DISSERTATION

HOW STRESS AFFECTS RICE: A CHARACTERIZATION OF THE RICE TRANSCRIPTOME DURING SINGLE AND SIMULTANEOUS ABIOTIC AND BIOTIC STRESSES

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ABSTRACT

HOW STRESS AFFECTS RICE: A CHARACTERIZATION OF THE RICE TRANSCRIPTOME DURING SINGLE AND SIMULTANEOUS ABIOTIC AND BIOTIC STRESSES

Environmental stresses, both abiotic and biotic, are large contributors to pre-harvest crop loss. Abiotic stresses, such as drought, salinity, non-optimal temperature and others, are non-living factors in the environment that have a negative effect on plants. Biotic stresses are biological factors that can harm plants, including pathogens, pests and competition from other plants. With climate change increasing the incidence of abiotic stresses and the constant pressures of pests and pathogens, it is critical to world agriculture that varieties of plants broadly tolerant to stresses are developed. For this, it is necessary to understand how plants respond to multiple simultaneous stresses. The goal of this work is to characterize the stress response of the global staple food plant rice.

Here, I present the results of two comprehensive transcriptome studies. In the first, I characterize how the rice transcriptome changes in response to simultaneous heat stress and infection by the bacterial pathogen *Xanthomonas oryzae* (Xo). Xo includes the causal agent for the economically important bacterial blight disease of rice, Xo pathovar *oryzae* (Xoo). Bacterial blight is more severe during abiotic stresses such as high temperature and drought. Most rice resistance (R) genes that target Xoo lose function at high temperature; however, function of the R-gene *Xa7* is enhanced when the host is subjected to abiotic stresses. Understanding why *Xa7* is more effective during heat stress gives insight into host processes that are important during combined abiotic and biotic stresses. The major finding of this study was that the abscisic acid (ABA) pathway is a node of cross-talk in the interactions between heat stress and pathogen attack, during both susceptible and resistant interactions.

In the second comprehensive study, I characterize how the rice transcriptome is universally regulated by all stresses. Understanding universalities in rice stress response transcriptomes provides insight into how plants endure a wide variety of stresses in the field. To explore the universal rice transcriptome response, I developed a custom workflow to analyze publicly available RNA-Seq data from rice stress response studies, including the abiotic stresses drought, salinity, heat and cold, and the biotic stresses bacterial leaf streak, bacterial blight, rice blast, and two viral diseases. From this study, I concluded that the rice stress response is a robust system with many overlapping features. This core response includes down-regulation of photosynthetic processes and up-regulation of downstream signaling of the hormones ABA, salicylic acid and jasmonic acid.

Within this dissertation, I present networks of gene regulation in four major rice responses: (1) response to a susceptible interaction with Xo during high temperature, (2) response to a resistant interaction with Xo during high temperature, (3) core response to abiotic stresses and (4) core response to biotic stresses. Common among all of these pathways are the pathways upstream and downstream of the plant hormone ABA. ABA-related processes are universally up-regulated by abiotic and biotic stresses, and are only repressed during the enhanced *Xa7* response at high temperature. Because ABA signaling is critical for stress response, we need a thorough understanding of how genes in the ABA response network interact to most efficiently improve rice to be tolerant to multiple and simultaneous stresses. The gene networks I have characterized can be integrated with genome and transcriptome data from stress-tolerant rice varieties. By having a complete understanding of the rice stress response, we can develop an informed approach for developing new varieties of rice that are resistant to stress.

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CHAPTER 1. Introduction[1]

1.1 The effects of abiotic and biotic stresses on crop plants

Stress is a large contributor to pre-harvest crop loss. The variety of stresses affecting crop plants can be broken into two broad categories: abiotic and biotic (Fig 1.1). Abiotic stresses are non-living factors in the environment that have a negative effect on plants. Some examples of abiotic stresses are drought, salinity, non-optimal temperature and limited nutrients. Biotic stresses are biological factors in the environment, such as pathogens, pests and competition, that can harm plants. With climate change increasing the incidence of abiotic stresses and the constant pressures of pests and pathogens, it is critical to world agriculture that broadly stress-tolerant crop varieties are developed. For this, it is necessary to understand how plants respond to stresses alone and in combination.

Abiotic stresses cause many similar effects on plants, independent of stress type. Yield reduction is a particularly severe effect of abiotic stress (Boyer 1982). Both cultivated wheat and its wild relatives experience major penalties to yield in response to drought and heat stress, with yield reduced roughly by half (Kilic and Yagbasanlar 2010; Pour-Aboughadareh et al. 2017; Vignjevic et al. 2015). Two other major staple cereal crops, rice and maize, also experience major yield penalties due to abiotic stresses, including salinity and heat stress (Joshi et al. 2018; Ordóñez et al. 2015; Thitisaksakul et al. 2015). In field experiments, each 1 °C of increased nighttime temperature reduces rice grain yield by 10% (Peng et al. 2004). These yield losses

[1]Parts of this chapter are adapted from the book chapter submitted for review as: Huerta, A. I., Cohen, S. P., Verdier, V., and Leach, J. E. Submitted: 2018. Molecular genetics of bacterial blight and bacterial leaf streak and their impact on future control strategies. in: E-book: Rice diseases: Biology and selected management practices. T. W. Mew, H. Hibino, S. Savary, C. M. Vera Cruz, R. Opulencia and G. P. Hettel, eds. International Rice Research Institute, Los Baños, Philippines.

due to moderate nighttime temperature increase, which are predicted to increase over the coming decades (Welch et al. 2010), will increase greenhouse-gases by 11.8% per 1 °C (Van Groenigen et al. 2013).

Abiotic stress-related yield loss is not specific to cereal crops; soybean, canola, cotton, the bioenergy grass *Miscanthus* × *giganteus*, and others also lose yield and biomass in response to abiotic stresses (Din et al. 2011; Dong et al. 2012; Hamayun et al. 2010; Stavridou et al. 2017). While climate change may bring positive effects on plant yield due to the increase in atmospheric CO_2 (AbdElgawad et al. 2016), the net effect on yield is projected to be negative in multiple crop systems due to high temperature and water deficits (Deryng et al. 2014; Hatfield and Prueger 2015). The projected crop losses due to a global mean temperature increase above 2 °C is predicted to impact 1.8 billion people, with the world's poorest people disproportionately impacted (Hoegh-Guldberg et al. 2018).

These yield losses are likely due to physiological changes in the plants during stress. For example, leaf senescence, which reduces the amount of productive plant tissue, is often caused by drought, salinity, and heat stress (Ghanem et al. 2008; Lobell et al. 2012; Lutts et al. 1996; Wehner et al. 2016). Abiotic stresses also have a negative effect on seed fertility, reducing grain filling and weight (Mohammed and Tarpley 2009; Rang et al. 2011; Thitisaksakul et al. 2015). Abiotic stresses can cause water loss, with an associated accumulation of osmoprotectants such as aquaporins and the amino acid proline in the plant (Afzal et al. 2016; Din et al. 2011; Harb et al. 2010; Kaur and Asthir 2015; Stavridou et al. 2017). Reduced chlorophyll and photosynthetic efficiency is also common in numerous plant species in response to multiple abiotic stresses (Din et al. 2011; Ghanem et al. 2008; Hamayun et al. 2010; Lutts et al. 1996; Utsumi et al. 2012). Because chlorophyll is reduced in abiotic stress-susceptible plants, green leaf area can serve as a screening parameter for selecting stress-tolerant plants in the field (Kalaji et al. 2016). Stress-tolerant plants are often more photosynthetically efficient than stress-susceptible plants, suggesting that maintaining green tissue during stress is a necessary

component of plant defense against abiotic stress (Arjenaki et al. 2012; Joshi et al. 2018; Vignjevic et al. 2015).

Plants mediate regulation of abiotic stress responses via phytohormones (Verma et al. 2016). Chief among plant hormones involved in abiotic stress response is abscisic acid (ABA), a hormone that accumulates in vegetative tissue in response to most abiotic stresses (Finkelstein 2013; Hamayun et al. 2010; Harb et al. 2010; Utsumi et al. 2012). ABA mediates abiotic stress response by causing both short- and long-term responses, such as stomatal closure and induction of dehydration response, respectively (Abe et al. 2003; McAinsh et al. 1990). Salicylic acid (SA) is another major player in abiotic stress response. SA accumulates in plant tissue in response to abiotic stresses and plays a role in preventing oxidative damage to cell membranes (Horváth et al. 2007; Larkindale and Knight 2002). Exogenous SA treatment prevents yield reduction and reduced spikelet fertility in high temperature-treated rice (Mohammed and Tarpley 2009). Concentrations of other hormones, such as jasmonic acid (JA), gibberellic acid (GA), auxins, and cytokinins, are also altered during abiotic stress (Ding et al. 2016; Ghanem et al. 2008; Hamayun et al. 2010; Joshi et al. 2018). There is no single hormonal regulator of abiotic stress response and there is likely cross-talk among these hormone responses during abiotic stress.

Biotic stresses such as weeds, animal pests and pathogens have a range of effects on plants. The amount of crop lost to biotic stresses varies widely among plants, regions and crop protections deployed (Oerke 2006). Average global pathogen- and pest-caused losses of the staples wheat, rice, maize, potato and soybean are between 17% and 30%, with maximum losses in food security hotspots ranging between 21% and 41% (Savary et al. 2019). For weeds and, to some extent, insects, effective and sustainable control strategies are generally well-established (Juraimi et al. 2013; Raybould and Quemada 2010; Savary et al. 2012). However, chemical control of plant pathogens is either costly and not sustainable, as with fungicide control of fungal pathogens, or not effective or widely deployed, as with chemical control of bacterial

and viral pathogens (Khoury and Makkouk 2010; Stuthman et al. 2007; Vidaver 2002). For many plant pathogens, breeding crops for durable resistance is the most sustainable control practice (Brown 2015; Mundt 2014; Stuthman et al. 2007). In this work, the term 'biotic stress' will hereafter refer to plant pathogens, unless otherwise specified.

Like with abiotic stresses, biotic stresses cause a reduction in photosynthetic activity in plants. Reduction in photosynthesis is part of a rapid host metabolic change in response to biotic stresses that also includes increases in respiration, photorespiration and sugar breakdown (Berger et al. 2007). This localized host change occurs in response to pathogens with different infection strategies, such as biotrophic bacteria, biotrophic fungi, necrotrophic fungi and viruses (Berger et al. 2004; Bilgin et al. 2010; Stare et al. 2015). The cause of this metabolic shift is unclear, though it may be a component of the plant's defense response. For example, during resistance, to quickly power reactions needed for defense, plants may switch from assimilating to catabolizing carbon (Scharte et al. 2005). Furthermore, because some pathogens hijack host sugars and sugar transporters, disabling photosynthesis may be a host strategy for depriving pathogens of nutrition to slow down infection (Chen et al. 2010).

Plant hormones play important roles during plant-pathogen interactions, and just as with abiotic stress response, there is no single hormone regulator of biotic stress response (Bari and Jones 2009; Shigenaga and Argueso 2016). SA is widely studied for its role in resistance against pathogens, especially biotrophs and hemi-biotrophs (Loake and Grant 2007). Host SA accumulation is necessary for both localized and systemic resistance to some pathogens (Delaney et al. 1994). JA is also important for localized and systemic defense, primarily against necrotrophic pathogens (Antico et al. 2012; Cohen et al. 1993). In dicots, SA and JA pathways are mutually antagonistic, with SA-induced pathways down-regulated by JA and vice versa (Caarls et al. 2015). In the monocot rice, JA and SA act synergistically and activate many of the same genes (De Vleesschauwer et al. 2016; Tamaoki et al. 2013). Ethylene, which acts

synergistically with JA (Huang et al. 2015), activates plant defense against necrotrophs (Zhu et al. 2014).

The role of ABA during plant-pathogen interactions is complex, with both negative and positive effects on plant defense (Lievens et al. 2017). ABA interacts antagonistically with SA signaling in rice and Arabidopsis (Jiang et al. 2010; Xu et al. 2013a; Yasuda et al. 2008). *Pseudomonas syringae* pv. *tomato* (Pst) uses effectors to target and induce plant ABA biosynthesis, which increases host susceptibility (de Torres-Zabala et al. 2007). Contrary to the findings of de Torres-Zabala et al. (2007), Seo and Park (2010) found that ABA signaling induced salicylic acid and, thus, host resistance to Pst. Stomatal pores on leaf surfaces, which are entry points for some pathogens, are closed by both ABA and SA (Khokon et al. 2011; Montillet et al. 2013). ABA also interacts antagonistically with JA and ethylene (Anderson et al. 2004), with ABA-induced resistance to the brown spot pathogen in rice mediated via ethylene repression (De Vleesschauwer et al. 2010). There are more plant genes responsive to ABA than to any other hormone (Garg et al. 2012; Nemhauser et al. 2006). Perhaps this magnitude of transcriptional reprogramming explains some of the complexity in ABA effects on biotic response.

1.2 Combinations of stress have unpredictable results

Crop improvement programs and research laboratories tend to focus on making their crop of choice more tolerant to a single stress (Mickelbart et al. 2015). This approach, while successful at increasing tolerance to one stress, neglects the biological reality that plants often experience multiple stresses in the field. This can inadvertently lead to varieties that can withstand one stress at the expense of susceptibility to another (Atkinson and Urwin 2012). Because plants perceive and respond to simultaneous stresses as a new stress, independent of either single stress, the effects of multiple simultaneous stresses on a plant are unpredictable (Gupta and Senthil-Kumar 2017). This unpredictable nature of response to simultaneous

stresses may be due to cross-talk among components of the individual pathways (Fujita et al. 2006).

Stress response components such as transcription factors (TFs), kinases, phytohormones and reactive oxygen species may act as nodes of antagonistic cross-talk, favoring one stress response at the expense of another. For example, overexpression of the Arabidopsis gene *NPR1* induces resistance to fungal and bacterial pathogens, but increases plant sensitivity to salt and drought stress (Quilis et al. 2008). Similarly, overexpression of *OsWRKY76*, a rice gene encoding a transcriptional repressor, increases tolerance to cold stress at the cost of greatly increased susceptibility to the rice blast fungus (Yokotani et al. 2013). The *N* gene, a tobacco resistance gene to tobacco mosaic virus, loses function at elevated temperatures (Zhu et al. 2010). Alternatively, some resistance genes gain function during abiotic stress, such as the wheat gene *Yr36* and the rice gene *Xa7*, which provide enhanced resistance at moderately high temperatures against stripe rust and bacterial blight, respectively (Fu et al. 2009; Webb et al. 2010). With this potential cross-talk among stress response pathways, the best strategy for developing broadly stress-resilient plants is unclear.

1.3 Bacterial blight of rice is more severe during abiotic stress

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a devastating bacterial disease of rice (Mew 1993). Early symptoms of BB are water-soaked streaks that first appear on the margins and tips of rice blades. As infection progresses, the streaks elongate and expand along the veins culminating in tannish-gray to white lesions. When BB occurs, typical rice yield reductions range between 20-50%, but under heavy disease pressure, conducive environmental conditions, and lack of disease resistance in deployed varieties, yield losses may reach 70% (Mew and Misra 1994; Ou 1985; Reddy et al. 1979). To effectively and sustainably manage this bacterial disease, farmers practice culture- and region-specific disease management tactics. These tactics include good water drainage, optimal plant spacing, timely

fertilizer application, routine field sanitation practices and rational deployment of resistant varieties (Leung et al. 2003; Mew et al. 2004). Chemical control tactics for BB are limited, expensive and unreliable, especially in the tropics where heavy rainfall, temperatures and high humidity limit efficacy (Chaudhary et al. 2012; Devadath 1989; Gnanamanickam et al. 1999).

During simultaneous abiotic and biotic stress, development of BB is more severe. In a comprehensive panel of rice varieties, researchers found that in almost all interactions tested, lesions were longer at high temperature, 35 °C, than normal temperature, 29 °C (Horino et al. 1982). In this study, both pathogen resistant and susceptible rice varieties developed more disease. In another study, several rice BB resistance genes lost function at 35 °C (Webb et al. 2010), and yet another study demonstrated that the rice BB resistance gene Xa21, which is fully active at 27 °C, lost all function when rice was grown at 31 °C (Chen et al. 2018). There is similar evidence that rice is more susceptible to BB during periods of drought, with even drought tolerance seemingly contributing to breakdown of BB resistance (Dossa et al. 2016b). Interestingly, two BB resistance genes, *Xa7* and an unknown gene from African rice, retain function during abiotic stresses (Dossa et al. 2016a; Dossa et al. 2017; Webb et al. 2010). These results show the need for understanding how abiotic stresses affect BB development, both phenotypically and mechanistically, in a range of stress tolerant and sensitive rice varieties. This information will support the development of rice varieties that can withstand BB, regardless of environmental conditions.

Some of the molecular mechanisms underlying interactions between rice response to abiotic stress and Xoo infection have been characterized. Transcriptomics and proteomics have identified genes induced by both abiotic and biotic stress, indicating that rice stress response is highly similar, regardless of stress (Kumar et al. 2015; Narsai et al. 2013). Functional studies have pointed to the importance of signaling and regulatory molecules in response to abiotic and biotic stresses; for example, WRKY45 is a well-characterized defense-response TF that is induced by both abiotic and biotic stresses, including Xoo, in rice (Qiu and Yu 2009). While

over-expression of this gene in Arabidopsis enhances both abiotic stress tolerance and disease resistance, overexpression in rice negatively regulates response to abiotic stress (Tao et al. 2011). OsNAC6 is a TF in the SNAC-A TF family that positively regulates resistance to abiotic and biotic stresses (Nakashima et al. 2012; Nakashima et al. 2007). Rice stress-activated protein kinases and valine-glutamine motif-containing proteins also act positively in both abiotic stress tolerance and Xoo resistance (Kim et al. 2013; Xu et al. 2013b). GF14 family 14-3-3 genes, which are induced by abiotic stress and effector-triggered resistance to Xoo and the blast fungus *Magnaporthe grisea*, negatively regulate Xoo resistance-associated cell death (Chen et al. 2006; Manosalva et al. 2011). Many of these genes contain similar *cis*-regulatory elements in their promoters, such as the biotic-responsive W and GCC boxes and the abiotic-responsive ABRE, MYB and MYC boxes, indicating that there are shared regulatory mechanisms underlying their expression. Ultimately, the mechanisms underlying rice responses to abiotic stresses and Xoo infection are complex and overlapping, necessitating the need for new technologies to understand these responses at a systems level.

1.4 The "RNA-Seq revolution" in plant research

RNA-Seq is the use of high-throughput DNA sequencing technology for transcriptome profiling (Wang et al. 2009). When RNA-Seq was introduced, the prevailing transcriptome profiling technique was DNA hybridization-based microarrays. Microarrays, which are still widely used for transcriptome profiling, use probes of known transcript sequences to detect complementary DNA generated from a population of RNA (Malone and Oliver 2011). While more expensive than microarrays, RNA-Seq overcomes some of their limitations. For example, with RNA-Seq, it is possible to detect splice variants and novel sequences (Mantione et al. 2014). RNA-Seq is thus a powerful tool for generating hypotheses about and investigating transcriptomes.

Since its introduction, RNA-Seq has become an invaluable and ubiquitous tool for research in plant sciences. The NCBI Sequence Read Archive (SRA) is a public repository for high-throughput sequencing data (Leinonen et al. 2010). SRA allows users to submit sequence reads generated in RNA-Seq experiments, a common requirement for publishing RNA-Seq results. Over 60,000 samples have been submitted to SRA from plant-related RNA-Seq experiments (Fig 1.2a, Table 1.1). Together, these studies contain over 200 petabases. Furthermore, the average amount of bases sequenced per experiment is generally increasing, from a mean of 743 megabases per sample (median 349) in 2010 to 3,297 (median 2,118) in 2017 (Fig 1.2b, Table 1.1), likely because of improvements and cost savings in high-throughput sequencing technologies. Some of the uses of RNA-Seq in plant sciences, detailed below, include (1) detailed elucidation of plant transcriptomes, (2) improvement of existing genome annotations, (3) generation of genomes for non-model plants and (4) characterization of gene regulation networks (Martin et al. 2013).

One of the most common uses of RNA-Seq in plant research is for elucidating plant transcriptomes in different tissues or under different stimuli. The standard units of transcriptome change are differentially expressed genes (DEGs). With RNA-Seq, a gene is considered a DEG if the treatment of interest changes the number of transcript reads, either positively or negatively, in a statistically significant manner (Anjum et al. 2016). An early RNA-Seq study in 2011 provided the rice research community with a comprehensive rice expression atlas (Shen et al. 2011). Rice DEGs in ten rice tissues, and response to six and thirteen abiotic and biotic stresses, respectively, were characterized in this study. While this study was large, it was not exhaustive, and sequencing technologies have improved in the years since. Therefore, many additional studies have been conducted to quantify the rice response to various abiotic and biotic stresses. For a more detailed exploration of the rice transcriptome response to stresses, see chapter 3 of this text.

RNA-Seq is a powerful technique for improving existing genome annotations. The rice Nipponbare reference genome was established by the International Rice Genome Sequencing Project in 2005 and has been incrementally improved over the years (Kawahara et al. 2013). Several RNA-Seq-based approaches have been important in this improvement. Long read RNA-Seq was used to identify over 5,000 novel splice variants not present in the reference genome, including several hundred multi-exon long non-coding RNAs (Zhang et al. 2018a). In a similar work, publicly available RNA-Seq data was paired with proteomic data to identify over 1,584 novel rice peptides, clustered into 692 genomic loci (Ren et al. 2018). Another work used RNA-Seq of rice plants experiencing mineral nutrient stress to identify 14 times more alternative splicing events than were previously known (Dong et al. 2018). RNA-Seq technologies have allowed researchers to identify several thousand novel stress-responsive long non-coding RNAs in rice (Li et al. 2018b; Shin et al. 2018; Yuan et al. 2018).

Annotation of other plant genomes has also been improved with RNA-Seq technologies. A proprietary 3'-end RNA-Seq approach was recently used to better annotate more than 45% of existing gene models in tomato (Tzfadia et al. 2018). RNA-Seq has allowed researchers to better annotate small and long non-coding RNAs in Arabidopsis, *Brassica napus*, and wild banana (Liu et al. 2018a; Polydore and Axtell 2018; Shen et al. 2018). Innovative applications of high-throughput sequencing technologies like these are allowing researchers to develop a more complete understanding of current plant genomes.

These technologies also allow researchers working on non-model species, when reference genomes are often lacking, to generate high quality genomic resources in the form of *de novo* transcriptomes. Beyond simply developing genomic resources, this approach allows researchers to identify genes in processes of interest. For example, *de novo* transcriptomics has been used to identify genes involved in catechin metabolism, fluoride accumulation and wound response in the non-model tea plant *Camellia sinensis* (Li et al. 2018a; Li et al. 2018c; Zhang et al. 2018c). *De novo* transcriptomes have been developed for many other non-model plants,

including the medicinal plant *Pfaffia glomerata*, the water chickweed *Myosoton aquaticum*, the broadleaf plaintain *Plantago major*, the tuberose *Polianthes tuberosa* and the zucchini *Cucurbita pepo* (Batista et al. 2018; Huang et al. 2018; Liu et al. 2018b; Madhavan et al. 2018; Vitiello et al. 2018). These studies, among the countless others not cited here, show that using RNA-Seq to generate *de novo* transcriptomes is a valid and powerful approach to studying plant species with few genomic resources.

Co-expression analysis of transcriptome data is a powerful inductive approach to identifying networks of gene interactions (Le Novere 2015; Ruprecht et al. 2017). Using RNA-Seq data from rice varieties resistant and susceptible to the fungal pathogens *Rhizoctonia solani* and *Tilletia horrida*, two groups built rice gene co-expression networks, allowing the authors to identify key gene interactions during resistance (Wang et al. 2018; Zhang et al. 2018b). Studies like these offer insights into differences in regulatory mechanisms among stress tolerant and sensitive plant varieties, which allow researchers to identify putative regulatory hubs that can be improved through targeted breeding. For example, the transcription factor HIGHER YIELD RICE, identified through gene co-expression analysis, is a master regulator of photosynthesis that increases rice yield stability under abiotic stresses (Ambavaram et al. 2014).

Other uses of RNA-Seq in plant research include transcriptomics applications to *in planta* microbe, single cell, and chloroplast gene expression. Analysis of the *in planta* microbe transcriptome gives insight into both how the host genotype affects gene expression of pathogens and the strategies employed by virulent pathogens (Chatnaparat et al. 2016; Khokhani et al. 2017; Ma et al. 2018; Nobori et al. 2018). Single cell and chloroplast transcriptome profiling gives spatial information on gene expression within plant tissue and organelles, respectively (Han et al. 2017; Michel et al. 2018; Sakai et al. 2018). These approaches demonstrate the power of RNA-Seq in giving researchers a holistic view of how plants and their environment interact.

In all these examples, the use of RNA-Seq provides a more comprehensive understanding of gene expression. With a greater understanding of how plant transcriptomes change in response to stresses, researchers can generate systems level hypotheses about how to improve crop varieties in light of those stresses. Two chapters within this dissertation (chapters 2 and 3) utilize RNA-Seq to generate hypotheses for how the rice transcriptome responds to abiotic and biotic stresses.

1.5 About this dissertation

Because environmental stresses are large contributors to yield loss in food crops, it is critical to understand how plants respond to stress. The purpose of this dissertation is to explore how the rice transcriptome changes in response to (1) simultaneous abiotic and biotic stress, (2) all abiotic stresses, (3) all biotic stresses and (4) all abiotic and biotic stresses. Rice is an ideal system for this study because it is an important global food staple and there are robust genomic resources available.

In chapter 2, I present the results of an RNA-Seq study of rice experiencing simultaneous heat stress and *Xanthomonas oryzae* (Xo) infection. As mentioned previously, Xo infections are more severe and many rice resistance genes that target Xo fail at high temperature. However, the resistance gene *Xa7* functions better at high temperature. To better understand the rice responses underlying both increased susceptibility and enhanced *Xa7*-mediated resistance at high temperature, we conducted a transcriptomics experiment. We conducted disease assays with rice cultivar IRBB61, containing *Xa7*, and two strains of Xo, one containing a plasmid with the gene for the elicitor of *Xa7* and the other with an empty vector.

In chapter 3, I explore the rice broad response to abiotic and biotic stresses via meta-analysis of publicly available rice transcriptome data. Crop improvement programs often focus on developing varieties tolerant to a single stress. While these varieties are tolerant to the chosen stress, whether they will be tolerant or susceptible to other stresses is unpredictable.

For the informed development of broadly stress-tolerant varieties of crops, it is necessary to identify genes and pathways that are universally regulated by multiple stresses. To identify universally regulated genes and pathways in rice, RNA-Seq data was analyzed from publicly available studies on drought, salinity, high temperature, cold, *Xanthomonas oryzae* pvs. *oryzae* and *oryzicola*, *Magneporthe oryzae*, rice stripe virus and rice dwarf virus.

In chapter 4, I present a discussion and conclusions on the work conducted. This chapter includes a summary of the novel findings in this dissertation, preliminary data on temperature-*Xa7*-ABA interactions and a commentary on the research I envision as necessary follow-up to these studies. Finally, in the appendix, I include tables, figures and methods that are supplementary to chapters 2 – 4.

1.6 Tables and Figures

Table 1.1: Summary of plant-related RNA-Seq experiments available on the NCBI Sequence Read Archive.

Year	Number of Samples	Total Bases*‡	Mean Bases per Sample‡	Median Bases per Sample‡
2010	19	14,126	743	359
2011	97	160,324	1,653	593
2012	288	608,503	2,113	1,437
2013	1,060	3,965,183	3,741	2,870
2014	4,261	11,713,484	2,749	1,394
2015	11,442	34,026,129	2,974	1,986
2016	16,824	61,192,575	3,637	2,771
2017	26,850	88,533,736	3,297	2,118
Total	60,841	200,214,060	-	-

Meta-data for Table 1.1 was downloaded from NCBI SRA on 2018-09-24. *Yearly totals are non-cumulative; ‡bases are in megabases

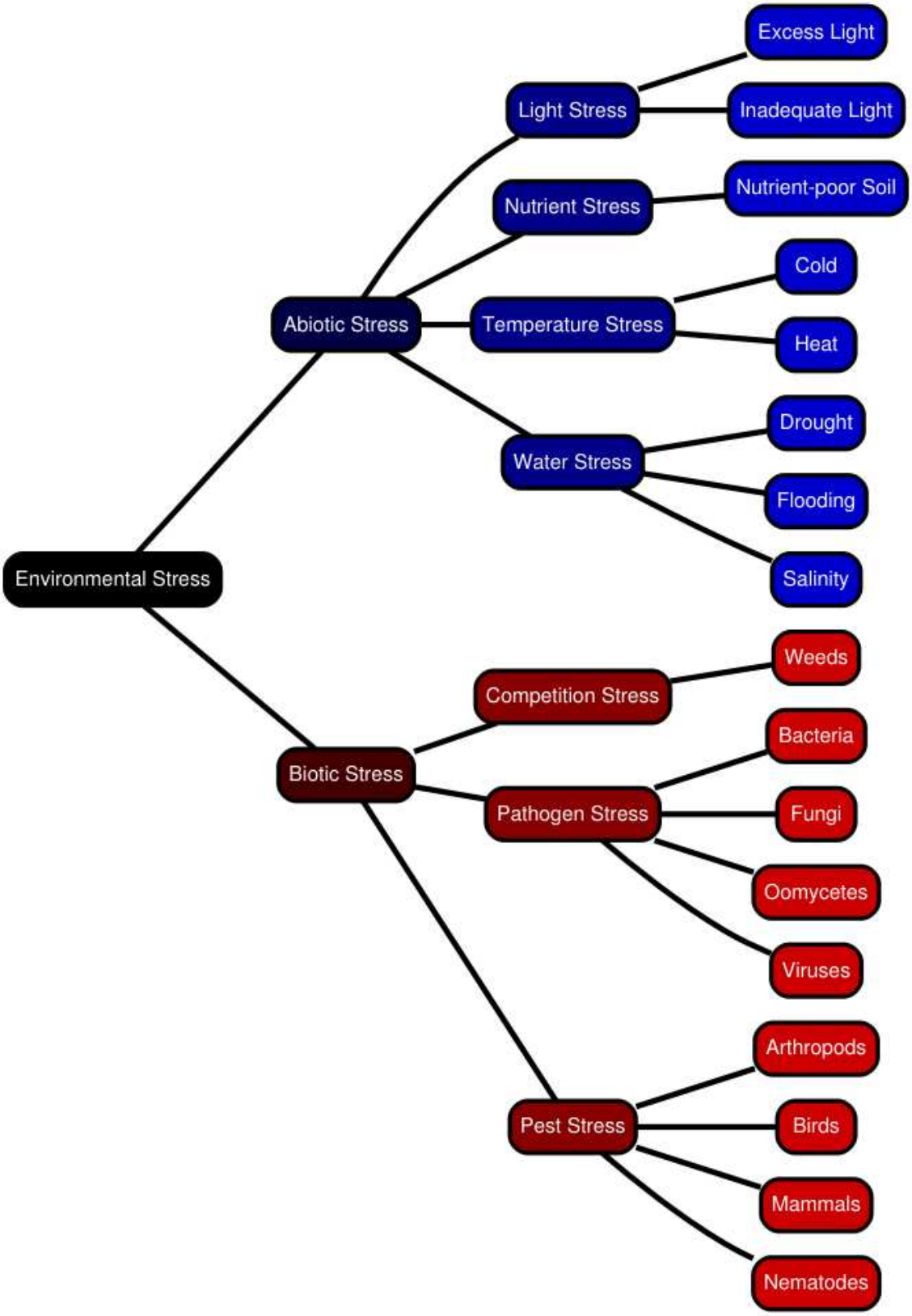

Fig 1.1: The most common environmental factors that cause plant stress. Abiotic (blue) and biotic (red) stresses are shown from general (left) to specific (right).

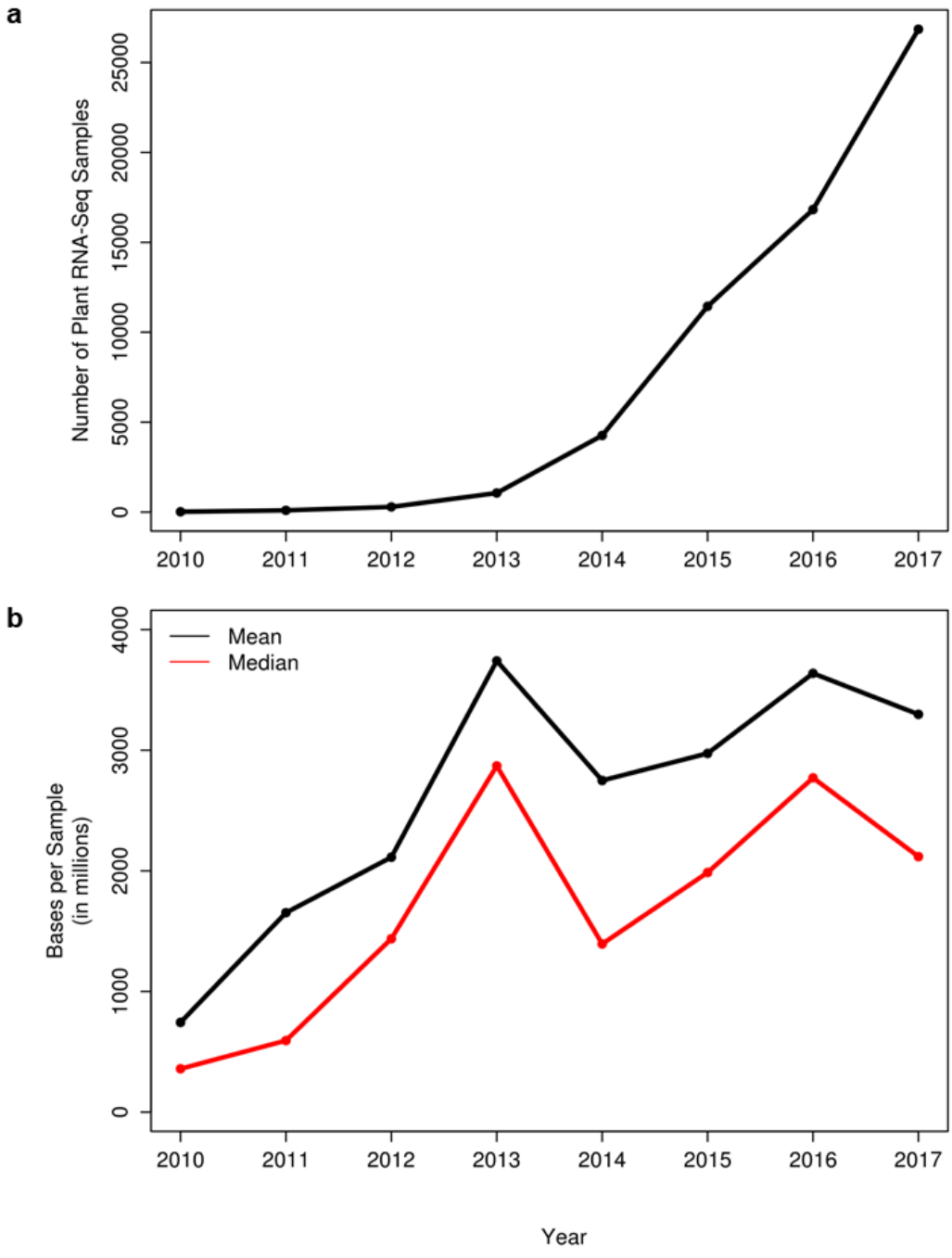

Fig 1.2: The amount of plant-related RNA-Seq experiments being conducted is increasing. (a) Number of plant-related RNA-Seq samples available on the NCBI SRA by year. (b) Mean (black) and median (red) number of bases per NCBI SRA RNA-Seq sample by year in millions of bases. Meta-data for Fig 1.2 was downloaded from NCBI SRA on 2018-09-24. Yearly data is non-cumulative.

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CHAPTER 2. RNA-Seq analysis reveals insight into enhanced rice Xa7-mediated bacterial blight resistance at high temperature[2]

2.1 Introduction

Plant diseases are a major detriment to global food production, accounting for an estimated 10% or more of crop yield loss each year (Oerke 2006). The disease phenotype is mediated by pathogen and host genotypes as well as environmental conditions, and these factors ultimately determine whether a plant succumbs to disease (Madgwick et al. 2011; Scholthof 2007). Environmental stresses can negatively impact a plant's ability to respond to pathogen attack, increasing disease severity (Mohr and Cahill 2003; O'Hara et al. 2016). This is due in part to cross-talk among the highly complex and intertwined plant stress signaling pathways (Prasch and Sonnewald 2013; Yasuda et al. 2008). Heat stress can reduce the effectiveness of plant disease resistance, rendering agriculturally important plants susceptible to attack (de Jong et al. 2002; Li et al. 2016; Whitham et al. 1996; Zhao et al. 2016; Zhu et al. 2010). While this phenomenon could pose a serious risk to food security in light of climate variability and global warming trends, current insight into specific underlying mechanisms of increased disease and/or loss of disease resistance at high temperature is lacking. Elucidation of these mechanisms would inform novel crop breeding strategies and reduce global food losses due to temperature-induced disease.

Bacteria in the *Xanthomonas oryzae* (Xo) group are pathogenic to rice and cause considerable yield loss every year (Reddy et al. 1979). Xo is most effectively controlled through the development of resistant rice varieties, particularly through deployment of single gene

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resistance (Suh et al. 2013). However, many rice resistance (*R*) genes lose function at higher temperatures, leading to increased bacterial blight disease caused by the Xo pathovar *oryzae* (Webb et al. 2010). Resistance genes in other plants similarly lose function, such as the Arabidopsis *R*-like gene *SNC1* and the tobacco *N* gene, an *R*-gene to tobacco mosaic virus (Zhu et al. 2010). One rice bacterial blight *R*-gene (*Xa7*) retains function at high temperature (Webb et al. 2010). Unusually, *Xa7* not only retains function, but also functions better at high temperature, both in long-lasting field trials, and at least up to 14 days post-inoculation in laboratory experiments. When triggered by the cognate pathogen effector protein AvrXa7, *Xa7* induces the hypersensitive response, a rapid, localized host cell death that reduces pathogen spread in the host plant (Hopkins et al. 1992). In addition to functioning better at high temperature, *Xa7* also retains function during drought stress, a condition in which other rice *R*-genes fail to function (Dossa et al. 2016; Dossa et al. 2017), suggesting that the underlying mechanism of *Xa7* can overcome general abiotic stresses. Because *Xa7* is a durable, long-lasting resistance gene that is effective in growth chamber, greenhouse, and field studies (Cruz et al. 2000; Webb et al. 2010), understanding the mechanism underlying enhanced resistance at high temperature will be an asset to agricultural researchers and crop breeders.

Plants are sessile, so they must be versatile in their ability to adapt to a wide range of abiotic and biotic stresses (Verma et al. 2016). Phytohormones are important regulators of plants' abilities to detect and respond to stresses (Nguyen et al. 2016; Shigenaga and Argueso 2016; Verma et al. 2016). One critical phytohormone involved in plant adaptation to abiotic stresses is abscisic acid (ABA), which acts as a generic regulator for abiotic stress response (Tuteja 2007). During abiotic stress, ABA primarily regulates plant osmotic stress tolerance, through mechanisms such as closure of stomata or expression of dehydration tolerance genes. During the rice-Xo interaction, exogenous treatment of ABA promotes rice susceptibility to Xo and acts as a cross-kingdom signal to promote bacterial swimming (Xu et al. 2013; Xu et al. 2015). The hormone salicylic acid (SA) plays an important role in rice defense against Xo, and

exogenous application of SA promotes both basal defense and the hypersensitive response during the rice-Xo interaction (Le Thanh et al. 2017). Intriguingly, ABA and SA play antagonistic roles in rice (Jiang et al. 2010; Xiong and Yang 2003), suggesting a possible regulatory conflict during simultaneous abiotic and biotic stresses. Here we report the results of a transcriptomics study designed to determine early host changes during *Xa7*-mediated resistance in an effort to elucidate the mechanisms underlying enhanced resistance at moderately high temperatures.

2.2 Results

Prior exposure to high temperature stress increases the effectiveness of *Xa7*-mediated resistance

Plants of rice line IRBB61, which carries the bacterial blight resistance gene *Xa7*, were grown for 4 weeks after germination in a greenhouse under normal conditions (approximately 28°/24°C day/night, 75-85% relative humidity). These plants were evenly transferred to growth chambers set to normal (29/23°C day/night) and high (35/29°C day/night) temperature regimes for one week. These plants were inoculated with *Xanthomonas oryzae* (Xo) strain X11-5A, a generally low virulence strain of Xo (Triplett et al. 2011), carrying either an empty plasmid vector or a vector encoding AvrXa7, the *Xa7*-mediated resistance inducing protein (Table 2.1). Plants in the susceptible interaction showed chlorosis, with a stronger yellowing in the high temperature plants (Fig 2.1a). During the resistant interactions, plants showed a browning indicative of the hypersensitive response associated with resistance to Xo harboring *avrXa7*, with a stronger response at high temperature. Plants at high temperature in the resistant interaction also showed reduced bacterial numbers due to *Xa7*-mediated defense as early as 12 h post-inoculation (hpi), while plants at normal temperature showed reduced bacterial numbers by 24 hpi (Fig 2.1b). By 48 hpi, during the resistant interaction, the plants at high temperature showed greatly reduced bacterial numbers when compared to the plants at normal temperature. During the susceptible interaction, bacterial numbers showed no differences due to high

temperature. These observations confirmed that the *Xa7*-mediated resistance was stronger and faster at high temperature than at low temperature.

Prior exposure to high temperature alters the rice transcriptome

To address the impact of prior exposure to high temperature on the rice transcriptome, we conducted an RNA-seq experiment using leaves from mock-inoculated plants grown in normal and high temperature conditions as described above (see Supplemental Table A1.1 for next generation summary statistics). Differential gene expression analysis was conducted, with genes having FDR-corrected p-values of <= 0.01 considered differentially expressed; this analysis revealed 1,511 differentially expressed genes (DEGs), with the majority of DEGs being up-regulated by high temperature (Fig 2.2a). Exposure of mock-treated plants to high temperature led to the upregulation of genes involved in many annotated biological processes (Fig 2.3, Supplemental Table A1.2). Stress-responsive gene ontology (GO) terms were enriched and over-represented in genes up-regulated by high temperature, including the following terms: 'response to stress', 'response to abiotic stimulus', 'response to biotic stimulus', and 'response to endogenous stimulus'. The GO terms 'response to stress' and 'response to biotic stimulus' were also over-represented in genes down-regulated by high temperature, but the median $\log_2$ fold change for genes annotated with these terms was positive, indicating that there were more genes with these annotations being up-regulated than down-regulated. GO terms associated with metabolic processes were enriched in genes up-regulated by high temperatures, including biosynthetic, carbohydrate metabolic, catabolic, lipid metabolic, and secondary metabolic processes. The DEGs associated with these metabolic terms had positive median $\log_2$ fold change, indicating that metabolic processes were generally up-regulated in mock-treated plants at high temperature. DEGs associated with energy metabolism terms, including generation of precursor metabolites and energy and photosynthesis, were enriched in genes down-regulated by high temperature and had negative $\log_2$ fold changes, indicating that energy metabolism was generally down-regulated at high temperature. Enriched GO terms for cellular processes

included ‘signal transduction’, ‘transport’, ‘cell differentiation’, ‘cell death’, and ‘cell growth’. DEGs for all of these processes were generally more up-regulated in mock-treated plants at high temperature. The GO term ‘translation’ was under-represented in genes up-regulated by high temperature with a negative median $\log_2$ fold change, indicating that gene translation was down-regulated by high temperature. To confirm that this transcriptomic response was due to temperature and not caused by a temperature-dependent wound response from the inoculation method, expression of VSP2, a gene responsive to a mediator of wound responses (jasmonic acid), was measured via qRT-PCR. Expression of VSP2 was not significantly changed by high temperature at 3, 6, and 12 h post-mock inoculation (Supplemental Fig A1.1). In addition, from a panel of 100 rice wound response genes in our RNA-Seq data, 96 were not differentially expressed at high temperature (Dataset 1.1). Thus, plants subjected to high temperature stress have dramatically altered transcriptomic profiles when compared to plants at normal temperature, and wound response from inoculation is not exacerbated at high temperature.

Plants respond uniquely to temperature in the resistant and susceptible interactions

To assess the plant transcriptomic response to high temperature during the susceptible and resistant interactions, gene expression profiles were determined from plants inoculated with the same strains as previously described at 3, 12, and 24 hpi (Table 2.1, Supplemental Table A1.1). The transcriptome of all pathogen-treated plants was altered at high temperature relative to normal temperature, but plant transcriptomes in the resistant interaction showed more differentially expressed genes (Fig 2.2a). A total of 8,499 DEGs were differentially regulated by high temperature in all biotic treatment conditions. While there was some overlap in DEGs per time point, the majority of these DEGs were unique to a single time point (Fig 2.2b), indicating that time was the strongest factor influencing transcriptome response. Within each time point, there were shared and unique transcriptomic responses in both the resistant and susceptible interactions (Fig 2.2c-e). Most shared DEGs were similarly regulated between both interactions, with only a few genes oppositely regulated based on pathogen treatment. At all time points, the

number of DEGs unique to the resistant interaction was roughly one order of magnitude greater than the number of DEGs unique to the susceptible interaction. This indicated that while exposure to high temperature caused similar transcriptome responses in both the susceptible and resistant interactions, more changes were observed in the resistant interaction, and most of these were unique to that response.

In general, biological processes were up-regulated in the susceptible interaction and down-regulated in the resistant interaction (Fig 2.3, Supplemental Table A1.2). Genes annotated with the GO term 'response to abiotic stimulus' showed opposite trends in the susceptible interaction; while genes annotated with this term were generally up-regulated in the susceptible interaction, they were generally down-regulated in the resistant interaction. Surprisingly, genes annotated with 'response to biotic stimulus' were generally down-regulated by high temperature in both the susceptible and resistant interactions. Genes annotated with 'response to stress' were up-regulated in the susceptible interaction at 3 and 24 hpi, and down-regulated in the resistant interaction at 3 and 12 hpi. Genes annotated with the GO terms 'biosynthetic process', 'carbohydrate metabolic process', and 'cellular process' showed similar trends, being generally up-regulated by high temperature in the susceptible interaction and down-regulated by high temperature in the resistant interaction, while genes annotated with the GO terms 'metabolic process' and 'lipid metabolic process' showed the opposite trend. Regulation of genes associated with GO terms in plants in the susceptible interaction responded to high temperature in a way similar to the mock-treated plants, while these processes were generally oppositely regulated in plants in the resistant interaction. This suggested that the plants undergoing *Xa7*-mediated resistance at high temperature were responding to high temperature by regulating many biological processes in a way opposite to both uninoculated plants and the plants in the susceptible interaction.

Rice plants experiencing heat stress alter hormone synthesis and downstream signaling

Hormones are key regulators of plant responses to both biotic and abiotic stresses (Nguyen et al. 2016; Shigenaga and Argueso 2016). Many transcripts encoding genes directing phytohormone biosynthesis were in the set of all DEGs. All hormone biosynthesis gene families were differentially expressed in response to high temperature, in all mock and pathogen treatments, suggesting that plants experiencing high temperature stress fundamentally alter endogenous hormone balance. There was also considerable overlap in hormone biosynthesis DEGs in the susceptible and resistant interaction, counter to the noted earlier trend of mostly unique transcriptomic responses (Fig 2.4a, Dataset 1.2). The expression patterns of hormone biosynthesis genes in the mock-treated plants most closely resembled that of plants in the susceptible interaction at all time points, especially in the ABA, auxin, and cytokinin biosynthesis pathways (Fig 2.4a). Genes involved in ABA biosynthesis were strongly up-regulated at high temperature in both mock-treated plants and plants during the susceptible interaction at 3 hpi, and strongly down-regulated in plants undergoing resistance responses at all time points. Genes predicted to contribute to biosynthesis of salicylic acid (SA), a pathogen-responsive hormone important in defense responses, were regulated independent of biotic treatment – being up-regulated by high temperature in the mock treatment, down-regulated by high temperature in both biotic interactions at 3 and 12 hpi, and up-regulated by high temperature in both biotic interactions at 24 hpi. This trend suggests that during resistant interactions at high temperature, plants enact transcriptional control of hormone metabolism that is unique from uninoculated plants in response to high temperature, while plants progressing towards a diseased state closely resemble uninoculated plants.

To further address the role of plant hormones in this response, analysis was conducted to examine how known hormone-responsive genes behave during simultaneous pathogen and temperature stresses. Many hormone-responsive DEGs were perturbed at high temperature in all mock- and pathogen-treated plants (Fig 2.4b, Supplemental Table A1.3). ABA-responsive

genes made up the largest proportion of hormone-responsive DEGs in all treatments. At each time point, a larger number of DEGs, but a smaller proportion of total DEGs, in the resistant interaction were hormone-responsive relative to the susceptible interaction at the same time point. Regardless of pathogen treatment, rice plants greatly altered hormone-regulated genes and downstream signaling in response to high temperature.

Rice plants expressing *Xa7*-mediated resistance suppress expression of ABA-responsive genes at high temperature

The fold changes of known ABA-up-regulated genes, identified as being induced 2-fold or greater following ABA treatment by a previous microarray study (Garg et al. 2012), were inspected to give insight into the associated regulatory trends. ABA-up-regulated genes were mostly up-regulated at high temperatures in the mock-treated plants (Fig 2.5a, Dataset 1.3). The transcriptome of plants in the susceptible interaction showed the same trend at 3 and 24 hpi, with the opposite trend at 12 hpi (Fig 2.5b, Dataset 1.3). In the resistant interaction, ABA-up-regulated genes were down-regulated at all time points, suggesting that during resistance, plants suppressed ABA downstream responses. Expression of the ABA-responsive master regulators bZIP23 and bZIP72 was tested using quantitative reverse transcriptase PCR. In susceptible plants at high temperature, expression of bZIP23 was increased approximately two-fold compared to the low temperature, mock-inoculated control at 3 and 6 hpi, while expression was reduced two-fold in resistant plants at 6 hpi (Fig 2.6a). Interestingly, while bZIP72 was suppressed by high temperature in the resistant interaction, it was also suppressed during the susceptible interaction (Fig 2.6b). In agreement with the findings for SA biosynthetic genes, genes responsive to SA were up-regulated by high temperature in the mock-inoculated plants (Supplemental Fig A1.2a, Dataset 1.4). Conversely, SA-responsive genes were down-regulated by high temperature at 3 and 12 hpi in the susceptible interaction, and at all time points in the resistant interaction (Supplemental Fig A1.2b, Dataset 1.4). These trends indicate that during high temperature stress, rice plants undergo significant changes in not just ABA-responsive

gene expression but in the regulatory networks that drive ABA-responsive gene expression, and that enhanced *Xa7*-mediated resistance at high temperature is likely independent of SA.

An ABA responsive element-like motif was enriched in the promoters of DEGs

Motif analysis was conducted on the upstream promoter sequences of DEGs for discovery of *cis*-regulatory elements that might give insight into the observed gene expression patterns. A motif was identified that resembled the ABA responsive element (ABRE), a G-box family motif recognized by bZIP family transcription factors that is found in the promoters of many ABA responsive genes (Gómez-Porras et al. 2007). This ABRE-like element was enriched in the promoters of genes up-regulated in the susceptible interaction at high temperature at 3 hpi, genes down-regulated in the susceptible interaction at 24 hpi, and genes down-regulated in the resistant interaction at all time points (Fig 2.7, Supplemental Table A1.4). Several other motifs identified from the Plant *cis*-acting Regulatory DNA Elements database (Higo et al. 1999) were also enriched in the DEGs, including motifs resembling the TATA box, the light-responsive IBOXCORENT, the anaerobic-responsive GCBP2ZMGAPC4, the root growth-related TELOBOXATEEF1AA1, and the axillary growth-related UP2ATMSD (Fig 2.7, Supplemental Table A1.4). The enrichment trends of these motifs may give insight into the rice processes perturbed by high temperature stress over the course of a 24 h day. Most importantly, the trends observed in the enrichment of ABRE-like motifs are evidence that plants activated the ABA response at high temperature early during the susceptible interaction, and suppressed the ABA response at high temperature during the resistant interaction.

2.3 Discussion

During periods of high temperature stress, *Xa7*-mediated rice resistance to Xo is enhanced, while resistance regulated by other *R* genes is generally repressed (Webb et al. 2010), but the underlying cause of this phenomenon is heretofore not understood. To provide insights into how *Xa7*-mediated resistance is enhanced at high temperature, we conducted a

transcriptomics experiment with RNA-Seq technology to identify the transcriptomic changes in rice during *Xa7*-mediated resistance at high temperature. A set of 8,499 DEGs was identified as temperature responsive in one rice cultivar, IRBB61, experiencing both a susceptible interaction with Xo strain X11-5A and a resistant interaction with Xo strain X11-5A carrying a plasmid encoding the *Xa7*-inducing effector protein AvrXa7 across three time points.

Under all treatments, expression of genes involved in metabolic processes was altered by high temperature. Genes annotated with GO terms related to metabolism were generally up-regulated by high temperature in plants treated with mock inoculation and in the susceptible interaction, while these genes were generally down-regulated in the resistant interaction. However, genes annotated with the GO term 'photosynthesis' showed the opposite trend. Photosynthesis is generally inhibited during high temperature stress, and reduced primary metabolism is associated with thermotolerance in plants (Barnabás et al. 2008; Zhang et al. 2005). Reduced photosynthesis is also associated with pathogen attack in both susceptible and resistant interactions (Berger et al. 2007). It is therefore surprising that enhanced *Xa7*-mediated resistance at high temperature is associated with the upregulation of photosynthesis-related genes. Additional experimentation is needed to explore the dynamics of primary metabolism at high temperature during *Xa7*-mediated resistance.

Genes in the ABA pathway were notably perturbed by high temperature during biotic stresses. ABA biosynthesis and ABA-responsive genes were induced by high temperature in both mock-treated plants and plants in the susceptible interaction at 3 and 24 hpi, and suppressed by high temperature in plants in the resistant interaction at all time points. ABA-responsive genes were down-regulated in the susceptible interaction at 12 hpi, possibly due to diurnal effects. SA-responsive genes were also induced by high temperature in mock-treated plants, but generally repressed by high temperature in all biotic interactions. This trend suggests that regulatory differences in ABA responsiveness are important to the rice resistance phenotype during a plant's response to simultaneous heat stress and Xo attack, and raises the

interesting hypothesis that enhanced *Xa7*-mediated resistance at high temperature is independent of SA.

ABA is a developmental plant hormone that is active in triggering plant physiological changes for acclimation to abiotic stresses such as drought, cold, heat, and salt stresses (Baron et al. 2012; Hu et al. 2008; Suzuki et al. 2016; Zandalinas et al. 2016). ABA also plays a complex role during plant response to biotic stresses. For example, ABA signaling can lead to closure of stomata, a common entry point for plant pathogens (Lim et al. 2015). However, ABA generally plays a negative role in plant defense responses to biotic stresses through antagonistic interactions with defense response pathways. In Arabidopsis, ABA treatment suppresses the induction of both systemic acquired resistance, a plant immune response effective against a broad range of pathogens, and the hypersensitive response (Mang et al. 2012; Yasuda et al. 2008). In light of these previous studies, our results suggest that suppression of ABA response is vital for the hypersensitive response associated with *Xa7*-mediated resistance.

In rice, ABA interacts antagonistically with the defense response hormone SA, leading to reduced resistance to blast disease and increased bacterial blight disease severity (Jiang et al. 2010; Xiong and Yang 2003). However, our results suggest that ABA and SA were regulated independently instead of antagonistically. While another study also showed that ABA enhanced rice susceptibility to Xo by antagonizing SA, when plants were treated with the ABA biosynthesis inhibitor fluridone, the resulting resistance to the pathogen was independent of SA (Xu et al. 2013). This suggests that there is some SA-independent mechanism of resistance to Xo upon depletion of ABA, which might explain why genes annotated with the GO term 'response to biotic stress' were down-regulated in the resistant interaction in this study. In agreement with our findings, ABRE motifs have previously been identified in the promoters of Xo-responsive genes in rice (Narsai et al. 2013). Interestingly, exogenous ABA has been linked to enhanced swimming ability in Xo (Xu et al. 2015), suggesting that the Xo-rice interaction has

been evolutionarily shaped by ABA. The results presented in our study further indicate that ABA response and plant defense are inversely regulated.

In addition to functioning better at high temperature, *Xa7* is also more effective during drought stress (Dossa et al. 2017). The plant transcriptional responses to drought and heat stress are drastically different, with many distinct genes triggered by each stress (Mittler 2006; Rizhsky et al. 2004), but a regulatory mechanism shared by both stresses is the accumulation of ABA. It is therefore tempting to speculate that *Xa7* activity directly represses ABA biosynthesis, signaling, or both. In fact, the ABRE-like motif identified in this study might serve as a binding element for a transcriptional repressor during defense. If this turns out to be the case, this could inform rice breeders on selection strategies for enhancing disease resistance at high temperature; for example, promoter regions for susceptibility and resistance genes could be screened for this motif across multiple varieties. However, further experimentation is needed to conclusively show whether the repression of ABA response is actively triggered during *Xa7*-mediated resistance or if it is a side effect of resistance to Xo. Additional work is needed to explore whether downregulation of abiotic response by *Xa7*-mediated resistance impacts heat tolerance. In a natural interaction between rice and Xo pathovar *oryzae*, the bacterial blight pathogen, the pathogen proliferates within the rice xylem, limiting water availability to rice leaf cells. The reduction of ABA signaling in resistant plants at high temperature may therefore be due in part to the reduced water stress associated with the limitation on bacterial proliferation induced by *Xa7*. Further studies are necessary to tease apart the role of the ABA signaling pathway to *Xa7*-mediated resistance.

In conclusion, this study presents novel results of a transcriptomic analysis of rice during simultaneous heat stress and Xo infection, with plant responses during both susceptible and resistant interactions. The results revealed that the ABA pathway was activated during both high temperature stress and the susceptible interaction at high temperature, and was repressed during *Xa7*-mediated defense at high temperature. The SA pathway was also down-regulated at

high temperature in both the susceptible and resistant interactions, suggesting that enhanced *Xa7*-mediated resistance is likely independent of SA signaling. A novel sequence motif that was similar to the ABRE was identified in the promoters of genes up-regulated by high temperature during the susceptible interaction and down-regulated by high temperature during the resistant interaction. These results suggest that ABA is an important node for cross-talk between plant transcriptional response pathways to high temperature stress and pathogen attack. This pathway represents an important area of study for future research in understanding how plants deal with combined abiotic and biotic stresses.

2.4 Materials and Methods

Plant materials and growth conditions

Seeds of rice NIL IRBB61 (Vera Cruz CM et al. unpublished) were germinated on wet filter paper under constant light at 28°C. After emergence, the seedlings were transplanted in soil in a greenhouse (approximately 28°/24°C day/night, 75-85% relative humidity) and grown for three weeks. The plants were evenly distributed to two growth chambers set to normal (29°/23°C day/night) and high (35°/29°C day/night) temperature regimes, 85% relative humidity and 14/10 h day/night light regimes. Plants were transferred to growth chambers one week before inoculations.

Bacterial strains, inoculations, and bacterial quantification

Cultures of Xo strain X11-5A carrying pKEB31 plasmids containing ORFs for *avrXa7* and *talΔCRR* (a non-functional TAL effector lacking the DNA binding region), described in Verdier et al. (2012) and Triplett et al. (2016) respectively, were grown at 28°C on peptone sucrose agar (PSA) (Karganilla et al. 1973) with 2 ug/mL tetracycline overnight and diluted in sterile distilled water to 10^8 cfu/mL. The first fully expanded leaves were inoculated with dilutions of both strains and water (for mock inoculations) using a needleless syringe (Reimers and Leach 1991). Leaves designated for RNA extractions and bacterial quantification were inoculated along a 4

cm section with six infiltration sites, while leaves designated for symptom observation were inoculated along an approximately 10 cm section with four infiltration sites. Inoculations were conducted approximately 3 h after growth chamber lights reached full intensity in the morning. Inoculated tissue was collected at full light (3 h, 24 h pathogen-inoculated, 6 h mock-inoculated) and full dark (12 h pathogen-inoculated) light stages. For bacterial quantification, inoculated leaf tissue was surface sterilized with 10% bleach and rinsed three times with sterile water, then ground in 1mL of sterile water in a tissue macerator (Qiagen TissueLyser II). The extract was plated in a dilution series on PSA with 2 ug/mL tetracycline and incubated overnight at 28°C. Pairwise analysis of bacterial numbers was performed in R (R Core Team 2016).

RNA extraction, sequencing, and qRT-PCR

Total RNA was extracted from plant tissue at the site of inoculation using a Sigma Aldrich Spectrum Plant Total RNA Kit as per kit instructions. RNA was collected for two biological replicates for each condition. RNA from mock-treated leaves was submitted to the University of North Carolina High-Throughput Sequencing Facility for cDNA generation via TruSeq RNA library construction kits with multiplex adapter primers and single-end 50 bp sequencing via Illumina HiSeq 2500. RNA from pathogen-treated leaves was submitted to Michigan State University Genomics Core for TruSeq mRNA library preparation with multiplex barcodes and sequencing via Illumina HiSeq 50 bp single read sequencing. For qRT-PCR, cDNA was generated from the previously collected RNA using Quantabio qScript cDNA SuperMix kit. Primers and thermal cycler conditions for qRT-PCR follow Lu et al. (2009) for bZIP23, bZIP72 and Lee et al. (2015) for VSP2. Data was analyzed using the ΔΔCT method (Livak and Schmittgen 2001).

Gene expression analyses

Sequence reads were processed with FASTX Toolkit 0.0.13 (Gordon and Hannon 2010) to remove low quality reads. The high-quality reads were aligned to the MSU RGAP 7.0 rice reference genome (Kawahara et al. 2013) using TopHat 2.1.1 (Kim et al. 2013) and counted

using HTSeq 0.6.1 (Anders et al. 2015). Sequence reads and gene counts are available in the Gene Expression Omnibus repository under accession number GSE95668. Differential gene expression analyses were conducted using the Bioconductor package edgeR (McCarthy et al. 2012; Robinson et al. 2010). Genes were considered differentially expressed in a condition if FDR-corrected p-value was less than or equal to 0.01. Rice wound response genes were identified from publicly available microarray data (NCBI Gene Expression Omnibus Accession GSE77097). The top 100 genes were chosen from this data by fold change. Fisher's exact test was used for GO term enrichment analysis. A GO term was considered statistically significant if FDR-corrected p-value was <= 0.05. Heatmaps were prepared using the heatmap.2 function from the R package gplots (Gregory R. Warnes 2016). Hierarchical clustering of hormone biosynthesis genes was performed with the hclust R function using the WPGMA method (R Core Team 2016). Hormone-responsive genes used in analysis were identified from a microarray study (Garg et al. 2012). Kernel density estimates were prepared with the density function from the R core package (R Core Team 2016). DREME (Bailey 2011) was used for motif discovery with the 1000 bp sequences upstream of putative transcription start sites from the reference genome. STAMP (Mahony and Benos 2007) was used for DNA motif matching.

2.5 Tables and Figures

Table 2.1: Experimental design for transcriptomics experiment involving rice undergoing heat/Xo stresses.

Host Plant	Temperature Regime	Pathogen	Plant Response
IRBB61 rice	Normal	Xo X11-5A empty vector	Susceptible
IRBB61 rice	High	Xo X11-5A empty vector	Susceptible
IRBB61 rice	Normal	Xo X11-5A *avrXa7*	Resistant
IRBB61 rice	High	Xo X11-5A *avrXa7*	More resistant

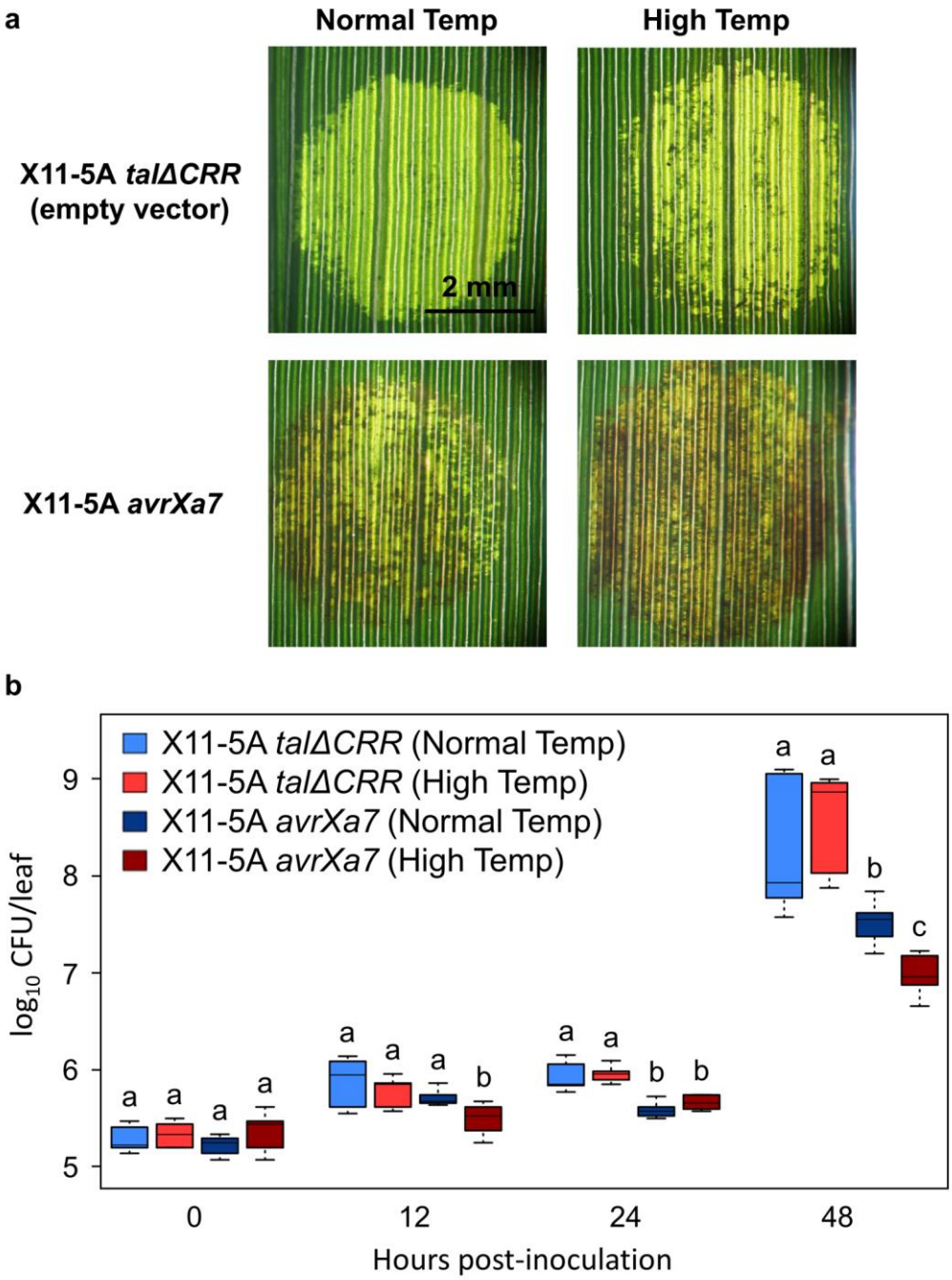


Fig 2.1: Rice displaying *Xa7*-mediated resistance is more resistant at high temperature. (a) Rice leaves displaying response to Xo strain X11-5A carrying either an empty vector (*talΔCRR*) or a vector with the gene encoding the *Xa7*-inducing effector (*avrXa7*) at normal and high-temperature at 72 hpi. Scale is indicated by the black bar. (b) Box plots of $\log_{10}$ transformed bacterial quantity of rice leaves inoculated with Xo X11-5A *talΔCRR* and Xo X11-5A *avrXa7* at normal and high temperature. One-way ANOVA revealed differences among treatments within all time points except 0 hpi ($p < 0.0005$). Within time points, letters indicate differences as determine by two-tailed pairwise t-test (FDR-adjusted p-value < 0.05).

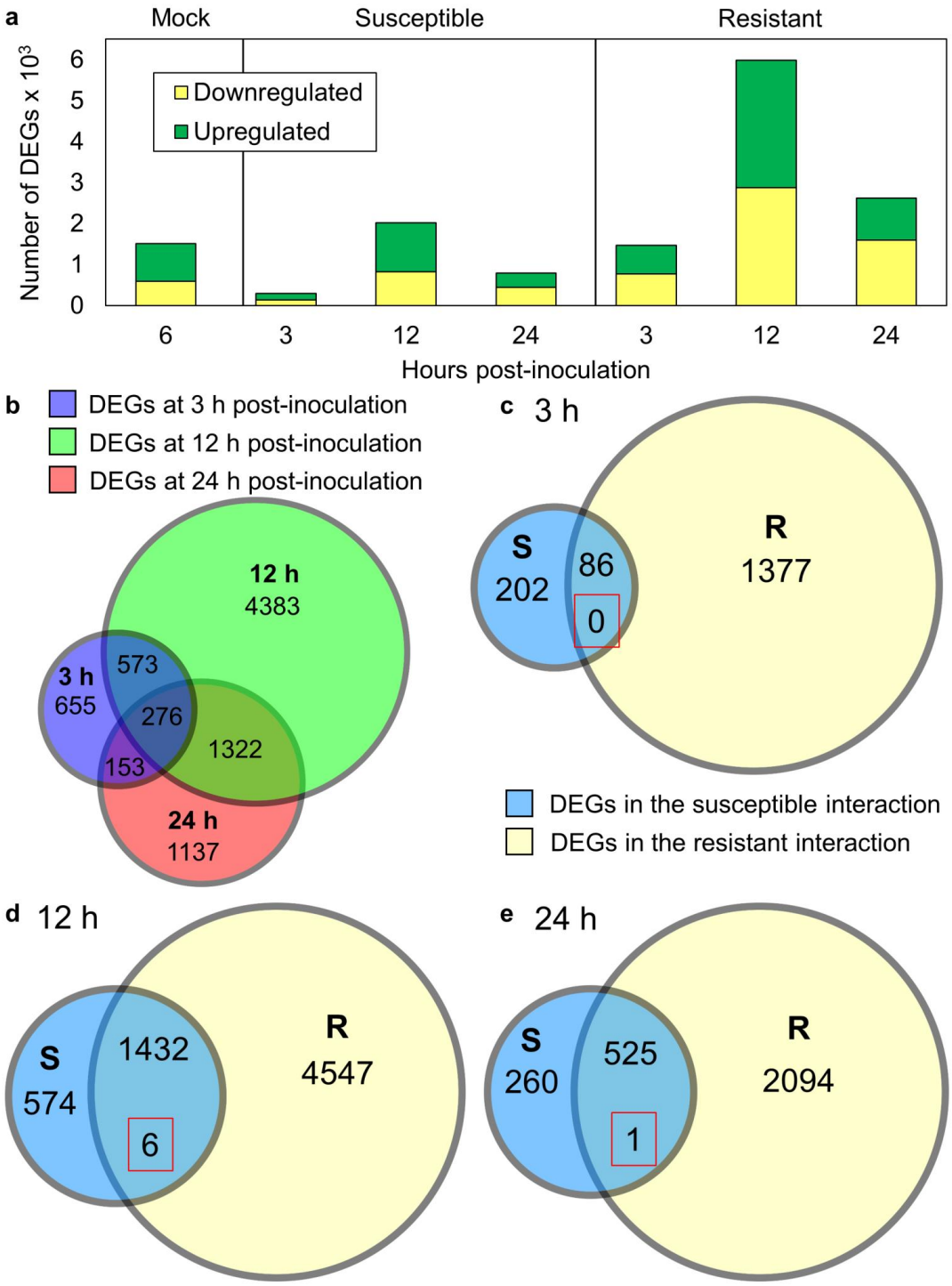


Fig 2.2: Differential gene expression analysis. (a) Genes differentially up and down-regulated at high relative to normal temperature in mock-inoculated plants, and during susceptible and resistant interactions. (b) Number of DEGs per time point, with DEGs from the susceptible and resistant interactions combined per each time point. (c – e) Number of DEGs up or down-regulated by high temperature in plants in the susceptible (S) plants or resistant (R) interaction at (c) 3 h, (d) 12 h, and (e) 24 hpi. The red-squared number represents DEGs which were oppositely regulated by susceptibility/resistance.

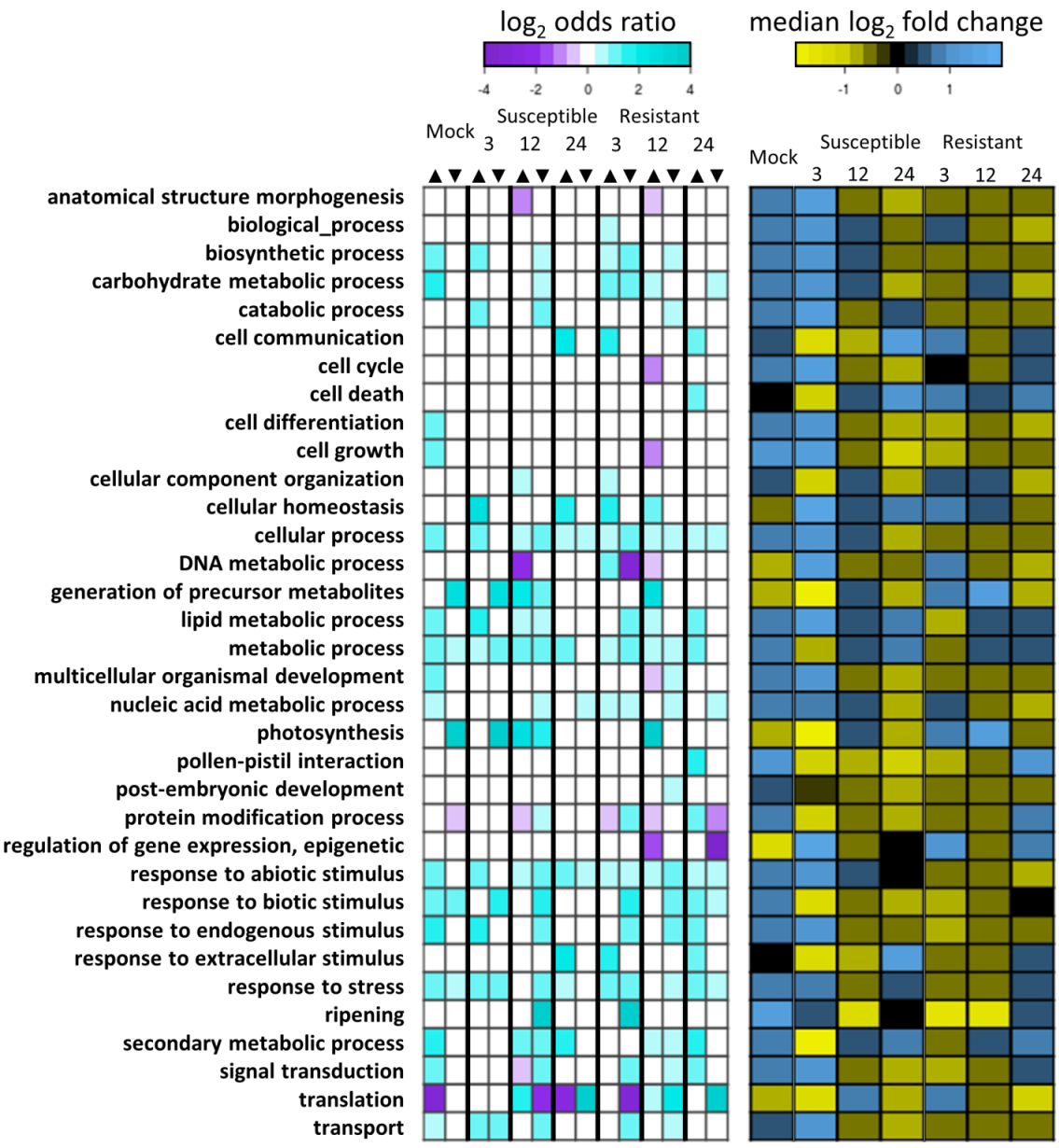


Fig 2.3: GO term analysis. (left) $\log_2$ odds ratio of GO annotated genes regulated by high temperature to genes not regulated by high temperature. Positive value (cyan) indicates the GO term is over-represented in regulated genes, while negative value (magenta) indicates the term is under-represented in regulated genes (Fisher's exact test, FDR-corrected p-value < 0.05; white = not enriched). The arrows indicate the genes were either up-regulated or down-regulated by high temperature within treatments. (right) median $\log_2$ fold change per GO term. Positive value (blue) indicates more genes annotated with the term are up-regulated, while negative value (yellow) indicates more genes annotated with the term are down-regulated.

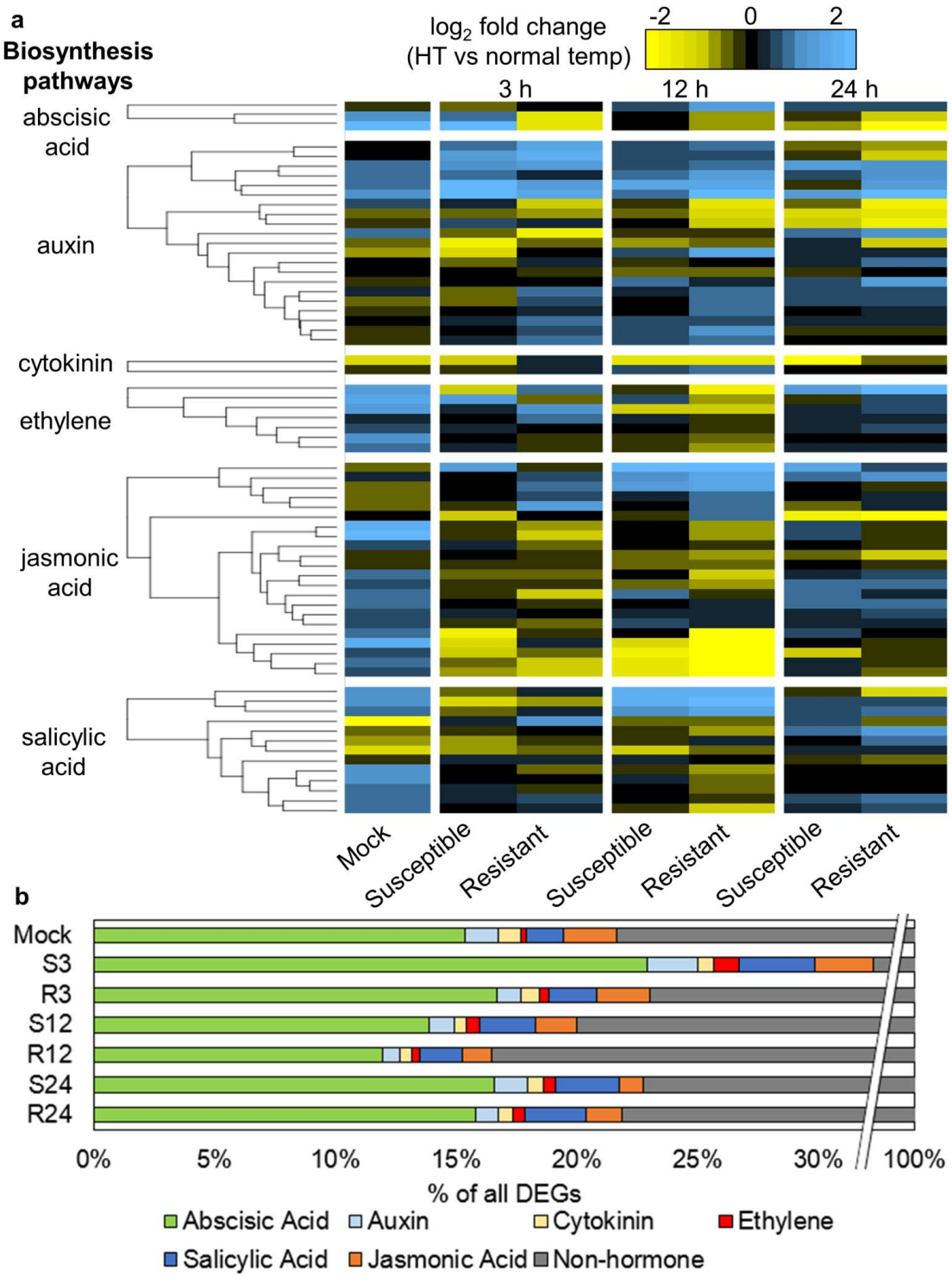


Fig 2.4: Differential expression of upstream and downstream hormone genes at high temperature. (a) Fold change for hormone biosynthesis genes at high temperature relative to normal temperature is represented for mock-inoculated plants, and plants during susceptible and resistant interactions. Hormone biosynthesis genes were selected for display only if they were differentially expressed in at least one column. (b) Downstream hormone-responsive genes represented as proportions of total DEGs for mock-inoculated plants, and plants during susceptible and resistant interactions.

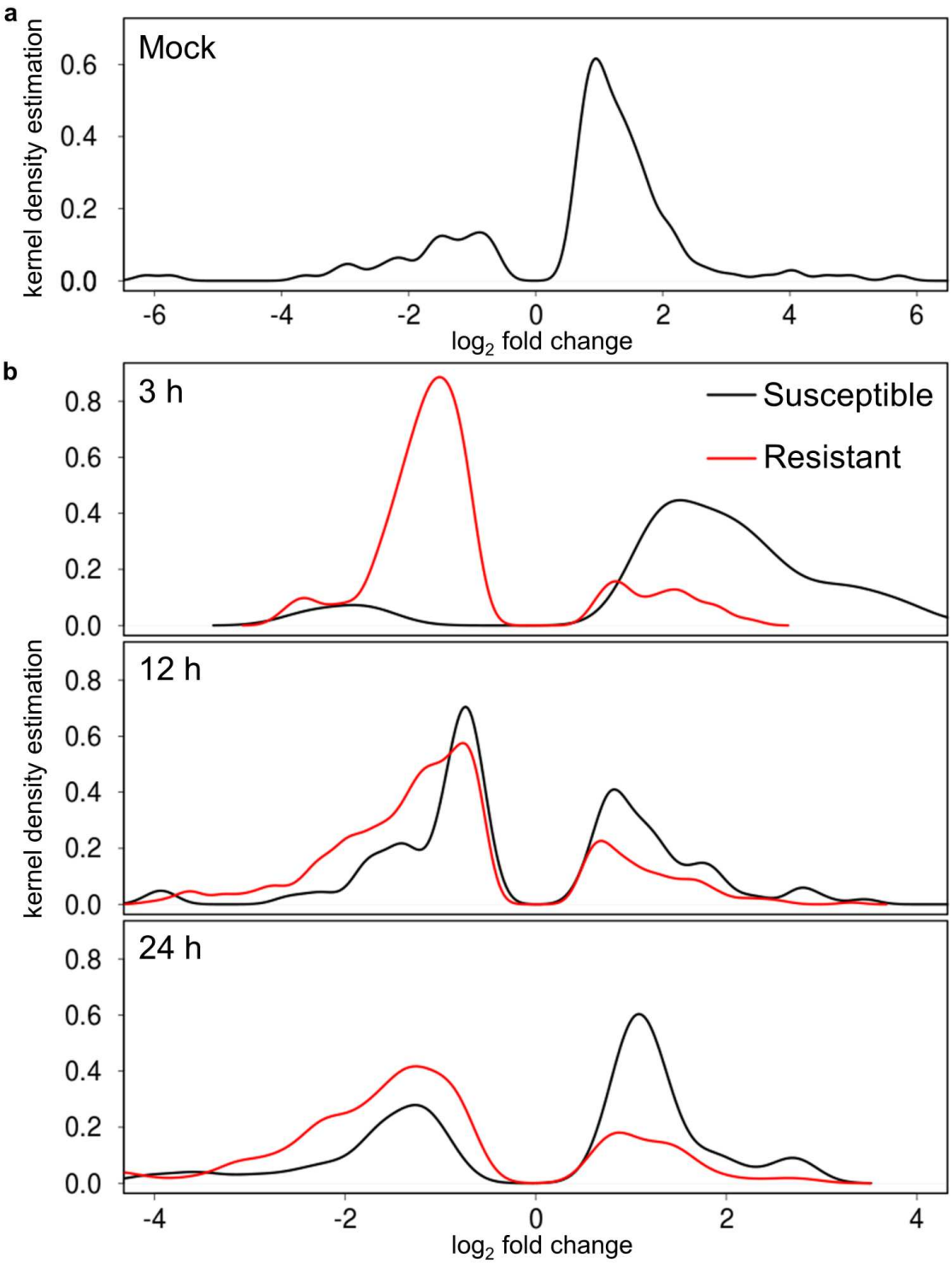


Fig 2.5: Differential expression of ABA up-regulated genes at high temperature. (a) Kernel density estimate of log_2 fold change for ABA up-regulated genes differentially regulated in mock-inoculated plants. (b) Kernel density estimates of log_2 fold change for ABA up-regulated genes differentially regulated in plants during susceptible and resistant interactions at 3, 12, and 24 hpi.

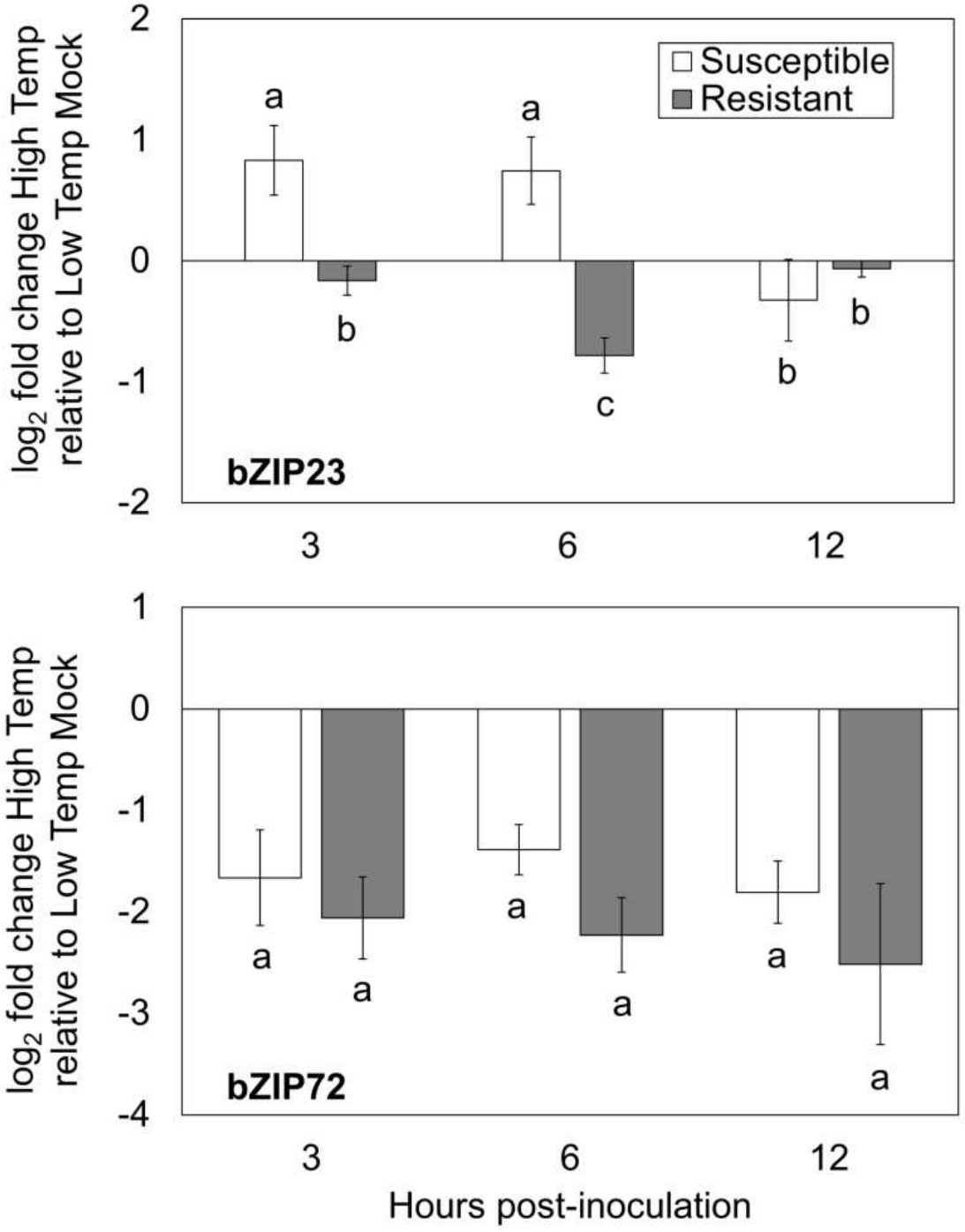


Fig 2.6: Analysis of ABA marker gene expression. Bars represent mean $\log_2$ fold changes of ABA marker genes (a) bZIP23 and (b) bZIP72 in plants during susceptible and resistant interactions at high temperature relative to the normal temperature mock-inoculated control at each time point as measured by qRT-PCR. Error bars represent standard error of the mean, with n = 8 and 4 for (a) and (b) respectively, and letters indicate pairwise groupings (pairwise t test, $p < 0.05$).

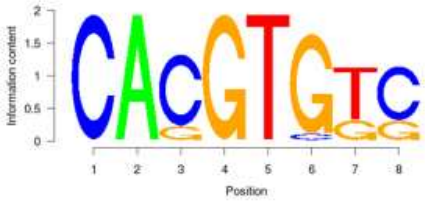
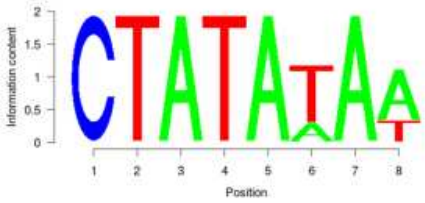
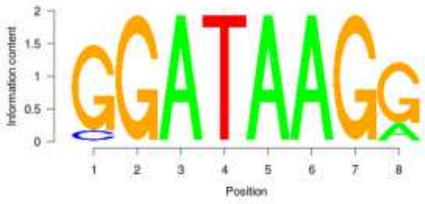
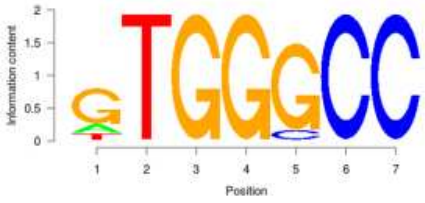
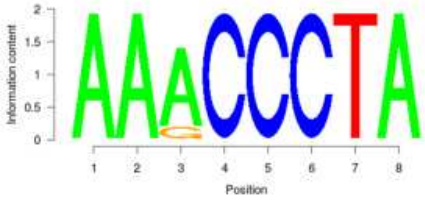
Consensus Motif Logo	Regulatory Trend	Comment
	Susceptible 3▲, 12n.e., 24▼ Resistant 3▼, 12▼, 24▼	ABRE-like [ABA-responsive element]
	Susceptible 3▲, 12▼, 24▼ Resistant 3▼, 12▼, 24▲▼	TATA Box-like [TATA binding protein]
	Susceptible 3▼, 12n.e., 24n.e. Resistant 3n.e., 12▲, 24n.e.	IBOXCORENT [light-responsive]
	Susceptible 3n.e., 12▼, 24▼ Resistant 3n.e., 12▼, 24▼	GCBP2ZMGAPC4 [anaerobic-responsive]
	Susceptible 3n.e., 12n.e., 24▼ Resistant 3n.e., 12n.e., 24▼	TELOBOXATEEF1AA1 UP2ATMSD [root and axillary-growth related]

Fig 2.7: Analysis of *cis*-regulatory element enrichment in DEGs. Up arrows indicate the motifs are enriched in up-regulated DEGs in the given time point, down arrows indicate enrichment in down-regulated DEG, and *n.e.* indicates no significant enrichment as determined by Fisher's exact test ($p < 0.05$). Similarity to motifs in PLACE database (Higo et al. 1999) is indicated.

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CHAPTER 3. Abiotic and biotic stresses induce a core transcriptome response in rice[3]

3.1 Introduction

Because plants are immobile, they must respond to and endure a wide variety of environmental and biotic stresses in the field. Both abiotic and biotic stresses cause major yield losses to crops (Atkinson and Urwin 2012; Lobell et al. 2011; Mafakheri et al. 2010; Semenov and Shewry 2011). It is therefore not surprising that many crop improvement programs focus on developing stress tolerant plant varieties (Ashraf and Akram 2009; Fukuoka et al. 2015; Jongdee et al. 2006). Breeding tolerance for a single stress (e.g. drought, salinity, pathogen, etc.) or a single stress type (e.g. abiotic or biotic) may be risky because plants respond uniquely to different or simultaneous stresses, and increasing tolerance to one stress may be at the expense of tolerance to another (Atkinson and Urwin 2012; Mittler 2006). With climate change, more extreme weather events are occurring, increasing the likelihood that plants experience multiple stresses in the field, including additional pressure from plant diseases (Garrett et al. 2006). There is, therefore, a need to understand the similarities and differences among stress response pathways to best optimize targeted crop improvement.

Plants respond to stress in a variety of ways. Common plant responses to avoid or tolerate abiotic stresses include stomatal closure, reduced photosynthesis, increased reactive oxygen scavenging activity, reduced leaf growth and increased root length (Maiti and Satya 2014). Biotic stresses such as pathogens also cause plants to close stomata and reduce photosynthesis (Bilgin et al. 2010; Melotto et al. 2006). Other plant responses to pathogens

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include production of toxic compounds, including phytoalexins and reactive oxygen species, and induction of localized cell death (Wojtaszek 1997). Many of these responses are coordinated by phytohormones (Nguyen et al. 2016; Shigenaga and Argueso 2016). The hormones abscisic acid (ABA) and jasmonic acid (JA) are critical regulators of tolerance to abiotic stresses. For immunity to pathogens, plants primarily rely on salicylic acid (SA), JA and ethylene signaling. The abiotic stress response is generally regulated by ABA-induced basic leucine zipper (bZIP) transcription factors (TFs) (Banerjee and Roychoudhury 2017; Yoshida et al. 2015). These TFs induce stomatal closure, expression of dehydration tolerance genes, and other adaptive physiological responses (Ghosh et al. 2016; Huang et al. 2018; Kim et al. 2010; Silva et al. 2018; Wang et al. 2018). However, ABA often increases plant susceptibility in biotic interactions (Lievens et al. 2017; Peskan-Berghöfer et al. 2015; Xiong and Yang 2003; Xu et al. 2013; Yasuda et al. 2008) and frequently acts antagonistically with SA (de Torres Zabala et al. 2009; Ding et al. 2016; Jiang et al. 2010).

With this study, we explore the rice transcriptome for a more thorough understanding of how rice regulates responses to multiple abiotic and biotic stresses. Previous studies have explored broad plant stress response by analyzing microarray data (Hahn et al. 2013; Narsai et al. 2013). We expand on these studies with robust meta-analysis of publicly available rice RNA-Seq data sets. Our results reveal universally stress-regulated pathways, which we call the rice core stress response. The network of core stress-responsive genes presented here can be further explored for rice improvement in light of the need for tolerance to multiple environmental stresses. In addition to the valuable predictive transcriptome analysis for an important crop system, our approach can be easily expanded to other plant and crop systems.

3.2 Results

Meta-analysis of publicly available RNA-Seq data reveals the rice core stress response

To investigate the rice response to stress, we downloaded and analyzed publicly available RNA-Seq data sets representing rice transcriptome response to diverse abiotic and biotic stresses. These stresses include drought (Galbiati et al. 2016), salt (Wang et al. 2017), high and low temperature (Cohen et al. 2017; Shen et al. 2011), and infection with diverse pathogens, including *Xanthomonas oryzae* pathovars *oryzicola* (Xoc) and *oryzae* (Xoo) (Wilkins et al. 2015; Zhang et al. 2015), *Magnaporthe oryzae* (Huang et al. 2017), and Rice Stripe (RSV) and Rice Dwarf (RDV) viruses (Yang et al. 2016; Zhao et al. 2017) (Table 3.1). All selected studies used stress-sensitive rice varieties. Four technical considerations were applied to choose RNA-Seq data sets: (1) there must be at least two replicates per treatment, (2) there must be untreated controls for each treatment, (3) tissue type was primarily above-ground, and (4) varieties were non-transgenic and non-mutant.

A standard pipeline for consistently processing all raw sequencing data files was used (Fig 3.1a). Included in this pipeline were steps for removing low quality reads, aligning to the reference genome, and counting reads. The proportions of reads mapped to the reference genome were generally high, with a mean of 77.4% total reads mapped to loci across all samples (Supplemental Table A2.1). We conducted differential gene expression analysis separately on each experiment (Fig 3.1a). The number of differentially expressed genes (DEGs) varied widely depending on stress treatment, and ranged from 1,220 to 11,644 DEGs (Fig 3.1b-c, Supplemental Table A2.2).

To explore the rice core response to abiotic stress, we used a meta-analysis to combine the results from all abiotic stress experiments (Fig 3.1a). We found 5,863 meta-analysis-identified DEGs (metaDEGs) that were generally responsive to all abiotic stresses (Fig 3.1d, Dataset 2.1). We repeated this process to explore the core response to biotic stress, and found

2,154 metaDEGs generally responsive to all biotic stresses (Fig 3.1d, Dataset 2.1). Of the DEGs identified in the individual analyses, 10 to 43% were retained as metaDEGs (Supplemental Table A2.2). The expression trends of the metaDEGs within individual studies followed the trends identified in the meta-analysis; that is, up- and down-regulated metaDEGs were mostly up- and down-regulated, respectively, within individual studies (Supplemental Fig A2.1). Therefore, this approach was valid for investigating rice core responses to abiotic and biotic stress.

To identify the rice response to all stresses, we investigated the overlap in expression patterns of the two sets of metaDEGs (Fig 3.1e). We found all possible patterns of gene expression between abiotic and biotic stresses, including metaDEGs that were uniquely regulated by one stress type (abiotic or biotic), similarly regulated by both stress types, and oppositely regulated by both stress types (Fig 3.1e, Supplemental Table A2.3). Most metaDEGs were uniquely regulated by either abiotic or biotic stress. Interestingly, there were many more metaDEGs regulated similarly by both stress types (913 metaDEGs) than oppositely (88 metaDEGs). Taken together, these results indicate there are: (1) genes responsive to a single stress type (abiotic or biotic), and (2) genes responsive to all stresses.

Stress altered regulation of photosynthesis-related genes in rice

To investigate the rice biological processes (BP) altered during stress, we evaluated the enrichment patterns of the 45 BP gene ontology (GO) terms in abiotic and biotic up- and down-regulated metaDEGs (Supplemental Table A2.4). The GO terms ‘catabolic process’, ‘cell communication’, ‘embryo development’, ‘reproduction’, and ‘response to extracellular stimulus’ were all enriched within metaDEGs (relative to background genes) up-regulated by both abiotic and biotic stress (Fisher’s exact test FDR-corrected $p \leq 0.01$, Table 3.2). The GO terms ‘photosynthesis’, ‘protein modification process’, and ‘response to external stimulus’ were all enriched within metaDEGs down-regulated by both stresses. Several GO terms were enriched

exclusively in abiotic or biotic metaDEGs, but no GO terms were enriched in genes oppositely regulated by stress type.

There were 85 metaDEGs annotated with the GO term 'photosynthesis'. These metaDEGs were generally down-regulated in individual transcriptome studies (Supplemental Fig A2.2). Rice down-regulated photosynthesis-annotated metaDEGs in response to drought, heat, cold, Xoc, *M. oryzae*, and RDV. Conversely, salt and RSV did not regulate these metaDEGs, and, in the study used, Xoo up-regulated them. These results indicate that altered regulation of photosynthetic pathways is a common rice response to stress.

Stress up-regulated rice phytohormone-induced genes

Because phytohormones are regulators of plant responses, we investigated how stress responses influenced phytohormone-induced genes. Abiotic metaDEGs responsive to ABA, auxin, JA and SA were more up-regulated than expected by random chance as determined by the χ^2 goodness of fit test ($p \leq 0.05$, Fig 3.2, Supplemental Table A2.5). Biotic metaDEGs in all hormone-responsive pathways were more up-regulated than expected. Response to ABA was the most significantly up-regulated hormone pathway in both abiotic and biotic metaDEGs, indicating that ABA signaling is likely important to the core stress response.

There were 408 and 228 genes responsive to JA and/or SA in abiotic and biotic metaDEGs, respectively (Fig 3.3, Dataset 2.2). The expression of these genes indicate that during either stress type, JA and SA signaling are increased (Fig 3.3). Only three small clusters identified within abiotic metaDEGs (Fig 3.3a, labeled C1-C3) and two small clusters within biotic metaDEGs (Fig 3.3b, labeled C4-C5) did not follow this trend; genes in these clusters were regulated oppositely by stress and hormones (JA and/or SA). Interestingly, in the Xoo study used for this validation, the expression of JA- and SA-responsive genes was generally opposite of all other biotic stress responses. JA and SA response were a larger component of the biotic stress response (10.6% of biotic metaDEGs) than of the abiotic stress response (7.0% of abiotic metaDEGs). Many of the JA- and/or SA-responsive genes were also responsive to ABA (Fig

3.3). Genes responsive to JA and/or SA, but not responsive to ABA, were still up-regulated more than expected by random chance (Supplemental Table A2.5). However, this was a much smaller proportion of metaDEGs (1.4 and 3.5% of abiotic and biotic metaDEGs, respectively). Taken together, these results indicate that in response to any stress, rice orchestrates responses via phytohormones.

Discovery of promoter motifs important to the stress response

We performed d*e novo* promoter motif enrichment analysis to identify potential stress-responsive regulatory elements. There were 22 and 17 motifs discovered in the abiotic and biotic metaDEGs, respectively (Supplemental Table A2.6). GO term analysis revealed six motifs that are likely to be involved in stress-responsive pathways (Fig 3.4). Many of these motifs contained a sequence similar to the ACGT core sequence of the ABA responsive element (ABRE), an upstream bZIP TF binding sequence (Gomez-Porras et al. 2007), indicating a possible role for bZIP TFs in the core stress response. Of the 21 bZIP TFs we identified as metaDEGs, 17 were up-regulated in response to abiotic stress (Dataset 2.1), including bZIP23 (MSU: LOC_Os02g52780) and bZIP46 (MSU: LOC_Os06g10880), which are key players in ABA response (Lu et al. 2009; Xiang et al. 2008). Biotic stress only up-regulated three bZIP TFs. The enrichment of ABRE-like motifs in the promoters of biotic stress-induced metaDEGs suggests that even though there are fewer bZIP TFs responsive to biotic stresses than abiotic stresses, bZIP TFs may still act as critical regulators of response to biotic stress. One bZIP TF (MSU: LOC_Os08g38020) was a potential node of antagonistic cross-talk, up-regulated by abiotic stress and down-regulated by biotic stress. Taken together, these results indicate that rice utilizes ACGT-bZIP TF to regulate response to both abiotic and biotic stress, and identify bZIP elements as key nodes for further studies.

Pre-processed publicly available gene expression data validates meta-analysis results

To validate the results of the meta-analysis, nine publicly available pre-processed gene expression studies were examined for the trends expected from our previous analysis (Table

3.3) (Bidzinski et al. 2016; Fu et al. 2017; Garg et al. 2015; Huang et al. 2014; Jung et al. 2016; Tran et al. 2018; Wang et al. 2016; Wilkins et al. 2015; Zong et al. 2016). With one exception, all studies fit the expected trends; i.e., up- and down-regulated metaDEGs were more up- and down-regulated than expected by random chance, respectively, as determined by the χ^2 goodness of fit test ($p \leq 0.05$, Fig 3.5a-b, Supplemental Table A2.7). The study that did not fit the expected trend (GSE57950 drought) had two time-points, with the earlier time-point (1 d after stress) not fitting the expected trend in down-regulated metaDEGs. As in the meta-analysis, photosynthesis genes were mostly down-regulated (Fig 3.5c, Supplemental Table A2.8). Three studies did not significantly alter photosynthesis gene expression (GSE42096 heat, GSE74465 drought 1 h, GSE107425 drought), and one study up-regulated this pathway (GSE57950 drought 1 d). In the later time-point of the latter study (GSE57950 drought 3d), plants down-regulated photosynthesis-annotated genes, suggesting there may be some temporal effects of drought on altered regulation of photosynthesis, particularly as leaves dehydrate after continued drought. In study GSE108504, rice strongly down-regulated photosynthesis-annotated genes in response to Xoo (Fig 3.5c), opposite to the set used in the training data, where these genes were up-regulated by Xoo (Supplemental Fig A2.2). These results validate our meta-analysis approach to finding the rice core stress response.

3.3 Discussion

A variety of environmental stresses affect plants in the field and can limit crop yield. To endure these stresses, plants respond with coordinated changes to their transcriptome. While these changes are dependent on the specific stress experienced, our results indicate that there is a rice core response to all stresses. With our meta-analysis of publicly available RNA-Seq data of rice experiencing various abiotic and biotic stresses, we identified 5,863 and 2,154 genes that are differentially regulated by abiotic stress and biotic stress, respectively (Fig 3.1, Dataset 2.1). Of these, 913 genes were similarly regulated by both abiotic and biotic stress,

while 88 were regulated oppositely (Supplemental Table A2.3). A different study utilized differential expression analysis of rice microarray data to identify genes commonly regulated by abiotic and biotic stresses, and found 240 rice genes that were responsive to both abiotic and biotic stresses (Narsai et al. 2013). Our meta-analysis of RNA-Seq data identified more of the rice core stress response than this previous comparative microarray analysis. We also validated our meta-analysis approach using additional publicly available studies not used in the training sets; through this validation, we identified sets of stress-responsive genes similar to those found in the meta-analysis (Fig 3.5, Supplemental Tables A2.7 and A2.8).

Although the reference genome is annotated with only 45 BP GO terms, we identified several BPs that were altered by stress, including 'catabolic process', 'cell communication', 'embryo development', 'reproduction', and 'response to extracellular stimulus', which were all up-regulated by stress, and 'photosynthesis', 'protein modification process', and 'response to external stimulus', which were all down-regulated by stress. Photosynthesis is known to be down-regulated by abiotic stresses such as drought, cold, and heat stress (Brestic et al. 2016; Maruyama et al. 2014; Pandey et al. 2013; Todaka et al. 2017). This is likely a protective mechanism against plant photooxidative damage during stress (Brestic et al. 2016; Yan et al. 2013). In stress tolerant varieties of rice, photosynthetic efficiency is restored, and up-regulation of photosynthesis is physiologically important for yield stability (Li et al. 2017; Zhang et al. 2016). Consistent with these findings, overexpression of a master regulator of photosynthesis enhanced rice tolerance to drought (Ambavaram et al. 2014). A range of biotic stresses, including bacterial, viral, and fungal pathogens, also inhibit photosynthesis in plants (Akimoto-Tomiyama et al. 2018; Cheng et al. 2016; Ghosh et al. 2017; Girija et al. 2017; Pérez-Clemente et al. 2015). It is hypothesized that the photosynthesis pathway is a hub of cross-talk in growth and defense trade-offs during plant-pathogen interactions (Kangasjärvi et al. 2014). Studying the roles of the photosynthesis-regulated metaDEGs identified in this study may facilitate the development of stress tolerant varieties of rice.

Various stresses positively induced phytohormone pathways (Fig 3.2). Abiotic stress up-regulated genes responsive to ABA, auxin, JA, and SA, while biotic stress up-regulated genes responsive to the same hormones plus cytokinin and ethylene. The ABA, JA, and SA pathways were the most significantly up-regulated hormone pathways in both abiotic and biotic stress. ABA, JA and SA signaling regulate response to abiotic stresses (Hahn et al. 2013; Maruyama et al. 2014; Sah et al. 2016; Todaka et al. 2017). While JA and SA are positive regulators, ABA tends to be a negative regulator of resistance to pathogens (Creelman and Mullet 1995; Klessig et al. 2018; Klessig and Malamy 1994; Lievens et al. 2017). ABA is also important to inter-kingdom signaling among pathogens and plants. For example, synthesis of ABA by the fungal pathogen *M. oryzae* during interactions with rice is necessary for pathogen virulence (Spence et al. 2015). Plant-synthesized ABA promotes rice susceptibility to the bacterial pathogen *X. oryzae* pv. *oryzae* (Xoo) and even induces swimming in the bacteria (Xu et al. 2013; Xu et al. 2015). While our results show that both ABA and SA are induced during response to biotic stress, ABA-induced susceptibility to Xoo is due to ABA suppressing SA-mediated defense (Xu et al. 2013). We previously hypothesized that ABA is a node of cross-talk in the rice response to simultaneous high temperature stress and *X. oryzae* infection (Cohen et al. 2017). The results from our current study show that cross-talk among ABA, JA and SA response pathways makes the contribution of each hormone to the rice transcriptome unclear (Fig 3.3). Notably, ABA-regulated genes appear to dominate the hormone response during stress. That is, of the metaDEGs responsive to JA and SA, most were also responsive to ABA (Fig 3.3, Supplemental Table A2.5). These intertwined pathways are critical to plant stress responses, which frequently occur simultaneously, emphasizing that additional study of hormonal cross-talk is needed to provide insights into how to improve plant health.

Our results open the path to future avenues of research, including both *in silico* and *in planta* studies. We immediately provide candidate genes for studying multiple stress responses in rice. For example, the prevalence of enriched ABRE-like promoter motifs suggest that the bZIP TFs

identified here are good candidate regulators of stress responses (Fig 3.4, Dataset 2.1). Our analysis only used studies with rice plants that were sensitive (susceptible) to the different stresses. Future researchers can expand on this work by analyzing the regulation of metaDEGs in studies with stress-tolerant rice varieties. We only found 88 oppositely regulated metaDEGs between abiotic and biotic stresses, but it is likely that stress tolerance and sensitivity oppositely regulate many more genes. The resources and approach provided with this work will allow for a deeper understanding of rice strategies for overcoming stresses.

We present this work as a proof of concept: meta-analysis of diverse transcriptomic data sets is a valid and robust approach to develop hypotheses for how plants respond to stress in general. It is also possible to expand our approach into other systems. For example, with the wealth of publicly available Arabidopsis transcriptome data, researchers can repeat this analysis to identify candidate regulators of Arabidopsis stress response. In systems with few or no publicly available transcriptome studies, the analysis we describe enables researchers to design transcriptome studies from the ground up to study stress response in their systems. Even while limited by the available rice stress-responsive transcriptome data, with multiple tissue types, host cultivars, and few replicates per treatment (Table 3.2), real trends were identified, indicating it is possible to design experiments in less well-studied plant systems to use with our approach.

To summarize, publicly available rice transcriptome data were used to identify genes and pathways regulated by abiotic stress, biotic stress, and both stress types. We confirmed that photosynthesis is a generally down-regulated pathway in response to all stress types. We also identified stress-induced plant hormone-responsive genes, particularly genes downstream of ABA, JA and SA. With this work, we provide a list of candidate genes to study for improving rice stress tolerance, and thus yield, in light of environmental stresses. This study provides a valid approach to ask additional questions with respect to how plants respond to stress, including but

not limited to (1) how tolerant rice varieties respond to stress and (2) how other plants respond to stress.

3.4 Materials and Methods

RNA-Seq Data Acquisition and Processing

Raw sequence data for all accessions were downloaded from NCBI Sequence Read Archive using the SRA Toolkit (https://github.com/ncbi/sra-tools). Adapter sequences and low quality reads were removed with Trimmomatic v0.36 (Bolger et al. 2014). Reads were mapped to the MSU RGAP 7.0 rice reference genome (Kawahara et al. 2013) with STAR v2.5 (Dobin et al. 2013) and counted using HTSeq v0.9.1 (Anders et al. 2015).

Differential Gene Expression and Meta-Analyses

Differential gene expression analyses were conducted using the Bioconductor package edgeR (McCarthy et al. 2012; Robinson et al. 2010). For single analyses, genes were considered differentially expressed if the FDR-adjusted p-values were ≤ 0.01. For meta-analyses, Fisher's sum of logs method, as discussed by Rau et al. (2014) and implemented in the R package metap v0.8 (https://cran.r-project.org/web/packages/metap/index.html), was used to combine unadjusted p-values. The p.adjust function in R was used to adjust the combined p-values for multiple testing with the 'fdr' method (R Core Team 2016). Genes were considered differentially expressed in meta-analyses if the adjusted p-values were ≤ 0.01 and the absolute value of the median $\log_2$ fold change for all studies within the analysis was ≥ 1. GO terms were considered enriched within a metaDEG set if the odds ratio estimates relative to background genes was > 1 and the FDR-corrected p-values from Fisher's exact test were ≤ 0.01.

Phytohormone-responsive Gene Analysis

Known hormone-responsive genes were from Garg et al. (2012). The chisq.test function in R was used for χ^2 goodness of fit test, with a p-value threshold of 0.05, to determine if

number of hormone-responsive up- and down-regulated genes were as expected due to random chance. For the χ^2 tests, the expected number of up- and down-regulated genes was proportional to the total number of up- and down-regulated genes in the background set.

De novo Promoter Motif Discovery

Promoter motifs and associated GO terms were discovered with DREME and GOMo respectively using 500 bp regions upstream of putative transcription start sites (Bailey 2011; Buske et al. 2010). Fisher's exact test with a p-value threshold of 0.01 was used to determine whether motifs were enriched in metaDEG sets.

Validation with Pre-processed Gene Expression Studies

Pre-processed gene expression studies were acquired from NCBI Gene Expression Omnibus. Because many of these studies lacked replicates, regulatory patterns of genes were estimated by finding the ratio of normalized expression value of treatment to control, disregarding $\log_2$ fold changes with absolute value < 1. Studies GSE67588 and GSE108504 were normalized by calculating number of gene reads per millions of total reads. The χ^2 goodness of fit test with a p-value threshold of 0.05 was used to determine whether the counts of up- and down-regulated were as expected by random chance. For the χ^2 tests, the expected number of up- and down-regulated genes was proportional to the total number of up- and down-regulated genes in the background set.

3.5 Tables and Figures

Table 3.1: Overview of NCBI SRA RNA-Seq accessions analyzed in this study.

Accession	Stress	Cultivar	Tissue	Time after stress	Replicates per Sample	Study Location
SRP071248	Drought long day Drought short day	Nipponbare	Leaf	13 d	3	Growth chamber
SRP052306	Drought	Nipponbare	Leaf	10 d	2	Greenhouse
SRP113286	Salt	9311	Seedling	1 h	3	Greenhouse
SRP101342	High Temperature	IRBB61	Leaf	6 h	2	Growth chamber
SRP004651	Cold	Nipponbare	Leaf	14 d	2	Growth chamber
SRP056884	Xoc BLS256 Xoc RS105 Xoc CFBP7331	Nipponbare	Leaf	2 d	3	Growth chamber
SRP049040	Xoo PXO349 1 dpi Xoo PXO349 2 dpi	Huanghuazhan	Leaf	1 d 2 d	3	Screenhouse
SRP076382	*M. oryzae* Guy11	Kasalath	Shoot	2 d	3	Unspecified
SRP049444	*M. oryzae* ZB13	Pid3	Leaf	1 d	2	Greenhouse
SRP065503	Rice Stripe Virus	Wuyujing 3	Leaf	7 d	3	Growth room
SRP115030	Rice Dwarf Virus	Zhonghua 11	Seedling	28 d	3	Greenhouse

Xoc indicates *X. oryzae* pv. *oryzicola*; Xoo indicates *X. oryzae* pv. *oryzae*

Table 3.2: Biological process GO terms exclusively enriched in up- or down-regulated metaDEGs.

GO Term	Abiotic	Biotic
catabolic process	**Up**	**Up**
cell communication	**Up**	**Up**
embryo development	**Up**	**Up**
reproduction	**Up**	**Up**
response to extracellular stimulus	**Up**	**Up**
photosynthesis	**Down**	**Down**
protein modification process	**Down**	**Down**
response to external stimulus	**Down**	**Down**
flower development	Up	*n.e.*
cell death	Down	Both
anatomical structure morphogenesis	Down	*n.e.*
cell differentiation	Down	*n.e.*
cell growth	Down	*n.e.*
cellular component organization	Down	*n.e.*
growth	Down	*n.e.*
ripening	Down	*n.e.*
tropism	Down	*n.e.*
multicellular organismal development	Both	Up
nucleic acid metabolic process	Both	Up
pollen-pistil interaction	Both	Up
post-embryonic development	Both	Up
response to endogenous stimulus	Both	Up
carbohydrate metabolic process	Both	Down

Terms indicated in bold are similarly enriched in both abiotic and biotic metaDEGs; Up, Down, and Both indicate terms are enriched in up-regulated, down-regulated, or both up- and down-regulated metaDEGs respectively; *n.e.* = not enriched

Table 3.3: NCBI GEO accessions analyzed to validate meta-analysis.

Accession	Stress	Cultivar	Tissue	Time after stress
GSE42096	High temperature	Zhongxian 3037	Leaf	18 d
GSE57950	Drought	Huanghuazhan	Leaf	1 d 3 d
GSE60287	Desiccation Salinity	IR64	Seedling	Unspecified
GSE74465	Drought	Nipponbare	Whole plant	1 h 6 h
GSE81462	Drought	Zhonghua 11	Above-ground	Unspecified
GSE107425	Drought	Zhonghua 11	Shoot	4 d
GSE67588	Xoc BLS279 Xoc CFBP7342	Nipponbare	Leaf	2 d
GSE84800	*M. oryzae* Fr13	Nipponbare	Shoot	4 d
GSE108504	Xoo MAI1	Nipponbare	Leaf	1 d

Xoc indicates *X. oryzae* pv. *oryzicola*; Xoo indicates *X. oryzae* pv. *oryzae*

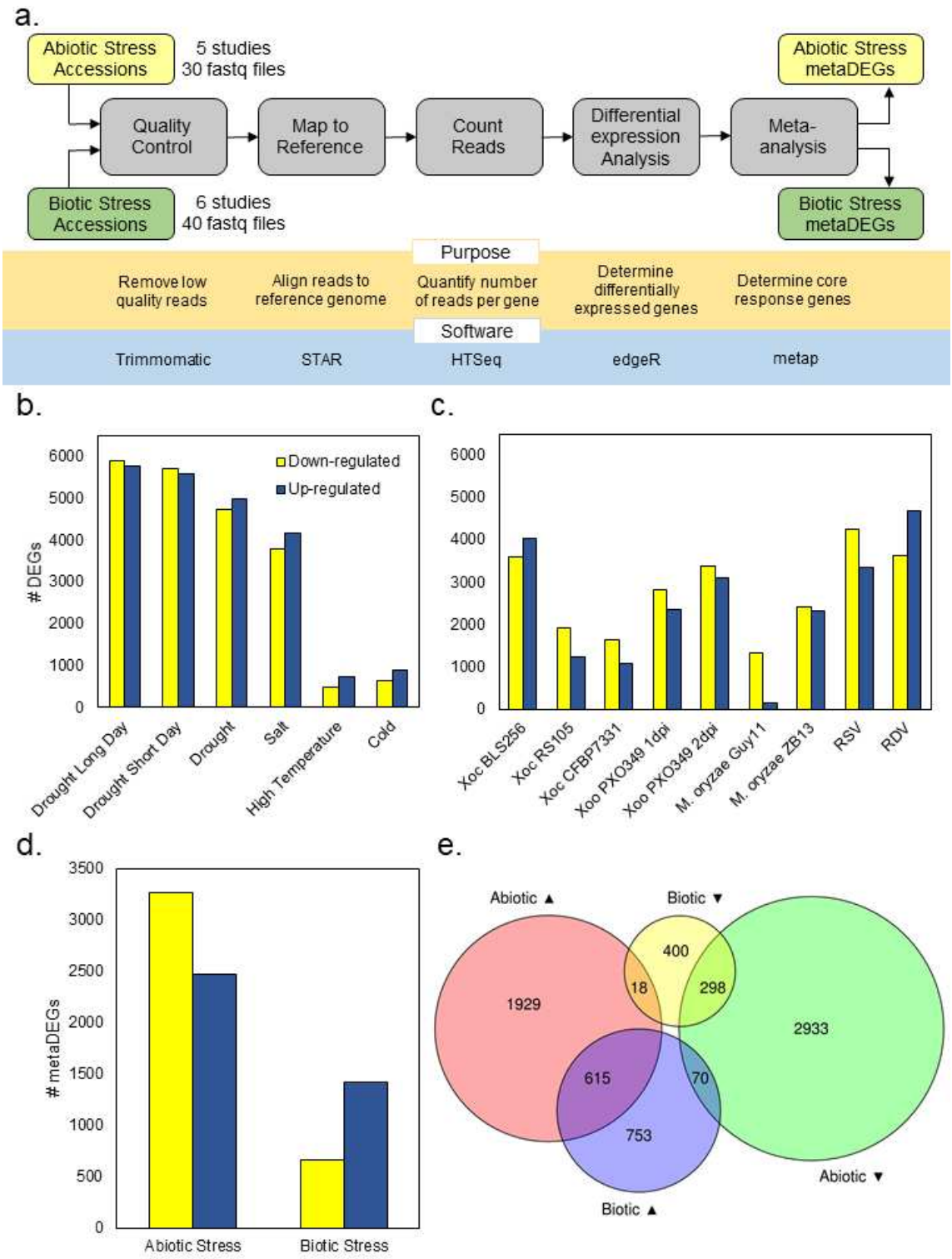


Fig 3.1: Analyses reveal rice core stress responses to abiotic and biotic stresses. (a) Analysis pipeline used to conduct differential gene expression analysis and meta-analysis on publicly available data sets. Number of DEGs identified in all (b) abiotic and (c) biotic stress experiments. (d) MetaDEGs identified from meta-analyses. (e) Number of metaDEGs unique and common in abiotic and biotic meta-analyses up- (up arrow) and down-regulated (down arrow).

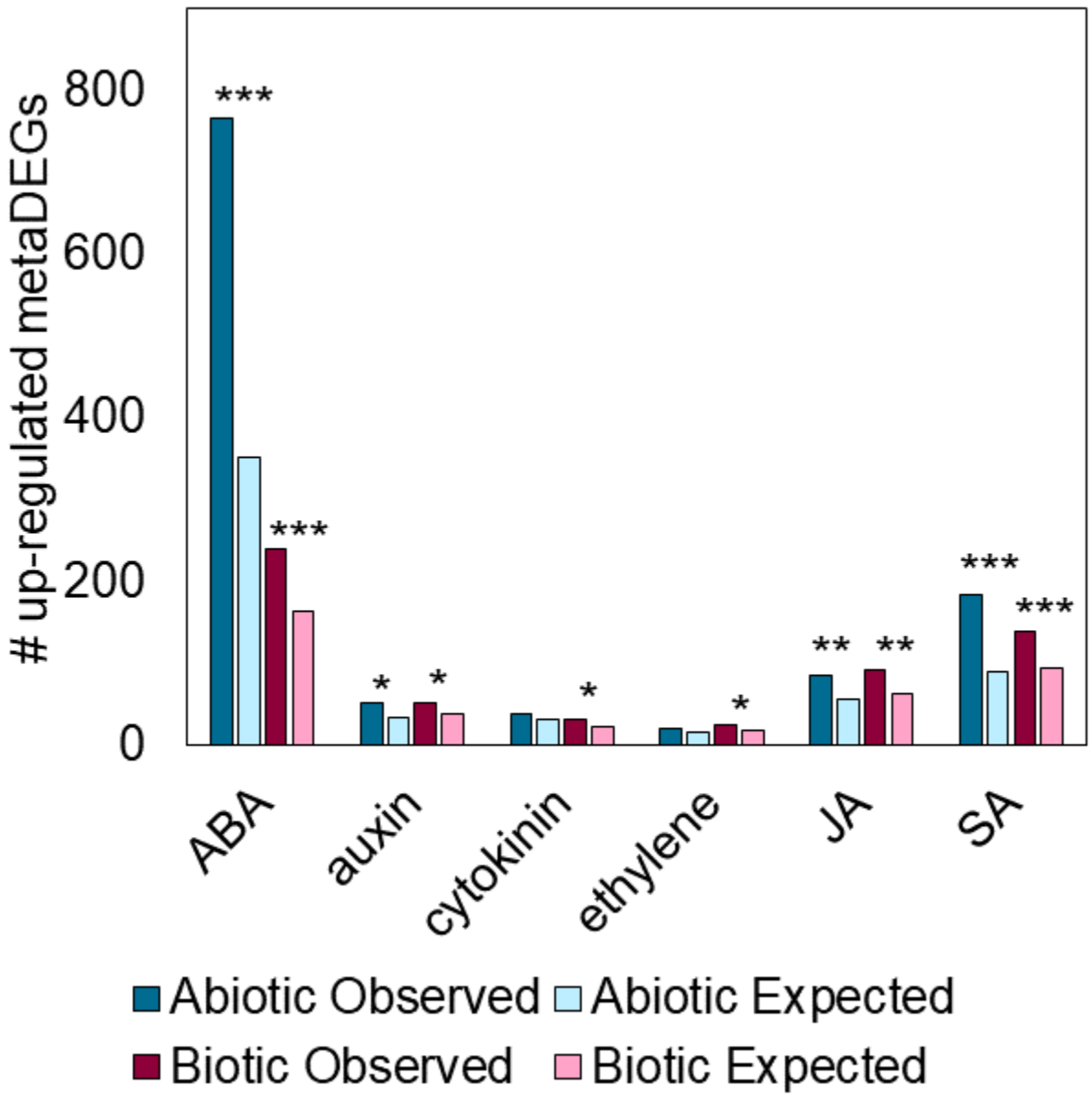


Fig 3.2: Rice hormone-responsive genes were generally up-regulated by stress. Observed number of up-regulated hormone-responsive metaDEGs is shown vs. the number expected to be up-regulated by random chance. Asterisks denote numbers observed differed significantly from numbers expected as determined by the χ^2 goodness of fit test (*** $p < 10^{-14}$, ** $p < 10^{-6}$, * $p < 0.005$, see Table S5 for all p-values).

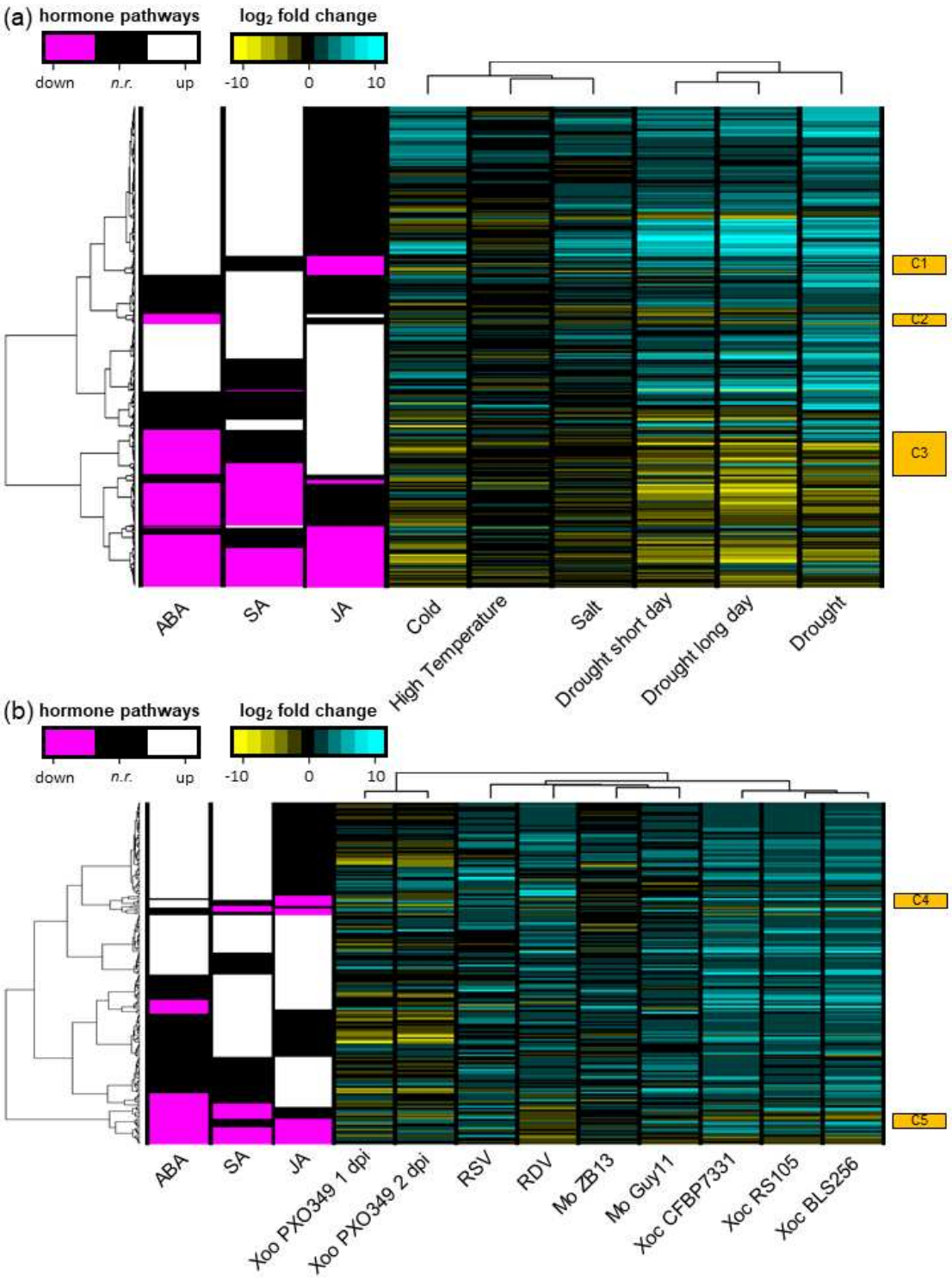


Fig 3.3: Signaling downstream of JA and SA is increased during stress. Gene expression (log_2 fold changes) of JA- and SA-responsive metaDEGs for (a) abiotic stress and (b) biotic stresses relative to controls (columns) are shown on the right in yellow (down-regulated), black (not regulated) and cyan (up-regulated). Hormone regulatory patterns of JA- and SA-responsive metaDEGs are shown on the left in magenta (down-regulated), black (not regulated; *n.r.*) and white (up-regulated). Clusters of genes regulated oppositely of hormone pathways are indicated by the orange squares (C1 through C5).

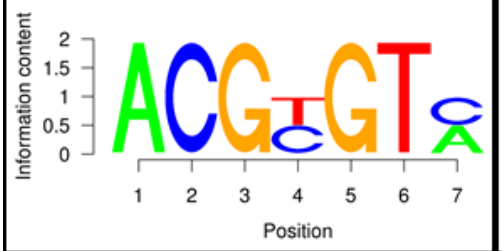
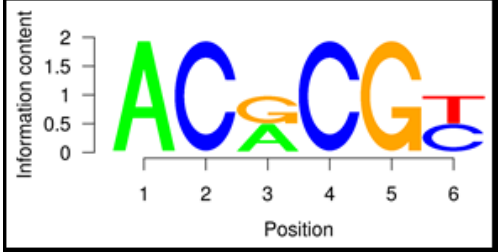
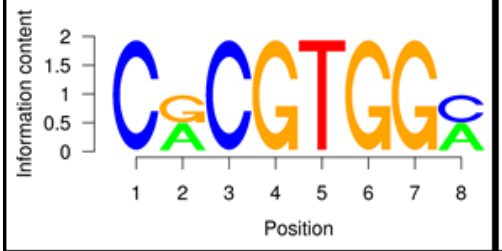
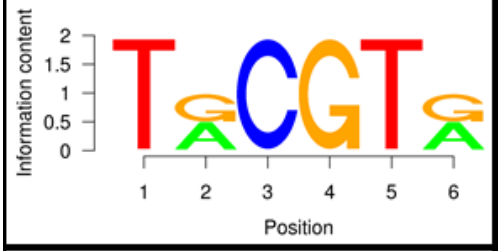
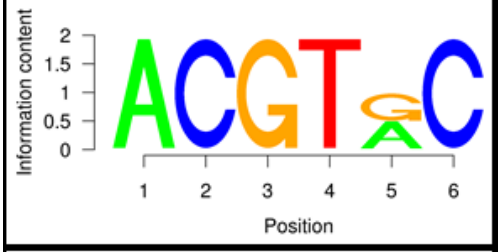
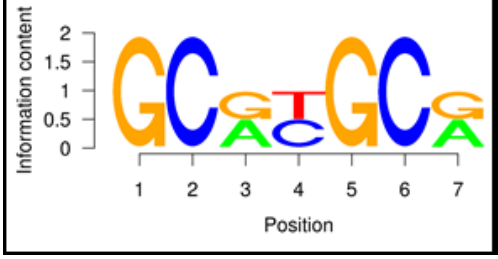
DREME Motif	GOMo Annotations	Enrichment
	• Response to abscisic acid stimulus • Response to cold • Response to desiccation	Abiotic Up Biotic Up
	• Response to water deprivation • Response to wounding	Abiotic Up Biotic Up
	• Response to abscisic acid stimulus • Response to cold	Abiotic Up
	• Response to wounding	Biotic Up
	• Peroxidase activity • Response to wounding	Biotic Up
	• Secondary cell wall biogenesis	Biotic Up

Fig 3.4: De novo discovered promoter motifs. Sequence logos for motifs discovered via DREME, associated GO term annotations discovered via GOMo, and enrichment within metaDEG sets as determined by Fisher's exact test ($p \leq 0.05$).

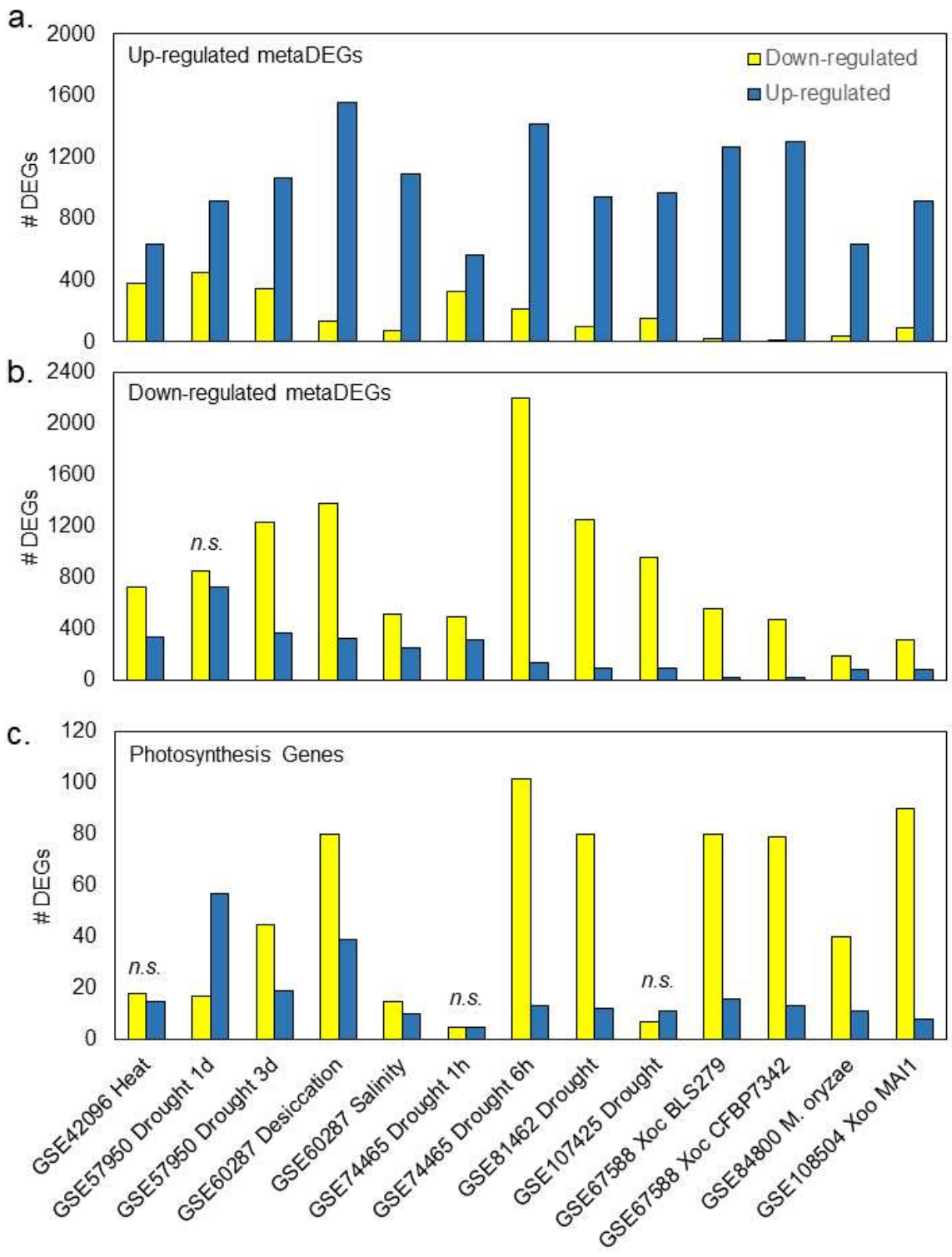


Fig 3.5: Publicly available gene expression studies validated meta-analysis results. (a) Up- and (b) Down-regulated metaDEGs and (c) photosynthesis-annotated genes generally followed expected trends in pre-processed publicly available gene expression datasets. *n.s.* indicates the counts observed did not differ significantly from counts expected as determined by the χ^2 goodness of fit test ($p > 0.05$, see Tables S7 and S8 for all p-values).

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CHAPTER 4. Discussion

4.1 During enhanced *Xa7* resistance at high temperature, rice suppresses the abscisic acid (ABA) response pathway

Plant disease, a major challenge to agriculture worldwide, is often exacerbated by abiotic environmental factors. During some plant-pathogen interactions, such as the rice interaction with *Xanthomonas oryzae* (Xo), heat stress allows pathogens to overcome host resistance. This phenomenon may severely reduce crop productivity over the coming decades in light of the global warming trends associated with climate change. In chapter 2, I presented the results of an RNA-Seq study conducted to understand rice transcriptome responses during simultaneous heat and susceptible or resistant rice-Xo interactions. The major finding of this work was that the ABA pathway was up-regulated during both high temperature stress and the susceptible interaction at high temperature, and was repressed during *Xa7*-mediated resistance at high temperature. These trends were confirmed by both the expression patterns of known ABA-responsive genes (Fig 2.4–2.6) and the identification of a novel ABA responsive element-like motif in the promoters of differentially regulated genes (Fig 2.7). These results suggest that there is interplay between *Xa7* and ABA signaling in rice during high temperature-enhanced resistance.

This study was the first of its kind to explore simultaneous high temperature stress and Xo infection in rice. It had a robust design, with two replicates per treatment over three time points each in susceptible and resistant interactions. The sequence read files generated, 28 in total, and the unprocessed read counts were submitted to the scientific community via the NCBI Sequence Read Archive and Gene Expression Omnibus. The high temperature data generated in this study were also used in the chapter 3 study.

This study is an example of what I described in chapter 1 as the most common use of RNA-Seq – the elucidation of plant transcriptome changes under different stimuli. Visualization of big data is difficult and for this, I used some uncommon visualization approaches. For example, to my knowledge, no other RNA-Seq study has presented gene ontology term results with both $\log_2$ odds ratio and median $\log_2$ fold change per term as I did with Fig 2.3. This figure allows a reader to identify regulatory trends of biological processes; for example, one can conclude that photosynthesis is down-regulated by high temperature because the $\log_2$ odds ratio in mock-down-regulated genes for the term 'photosynthesis' is positive and the median $\log_2$ fold change for 'photosynthesis'-annotated genes in mock-treated plants is negative (Fig 2.3).

Another uncommon visualization I used was kernel density estimation (KDE) to show $\log_2$ fold change of ABA- and salicylic acid-responsive genes (Fig 2.5, Supplemental Fig A1.2). KDE is a method to estimate the probability function of a variable – in this case, $\log_2$ fold change of genes. The output is similar to a proportional histogram displayed as a smooth curve. With KDE plots, multiple treatments can be overlaid on the same plot with different line colors. This allows for quick comparison of regulatory trends, i.e. up-regulation, down-regulation or no regulation, and of the extremity of the fold change in the displayed pathway.

The primary advantage of both of these uncommon visualization techniques was that they allowed discovery of the trends in the data in the early stages of the analysis. Conducting a transcriptome study is relatively easy; it requires good study design and laboratory technique. Available transcriptomics software suites allow rapid and straightforward mapping of sequence reads and differential gene expression analysis. However, this process generates a large list of genes, their fold changes and the associated p-values. By using the visualization techniques I discussed, I could rapidly generate dozens of visualizations to identify potentially interesting trends, breaking down the data to find meaningful information. The processes and tools used in this work were further refined for the next work.

4.2 Rice responds to abiotic and biotic stresses via universal transcriptome changes

Plants experience a range of abiotic and biotic stresses in the field. These stresses are unavoidable and greatly limit crop yield worldwide. Plants must overcome these stresses and one approach to do so is via physiological changes allowed by transcriptome versatility. Understanding commonalities among transcriptome responses to different stresses will provide fundamental information that informs strategies to develop broadly stress-tolerant plant lines. In chapter 3, I presented the results of a meta-analysis of publicly available rice stress response transcriptomes. The data analyzed included many abiotic and biotic stresses. The major finding of this work was that there are universal regulatory trends among all abiotic stresses, among all biotic stresses and among all stresses. The core response to abiotic and biotic stresses includes the down-regulation of photosynthesis and up-regulation of hormone-responsive genes.

This study provides a list of candidate genes for future study to improve rice stress tolerance. The full list of genes is fairly broad with 5,863 abiotic stress-responsive, 2,154 biotic stress-responsive genes and 913 genes responsive to both stress types. To narrow the list down to a more manageable number of genes, one could examine the expression of these genes in stress tolerance and disease resistance studies. I hypothesize that in tolerance studies, the meta-analysis-identified genes will show all of the following expression patterns: (1) similar expression in both meta-analysis and tolerance studies, (2) opposite expression in meta-analysis and tolerance studies, (3) meta-analysis genes which are not differentially expressed in tolerance studies and (4) genes differentially expressed in tolerance studies which were not identified in meta-analysis. After narrowing down the list of rice genes to study by comparing to tolerance studies, future researchers could design functional studies using genome editing or similar approaches.

This study also serves as an important proof of concept that meta-analysis of diverse transcriptome data is a valid approach to develop hypotheses for how plants respond to stress. Using this work as a guide, future researchers can design new meta-analysis studies based on either pre-existing data, where available, or newly-generated data in lesser studied or non-model systems. The data used in this study had a wide range of quality, from poor to robust. The number of high quality reads ranged from two million to 86 million reads (Supplemental Table A2.1). This diversity of quality demonstrates that robustness of transcriptome data is not a major limiting factor when conducting this style of meta-analysis. Importantly, this work also gave new life to abandoned studies; the data generators had previously abandoned many of the studies used here after mining it for a single publication or hypothesis. Many such abandoned transcriptome studies exist in public data repositories, giving future researchers an avenue to conduct new meta-analyses with no new sequencing costs.

4.3 Preliminary results suggest host ABA response enhances *Xa7* function

ABA is an important hormonal regulator of rice response to abiotic and biotic stresses. In susceptible rice-Xo interactions, ABA-responsive genes are generally up-regulated, suggesting that disease development favors ABA signaling. Abiotic stresses trigger biosynthesis and downstream signaling of ABA. Because development of Xo-caused disease favor abiotic stresses, ABA may play a central role in reducing rice resistance to Xo-caused disease during high temperature. However, *Xa7*, which is more effective at high temperature, suppresses ABA biosynthesis and signaling in rice. I conducted preliminary experiments to better understand the dynamics of how ABA signaling influences *Xa7* in rice (Supplemental Methods A3.1–A3.3).

To determine if ABA influences the outcome of rice responses to Xo during *Xa7*-mediated resistance, rice plants of variety IRBB61 (*Xa7*) were inoculated with Xo strains X11-5A *avrXa7*, carrying a plasmid with the gene for the *Xa7* elicitor AvrXa7, and X11-5A ΔCRR,

carrying a non-functional mutant of *avrXa7*, via leaf infiltration with a needleless syringe. Inoculations were co-infiltrated with 100 uM ABA in 0.095% ethanol or 0.095% ethanol alone. One week after inoculation, bacterial numbers in the entire inoculated leaf were quantified. At this time point, there was not yet a significant difference between the bacterial numbers in the susceptible and resistant interactions (Supplemental Fig A3.1). However, bacterial number was lower with co-infiltration of bacteria and ABA in the resistant interaction. This outcome suggests that co-infiltration with Xo and ABA enhanced *Xa7* activity.

To asses whether ABA influences the outcome of rice responses to Xo during high temperature stress and *Xa7*-mediated resistance, rice plants of near-isogenic varieties IR24 (no resistance) and IRBB7 (*Xa7*) were grown for 4 weeks after germination in a growth chamber under normal conditions. After three weeks, the plants were evenly split into normal temperature and high temperature chambers. Four days after temperature exposure, plants were sprayed with either 100 uM ABA (in 0.02% Tween 20) or 0.02% Tween 20 alone (no treatment). Three days after chemical treatment, plants were inoculated with Xo strain X11-5A *avrXa7* via scissor clip inoculation. Bacterial number in the 5 cm tip of inoculated leaves was quantified one week after inoculation. In the non-resistant plants, there were no differences among all treatments (Supplemental Fig A3.2a). In resistant plants, bacterial populations were reduced by high temperature, ABA and combined high temperature and ABA (Supplemental Fig A3.2b). These results suggest that foliar treatment of ABA enhances *Xa7* function to a similar level as high temperature.

To assess whether ABA had a direct effect on the growth of Xo strain X11-5A, *in vitro* growth curves were conducted in a plate reader (Supplemental Fig A3.3). There were no differences between untreated and 100 uM ABA-treated bacteria in 73 time points over 72 h ($p > 0.05$, student's t-test). Because ABA had no direct effect on the growth of Xo, it is likely that the differences seen in the *in planta* experiments were due to host responses.

Together, these preliminary experiments show an unexpected result: exogenous treatment with ABA may enhance *Xa7* (Supplemental Fig A3.4). This is unexpected because, as discussed in chapter 2, enhanced *Xa7* at high temperature suppresses ABA biosynthesis and downstream signaling. If depletion of ABA is core to the *Xa7* mechanism, exogenous treatment with ABA would be expected to reduce *Xa7* function rather than enhance it. However, ABA enhancement of *Xa7* is intuitive, because ABA signaling is a common denominator in rice response to heat and drought, both of which enhance *Xa7*.

To tease apart the interactions between *Xa7* and ABA signaling in rice, additional work is needed. To conduct this work, I will use a passive hydroponics system for growing and assaying rice (Supplemental Methods A3.4). Using this soil-free system allows for two improvements over a standard soil-based plant growth system: (1) the application of exogenous chemicals with accurate concentrations and (2) the execution of fast disease assays with a large replication number in a limited space. In addition to confirming that ABA enhances *Xa7* function, I will test other rice resistance genes that target Xo, including *xa5* and *Xa10*, which lose function at high temperature. To test the effects of ABA depletion on these interactions, I will treat plants with the ABA biosynthesis inhibitor fluridone.

4.4 Conclusion

Environmental stresses, both abiotic and biotic, are large contributors to yield loss in food crops. With climate change increasing the incidence of abiotic stresses and the constant pressures of pests and pathogens, the development of broadly stress-tolerant plant varieties is critical to food security. A thorough understanding of how plants respond to stresses is necessary for the development of these varieties. This work characterized how the transcriptome of the global staple food plant rice changes in response to abiotic stresses, biotic stresses and simultaneous abiotic and biotic stress.

The rice genome, with over 55,000 total loci, is an enormously complex system. The challenge of understanding how these genes are regulated during stress response is impossible with a single transcriptome study. In chapters 2 and 3, I characterized networks of gene regulation in four major rice responses: (1) response to a susceptible interaction with Xo during high temperature, (2) response to a resistant interaction with Xo during high temperature, (3) core response to abiotic stresses and (4) core response to biotic stresses. These data provide much of the necessary groundwork for beginning to understand the complex regulatory trends that underlie the rice response to single or multiple stresses. Our contributions to the understanding of stress-response networks have enabled the development of hypotheses to test the roles of candidate genes and pathways in plant stress defense. The long-term goal will be to inform approaches to selection of target genes and pathways for crop improvement research.

APPENDIX

A.1 CHAPTER 2 Supplemental Information

Supplemental Table A1.1: Sequencing reads and mapping summary statistics.

Samples	Repeat	Raw Reads			Mapped Reads		Mapped to Genes	
		Total	High-quality	%	Total	%	Total	%
Mock	1	28856641	28797259	99.79	27803387	96.35	25304614	87.69
NT 6 hpi	2	30695442	30638812	99.82	29600586	96.43	26848926	87.47
Mock	1	41141433	41070267	99.83	39789983	96.72	36328352	88.30
HT 6 hpi	2	30123157	30054334	99.77	28809619	95.64	26283199	87.25
Susceptible	1	18821068	18655171	99.12	18152259	96.45	16166375	85.90
NT 3 hpi	2	13444900	13408422	99.73	13058055	97.12	11616326	86.40
Susceptible	1	22988953	22926231	99.73	22301041	97.01	20033428	87.14
NT 12 hpi	2	18575425	18526120	99.73	18001933	96.91	16199997	87.21
Susceptible	1	23032633	22976160	99.75	22291085	96.78	19999269	86.83
NT 24 hpi	2	12956182	12924337	99.75	12517108	96.61	11153937	86.09
Susceptible	1	23971411	23908160	99.74	23267192	97.06	20852465	86.99
HT 3 hpi	2	21613616	21555636	99.73	20975915	97.05	18787524	86.92
Susceptible	1	27982082	27894994	99.69	27068780	96.74	24365034	87.07
HT 12 hpi	2	23750702	23678271	99.70	22952651	96.64	20657166	86.97
Susceptible	1	12757097	12721172	99.72	12310495	96.50	10965566	85.96
HT 24 hpi	2	18194844	18140633	99.70	17532615	96.36	15647360	86.00
Resistant	1	23216413	23154090	99.73	22531147	97.05	20277909	87.34
NT 3 hpi	2	17686011	17640774	99.74	17163755	97.05	15428030	87.23
Resistant	1	13651450	13615929	99.74	13229846	96.91	11903526	87.20
NT 12 hpi	2	22218398	22157390	99.73	21529837	96.90	19382683	87.24
Resistant	1	22393658	22329613	99.71	21673815	96.79	19035385	85.00
NT 24 hpi	2	14395418	14360572	99.76	13919943	96.70	12372688	85.95
Resistant	1	22577109	22517295	99.74	21889456	96.95	19612136	86.87
HT 3 hpi	2	25936686	25856236	99.69	25140318	96.93	22637373	87.28
Resistant	1	17682014	17631071	99.71	17117963	96.81	15248118	86.24
HT 12 hpi	2	24896378	24828591	99.73	24115576	96.86	21627216	86.87
Resistant	1	26980561	26721295	99.04	25723891	95.34	22705900	84.16
HT 24 hpi	2	20501057	20440854	99.71	19770695	96.44	17521343	85.47

NT = normal temperature; HT = high temperature; hpi = hours post-inoculation; percentages are per total raw reads per row

Supplemental Table A1.2: GO term enrichment analysis for genes differentially expressed by high temperature.

Mock Up-regulated GO Terms	# Regulated	# Not Regulated	log odds ratio	FDR-corrected p-value
biosynthetic process	205 / 917	3491 / 26832	0.945671	9.80E-13
response to endogenous stimulus	87	1024	1.402012	3.13E-12
cellular process	291	5655	0.80055	4.56E-12
metabolic process	285	5503	0.806354	4.68E-12
response to stress	162	2576	1.015332	4.88E-12
carbohydrate metabolic process	65	724	1.460453	3.61E-10
response to abiotic stimulus	104	1588	1.024647	1.62E-08
response to biotic stimulus	63	785	1.291966	5.36E-08
secondary metabolic process	29	300	1.530591	1.94E-05
translation	2	550	-3.24492	2.89E-05
multicellular organismal development	66	1094	0.868266	0.000197
signal transduction	66	1113	0.84242	0.00031
transport	94	1876	0.604408	0.002109
lipid metabolic process	44	736	0.838311	0.004324
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	125	2772	0.455095	0.010172
cell growth	22	309	1.077804	0.012999
cell differentiation	23	378	0.849111	0.040416
Mock Down-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
photosynthesis	33 / 594	140 / 27155	3.50486	5.14E-20
generation of precursor metabolites and energy	28	233	2.51504	6.54E-11
metabolic process	165	5623	0.559515	0.000396
response to stress	86	2652	0.646269	0.001977
response to biotic stimulus	33	815	0.927582	0.005596
protein modification process	33	2326	-0.66904	0.030064
Susceptible 3 h Up-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
response to endogenous stimulus	18 / 153	1093 / 27596	1.693224	0.000341
response to stress	32	2706	1.283078	0.000396
cellular homeostasis	7	184	2.836594	0.000706
response to abiotic stimulus	20	1672	1.221842	0.005964
biosynthetic process	35	3661	0.956224	0.006095
lipid metabolic process	12	768	1.572274	0.006948

cellular process	50	5896	0.838013	0.007315
metabolic process	47	5741	0.756078	0.017197
transport	21	1949	1.066544	0.017207
catabolic process	14	1205	1.14204	0.034787
Susceptible 3 h Down-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
photosynthesis	9 / 135	164 / 27614	3.579208	2.37E-06
metabolic process	50	5738	1.165798	0.000143
generation of precursor metabolites and energy	7	254	2.558439	0.002049
response to stress	26	2712	1.131691	0.004598
response to biotic stimulus	12	836	1.644191	0.005297
transport	18	1952	1.016838	0.039546
Susceptible 12 h Up-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
photosynthesis	40 / 1192	133 / 26557	2.786277	7.84E-17
metabolic process	374	5414	0.83711	1.80E-16
generation of precursor metabolites and energy	37	224	1.913428	3.45E-09
translation	57	495	1.403305	2.67E-08
cellular process	329	5617	0.508111	2.86E-06
response to abiotic stimulus	116	1576	0.773817	7.17E-06
secondary metabolic process	32	297	1.286863	0.000203
protein modification process	65	2294	-0.7107	0.000396
cellular component organization	79	1120	0.689756	0.001344
DNA metabolic process	4	379	-2.09807	0.003226
lipid metabolic process	54	726	0.756408	0.003857
anatomical structure morphogenesis	14	633	-1.03589	0.01928
signal transduction	34	1145	-0.61569	0.046676
Susceptible 12 h Down-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
metabolic process	288 / 820	5500 / 26929	1.077367	6.45E-20
response to stress	156	2582	1.148121	2.61E-14
cellular process	271	5675	0.887225	2.11E-13
response to biotic stimulus	70	778	1.649835	3.49E-13
response to abiotic stimulus	93	1599	1.019617	1.12E-07
biosynthetic process	167	3529	0.762756	2.05E-07
catabolic process	69	1150	1.042995	2.93E-06
transport	97	1873	0.84455	8.41E-06
response to endogenous stimulus	60	1051	0.959631	7.09E-05

signal transduction	62	1117	0.919233	0.000122
photosynthesis	15	158	1.65898	0.00144
translation	4	548	-2.0771	0.003257
generation of precursor metabolites and energy	18	243	1.301911	0.006334
secondary metabolic process	21	308	1.184424	0.007211
ripening	2	2	4	0.021854
protein modification process	92	2267	0.460225	0.026131
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	109	2788	0.409728	0.035725
lipid metabolic process	36	744	0.69336	0.037443
carbohydrate metabolic process	36	753	0.675527	0.039403
Susceptible 24 h Up-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
metabolic process	119 / 355	5669 / 27394	0.950988	3.45E-07
secondary metabolic process	14	315	1.819591	0.00072
cell communication	9	160	2.146664	0.002287
response to extracellular stimulus	9	172	2.041607	0.003613
response to stress	55	2683	0.756591	0.004922
cellular process	102	5844	0.573038	0.005964
response to abiotic stimulus	36	1656	0.81142	0.015777
translation	1	551	-2.85057	0.044306
cellular homeostasis	7	184	1.57306	0.044482
Susceptible 24 h Down-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
translation	94 / 440	458 / 27309	3.993133	1.29E-66
cellular process	132	5814	0.664888	0.000197
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	69	2828	0.687915	0.004183
response to abiotic stimulus	41	1651	0.676164	0.027297
Resistant 3 h Up-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
response to stress	112 / 694	2626 / 27055	0.840902	2.49E-06
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	112	2785	0.746727	2.89E-05
metabolic process	195	5593	0.585462	4.01E-05
biosynthetic process	133	3563	0.645321	0.000114
DNA metabolic process	21	362	1.202742	0.004627

cellular homeostasis	13	178	1.527673	0.005964
cell communication	12	157	1.592287	0.006194
carbohydrate metabolic process	34	755	0.844304	0.011597
response to abiotic stimulus	62	1630	0.614618	0.01347
cellular process	181	5765	0.382906	0.014815
response to extracellular stimulus	11	170	1.349301	0.025769
protein modification process	40	2319	-0.61395	0.02932
biological_process	195	6437	0.325023	0.039403
cellular component organization	44	1155	0.603026	0.049575
Resistant 3 h Down-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
biosynthetic process	186 / 769	3510 / 26980	1.093712	8.14E-15
cellular process	260	5686	0.93644	3.32E-14
metabolic process	246	5542	0.86427	6.81E-12
signal transduction	74	1105	1.31851	1.34E-09
response to biotic stimulus	56	792	1.377337	4.81E-08
response to stress	123	2615	0.827826	1.02E-06
response to endogenous stimulus	61	1050	1.089909	5.80E-06
protein modification process	106	2253	0.812007	8.05E-06
transport	92	1878	0.8618	1.10E-05
lipid metabolic process	46	734	1.186359	2.06E-05
translation	2	550	-2.98527	0.000305
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	116	2781	0.629213	0.000354
response to abiotic stimulus	74	1618	0.739794	0.000866
carbohydrate metabolic process	40	749	0.943031	0.001753
DNA metabolic process	1	382	-3.44727	0.002853
ripening	2	2	4	0.019673
Resistant 12 h Up-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
photosynthesis	117 / 3107	56 / 24642	4	5.52E-67
generation of precursor metabolites and energy	106	155	2.4804	1.55E-32
metabolic process	873	4915	0.650226	2.01E-22
cellular process	820	5126	0.45003	6.01E-11
lipid metabolic process	126	654	0.633475	0.000203
response to abiotic stimulus	233	1459	0.366461	0.004325
cellular homeostasis	37	154	0.939214	0.004616
biosynthetic process	474	3222	0.260376	0.005389
anatomical structure morphogenesis	47	600	-0.69791	0.005596

multicellular organismal development	96	1064	-0.49914	0.005596
carbohydrate metabolic process	117	672	0.482069	0.008022
cell cycle	18	277	-0.96138	0.017389
DNA metabolic process	26	357	-0.79821	0.019005
secondary metabolic process	54	275	0.649111	0.020539
protein modification process	224	2135	-0.28616	0.024521
cell growth	22	309	-0.82987	0.026929
regulation of gene expression, epigenetic	4	110	-1.79338	0.027297
translation	82	470	0.480375	0.031394
Resistant 12 h Down-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
translation	192 / 2878	360 / 24871	2.283178	1.23E-52
cellular process	809	5137	0.5879	2.22E-17
response to stress	415	2323	0.710554	8.83E-15
response to abiotic stimulus	280	1412	0.841171	3.32E-14
transport	305	1665	0.725172	7.58E-12
response to endogenous stimulus	190	921	0.87891	6.18E-11
response to biotic stimulus	148	700	0.905197	3.86E-09
metabolic process	731	5057	0.416852	1.04E-08
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	395	2502	0.509178	6.43E-08
biosynthetic process	479	3217	0.427565	9.31E-07
multicellular organismal development	156	1004	0.447174	0.004183
secondary metabolic process	53	276	0.742262	0.006948
signal transduction	155	1024	0.407732	0.010172
catabolic process	158	1061	0.38355	0.015688
biological_process	745	5887	0.172863	0.035975
post-embryonic development	119	798	0.380862	0.0426
Resistant 24 h Up-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
metabolic process	343 / 1022	5445 / 26727	0.982175	5.14E-20
protein modification process	162	2197	1.073233	1.98E-13
cellular process	311	5635	0.712123	1.33E-10
response to stress	163	2575	0.832484	1.04E-08
secondary metabolic process	32	297	1.524688	7.51E-06
signal transduction	77	1102	0.922715	1.21E-05
response to endogenous stimulus	72	1039	0.906732	4.01E-05
response to biotic stimulus	57	791	0.954347	0.000143

response to abiotic stimulus	96	1596	0.707874	0.000206
lipid metabolic process	49	731	0.841423	0.002126
cell death	22	275	1.082071	0.012924
pollen-pistil interaction	9	75	1.658983	0.017207
cell communication	14	155	1.252102	0.024684
response to extracellular stimulus	14	167	1.143942	0.034011
Resistant 24 h Down-regulated GO Terms	**# Regulated**	**# Not Regulated**	**log odds ratio**	**FDR-corrected p-value**
translation	220 / 1598	332 / 26151	3.633829	8.05E-125
protein modification process	82	2277	-0.8157	2.09E-06
response to abiotic stimulus	149	1543	0.714487	2.49E-06
response to stress	207	2531	0.474825	0.000313
cellular process	396	5550	0.291467	0.005497
regulation of gene expression, epigenetic	0	114	-4	0.009868
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	201	2696	0.32507	0.02071
carbohydrate metabolic process	62	727	0.498315	0.048263
response to biotic stimulus	66	782	0.483949	0.04917

The columns indicate GO term, number of annotated genes upregulated by high temperature, number of annotated genes not upregulated, $\log_2$ odds ratio, and FDR-corrected p-value. The first row includes the total number of genes upregulated and not upregulated.

Supplemental Table A1.3: Hormone response genes differentially expressed at high temperature.

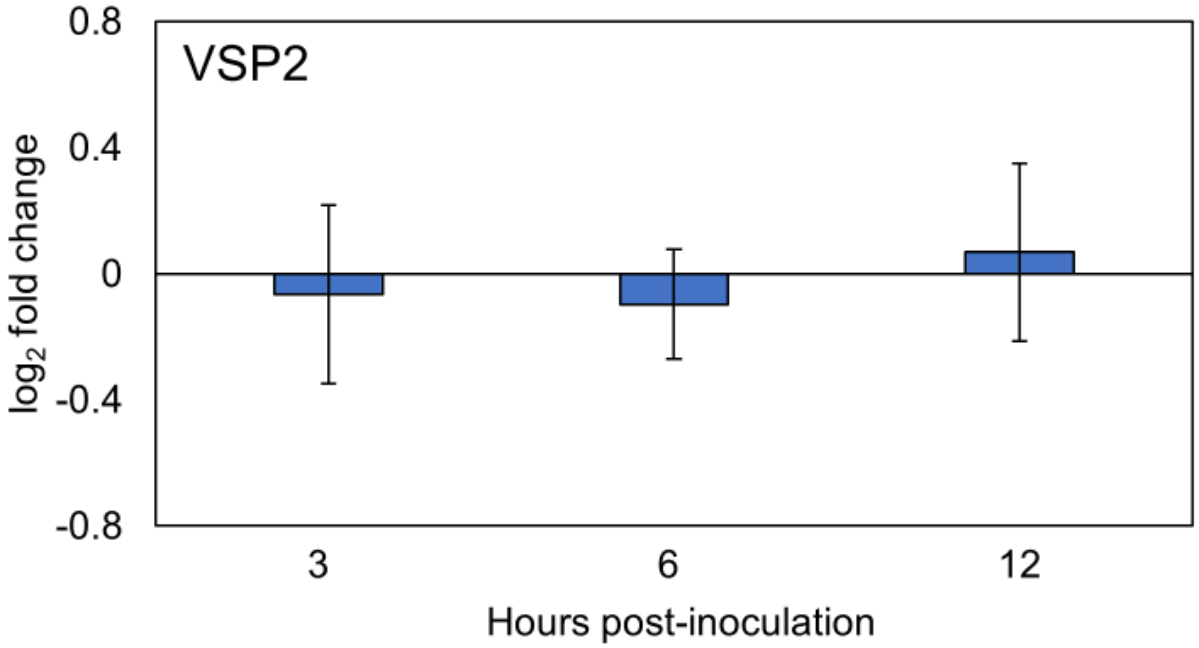
	6 h	3 h		12 h		24 h	
	Mock	**Susceptible**	**Resistant**	**Susceptible**	**Resistant**	**Susceptible**	**Resistant**
Total DEGs	1511	288	1463	2012	5985	795	2620
Auxin genes	21 1.4%	6 2.1%	15 1.0%	21 1.0%	44 0.7%	11 1.4%	24 0.9%
Cytokinin genes	14 0.9%	2 0.7%	11 0.8%	11 0.6%	31 0.5%	5 0.6%	16 0.6%
ABA genes	232 15.4%	66 22.9%	244 16.7%	279 13.9%	715 12.0%	132 16.6%	415 15.8%
Ethylene genes	4 0.3%	3 1.0%	6 0.4%	11 0.6%	19 0.3%	4 0.5%	13 0.5%
SA genes	23 1.5%	9 3.1%	29 2.0%	46 2.3%	106 1.8%	21 2.6%	66 2.5%
JA genes	33 2.2%	7 2.4%	32 2.2%	35 1.7%	71 1.2%	8 1.0%	39 1.5%

The whole numbers indicate the number of DEGs that are downstream of hormone response in each treatment, and the percentages indicate the percentage of total DEGs. Genes which were responsive to more than one hormone were equally divided among the hormones they were responsive to, with the total number of genes being rounded the nearest whole number.

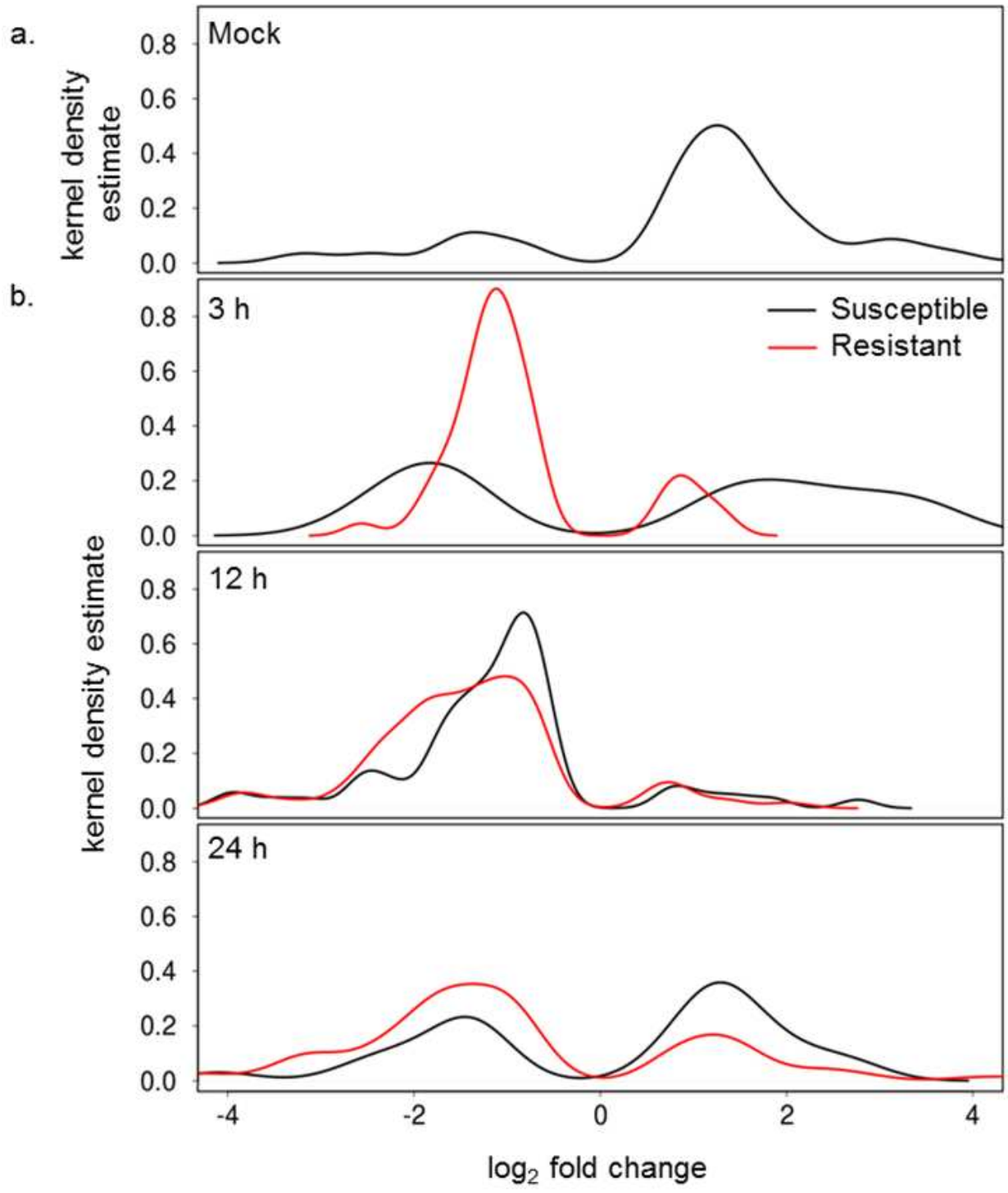
Supplemental Table A1.4: Odds ratios of promoter motifs in the promoters of different gene sets.

		ABRE	TATA	IBOX	GCBP2	TELO
3 hpi	Sus up	1.47	1.64	*n.e.*	*n.e.*	*n.e.*
	Sus dn	0.61	*n.e.*	2.03	*n.e.*	*n.e.*
	Res up	*n.e.*	*n.e.*	*n.e.*	*n.e.*	0.72
	Res dn	1.39	1.31	*n.e.*	*n.e.*	0.68
12 hpi	Sus up	*n.e.*	*n.e.*	*n.e.*	*n.e.*	0.76
	Sus dn	*n.e.*	1.23	*n.e.*	1.18	0.70
	Res up	*n.e.*	0.86	1.22	*n.e.*	0.68
	Res dn	1.14	1.23	0.83	1.12	*n.e.*
24 hpi	Sus up	*n.e.*	*n.e.*	*n.e.*	*n.e.*	0.38
	Sus dn	1.27	1.40	*n.e.*	1.25	1.87
	Res up	*n.e.*	1.18	*n.e.*	0.87	0.50
	Res dn	1.21	1.19	*n.e.*	1.26	1.32

An odds ratio >1 indicates that the motif was significantly enriched in the upstream promoters of the genes in the given set (Sus = susceptible, Res = resistant, up = upregulated genes, dn = downregulated genes) when compared to a random set of 10,000 rice genes, while an odds ratio <1 indicates that's the motif was significantly underrepresented in the promoters of the genes in the given set when compared to the random set of 10,000 rice genes. n.e. = not statistically enriched relative to the random set of genes. Statistical enrichment was determined by Fisher's exact test ($p < 0.05$).



Supplemental Fig A1.1: Mean log_2 fold change of VSP2 in mock treated plants at high temperature as measured by qRT-PCR. There were no significant differences in expression of the wound-responsive jasmonic acid marker VSP2 at 3, 6, and 12 h post-mock inoculation at high temperature relative to low temperature (Student's t-test, $p > 0.05$). Error bars represent SEM ($n = 6$).



Supplemental Fig A1.2: Differential expression of SA up-regulated genes at high temperature. (a) Kernel density estimate of log_2 fold change for SA up-regulated genes differentially regulated by high temperature in mock inoculated plants. (b) Kernel density estimates of log_2 fold change for SA up-regulated genes differentially regulated by high temperature in plants during susceptible and resistant interactions at 3, 12, and 24 hpi.

A.2 CHAPTER 3 Supplemental Information

Supplemental Table A2.1: Sequence reads and mapping summary statistics for studies used in this analysis.

SRA Accession	SRA File	Raw Reads			Mapped Reads		Mapped to Genes	
		Total	High-quality	%	Total	%	Total	%
SRP004651	SRR074138	2846609	2081083	73.1	1639951	57.6	647117	22.7
	SRR074139	4275874	4098121	95.8	3123652	73.1	1260614	29.5
	SRR074152	2813770	2102466	74.7	1545280	54.9	589689	21.0
	SRR074153	4100071	3965068	96.7	2800270	68.3	1112812	27.1
SRP049040	SRR1615264	17789340	15830450	89.0	15063183	84.7	13399817	75.3
	SRR1615265	18653720	16503925	88.5	15939728	85.5	14567482	78.1
	SRR1615270	20593774	18494227	89.8	17957374	87.2	16293467	79.1
	SRR1615271	22838476	19933545	87.3	19358889	84.8	18026902	78.9
	SRR1615276	22912622	19997866	87.3	19337019	84.4	17859494	77.9
	SRR1615277	22824036	19912550	87.2	19178057	84.0	17560005	76.9
SRP049444	SRR1636849	19092472	17838233	93.4	17531136	91.8	15874146	83.1
	SRR1636850	19230528	18060948	93.9	17732822	92.2	16024851	83.3
	SRR1636851	19145050	17972749	93.9	17568563	91.8	15800857	82.5
	SRR1636852	19204242	18077313	94.1	17655624	91.9	15947097	83.0
SRP052306	SRR1761528	14664192	14336202	97.8	14126322	96.3	12561495	85.7
	SRR1761529	16490701	16079832	97.5	15930835	96.6	14136931	85.7
	SRR1761530	14302686	13957683	97.6	13854692	96.9	12341694	86.3
	SRR1761531	14857078	14499467	97.6	14378412	96.8	12714596	85.6
SRP056884	SRR1952778	24830499	23223823	93.5	22989791	92.6	20741839	83.5
	SRR1952779	4883837	4628841	94.8	4569527	93.6	4173349	85.5
	SRR1952780	20256732	18175744	89.7	18025351	89.0	16268893	80.3
	SRR1952793	42745641	39611721	92.7	39235112	91.8	35101114	82.1
	SRR1952794	21223770	20138835	94.9	19936854	93.9	18145834	85.5
	SRR1952795	23224262	21169649	91.2	20983836	90.4	18960452	81.6
	SRR1952799	11473000	10789043	94.0	10307039	89.8	9175002	80.0
	SRR1952800	7052129	5782355	82.0	5735075	81.3	5189344	73.6
	SRR1952801	94865601	86940356	91.7	85208991	89.8	76480884	80.6

	SRR1952808	26656102	24944374	93.6	24623033	92.4	22051552	82.7
	SRR1952809	11353463	9116423	80.3	9042009	79.6	8204216	72.3
	SRR1952810	21537709	19914067	92.5	19762897	91.8	17545990	81.5
SRP065503	SRR2862232	10881310	10666806	98.0	10476630	96.3	8796680	80.8
	SRR2862233	11549952	11335079	98.1	11184408	96.8	9652944	83.6
	SRR2862234	14669472	14400690	98.2	14196515	96.8	12212313	83.2
	SRR2862235	14835261	13831522	93.2	12759480	86.0	9019246	60.8
	SRR2862236	14492224	13457128	92.9	12427566	85.8	8693814	60.0
	SRR2862237	14732837	13866180	94.1	13034861	88.5	9616513	65.3
SRP071248	SRR3209769	33012246	32544030	98.6	32183678	97.5	28458579	86.2
	SRR3209770	28693929	28272609	98.5	27919697	97.3	24568753	85.6
	SRR3209771	33738285	32592932	96.6	32322917	95.8	28552310	84.6
	SRR3209772	31951497	31491434	98.6	31136484	97.4	27552347	86.2
	SRR3209773	34717753	34209223	98.5	33932453	97.7	29807973	85.9
	SRR3209774	28803587	28402703	98.6	28138752	97.7	24721147	85.8
	SRR3209775	32798250	32317710	98.5	31714289	96.7	27963729	85.3
	SRR3209776	33838322	33335561	98.5	32500089	96.0	28722587	84.9
	SRR3209777	32797025	32257404	98.4	32045115	97.7	28278419	86.2
	SRR3209778	35848188	35304074	98.5	34446373	96.1	30406765	84.8
	SRR3209779	37177413	36585163	98.4	36146494	97.2	31734877	85.4
	SRR3209780	24165701	23716826	98.1	23503601	97.3	20649808	85.5
SRP076382	SRR3657371	22197650	20998530	94.6	20639867	93.0	18214810	82.1
	SRR3657372	22268264	21738274	97.6	21537816	96.7	18805709	84.5
	SRR3657373	22266057	21732275	97.6	21385244	96.0	18725054	84.1
	SRR3657374	64675050	63208865	97.7	61855799	95.6	53979022	83.5
	SRR3657375	64439195	62839529	97.5	61104240	94.8	51675412	80.2
	SRR3657376	64563225	63079703	97.7	61465550	95.2	52331655	81.1
SRP101342	SRR5311338	28856641	28556116	99.0	28045305	97.2	25119042	87.0
	SRR5311339	30695442	30362383	98.9	29840798	97.2	26626693	86.7
	SRR5311340	41141433	40804827	99.2	40082712	97.4	36075724	87.7
	SRR5311341	30123157	29815532	99.0	29102390	96.6	26153865	86.8
	SRR5856927	45759126	42925774	93.8	36858231	80.5	33434870	73.1

SRP113286	SRR5856928	42646908	39885840	93.5	34578376	81.1	31388151	73.6
	SRR5856929	62531390	58566201	93.7	49797889	79.6	45432151	72.7
	SRR5856930	48560300	45410331	93.5	40126712	82.6	36866849	75.9
	SRR5856931	53548032	50082357	93.5	45393291	84.8	41719887	77.9
	SRR5856932	42810176	40099526	93.7	35954083	84.0	32689730	76.4
SRP115030	SRR5909330	30885880	29633997	95.9	28103798	91.0	23947753	77.5
	SRR5909331	43120834	41191189	95.5	38723981	89.8	31260292	72.5
	SRR5909332	34017864	32708190	96.2	30951045	91.0	25933263	76.2
	SRR5909333	25897684	24806086	95.8	22676004	87.6	16798889	64.9
	SRR5909334	31044302	29575566	95.3	28524764	91.9	24513853	79.0
	SRR5909335	32654458	31285854	95.8	30187414	92.4	26092712	79.9

All percentages are per total raw reads per row.

Supplemental Table A2.2: Total number of DEGs identified in each study and the amount of DEGs per study retained after meta-analysis.

Stress	# DEGs	# Retained	% Retained
Drought long day	11644	4531	39
Drought short day	11292	4393	39
Drought	9710	3925	40
Salt	7950	2438	31
High Temperature	1220	505	41
Cold	1545	663	43
Xoc BLS256	7625	1194	16
Xoc RS105	3163	807	26
Xoc CFBP7331	2684	754	28
Xoo PXO349 1 dpi	5164	559	11
Xoo PXO349 2 dpi	6477	673	10
M. oryzae ZB13	1465	436	30
M. oryzae Guy11	4756	488	10
Rice Stripe Virus	7620	881	12
Rice Dwarf Virus	8320	1108	13

Xoc indicates *X. oryzae* pv. *oryzicola*; Xoo indicates *X. oryzae* pv. *oryzae*

Supplemental Table A2.3: Number and percentage of metaDEGs identified with all possible expression patterns.

Expression Pattern	metaDEGs	Abiotic metaDEGs (%)	Biotic metaDEGs (%)	All metaDEGs (%)
Abiotic Up	1929	32.9	0	27.5
Abiotic Up Biotic Up	615	10.5	28.6	8.8
Abiotic Up Biotic Down	18	0.3	0.8	0.3
Abiotic Down	2933	50.0	0	41.8
Abiotic Down Biotic Up	70	1.2	3.2	1.0
Abiotic Down Biotic Down	298	5.1	13.8	4.2
Biotic Up	753	0	35.0	10.7
Biotic Down	400	0	18.6	5.7

Supplemental Table A2.4: Biological process GO terms significantly enriched within metaDEG sets.

Abiotic Up-regulated GO Term	# in pattern	# in background	p-value
biological process	797	10051	7.60E-29
biosynthetic process	557	5922	3.01E-35
carbohydrate metabolic process	152	1287	2.46E-16
catabolic process	196	1812	3.59E-17
cell communication	34	326	0.001511
cellular homeostasis	35	308	0.000287
cellular process	761	9396	8.09E-30
embryo development	70	740	7.43E-05
lipid metabolic process	140	1236	7.11E-14
metabolic process	863	9239	2.41E-58
multicellular organismal development	177	1861	3.66E-11
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	377	4604	3.29E-14
pollen-pistil interaction	14	104	0.006178
post-embryonic development	117	1456	0.000158
reproduction	106	1092	2.00E-07
response to abiotic stimulus	322	2703	3.01E-35
response to biotic stimulus	133	1272	5.04E-11
response to endogenous stimulus	198	1818	1.20E-17
response to extracellular stimulus	37	356	0.001101
response to stress	466	4198	7.52E-45
secondary metabolic process	52	531	0.000335
signal transduction	143	1810	6.36E-05
transport	283	2997	2.88E-17
Abiotic Down-regulated GO Term	**# in pattern**	**# in background**	**p-value**
anatomical structure morphogenesis	145	996	1.75E-13
biological process	906	9942	3.07E-15
biosynthetic process	629	5850	7.77E-24

carbohydrate metabolic process	149	1290	1.47E-07
cell differentiation	77	634	4.64E-05
cell growth	85	482	5.00E-12
cellular component organization	197	1739	3.81E-09
cellular homeostasis	48	295	2.13E-06
cellular process	1076	9081	1.11E-64
generation of precursor metabolites and energy	115	365	2.63E-33
lipid metabolic process	173	1203	1.57E-15
metabolic process	1275	8827	8.80E-139
multicellular organismal development	187	1851	1.06E-05
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	405	4576	1.71E-05
photosynthesis	120	204	1.76E-56
pollen-pistil interaction	19	99	0.000463
post-embryonic development	142	1431	0.000285
protein modification process	456	3522	1.93E-31
response to abiotic stimulus	377	2648	7.78E-33
response to biotic stimulus	174	1231	4.58E-15
response to endogenous stimulus	226	1790	2.16E-14
response to external stimulus	33	147	2.08E-07
response to stress	455	4209	3.30E-17
ripening	3	3	0.007739
secondary metabolic process	79	504	3.76E-09
signal transduction	245	1708	1.47E-21
transport	353	2927	1.87E-19
tropism	28	103	6.94E-08
Biotic Up-regulated GO Term	**# in pattern**	**# in background**	**p-value**
biological process	391	10457	3.33E-06
biosynthetic process	299	6180	6.03E-16
catabolic process	83	1925	0.00306
cell death	27	451	0.00351

cellular process	374	9783	8.00E-07
generation of precursor metabolites and energy	27	453	0.00351
lipid metabolic process	85	1291	6.46E-10
metabolic process	500	9602	3.54E-37
multicellular organismal development	92	1946	9.60E-05
nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	219	4762	1.94E-09
pollen-pistil interaction	10	108	0.005523
post-embryonic development	74	1499	0.000162
reproduction	54	1144	0.003885
response to abiotic stimulus	181	2844	1.49E-19
response to biotic stimulus	119	1286	2.86E-24
response to endogenous stimulus	146	1870	4.65E-23
response to extracellular stimulus	25	368	0.001114
response to stress	310	4354	1.70E-43
secondary metabolic process	46	537	8.11E-09
signal transduction	90	1863	6.89E-05
Biotic Down-regulated **GO Term**	**# in pattern**	**# in background**	**p-value**
biological process	193	10655	0.00389
biosynthetic process	124	6355	0.003655
carbohydrate metabolic process	48	1391	1.19E-06
cellular process	198	9959	2.09E-05
generation of precursor metabolites and energy	21	459	6.43E-05
metabolic process	221	9881	3.51E-10
photosynthesis	29	295	3.55E-13
response to biotic stimulus	41	1364	0.00018
response to external stimulus	10	170	0.001686
response to stress	94	4570	0.003655

Supplemental Table A2.5: Number of observed and expected phytohormone-induced metaDEGs.

Abiotic metaDEGs					
Pathway	**Up-regulated**	**Expected Up-regulated**	**Down-regulated**	**Expected Down-regulated**	**p-value***
ABA	765	351	38	452	1.1E-190
auxin	52	35	28	45	1.3E-04
cytokinin	39	33	36	42	1.6E-01
ethylene	21	16	15	20	9.4E-02
JA	85	56	44	73	2.6E-07
SA	184	90	23	117	1.2E-39
JA‡	21	11	5	15	7.2E-05
SA‡	34	16	2	20	1.6E-09
Biotic metaDEGs					
Pathway	**Up-regulated**	**Expected Up-regulated**	**Down-regulated**	**Expected Down-regulated**	**p-value***
ABA	239	165	8	82	1.5E-23
auxin	53	38	4	19	2.5E-05
cytokinin	32	23	3	12	1.4E-03
ethylene	26	19	2	9	4.6E-03
JA	93	64	3	32	3.4E-10
SA	139	95	3	47	4.3E-15
JA‡	40	29	4	15	4.7E-04
SA‡	45	31	2	16	1.6E-05

*p-values are calculated with χ^2 goodness of fit test; ‡ indicates genes not responsive to ABA

Supplemental Table A2.6: All *de novo* discovered promoter motifs.

Motif	Discovered In	Enriched In*			
		Abiotic Up	Abiotic Down	Biotic Up	Biotic Down
ACGYGTM	Abiotic Up	Yes	No	Yes	No
TRCGTR	Abiotic Up	Yes	Yes	Yes	No
CTATAWA	Abiotic Up	Yes	Yes	Yes	Yes
CRCGTGGM	Abiotic Up	Yes	No	No	No
AAAAADA	Abiotic Up	Yes	Yes	Yes	Yes
AGTASTA	Abiotic Up	Yes	Yes	Yes	Yes
AAACG	Abiotic Up	Yes	Yes	Yes	No
CACGNCAC	Abiotic Up	Yes	No	Yes	No
DAAAAAAH	Abiotic Down	Yes	Yes	Yes	Yes
TAGCTR	Abiotic Down	Yes	Yes	Yes	Yes
AMTRTA	Abiotic Down	Yes	Yes	No	Yes
AATTW	Abiotic Down	Yes	Yes	Yes	Yes
MTGMAA	Abiotic Down	Yes	Yes	Yes	Yes
STAGTA	Abiotic Down	Yes	Yes	Yes	No
TKCAGW	Abiotic Down	Yes	Yes	Yes	Yes
DCCACACA	Abiotic Down	No	Yes	Yes	No
TAYATR	Abiotic Down	Yes	Yes	Yes	Yes
ATGTKW	Abiotic Down	No	Yes	No	Yes
ACKTACG	Abiotic Down	Yes	Yes	Yes	Yes
AYGMATG	Abiotic Down	Yes	Yes	Yes	Yes
AAAT	Abiotic Down	Yes	Yes	Yes	Yes
CAGYA	Abiotic Down	Yes	Yes	Yes	Yes
ACGTRC	Biotic Up	Yes	Yes	Yes	No
ACRCGY	Biotic Up	Yes	No	Yes	No
GCRYGCR	Biotic Up	Yes	Yes	Yes	No
SCTATAWA	Biotic Up	Yes	Yes	Yes	Yes
CGATCRW	Biotic Up	Yes	No	Yes	No
YAGCTR	Biotic Up	Yes	Yes	Yes	Yes
GTTTGAM	Biotic Up	Yes	No	Yes	No
AGTASTAB	Biotic Up	Yes	Yes	Yes	No
TGCABA	Biotic Down	No	Yes	No	Yes
AGCTASY	Biotic Down	Yes	Yes	Yes	Yes

ADAAAAA	Biotic Down	Yes	Yes	Yes	Yes
ATAWATA	Biotic Down	Yes	Yes	Yes	Yes
TGCAW	Biotic Down	Yes	Yes	No	Yes
CWCACW	Biotic Down	Yes	Yes	Yes	Yes
CAGTD	Biotic Down	No	Yes	No	Yes
ATWTA	Biotic Down	Yes	Yes	Yes	Yes
CATYTTGC	Biotic Down	No	No	No	Yes

*Enrichment determined by Fisher's exact test ($p \leq 0.05$)

Supplemental Table A2.7: Number of metaDEGs up- and down-regulated within pre-processed gene expression studies.

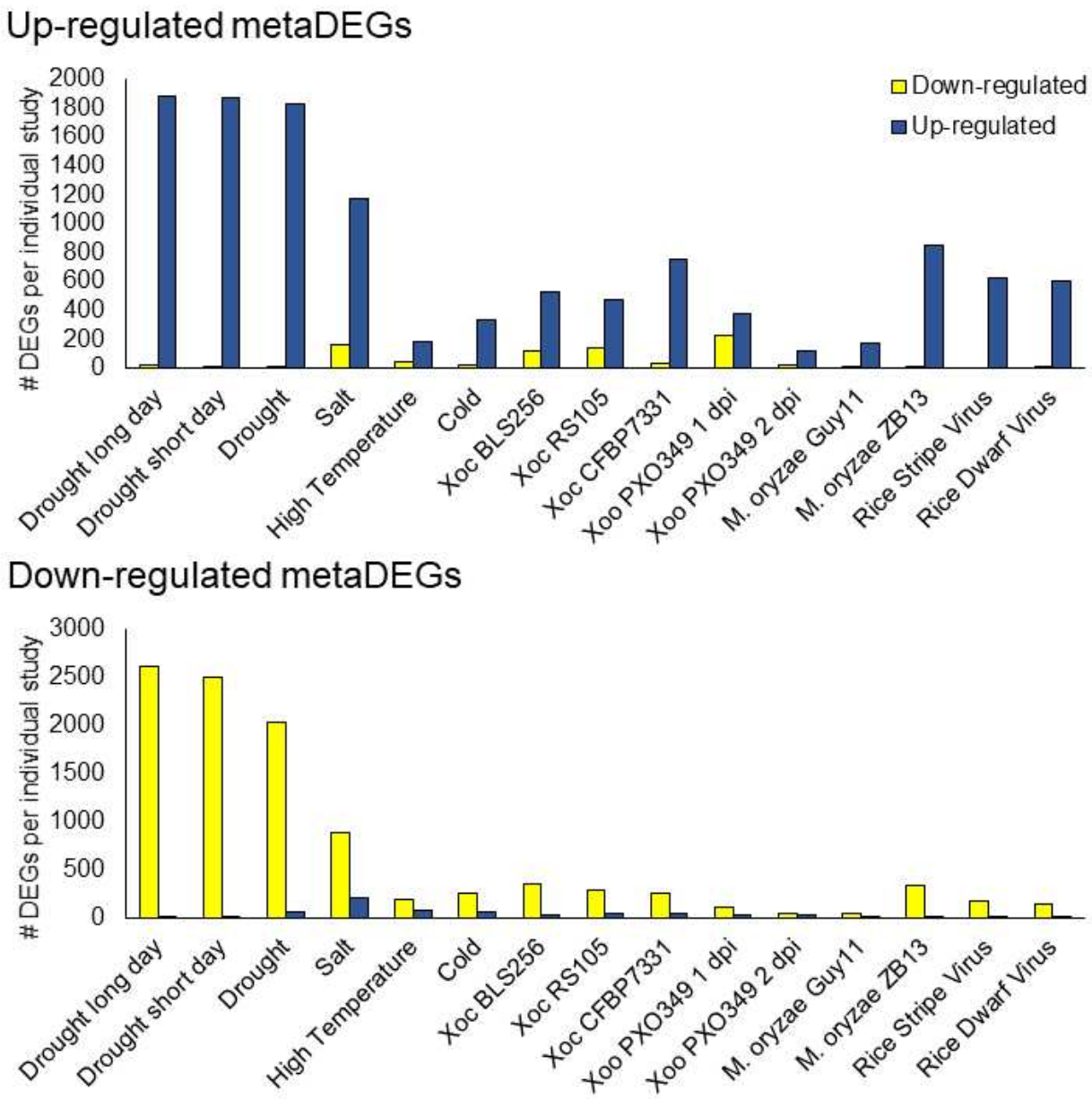
Abiotic Stress Up-regulated metaDEGs					
Study	**Up-regulated metaDEGs**	**Expected Up-regulated**	**Down-regulated metaDEGs**	**Expected Down-regulated**	**p-value***
GSE42096	632	501	380	511	1.8E-16
GSE57950 1 d	914	643	453	724	8.0E-49
GSE57950 3 d	1062	620	349	791	3.0E-124
GSE60287 dess.	1557	929	139	767	3.8E-206
GSE60287 salinity	1095	697	77	475	5.8E-124
GSE74465 1 h	567	404	331	494	7.8E-28
GSE74465 6 h	1417	504	217	1130	0
GSE81462	945	405	98	638	5.9E-258
GSE107425	973	431	157	699	1.3E-241
Abiotic Stress Down-regulated metaDEGs					
Study	**Up-regulated metaDEGs**	**Expected Up-regulated**	**Down-regulated metaDEGs**	**Expected Down-regulated**	**p-value***
GSE42096	342	530	728	540	1.4E-30
GSE57950 1 d	729	743	850	836	0.48
GSE57950 3 d	374	705	1230	899	2.9E-62
GSE60287 dess.	328	937	1381	772	1.4E-192
GSE60287 salinity	257	463	522	316	4.6E-51
GSE74465 1 h	319	369	500	450	4.5E-4
GSE74465 6 h	136	722	2206	1620	1.5E-151
GSE81462	97	523	1250	824	2.3E-125
GSE107425	97	405	965	657	2.5E-84
Biotic Stress Up-regulated metaDEGs					
Study	**Up-regulated metaDEGs**	**Expected Up-regulated**	**Down-regulated metaDEGs**	**Expected Down-regulated**	**p-value***
GSE67588 BLS279	1267	804	20	483	1.6E-156
GSE67588 CFBP7342	1300	874	10	436	1.1E-137
GSE84800	632	465	40	207	3.0E-44
GSE108504	918	576	90	432	4.7E-105
Biotic Stress Down-regulated metaDEGs					
Study	**Up-regulated metaDEGs**	**Expected Up-regulated**	**Down-regulated metaDEGs**	**Expected Down-regulated**	**p-value***
GSE67588 BLS279	25	367	562	220	6.1E-187
GSE67588 CFBP7342	25	335	478	168	5.7E-190
GSE84800	81	188	190	83	3.7E-45
GSE108504	90	232	316	174	5.1E-46

*p-values are calculated with χ^2 goodness of fit test; dess. = dessication

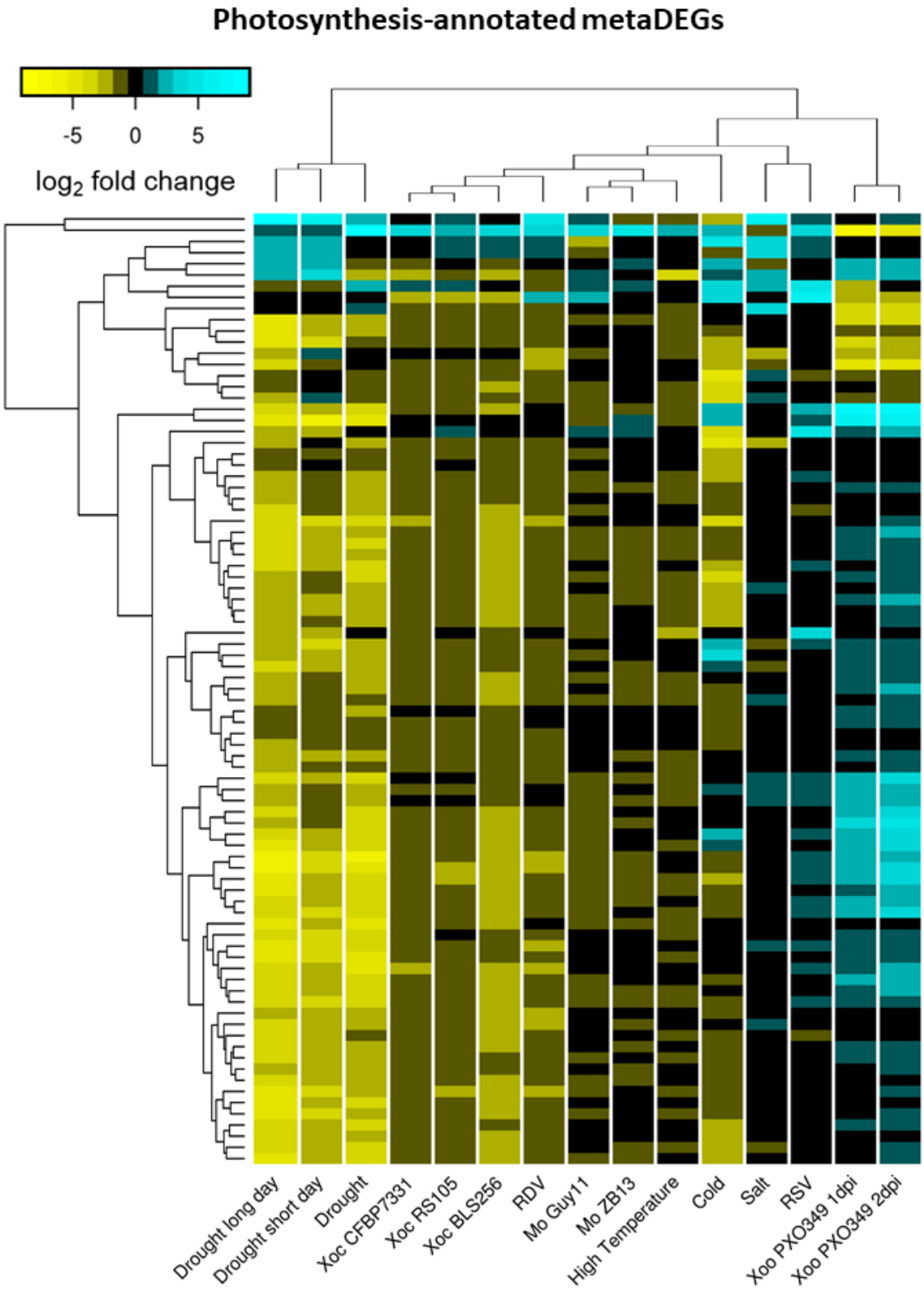
Supplemental Table A2.8: Number of photosynthesis-annotated genes differentially regulated in pre-processed gene expression studies.

Study	Up-regulated	Expected Up-regulated	Down-regulated	Expected Down-regulated	p-value*
GSE42096	15	16	18	17	0.73
GSE57950 1 d	57	35	17	39	3.0E-7
GSE57950 3 d	19	28	45	36	0.023
GSE60287 dess.	39	65	80	54	1.7E-6
GSE60287 salinity	10	15	15	10	0.041
GSE74465 1 h	5	5	5	5	1
GSE74465 6 h	13	35	102	80	8.3E-6
GSE81462	12	36	80	56	2.9E-7
GSE107425	11	7	7	11	0.053
GSE67588 BLS279	16	60	80	36	1.8E-20
GSE67588 CFBP7342	13	61	79	31	3.4E-26
GSE84800	11	35	40	16	4.4E-13
GSE108504	8	56	90	42	1.1E-22

*p-values are calculated with χ^2 goodness of fit test; dess. = dessication

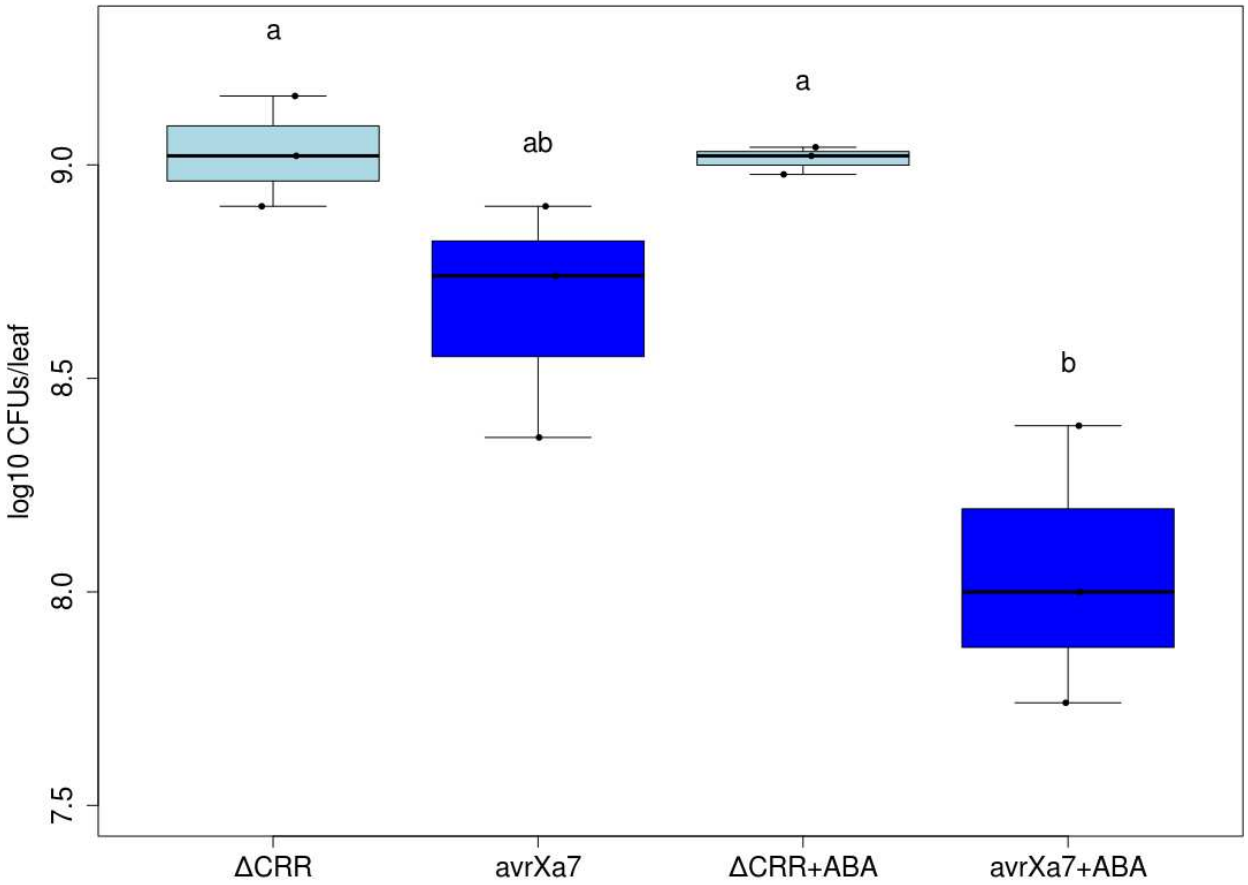


Supplemental Fig A2.1: DEGs per study retained in meta-analysis followed the expected regulatory trends. The DEGs retained as up-regulated (top) and down-regulated (bottom) metaDEGs were mostly up- and down-regulated, respectively, within each individual study.

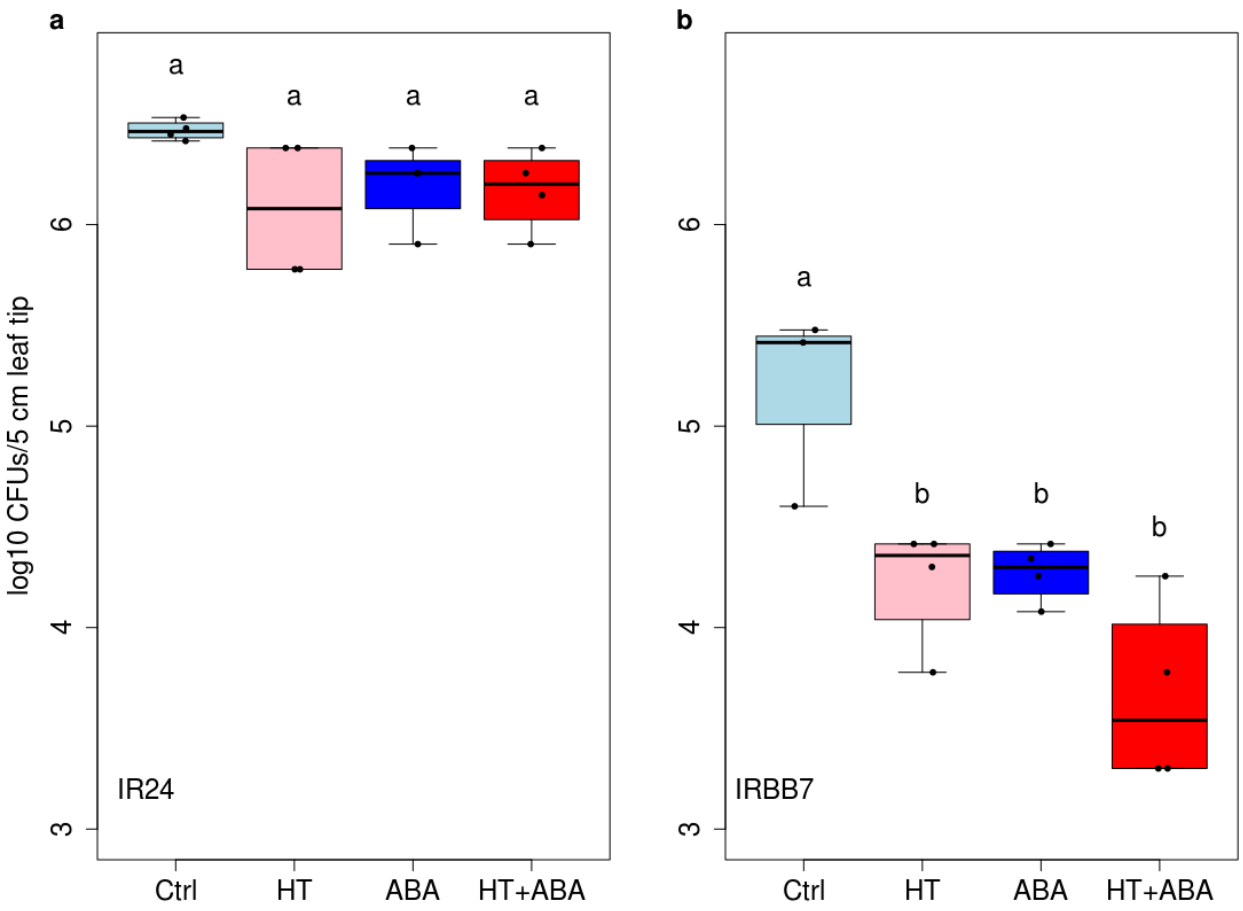


Supplemental Fig A2.2: Stress down-regulated photosynthesis in rice. Gene expression (log_2 fold changes) for stresses relative to controls (columns) are shown for photosynthesis-annotated metaDEGs (rows).

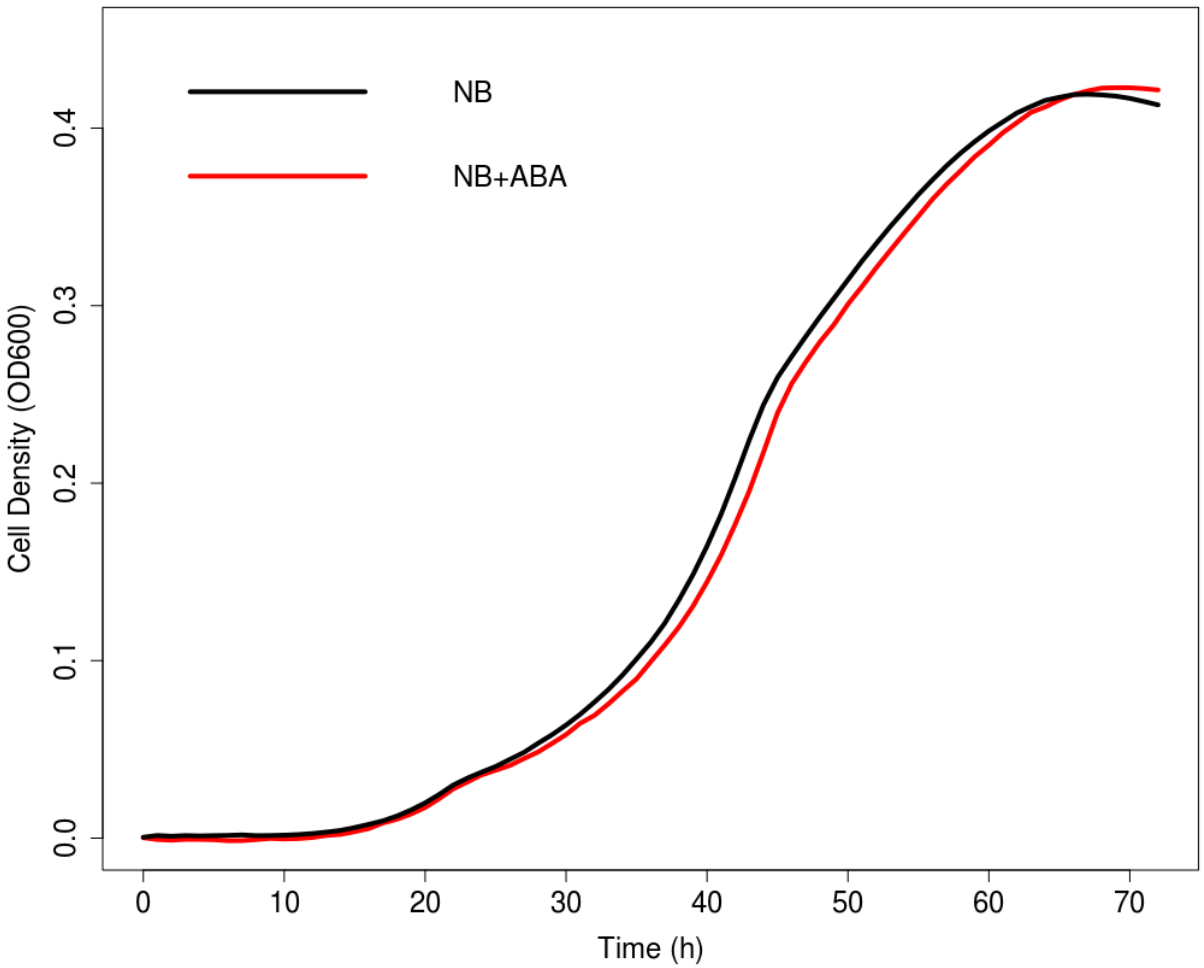
A.3 CHAPTER 4 Supplemental Information



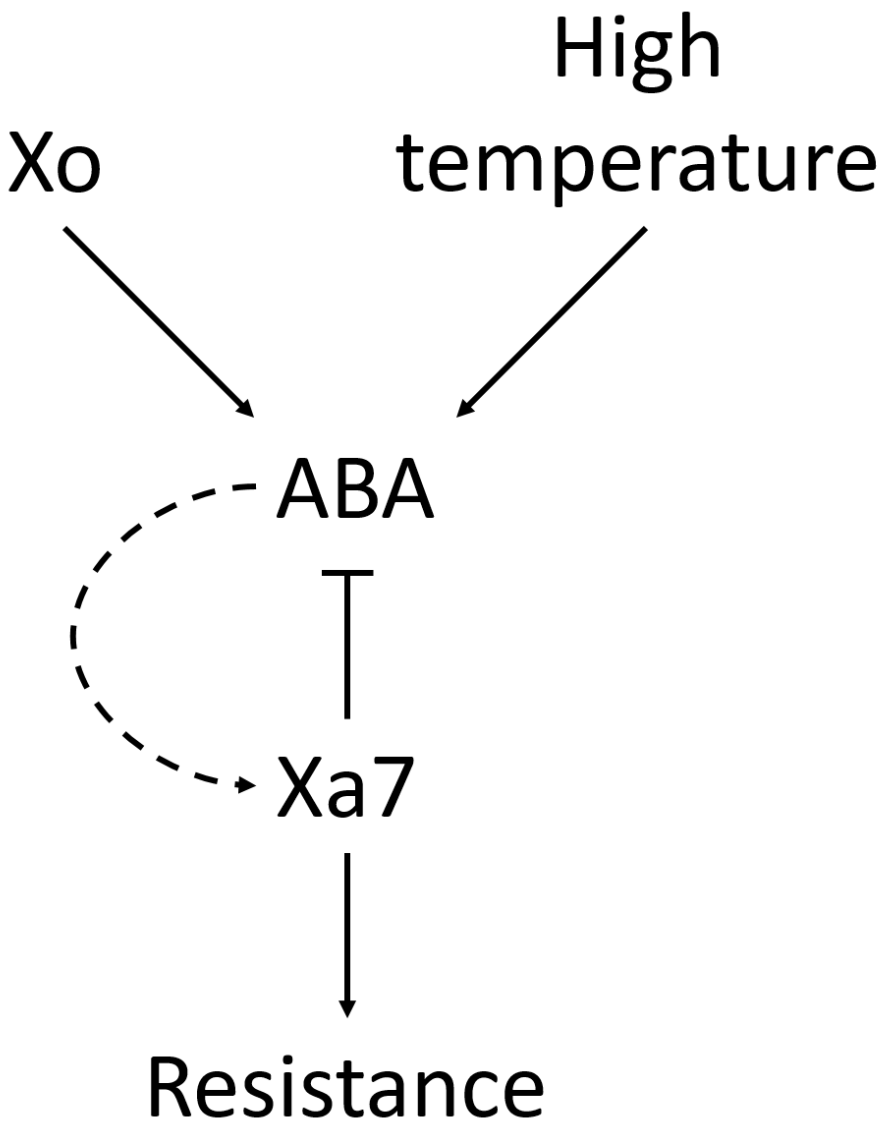
Supplemental Fig A3.1: Co-infiltration with Xo strain X11-5A avrXa7 and ABA enhances *Xa7* resistance in rice. Plants were inoculated with Xo strains X11-5A ΔCRR (empty vector) or X11-5A avrXa7 (a plasmid carrying the gene for the elicitor of *Xa7*). Bacterial populations were quantified at 7 d post-inoculation. Contrary to the resistant interaction alone, co-infiltration with the resistance-inducing bacteria and ABA was detectable. ANOVA was used to determine if there were differences among means ($p < 0.05$) and a post-hoc Tukey test was used to determine groups (alpha = 0.05).



Supplemental Fig A3.2: Foliar treatment with ABA enhances *Xa7* resistance to a similar level as high temperature. Plants were treated with nothing (Ctrl), high temperature (HT), ABA or HT and ABA and inoculated with Xo strain X11-5A avrXa7. In non-resistant IR24 plants (a), bacterial numbers were the same in all treatments. In resistant IRBB7 plants, (b) bacterial numbers were reduced by all treatments relative to the Ctrl. ANOVA was used to determine if there were differences among means ($p < 0.05$) and a post-hoc Tukey test was used to determine groups (alpha = 0.05).



Supplemental Fig A3.3: ABA has no effect on the growth of *Xo* strain X11-5A. There were no differences between untreated and ABA-treated *Xo* strain X11-5A in 72 time points within 72 h ($p > 0.05$, student's t-test). Lines shown are the means of three biological replicates per time point (n = 12 technical replicates each).



Supplemental Fig A3.4: Putative model for *Xa7*/ABA interactions. Xo infection and high temperature treatment induce ABA biosynthesis and signaling in rice. *Xa7*, which induces host resistance, suppresses ABA biosynthesis and signaling. ABA enhances *Xa7* activity; arrows = activation, blunt arrow = suppression, dotted line arrow = putative activation.

Supplemental Methods A3.1: Bacterial culturing and growth

For all bacteria experiments, bacteria were revived from glycerol stock on peptone sucrose agar (PSA) and grown in an incubator at 28°C. After approximately three days, bacteria were restreaked on fresh PSA and grown overnight. Overnight cultures were used as starter cultures in *in vitro* experiments and as inoculum in all *in planta* experiments. For the *in vitro* growth curves, the starter cultures were diluted to approximately 2×10^5 CFU/mL in 200 uL of nutrient broth (NB; Difco™) in a 96-well culture plate. NB was supplemented with either 0.095% ethanol or 100 uM ABA (Sigma-Aldrich®, CAS Number: 14375-45-2) in 0.095% ethanol. Plates were shaken at 225 RPM at 28°C with readings taken every hour in a plate reader (BioTek® PowerWave HT). Student's t-test was used to detect differences at each time point with a threshold of $p = 0.05$.

Supplemental Methods A3.2: Plant growth and inoculations

For the co-infiltration assay, rice seeds of IRBB61 (*Xa7*) were placed directly into a custom soil-free growing system in the greenhouse (approximately 24–30°C, 14 h days, >80% relative humidity). This system is a passive hydroponics system using 10 L opaque black plastic bins with holes drilled in the lid to accommodate 15 mL conical tubes. The bottoms of the conical tubes were cut open and plugged with cheesecloth. The bins were filled with greenhouse tap water to the level where the tube bottoms were barely submerged (about 5 L). The ungerminated seeds were placed directly onto the cheesecloth. After seedling emergence and 5 cm of growth (approximately one week), the greenhouse water was replaced with Peters Excel® 13-2-13 fertilizer, final concentration 300 ppm N, supplemented with iron chelate solution, final concentrations 27.8 mg/L ferrous sulfate heptahydrate and 37.3 mg/L of EDTA disodium salt, as in standard MS media. Three weeks after germination, plants were inoculated with Xo strains X11-5A avrXa7-pKEB31 and X11-5A ΔCRR-pKEB31 resuspended in sterile water to a concentration of 2×10^8 CFU/mL via infiltration with a needleless syringe. For bacterial quantification, inoculated leaves were collected one week after inoculation, flash frozen with

liquid nitrogen and ground with a tissue macerator (Qiagen TissueLyser II). The extract was resuspended in 1 mL of sterile water, 10-fold serially diluted and plated on PSA + 100 mg/L cycloheximide.

For the foliar treatment assay, rice seeds of near-isogenic varieties IR24 (no resistance) and IRBB7 (*Xa7*) were germinated on wet filter paper (Whatman No.1) under constant light at 28°C. After seedling emergence, plants were transplanted in a custom soil mixture (50% potting soil, 50% Greens Grate™) in a growth chamber and grown for 4 weeks after germination under a standard growing regime (28°C/24°C day/night, 12 h days, >70% relative humidity). After three weeks, half of the plants of both varieties were transferred to a high temperature growing regime (35°C/29°C day/night, 12 h days, >70% relative humidity). Four days after transfer, leaves were treated with 100 uM ABA in 0.02% Tween 20 or 0.02% Tween 20 alone via spraying. Three days after chemical treatment, the largest fully expanded leaves were inoculated with Xo strain X11-5A avrXa7-pKEB31 resuspended in sterile water to a concentration of 2×10^8 CFU/mL via scissor clip inoculation. Inoculated leaf tips (5 cm) were collected one week after inoculation and bacterial numbers were quantified as above.

Supplemental Methods A3.3: Statistical analyses and figure generation

ANOVA was used to detect differences among means within *in planta* bacterial number experiments (threshold, $p < 0.05$), via the anova function in R (https://www.r-project.org). If differences were detected, the post-hoc Tukey HSD test was used to determine with significance groups, via the HSD.test function from the R library agricolae (https://cran.r-project.org/package=agricolae). For the *in vitro* growth curve, 12 technical replicates were averaged per biological replicate at each time point, with 3 biological replicates. The Student's t-test was used to detect differences in the means between treatments at each time-point. Despite the high number of t-tests, no p-value correction was applied because no p-values were significant. Boxplots and line charts were generated via the boxplot and plot functions, respectively, in R.

Supplemental Methods A3.4: Protocol: a rapid assay for rice disease assays using a passive hydroponics system

Non-skirted 96-well PCR plates are prepared by cutting an approximately 2 mm hole in the bottom of all tubes with sharp scissors. Rice seeds are sterilized in 20% Clorox® Regular Bleach for 30 minutes on a rocker then washed five times with sterile, deionized water for 10 seconds per wash. Seeds are then placed into the PCR plates, one seed per tube, germ-side up. The PCR plate is placed in a sterile inverted pipette tip box lid filled with roughly 3 mm of deionized water. The PCR plate and box lid are placed in a sterile, sealed plastic bag, and then transferred to a germinating growth chamber (28 °C, 24 h light) for four days. The deionized water is replaced with iron-supplemented Peters Excel® 13-2-13 fertilizer as described in Supplemental Methods A3.2. PCR plate and box lid are placed 30 cm from the light in a growth chamber set for standard rice growth (28 / 24 °C, >70% humidity, 14 / 10 h day / night) for approximately seven days. When seedlings are 10 to 15 cm tall, they are transferred to a high temperature growth chamber (35 / 29 °C) for seven days. One week after temperature treatment, they are inoculated with Xo via clip inoculation. Tissue for RNA extraction is collected as needed. Lesions and bacterial number are quantified 11 days after inoculation. Plants must be watered with the fertilizer solution daily.