin 2010; Caldwell et al. 2017). Once in the xylem, RS multiplies and moves into the plant shoot vasculature (Genin 2010). RS produces many virulence factors to aid its invasion, including Type-III effectors, cell-wall degrading enzymes and extracellular polysaccharides (EPS) (Genin 2010). RS eventually produces enough EPS to stop water flow through the xylem, leading to chlorosis, wilting and death of the host plant (Genin 2010).

Management strategies

RS is primarily managed through the use of resistant cultivars. Grafting of susceptible scions onto resistant rootstocks is an effective management strategy, though the cost may be

prohibitive unless fields are highly infested (Rivard et al. 2012; McAvoy et al. 2012). RS is resistant to many other management approaches, as it persists in the soil for long periods of time and can survive in even pure water, though it is susceptible to cold temperatures (Denny 2006). Previously, soil fumigation was used to disinfect RS infested fields, but the high toxicity of soil fumigants, like methyl bromide, has resulted in their ban for agricultural use (Rosskopf et al. 2005). Various biocontrol agents and soil amendments have also been explored for their potential use in managing RS, but few field trials have been performed to evaluate their effectiveness under field conditions (Kiirika et al. 2013; Yuliar et al. 2015).

Mechanisms of resistance

Resistance to BW is primarily quantitative, consisting of many genes each contributing a small effect. In fact, the only known source of qualitative (single gene) resistance to BW is found in Arabidopsis (RRS1), though there is debate over whether Arabidopsis can be considered a true host of RS (Denny 2006). In tomato, quantitative trait loci (QTL) for resistance have only been coarsely mapped, and include both broad-spectrum and strain-specific resistance traits (Danesh et al. 1994; Thoquet et al. 1996a, b; Mangin et al. 1999; Wang et al. 2000; Carmeille et al. 2006). Most QTL for tomato resistance have been identified in the Hawaii7996 x West Virginia 700 QTL population (Danesh et al. 1994; Thoquet et al. 1996a, b; Mangin et al. 1999; Wang et al. 2000; Carmeille et al. 2006). H7996 is the resistant cultivar and contains resistance to a variety of RS strains.

The plant root is the first line of defense against soilborne pathogens, as it is the first point of contact between the pathogen and the host plant. Roots have been shown to be important for resistance because susceptible scions grafted onto resistant rootstocks are resistant to BW (Rivard et al. 2012; McAvoy et al. 2012). However, resistant plants are still colonized and can sustain latent infections with high pathogen loads (Caldwell et al. 2017). Caldwell et al. (2017) provided important insights into root-mediated resistance by showing that in the resistant cultivar H7996, root colonization by RS is delayed compared to WV. RS was able to colonize the vasculature of WV by 24 hours after infection but did not enter the vasculature of H7996 until between 24 and 48 hours. Colonization was also spatially restricted in H7996 compared to WV. In H7996 at 144 hours, RS was only found in some xylem vessels, while at the same time point in WV, RS could be found throughout the root vasculature, including the cortex. These data

show the importance of the root in resistance to RS, but the underlying molecular responses that govern resistance in the roots are still poorly understood.

Transcriptional responses to RS infection

One way to understand the molecular basis for resistance to a pathogen is through transcriptional profiling of resistant and susceptible genotypes of a host plant in response to infection with a pathogen. Several transcriptional studies have been carried out to examine RS resistance responses in leaves in various hosts including Arabidopsis (Hu et al. 2008), ginger (Prasath et al. 2014), and tomato (Ghareeb et al. 2011; Milling et al. 2011; Ishihara et al. 2012; Kiirika et al. 2013). Two studies looked at transcriptional profiles of infection in roots – in peanut (Chen et al. 2014) and *S. commersonii* (wild potato) (Zuluaga et al. 2015). Overall, some observed higher and/or earlier upregulation of defense-related genes (Milling et al. 2011; Ishihara et al. 2012; Prasath et al. 2014), though Zuluaga et al. (2015) observed more upregulation of biotic stress related genes in the susceptible wild potato accession compared to the resistant accession. In peanut roots, downregulation of defense genes such as leucine-rich-repeat (LRR) kinase and R genes was observed in both resistant and susceptible cultivars (Chen et al. 2014). Interestingly, Ishihara et al. (2012) observed no differential regulation in the shoots of susceptible tomato cultivar Ponderosa, though genes in defense, hormone, and lignin pathways were upregulated in the resistant cultivar LS-89. Additionally, some studies observed down-regulation of developmental and metabolism-related genes, though primary metabolism was inhibited more in the resistant cultivar in peanut (Chen et al. 2014) and more in the susceptible cultivar in *S. commersonnii* (Zuluaga et al. 2015).

Most of these studies observed differential expression in plant defense hormones, including salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and even auxin, though no consistent pattern of up- or down-regulation in the resistant or susceptible cultivars emerged. In tomato shoots (Ishihara et al. 2012) and ginger leaves (Prasath et al. 2014), genes related to ET and JA were upregulated in the resistant cultivar more than the susceptible, but this pattern reversed in wild potato with ET and JA being more upregulated in the susceptible cultivar (Zuluaga et al. 2015). More SA related genes were downregulated in the susceptible wild potato (Zuluaga et al. 2015). Interestingly, auxin-related genes were upregulated in both wild potato and tomato shoots in response to infection, but only in the resistant tomato cultivar (Ishihara et al.

2012; Zuluaga et al. 2015). Together these studies suggest species-specific differences in root-mediated resistance, though differences may also be due to differences in tissue examined, time after infection, infection methods, and RS strain used.

Summary and study focus

RS is a highly important bacterial pathogen affecting economically important Solanaceous crops like tomato. Further work is needed to elucidate the roles of plant growth and defense hormones in resistance to RS. Thus far, no studies have been undertaken to look at root transcriptional responses to RS in tomato. Therefore, the aim of the study described in Chapter 2 was to examine the root transcriptional responses to *R. solanacearum* in resistant cultivar H7996 and susceptible cultivar WV.

Host Control Of The Root Microbiome: Introduction

Plants live in close association with the abundant diversity of microbes found in the soil. Plant roots release carbon-rich root exudates, which support a high level of microbial growth in the rhizosphere- the zone of soil immediately surrounding plant roots. Additionally, some of these microbes actually enter the root and live inside the plant itself, known as the endosphere. Together, this root microbiome serves as a "second genome" to the plant, providing important benefits such as growth promotion and defense against pathogens (Berendsen et al. 2012). Multiple studies have revealed differences between root microbiomes of different species (Olivares et al. 1996; Weber et al. 1999; Dong et al. 2003; Fitzpatrick et al. 2018), and even differences in closely-related and genotypes within species (Table 1.1). These differences can be both phylogenetic or functional (Table 1.1).

Table 1.2 Summary of literature on host genetic differences in the root microbiome

Host	Compartment	Methods	Species/Cultivar	Findings	Reference
Rice	Rhizosphere	16S sequencing	Wild vs. cultivated	PCoA of Unifrac distances show 6.1% of bacterial community diversity accounted for by differences between wild and cultivated species. Anaerolinae overrepresented in wild species compared to domesticated. Some methanotrophs more abundant in early divergent wild rhizospheres. Lower alpha diversity in wild relatives.	(Shenton et al. 2016)
Rice	Endosphere	PCR-DGGE	Aromatica, sativa, and japonica cultivars	Genotypic group explained 48.8% of variability in overall DGGE profiles and 68.4% of variability in Alphaproteobacteria profiles.	(Hardoim et al. 2011)
Potato	Rhizosphere	PCR-DGGE and substrate use profiles	Cultivars	No effect on overall community (compared to effect of soil), but cultivar influenced carbon, phosphorus, and sulfur utilization profiles.	(İnceoğlu et al. 2012)
Potato	Endosphere	B-ARISA and 16S sequencing	Cultivars	Cultivars harbored different communities based on both weighted and unweighted Unifrac distances.	(Manter et al. 2010)
Arabidopsis	Rhizo- and endosphere	16S sequencing	Accessions	Abundance of 12 OTUs of 778 total differed quantitatively among 8 Arabidopsis accessions in endosphere	(Lundberg et al. 2012)

Table 3.1 Continued. Summary of literature on host genetic differences in the root microbiome

Host	Compartment	Methods	Species/Cultivar	Findings	Reference
Common bean	Rhizosphere	16S sequencing	Wild, landrace, domesticated	Differences in bacterial communities associated with root traits. Differences in rel. ab. of some bacterial families along domestication gradient. 13.5% of variation in beta-diversity explained by genotype.	(Pérez-Jaramillo et al. 2017)
Maize	Rhizosphere	tRFLP and fatty acid methyl ester analysis.	Wild vs. cultivated (Balsas teosinte vs. sweet corn and popping corn)	Lower bacterial diversity in sweet corn compared to Teosinte and popping corn. MRPP analysis of tRFLP profiles showed genotype specific differences.	(Szoboszlay et al. 2015)
Arabidopsis	Root and rhizosphere	16S sequencing	Wild vs. ecotypes	9 of 70 root community members differ between A. thaliana and divergent C. hirsuta grown under natural conditions. Variation between hosts largely quantitative in greenhouse grown plants. Greater variation between than within species.	(Schlaeppi et al. 2014)
Maize	Rhizosphere	PFLA analysis, Microbial growth	Cultivars differing in C allocation	Changes in fungal and gram-positive bacterial biomass between genotypes.	(Aira et al. 2010)
Maize		16S sequencing	Cultivars	19.1% of variation in OTU richness and 5% (unweighted Unifrac) and 7.7% (weighted Unifrac) of variation could be explained by genotype across plots and field environment, but this effect was different depending on the field environment.	(Peiffer et al. 2013)

Table 4.1 Continued. Summary of literature on host genetic differences in the root microbiome

Host	Compartment	Methods	Species/Cultivar	Findings	Reference
Wheat	Rhizosphere	Culturable analysis, Functional gene analysis	Cultivars	One wheat cultivar harbored higher abundances of DAPG producing Pseudomonads.	(Mazzola, M; Hewavitharana, SS, Strauss 2014)
Boechera stricta (wild mustard)	Leaf and root-associated	16S sequencing	Genotypes	Genotype affected Chao1 diversity in leaves but not roots.	(Wagner et al. 2016)
Potato	Rhizosphere and bulk	Functional gene PCR-DGGE, qPCR, library sequencing	Cultivars	Cultivar influenced structure of ammonia oxidizing communities	(Dias et al. 2012)
Blackberry and Blueberry	Rhizosphere	16S sequencing	Species and Cultivars of blueberry	Differences in phylum abundance between Blackberry and blueberry rhizospheres. Differences in Beta-diversity between Blackberry and blueberry as well as between cultivars of blueberry.	(Jiang et al. 2017)
Pearl millet	Rhizosphere	16S sequencing	Inbred lines	Alpha diversity correlated positively with root associated soil/root weight ratio (RAS/RT). Abundance of Rhizopbiales and Bacillales correlated with RAS/RT.	(Ndour et al. 2017)
Echinaceae	Rhizosphere, root, leaf/stem	Culturables, functional analysis	Closely related species	The two species harbor different culturable communities with differences in extracellular enzyme activity and antibiotic resistance.	(Chiellini et al. 2015; Maggini et al. 2018)

Together these studies (Table 1.1) suggest a minor role of plant genotype in selection of the root microbial community, generally accounting for between 5-20% of root microbiome variability. In a survey of 8 Arabidopsis accessions, Lundberg et al. (2012) showed that 12 out of 778 total OTUs varied quantitatively between the accessions. More recently, Perez-Jaramillo et al. (2017) examined the root microbial communities of a domestication gradient of common bean, using wild, landrace, and domesticated accessions and found differences in abundance of a number of bacterial families along the bean domestication gradient.

Though these studies reveal a small impact of genotype on the root microbiome, recent evidence suggests that even these small changes can have ecologically relevant impacts. Haney et al. (2015) found differences in colonization levels of a subset of Pseudomonads between Arabidopsis accessions. Col-0 harbored these *Pseudomonas* isolates and showed growth promotion in their presence, but also increased susceptibility to biotrophic pathogen *P. syringae* DC3000. However, some accessions that actively inhibited colonization did not show growth promotion in the presence of these *Pseudomonas* isolates, but also did not show increased susceptibility to *P. syringae*, suggesting a fitness trade-off to harboring these Pseudomonads.

Additionally, this study suggests a genetic basis for interactions with root-associated bacteria as they observed quantitative variation for colonization of *Pseudomonas* isolates (Haney et al. 2015). Several studies have examined the genetic basis for positive plant-microbe or microbiome interactions, in order to discover candidate genes involved in these interactions. In tomato, multiple QTL were discovered for disease-suppression by a *Bacillus cereus* in an RIL population (Smith et al. 1999) as well as candidate genes using a GWAS with plant-growth promoting *P. simiae* WCS417r and Arabidopsis (Wintermans et al. 2016). Many of these candidate genes were associated with plant-growth processes like photosynthesis, cytokinin transport, and secondary metabolism.

A few studies have looked at a genetic basis for selection of the entire microbiome, rather than focusing on interactions with a single isolate, though these have only been performed for leaves. Balint-Kurti et al. (2010) discovered QTL that underlie differences in alpha diversity on maize leaves that colocalize with QTL for resistance to fungal disease Southern leaf blight. They also found differences in glutamate decarboxylase enzyme activity between high and low bacterial diversity genotypes, corresponding to one of their candidate genes, a glutamate decarboxylase. A GWAS study performed on leaf communities of Arabidopsis accessions

discovered a number of candidate genes associated with defense and cell wall integrity (Horton et al. 2015). To our knowledge, no candidate genes have been discovered for bacterial community diversity in plant roots thus far.

Though associations with the microbiome clearly have a genetic basis, our understanding of the plant's role in assembling the root endophytic microbiome is still poorly understood. Soil and environmental factors play a decisive role in determining the microbial community in the bulk soil that is available to plants for recruitment to the rhizosphere and ultimately the endosphere (Bulgarelli et al. 2013). Plant host factors such as root exudation, root architecture, and innate immunity are known to play roles in root microbiome assembly.

Root exudates

Plant roots produce a variety of root exudates, consisting of organic acids, sugars, amino acids, and secondary metabolites, which may attract different microorganisms depending on their substrate preferences or repel invaders (Neal et al. 2012; Strehmel et al. 2014; Zhalnina et al. 2018). Mutant studies have revealed that alterations in the root exudate profiles due to mutations in transporters (Badri et al. 2009) or hormone pathways that affect exudation affect the root microbial community (Carvalhais et al. 2015).

A few studies have revealed that plant roots release compounds that preferentially attract microbes with desirable traits. For example, young maize roots release benzoaxinoid compound 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA), which normally exerts allelochemical or antimicrobial effects (Neal et al. 2012). However, DIMBOA preferentially attracts beneficial *Pseudomonas putida* strain KT2440 to its rhizosphere (Neal et al. 2012). Interestingly, plants can also increase exudation of particular compounds in response to pathogen attack in order to recruit beneficial bacteria that may help protect them from pathogen attack. Arabidopsis roots increase exudation of malic acid in response to foliar pathogen *Pseudomonas syringae* pv. *tomato* (*Pst* DC3000) and recruit beneficial *Bacillus subtilis* FB17 (Rudrappa et al. 2008).

Different plant species produce root exudates of differing composition, which may affect the structure of their rhizosphere microbiome (Kowalchuk et al. 2002; Högberg et al. 2007). Even cultivars within species can differ in their root exudate profiles, affecting root microbiome composition (Micallef et al. 2009), though the underlying genetic basis for this is not understood.

QTL analysis in *Phaseolus vulgaris* roots revealed 9 QTL linked to traits involved in acid exudation from roots (Yan et al. 2004). A cloned QTL for Al tolerance was found to encode a citrate transporter that responded to high levels of Al with increased citrate exudation (Maron et al. 2010). Though these QTL were not directly linked to effects on the rhizosphere microbiome, they reveal a genetic basis for differences in root exudation, which may lead to differences in the microbiome. Though not a direct measurement of root exudation, Ndour et al (2017) measured rhizosphere soil aggregation in inbred lines of pearl millet and showed a positive correlation between soil aggregation and alpha diversity as well as correlations between aggregation and abundance of specific bacterial taxa (Table 1).

Root architecture

Root architecture may also play a role in host control of the root microbiome, though this has not been well explored. Known endophytes colonize heavily at lateral root emergence sites, indicating that differences in lateral root structure may affect endosphere colonization (Chi et al. 2005; Lundberg et al. 2012). Perez-Jaramillo et al. (2017) showed that differences in the rhizosphere microbiomes of wild and domesticated bean accessions correlate with differences in specific root length, and that SRL correlates with abundance of families of Bacteroidetes. *Balsas teosinate* has been shown to have different root architecture than modern maize cultivars, as well as differences in root microbial communities (Szoboszlay et al. 2015) (Table 1), though the correlation between these traits was not explored in the study. More research is required to further examine the role of root architecture in assembly of the root microbiome.

Innate immunity

The role of innate immunity in assembly of plant-associated bacterial communities has recently begun to be explored. Knocking out multiple pathways of the innate immune system resulted in disrupted homeostasis of the Arabidopsis leaf endophytic community at high relative humidity levels (Xin et al. 2016). Endophytic colonization increased in quadruple mutants *min7 fls2 efr cerk1* and *min7 bak1-5 bkk1-1 cerk1*, both defective in pattern-triggered immunity and lacking *AtMin7*, a host target of *Pseudomonas syringae* HopM1, thus demonstrating a role for innate immunity in restricting colonization levels of commensal bacteria in the phyllosphere. In a seminal paper, Lebeis et al. (2015) examined the root endophytic bacterial communities of a

panel of Arabidopsis mutants representing various components of the innate immune system. Strikingly, they discovered differences in beta-diversity or relative abundance of bacterial Phyla in six of the ten mutants examined. Focusing on *cpr5*, a constitutively active salicylic acid (SA) pathway mutant, in which Proteobacteria were enriched and Actinobacteria were depleted, they showed that some members of Proteobacteria utilize salicylic acid as a carbon source, which may explain their overrepresentation in *cpr5* compared to wild-type Columbia-0.

Using a similar mutant approach with a simplified synthetic community consisting of seven members naturally abundant on leaves, Bodenhausen et al. (2014) demonstrated a role for ethylene in proper assembly of the Arabidopsis phyllosphere bacterial community. Bray-Curtis index - a measure of beta-diversity - and total colonization increased in the *ein-2* mutant compared to wild-type Col-0. These differences were attributed to an increase in the abundance of the *Variovorax spp.* isolate. In contrast, a reduction of culturable bacterial abundance was observed in the rhizosphere of the *ein-2* Arabidopsis mutant, though this reduction was not associated with differences in community structure (Doornbos et al. 2011). However, wild tobacco *Nicotiana attenuata* mutants for ethylene biosynthesis and signaling showed a small reduction in culturable bacterial diversity in the root endosphere compared to wild type (Long et al. 2010).

In wheat, exogenous application of methyl jasmonate to activate jasmonic acid (JA) signaling affected bacterial community structure in the root endophytic compartment but had no effect on the rhizosphere or shoot endosphere (Liu et al. 2017). Active JA signaling reduced root endophyte bacterial richness and affected the abundance of several OTUs. In Arabidopsis, activation of JA signaling affected bacterial community composition in the rhizosphere, but not richness or evenness (Carvalhais et al. 2013). Interestingly, upregulation of the JA pathway also plays a role in restricting colonization of incompatible strains of nitrogen-fixing *Azoarcus* in *Oryza sativa* (Miché et al. 2006) and suppressing nodulation of *Lotus japonicus* (Nakagawa and Kawaguchi 2006), indicating a role for JA in restricting non-pathogenic bacterial colonization.

Summary and study focus

Taken together these studies suggest that innate immunity regulated by defense hormones is essential for proper assembly of plant-associated bacterial communities. However, the role that each hormone plays in community assembly appears to depend on both plant species

as well as plant compartment. Further research is needed in additional model organisms to tease out the roles of various components of the innate immune system on bacterial communities in multiple plant compartments. Thus, the study described in Chapter 3 aimed to identify the role of defense hormones in tomato root microbiome assembly.

References

Aira M, Gómez-Brandón M, Lazcano C, et al (2010) Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. Soil Biol Biochem 42:2276–2281. doi: 10.1016/j.soilbio.2010.08.029

Artiola JF, Rasmussen C, Freitas R (2012) Effects of a Biochar-Amended Alkaline Soil on the Growth of Romaine Lettuce and Bermudagrass. Soil Sci 177:561–570. doi: 10.1097/SS.0b013e31826ba908

Badri D V., Quintana N, El Kassis EG, et al (2009) An ABC Transporter Mutation Alters Root Exudation of Phytochemicals That Provoke an Overhaul of Natural Soil Microbiota. Plant Physiol 151:2006–2017. doi: 10.1104/pp.109.147462

Balint-Kurti P, Simmons SJ, Blum JE, et al (2010) Maize Leaf Epiphytic Bacteria Diversity Patterns Are Genetically Correlated with Resistance to Fungal Pathogen Infection. Mol Plant-Microbe Interact 23:473–484. doi: 10.1094/MPMI-23-4-0473

Bargmann I, Rillig MC, Buss W, et al (2013) Hydrochar and Biochar Effects on Germination of Spring Barley. J Agron Crop Sci 199:360–373. doi: 10.1111/jac.12024

Baronti S, Alberti G, Vedove GD, et al (2010) The biochar option to improve plant yields: First results from some field and pot experiments in Italy. Ital J Agron 5:3–11. doi: 10.4081/ija.2010.3

Belz RG, Cedergreen N (2010) Parthenin hormesis in plants depends on growth conditions. Environ Exp Bot 69:293–301. doi: 10.1016/j.envexpbot.2010.04.010

Berendsen RL, Pieterse CMJ, Bakker P a HM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–86. doi: 10.1016/j.tplants.2012.04.001

Berens ML, Berry HM, Mine A, et al (2017) Evolution of Hormone Signaling Networks in Plant Defense.

Bhattarai KK, Xie Q-G, Mantelin S, et al (2008) Tomato Susceptibility to Root-Knot Nematodes Requires an Intact Jasmonic Acid Signaling Pathway. Mol Plant-Microbe Interact 21:1205–1214. doi: 10.1094/MPMI-21-9-1205

Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy 5:202–214. doi: 10.1111/gcbb.12037

Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA (2014) A Synthetic Community Approach Reveals Plant Genotypes Affecting the Phyllosphere Microbiota. PLoS Genet. doi: 10.1371/journal.pgen.1004283

Bouzroud S, Gouiaa S, Hu N, et al (2018) Auxin response factors (ARFs) are potential mediators of auxin action in tomato response to biotic and abiotic stress (Solanum lycopersicum). PLoS One 13:1–20. doi: 10.1371/journal.pone.0193517

Bulgarelli D, Schlaeppi K, Spaepen S, et al (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–38. doi: 10.1146/annurev-arplant-050312-120106

Calabrese EJ, Blain RB (2009) Hormesis and plant biology. Environ Pollut 157:42–48. doi: 10.1016/j.envpol.2008.07.028

Caldwell D, Kim B, Iyer-pascuzzi AS (2017) Ralstonia solanacearum Differentially Colonizes Roots of Resistant and Susceptible Tomato Plants. 528–536.

Carmeille A, Caranta C, Dintinger J, et al (2006) Identification of QTLs for Ralstonia solanacearum race 3-phylotype II resistance in tomato. Theor Appl Genet. doi: 10.1007/s00122-006-0277-3

Carvalhais LC, Dennis PG, Badri D V., et al (2013) Activation of the Jasmonic Acid Plant Defence Pathway Alters the Composition of Rhizosphere Bacterial Communities. PLoS One 8:1–5. doi: 10.1371/journal.pone.0056457

Carvalhais LC, Dennis PG, Badri D V, et al (2015) Linking Jasmonic Acid Signaling , Root Exudates , and Rhizosphere Microbiomes. 28:1049–1058.

Carvalhais LC, Schenk PM, Dennis PG (2017) Jasmonic acid signalling and the plant holobiont. Curr Opin Microbiol 37:42–47. doi: 10.1016/j.mib.2017.03.009

Chen Y, Ren X, Zhou X, et al (2014) Dynamics in the resistant and susceptible peanut (Arachis hypogaea L.) root transcriptome on infection with the Ralstonia solanacearum. BMC Genomics 15:1078. doi: 10.1186/1471-2164-15-1078

Chen Z, Agnew JL, Cohen JD, et al (2007) Pseudomonas syringae type III effector AvrRpt2 alters Arabidopsis thaliana auxin physiology. Proc Natl Acad Sci 104:20131–20136. doi: 10.1073/pnas.0704901104

Chi F, Shen S, Cheng H, et al (2005) Ascending Migration of Endophytic Rhizobia , from Roots to Leaves , inside Rice Plants and Assessment of Benefits to Rice Growth Physiology. 71:7271–7278. doi: 10.1128/AEM.71.11.7271

Chiellini C, Maida I, Emiliani G, et al (2015) Endophytic and rhizospheric bacterial communities isolated from the medicinal plants echinacea purpurea and echinacea angustifolia. Int Microbiol 17:165–174. doi: 10.2436/20.1501.01.219

Colebrook EH, Thomas SG, Phillips AL, Hedden P (2014) The role of gibberellin signalling in plant responses to abiotic stress. J Exp Biol 217:67–75. doi: 10.1242/jeb.089938

Copley T, Bayen S, Jabaji S (2017) Biochar amendment modifies expression of soybean and Rhizoctonia solani genes leading to increased severity of Rhizoctonia Foliar Blight. Front Plant Sci 8:1–15. doi: 10.3389/fpls.2017.00221

Copley TR, Aliferis KA, Jabaji S (2015) Maple bark biochar affects Rhizoctonia solani metabolism and increases damping-off severity. Phytopathology 105:1334–1346. doi: 10.1094/PHYTO-08-14-0231-R

Dai Z, Hu J, Xu X, et al (2016) Sensitive responders among bacterial and fungal microbiome to pyrogenic organic matter (biochar) addition differed greatly between rhizosphere and bulk soils. Sci Rep 6:36101. doi: 10.1038/srep36101

Danesh D, Aarons S, McGill GE, Young ND (1994) Genetic dissection of oligogenic resistance to bacterial wilt in tomato.

De Tender C, Haegeman A, Vandecasteele B, et al (2016a) Dynamics in the Strawberry Rhizosphere Microbiome in Response to Biochar and Botrytis cinerea Leaf Infection. Front Microbiol 7:1–14. doi: 10.3389/fmicb.2016.02062

De Tender CA, Debode J, Vandecasteele B, et al (2016b) Biological , physicochemical and plant health responses in lettuce and strawberry in soil or peat amended with biochar. Appl Soil Ecol 107:1–12. doi: 10.1016/j.apsoil.2016.05.001

Denancé N, Ranocha P, Oria N, et al (2012) Arabidopsis wat1 (walls are thin1)-mediated resistance to the bacterial vascular pathogen, Ralstonia solanacearum, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism. Plant J. doi: 10.1111/tpj.12027

Denny T (2006) Plant Pathogenic Ralstonia species. In: Gnanamanickam SS (ed) Plant-associated Bacteria. Springer, pp 573–644

Di X, Gomila JO, Takken FLW (2017) Involvement of salicylic acid , ethylene and jasmonic acid signalling pathways in the susceptibility of tomato to Fusarium oxysporum. 8:1024–1035.

Dias ACF, Hoogwout EF, Pereira e Silva MDC, et al (2012) Potato cultivar type affects the structure of ammonia oxidizer communities in field soil under potato beyond the rhizosphere. Soil Biol Biochem 50:85–95. doi: 10.1016/j.soilbio.2012.03.006

Ding X, Cao Y, Huang L, et al (2008) Activation of the Indole-3-Acetic Acid-Amido Synthetase GH3-8 Suppresses Expansin Expression and Promotes Salicylate- and Jasmonate-Independent Basal Immunity in Rice. Plant Cell Online 20:228–240. doi: 10.1105/tpc.107.055657

Dong Y, Iniguez AL, Triplett EW (2003) Quantitative assessments of the host range and strain specificity of endophytic colonization by Klebsiella pneumoniae 342. Plant Soil 257:49–59. doi: 10.1023/A:1026242814060

Doornbos RF, Geraats BPJ, Kuramae EE, et al (2011) Effects of Jasmonic Acid, Ethylene, and Salicylic Acid Signaling on the Rhizosphere Bacterial Community of Arabidopsis thaliana. Mol Plant-Microbe Interact 24:395–407. doi: 10.1094/mpmi-05-10-0115

Dubrovsky JG, Sauer M, Napsucialy-Mendivil S, et al (2008) Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. Proc Natl Acad Sci 105:8790–8794. doi: 10.1073/pnas.0712307105

Ebrahimi N, Viaene N, Vandecasteele B, et al (2016) Traditional and new soil amendments reduce survival and reproduction of potato cyst nematodes, except for biochar. Appl Soil Ecol 107:191–204. doi: 10.1016/j.apsoil.2016.06.006

Eizenberg H, Plakhine D, Ziadne H, et al (2017) Non-chemical Control of Root Parasitic Weeds with Biochar. Front Plant Sci 8:1–9. doi: 10.3389/fpls.2017.00939

Elad Y, David DR, Harel YM, et al (2010) Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. Phytopathology 100:913–21. doi: 10.1094/PHYTO-100-9-0913

Elmer WH (2012) Influence of biochar and earthworms on plant growth, fusarium crown and root rot, and mycorrhizal colonization of asparagus. In: Acta Horticulturae. pp 263–270

Elmer WH, Pignatello JJ (2011) Effect of Biochar Amendments on Mycorrhizal Associations and Fusarium Crown and Root Rot of Asparagus in Replant Soils. Plant Dis 95:960–966. doi: 10.1094/PDIS-10-10-0741

Etemadi M, Gutjahr C, Couzigou J-M, et al (2014) Auxin Perception Is Required for Arbuscule Development in Arbuscular Mycorrhizal Symbiosis. Plant Physiol 166:281–292. doi: 10.1104/pp.114.246595

Fitzpatrick CR, Copeland J, Wang PW, et al (2018) Assembly and ecological function of the root microbiome across angiosperm plant species. Proc Natl Acad Sci 201717617. doi: 10.1073/pnas.1717617115

Fracetto GGM, Peres LEP, Lambais MR (2017) Gene expression analyses in tomato near isogenic lines provide evidence for ethylene and abscisic acid biosynthesis fine-tuning during arbuscular mycorrhiza development. Arch Microbiol 199:787–798. doi: 10.1007/s00203-017-1354-5

Free HF, McGill CR, Rowarth JS, Hedley MJ (2017) The effect of biochars on maize (Zea mays) germination. New Zeal J Agric Res 53:1–4. doi: 10.1080/00288231003606039

Fu J, Wang S (2011) Insights into Auxin Signaling in Plant?Pathogen Interactions. Front Plant Sci 2:1–7. doi: 10.3389/fpls.2011.00074

Gaion LA, Monteiro CC, Cruz FJR, et al (2018) Constitutive gibberellin response in grafted tomato modulates root-to-shoot signaling under drought stress. J Plant Physiol 221:11–21. doi: 10.1016/j.jplph.2017.12.003

Gale N V., Sackett TE, Thomas SC (2016) Thermal treatment and leaching of biochar alleviates plant growth inhibition from mobile organic compounds. PeerJ 4:e2385. doi: 10.7717/peerj.2385

Gascó G, Cely P, Plaza C, Méndez A (2017) Relation between biochar properties and effects on seed germination and plant development. Biol Agric Hortic 32:237–247. doi: 10.1080/01448765.2016.1166348

Gaskin JW, Speir RA, Harris K, et al (2010) Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status, and yield. Agron J 102:623–633. doi: 10.2134/agronj2009.0083

Genin S (2010) Molecular traits controlling host range and adaptation to plants in Ralstonia solanacearum. New Phytol 187:920–8. doi: 10.1111/j.1469-8137.2010.03397.x

Genin S, Denny TP (2012) Pathogenomics of the Ralstonia solanacearum species complex. Annu Rev Phytopathol 50:67–89. doi: 10.1146/annurev-phyto-081211-173000

George C, Kohler J, Rillig MC (2016) Biochars reduce infection rates of the root-lesion nematode Pratylenchus penetrans and associated biomass loss in carrot. Soil Biol Biochem 95:11–18. doi: 10.1016/j.soilbio.2015.12.003

Gharbi E, Lutts S, Dailly H, Quinet M (2018) Comparison between the impacts of two different modes of salicylic acid application on tomato (*Solanum lycopersicum*) responses to salinity. Plant Signal Behav 00:1–9. doi: 10.1080/15592324.2018.1469361

Ghareeb H, Bozsó Z, Ott PG, et al (2011) Transcriptome of silicon-induced resistance against Ralstonia solanacearum in the silicon non-accumulator tomato implicates priming effect. Physiol Mol Plant Pathol 75:83–89. doi: 10.1016/j.pmpp.2010.11.004

Graber ER, Frenkel O, Jaiswal a. K, Elad Y (2014) How may biochar influence severity of diseases caused by soilborne pathogens? Carbon Manag 5:169–183. doi: 10.1080/17583004.2014.913360

Graber ER, Tsechansky L, Mayzlish-Gati E, et al (2015) A humic substances product extracted from biochar reduces Arabidopsis root hair density and length under P-sufficient and P-starvation conditions. Plant Soil 395:21–30. doi: 10.1007/s11104-015-2524-3

Gravel V, Dorais M, Ménard C (2013) Organic potted plants amended with biochar: its effect on growth and Pythium colonization. Can J Plant Sci 93:1217–1227. doi: 10.4141/cjps2013-315

Gu Y, Hou Y, Huang D, et al (2016) Application of biochar reduces Ralstonia solanacearum infection via effects on pathogen chemotaxis , swarming motility , and root exudate adsorption. Plant Soil. doi: 10.1007/s11104-016-3159-8

Hafez AAR, Stout PR, DeVay JE (1975) Potassium Uptake by Cotton in Relation to Verticillium Wilt1. Agron J 67:359.

Haney CH, Samuel BS, Bush J, Ausubel FM (2015) Associations with rhizosphere bacteria can confer an adaptive advantage to plants. Nat Plants 1:15051. doi: 10.1038/nplants.2015.51

Hardoim PR, Andreote FD, Reinhold-Hurek B, et al (2011) Rice root-associated bacteria: Insights into community structures across10 cultivars. FEMS Microbiol Ecol 77:154–164. doi: 10.1111/j.1574-6941.2011.01092.x

Harter J, Weigold P, El-Hadidi M, et al (2016) Soil biochar amendment shapes the composition of N2O-reducing microbial communities. Sci Total Environ 562:379–390. doi: 10.1016/j.scitotenv.2016.03.220

Harvey OR, Herbert BE, Kuo LJ, Louchouarn P (2012) Generalized two-dimensional perturbation correlation infrared spectroscopy reveals mechanisms for the development of surface charge and recalcitrance in plant-derived biochars. Environ Sci Technol 46:10641–10650. doi: 10.1021/es302971d

Hase S, Takahashi S, Takenaka S, et al (2008) Involvement of jasmonic acid signalling in bacterial wilt disease resistance induced by biocontrol agent Pythium oligandrum in tomato. Plant Pathol 57:870–876. doi: 10.1111/j.1365-3059.2008.01858.x

Hedden P, Sponsel V (2015) A Century of Gibberellin Research. J Plant Growth Regul 34:740–760. doi: 10.1007/s00344-015-9546-1

Herrera-Medina MJ, Tamayo MI, Vierheilig H, et al (2008) The jasmonic acid signalling pathway restricts the development of the arbuscular mycorrhizal association in tomato. J Plant Growth Regul 27:221–230. doi: 10.1007/s00344-008-9049-4

Hille M, den Ouden J (2005) Charcoal and activated carbon as adsorbate of phytotoxic compounds - A comparative study. Oikos 108:202–207. doi: 10.1111/j.0030-1299.2005.13482.x

Högberg MN, Högberg P, Myrold DD (2007) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150:590–601. doi: 10.1007/s00442-006-0562-5

Horton MW, Bodenhausen N, Beilsmith K, et al (2015) Genome-wide association study of Arabidopsis thaliana's leaf microbial community. Nat Commun. doi: 10.1038/ncomms6320.Genome-wide

Hu J, Barlet X, Deslandes L, et al (2008) Transcriptional responses of Arabidopsis thaliana during wilt disease caused by the soil-borne phytopathogenic bacterium, Ralstonia solanacearum. PLoS One. doi: 10.1371/journal.pone.0002589

Huang W, Ji H, Gheysen G, et al (2015) Biochar-amended potting medium reduces the susceptibility of rice to root-knot nematode infections. BMC Plant Biol 15:267. doi: 10.1186/s12870-015-0654-7

Huot B, Yao J, Montgomery BL, He SY (2014) Growth-defense tradeoffs in plants: A balancing act to optimize fitness. Mol Plant 7:1267–1287. doi: 10.1093/mp/ssu049

Ibort P, Imai H, Uemura M, Aroca R (2018) Proteomic analysis reveals that tomato interaction with plant growth promoting bacteria is highly determined by ethylene perception. J Plant Physiol 220:43–59. doi: 10.1016/j.jplph.2017.10.008

Ibort P, Molina S, Núñez R, et al (2017) Tomato ethylene sensitivity determines interaction with plant growth-promoting bacteria. Ann Bot 120:101–122. doi: 10.1093/aob/mcx052

İnceoğlu Ö, Falcão Salles J, van Elsas JD (2012) Soil and cultivar type shape the bacterial community in the potato rhizosphere. Microb Ecol 63:460–70. doi: 10.1007/s00248-011-9930-8

Ishihara T, Mitsuhara I, Takahashi H, Nakaho K (2012) Transcriptome Analysis of Quantitative Resistance-Specific Response upon Ralstonia solanacearum Infection in Tomato. PLoS One. doi: 10.1371/journal.pone.0046763

Jaiswal AK, Elad Y, Graber ER, Frenkel O (2014) Rhizoctonia solani suppression and plant growth promotion in cucumber as affected by biochar pyrolysis temperature, feedstock and concentration. Soil Biol Biochem 69:110–118. doi: 10.1016/j.soilbio.2013.10.051

Jaiswal AK, Elad Y, Paudel I, et al (2017) Linking the Belowground Microbial Composition, Diversity and Activity to Soilborne Disease Suppression and Growth Promotion of Tomato Amended with Biochar. Sci Rep 7:44382. doi: 10.1038/srep44382

Jaiswal AK, Frenkel O, Elad Y, et al (2015) Non-monotonic influence of biochar dose on bean seedling growth and susceptibility to Rhizoctonia solani: the “Shifted Rmax-Effect.” Plant Soil 395:125–140. doi: 10.1007/s11104-014-2331-2

Jamieson T, Sager E, Gu??guen C (2014) Characterization of biochar-derived dissolved organic matter using UV-visible absorption and excitation-emission fluorescence spectroscopies. Chemosphere 103:197–204. doi: 10.1016/j.chemosphere.2013.11.066

Jeffery S, Abalos D, Prodana M, et al (2017a) Biochar boosts tropical but not temperate crop yields. Environ Res Lett 12:053001. doi: 10.1088/1748-9326/aa67bd

Jeffery S, Memelink I, Hodgson E, et al (2017b) Initial biochar effects on plant productivity derive from N fertilization. Plant Soil. doi: 10.1007/s11104-016-3171-z

Jeffery S, Verheijen FG a., van der Velde M, Bastos a. C (2011) A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agric Ecosyst Environ 144:175–187. doi: 10.1016/j.agee.2011.08.015

Jenkins JR, Viger M, Arnold EC, et al (2016) Biochar alters the soil microbiome and soil function: Results of next-generation amplicon sequencing across Europe. GCB Bioenergy 1–22. doi: 10.1111/gcbb.12371

Jiang Y, Li S, Li R, et al (2017) Plant cultivars imprint the rhizosphere bacterial community composition and association networks. Soil Biol Biochem 109:145–155. doi: 10.1016/j.soilbio.2017.02.010

Jogaiah S, Abdelrahman M, Tran LSP, Ito SI (2018) Different mechanisms of Trichoderma virens-mediated resistance in tomato against Fusarium wilt involve the jasmonic and salicylic acid pathways. Mol Plant Pathol 19:870–882. doi: 10.1111/mpp.12571

Jones R, Ougham H, Thomas H, Waaland S (2012) Molecular Life of Plants. Wiley-Blackwell, Chichester

Jung JK, McCouch S (2013) Getting to the roots of it: Genetic and hormonal control of root architecture. Front Plant Sci 4:186. doi: 10.3389/fpls.2013.00186

Kazan K, Manners JM (2009) Linking development to defense: auxin in plant-pathogen interactions. Trends Plant Sci 14:373–382. doi: 10.1016/j.tplants.2009.04.005

Kidd BN, Kadoo NY, Dombrecht B, et al (2011) Auxin signaling and transport promote susceptibility to the root-infecting fungal pathogen Fusarium oxysporum in Arabidopsis. Mol Plant Microbe Interact MPMI 24:733–748. doi: 10.1094/MPMI-08-10-0194

Kiirika LM, Stahl F, Wydra K (2013) Phenotypic and molecular characterization of resistance induction by single and combined application of chitosan and silicon in tomato against Ralstonia solanacearum. Physiol Mol Plant Pathol 81:1–12. doi: 10.1016/j.pmpp.2012.11.002

Kochanek J, Long RL, Lisle AT, Flematti GR (2016) Karrikins identified in biochars indicate post-fire chemical cues can influence community diversity and plant development. PLoS One 11:e0161234. doi: 10.1371/journal.pone.0161234

Kolton M, Graber ER, Tsehansky L, et al (2016) Biochar-stimulated plant performance is strongly linked to microbial diversity and metabolic potential in the rhizosphere. New Phytol 213:1393–1404. doi: http://dx.doi.org/10.1111/nph.14253

Kolton M, Meller Harel Y, Pasternak Z, et al (2011) Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Appl Environ Microbiol 77:4924–30. doi: 10.1128/AEM.00148-11

Kowalchuk G, Buma D, de Boer W, et al (2002) Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms RID C-4298-2011 RID C-2737-2011 RID C-3697-2011. Antonie Van Leeuwenhoek Int J Gen Mol Microbiol 81:509–520. doi: 10.1023/A:1020565523615

Kyndt T, Goverse A, Haegeman A, et al (2016) Redirection of auxin flow in Arabidopsis thaliana roots after infection by root-knot nematodes. J Exp Bot 67:4559–4570. doi: 10.1093/jxb/erw230

Laird DA, Brown RC, Amonette JE, Lehmann J (2009) Review of the pyrolysis platform for coproducing bio-oil and biochar. Biofuels, Bioprod Biorefining 6:547–562. doi: 10.1002/bbb.169

Laird DA, Novak JM, Collins HP, et al (2017) Multi-year and multi-location soil quality and crop biomass yield responses to hardwood fast pyrolysis biochar. Geoderma 289:46–53. doi: 10.1016/j.geoderma.2016.11.025

Lebeis SL, Sur Herrera P, Lundberg DS, et al (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science (80-) 349:860–864. doi: 10.5061/dryad.238b2

Li S, Song L, Jin Y, et al (2016) Linking N2O emission from biochar-amended composting process to the abundance of denitrify (nirK and nosZ) bacteria community. AMB Express 6:37. doi: 10.1186/s13568-016-0208-x

Li Y, Shen F, Guo H, et al (2015) Phytotoxicity assessment on corn stover biochar, derived from fast pyrolysis, based on seed germination, early growth, and potential plant cell damage. Environ Sci Pollut Res 22:9534–9543. doi: 10.1007/s11356-015-4115-5

Liao S, Pan B, Li H, et al (2014) Detecting free radicals in biochars and determining their ability to inhibit the germination and growth of corn, wheat and rice seedlings. Environ Sci Technol 48:8581–8587. doi: 10.1021/es404250a

Lin Y, Munroe P, Joseph S, et al (2012) Water extractable organic carbon in untreated and chemical treated biochars. Chemosphere 87:151–157. doi: 10.1016/j.chemosphere.2011.12.007

Liu H, Carvalhais LC, Schenk PM, Dennis PG (2017) Effects of jasmonic acid signalling on the wheat microbiome differ between body sites. Sci Rep 7:41766. doi: 10.1038/srep41766

Llorente F, Muskett P, Sánchez-Vallet A, et al (2008) Repression of the auxin response pathway increases Arabidopsis susceptibility to necrotrophic fungi. Mol Plant 1:496–509. doi: 10.1093/mp/ssn025

Long HH, Sonntag DG, Schmidt DD, Baldwin IT (2010) The structure of the culturable root bacterial endophyte community of Nicotiana attenuata is organized by soil composition and host plant ethylene production and perception. New Phytol 185:554–567. doi: 10.1111/j.1469-8137.2009.03079.x

Ludwig-Müller J (2015) Bacteria and fungi controlling plant growth by manipulating auxin: Balance between development and defense. J Plant Physiol 172:4?12. doi: 10.1016/j.jplph.2014.01.002

Lund ST, Stall RE, Klee HJ (1998) Ethylene regulates the susceptible response to pathogen infection in tomato. Plant Cell 10:371–382. doi: 10.1016/S1369-5266(98)80080-0

Lundberg DS, Lebeis SL, Paredes SH, et al (2012) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90. doi: 10.1038/nature11237

Maggini V, Elisangela M, Fagorzi C, et al (2018) Antagonism and antibiotic resistance drive a species-specific plant microbiota differentiation in Echinacea spp. FEMS Microbiol Ecol 94:fiy118. doi: https://doi.org/10.1093/femsec/fiy118

Mandal S, Mallick N, Mitra A (2009) Salicylic acid-induced resistance to Fusarium oxysporum f. sp. lycopersici in tomato. Plant Physiol Biochem 47:642–649. doi: 10.1016/j.plaphy.2009.03.001

Mangin B, Thoquet P, Olivier J, Grimsley NH (1999) Temporal and multiple quantitative trait loci analyses of resistance to bacterial wilt in tomato permit the resolution of linked loci.

Mansfield J, Genin S, Magori S, et al (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol 13:614–29. doi: 10.1111/j.1364-3703.2012.00804.x

Manter DK, Delgado JA, Holm DG, Stong RA (2010) Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. Microb Ecol 60:157–166. doi: 10.1007/s00248-010-9658-x

Maron LG, Piñeros MA, Guimarães CT, et al (2010) Two functionally distinct members of the MATE (multi-drug and toxic compound extrusion) family of transporters potentially underlie two major aluminum tolerance QTLs in maize. Plant J 61:728–740. doi: 10.1111/j.1365-313X.2009.04103.x

Marris E (2006) Putting the carbon back: black is the new green. Nature 442:624–6. doi: 10.1038/442624a

Matsubara Y, Hasegawa N, Fukui H (2002) Incidence of Fusarium Root Rot in Asparagus Seedlings Infected with Arbuscular Mycorrhizal Fungus as Affected by Several Soil Amendments. Engei Gakkai zasshi 71:370–374. doi: 10.2503/jjshs.71.370

Mazzola, M; Hewavitharana, SS, Strauss S (2014) Brassica seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen re-infestation. Phytopathology 1–48.

McAvoy T, Freeman JH, Rideout SL, et al (2012) Evaluation of Grafting Using Hybrid Rootstocks for Management of Bacterial Wilt in Field Tomato Production. HortScience 47:621–625.

Mehari ZH, Elad Y, Rav-David D, et al (2015) Induced systemic resistance in tomato (Solanum lycopersicum) against Botrytis cinerea by biochar amendment involves jasmonic acid signaling. Plant Soil 395:31–44. doi: 10.1007/s11104-015-2445-1

Meller Harel Y, Elad Y, Rav-David D, et al (2012) Biochar mediates systemic response of strawberry to foliar fungal pathogens. Plant Soil 357:245–257. doi: 10.1007/s11104-012-1129-3

Merchante C, Alonso JM, Stepanova AN (2013) Ethylene signaling: Simple ligand, complex regulation. Curr Opin Plant Biol 16:554–560. doi: 10.1016/j.pbi.2013.08.001

Micallef SA, Shiaris MP, Colón-Carmona A (2009) Influence of Arabidopsis thaliana accessions on rhizobacterial communities and natural variation in root exudates. J Exp Bot 60:1729–1742. doi: 10.1093/jxb/erp053

Miché L, Battistoni F, Gemmer S, et al (2006) Upregulation of jasmonate-inducible defense proteins and differential colonization of roots of Oryza sativa cultivars with the endophyte Azoarcus sp. Mol Plant Microbe Interact 19:502–511. doi: 10.1094/MPMI-19-0502

Milling A, Babujee L, Allen C (2011) Ralstonia solanacearum extracellular polysaccharide is a specific elicitor of defense responses in wilt-resistant tomato plants. PLoS One 6:e15853. doi: 10.1371/journal.pone.0015853

Nair A, Kolet SP, Thulasiram H V., Bhargava S (2015a) Systemic jasmonic acid modulation in mycorrhizal tomato plants and its role in induced resistance against Alternaria alternata. Plant Biol 17:625–631. doi: 10.1111/plb.12277

Nair A, Kolet SP, Thulasiram H V, Bhargava S (2015b) Role of methyl jasmonate in the expression of mycorrhizal induced resistance against Fusariumoxysporum in tomato plants. Physiol Mol Plant Pathol 92:139–145. doi: 10.1016/j.pmpp.2015.10.002

Nakagawa T, Kawaguchi M (2006) Shoot-applied MeJA suppresses root nodulation in Lotus japonicus. Plant Cell Physiol 47:176–180. doi: 10.1093/pcp/pci222

Naseem M, Kaltdorf M, Dandekar T (2015) The nexus between growth and defence signalling: auxin and cytokinin modulate plant immune response pathways. J Exp Bot 66:4885–4896. doi: 10.1093/jxb/erv297

Navarro L, Dunoyer P, Jay F, et al (2016) A Plant miRNA Contributes to Antibacterial Resistance by Repressing Auxin Signaling. Science (80-) 312:436–439.

Ndour PMS, Gueye M, Barakat M, et al (2017) Pearl Millet Genetic Traits Shape Rhizobacterial Diversity and Modulate Rhizosphere Aggregation. 8:1–14. doi: 10.3389/fpls.2017.01288

Neal AL, Ahmad S, Gordon-Weeks R, Ton J (2012) Benzoxazinoids in root exudates of maize attract Pseudomonas putida to the rhizosphere. PLoS One 7:e35498. doi: 10.1371/journal.pone.0035498

Nelson DC, Flematti GR, Ghisalberti EL, et al (2012) Regulation of Seed Germination and Seedling Growth by Chemical Signals from Burning Vegetation. Annu Rev Plant Biol 63:107–130. doi: 10.1146/annurev-arplant-042811-105545

Nojiri H, Sugimori M, Yamane H, et al (1996) lnvolvement of Jasmonic Acid in Elicitor-lnduced Phytoalexin. Plant Physiol 110:387–392.

Olivares FL, Baldani VLD, Reis VM, et al (1996) Occurrence of the endophytic diazotrophs Herbaspirillum spp. in roots, stems, and leaves, predominantly of Gramineae. Biol Fertil Soils 21:197–200. doi: 10.1007/BF00335935

Peiffer JA, Spor A, Koren O, et al (2013) Diversity and heritability of the maize rhizosphere microbiome under fi eld conditions. doi: 10.1073/pnas.1302837110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1302837110

Pérez-Jaramillo JE, Carrión VJ, Bosse M, et al (2017) Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. ISME J 11:2244–2257. doi: 10.1038/ismej.2017.85

Pieterse CMJ, Van der Does D, Zamioudis C, et al (2012) Hormonal Modulation of Plant Immunity. Annu Rev Cell Dev Biol 28:489–521. doi: 10.1146/annurev-cellbio-092910-154055

Pieterse CMJ, Zamioudis C, Berendsen RL, et al (2014) Induced Systemic Resistance by Beneficial Microbes. Annu Rev Phytopathol 52:347–375. doi: 10.1146/annurev-phyto-082712-102340

Poór P, Kovács J, Borbély P, et al (2015) Salt stress-induced production of reactive oxygen- and nitrogen species and cell death in the ethylene receptor mutant Never ripe and wild type tomato roots. Plant Physiol Biochem 97:313–322. doi: 10.1016/j.plaphy.2015.10.021

Prasath D, Karthika R, Habeeba NT, et al (2014) Comparison of the transcriptomes of ginger (Zingiber officinale Rosc.) and mango ginger (Curcuma amada Roxb.) in response to the bacterial wilt infection. PLoS One 9:e99731. doi: 10.1371/journal.pone.0099731

Qi L, Yan J, Li Y, et al (2012) Arabidopsis thaliana plants differentially modulate auxin biosynthesis and transport during defense responses to the necrotrophic pathogen Alternaria brassicicola. New Phytol 195:872–882. doi: 10.1111/j.1469-8137.2012.04208.x

Rajkovich S, Enders A, Hanley K, et al (2012) Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. Biol Fertil Soils 48:271–284. doi: 10.1007/s00374-011-0624-7

Ribaudo CM, Krumpholz EM, Cassán FD, et al (2006) Azospirillum sp. Promotes root hair development in tomato plants through a mechanism that involves ethylene. J Plant Growth Regul 25:175–185. doi: 10.1007/s00344-005-0128-5

Rivard CLL, O'Connell S, Peet MMM, et al (2012) Grafting Tomato to Manage Bacterial Wilt Caused by Ralstonia solanacearum in the Southeastern United States. Plant Dis. doi: 10.1094/PDIS-12-10-0877

Robert-Seilaniantz A, Grant M, Jones JDG (2011) Hormone Crosstalk in Plant Disease and Defense: More Than Just JASMONATE-SALICYLATE Antagonism. Annu Rev Phytopathol 49:317–343. doi: 10.1146/annurev-phyto-073009-114447

Rondon M a., Lehmann J, Ramírez J, Hurtado M (2007) Biological nitrogen fixation by common beans (Phaseolus vulgaris L.) increases with bio-char additions. Biol Fertil Soils 43:699–708. doi: 10.1007/s00374-006-0152-z

Rosskopf EN, Chellemi DO, Kokalis-Burelle N, Church GT (2005) Alternatives to Methyl Bromide: A Florida Perspective. Plant Manag Prog. doi: 10.1094/PHP-2005-1027-01-RV

Rudrappa T, Czymmek KJ, Paré PW, Bais HP (2008) Root-secreted malic acid recruits beneficial soil bacteria. Plant Physiol 148:1547–56. doi: 10.1104/pp.108.127613

Schlaeppi K, Dombrowski N, Oter RG, et al (2014) Quantitative divergence of the bacterial root microbiota in Arabidopsis thaliana relatives. Proc Natl Acad Sci U S A 111:585–92. doi: 10.1073/pnas.1321597111

Senaratna, T., Touchell, D., Bunn, E., and Dixon K (2000) Acetyl salicylic acid (Asprin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regul 30:157–161.

Shenton M, Iwamoto C, Kurata N, Ikeo K (2016) Effect of Wild and Cultivated Rice Genotypes on Rhizosphere Bacterial Community Composition. Rice (N Y) 9:42. doi: 10.1186/s12284-016-0111-8

Shoaf N, Hoagland L, Egel DS (2016) Suppression of phytophthora blight in sweet pepper depends on biochar amendment and soil type. HortScience 51:518–524.

Smith KP, Handelsman J, Goodman RM (1999) Genetic basis in plants for interactions with disease-suppressive bacteria. Proc Natl Acad Sci U S A 96:4786–90. doi: 10.1073/pnas.96.9.4786

Solaiman ZM, Murphy D V, Abbott LK (2012) Biochars influence seed germination and early growth of seedlings. Plant Soil 353:273–287. doi: 10.1007/s11104-011-1031-4

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448. doi: 10.1111/j.1574-6976.2007.00072.x

Spletzer ME, Enyedi a J (1999) Salicylic Acid Induces Resistance to Alternaria solani in Hydroponically Grown Tomato. Phytopathology 89:722–727. doi: 10.1094/PHYTO.1999.89.9.722

Spokas KA, Baker JM, Reicosky DC (2010) Ethylene: potential key for biochar amendment impacts. Plant Soil 333:443–452. doi: 10.1007/s11104-010-0359-5

Spokas KA, Cantrell KB, Novak JM, et al (2012) Biochar: a synthesis of its agronomic impact beyond carbon sequestration. J Environ Qual 41:973–89. doi: 10.2134/jeq2011.0069

Spokas KA, Novak JM, Stewart CE, et al (2011) Qualitative analysis of volatile organic compounds on biochar. Chemosphere 85:869–882. doi: 10.1016/j.chemosphere.2011.06.108

Stevens J, Senaratna T, Sivasithamparam K (2006) Salicylic acid induces salinity tolerance in tomato (Lycopersicon esculentum cv. Roma): Associated changes in gas exchange, water relations and membrane stabilisation. Plant Growth Regul 49:77–83. doi: 10.1007/s10725-006-0019-1

Strehmel N, Böttcher C, Schmidt S, Scheel D (2014) Profiling of secondary metabolites in root exudates of Arabidopsis thaliana. Phytochemistry 108C:35–46. doi: 10.1016/j.phytochem.2014.10.003

Suzuki A, Suriyagoda L, Shigeyama T, et al (2011) Lotus japonicus nodulation is photomorphogenetically controlled by sensing the red/far red (R/FR) ratio through jasmonic acid (JA) signaling. Proc Natl Acad Sci 108:16837–16842. doi: 10.1073/pnas.1105892108

Szepesi Á, Csiszár J, Gémes K, et al (2009) Salicylic acid improves acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and abscisic acid accumulation, and increases Na+content in leaves without toxicity symptoms in Solanum lycopersicum L. J Plant Physiol 166:914–925. doi: 10.1016/j.jplph.2008.11.012

Szoboszlay M, Lambers J, Chappell J, et al (2015) Comparison of root system architecture and rhizosphere microbial communities of Balsas teosinte and domesticated corn cultivars. Soil Biol Biochem 80:34–44. doi: 10.1016/j.soilbio.2014.09.001

Tejeda-Sartorius M, Martínez De La Vega O, Délano-Frier JP (2008) Jasmonic acid influences mycorrhizal colonization in tomato plants by modifying the expression of genes involved in carbohydrate partitioning. Physiol Plant 133:339–353. doi: 10.1111/j.1399-3054.2008.01081.x

Thaler JS (1999) Induced resistance in agricultural crops: Effects of jasmonic acid on\rherbivory and yield in tomato plants. Environ Entomol 28:30–37.

Thoquet P, Olivier J, Sperisen C, et al (1996a) Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii7996. Mol. Plant. Microbe. Interact. 9:826–836.

Thoquet P, Olivier J, Sperisen C, et al (1996b) Polygenic resistance of tomato plants to bacterial wilt in the French West Indies.

Tiryaki I (2002) An Arabidopsis Mutant Defective in Jasmonate Response Is Allelic to the Auxin-Signaling Mutant axr1. Plant Physiol 130:887–894. doi: 10.1104/pp.005272

Van de Poel B, Smet D, Van Der Straeten D (2015) Ethylene and Hormonal Cross Talk in Vegetative Growth and Development. Plant Physiol 169:61–72. doi: 10.1104/pp.15.00724

van Zwieten L, Kimber S, Morris S, et al (2010) Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant Soil 327:235–246. doi: 10.1007/s11104-009-0050-x

Vidoz ML, Loreti E, Mensuali A, et al (2010) Hormonal interplay during adventitious root formation in flooded tomato plants. Plant J 63:551–562. doi: 10.1111/j.1365-313X.2010.04262.x

Viger M, Hancock RD, Miglietta F, Taylor G (2014) More plant growth but less plant defence? First global gene expression data for plants grown in soil amended with biochar. GCB Bioenergy 7:658–672. doi: 10.1111/gcbb.12182

Wagner MR, Lundberg DS, del Rio TG, et al (2016) Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat Commun 7:1–15. doi: 10.1038/ncomms12151

Wang J-F, Olivier J, Thoquet P, et al (2000) Resistance of Tomato Line Hawaii7996 to *Ralstonia solanacearum* Pss4 in Taiwan Is Controlled Mainly by a Major Strain-Specific Locus. Mol Plant-Microbe Interact. doi: 10.1094/MPMI.2000.13.1.6

Wang X, Zhou W, Liang G, et al (2015a) Characteristics of maize biochar with different pyrolysis temperatures and its effects on organic carbon, nitrogen and enzymatic activities after addition to fluvo-aquic soil. Sci Total Environ 538:137–144. doi: 10.1016/j.scitotenv.2015.08.026

Wang Z, Zong H, Zheng H, et al (2015b) Reduced nitrification and abundance of ammonia-oxidizing bacteria in acidic soil amended with biochar. Chemosphere 138:576–583. doi: 10.1016/j.chemosphere.2015.06.084

Weber OB, Baldani VLD, Teixeira KRS, et al (1999) Isolation and characterization of diazotrophic bacteria from banana and pineapple plants. Plant Soil 210:103–113. doi: 10.1023/A:1004623523179

Wintermans PCA, Bakker PAHM, Pieterse CMJ (2016) Natural genetic variation in Arabidopsis for responsiveness to plant growth-promoting rhizobacteria. Plant Mol Biol 90:623–634. doi: 10.1007/s11103-016-0442-2

Woods WI, McCann JM (1999) The anthropogenic origin and persistence of Amazonian dark earths. Yearb Conf Lat Am Geogr 25:7–14. doi: 10.2307/25765871

Xiang Y, Deng Q, Duan H, Guo Y (2017) Effects of biochar application on root traits: a meta-analysis. GCB Bioenergy. doi: 10.1111/ijlh.12426

Xiao Q, Zhu LX, Zhang HP, et al (2016) Soil amendment with biochar increases maize yields in a semi-arid region by improving soil quality and root growth. Crop Pasture Sci 67:495–507. doi: 10.1071/CP15351

Xin X-F, Nomura K, Aung K, et al (2016) Bacteria establish an aqueous living space in plants crucial for virulence. Nature 539:524–529. doi: 10.1038/nature20166

Yan X, Liao H, Beebe SE, et al (2004) QTL mapping of root hair and acid exudation traits and their relationship to phosphorus uptake in common bean. Plant Soil 265:17–29. doi: 10.1007/s11104-005-0693-1

Yan Z, Reddy MS, Ryu C-M, et al (2002) Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. Phytopathology 92:1329–33. doi: 10.1094/PHYTO.2002.92.12.1329

Yuliar, Nion YA, Toyota K (2015) Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes Environ 30:1–11. doi: 10.1264/jsme2.ME14144

Zhalnina K, Louie KB, Hao Z, et al (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nat Microbiol 3:470–480. doi: 10.1038/s41564-018-0129-3

Zhang C, Lin Y, Tian X, et al (2016) Tobacco bacterial wilt suppression with biochar soil addition associates to improved soil physiochemical properties and increased rhizosphere bacteria abundance. Appl Soil Ecol 112:90–96. doi: 10.1016/j.apsoil.2016.12.005

Zhang H, Hu Z, Lei C, et al (2018) A Plant Phytosulfokine Peptide Initiates Auxin-Dependent Immunity through Cytosolic Ca2+ Signaling in Tomato. Plant Cell tpc.00537.2017. doi: 10.1105/tpc.17.00537

Zuluaga AP, Lu H, Góngora-Castillo E, et al (2015) Transcriptome responses to Ralstonia solanacearum infection in the roots of the wild potato Solanum commersonii. BMC Genomics 1–16. doi: 10.1186/s12864-015-1460-1

Zwart DC, Kim SH (2012) Biochar amendment increases resistance to stem lesions caused by Phytophthora spp. in tree seedlings. HortScience 47:1736–1740. doi: 10.4236/ojss.2015.59019

CHAPTER 2: A ROLE FOR THE GIBBERELLIN PATHWAY IN BIOCHAR-MEDIATED GROWTH PROMOTION*

Abstract

Biochar is a promising, black carbon soil amendment that often promotes crop growth. Understanding the molecular basis for biochar-mediated crop growth is critical for optimal biochar application and breeding biochar-responsive plants. In this study, we investigate the species- and within-species-specific effects of biochar on shoot growth and the molecular basis for biochar-mediated growth promotion in tomato. A pot experiment was performed in which two cultivars of tomato (*Solanum lycopersicum)*, and two wild relatives of tomato, *Solanum pimpinellifolium*, and *Solanum pennelli*, were grown with two types of biochar at 0 and 4% amendment. RNAseq was used to identify candidate molecular pathways, which were further investigated using functional assays. Biochar promoted shoot growth in all genotypes independent of biochar type. RNAseq analysis revealed downregulation of defense genes, as well as differential regulation of genes involved in gibberellin (GA) and brassinosteroid hormone pathways. Germination tests, exogenous GA_4 application and Arabidopsis mutant analysis confirmed the role of GA in biochar-mediated plant growth promotion. Our work identified genotype-specific effects of biochar on germination and interaction with exogenous GA. Our results suggest a role for GA signaling in biochar growth promotion in tomato.

Introduction

Biochar is a carbon negative soil amendment produced from pyrolysis of organic material. Its potential for mitigating climate change and improving agricultural soils was recognized after the discovery of Terra Preta soil in the Amazon, where soils conditioned with black carbon additions thousands of years ago by native residents continue to be more fertile and carbon-rich than surrounding soils even today (Woods and McCann 1999; Marris 2006). Today, soil amendment

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with biochar is being evaluated as a strategy for improving soil fertility, while simultaneously sequestering carbon and reducing greenhouse gas emissions (Woolf et al. 2010). Overall, modern biochars appear to promote plant growth (Biederman and Harpole 2013), though some studies have documented mixed or even negative effects of biochar (Marks et al. 2014; Sun et al. 2014; Smider and Singh 2014; Borchard et al. 2014; Haider et al. 2015; Nelissen et al. 2015; Butnan et al. 2015; Carvalho et al. 2016; Egamberdieva et al. 2016).

Research on how biochar promotes plant growth has focused on its positive impacts on soil characteristics, nutrient availability (reviewed in Spokas et al. 2012; Biederman and Harpole 2013) and the soil microbial community (reviewed in Lehmann et al. 2011). Additionally, several groups have demonstrated that biochar is effective in controlling foliar and soil-borne pathogens with both biotrophic and necrotrophic lifestyles on multiple host crops (reviewed in Elad et al. 2011; Graber et al. 2014). However, others have shown a neutral or negative effect of biochar on disease progress (Shoaf et al. 2016; Ebrahimi et al. 2016; Copley et al. 2015; Copley et al. 2017). Evidence indicates that differences in environment as well as biochar feedstock and production conditions each play a role in biochar's effectiveness (Biederman and Harpole 2013). A recent meta-analysis of biochar studies showed that biochar's effect on total plant biomass differs between annual and perennial crops (Biederman and Harpole 2013), suggesting that the effect of biochar on crop growth promotion may by dependent on crop species. Although the complexity of determinants underlying the agricultural outcomes of biochar amendment is not fully understood, further insight into the differences in biochar response both between and within species could improve biochar's use in agriculture and lead to breeding for biochar responsiveness.

The bulk of biochar research on agricultural productivity has thus far focused on biochar's effects on overall shoot growth and yield in major crops (Major et al. 2010; Zhang et al. 2012; Biederman and Harpole 2013; Dong et al. 2014; Olmo et al. 2014; Zhao et al. 2014; Liang et al. 2014; Rogovska et al. 2014; Martinsen et al. 2014). Fewer studies have examined the effects of biochar on particular growth traits that impact yield, such as germination, shoot or root architecture (Solaiman et al. 2012; Prendergast-Miller et al. 2014; Abiven et al. 2015; Olmo et al. 2016; Gascó et al. 2017). Understanding how specific growth traits are impacted by biochar addition will lead to improved uses for biochar, such as in germination potting mixes for use in greenhouse applications.

The applicability of such data is maximized by understanding the molecular mechanisms underlying the positive effects of biochar on plant growth. Microarray-based genome-wide transcriptional analysis in Arabidopsis (Viger et al. 2014) demonstrated that biochar application increased transcription of auxin- and brassinosteroid- related genes with a concurrent decrease in defense-related genes, which the authors suggested indicated a tradeoff between growth and defense in biochar-grown plants. Further elucidation of the molecular basis for how biochar functions to promote growth and influence disease will lead to improved practices for biochar use and potentially new synergistic applications with other horticultural or agronomic practices.

In this study we aimed to understand the species-specific and within-species (e.g. cultivar) – specific aspects of biochar-mediated plant growth promotion, as well as the molecular basis for biochar growth promotion. We first examined the effect of two different biochars on the growth of two cultivars of tomato (*S. lycopersicum*) and two species that are wild tomato relatives (*S. pimpinellifolium* and *S. pennellii*) in glasshouse conditions. We next examined the molecular basis underlying these responses via next-generation RNA sequencing (RNAseq) and identified hormone and defense pathways involved in biochar-mediated plant growth promotion and interaction with pathogens. Finally, we used hormone and disease assays and mutant analysis to confirm the functional role of the identified transcriptional pathways in biochar-induced growth promotion and disease interaction.

Materials and Methods

Biochar and leaf tissue analysis

Premium and Ultra biochars were obtained from Black Owl Biochar in Washington state (http://www.biocharsupreme.com/). Both were produced from sustainably managed pine under different commercial production conditions. Biochars were chemically analyzed by the Cornell Nutrient Analysis Laboratory following methods from the Soil Survey Laboratory Methods Manual created by the National Soil Survey Center (Soil Survey Staff, 2014).

Seed sterilization

For all experiments, tomato seeds were sterilized by shaking in 10% bleach for 10 minutes, and then rinsed six times in sterile double distilled water (ddH_2O). Seeds were then left

in sterile water overnight in a 4°C refrigerator to imbibe. *Arabidopsis thaliana* seeds were prepared by allowing them to stratify in sterile water in a 4°C refrigerator for five days. Seeds were then sterilized by shaking in 1 mL 50% bleach and 1 µL Tween for five minutes and then rinsing in sterile ddH_2O five times.

Tomato growth in biochar

A full-factorial glasshouse experiment was designed to test the effects of biochar addition on the growth response of two *Solanum lycopersicum* (tomato) cultivars and two wild tomato species, *S. pimpinellifolium* and *S. pennellii*. The tomato cultivars used were Hawaii7996 (H7996), known for its disease resistance (Kim et al. 2016) and M82, an inbred processing tomato cultivar (Eshed and Zamir 1995). The *Solanum pimpinellifolium* accession used was West Virginia700 (WV), known for its susceptibility to the bacterial pathogen *Ralstonia solanacearum* (Thoquet et al. 1996). The *Solanum pennellii* (SP) accession was LA0716, which was recently sequenced (Bolger et al. 2014). For simplicity, the two tomato cultivars and two wild species will be referred to as four genotypes. Two types of biochar made from the same feedstock under different production conditions were used: Premium and Ultra. A custom soilless potting mix was made that consisted of a 1:1 (volume volume^{-1}) ratio of peat to Turface MVP (Turface Athletics, Buffalo Grove, IL, USA). Biochars were amended into the potting mix at a rate of 4% biochar (weight weight^{-1}) and mixed by hand. All four genotypes were also grown in control pots not amended with biochar.

Sterile tomato seeds were planted into classic 300 size pots (about 2.5 L) (Nursery Supplies, Inc., Chambersburg, Pennsylvania, USA) and were grown in a light and temperature-controlled glasshouse (temperature setting 75-84°F) that was regularly maintained for pests. Lights operated on a 16 hour on, 8 hour off long day cycle. Pots were watered two to five minutes, one to three times per day by drip irrigation to maintain adequate water status and fertilized with a solution of Peter's Excel 15-5-15 NPK Cal-Mag Special (Hummert's International, Earth City, Missouri, USA) at 80 ppm nitrogen (N) with every watering after plants reached first true leaf stage. Pots were organized into five randomized, complete blocks for statistical analysis. Each biochar treatment (0 or 4%) and tomato genotype combination had five replicates, and the full experiment was repeated in two independent trials. The first trial was from Dec 2014 – Feb 2015 and the second from March – May 2015. In each trial, plants were harvested 8 weeks after planting and measured for shoot length and fresh weight. Days to

flowering and number of open flowers were counted for the second trial only. Days to flowering was counted as number of days from germination to first open flowers.

Results were analyzed by three-way ANOVA with a general linear mixed model (PROC GLIMMIX) in SAS 9.4. Biochar treatment, genotype, and trial were included as fixed effects with all possible interactions between the three effects, and block was included as a random effect. Post-hoc tests were performed with Tukey's honest significant differences test. No transformations were necessary to meet the homogeneity of variance and normality assumptions.

RNA extraction

Two mature leaves (from leaves 4-8) were harvested from each of the five H7996 plants from the control and the 4% Premium biochar in both trial 1 and trial 2. Only H7996 leaf tissue from the control and Premium biochar was collected because the growth-promoting effect of biochar was genotype-independent in this study. Leaf tissue samples were ground into a powder using a mortar and pestle under liquid nitrogen. Total RNA was obtained from ~50 mg of each ground, frozen tissue sample using the Norgen Biotek Corporation Total RNA Purification Kit (Norgen Biotek Corp, Ontario, Canada). RNA purity and concentration were measured by NanoDrop 2000C (ThermoScientific), and RNA integrity was verified by agarose gel electrophoresis.

mRNA sequencing and bioinformatics pipeline

For each biochar treatment (0 and 4%), the five H7996 RNA samples within a trial were separated into two samples in equal amounts by mass: one sample consisted of the total RNA from three H7996 plants, and the other sample consisted of total RNA from two H7996 plants (see Appendix Fig. A2.1 for RNAseq workflow). This resulted in 2 samples of 0% biochar and two samples of 4% biochar for each trial. RNA quality was then confirmed with the Agilent 2100 Bioanalyzer (Agilent Technologies) at the Purdue Genomics Facility. All samples had a RIN score greater than 6. At the Purdue Genomics Facility, stranded complementary DNA (cDNA) libraries were made from 3 µg of total RNA using the Illumina TruSeq Stranded mRNA HT Sample Prep Kit (Illumina, San Diego, CA) with the high-throughput protocol. cDNA libraries were then pooled in equal amounts for paired end, 100 base pair sequencing in an Illumina HiSeq2500 at the Purdue Genomics Core (Purdue University). Quality control and

filtering was performed with Tophat v2.1.0 with a quality score filter > 30. Reads were mapped to *Solanum lycopersicum* reference genome v. ITAG2.4 also using Tophat v2.1.0. Reads were then filtered to keep only the first mate in uniquely mapped paired reads. Trials were analyzed separately. First, low counts were filtered to keep only genes with at least one count per million reads (CPM) in at least two samples. Bioconductor package *edgeR* (Robinson et al. 2009) was used to normalize libraries and perform differential expression analysis. Genes were considered differentially expressed if they met a log fold change cut off of 0.585 (|fold change| > 1.5) and an adjusted p-value < 0.05 (Benjamini and Hochberg 1995). As no genes were differentially expressed between the Control and Premium biochar treatments in the second trial, the downstream analysis reflects differentially expressed genes from the first trial only. Annotations from Plant Ensembl (plants.ensembl.org) downloaded July 2016 were used to categorize genes by gene ontology categories. Sequencing data has been deposited in the Short Read Archive (https://www.ncbi.nlm.nih.gov/sra/) and can be accessed through the study number SRP078247.

Validation of RNAseq by quantitative RT-PCR

For real-time quantitative PCR (qPCR) confirmation, RNA was reisolated from the same leaf tissue samples as used for RNAseq, and RNA was pooled in the same manner to form two biological replicates each of control and biochar-treated samples from Trial 1. cDNA was reverse-transcribed from 1 µg pooled RNA using the NEB AMV first strand cDNA synthesis kit (New England BioLabs, Ipswich, MA, USA) as per manufacturer instructions. Primers for 10 selected differentially expressed genes were designed with NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primer sequences, melting temperatures, and expected product sizes are listed in Appendix Table A2.3. qPCR was performed in a BioRad CFX Connect Real Time PCR Detection System. The following PCR program was used: 95°C for 2 min, 40 cycles of 95°C for 10 s, 60°C for 30 s followed by a 5 min extension at 72°C. A melting curve (65-95°C in 0.5°C increments over 5 min with a continuous fluorescence measurement) was performed after each run to check for alternate PCR products. Each qPCR reaction was performed in a total volume of 20 µL. qPCR for each primer set with a ten-fold dilution series of cDNA from 10^0 to 10^{-4} was performed to evaluate primer efficiency. R^2 values fell between 97-99% for all primer sets. Three technical replicates were performed for each

biological replicate. *GAPDH* (*Solyc03g111010.2*) was used as the gene for normalization, and the ΔΔCt method (Livak and Schmittgen 2001) was used to calculate fold changes.

Fusarium oxysporum f. sp. *lycopersici inoculation*

Resistant tomato cultivar M82 and susceptible *S. pimpinellifolium* WV were grown in 36 pot flats in the glasshouse in either peat:turface or peat:turface amended with 4% Premium biochar until plants were between 3rd and 4th leaf stage (about 18 days). Inoculum was prepared by plating an infected corn kernel onto a potato dextrose agar (PDA) plate with 20-30 sterile corn kernels and incubating it at 28°C for seven days to allow infection of all kernels. After seven days, individual infected kernels were plated onto new PDA plates and incubated for an additional seven days. Then, approximately 5 mL of sterile ddH_2O was added to each plate. Mycelial mats and liquid were scraped from the surface of each plate using a sterile glass slide. Mycelium and liquid were then filtered through miracloth to collect conidia. Conidia were quantified using a hemacytometer and inoculum was adjusted to a concentration of 1 x 10^6 conidia ml^{-1}. Eighteen-day old plants were then inoculated by removing plants from potting mix, rinsing them off in water, and then dipping the roots for five seconds in either sterile ddH_2O (mock) or inoculum. After dipping, plants were repotted into the flat using the same potting mix. Plants were then placed back in the glasshouse and evaluated for disease for 21 days. Disease was scored as follows: $Percent\ disease\ coverage = \frac{1\times(number\ of\ wilted\ leaves)+0.5\times(number\ of chlorotic\ or\ partially\ wilted\ leaves)}{total\ number\ of\ leaves} \times 100$. Plants were watered as needed and fertilized once weekly with a solution of 80 ppm N 15-5-15 fertilizer. Experiment was repeated three times with three to six inoculated M82 and six WV plants per trial. Six mock-inoculated plants of each genotype were also included in each trial, and no disease symptoms were observed on any mock-inoculated plant.

Germination in potting mix

In order to determine the effect of Premium biochar amendment on germination, individual sterile seeds of each of the four genotypes (H7996, M82, WV, and SP) were planted into pots in 36 pot flats containing either 1:1 peat/turface potting mix or 1:1 peat/turface amended with 4% Premium biochar by weight. Fifty-four seeds per treatment and genotype were planted. Days to germination was defined as the number of days to cotyledon expansion. Germination was measured once per day for 10 days. The germination experiment was fully

replicated in four trials. Percent germination was calculated as follows: $Percent\ germination = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ planted\ seeds} \times 100$. Area under the germination progress curve (AUGPC) was calculated by the trapezoidal integration method (Campbell and Madden, 1990). Statistical analysis was performed in JMP12 to compare AUGPC values between Premium biochar-amended and un-amended pots within each species with a linear mixed model with biochar treatment as a fixed effect and trial as a random effect. The effect of biochar treatment was considered significantly different at $p < 0.05$.

Exogenous GA spray

To determine the effect of bioactive gibberellin (GA_4) treatment on biochar-treated vs. untreated tomato plants, approximately 50 plants of each of the four genotypes were grown in +/- biochar potting mix in 36 pot flats. When plants were two weeks old, they were divided into two sets (between 12-27 individuals, depending on germination rates). Once per day for five days, one set was sprayed with 7.5 mg ml^{-1} GA_4, while the other set was sprayed with water. Shoot length measurements were taken at the beginning (Day 1) and end of the experiment (Day 8), and shoot weights were also taken at the end (eight days after initial spray). The entire experiment was repeated four times. Shoot weight and shoot length after eight days were used for statistical analysis. Statistical analysis was performed using PROC GLIMMIX in SAS 9.4. Fixed effects included in the model were biochar treatment and GA_4 treatment with their interaction, and trial was included as a random factor. Data were examined for homogeneity of variance and normality. Shoot weight and length values were square root transformed to meet the homogeneity of variance assumption. Differences in the biochar*GA_4 interaction model effect were considered significant at $p < 0.05$.

Arabidopsis *ga3ox1-3* mutant analysis

The *ga3ox1-3* mutant was used for analysis (Mitchum et al. 2006). This mutant is defective in Gibberellin 3-Oxidase 1 (*At1g15550*), which catalyzes the production of bioactive gibberellin GA_4 during the vegetative growth stage (Mitchum et al. 2006). *ga3ox1-3* was obtained from the Arabidopsis Biological Resource Center (CS6943) and the homozygous mutant was confirmed using TDNA insertion PCR using primers from Mitchum et al. (2006).

Water extracts of Premium biochar were made by stirring 50 g of biochar in 1 L of ddH_2O overnight at room temperature. After stirring, extract was filtered first using vacuum filtration with Whatman 42 filter paper to remove large particles, and then filter-sterilized with a 0.22 µM filter. Treated 1% agar plates were prepared by applying 2 mL of sterile ddH_2O (control) or sterile biochar filtrate to the plate surface and allowing the liquid to sink into the plate. For Arabidopsis mutant growth assays on plates, approximately 30 sterile seeds each of WT (Col-0) and *ga3ox1-3* were plated onto treated 1% agar plates in a single row and placed upright in a growth chamber set to 24° C, 16-hour day and 8-hour night cycle and average of 80 µmol m^{-2} s^{-1} light. After eight days, plates were scanned and measured in ImageJ for hypocotyl length. The entire experiment was replicated twice. Statistical analysis was performed using PROC GLIMMIX in SAS 9.4. Fixed effects included in the model were biochar treatment, genotype, and trial with their interactions, and plate was included as a random factor. Hypocotyl length values were square root transformed to meet homogeneity of variance and normality assumptions.

Solanum pennellii seedling growth on plates

Control and Premium biochar extract treatments were prepared as for the Arabidopsis *ga3ox1-3* assays. For seedling growth experiments, 10 sterile *S. pennellii* seeds were plated onto treated plates in a single row and placed upright in a growth chamber set to 24 °C, 16-hour day and 8-hour night cycle and an average of 80 µmol m^{-2} s^{-1} light. After six days, plates were scanned and measured in ImageJ for root length and hypocotyl length. After scanning, seedlings from each plate were pooled and weighed together. Statistical analysis was performed in JMP12. Statistical differences were tested by ANOVA with treatment as a fixed effect and plate as a random effect, followed by a post-hoc Tukey test with a significance cut-off of $p < 0.05$. No transformations were necessary to meet the homogeneity of variance and normality assumptions. The experiment was repeated four times with similar results.

KAR1 extraction and GC-MS analysis

Premium biochar extraction was performed by first grinding 25 g biochar in a mortar and pestle. After grinding, biochar was stirred at room temperature with 100 ml ethyl acetate for two hours. Then biochar extract was filtered by vacuum filtration with Whatman 42 filter paper.

Filtered extracts were then submitted along with 1 mM KAR1 standard to the Purdue Campus-Wide Mass Spectrometry Center. 1 µl samples were analyzed on an Agilent 5975C gas chromatograph-mass spectrometer (GC-MS). Sample separation was done on a DB-5 column 30 m in length, with a 0.25 mm inner diameter and 0.25 µm film thickness. The injection temperature was set to 250 °C. The temperature separation program for the GC was as follows: 100 °C for 0.1 min, ramped at 10 °C to 320 °C. Mass spectra were scanned in the range of 42-300 amu. Chromatograms were visualized using OpenChrom 1.1.0 (Wenig and Odermatt 2010).

Results

Biochar promotes shoot growth in a trait-dependent, but genotype-independent manner

In order to test the effects of biochar addition on different tomato genotypes, we performed a full factorial glasshouse experiment on two *S. lycopersicum* cultivars, *S. pimpinellifolium*, and *S. pennellii* with two different types of biochar at 4% weight weight^{-1} and a control 0% application rate. The two biochars significantly differed in their pH, organic matter content, and mineral composition (Table 2.1). We measured shoot growth traits after eight weeks of growth. For simplicity, the two *S. lycopersicum* cultivars and two wild species will be referred to as four genotypes. Biochar was a significant effect in the model for both shoot length and fresh weight (Table 2.2). Genotype was also significant for both shoot growth parameters, reflecting the different growth patterns between genotypes (Table 2.2). The biochar*genotype interaction was not significant for either model, indicating that the effects of biochar on growth are genotype-independent.

Table 2.1 Biochar Characteristics

	Units	Premium	Ultra
Moisture	%	6.41[1] (± 0.275)a[2]	6.02 (± 0.158)a
pH		10.22 (± 0.012)a	10.09 (± 0.020)b
LOI[3]	%	91.97 (± 0.410)b	96.06 (± 0.217)a
Org. matter	%	64.15 (± 0.287)b	67.01 (± 0.152)a
Nitrogen	**%**	0.19 (± 0.027)a	0.33 (± 0.067)a
Carbon	**%**	75.78 (± 1.204)b	80.79 (± 1.005)a
Aluminum	mg kg^{-1}	24.99 (± 0.330)a	15.92 (± 0.111)b
Arsenic	mg kg^{-1}	1.32 (± 0.123)b	8.26 (± 1.583)a
Boron	mg kg^{-1}	9.71 (± 0.114)b	11.57 (± 0.342)a
Barium	mg kg^{-1}	38.53 (± 0.600)a	38.27 (± 0.299)a
Calcium	mg kg^{-1}	8,151.66 (± 146.05)a	5,217.97 (± 56.99)b
Cadmium	mg kg^{-1}	0.14 (± 0.004)a	0.03 (± 0.004)b
Cobalt	mg kg^{-1}	0.05 (± 0.003)a	0.07 (± 0.010)a
Chromium	mg kg^{-1}	0.12 (± 0.005)a	0.13 (± 0.008)a
Copper	mg kg^{-1}	0.00 (± 0.000)b	1.06 (± 0.228)a
Iron	mg kg^{-1}	1.36 (± 0.020)a	0.70 (± 0.044)b
Potassium	mg kg^{-1}	3,499.82 (± 25.75)b	3,696.03 (± 12.58)a
Magnesium	mg kg^{-1}	695.78 (± 12.68)a	704.82 (± 18.29)a
Manganese	mg kg^{-1}	350.32 (± 5.791)a	220.29 (± 2.114)b
Molybdenum	mg kg^{-1}	0.02 (± 0.001)a	0.02 (± 0.005)a
Sodium	mg kg^{-1}	1,028.96 (± 8.359)b	1,112.95 (± 8.587)a
Nickel	mg kg^{-1}	0.26 (± 0.009)a	0.18 (± 0.016)b
Phosphorus	mg kg^{-1}	544.85 (± 9.443)a	356.24 (± 5.590)b
Lead	mg kg^{-1}	1.36 (± 0.091)a	0.20 (± 0.031)b
Sulfur	mg kg^{-1}	631.44 (± 11.69)a	82.83 (± 1.555)b
Selenium	mg kg^{-1}	0.25 (± 0.043)a	0.24 (± 0.051)a
Silicon	mg kg^{-1}	110.64 (± 3.215)a	47.51 (± 1.335)b
Strontium	mg kg^{-1}	28.27 (± 0.397)a	24.66 (± 0.407)b
Titanium	mg kg^{-1}	0.19 (± 0.003)a	0.08 (± 0.007)b
Vanadium	mg kg^{-1}	0.00 (± 0.005)a	0.00 (± 0.001)a
Zinc	mg kg^{-1}	6.57 (± 0.076)a	1.63 (± 0.133)b

[1]Values indicate averages of three technical replicates ± (standard error)
[2]Differing letters indicate differences between biochars by t-test at $p < 0.05$
[3]LOI = loss on ignition

Table 2.2 General linear mixed model results of effects of biochar treatment, genotype, trial and their interactions on shoot weight and length

	Effect	Num DF[1]	Den DF	F Value	Pr > F
Shoot Length (cm)	Genotype	3	88	177.78	**<0.0001**
	Biochar	2	88	6.83	**0.0017**
	Trial	1	88	0.15	0.7010
	Genotype*Biochar	6	88	0.64	0.6975
	Genotype*Trial	3	88	2.16	0.0984
	Biochar*Trial	2	88	0.73	0.4838
	Genotype*Biochar*Trial	6	88	0.47	0.8325
Shoot Fresh Weight (g)	Genotype	3	88	14.63	**<0.0001**
	Biochar	2	88	39.79	**<0.0001**
	Trial	1	88	1.21	0.2739
	Genotype*Biochar	6	88	0.43	0.8551
	Genotype*Trial	3	88	2.54	0.0617
	Biochar*Trial	2	88	0.85	0.4324
	Genotype*Biochar*Trial	6	88	0.83	0.5488

[1]Degrees of Freedom

Overall, both types of biochar (Premium and Ultra) significantly promoted shoot weight and shoot length over the control plants (Table 2.2; Fig. 2.1). Premium biochar increased shoot weight by an average of 33.1% (±6.9%) and shoot length by 8.0% (±2.6%). Ultra biochar increased shoot weight by 50.9% (±11.6%) and shoot length by 9.2% (±3.1%). Ultra biochar promoted shoot weight, but not shoot length, significantly more than Premium biochar (Fig. 2.1).

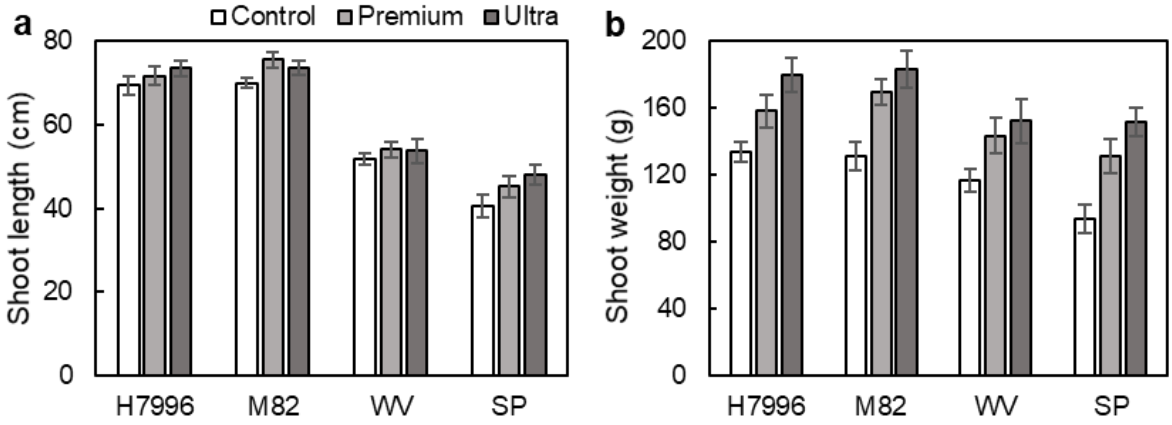


Fig. 2.1 Biochar promotes shoot length and shoot weight in a genotype-independent manner. Graphs of a) shoot length and b) shoot fresh weight, showing the effects of biochar addition and genotype. 'Control' indicates no biochar addition, while 'Premium' and 'Ultra' represent 4% (w w^{-1}) soil amendment with indicated biochar. Because a significant interaction between genotype and biochar addition was not observed (Table 2.2), here we compared the effect of different biochar types with the control. For this, data from different genotypes were pooled within each biochar and within the control. A post-hoc Tukey's honestly significant differences test comparing biochar types and the control indicated significant differences between control and biochar amendments for both shoot length and weight. Ultra had a significantly greater effect than Premium on shoot weight only.

Biochar amendment suppresses defense pathways in tomato

To understand the molecular basis of biochar-mediated plant growth promotion, we performed global gene expression profiling using RNA sequencing (RNAseq). Because both Premium and Ultra biochars promoted shoot growth similarly across all four genotypes, only leaf tissue samples of Hawaii7996 grown in control potting mix (no biochar) and in 4% Premium biochar were used for RNAseq. As described in the materials and methods, two RNA samples from each of 0% and 4% biochar were sequenced from each trial (diagram in Appendix Fig. A2.1). About 50 million high quality mapped reads were obtained from each sample (Appendix Table A2.1). Out of the total 34,725 annotated tomato genes, 24,999 transcripts were detected, though only 18,073 genes were retained for differential expression analysis after filtering for low expression. Overall, 236 differentially expressed genes were detected, 130 down-regulated and 106 up-regulated between treatments from the first trial (Appendix Table A2.2). Surprisingly, no genes were found to be differentially expressed between treatments in the second trial using a false discovery rate (FDR) < 0.05 and a |fold change| > 1.5. Therefore, all downstream analysis focused on differentially regulated genes from the first trial. Ten genes were chosen for

quantitative RT-PCR to confirm RNAseq analysis (Appendix Fig. A2.2, primer sequences are listed in Appendix Table A2.3). All 10 genes were expressed in the same direction as expected from the RNAseq analysis.

To give a general overview of categories of differentially expressed genes, the top 40 biological process gene ontology (GO) categories were plotted (Appendix Fig. A2.3, see Appendix Tables A2.4 and A2.5 for all categories represented). The categories with the highest total number of genes (both up- and down-regulated) include transport, metabolic process, oxidation-reduction process, defense response, biosynthetic process, and DNA-templated transcription. Several categories representing primary metabolism such as lipid metabolic process, carbohydrate metabolic process, lipid catabolic process, and proteolysis were identified (Appendix Fig. A2.3).

Many plant defense processes were found in the top 40 GO categories, including several related to hormone-mediated defense signaling, particularly salicylic acid (SA), jasmonic acid (JA), and ethylene (Appendix Fig. A2.3). Unexpectedly, the majority of these defense-related genes were down-regulated (Fig. 2.2). These included many pathogenesis-related (PR) proteins – *PR-1b*, *PR-1a*, chitinases, subtilisin-like protease, osmotin-like proteins, thaumatin-like proteins, and beta-glucanases – as well as defense signaling genes enhanced disease susceptibility 1 (*EDS1*), phytoalexin deficient 4 (*PAD4*), and mildew locus O-like 3 (*MLO-like 3*), all known to play integral roles in plant defense responses. Moreover, many signaling genes with putative functions in defense signaling were down-regulated due to biochar treatment, including eight leucine rich repeat-receptor like kinases (LRR-RLKs), nine additional serine-threonine kinases and two serine-threonine kinase receptors (Fig. 2.2, see Appendix Table A2.6 for genes and log fold changes of all genes in each category of Fig. 2.2). Several WRKY transcription factors with putative roles in defense were down-regulated (Fig. 2.2; Appendix Table A2.6). Lipoxygenase 1 (*LOX1*), a key jasmonic acid (JA) biosynthesis gene, was down-regulated in biochar-treated plants (Fig. 2).

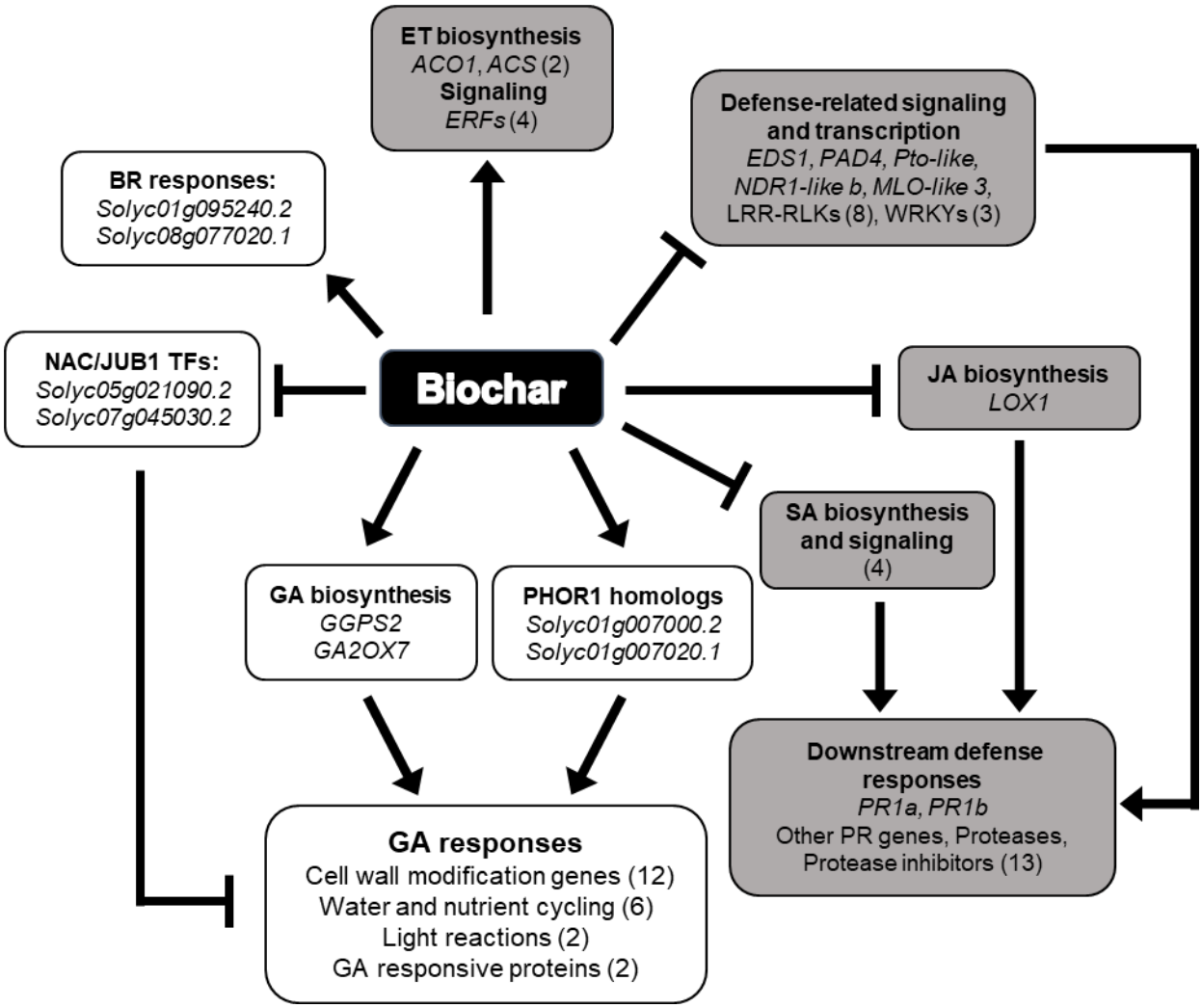


Fig. 2.2 Biochar amendment differentially regulates genes in the gibberellin (GA) pathway and downregulates defense-related genes. White boxes represent categories related to GA/growth signaling. Gray boxes represent categories involved in defense. Arrows indicate a positive relationship, and the T-bar indicates an inhibitory (negative) relationship. Abbreviations: BR – brassinosteroid. NAC - NAM, ATAF1/2, CUC2. JUB1 - JUNGBRUNNEN1. PHOR1 - Photoperiod Regulated 1. JA – jasmonic acid. ET – ethylene. SA – salicylic acid. PR – pathogenesis-related. ERF – ethylene response factor. LRR-RLK – leucine rich repeat-receptor like kinase.

On the other hand, ethylene biosynthesis genes 1-aminocyclopropane-1-carboxylate oxygenase 1 (*ACO1*) and two 1-aminocyclopropane-1-carboxylic acid synthase (*ACS*) genes were up-regulated (Fig. 2.2), though *ACO4* was down-regulated. Consequently, several downstream ethylene response factors (ERFs) were also up-regulated: *ERF1a*, *ERF2b*, *ERF3a*, and *ERF4* (Fig. 2.2). Interestingly, several proteases and protease inhibitors were also found to be up-regulated in biochar-treated plants (Appendix Table A2.6).

Because biochar down-regulated many genes related to defense, we tested the role of biochar in disease development. We chose to examine Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* both because of its economic importance to tomato growers and because biochar amendment downregulated genes in the SA pathway. This pathway is important for defense against *F. oxysporum* (Edgar et al. 2006). However, addition of 4% Premium biochar had no effect on disease progress in either resistant M82 or susceptible WV (Fig. 2.3).

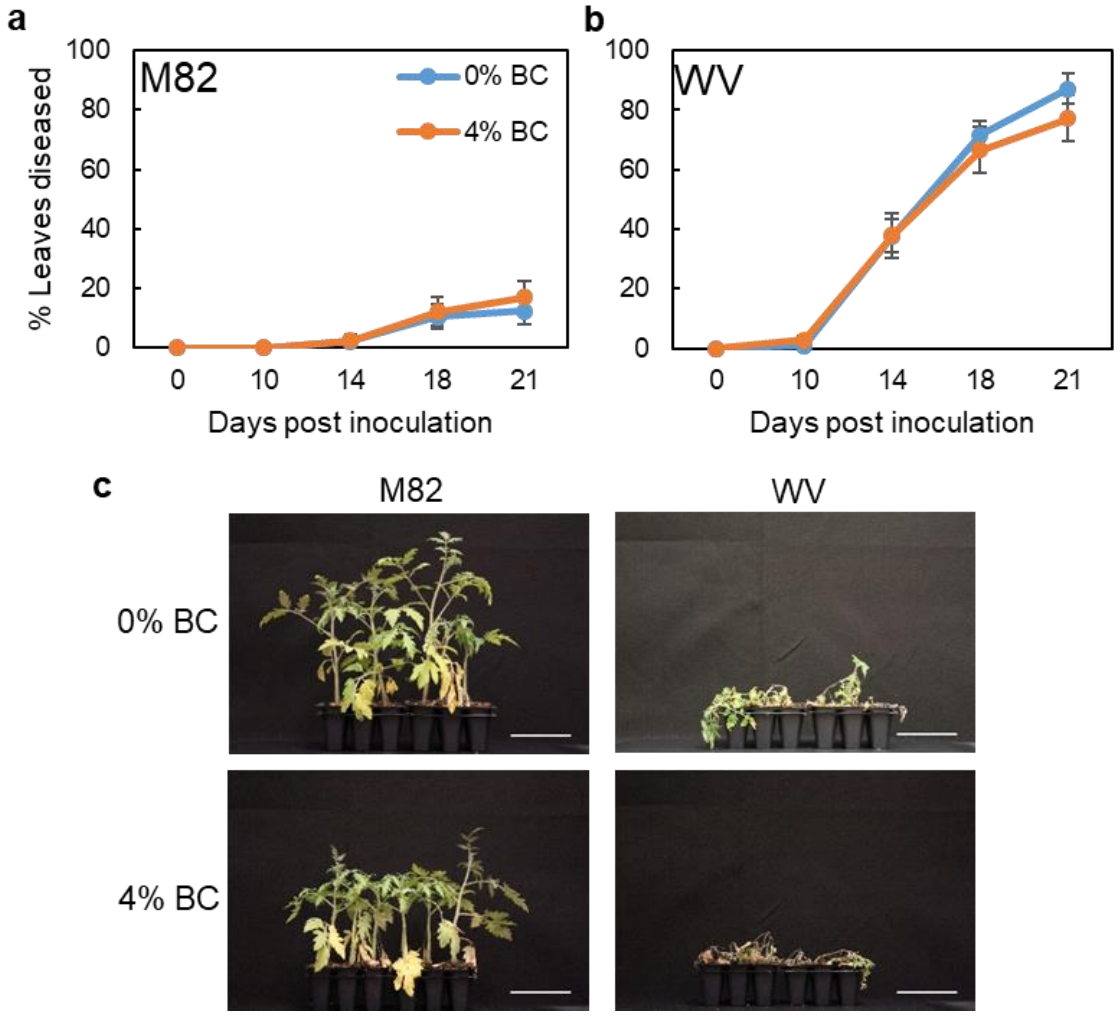


Fig. 2.3 Premium biochar (BC) at 4% addition has no effect on Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. Effect of 4% Premium BC on disease progress in (a) M82, a *Solanum lycopersicum* cultivar resistant to Fusarium wilt, and (b) WV, a *Solanum pimpinellifolium* susceptible to Fusarium wilt. Disease progess measured as percent of leaves showing wilt symptoms. Error bars represent one standard error. c) Representative images of inoculated plants from Trial 3, 21 days after inoculation with *F. oxysporum* f. sp. *lycopersici.* Scale bar represents 10 cm.

Biochar amendment upregulates genes in the gibberellin (GA) pathway and interconnected pathways in growth and development

In biochar amended plants, geranylgeranyl pyrophosphate synthase 2 (*GGPS2*) (*Solyc04g079960.1*), an early enzymatic step of gibberellin (GA) biosynthesis is upregulated (Fig. 2.2). One gene encoding a GA deactivation enzyme – GA 2-beta-dioxygenase 7 (*GA2OX7*) (*Solyc02g080120.1*) was also upregulated, though this inactivation gene can be upregulated by high levels of endogenous GA (Hedden and Phillips 2000) (Fig. 2.2). Two U-box ubiquitin ligases homologous to Arabidopsis *PHOR1* ubiquitin ligases, were upregulated in biochar treated plants (Fig. 2.2). *Solanum tuberosum* (potato) *PHOR1* is an E3 ubiquitin ligase known as a positive regulator of GA signaling (Amador et al. 2001; Monte et al. 2003).

Two NAM, ATAF1/2, CUC2 (NAC) transcription factors (*Solyc05g021090.2*, *Solyc07g045030.2*) were downregulated in biochar-treated plants (Fig. 2.2). These two NACs are closely related to a tomato NAC that is downregulated in response to exogenous GA treatment (Kou et al. 2014). Both NACs are homologous to Arabidopsis *JUB1*, which plays a role in suppressing GA biosynthesis and activating DELLA genes (Shahnejat-Bushehri et al. 2016), which are transcriptional repressors of the GA biosynthesis pathway (Xu et al. 2014).

Consequently, a number of genes involved in downstream GA responses were upregulated in biochar treated plants (Fig. 2.2). Most strikingly, 12 cell wall growth genes and modifiers were up-regulated. These included a cellulose synthase-like gene, two expansins, pectate lyase, Polygalactonurase 2 (*PG2*), two pectinesterases, and a COBRA-like gene (Fig. 2.2). In addition, two genes annotated as “Gibberellin regulated proteins” were also up-regulated in biochar-treated leaves. Upregulation of these genes fits well with the biochar-mediated growth promotion phenotype as increased cell expansion may lead to larger plant size, and increased cell expansion is a hallmark of GA-induced growth (Xu et al. 2014).

Consistent with our findings that biochar promotes growth and activates the GA signaling pathway, several light, water- and nutrient-cycling related genes were found to be up-regulated: carbonic anhydrase, an aquaporin, a chlorophyll a-b binding protein, ascorbate peroxidase, and a cyclic nucleotide gated ion channel, annotated for potassium ion transport (Fig. 2.2). Two genes involved in sulfur assimilation were upregulated - phosphoadenosine phosphosulfate reductase (*Solyc03g031620.2*) and a sulfotransferase (*Solyc05g013010.2*) (Fig. 2.2). This result is consistent with the high levels of sulfur found in Premium biochar (Table 2.1). A few nutrient

cycling genes were down-regulated, including an inorganic phosphate transporter and an ammonium transporter (Appendix Table A2.6).

Biochar treatment also affected regulation of other hormone pathways that directly interact with GA. While no components of the brassinosteroid (BR) signaling pathway were differentially regulated in biochar-treated plants, 2 downstream BR-responsive genes were upregulated, suggesting a possible role for BR in biochar-plant promotion (Fig. 2.2).

Biochar amendment increases response to exogenous GA in one tomato genotype

Analysis of global gene expression differences between biochar-treated and untreated plants suggested that biochar promotes growth at least in part through the GA pathway. Therefore, we chose to focus on the interaction of biochar with the GA pathway. We examined the effects of exogenous GA_4 treatment on shoot growth in all four tomato genotypes grown with or without Premium biochar (Fig. 2.4). Our results show a positive, interactive effect of biochar and GA_4 treatment on both shoot fresh weight and shoot length in one genotype, M82, suggesting that biochar stimulates the GA pathway (Fig. 2.4c,g). This effect appears to be within-species specific as the interaction effect was observed in M82 and not H7996, and both *S. lycopersicum* cultivars (Fig. 2.4a,c,e,g). Full model results are in Appendix Table A2.7.

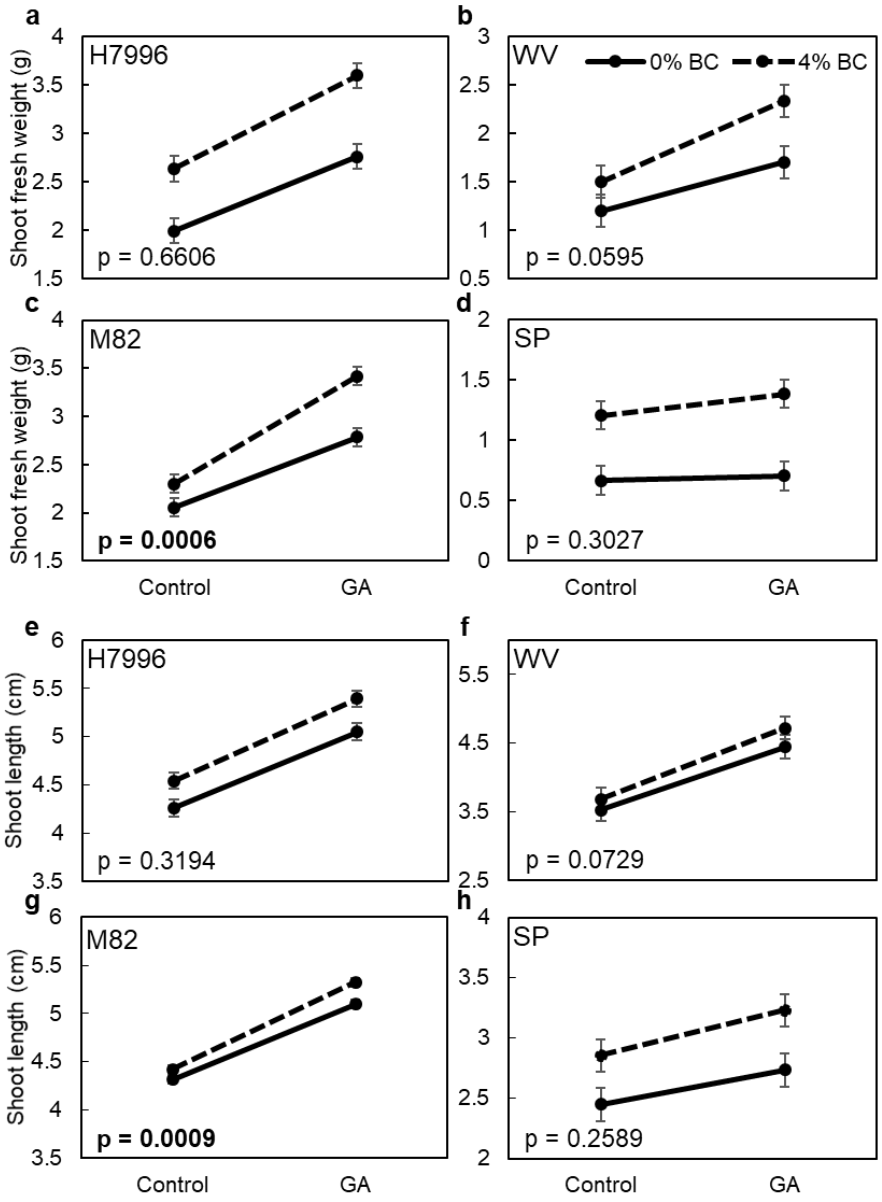
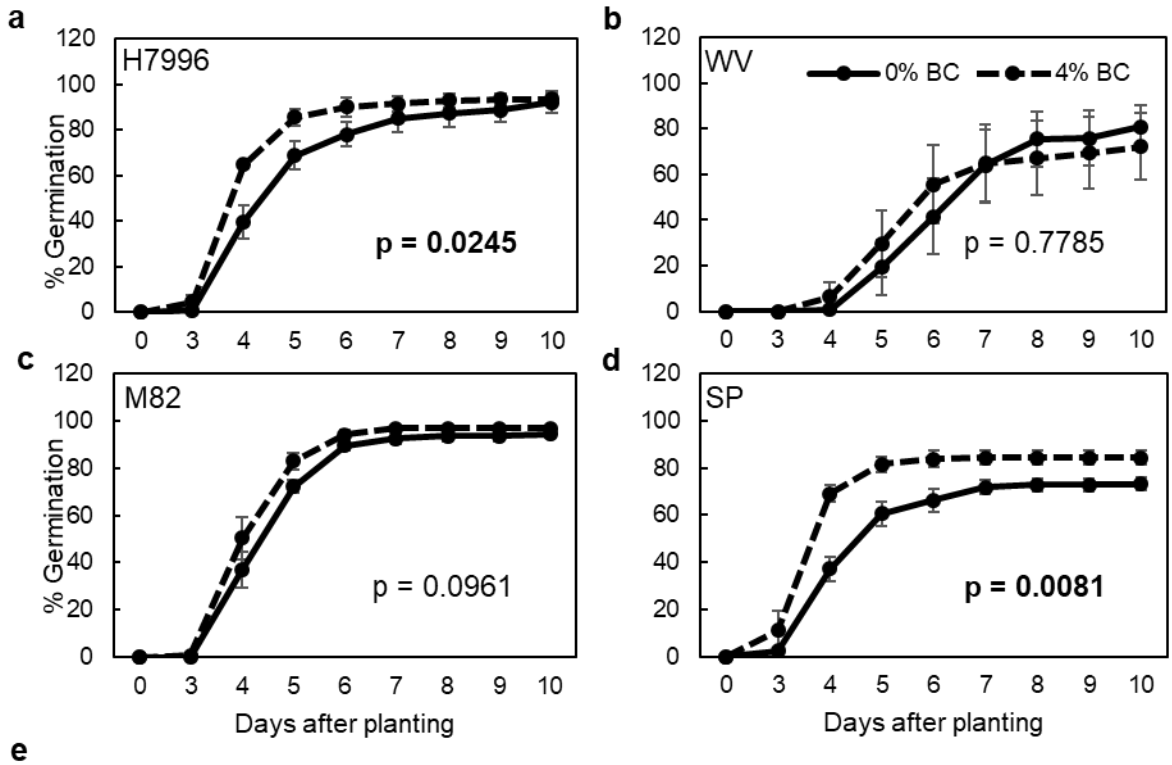


Fig. 2.4 Exogenous gibberellin (GA_4) application and Premium biochar (BC) amendment interact synergistically to increase the shoot biomass and length of M82 tomato plants. Least Square (LS) Mean interaction plots for each species show relationship between GA_4 application and BC amendment for a-d) shoot fresh weight and e-f) shoot length. The experiment was repeated in four trials, and trial was included as a random factor in the model. Shoot weight and length values were square root transformed to meet homogeneity of variance and normality assumptions. Values represented in the figure are not transformed. P values represent significance of BC x GA_4 interaction in the full model analysis. The interaction effect was considered statistically different at $p < 0.05$. Error bars represent one standard error. Full model results are in Appendix Table A2.7.

Biochar amendment affects GA-related traits, including germination and time to flowering in a genotype-specific manner

In order to further investigate biochar's role in other traits that require GA signaling, we decided to examine the effect of biochar on germination. Seeds from all four genotypes were planted with or without 4% Premium biochar and were measured for germination over a 10-day period. Area under the germination progress curves (AUGPC) for each of the four genotypes revealed that Premium biochar increased germination rate in H7996 and SP (Fig. 2.5a,d). Overall percent germination increased for SP by nearly 20% (Fig. 2.5d). AUGPC differences for germination in M82 between biochar-grown and control seeds were significant at $p < 0.1$, suggesting a possibly similar, but weaker trend to H7996. These results suggest a species-specific effect of biochar on germination, as only 2 (*S. lycopersicum* and *S. pennellii*) out of 3 species showed a germination phenotype with biochar addition. Consistent with our observations that biochar affects GA-related traits, we observed both a decrease in days to flowering and an increase in the number of flowers at eight weeks in the second trial of the glasshouse experiment (Appendix Fig. A2.4). A summary of the effect of biochar in different *Solanum* species and within a given *Solanum* species is in Fig. 2.5e.



e

	Shoot Biomass	Shoot Length	Exogenous GA Application	Germination	Days to flowering	# of open flowers
H7996						
M82						
WV						
SP					N/A	N/A

Fig. 2.5 Premium biochar reduces time to germination and increases germination percentage in two tomato genotypes. a-d) Percent germination over time in genotypes H7996, WV, M82, and SP, respectively, in 0 and 4% Premium biochar amended potting mix. Results in a-d are the averages of 54 seeds/treatment with four biological replicates. Error bars represent one standard error. P value represents mixed model ANOVA comparing Area Under the Germination Curve (AUGPC) values between biochar-treated and un-treated pots for each genotype. P value was considered significant at $p < 0.05$ (bold). e) Table summarizing the effects of biochar on growth traits, separated by plant genotype. Gray boxes indicate that biochar had a positive effect on that trait for that genotype. White boxes indicate no effect. Flowering traits for SP are marked N/A because control SP plants had not flowered by the end of the experiment, so no statistical analysis could be performed.

Biochar water extracts affect *S. pennellii* seedling growth traits

After observing the effect of biochar on growth under controlled growth conditions and its effects on gene expression, we hypothesized that this phenotype was due to a direct effect of chemicals present in the biochar. In order to test this hypothesis, we made water extracts of both biochars and measured growth of *S. pennellii* (SP) on agar plates (Appendix Fig. A2.5). We chose SP because it is the most amenable to growth on agar plates and responded highly to biochar amendment. Seedlings treated with Ultra and Premium biochar extracts exhibited higher seedling weight and longer hypocotyls compared to the control (Appendix Fig. A2.5a-b). Root length was only affected by Premium water extracts compared to the control (Appendix Fig. A2.5c). These results suggest that biochar's growth promoting effects in the glasshouse trials come at least in part from water-soluble compounds present in the biochar.

We hypothesized that Premium biochar may contain karrikins, germination promoting compounds found in smoke and other biochars (Kochanek et al. 2016) that require the GA pathway to promote germination (Nelson et al. 2009). GC-MS analysis was performed on ethyl acetate extracts of ground Premium biochar to detect the presence of karrikins. However, no karrikins were detected in Premium biochar (Appendix Fig. A2.6).

Biochar induced growth promotion requires an intact GA biosynthesis pathway in *Arabidopsis thaliana*

In order to further evaluate the role of GA in biochar induced growth promotion, mutant analysis was performed. Because few tomato GA mutants without severe growth defects exist, the Arabidopsis GA biosynthesis mutant *ga3ox1-3* was used. *ga3ox1-3* is defective in Gibberellin 3-oxidase 1 *(At1g15550),* which is involved in the production of bioactive GA_4 during the vegetative growth stage (Mitchum et al. 2006). Growth assays on plates with biochar extracts showed that the GA pathway is required for a hypocotyl growth response to water extracts of biochar (Fig. 2.6, Appendix Table A2.8). Premium biochar extracts promoted hypocotyl growth in Col-0, the wild-type background for *ga3ox1-3* (Fig. 2.6). However, no significant difference was found in hypocotyl growth between control and Premium biochar extract-treated *ga3ox1-3* seedlings (Fig. 2.6). This result suggests that GA is at least partially responsible for biochar growth promotion in Arabidopsis.

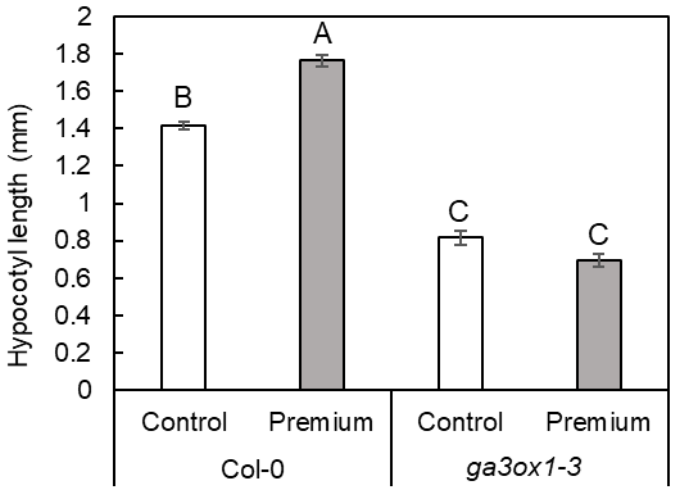


Fig. 2.6 Premium biochar water extracts promote hypocotyl growth in WT Col-0, but not in *ga3ox1-3* mutant. Hypocotyl length of Col-0 or *ga3ox1-3* seedlings plated with Premium biochar water extract vs. sterile water treatment. Results represent the averages of three plates/treatment of 7-29 seeds/plate with two biological replicates performed. Square root transformed values were used for statistical analysis to meet homogeneity of variance assumption. Values represented in the figure are not transformed. Significant differences between all genotype and treatment combinations determined by Tukey's honest significant differences at $p < 0.05$ indicated by differing letters. Error bars represent one standard error. Full model results can be found in Appendix Table A2.8.

Discussion

In this study we showed for the first time a within-species specific effect of biochar for some growth traits, including germination. We also showed that biochar-mediated growth promotion works in part through the GA pathway. We demonstrated that biochar and exogenous GA application acted synergistically to affect tomato shoot growth, but only in M82. Though we observed similar increases in shoot growth for all four genotypes tested, we only observed a positive effect of Premium biochar on germination in SP, a wild relative of tomato, as well as on H7996, a *S. lycopersicum* cultivar, though not in WV, another wild tomato relative or M82, a *S. lycopersicum* processing cultivar.

Our study highlights the complexity of biochar-plant interactions and helps explain some of the apparent contradictions in the biochar literature. Differences in growth or disease outcomes are commonly reported for different types and rates of biochar (Zwart and Kim 2012;

Solaiman et al. 2012; Meller Harel et al. 2012; Dong et al. 2014; Jaiswal et al. 2014, 2015; Backer et al. 2016; Egamberdieva et al. 2016; Olmo et al. 2016; Shoaf et al. 2016; Gascó et al. 2017). Additionally, previous studies have observed the full range of germination responses to biochar, from an inhibitory to a stimulatory effect (Gascó et al. 2017; Free et al. 2017; Gravel et al. 2013; Bargmann et al. 2013; Jones et al. 2012; Solaiman et al. 2012). One reason for these discrepancies could be due to the differential effects of biochar on different species and cultivars within a species. We showed that while biochar affected overall shoot growth across all genotypes, germination was only positively affected in two of the studied genotypes.

The trait-dependent effects of biochar have not been well characterized (Solaiman et al. 2012; Backer et al. 2016; De Tender et al. 2016; Gascó et al. 2017). Here we show that, depending on the genotype, biochar impacts some growth traits but not others (germination or response to GA application, for example). Solaiman et al., (2012) tested the effects of different levels of five different biochars on three plant species and observed differing effects dependent on trait observed, plant species, biochar amendment level, and biochar type. For example, in wheat, biochar amendment increased germination and seedling growth at low amendment levels, but had negative effects at higher concentrations (Solaiman et al. 2012). For mung bean and clover, biochar amendment had a negative effect on germination regardless of amendment level. Providing further evidence that biochar affects plant growth in a trait-specific manner, biochar did have positive effects on mung bean and clover growth at low concentrations, despite its negative effect on germination in these species (Solaiman et al. 2012). While it is still unclear why the effects of biochar depend on the trait, species and cultivar examined, our discovery of within-species (e.g. cultivar) variation opens the door to the potential for breeding for a positive biochar response.

Evaluation of global transcriptional differences between H7996 leaves from plants grown in 4% Premium biochar and control plants revealed differential regulation of genes in the GA and interconnected hormone pathways, suggesting multiple mechanisms for biochar-mediated growth with a role for GA. Differential regulation of genes involved in carbon flux, lipid metabolism, and protein turnover suggests that biochar amendment affects primary metabolism. A microarray study comparing global gene expression between biochar-grown and control Arabidopsis plants observed upregulation of genes involved in cell wall expansion and modification, water and nutrient transport, similarly to our study (Viger et al. 2014). Soybean

plants grown in 5% biochar exhibited upregulated transcription of β-1,4-glucanase, also involved in cell wall expansion (Copley et al. 2017). These results together suggest that biochar induces shoot growth through increases in cell wall growth and modifications of water and nutrient transport within the plant. Viger et al., (2014) observed upregulation of auxin and brassinosteroid (BR) signaling and biosynthesis genes, two major hormones involved in growth and development. We observed differential regulation for some genes in the BR pathway, but not in the auxin pathway. In our study, genes involved in the gibberellin (GA) pathway were differentially regulated in tomato plants grown in biochar. GA and BR act together to promote plant growth (Huot et al. 2014), which suggests that GA and BR may both be involved in biochar-mediated plant growth promotion.

To test the hypothesis that GA is involved in biochar-mediated plant growth promotion, we examined biochar response traits related to GA and performed mutant analysis. Exogenous GA_4 application revealed a positive, interactive effect of Premium biochar and GA application on shoot growth in M82, indicating that biochar stimulates the GA pathway. Similarly, Premium biochar promoted germination in H7996 and SP with the largest effect occurring in SP with approximately a 20% increase in percent germination over the control. Our experiments with the *ga3ox1-3* Arabidopsis mutant for GA biosynthesis confirmed the involvement of the GA pathway in biochar-mediated plant growth promotion. Our study supports evidence for a model by which biochar promotes tomato growth partly through stimulation of germination and growth through the GA pathway. Future work should focus on measuring hormone levels and transport in biochar-treated plants to further understand biochar's influence on hormone pathways.

Our transcriptional analysis revealed downregulation of defense genes. Similar to our observations, Viger et al., (2014) showed a general downregulation of many defense-related genes in biochar-grown Arabidopsis. Copley et al., (2017) also observed downregulation of *PR1* and *LOX10* of biochar-treated soybeans prior to infection with *Rhizoctonia solani*. Biochar addition increased soybean susceptibility to *R. solani* infection at 5% amendment, but no differences in disease progress were observed at 1 or 3% amendment (Copley et al. 2017). In our study, evaluation of biochar's role in disease progress revealed no effect of biochar on Fusarium wilt in either resistant M82 or susceptible WV. This was consistent with other studies that have shown null effects of biochar on disease suppression at similar amendment rates (Zwart and Kim 2012; Jaiswal et al. 2015; Shoaf et al. 2016). Some of these studies reported a 'u-shaped curve'

of biochar's effects on disease suppression where low concentrations of biochar increase resistance, but high concentrations reduce resistance (Zwart and Kim 2012; Jaiswal et al. 2014, 2015). Together, these studies suggest that at relatively high levels of biochar addition (>3% w w^{-1}), genes involved in defense are down-regulated, and plant immunity is thus compromised. Future research will be needed to analyze the costs versus benefits of biochar use.

Biochar's positive effects on shoot growth and germination, even under well-watered and fertilized conditions, suggest a direct effect of compounds present in the biochar on the plant. Our results showing that water-extracts of biochar promoted SP seedling growth confirmed this hypothesis. Though we were unable to identify karrikins in the biochar used here, recent studies have demonstrated that biochars contain bioactive compounds, including karrikins and humic substances products (HSP) which have been shown to have hormone-like effects on plant growth (Lin et al. 2012; Graber et al. 2015; Kochanek et al. 2016). Alternatively, biochar's effects on plant growth and defense may occur indirectly through its impacts on the soil microbial community. Biochar has been shown to shift root-associated communities toward microbes with plant-growth promoting or defense-promoting capabilities (Egamberdieva et al. 2016; Kolton et al. 2011, 2016; Fox et al. 2016). These altered communities may, in turn, impact plant growth and defense.

In this study, we have demonstrated that biochar application stimulates the GA pathway in tomato shoots, and that GA biosynthesis is required for increased hypocotyl growth in Arabidopsis. This improved understanding of the molecular basis for biochar-mediated growth promotion may lead to new potential applications for biochar, such as enhancing current horticultural practices like spraying exogenous GA on grapes for larger fruit production. We have also shown that while biochar generally promotes tomato shoot growth under controlled glasshouse conditions, it affects specific traits such as germination in a genotype- and trait-specific manner. Our data suggests that direct interaction between the plant and compounds present in biochar may play a role in biochar's effects on plant hormone pathways. Future studies are needed to better understand how biochar affects plant hormone pathways and to examine how genetic differences influence plant response to biochar in order to breed for more biochar-responsive crops. This study illuminates the complexity of plant-biochar interactions and highlights the importance of weighing potential negative impacts of biochar addition, like downregulation of defense-related genes against its positive impacts on plant growth.

References

Abiven S, Hund A, Martinsen V, Cornelissen G (2015) Biochar amendment increases maize root surface areas and branching: a shovelomics study in Zambia. Plant Soil 395:45–55. doi: 10.1007/s11104-015-2533-2

Amador V, Monte E, García-Martínez JL, Prat S (2001) Gibberellins signal nuclear import of PHOR1, a photoperiod-responsive protein with homology to Drosophila armadillo. Cell 106:343–354. doi: 10.1016/S0092-8674(01)00445-7

Backer RGM, Schwinghamer TD, Whalen JK, et al (2016) Crop yield and SOC responses to biochar application were dependent on soil texture and crop type in southern Quebec, Canada. J Plant Nutr Soil Sci 179:399–408. doi: 10.1002/jpln.201500520

Bargmann I, Rillig MC, Buss W, et al (2013) Hydrochar and Biochar Effects on Germination of Spring Barley. J Agron Crop Sci 199:360–373. doi: 10.1111/jac.12024

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 57:289–300. doi: 10.2307/2346101

Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy 5:202–214. doi: 10.1111/gcbb.12037

Bolger A, Scossa F, Bolger ME, et al (2014) The genome of the stress-tolerant wild tomato species Solanum pennellii. Nat Genet 46:1034–1038. doi: 10.1038/ng.3046

Borchard N, Siemens J, Ladd B, et al (2014) Application of biochars to sandy and silty soil failed to increase maize yield under common agricultural practice. Soil Tillage Res 144:184–194. doi: 10.1016/j.still.2014.07.016

Butnan S, Deenik JL, Toomsan B, et al (2015) Biochar characteristics and application rates affecting corn growth and properties of soils contrasting in texture and mineralogy. Geoderma 237–238:105–116. doi: 10.1016/j.geoderma.2014.08.010

Carvalho MTM, Madari BE, Bastiaans L, et al (2016) Properties of a clay soil from 1.5 to 3.5years after biochar application and the impact on rice yield. Geoderma 276:7–18. doi: 10.1016/j.geoderma.2016.04.013

Copley T, Bayen S, Jabaji S (2017) Biochar amendment modifies expression of soybean and Rhizoctonia solani genes leading to increased severity of Rhizoctonia Foliar Blight. Front Plant Sci 8:1–15. doi: 10.3389/fpls.2017.00221

Copley TR, Aliferis KA, Jabaji S (2015) Maple bark biochar affects Rhizoctonia solani metabolism and increases damping-off severity. Phytopathology 105:1334–1346. doi: 10.1094/PHYTO-08-14-0231-R

De Tender CA, Debode J, Vandecasteele B, et al (2016) Biological , physicochemical and plant health responses in lettuce and strawberry in soil or peat amended with biochar. Appl Soil Ecol 107:1–12. doi: 10.1016/j.apsoil.2016.05.001

Dong D, Feng Q, McGrouther K, et al (2014) Effects of biochar amendment on rice growth and nitrogen retention in a waterlogged paddy field. J Soils Sediments 15:153–162. doi: 10.1007/s11368-014-0984-3

Ebrahimi N, Viaene N, Vandecasteele B, et al (2016) Traditional and new soil amendments reduce survival and reproduction of potato cyst nematodes, except for biochar. Appl Soil Ecol 107:191–204. doi: 10.1016/j.apsoil.2016.06.006

Edgar CI, Mcgrath KC, Dombrecht B, et al (2006) Salicylic acid mediates resistance to the vascular wilt pathogen Fusarium oxysporum in the model host Arabidopsis thaliana. Australas Plant Pathol 35:581–591.

Egamberdieva D, Wirth S, Behrendt U, et al (2016) Biochar treatment resulted in a combined effect on soybean growth promotion and a shift in plant growth promoting rhizobacteria. Front Microbiol 7:1–11. doi: 10.3389/fmicb.2016.00209

Elad Y, Cytryn E, Harel YM, et al (2011) The Biochar Effect : plant resistance to biotic stresses. Phytopathol Mediterr 50:335–349.

Eshed Y, Zamir D (1995) An introgression line population of Lycopersicon pennellii in the cultivated tomato enables the identification and fine mapping of yield- associated QTL. Genetics 141:1147–1162.

Fox A, Gahan J, Ikoyi I, et al (2016) Miscanthus biochar promotes growth of spring barley and shifts bacterial community structures including phosphorus and sulfur mobilizing bacteria. Pedobiologia (Jena) 59:195–202. doi: 10.1016/j.pedobi.2016.07.003

Fox A, Kwapinski W, Griffiths BS, Schmalenberger A (2014) The role of sulfur- and phosphorus-mobilizing bacteria in biochar-induced growth promotion of Lolium perenne. FEMS Microbiol Ecol 90:78–91. doi: 10.1111/1574-6941.12374

Free HF, McGill CR, Rowarth JS, Hedley MJ (2017) The effect of biochars on maize (Zea mays) germination. New Zeal J Agric Res 53:1–4. doi: 10.1080/00288231003606039

Gascó G, Cely P, Plaza C, Méndez A (2017) Relation between biochar properties and effects on seed germination and plant development. Biol Agric Hortic 32:237–247. doi: 10.1080/01448765.2016.1166348

Graber ER, Frenkel O, Jaiswal a. K, Elad Y (2014) How may biochar influence severity of diseases caused by soilborne pathogens? Carbon Manag 5:169–183. doi: 10.1080/17583004.2014.913360

Graber ER, Tsechansky L, Mayzlish-Gati E, et al (2015) A humic substances product extracted from biochar reduces Arabidopsis root hair density and length under P-sufficient and P-starvation conditions. Plant Soil 395:21–30. doi: 10.1007/s11104-015-2524-3

Gravel V, Dorais M, Ménard C (2013) Organic potted plants amended with biochar: its effect on growth and Pythium colonization. Can J Plant Sci 93:1217–1227. doi: 10.4141/cjps2013-315

Haider G, Steffens D, Moser G, et al (2015) Biochar reduced nitrate leaching and improved soil moisture content without yield improvements in a four-year field study. Agric Ecosyst Environ 237:80–94. doi: 10.1016/j.agee.2016.12.019

Hedden P, Phillips AL (2000) Gibberellin metabolism: New insights revealed by the genes. Trends Plant Sci 5:523–530. doi: 10.1016/S1360-1385(00)01790-8

Huot B, Yao J, Montgomery BL, He SY (2014) Growth-defense tradeoffs in plants: A balancing act to optimize fitness. Mol Plant 7:1267–1287. doi: 10.1093/mp/ssu049

Jaiswal AK, Elad Y, Graber ER, Frenkel O (2014) Rhizoctonia solani suppression and plant growth promotion in cucumber as affected by biochar pyrolysis temperature, feedstock and concentration. Soil Biol Biochem 69:110–118. doi: 10.1016/j.soilbio.2013.10.051

Jaiswal AK, Frenkel O, Elad Y, et al (2015) Non-monotonic influence of biochar dose on bean seedling growth and susceptibility to Rhizoctonia solani: the “Shifted Rmax-Effect.” Plant Soil 395:125–140. doi: 10.1007/s11104-014-2331-2

Jones DL, Rousk J, Edwards-Jones G, et al (2012) Biochar-mediated changes in soil quality and plant growth in a three year field trial. Soil Biol Biochem 45:113–124. doi: 10.1016/j.soilbio.2011.10.012

Kim B-S, French EA, Caldwell D, et al (2016) Bacterial wilt disease: host resistance and pathogen virulence mechanisms. Physiol Mol Plant Pathol. doi: doi:10.1016/j.pmpp.2016.02.007

Kochanek J, Long RL, Lisle AT, Flematti GR (2016) Karrikins identified in biochars indicate post-fire chemical cues can influence community diversity and plant development. PLoS One 11:e0161234. doi: 10.1371/journal.pone.0161234

Kolton M, Graber ER, Tsehansky L, et al (2016) Biochar-stimulated plant performance is strongly linked to microbial diversity and metabolic potential in the rhizosphere. New Phytol 213:1393–1404. doi: http://dx.doi.org/10.1111/nph.14253

Kolton M, Meller Harel Y, Pasternak Z, et al (2011) Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Appl Environ Microbiol 77:4924–30. doi: 10.1128/AEM.00148-11

Kou X, Wang S, Wu M, et al (2014) Molecular characterization and expression analysis of NAC family transcription factors in tomato. Plant Mol Biol Report 32:501–516. doi: 10.1007/s11105-013-0655-3

Lehmann J, Rillig MC, Thies J, et al (2011) Biochar effects on soil biota – A review. Soil Biol Biochem 43:1812–1836. doi: 10.1016/j.soilbio.2011.04.022

Liang F, Li G, Lin Q, Zhao X (2014) Crop yield and soil properties in the first 3 years after biochar application to a calcareous soil. J Integr Agric 13:525–532. doi: 10.1016/S2095-3119(13)60708-X

Lin Y, Munroe P, Joseph S, et al (2012) Water extractable organic carbon in untreated and chemical treated biochars. Chemosphere 87:151–157. doi: 10.1016/j.chemosphere.2011.12.007

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2 -delta delta CT Method. Methods 25:402–408. doi: 10.1006/meth.2001.1262

Major J, Rondon M, Molina D, et al (2010) Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant Soil 333:117–128. doi: 10.1007/s11104-010-0327-0

Marks EAN, Alcañiz JM, Domene X (2014) Unintended effects of biochars on short-term plant growth in a calcareous soil. Plant Soil 385:87–105. doi: 10.1007/s11104-014-2198-2

Marris E (2006) Putting the carbon back: black is the new green. Nature 442:624–6. doi: 10.1038/442624a

Martinsen V, Mulder J, Shitumbanuma V, et al (2014) Farmer-led maize biochar trials: Effect on crop yield and soil nutrients under conservation farming. J Plant Nutr Soil Sci 177:681–695. doi: 10.1002/jpln.201300590

Meller Harel Y, Elad Y, Rav-David D, et al (2012) Biochar mediates systemic response of strawberry to foliar fungal pathogens. Plant Soil 357:245–257. doi: 10.1007/s11104-012-1129-3

Mitchum MG, Yamaguchi S, Hanada A, et al (2006) Distinct and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development. Plant J 45:804–818. doi: 10.1111/j.1365-313X.2005.02642.x

Monte E, Amador V, Russo E, et al (2003) PHOR1: A U-Box GA signaling component with a role in proteasome degradation? J Plant Growth Regul 22:152–162. doi: 10.1007/s00344-003-0029-4

Nelissen V, Ruysschaert G, Manka'Abusi D, et al (2015) Impact of a woody biochar on properties of a sandy loam soil and spring barley during a two-year field experiment. Eur J Agron 62:65–78. doi: 10.1016/j.eja.2014.09.006

Nelson DC, Riseborough J-A, Flematti GR, et al (2009) Karrikins discovered in smoke trigger Arabidopsis seed germination by a mechanism requiring gibberellic acid synthesis and light. Plant Physiol 149:863–873. doi: 10.1104/pp.108.131516

Olmo M, Alburquerque JA, Barrón V, et al (2014) Wheat growth and yield responses to biochar addition under Mediterranean climate conditions. Biol Fertil Soils 50:1177–1187. doi: 10.1007/s00374-014-0959-y

Olmo M, Villar R, Salazar P, Alburquerque JA (2016) Changes in soil nutrient availability explain biochar's impact on wheat root development. Plant Soil 399:333–343. doi: 10.1007/s11104-015-2700-5

Prendergast-Miller MT, Duvall M, Sohi SP (2014) Biochar-root interactions are mediated by biochar nutrient content and impacts on soil nutrient availability. Eur J Soil Sci 65:173–185. doi: 10.1111/ejss.12079

Robinson MD, McCarthy DJ, Smyth GK (2009) edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. doi: 10.1093/bioinformatics/btp616

Rogovska N, Laird D a., Rathke SJ, Karlen DL (2014) Biochar impact on Midwestern Mollisols and maize nutrient availability. Geoderma 230–231:340–347. doi: 10.1016/j.geoderma.2014.04.009

Shahnejat-Bushehri S, Tarkowska D, Sakuraba Y, Balazadeh S (2016) Arabidopsis NAC transcription factor JUB1 regulates GA/BR metabolism and signalling. Nat Plants 2:1–9. doi: 10.1038/nplants.2016.13

Shoaf N, Hoagland L, Egel DS (2016) Suppression of phytophthora blight in sweet pepper depends on biochar amendment and soil type. HortScience 51:518–524.

Smider B, Singh B (2014) Agronomic performance of a high ash biochar in two contrasting soils. Agric Ecosyst Environ 191:99–107. doi: 10.1016/j.agee.2014.01.024

Solaiman ZM, Murphy D V, Abbott LK (2012) Biochars influence seed germination and early growth of seedlings. Plant Soil 353:273–287. doi: 10.1007/s11104-011-1031-4

Spokas KA, Cantrell KB, Novak JM, et al (2012) Biochar: a synthesis of its agronomic impact beyond carbon sequestration. J Environ Qual 41:973–89. doi: 10.2134/jeq2011.0069

Sun Z, Bruun EW, Arthur E, et al (2014) Effect of biochar on aerobic processes, enzyme activity, and crop yields in two sandy loam soils. Biol Fertil Soils 50:1087–1097. doi: 10.1007/s00374-014-0928-5

Thoquet P, Olivier J, Sperisen C, et al (1996) Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii7996. Mol. Plant. Microbe. Interact. 9:826–836.

Viger M, Hancock RD, Miglietta F, Taylor G (2014) More plant growth but less plant defence? First global gene expression data for plants grown in soil amended with biochar. GCB Bioenergy 7:658–672. doi: 10.1111/gcbb.12182

Wenig P, Odermatt J (2010) OpenChrom: a cross-platform open source software for the mass spectrometric analysis of chromatographic data. BMC Bioinformatics 11:405. doi: 10.1186/1471-2105-11-405

Woods WI, McCann JM (1999) The anthropogenic origin and persistence of Amazonian dark earths. Yearb Conf Lat Am Geogr 25:7–14. doi: 10.2307/25765871

Woolf D, Amonette JE, Street-Perrott FA, et al (2010) Sustainable biochar to mitigate global

climate change. Nat Commun 1:56. doi: 10.1038/ncomms1053

Xu H, Liu Q, Yao T, Fu X (2014) Shedding light on integrative GA signaling. Curr Opin Plant Biol 21:89–95. doi: 10.1016/j.pbi.2014.06.010

Zhang A, Bian R, Pan G, et al (2012) Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: A field study of 2 consecutive rice growing cycles. F Crop Res 127:153–160. doi: 10.1016/j.fcr.2011.11.020

Zhao X, Wang J, Wang S, Xing G (2014) Successive straw biochar application as a strategy to sequester carbon and improve fertility: A pot experiment with two rice/wheat rotations in paddy soil. Plant Soil 378:279–294. doi: 10.1007/s11104-014-2025-9

Zwart DC, Kim SH (2012) Biochar amendment increases resistance to stem lesions caused by Phytophthora spp. in tree seedlings. HortScience 47:1736–1740. doi: 10.4236/ojss.2015.59019

CHAPTER 3: WHOLE ROOT TRANSCRIPTOMIC ANALYSIS SUGGESTS A ROLE FOR AUXIN PATHWAYS IN RESISTANCE TO *RALSTONIA SOLANACEARUM* IN TOMATO*

Note: Bong Suk Kim grew and inoculated plants, harvested root samples, and extracted RNA for RNAseq. I analyzed the RNAseq data, and Katherine Rivera performed auxin growth and disease assays.

Abstract

The soilborne pathogen *Ralstonia solanacearum* is the causal agent of bacterial wilt, and causes significant crop loss in the Solanaceae family. The pathogen first infects roots, which are a critical source of resistance in tomato (*Solanum lycopersicum* L.). Roots of both resistant and susceptible plants are colonized by the pathogen, yet rootstocks can provide significant levels of resistance. Currently, mechanisms of this 'root-mediated resistance' remain largely unknown. To identify the molecular basis of this resistance, we analyzed the genome-wide transcriptional response of roots of resistant (Hawaii7996) and susceptible (West Virginia700) tomatoes at multiple time points after inoculation with *R. solanacearum*. We found that defense pathways in roots of the resistant Hawaii7996 are activated earlier and more strongly than roots of susceptible West Virginia700. Further, auxin signaling and transport pathways are suppressed in roots of the resistant variety. Functional analysis of an auxin transport mutant in tomato confirmed a role for auxin pathways in bacterial wilt. Together, our results suggest that roots mediate resistance to *R. solanacearum* through genome-wide transcriptomic changes that result in strong activation of defense genes and alteration of auxin pathways.

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Introduction

The soilborne betaproteobacterium *Ralstonia solanacearum* is the causal agent of bacterial wilt and has been ranked as one of the top 10 most destructive plant bacterial pathogens of all time (Mansfield et al. 2012). The pathogen infects over 200 plant species in 50 families, but is particularly devastating to members of the Solanaceae family (Hayward 1991; Huet 2014). *R. solanacearum* is a vascular pathogen that first colonizes the root surface and subsequently enters the root of both resistant and susceptible plants through small natural wounds or root tips (Genin 2010). The bacterium secretes cell wall-degrading enzymes and eventually spreads into the vascular system where it moves to the shoot via the flow of xylem fluid (Genin 2010; Genin and Denny 2012). As bacteria multiply, they secrete exopolysaccharide (EPS) (Genin 2010; Genin and Denny 2012), which likely leads to physical xylem blockage, and aboveground wilting. Resistant plants are able to delay colonization of the root vasculature (Caldwell et al. 2017), but the molecular responses involved in this delay are not clear. Here we use RNA-seq and mutant analysis to understand responses to *R. solanacearum* in roots of resistant and susceptible tomato genotypes.

In tomato, resistance to *R. solanacearum* is quantitative (Danesh et al. 1994; Thoquet et al. 1996a; Thoquet et al. 1996b; Wang et al. 2000; Carmeille et al. 2006; Wang et al. 2013; Kim et al. 2016), but no quantitative trait loci (QTL) for resistance have been cloned. Microarray analysis of genes differentially expressed in tomato stems 24 hours after infection showed that *R. solanacearum* activates defense, hormone, and lignin pathways in resistant tomato stems (Ishihara et al. 2012). Surprisingly, no differentially expressed genes (fold change > 2) were identified in susceptible stems after infection (Ishihara et al. 2012).

Despite the prevalence of soilborne pathogens and root diseases, most work in plant-pathogen interactions has focused on the aboveground portion of the plant. This is likely due to the hidden nature of roots, and the visible aboveground disease phenotypes that often result from root infection. However, recent reports indicate that roots also have a robust immune system that functions to protect the plant from soilborne pathogens. For example, Arabidopsis roots can recognize microbe associated molecular patterns (MAMPs) from pathogenic bacteria (Millet et al. 2010). In addition, roots infected with nematodes, which colonize root cortex tissue, can activate both MAMP-triggered immunity (MTI) (Teixeira et al. 2016) and effector-triggered immunity (ETI) (Mitchum et al. 2013; Goverse and Smant 2014). Tomato roots also appear to

mount a defense response to *R. solanacearum* because resistant rootstocks grafted to susceptible scions result in scions that are resistant to *R. solanacearum* and do not wilt (McAvoy et al. 2012; Rivard et al. 2012).

One approach to uncover the mechanisms of resistance in tomato roots to *R. solanacearum* is the analysis of whole genome transcriptional responses. In resistant and susceptible accessions of a wild potato species, *Solanum commersonii*, transcriptome analysis 3 – 4 days after inoculation with *R. solanacearum* identified 221 genes in the resistant accession and 644 genes in the susceptible that respond to infection (Chen et al. 2014; Zuluaga et al. 2015). In both accessions, genes that function in development were primarily downregulated, while those in the gene ontology category 'biotic stress' were mainly upregulated after infection (Zuluaga et al. 2015). In contrast, in a time-course of peanut root infection the expression patterns of many defense genes, including LRR-Kinases and *R* genes were mainly downregulated in both resistant and susceptible peanut genotypes (Chen et al. 2014). Carbohydrate metabolism was repressed after infection in roots of both resistant and susceptible peanut roots, but more strongly inhibited in resistant roots (Chen et al. 2014). This suggests that the mechanisms of root-mediated resistance may differ among plant species.

The plant hormone auxin can have both positive and negative effects on plant defense, (reviewed in (Kazan and Manners 2009; Fu and Wang 2011; Ludwig-Muller 2015)). Plant resistance to some necrotrophic pathogens requires auxin signaling (Tiryaki and Staswick 2002; Llorente et al. 2008; Qi et al. 2012), but multiple reports have revealed a relationship between plant susceptibility to biotrophic pathogens and increased auxin accumulation or signaling (O'Donnell et al. 2003; Navarro et al. 2006; Chen et al. 2007; Ding et al. 2008; Fu and Wang 2011). Many phytopathogens produce auxin (Spaepen et al. 2007; Ludwig-Muller 2015), and this probably includes *R. solanacearum* (Valls et al. 2006). Exogenous treatment with auxin or auxin analogs increases disease symptoms caused by *Pseudomonas syringae* in Arabidopsis (Navarro et al. 2006; Chen et al. 2007) and increases rice susceptibility to *Xanthomonas oryzae* pv. *oryzae* (Ding et al. 2008), *X. oryzae* pv. *oryzicola* and *Magnaporthe grisea* in rice (Fu and Wang 2011). In Arabidopsis, overexpression of the AvrRpt2 type III effector from *Pseudomonas syringae* changes auxin-related developmental phenotypes (Chen et al. 2007) through the ability of AvrRpt2 to promote degradation of an AUX/IAA transcription factor, AXR2/IAA7, which represses auxin responses (Cui et al. 2013).

Suppression of auxin signaling may be particularly important in plant defense against vascular wilt pathogens. Several Arabidopsis auxin signaling and transport mutants are resistant to the soilborne vascular wilt pathogen *Fusarium oxysporum* (Kidd et al. 2011), and the walls are thin (*wat1*) mutant of Arabidopsis is resistant to multiple vascular wilt pathogens, including *R. solanacearum* (Denance et al. 2012). The *wat1* mutant has decreased levels of auxin in roots (Denance et al. 2012) and the base of stems (Ranocha et al. 2013), and the gene was recently shown to encode a vacuolar auxin transporter . *WAT1* is expressed in the root pericycle and lateral root primordium (Denance et al. 2012), suggesting that auxin homeostasis within these tissues is particularly important for bacterial wilt resistance.

In this study, we aimed to identify the transcriptional response of resistant and susceptible tomato roots to *R. solanacearum* infection at 24 hours and 48 hours post inoculation (hpi). We identified the responsive genes in resistant and susceptible accessions independently and compared the responses. We show that resistant tomato roots activate defense pathways and terpene biosynthesis genes, and suppress auxin signaling and transport pathways in response to *R. solanacearum*. In contrast, susceptible tomato roots, activate defense response marker genes later, and at a lower fold-change, and genes required for root growth are suppressed by 48 hours post inoculation. Consistent with our finding that auxin pathways are suppressed in resistant roots, we show that an auxin transport mutant in a susceptible tomato wild-type background is resistant to *R. solanacearum*. Our data suggest that tomato roots mediate resistance to *R. solanacearum* in part through the suppression of auxin pathways.

Materials and Methods

Plant growth and *R. solanacearum* K60 inoculation

Resistant tomato (*Solanum lycopersicum* L.) accession Hawaii7996 (H7996), susceptible *S. pimpinellifolium* West Virginia700 (WV), *diageotropica* (*dgt1-1; S. lycopersicum*), and Ailsa Craig (AC; *S. lycopersicum*) were grown in Propagation Mix (Sun Gro Horticulture) in square pots containing 25-27g of soil and grown under 16:8 h light, 28° - 30°C in a growth chamber. The *dgt1-1* mutant has been previously reported (Oh et al. 2006), and we confirmed that the mutation was present by sequencing the gene. Growth and inoculation of *R. solanacearum* was as described in (Caldwell et al. 2017). Briefly, *R. solanacearum* strain K60 (phylotype IIA,

sequevar 7) was recovered from a glycerol stock and grown for two days on Casamino Peptone Agar (CPG) containing 1% triphenyltetrazolium chloride (TZC) at 28°C. Bacteria were harvested with sterile water and resuspended to 1.0 x 10^8 CFU/ml. At the three-leaf stage (approximately 14 – 17 days after planting), tomato plants were root-inoculated by gently lifting plants from their growth containers, and then soaking in either inoculum or water to the root-shoot soil line (approximately 40 ml per plant) (as in Caldwell et al. 2017). After soaking for 5 min, seedlings were transferred back to their growth containers and placed back into a growth chamber with the conditions above. Dilution plating was used to confirm the concentration of inoculum after each set of inoculations.

For *dgt* and AC resistance tests, wilting was rated daily and scored as the percentage of leaves per plant wilted. For each of soil-soak and petiole inoculation, average wilting with standard error are shown for three independent experiments. For soil soak inoculation, each independent experiment had 8 - 9 plants per genotype, and for petiole inoculation, each experiment had 3 – 9 plants per genotype. The Area Under the Disease Progress Curve (AUDPC) was calculated according to (Madden et al. 2007) with percent leaf wilting used as the disease measure.

Plant colonization assays

Individual plants from both mock and *R. solanacearum* inoculations were removed from pots, and the soil was gently washed off in a tray of sterilized distilled water. Roots of each plant were transferred into a 50 ml Falcon tube containing 45 ml of sterilized distilled water, and further cleaned to remove residual soil by shaking the Falcon tube for 1 minute. This wash was repeated 5 times. Water from cleaned roots was removed with a dried paper towel and roots were weighed. Washed, cleaned roots were surface sterilized by dipping in 100 % ethanol for 30 seconds, and then flamed quickly to remove residual ethanol. Each surface sterilized root was ground in 1 ml ddH_2O with a mortar and pestle, the lysate was centrifuged briefly, and the supernatant was used to determine *R. solanacearum* K60 titer with serial dilutions in ddH_2O. 100 µl of diluent was plated on CPG plates containing 1% TZC and incubated at 28°C for 48 hours. Colonies were counted and *R. solanacearum* K60 titer was determined as CFU/g of tissue. Colonization assays were performed in three independent experiments with three plants per genotype and time point per experiment. Statistics were performed in RStudio version 0.99.484.

Total RNA extraction and RNA-seq sample preparation

Whole roots from 10 plants of each genotype (H7996 and WV) were harvested at each time point (0 hour mock-inoculation, 24 hpi, and 48 hpi). Roots from these 10 plants were pooled for each genotype at each time point in each replicate. Three independent replicates were performed. Samples were ground into a powder using a mortar and pestle under liquid nitrogen. 100 mg of ground root tissue from each sample was used for total RNA extraction using Trizol following the manufacturer's instructions (Invitrogen, CA). 50 µg of extracted total RNA was subjected to RNAse-free DNase (Omega, GA) treatment. DNAse treated total RNA was further cleaned using a Nortek column following the manufacturer's instructions (Norgen BioTek Corp., Canada). Two µg from each of 18 samples (three time points x two genotypes x three replicates) were submitted to the Purdue Genomic Center for RNA-seq on the Illumina HiSeq 2500. RNA quality was determined using an Agilent Nanochip (Agilent, CA) and all samples had a RIN score of at least 7.8. Stranded mRNA libraries were constructed at the Purdue Genomics Facility using Illumina's TruSeq Stranded mRNA Sample Preparation kit (Revision E, Oct 2013) according to the manufacturer's instructions.

RNA-seq data analysis

Illumina paired-end 100 bp RNA sequencing was performed on all samples. A total of 967,730,337 reads were generated after quality filtering and mapping (Table B3.1). Reads for each of the 18 samples were aligned by the Purdue Genomics Facility to the ITAG2.4 *S. lycopersicum* reference genome using Tophat2 version 2.0.14 (Trapnell et al. 2009). Library type was set to strand-specific (first strand), mate inner distribution to 300, and mate standard deviation to 150. Gene expression was measured as the total number of reads for each sample that uniquely mapped to the reference, binned by gene. Each sample averaged about 54 million high quality, uniquely aligned reads (Appendix Table B3.1). After filtering for low counts such that at least 3 of the 18 samples had at least 3 counts per million (CPM) per row, a total of 20,641 genes remained for differential expression analysis. Differential gene expression analysis was performed using the edgeR package (Robinson et al. 2010) in Bioconductor version 3.3. The edgeR function calcNormFactors was used for library normalization with the default edgeR trimmed mean of M-values (TMM) method. Normalized library sizes are listed in Appendix

Table B3.2. Differentially expressed genes were identified using the glm (General Linear Model) pipeline in edgeR according to the edgeR documentation. The design matrix was created with coefficients for the expression level of each group. A group consisted of genotype and timepoint (H7996_0 hour = group 1, H7996_24 hour = group 2, etc). Common and tagwise dispersions were estimated with the function estimateDisp function. Multidimensional scaling (MDS) analysis revealed no batch effect of different replicates (Appendix Figure B3.1). Pairwise comparisons were performed between mock 0 hour and 24 hpi, and between mock 0 hour and 48 hpi within each of H7996 and WV using the contrast argument in the glmLRT function. Differential expression was determined using the Benjamini-Hochberg false discovery rate (FDR) multiple testing correction (Benjamini and Hochberg 1995) with an adjusted P-value of 0.05 and a log2 fold change > |0.585| (corresponds to a fold change of > |1.5|). Venn diagrams were generated using VENNY 2.1 (Oliveros 2007-2015). Gene ontology (GO) analysis was performed using the PANTHER GO analysis tool (http://www.pantherdb.org/) (Huaiyu et al. 2016). GO terms are derived from annotations of the sequenced *S. lycopersicum* genome, Heinz1706 (Tomato Genome Sequencing Consortium 2012). All GO categories shown are for 'biological process'. Heat maps, including those for GO figures were visualized with Multiple Experiment Viewer from TM4 (Saeed et al. 2003; Saeed et al. 2006).

cDNA synthesis and qRT-PCR

Total RNA extraction was performed as above from root tissue used in the RNA-seq analysis. cDNA synthesis and qRT-PCR was performed as in (Kim et al. 2017). Two biological replicates were used for validation. Briefly, cDNA was reverse-transcribed from 1 µg RNA using the NEB AMV first strand cDNA synthesis kit as per manufacturer's instructions. Quantitative RT-PCR was performed with 1µl of cDNA on a Roche Light Cycler (Roche, CA) with the following amplification protocol: 50°C for 2 min and 95°C for 2 min followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. PCR efficiency of the primers ranged from 95 % to 105 %. *ACTIN* (*Solyc11g005330*) was used as the gene for normalization. *Solyc11g005330* was not differentially expressed in either H7996 or WV at either time point (Appendix Table B3.4). The ΔΔCt method (Livak and Schmittgen 2001) was used to calculate fold changes relative to the internal control and the mock-inoculated control plant. Primer sequences are listed in Appendix Table B3.5.

Root architecture measurements

Roots were harvested from mock and *R. solanacearum* -inoculated plants at 6 dpi (AC and *dgt1-1*) or 10 dpi (WV and H7996). Whole root systems were washed gently in water and scanned with a calibrated color optical scanner from Regent Instruments, Inc (Quebec, Canada) and measured using software in the WinRHIZO V. 2016a system (Regent Instruments Inc, Quebec, Canada) (Arsenault et al. 1995). Two independent biological replicates with at least six plants per treatment and genotype were performed for AC and *dgt1-1*. Three independent biological replicates with at least 5 roots per treatment and genotype were performed for WV and H7996. Representative images are shown.

Results

Roots of resistant and susceptible tomato plants have a strong transcriptional response to *R. solanacearum* infection

We utilized resistant Hawaii7996 and susceptible West Virginia (WV) for our analyses. H7996 is a variety of cultivated tomato (*S. lycopersicum*) that is resistant to many different *R. solanacearum* strains (Lebeau et al. 2011). WV is an accession of *S. pimpinellifolium*, the closest wild relative to *S. lycopersicum* (Tomato Genome Sequencing Consortium 2012), and is highly susceptible to *R. solanacearum*. We chose these genotypes for transcriptomic analysis because they are the parents of a recombination inbred line population that has been used in multiple QTL (quantitative trait loci) studies (Thoquet et al. 1996a; Wang et al. 2000; Carmeille et al. 2006; Wang et al. 2013) for resistance to *R. solanacearum*. Transcriptomic data may be useful towards the further identification of genes underlying resistance QTL.

We hypothesized that transcriptional events that promoted defense responses in roots of resistant plants would occur early, before wilting, but would be non-existent or diminished in roots of susceptible plants. We inoculated roots using our previously established soil-soak inoculation method (Caldwell et al. 2017), in which wilting typically begins at 72 – 96 hpi in WV. We previously performed light and scanning electron microscopy and showed that bacteria colonize the root of both resistant H7996 and susceptible WV at 24 hpi and 48 hpi at 2.5 cm below the root-shoot junction (Caldwell et al. 2017). Here, we first tested whole roots to confirm that *R. solanacearum* colonizes roots of resistant H7996 and susceptible WV at 24 and 48 hpi

(Fig. 3.1). Plants were grown in potting mix and inoculated with 10^8 CFU/ml *R. solanacearum* K60 at the three-leaf stage as in (Caldwell et al. 2017). Consistent with our previous results, in three independent experiments, bacteria colonized roots of both resistant H7996 and susceptible WV at 24 and 48 hpi (Fig. 3.1). We then used genome wide-RNA seq analysis to identify the *R. solanacearum*-responsive transcriptome of whole roots in resistant H7996 and susceptible WV tomatoes prior to the onset of wilting at 0, 24, and 48 hpi. Plants were grown and root inoculated as above. Whole roots were harvested at 0, 24 and 48 hpi. Total RNA from 10 roots was pooled for each genotype at each timepoint and was sent to the Purdue Genomics Facility for library creation and sequencing on the Illumina HiSeq2500 (see Materials and Methods). Reads were mapped by the Purdue Genomics Facility using TopHat2 version 2.0.14 to the *S. lycopersicum* genome (ITAG2.4).

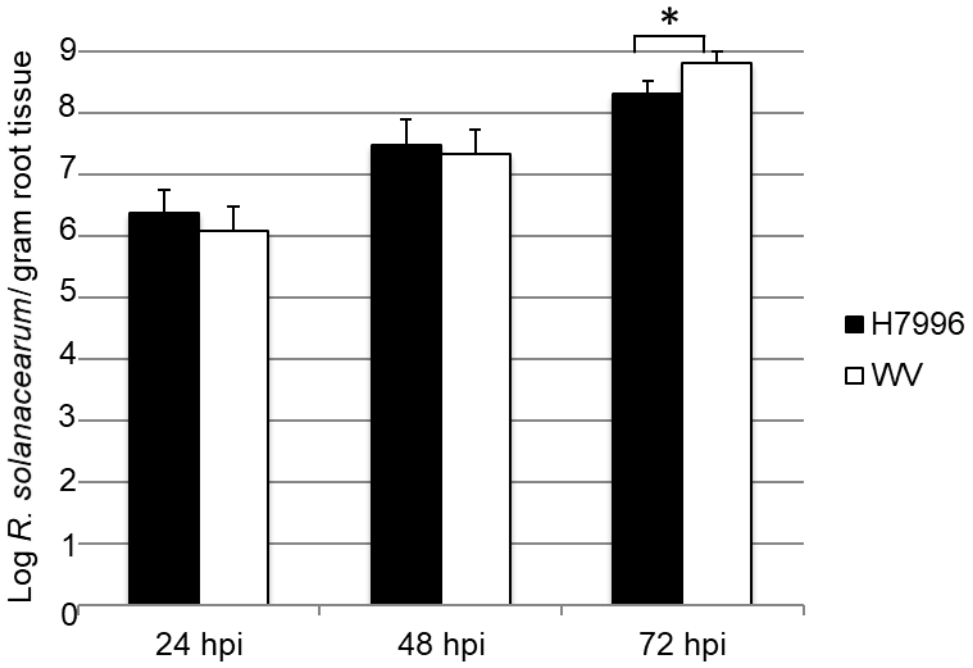


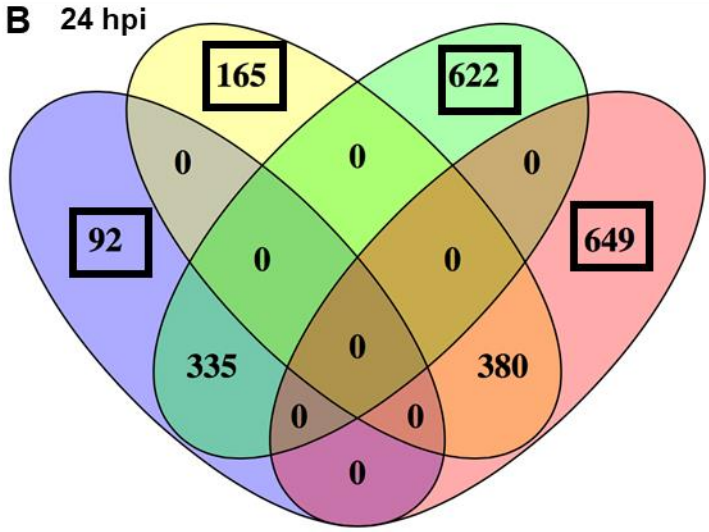
Fig 3.1 Root colonization of *R. solanacearum* K60 in whole roots of resistant H7996 and susceptible WV. Plants were grown in potting mix and root inoculated via soil soaking at the three-leaf stage. The average of three independent replicates, each with roots of three plants per genotype and timepoint, is shown. Error bars show standard deviation. * = $P < 0.05$ with the Mann Whitney Wilcoxon test.

Pairwise comparisons were made between each time point and 0 hpi (mock inoculated control) to identify transcriptional responses to *R. solanacearum* infection within each genotype. We classified responsive genes as those that showed a log2 fold change > |0.585| and a false

discovery rate (FDR) < 0.05. Because resistant H7996 (*S. lycopersicum*) and susceptible WV (*S. pimpinellifolium*) are different species, we first identified the response within each species by comparing each timepoint to the 0 hpi control for each genotype. To understand how the response to *R. solanacearum* infection in resistant and susceptible roots differed, the differentially expressed genes (DEGs) at each time point within a genotype were then compared between genotypes (Fig. 3.2). The mapping summary is in Appendix Table B3.1, raw counts are listed in Appendix Table B3.2, and processed edgeR gene expression results are in Appendix Table B3.3. Differential expression analysis showed that within susceptible roots at 24 hpi, 427 genes were upregulated and 545 downregulated, while within resistant roots at that time point almost twice as many genes were differentially expressed (957 up and 1029 down). At 48 hpi, 1316 genes were upregulated in susceptible roots and 1571 were downregulated compared to 1265 upregulated in resistant roots and 1419 downregulated. We used quantitative RT-PCR to validate the differential expression of fifteen genes. These showed similar expression patterns as identified in our RNA-seq analysis (Appendix Fig. B3.1).

A

Comparison	log2FC > 0.585, FDR < 0.05	
	UP	DN
WV 24-0h	427	545
WV 48-0h	1316	1571
Exclusive WV 24h	92	165
Exclusive WV 48h	594	808
H7996 24-0h	957	1029
H7996 48-0h	1265	1419
Exclusive H7996 24h	622	649
Exclusive H7996 48h	543	656



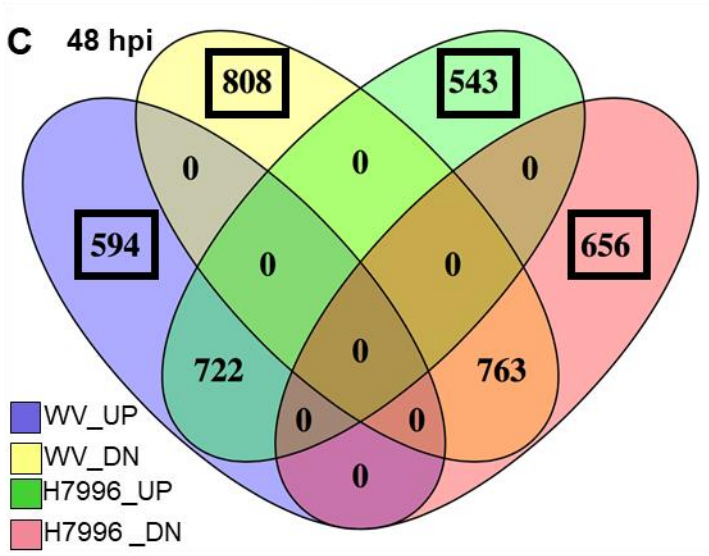


Fig. 3.2 Summary of DEGs from pairwise comparisons between timepoints within each genotype (H7996 or WV). A) Numbers of DEGs at each pairwise comparison within each genotype. Threshold for differential expression is log2 fold change > |0.585|, False Discovery Rate (FDR) < 0.05. (B and C) Venn Diagram of up- and downregulated DEGs at 24 hpi (B) and 48 hpi (C) showing overlap between the responses of resistant H7996 and susceptible WV. Boxed numbers show 'exclusive' genes at each timepoint.

At each time point, we also examined genes that were up or downregulated only within resistant H7996 or susceptible WV roots (Fig. 3.2b, 3.2c, boxed numbers). We call these genes 'exclusive' genes. Major shifts in numbers of exclusive DEGs were observed in susceptible roots between 24 and 48 hpi. For example, at 24 hpi, only 92 genes were exclusively upregulated in susceptible WV roots, compared to 622 genes in resistant H7996 roots. However, by 48 hpi, this number rose to 594 genes in susceptible WV roots compared to 543 in resistant H7996 roots (Fig. 3.2). We did not identify any significant DEGs whose expression was upregulated in roots of resistant H7996 and simultaneously downregulated in susceptible WV (or vice versa) at either time point.

We used Gene Ontology (GO) analysis to understand what biological processes were affected within roots of resistant H7996 and susceptible WV plants after inoculation. GO analysis using PANTHER (Huaiyu et al. 2016) showed that in susceptible WV at 24 hpi, only seven GO terms for biological process are overrepresented ($P < 0.05$) among the 427 genes upregulated (Table B3.4). These include 'response to stress' (GO:0006950; $P = 9.76 \times 10^{-3}$) and 'response to stimulus' (GO:0050896; $P = 2 \times 10^{-2}$). In contrast, at 24 hpi in roots of the resistant H7996, 27 biological process GO terms were overrepresented in the 957 upregulated genes (Fig. 3.3 shows a subset of overrepresented GO categories, all overrepresented GO categories for all comparisons are in Appendix Table B3.4). These included 'reactive oxygen species metabolic process' (GO:0072593; $P = 6.3 \times 10^{-6}$) and 'cellular detoxification' (GO:1990748; $P = 8.7 \times 10^{-6}$). Not unexpectedly, the GO category 'defense responses' (GO: 0006952; $P = 2.45 \times 10^{-5}$) was identified in upregulated genes in roots of the resistant plant at 24 hpi (Fig. 3.3) but was not present in upregulated genes of susceptible roots at this time point.

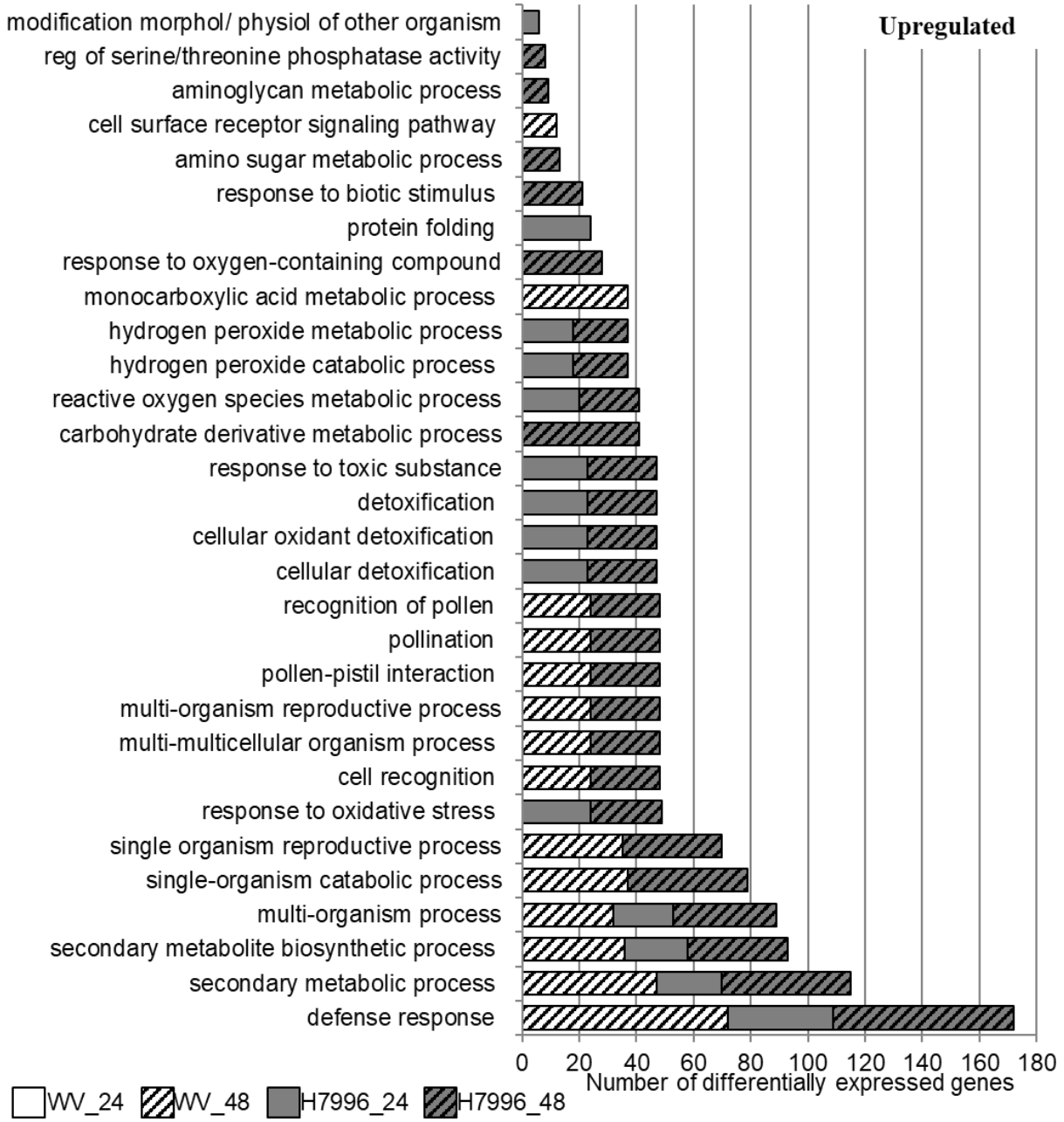


Fig. 3.3 GO categories overrepresented (corrected P-value < 0.05) in the set of upregulated genes at each timepoint. Only categories that contain less than 600 total *S. lycopersicum* genes are shown in the figure (all categories are in Appendix Table B3.5). WV 24 = 24 – 0 hpi comparison, WV 48 = 48 – 0 hpi comparison etc. No GO categories with less than 600 total genes are overrepresented in WV_24 upregulated genes.

Twenty-five biological process GO terms are overrepresented in the 545 downregulated genes at 24 hpi in susceptible WV roots, including 'plant-type cell wall organization or biogenesis' (GO:0071669; $P = 2.38 \times 10^{-2}$), 'reactive oxygen species metabolic process'

(GO:0072593; P = 3.34 x 10^{-3}), and ‘cellular detoxification’ (GO:1990748; P = 1.69 x 10^{-4}) (Fig. 3.4 and Appendix Table B3.4). Notably, and as stated above, the latter two GO categories were both overrepresented in *upregulated* genes in resistant roots at this time point. GO overrepresentation in downregulated H7996 genes at 24 hpi included ‘regulation of jasmonic acid (JA) mediated signaling pathway’ (GO:2000022; P = 1.26 x 10^{-6}) (Fig. 3.4), consistent with the downregulation of JA responses in resistant plants after infection with some biotrophic pathogens (Spoel et al. 2003; Glazebrook 2005; Spoel et al. 2007; Koornneef et al. 2008; Koornneef and Pieterse 2008).

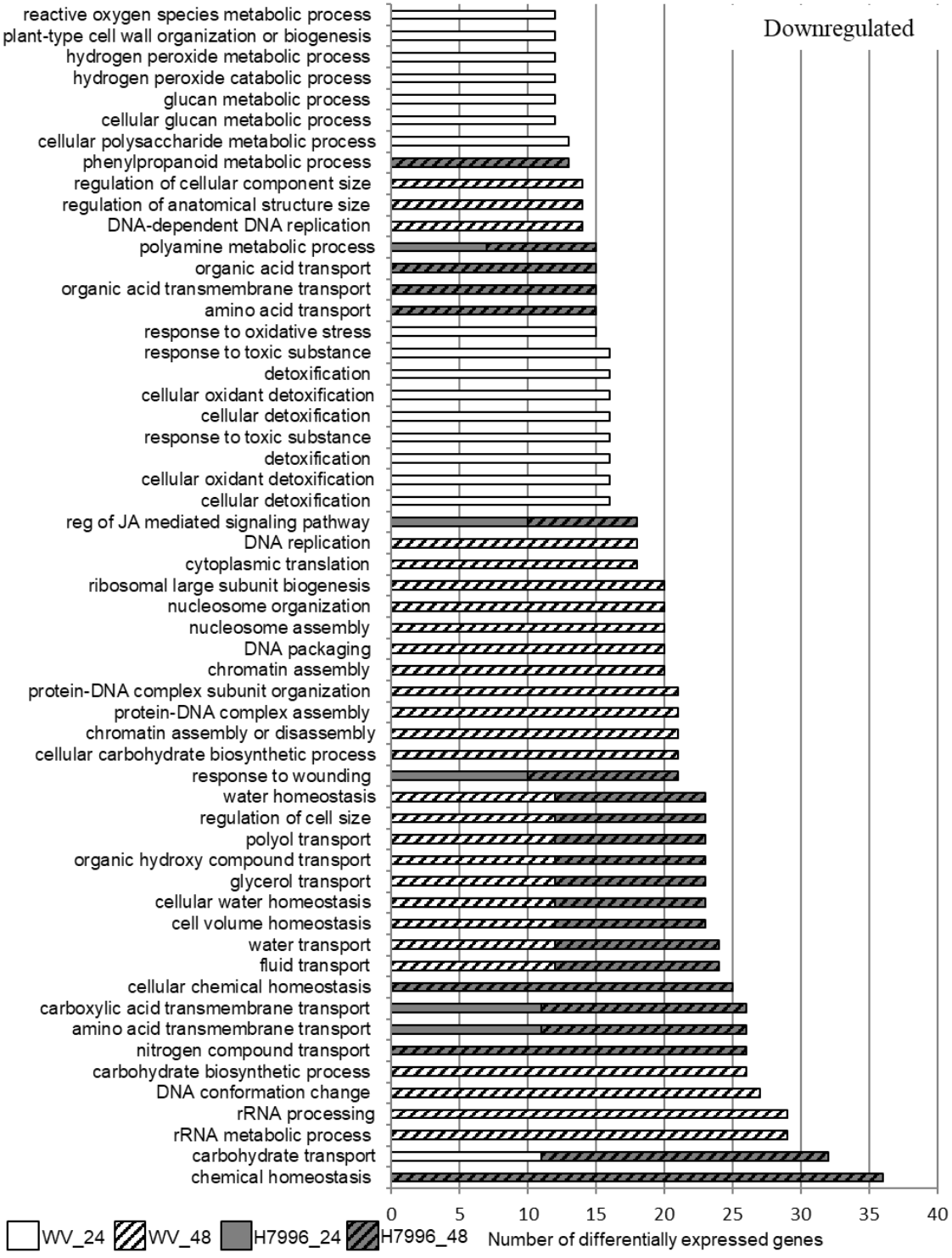


Fig. 3.4 GO categories overrepresented (corrected P-value < 0.05) in the set of downregulated genes at each timepoint. Only categories that contain less than 300 total *S. lycopersicum* genes are shown in the figure (all categories are in Appendix Table B3.5). WV 24 = 24 – 0 hpi comparison, WV 48 = 48 – 0 hpi comparison etc.

Many of the same trends in GO terms were observed at 48 hpi as at 24 hpi in each genotype. For example, 'Reactive oxygen species metabolic process' and 'cellular detoxification' categories were still overrepresented in upregulated genes in the resistant H7996 root at 48 hpi (Fig. 3.3) ($P = 5.41 \times 10^{-5}$ and $P = 3.47 \times 10^{-4}$, respectively), but were not overrepresented in upregulated genes of the susceptible WV root at either time point (Fig. 3.3). The GO category 'defense response' continued to be overrepresented in upregulated genes of the resistant H7996 root at 48 hpi ($P = 2.98 \times 10^{-15}$) (Fig. 3). While the 'defense response' category was not overrepresented at 24 hpi in the root of susceptible WV, it was identified at 48 hpi ($P = 4.27 \times 10^{-20}$) in upregulated genes of the susceptible WV root (Fig. 3.3). In downregulated genes, 'Cell wall organization or biogenesis (GO:0071554)' was overrepresented in susceptible roots at 48 hpi ($P = 1.46 \times 10^{-4}$) (see Appendix Table B3.4), while 'JA mediated signaling pathway' continued to be overrepresented in the resistant H7996 plant at 48 hpi ($P = 3.78 \times 10^{-3}$) (Fig. 3.4).

Defense gene activation occurs earlier and is stronger in roots of resistant tomato plants

Our GO analysis of genes up and downregulated at each time point showed that roots of resistant plants activated genes enriched for immune GO categories (such as 'response to biotic stimulus', response to oxidative stress', 'defense response', and response to stimulus) earlier in the resistant H7996 root than in the susceptible WV root (Fig. 3.3 and 3.4).

To examine this more carefully, we next focused on the expression of specific defense marker genes in classic defense hormone pathways. We examined genes previously used as markers for defense responses in resistant H7996 (Milling et al. 2011). The ethylene (ET) marker gene *PR-1b* was upregulated only in the resistant H7996 genotype, while *Osmotin* was activated earlier and with a higher fold-change compared to 0 hpi in H7996 compared to WV (Fig. 3.5a). SA marker genes were similarly regulated, with *PR-1a* being exclusively activated in H7996 at 48 hpi, and *Glu-A* was activated more strongly in H7996 compared to susceptible WV at both 24 and 48 hpi.

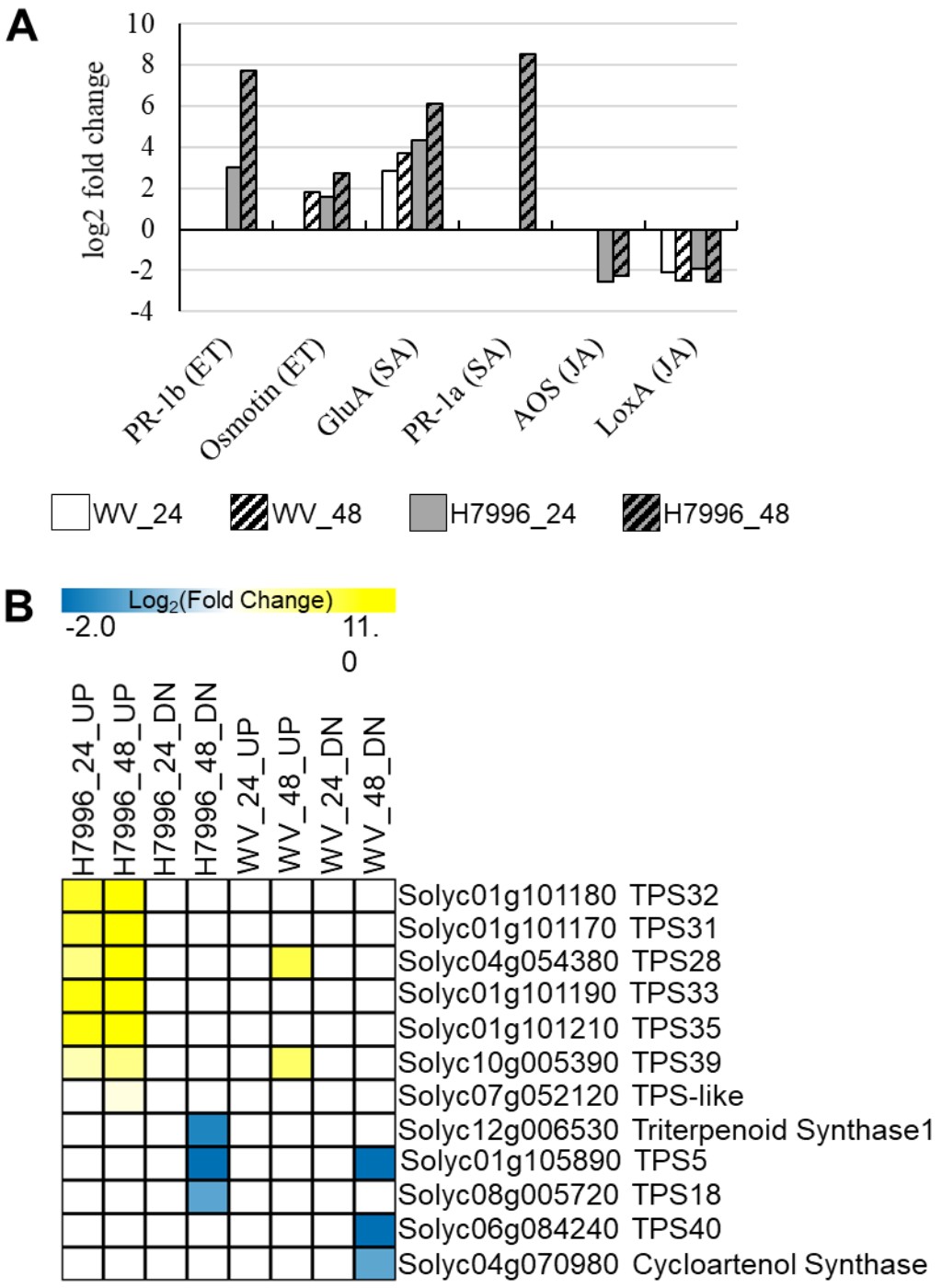


Fig 3.5 Defense responses are activated earlier and with higher fold changes in the root of resistant H7996. A) log fold changes in RNA-seq data of marker genes for classic defense hormones, B) Heat map showing log fold changes of genes in the 'terpenoid' bin in MapMan software (Thimm et al. 2004). More terpene synthase (TPS) genes are activated in roots of resistant plants and at an earlier timepoint.

Consistent with JA – SA antagonism (Robert-Seilaniantz et al. 2011; Derksen et al. 2013), and our GO analysis above, marker genes for JA defense responses were repressed in both resistant H7996 and susceptible WV, but showed greater fold-change repression in roots of the resistant H7996 plants. *ALLENE OXIDE SYNTHASE* (*AOS*) and *LIPOXYGENASE* (*LoxA*) were both downregulated in resistant H7996 after both time points, *LoxA* was also downregulated in WV (Fig. 3.5a). This corresponded to the GO enrichment analysis that showed that regulation of JA mediated signaling was overrepresented in downregulated genes only for resistant H7996 (Fig. 3.4). Together, these results reveal activation of SA- and ET- dependent defense pathways earlier in roots of the resistant plant H7996, as well as an earlier deactivation of JA-dependent defense signaling in resistant H7996.

In addition to these classic defense pathways, we observed strong upregulation of terpene synthases in resistant tomato roots (Fig. 3.5b). Terpenoids are a large class of compounds composed of five carbon isoprene units and are building blocks of some plant hormones and of specialized secondary metabolites (Falara et al. 2011). Tomato has 44 terpene synthase (TPS) genes, of which 29 are functional and are divided into 5 clades (Falara et al. 2011). In roots of resistant plants, five TPS genes in the alpha clade, which encode sesquiterpene synthases (TPS28, 31, 32, 33, 35), a TPS-like gene, and a linadool/nerolidol synthase (TPS39) are strongly upregulated at 24 hpi and 48 hpi (Fig. 3.5b). In contrast, only one sesquiterpene synthase, TPS28, and the linadool/nerolidol TPS39 are upregulated in susceptible roots at 48 hpi (Fig. 3.5b). Terpenoids act as antimicrobial or anti-insect compounds, and the strong upregulation observed in roots of resistant plants may contribute to resistance.

Roots of susceptible tomato plants downregulate genes required for organ growth at 48 hpi

To have a better understanding of the response within roots of each genotype, we focused on genes that were exclusively responsive within each time point in each genotype (i.e. genes that were activated or repressed only within H7996 or WV at each time point, boxed numbers in Fig. 3.2b and c). All nine GO terms that overlapped among exclusive genes in WV and H7996 were related to defense and detoxification (Appendix Fig. B3.2). Consistent with earlier and larger fold- change defense responses in the resistant H7996 root, all but one of these categories were found both in genes upregulated in the resistant H7996 root at 24 hpi and genes downregulated in the susceptible WV root at 24 hpi (Appendix Fig. B3.2).

Analysis of the 808 genes exclusively downregulated at 48 hpi in susceptible WV roots revealed several GO categories with known roles in root growth. These included GO categories 'DNA replication' (GO: 0006260; P = 8.7 x 10^{-7}) (Ni et al. 2009; Jia et al. 2016), DNA packaging (GO:0006323; P = 4.4 x 10^{-10}), chromatin assembly (GO:0031497, P = 9.7 x 10^{-11}) (Shen and Xu 2009; Aichinger et al. 2011; Sang et al. 2012), and translation (GO: 0006412; P = 3.7 x 10^{-31}) (Wieckowski and Schiefelbein 2012) (Fig. 3.6). Genes repressed in these categories included DNA replication helicases *MCM3* (*Solyc02g070780*), *MCM4* (*Solyc01g110130*), *MCM5* (*Solyc07g005020*) and *MCM7* (*Solyc01g079500*), ribosomal proteins and histones. In Arabidopsis, *MCM2* is involved in DNA replication and is important for root meristem maintenance (Ni et al. 2009), and mutations in a DNA helicase/nuclease result in very short roots (Jia et al. 2016). Further, mutation of AtMDN1, an AAA-ATPase that is a component of the pre-60S ribosome, results in several developmental defects including a shorter root (Li et al. 2016). Histone modifications have also been shown to be critical for proper root growth and development (reviewed in (Takatsuka and Umeda 2015)). None of these GO categories were identified within differentially expressed genes in the resistant H7996 root (Fig. 3.6).

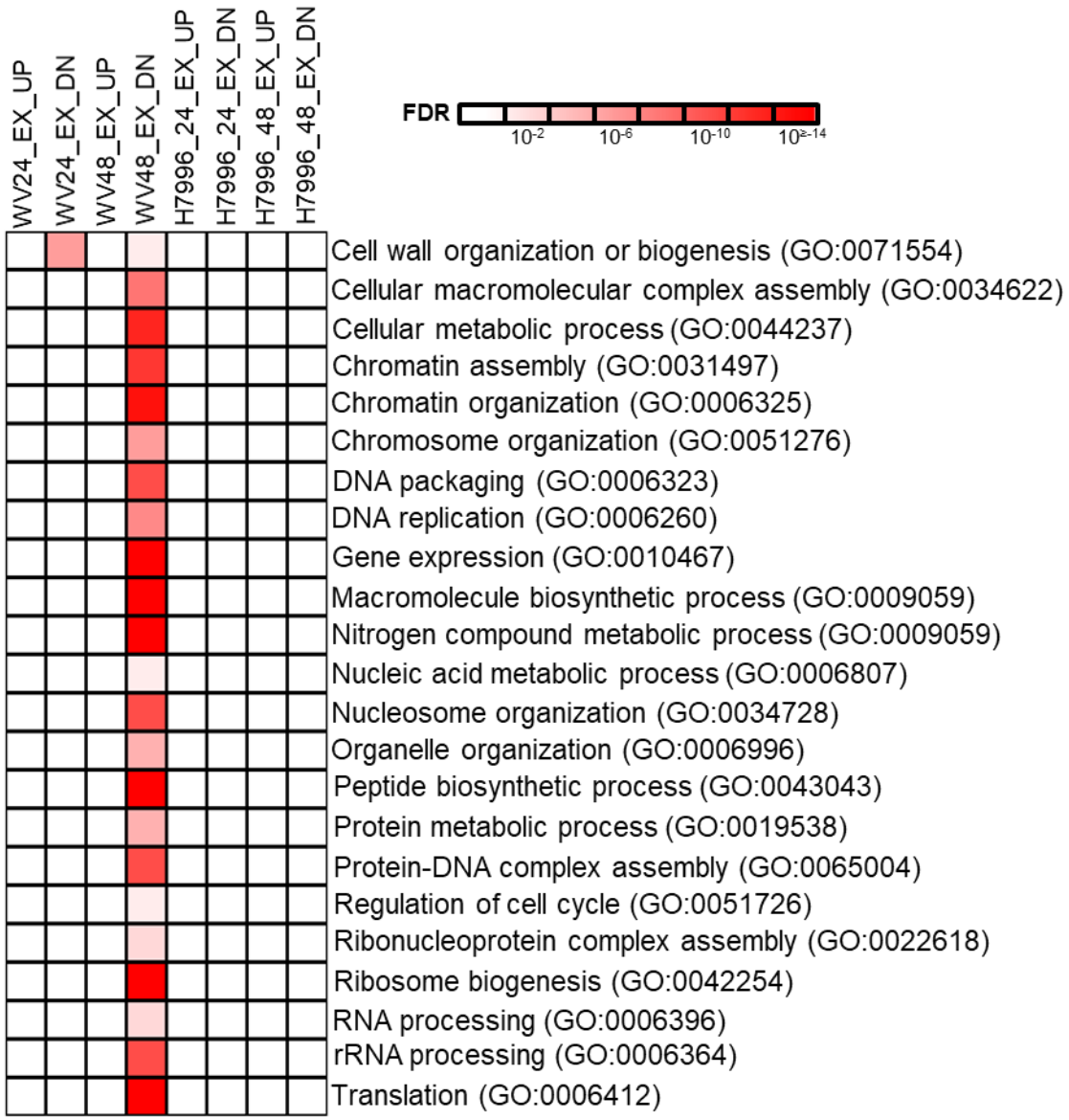


Fig 3.6 Roots of susceptible plants strongly repress pathways required for organ growth at 48 hpi. Heatmap of selected overrepresented GO categories (corrected $P < 0.05$) in up- and downregulated genes in roots of susceptible WV at 24 and 48 hpi. All GO categories in Appendix Table C3.5. No overrepresented categories were observed in WV24_EX_UP.

These data suggested that roots of susceptible plants slow growth after infection. To test this, we quantified root growth of H7996 and WV at 10 dpi. Plants were removed from pots, and the root systems were gently washed with water to remove soil. Cleaned roots were scanned and surface area quantified using a WinRHIZO root scanning and quantification system (Arsenault et al. 1995). We find that roots of WV have significantly decreased surface area after inoculation

compared to mock-inoculated controls (Fig. 3.7). In contrast, *R. solanacearum* inoculated roots of resistant H7996 have no difference in surface area compared to mock-inoculated resistant roots (Fig. 3.7). The differential root growth response to *R. solanacearum* between resistant and susceptible accessions is consistent with the transcriptional changes that we observed.

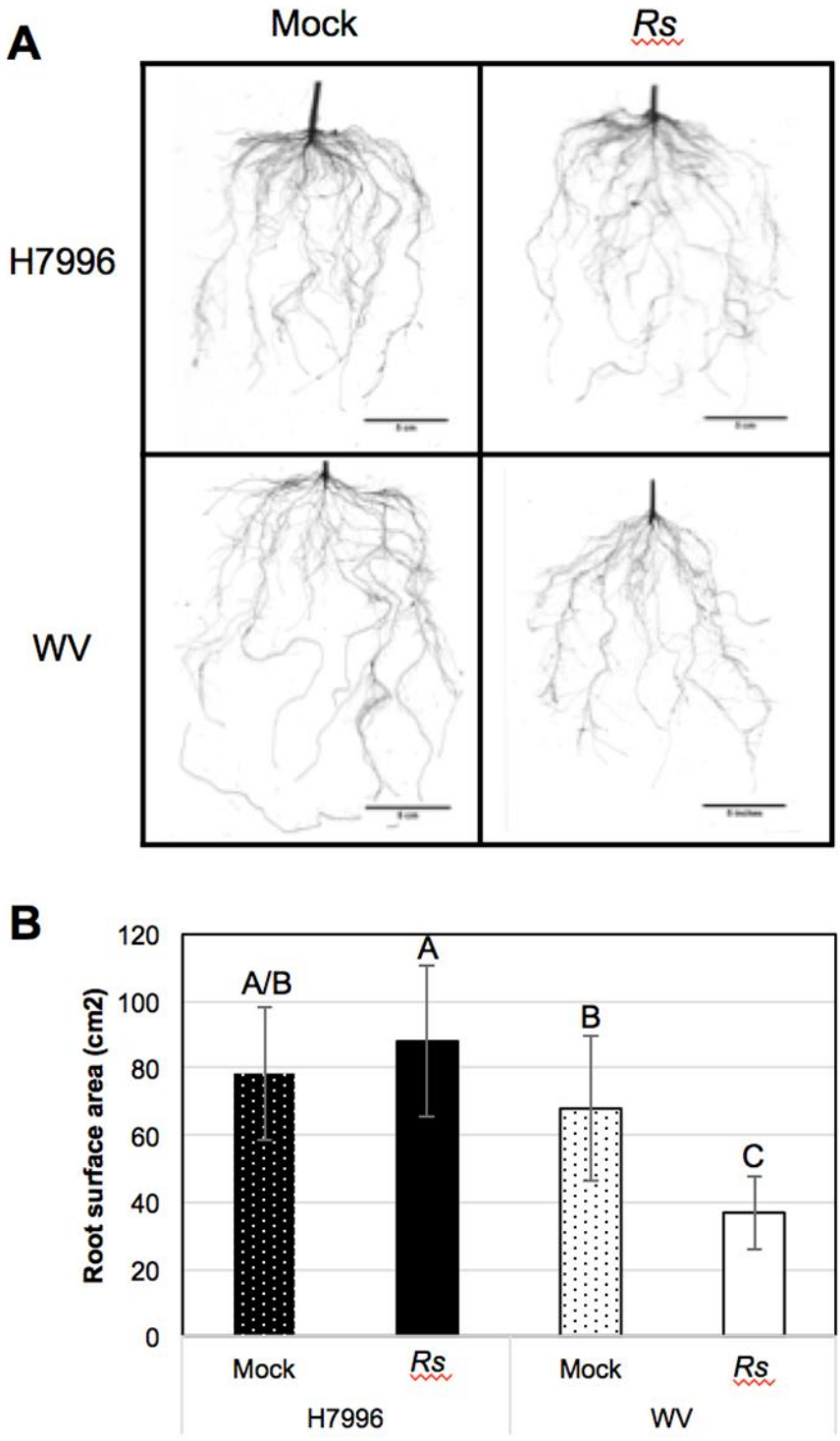


Fig 3.7 Root architecture of resistant H7996 and susceptible WV at 10 dpi. A) *R. solanacearum* (*Rs*) and mock-inoculated roots at 10 dpi imaged with a flatbed scanner. Representative images from three independent experiments, each with at least five roots per genotype and treatment, are shown, B) Quantification of whole root surface area using the WinRhizo software image analysis system (Arsenault et al. 1995). Letters indicate significantly different means ($P < 0.05$) with a two-way ANOVA and Tukey's HSD test.

Consistent with the hypothesis that the susceptible WV root, responds to *R. solanacearum* with growth suppression, far fewer GO categories were overrepresented in the set of exclusively upregulated genes in WV roots at 48 hpi (Appendix Table B3.4). Three GO categories were identified among the 594 number of genes exclusively upregulated in WV, compared to 72 categories identified among the 808 downregulated genes. Among the three GO categories overrepresented in the exclusively upregulated genes in WV at 48 hpi was 'defense response' (GO: 0006952; $P = 1.01 \times 10^{-4}$) (Appendix Table B3.4). Together these results show that although roots of the susceptible WV plant do eventually activate defense responses, they are also initiating processes that limit root growth.

Auxin response pathways are altered in roots of resistant plants

GO analysis of genes that were exclusively expressed in roots of the resistant variety H7996 at each time point revealed that the categories 'auxin-activated signaling pathway' (GO:0009734; $P = 4.3 \times 10^{-2}$) and 'cellular response to auxin stimulus' (GO: 0071365, $P = 4.3 \times 10^{-2}$) were overrepresented in genes exclusively downregulated in the resistant H7996 at 48 hpi (Fig. 3.8).

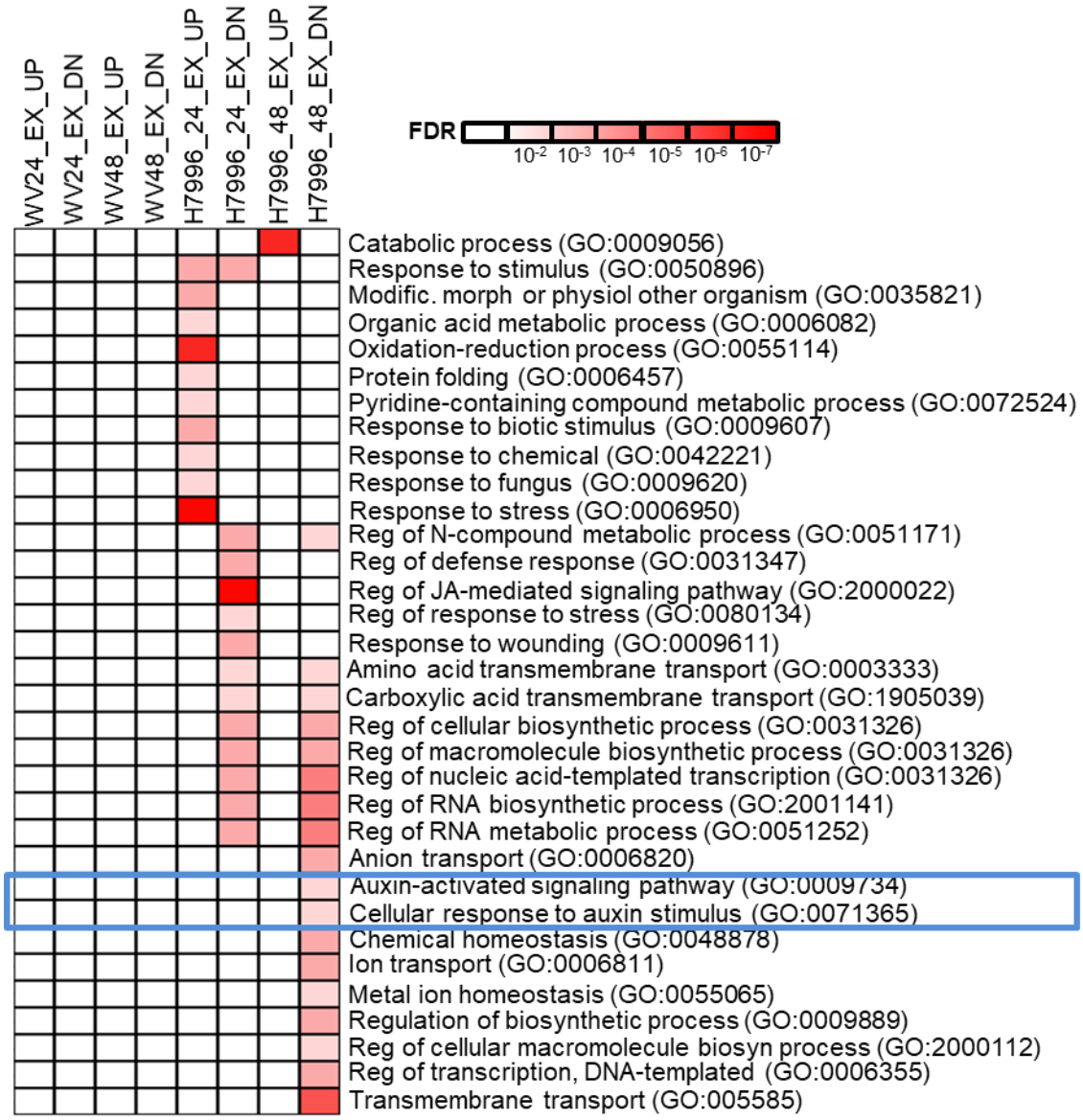


Fig 3.8 Auxin-related and lateral root development genes are differentially expressed in the resistant root at 48 hpi. Selected GO categories overrepresented among genes exclusively differentially expressed in H7996 at each of the timepoints shown. The blue box highlights auxin-related GO categories. The nine categories that overlapped between H7996 and WV are shown in Appendix Fig B3.2 and are not shown here.

Examination of the eight genes within these categories identified three genes encoding transcription factors known as *AUXIN RESPONSE FACTORs* (*ARF*s), which have both positive and negative roles in auxin signaling. These included two *S. lycopersicum* orthologs (*Solyc12g042070* and *Solyc03g118290*) of Arabidopsis *ARF2*, and the *S. lycopersicum* ortholog of Arabidopsis *ARF4* (*Solyc11g069190*). Of the other five genes within the 'auxin response' GO

category, one encoded a PIN auxin transporter (*Solyc10g080880*), three were AUXIN/INDOLE-3-ACETIC-ACID (AUX/IAA) transcription factors (*Solyc06g008590*, *Solyc06g008580*, *Solyc01g097290*), and another encoded an uncharacterized gene (*Solyc02g036370*) related to the REVEILLE1 transcription factor in Arabidopsis.

The tomato auxin transport mutant *diageotropica* (*dgt*) is resistant to *R. solanacearum*

One of the genes within the auxin response GO category above was *Solyc10g080880,* which encodes a PIN auxin efflux transporter known as SISTER OF PIN1b (SlSoPIN1b). PIN proteins are the primary auxin efflux transporters in plants and are responsible for polar auxin transport (Krecek et al. 2009; Adamowski and Friml 2015). In Arabidopsis, mutations in several auxin transporters, including *PIN2,* lead to decreased disease symptoms caused by *Fusarium oxysporum* (Kidd et al. 2011). We hypothesized that tomato genes required for polar auxin transport function in resistance to *R. solanacearum*. To test this, we examined resistance of the tomato mutant *diageotropica* (*dgt*) to *R. solanacearum*. *DGT* encodes a cyclophilin that negatively regulates PIN auxin efflux transporters in tomato (Ivanchenko et al. 2015). Mutations in *DGT* lead to altered auxin transport and changes to the transcription and/or protein localization of PINs (Ivanchenko et al. 2015). Root inoculation of the *dgt1-1* mutant and its susceptible wild type parent, Ailsa Craig (AC), showed that *dgt* was highly resistant to *R. solanacearum* compared to the wild type parent (Fig. 3.9). Three independent biological replicates revealed that mutant plants had consistently less than 10% wilting at 12 dpi. In contrast, the wild type parent had almost 80% wilting at the same time point.

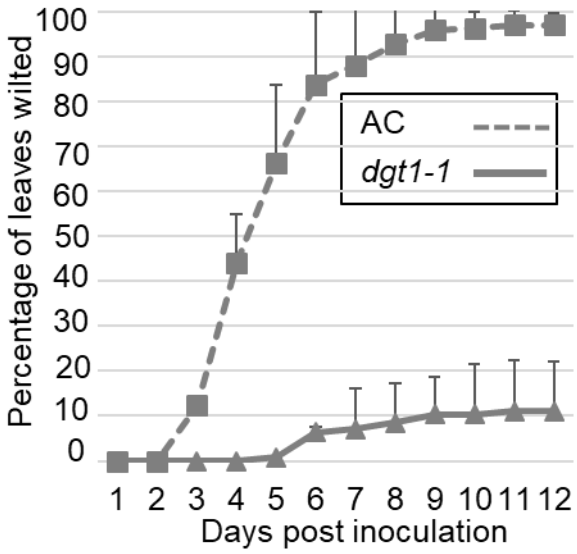


Fig 3.9 The *dgt* mutant shows enhanced resistance to *R. solanacearum* compared to its wild type control AC with root soaking inoculation. Wilting was scored daily based on the percentage of leaves wilted per plant. Each point represents the average of three independent experiments, each with 8 - 9 plants per genotype. Area Under the Disease Progress Curve (AUPDC) for AC = 725.2 ± 85.2 and for *dgt1-1* = 60 ± 64.2 ($P < 0.001$ with a two-tailed t-test). Error bars indicate standard deviation.

The increased resistance of *dgt1-1* is not due solely to alterations in root architecture

The *dgt1-1* mutant has been previously described as lacking lateral roots (Muday et al. 1995; Oh et al. 2006; Ivanchenko et al. 2015). Because *R. solanacearum* enters the root system in part through wounds created as lateral roots emerge from the primary root, we questioned whether the decreased colonization of *R. solancearum* in *dgt1-1* was due to deficiencies in lateral root emergence. Previous work showing a lack of lateral roots in *dgt1-1* used plants grown in agar (Ivanchenko et al. 2015). However, examination of root systems of *dgt1-1* grown in potting mix revealed that the mutant does produce lateral roots in these conditions (Fig. 3.10, arrows), although roots of *dgt1-1* were still significantly smaller compared to the wild-type parent AC (Fig. 3.10).

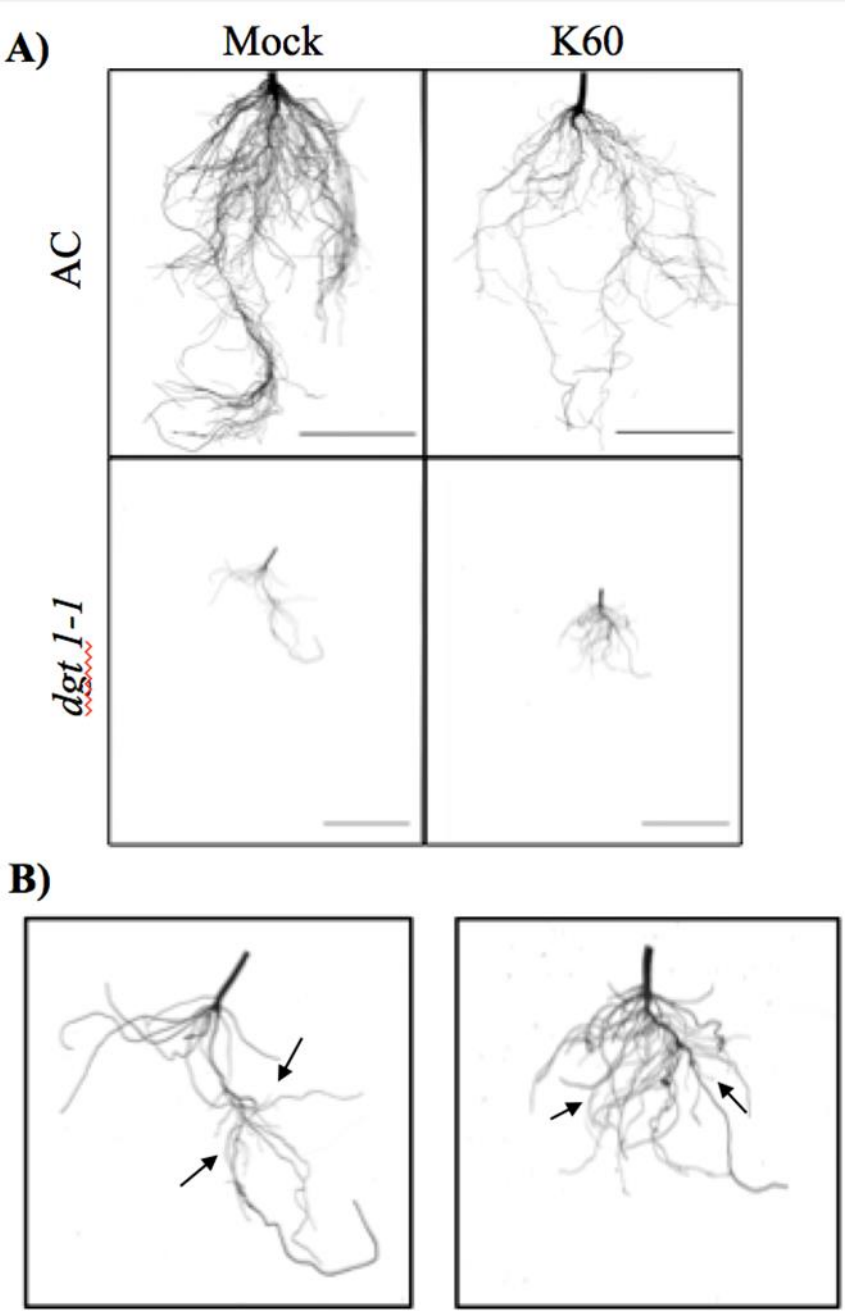


Fig 3.10 Root architecture of susceptible AC and resistant *dgt1-1* plants at 6 dpi grown in potting mix and soil-soak inoculated with water (mock) or *R. solanacearum* strain K60 (*Rs*). A) Plants were grown in potting mix and roots imaged with a flatbed scanner, B) Close-up images of *dgt1-1* in (A). Arrows point to examples of lateral roots. Images are representative of those from two independent biological replicates with six plants per replicate per treatment and genotype. Scale bars = 5 cm.

To examine whether the altered root structure was the underlying basis for the increased resistance, we used petiole inoculation of *R. solanacearum* in the *dgt1-1* and AC mutant. This

method bypasses the root system by directly injecting bacteria into the petiole vasculature (Tans-Kersten et al. 2001; Dalsing and Allen 2014). If decreased lateral root emergence in the *dgt1-1* mutant were the primary reason for resistance, we would expect that the *dgt1-1* mutant would show an increased susceptibility using this method. Using petiole inoculation, the *dgt1-1* mutant did not wilt by 12 dpi, compared to approximately 90% wilting in the wild type AC control (Fig. 3.11). Together, these results suggest that the enhanced resistance to *R. solanacearum* in the *dgt1-1* mutant is due to modulation of auxin transport.

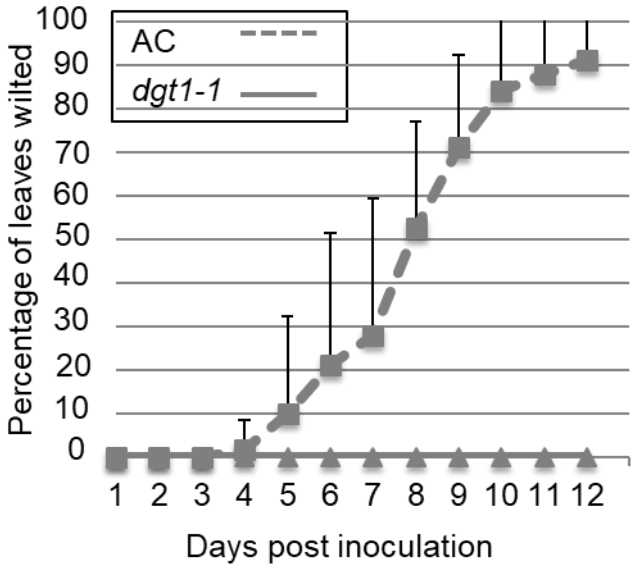


Fig 3.11 The *dgt* mutant shows enhanced resistance to *R. solanacearum* compared to its wild type susceptible parent AC with petiole inoculation. Wilting was scored daily based on the percentage of leaves wilted per plant. The experiment was repeated three times with 3 – 9 plants of each genotype per experiment. The average of three experiments is shown. The average Area Under the Disease Progress Curve (AUDPC) for AC = 401.6 ± 154.8; average AUDPC for *dgt1-1* = 0 ± 0 ($P < 0.01$; two-tailed t-test with unequal variance). Error bars represent standard deviation.

Discussion

In this manuscript we show that infection with the soilborne pathogen *R. solanacearum* leads to a strong defense response in tomato roots that includes alteration of auxin pathways. Analysis of a tomato mutant with defective auxin transport confirmed a role for auxin pathways in resistance. Susceptible tomato roots are stunted at 6 dpi, and consistent with this, we find significant suppression of genes required for growth and cellular homeostasis at 24 and 48 hpi.

Additionally, roots of the susceptible variety are slower to activate defense responses, and their defense responses are lower in magnitude compared to resistant roots.

Genome-wide transcriptional responses to *R. solanacearum* in tomato have been previously examined primarily in aboveground regions of the plant. (Ghareeb et al. 2011; Ishihara et al. 2012; Kiirika et al. 2013). Ishihara *et al*. 2012 used tomato microarrays to examine gene expression changes 24 hpi with *R. solanacearum* strain 8107S in stems and leaves of susceptible tomato cultivar Ponderosa and resistant LS-89. They did not identify any changes in gene expression at 24 hpi in the susceptible cultivar, and only 143 genes were differentially expressed in leaves of the resistant cultivar compared to the mock-inoculated controls. Differences in our results can be explained in part by the region of the plant sampled (aboveground vs. belowground), inoculation method, or the result of differences in the gene expression profiling method used in each study (microarray vs. RNA-seq). Despite these differences, several of the genes upregulated in resistant tomato stems were found in similar pathways as those we identified in roots of resistant H7996, including *PR* genes. In line with the idea of some overlap in defense responses between below and aboveground regions to *R. solanacearum*, defense marker gene expression in aboveground regions of resistant tomato plants also occurred earlier and more strongly in resistant H7996 compared to susceptible variety Bonnie Best (Milling et al. 2011). Together, these data suggest that root defense responses partially overlap with those in the shoot, but also have unique responses to pathogen attack.

We observed a strong upregulation of terpene synthase genes specifically in roots of resistant plants. Analysis of ginger leaves after rhizome infection with *R. solanacearum* revealed a similar upregulation of terpene synthases in resistant plants (Prasath et al. 2014). A previous report (Lin et al. 2014) used virus-induced gene silencing in resistant H7996 to knock down expression of four TPS genes (*TPS31*, *TPS32*, *TPS33*, and *TPS35*) that were highly upregulated in our dataset. They found that more silenced plants were colonized by *R. solanacearum* in the stem, suggesting that TPS silenced lines had decreased tolerance to *R. solanacearum*. These data suggest that upregulation of TPS genes may contribute to resistance in tomato and ginger. However, this does not appear to be a mechanism used in all crops, as in peanut, terpenoid synthase genes were downregulated at 12 hpi after infection in both resistant and susceptible genotypes (Chen et al. 2014). Indeed, resistance in peanut may operate through different mechanisms than in tomato, as evidenced in the root of a resistant peanut genotype, in which

many NBS-LRR type resistance genes and genes encoding proteins with a LRR-LRK motif were mainly downregulated (Chen et al. 2014).

Our data show both commonalities and differences in resistance between tomato variety H7996 and wild potato species *S. commersonii* (Zuluaga et al. 2015). In resistant roots of both species, more genes with roles in biotic stress were upregulated than downregulated. However, in contrast to our results, which found overrepresentation of the JA pathway in downregulated genes of resistant roots, no genes in the JA pathway were downregulated in roots of resistant potato plants (Zuluaga et al. 2015). Additionally, in resistant wild potato roots, genes in the auxin pathway were upregulated and none were repressed (Zuluaga et al. 2015), while we observed overrepresentation of auxin pathways in downregulated genes in resistant tomato roots. These differences could be the result of differences in species, or to time of inoculation, as we sampled our plants at an earlier time point (24 and 48 hpi compared to 3 – 4 days).

Suppression of auxin biosynthesis, responses and signaling has been associated with plant resistance to biotrophic or hemi-biotrophic pathogens in multiple pathosystems (reviewed in (Fu and Wang 2011; Ludwig-Muller 2015)). In Arabidopsis, mutations in several auxin transporters, including *PIN2* and *AUX1*, reduce disease severity caused by the pathogenic fungus *Fusarium oxysporum* (Kidd et al. 2011). The *walls are thin* (*wat1*) mutant of Arabidopsis is resistant to *R. solanacearum*, has decreased auxin content in roots, suppressed indole metabolism, and decreased tryptophan in roots at 4 dpi (Denance et al. 2012). *WAT1* encodes a vacuolar auxin transporter (Ranocha et al. 2013) and appears to modulate both cellular auxin levels within the vascular tissues as well as whole organ levels of auxin in the root and stem. Intriguingly, *wat1* is resistant to multiple pathogens that, like *R. solanacearum*, colonize the vasculature, but not to non-vascular pathogens such as *Pseudomonas syringae* pv. *tomato* (Denance et al. 2012). Resistance to *R. solanacearum* was dependent on SA, because *wat1 NahG* plants showed comparable levels of disease to wild type Arabidopsis. The *wat1* mutant was first identified due to a defect in secondary cell wall biosynthesis (Ranocha et al. 2010). Mutations in genes required for secondary cell wall formation including *CELLULOSE SYNTHASE4* (*CESA4*)/*IRREGULAR XYLEM5* (*IRX5*), *CESA7*/*IRX3*, and *CESA8*/*IRX1*, also lead to enhanced resistance to *R. solanacearum* in Arabidopsis (Hernandez-Blanco et al. 2007). However, in these mutants, resistance is independent of the SA pathway, but dependent on ABA responses (Hernandez-Blanco et al. 2007).

Here we showed that genes in auxin pathways, including *SlSoPIN1b*, a homolog of the PIN1 auxin transporter, are overrepresented in exclusively downregulated genes in resistant tomato roots after *R. solanacearum* infection. We find that a tomato mutant with altered auxin transport is resistant to *R. solanacearum*. Mutations in tomato *DGT* lead to changes in polar auxin transport that result in abnormal auxin distribution along the root (Ivanchenko et al. 2006). Polar auxin transport is crucial for plant development and is mediated by PIN auxin transporters (reviewed in (Krecek et al. 2009; Adamowski and Friml 2015). Roots are composed of multiple cell types and tissues that differ in auxin levels (Petersson et al. 2009). In Arabidopsis, most PIN transporters localize to the plasma membrane on specific faces of the cell, and their localization varies depending on root cell type (Blilou et al. 2005). The tomato DGT protein regulates levels and localization of PIN1 and PIN2 transporters in the root (Ivanchenko et al. 2015). In wild type tomato roots, PIN1 localizes to the rootward face of cells in the root stele (Ivanchenko et al. 2015). The *dgt* mutation leads to decreased PIN1 protein in the stele of root tips. In addition, expression of *PIN2* is significantly decreased in root tips of the *dgt* mutant and the PIN2 protein localization is altered (Ivanchenko et al. 2015). Although auxin levels in whole roots of the *dgt* mutant are greater than those in wild-type plants (Ivanchenko et al. 2006), auxin responses and signaling in the root vasculature are decreased (Ivanchenko et al. 2015) due to the altered localization of PIN1 and PIN2. How mutations in *DGT* lead to resistance is not entirely clear. One possibility is that resistance is due to antagonism between auxin and SA. Alternatively, like *wat1* and other Arabidopsis mutants, *dgt* may have altered secondary cell wall structure that enhances resistance or may be altered in another auxin-related process that results in enhanced resistance.

Understanding mechanisms of root-mediated resistance is an important step in developing crops with resistance to soilborne pathogens. Like many other bacterial pathogens, *R. solanacearum* produces auxin (Valls et al. 2006). Whether resistant plants downregulate auxin pathways to overcome pathogen auxin production, and whether the alteration of auxin transport is a general feature of root-mediated resistance are intriguing questions whose answers may lead to new insights into enhancing crop resistance.

References

Adamowski, M., and Friml, J. 2015. PIN-dependent auxin transport: action, regulation, and evolution. Plant Cell 27:20-32.

Aichinger, E., Villar, C.B., Di Mambro, R., Sabatini, S., and Kohler, C. 2011. The CHD3 chromatin remodeler PICKLE and polycomb group proteins antagonistically regulate meristem activity in the Arabidopsis root. Plant Cell 23:1047-1060.

Arsenault, J.-L., Pouleur, S., Messier, C., and Guay, R. 1995. WinRHIZO, a root-measuring system with a unique overlap correction method. HortScience 30:906.

Benjamini, Y., and Hochberg, Y. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. Royal Stat. Soc. Series B (Methodological) 57:289-300.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K., and Scheres, B. 2005. The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature 433:39-44.

Caldwell, D., Kim, B.S., and Iyer-Pascuzzi, A.S. 2017. *Ralstonia solanacearum* Differentially Colonizes Roots of Resistant and Susceptible Tomato Plants. Phytopathology 107:528-536.

Carmeille, A., Caranta, C., Dintinger, J., Prior, P., Luisetti, J., and Besse, P. 2006. Identification of QTLs for *Ralstonia solanacearum* race 3-phylotype II resistance in tomato. Theor. Appl. Genet. 113:110-121.

Chen, Y., Ren, X., Zhou, X., Huang, L., Yan, L., Lei, Y., Liao, B., Huang, J., Huang, S., Wei, W., and Jiang, H. 2014. Dynamics in the resistant and susceptible peanut (Arachis hypogaea L.) root transcriptome on infection with the *Ralstonia solanacearum*. BMC genomics 15:1078.

Chen, Z., Agnew, J.L., Cohen, J.D., He, P., Shan, L., Sheen, J., and Kunkel, B.N. 2007. *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. Proc Natl Acad Sci U S A 104:20131-20136.

Cui, F., Wu, S., Sun, W., Coaker, G., Kunkel, B., He, P., and Shan, L. 2013. The *Pseudomonas syringae* type III effector AvrRpt2 promotes pathogen virulence via stimulating Arabidopsis auxin/indole acetic acid protein turnover. Plant Physiol. 162:1018-1029.

Dalsing, B.L., and Allen, C. 2014. Nitrate assimilation contributes to *Ralstonia solanacearum* root attachment, stem colonization, and virulence. J Bacteriol 196:949-960.

Danesh, D., Aarons, S., McGill, G.E., and Young, N.D. 1994. Genetic Dissection of Oligogenic Resistance to Bacterial Wilt in Tomato. Mol Plant Microbe Interact 7:464-471.

Denance, N., Ranocha, P., Oria, N., Barlet, X., Riviere, M.P., Yadeta, K.A., Hoffmann, L., Perreau, F., Clement, G., Maia-Grondard, A., van den Berg, G.C., Savelli, B., Fournier, S., Aubert, Y., Pelletier, S., Thomma, B.P., Molina, A., Jouanin, L., Marco, Y., and Goffner, D. 2012. Arabidopsis *wat1* (*walls are thin1*)-mediated resistance to the bacterial vascular pathogen, *Ralstonia solanacearum*, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism. Plant J.

Derksen, H., Rampitsch, C., and Daayf, F. 2013. Signaling cross-talk in plant disease resistance. Plant Sci. 207:79-87.

Ding, X., Cao, Y., Huang, L., Zhao, J., Xu, C., Li, X., and Wang, S. 2008. Activation of the indole-3-acetic acid-amido synthetase GH3-8 suppresses expansin expression and promotes salicylate- and jasmonate-independent basal immunity in rice. Plant Cell 20:228-240.

Falara, V., Akhtar, T.A., Nguyen, T.T., Spyropoulou, E.A., Bleeker, P.M., Schauvinhold, I., Matsuba, Y., Bonini, M.E., Schilmiller, A.L., Last, R.L., Schuurink, R.C., and Pichersky, E. 2011. The tomato terpene synthase gene family. Plant Physiol. 157:770-789.

Fu, J., and Wang, S. 2011. Insights into auxin signaling in plant-pathogen interactions. Front Plant Sci 2:74.

Genin, S. 2010. Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. New Phytol. 187:920-928.

Genin, S., and Denny, T.P. 2012. Pathogenomics of the Ralstonia solanacearum species complex. Annu. Rev. Phytopathol. 50:67-89.

Ghareeb, H., Bozso, Z., Ott, P.G., Repenning, C., Stahl, F., and Wydra, K. 2011. Transcriptome of silicon-induced resistance against *Ralstonia solanaceaerum* in the silicon non-accumulator tomato implicates priming effect. Physiol. Mol. Plant Pathol. 75:83-89.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43:205-227.

Goverse, A., and Smant, G. 2014. The activation and suppression of plant innate immunity by parasitic nematodes. Annu. Rev. Phytopathol. 52:243-265.

Hayward, A.C. 1991. Biology and Epidemiology of Bacterial Wilt Caused by Pseudomonas-Solanacearum. Annu. Rev. Phytopathol. 29:65-87.

Hernandez-Blanco, C., Feng, D.X., Hu, J., Sanchez-Vallet, A., Deslandes, L., Llorente, F., Berrocal-Lobo, M., Keller, H., Barlet, X., Sanchez-Rodriguez, C., Anderson, L.K., Somerville, S., Marco, Y., and Molina, A. 2007. Impairment of cellulose synthases required for Arabidopsis secondary cell wall formation enhances disease resistance. Plant Cell 19:890-903.

Huaiyu, A., Poudel, S., Muruganujan, A., Casagrande, J.T., and Thomas, P.D. 2016. PANTHER version 10: expanded protein families and functions, and analysis tools. Nucleic Acids Res. 44(D1):D336-42.

Huet, G. 2014. Breeding for resistances to *Ralstonia solanacearum*. Front Plant Sci. 5:715.

Ishihara, T., Mitsuhara, I., Takahashi, H., and Nakaho, K. 2012. Transcriptome analysis of quantitative resistance-specific response upon *Ralstonia solanacearum* infection in tomato. PLoS One 7:e46763.

Ivanchenko, M.G., Coffeen, W.C., Lomax, T.L., and Dubrovsky, J.G. 2006. Mutations in the *Diageotropica* (*Dgt*) gene uncouple patterned cell division during lateral root initiation from proliferative cell division in the pericycle. Plant J. 46:436-447.

Ivanchenko, M.G., Zhu, J., Wang, B., Medvecka, E., Du, Y., Azzarello, E., Mancuso, S., Megraw, M., Filichkin, S., Dubrovsky, J.G., Friml, J., and Geisler, M. 2015. The cyclophilin A *DIAGEOTROPICA* gene affects auxin transport in both root and shoot to control lateral root formation. Development 142:712-721.

Jia, N., Liu, X., and Gao, H. 2016. A DNA2 Homolog Is Required for DNA Damage Repair, Cell Cycle Regulation, and Meristem Maintenance in Plants. Plant Physiol. 171:318-333.

Kazan, K., and Manners, J.M. 2009. Linking development to defense: auxin in plant-pathogen interactions. Trends Plant Sci. 14:373-382.

Kidd, B.N., Kadoo, N.Y., Dombrecht, B., Tekeoglu, M., Gardiner, D.M., Thatcher, L.F., Aitken, E.A., Schenk, P.M., Manners, J.M., and Kazan, K. 2011. Auxin signaling and transport promote susceptibility to the root-infecting fungal pathogen *Fusarium oxysporum* in Arabidopsis. Mol Plant Microbe Interact 24:733-748.

Kiirika, L.M., Stahl, F., and Wydra, K. 2013. Phenotypic and molecular characterization of resistance induction by single and combined application of chitosan and silicon in tomato against *Ralstonia solanacearum*. Physiol. Mol. Plant Pathol. 81:1-12.

Kim, B.S., French, E., Caldwell, D., Harrington, E.J., and Iyer-Pascuzzi, A.S. 2016. Bacterial wilt disease: Host resistance and pathogen virulence mechanisms. Physiol. Mol. Plant Pathol. 95:37-43.

Kim, H., Kim, B.S., Shim, J.E., Hwang, S., Yang, S., Kim, E., Iyer-Pascuzzi, A.S., and Lee, I. 2017. TomatoNet: A Genome-wide Co-functional Network for Unveiling Complex Traits of Tomato, a Model Crop for Fleshy Fruits. Mol. Plant 10:652-655.

Koornneef, A., and Pieterse, C.M. 2008. Cross talk in defense signaling. Plant Physiol. 146:839-844.

Koornneef, A., Leon-Reyes, A., Ritsema, T., Verhage, A., Den Otter, F.C., Van Loon, L.C., and Pieterse, C.M. 2008. Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. Plant Physiol. 147:1358-1368.

Krecek, P., Skupa, P., Libus, J., Naramoto, S., Tejos, R., Friml, J., and Zazimalova, E. 2009. The PIN-FORMED (PIN) protein family of auxin transporters. Genome Biol. 10:249.

Lebeau, A., Daunay, M.C., Frary, A., Palloix, A., Wang, J.F., Dintinger, J., Chiroleu, F., Wicker, E., and Prior, P. 2011. Bacterial wilt resistance in tomato, pepper, and eggplant: genetic resources respond to diverse strains in the *Ralstonia solanacearum* species complex. Phytopathol. 101:154-165.

Li, P.C., Yu, S.W., Li, K., Huang, J.G., Wang, X.J., and Zheng, C.C. 2016. The Mutation of Glu at Amino Acid 3838 of AtMDN1 Provokes Pleiotropic Developmental Phenotypes in Arabidopsis. Sci. Rep. 6:36446.

Lin, Y.M., Shih, S.L., Lin, W.C., Wu, J.W., Chen, Y.T., Hsieh, C.Y., Guan, L.C., Lin, L., and Cheng, C.P. 2014. Phytoalexin biosynthesis genes are regulated and involved in plant response to *Ralstonia solanacearum* infection. Plant Sci. 224:86-94.

Livak, K.J., and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408.

Llorente, F., Muskett, P., Sanchez-Vallet, A., Lopez, G., Ramos, B., Sanchez-Rodriguez, C., Jorda, L., Parker, J., and Molina, A. 2008. Repression of the auxin response pathway increases Arabidopsis susceptibility to necrotrophic fungi. Mol. Plant 1:496-509.

Ludwig-Muller, J. 2015. Bacteria and fungi controlling plant growth by manipulating auxin: balance between development and defense. J. Plant Physiol. 172:4-12.

Madden, L.V., Hughes, G., and Van Den Bosh, F. 2007. The Study of Plant Disease Epidemics. APS Press, Saint Paul, USA.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A., Toth, I., Salmond, G., and Foster, G.D. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol. Plant Pathol. 13:614-629.

McAvoy, T., Freeman, J.H., Rideout, S.L., Olson, S.M., and Paret, P.L. 2012. Evaluation of Grafting Using Hybrid Rootstocks for Managment of Bacterial Wilt in Field Tomato Production. HortScience 47:621-625.

Millet, Y.A., Danna, C.H., Clay, N.K., Songnuan, W., Simon, M.D., Werck-Reichhart, D., and Ausubel, F.M. 2010. Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. Plant Cell 22:973-990.

Milling, A., Babujee, L., and Allen, C. 2011. *Ralstonia solanacearum* extracellular polysaccharide is a specific elicitor of defense responses in wilt-resistant tomato plants. PLoS One 6:e15853.

Mitchum, M.G., Hussey, R.S., Baum, T.J., Wang, X., Elling, A.A., Wubben, M., and Davis, E.L. 2013. Nematode effector proteins: an emerging paradigm of parasitism. New Phytol. 199:879-894.

Muday, G.K., Lomax, T.L., and Rayle, D.L. 1995. Characterization of the growth and auxin physiology of roots of the tomato mutant, *diageotropica*. Planta 195:548-553.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O., and Jones, J.D. 2006. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science 312:436-439.

Ni, D.A., Sozzani, R., Blanchet, S., Domenichini, S., Reuzeau, C., Cella, R., Bergounioux, C., and Raynaud, C. 2009. The Arabidopsis MCM2 gene is essential to embryo development and its over-expression alters root meristem function. New Phytol. 184:311-322.

O'Donnell, P.J., Schmelz, E.A., Moussatche, P., Lund, S.T., Jones, J.B., and Klee, H.J. 2003. Susceptible to intolerance--a range of hormonal actions in a susceptible Arabidopsis pathogen response. Plant J 33:245-257.

Oh, K., Ivanchenko, M.G., White, T.J., and Lomax, T.L. 2006. The diageotropica gene of tomato encodes a cyclophilin: a novel player in auxin signaling. Planta 224:133-144.

Oliveros, J.C. 2007-2015. Venny. An interactive tool for comparing lists with Venn's diagrams. . http://bioinfogp.cnb.csic.es/tools/venny/index.html.

Petersson, S.V., Johansson, A.I., Kowalczyk, M., Makoveychuk, A., Wang, J.Y., Moritz, T., Grebe, M., Benfey, P.N., Sandberg, G., and Ljung, K. 2009. An auxin gradient and maximum in the Arabidopsis root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. Plant Cell 21:1659-1668.

Prasath, D., Karthika, R., Habeeba, N.T., Suraby, E.J., Rosana, O.B., Shaji, A., Eapen, S.J., Deshpande, U., and Anandaraj, M. 2014. Comparison of the transcriptomes of ginger (*Zingiber officinale* Rosc.) and mango ginger (*Curcuma amada* Roxb.) in response to the bacterial wilt infection. PLoS One 9:e99731.

Qi, L., Yan, J., Li, Y., Jiang, H., Sun, J., Chen, Q., Li, H., Chu, J., Yan, C., Sun, X., Yu, Y., Li, C., and Li, C. 2012. Arabidopsis thaliana plants differentially modulate auxin biosynthesis and transport during defense responses to the necrotrophic pathogen *Alternaria brassicicola*. New Phytol. 195:872-882.

Ranocha, P., Denance, N., Vanholme, R., Freydier, A., Martinez, Y., Hoffmann, L., Kohler, L., Pouzet, C., Renou, J.P., Sundberg, B., Boerjan, W., and Goffner, D. 2010. Walls are thin1 (WAT1), an Arabidopsis homolog of *Medicago truncatula* NODULIN21, is a tonoplast-localized protein required for secondary wall formation in fibers. Plant J. 63:469-483.

Ranocha, P., Dima, O., Nagy, R., Felten, J., Corratge-Faillie, C., Novak, O., Morreel, K., Lacombe, B., Martinez, Y., Pfrunder, S., Jin, X., Renou, J.-P., Thibaud, J.-P., Ljung, K., Fischer, U., Martinoia, E., Boerjan, W., and Goffner, D. 2013. Arabidopsis WAT1 is a vaculolar auxin transport facilitator required for auxin homeostasis. Nat. Commun. 2625.

Rivard, C.L., O'Connell, S., Peet, M.M., Welker, R.M., and Louws, F.J. 2012. Grafting Tomato to Manage Bacterial Wilt Caused by *Ralstonia solanacearum* in the Southeastern United States. Plant Dis. 96:973-978.

Robert-Seilaniantz, A., Grant, M., and Jones, J.D. 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu. Rev. Phytopathol. 49:317-343.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139-140.

Saeed, A.I., Bhagabati, N.K., Braisted, J.C., Liang, W., Sharov, V., Howe, E.A., Li, J., Thiagarajan, M., White, J.A., and Quackenbush, J. 2006. TM4 microarray software suite. Methods Enzymol. 411:134-193.

Saeed, A.I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M., Currier, T., Thiagarajan, M., Sturn, A., Snuffin, M., Rezantsev, A., Popov, D., Ryltsov, A., Kostukovich, E., Borisovsky, I., Liu, Z., Vinsavich, A., Trush, V., and Quackenbush, J. 2003. TM4: a free, open-source system for microarray data management and analysis. BioTechniques 34:374-378.

Sang, Y., Silva-Ortega, C.O., Wu, S., Yamaguchi, N., Wu, M.F., Pfluger, J., Gillmor, C.S., Gallagher, K.L., and Wagner, D. 2012. Mutations in two non-canonical Arabidopsis SWI2/SNF2 chromatin remodeling ATPases cause embryogenesis and stem cell maintenance defects. Plant J. 72:1000-1014.

Shen, W.H., and Xu, L. 2009. Chromatin remodeling in stem cell maintenance in Arabidopsis thaliana. Mol. Plant 2:600-609.

Spaepen, S., Vanderleyden, J., and Remans, R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol. Rev. 31:425-448.

Spoel, S.H., Johnson, J.S., and Dong, X. 2007. Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc. Natl. Acad. Sci. U. S. A. 104:18842-18847.

Spoel, S.H., Koornneef, A., Claessens, S.M., Korzelius, J.P., Van Pelt, J.A., Mueller, M.J., Buchala, A.J., Metraux, J.P., Brown, R., Kazan, K., Van Loon, L.C., Dong, X., and Pieterse, C.M. 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 15:760-770.

Takatsuka, H., and Umeda, M. 2015. Epigenetic Control of Cell Division and Cell Differentiation in the Root Apex. Front Plant Sci. 6:1178.

Tans-Kersten, J., Huang, H., and Allen, C. 2001. *Ralstonia solanacearum* needs motility for invasive virulence on tomato. J Bacteriol. 183:3597-3605.

Teixeira, M.A., Wei, L., and Kaloshian, I. 2016. Root-knot nematodes induce pattern-triggered immunity in *Arabidopsis thaliana* roots. New Phytol. 211:276-287.

Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Muller, L.A., Rhee, S.Y., and Stitt, M. 2004. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J. 37:914-939.

Thoquet, P., Olivier, J., Sperisen, C., Rogowsky, P., Laterrot, H., and Grimsley, N. 1996a. Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii7996. Mol. Plant-Microbe Interact. 9:826-836.

Thoquet, P., Olivier, J., Sperisen, C., Rogowsky, P., Prior, P., Anais, G., Mangin, B., Bazin, B., Nazer, R., and Grimsley, N. 1996b. Polygenic resistance of tomato plants to bacterial wilt in the French West Indies. Mol. Plant-Microbe Interact. 9:837-842.

Tiryaki, I., and Staswick, P.E. 2002. An Arabidopsis mutant defective in jasmonate response is allelic to the auxin-signaling mutant axr1. Plant Physiol. 130:887-894.

Tomato Genome Consortium. 2012. The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485:635-641.

Trapnell, C., Pachter, L., and Salzberg, S.L. 2009. TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25:1105-1111.

Valls, M., Genin, S., and Boucher, C. 2006. Integrated regulation of the type III secretion system and other virulence determinants in *Ralstonia solanacearum*. PLoS Pathog. 2:e82.

Wang, J.-F., Ho, F.-I., Truong, H.T.H., Hugan, S.-M., Balatero, C.H., Dittapongpitch, V., and Hidayati, N. 2013. Identification of major QTLs associated with stable resitance of tomato cultivar 'Hawaii 7996' to *Ralstonia solanacearum*. Euphytica 190:241-252.

Wang, J.F., Olivier, J., Thoquet, P., Mangin, B., Sauviac, L., and Grimsley, N.H. 2000. Resistance of tomato line Hawaii7996 to *Ralstonia solanacearum* Pss4 in Taiwan is controlled mainly by a major strain-specific locus. Mol. Plant-Microbe Interact. 13:6-13.

Wieckowski, Y., and Schiefelbein, J. 2012. Nuclear ribosome biogenesis mediated by the DIM1A rRNA dimethylase is required for organized root growth and epidermal patterning in Arabidopsis. Plant Cell 24:2839-2856.

Zuluaga, A.P., Sole, M., Lu, H., Gongora-Castillo, E., Vaillancourt, B., Coll, N., Buell, C.R., and Valls, M. 2015. Transcriptome responses to *Ralstonia solanacearum* infection in the roots of the wild potato *Solanum commersonii*. BMC Genomics 16:246.

CHAPTER 4: LINKING SALICYLIC ACID AND THE ETHYLENE PATHWAY TO ROOT MICROBIOME ASSEMBLY AND STRUCTURE IN *SOLANUM* SPP.

Abstract

Plant roots live in close association with the abundant diversity of microbes, both beneficial and pathogenic, found in the soil. Roots are able to mount defense responses to restrict colonization by pathogenic microbes, but communities of non-pathogenic microbes are able to colonize roots internally without significant challenge from the plant. Defense hormone pathways and the innate immune system are required for normal microbiome assembly in Arabidopsis, but their function to maintain community homeostasis is not understood and has not been studied in crop plants. We hypothesized that quantitative differences in defense hormones alter root microbiome assembly in tomato. 16S rRNA amplicon library sequencing showed that salicylic acid (SA) and the ethylene (ET) precursor 1-aminocyclopropane-1-carboxylic acid (ACC) are required for microbiome assembly in the tomato root. Root endospheres of the SA-deficient *NahG* transgenic and ACC-deficient *ACD* mutant had significantly less diversity than their wild type parents and were more alike in terms of alpha and beta diversity than those of other mutants or wild type plants. Amplicon sequence variants (ASVs) enriched within these mutants were similar, but ASV depletion profiles were not, suggesting that the similarity in diversity indexes between *NahG* and *ACD* was due primarily to similar enrichment profiles. These enrichment profiles were driven by two taxa, which were responsible for approximately 30% and 45% of the root endomicrobiome in *NahG* and *ACD*, respectively. In microbial growth analyses, an isolate similar to one enriched in *NahG* was more sensitive to SA exposure compared to a non-enriched microbe, suggesting that the lack of SA in *NahG* was functionally important for microbial colonization. We hypothesized that *NahG*/*ACD*-enriched taxa prevented colonization by other ASVs. To test this hypothesis, we examined the relative abundance of the *NahG*/*ACD* enriched taxa in a panel of recombinant inbred lines that differed quantitatively in Shannon diversity. We find that the relative abundance of *NahG*- and *ACD*-enriched taxa correlates negatively with Shannon Diversity, suggesting that the highly enriched *NahG*/*ACD* ASVs prevent root colonization by other ASVs. Analysis of SA content in roots of eight tomato

or wild tomato genotypes found a negative correlation between root SA content and relative abundance of *NahG*/*ACD* enriched taxa. Together our data show that root microbiome assembly in tomato requires SA and ACC and suggests that these hormones maintain a balanced root microbiome by promoting microbial diversity and preventing overcolonization of a small number of ASVs.

Introduction

Plants live in close association with the abundant diversity of microbes found in the soil. Plant roots release carbon-rich root exudates, which support a high level of microbial growth in the rhizosphere - the zone of soil immediately surrounding plant roots. Additionally, some of these microbes actually enter the root and live inside the plant itself, known as the endosphere (Berendsen et al. 2012). Together, this root microbiome serves as a "second genome" to the plant, providing important benefits such as growth promotion, abiotic stress relief, and defense against pathogens (Berendsen et al. 2012). Multiple studies have revealed differences between root microbiomes of highly divergent species (Olivares et al. 1996; Weber et al. 1999; Dong et al. 2003; Fitzpatrick et al. 2018). Recently, a survey of the root rhizosphere and endosphere bacterial communities of thirty species spanning 140 Mya of evolution observed that 40% of endosphere community diversity could be attributed to species and these differences correlated with plant phylogenetic distance (Fitzpatrick et al. 2018). Interestingly, only 17% of rhizosphere diversity was accounted for by species, with no correlation to phylogenetic distance.

Recent studies examining closely-related species have also uncovered differences in root microbiome communities (Schlaeppi et al. 2014; Chiellini et al. 2015; Szoboszlay et al. 2015; Shenton et al. 2016; Jiang et al. 2017; Pérez-Jaramillo et al. 2017; Maggini et al. 2018). In a comparison of wild and domesticated rice cultivars, Shenton et al. (2016) found that about 6.1% of the variability in beta-diversity could be attributed to domestication status. More recently, Perez-Jaramillo (2017) examined the root microbial communities of a domestication gradient of common bean, using wild, landrace, and domesticated accessions and found differences in abundance of several bacterial families along the bean domestication gradient. Though the overall effect of species in studies examining closely-related species on the root microbiome is small compared to the effects of soil type, recent evidence suggests that even small differences in

root microbiome communities can impact plant fitness outcomes (Haney et al. 2015; Fitzpatrick et al. 2018).

Our understanding of host factors that may play roles in these species-specific differences in root microbiome assembly is limited. Soil and environmental factors play a decisive role in determining the microbial community in the bulk soil that is available to plants for recruitment to the rhizosphere and ultimately the endosphere (Bulgarelli et al. 2013). Plant host factors such as root exudation, root architecture, and innate immunity are known to play roles in root microbiome assembly.

The role of innate immunity in assembly of plant-associated bacterial communities has recently begun to be explored. Quadruple mutants for multiple signaling components of the Arabidopsis innate immune system demonstrated a role for innate immunity in restricting colonization levels of commensal bacteria in the phyllosphere (Xin et al. 2016). Mutant analysis has also uncovered roles for salicylic acid in Arabidopsis roots (Lebeis et al. 2015), ethylene in Arabidopsis leaves (Bodenhausen et al. 2014), rhizosphere (Doornbos et al. 2011), and tobacco root endospheres (Long et al. 2010). Exogenous methyl jasmonate application has implicated JA in root endophyte community assembly in wheat (Liu et al. 2017) and Arabidopsis rhizospheres (Carvalhais et al. 2013b), as well as in restricting colonization of incompatible nitrogen-fixing bacteria in rice (Miché et al. 2006) and nodulation in *Lotus japonicus* (Nakagawa and Kawaguchi 2006).

Taken together, these studies suggest that innate immunity regulated by defense hormones is essential for proper assembly of plant-associated bacterial communities. However, the role that each hormone plays in community assembly appears to depend on both plant species and plant compartment. Thus, further research is needed in additional model organisms to tease out the roles of various components of the innate immune system on bacterial communities in multiple plant compartments. Host species-associated differences in innate immune regulation may play a role in root microbial community assembly.

Therefore, we selected a panel of tomato mutants for the SA, JA, and ET pathways in order to examine differences in their root microbiomes and compare them to the effects of natural differences in innate immunity. The *NahG* mutant is a transgenic in the Money Maker cultivar that constitutively degrades salicylic acid (Brading et al. 2000). *def1* is a JA biosynthesis mutant in the Castlemart II background (Howe et al. 1996). *Nr* is a spontaneous ethylene

perception mutant in the near isogenic line Pearson (Lanahan et al. 1994), and *ACD* is a transgenic for constitutive degradation of ACC, the precursor to ethylene, in the UC82B background (Klee et al. 1991).

Solanum lycopersicum cv. Hawaii7996 (H7996) is a domesticated tomato cultivar exhibiting quantitative resistance to *Ralstonia solanacearum*, causal agent of bacterial wilt (Danesh et al. 1994; Wang et al. 2000). *Solanum pimpinellifolium* cv. WestVirginia700 (WV) is a closely-related wild relative of tomato highly susceptible to bacterial wilt. These two lines are the parents of a recombinant inbred line (RIL) population used to uncover quantitative trait loci (QTL) for resistance to bacterial wilt (Danesh et al. 1994; Thoquet et al. 1996; Mangin et al. 1999; Wang et al. 2000). My work from Chapter 2 has shown that roots of these two genotypes vary in their innate immune responses to early infection with *R. solanacearum* (French et al. 2018). H7996 exhibits stronger and early upregulation of genes in the SA and ET pathways, and stronger downregulation of JA pathways compared to WV in response to pathogen infection. We hypothesized that these natural differences in innate immune responses would influence their root microbiome communities.

For this study, we hypothesize that 1) intact hormone pathways are crucial for proper root bacterial community assembly in *Solanum lycopersicum*, and 2) natural differences in defense hormone pathways would result in quantitative differences between the root bacterial community between *S. lycopersicum* H7996 and *S. pimpinellifolium* WV.

Materials and Methods

Soil mix

The soil mix for all growth experiments was prepared by hand-mixing autoclave-sterile potting mix and field soil in a 2:1 ratio by volume. Potting mix used was the Fafard germination mix, custom blend with 56.69% spaghnum peat moss, composted bark, perlite, vermiculite, dolomite lime, wetting agent and 0001% silicon dioxide (SKU code 8269028, lot Q17.05). The potting mix was autoclaved for 30 min at 122.8 °C. The field soil was a sandy loam collected from the top 10 cm of a conventional agricultural field at Throckmorton Purdue Agricultural Center in three batches from April – June 2017, ground, sieved to 4 mm, dried to a constant weight, and mixed to homogenize the three batches. Samples of field soil and potting mix/field

soil mixture were sent to A&L Great Lakes Laboratories (Fort Wayne, IN) for determination of % organic matter, Bray-1 phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), pH, cation exchange capacity (CEC), and nitrate (Appendix Table C4.1). Three technical replicates were performed for each sample.

Plant growth and harvest

Seeds of each genotype were surface sterilized by incubating with gentle rocking in 50% bleach for ten minutes and then rinsing 5-6 times in sterile ddH_2O. Seeds were then stored at 4 °C overnight. Surface-sterile seeds were planted in randomized complete blocks in 36-pot flats with 1 unplanted cell per block to represent the bulk soil control. Three seeds were planted to each pot and thinned to one plant after germination. Eight full biological replicate blocks were planted to account for any issues with germination. Flats were fertilized once per week with 500mL of 150 ppm N standard MiracleGro fertilizer.

Five blocks were harvested after seedlings reached 4-leaf stage (approximately 2.5 weeks). Rhizosphere and endosphere samples were collected from each genotype except RILs for which only endosphere samples were collected (see Appendix tables C4.2 and C4.3 for list of all genotypes included in each experiment). For rhizosphere sampling, roots were removed from pot and excess soil was removed gently under aseptic conditions until only soil within 1mm from the root surface remained (Lundberg et al. 2013). Roots were then placed into 15 mL conical tubes containing sterile 1X PBS. Manual shaking as performed to remove rhizosphere soil. Roots were removed and placed in a new 15mL conical tube for surface sterilization. Conical tubes containing the rhizosphere soil were spun down at 5000 rpm for 5 min. Excess liquid was decanted, soil pellets were resuspended and transferred to a 1.5 mL sterile Eppendorf tube. The Eppendorf tube was then spun at max speed for 10 min, supernatant decanted and rhizosphere soil was frozen in LN_2 and stored at -80 °C until DNA extraction. These samples were designated the rhizosphere compartment samples.

Roots were cleaned by performing an additional rinse to remove any remaining soil. Then roots were surface sterilized by incubating in 5% bleach with gentle shaking for 2 min, then rinsed 3 times in sterile ddH_2O before freezing in LN_2 and storing at -80 °C until DNA extraction.

DNA extraction and library preparation

Frozen roots were ground under LN_2 in sterile mortars and pestles before DNA extraction. These samples were designated the endosphere compartment samples. DNA was extracted from all samples using Norgen Biotek Soil DNA extraction kits (Ontario, Canada). DNA concentration and purity were measured by Nanodrop3000 (ThermoFisher Scientific) before library preparation. Library preparation was performed using the Illumina 16S Metagenomics Sequencing Library Preparation protocol with a few modifications. In brief, two step PCR was performed to amplify the V5 through V7 region of the 16S rRNA gene with the chloroplast excluding primer pair 799F-1193R (Chelius and Triplett 2001; Beckers et al. 2016) and add Illumina Nextera XT indices.

First, all DNA samples were diluted to 5 ng/uL. One negative water control and one mock community DNA control (Zymobiomics) were included on each plate. 25 uL PCR reactions were performed with 2.5 uL genomic DNA, 12.5 uL 2X KAPA HiFi HotStart ReadyMix and 5 uL each of the forward and reverse primers (1 uM) in two 96 well plates. Primer sequences with adapters purchased from IDT were as follows: 799F + Nextera adapter 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACMGGATTAGATACCCKG-3' and 1193R + Nextera adapter 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGTCATCCCCACCTTCC-3', which produces a ~480 bp product. Underlined portions indicate the 16S primer portion. PCR cycle performed as follows: 95 °C for 3 min, [95 °C for 30 sec, 55 °C for 30 sec, 72 °C for 30 sec] x 27 cycles, 72 °C for 5 min.

After the initial PCR step, PCR products were run on 1.5% agarose gels. The ~480 bp band was excised and gel extracted with the Invitrogen PureLink gel purification kit. This step was performed to exclude the larger mitochondrial band amplified by the 16S primers. Gel purified PCR products were used for the second PCR step to attach dual indices and sequencing adapters. For this step, 50 ul PCR reactions were performed with 5 uL purified Step 1 PCR product, 25 uL 2X KAPA HiFi HotStart ReadyMix, 10 uL of sterile ddH2O and 5 uL each of the Index 1 and Index 2 Nextera XT Primers (set A and B) in two 96 well plates. PCR cycle performed as follows: 95 °C for 3 min, [95 °C for 30 sec, 55 °C for 30 sec, 72 °C for 30 sec] x 8

cycles, 72 °C for 5 min. Standard AmpureXP bead purification was performed on the Step 2 PCR products.

Step 2 PCR products were quantified at the Purdue Genomics Core by mixing 1 uL of each library into a pool and sequencing as 10% of a MiSeq paired end 250 bp run. The library sizes were then estimated from the number of reads obtained from each library and used to calibrate library concentrations for the final pool. All 188 samples were multiplexed into a single pool in equivalent concentrations. The pool was run on an Agilent bioanalyzer chip to confirm library size and purity. The pool was sequenced using Illumina MiSeq V2 chemistry with paired end 250 bp sequencing.

Sequence processing

Demultiplexing was performed by Genomics Core with Illumina software. Adapter removal and primer clipping performed with trimmomatic (v 0.36) and cutadapt (v 1.13). Reads were then processed through the DADA2 (v 1.8.0) pipeline by filtering and trimming based on read quality, inferring error rates, merging paired end reads, removing chimeras, and assigning taxonomy with the Silva reference database v. 132 (Callahan et al. 2016). Likely contaminant sequences were removed with the decontam package (v 1.0.0) using negative controls to infer likely contaminants (Davis et al. 2018). Very low abundance sequences (fewer than 2 reads in 10% of the samples) were removed. Samples with alpha beta diversity measurements more than 1.5x outside the interquartile range were considered outliers and removed. Alpha diversity measurements performed with the Phyloseq (v 1.24.0) package after subsampling to the smallest library size (2,569 reads) 100 times and averaging the results (McMurdie and Holmes 2013). Beta diversity measurements were performed with Phyloseq and vegan (v 2.5-2) packages with reads proportionally scaled to the smallest library size (code courtesy of Denef lab tutorial - http://deneflab.github.io/MicrobeMiseq/). Normalization and differential abundance analysis performed with DESeq2 (v 1.20.0) (Love et al. 2014). All plots were made with the ggplot2 (Wickham 2009) package and arranged in Inkscape (v 0.92.3). All code for analysis and figure generation can be found here: https://github.rcac.purdue.edu/AnjaliIyerpascuzziGroup/Tomato-Root-16S-Sequencing.

Total salicylic acid quantification

For the estimation of total salicylic acid from tomato roots, 300 mg (±10) of fresh tissue (powdered in liquid nitrogen) was mixed with 3 mL 80% (v/v) methanol and 80 µL of 4-chlorobenzoic acid at the concentration of 5 µg/mL, and extracted overnight by shaking (200 rpm) at 4 °C. Later, the tubes were centrifuged at 5000 x g at 4oC for 3min, the supernatant was removed and 1500 µL of supernatant was evaporated under nitrogen till near dryness and processed further using the protocols as follows. Then, 500 µL 2.5 N HCl was added to the pre-evaporated tubes and incubated at 80°C for 1 Hr.

LC-MS analysis was performed using an Agilent 1290 Infinity II UHPLC system (Palo Alto, CA, USA) coupled with diode array detection and a 6135 single quadrupole mass spectrometer equipped with a Jet Stream electrospray ionization (ESI) source. 20 µl of sample injected and separated on a Zorbax SB-C18 column (1.8-µm, 2.1x1.8mm; Agilent) connected to a Zorbax SB-C18 analytical guard column (5-µm, 4.6 x 12.5mm; Agilent), 0.1% formic acid (v/v) in LC-MS grade water (A) and 0.1% formic acid (v/v) in acetonitrile (B) were used as the mobile phases. After sample injection, the column was eluted with 20% B for 1 min, followed by a linear gradient to 95% B over 8 min with 4 min hold, at 13 min the solvents were ramped back to 5% B with final hold of 1 min, the flow rate was maintained at 300 µL min-1 and column temperature at 40 °C. The MS was operated in negative ion mode with the nozzle and capillary voltages at 2000 V and 4000 V, respectively. Sheath gas temperature was kept at 360 °C with a flow of 12 mL min-1 and drying gas temperature kept at 350 °C with the flow of 13 mL min^{-1}. Detection the compounds salicylic acid and 4-chlorobenzoic acid was performed in selected ion monitoring (SIM) mode with ions 137 m/z (M-H)- and 155 m/z (M-H)- respectively.

The data was analyzed using ChemStation software (version C.01.08). Identification was performed based on retention times and masses of salicylic acid and 4-chlorobenzoic acid standards (Sigma). The amount of salicylic acid was calculated on the basis of its relative response factor of the internal standard 4-chlorobenzoic acid at 1000ng/mL. Statistical significance of differences between genotypes was tested with one-way analysis of variance (ANOVA) in JMP 13, followed by Tukey's honest significant differences post-hoc tests. Values were log transformed to meet homogeneity of variance assumption.

Growth assay in salicylic acid

The HA-64 bacterial isolate was originally isolated from surface-sterilized roots of a mature H7996 plant grown ~5 months in the field under conventional growing conditions. This isolate was selected based on 16S sequence identity to ASV1 (95.8% identity). To test its tolerance to salicylic acid, HA-64 was cultured in liquid minimal media +/- salicylic acid (3.4 g KH_2PO_4 , 0.5g $(NH_4)_2SO_4$, 100 uL 1.25mg/ml solution $FeSO_4 \cdot 7H_2O$, and 516 uL of 1M $MgSO_4$ per liter of media, pH 7.2, supplemented with 10 mL of 20% filter-sterile glucose added after autoclaving). +SA Media was then supplemented with 10 mL of filter-sterile 50 mM salicylic acid after cooling. Isolates were primary cultured in 2 mL of LB overnight from glycerol stocks with shaking at 28C. Then 100 uL of primary culture was pipetted into 20 mL of minimal media +/- SA. Growth was measured by optical density (OD) at 600 nm every four hours for 16 hours and then at 24 hours. 5 biological replicates were included for each treatment. When needed, samples for measurement were diluted to readable range (OD600 < 1) and actual OD back-calculated. Area under the growth progress curve (AUGPC) was calculated by the trapezoidal integration method (Campbell and Madden 1990). Two-way ANOVA to test the effects of isolate and SA treatment with posthoc Tukey's test were performed in JMP 13.

Results

SA and ACC are required for root microbiome assembly in tomato

To determine the role of tomato defense hormones in root microbiome assembly, we used four tomato transgenics or mutants defective in salicylic acid (SA), jasmonic acid (JA), ethylene (ET), or the ET precursor ACC, and their respective wild-type backgrounds (Table S2 for genotype information). *NahG* plants constitutively break down SA, *def1* mutants are defective in JA biosynthesis, *never ripe* (*nr*) mutants are defective in ET perception and signaling, and *ACD* transgenics constitutively break down ACC. Plants were grown in a natural soil mix in the greenhouse (Appendix Table C4.1 for soil characteristics) and DNA was harvested from bulk soil, the rhizosphere and root endosphere of 4 replicates of each genotype.

The V5-V7 region of the 16S rRNA from these samples was sequenced by paired-end 250bp MiSeq Illumina sequencing, resulting in 2.9 million high quality sequences after quality filtering and chimera, non-target sequence (mitochondria, chloroplast, archaea), and likely

contaminant removal (Appendix Table C4.4). These sequences corresponded to 23,833 amplicon sequence variants (ASVs). After filtering for low abundance ASVs (fewer than 2 reads in less than 10% of the samples), samples with fewer than 2000 total reads, and outliers, our final data set for analysis consisted of approximately 1.8 million reads and 901 ASVs across 186 samples with an average of 10,185 reads per sample.

Across all samples and consistent with other rhizosphere studies, Bray-Curtis beta diversity patterns showed separation between the endosphere samples and the rhizosphere/bulk samples along the first axis (27.2%) and separation between the rhizosphere and bulk soil samples along the second axis (8.9%) (Appendix Fig C4.1) (PERMANOVA, compartment: $F_{(2, 154)} = 21.96$, $p < 0.001$). Because rhizosphere samples overall showed very little variation in beta-diversity (Appendix Fig C4.1), we chose to focus on endosphere samples for further beta-diversity comparisons.

ACD and *NahG* mutant root endosphere communities exhibit significant reductions in both richness and Shannon diversity compared to their respective wild-type backgrounds (Fig 4.1A). No significant differences in either measure of alpha diversity were observed between rhizosphere samples. In contrast, neither the ET signaling and perception mutant *Nr* nor the biosynthesis mutant *def1* were significantly different than their wild type controls. These data show that SA and ACC are required for wild-type root microbiome assembly.

A non-metric multidimensional scaling (NMDS) analysis based on Bray-Curtis dissimilarity of root endosphere samples revealed similar patterns as the alpha diversity. Both *ACD* and *NahG* separate from their wild-type backgrounds along the first axis (Fig. 4.1B). A PERMANOVA revealed a significant difference between wild type and mutant endospheres and a significant interaction between wild types and mutants by their mutant group (PERMANOVA Mutant Group – $F_{(3, 18)} = 1.17$, $p = 0.159$. Defense – $F_{(1, 18)} = 2.38$, $p = 0.004$, Mutant Group:Defense – $F_{(3, 18)} = 1.46$, $p = 0.030$) (Fig 4.1B).

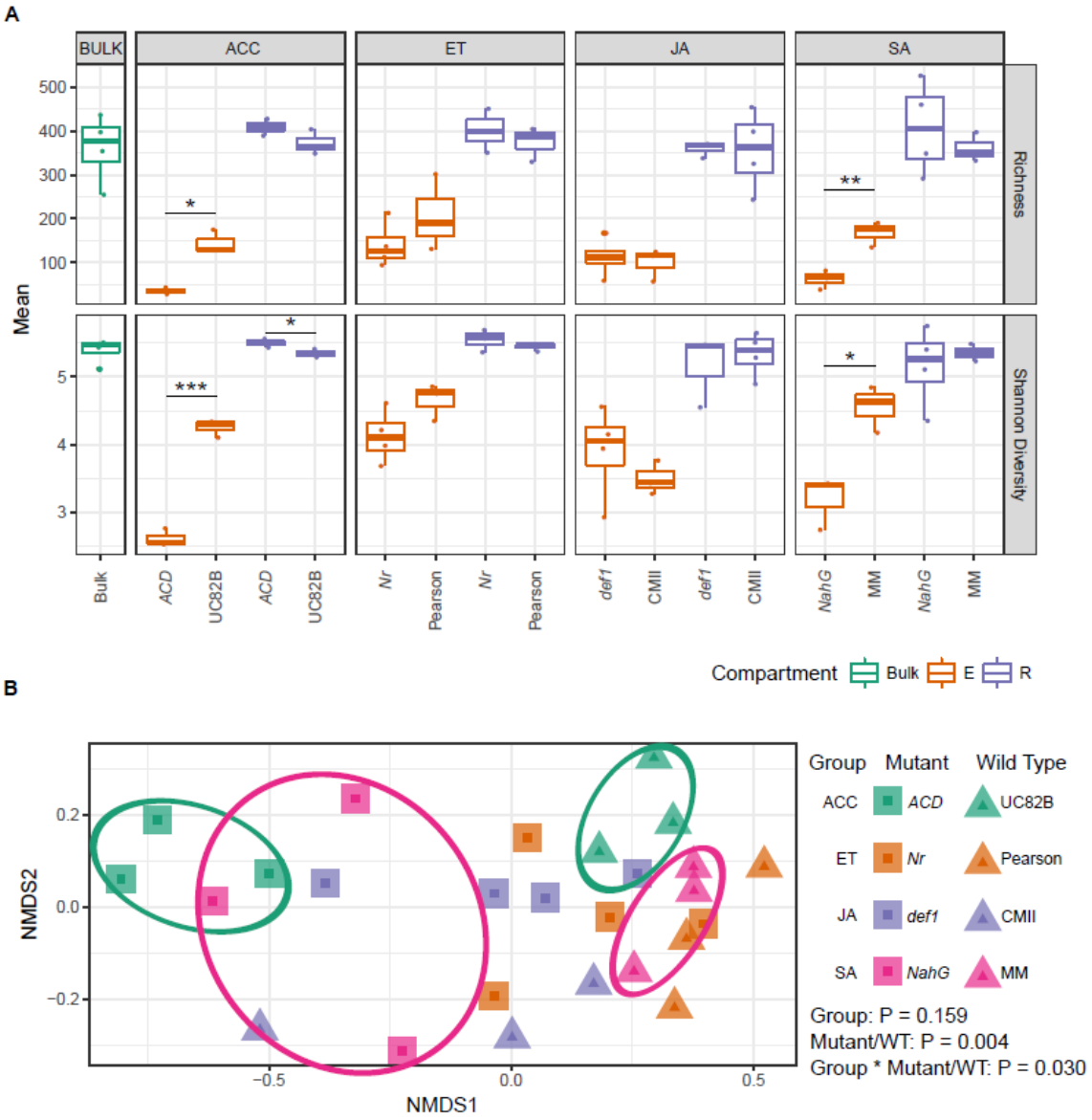


Fig 4.1 *ACD* and *NahG* mutant endospheres differ from their wild type backgrounds in alpha and beta diversity. A) Boxplots of bacterial richness and Shannon diversity of rhizospheres and endospheres of all four mutants and their respective backgrounds and bulk (unplanted) soil. Asterisks indicate significant differences by t test. * P < 0.05. ** P < 0.01. *** P < 0.001. B) Principle coordinate analysis of endosphere bacterial communities of all four mutants and their wild type backgrounds. Two-way PERMANOVA results indicated on graph. Squares indicate mutant samples, and triangles indicate wild type samples. Colored ellipses added for emphasis. E – endosphere. R – rhizosphere. ET – ethylene. JA – jasmonic acid. SA – salicylic acid. *ACD* – ACC-deaminase transgenic mutant. UC82B – *S. lycopersicum* cv. UC82B. *Nr* – *Never ripe* mutant. Pearson – *S. lycopersicum* cv. Pearson. *def1* – *defenseless1*. CMII – *S. lycopersicum* cv. Castlemart II. *NahG* – salicylate hydroxylase transgenic mutant. MM – *S. lycopersicum* cv. Money Maker.

Beta diversity patterns in root endospheres of *NahG* and *ACD* transgenics appear more similar to each other than to their wild-type parents/isolines

The Bray-Curtis data in Fig 4.1B suggested that root endospheres of *NahG* and *ACD* transgenics may be more similar to each other than to their wild-type parents. To test this, we performed an NMDS with only *NahG*, *ACD* and their respective wild types. The mutant (*NahG*/*ACD*) endospheres separated from the wild type genotypes along the first axis, and a PERMANOVA indicated that samples separated significantly between mutants and wild types, but not between mutant groups, indicating that *NahG* and *ACD* endospheres are different from their wild type backgrounds in a similar (PERMANOVA Mutant Group – $F_{(1, 8)} = 1.12$, $p = 0.251$. Defense – $F_{(1, 8)} = 3.71$, $p = 0.002$, Mutant Group:Defense – $F_{(1, 8)} = 1.31$, $p = 0.160$) (Fig 4.2).

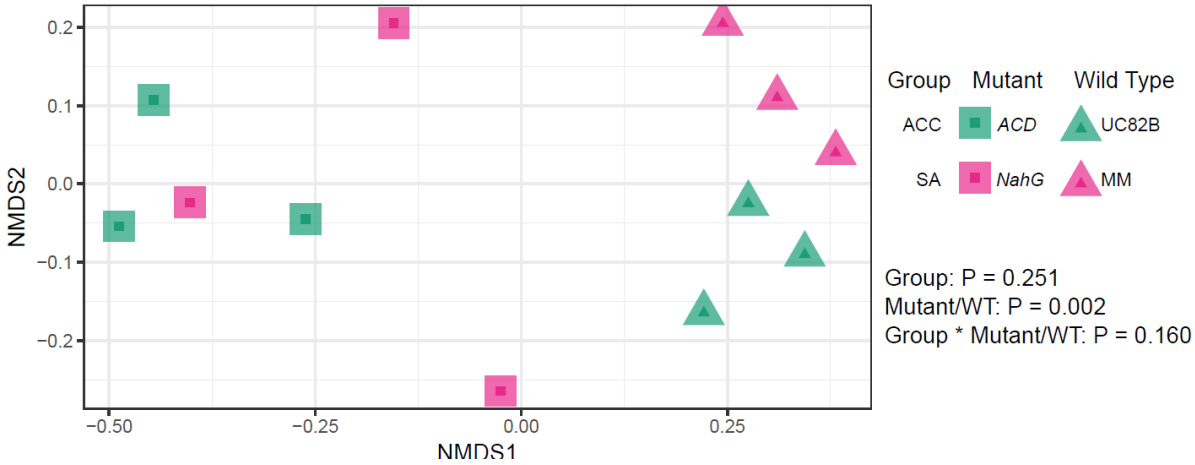


Fig 4.2 *ACD* and *NahG* differ from their respective wild types in beta diversity. Nonmetric multidimensional scaling (NMDS) analysis of endosphere bacterial communities of *ACD* and *NahG* along with their respective wild types UC82B and MM. One-way PERMANOVA results indicated on graph. E - endosphere. R – rhizosphere.

Together, results from in Fig 4.1 and 4.2 indicate that SA and ACC are required for normal root endosphere bacterial community assembly in tomato and suggest that these hormones play a role in promoting bacterial diversity in the root endosphere.

NahG and *ACD* rhizosphere to endosphere differential abundance profiles show similarities in enrichment but not depletion

The endospheres of *NahG* and *ACD* roots appeared to play a role in selection of the microbiome from the rhizosphere. We compared the rhizosphere to endosphere differential abundance profiles in each of the mutants and their respective wild type controls. We used DESeq2 to determine differentially abundant ASVs between the rhizosphere and endosphere for each genotype. We found 178 ASVs of the total 901 that were differentially abundant between the rhizosphere and endosphere of any of the eight genotypes tested (including mutants).

We categorized the differentially abundant ASVs into endosphere-enriched and endosphere-depleted (See Appendix Table C4.5 for summary and Appendix Tables C4.6-13 for full results for each genotype). More depleted ASVs were observed in mutant lines *ACD* (133) and *NahG* (109) than in their respective wild types (UC82B = 36, MM = 32) (Fig 4.3A, B, Appendix Table C4.5). The additional ASVs depleted in the transgenics are consistent with the reduced alpha diversity observed in the endosphere of these plants. In general, most of the endosphere-depleted ASVs in either wild type were also found in the mutant lines *ACD* and *NahG* (Fig 4.3A-B). For example, a total of 133 ASVs were endosphere-depleted in *ACD*, compared to 36 in the wild-type parent UC82B. Of these 36 ASVs, 28, or over 77%, were also depleted in *ACD*. *Nr* and *def1* had fewer depletions and did not share as many ASVs with their respective wild types (Fig 4.3C-D). Taxonomically, the main three phyla observed in the differentially abundant ASVs across all eight genotypes were Proteobacteria (enriched = 72.8%, depleted = 66.2%), Actinobacteria (enriched = 14.4%, depleted = 22.7%), and Firmicutes (enriched = 12.8%, depleted = 0%) (Appendix Table C4.5).

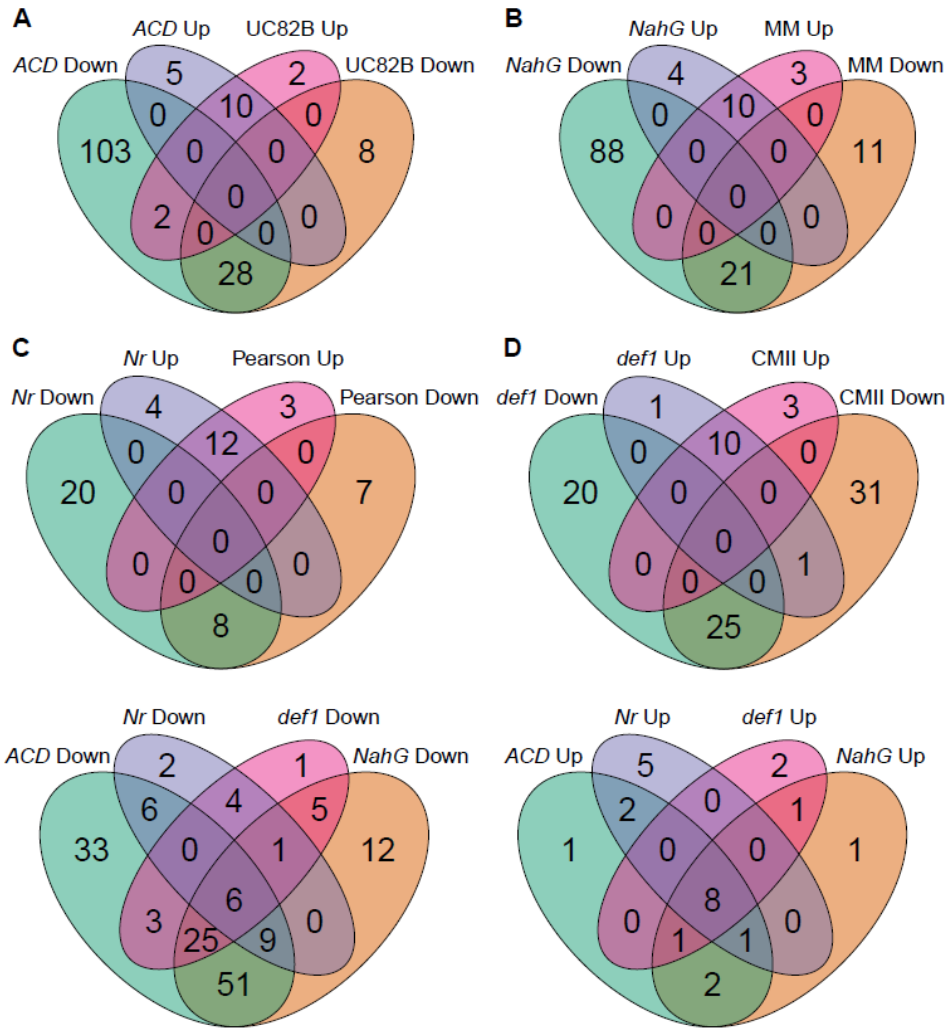


Fig 4.3 Rhizosphere to endosphere differential abundance (DA) profiles reveal similarities between *ACD* and NahG mutants compared to their wild types. Venn diagrams of enriched (Up) and depleted (Down) differentially abundant ASVs from the rhizosphere to endosphere in A) *ACD* and wild-type background UC82B, B) *NahG* and wild-type background MM, C) *Nr* and Pearson, D) *def1* and CMII. Venn diagrams comparing F) depleted ASVs and G) enriched ASVs among *NahG*, *ACD*, *def1*, and *Nr*.

Because the beta diversity pattern in Fig 4.2 suggested that taxa in the *ACD* and *NahG* root endospheres were more similar to each other than to wild type, we also compared the endosphere-depleted (Fig 4.3E) and enriched ASVs (Fig 4.3F) between these two genotypes. Interestingly, *ACD* and *NahG* alone shared about 66% of depleted ASVs and 71% of enriched ASVs. These data suggest that *NahG* and *ACD* have overlapping effects on the tomato root microbiome.

Two *NahG* and *ACD* endosphere-enriched ASVs drive enrichment profiles

Across all eight genotypes, we found a total of 27 endosphere-enriched ASVs and 160 endosphere-depleted ASVs, though 2 depleted ASVs had no counts in the endosphere across all genotypes and were excluded from further analysis. Some ASVs overlapped between the enriched and depleted datasets because they were enriched in one genotype and depleted in another. For simplicity, these ASVs were included in both datasets. We then clustered the genotypes and ASVs based on the average relative abundance of enriched and depleted ASVs in the endosphere using Bray-Curtis distance and average hierarchical clustering (Fig 4.4). Clustering on genotype revealed three major clusters for the enrichment profile, with the *NahG* and *ACD* transgenics clustered together, *Nr*, *def1* and CMII clustered together, and the remaining wild-type parents made a third cluster (Fig 4.4A). The depletion profiles grouped in three clusters – *ACD* by itself, *NahG* and CMII together, and the remaining genotypes clustered together (Fig 4.4B). This data suggests that the similarities between the two hormone mutants are driven by similarities in their enrichment, but not their depletion, profiles.

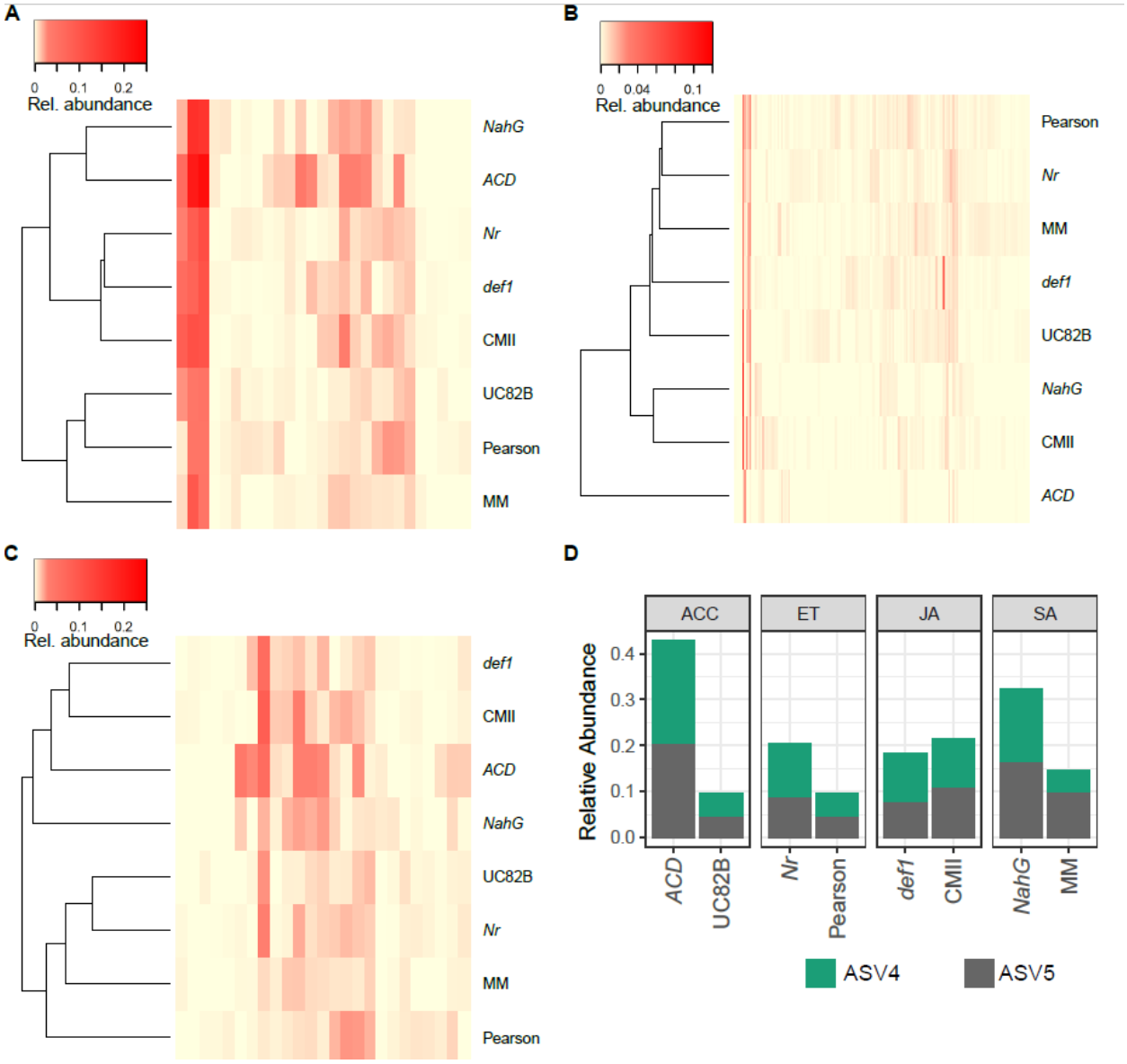


Fig 4.4. The rhizosphere to endosphere differential abundance profiles of the genotypes show similarities in enrichment but not depletion profiles between WV and hormone mutants. A-C) Hierarchical clustered heatmaps of average relative abundance of rhizosphere-to-endosphere A) enriched and B) depleted ASVs by genotype. C) Clustered heatmap of average relative abundance of rhizosphere-to-endosphere enriched ASVs without ASV4 and 5. D) Average relative abundance of ASV4 (*Anaerobacillus*) and ASV5 (*Delftia*) across all eight genotypes.

This result was unexpected because the hormone mutants show reductions in their alpha diversity and share a larger percentage of endosphere-depleted ASVs compared to endosphere-enriched ASVs. Examination of the endosphere-enriched ASV clusters showed that two highly abundant ASVs appeared to be more abundant in the endospheres of *NahG* and *ACD* compared to the other genotypes (Fig 4.4A). In fact, the abundance of these ASVs appears to drive the

clustering of the enrichment profiles, as the clustering of *NahG* and *ACD* disappears when they are removed from the analysis (Fig 4.4C). ASV4 is classified in the genus *Anaerobacillus* (Phylum: Firmicutes) and ASV5 is in the genus *Delftia* (Phylum: Proteobacteria). These two ASVs appear to show increased relative abundance in *NahG* and *ACD* compared to their respective background genotypes with these two ASVs making up between 30-45% of the total community of *NahG*, *ACD* and only 10-20% of the total community of the other five genotypes (Fig 4.4D).

The increased relative abundance of ASV4 and 5 may be a factor in the decreased richness and Shannon Diversity of the root endospheres of the *NahG* and *ACD* transgenics compared to wild-type parents. These data suggest that a small number of ASVs typically enriched in the root endosphere of wild-type roots are more highly enriched in SA and ACC-deficient plants.

Relative abundance of ASV4 and ASV5 is negatively correlated with Shannon Diversity

Because *NahG* and *ACD* roots have significantly lower alpha diversity (as measured by Richness and Shannon diversity) than their wild-type parents (Fig 4.1) and their endosphere enrichment profiles are driven by two ASVs (Fig 4.4), we hypothesized that these highly enriched ASVs 'crowd out' colonization of normally low abundance endosphere bacteria. If this hypothesis were correct, then the relative abundance of these ASVs may be negatively correlated with alpha diversity in the root endospheres of additional tomato genotypes. To examine this hypothesis, we sequenced the root microbiome of two additional *Solanum lycopersicum*, Hawaii7996 (H7996) and Bonny Best (BB), and two *S. pimpinellifolium* genotypes WestVirginia700 (WV) and LA2093, and twenty recombinant inbred lines (RILs) derived from H7996 and WV. Four replicates of each genotype were grown in the same conditions as the hormone mutants and their wild-type parents. DNA was extracted from the bulk soil, rhizosphere, and root endosphere of four replicates of each genotype. 16S rRNA amplicon sequencing was performed as above (sequencing data is summarized in Appendix Table C4.4). The relative abundance of ASV4 and ASV5 varied quantitatively across genotypes (Fig 4.5A and B), as did Shannon Diversity (Appendix Fig C4.3). In support of our hypothesis, the relative abundance of both ASV4 and ASV5 correlated negatively with Shannon Diversity (Fig 4.5C and D, ASV4, $R^2 = 0.49$; ASV5 $R^2 = 0.57$). This suggests that ASVs highly enriched in SA and

ACC-deficient mutants ‘crowd out’ root endosphere colonization of other taxa, resulting in lower alpha diversity in these mutants.

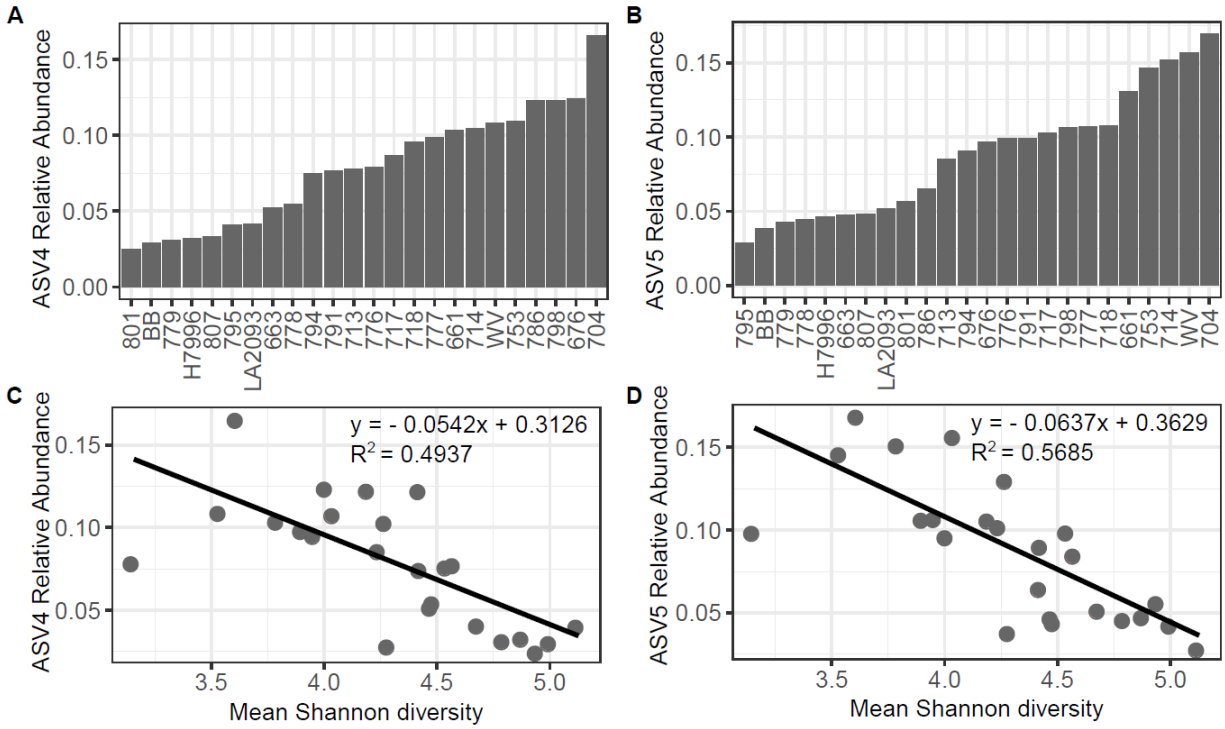


Fig 4.5 The relative abundance of ASVs 4 and 5 varies quantitatively in *S. lycopersicum* and *S. pimpinellifolium* RILs, and correlates negatively with Shannon Diversity. Relative abundance of A) ASV4 and B) ASV5 across H7996, WV, BB, LA2093 and 20 H7996 x WV RILs. Linear correlation between Shannon Diversity and C) ASV4 or D) ASV5. Equations and R^2 values for each trendline represented on each plot in C and D.

SA levels negatively correlate with relative abundance of ASV4 and ASV5

Because SA and ACC-deficient mutants recruit bacteria that appear to crowd out other taxa, we proposed that SA and ACC modulate microbial diversity in the root endosphere. We measured total SA levels in *NahG*, MM, *ACD*, UC82B, H7996, WV, BB and LA2093. We found a negative correlation between total SA levels and the average relative abundance of ASV4 (Fig 4.6A; $R^2 = 0.39$) and ASV5 (Fig 4.6B, $R^2 = 0.43$) and a positive correlation ($R^2 = 0.39$) between total SA levels and Shannon diversity (Fig C4.4), indicating that SA promotes microbial diversity in the root endosphere.

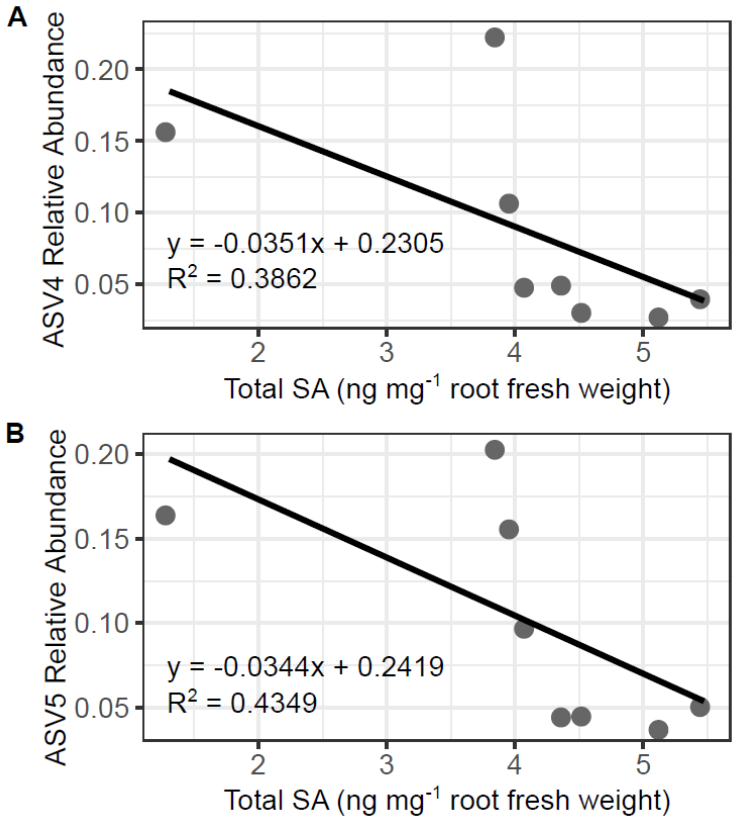


Fig 4.6 The relative abundance of ASVs driving differences in enrichment profiles show a negative association with SA content. Scatterplots with fitted trendlines comparing the average relative abundance of A) ASV4 (*Anaerobacillus*) and B) ASV5 (*Delftia*) versus the SA content in nanograms (ng) per milligram (mg) fresh weight (FW) of frozen, ground roots grown in potting mix/field soil. Equations and R^2 values for each trendline represented on each plot.

A bacterial strain similar to ASV4 has low tolerance to SA

Finally, we hypothesized that ASVs more enriched in the endospheres of *NahG* and *ACD* may be less tolerant to SA as their relative abundance was negatively correlated with SA content. Their colonization would thus be more restricted in genotypes containing higher levels of SA. We have a collection of over 400 bacterial taxa isolated from tomato root endospheres. From this collection we selected an isolate, HA-64, that was most similar in its 16S sequence to ASV4. As describe above, ASV4 is an endosphere-enriched *Anaerobacillus* ASV that was more enriched in *NahG* and *ACD* compared to the other genotypes (Fig 4.4D). The 16S sequence of HA-64 was 95.8% similar to ASV4. HA-64 was isolated from roots of field-grown, mature H7996 plants in the summer of 2017. We measured growth of HA-64 and HA-22, an isolate with low similarity

to either ASV4 or 5 (Fig 4.7). Growth was measured over 24 hours in minimal medium containing 0 or 1 mM salicylic acid. An ANOVA comparing the Area Under the Growth Curve (AUGPC) values for isolate and treatment revealed significant effects of SA treatment ($p<0.0001$), isolate ($p < 0.0001$) and their interaction ($p<0.0113$). Isolate HA-64, similar to the endosphere-enriched *Anaerobacillus* ASV4, exhibited low tolerance to SA based on a post-hoc Tukey test (Fig 4.7). In contrast isolate HA-22 was less affected by SA.

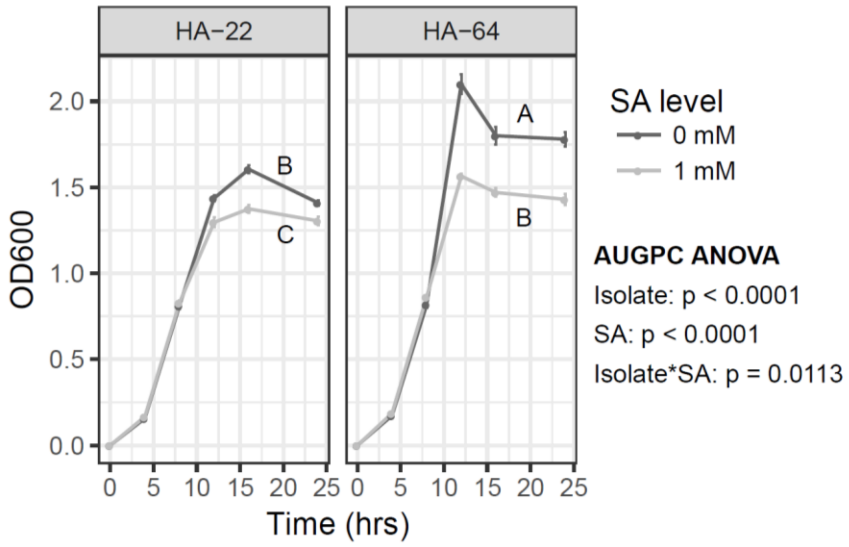


Fig 4.7 Bacterial isolate HA-64 similar to *Anaerobacillus* ASV4 shows low tolerance to salicylic acid compared to non-similar isolate HA-22 *in vitro*. Growth curve of the average of five replicates of HA-22 and HA-64 grown in liquid minimal media +/- 1 mM salicylic acid (SA) over 24 hours. ANOVA results for the effects of Isolate, SA level, and their interaction on Area Under the Progress Curve (AUGPC) OD600 displayed in figure. Differing letters indicate significant differences in AUGPC at $p < 0.05$ by posthoc Tukey's test.

Discussion

Overall, our study is the first to reveal a role for the ET and SA pathways for normal bacterial community assembly in tomato. Our results suggest that they normally function to restrict the growth of a few highly abundant taxa in the tomato endosphere, which fits the hypothesis that the plant immune system acts to maintain microbial homeostasis and control the microbial load of commensal microbes (Hacquard et al. 2017).

Salicylic acid (SA) has been shown to significantly affect both the leaf (Kniskern et al. 2007) and root (Lebeis et al. 2015) bacterial community of Arabidopsis. On the other hand,

Bodenhausen et al. (2014) showed no effect of SA on the Arabidopsis leaf community, though they were examining a limited number of taxa in a synthetic community. While Lebeis et al. (2015) revealed a key role for SA in proper assembly of the Arabidopsis microbiome, their results differed from ours in some key respects, indicating that SA may play species-specific roles on root microbiome assembly. First, they observed a reduction in alpha-diversity in Arabidopsis mutant *cpr5*, which has a constitutively active SA pathway. Consistent with this result, exogenous application of SA resulting in induction of SA mediated defense reduced bacterial diversity on Arabidopsis leaves (Kniskern et al. 2007); however, these effects were not observed on the rhizosphere community (Doornbos et al. 2011). We also observed a reduction in alpha diversity, but in constitutive SA degradation transgenic *NahG*. When examining enrichment and depletion profiles in the endosphere, Lebeis et al. (2015) observed an increased abundance of normally endosphere-depleted bacteria in the SA mutants, which differs from our result that normally endosphere-depleted microbes were also depleted in the mutants along with additional depletions, while two endosphere-enriched taxa were more enriched in the mutant. However, due to our low sequencing depth, we cannot rule out the possibility that these depleted, low abundance ASVs were still present in the samples and were not represented in the sequencing. We observed a negative relationship between the relative abundance of the two *NahG/ACD* endosphere-enriched ASVs and SA content. Both studies showed direct effects of SA on bacterial community members consistent with their enrichment or depletion in a given mutant.

Using a mutant approach with a simplified synthetic community consisting of seven members naturally abundant on leaves, Bodenhausen et al. (2014) demonstrated a role for ethylene in proper assembly of the Arabidopsis phyllosphere bacterial community. Bray-Curtis index and total colonization increased in the *ein-2* mutant compared to wild-type Col-0. In contrast, a reduction of culturable bacterial abundance was observed in the rhizosphere of the *ein-2* Arabidopsis mutant, though this reduction was not associated with difference in community structure (Doornbos et al. 2011). Although we did not measure absolute colonization levels, we did not observe significant differences in either alpha or beta diversity between the *Nr* ethylene perception mutant and its wild type background. However, wild tobacco *Nicotiana attenuata* mutants for ethylene biosynthesis and signaling showed a small reduction in culturable bacterial diversity in the root endosphere compared to wild type (Long et al. 2010), which fits

our observation of reduced alpha-diversity in the tomato endosphere of ACC biosynthesis transgenic mutant ACD.

Interestingly, the ACC and SA pathways appear to have overlapping effects on the tomato endosphere community. This may be due to a synergistic or redundant role of these two pathways in maintaining the root microbial community, suggesting that a common downstream component of both pathways functions to maintain balance in the root microbiome. SA and ET pathways have been shown to interact either positively or negatively in different plant-pathogen interactions (Pieterse et al. 2012), so they may play a role together in structuring the commensal bacterial community. Other studies have demonstrated a role for JA in microbiome assembly in the root endosphere of wheat (Liu et al. 2017), *Arabidopsis* rhizosphere (Carvalhais et al. 2013a), as well as in interactions with nitrogen fixing bacteria in rice (Miché et al. 2006) and *Lotus japonicus* (Nakagawa and Kawaguchi 2006), but our study revealed no role for JA in assembly of the tomato root microbiome.

NahG/ACD enriched ASVs 4 and 5 were classified into genus *Anaerobacillus* (Phylum: Firmicutes) and *Delftia* (Phylum: Proteobacteria), respectively. *Delftia* is a highly diverse root-colonizer, and some isolates have been well-characterized for their biocontrol and plant-growth promotion (PGP) properties (Morel et al. 2015; Agafonova et al. 2017). A *Delftia* isolate has even been shown to have antagonistic properties towards *R. solanacearum* (Huang et al. 2013). *Anaerobacillus* is not a well characterized genus (Zavarzina et al. 2009), but this ASV is also identical to some isolates from the *Bacillus* genus, which is well known for its biocontrol and PGP properties (Santoyo et al. 2012, 2016; Takahashi et al. 2014; Xiong et al. 2015; Cao et al. 2018). Because these ASVs were more abundant in the endospheres of *ACD* and *NahG* mutants, this suggests that the SA and ET pathways play a role in restricting their colonization. Future work is needed to determine the functional and ecological purpose that restricting or permitting colonization by these particular ASVs might play. It is possible that there are ecological tradeoffs to their colonization in natural systems, as shown by Haney et al. (2015; 2017) with some Pseudomonas isolates and Arabidopsis. These tradeoffs may be a product of adaptation to local environments (Hacquard et al. 2017). Future work will also need to be performed to determine the total bacterial load.

We observed quantitative differences in alpha diversity and relative abundance of ASVs 4 and 5 of root endosphere bacterial communities in a panel of tomato cultivars, wild relatives, and

RILs derived from a cross between Hawaii7996, a tomato cultivar known for resistance to *Ralstonia solanacearum*, and WestVirginia700, a *Solanum pimpinellifolium* susceptible to *R. solanacearum*. Previous studies have shown species-level differences in the root microbiome (Ofek-lalzar et al. 2014; Fitzpatrick et al. 2018) including differences in closely-related species along a domestication gradient (Pérez-Jaramillo et al. 2015; Szoboszlay et al. 2015; Shenton et al. 2016). Similar to our study, Shenton et al. (2016) observed lower alpha diversity in wild rice varieties compared to domesticated cultivars. Conversely, teosinte showed higher alpha diversity in its rhizosphere than a variety of sweet corn (Szoboszlay et al. 2015). Pérez-Jaramillo et al. (2017) was able to show quantitative variation in relative abundance of certain taxa in wild, domesticated, and landrace bean genotypes and relate them to differences in root traits between the genotypes. Our results suggest a genetic basis for host plant control of alpha diversity in tomato root endosphere bacterial communities, though further work will be required to discover QTL underlying this trait as well as the ecological costs or benefits that may be associated with differences in root community alpha diversity.

A few studies thus far have linked plant disease resistance traits to differences in root (Yao and Wu 2010; Mendes et al. 2018) or leaf (Balint-Kurti et al. 2010) microbiomes. Cucumber (Yao and Wu 2010) and bean cultivars (Mendes et al. 2018) with higher resistance to *Fusarium oxysporum* both showed higher diversity in their rhizospheres compared to susceptible cultivars, which fits with the hypothesis in the field that higher bacterial diversity is related to pathogen resistance (Wei et al. 2015). Balint-Kurti (2010) showed the opposite effect with leaf epiphytes on maize – resistance to Southern leaf blight was associated with lower leaf bacterial diversity. We observed that WV, susceptible to *R. solanacearum*, has a lower alpha diversity than H7996, which is resistant to *R. solanacearum*. However, Bonnie Best is also moderately susceptible to *R. solanacearum*, but its alpha diversity did not differ from resistant lines H7996 and LA2093. Together, this data suggests that the effects of resistance or susceptibility on the microbiome diversity may depend on the mechanism of resistance.

Previous work from our lab has demonstrated that H7996 and WV regulate their SA and ET pathways differently in response to bacterial pathogens (French et al. 2018), opening the possibility that they may regulate these pathways differently in response to non-pathogenic commensal bacteria. ET has a known role in general root responses to bacteria (Millet et al. 2010) as well as a role in the establishment of induced systemic resistance and colonization by

ISR-inducing microbes (Broekgaarden et al. 2015). Our lab has also shown that H7996 restricts bacterial pathogen colonization compared to WV (Caldwell et al. 2017), which suggests either root structural differences or differences in defense responses that may also lead to restriction of colonization of non-pathogenic microbes. Other studies comparing SA responses to different stresses have shown species-specific differences in tomato and wild relatives, particularly to salinity stress (Sun et al. 2010) and a tripartite interaction between beneficial fungi Trichoderma and pathogen *Botrytis cinerea* (Tucci et al. 2011). Together these studies suggest natural variations in hormonal pathways that affect how these plants respond to stress and may affect their ability to recruit or restrict root-colonizing microbes. Characterization of the root communities and SA/ET responses of the full RIL panel derived from H7996 and WV will help to answer this question.

Many previous studies have focused on rhizosphere bacterial communities. Interestingly, we saw no striking effects of hormone pathways on rhizosphere bacterial communities, but this may have been due to the young age of our plants, as plant age plays a role in determining the effects on the root microbiome (Wagner et al. 2016). However, we did see large effects on the endosphere, which points to a role for the plant immune system on endosphere community assembly early on in the plant life cycle. Consistent with our finding of microbiome differences in the endosphere but not rhizosphere compartment, Fitzpatrick et al. (2018) showed a phylogenetic signal for species-level differences in root endospheres, but not rhizospheres of a large panel of species along an evolutionary gradient.

Overall, our study has shown an important role for SA and ET in the normal assembly of the tomato endophytic microbiome, as well as natural variation in the microbiomes of close wild relatives that relate to their ability to restrict the colonization of specific ASVs. These findings are important for our basic understanding of how the plant is able to regulate colonization by non-pathogenic microbes and opens the door for the possibility of breeding tomatoes for improved associations with beneficial root microbe communities.

References

Agafonova N V., Doronina N V., Kaparullina EN, et al (2017) A novel Delftia plant symbiont capable of autotrophic methylotrophy. Microbiology 86:96–105. doi: 10.1134/S0026261717010039

Balint-Kurti P, Simmons SJ, Blum JE, et al (2010) Maize Leaf Epiphytic Bacteria Diversity Patterns Are Genetically Correlated with Resistance to Fungal Pathogen Infection. Mol Plant-Microbe Interact 23:473–484. doi: 10.1094/MPMI-23-4-0473

Beckers B, Op De Beeck M, Thijs S, et al (2016) Performance of 16s rRNA primer pairs in the study of rhizosphere and endosphere bacterial microbiomes in metabarcoding studies. Front Microbiol. doi: 10.3389/fmicb.2016.00650

Berendsen RL, Pieterse CMJ, Bakker P a HM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–86. doi: 10.1016/j.tplants.2012.04.001

Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA (2014) A Synthetic Community Approach Reveals Plant Genotypes Affecting the Phyllosphere Microbiota. PLoS Genet. doi: 10.1371/journal.pgen.1004283

Brading P a., Hammond-Kosack KE, Parr A, Jones JDG (2000) Salicylic acid is not required for Cf-2- and Cf-9-dependent resistance of tomato to Cladosporium fulvum. Plant J 23:305–318. doi: 10.1046/j.1365-313X.2000.00778.x

Broekgaarden C, Caarls L, Vos IA, et al (2015) Ethylene : Traf fi c Controller on Hormonal Crossroads to Defense 1. 169:2371–2379. doi: 10.1104/pp.15.01020

Bulgarelli D, Schlaeppi K, Spaepen S, et al (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–38. doi: 10.1146/annurev-arplant-050312-120106

Caldwell D, Kim B, Iyer-Pascuzzi AS (2017) Ralstonia solanacearum Differentially Colonizes Roots of Resistant and Susceptible Tomato Plants. 528–536.

Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. doi: 10.1038/nmeth.3869

Campbell CL, Madden L V. (1990) Introduction to plant disease epidemiology.

Cao Y, Pi H, Chandrangsu P, et al (2018) Antagonism of Two Plant-Growth Promoting Bacillus velezensis Isolates Against Ralstonia solanacearum and Fusarium oxysporum. Sci Rep 8:1–14. doi: 10.1038/s41598-018-22782-z

Carvalhais LC, Dennis PG, Badri D V., et al (2013a) Activation of the Jasmonic Acid Plant Defence Pathway Alters the Composition of Rhizosphere Bacterial Communities. PLoS One 8:1–5. doi: 10.1371/journal.pone.0056457

Carvalhais LC, Dennis PG, Fan B, et al (2013b) Linking plant nutritional status to plant-microbe interactions. PLoS One 8:e68555. doi: 10.1371/journal.pone.0068555

Chelius MK, Triplett EW (2001) The diversity of archaea and bacteria in association with the roots of Zea mays L. Microb Ecol 41:252–263. doi: 10.1007/s002480000087

Chiellini C, Maida I, Emiliani G, et al (2015) Endophytic and rhizospheric bacterial communities isolated from the medicinal plants echinacea purpurea and echinacea angustifolia. Int Microbiol 17:165–174. doi: 10.2436/20.1501.01.219

Danesh D, Aarons S, McGill GE, Young ND (1994) Genetic dissection of oligogenic resistance to bacterial wilt in tomato.

Davis NM, Proctor D, Holmes SP, et al (2018) Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. bioRxiv. doi: 10.1101/221499

Dong Y, Iniguez AL, Triplett EW (2003) Quantitative assessments of the host range and strain specificity of endophytic colonization by Klebsiella pneumoniae 342. Plant Soil 257:49–59. doi: 10.1023/A:1026242814060

Doornbos RF, Geraats BPJ, Kuramae EE, et al (2011) Effects of Jasmonic Acid, Ethylene, and Salicylic Acid Signaling on the Rhizosphere Bacterial Community of Arabidopsis thaliana. Mol Plant-Microbe Interact 24:395–407. doi: 10.1094/mpmi-05-10-0115

Fitzpatrick CR, Copeland J, Wang PW, et al (2018) Assembly and ecological function of the root microbiome across angiosperm plant species. Proc Natl Acad Sci 201717617. doi: 10.1073/pnas.1717617115

French E, Kim B, Rivera-Zuluaga K, Iyer-Pascuzzi AS (2018) Whole Root Transcriptomic Analysis Suggests a Role for Auxin Pathways in Resistance to Ralstonia solanacearum in Tomato. Mol Plant-Microbe Interact 31:432–444. doi: 10.1094/MPMI-08-17-0209-R

Hacquard S, Spaepen S, Garrido-Oter R, Schulze-Lefert P (2017) Interplay Between Innate Immunity and the Plant Microbiota. Annu Rev Phytopathol 55:annurev-phyto-080516-035623. doi: 10.1146/annurev-phyto-080516-035623

Haney CH, Samuel BS, Bush J, Ausubel FM (2015) Associations with rhizosphere bacteria can confer an adaptive advantage to plants. Nat Plants 1:15051. doi: 10.1038/nplants.2015.51

Haney CH, Wiesmann CL, Shapiro LR, et al (2017) Rhizosphere-associated Pseudomonas induce systemic resistance to herbivores at the cost of susceptibility to bacterial pathogens. Mol Ecol. doi: 10.1111/mec.14400

Howe GA, Lightner J, Browse J, Ryan CA (1996) An Octadecanoid Pathway Mutant (JL5) of Tomato is Compromised in Signaling for Defense against lnsect Attack. Plant Cell Am Soc Plant Physiol 8:2067–2077. doi: 10.1105/tpc.8.11.2067

Huang J, Wei Z, Tan S, et al (2013) The rhizosphere soil of diseased tomato plants as a source for novel microorganisms to control bacterial wilt. Appl Soil Ecol 72:79–84. doi: 10.1016/j.apsoil.2013.05.017

Jiang Y, Li S, Li R, et al (2017) Plant cultivars imprint the rhizosphere bacterial community composition and association networks. Soil Biol Biochem 109:145–155. doi: 10.1016/j.soilbio.2017.02.010

Klee HJ, Hayford MB, Kretzmer K a, et al (1991) Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. Plant Cell 3:1187–1193. doi: 10.1105/tpc.3.11.1187

Kniskern JM, Traw MB, Bergelson J (2007) Salicylic Acid and Jasmonic Acid Signaling Defense Pathways Reduce Natural Bacterial Diversity on Arabidopsis thaliana. http://dx.doi.org/101094/MPMI-20-12-1512 20:1512–1522. doi: 10.1094/MPMI-20-12-1512

Lanahan MB, Yen H-C, Giovannoni JJ, Klee HJ (1994) The Never Ripe Mutation Blocks Ethylene Perception in Tomato. Plant Cell Online 6:521–530. doi: 10.1105/tpc.6.4.521

Lebeis SL, Sur Herrera P, Lundberg DS, et al (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science (80-) 349:860–864. doi: 10.5061/dryad.238b2

Liu H, Carvalhais LC, Schenk PM, Dennis PG (2017) Effects of jasmonic acid signalling on the wheat microbiome differ between body sites. Sci Rep 7:41766. doi: 10.1038/srep41766

Long HH, Sonntag DG, Schmidt DD, Baldwin IT (2010) The structure of the culturable root bacterial endophyte community of Nicotiana attenuata is organized by soil composition and host plant ethylene production and perception. New Phytol 185:554–567. doi: 10.1111/j.1469-8137.2009.03079.x

Love MI, Anders S, Huber W (2014) Differential analysis of count data - the DESeq2 package.

Lundberg DS, Lebeis SL, Paredes SH, et al (2013) Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90. doi: 10.1038/nature11237

Maggini V, Elisangela M, Fagorzi C, et al (2018) Antagonism and antibiotic resistance drive a species-specific plant microbiota differentiation in Echinacea spp. FEMS Microbiol Ecol 94:fiy118. doi: https://doi.org/10.1093/femsec/fiy118

Mangin B, Thoquet P, Olivier J, Grimsley NH (1999) Temporal and multiple quantitative trait loci analyses of resistance to bacterial wilt in tomato permit the resolution of linked loci.

McMurdie PJ, Holmes S (2013) Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One. doi: 10.1371/journal.pone.0061217

Mendes LW, Raaijmakers JM, De Hollander M, et al (2018) Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. ISME J 12:212–224. doi: 10.1038/ismej.2017.158

Miché L, Battistoni F, Gemmer S, et al (2006) Upregulation of jasmonate-inducible defense proteins and differential colonization of roots of Oryza sativa cultivars with the endophyte Azoarcus sp. Mol Plant Microbe Interact 19:502–511. doi: 10.1094/MPMI-19-0502

Millet YA, Danna CH, Clay NK, et al (2010) Innate Immune Responses Activated in Arabidopsis Roots by Microbe-Associated Molecular Patterns. Plant Cell 22:973–990. doi: 10.1105/tpc.109.069658

Morel MA, Cagide C, Minteguiaga MA, Dardanelli MS (2015) The Pattern of Secreted Molecules During the Co-Inoculation of Alfalfa Plants With Sinorhizobium meliloti and Delftia sp . strain JD2 : An Interaction That Improves Plant Yield. 28:134–142.

Nakagawa T, Kawaguchi M (2006) Shoot-applied MeJA suppresses root nodulation in Lotus japonicus. Plant Cell Physiol 47:176–180. doi: 10.1093/pcp/pci222

Ofek-lalzar M, Sela N, Goldman-voronov M, et al (2014) Niche and host-associated functional signatures of the root surface microbiome. Nat Commun 5:1–9. doi: 10.1038/ncomms5950

Olivares FL, Baldani VLD, Reis VM, et al (1996) Occurrence of the endophytic diazotrophs Herbaspirillum spp. in roots, stems, and leaves, predominantly of Gramineae. Biol Fertil Soils 21:197–200. doi: 10.1007/BF00335935

Pérez-Jaramillo JE, Carrión VJ, Bosse M, et al (2017) Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. ISME J 11:2244–2257. doi: 10.1038/ismej.2017.85

Pérez-Jaramillo JE, Mendes R, Raaijmakers JM (2015) Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol Biol 90:635–644. doi: 10.1007/s11103-015-0337-7

Pieterse CMJ, Van der Does D, Zamioudis C, et al (2012) Hormonal Modulation of Plant Immunity. Annu Rev Cell Dev Biol 28:489–521. doi: 10.1146/annurev-cellbio-092910-154055

Santoyo G, del Orozco-Mosqueda MC, Govindappa M (2012) Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of Bacillus and Pseudomonas: A review. Biocontrol Sci Technol 22:855–872. doi: 10.1080/09583157.2012.694413

Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99. doi: 10.1016/j.micres.2015.11.008

Schlaeppi K, Dombrowski N, Oter RG, et al (2014) Quantitative divergence of the bacterial root microbiota in Arabidopsis thaliana relatives. Proc Natl Acad Sci U S A 111:585–92. doi: 10.1073/pnas.1321597111

Shenton M, Iwamoto C, Kurata N, Ikeo K (2016) Effect of Wild and Cultivated Rice Genotypes on Rhizosphere Bacterial Community Composition. Rice (N Y) 9:42. doi: 10.1186/s12284-016-0111-8

Sun W, Xu X, Zhu H, et al (2010) Comparative transcriptomic profiling of a salt-tolerant wild tomato species and a salt-sensitive tomato cultivar. Plant Cell Physiol 51:997–1006. doi: 10.1093/pcp/pcq056

Szoboszlay M, Lambers J, Chappell J, et al (2015) Comparison of root system architecture and rhizosphere microbial communities of Balsas teosinte and domesticated corn cultivars. Soil Biol Biochem 80:34–44. doi: 10.1016/j.soilbio.2014.09.001

Takahashi H, Nakaho K, Ishihara T, et al (2014) Transcriptional profile of tomato roots exhibiting Bacillus thuringiensis-induced resistance to Ralstonia solanacearum. Plant Cell Rep 33:99–110. doi: 10.1007/s00299-013-1515-1

Thoquet P, Olivier J, Sperisen C, et al (1996) Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar Hawaii7996. Mol. Plant. Microbe. Interact. 9:826–836.

Tucci M, Ruocco M, Masi LDE, et al (2011) The beneficial effect of Trichoderma spp . on tomato is modulated by the plant genotype. 12:341–354. doi: 10.1111/J.1364-3703.2010.00674.X

Wagner MR, Lundberg DS, del Rio TG, et al (2016) Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. Nat Commun 7:1–15. doi: 10.1038/ncomms12151

Wang J-F, Olivier J, Thoquet P, et al (2000) Resistance of Tomato Line Hawaii7996 to *Ralstonia solanacearum* Pss4 in Taiwan Is Controlled Mainly by a Major Strain-Specific Locus. Mol Plant-Microbe Interact. doi: 10.1094/MPMI.2000.13.1.6

Weber OB, Baldani VLD, Teixeira KRS, et al (1999) Isolation and characterization of diazotrophic bacteria from banana and pineapple plants. Plant Soil 210:103–113. doi: 10.1023/A:1004623523179

Wei Z, Yang T, Friman V-P, et al (2015) Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. Nat Commun 6:8413. doi: 10.1038/ncomms9413

Wickham H (2009) ggplot2: Elegant graphics for data analysis. Springer

Xin X-F, Nomura K, Aung K, et al (2016) Bacteria establish an aqueous living space in plants crucial for virulence. Nature 539:524–529. doi: 10.1038/nature20166

Xiong H, Li Y, Cai Y, et al (2015) Isolation of Bacillus amyloliquefaciens JK6 and identification of its lipopeptides surfactin for suppressing tomato bacterial wilt. RSC Adv 5:82042–82049. doi: 10.1039/C5RA13142A

Yao H, Wu F (2010) Soil microbial community structure in cucumber rhizosphere of different resistance cultivars to fusarium wilt. FEMS Microbiol Ecol 72:456–63. doi: 10.1111/j.1574-6941.2010.00859.x

Zavarzina DG, Tourova TP, Kolganova T V., et al (2009) Description of Anaerobacillus alkalilacustre gen. nov., sp. nov.—Strictly anaerobic diazotrophic bacillus isolated from soda lake and transfer of Bacillus arseniciselenatis, Bacillus macyae, and Bacillus alkalidiazotrophicus to Anaerobacillus as the new combinations A. arseniciselenatis comb. nov., A. macyae comb. nov., and A. alkalidiazotrophicus comb. nov. Microbiology 78:723–731. doi: 10.1134/S0026261709060095

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

Plant hormones are key regulators of plant responses to their environment, from soil conditions to biotic stresses to interactions with the soil microbial community. In this dissertation, I have revealed roles for plant growth and defense hormones in *Solanum* spp. responses to 1) biochar, a black carbon soil amendment (Chapter 2), 2) infection with *Ralstonia solanacearum*, an economically important soilborne pathogen causing bacterial wilt (Chapter 3), and 3) endophytic colonization by the soil bacterial community (Chapter 4).

In Chapter 2, I utilized RNAseq to show that biochar upregulates genes consistent with stimulation of the GA pathway with concomitant downregulation of genes in the SA and JA defense pathways. I showed that exogenous application of GA stimulated growth of plants grown in biochar more than with either biochar or GA alone, and this effect was species- and cultivar-specific. Germination and time to flowering, both GA-related traits, were also affected by biochar in a species- and cultivar- specific manner. Furthermore, an intact GA biosynthesis pathway was required for growth promotion of Arabidopsis seedlings by biochar water extracts. Lastly, we showed, contrary to findings by other groups (reviewed in Elad et al. 2011), that biochar application had no effect on Fusarium wilt disease symptoms, consistent with our observation of downregulation of SA and JA defense pathways.

In Chapter 3, I compared global transcriptional responses in roots of two closely related Solanum spp. Hawaii7996 (H7996 - *S. lycopersicum*) and WestVirginia700 (WV - *S. pimpinellifolium*) to *R. solanacearum*, an economically important soilborne bacterial pathogen. Consistent with the literature examining tomato shoot responses to *R. solanacearum*, I found earlier and stronger upregulation of SA and ET marker genes in the resistant plant H7996 than in the susceptible plant WV. Secondly, I found that auxin signaling genes were downregulated in roots of the resistant plant in response to pathogen infection. Further, we showed that auxin signaling mutant *dgt* is highly resistant to *R. solanacearum*, thus confirming a role for auxin in resistance to bacterial wilt.

In Chapter 4, I compared 16S sequence profiles of the endospheres and rhizospheres of a panel of defense hormone mutants in tomato. I found that SA and ACC/ET are essential for

promoting normal endophytic bacterial community alpha diversity. These two hormones play a role in restricting the colonization of two ASVs – classified as an *Anaerobacillus* and a *Delftia* – which were enriched in *NahG/ACD* endospheres more than in other genotypes tested. The relative abundance of these two *NahG/ACD*-enriched ASVs was negatively correlated with Shannon diversity in a panel of two *S. lycopersicums* (H7996 and Bonnie Best), two *S. pimpinellifoliums* (WV and LA2093), and 20 RILs derived from a cross between H7996 and WV, suggesting that the proliferation of these taxa in the root prevents colonization by other microbes. I further elucidated the role of SA in tomato root community assembly by showing that 1) a *Bacillus* isolate similar to the *NahG/ACD*-enriched *Anaerobacillus* ASV showed lower tolerance to SA in vitro than a non-similar isolate, and 2) root total SA content was negatively correlated with the relative abundance of the two ASVs. Together, this data suggests a functional role for SA in restricting the colonization of a few taxa in order to promote overall diversity in the tomato root endosphere.

In conclusion, in my dissertation I showed novel roles for plant growth and defense hormones in 1) modulating responses to the soil amendment biochar, 2) responding to invasion by the root bacterial pathogen *R. solanacearum* and 3) maintaining root bacterial community homeostasis. In addition, I showed that these effects are species-specific within closely related *Solanum* species.

Future Directions

In this dissertation, I was able to show variation for new traits that could be selected on in a breeding program. In Chapter 2, I showed genetic variation for response to biochar in germination traits and growth response to exogenous application of GA. This variation in important agricultural traits could be exploited both for incorporating the use of biochar into existing agricultural practices (i.e. spraying crops with GA to increase growth) or for breeding crops that respond more strongly to biochar. In Chapter 4, I showed quantitative genetic variation for root endosphere alpha diversity in a panel of RILs. Additional research could reveal QTLs associated with alpha-diversity for incorporation into a breeding program, though further work is needed to determine an ecological benefit or cost to differences in alpha diversity in the root endosphere.

Additionally, I was able to show a novel role for auxin in root responses to bacterial pathogens. Further work is needed to elucidate the role of auxin in resistance to *R. solanacearum*, especially its interaction with other defense hormones in the production of plant defense responses.

APPENDIX A. CHAPTER 2 SUPPLEMENTAL FIGURES AND TABLES

FIGURES

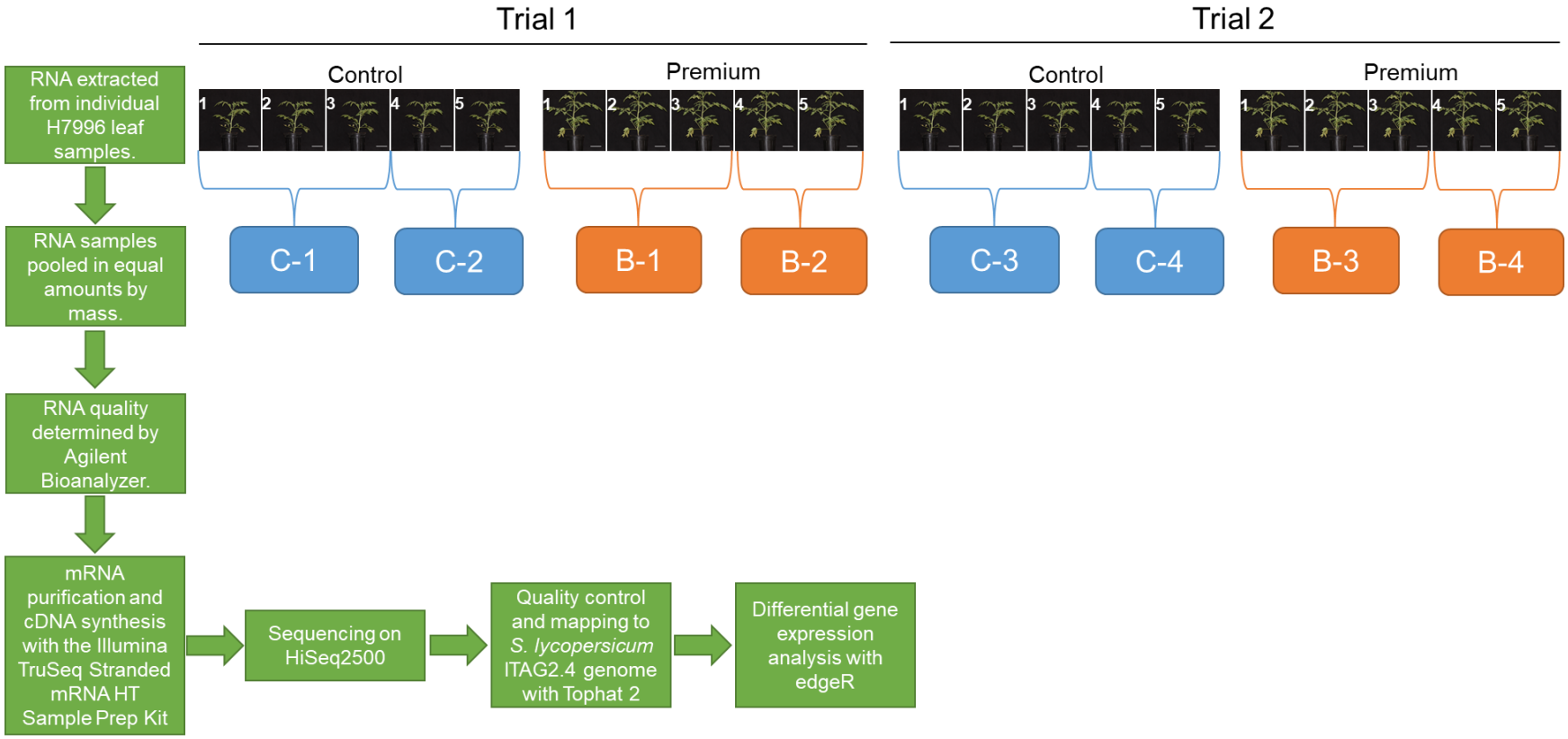


Fig A2.1 RNAseq workflow, showing sample collection scheme.

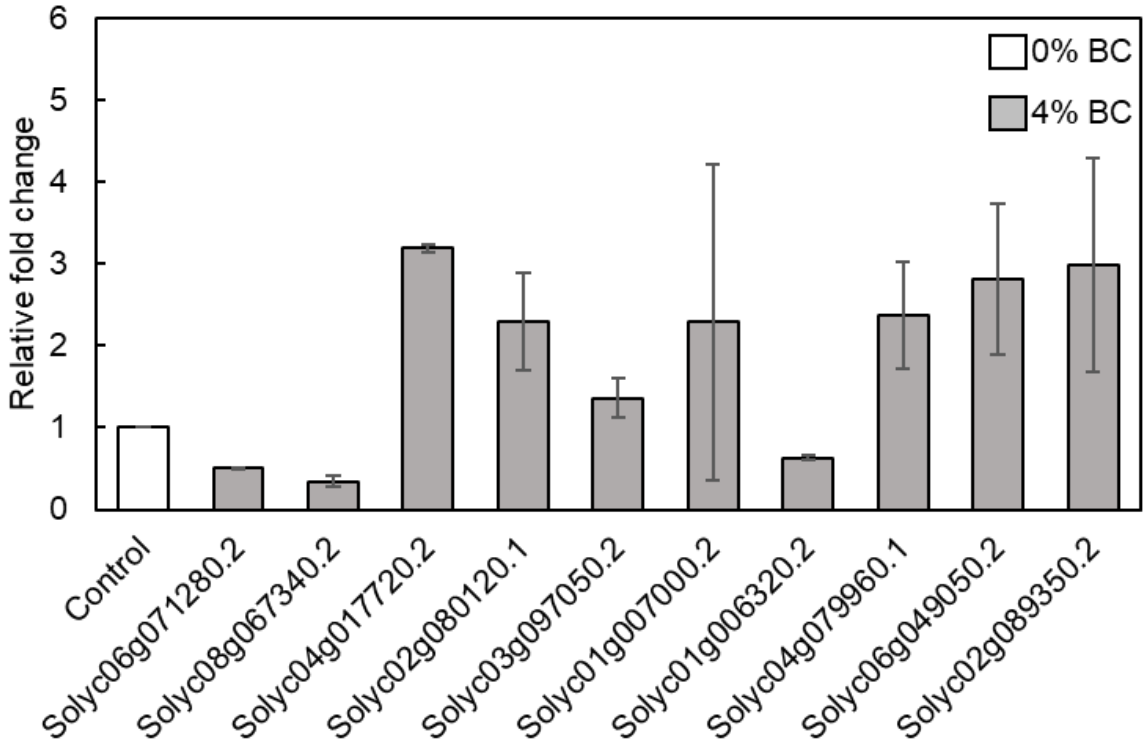


Fig. A2.2 qRT-PCR confirmation of RNAseq analysis. 10 genes differentially expressed between control and Premium biochar (BC) grown leaves from the RNAseq analysis were chosen for qRT-PCR confirmation. Expression of housekeeping gene *GAPDH* was used for normalization. Fold changes are relative to expression in the control plant samples. Relative fold change < 1 indicates down-regulation; > 1 indicates up-regulation. All 10 genes were up- or down-regulated in the same direction as in the RNAseq analysis. Values represent relative fold change calculated by the delta-delta CT method from averages of two biological replicates with three technical replicates each. Error bars represent one standard error.

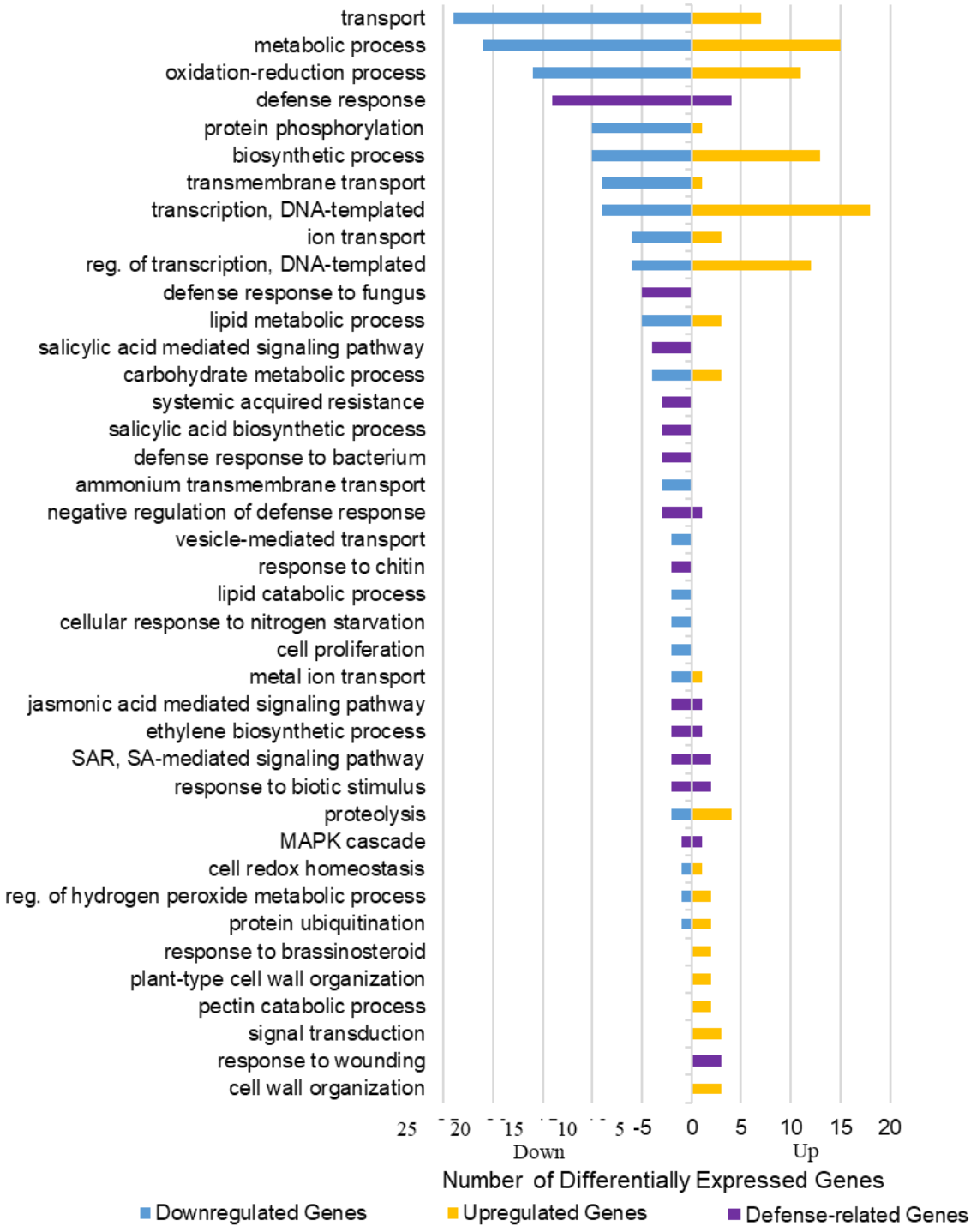


Fig. A2.3 Premium biochar amendment results in changes to gene expression. This table shows the top 40 Biological Process gene ontology (GO) categories found in the differentially expressed genes. Genes were considered differentially regulated if they met a false discovery rate < 0.05 and a fold change > 1.5. Yellow bars indicate up-regulated genes. Blue bars indicate down-regulated genes, and purple bars indicate either up- or down-regulated genes that are involved in biological process categories relating to defense. Abbreviations: SA – salicylic acid. MAPK – mitogen-activated protein kinase. SAR – systemic acquired resistance. Reg. – regulation.

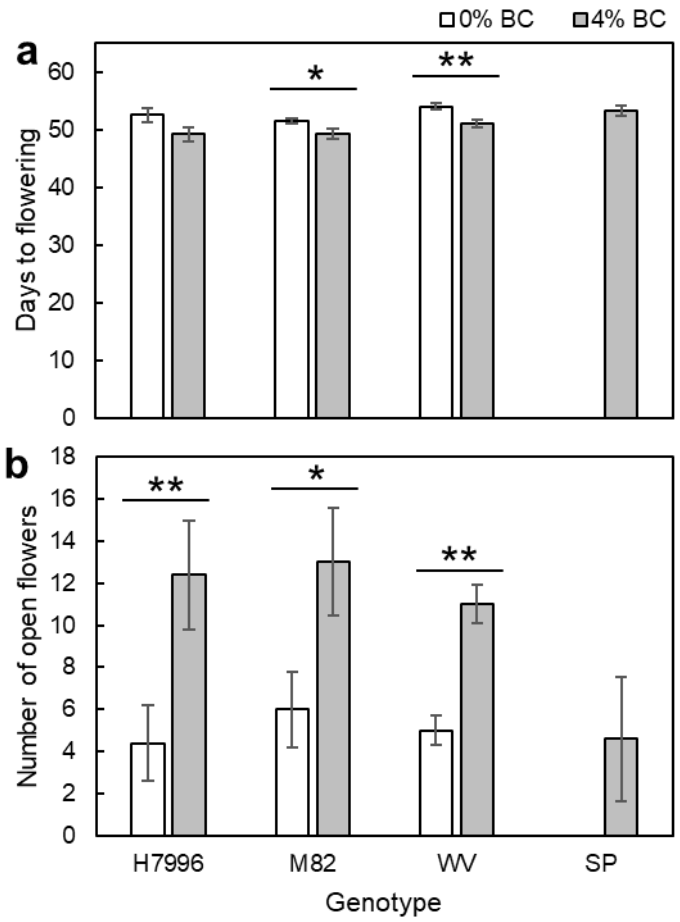


Fig. A2.4 Premium biochar (BC) reduces days to flowering and increases number of flowers. a) Average number of days to flowering in all 4 genotypes grown in 0% BC or 4% Premium BC. b) Average number of open flowers in all 4 genotypes grown in 0% or 4% Premium BC. Asterisks indicate significance by t-test at the following levels: * $p < 0.1$, ** $p < 0.05$. Error bars represent one standard error. No statistical analysis performed on SP as no control plants had flowered by the end of the experiment (56 days after planting).

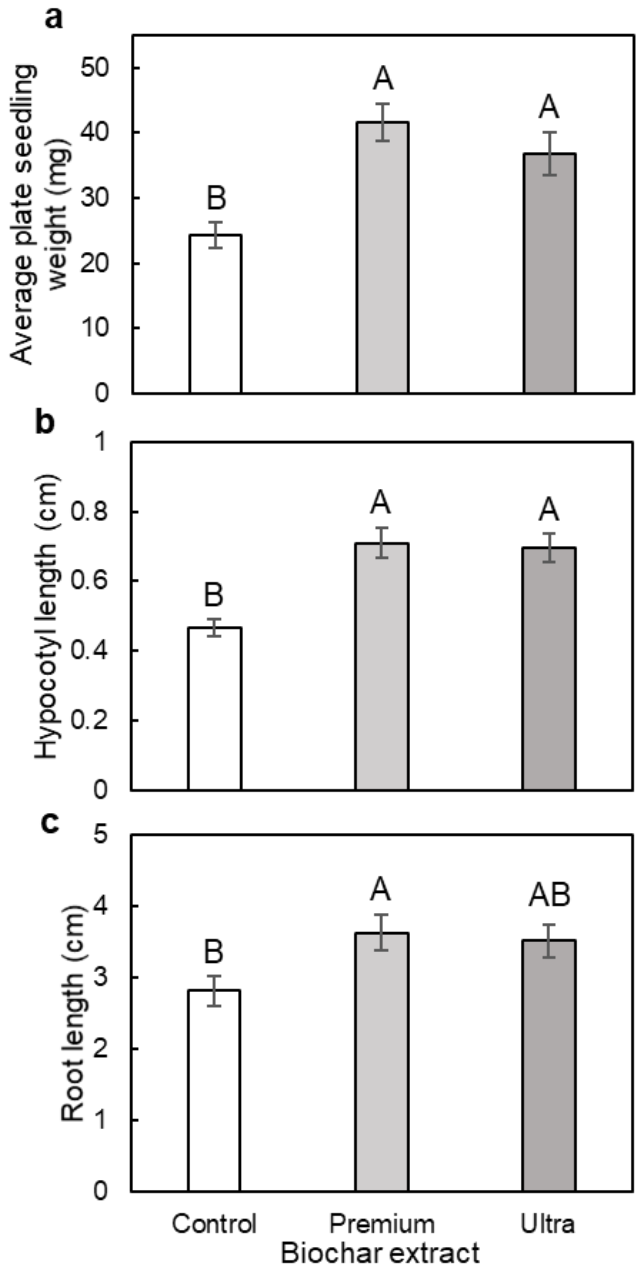


Fig. A2.5 Biochar water extracts positively affect seedling growth in *Solanum pennellii*. a) average weight of pooled seedlings from each plate in milligrams (mg) b) average hypocotyl length in centimeters (cm) c) average root length in cm and of *S. pennellii* seeds plated with water (Control) or water extracts of the two biochars. Results represent the averages of five plates/treatment of 10 seeds/plate. Data displayed from first biological replicate. Experiment was performed four times with similar results. Significant differences between biochar treatments at $p < 0.05$ as determined by Tukey's honest significant differences indicated by differing letters for a-c. Hypocotyl length values were square root transformed to meet homogeneity of variance assumption. Values displayed are untransformed. Error bars represent one standard error.

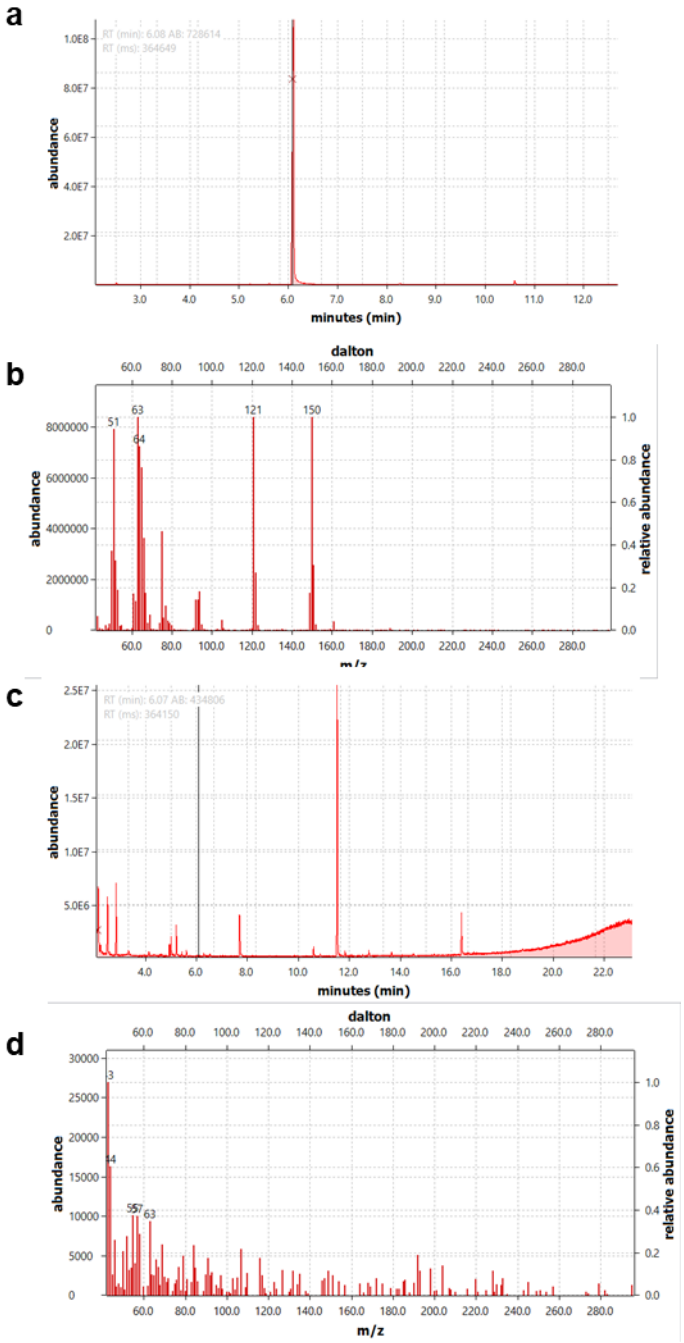


Fig. A2.6 Gas Chromatography-Mass Spectrometry (GC/MS) of ethyl acetate extracts of Premium biochar (BC). a) Chromatogram of 1 mM karrikin 1 (KAR1) in ethyl acetate. Retention time ~6 min. B) Mass spectrum of KAR1 at 6.08 min retention time (marked as X in a). C) Chromatogram of ethyl acetate Premium BC extract. D) Mass spectrum at 6.09 min retention time.

TABLES

Table A2.1 RNA sequencing and alignment summary.

	Raw reads		Quality & adapter clipped reads				Tophat 2 mapping				
Sample name	Total reads	Bases	Total reads	Bases	%seqs[1] lost	%bases lost	All mapping seqs	Mapped Seqs	High map quality seqs	Quality Mapped%	X-Coverage
C-1	59,748,074	6,034,555,474	58,439,012	5,820,481,587	2	3	57,485,379	57,485,379	56,492,300	96	6
C-2	52,315,688	5,283,884,488	51,152,408	5,095,437,169	2	3	50,565,196	50,565,196	49,680,567	97	6
C-3	69,307,334	7,000,040,734	67,232,438	6,705,307,473	2	4	66,438,008	66,438,008	65,256,047	97	8
C-4	59,933,378	6,053,271,178	58,858,422	5,870,565,758	1	3	58,138,960	58,138,960	57,184,562	97	7
B-1	67,745,816	6,842,327,416	66,564,704	6,637,482,226	1	2	65,620,591	65,620,591	64,519,954	96	7
B-2	68,208,396	6,889,047,996	66,799,518	6,660,129,791	2	3	65,984,340	65,984,340	64,861,113	97	7
B-3	70,082,612	7,078,343,812	68,276,614	6,795,822,312	2	3	67,469,744	67,469,744	66,288,941	97	8
B-4	59,308,366	5,990,144,966	58,007,668	5,783,063,629	2	3	57,333,324	57,333,324	56,224,741	96	6

[1]seqs - sequences

***Table A2.2** List of all differentially expressed genes from edgeR with FDR < 0.05, |logFC| > 0.585

Table A2.3 qRT-PCR primers for confirmation of RNAseq results

Gene ID	LogFC[1] (RNAseq)	F Primer	Tm (°C)	R Primer	Tm (°C)
Solyc04g079960.1	1.06	GCTGCCTGTGAACTTGTTGG	60	GACGGAGAAACTCCGGTTGT	60
Solyc02g080120.1	1.12	CCAACAACCCTAAGCTCCAC	58.5	GGAGAAATAGGGCATGCTGGA	59.9
Solyc02g089350.2	1.17	AAATCAGGTTTCAAGGGCCAAC	59.6	GGTACCAGCAGGAACACACA	59.9
Solyc04g017720.2	1.66	TTCTTCTTGTTGGAACTCATGCAA	59.4	GTCCCAGCAGGAACACAGAG	60.3
Solyc01g007000.2	2.06	TGGAATAACGTATGATCGTGAAAAT	57.2	TTTGGGGTCAACTCTATGCCC	60
Solyc01g006320.2	-0.98	TCTCTGACTTTTCAACATGGCG	59.2	ATGGTTGTCACGCTTCGTCT	60
Solyc03g097050.2	1.05	AACCATGGAGGCCACTTACG	60	CCCACAACCAAACTGCATCG	60
Solyc06g049050.2	1.24	AGCCGGTATCGTCCCTGTAT	60.2	GACATTGCTTGCCATCCAGT	58.8
Solyc06g071280.2	-0.86	GGCTGTTGCACGATCAAGTC	59.8	TGCAGAACAGCATCAGGGTT	59.9
Solyc08g067340.2	-1.33	TGCACCATCATGTCCAGTCA	59.3	TTCGACAACGAAGACGCCAT	60.3

[1]Log fold change

***Table A2.4** All Gene Ontology (GO) terms represented in down-regulated genes

***Table A2.5** All Gene Ontology (GO) terms represented in up-regulated genes

***Table** A2.6 List of genes represented in Fig 2, divided by category

Table A2.7 Generalized linear models of the effects of Premium biochar treatment (BC) and gibberellin treatment (GA_4) on shoot weight and length, separated by genotype.

Type III Tests of Fixed Effects						
Genotype	Response Variable	Effect	Num DF[1]	Den DF	F Value	Pr > F
H7996	Shoot Fresh Weight (g)[2]	BC	1	3	24.25	**0.0161**
		GA	1	3	66.66	**0.0038**
		BC*GA	1	3	1.09	0.3723
	Shoot Length (cm)[3]	BC	1	3	13.67	**0.0344**
		GA	1	3	79.1	**0.003**
		BC*GA	1	3	0.37	0.5844
M82	Shoot Fresh Weight (g)	BC	1	3	35.59	**0.0094**
		GA	1	3	154.38	**0.0011**
		BC*GA	1	3	12.11	**0.0401**
	Shoot Length (cm)	BC	1	3	54	**0.0052**
		GA	1	3	206.18	**0.0007**
		BC*GA	1	3	6.82	0.0795
WV	Shoot Fresh Weight (g)	BC	1	3	4.96	0.1123
		GA	1	3	64.58	**0.004**
		BC*GA	1	3	3.92	0.142
	Shoot Length (cm)	BC	1	3	2.96	0.1841
		GA	1	3	260.06	**0.0005**
		BC*GA	1	3	0.97	0.3971
SP	Shoot Fresh Weight (g)	BC	1	3	82.92	**0.0028**
		GA	1	3	4	0.1395
		BC*GA	1	3	1.64	0.2903
	Shoot Length (cm)	BC	1	3	41.19	**0.0077**
		GA	1	3	35.52	**0.0095**
		BC*GA	1	3	1.41	0.3208

[1]Degrees of Freedom
[2]Shoot weight - not transformed for analysis.
[3]Shoot length - square root transformed for analysis.

Table A2.8 Generalized linear model of the effects of Arabidopsis genotype and Premium biochar (BC) extract treatment on hypocotyl length.

Type III Tests of Fixed Effects					
Response Variable	Effect	Num DF	Den DF	F Value	Pr > F
Hypocotyl length (mm)	Genotype	1	1	7.25	0.2264
	BC	1	15.52	10.5	**0.0053**
	Genotype*BC	1	15.52	29.74	**<.0001**

[1]Hypocotyl length – square root transformed for analysis

***Tables A2.2, A2.4, A2.5, and A2.6 are too large to be included in this document. They are available online at** https://doi.org/10.1038/s41598-018-23677-9

and are labeled Supplementary Tables 2, 4, 5, and 6, respectively.

APPENDIX B. CHAPTER 3 SUPPLEMENTAL FIGURES AND TABLES

FIGURES

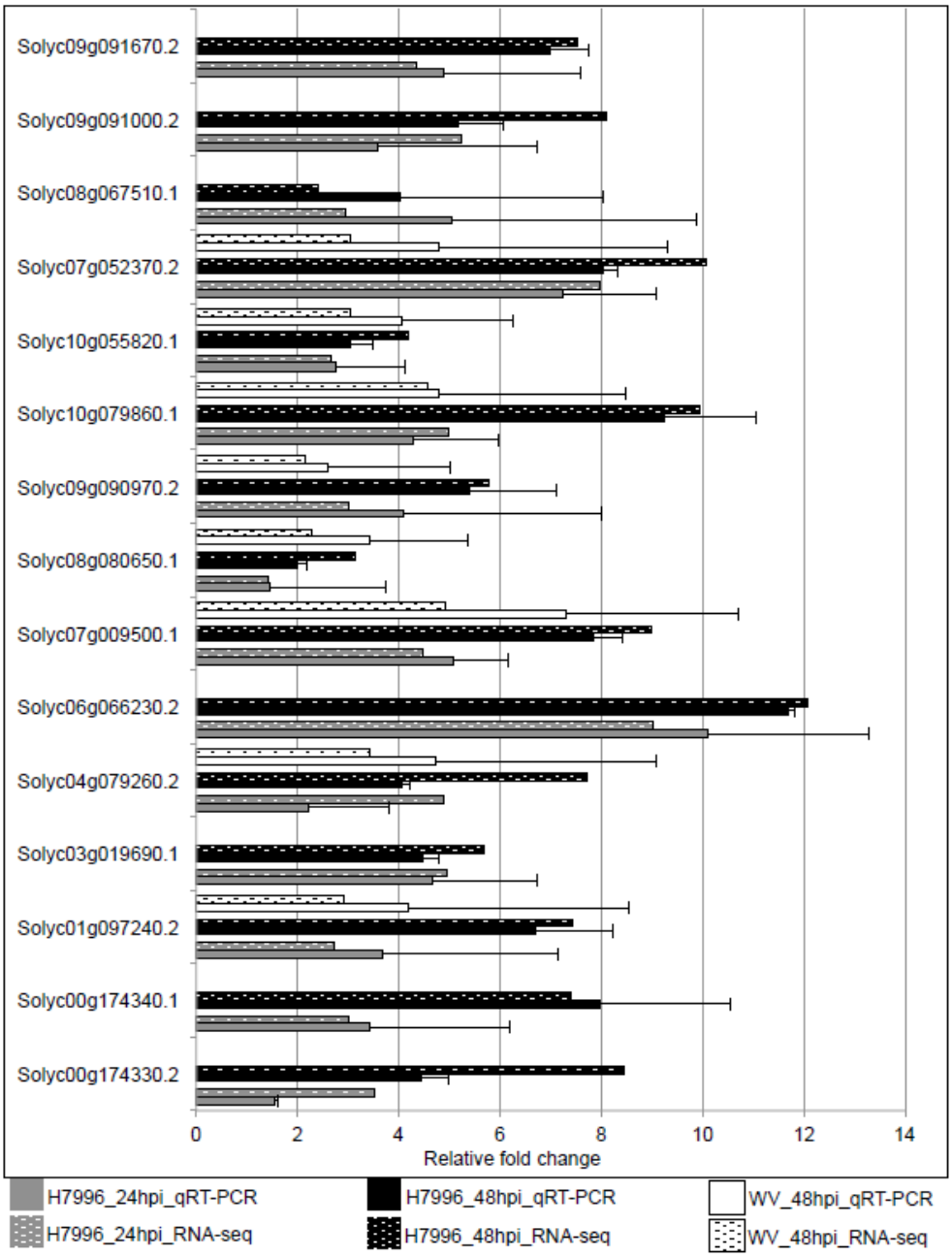


Fig B3.1 Comparison of qRT-PCR and RNA-seq data at 24 and 48 hpi after inoculation in H7996 and WV. Two biological replicates were used for the qRT-PCR analysis. Expression of genes is shown for both genotypes and timepoints only if the gene expression was significant (FC > 1.5, q < 0.05) in the RNA-seq data. Error bars show standard deviation for qRT-PCR data.

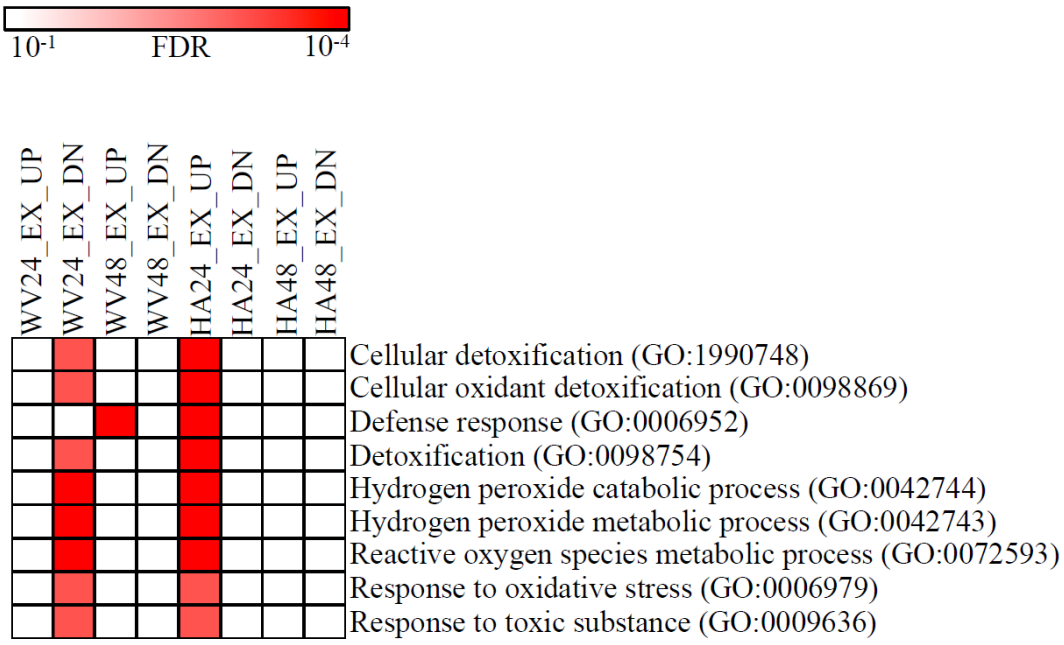


Fig B3.2 GO categories that are found in more than one 'Exclusive gene' list.

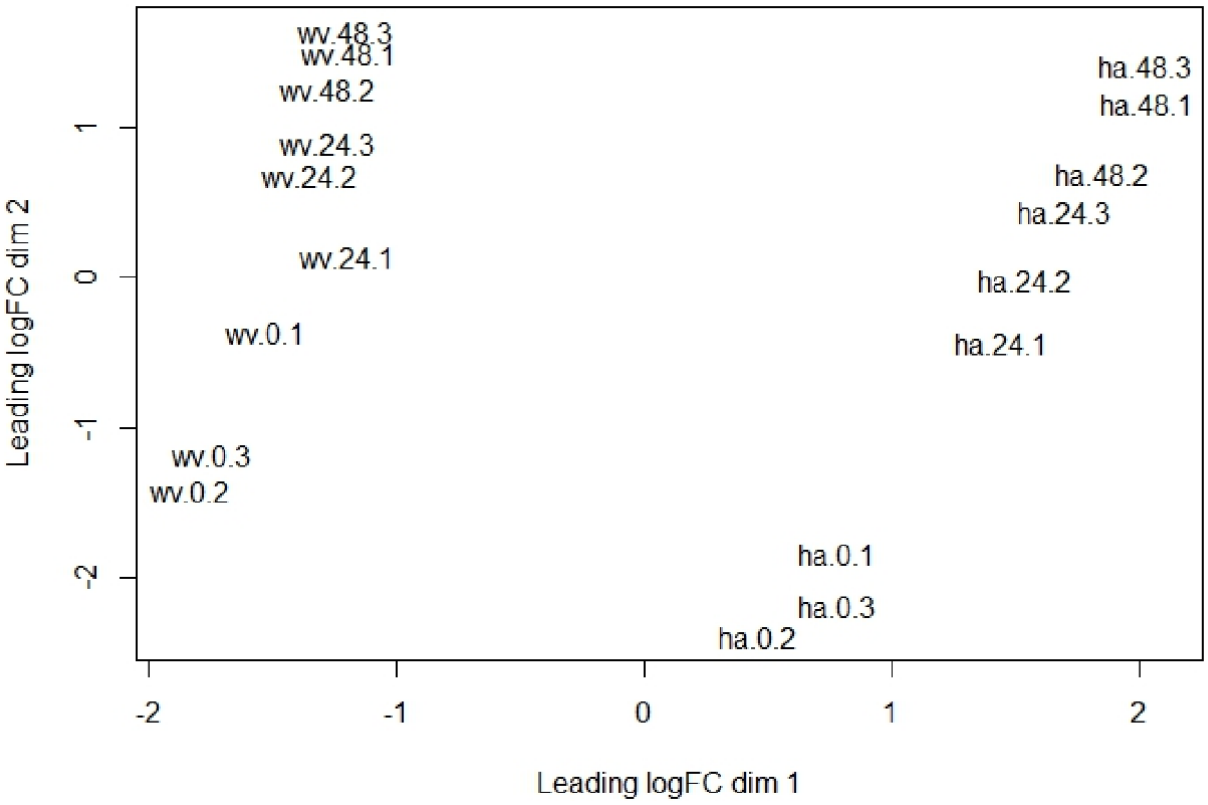


Fig B3.3 MDS plots of samples from edgeR. To examine the tomato root response to R. solanacearum infection within resistant H7996 and susceptible WV, differentially expressed genes were identified from two comparisons within each genotype: 1) 24 hpi to 0 hour and 2) 48 hpi to 0 hour.

TABLES

Table B3.1 Mapping summary showing numbers of raw reads, quality and adapter clipped reads, Tophat mapping percentage for each of the 18 RNA-seq samples and specified options in Tophat

Sample name	Raw reads		Quality & adapter clipped reads							Tophat					
	Total reads	Bases	Total reads	Bases	MinLen	MeanLen	MaxLen	%seqs lost	%bases lost	All mapping seqs	Mapped Seqs	High map quality seqs	Quality Mapped %	X-Coverage	NZ%
1-I-0H-HA	54,957,080	5,550,665,080	54,322,890	5,420,694,605	30	99	101	1	2	53,687,966	52,871,099	52,274,810	96	6	67
2-I-0H-WV	53,380,030	5,391,383,030	52,765,410	5,265,613,422	30	99	101	1	2	52,093,116	51,366,740	50,828,793	96	6	67
3-I-24H-HA	56,658,540	5,722,512,540	56,060,952	5,594,525,573	30	99	101	1	2	55,274,878	54,520,997	53,930,435	96	6	68
4-I-24H-WV	59,015,180	5,960,533,180	58,189,386	5,800,766,549	30	99	101	1	2	57,242,018	56,614,266	56,128,518	96	6	68
5-I-48H-HA	52,887,034	5,341,590,434	52,224,296	5,208,546,052	30	99	101	1	2	51,426,228	50,575,883	49,896,688	95	6	68
6-I-48H-WV	59,780,136	6,037,793,736	59,171,196	5,907,029,796	30	99	101	1	2	58,425,109	57,469,212	56,732,881	95	7	68
7-II-0H-HA	59,166,948	5,975,861,748	58,501,448	5,838,149,366	30	99	101	1	2	57,711,849	57,033,771	56,545,170	96	6	68
8-II-0H-WV	63,099,904	6,373,090,304	62,301,648	6,214,539,110	30	99	101	1	2	61,536,055	60,721,856	60,136,671	96	7	68
9-II-24H-HA	57,622,614	5,819,884,014	56,946,532	5,681,967,922	30	99	101	1	2	56,189,750	55,401,866	54,799,009	96	6	68
10-II-24H-WV	57,092,544	5,766,346,944	56,438,650	5,631,990,355	30	99	101	1	2	55,876,971	54,883,646	54,198,371	96	6	68
11-II-48H-HA	73,064,894	7,379,554,294	72,136,388	7,192,481,391	30	99	101	1	2	71,060,746	69,931,806	69,049,316	95	8	68
12-II-48H-WV	55,465,320	5,601,997,320	54,767,992	5,462,764,705	30	99	101	1	2	54,070,452	53,271,522	52,693,126	96	6	67
13-III-0H-HA	54,947,770	5,549,724,770	54,022,214	5,379,116,042	30	99	101	1	3	53,384,859	52,630,325	52,101,991	96	6	68
14-III-0H-WV	52,852,916	5,338,144,516	52,274,426	5,217,324,914	30	99	101	1	2	51,467,339	50,719,673	50,180,465	95	6	68
15-III-24H-HA	52,757,226	5,328,479,826	51,958,430	5,178,025,404	30	99	101	1	2	51,224,178	50,508,891	49,961,412	96	6	68
16-III-24H-WV	51,683,342	5,220,017,542	50,958,894	5,079,524,793	30	99	101	1	2	50,329,976	49,433,542	48,821,076	95	6	67
17-III-48H-HA	53,951,092	5,449,060,292	53,422,692	5,334,249,917	30	99	101	0	2	52,356,620	51,607,580	51,014,536	95	6	68
18-III-48H-WV	51,089,034	5,159,992,434	50,518,210	5,041,443,512	30	99	101	1	2	49,898,765	49,055,116	48,437,069	95	6	68
Sum	1,019,471,604	102,966,632,004	1,006,981,654	100,448,753,428						993,256,875	978,617,791	967,730,337			
Average	56,637,311	5,720,368,445	55,943,425	5,580,486,302	30	99	101	1	2	55,180,938	54,367,655	53,762,797	96	6	68

TopHat Specified Options

library-type	fr-firststrand
num-threads	8
output-dir	tophat_with_gtf
GTF	ITAG2.4_gene_models.gff
tmp-dir	tophat_with_gtf.tmp
mate-inner-dist	300
mate-std-dev	150

Table A3.2 Normalized library sizes

sample	library size	normalization factors	Normalized library size
H7996.0H.1	21910860	0.8558073	18751473.94
H7996.0H.2	23637799	1.0664308	25208076.9
H7996.0H.3	21106140	0.9849515	20788524.25
H7996.24H.1	22649288	1.0398322	23551458.97
H7996.24H.2	22807153	1.0564344	24094261
H7996.24H.3	20570154	1.0148387	20875388.34
H7996.48H.1	20756299	0.9700082	20133780.23
H7996.48H.2	28621109	0.968652	27723894.48
H7996.48H.3	21499722	1.0158526	21840548.49
WV.0H.1	21137280	0.9578474	20246288.69
WV.0H.2	25064139	1.0247961	25685631.9
WV.0H.3	20777875	1.0176952	21145543.65
WV.24H.1	23000897	1.0943429	25170868.33
WV.24H.2	22326607	0.9975427	22271743.83
WV.24H.3	20099037	0.9497958	19089980.93
WV.48H.1	23711599	0.9936072	23560015.49
WV.48H.2	21433822	1.0229955	21926703.45
WV.48H.3	19762884	0.9929896	19624338.28

***Tables A3.3, A3.4, and A3.5 are too large to be included in this document. They are available online at** https://doi.org/10.1094/MPMI-08-17-0209-R **and are labeled Supplementary Tables 2, 3, 4, and 5, respectively.**

APPENDIX C. CHAPTER 4 SUPPLEMENTAL FIGURES AND TABLES

FIGURES

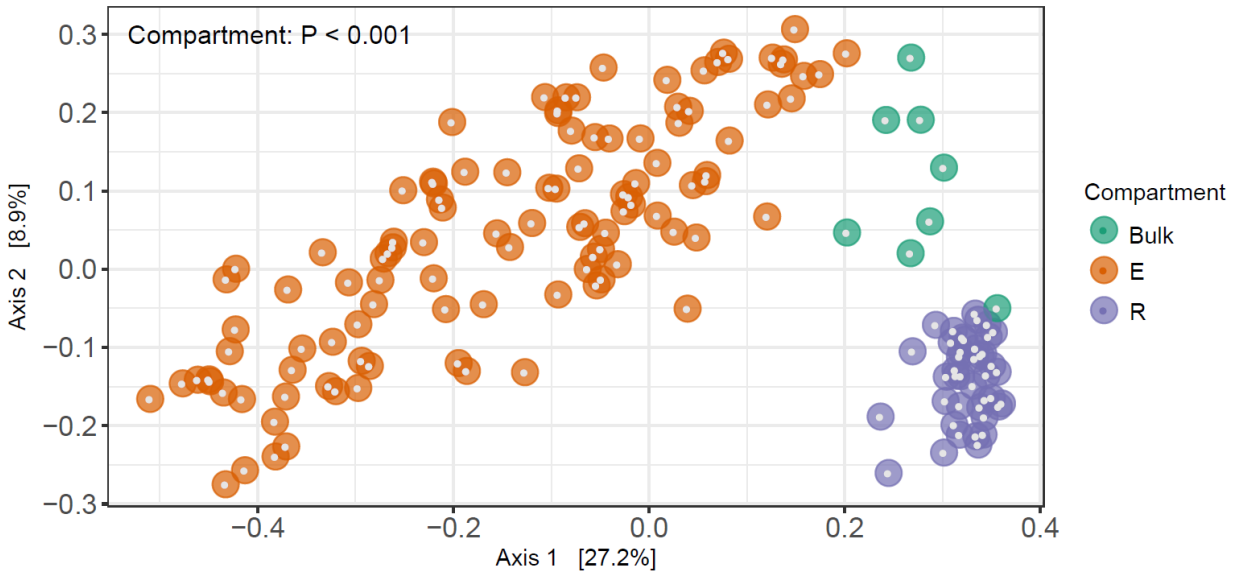


Fig C4.1. Overall patterns of beta diversity across all samples cluster by compartment. Principle coordinate analysis of all samples colored by compartment. Bulk indicates unplanted soil samples. One-way PERMANOVA results indicated on graph. E - endosphere. R – rhizosphere.

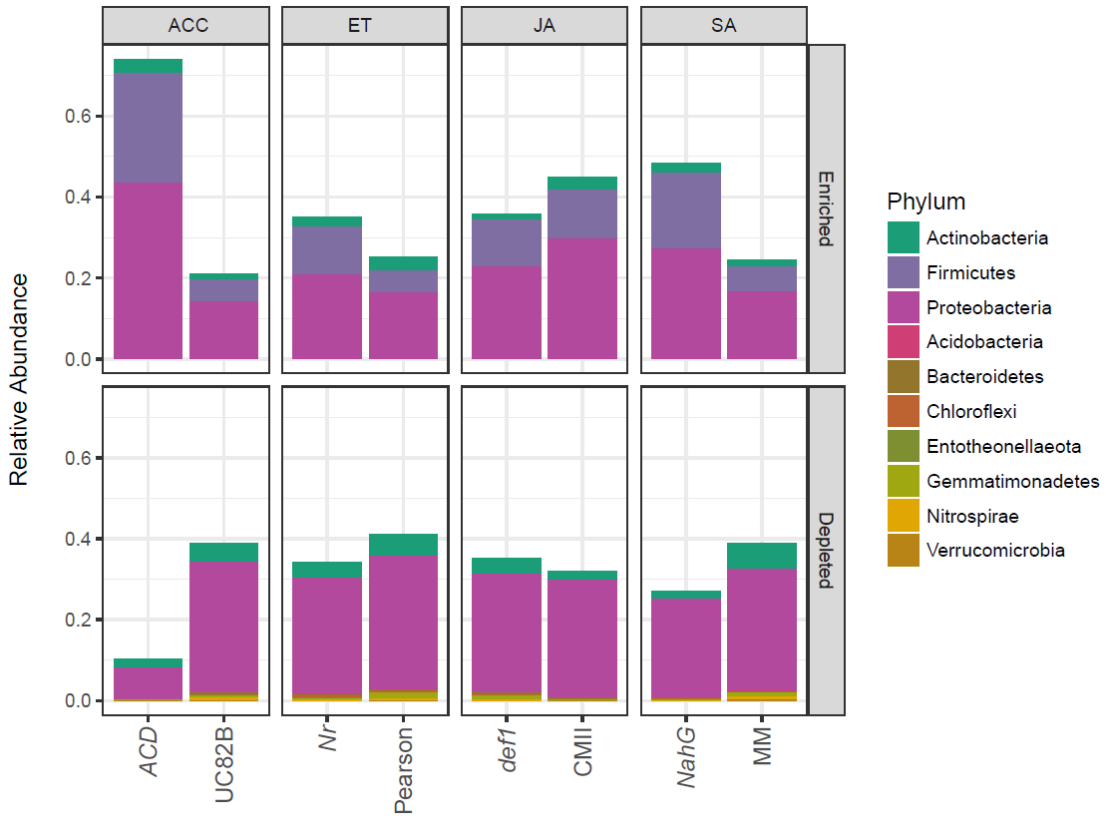


Fig C4.2 *ACD* and *NahG* endosphere-enriched ASVs are more enriched and depleted ASVs are more depleted than in other genotypes. Stacked barplots of relative abundance of all endosphere-enriched (top panel) and all endosphere-depleted ASVs in the endosphere (bottom panel). Enriched and depleted datasets generated by grouping all ASVs significantly enriched/depleted in the endosphere compared to the rhizosphere across any of the eight genotypes. Barplot colors indicate relative abundance of each phyla.

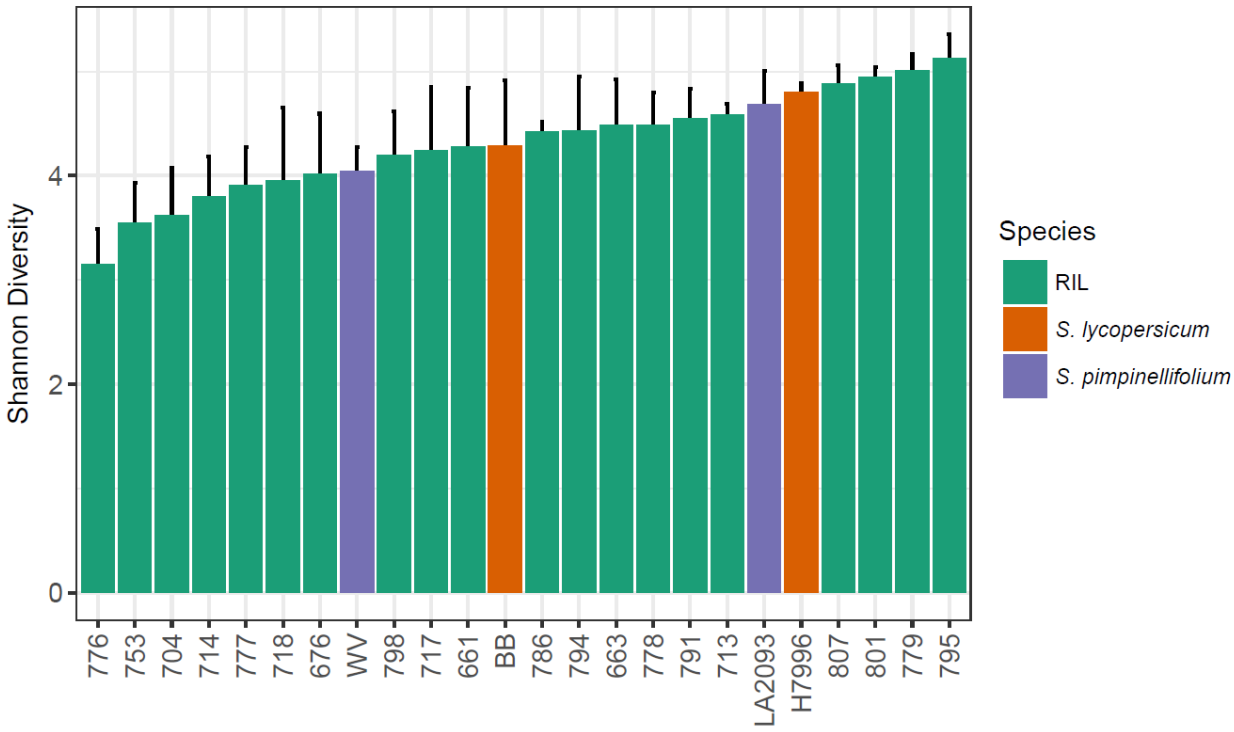


Fig C4.3 Quantitative variation in Shannon diversity of root endophyte bacterial community of panel of H7996xWV RILs. Bar graph of Shannon diversity of H7996, WV, 20 H7996xWV RILs, LA2093, and BB. Error bars represent standard error. Green bars represent RIL lines, orange bars represent *S. lycopersicum* cultivars and purple bars represent *S. pimpinellifolium* accessions. RIL – recombinant inbred line. H7996 - *Solanum lycopersicum* cv. Hawaii7996. WV - *S. pimpinellifolium* accession West Virginia700. BB - *S. lycopersicum* cv. Bonnie Best. LA2093 - *S. pimpinellifolium* accession LA2093.

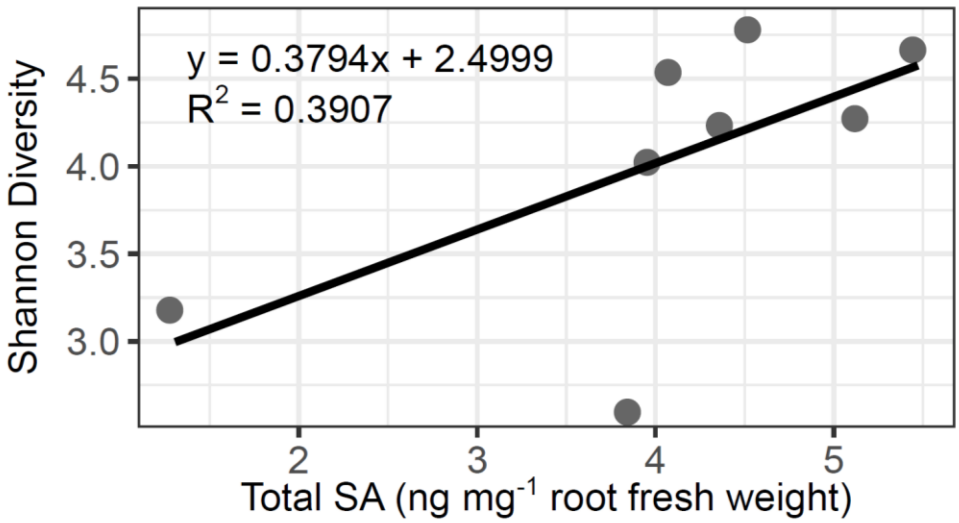


Fig C4.4 Shannon diversity and total SA content in roots are positively correlated. Linear correlation of Shannon diversity and total root SA content in nanograms (ng) per milligram (mg) fresh weight (FW) of frozen, ground roots from eight genotypes – ACD, UC82B, NahG, MM, H7996, WV, BB, LA2093. Equation and R^2 value for the trendline represented on the plot. SA – salicylic acid. *ACD* – ACC-deaminase transgenic mutant. UC82B – *S. lycopersicum* cv. UC82B. *NahG* – salicylate hydroxylase transgenic mutant. MM – *S. lycopersicum* cv. Money Maker. H7996 – *S. lycopersicum* cv. Hawaii7996. WV – *S. pimpinellifolium* WV. BB – *S. lycopersicum* cv. Bonnie Best. LA2093 – *S. pimpinellifolium* accession LA2093.

TABLES

Table C4.1. Soil characteristics.

Soil	*% OM*	*Bray-1 P (ppm)*	*K (ppm)*	*Mg (ppm)*	*Ca (ppm)*	*pH*	*CEC (meq/100 g)*	*NO^3-N (ppm)*
Potting Mix/Field soil	6.8 (±0.4)	40.3 (±0.3)	137 (±4.5)	285 (±2.9)	1483 (±33.3)	6.8 (±0.03)	10.7 (±0.3)	26 (±0.6)
Field soil	2.6 (±0.1)	61.7 (±1.2)	173 (±3.8)	243 (±3.3)	1567 (±33.3)	7.4 (±0.03)	10.3 (±0.2)	7 (±0.0)

Table C4.2. Mutant lines and backgrounds

Mutant	**Background**	**Pathway**	**Rhizo?**	**Endo?**
ACD	UC82B	Ethylene	✓	✓
Nr	Pearson	Ethylene	✓	✓
NahG	Money Maker (MM)	Salicylic acid	✓	✓
def1	Castlemart II (CMII)	Jasmonic acid	✓	✓

Table C4.3. *S. lycopersicum*, *S. pimpinellifolium* lines and RILs

Genotype	Species/RIL	Resistance	Rhizo?	Endo?
H7996	*Solanum lycopersicum*	R	✓	✓
WV	*S. pimpinellifolium*	S	✓	✓
LA2093	*S. pimpinellifolium*	R	✓	✓
BB	*S. lycopersicum*	S	✓	✓
676	H7996xWV RIL	R		✓
713	H7996xWV RIL	R		✓
718	H7996xWV RIL	R		✓
663	H7996xWV RIL	R		✓
717	H7996xWV RIL	R		✓
661	H7996xWV RIL	R		✓
714	H7996xWV RIL	R		✓
807	H7996xWV RIL	R		✓
704	H7996xWV RIL	R		✓
801	H7996xWV RIL	R		✓
753	H7996xWV RIL	S		✓
779	H7996xWV RIL	S		✓
777	H7996xWV RIL	S		✓
786	H7996xWV RIL	S		✓
791	H7996xWV RIL	S		✓
795	H7996xWV RIL	S		✓
776	H7996xWV RIL	S		✓
778	H7996xWV RIL	S		✓
794	H7996xWV RIL	S		✓
798	H7996xWV RIL	S		✓

Table C4.4 Sequencing Summary.

	Total paired reads	# of ASVs
Initial reads	8,155,368	NA
After adapter/primer clipping	8,154,145	NA
dada2 quality filtering	7,420,024	NA
Error correction, merged F and R reads, length filtering	6,241,313	37,189
Chimera removal	6,139,131	30,630
Non-target sequence removal	3,073,541	24,033
Likely contaminant removal	2,907,351	23,833
Low abundance filtering	1,838,154	901

Table C4.5 Summary of differential abundance from rhizosphere to endosphere by genotype.

	ACD		UC82B		*NahG*		MM		*Nr*		Pearson		*def1*		CMII		Average	
	En	Dep	En	Dep	En	Dep	En	Dep	En	Dep	En	Dep	En	Dep	En	Dep	En	Dep
Total (#)	15	133	14	36	14	109	13	32	16	28	15	15	12	45	13	57	14	57
Actinobacteria (%)	13.3	19.5	14.3	19.4	14.3	21.1	15.4	15.6	12.5	25	13.3	33.3	16.7	24.4	15.4	22.8	14.4	22.7
Bacteroidetes (%)	0	2.3	0	5.6	0	0.9	0	9.4	0	7.1	0	6.7	0	6.7	0	0	0	4.8
Firmicutes (%)	13.3	0	7.1	0	21.4	0	15.4	0	6.3	0	6.7	0	16.7	0	15.4	0	12.8	0
Proteobacteria (%)	73.3	72.2	78.6	72.2	64.3	71.6	69.2	65.6	81.3	64.3	80	53.3	66.7	62.2	69.2	68.4	72.8	66.2

Table C4.6 Full differential abundance results for *ACD*

Table C4.7 Full differential abundance results for UC82B

Table C4.8 Full differential abundance results for *NahG*

Table C4.9 Full differential abundance results for MM

Table C4.10 Full differential abundance results for *Nr*

Table C4.11 Full differential abundance results for Pearson

Table C4.12 Full differential abundance results for *def1*

Table C4.13 Full differential abundance results for CMII

***Tables C4.6-13 are too large to be included in this document. They are available online at**

https://github.rcac.purdue.edu/AnjaliIyerpascuzziGroup/Tomato-Root-16S-Sequencing

and are labeled Supplementary Tables 6-13, respectively.