THESIS

GENE EXPRESSION REGULATION BY A STRESS-RESPONSIVE TRANSCRIPTION FACTOR IN RICE SEEDLINGS

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ABSTRACT

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Stress physiology is an inherently complex field. As plants cannot leave their environment when it becomes unfavorable, they have developed multiple mechanisms to cope with stresses. Many of these are unique to plants compared to mobile organisms. Plant stress physiology is of interest not only for this reason, but because the human population relies on agriculture for food. Additionally, our ecosystem relies on plants as primary producers as an integral component of life on earth.

Plant stress physiology at the molecular level involves a symphony of signaling cascades that reshape cell physiology and communicate the stress signal to the whole plant and even nearby organisms. Over the last thirty years, enormous progress has been made to identify key genes, hormones, and signaling pathways that are involved in plant stress responses. To this end, we have yet to understand a cohesive picture of how plants respond to a combination of stresses. Given the variety of biotic stresses from bacteria, fungi, viruses, nematodes, and herbivores and their interaction with abiotic stresses including environmental extremes and resource availability, continued efforts are needed to understand the molecular nuances of plant stress responses. Not only are stresses variable and unique, plants have evolved to thrive in specific habitats, thereby developing unique strategies to cope with local environments. For example, rice grows well in flooded soils which would induce a stress-response in typical, non-aquatic organisms. Therefore, stress response will need to be decoded at the level of the organism.

The goal of this work is to better elucidate stress response in rice. Specifically, I have looked at the influence of a transcription factor, SIGNAL RESPONSIVE 1 (*Os*SR1), that is regulated by Ca2+/CaM and known to be a dynamic regulator in a myriad of stresses in *Arabidopsis*. I have generated complemented lines of *Ossr1* mutant and *OsSR1* overexpressor transgenic rice lines. When compared with WT and mutant lines, these lines showed a range of *OsSR1* expression. These lines will be of great help in deciphering the action of this transcription factor. Homozygous *SR1* complemented and overexpressor lines along with WT and *Ossr1* mutant will be used in future studies to better understand the action of SR1 in stress response in rice.

Additionally, I performed a factorial global gene expression analysis using RNA-seq with WT and *Ossr1* lines at the seedling stage in control and drought conditions, which will serve as a breeding ground for hypothesis generation and testing in future studies. Significant differentially expressed (DE) genes show down-regulation of genes encoding serine threonine-protein kinase receptor (SRK)-receptors, kinases, TCP family transcription factor, cytokinin-modifying enzyme and up-regulation of aquaporin, sucrose synthase, G-protein-related, and ferredoxin-nitrate reductase in the mutant when compared to WT. In response to polyethylene glycol (PEG)-induced drought stress, the mutant up-regulated transcription factors (homeobox [HOX]-containing TFs, WRKY, and DIVARICATA), signaling proteins (protein phosphatases), late embryogenesis abundant protein 1 (LEA1), nodulin-related genes, and senescence-associated gene 21 (SAG21), while down-regulating a CaM-dependent protein kinase, efflux transporters, peroxidases, aquaporins, and disease-related genes including Pathogenesis-related protein PRB1-2, disease resistance protein RPS2, and NB-ARC domain-containing protein. Lastly, significant DE genes in the WT illuminate how this important crop plant responds when exposed to PEG-

induced drought. Drought induced the expression of MAPKKKs, ethylene-responsive transcription factors (ERFs), HOX TFs, as well as zinc-finger proteins and protein phosphatase 2Cs. In drought, WT down-regulated glycol-lipid transfer proteins, aquaporins, and salt stress-induced proteins. Gene ontology (GO) analysis of significant DE genes showed enrichment of GO terms related to membranes, oxidative stress, response to stimulus, and transcription regulation in both the WT and mutant when exposed to PEG. Future work will analyze the promoters of candidate genes for the *Os*SR1 DNA-binding motif (CG-1) to identify direct targets of *Os*SR1.

Rice is the model organism for monocots and provides 15% of the calories consumed by humans. This study and other studies based on this work will help in elucidating the functions of this stress-responsive transcription factor, *Os*SR1, in this important crop plant.

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Introduction

Plants experience stress

Life only occurs in a specific range of conditions, and within this range, each organism must adjust its internal or external conditions to maintain homeostasis. Many living organisms are able to move to more conducive local surroundings, however, plants, rooted where they germinate, are not. Plants instead, must maintain homeostasis as their local environment shifts and their physiology must account for the lack of motility. Plant stress responses are of interest not only because they differ from mobile organisms, but also because we depend on plants for food and a functioning ecosystem. As primary producers, plants fix inorganic carbon and provide energy to herbivores and heterotrophs and oxygen to the atmosphere (Crowe et al., 2013). Understanding the genetic, molecular, and physiological mechanisms of plant stress response will allow us to breed more resilient crops and care for the environment that we depend on.

Plant stress has been defined in multiple ways. Lichtenthaler defined stress as “Any unfavorable condition or substance that affects or blocks a plant's metabolism, growth or development, is to be regarded as stress” (1996). Larcher classified stress as a change in physiology when a species is exposed to extraordinary unfavorable conditions that do not kill the organism, but elicit a response (2003). Gaspar further distinguished stress from strain, noting that stress initiates with an unpredictable fluctuation hindering normal metabolism and resulting in aberrant physiology. “Stress is the altered physiological condition caused by factors that tend to alter an equilibrium. Strain is any physical and/or chemical change produced by a stress, i.e. every established condition, which forces a system away from its thermodynamic optimal state” (2002). Akram went even further to distinguish stress as any factor, which reduces “growth and

productivity less than the actual genotype's potential" (2019). The nature of any physiological condition is relative and thus difficult to define. However, it is clear that stress is a hindrance to growth when an organism is pushed beyond the normal or optimal range of growth conditions in its environment.

Plant stress can be caused by a number of biotic and abiotic factors, occurring independently or in concert and causing a localized or systemic effect. Biotic factors include pathogen attack or herbivory, and both can elicit not only a localized and systemic response, but volatiles have been shown to transmit the stress signal between plants (Bozorov et al., 2017; Toyota et al., 2018; Farmer and Ryan, 1990; Muroi et al., 2011). Abiotic factors include extreme temperatures, salinity, water (in extremes of drought, flooding, and submergence), nutrient deficiency, and toxins. Stresses are often paired, such as heat and drought, drought and salt, drought and nutrient deficiency, or heat and pathogen attack (Cohen and Leach, 2019; Barah et al., 2016; Shinozaki et al., 2000).

The experience of plant stress at the cellular level occurs in three stages: 1) perception of a change in environment: the sensor, 2) relay throughout the organism or cell: the signal/messenger, and 3) modifications to cope with the stress: the response.

It is thought that unique stressors induce/activate unique sensors. For example, the receptors/sensors involved in recognizing pathogen-associated molecular patterns (PAMPs) that elicit basal immunity or PAMP-triggered immunity (PTI) are localized on the plasma membrane (e.g, FLS2, a leucine-rich repeat receptor-like kinase LRR-RLK) (Gomez-Gomez and Boller, 2000). OSCA1, a hyperosmolality-gated calcium-permeable channel, is proposed as an osmotic sensor (Zhu, 2016) and COLD1, a cold sensor that regulates G-protein signaling, confers chilling tolerance in rice (Ma et al., 2015). Transmembrane proteins link each cell to the whole organism

and are therefore probable targets for sensors, however, protein cleavages or mis-folding of proteins in the ER in response to an environmental shift can also function as putative sensors, initiating a signal in response to the change in protein shape (Zhu, 2016).

The sensor must relay the stress signal, and ideally, this is not limited to a single cell but relayed throughout the entire organism. Studies propose that small molecule second messengers such as Ca^{2+} and reactive oxygen species (ROS) are efficient at relaying the stress signal.

Calcium as a second messenger acts as a powerful and dynamic signal that is involved in growth and development as well as stress responses (Reddy, 2001; Poovaiah et al., 1987). Calcium concentrations are highly regulated across plant cell membranes. Calcium fluctuations are of particular interest because they have the capacity to relay specific signals throughout the entire organism quickly (Choi et al., 2014). Increases in cytosolic Ca^{2+} levels occur in waves with specific duration and frequency and have the capacity to travel long distances throughout a plant quickly. A number of membrane channels and gates regulate this signal. Plants maintain tight control on Ca^{2+} concentration, maintaining concentrations of 100-200nM in the cytoplasm compared to increased concentrations of 0.2-10mM in the vacuole, 1mM in the endoplasmic reticulum, and 10mM in the apoplast (Sanders et al., 1999). Ion channels are activated to moderate the flow of Ca^{2+} across membranes and the signal can be relayed in seconds. Once the cytoplasmic levels increase, Ca^{2+} has a number of effects. Proteins that contain EF-hand motifs readily bind Ca^{2+}, which generally induces a conformational change in calcium-binding proteins (Zhang et al., 2012). There are three classes of EF-hand Ca^{2+} sensors in plants: calmodulins (CaMs) including calmodulin-like proteins (CMLs), calcium-dependent protein kinases (CDPKs), and calcineurin B-like proteins (CBLs) (DeFalco et al., 2010; Day et al., 2002). Upon

activation, these calcium sensors interact with diverse proteins ranging from transcription factors to molecular motors and modulate their activity (Reddy et al., 2011, 2004).

Plants not only consume but produce O_2, thus they must manage oxygen in both capacities (De Gara et al., 2010). ROS concentrations are directly tied to plant metabolism, and they function as key messengers in mediating multiple stress responses. The chloroplast is the site of ROS production, and anterograde and retrograde signaling between the three (nuclear, mitochondrial and plastid) genomes involves ROS (Zhu, 2016).

Second messengers must effectively relay the stress signal. Signaling molecules initiate specific signaling cascades, which modify chromatin, change gene expression, and restructure the molecular composition of the cell. A variety of signal cascades are involved in plant stress response: MITOGEN-ACTIVATED PROTEIN KINASE (MAPK), HIGH AFFINITY POTASSIUM TRANSPORTERS (HKT), SALT OVERLY SENSITIVE (SOS), PYRABACTIN RESISTANCE 1 LIKE/ REGULATORY COMPONENTS OF ABA RECEPTORS (PYL/RCARs), V-MYB AVIAN MYELOBLASTOSIS VIRAL ONCOGENE HOMOLOG (MYB), CaM, and ENHANCED DISEASE SUSCEPTIBILITY 1/PHYTOALEXIN DEFICIENT4 (EDS1/PAD4) (Shi et al., 2002; Laluk et al., 2012; Kim et al., 2014; Wang et al., 2011; Rus et al., 2001). These cascades crosstalk with hormones like salicylic acid, ethylene, or auxin and are linked to defense and development (Brader et al., 2007; Pieterse et al., 1998; Karasov et al., 2017). These signals act to remodel cell wall composition, shift solute concentrations in the vacuole and peroxisome, or induce localized cell death.

The inherent complexity of physiology requires living organisms to sense and respond on a sliding scale involving balance, complementation, and dynamic adjustment. For example, plants do not experience freezing conditions without first experiencing cold. In light of this,

plants exercise stress acclimation whereby cold induces a preparatory response for freezing (Novillo et al., 2007). The dynamic nature of stress involves a concert of factors that constantly interact and communicate.

Stress response mechanisms in plants have also developed through speciation. Extremophiles express an incredible diversity of mechanisms to tolerate severe environments. From mangroves growing in hypersaline, brackish water to frost-tolerant plants in the arctic, to desiccation-tolerant species, some plants have developed mechanisms to handle these conditions. In contrast, cultivated species have been pampered for centuries. Crops are fertilized, irrigated, and weeded, experiencing optimal growing conditions. Additionally, crops have been selected by humans for desirable traits, such as grain protein content or shelf life, but not for traits that promote plants to flourish in changing environments. It is paramount to develop crops that withstand various environmental conditions. If we can learn how plants cope with stress, we can better understand what mechanisms to breed for to broaden the range of environments a plant can survive in. It is the focus of this work to describe abiotic stress response in plants at the genetic level, specifically focusing on transcriptional regulation in *Arabidopsis* as the model species and rice as the crop.

Stress responses in *Arabidopsis thaliana*

Genetic, molecular, and phenotypic aspects of plant stress have been studied extensively in the model plant, *Arabidopsis thaliana*. Second messengers, phosphorylation cascades, changes in gene expression, and phytohormone signaling are all known to be involved in molecular plant stress response (Xiong et al., 2002; Zhu, 2016; Suzuki and Katano, 2018).

Sensors are difficult to identify because their action may be brief and isolated. A few sensors have been identified by forward genetic screen using stress-related signatures. For instance, OSCA1, is a putative osmo-regulator (Yuan et al., 2014). *Osca1* did not show a drought or salt stress phenotype, however, it showed reduced cytosolic Ca^{2+} influx in guard and root cells in response to osmotic stress. This sensor was found in a calcium-imaging assay that is sensitive enough to relay a signal after only 5 seconds of stimulus and returns to equilibrium after 5 minutes. OSCA1 is in the EARLY RESPONSE TO DEHYDRATION 4 (ERD4) family of proteins, however knockout mutation of *ERD4* does not elicit the same osmotic response as *osca1*. Very few Ca^{2+} ion channels have been characterized as stress-responsive. The field of stress sensing has abundant potential to be realized.

Before high-throughput sequencing, genes and proteins involved in stress were identified by isolating genes that are regulated by hormones and second messengers. Abscisic acid, Ca^{2+}, and phytohormones like ethylene, auxin, and salicylic acid were understood to be involved in stress response, but their affiliates were unknown.

Abscisic acid (ABA) has long been known to be a key indicator of stress. Studies in the 1990s focused on this hormone and both ABA-dependent and ABA-independent pathways emerged (Yamaguchi-Shinozaki et al., 1995). The dehydration-responsive element (DRE), TACCGACAT, in promoters is involved in ABA-independent pathways and the ABA response element (ABRE), PyACGTGGC, is involved in ABA-dependent pathways (Yamaguchi-Shinozaki et al., 1995). Dehydration-induced genes, specifically *RD29A* and *RD29B* became stress markers. *RD29A* and *B* are 55% identical and coded contiguously on the same sense strand on chromosome five (Jia et al., 2012). These genes code for small, hydrophobic proteins that aid in modifying membranes to cope with the physical strain induced by osmotic stress (Jia et al.,

2012), however, it was found that *RD29B* is inducible in salt stress, while *RD29A* was more often induced in cold or drought stress (Msanne et al., 2011).

The Ca^{2+} signal influences a number of down-stream molecules (Ranty et al., 2016). Ca^{2+} may bind to an EF-hand containing protein, such as CaMs, CMLs, CDPKs, or CBLs. Ca^{2+}/CaM is implicated in abiotic stress tolerance. In a recent study by Yoo et. al., it was shown that Ca^{2+}/CaM interacts with the second helix of GTL1 N-terminal trihelix binding domain, causing an allosteric change of the third helix that results in inhibition of GTL1 binding to the *STOMATAL DENSITY AND DISTRIBUTION 1* (*SDD1*) promoter. SDD1 is responsible for stomatal development, thus Ca^{2+}/CaM transduces the osmotic stress signal to repress stomatal development and manage water use efficiency (2019).

Other CaM targets have been identified by screening expression libraries with ^{35}S-labeled CaM under a variety of conditions. ^{35}S-CaM was found to bind *At*CAMTA (calmodulin transcriptional activator, also called *At*SR and *At*EICBP) in the presence of Ca^{2+} and ethylene (Reddy et al., 2000; Yang and Poovaiah, 2002). This protein not only contains a CaM-binding domain but also a nuclear localization signal and DNA binding domain, making it of particular interest as a Ca^{2+}/CaM responsive transcription regulator (Reddy et al., 2000). The SRs have been shown to be involved in a number of stress response pathways in *Arabidopsis* and other organisms (Du et al., 2009; Laluk et al., 2012; Li et al., 2014; Rahman et al., 2016a; Yang et al., 2015; Kakar et al., 2018). SR activity in stress responses in *Arabidopsis* and then in rice will be the focus of this discussion.

The SIGNAL RESPONSIVE (SR) or CALMODULIN TRANSCRIPTION ACTIVATORS (CAMTA) family of proteins are characterized by three domains: 1) A DNA binding domain called CG-1, including one or multiple nuclear localization signals, 2) CaM-

binding domain, and 3) transcription factor immunoglobulin (TIG) domain and several ankyrin repeat domains (Figure 1). The CG-1 DNA binding domain binds CGCG or CGTG core motifs, which were first identified as transcription factor binding domains in parsley (*Petroselinum crispum*) (da Costa Silva, 1994). This core binding motif is part of a rapid stress response element (RSRE – *VCGCGB*). RSRE is a *cis*-element in the promoters of genes that are rapidly activated by both biotic and abiotic stress responses (Walley et al., 2007).

SRs are conserved across eukaryotes. Six SRs have been identified in *Arabidopsis* and rice (Choi et al., 2005), with members identified in sorghum, rapeseed (Rahman et al., 2016a), tomato (Yang et al., 2012), grapevine (Shangguan et al., 2014), soybean (Wang et al., 2015), *Zea mays* (Yue et al., 2015), and alfalfa (Yang et al., 2015). Following the discovery of this family of transcription factors first in plants, homologs have been identified in animals. One *SR* was found in *Drosophila* and implicated in photoreception and one in *Caenorhabditis elegans* (Han et al., 2006; Song et al., 2006a). SR from fruit fly (*dSR*) is involved in regulating deactivation of rhodopsin, a visual G protein-coupled receptor (Han et al., 2006), while SR2 from mouse activates atrial natriuretic factor (ANF) expression and promotes cardiomyocyte hypertrophy (Song et al., 2006b).

SRs were first identified as being up-regulated in response to ethylene in *Arabidopsis* and tobacco (Reddy et al., 2000; Yang and Poovaiah, 2000). The first *SR* gene identified with a DNA binding domain was in *Arabidopsis* and consisted of 4,485 nt and 13 exons (Reddy et al., 2000). *AtSRs* were later found to have on average 11-12 exons (Rahman et al., 2016b). The full-length *AtSR1* cDNA encodes a 1032aa protein with 10% serine residues. The six *SR*s in

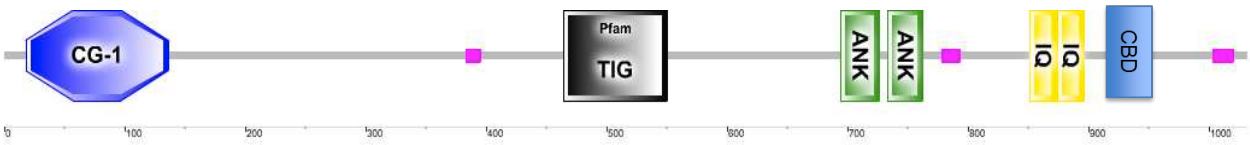


Figure 1. Schematic diagram showing the organization of *Os*SR1. CG-1, DNA binding domain; TIG, a non-specific DNA binding domain; ANK, ankyrin repeat; IQ, Ca^{2+}-independent CaM-binding domain; CBD, Ca^{2+}-dependent CaM-binding domain.

Arabidopsis are located on four different chromosomes (At2g22300, At5g09410, At3g16940, At5g64220, At1g67310, and At4g16150), and while the three common domains are relatively well conserved, the middle region (aa 164-469) is not (Choi et al., 2005; Reddy et al., 2000). The DNA binding domain is in the N-terminus and contains two overlapping nuclear localization sequences (aa 71-87 and 85-88). SR*s* contain various numbers of ankyrin repeat domains, between the TIG and CBD domains (see Figure 1) The CaM binding domain occurs at ~ aa 917-929, which is then followed by an acidic domain near the C-terminus (Reddy et al., 2000).

*At*SR binds CaM only in the presence of Ca^{2+} (Bouché et al., 2002), suggesting SRs role in stress response, which has since been thoroughly supported in biotic and abiotic stress tolerance assays. Bacterial resistance studies have shown that a loss-of-function mutant of *Atsr1* is more resistance to virulent and avirulent strains of *Pseudomonas syringae DC3000* and *Xanthomonas oryzae* pv. *oryzae* than WT (Du et al., 2009; Galon et al., 2008; Rahman et al., 2016b). Studies with *Botrytis cinerea, Sclerotinia sclerotiorum*, and *Golovinomyces cichoracearum,* two necrotropic and one biotrophic fungi, respectively, again suggest that *At*SR1 is a negative regulator of plant immunity as the mutant is resistant to these pathogens (Du et al., 2009; Nie et al., 2012; Rahman et al., 2016a, 2016b). *At*SR1 was also shown to be involved in regulating defense gene expression by binding to the promoter of *EDS1* and *NON-RACE-SPECIFIC DISEASE RESISTANCE* (*NDR) 1* and suppressing their expression (Du et al., 2009; Nie et al., 2012). EDS1 is involved in salicylic acid biosynthesis, and as a negative regulator of *EDS1*, *At*SR1 inhibits salicylic acid production (Du et al., 2009). Interestingly, AtSR1 is a positive regulator of herbivory and wound-induced responses (Qiu, Xi, Du, Suttle, & Poovaiah, 2012). Susceptibility to insects in the mutant is associated with jasmonate accumulation and decreased glucosinolate levels (Laluk et al., 2012).

*At*SR1 is a nuanced regulator in response to temperature changes. *Atsr1* and WT display similar phenotypes when grown in optimal conditions at 25°C, however, the mutant displays stunted growth at 19°C (Du et al., 2009). Interestingly, when plants are grown at 22°C, *At*SR1 represses SA biosynthesis but cold treatment at 4°C for one week overcomes this repression (Kim et al., 2017). Doherty et. al. showed that *At*SR1 (CAMTA3) acts with CAMTA 1 to enhance freezing tolerance, but only after cold treatment, thus this CAMTA duo is involved as a positive regulator specifically in cold acclimation (2009). *At*SR1 has been shown to be a negative regulator of salt stress where mutant germination rates and seedling root growth is considerably greater at both 100 mM and 150 mM NaCl than WT (Prasad et al., 2016).

It is clear that the SRs are dynamic regulators. Recently, Shkolnik et. al. showed that CAMTA6 can be a positive regulator of salt stress during germination but switch to be a negative regulator of salt stress of seedling growth only days later. *Camta6* mutants showed enhanced germination rates in the presence of salt, however, these same mutant seedlings showed chlorosis in salt conditions in three-day-old seedlings while the WT was healthy (Shkolnik et al., 2019). The opposing functions of *SRs* depending on the developmental stage of a plant or the type of stress indicate the difficulty in deciphering their role, however, this family of transcription activators holds promise for resolving the inherently complex regulation of plant physiology.

Transcription factors are the key regulators of gene expression at the transcriptional level, thus they are attractive for manipulation as singular elements that can have a broad effect. Identifying crucial transcriptional regulators in stress response in *Arabidopsis* paved the way for further research in crop species (Zhang et al., 2004). *Arabidopsis* and rice diverged 130-200 million years ago (Krom and Ramakrishna, 2008; Chang et al., 2004; Wolfe et al., 1989). While the rice genome is almost three times the size of the Arabidopsis genome (420 to 155 Mbp,

respectively), these species share 55-77% homologous genes (Movahedi et al., 2011; Ma et al., 2005). Homologous genes do not imply homologous gene expression, however. Movahedi calculated expression coherence coefficients (ECC) for 19,937 *Arabidopsis* and 32,004 rice genes (2011). Using GO annotations from expression patterns, 4,630 orthologous gene pairs showed 77% conserved expression. As expected, housekeeping functions such as photosynthesis, plastid organization, DNA replication, and cell division, returned highly conserved expression contexts. Interestingly, transcription factor activity, cell communication, response to salt stress, and hormone stimulus were significantly underrepresented in ECC conserved genes (Movahedi et al., 2011). While work in *Arabidopsis* stands as a foundation for future work in rice and other crop species, transcription factors, particularly those involved in stress response, will need to be tested and functionally understood at the level of the organism.

Importance of rice as a crop

Rice accounts for 20% of all cereal production and is the second leading staple grain, following *Zea mays* (corn), and the first in human consumption (Timmer, 2010). Rice provides over 60% of the calories consumed by over a quarter of the world's population, making this one species responsible for 15% of the calories consumed by humans on a daily basis (Mohanty, 2013). As of 2017, eight Asian countries produced 80% of the world's rice and hold 46.6% of the world's population (Chauhan Khawar et al., 2017). Rice provides 30% of the calories to people in China and 75% of calories to people in Bangladesh. Rice consumption is the highest in developing countries with dense populations, where dietary diversity is limited and malnutrition is prevalent. Rice consumption in China has decreased since the 1960s as dietary diversification

has increased with dairy and meat products available to a higher percentage of the population. Rice consumption is predicted to increase by 1% per year (Chauhan Khawar et al., 2017).

Rice is a unique cereal in that it grows in flooded conditions but does not become oxygen-deprived. Oxygen is not only a requirement for respiration, but for iron uptake at the roots (Chauhan Khawar et al., 2017). In seedling development, programmed cell death occurs to create specialized structures called aerenchyma. Gas enters nodes and internodes above floodwaters and travels within aerenchyma to provide oxygen to submerged tissue. Irrigated rice requires on average 1300-1500 mm of water, which accounts for 24-30% of the world's developed freshwater resources (Chauhan Khawar et al., 2017), however, water input can vary from 660 – 5280 mm depending on the climate, season, soil type, and hydrologic conditions (Tuong and Bouman, 2003).

Aerobic rice has been bred in China. These varieties double in water use efficiency and produce 4.5-6.5 tons/ha, which is double that of what is generally harvested from upland varieties and 25% less than that obtained from lowland varieties. Studies comparing natural variation between upland and lowland varieties hold promise for finding stress-related genes and targets (Ali et al., 2018).

The rice genome is diploid, composed of 420 Mbp on 12 chromosomes (Movahedi et al., 2011). The genome has been sequenced multiple times and is well annotated (Kawahara et al., 2013). Rice can be transformed, making it an excellent model crop species (Han et al., 2012). *Oryza sativa* has three main lineages: the *japonica* type, *indica* type, and *Oryza glaberrima,* an African type. While both the *japonica* and *indica* subspecies contain a mutation on the *sh4* gene making their grain heads shatter-resistant and good for cultivation, these subspecies are more closely related to wild relatives than to one another (Chauhan Khawar et al., 2017).

Stress responses in rice

Rice is susceptible to a range of stresses including biotic stresses such as bacterial blight and abiotic stresses such as submergence, drought, and heat. At the cellular level, the stress signal in rice, like all plants, involves a myriad of second messengers, enzyme cascades, phosphorylation events, and transcriptional changes. The severity of the stress can result in phenotypic changes ranging from decreased plant growth and decreased yield to senescence and loss of a season's harvest. To better understand stress responses in rice, molecular and genetic trends seen in *Arabidopsis* stress tolerance can be evaluated for similarity in rice.

There is considerable overlap in abiotic stress tolerance mechanisms between *Arabidopsis* and rice. For instance, one class of APETALA (AP) 2 transcription factors, the CRT binding factors, also known as CBFs or DREB1s, occur in *Arabidopsis* and rice (Todaka et al., 2012). They are involved in ABA-dependent and -independent pathways in cold and drought tolerance in both species (Doherty et al., 2009; Novillo et al., 2004; Ito et al., 2006; Chen et al., 2008). Constitutive expression of the *CBF* rice homolog, *OsDREB1,* in *Arabidopsis* resulted in salt, cold, and drought tolerance (Dubouzet et al., 2003).

Similar to *Arabidopsis*, rice contains six *SR* (or *CAMTA*) genes (Os01g69910, Os03g09100, Os03g27080, Os04g31900, Os07g43030, and Os10g22950) that all contain the three structural domain common to *SR*s (Choi et al., 2005). *OsCaM*s are involved in stress response signaling (Chinpongpanich et al., 2012). *OsCBT*, an *OsSR* member, shows enhanced resistance to rice blast fungus, *Magnaporthe grisea,* as well as the bacterial pathogen, *Xanthomonas oryzae* pv. *oryzae* (Koo et al., 2009). Considering the similarities found in stress response mechanisms in rice and *Arabidopsis* and the similarity in the *SR* family, there is considerable potential for *Os*SRs to be involved in rice biotic and abiotic stress response.

Potential of genetic/molecular tools for developing resilient crops

Elucidating how plants sense, signal, and respond to stress is a complex task overlaying genetic and molecular modifications with the physiological response and phenotypic evaluations. Up until the development of high throughput sequencing technology, this work was done by piece-meal modification of a gene or protein sequence, followed by comparing the resulting phenotype of a mutant and WT. Now, full genome sequencing, annotation, and transcriptomic analysis can give us insight into global changes in gene expression and permit network-scale interaction analysis.

Transcriptomic profiling is a powerful method to identify organismal responses at the level of gene expression. Differentially expressed genes paint a picture of genetic regulation at a moment in time. From this, hypotheses can be generated to test and develop a greater understanding of physiological responses to stresses. Ultimately, any hypothesis will need to be tested phenotypically. Understanding the role of a transcription factor involved in stress response will need to have phenotypically measurable differences if improvements to plant health and agriculture are to be made.

Weeds are called such because of their incredible propensity to grow. They often withstand extreme conditions that other plants cannot. The potential for plants to survive variable and extreme conditions exists, however many high-yielding crop plants have not experienced the selection pressure that results in stress-tolerance. Understanding stress response at the molecular level in plants will result in breeding practices that can stack multiple traits into one organism, giving tolerance to multiple stresses and extreme environmental conditions (Arora et al., 2018). This resilience will result in more efficient crop production, fewer chemical inputs, and a greater understanding of how these crucial primary producers survive in the ecosystem.

MATERIALS AND METHODS

1. Generation of Transgenic Lines

1.1 Construct Preparation

1.1.1 *OsSR1* Cloning

Total RNA was isolated from 30-day-old rice seedlings of wild type (WT) *O. sativa* L. ssp *japonica* cv. Dongjin seedlings using TRIzol reagent (Invitrogen, USA) and treated with RNase-free DNase (Promega, USA) to remove any genomic DNA contamination. Two μg of the DNAse-treated RNA was used for cDNA synthesis using oligo dT primer and Superscript III reverse transcriptase (Invitrogen, USA) per manufacturer instructions. To amplify full-length *OsSR1* transcript gene-specific forward and reverse primers with *Asc*I and *Bam*HI restriction sites, respectively, were used. Two μl of cDNA was used as a template for amplification using PrimeStar HS Taq polymerase (Takara, USA). (See Table 1 for primer sequences and Table 2 for PCR conditions.) The amplified products were separated on 1.0% agarose gel in 1X TAE buffer and the amplicon was excised from the gel and purified with a gel extraction kit (Qiagen, USA).

About 300 ng of blunt end amplified product was used for A-tailing reaction. For A-tailing, purified amplified product was incubated at 72^{0}C for 20 min in Ex-Taq reaction buffer consisting of dATP along with 1 unit of Ex-Taq DNA polymerase (Takara, USA). The amplified product was column purified and cloned into the "TA" cloning vector using the pGEMT-Easy system (Promega, USA) per manufacturer's instruction. The 10 μl ligation mixture was transformed into Top10 *E. coli* competent cells and plated on selection medium that enabled blue-white selection.

Table 1. Names, sequences and annealing temperatures of primers used.

Name	Primer No.	Sequence, 5' to 3'	Tm (°C)	Ordered
Rice *CAMTA3* FW-SEQ	1F	TTGGGATAGTGGAGAAAGTTATATTG	52.6	8/8/15
Rice CAMTA3 REV -SEQ	1R	TACATCAGCACCTGCAGCACCTATAT	59.6	8/8/15
Ubi Pro *Stu*I -FW	2F	GAAGGCCTTCCTGCAGTGCAGCGTGACCCGGT	71.9	11/2/16
Ubi Pro *Asc*I-RW	2R	TTGGCGCGCCAACTGCAGAAGTAACACCAAACAACA	68.2	11/2/16
Native *SR1* pro-FW	3F	TCCCCCGGGATAATATGCTAATAACCTCTCTTATTTTGCACA	64.1	12/21/16
Native *SR1* pro-RW-*Asc*I	3R	TTGGCGCGCCGGCTGTGGTGGGGGGTAT	74.3	12/21/16
OsSR1 gDNA FWD Set 1	4F	GCAGCTAGGTGAGCATAACA	54.8	12/19/18
OsSR1 gDNA REV Set 1	4R	ATCACTGGAGCAATGGACTAAA	54.0	12/19/18
CAMTA3 Rice Fwd 1	5F	TTCGCGGTCGTGCCGCAGCTA	66.3	4/18/17
pFGC5941 ocs term	5R	AACCGGCGGTAAGGATCTGAGCTACAC	63.5	2/16/16
BAR FWD Set 1	6F	GAAGTCCAGCTGCCAGAAA	55.2	7/14/17
BAR REV Set 1	6R	CACCATCGTCAACCACTACAT	54.8	7/14/17
HPH-FW	7F	ATGAAAAAGCCTGAACTCAGGGC	58.0	7/27/16
HPH-RW	7R	CTATTCCTTTGCCCTCGGACGAG	59.3	7/27/16
GAPDH FWD	8F	CTGCAACTCAGAAGACCGTTG	~55	12/31/13
GAPDH REV	8R	CCTGTTGTCACCCTGGAAGTC	~55	12/31/13
OsSR1 Primer FWD Set 2	9F	CTAACGGACATAAGGGCATCTC	55.0	7/24/17
OsSR1 Primer REV Set 2	9R	ATCCCTTGCTTCTGGGTATTG	54.8	7/24/17
CAMTA3 Rice Fwd 1	10F	TTCGCGGTCGTGCCGCAGCTA	66.3	4/18/17
CAMTA3 Rice REV Set 3	10R	CAGGATGAACAGGAGGTTGTAG	54.9	4/18/17
DREB1C:LOC_Os06g03670 FWD Set 4	11F	TGGTAGGGAGTTCTAGAAGGAG	54.8	12/11/17
DREB1C:LOC_Os06g03670 REV Set 4	11R	GAGAGAGAGAGAGCATAGCAATTAT	53.8	12/11/17
QPCR-*OsCAMTA3* FWD Set 1	12F	GGAGATTCCTTAGGTGCTGTTC	55.2	10/18/17
QPCR-*OsCAMTA3* REV Set 1	12R	CCACCTTTGTCACCCTCATATT	54.8	10/18/17

Table 2. PCR conditions that were used for different primer sets.

Gene/Transcript	Product Size	Primer Set	Initial Denaturation	Cycles	Amplification	Extension
OsSR1 gene for cloning	-	1	98ºC for 2 min	35	98ºC for 10 sec., 56ºC for 30 sec., 72ºC for 3 min.	72ºC for 10 min.
Ubiquitin Pro	-	2	98ºC for 2 min	35	98ºC for 10 sec., 62ºC for 30 sec., 72ºC for 2 min.	72ºC for 10 min.
OsSR1 Native Pro	-	3	98ºC for 2 min	35	98ºC for 10 sec., 60ºC for 10 sec., 72ºC for 1.5 min.	72ºC for 10 min.
OsSR1 genomic	400 bp	4	98ºC for 3 min	35	98ºC for 30 sec., 55ºC for 30 sec., 72ºC for 30 sec.	72ºC for 10 min.
OsSR1 transgene	3 kbp	5	95ºC for 2 min	35	98ºC for 30 sec., 57ºC for 30 sec., 72ºC for 3 min.	72ºC for 10 min.
Basta resistance	500 bp	6	96ºC for 2 min	35	98ºC for 30 sec., 55ºC for 30 sec., 72ºC for 30 sec.	72ºC for 10 min.
GAPDH genomic	978 bp	8	96ºC for 2 min	35	98ºC for 30 sec., 55ºC for 30 sec., 72ºC for 45 sec.	72ºC for 10 min.
GAPDH cDNA	329 bp	8	96ºC for 2 min	30	98ºC for 30 sec., 55ºC for 30 sec., 72ºC for 30 sec.	72ºC for 10 min.
OsSR1 cDNA 1	734 bp	9	95ºC for 2 min	30	98ºC for 30 sec., 55ºC for 30 sec., 72ºC for 30 sec.	72ºC for 10 min.
OsSR1 cDNA 2	668 bp	10	95ºC for 2 min	30	98ºC for 30 sec., 60ºC for 30 sec., 72ºC for 30 sec.	72ºC for 10 min.
OsDREB1C cDNA	106 bp	11	95ºC for 2 min	25	98ºC for 10 sec., 60ºC for 10 sec., 72ºC for 10 sec.	72ºC for 5 min.
OsDREB1C qRT-PCR	N/A	11	I-96 well SYBR Green standard protocol on Roche 480			
OsSR1 qRT-PRC	N/A	12	I-96 well SYBR Green standard protocol on Roche 480			

Colonies that were grown on selection medium were inoculated in 5 ml of LB medium with 100 mg/L ampicillin and incubated at 37^0C on an orbital shaker (200 rpm) for 16 hrs. Plasmid was isolated using the Qiagen plasmid purification kit and used for the sequencing with *T7*, *SP6* promoters and *OsSR1*-specific primers (Primer Set 1, see Table 1). The clones that did not exhibit any errors were digested with *Asc*I and *Bam*HI restriction enzymes and a 3.2 kbp fragment was gel purified and cloned into the previously digested *pFGC5941* binary vector at *Asc*I and *Bam*HI sites using T4 DNA ligase down-stream to *CaMV35S* promoter.

1.1.2 *Ubiquitin* Promoter Cloning

For cloning of full-length *Zea mays Ubiquitin* promoter, pCAMBIA1300 plasmid was digested with *Hind*III and *Sac*I restriction enzymes and ~2kb fragment consisting of *Ubiquitin* promoter with 1st exon and intron was ligated into pBluescript SK(+) at *Hind*III and *Sac*I sites present in the multiple cloning site using T4 DNA ligase. The resultant plasmid (pBluescript SK(+) *Ubiquitin* Pro) was sequenced with *T7* and *T3* promoter primers for determination of the authenticity of the *Ubiquitin* promoter sequence. The full-length *Ubiquitin* promoter was PCR amplified using *Ubiquitin*-end primers to avoid extraneous sequences. For PCR amplification, PrimeStar HS Taq polymerase (Takara, USA) was used along with forward and reverse primers bearing *Stu*I and *Asc*I sites, respectively. The amplified products were separated on 1.0% agarose gel in 1X TAE buffer and the amplicon was excised from the gel and purified with a gel extraction kit (Qiagen, USA) and re-sequenced. Gel purified amplicon was digested with *Stu*I and *Asc*I restriction enzymes and subsequently column purified. The digested *Ubiquitin* promoter fragment was subsequently ligated into the previously digested pFGC5941 binary vector at *Stu*I and *Asc*I sites using T4 DNA ligase. The ligation mixture was transformed into *E.*

coli and plated on LB medium supplemented with kanamycin 50mg/L and incubated at 37^{0}C. A few colonies that grew on selection plate were individually inoculated in 5 ml of LB liquid medium supplemented with 50mg/L kanamycin and incubated on orbital shaker maintained at 37^{0}C for 16h. Plasmid was isolated from the bacterial culture using a plasmid purification kit (Qiagen, USA). The presence of the insert was confirmed by digesting the plasmid with *Stu*I and *Asc*I.

In a separate reaction, binary vector plasmids pFGC5941 containing *CAMV35S: OsSR1: OCS* and pFGC5941 containing *Ubiquitin* Promoter were digested with *Asc*I and *BamH*I restriction enzymes and fragments were separated on 1% Agarose gel. A ~3.2 kb fragment corresponding to *OsSR1* cDNA and ~11kb vector backbone containing *Ubiquitin* promoter, were excised from the gel and purified using gel extraction kit (Qiagen, USA). Both of these excised fragments were ligated together using T4 DNA ligase (NEB, USA). The ligation mixture was transformed into *E. coli* and plated on LB medium supplemented with kanamycin 50mg/L and incubated at 37^{0}C. A few colonies were individually inoculated in 5 ml of LB liquid medium supplemented with 50mg/L kanamycin and incubated on orbital shaker maintained at 37^{0}C for 16h. Plasmid was isolated from the bacterial culture using a plasmid purification kit (Qiagen, USA). Confirmation digestions with *Stu*I, *Asc*I and *BamH*I were performed to ascertain successful cloning of *OsSR1* cDNA downstream of *Ubiquitin* promoter in the pFGC5941 plasmid. The positive clones of pFGC5941 containing *Ubiquitin pro*: *OsSR1: OCS* were verified by sequencing.

1.1.3 *OsSR1* Promoter Cloning

To clone *OsSR1* promoter from Dongjin variety, primers (3F and 3R, Table 1) were designed to amplify the 1.4kb genomic region encompassing the upstream sequence from ATG codon of LOC_Os10g22950 to the end of 3'UTR of LOC_Os10g22940. The genomic DNA isolated from 30d-old-seedlings using Plant DNAeasy Kit (Qiagen, USA) was used as a template for amplification of *OsSR1* promoter. For PCR amplification, PrimeStar HS Taq polymerase (Takara, USA) was used along with forward and reverse primers bearing *SmaI* and *AscI* restriction enzymes sites, respectively (primer set 3). The 1.4 kbp amplified product was separated on 1.0% agarose gel in 1X TAE buffer and the amplicon was excised from the gel and purified with a gel extraction kit (Qiagen, USA). Gel purified amplicon was digested with *Sma*I and *Asc*I restriction enzymes and subsequently column purified. The digested *OsSR1* promoter fragment was ligated into the previously digested pFGC5941 binary vector at *Stu*I and *Asc*I sites using T4 DNA ligase to obtain pFGC5941: *OsSR1Pro*.

As described above, in a separate reaction, binary vector plasmids pFGC5941: *CAMV35S: OsSR1: OCS* and pFGC5941:*OsSR1* Pro were digested with *Asc*I and *BamH*I restriction enzymes and fragments were separated on 1% Agarose gel. ~3.2 kb fragment corresponding to *OsSR1* cDNA and ~11.5 kb vector backbone containing *OsSR1* Promoter were excised from the gel and purified using gel extraction kit (Qiagen, USA). Both these excised fragments were ligated together using T4 DNA ligase (NEB, USA). The ligation mixture was transformed into *E. coli* and plated on LB medium supplemented with kanamycin 50mg/L and incubated at 37^0C. A few colonies were individually inoculated in 5 ml of LB liquid medium supplemented with 50mg/L kanamycin and incubated in an orbital shaker maintained at 37^0C for 16h. Plasmid was isolated

from the bacterial culture using a plasmid purification kit (Qiagen, USA). Confirmation digestions with *Stu*I, *Asc*I and *BamH*I were performed to ascertain successful cloning of *OsSR1* downstream of *OsSR1* promoter in pFGC5941. The positive clones of pFGC5941: *OsSR1 Pro*: *OsSR1: OCS* that exhibited correct digestion pattern were selected for further use.

1.1.4 Transformation of *Agrobacterium tumefaciens*

To generate stably transformed lines from rice embryogenic callus, *Agrobacterium tumefaciencs* LBA4404 was used. Chemical competent cells of LBA4404 were transformed with pFGC5941: *OsSR1 Pro*: *OsSR1: OCS* or pFGC5941: *Ubiquitin Pro*: *OsSR1:OCS* plasmids using temperature shock method. Briefly, 3μg of transformation vector was added to the 100 μl of competent cells and incubated on ice for 30 min. Subsequently, they were flash frozen in liquid nitrogen for 1min and thawed at 37^{0}C for 2 min. This freezing and thawing step was repeated once more, 1ml of LB medium was added to the cells and incubated on orbital shaker maintained at 28^{0}C with 150 rpm for 4 to 5h. Subsequently, the cells were pelleted by centrifugation at 8000 rpm for 2min and plated on solid LB medium supplemented with kanamycin 50 mg/L+ rifampicin 50 mg/L and incubated at 28^{0}C for 36 to 48 hrs.

Successful transformation of LBA4404 was determined by inoculating a single colony obtained on selection plates into liquid LB medium supplemented with kanamycin 50 mg/L+ rifampicin 50 mg/L and placed on orbital shaker maintained at 28^{0}C, 200 rpm for 24 to 36 hrs. The cells from the culture were pelleted and plasmid was isolated using plasmid isolation kit (Qiagen, USA). About 10 to 15 μl of purified plasmid was used for transformation of 50 μl of chemical competent *E. coli* cells using the heat shock method. A few colonies obtained on selection media were inoculated in liquid LB medium supplemented with kanamycin 50 mg/L+ rifampicin 50

mg/L and placed on orbital shaker maintained at 37^0C, 200 rpm for 12 to 16 hrs. The plasmid was isolated from this bacterial culture using plasmid isolation kit (Qiagen, USA). The plasmid was verified by restriction digestions. Only those clones of LBA4404 bearing transformation vector that were determined to be error-free were used for generation of transgenic lines.

1.2 *Agrobacterium tumefaciens*-mediated Transformation of *Oryza sativa* L. *japonica* cv. Dongjin

1.2.1 Callus Induction

Oryza sativa L. *japonica* cv. Dongjin wild type and *Ossr1* mutant seeds were dehulled and sterilized by rinsing in 70% EtOH for 1 min and in sterile water. The seeds were then incubated with agitation in 6.7% bleach with 0.02% Tween 20 for 40min. Seeds were rinsed for 5 minutes in sterile water six times, plated on 2N6 medium (see Table 3 for the composition of all media), and incubated at 28°C in the dark. After five days, roots and shoots were removed with a sterile scalpel and seeds were returned to the incubator for another five days in the dark. NAA in 2N6 medium is auxin for induction of callus growth.

1.2.2 Transformation of Callus

Agrobacterium tumefaciens strain LBA4404 containing pFGC5941: *OsSR1 Pro*: *OsSR1: OCS* or pFGC5941: *Ubiquitin Pro*: *OsSR1:OCS* was grown on AB medium with kanamycin 50µg/ml, streptomycin 50µg/ml and rifampicin 50µg/ml at 28°C for 3 days. Rice transformation was performed following the protocol described by Han et.al. 2012 (Han et al., 2012).

AAM liquid medium, acetosyringone (AS) stocks (Table 4), and 2N6-AS plates were made in advance and warmed to RT before use. 25ml AAM + 25µl AS was added to a 50ml conical tube, inverted to mix, and then 5ml was transferred to a second tube. One large colony of

Table 3. Composition of media used for *Agrobacterium tumefaciens*-mediated transformation.

Medium	Composition
AB	NH_4Cl 1 g/L, $NaH_2PO_4 \cdot 2H_2O$ 1.3 g/L, K_2HPO_4 3 g/L, $MgSO_4 \cdot 7H_2O$ 296 mg/L, $CaCl_2 \cdot 2H2O$ 10 mg/L, KCl 150 mg/L, $FeSO_4 \cdot 7H_2O$ 2.5 mg/L, glucose 5 g/L, add antibiotics (50mg/ml each rifampicin, kanamycin, streptomycin), bacto agar 15 g/L, pH7.2
AAM	AA salt and amino acids (Toriyama and Hinata, 1985), MS w/vitamins 4.4 g/L (Murashige and Skoog, 1962), casamino acid 300 mg/L, sucrose 68.5 g/L, glucose 26 g/L, pH5.8
2N6	N6 w/vitamins 4 g/L, casamino acid 300 mg/L, proline 500 mg/L, sucrose 30 g/L, glutamine 500 mg/L, mes 500 mg/L, myo-inositol 10 mg/L, 2,4-D 2 mg/L, phytoblend 8 g/L, pH5.8
2N6-AS	2N6 medium and glucose 10 g/L, acetosyringone 100 uM/L, phytoblend 8g/L, pH 5.2
2N6-CH	2N6 medium and cefotaxime 250 mg/L, hygromycin B 40 mg/L or Basta 5 mg/L, pH 5.8
MSR-CH	MS w/vitamins 4.4 g/L, sorbitol 30 g/L, maltose 20 g/L, mes 500 mg/L, NAA 0.1 mg/L, kinetin 2 mg/L, cefotaxime 250 mg/L, hygromycin B 40 mg/L or Basta 5 mg/L, phytoblend 8 g/L, pH 5.8
REIII-CH	MS macro and micro salt, MS vitamins, sorbitol 30 g/L, sucrose 30 g/L, casamino acid 2 g/L, NAA 0.02 mg/L, kinetin 2 mg/L, cefotaxime 250 mg/L, hygromycin B 50 mg/L or Basta 5 mg/L, phytoblend 8 g/L, pH 5.8

Table 4. Antibiotic and hormone stocks.

Antibiotic / Hormone	Composition
Cefotaxime	100mg/mL: 1mg/ml in DI water, sterile filter, store at -20°C
Basta	10 mg/mL in DI water, sterile filter, store at -20°C
Kinetin	1mg/mL: Dissolve 40mg in 1M KOH, bring to 40ml with DI water, filter sterilize, store 1mg/mL aliquots at -20°C
NAA	1mg/mL: Dissolve 20mg in a small amount of 1N NaOH. Bring to 20ml with DI water, store at 4°C
Rifampicin	50mg/mL in DMSO, store at -20°C, light sensitive
Kanamycin	50 mg/mL in DI water, sterile filter, store at -20°C
Streptomycin	50 mg/mL in DI water, store at 20°C
Acetosyringone	100mM in DMSO, store at -20°C, light sensitive

Agrobacterium containing the desired vector was transferred to 5ml of liquid medium and vortexed in short bursts until it was dispersed evenly. A spectrophotometer was blanked with AAM+AS without culture and then absorbance of the suspension was determined and brought to 0.02. The liquid culture was incubated with shaking in AAM+AS for 1hr at 28°C.

Any additional root and shoot that grew were removed from callus. Calli were transfected in AAM+AS containing *Agrobacterium* harboring the desired plasmid for 15 minutes at RT, swirling frequently. Calli were separated from the liquid culture and excess liquid was removed using filter paper. A piece of sterile filter paper was placed on each 2N6-AS plate and 1ml of AAM+AS was used to wet the filter paper. Five to six calli per plate were transferred and incubated at 24-25°C in the dark for seven days. Plates and callus were checked for contamination after three and six days of incubation. Calli were transferred to 2N6-CH plates on day six (if excessive bacterial growth was seen) or seven. Callus was sub-cultured onto medium so each nodule of callus contacted the media. Plates were incubated at 28°C in the dark for 10-14 days. Calli were then transferred to 2MSR-CH medium and incubated at 28°C with 16hr light for two weeks. Calli were then transferred to new 2MSR-CH every two weeks until shoot and root growth was observed. Callus with root and shoot growth was transferred onto selective regeneration media (REIII-CH) and after significant root and shoot growth, transferred to REIII-CH medium in upright tubes. Transformation procedure was repeated every two weeks until at least six independent insertion events (identified by shoots and roots developing from independent callus) had occurred.

Putative transgenic seedlings that showed significant root and shoot growth were transferred from upright tubes to soil consisting of a 3:5 mixture of Greens Grade and ProMix BX in 4" pots. Seedlings were insulated by placing skewers in corners of the pot and covering the pot with a clear, plastic bag. Seedlings were grown in a growth chamber at 27°C with 12hr light. After 3-4 days, plastic bags were opened for increased air exchange and removed after one week. Seedlings were either grown to full maturity in the chamber or transferred after reaching the 3-leaf stage to the greenhouse and grown at 27°C with 12hr light. Four to five weeks after seedlings transfer, the soil was supplemented with 1 teaspoon of iron per pot and top-watered 24 hours after application. If any yellowing was observed from seedlings before reaching full vegetative maturity a second application of iron was given. Plants were kept in water at all times and fertilized once a week.

Putative transgenic seedlings were grown along with the wild type and *Ossr1* lines in the greenhouse or chamber until maturation. T_0 seeds were harvested, dried, and stored at RT. T_1 seeds for the next generation were dehulled, sterilized, (as described above) and plated on selective medium. T_1 seedlings showing healthy germination were transferred to soil when they showed significant root and shoot growth. T_1 plants were grown with WT and *Ossr1* lines in the greenhouse or chamber until maturation. T_2 seeds were harvested, dried, and stored at RT. T_2 seeds were plated on selective media. Germination rates after 4-7 days were noted and a chi-square test was performed to determine if lines were homozygous. Again, healthy seedlings with germination rates suggesting homozygosity were transferred to soil and grown with WT and *Ossr1* lines.

1.3 Confirmation of Transgenic Lines

1.3.1 Confirmation of Transgenic Lines by Genomic PCR

1.3.1.1 DNA Extraction

DNA from ground leaf tissue was extracted using C-TAB. C-TAB extraction buffer contained 2% CTAB, 100mM Tris pH9.5, 1.4M NaCl, 1% PEG6000, 20mM EDTA, and 2% PVP. 24:1 chloroform:isoamyl alcohol, 10mM Tris HCl pH 8 Elution Buffer, and 25:24:1 phenol:chloroform:isoamyl alcohol pH 6.7 were made. 20µl ßME was added to 20ml CTAB before use. One ml of CTAB+ßME was added to 200mg of frozen, ground tissue. Samples were vortexed to mix and incubated at 65°C for 1 hr., inverting every 15 min. At RT, 1ml of phenol:chloroform:isoamyl alcohol was added and samples were inverted to mix. Samples were centrifuged at 8000rpm for 12 min at RT. The supernatant was removed and saved. An equal volume of 24:1 chloroform:isoamyl alcohol was added and samples were centrifuged at 8000rpm for 12 min at RT. The top aqueous layer was removed and saved. 0.7 volume of isopropanol was added and mixed by inversion. Samples were centrifuged at 7500rpm for 7min. Liquid was removed, keeping faint, white pellet. 500µl 70% EtOH was added and samples were centrifuged at 7500rpm for 7min. Liquid was removed and the pellet was dried and resuspended in 50µl Elution buffer. DNA integrity was checked on 0.7% agarose gel.

1.3.1.2 Genomic PCR

The presence or absence of *OsSR1* and herbicide resistance genes in WT, *Ossr1,* and putative transgenic lines was verified by PCR using gene-specific primer 4F and 4R (Table 1). Amplified products were separated on 1% agarose gel electrophoresis. Expected size bands were confirmed in selected lines.

1.3.2 Analysis of Transgene Expression

1.3.2.1 RNA Isolation

Putative transgenic lines were tested for *OsSR1* expression. Leaf tissue was harvested, immediately frozen in liquid nitrogen, and stored at -80°C. Tissue was ground by hand using mortar and pestle or with TissueLyser using a sterile, stainless steel ball, and shook for 30-40 seconds, 1-2 times at a rate of 30/sec. RNA was extracted from leaf tissue using TRIzol. One ml of cold TRIzol was added to 200mg of frozen, ground tissue and vortexed until the tissue was resuspended uniformly. 200µl of chloroform was added and tubes were centrifuged at 12,000rpm for 10 minutes at 4°C. The supernatant was transferred to new tubes and 500µl isopropanol was added. Tubes were mixed by inversion and left to sit at RT for 5 minutes. Samples were centrifuged at 12,000rpm for 10 minutes at 4°C and the supernatant was discarded, keeping the pellet. The pellet was washed with 1ml of 80% EtOH and centrifuged at 12,000rpm for 6 minutes at RT. The liquid was removed and the pellet was left to dry until clear. RNA was dissolved in 50µl of DEPC-water and concentration was determined using the NanoDrop. RNA was stored at -80°C.

1.3.2.2 cDNA Preparation

RNA was treated with DNase and converted into cDNA with MMLV-RT following the Invitrogen, USA protocol. Two µg of RNA was incubated with DNase1 buffer, $MgCl_2$, DNase, and DEPC water in a final volume of 10µl for 30 minutes at 37°C. DNase reaction was quenched with 1µl of 50mM EDTA. One µl of each 10mM dNTPs and 0.5µg/µl Oligo dT 12-18bp primer was added and samples were incubated at 65°C for 10min then cooled on ice for 5 minutes. A master mix of n+1 reactions was prepared during the incubation containing 4µl 5X First-Strand

Buffer, 2µl 0.1M DTT, and 1µl RNase inhibitor (RiboLock RI). Seven µl of the master mix was added to each tube and samples were incubated at 37°C for 2 minutes. 1µl of the M-MLV RT enzyme was added to each tube and samples were incubated for 60 minutes at 37°C. The reaction was quenched by incubating the tubes for 15 min at 70°C. A subsample of cDNA was checked for integrity using 1% agarose gel electrophoresis. cDNA was stored at -20°C.

1.3.2.3 RT-PCR

OsSR1 expression was checked using semi-quantitative RT-PCR. Transcript specific primers were used to amplify *OsSR1* and the presence or absence of transcript abundance was observed.

1.3.2.4 qRT-PCR

OsSR1 expression was determined via qRT-PCR using SYBR Green and gene-specific primers on the Roche480. cDNA was diluted 1:7 with sterile DI water. A 96-well plate was prepared with 1.5µl of cDNA, 1µl of each the forward and reverse qRT-PCR specific primers, 5µl SYBR Green mix (added last and covered with aluminum foil to preserve light reactivity), and 1.5µl of sterile DI water. All samples were run in technical triplicates. Every run included negative controls (without cDNA template). Expression was normalized to GAPDH, and fold change in expression of selected genes were compared to expression levels in WT.

2. Transcriptomic Analysis of WT and *Ossr1*

2.1 Seedling Drought Stress Treatment

1.5L of ½ MS medium was made using 3.1g MS (M524, PhytoTechology Laboratory) and 4.3g MES. The solution was brought to pH 5.7 and autoclaved. Autoclaved glass tubes with filter

paper bridges were filled with 11mls of ½ MS solution. Fifty-five of each WT and *Ossr1* seeds (harvested on 9/19/2017) were dehulled and sterilized. Seeds were rinsed in 70% EtOH for 1 min, rinsed in sterile water, then incubated with agitation in 6.7% bleach with 0.02% Tween 20 for 40min. Seeds were rinsed in sterile water six times, for 5 min each time. Sterile seeds were placed on sterile filter paper in glass tubes at 28°C and 16hr light (Day 0) (Figure 2A).

Seeds germinated by Day 3 (Figure 2B) and had >2" high shoots by day 5 (Figure 2C). On the evening of Day 5, healthy seedlings were separated from seeds that had not germinated and returned to the chamber (Figure 2D; Figure 3B). ½ MS + 40% PEG 8000 was made by adding 40% final volume of PEG 8000 (Cat.No. P5413, Sigma Aldrich) immediately after ½ MS was removed from the autoclave. Note that PEG % in the original PEG-infused media instructions do not account for the additional volume of the PEG crystals and the difference in the final concentration of the PEG should be noted.

Two hours after sunrise on Day 6 (Figure 3C), 24 WT and 24 *Ossr1* plants were removed from the chamber and their liquid media was replaced with ½ MS + 40% PEG (Figure 3C). Plants were returned to the 28°C chamber. One hour after PEG treatment, half of the treated and half of the control plants were harvested (Figure 3D). Whole seedlings were dipped in sterile water to remove residual MS and/or PEG and dried on Kimwipes. A sterile scalpel was used to cut the root and shoot, removing the embryo and endosperm. Root and shoot tissue of three seedlings was pooled into one-2ml sample tube with a sterile stainless-steel ball and frozen immediately in liquid N (Figure 4). Three hours after PEG treatment, remaining samples were harvested and

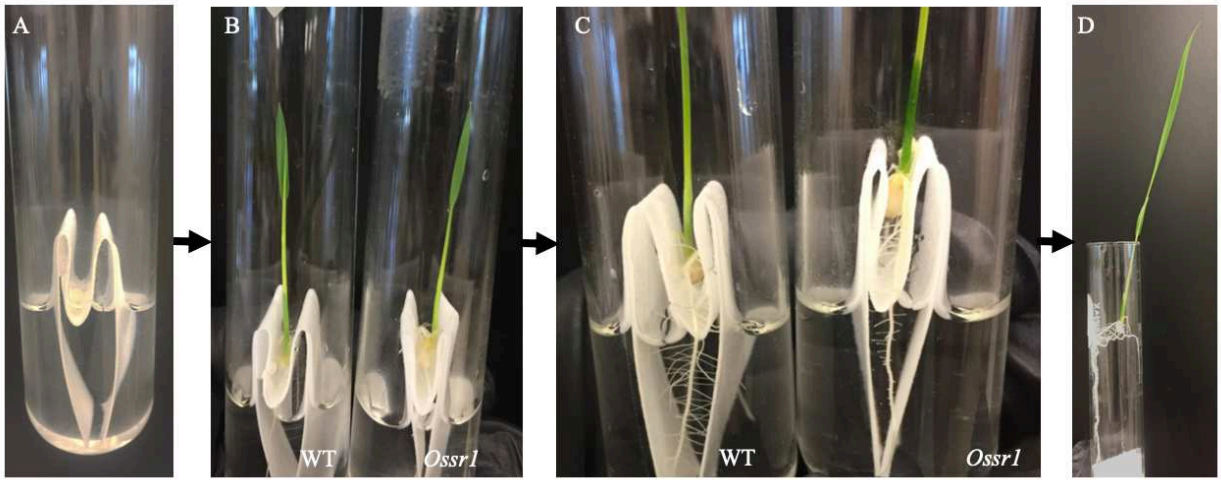


Figure 2. Experimental setup used for germination and growth of rice seedlings before treatment. A) Dehulled seeds were sterilized and placed on filter-paper bridges in ½ MS medium in upright tubes. B) Seedlings germinated by day 3. WT and *Ossr1* seedlings show similar growth. C) Lateral root hairs developed by day 4. If roots pushed seedling up, seedlings were gently placed down so that roots were submerged in the liquid medium. D) Seedling before treatment on day 6 showed significant root and shoot growth. The medium was exchanged in treated samples. Seedlings were placed so that roots were submerged in the liquid medium.

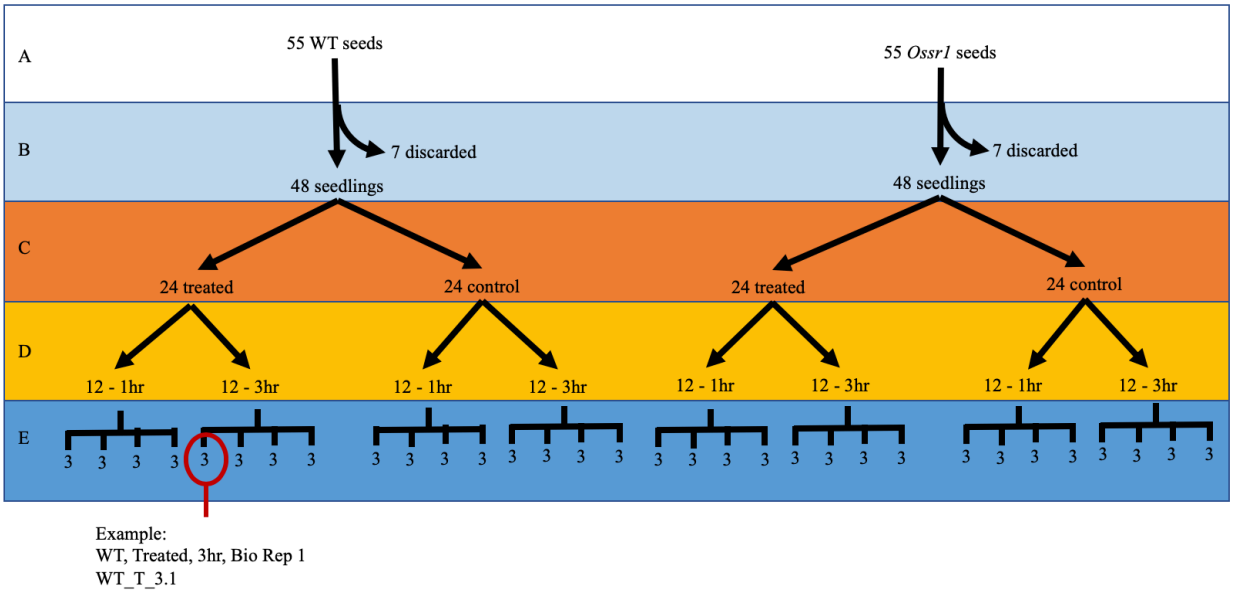


Figure 3. Experimental design of RNA-seq study. A) Fifty-five seeds of each genotype, WT and mutant, were dehulled and sterilized, B) Forty-eight healthy seedlings were selected and seven small/un-germinated seeds were discarded, C) Equal number of seedlings (24) was used for control and treatment, D) Twelve seedlings were collected for each time point, E) Root and shoot tissue of three seedlings were pooled into one biological replicate; four biological replicates per genotype, treatment and timepoint were collected.

prepared in the same manner as the first time point (Figure 3D). In total, three seedlings made up each biological replicate. There were three biological replicates per line (WT or *Ossr1*), treatment (control or PEG), and time point (1 or 3 hours after PEG treatment) (Figure 3E). Samples were handled in repeated order in all steps to keep treatment time as consistent as possible. Samples were stored at -80°C.

2.2 RNA-seq Sample Preparation

Samples were ground using the TissueLyzer for 30-40 seconds, 1-2 times until tissue became a fine powder but not thawed. Tissue from each sample was separated into two tubes using liquid nitrogen to keep samples frozen. Additional samples were stored at -80°C. RNA was extracted using the Qiagen RNeasy Plant Mini Kit, QiaShredder column, and treated with DNase using on-column digestion. RNA intactness was determined for 14/26 samples using the Agilent 2200 TapeStation High Sensitivity, eukaryotic RNA protocol and screen tape. RIN values ranged from 7.2 – 8. Two µg of cDNA was made from all samples using MMLV-RT. Samples were verified as WT or mutant using PCR with *OsSR1*-specific primers. Expression of drought-responsive element binding factor (*DREB 1C*) was determined using SYBR Green qRT-PCR on the Roche 480 and normalized to GAPDH expression.

After RNA quality, RNA concentration, and genotype of all samples had been verified, 24 samples were sent on dry ice to Novogene for Illumina sequencing and analysis. After further analysis of *DREB1C* expression using endpoint PCR, sample three was replaced with a different biological replicate (Sample 26). Novogene found the replacement sample to have low concentration and poor-quality RNA so a third sample (Sample 29) of this same biological

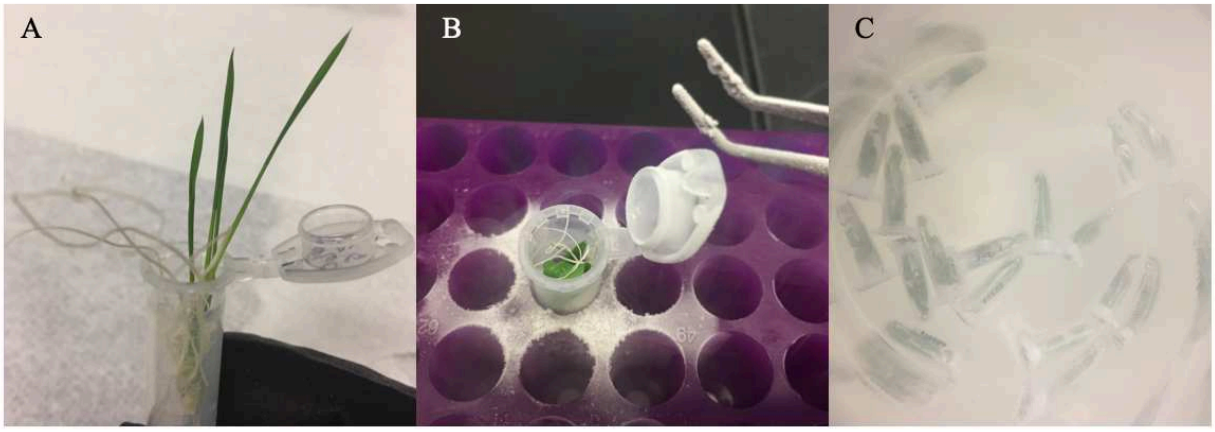


Figure 4. Harvesting and freezing of seedlings for RNA-seq analysis. A) Three seedlings were pooled into one 2-ml sterile tube. B) Seedlings were frozen by submerging seedlings in liquid nitrogen and the tubes were capped after the liquid nitrogen was evaporated. C) Tubes were kept in liquid nitrogen until sampling was completed. Tubes were then moved to the -80°C freezer and stored.

replicate was sent to replace sample three.

Novogene assessed RNA degradation and contamination using 1% agarose gels. They determined purity on a NanoPhotometer spectrophotometer and integrity and quantity using an RNA Nano 6000 Assay Kit from the Bioanalyzer 2100 system.

2.3 Library Preparation and Sequencing

NEBNext Ultra RNA Library Prep Kit for Illumina was used to generate RNA libraries. mRNA from one μg of total RNA from each sample was enriched using poly-T oligo-attached magnetic beads and fragmented using divalent cations at elevated temperatures in NEBNext First-strand Synthesis Reaction Buffer (5X). cDNA was synthesized using random hexamer primers and MuLV-RT without RNaseH. Overhangs were blunted, 3' ends were polyadenylated and NEBNext adaptors with hairpin loop structure were ligated. Fragments were purified with AMPure XP system and size selected for 150-200 bp length. Three μl USER Enzyme was used with adaptor-ligated cDNA at 15 minutes and then 5 minutes at 95°C before PCR. The product was amplified using Phusion High-Fidelity DNA polymerase, universal PCR primers, and the index primer. PCR product quality was assessed on the Agilent Bioanalyzer 2100 system. Samples were clustered using a cBot Cluster Generation System using PE Cluster Kit cBot-HS. All samples were sequenced on a single flow-cell on an Illumina platform to generate 125bp/150bp paired-end reads.

2.4 RNA-seq Analysis Pipelines

2.4.1 Analysis by Novogene: Pipeline 1

2.4.1.1 Read Cleaning

Reads were cleaned using Novogene's in-house perl scripts. Reads containing poly-N, and low quality were removed from the data. Quality was reported in terms of the Q20 or Q30 phred score.

2.4.1.2 Read Mapping and Counting

The reference genome and annotations were accessed and downloaded from solgenomics: IRGSPb.5 solgenomics.net/genomes/Oryza_sativa/assembly/build_5.00/ and with annotations from RAP-DP https://rapdb.dna.affrc.go.jp/index.html. Index of the reference genome was built using Bowtie v2.2.3, and reads were aligned using TopHatv2.0.12.

HTSeq v0.6.1 was used to count the read numbers mapped to each gene (Table 2). Fragments Per Kilobase of transcript sequence per Million base pairs sequenced (FPKM) normalizes differences in read depth in different samples and gene length. FPKM was calculated to aid in estimating expression levels (Trapnell et al., 2010).

2.4.1.3 Differential Expression Analysis

Differential expression of genes between genotypes and treatments was assessed using DESeq R package (1.18.0) against a negative binomial distribution. p-values were adjusted using Benjamini and Hocherg's approach to control the false discovery rate. Genes with adjusted p-value < 0.05 were assigned as differentially expressed (Anders and Huber, 2010).

Gene Ontology (GO) enrichment analysis of differentially expressed genes was performed using GOseq R package (version unknown) with a p-value threshold of significance < 0.05.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to decipher high-level functions of the biological system. KOBAS software was used to test the enrichment of DE genes in KEGG pathways (https://www.genome.jp/kegg/. (Results available upon request.)

Using the STRING database, protein-protein interaction (PPI) network of DE genes was constructed. Blast v2.2.28 was used to align the target gene sequence to the selected reference protein sequence and networks were built according to the known interaction of selected reference species. (Results available upon request.)

2.4.2 In-house Analysis: Pipeline 2

Raw reads were accessed via the ftp client from Novogene and uploaded onto the Summit Supercomputer (https://www.acns.colostate.edu/hpc/) as zipped files. Reads were cleaned, aligned, and counted on Summit. Analysis programs were accessed using Dr. David King's Summit environment. Expression analysis of counts was performed in R. See supplementary PDF for code and all the parameters used (Supplementary Data 1).

2.4.2.1 Read Cleaning

Raw reads were cleaned using Trimmomatic v0.36. Leading and trailing sequences were cut when quality scores were below 3. A sliding window of 4:15 was used to view 4 bases at a time cutting when average quality per base drops below 15. A minimum read length was set to 36 and reads of less than 36 bases were dropped.

2.4.2.2 Read Mapping and Counting

The *Oryza sativa* pseudomolecule version 7.0 genome and annotations were accessed and downloaded from the Rice Genome Annotation Project (RGAP) (http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_7.0/). STAR v2.5.3a was used to align cleaned reads (Dobin et al., 2013). An index was generated in STAR and trimmed reads were aligned with a maximum intron length of 10,000 nt (see Appendix 1 for code). STAR was built to be used with animal genomes and the default maximum intron length is 1,000,000.

HTSeq v0.11.2 was used to count reads using bam files, non-stranded reads, and counting to gene IDs (as opposed to exons or other gene features available in the annotation). Alignment statistics for each sample along with counts were transferred from Summit.

2.4.2.3 Differential Expression Analysis

EdgeR v3.26.5 was used in R v3.6.0 to statistically determine differentially expressed genes between sample groups (Robinson et al., 2010; McCarthy et al., 2012; Lun et al., 2016; Chen et al., 2014). Raw counts from each sample were assigned to a design matrix identifying the variety, treatment, timepoint, and biological replicate. Gene IDs were imported from the RGAP annotation file. Group, gene ID and sample counts were made into a DGEList and logFC dimensions were plotted in an MDS plot to determine the relatedness of samples (Figure 5). EdgeR uses counts per million without calculating FPKM. A general linear model using the quasi-likelihood F-test was used to fit the data. Comparisons across sample groups were made to determine significant up- and down-regulated genes using FDR adjusted p-value threshold of <0.05.

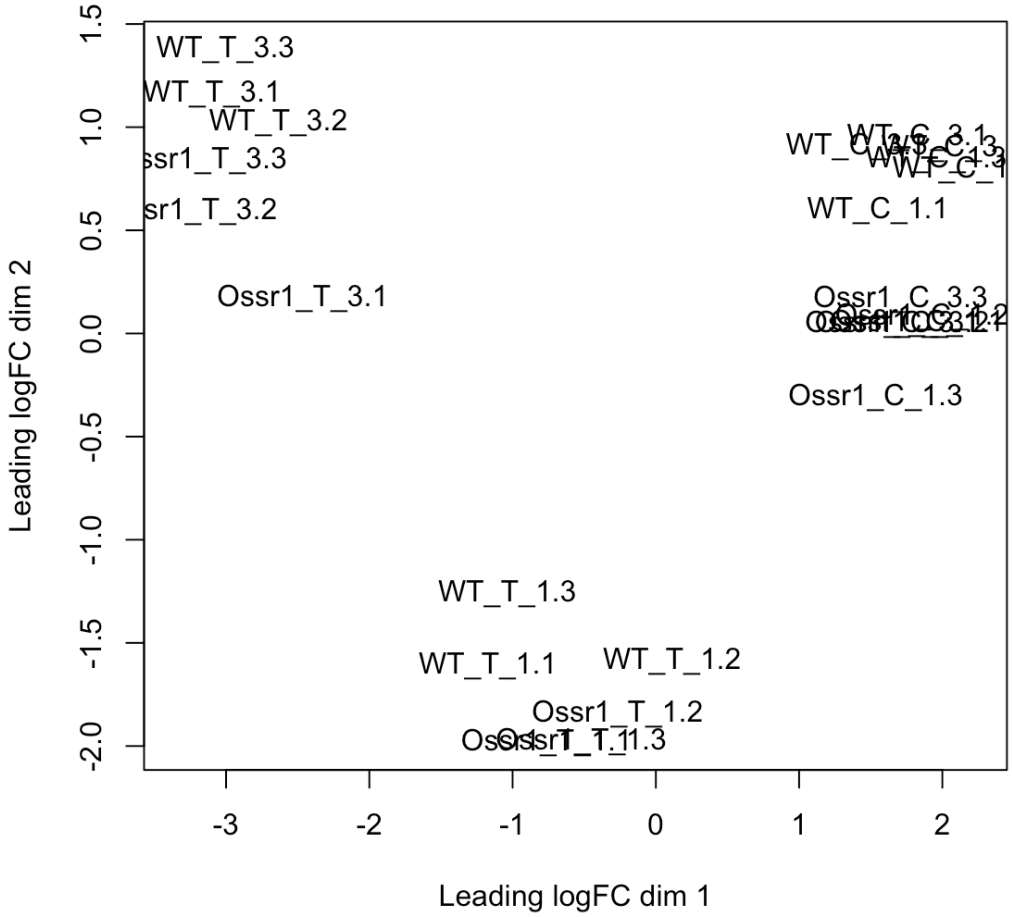


Figure 5. Pipeline 2: Multidimensional scaling plot (MDS). MDS plot showing the relatedness of samples across two dimensions of logFC. WT_C samples group in upper-right with *Ossr1*_C grouping just below. All treated, 1hr sample group at bottom center, and all treated, 3hr samples group at upper-left.

Gene ontology singular enrichment analysis (GO) was performed using AgriGO v2.0 (Tian et al., 2017). GO terms of gene groups >10 are listed in the categories of Biological Process (P), Cellular Component (C), and Molecular Function (F), significant at a p-value <0.05.

2.4.3 Comparison of RNA-seq Analyses

Significant DE genes from the Novogene analysis and the in-house analysis were compared. IRGSP (the reference and annotation used by Novogene) gene IDs correspond to RAP-DB nomenclature, while RGAP uses MSU nomenclature. Gene IDs were converted using the RAP-DB ID Converter (https://rapdb.dna.affrc.go.jp/tools/converter/run) and MSU gene IDs were used for GO analysis. The intersection of DE genes in both analysis pipelines for genotype by treatment comparisons, averaging across timepoints, was analyzed. GO terms were determined for these genes using AgriGO v2.0.

Chapter 1: COMPLEMENTATION OF *OsSR1* MUTANT AND OVEREXPRESSION OF *OsSR1* IN WT AND *Ossr1*

INTRODUCTION

Mutant and WT functional analysis can provide valuable insight into the action of a single gene. Mutants are generated by genomic modifications or induced with RNAi techniques, and off-target effects are numerous. To confirm the action of a gene, a complemented line expressing a functional copy of the gene-of-interest in the mutant background can be used to validate any phenotypic differences observed between WT and mutant lines. Additionally, over-expression of a gene can further clarify the action of the gene when compared to mutant, complemented, and WT lines.

To understand the function of *Os*SR1 in rice, we have a WT and knock-out mutant line that can be compared in a variety of conditions, but without complementation, we will not be able to confirm that an observed difference is due to the mutation and not due to an extraneous effect due to another mutation in the genome. I have generated three additional transgenic lines to verify the action of *Os*SR1. These lines will be used in future studies to understand the function of this dynamic stress regulator, SR1, in rice.

RESULTS

1. Generation of Transgenic Rice

1.1 Complementation of *Ossr1* and *OsSR1* Overexpression in WT and *Ossr1*

To determine the action of *Os*SR1 in stress responses in rice, a full knock-out mutant and WT line can be tested against a battery of stresses. To confirm that the differences in response are due to *Ossr1* and not due to any other unknown second mutation, complemented lines are needed. In addition, to test if the levels of *OsSR1* expression affect SR1-mediated responses, overexpressor lines in the mutant and wild type background would be needed. To address this, I have generated four different types of transgenic lines that will be valuable in addressing the function of SR1 in rice.

The rice *SIGNAL RESPONSIVE 1 (OsSR1*, also called *OsCAMTA3,* LOC_Os10g22950*)*, is the gene with the highest sequence similarity to *Arabidopsis SR1* at the amino acids level. *OsSR1* is 6881nt long with 12 introns, producing a 3072 nt long transcript, which encodes a 116 kDa protein. The full knock-out mutant, *Ossr1*, was obtained from a Korean collaborator and confirmed to be a homozygous loss-of-function mutant (Figure 6). In the mutant, there is no transcript of *OsSR1* due to an insertion in the first intron, which is located 391 bp from the transcription start site (Figure 6). Absence of intact *OsSR1* gene was confirmed by genomic PCR and absence of *OsSR1* transcript was confirmed using semi-quantitative RT-PCR and quantitative RT-PCR (Figure 7). Expression of related *OsSR* family members (*OsSR/CAMTA*1,2,5 and 6) in the *Ossr1* mutant was confirmed (Figure 8, Mohammed and Reddy, unpublished), thus the mutation only affects *OsSR1* (*OsCAMTA3*) expression and does not significantly alter the expression of other *OsSRs*.

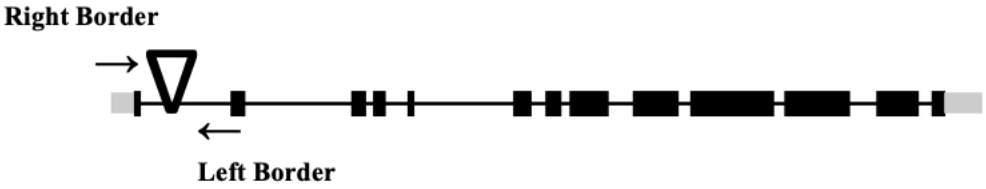


Figure 6. *Ossr1* knockout mutant gene. T-DNA Insertion (triangle) at 391 nt from transcription start site in the first intron of *OsSR1* caused a full knock-out of this gene. Boxes represent exons and introns are indicated by lines between the exons. The position of primers used in genomic PCR is shown with arrows.

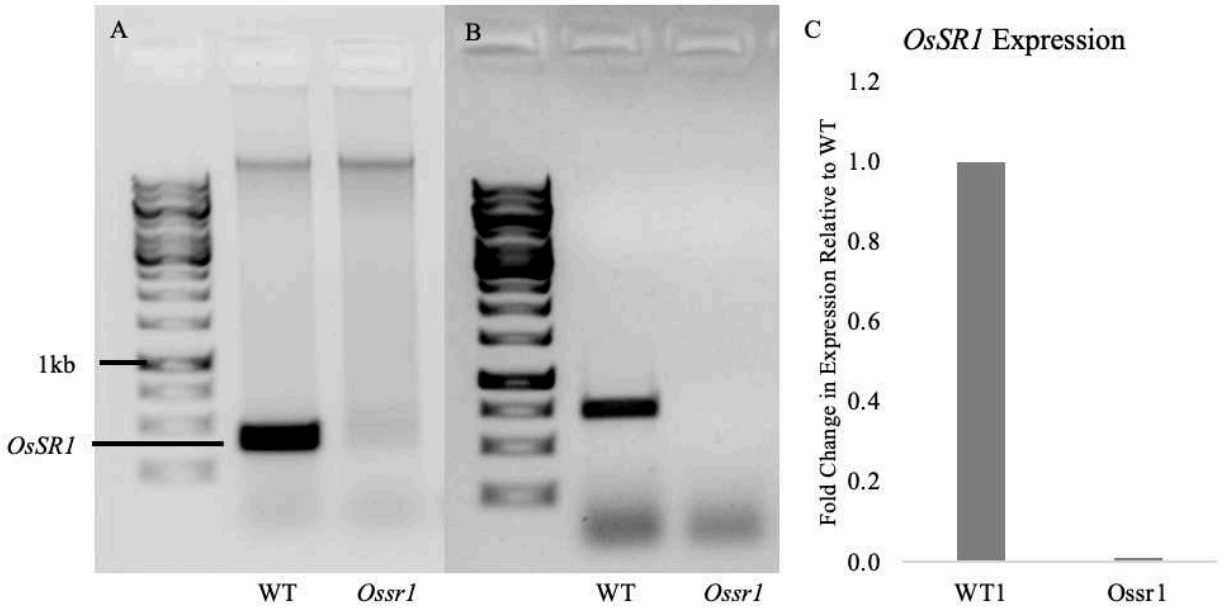


Figure 7. Verification of *Ossr1* mutation using genomic PCR, RT-PCR, and qRT-PCR. A) Genomic PCR of WT and *Ossr1* using primers flanking the T-DNA insertion (See Fig 7 for primer locations). Expected *OsSR1* amplicon size is 400bp. B) RT-PCR of *OsSR1* expression in WT and *Ossr1*. Expected *OsSR1* amplicon size is 736bp. C) Expression of *OsSR1* via qRT-PCR.

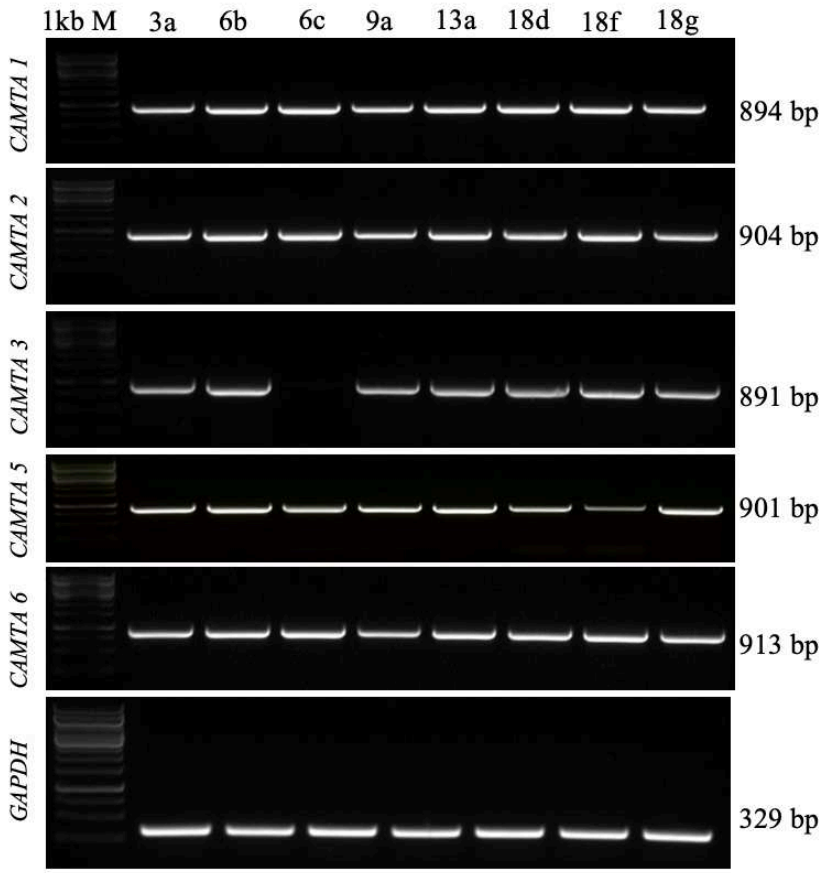


Figure 8. Expression analysis of *OsCAMTAs* in *Ossr1* mutant (6c) via RT-PCR. Gene-specific primers for *SRs/CAMTA*s (1,2,3,5 & 6) were used. All lines except 6c are wild type for *OsSR*1 (*OsCAMTA3)* gene. *OsCAMTA*1,2, 5 and 6 expression is evident in all wild type plants and *Ossr1* mutant. *GAPDH* was used as a control.

In our ongoing efforts to investigate *Os*SR1 transcription factor function in rice, I generated *OsSR1* complemented and overexpressing transgenic lines using *Agrobacterium tumefaciens*-mediated transformation (Han, et.al., 2012). *Ossr1* mutant was generated by a T-DNA insertion and contains hygromycin B phosphotransferase gene, conferring hygromycin resistance, therefore, a different selectable marker gene must be used to complement the mutant with *OsSR1*. Transgenic lines in the mutant background were generated using a plasmid vector that contains the *BAR* gene, conferring Basta resistance. Three vectors (see below for details) were constructed and introduced into *Agrobacterium* strain LB4404 for stable transformation of *Ossr1* or WT (Figure 9). A) A plasmid construct with *OsSR1* driven by the *Zea mays Ubiquitin* promoter with hygromycin resistance gene (Figure 9A) was used for constitutive overexpression of *OsSR1* in WT; B) A construct with *OsSR1* driven by the *Zea mays Ubiquitin* promoter with Basta resistance was used for overexpression of *OsSR1* in the mutant (Figure 9B); and C) A construct with *OsSR1* driven by the *OsSR1* native promoter with Basta resistance (Figure 9C) was used to complement the mutant. The native promoter consists of the nucleotide sequence 1.4kbp upstream of the *OsSR1* transcription start site. For constitutive expression using the *Ubiquitin* promoter, the first exon and intron of the *Ubiquitin* gene are included before the *OsSR1* start codon (Christensen et al., 1992). After the 900 nt *Ubiquitin* promoter, the transcription initiation start site is followed by an 83 nt untranslated exon, and a 1017 nt intron of the *Ubiquitin* gene.

1.2 Generation of Transgenic Plants

Oryza sativa L. *japonica* ssp. Dongjin WT and *Ossr1* mutant were transformed using the calli generated from embryos following the Han et. al. protocol (Han et al., 2012).

Transformation involves inducing callus formation on auxin-containing medium and then culturing with *Agrobacterium* containing the desired construct for transformation. Cultured calli are grown on selective media (Figure 9A). Non-transformed calli died, while transformed callus produced root and shoot growth (Figure 9A, B). Individuals showing root and shoot growth are transferred to regeneration media where they continue to develop into seedlings (Figure 9C). Plantlets are transferred to regeneration media in upright tubes (Figure 9D). After establishing significant root and shoot growth (Figure 9E), plantlets are transferred to soil (Figure 9F). Plants are grown in the greenhouse or chamber, with proper nutrient and fertilizer additions, kept well-watered until plants are at full maturity (Figure 10G) and seeds are harvested. Leaf tissue at vegetative or seedling stage was used to verify genotype and *OsSR1* expression.

To overexpress *OsSR1* in the WT background, transgenic lines were generated with vector A containing *OsSR1* driven by the *Zea mays Ubiquitin* promoter and hygromycin resistance gene (Figure 9A). Twenty independent insertion lines were generated on a selective medium. From here on we refer to these transgenic lines as *OsSR1* overexpressors in WT (OE-WT) 1-20. *OsSR1* expression levels in these transgenic lines should be greater than levels seen in WT, and this was confirmed in 17 out of 20 T_0 putative transgenics by qRT-PCR analysis using leaf tissue (Figure 11). Five lines, OE-WT 4, 8, 12, 14, and 16, showed 9 to 25-fold increase in *OsSR1* expression as compared to WT (Figure 11). These lines were selected to generate homozygous lines in the next generation.

To confirm the action of *Os*SR1 in rice, a WT phenotype would need to be observed in a complemented line in the *Ossr1* mutant background. *Ossr1* was complemented by expressing *OsSR1* with either a constitutive promoter (construct B) or the *OsSR1* native promoter (construct C) (Figure 9). Complementation with construct B is expected to produce *OsSR1* expression

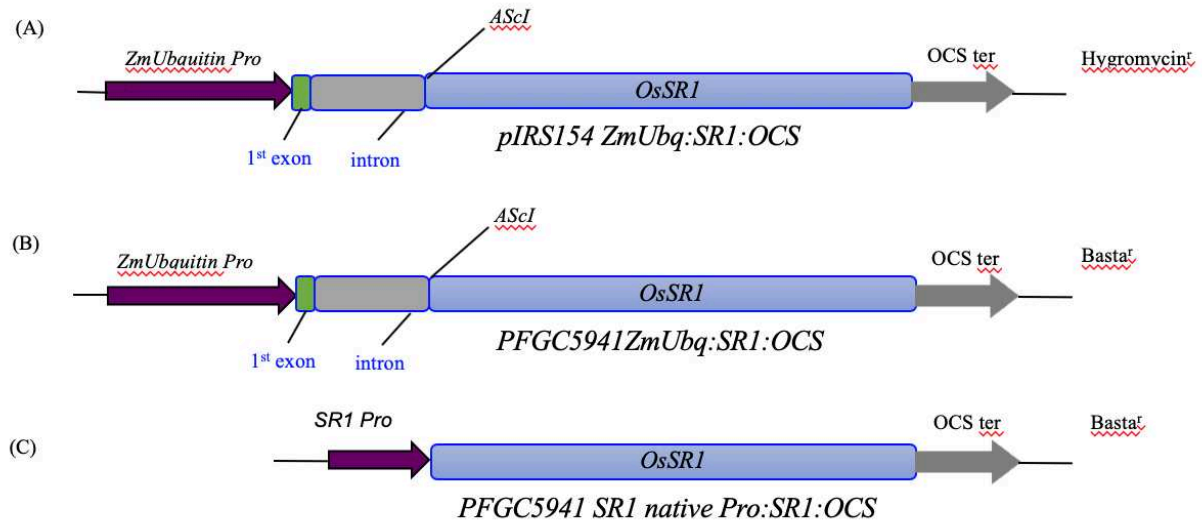


Figure 9. Diagram of constructs used in transformation with *Agrobaterium tumefaciens*. A) *OsSR1* driven by *Zea mays Ubiquitin* promoter, first exon, and intron. Construct contains OCS terminator and hygromycin resistance gene for transforming WT: OE-WT B) *OsSR1* driven by the *Zea mays Ubiquitin* promoter as in A. Construct contains OCS terminator and *BAR* gene (Basta resistance) for transforming *Ossr1*: OE-*Ossr1* C) *OsSR1* driven by the native promoter (1.4kb upstream of the transcription start site. The construct contains OCS terminator and *BAR* gene for transforming *Ossr1*: NP-*Ossr1*.

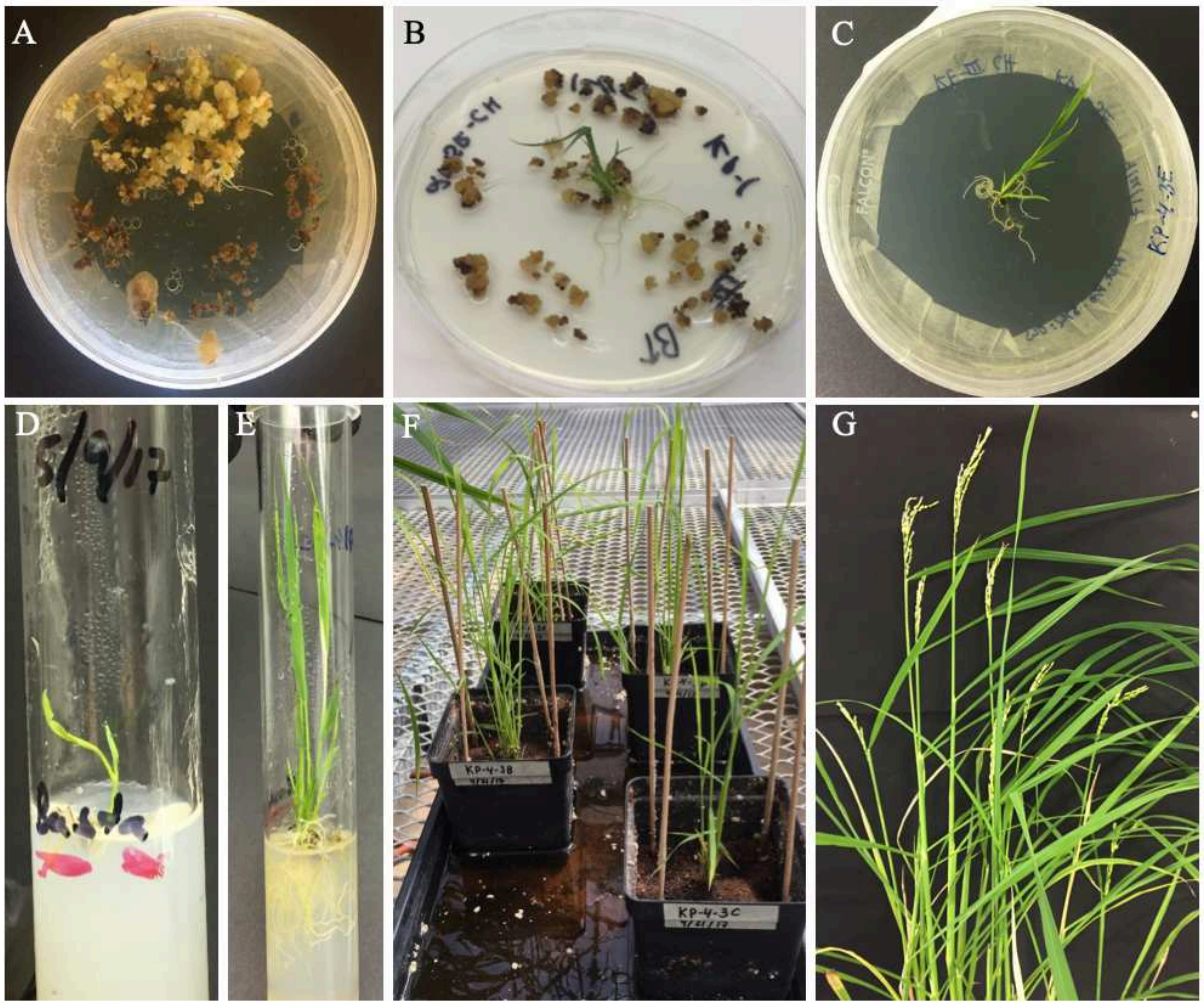


Figure 10. Transformation of rice. A) Callus cultured with Agrobacterium were grown on MSR-CH selective medium. Callus containing the transgene (including the resistance gene) grew (top half of plate) while calli that are not transformed died (bottom half of plate). Excess *Agrobacterium* can be seen growing on dying callus at 7 o'clock. Root growth is noticeable on live callus at the top of the plate: one at 11 o'clock and the other near the center of the plate. B) Resistant plantlets grew as non-transformed, susceptible callus died. C) Putative transgenic seedlings were transferred from MSR-CH medium to REIII-CH selective, regeneration medium and produced healthy roots and shoots. D) Putative transgenic plantlets were transferred from REIII-CH medium plates to REIII-CH medium upright tubes. E) Plantlets established significant root and shoot growth in selective regeneration medium. F) Putative transgenic seedlings were transferred to soil and grown at 27ºC, 16hr light, 75% humidity in the greenhouse (shown) or chamber (not shown). G) Transgenic plants were grown to full maturity and developed seeds.

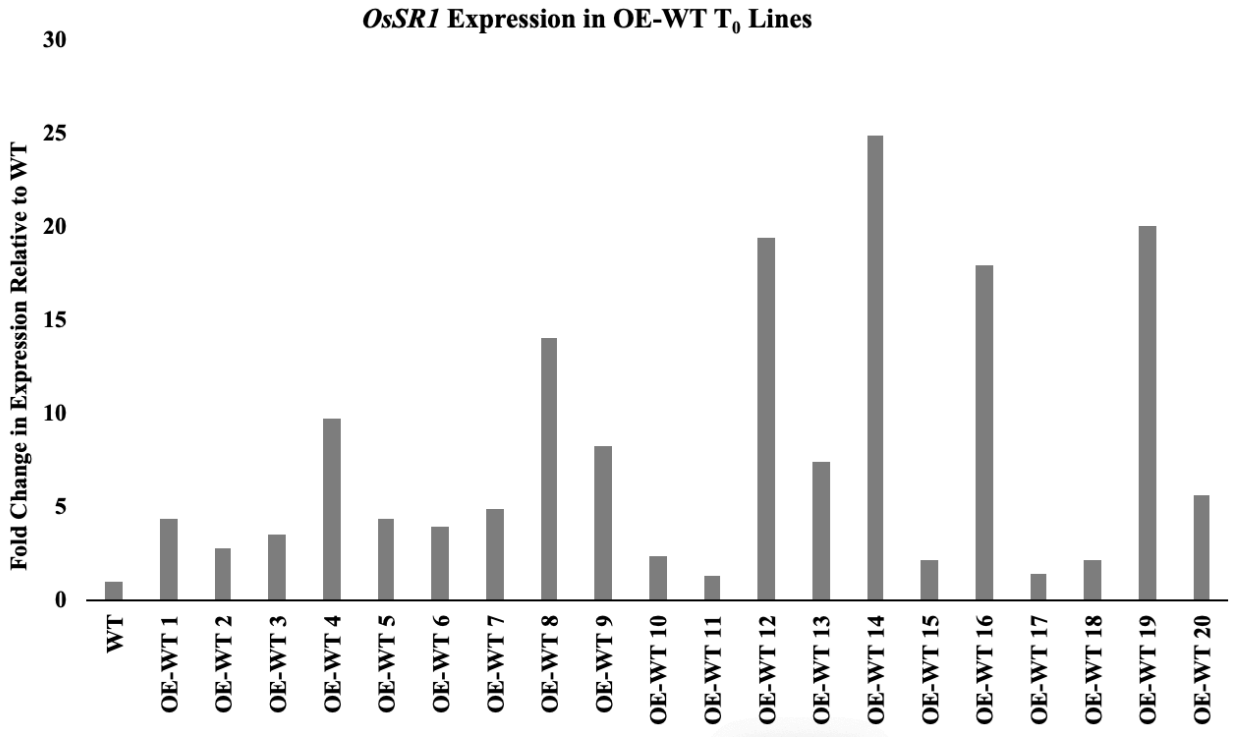


Figure 11. *OsSR1* expression in OE-WT 1-20 T_0 lines. Expression of *SR1* is normalized to *GAPDH* as the housekeeping gene. Fold change in expression relative to WT is shown. *SR1* expression in OE lines is expected to be above that of WT levels.

levels greater than the levels seen in WT, and complementation with construct C is expected to produce *OsSR1* expression levels equivalent to those seen in WT.

Eight independent transgenic lines were generated with construct B, and these are referred to as overexpressor in *Ossr1* (OE-*Ossr1*) 1-8. RNA was extracted from T_0 leaf tissue and *OsSR1* expression was assessed via RT-PCR (Figure 12) and qRT-PCR (Figure 13). Five of the eight transgenic lines, (OE-*Ossr1* 4-8) showed *OsSR1* expression in both RT-PCR and qRT-PCR, at levels greater than WT (Figures 13 and 14) and were carried onto the T_1 generation.

Eight independent transgenic lines were obtained with construct C, and these lines are referred to as native promoter in *Ossr1* (NP-*Ossr1*) 1-8. DNA was extracted and tested for the presence of *OsSR1* and the resistance gene using genomic PCR with T_0 leaf tissue (Figures 15 and 16). Six of the eight putative transgenic lines, NP-*Ossr1* 1, 3, 4, 6-8, contained both the *OsSR1* transgene (Figure 14) and the expressed *OsSR1* and the resistance gene (Figure 15). RNA was extracted from leaf tissue of these six T_0 NP-*Ossr1* lines. *OsSR1* expression was confirmed by RT-PCR in the six transgenic lines that had shown the transgene in genomic PCR (Figure 15). Ideally, NP-*Ossr1* lines would express *OsSR1* at the level of WT. Quantification of *OsSR1* expression in these six lines showed *OsSR1* expression 20 to 60% of WT level (Figure 16). Seeds from NP-*Ossr1* 1, 3, 4, 6-8 transgenics were harvested and carried on to the T_1 stage.

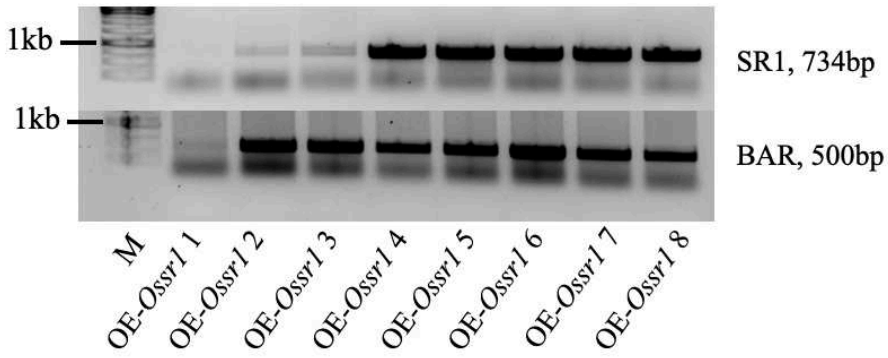


Figure 12. Presence of *SR1* transcript in T_0 OE-*Ossr1* 1-8 via RT-PCR. *BAR* transcript signifies Basta resistance. Presence of the expected amplicon size for the *BAR* gene (500bp) confirms the presence and expression of the transgene. *OsSR1* amplicon expected size is 734bp. *OsSR1* is present in 5 out of 8 putative transgenic lines: OE-*Ossr1* 4-8.

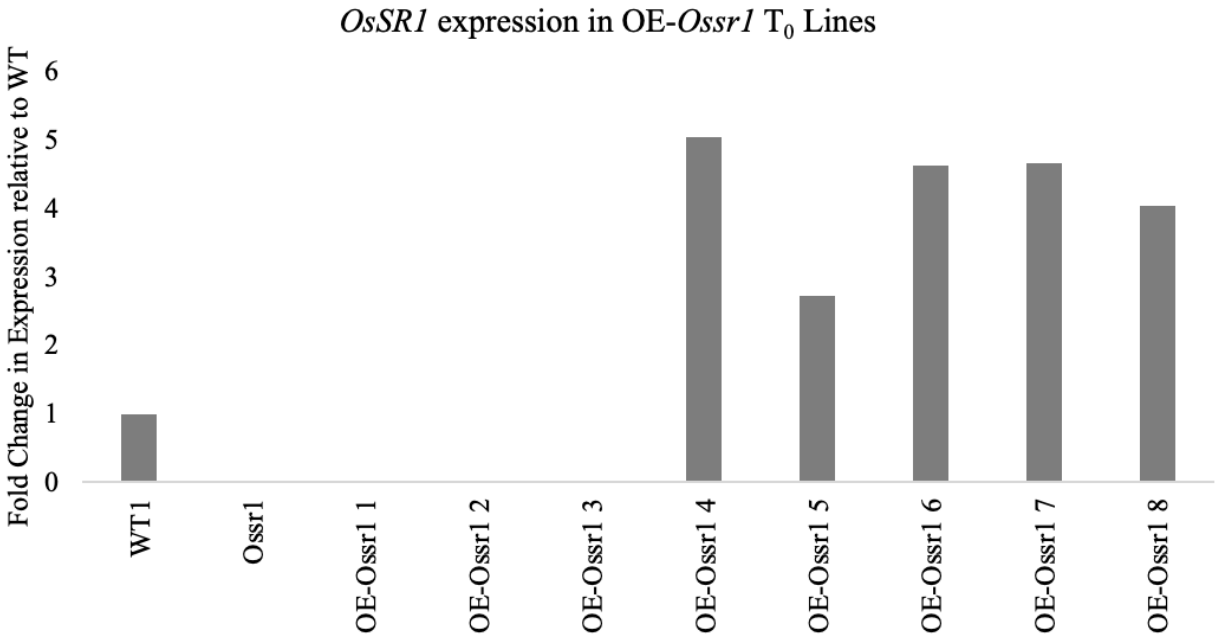


Figure 13. Analysis of *OsSR1* expression in OE-*Ossr1* 1-8 using qRT-PCR. *OsSR1* expression was normalized to GAPDH as the housekeeping gene. Fold change is relative to WT level. *Ossr1* does not show *OsSR1* expression. OE-*Ossr1* 4-8 all show expression greater than WT as expected.

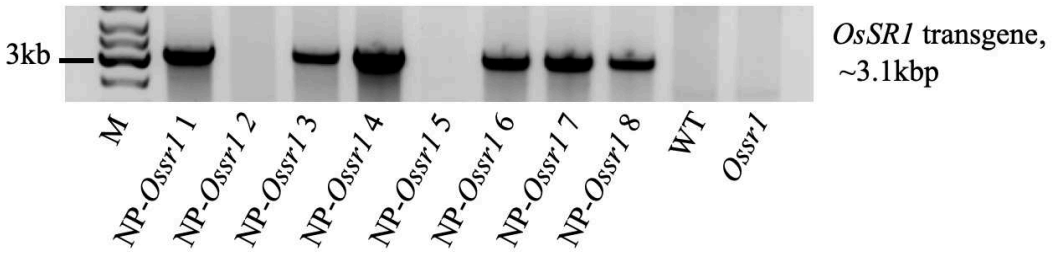


Figure 14. Genomic PCR for the presence of *OsSR1* in WT, *Ossr1*, and T_0 NP-*Ossr1*. Primer 5F and 5R, corresponding to the *OsSR1* gene and *ocs* terminator, were used for PCR amplification. *OsSR1* was not amplified in WT or *Ossr1* because they do not contain the *OCS* terminator sequence (or the transgene).

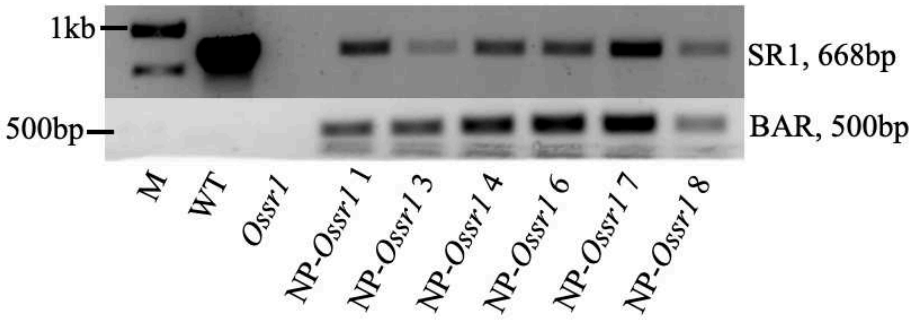


Figure 15. *OsSR1* expression in WT, *Ossr1*, and T_0 NP-*Ossr1* lines 1-4,6-8 via RT-PCR. *OsSR1* amplicon is 668 bp. *OsSR1* expression is apparent in WT and all NP lines. *BAR* gene expression is clear in NP lines, as expected (*Ossr1* contains hygromycin resistance).

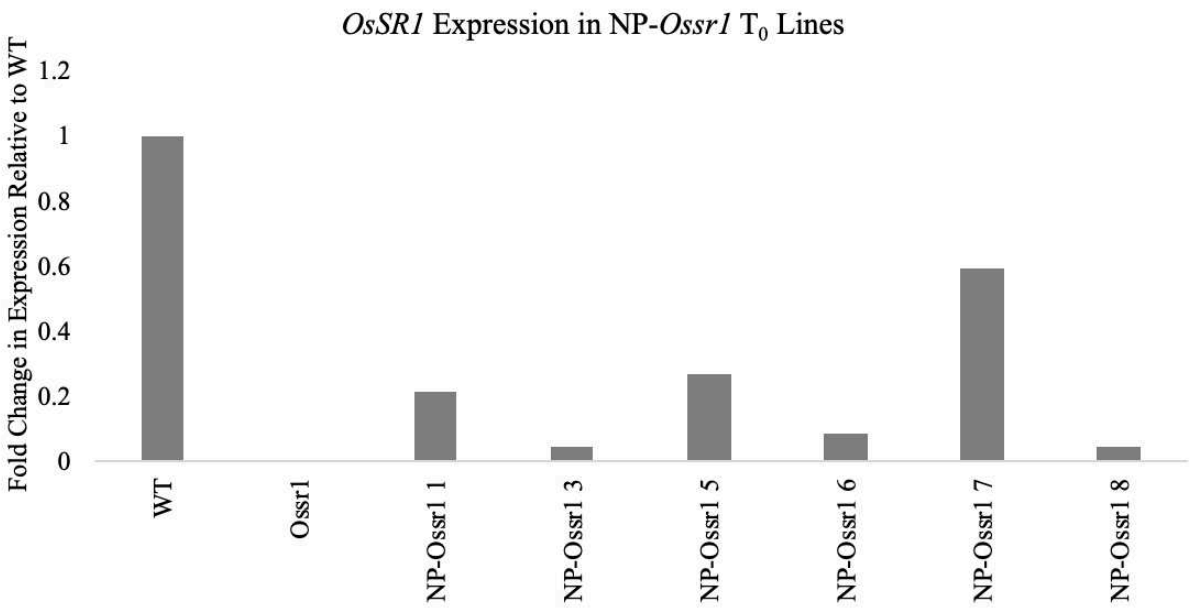


Figure 16. Analysis of *OsSR1* expression in NP-*Ossr1* lines using qRT-PCR. Expression levels of lines 1, 5, and 7 are 20-60% that of WT. All tissue was collected at the vegetative stage however, WT and mutant tissues were not collected along with NP lines.

1.3 Breeding Homozygous Transgenic Lines

Callus cultured with *Agrobacterium* and growing on selective media are referred to as T_0 plants. Unless the transgene inserted itself into multiple locations, all T_0 transgenic plants can be assumed to be heterozygous with one copy of the transgene present. When grown to full maturity, T_0 plants self-pollinate and produce T_1 seeds which are expected to have a 3:1 phenotypic ratio for the presence of the transgene, with ¼ homozygous and ½ heterozygous offspring. T_1 seeds containing one or two copies of the transgene will germinate on selective medium, thus there should be a 3:1 (resistant vs sensitive) segregation at the T_1 stage. T_1 plants grew to full maturity, self-pollinated, and produced T_2 seeds. T_1 plants will again have a 3:1 ratio for the presence of the transgene in their T_2 seeds. T_2 seeds were again germinated on selective medium. When germination rates on selective media near 100%, a line can be assumed to be homozygous for the transgene. Confirmation of homozygosity can also be done using a χ^2 test for segregation. Germination rates were noted and segregation ratios of resistant versus sensitive seedlings were calculated (Table 5).

Table 5. Germination rates and χ^2 values for T_2 seeds from different plants germinating on selective medium. With one degree of freedom, the χ^2 value for significance at a p-value <0.05 is 3.84 or greater. Lines with χ^2 values greater than 3.84 are either not segregating (homozygous for the transgene) or do not contain the transgene as indicated by the germination rate. Fifteen seeds per line were plated. OE-*Ossr1* transgenic plants were generated a year after OE-WT and NP-*Ossr1* lines. Rates and values for T_2 OE-WT and NP-*Ossr1* lines are shown.

	Line	Germination Rate	χ^2	
OE-WT 4	1	0.933	2.69	Segregating
	2	0.667	0.56	Segregating
	3	0.933	2.69	Segregating
	4	0.733	0.02	Segregating
	5	0.867	1.09	Segregating
	6	0.467	6.42	No Transgene
	7	0.600	1.80	Segregating
OE-WT 8	1	1.000	5.00	Homozygous
	2	0.800	0.20	Segregating
	3	0.733	0.02	Segregating
	4	0.600	1.80	Segregating
	5	0.400	9.80	No Transgene
	6	0.667	0.56	Segregating
	7	0.800	0.20	Segregating
OE-WT 12	1	0.733	0.02	Segregating
	2	0.667	0.56	Segregating
	3	0.867	1.09	Segregating
	4	0.667	0.56	Segregating
	5	0.867	1.09	Segregating
	6	0.933	2.69	Segregating
	7	0.533	3.76	Segregating
OE-WT 14	1	1.000	5.00	Homozygous
	2	0.733	0.02	Segregating
	3	0.867	1.09	Segregating
	4	0.533	3.76	Segregating
	5	0.867	1.09	Segregating
	6	0.667	0.56	Segregating
	7	0.600	1.80	Segregating
OE-WT 16	1	1.000	5.00	Homozygous
	2	0.733	0.02	Segregating
	3	0.667	0.56	Segregating
	4	0.800	0.20	Segregating
	5	0.533	3.76	Segregating
	6	0.600	1.80	Segregating
	7	0.533	3.76	Segregating

	Line	Germination Rate	χ^2	
NP-Ossr1 1	1	0.867	1.09	Segregating
	2	0.733	0.02	Segregating
	3	1.000	5.00	Homozygous
	4	0.933	2.69	Segregating
	5	0.933	2.69	Segregating
	6	0.786	0.10	Segregating
	7	0.667	0.56	Segregating
NP-Ossr1 6	1	0.800	0.20	Segregating
	2	0.533	3.76	Segregating
	3	0.867	1.09	Segregating
	4	0.429	7.71	No Transgene
	5	0.733	0.02	Segregating
	6	0.600	1.80	Segregating
	7	0.667	0.56	Segregating
NP-Ossr1 7	1	0.933	2.69	Segregating
	2	0.667	0.56	Segregating
	3	0.600	1.80	Segregating
	4	0.933	2.69	Segregating
	5	0.733	0.02	Segregating
NP-Ossr1 8	1	0.600	1.80	Segregating
	2	0.533	3.76	Segregating
	3	0.467	6.42	No Transgene
	4	0.667	0.56	Segregating
	5	0.667	0.56	Segregating
	6	0.867	1.09	Segregating
	7	0.923	2.08	Segregating

1.4 Expression Validation of Transgenic Lines

OsSR1 expression was assessed in WT, *Ossr1* and selected T_3, and T_2 transgenic lines at the late vegetative stage before flowering by RT-PCR (Figure 17). The *OsSR1* transcript is present in all lines except *Ossr1* as expected though bands are faint in complemented lines, suggesting low expression levels. The same tissue was then used to assess *OsSR1* expression using qRT-PCR (Figure 18). Complemented lines showed almost no *OsSR1* expression. OE-WT lines showed 1 to 6-fold *OsSR1* expression over WT.

OsSR1 expression at the seedling stage was assessed in WT, *Ossr1*, and select transgenic lines. Seeds were germinated on non-selective medium to assess *OsSR1* expression in select T_3 OE-WT and NP-*Ossr1* and T_2 OE-*Ossr1* tissue from seedlings (Figure 19). Expression in OE-WT 4, 12, and 14 is 4 to 7-fold greater than in WT. OE-*Ossr1* 6 shows 3 times the expression of *OsSR1* in WT, confirming the presence of an over-expressed and complemented line. NP-*Ossr1* 1 and 7 show almost no *OsSR1* expression.

1.5 Phenotypic Comparison of WT, *Ossr1,* and Transgenic Lines

Over- or under-expression of a gene can often lead to changes in phenotype under normal conditions. WT, *Ossr1*, and transgenic lines appeared to develop with similar progress (Figures 20 and 21). At full vegetative stage, transgenics showed less dense architecture and more erect panicles (Figure 21). Future studies will require quantitative phenotypic measurements to confirm these qualitative, visual observation.

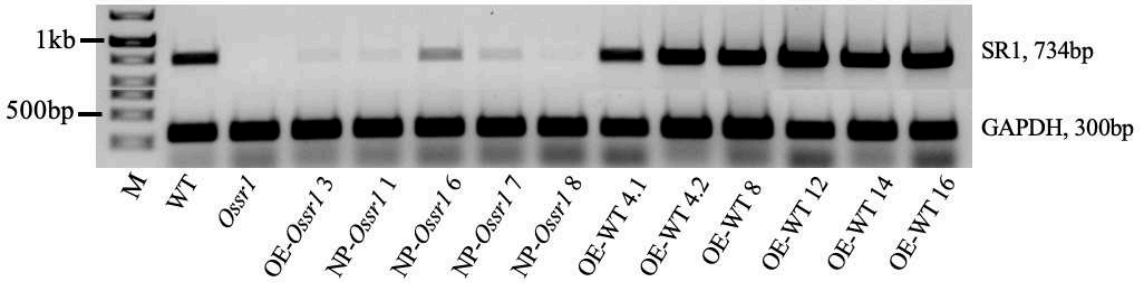


Figure 17. RT-PCR of *OsSR1* amplification in late-vegetative tissue. WT, *Ossr1*, and select T_1 OE-*Ossr1*, T_2 NP-*Ossr1,* and T_2 OE-WT transgenics are shown. *OsSR1* amplicon is 734bp. No expression was observed in *Ossr1*, as expected. Amplification in OE-*Ossr1* and NP-*Ossr1* was present but at lower levels than seen in WT and OE-WT lines.

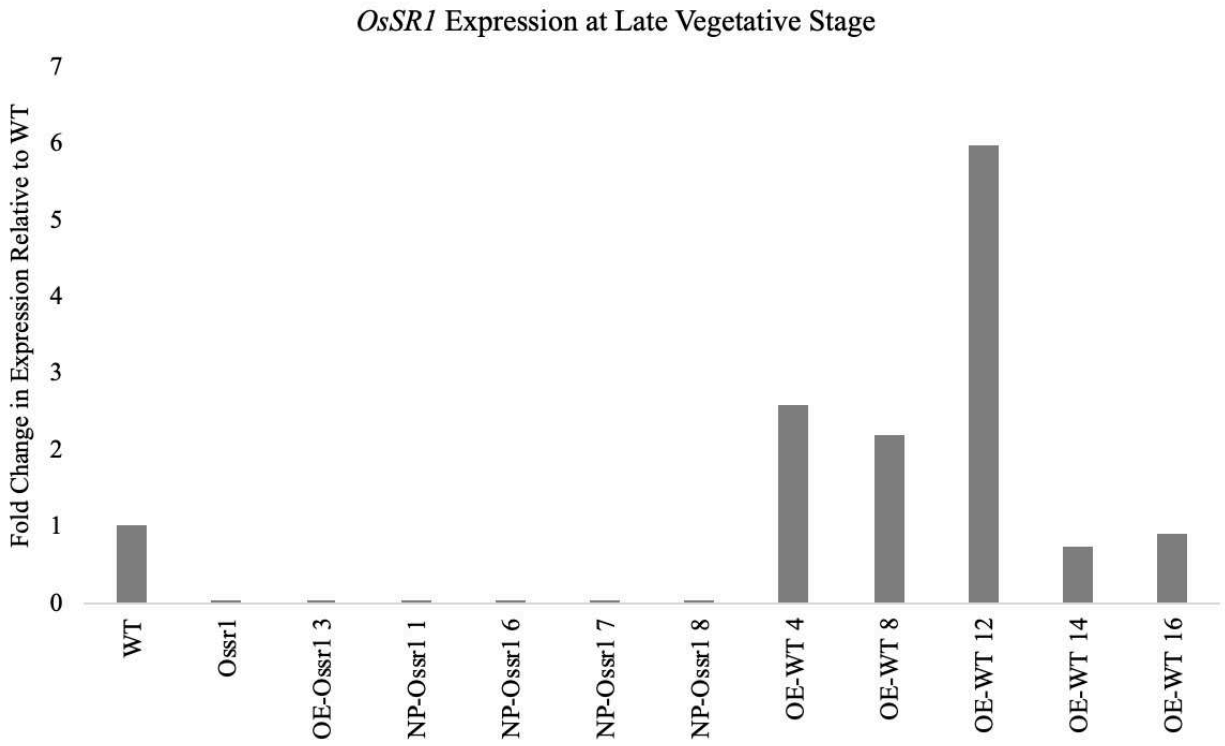


Figure 18. *OsSR1* expression in T_1 OE-*Ossr1*, T_2 NP-*Ossr1*, and T_2 OE-WT lines in leaf tissue at the late vegetative stage. *OsSR1* expression is normalized to the *GAPDH* house-keeping gene. Fold change is relative to WT. No *OsSR1* expression is observed in complemented lines. OE-WT 4 and 8 show 2 to 3-fold increase over WT, OE-WT 12 shows 6-fold over WT, and OE-WT 14 and 16 show similar levels to WT.

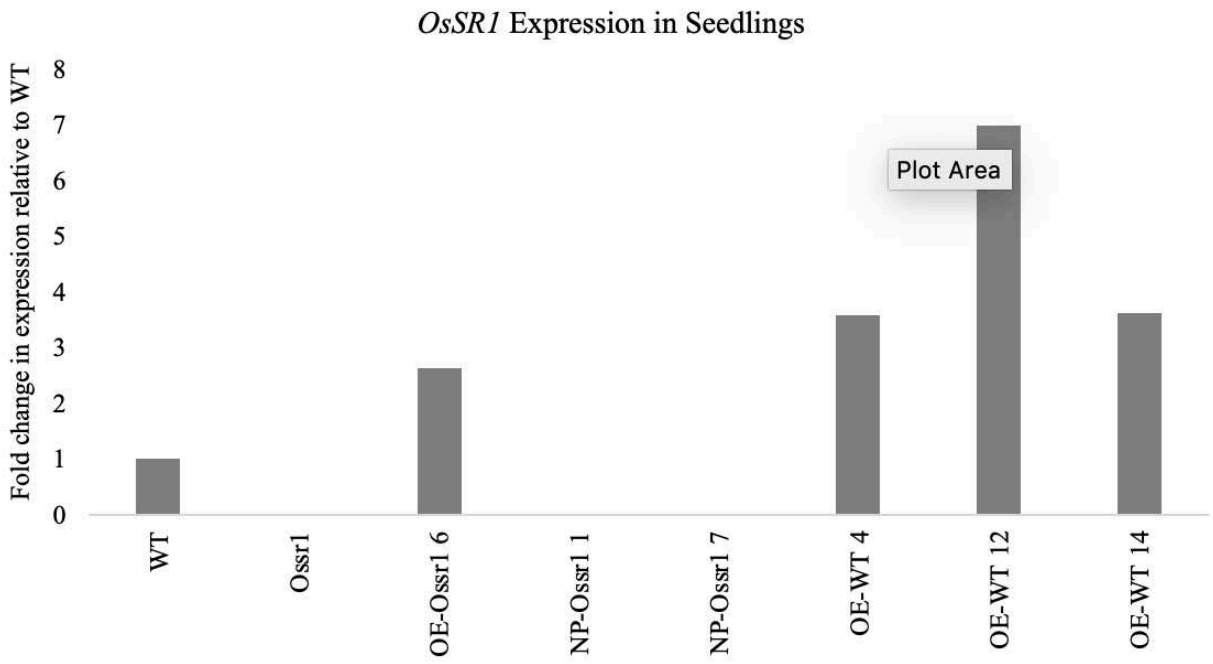


Figure 19. qRT-PCR of *OsSR1* expression in WT, *Ossr1*, and select transgenics at the seedling stage. Expression of *SR1* is normalized to *GAPDH* housekeeping gene expression and fold change is relative to WT levels. T_2 OE-*Ossr1* 6 and T_3 OE-WT 5, 12, and 14 show expression greater than WT levels. No expression is observed in T_3 NP lines, similar to *Ossr1*.

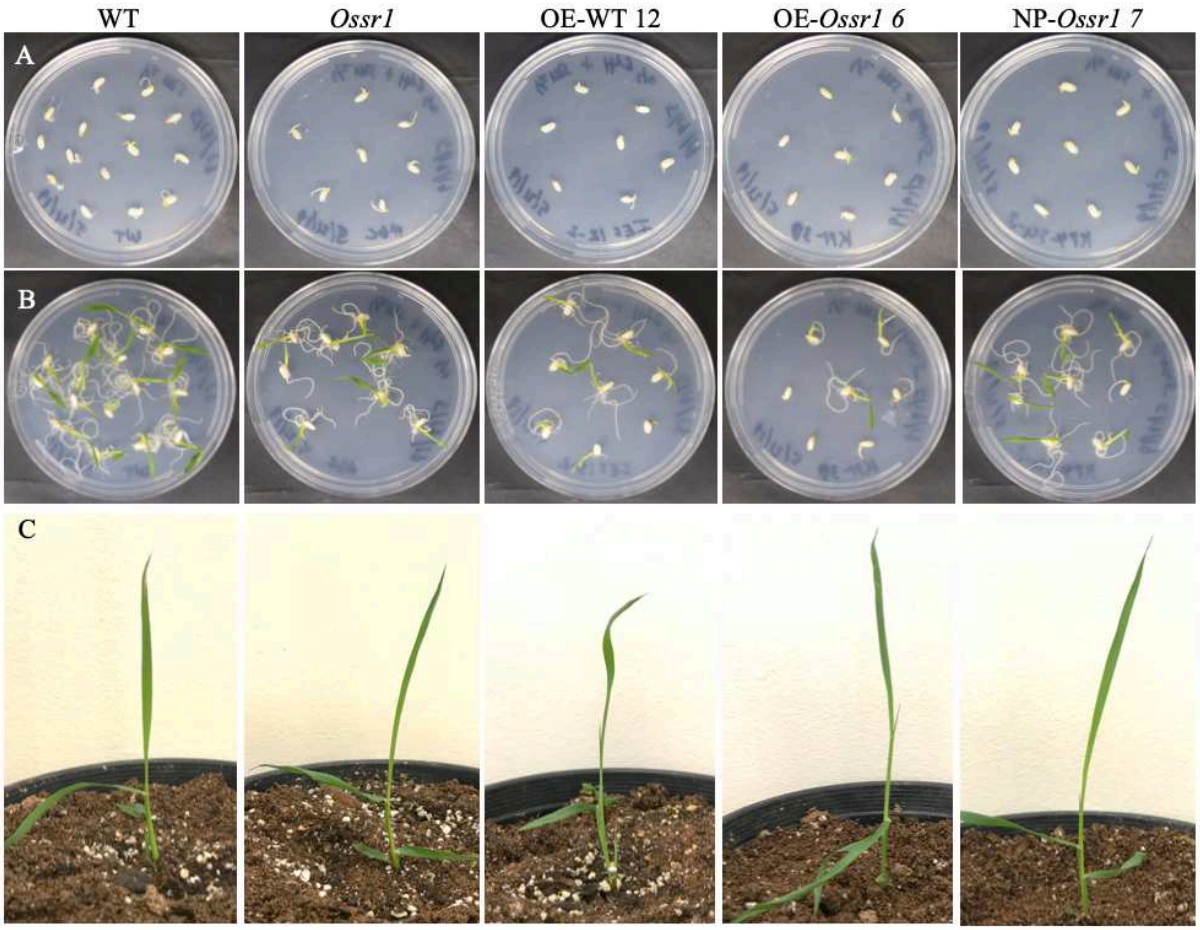


Figure 20. Rice WT, *Ossr1,* and transgenics at germination and seedling stage. WT, *Ossr1*, T_3 OE-WT 12, T_2 OE-*Ossr1* 6, and T_3 NP-*Ossr1* 7 are shown. A) Seeds were germinated on selective medium. B) Seedlings (7-days-old) show root and shoot growth. Germination rates were noted. C) Healthy seedlings were transferred to soil. No apparent phenotypic difference between WT and transgenics can be seen at this stage.

Figure 21. Rice WT, *Ossr1,* and transgenics at flowering. WT, *Ossr1*, T_3 OE-WT 12, T_2 OE-*Ossr1* 6, and T_3 NP-*Ossr1* 7 are shown. A) Panicles developed seeds in all lines. B) Plants have multiple tillers and panicles. Plant architecture is less dense and panicles are more erect in transgenics compared to WT and *Ossr1*.

DISCUSSION

Oryza sativa L. ssp. *japonica cv.* Dongjin WT and *Ossr1* mutant lines were transformed with *Agrobaterium* to generate *SR1* overexpression and complemented lines. Complementation is necessary to confirm the action of *Os*SR1 in future phenotypic studies. Rescue of a mutant phenotype validates that any difference seen between genotypes is due to the action of the gene of interest and not due to a second mutation or insertional effect.

Twenty overexpressor lines in the WT background were generated (OE-WT 1-20) that showed a range of *OsSR1* expression. Variable expression from independent transgenic insertion events is common (Kanzaki et al., 2002; Katiyar-Agarwal et al., 2003; Hernandez-Garcia et al., 2010). One line, OE-WT 12, consistently expressed *OsSR1* at >6-fold that of WT at seedling (Figure 19), vegetative (Figure 11), and late vegetative (Figure 18) stages. This line was carried on to future generations and will be useful to study the function of *Os*SR1 at overexpressed levels.

Five overexpressor lines in the *Ossr1* background were generated (OE-*Ossr1* 4-8). OE-*Ossr1* 6 consistently expressed *OsSR1* at >3-fold WT levels at seedling (Figure 19) and vegetative (Figure 13) stages (Line 6 not tested at the late vegetative stage) and will be carried onto future generations as the complemented, over-expressing line.

Six lines expressing *OsSR1* driven by the native promoter were generated (NP-*Ossr1* 1,2,4,6-8), and are phenotypically similar to WT (Figure 21). Ideally, NP-*Ossr1* lines are expected to express *OsSR1* at levels seen in WT, however these lines expressed 0-60% of WT levels at vegetative stage (Figure 16). NP-*Ossr1* 7 is being carried onto future generations, as it appears to be the most promising NP-*Ossr1* line. T_0 NP-*Ossr1* 7 showed 60% of the expression

seen in WT (the highest of all NP lines) at vegetative (Figure 16) and while no *OsSR1* expression was seen in T_3 seedling tissue (Figure 19), *OsSR1* was amplified from late vegetative tissue (Figure 17). The low level of expression in these transgenics could be due to an insertional effect, as is often an issue in transgenic lines (Schnell et al., 2015). However, this is an unlikely possibility since several independent lines showed relatively low expression compared to WT. Another possibility is that the *OsSR1* expression is regulated by DNA elements away from the promoter (enhancers, introns and/or 3' region of the upstream gene). Introns, especially the first intron, have been shown to enhance gene expression of plant, animal and fungal genes (Rose, 2008; Parra et al., 2011). Furthermore, the 3' end of an upstream gene has been shown to regulate the expression of a downstream gene in plants (Reddy and Reddy, 2004). The promoter that we used was generated using the 1.4kbp sequence upstream of the *OsSR1* ATG start site, up to the end of the previous gene sequence. It could be that *OsSR1* expression is dependent on its introns and/or other *cis*-regulatory regions away from the promoter.

Interestingly, all three transgenic lines expressed greater levels of the transgene in the T_0 generation than in subsequent generations. Tissue from the T_0 generation was taken at the vegetative stage, whereas tissue at the T_1 and T_2 generations was taken at the seedling or late vegetative (just before flowering) stages. It is possible that *OsSR1* expression varies developmentally. The expression of *SR1* will need to be quantified over different stages of development in future studies.

Additionally, while transgenics developed at similar rates as WT and *Ossr1,* they showed less dense architecture and more erect panicles (Figure 20 and 21). Quantitative phenotypic measurements will need to be taken to understand the effect of *Os*SR1 on rice morphology.

In sum, we now have WT, full knock-out mutant *Ossr1*, OE-WT, OE-*Ossr1*, and NP-*Ossr1* lines to use in future studies to determine the action of *OsSR1* in rice. I look forward to seeing how this dynamic regulator works in this important crop.

Chapter 2: GLOBAL ANALYSIS OF SR1-REGULATED GENES UNDER NORMAL CONDITION AND IN RESPONSE TO DROUGHT STRESS IN RICE SEEDLINGS

INTRODUCTION

RNA-sequencing makes use of high-throughput sequencing to capture a picture of the entire transcriptome at a moment in time. While this technology requires bioinformatics to interpret gene transcript counts and statistics to adjust for false discovery rate, it holds promise for identifying relationships that may otherwise be difficult to resolve.

There are three overarching steps in analyzing a transcriptomic data set: 1) cleaning of reads, aligning the reads to a reference genome, and counting the reads aligned to each gene, 2) using statistical analysis to determine differential expression (DE) of significantly up- and down-regulated genes in comparisons between sample groups, and 3) interpreting the DE genes to make meaningful inference based on the question asked in the sample comparison. Analysis of DE genes can be done a number of ways but gene ontology (GO) (chosen in this study), Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein-protein interactions are commonly used methods to gain understanding about trends at the transcription level. In transcriptomic studies, the term "differential expression" refers to transcript abundance and not transcription rates. RNA is purified from sample tissue and mRNA is selected using poly-T magnetic beads, thus, the sample captures all mRNA in the cell. The observed differences in transcript levels between genotypes/conditions could be due to altered rates of transcription and/or mRNA stability. For simplicity, this discussion will continue to use "differential expression" when referring to transcript levels.

GO enrichment groups genes that have a related function and identifies the GO terms that are overrepresented in the DE genes. GO terms are calculated within three categories: Biological Process, Molecular Function, and Cellular Component.

Transcriptomic profiling with *Arabidopsis sr1* mutant (WT compared to *Atsr1*) was performed. This analysis showed a change in regulation of over 3,000 genes (Prasad et al., 2016) when SR1 was not present (WT versus *Atsr1)*. Not surprisingly, GO analysis returned significant terms involving biotic and abiotic stress. Specifically, a number of genes conferring drought and salt stress were upregulated in the mutant, supporting that *At*SR1 regulates salt and drought stresses. Additionally, a number of these genes contain *At*SR1 binding motifs in their promoter regions.

To gain a foundational understanding of *Os*SR1, we performed an RNA-sequencing study of *Oryza sativa* L. *japonica* cv. Dongjin WT and an *Ossr1* mutant. We hope to reveal trends in this transcriptomic profile that give insight to the action of *Os*SR1 in rice.

RESULTS

2. RNA-seq of WT and *Ossr1* Seedlings

SIGNAL RESPONSIVE (SR) proteins (also known as CAMTAs) are a small family of highly conserved transcription factors in eukaryotes (Reddy et al., 2011; Rahman et al., 2016b). In *Arabidopsis,* there are six *SR* genes that are highly responsive to diverse abiotic and biotic signals (Reddy et al., 2000; Yang and Poovaiah, 2002; Bouché et al., 2002). Among the *At*SRs, *AtSR1* has been relatively well studied (Reddy et al., 2011; Yuan et al., 2018). *AtSR1* is an upstream regulator involved in multiple stress responses. This dynamic transcription factor acts as both a positive and negative regulator of different biotic and abiotic stresses (Du et al., 2009; Galon et al., 2010; Prasad et al., 2016; Laluk et al., 2012; Kim et al., 2013). Rice contains six *SR* genes, and the function of *Os*SR1 in rice is unknown. RNA-sequencing is a hypothesis-generating method. To gain a foundational understanding of the action of the SR1 transcription factor in rice, we performed an RNA-seq study of 6-day old seedling root and shoot tissue. Comparing WT to *Ossr1* should provide insights into the action of SR1 in rice.

*At*SR1 is involved in stress responses, so we chose to perform RNA-seq of WT and *Ossr1* seedlings in response to a stress treatment. Rice is an important crop and requires ample water for healthy growth and development. Droughts reduce rice yield significantly (Chauhan Khawar et al., 2017). Drought induces osmotic stress, causing a loss of turgor pressure and cavitation. Heat, salt, cold, and freezing also cause these physical effects, thus, for a foundational study on the action of SR1 in rice, we chose drought stress. Drought was induced with a 40% polyethylene glycol (PEG) 8000 solution (Lagerwerff et al., 1961; Pandey et al., 2004). PEG 8000 is a polymer composed of carbon, hydrogen, and oxygen to a molecular weight of 8000,

making it particularly hydrophilic (Janes, 1974). A 40% weight to volume solution of PEG 8000 modifies the surrounding pressure potential to -18 bars or -1.8MPa (Michel, 1983). Subjecting plant roots to a PEG solution induces an effective drought response; leaf curl was induced after 3 hours of 40% PEG 8000 treatment (Figure 22). Not only can we compare WT to *Ossr1* to glean insight into the action of SR1 in rice, we can compare WT to *Ossr1* under drought to identify the action of SR1 in osmotic stress.

This RNA-seq study involves two genotypes (also referred to as lines), WT and *Ossr1,* and two treatments, drought stress-treated (40% PEG 8000) and control. Because *At*SR1 is known to be an upstream regulator in stress studies with *Arabidopsis,* we were also interested in identifying drought-regulated genes in WT and *Ossr1* at early (1 hr) and late (3 hr) time points following the stress treatment. We collected tissue at 1 hr and 3 hr after drought stress treatment, giving rise to a 2x2x2 factorial experiment. In the following analysis, samples are referred to by line - WT or *Ossr1*/Mu, treatment - treated (T) or control (C), timepoint -1 hr or 3 hr, and biological replicate, 1, 2, or 3. For example, the second biological replicate of a WT sample treated with PEG for 1 hour will be written: WT_T_1.2.

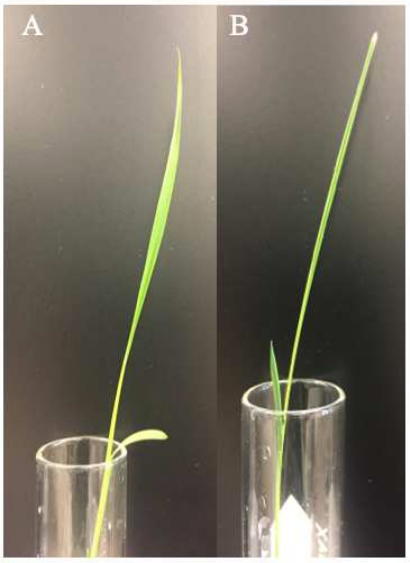


Figure 22. Six-day-old control and PEG-treated seedlings. A) Seedling in normal condition shows healthy open leaf. B) Seedling after 3hrs of 40% PEG 8000 treatment shows leaf rolling, an indication of drought stress.

2.1 Sample Preparation

2.1.1 Design of Experiment

RNA-seq was performed on WT and *Ossr1* six-day-old seedlings to determine the effect of loss of *Os*SR1 on the transcriptome in a factorial experiment. Two varieties, WT and *Ossr1*, were treated with a 40% PEG 8000 solution or control solution, and harvested at two time points, 1 or 3 hours after PEG treatment as described in methods (also see Figures 2-5 in methods section). One and three-hour time points have been used previously to show early and late responses (Doherty et al., 2009). Three seedlings consisting of root and shoot tissue (avoiding the embryo and starchy endosperm) were collected and pooled per sample (Figure 3 in Methods section). Four biological replicates per variety, timepoint, and treatment were collected. Three biological replicates were used for RNA-seq analysis with one biological replicate preserved if one of the other replicates was damaged or lost.

2.1.2 Verification of RNA quality and efficacy of stress treatment

RNA was extracted and tested for purity and quality. Samples were confirmed for correct genotype by assessing *OsSR1* expression (Figure 23 A, B). *OsDREB*s are known to be highly induced in response to drought stress (Dubouzet et al., 2003). While there are 10 *DREB* genes in rice, *OsDREB1-10*, previous qRT-PCR data from our lab showed the greatest fold change in expression in *OsDREB1C* in response to drought stress, thus, *OsDREB1C* expression was used to assess the effectiveness of drought stress treatment. Expression of *OsDREB1C* in treated but not control samples (with the exception of one *Ossr1*_C_1 sample, which was replaced with a different biological replicate) verified the efficacy of the 40% PEG 8000 drought stress (Figure 23C and 24). Verified RNA samples were sent to Novogene for additional quality testing followed by library preparation and sequencing.

2.2 Analysis of RNA-seq Data

Large amounts of sequencing data require bioinformatic pipelines to make meaningful inference. While high-throughput sequencing technology gives us access to large data, accuracy in interpreting the data is paramount. Bioinformatic pipelines gain popularity and are often chosen for their ubiquitous use. Novogene not only performed RNA-seq, but also performed an RNA-sequencing analysis using default parameters, not specific for plants (pipeline 1). I performed a second sequencing analysis (pipeline 2) using alternative software in all steps except for counting reads which was performed with HTseq in both pipelines.

2.2.1 Analysis Pipelines

Single-indexed, paired-end reads were received from Novogene. Two analyses of the data were conducted: Pipeline 1) Novogene performed an analysis aligning reads using Bowtie and TopHat, counting reads using HTSeq, and determining differential expression using DESeq against the Nipponbare reference genome from solgenomics. Pipeline 2) A second analysis was performed using Trimmomatic, a STAR alignment with a maximum intron length of 10,000 nt, again counting reads using HTSeq, and then determining differential expression using edgeR against the Nipponbare reference genome from the Rice Genome Annotation Project (RGAP) database, version 7 pseudomolecule.

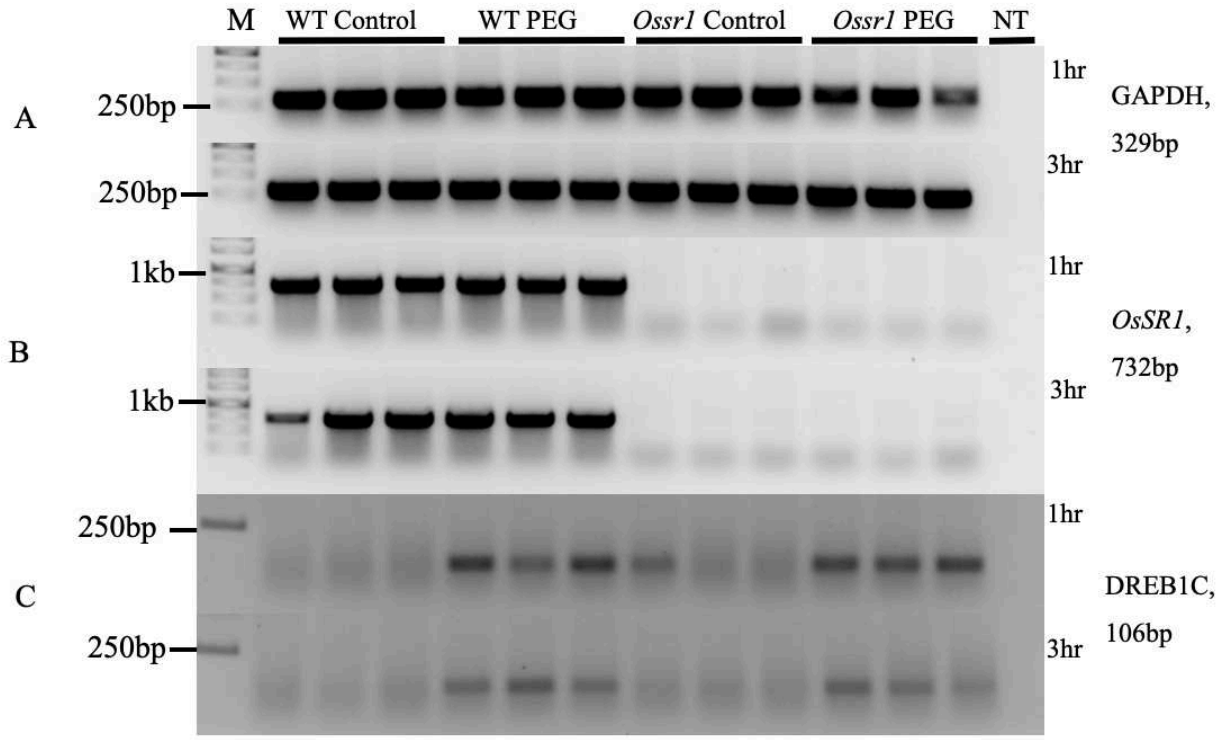


Figure 23. Validation of genotypes of all samples and efficacy of PEG treatment using RT-PCR prior to RNA-sequencing. A) All samples expressed *GAPDH*, 329bp amplicon. B) Only WT samples expressed *OsSR1*, 734bp amplicon. C) Only PEG-treated samples expressed *DREB1C*, 106bp amplicon, with the exception of the first sample of *Ossr1*_C at 1hour. This sample was replaced with a different *Ossr1*_C_1 biological replicate.

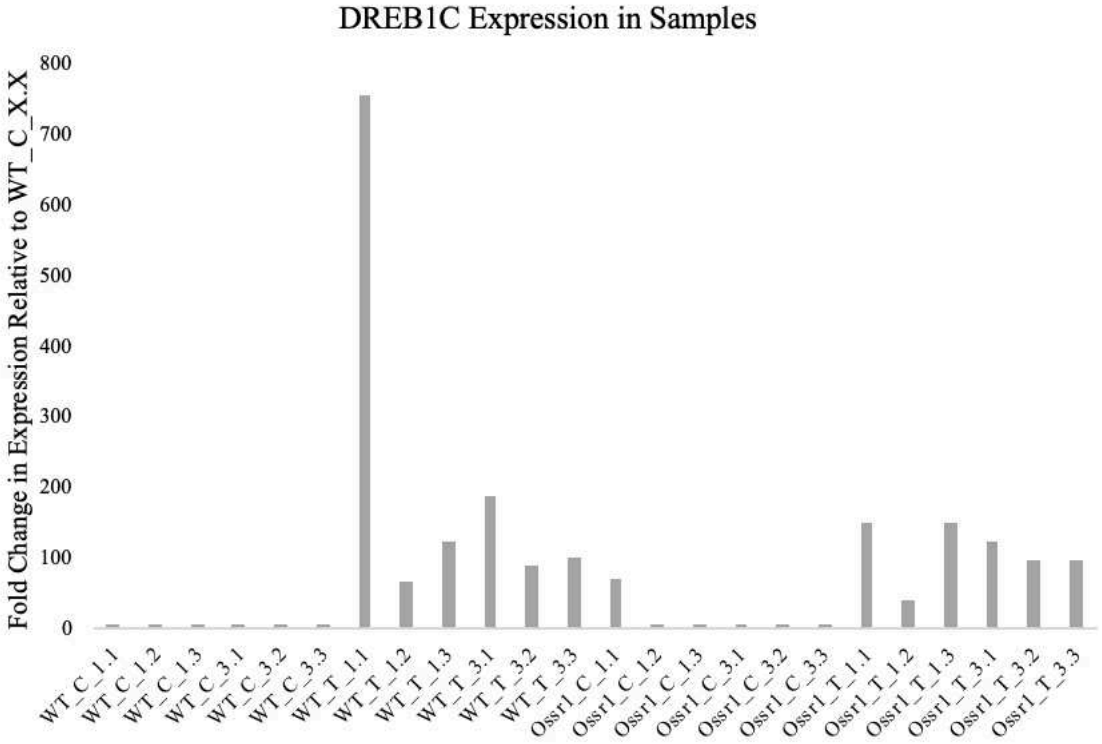


Figure 24. qRT-PCR of *OsDREB1C* expression is apparent in all treated WT and *Ossr1* samples. *Ossr1*_C_1.1 shows *OsDREB1C* expression and this sample was replaced with a different biological replicate. *OsDREB1C* expression is not evident in the control samples. The expression of *OsDREB1C* was normalized to *GAPDH* as the housekeeping gene. Fold change in expression relative to WT was calculated using the expression value of the corresponding WT_C_X.X sample.

2.2.2 Pipeline 1 Analysis

Novogene cleaned and assessed the quality of reads from all 24 samples (Table 6). A Phred score was used to measure the quality per base where a score of Q20 means that the chance that this base is called incorrectly is 1 in 100. Table 6 shows the percentage of the reads that had a Phred Q20 or greater. Reads from all samples had at least 97.79% of bases with a Q20 score and an error rate of 0.02%. Clean reads ranged from about 39 to 78 million per sample. Average correlation among biological replicates ranged 0.956-0.983 (Figure 25 and Table 7). About 78 to 90% of reads were uniquely mapped to the reference genome (Table 8). The range of FPKM values in all samples is presented in Table 9.

Differential expression (DE) of genes was assessed using DESeq across all variety, treatment, time point combinations (Table 10, Pipeline 1). Analysis returned total DE genes ranging from 53-6,664 per comparison. WT vs. *Ossr1* comparisons ranged from 53-146 DE genes. Treatment comparisons ranged from 1,334-6,664 DE genes. Timepoint comparison ranged from 158-3,708 DE genes. Timepoint comparisons differ greatly between treated and control conditions. DE genes in treated conditions ranged 3661-3708, while DE genes in control conditions were in the range of 158-559. In all comparison groups, there were more DE genes at the 3 hr time point than at the 1 hr time point. Top 20 annotated DE genes for comparisons averaging over timepoints (comparisons 13-16, Table 10) are shown in Tables 11-14. Top 100 DE genes are included in supplementary tables (Supplementary Table 1).

Table 6. **Pipeline 1: Raw and cleaned read numbers including quality score after Novogene perl scripts and before alignment.** Q20 refers to a Phred score of 20. In the case of a sample with a Q20 of 98.27%, 98.27% of bases were called with 99/100 certainty.

Sample Description	Raw reads	Clean reads	Raw bases	Clean bases	Error rate(%)	Q20(%)
Ossr1_C_1.1	63077082	62218078	9.5G	9.3G	0.02	98.27
Ossr1_C_1.2	48617228	47920848	7.3G	7.2G	0.02	98.94
Ossr1_C_1.3	44061808	43442728	6.6G	6.5G	0.02	98.94
Ossr1_C_3.1	69720472	68784906	10.5G	10.3G	0.02	98.36
Ossr1_C_3.2	64672326	63705888	9.7G	9.6G	0.02	98.3
Ossr1_C_3.3	63717120	62743322	9.6G	9.4G	0.02	98.33
Ossr1_T_1.1	77134590	75909334	11.6G	11.4G	0.02	98.39
Ossr1_T_1.2	67089192	65998232	10.1G	9.9G	0.02	98.4
Ossr1_T_1.3	69156426	68006706	10.4G	10.2G	0.02	98.49
Ossr1_T_3.1	65565546	64655232	9.8G	9.7G	0.03	97.79
Ossr1_T_3.2	39585916	39096922	5.9G	5.9G	0.02	98.88
Ossr1_T_3.3	39392240	38776612	5.9G	5.8G	0.02	98.95
WT_C_1.1	75527964	74519150	11.3G	11.2G	0.02	98.39
WT_C_1.2	44277174	43701850	6.6G	6.6G	0.02	98.92
WT_C_1.3	69996650	68999586	10.5G	10.3G	0.02	98.47
WT_C_3.1	79346876	78336488	11.9G	11.8G	0.02	98.33
WT_C_3.2	62667956	61679752	9.4G	9.3G	0.02	98.45
WT_C_3.3	69293310	68293796	10.4G	10.2G	0.02	98.4
WT_T_1.1	43688330	43037148	6.6G	6.5G	0.02	98.83
WT_T_1.2	74213470	73236980	11.1G	11G	0.02	98.32
WT_T_1.3	70233932	68996930	10.5G	10.3G	0.02	98.49
WT_T_3.1	70794542	69845458	10.6G	10.5G	0.02	98.13
WT_T_3.2	69829476	68781956	10.5G	10.3G	0.02	98.25
WT_T_3.3	58577990	57739862	8.8G	8.7G	0.02	98.4

Table 7. **Pipeline 1: Average Pearson correlation coefficients by the group.**

Sample Group	Average R^2
*Ossr1*_C_1	0.969
*Ossr1*_C_3	0.983
*Ossr1*_T_1	0.981
*Ossr1*_T_3	0.956
WT_C_1	0.975
WT_C_3	0.974
WT_T_1	0.962
WT_T_3	0.973

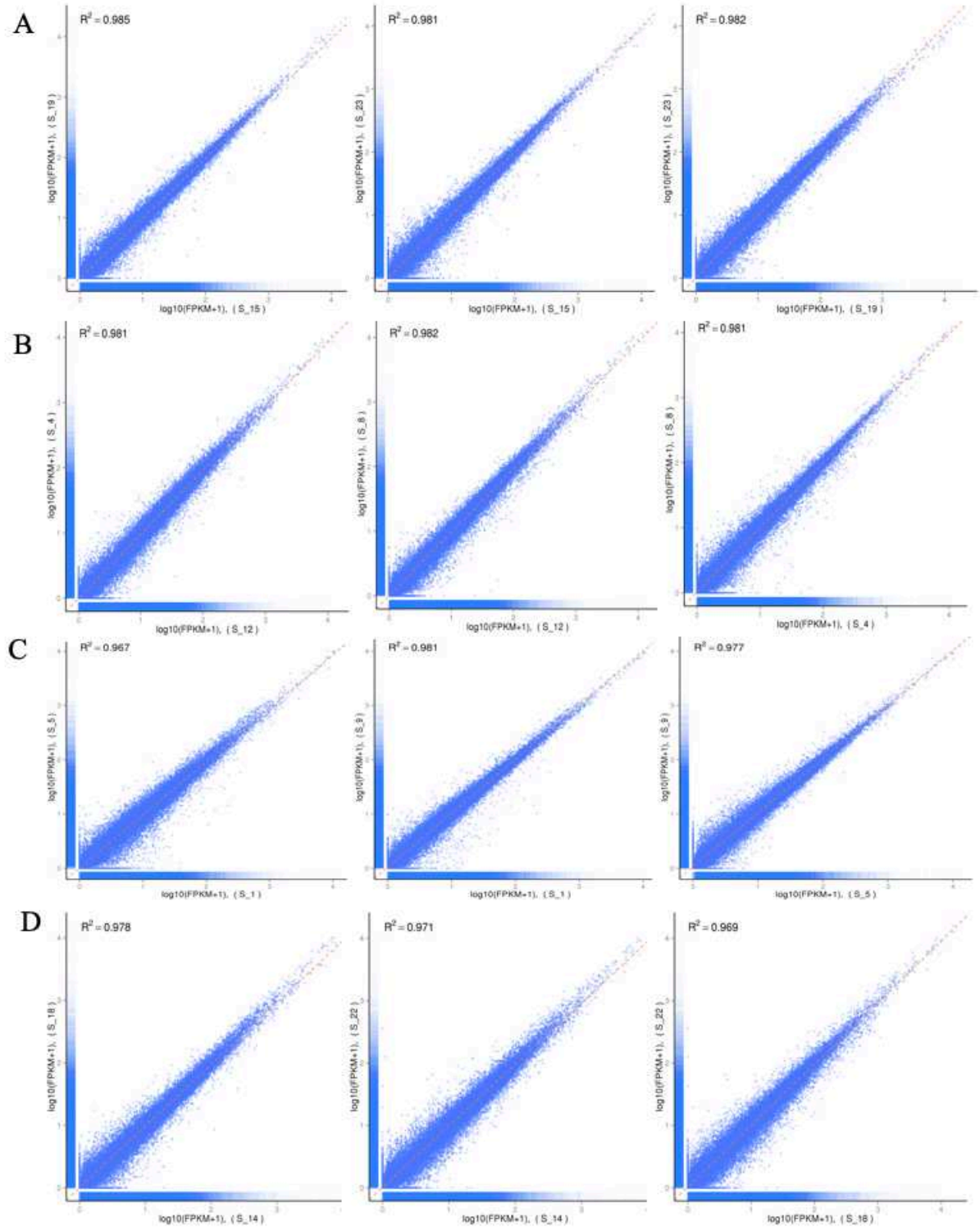


Figure 25. Pipeline 1: Pearson correlations of biological replicates. A) Correlation between *Ossr1*_C_3 samples: S_15, 19, and 23. R^2 values range 0.981-0.985. B) Correlation between *Ossr1*_T_1 samples: S_4, 8, and 12. R^2 values range 0.981-0.982. C) Correlation between WT_C_1 samples: S_1, 5, and 9. R^2 values range 0.967-0.981. D) Correlation between WT_T_3 samples: S_14, 18, and 22. R^2 values range 0.969-0.978.

Table 8. Pipeline 1: Mapped reads aligned to the IRGSPb.5 reference genome using TopHat.

Sample Description	Total reads	Total mapped	Multiple mapped	Uniquely mapped	Non-splice reads	Splice reads
Ossr1_C_1.1	62218078	56795902 (91.29%)	2546243 (4.09%)	54249659 (87.19%)	36094472 (58.01%)	18155187 (29.18%)
Ossr1_C_1.2	47920848	41967304 (87.58%)	4620524 (9.64%)	37346780 (77.93%)	24472530 (51.07%)	12874250 (26.87%)
Ossr1_C_1.3	43442728	38171622 (87.87%)	2090019 (4.81%)	36081603 (83.06%)	23942650 (55.11%)	12138953 (27.94%)
Ossr1_C_3.1	68784906	62243592 (90.49%)	2380699 (3.46%)	59862893 (87.03%)	39939011 (58.06%)	19923882 (28.97%)
Ossr1_C_3.2	63705888	57878401 (90.85%)	2366240 (3.71%)	55512161 (87.14%)	37289820 (58.53%)	18222341 (28.6%)
Ossr1_C_3.3	62743322	55773170 (88.89%)	3401472 (5.42%)	52371698 (83.47%)	34784937 (55.44%)	17586761 (28.03%)
Ossr1_T_1.1	75909334	68626275 (90.41%)	3217289 (4.24%)	65408986 (86.17%)	42400756 (55.86%)	23008230 (30.31%)
Ossr1_T_1.2	65998232	59883254 (90.73%)	3244620 (4.92%)	56638634 (85.82%)	36689126 (55.59%)	19949508 (30.23%)
Ossr1_T_1.3	68006706	62523796 (91.94%)	1309779 (1.93%)	61214017 (90.01%)	39491399 (58.07%)	21722618 (31.94%)
Ossr1_T_3.1	64655232	58366936 (90.27%)	2446769 (3.78%)	55920167 (86.49%)	37281546 (57.66%)	18638621 (28.83%)
Ossr1_T_3.2	39096922	35580208 (91.01%)	2711907 (6.94%)	32868301 (84.07%)	21735250 (55.59%)	11133051 (28.48%)
Ossr1_T_3.3	38776612	35315244 (91.07%)	1331754 (3.43%)	33983490 (87.64%)	22421784 (57.82%)	11561706 (29.82%)
WT_C_1.1	74519150	67585850 (90.7%)	3024622 (4.06%)	64561228 (86.64%)	42945000 (57.63%)	21616228 (29.01%)
WT_C_1.2	43701850	38814107 (88.82%)	3129162 (7.16%)	35684945 (81.66%)	23594366 (53.99%)	12090579 (27.67%)
WT_C_1.3	68999586	63713418 (92.34%)	3066000 (4.44%)	60647418 (87.9%)	39808164 (57.69%)	20839254 (30.2%)
WT_C_3.1	78336488	71731953 (91.57%)	2424237 (3.09%)	69307716 (88.47%)	46469923 (59.32%)	22837793 (29.15%)
WT_C_3.2	61679752	56309349 (91.29%)	2928517 (4.75%)	53380832 (86.55%)	35425801 (57.44%)	17955031 (29.11%)
WT_C_3.3	68293796	61208271 (89.62%)	4129644 (6.05%)	57078627 (83.58%)	38106773 (55.8%)	18971854 (27.78%)
WT_T_1.1	43037148	38512490 (89.49%)	1932916 (4.49%)	36579574 (85%)	24221343 (56.28%)	12358231 (28.72%)
WT_T_1.2	73236980	66205995 (90.4%)	4401573 (6.01%)	61804422 (84.39%)	40506767 (55.31%)	21297655 (29.08%)
WT_T_1.3	68996930	63313724 (91.76%)	2340692 (3.39%)	60973032 (88.37%)	40673625 (58.95%)	20299407 (29.42%)
WT_T_3.1	69845458	62920833 (90.09%)	3915301 (5.61%)	59005532 (84.48%)	38674668 (55.37%)	20330864 (29.11%)
WT_T_3.2	68781956	61511605 (89.43%)	3624611 (5.27%)	57886994 (84.16%)	38431648 (55.87%)	19455346 (28.29%)
WT_T_3.3	57739862	52911775 (91.64%)	1995620 (3.46%)	50916155 (88.18%)	33775740 (58.5%)	17140415 (29.69%)

Table 9. **Pipeline 1: FPKM value ranges by sample.**

Sample Description	FPKM Interval	0~1	1~3	3~15	15~60	>60
	Ossr1_C_1.1	19297(45.85%)	3394(8.06%)	7660(18.20%)	7742(18.39%)	3995(9.49%)
	Ossr1_C_1.2	19702(46.81%)	3335(7.92%)	7544(17.92%)	7498(17.82%)	4009(9.53%)
	Ossr1_C_1.3	19823(47.10%)	3383(8.04%)	7700(18.30%)	7319(17.39%)	3863(9.18%)
	Ossr1_C_3.1	19447(46.21%)	3452(8.20%)	7714(18.33%)	7523(17.87%)	3952(9.39%)
	Ossr1_C_3.2	19538(46.42%)	3392(8.06%)	7772(18.47%)	7496(17.81%)	3890(9.24%)
	Ossr1_C_3.3	19492(46.31%)	3331(7.91%)	7680(18.25%)	7552(17.94%)	4033(9.58%)
	Ossr1_T_1.1	19727(46.87%)	3494(8.30%)	7516(17.86%)	7386(17.55%)	3965(9.42%)
	Ossr1_T_1.2	19587(46.54%)	3356(7.97%)	7657(18.19%)	7512(17.85%)	3976(9.45%)
	Ossr1_T_1.3	19446(46.20%)	3318(7.88%)	7509(17.84%)	7679(18.25%)	4136(9.83%)
	Ossr1_T_3.1	19793(47.03%)	3511(8.34%)	7723(18.35%)	7106(16.88%)	3955(9.40%)
	Ossr1_T_3.2	20179(47.94%)	3577(8.50%)	7475(17.76%)	6923(16.45%)	3934(9.35%)
	Ossr1_T_3.3	20616(48.98%)	3425(8.14%)	7398(17.58%)	6697(15.91%)	3952(9.39%)
	WT_C_1.1	19764(46.96%)	3575(8.49%)	7745(18.40%)	7190(17.08%)	3814(9.06%)
	WT_C_1.2	20074(47.70%)	3474(8.25%)	7670(18.22%)	7116(16.91%)	3754(8.92%)
	WT_C_1.3	19545(46.44%)	3439(8.17%)	7662(18.20%)	7516(17.86%)	3926(9.33%)
	WT_C_3.1	19729(46.88%)	3453(8.20%)	7890(18.75%)	7282(17.30%)	3734(8.87%)
	WT_C_3.2	19489(46.31%)	3327(7.90%)	7750(18.41%)	7562(17.97%)	3960(9.41%)
	WT_C_3.3	19614(46.60%)	3345(7.95%)	7634(18.14%)	7558(17.96%)	3937(9.35%)
	WT_T_1.1	20527(48.77%)	3663(8.70%)	7473(17.76%)	6708(15.94%)	3717(8.83%)
	WT_T_1.2	19761(46.95%)	3499(8.31%)	7609(18.08%)	7415(17.62%)	3804(9.04%)
	WT_T_1.3	20147(47.87%)	3491(8.29%)	7628(18.12%)	7010(16.66%)	3812(9.06%)
	WT_T_3.1	20300(48.23%)	3609(8.57%)	7502(17.82%)	6788(16.13%)	3889(9.24%)
	WT_T_3.2	20486(48.67%)	3589(8.53%)	7549(17.94%)	6679(15.87%)	3785(8.99%)
	WT_T_3.3	20423(48.52%)	3611(8.58%)	7483(17.78%)	6714(15.95%)	3857(9.16%)

Table 10. Differentially expressed (DE) gene comparisons between sample groups. Up- and down-regulated and total DE genes are shown for each comparison. Up- and -down refers to expression in the mutant or treatment group. Comparisons 1-4 are at 1 hour, comparisons 5-8 are at 3 hours, comparisons 9-12 compare 1 hr and 3 hr timepoints, and comparisons 13-16 average over timepoints. Comparisons that "average over" timepoints do not consider biological replicates at 1 hr and 3 hrs to be different.

				Pipeline 1			Pipeline 2		
		Control Group	Treatment Group	Up	Down	Total	Up	Down	Total
Comparison	1	WT_C_1	Ossr1_C_1	69	73	142	55	14	69
	2	WT_T_1	Ossr1_T_1	34	19	53	8	17	25
	3	Ossr1_C_1	Ossr1_T_1	1462	1719	3181	1697	2171	3868
	4	WT_C_1	WT_T_1	570	764	1334	2505	1973	4478
	5	WT_C_3	Ossr1_C_3	94	30	124	67	20	87
	6	WT_T_3	Ossr1_T_3	52	10	62	83	28	111
	7	Ossr1_C_3	Ossr1_T_3	2365	2711	5076	5191	4686	9877
	8	WT_C_3	WT_T_3	3309	3355	6664	5436	5122	10558
	9	Ossr1_C_1	Ossr1_C_3	348	211	559	142	133	275
	10	Ossr1_T_1	Ossr1_T_3	1824	1873	3661	4724	3953	8677
	11	WT_C_1	WT_C_3	73	85	158	61	59	120
	12	WT_T_1	WT_T_3	1931	1777	3708	4589	4272	8861
	13	WT_C	Ossr1_C	103	43	146	132	72	204
	14	WT_T	Ossr1_T	56	11	67	163	152	315
	15	Ossr1_C	Ossr1_T	1243	1743	2986	5001	4729	9730
	16	WT_C	WT_T	1786	1605	3391	5485	5202	10687

Table 11. **Pipeline 1: Top 20 up- and down-regulated annotated DE genes in WT_C vs *Ossr1*_C.** Up and down refers to the expression of genes in the mutant as compared to WT. The most down-regulated gene in the mutant is the calmodulin-binding transcription activator *SR1*.

		Gene ID	Adjusted p-value	Annotation
Down	1	Os10t0375600-01	0.00E+00	CMTA_ARATH Calmodulin-binding transcription activator OS
	2	Os04t0103700-01	3.94E-64	Y2913_ARATH G-type lectin S-receptor-like serine/threonine-protein kinase At2g19130 OS
	3	Os01t0847600-01	1.06E-26	AKRCA_ARATH Aldo-keto reductase family 4 member C10 OS
	4	Os06t0665800-01	5.69E-14	HMA9_ORYSJ Cation-transporting ATPase HMA5 OS
	5	Os09t0521300-00	4.59E-12	PCF2_ORYSJ Transcription factor PCF2 OS
	6	Os04t0125700-01	1.08E-06	LRK91_ARATH L-type lectin-domain containing receptor kinase IX.1 OS
	7	Os05t0160600-01	1.08E-06	DGP14_ARATH Putative protease Do-like 14 OS
	8	Os11t0529900-00	3.63E-06	CUS_ORYSJ Bisdemethoxycurcumin synthase OS
	9	Os08t0290700-01	3.41E-05	Y1103_ORYSJ Flavonoid O-methyltransferase-like protein Os11g0303600 OS
	10	Os04t0357300-01	1.04E-04	PIF1_DANRE ATP-dependent DNA helicase PIF1 OS
	11	Os04t0112200-00	1.74E-04	E2FB_ARATH Transcription factor E2FB OS
	12	Os04t0116800-01	4.56E-04	KCS6_ARATH 3-ketoacyl-CoA synthase 6 OS
	13	Os09t0403300-00	9.56E-04	C7351_ARATH Cytokinin hydroxylase OS
	14	Os09t0505400-00	1.75E-03	PIN5B_ORYSJ Probable auxin efflux carrier component 5b OS
	15	Os07t0127700-01	1.88E-03	PR13_HORVU Pathogenesis-related protein PRB1-3 OS
	16	Os04t0445100-01	3.75E-03	HS232_ORYSJ 23.2 kDa heat shock protein OS
	17	Os11t0641800-01	6.94E-03	LAC20_ORYSJ Laccase-20 OS
	18	Os03t0369000-01	8.49E-03	PER2_MAIZE Peroxidase 2 OS
	19	Os03t0667500-01	8.56E-03	IRT1_ORYSJ Fe(2+) transport protein 1 OS
	20	Os06t0564700-01	1.23E-02	CYSKP_SPIOL Cysteine synthase, chloroplastic/chromoplastic OS
Up	1	Os02t0765900-00	6.68E-75	NIR_ORYSJ Ferredoxin--nitrite reductase, chloroplastic OS
	2	Os04t0510000-01	1.60E-69	Y3643_ORYSJ B3 domain-containing protein Os03g0164300 OS
	3	Os09t0358000-00	8.00E-46	Y5189_ARATH Probable LRR receptor-like protein kinase At1g51890 OS
	4	Os02t0288600-01	8.37E-37	BURP4_ORYSJ BURP domain-containing protein 4 OS
	5	Os12t0228500-01	1.64E-30	GSTT3_ARATH Glutathione S-transferase T3 OS
	6	Os02t0258300-01	3.76E-23	XLG1_ARATH Extra-large guanine nucleotide-binding protein 1 OS
	7	Os01t0960500-01	2.41E-22	RGLG3_ARATH E3 ubiquitin-protein ligase RGLG3 OS
	8	Os04t0116600-01	4.27E-18	KCR1_ARATH Very-long-chain 3-oxoacyl-CoA reductase 1 OS
	9	Os07t0636600-01	5.71E-14	DIR5_ARATH Dirigent protein 5 OS
	10	Os11t0213000-01	1.80E-12	CRK29_ARATH Cysteine-rich receptor-like protein kinase 29 OS
	11	Os03t0861300-01	6.34E-12	PIP28_ORYSJ Probable aquaporin PIP2-8 OS
	12	Os04t0249500-00	9.94E-11	SUS7_ORYSJ Sucrose synthase 7 OS
	13	Os04t0204000-00	1.71E-10	UGT13_HORVV UDP-glucosyltransferase UGT13248 OS
	14	Os05t0472400-00	8.16E-10	ZIP9_ORYSJ Zinc transporter 9 OS
	15	Os04t0474900-01	1.74E-09	BGL13_ORYSJ Beta-glucosidase 13 OS
	16	Os10t0429400-00	1.87E-09	BPM1_ARATH BTB/POZ and MATH domain-containing protein 1 OS
	17	Os05t0175450-00	3.30E-08	Y3643_ORYSJ B3 domain-containing protein Os03g0164300 OS
	18	Os12t0554100-01	3.85E-08	TBL16_ARATH Protein trichome birefringence-like 16 OS
	19	Os04t0117900-01	7.62E-07	AMI1_ORYSJ Amidase 1 OS
	20	Os04t0117600-01	1.36E-06	DUS3L_ORYSJ tRNA-dihydrouridine(47) synthase [NAD(P)(+)]-like OS

Table 12. **Pipeline 1: Top 20 up- and down-regulated annotated genes in WT_T vs *Ossr1*_T.** Up and down refer to the expression of genes in the mutant as compared to WT. The most down-regulated gene in the mutant is the calmodulin-binding transcription activator *SR1*. 11 genes are down-regulated in the mutant and only 7 of those 11 are annotated.

		Gene ID	Adjusted p-value	Annotation
Down	1	Os10t0375600-01	2.98E-71	CMTA_ARATH Calmodulin-binding transcription activator OS
	2	Os04t0103700-01	6.99E-13	Y2913_ARATH G-type lectin S-receptor-like serine/threonine-protein kinase
	3	Os01t0847600-01	3.41E-07	AKRCA_ARATH Aldo-keto reductase family 4 member C10 OS
	4	Os04t0125700-01	6.43E-03	LRK91_ARATH L-type lectin-domain containing receptor kinase IX.1 OS
	5	Os11t0529900-00	1.48E-02	CUS_ORYSJ Bisdemethoxycurcumin synthase OS
	6	Os09t0521300-00	2.00E-02	PCF2_ORYSJ Transcription factor PCF2 OS
	7	Os09t0360500-01	2.37E-02	KCS17_ARATH 3-ketoacyl-CoA synthase 17 OS
Up	1	Os02t0288600-01	4.84E-18	BURP4_ORYSJ BURP domain-containing protein 4 OS
	2	Os09t0358000-00	7.36E-17	Y5189_ARATH Probable LRR receptor-like protein kinase At1g51890 OS
	3	Os02t0765900-00	2.98E-14	NIR_ORYSJ Ferredoxin--nitrite reductase, chloroplastic OS
	4	Os04t0510000-01	2.75E-09	Y3643_ORYSJ B3 domain-containing protein Os03g0164300 OS
	5	Os12t0228500-01	1.99E-06	GSTT3_ARATH Glutathione S-transferase T3 OS
	6	Os05t0175450-00	4.38E-06	Y3643_ORYSJ B3 domain-containing protein Os03g0164300 OS
	7	Os05t0472400-00	4.02E-05	ZIP9_ORYSJ Zinc transporter 9 OS
	8	Os06t0141400-01	4.69E-04	NO93_SOYBN Early nodulin-93 OS
	9	Os05t0166600-00	5.82E-04	Y2913_ARATH G-type lectin S-receptor-like serine/threonine-protein kinase
	10	Os10t0139700-01	1.66E-03	C89A2_ARATH Cytochrome P450 89A2 OS
	11	Os10t0429400-00	2.06E-03	BPM1_ARATH BTB/POZ and MATH domain-containing protein 1 OS
	12	Os04t0249500-00	4.78E-03	SUS7_ORYSJ Sucrose synthase 7 OS
	13	Os04t0204000-00	5.31E-03	UGT13_HORVV UDP-glucosyltransferase UGT13248 OS
	14	Os01t0267300-00	1.88E-02	SPZ12_ORYSJ Putative serpin-Z12 OS
	15	Os11t0620300-01	2.00E-02	NLTP2_MAIZE Probable non-specific lipid-transfer protein 2 OS
	16	Os03t0371000-01	2.41E-02	C70B2_ARATH Cytochrome P450 709B2 OS
	17	Os02t0240100-01	2.49E-02	PER70_MAIZE Peroxidase 70 OS
	18	Os11t0213000-01	2.65E-02	CRK29_ARATH Cysteine-rich receptor-like protein kinase 29 OS
	19	Os10t0469300-00	2.69E-02	Y1571_ARATH Probable leucine-rich repeat receptor-like protein kinase At1g35710
	20	Os01t0960500-01	4.10E-02	RGLG3_ARATH E3 ubiquitin-protein ligase RGLG3 OS

Table 13. **Pipeline 1: Top 20 up- and down-regulated annotated genes in *Ossr1*_C vs *Ossr1*_T.** Up and down refer to the expression of genes in the treated as compared to control.

		Gene ID	Adjusted p-value	Annotation
Down	1	Os07t0677300-01	2.40E-44	PER2_ORYSJ Peroxidase 2 OS
	2	Os03t0107300-01	7.56E-42	LSI2_ORYSJ Silicon efflux transporter LSI2 OS
	3	Os02t0745100-01	3.27E-34	NIP21_ORYSJ Aquaporin NIP2-1 OS
	4	Os11t0179700-00	1.63E-33	DIR2_ARATH Dirigent protein 2 OS
	5	Os07t0442900-01	1.65E-32	CSPL7_ORYSJ CASP-like protein 1D1 OS
	6	Os07t0635200-00	4.39E-30	C70B2_ARATH Cytochrome P450 709B2 OS
	7	Os02t0684100-00	6.04E-30	SOT15_ARATH Cytosolic sulfotransferase 15 OS
	8	Os01t0347600-01	1.13E-28	BROM1_ANACO Fruit bromelain OS
	9	Os01t0347800-00	1.25E-28	BROM1_ANACO Fruit bromelain OS
	10	Os07t0645300-01	3.53E-28	DMP1_ARATH Protein DMP1 OS
	11	Os06t0641500-00	3.39E-27	C7D55_HYOMU Premnaspirodiene oxygenase OS
	12	Os02t0218800-01	5.98E-27	C74A4_ORYSJ Allene oxide synthase 4 OS
	13	Os07t0104500-01	1.36E-26	PER1_ORYSJ Peroxidase 1 OS
	14	Os12t0259800-00	1.61E-26	LAC25_ORYSJ Laccase-25 OS
	15	Os05t0134800-00	1.14E-25	PER2_MAIZE Peroxidase 2 OS
	16	Os02t0650300-01	2.97E-25	YSL15_ORYSJ Iron-phytosiderophore transporter YSL15 OS
	17	Os03t0667500-01	4.89E-25	IRT1_ORYSJ Fe(2+) transport protein 1 OS
	18	Os10t0191300-01	8.30E-25	PR12_HORVU Pathogenesis-related protein PRB1-2 OS
	19	Os09t0311600-01	2.53E-24	RPS2_ARATH Disease resistance protein RPS2 OS
	20	Os10t0547500-01	3.29E-24	LSI3_ORYSJ Silicon efflux transporter LSI3 OS
Up	1	Os04t0244800-01	2.07E-29	HIP26_ARATH Heavy metal-associated isoprenylated plant protein 26 OS
	2	Os03t0125100-01	5.06E-28	BCH_GENLU Beta-carotene 3-hydroxylase, chloroplastic OS
	3	Os03t0820300-01	2.71E-27	ZAT7_ARATH Zinc finger protein ZAT7 OS
	4	Os03t0386000-01	2.61E-26	YGI3_SCHPO Uncharacterized WD repeat-containing protein C2A9.03 OS
	5	Os01t0868000-00	3.84E-21	EF110_ARATH Ethylene-responsive transcription factor ERF110 OS
	6	Os05t0494600-01	4.77E-21	EDL3_ARATH EID1-like F-box protein 3 OS
	7	Os01t0901600-01	6.34E-20	4CLL6_ORYSJ 4-coumarate--CoA ligase-like 6 OS
	8	Os09t0325700-01	6.92E-20	P2C68_ORYSJ Probable protein phosphatase 2C 68 OS
	9	Os01t0314800-01	3.24E-18	SAG21_ARATH Protein SENESCENCE-ASSOCIATED GENE 21, mitochondrial OS
	10	Os05t0541100-01	3.96E-18	IQD1_ARATH Protein IQ-DOMAIN 1 OS
	11	Os03t0268600-01	5.73E-18	P2C30_ORYSJ Probable protein phosphatase 2C 30 OS
	12	Os01t0863300-01	3.06E-17	DIV_ANTMA Transcription factor DIVARICATA OS
	13	Os07t0418500-01	4.84E-17	C70B2_ARATH Cytochrome P450 709B2 OS
	14	Os05t0583000-01	8.86E-17	WRK28_ARATH WRKY transcription factor 28 OS
	15	Os01t0609200-00	3.14E-16	AB35G_ORYSJ ABC transporter G family member 35 OS
	16	Os04t0541700-01	1.00E-15	HOX22_ORYSJ Homeobox-leucine zipper protein HOX22 OS
	17	Os01t0699100-01	4.65E-15	M3K17_ARATH Mitogen-activated protein kinase kinase kinase 17 OS
	18	Os12t0594000-00	1.05E-14	JGB_ARATH Protein JINGUBANG OS
	19	Os06t0652300-00	1.19E-14	FCL2_ORYSJ Putative GDP-L-fucose synthase 2 OS
	20	Os01t0128300-00	1.82E-14	MHZ4_ORYSJ Protein MAO HUZI 4, chloroplastic OS

Table 14. Pipeline 1: Top 20 up- and down-regulated annotated genes in WT_C vs WT_T. Up and down refer to the expression of genes in the treated as compared to control.

		Gene ID	Adjusted p-value	Annotation
Down	1	Os02t0650300-01	1.64E-54	YSL15_ORYSJ Iron-phytosiderophore transporter YSL15 OS
	2	Os06t0169001-01	8.63E-48	GOS9_ORYSJ Protein GOS9 OS
	3	Os09t0564200-01	4.44E-43	CYSP_HEMSP Thiol protease SEN102 OS
	4	Os11t0134900-01	2.47E-39	ZIFL1_ARATH Protein ZINC INDUCED FACILITATOR-LIKE 1 OS
	5	Os03t0107300-01	6.19E-34	LSI2_ORYSJ Silicon efflux transporter LSI2 OS
	6	Os02t0745100-01	2.41E-33	NIP21_ORYSJ Aquaporin NIP2-1 OS
	7	Os10t0323500-01	1.52E-26	BGL34_ORYSJ Beta-glucosidase 34 OS
	8	Os01t0355250-00	1.27E-25	SALT_ORYSJ Salt stress-induced protein OS
	9	Os07t0442900-01	6.26E-25	CSPL7_ORYSJ CASP-like protein 1D1 OS
	10	Os12t0137700-01	6.86E-25	SOT5_ARATH Cytosolic sulfotransferase 5 OS
	11	Os02t0218800-01	1.42E-24	C74A4_ORYSJ Allene oxide synthase 4 OS
	12	Os03t0667500-01	1.42E-24	IRT1_ORYSJ Fe(2+) transport protein 1 OS
	13	Os12t0260500-01	3.21E-24	SDR5_ARATH Short-chain dehydrogenase reductase 5 OS
	14	Os11t0593000-01	1.68E-23	NPC4_ARATH Non-specific phospholipase C4 OS
	15	Os01t0220100-01	2.00E-23	GUN2_ORYSJ Endoglucanase 2 OS
	16	Os02t0684100-00	1.83E-22	SOT15_ARATH Cytosolic sulfotransferase 15 OS
	17	Os03t0809000-01	2.35E-22	DIR15_ARATH Dirigent protein 15 OS
	18	Os02t0131800-01	1.03E-21	NRAT1_ORYSJ Metal transporter NRAT1 OS
	19	Os06t0641400-01	1.17E-21	C71DC_CATRO Tabersonine 16-hydroxylase 1 OS
	20	Os07t0677300-01	1.81E-21	PER2_ORYSJ Peroxidase 2 OS
Up	1	Os01t0858350-01	2.78E-31	C94C1_ARATH Cytochrome P450 94C1 OS
	2	Os09t0325700-01	2.79E-25	P2C68_ORYSJ Probable protein phosphatase 2C 68 OS
	3	Os05t0494600-01	5.09E-24	EDL3_ARATH EID1-like F-box protein 3 OS
	4	Os01t0699500-01	1.40E-22	M3K17_ARATH Mitogen-activated protein kinase kinase kinase 17 OS
	5	Os12t0168100-01	9.14E-22	EF110_ARATH Ethylene-responsive transcription factor ERF110 OS
	6	Os03t0125100-01	9.91E-22	BCH_GENLU Beta-carotene 3-hydroxylase, chloroplastic OS
	7	Os03t0820300-01	1.89E-20	ZAT7_ARATH Zinc finger protein ZAT7 OS
	8	Os04t0541700-01	2.77E-20	HOX22_ORYSJ Homeobox-leucine zipper protein HOX22 OS
	9	Os07t0418500-01	4.31E-20	C70B2_ARATH Cytochrome P450 709B2 OS
	10	Os04t0244800-01	4.26E-19	HIP26_ARATH Heavy metal-associated isoprenylated plant protein 26 OS
	11	Os07t0687900-01	7.46E-18	GOLS2_SOLLC Galactinol synthase 2 OS
	12	Os01t0802600-00	1.13E-17	EDL3_ARATH EID1-like F-box protein 3 OS
	13	Os01t0901600-01	1.17E-17	4CLL6_ORYSJ 4-coumarate--CoA ligase-like 6 OS
	14	Os03t0820400-01	1.35E-17	ZAT8_ARATH Zinc finger protein ZAT8 OS
	15	Os02t0649300-01	1.73E-17	HOX24_ORYSJ Homeobox-leucine zipper protein HOX24 OS
	16	Os06t0216350-00	1.97E-17	OPR1_ORYSJ 12-oxophytodienoate reductase 1 OS
	17	Os09t0555500-01	8.91E-17	PSY3_ORYSJ Phytoene synthase 3, chloroplastic OS
	18	Os01t0846300-01	1.38E-16	P2C09_ORYSJ Probable protein phosphatase 2C 9 OS
	19	Os11t0177400-01	2.08E-16	AB25G_ARATH ABC transporter G family member 25 OS
	20	Os02t0764700-01	1.29E-15	EF112_ARATH Ethylene-responsive transcription factor ERF112 OS

Gene ontology enrichment analysis was performed using GOSeq package in R with GO terms significant at a corrected p-value of <0.5. Figures 27-30 show significant up- and down-regulated GO terms (top 30 GO terms if more that 30 GO terms were found significant) for the same group comparisons given in Tables 11-14 of DE genes. GO terms appear in the two categories of Biological Process (top) and Molecular Function (bottom) (Figures 26-29).

2.2.3 Pipeline 2 Analysis

Reads were cleaned using Trimmomatic and aligned to the RGAP v.7 Nipponbare reference genome using STAR. Uniquely mapped reads ranged from 17 to 37 million (81.68-95.55% of total reads) and 488,797-1,582,585 (1.65-5.93%) reads mapped to multiple locations (Table 15). HTSeq was used to determine gene counts and the count matrix was piped to R.

Multidimensional scaling (MDS) plots can be used to visualize the relatedness of samples. Two-dimensional analysis of logFC of samples (see Figure 5 in Materials and Methods section) groups similar samples. All control samples grouped in the upper right. All treated 1hr samples group in the bottom center, again with tighter groupings by variety. All treated 3hr samples grouped in the upper left. Within these groups, same genotypes were also grouped together, suggesting closeness of biological replicates.

To test for DE, each gene requires a test, thus the issue of multiple testing must be addressed. EdgeR accounts for multiple testing in two ways. First, it filters out genes that are lowly expressed in multiple samples. It then looks at library sizes, which can vary up to 2-fold and calculates normalization factors (Table 16).

Figure 26. Pipeline 1: Most enriched down-regulated GO terms for WT_C_1 vs *Ossr1*_C_1. Down refers to expression of genes in the mutant. Significant terms at an adjusted p-value of <0.05 are indicated with an asterisk, *. No significant GO terms for up-regulated genes were observed. Additionally, no significant GO terms for comparisons averaging across timepoints were observed.

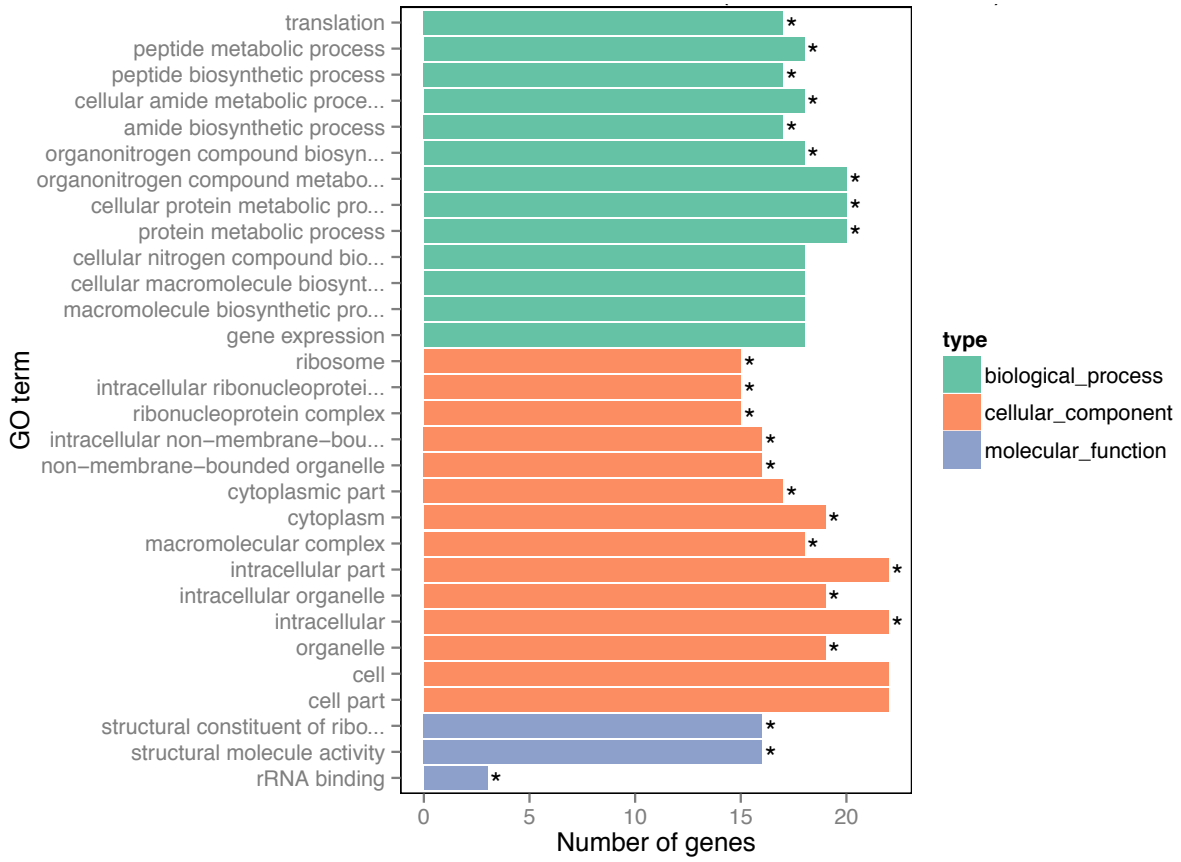
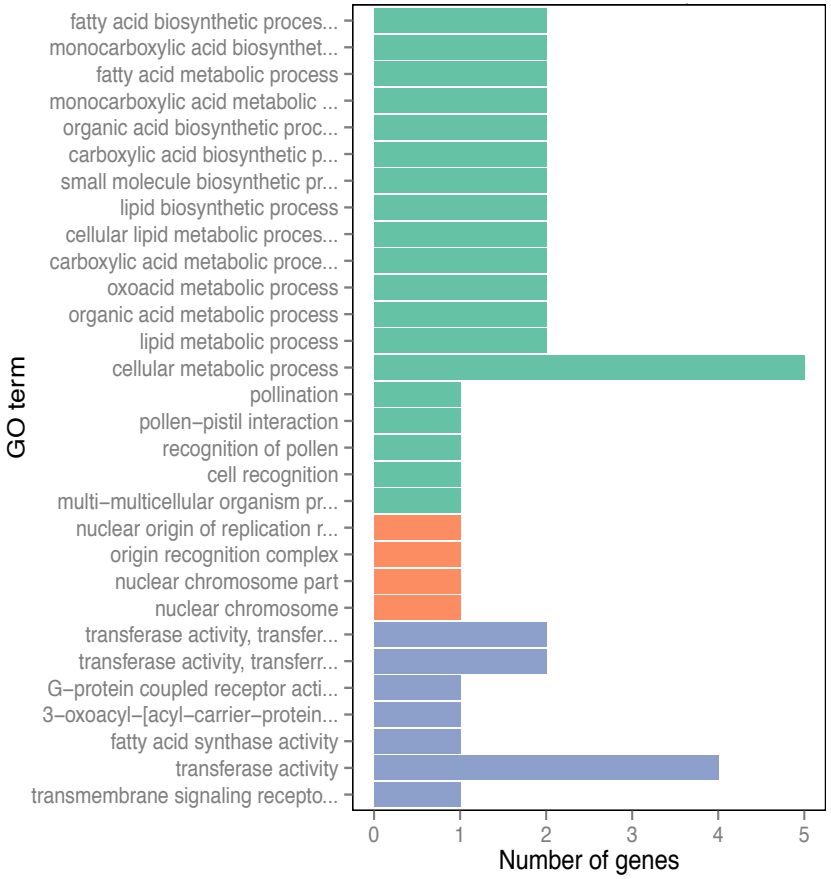


Figure 27. Pipeline 1: Most enriched GO terms for WT_T vs Mu_T. A) GO terms for down-regulated genes in the mutant. B) GO terms for up-regulated genes in the mutant. No significant terms for up- or down-regulated genes were observed (indicated by no asterisks). Instead, shown are terms with an over-represented p-value of <0.05.

A) Down-regulated



B) Up-regulated

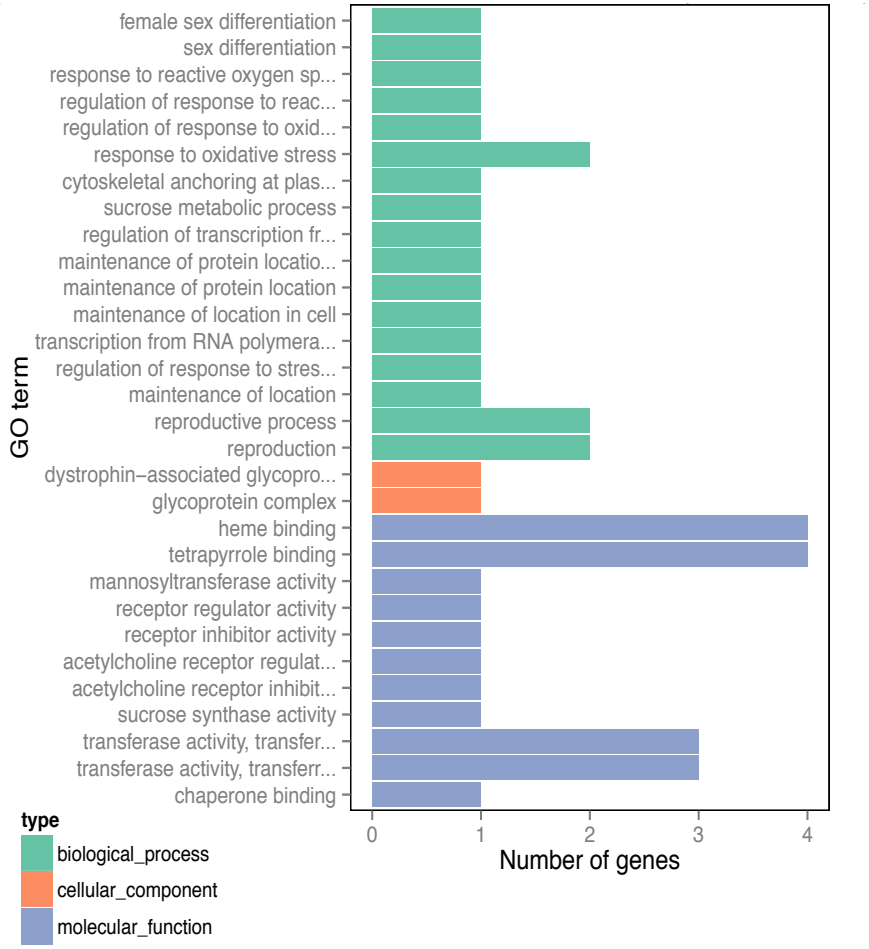


Figure 28. Pipeline 1: Most enriched GO terms for Mu_C vs Mu_T. A) GO terms for down-regulated genes in the mutant. B) GO terms for up-regulated genes in the mutant. Significant terms at an adjusted p-value of <0.05 are indicated with an asterisk, *.

A) Down-regulated

B) Up-regulated

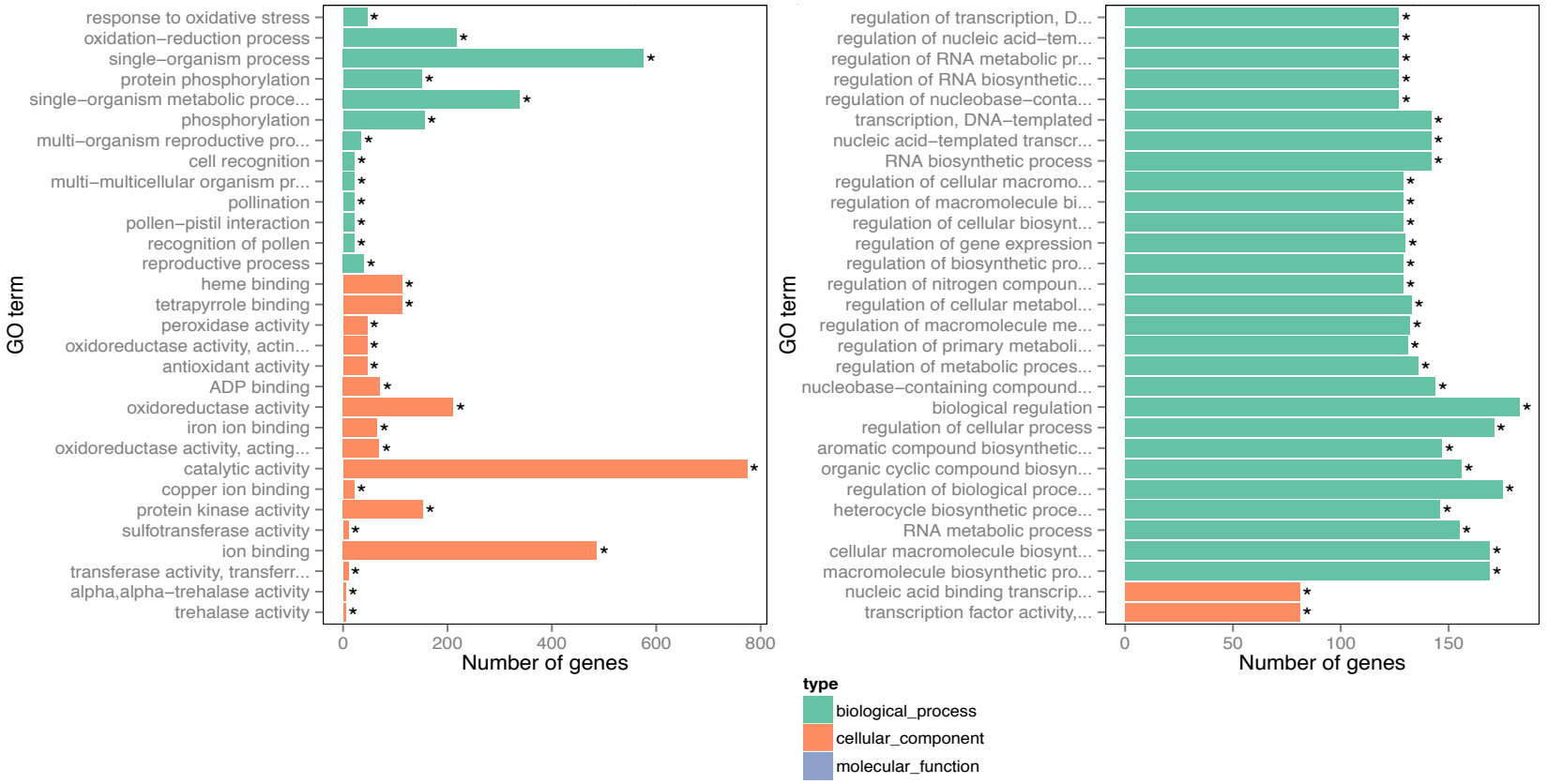


Figure 29. Pipeline 1: Most enriched GO terms for WT_C vs WT_T. A) GO terms for down-regulated genes in the mutant. B) GO terms for up-regulated genes in the mutant. Significant terms at an adjusted p-value of <0.05 are indicated with an asterisk, *.

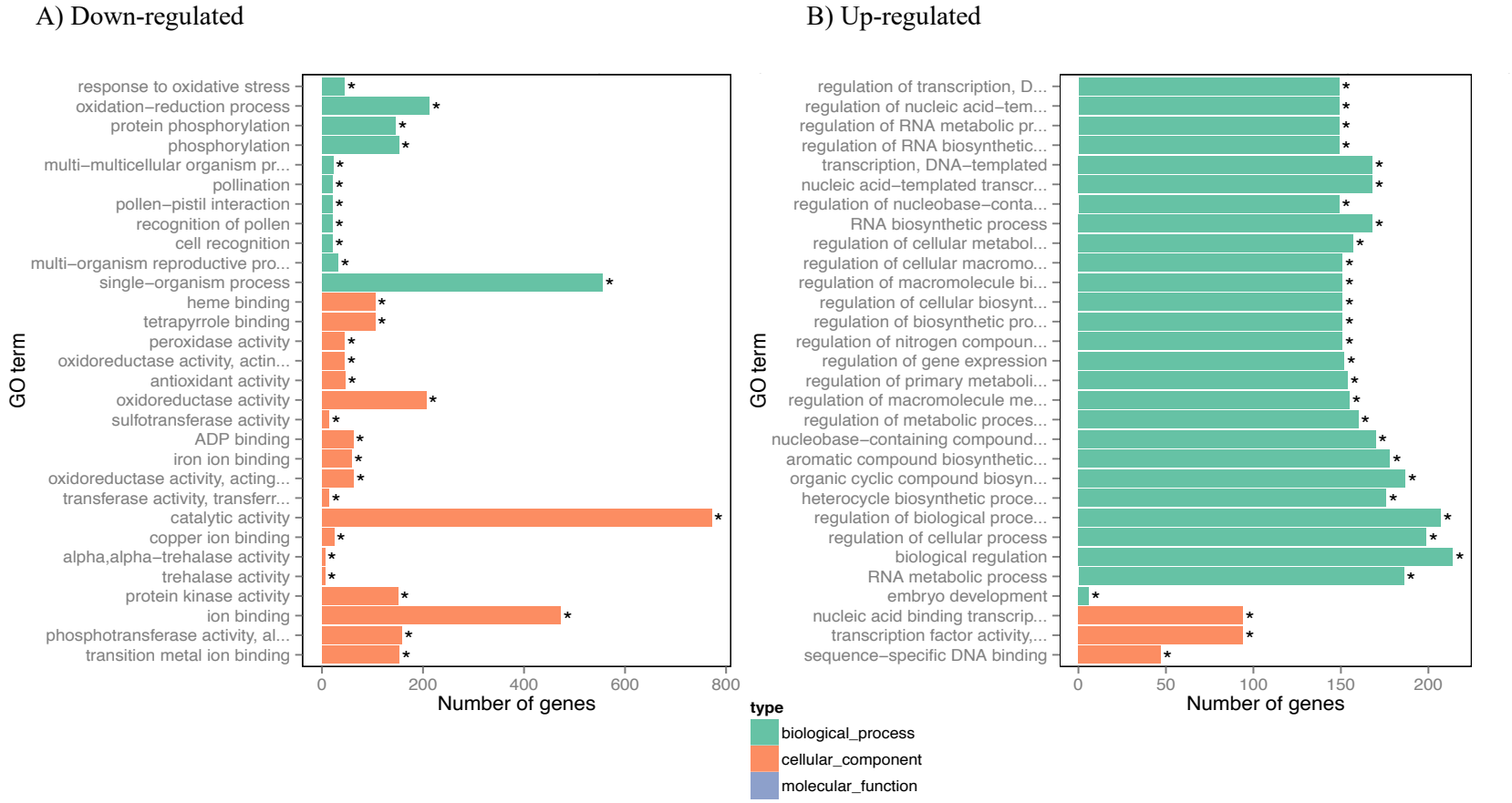


Table 15. Pipeline 2: Mapped reads aligned to the RGAP v.7 genome using STAR.

Sample Description	Input reads	Reads mapped per sample	Reads mapped %	Uniquely mapped	Uniquely mapped %	Multiple mapped	Multiple mapped %	Splice reads
Ossr1_C_1.1	30786760	29439991	95.63%	28523015	92.65%	916976	2.98%	20541372
Ossr1_C_1.2	23868325	20911433	87.61%	19495908	81.68%	1415525	5.93%	14530114
Ossr1_C_1.3	21633876	19692055	91.02%	18999255	87.82%	692800	3.20%	13646309
Ossr1_C_3.1	34051568	32364796	95.05%	31483230	92.46%	881566	2.59%	22519635
Ossr1_C_3.2	31497350	30083523	95.51%	29238506	92.83%	845017	2.68%	20657738
Ossr1_C_3.3	31032180	28643244	92.30%	27521192	88.69%	1122052	3.62%	19869649
Ossr1_T_1.1	37575064	35556016	94.63%	34427370	91.62%	1128646	3.00%	26126316
Ossr1_T_1.2	32685278	30977040	94.77%	29837372	91.29%	1139668	3.49%	22641834
Ossr1_T_1.3	33705565	32756936	97.19%	32200184	95.53%	556752	1.65%	24858057
Ossr1_T_3.1	31825489	30413746	95.56%	29566986	92.90%	846760	2.66%	21258329
Ossr1_T_3.2	19472207	18046113	92.68%	17165921	88.16%	880192	4.52%	12640081
Ossr1_T_3.3	19310181	18303775	94.79%	17814978	92.26%	488797	2.53%	13168700
WT_C_1.1	36869542	35079949	95.15%	33958802	92.11%	1121147	3.04%	24440980
WT_C_1.2	21761898	19638246	90.24%	18698077	85.92%	940169	4.32%	13626116
WT_C_1.3	34194246	32901214	96.22%	31894844	93.28%	1006370	2.94%	23614151
WT_C_3.1	38707414	37390160	96.60%	36487904	94.27%	902256	2.33%	25780839
WT_C_3.2	30545898	29011426	94.98%	27974576	91.58%	1036850	3.39%	20291814
WT_C_3.3	33810999	31344529	92.71%	30033962	88.83%	1310567	3.88%	21448115
WT_T_1.1	21414480	19900899	92.93%	19213803	89.72%	687096	3.21%	13934850
WT_T_1.2	36235169	34112147	94.14%	32529562	89.77%	1582585	4.37%	24074464
WT_T_1.3	34169272	33081323	96.82%	32206996	94.26%	874327	2.56%	22915354
WT_T_3.1	34482459	32413130	94.00%	31047641	90.04%	1365489	3.96%	23142854
WT_T_3.2	33980417	31821072	93.65%	30533270	89.86%	1287802	3.79%	22056184
WT_T_3.3	28593556	27517820	96.24%	26791107	93.70%	726713	2.54%	19502489

Table 16. Pipeline 2: Filtering of lowly expressed genes and library normalization by edgeR.

Sample	Group	Library size before filtering	Library size after filtering	Normalization Factors
Ossr1_C_1.1	Ossr1_C_1	49695630	49687965	1.0895735
Ossr1_C_1.2	Ossr1_C_1	34136728	34130884	1.0694864
Ossr1_C_1.3	Ossr1_C_1	32739114	32734261	1.024299
Ossr1_C_3.1	Ossr1_C_3	55108430	55100052	1.0593579
Ossr1_C_3.2	Ossr1_C_3	50807943	50800581	1.0285747
Ossr1_C_3.3	Ossr1_C_3	48340707	48332469	1.1026372
Ossr1_T_1.1	Ossr1_T_1	60126350	60114639	1.0296289
Ossr1_T_1.2	Ossr1_T_1	51612551	51604154	1.0583309
Ossr1_T_1.3	Ossr1_T_1	56337390	56328983	1.0988311
Ossr1_T_3.1	Ossr1_T_3	51744962	51736076	0.9673183
Ossr1_T_3.2	Ossr1_T_3	30583617	30578860	0.972238
Ossr1_T_3.3	Ossr1_T_3	31424051	31419281	0.9197016
WT_C_1.1	WT_C_1	58727456	58718265	0.9805174
WT_C_1.2	WT_C_1	32306704	32302420	0.987862
WT_C_1.3	WT_C_1	55368229	55359779	1.0499672
WT_C_3.1	WT_C_3	62976185	62966846	0.9737863
WT_C_3.2	WT_C_3	49120794	49110307	1.0925651
WT_C_3.3	WT_C_3	52342886	52333679	1.0426199
WT_T_1.1	WT_T_1	33287792	33281827	0.8752161
WT_T_1.2	WT_T_1	56335378	56325550	1.0086337
WT_T_1.3	WT_T_1	55544262	55536219	0.931022
WT_T_3.1	WT_T_3	55039111	55031528	0.9146178
WT_T_3.2	WT_T_3	53322190	53313326	0.8949883
WT_T_3.3	WT_T_3	47140817	47132025	0.8871113

The biological coefficient of variation (BCV) is used to estimate the variation among the samples. Figure 30 shows BCV against average gene abundance given by log counts per million (CPM). Estimated dispersion is 0.0736 and the square root of this is the common dispersion, 0.2713 (red line, Figure 30). Common dispersion should range between 0.2 and 0.4 and the dispersion in our samples is 0.2713. If it is greater than 0.5, this can greatly reduce the number of DE genes.

DE was assessed across all variety, treatment, and time point combinations (Table 10, Pipeline 2). Analysis returned total DE genes ranging from 25 - 10,687 depending on the comparison. WT vs. *Ossr1* comparisons ranged from 25 - 315 DE genes. Treatment comparisons ranged from 3,868 - 10,687 DE genes. Timepoint comparison ranged from 120 – 8,861 DE genes. Timepoint comparisons differ greatly between treated and control conditions. DE genes in treated condition ranged from 8,677 – 8,861, while DE genes in control condition ranged from 20-275. In all comparison groups, there are more DE genes at the 3hr time point than at the 1hr timepoint. Top 20 annotated DE genes are shown in Tables 17-20 for comparisons averaging across timepoints. Top 100 DE genes are included in Supplementary Table 2.

GO singular enrichment analysis (GO) was determined using AgriGO, a gene ontology database aimed for use with crop species. Top 10 significant GO terms (or fewer when less than 10 terms were significant) are shown for each comparison (Tables 21-24).

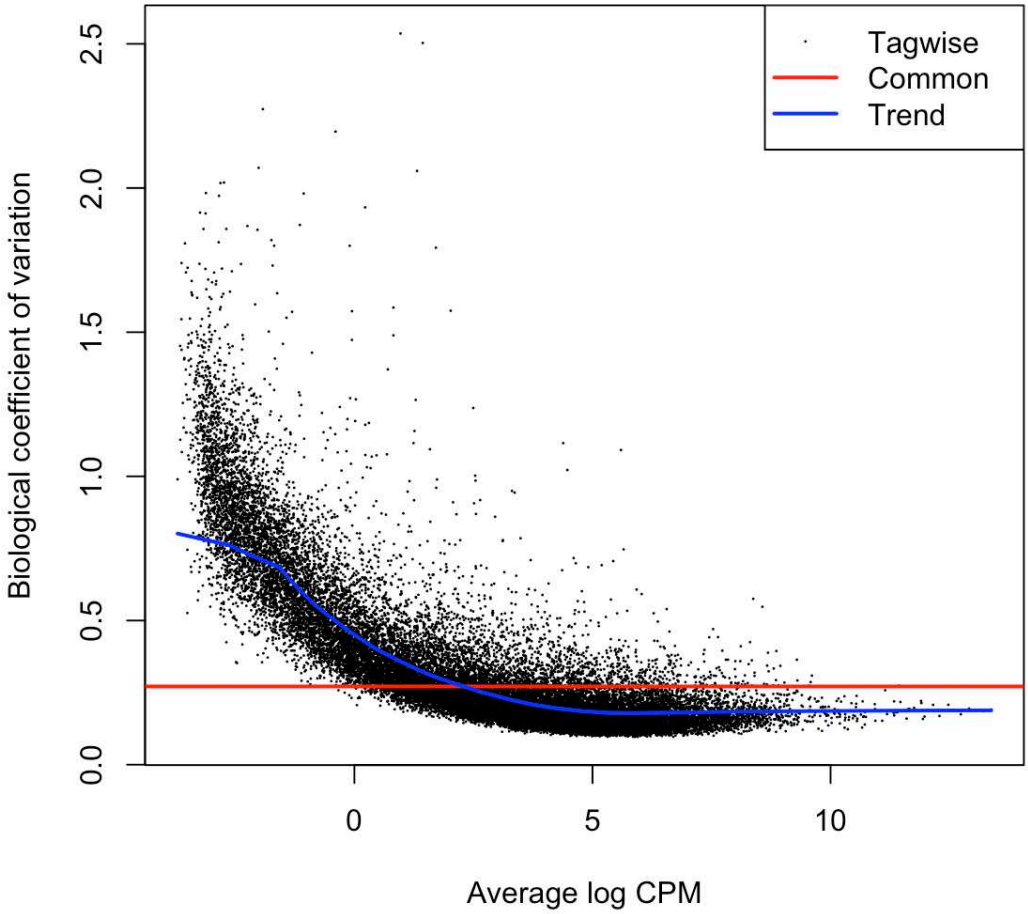


Figure 30. Pipeline 2: Biological coefficient of variation of samples. Common dispersion is 0.2713, within the desired range of 0.2-0.4.

Table 17. Pipeline 2: Top 20 up-and down-regulated annotated DE genes in WT_C vs *Ossr1*_C. Up and down refer to the expression of genes in the mutant as compared to WT. The most down-regulated gene in the mutant is the calmodulin-binding transcription activator *SR1*.

		Gene ID	Adjusted p-value	Annotation
Down	1	LOC_Os10g22950	4.37E-14	calmodulin-binding transcription activator, putative, expressed
	2	LOC_Os04g01330	2.19E-13	expressed protein
	3	LOC_Os04g01320	9.99E-11	serine threonine-protein kinase receptor precursor, putative, expressed
	4	LOC_Os02g34190	9.58E-09	expressed protein
	5	LOC_Os06g45500	4.29E-07	copper-transporting ATPase, putative, expressed
	6	LOC_Os04g02640	5.07E-06	3-ketoacyl-CoA synthase 6, putative, expressed
	7	LOC_Os01g62860	2.46E-05	oxidoreductase, aldo keto reductase family protein, putative, expressed
	8	LOC_Os10g35330	2.46E-05	expressed protein
	9	LOC_Os11g32610	3.21E-05	chalcone and stilbene synthases, putative, expressed
	10	LOC_Os04g03579	7.14E-05	protein kinase, putative, expressed
	11	LOC_Os07g23030	1.37E-04	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
	12	LOC_Os05g06814	1.47E-04	expressed protein
	13	LOC_Os09g34950	1.50E-04	TCP family transcription factor, putative, expressed
	14	LOC_Os10g09990	3.26E-04	cytokinin-O-glucosyltransferase 3, putative, expressed
	15	LOC_Os04g02140	5.27E-04	transposon protein, putative, CACTA, En Spm sub-class, expressed
	16	LOC_Os11g25220	1.22E-03	oxidoreductase, short chain dehydrogenase reductase domain containing protein, expressed
	17	LOC_Os03g36490	1.60E-03	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
	18	LOC_Os03g20740	1.76E-03	expressed protein
	19	LOC_Os02g34390	1.99E-03	expressed protein
	20	LOC_Os04g28820	1.99E-03	retrotransposon protein, putative, unclassified, expressed
Up	1	LOC_Os07g01904	4.20E-08	expressed protein
	2	LOC_Os07g10850	1.05E-07	retrotransposon protein, putative, unclassified, expressed
	3	LOC_Os01g73000	1.74E-07	copine, putative, expressed
	4	LOC_Os07g32710	4.29E-07	retrotransposon protein, putative, unclassified, expressed
	5	LOC_Os07g44250	6.57E-07	dirigent, putative, expressed
	6	LOC_Os03g64330	2.57E-06	aquaporin protein, putative, expressed
	7	LOC_Os03g51600	4.04E-06	tubulin FtsZ domain containing protein, putative, expressed
	8	LOC_Os04g14690	5.07E-06	flavin-containing monooxygenase family protein, putative, expressed
	9	LOC_Os07g27350	6.21E-06	atuA, putative, expressed
	10	LOC_Os02g52730	6.21E-06	ferredoxin--nitrite reductase, putative, expressed
	11	LOC_Os02g53240	6.48E-06	expressed protein
	12	LOC_Os04g18770	7.40E-06	retrotransposon protein, putative, unclassified, expressed
	13	LOC_Os02g15820	8.17E-06	extra-large G-protein-related, putative, expressed
	14	LOC_Os04g01850	9.26E-06	hypothetical protein
	15	LOC_Os04g02530	9.26E-06	expressed protein
	16	LOC_Os02g50140	1.07E-05	caleosin related protein, putative, expressed
	17	LOC_Os04g02754	1.67E-05	amidase family protein, putative, expressed
	18	LOC_Os05g23255	1.76E-05	expressed protein
	19	LOC_Os05g50390	1.89E-05	expressed protein
	20	LOC_Os03g43100	2.01E-05	expressed protein

Table 18. Pipeline 2: Top 20 up- and down-regulated annotated DE genes in WT_T vs *Ossr1*_T. Up and down refer to the expression of genes in the mutant as compared to WT. The most down-regulated gene in the mutant is the calmodulin-binding transcription activator *SR1*.

		Gene ID	Adjusted p-value	Annotation
Down	1	LOC_Os10g22950	5.14E-14	calmodulin-binding transcription activator, putative, expressed
	2	LOC_Os04g01330	2.33E-13	expressed protein
	3	LOC_Os04g01320	3.46E-10	serine threonine-protein kinase receptor precursor, putative, expressed
	4	LOC_Os02g34190	1.10E-08	expressed protein
	5	LOC_Os11g32610	3.77E-05	chalcone and stilbene synthases, putative, expressed
	6	LOC_Os08g19420	1.07E-04	O-methyltransferase, putative, expressed
	7	LOC_Os09g36700	1.07E-04	ribonuclease T2 family domain containing protein, expressed
	8	LOC_Os04g02640	1.19E-04	3-ketoacyl-CoA synthase 6, putative, expressed
	9	LOC_Os05g06814	1.31E-04	expressed protein
	10	LOC_Os10g09990	1.55E-04	cytokinin-O-glucosyltransferase 3, putative, expressed
	11	LOC_Os04g03579	3.27E-04	protein kinase, putative, expressed
	12	LOC_Os03g36490	3.35E-04	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
	13	LOC_Os01g62860	3.35E-04	oxidoreductase, aldo keto reductase family protein, putative, expressed
	14	LOC_Os03g36480	5.63E-04	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
	15	LOC_Os10g35330	5.84E-04	expressed protein
	16	LOC_Os10g04600	8.81E-04	OsFBX359 - F-box domain containing protein, expressed
	17	LOC_Os09g36680	1.03E-03	ribonuclease T2 family domain containing protein, expressed
	18	LOC_Os04g34610	1.37E-03	expressed protein
	19	LOC_Os06g15170	1.80E-03	3-ketoacyl-CoA synthase, putative, expressed
	20	LOC_Os07g32620	1.94E-03	anthocyanidin 5,3-O-glucosyltransferase, putative, expressed
Up	1	LOC_Os07g01904	4.79E-07	expressed protein
	2	LOC_Os03g64330	1.20E-06	aquaporin protein, putative, expressed
	3	LOC_Os07g10850	3.18E-06	retrotransposon protein, putative, unclassified, expressed
	4	LOC_Os01g73000	4.75E-06	copine, putative, expressed
	5	LOC_Os07g44250	7.37E-06	dirigent, putative, expressed
	6	LOC_Os10g05020	1.02E-05	cytochrome P450, putative, expressed
	7	LOC_Os02g14720	1.02E-05	expressed protein
	8	LOC_Os03g25490	2.27E-05	cytochrome P450 72A1, putative, expressed
	9	LOC_Os09g19350	2.27E-05	expressed protein
	10	LOC_Os07g32710	5.61E-05	retrotransposon protein, putative, unclassified, expressed
	11	LOC_Os05g50390	7.08E-05	expressed protein
	12	LOC_Os05g11900	8.88E-05	expressed protein
	13	LOC_Os07g27350	9.72E-05	atuA, putative, expressed
	14	LOC_Os04g02530	1.06E-04	expressed protein
	15	LOC_Os10g33104	1.14E-04	expressed protein
	16	LOC_Os02g52730	1.25E-04	ferredoxin--nitrite reductase, putative, expressed
	17	LOC_Os02g53240	1.28E-04	expressed protein
	18	LOC_Os04g02754	1.28E-04	amidase family protein, putative, expressed
	19	LOC_Os04g18770	1.31E-04	retrotransposon protein, putative, unclassified, expressed
	20	LOC_Os04g12710	1.55E-04	indole-3-acetate beta-glucosyltransferase, putative, expressed

Table 19. Pipeline 2: Top 20 up-and down-regulated annotated DE genes in *Ossr1*_C vs *Ossr1*_T. Up and down refer to the expression of genes in the treated as compared to control.

		Gene ID	Adjusted p-value	Annotation
Down	1	LOC_Os07g26110	1.55E-12	membrane associated DUF588 domain containing protein, putative, expressed
	2	LOC_Os02g51110	1.30E-11	aquaporin protein, putative, expressed
	3	LOC_Os09g33830	1.88E-11	solute carrier family 35 member F1, putative, expressed
	4	LOC_Os10g39980	2.03E-11	expressed protein
	5	LOC_Os06g47700	4.88E-11	serine threonine-protein kinase BRI1-like 2 precursor, putative, expressed
	6	LOC_Os07g26100	6.47E-11	expressed protein
	7	LOC_Os06g43410	6.89E-11	cytochrome P450, putative, expressed
	8	LOC_Os07g45080	7.02E-11	expressed protein
	9	LOC_Os01g68720	7.02E-11	keratin, type I cytoskeletal 9, putative, expressed
	10	LOC_Os02g36414	8.40E-11	transporter family protein, putative, expressed
	11	LOC_Os04g12010	8.48E-11	glycosyltransferase, putative, expressed
	12	LOC_Os03g22050	8.66E-11	CAMK_KIN1 SNF1 Nim1_like.16 - CAMK includes calcium calmodulin depedent protein kinases, expressed
	13	LOC_Os03g20420	8.66E-11	alpha-N-arabinofuranosidase A, putative, expressed
	14	LOC_Os03g01700	1.05E-10	expressed protein
	15	LOC_Os01g72360	1.08E-10	expressed protein
	16	LOC_Os07g12900	1.08E-10	cadmium zinc-transporting ATPase, putative, expressed
	17	LOC_Os10g33440	1.60E-10	NB-ARC domain containing protein, expressed
	18	LOC_Os01g48800	1.84E-10	purine permease, putative, expressed
	19	LOC_Os01g18744	1.87E-10	transferase family protein, putative, expressed
	20	LOC_Os05g35010	2.37E-10	cytochrome P450, putative, expressed
Up	1	LOC_Os02g43330	1.55E-12	homeobox associated leucine zipper, putative, expressed
	2	LOC_Os09g15670	1.55E-12	protein phosphatase 2C, putative, expressed
	3	LOC_Os03g20680	5.87E-12	late embryogenesis abundant protein 1, putative, expressed
	4	LOC_Os09g38320	8.05E-12	phytoene synthase, chloroplast precursor, putative, expressed
	5	LOC_Os03g44380	1.25E-11	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed
	6	LOC_Os02g30910	2.03E-11	nodulin MtN3 family protein, putative, expressed
	7	LOC_Os04g45810	2.03E-11	homeobox associated leucine zipper, putative, expressed
	8	LOC_Os01g62760	2.07E-11	protein phosphatase 2C, putative, expressed
	9	LOC_Os03g03370	2.22E-11	fatty acid hydroxylase, putative, expressed
	10	LOC_Os06g03930	5.46E-11	cytochrome P450 86A1, putative, expressed
	11	LOC_Os09g21120	6.29E-11	armadillo beta-catenin repeat family protein, putative, expressed
	12	LOC_Os07g05940	6.47E-11	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed
	13	LOC_Os05g38290	6.47E-11	protein phosphatase 2C, putative, expressed
	14	LOC_Os03g60580	6.89E-11	actin-depolymerizing factor, putative, expressed
	15	LOC_Os06g44190	6.89E-11	expressed protein
	16	LOC_Os03g14370	6.89E-11	ACT domain containing protein, expressed
	17	LOC_Os06g46740	7.02E-11	early nodulin 20 precursor, putative, expressed
	18	LOC_Os07g29750	9.69E-11	glycosyl hydrolases family 16, putative, expressed
	19	LOC_Os01g37832	9.94E-11	thioredoxin, putative, expressed
	20	LOC_Os01g50400	1.05E-10	STE_MEKK_ste11_MAP3K.5 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed

Table 20. Pipeline 2: Top 20 up-and down-regulated annotated genes in WT_C vs WT_T. Up and down refer to the expression of genes in the treated as compared to control.

		Gene ID	Adjusted p-value	Annotation
Down	1	LOC_Os07g26110	2.85E-12	membrane associated DUF588 domain containing protein, putative, expressed
	2	LOC_Os02g51110	1.75E-11	aquaporin protein, putative, expressed
	3	LOC_Os09g33830	1.75E-11	solute carrier family 35 member F1, putative, expressed
	4	LOC_Os03g22050	3.18E-11	CAMK_KIN1 SNF1 Nim1_like.16 - CAMK includes calcium calmodulin dependent protein kinases, expressed
	5	LOC_Os04g12010	3.62E-11	glycosyltransferase, putative, expressed
	6	LOC_Os01g68720	5.81E-11	keratin, type I cytoskeletal 9, putative, expressed
	7	LOC_Os03g20420	7.23E-11	alpha-N-arabinofuranosidase A, putative, expressed
	8	LOC_Os10g39980	8.72E-11	expressed protein
	9	LOC_Os07g45080	9.19E-11	expressed protein
	10	LOC_Os06g43410	9.42E-11	cytochrome P450, putative, expressed
	11	LOC_Os07g45370	1.01E-10	expressed protein
	12	LOC_Os05g33130	1.08E-10	CHIT17 - Chitinase family protein precursor, expressed
	13	LOC_Os03g25040	1.19E-10	GDSL-like lipase acylhydrolase, putative, expressed
	14	LOC_Os02g01220	1.32E-10	rhodanese-like domain containing protein, putative, expressed
	15	LOC_Os01g09080	1.63E-10	WRKY107, expressed
	16	LOC_Os10g33440	1.77E-10	NB-ARC domain containing protein, expressed
	17	LOC_Os02g36414	1.85E-10	transporter family protein, putative, expressed
	18	LOC_Os07g26100	1.92E-10	expressed protein
	19	LOC_Os08g01710	2.05E-10	GLTP domain containing protein, putative, expressed
	20	LOC_Os03g08720	2.05E-10	transferase family protein, putative, expressed
Up	1	LOC_Os09g15670	6.38E-13	protein phosphatase 2C, putative, expressed
	2	LOC_Os02g43330	8.16E-13	homeobox associated leucine zipper, putative, expressed
	3	LOC_Os03g20680	2.85E-12	late embryogenesis abundant protein 1, putative, expressed
	4	LOC_Os09g38320	3.58E-12	phytoene synthase, chloroplast precursor, putative, expressed
	5	LOC_Os03g44380	4.15E-12	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed
	6	LOC_Os06g03930	4.15E-12	cytochrome P450 86A1, putative, expressed
	7	LOC_Os03g03370	5.53E-12	fatty acid hydroxylase, putative, expressed
	8	LOC_Os01g62760	6.81E-12	protein phosphatase 2C, putative, expressed
	9	LOC_Os02g30910	7.65E-12	nodulin MtN3 family protein, putative, expressed
	10	LOC_Os04g45810	8.30E-12	homeobox associated leucine zipper, putative, expressed
	11	LOC_Os07g05940	9.41E-12	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed
	12	LOC_Os05g40010	1.10E-11	LTPL17 - Protease inhibitor seed storage LTP family protein precursor, expressed
	13	LOC_Os05g38290	1.17E-11	protein phosphatase 2C, putative, expressed
	14	LOC_Os06g44190	1.17E-11	expressed protein
	15	LOC_Os03g60580	1.42E-11	actin-depolymerizing factor, putative, expressed
	16	LOC_Os09g21120	1.75E-11	armadillo beta-catenin repeat family protein, putative, expressed
	17	LOC_Os06g46920	2.30E-11	dihydroflavonol-4-reductase, putative, expressed
	18	LOC_Os06g46740	2.57E-11	early nodulin 20 precursor, putative, expressed
	19	LOC_Os01g40094	3.18E-11	protein phosphatase 2C, putative, expressed
	20	LOC_Os02g10310	3.18E-11	fumarylacetoacetase, putative, expressed

Table 21. Pipeline 2: Top 10 significant GO enriched terms in each GO category for WT_C vs *Ossr1*_C. Up and down refer to the expression of genes in the mutant as compared to WT.

			GO term	Description	Adjusted p-value
Down	**Molecular Function**	1	GO:0016491	oxidoreductase activity	0.044
Up	**Molecular Function**	1	GO:0016757	transferase activity, transferring glycosyl groups	0.041
		2	GO:0016758	transferase activity, transferring hexosyl groups	0.045

Table 22. Pipeline 2: Top 10 significant GO enriched terms in each GO category for WT_T vs *Ossr1*_T. Up and down refer to the expression of genes in the mutant as compared to WT.

			GO term	Description	Adjusted p-value
Down	**Molecular Function**	1	GO:0016491	oxidoreductase activity	0.044
Up	**Molecular Function**	1	GO:0016757	transferase activity, transferring glycosyl groups	0.041
		2	GO:0016758	transferase activity, transferring hexosyl groups	0.045

Table 23. Pipeline 2: Top 10 significant GO enriched terms in each GO category for *Ossr1*_C vs *Ossr1*_T. Up and down refer to the expression of genes in the treated as compared to control.

			GO term	Description	Adjusted p-value
Down	Cellular Component	1	GO:0016020	membrane	3.40E-12
		2	GO:0031224	intrinsic to membrane	8.50E-05
		3	GO:0016021	integral to membrane	1.00E-04
		4	GO:0044425	membrane part	9.60E-03
		5	GO:0005576	extracellular region	1.20E-02
		6	GO:0048046	apoplast	2.50E-02
	Molecular Function	1	GO:0017076	purine nucleotide binding	2.10E-37
		2	GO:0001883	purine nucleoside binding	2.10E-37
		3	GO:0030554	adenyl nucleotide binding	2.10E-37
		4	GO:0001882	nucleoside binding	3.80E-37
		5	GO:0032555	purine ribonucleotide binding	8.20E-35
		6	GO:0032553	ribonucleotide binding	8.20E-35
		7	GO:0032559	adenyl ribonucleotide binding	2.20E-34
		8	GO:0000166	nucleotide binding	2.60E-34
		9	GO:0005524	ATP binding	2.60E-34
		10	GO:0016301	kinase activity	1.50E-23
	Biological Process	1	GO:0006468	protein amino acid phosphorylation	2.20E-22
		2	GO:0050896	response to stimulus	3.20E-20
		3	GO:0016310	phosphorylation	1.60E-19
		4	GO:0055114	oxidation reduction	3.80E-19
		5	GO:0043687	post-translational protein modification	5.90E-19
		6	GO:0006796	phosphate metabolic process	1.80E-18
		7	GO:0006793	phosphorus metabolic process	1.80E-18
		8	GO:0006464	protein modification process	1.40E-17
		9	GO:0012501	programmed cell death	2.00E-17
		10	GO:0006915	apoptosis	2.00E-17

			GO term	Description	Adjusted p-value
Up	Cellular Component	1	GO:0044464	cell part	1.20E-03
		2	GO:0008287	protein serine/threonine phosphatase complex	1.20E-03
		3	GO:0005623	cell	1.20E-03
		4	GO:0016020	membrane	1.20E-03
		5	GO:0016021	integral to membrane	1.70E-03
		6	GO:0031224	intrinsic to membrane	2.30E-03
		7	GO:0005654	nucleoplasm	4.50E-02
		8	GO:0031981	nuclear lumen	4.50E-02
		9	GO:0044451	nucleoplasm part	4.50E-02
		10	GO:0044428	nuclear part	4.90E-02
	Molecular Function	1	GO:0030528	transcription regulator activity	1.10E-17
		2	GO:0003700	transcription factor activity	1.80E-15
		3	GO:0043565	sequence-specific DNA binding	1.20E-08
		4	GO:0048037	cofactor binding	1.10E-06
		5	GO:0016491	oxidoreductase activity	2.40E-05
		6	GO:0016791	phosphatase activity	3.30E-04
		7	GO:0042578	phosphoric ester hydrolase activity	3.30E-04
		8	GO:0004722	protein serine/threonine phosphatase activity	3.40E-04
		9	GO:0050662	coenzyme binding	3.40E-04
		10	GO:0004721	phosphoprotein phosphatase activity	4.00E-04
	Biological Process	1	GO:0065007	biological regulation	7.00E-31
		2	GO:0050794	regulation of cellular process	9.80E-31
		3	GO:0050789	regulation of biological process	9.20E-30
		4	GO:0045449	regulation of transcription	1.10E-24
		5	GO:0019219	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	1.10E-24
		6	GO:0051171	regulation of nitrogen compound metabolic process	1.10E-24
		7	GO:0031323	regulation of cellular metabolic process	1.30E-24
		8	GO:0006350	transcription	1.10E-23
		9	GO:0010556	regulation of macromolecule biosynthetic process	1.20E-23
		10	GO:0009889	regulation of biosynthetic process	1.20E-23

Table 24. Pipeline 2: Top 10 significant GO enriched terms in each GO category for WT_C vs WT_T. Up and down refer to the expression of genes in treated as compared to control.

			GO term	Description	Adjusted p-value
Down	Cellular Component	1	GO:0016020	membrane	3.50E-09
		2	GO:0031224	intrinsic to membrane	3.80E-04
		3	GO:0048046	apoplast	4.30E-04
		4	GO:0005576	extracellular region	6.20E-04
		5	GO:0016021	integral to membrane	1.00E-03
		6	GO:0044425	membrane part	3.60E-03
		7	GO:0005737	cytoplasm	2.10E-02
	Molecular Function	1	GO:0017076	purine nucleotide binding	5.10E-34
		2	GO:0032555	purine ribonucleotide binding	1.70E-33
		3	GO:0032553	ribonucleotide binding	1.70E-33
		4	GO:0001883	purine nucleoside binding	3.60E-33
		5	GO:0030554	adenyl nucleotide binding	3.60E-33
		6	GO:0000166	nucleotide binding	4.70E-33
		7	GO:0001882	nucleoside binding	6.10E-33
		8	GO:0032559	adenyl ribonucleotide binding	2.90E-32
		9	GO:0005524	ATP binding	3.70E-32
		10	GO:0016491	oxidoreductase activity	1.10E-20
	Biological Process	1	GO:0050896	response to stimulus	2.70E-20
		2	GO:0055114	oxidation reduction	1.30E-18
		3	GO:0006950	response to stress	2.10E-17
		4	GO:0012501	programmed cell death	6.00E-16
		5	GO:0006915	apoptosis	6.00E-16
		6	GO:0008219	cell death	6.10E-16
		7	GO:0016265	death	6.10E-16
		8	GO:0006468	protein amino acid phosphorylation	2.00E-15
		9	GO:0006952	defense response	6.20E-14
		10	GO:0044281	small molecule metabolic process	1.30E-13

			GO term	Description	Adjusted p-value
Up	Cellular Component	1	GO:0016020	membrane	1.20E-06
		2	GO:0044464	cell part	5.30E-06
		3	GO:0005623	cell	5.30E-06
		4	GO:0016021	integral to membrane	2.60E-05
		5	GO:0031224	intrinsic to membrane	2.70E-05
		6	GO:0044425	membrane part	3.20E-04
		7	GO:0008287	protein serine/threonine phosphatase complex	1.30E-03
		8	GO:0044428	nuclear part	1.50E-03
		9	GO:0043234	protein complex	3.90E-03
		10	GO:0005654	nucleoplasm	7.70E-03
	Molecular Function	1	GO:0030528	transcription regulator activity	6.50E-21
		2	GO:0003700	transcription factor activity	1.90E-17
		3	GO:0043565	sequence-specific DNA binding	1.40E-09
		4	GO:0005515	protein binding	4.00E-06
		5	GO:0004721	phosphoprotein phosphatase activity	2.30E-05
		6	GO:0042578	phosphoric ester hydrolase activity	3.20E-05
		7	GO:0016791	phosphatase activity	5.60E-05
		8	GO:0004722	protein serine/threonine phosphatase activity	5.60E-04
		9	GO:0016818	hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	4.50E-03
		10	GO:0016817	hydrolase activity, acting on acid anhydrides	4.50E-03
	Biological Process	1	GO:0050794	regulation of cellular process	4.80E-29
		2	GO:0065007	biological regulation	4.80E-29
		3	GO:0050789	regulation of biological process	1.90E-28
		4	GO:0045449	regulation of transcription	8.90E-25
		5	GO:0031323	regulation of cellular metabolic process	8.90E-25
		6	GO:0019219	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	1.30E-24
		7	GO:0051171	regulation of nitrogen compound metabolic process	1.30E-24
		8	GO:0019222	regulation of metabolic process	2.60E-24
		9	GO:0006350	transcription	2.60E-24
		10	GO:0010556	regulation of macromolecule biosynthetic process	3.50E-24

2.2.4 Comparison of Analyses

Pipeline 1 used the *Oryza sativa* L. ssp. *japonica* cv. Nipponbare reference genome from solgenomics from the International Rice Genome Sequencing Project, IRGSP genome build 5.0 (https://rgp.dna.affrc.go.jp/IRGSP/) (Sasaki, 2005), with annotations from the Rice Annotation Project Database (RAP-DB) (https://rapdb.dna.affrc.go.jp/index.html). Pipeline 2 used the *Oryza sativa* L. ssp. *japonica* cv. Nipponbare reference genome from the Rice Genome Annotation Project (RGAP) from Michigan State University (Kawahara et al., 2013). As of the release of the RGAP7 pseudomolecule (October 31, 2011), the two genome builds should be identical. At the outset of Pipeline 2 development, sample 1 (WT_C_1.1) was mapped to both the IRGSP5.0 and the RGAP7 genome builds. After read cleaning and alignment, uniquely mapped reads only differed by 418 reads (33,959,220 for IRGSP5.0 and 33,958802 for RGAP7).

Annotations from RGAP and RAP-DB vary significantly, however. RGAP contains 55,986 total loci with 39,045 non-TE genes annotated with splice variants to total 49,066 gene models (http://rice.plantbiology.msu.edu/index.shtml). RAP-DB contains protein-coding loci for 23,943 full-length cDNA and 9,336 non-full-length cDNAs, totaling to 33,279. With 6,667 alternative variants, RAP-DB contains 39,946 gene models (https://rapdb.dna.affrc.go.jp/rice_docs/docs_genes_statistics.html).

Using two analysis pipelines begs the question of DE gene intersection. Because of the reduced number of annotations in the RAP-DB database, it is not informative to look at numbers of overlapping DE genes in group comparisons. The lack of annotation biases these numbers downward. Instead, GO terms (using AgriGO) of intersecting DE genes were used to interpret the overlap in datasets. In the DE analysis for individual pipelines (sections 2.2.2 and 2.2.3), line-by-treatment comparisons were made by averaging over timepoints. Here, intersecting genes

have been compared across timepoint groups and within timepoint groups. The top 10 GO terms for within line comparisons of treatment and control groups are shown (Tables 25-30).

Table 25. Pipeline Intersection: Top 10 significant GO enriched terms in each GO category for *Ossr1*_C_1 vs *Ossr1*_T_1. Up and down refer to the expression of genes in the treated as compared to control.

			GO term	Description	Adjusted p-value
Down	Cellular Component	1	GO:0016020	membrane	1.30E-08
		2	GO:0016021	integral to membrane	1.60E-08
		3	GO:0031224	intrinsic to membrane	1.60E-08
		4	GO:0044425	membrane part	5.30E-05
	Molecular Function	1	GO:0020037	heme binding	3.70E-20
		2	GO:0046906	tetrapyrrole binding	4.00E-20
		3	GO:0005506	iron ion binding	4.90E-19
		4	GO:0030554	adenyl nucleotide binding	4.00E-17
		5	GO:0001883	purine nucleoside binding	4.00E-17
		6	GO:0001882	nucleoside binding	4.70E-17
		7	GO:0017076	purine nucleotide binding	1.60E-15
		8	GO:0005524	ATP binding	2.60E-15
		9	GO:0032559	adenyl ribonucleotide binding	2.60E-15
		10	GO:0016491	oxidoreductase activity	8.90E-15
	Biological Process	1	GO:0050896	response to stimulus	2.70E-20
		2	GO:0006950	response to stress	1.90E-18
		3	GO:0006952	defense response	1.30E-14
		4	GO:0012501	programmed cell death	1.70E-14
		5	GO:0006915	apoptosis	1.70E-14
		6	GO:0016265	death	3.40E-14
		7	GO:0008219	cell death	3.40E-14
		8	GO:0055114	oxidation reduction	4.90E-13
		9	GO:0006979	response to oxidative stress	1.00E-12
		10	GO:0006810	transport	2.10E-11
Up	Cellular Component	1	GO:0008287	protein serine/threonine phosphatase complex	6.40E-06
		2	GO:0000151	ubiquitin ligase complex	2.90E-02
		3	GO:0043234	protein complex	2.90E-02
		4	GO:0005576	extracellular region	2.90E-02
	Molecular Function	1	GO:0030528	transcription regulator activity	1.20E-24
		2	GO:0003700	transcription factor activity	1.50E-22
		3	GO:0043565	sequence-specific DNA binding	1.10E-08
		4	GO:0004722	protein serine/threonine phosphatase activity	6.40E-07
		5	GO:0004721	phosphoprotein phosphatase activity	4.30E-05
		6	GO:0016791	phosphatase activity	9.10E-05
		7	GO:0042578	phosphoric ester hydrolase activity	1.40E-03
		8	GO:0004842	ubiquitin-protein ligase activity	1.90E-02
		9	GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	2.60E-02
	Biological Process	1	GO:0045449	regulation of transcription	1.60E-26
		2	GO:0019219	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	1.60E-26
		3	GO:0051171	regulation of nitrogen compound metabolic process	1.60E-26
		4	GO:0031323	regulation of cellular metabolic process	5.20E-26
		5	GO:0009889	regulation of biosynthetic process	7.70E-26
		6	GO:0010556	regulation of macromolecule biosynthetic process	7.70E-26
		7	GO:0031326	regulation of cellular biosynthetic process	7.70E-26
		8	GO:0010468	regulation of gene expression	1.40E-25
		9	GO:0019222	regulation of metabolic process	9.20E-25
		10	GO:0080090	regulation of primary metabolic process	1.40E-24

Table 26. Pipeline Intersection: Top 10 significant GO enriched terms in each GO category for *Ossr1*_C_3 vs *Ossr1*_T_3. Up and down refer to the expression of genes in the treated as compared to control.

			GO term	Description	Adjusted p-value
Down	Cellular Component	1	GO:0016020	membrane	1.90E-07
		2	GO:0031224	intrinsic to membrane	2.30E-03
		3	GO:0005576	extracellular region	2.30E-03
		4	GO:0016021	integral to membrane	3.50E-03
		5	GO:0044425	membrane part	2.30E-02
		6	GO:0048046	apoplast	2.50E-02
	Molecular Function	1	GO:0016491	oxidoreductase activity	1.70E-17
		2	GO:0017076	purine nucleotide binding	5.40E-17
		3	GO:0001883	purine nucleoside binding	5.40E-17
		4	GO:0030554	adenyl nucleotide binding	5.40E-17
		5	GO:0001882	nucleoside binding	6.80E-17
		6	GO:0020037	heme binding	3.90E-16
		7	GO:0046906	tetrapyrrole binding	7.80E-16
		8	GO:0032555	purine ribonucleotide binding	9.00E-16
		9	GO:0032553	ribonucleotide binding	9.00E-16
		10	GO:0032559	adenyl ribonucleotide binding	1.50E-15
	Biological Process	1	GO:0055114	oxidation reduction	4.40E-19
		2	GO:0042221	response to chemical stimulus	2.00E-13
		3	GO:0006468	protein amino acid phosphorylation	3.30E-13
		4	GO:0016310	phosphorylation	6.00E-12
		5	GO:0006979	response to oxidative stress	9.30E-12
		6	GO:0006796	phosphate metabolic process	9.30E-11
		7	GO:0006793	phosphorus metabolic process	9.30E-11
		8	GO:0043687	post-translational protein modification	2.10E-10
		9	GO:0050896	response to stimulus	3.20E-10
		10	GO:0006629	lipid metabolic process	3.60E-09
Up	C. C.	1	GO:0008287	protein serine/threonine phosphatase complex	1.40E-04
	Molecular Function	1	GO:0003700	transcription factor activity	7.40E-13
		2	GO:0030528	transcription regulator activity	1.10E-09
		3	GO:0016491	oxidoreductase activity	2.00E-09
		4	GO:0043565	sequence-specific DNA binding	5.00E-07
		5	GO:0004721	phosphoprotein phosphatase activity	3.40E-06
		6	GO:0016791	phosphatase activity	6.80E-06
		7	GO:0004722	protein serine/threonine phosphatase activity	8.20E-06
		8	GO:0042578	phosphoric ester hydrolase activity	1.60E-05
		9	GO:0048037	cofactor binding	1.70E-03
		10	GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	1.70E-03
	Biological Process	1	GO:0050794	regulation of cellular process	6.50E-12
		2	GO:0065007	biological regulation	2.00E-11
		3	GO:0050789	regulation of biological process	2.30E-11
		4	GO:0045449	regulation of transcription	1.30E-10
		5	GO:0019219	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	1.30E-10
		6	GO:0051171	regulation of nitrogen compound metabolic process	1.30E-10
		7	GO:0031323	regulation of cellular metabolic process	1.80E-10
		8	GO:0009889	regulation of biosynthetic process	2.70E-10
		9	GO:0010556	regulation of macromolecule biosynthetic process	2.70E-10
		10	GO:0031326	regulation of cellular biosynthetic process	2.70E-10

Table 27. Pipeline Intersection: Top 10 significant GO enriched terms in each GO category for WT_C_1 vs WT_T_1. Up and down refer to the expression of genes in the treated as compared to control.

			GO term	Descripton	Adjusted p-value
Down	Cellular Component	1	GO:0016020	membrane	5.50E-05
		2	GO:0005576	extracellular region	4.60E-03
		3	GO:0016021	integral to membrane	5.80E-03
		4	GO:0031224	intrinsic to membrane	5.80E-03
		5	GO:0048046	apoplast	7.90E-03
	Molecular Function	1	GO:0046906	tetrapyrrole binding	2.20E-17
		2	GO:0020037	heme binding	2.20E-17
		3	GO:0005506	iron ion binding	5.40E-16
		4	GO:0016491	oxidoreductase activity	1.90E-14
		5	GO:0016684	oxidoreductase activity, acting on peroxide as acceptor	1.10E-11
		6	GO:0004601	peroxidase activity	1.10E-11
		7	GO:0016209	antioxidant activity	1.50E-11
		8	GO:0004497	monooxygenase activity	8.60E-07
		9	GO:0005215	transporter activity	2.00E-06
		10	GO:0009055	electron carrier activity	6.30E-06
	Biological Process	1	GO:0055114	oxidation reduction	1.50E-12
		2	GO:0050896	response to stimulus	1.50E-12
		3	GO:0042221	response to chemical stimulus	1.50E-12
		4	GO:0006979	response to oxidative stress	9.30E-12
		5	GO:0006950	response to stress	6.30E-11
		6	GO:0006810	transport	6.70E-09
		7	GO:0051234	establishment of localization	6.70E-09
		8	GO:0051179	localization	8.30E-09
		9	GO:0055085	transmembrane transport	1.50E-08
		10	GO:0006952	defense response	7.60E-06
Up	C.C.	1	GO:0008287	protein serine/threonine phosphatase complex	1.40E-04
		2	GO:0005576	extracellular region	2.70E-02
	Molecular Function	1	GO:0030528	transcription regulator activity	5.90E-20
		2	GO:0003700	transcription factor activity	1.30E-16
		3	GO:0043565	sequence-specific DNA binding	1.50E-07
		4	GO:0004722	protein serine/threonine phosphatase activity	4.30E-04
		5	GO:0004721	phosphoprotein phosphatase activity	1.20E-02
	Biological Process	1	GO:0045449	regulation of transcription	8.80E-22
		2	GO:0019219	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	8.80E-22
		3	GO:0051171	regulation of nitrogen compound metabolic process	8.80E-22
		4	GO:0031323	regulation of cellular metabolic process	1.80E-21
		5	GO:0031326	regulation of cellular biosynthetic process	1.80E-21
		6	GO:0009889	regulation of biosynthetic process	1.80E-21
		7	GO:0010556	regulation of macromolecule biosynthetic process	1.80E-21
		8	GO:0010468	regulation of gene expression	2.50E-21
		9	GO:0019222	regulation of metabolic process	9.40E-21
		10	GO:0080090	regulation of primary metabolic process	1.10E-20

Table 28. Pipeline Intersection: Top 10 significant GO enriched terms in each GO category for WT_C_3 vs WT_T_3. Up and down refer to the expression of genes in the treated as compared to control.

			GO term	Description	Adjusted p-value
Down	Cellular Component	1	GO:0016020	membrane	1.70E-13
		2	GO:0031224	intrinsic to membrane	7.30E-07
		3	GO:0016021	integral to membrane	3.80E-06
		4	GO:0044425	membrane part	4.50E-05
		5	GO:0005576	extracellular region	8.20E-04
		6	GO:0048046	apoplast	5.50E-03
	Molecular Function	1	GO:0017076	purine nucleotide binding	7.90E-24
		2	GO:0001883	purine nucleoside binding	4.40E-23
		3	GO:0001882	nucleoside binding	4.40E-23
		4	GO:0030554	adenyl nucleotide binding	4.40E-23
		5	GO:0032555	purine ribonucleotide binding	2.10E-22
		6	GO:0032553	ribonucleotide binding	2.10E-22
		7	GO:0000166	nucleotide binding	1.20E-21
		8	GO:0032559	adenyl ribonucleotide binding	3.60E-21
		9	GO:0005524	ATP binding	3.60E-21
		10	GO:0016491	oxidoreductase activity	3.00E-20
	Biological Process	1	GO:0055114	oxidation reduction	1.30E-19
		2	GO:0006468	protein amino acid phosphorylation	1.40E-14
		3	GO:0016310	phosphorylation	3.70E-13
		4	GO:0006796	phosphate metabolic process	1.40E-12
		5	GO:0006793	phosphorus metabolic process	1.40E-12
		6	GO:0043687	post-translational protein modification	2.10E-12
		7	GO:0043412	macromolecule modification	3.50E-11
		8	GO:0006464	protein modification process	5.00E-11
		9	GO:0006979	response to oxidative stress	8.30E-11
		10	GO:0042221	response to chemical stimulus	1.50E-10

			GO term	Description	Adjusted p-value
Up	Cellular Component	1	GO:0008287	protein serine/threonine phosphatase complex	5.80E-03
		2	GO:0016020	membrane	5.80E-03
		3	GO:0031967	organelle envelope	2.00E-02
		4	GO:0031975	envelope	4.20E-02
		5	GO:0005740	mitochondrial envelope	4.20E-02
		6	GO:0044464	cell part	4.90E-02
		7	GO:0031224	intrinsic to membrane	4.90E-02
		8	GO:0016021	integral to membrane	4.90E-02
		9	GO:0044429	mitochondrial part	4.90E-02
		10	GO:0005739	mitochondrion	4.90E-02
	Molecular Function	1	GO:0003700	transcription factor activity	3.20E-12
		2	GO:0030528	transcription regulator activity	1.10E-11
		3	GO:0016491	oxidoreductase activity	9.70E-06
		4	GO:0043565	sequence-specific DNA binding	1.50E-05
		5	GO:0042578	phosphoric ester hydrolase activity	4.20E-05
		6	GO:0016791	phosphatase activity	7.40E-05
		7	GO:0004721	phosphoprotein phosphatase activity	2.10E-04
		8	GO:0048037	cofactor binding	3.30E-04
		9	GO:0004722	protein serine/threonine phosphatase activity	5.50E-04
		10	GO:0050662	coenzyme binding	1.90E-03
	Biological Process	1	GO:0050794	regulation of cellular process	2.70E-16
		2	GO:0050789	regulation of biological process	4.10E-16
		3	GO:0065007	biological regulation	4.10E-16
		4	GO:0045449	regulation of transcription	3.20E-14
		5	GO:0051171	regulation of nitrogen compound metabolic process	3.20E-14
		6	GO:0019219	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	3.20E-14
		7	GO:0031323	regulation of cellular metabolic process	4.10E-14
		8	GO:0009889	regulation of biosynthetic process	7.10E-14
		9	GO:0031326	regulation of cellular biosynthetic process	7.10E-14
		10	GO:0010556	regulation of macromolecule biosynthetic process	7.10E-14

Table 29. Pipeline Intersection: Top 10 significant GO enriched terms in each GO category for Mu_C vs Mu_T. Up and down refer to the expression of genes in the treated as compared to control.

			GO term	Description	Adjusted p-value
Down	Cellular Component	1	GO:0016020	membrane	4.20E-06
		2	GO:0005576	extracellular region	4.00E-05
		3	GO:0031224	intrinsic to membrane	8.80E-04
		4	GO:0016021	integral to membrane	9.10E-04
		5	GO:0048046	apoplast	9.10E-04
	Molecular Function	1	GO:0020037	heme binding	2.70E-25
		2	GO:0046906	tetrapyrrole binding	3.40E-25
		3	GO:0005506	iron ion binding	7.60E-25
		4	GO:0016491	oxidoreductase activity	3.30E-21
		5	GO:0030554	adenyl nucleotide binding	1.60E-20
		6	GO:0001883	purine nucleoside binding	1.60E-20
		7	GO:0001882	nucleoside binding	2.10E-20
		8	GO:0017076	purine nucleotide binding	1.30E-18
		9	GO:0005524	ATP binding	1.20E-17
		10	GO:0032559	adenyl ribonucleotide binding	1.20E-17
	Biological Process	1	GO:0055114	oxidation reduction	8.30E-20
		2	GO:0050896	response to stimulus	8.60E-18
		3	GO:0006950	response to stress	9.20E-16
		4	GO:0006468	protein amino acid phosphorylation	2.80E-15
		5	GO:0006979	response to oxidative stress	9.70E-15
		6	GO:0042221	response to chemical stimulus	3.40E-13
		7	GO:0016310	phosphorylation	6.50E-13
		8	GO:0043687	post-translational protein modification	2.80E-12
		9	GO:0006796	phosphate metabolic process	3.20E-11
		10	GO:0006793	phosphorus metabolic process	3.20E-11

			GO term	Description	Adjusted p-value
Up	C. C.	1	GO:0008287	protein serine/threonine phosphatase complex	1.20E-09
		2	GO:0000151	ubiquitin ligase complex	9.10E-03
		3	GO:0043234	protein complex	1.50E-02
	Molecular Function	1	GO:0003700	transcription factor activity	9.60E-23
		2	GO:0030528	transcription regulator activity	1.60E-22
		3	GO:0004722	protein serine/threonine phosphatase activity	7.70E-10
		4	GO:0043565	sequence-specific DNA binding	1.60E-07
		5	GO:0004721	phosphoprotein phosphatase activity	1.60E-07
		6	GO:0016791	phosphatase activity	1.70E-05
		7	GO:0042578	phosphoric ester hydrolase activity	3.00E-04
		8	GO:0004842	ubiquitin-protein ligase activity	1.60E-02
		9	GO:0050662	coenzyme binding	3.00E-02
		10	GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	3.90E-02
	Biological Process	1	GO:0045449	regulation of transcription	7.50E-28
		2	GO:0019219	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	7.50E-28
		3	GO:0051171	regulation of nitrogen compound metabolic process	7.50E-28
		4	GO:0009889	regulation of biosynthetic process	3.80E-27
		5	GO:0050794	regulation of cellular process	3.80E-27
		6	GO:0010556	regulation of macromolecule biosynthetic process	3.80E-27
		7	GO:0031326	regulation of cellular biosynthetic process	3.80E-27
		8	GO:0031323	regulation of cellular metabolic process	4.30E-27
		9	GO:0010468	regulation of gene expression	5.80E-27
		10	GO:0006350	transcription	2.30E-26

Table 30. Pipeline Intersection: Top 10 significant GO enriched terms in each GO category for WT_C vs WT_T. Up and down refer to the expression of genes in the treated as compared to control.

			GO term	Description	Adjusted p-value
Down	Cellular Component	1	GO:0005576	extracellular region	1.30E-06
		2	GO:0016020	membrane	1.80E-04
		3	GO:0031224	intrinsic to membrane	5.90E-03
		4	GO:0016021	integral to membrane	5.90E-03
		5	GO:0048046	apoplast	5.90E-03
	Molecular Function	1	GO:0020037	heme binding	6.20E-22
		2	GO:0046906	tetrapyrrole binding	7.20E-22
		3	GO:0005506	iron ion binding	1.10E-20
		4	GO:0016491	oxidoreductase activity	1.90E-20
		5	GO:0030554	adenyl nucleotide binding	8.50E-14
		6	GO:0001883	purine nucleoside binding	8.50E-14
		7	GO:0001882	nucleoside binding	1.00E-13
		8	GO:0016684	oxidoreductase activity, acting on peroxide as acceptor	2.80E-13
		9	GO:0004601	peroxidase activity	2.80E-13
		10	GO:0005524	ATP binding	3.60E-13
	Biological Process	1	GO:0055114	oxidation reduction	1.40E-18
		2	GO:0050896	response to stimulus	1.20E-13
		3	GO:0006979	response to oxidative stress	3.70E-13
		4	GO:0006950	response to stress	6.20E-13
		5	GO:0006468	protein amino acid phosphorylation	4.60E-12
		6	GO:0042221	response to chemical stimulus	4.60E-12
		7	GO:0016310	phosphorylation	3.90E-10
		8	GO:0006952	defense response	4.00E-09
		9	GO:0043687	post-translational protein modification	4.00E-09
		10	GO:0006796	phosphate metabolic process	5.70E-09

			GO term	Description	Adjusted p-value
Up	C. C.	1	GO:0008287	protein serine/threonine phosphatase complex	2.20E-05
	Molecular Function	1	GO:0030528	transcription regulator activity	1.40E-23
		2	GO:0003700	transcription factor activity	1.40E-23
		3	GO:0043565	sequence-specific DNA binding	2.60E-08
		4	GO:0004722	protein serine/threonine phosphatase activity	2.20E-06
		5	GO:0042578	phosphoric ester hydrolase activity	9.20E-06
		6	GO:0016791	phosphatase activity	9.20E-06
		7	GO:0004721	phosphoprotein phosphatase activity	3.40E-05
		8	GO:0016491	oxidoreductase activity	1.70E-03
		9	GO:0050662	coenzyme binding	1.20E-02
		10	GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	2.30E-02
	Biological Process	1	GO:0045449	regulation of transcription	2.80E-28
		2	GO:0019219	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	2.80E-28
		3	GO:0051171	regulation of nitrogen compound metabolic	2.80E-28
		4	GO:0009889	regulation of biosynthetic process	1.90E-27
		5	GO:0010556	regulation of macromolecule biosynthetic process	1.90E-27
		6	GO:0031326	regulation of cellular biosynthetic process	1.90E-27
		7	GO:0031323	regulation of cellular metabolic process	1.90E-27
		8	GO:0010468	regulation of gene expression	3.70E-27
		9	GO:0006350	transcription	7.60E-27
		10	GO:0050794	regulation of cellular process	4.20E-26

DISCUSSION

Drought Stress Response in Rice

Transcriptomic comparison of WT in control and PEG-treated conditions (WT_C versus WT_T) will tell the story of drought-responsive gene regulation in rice seedlings. In response to drought stress, rice seedlings up-regulated genes encoding membrane proteins, transcription factors and other proteins that are associated with sequence-specific DNA-binding, regulation of cellular processes, transcription, nucleic acid metabolic processes, and regulation of the biosynthesis of macromolecules (Table 24 and Figure 29). These DE genes encode protein phosphatase 2C, homeobox (HOX)-containing TFs, late embryogenesis abundant 1 (LEA1), nodulin family proteins, EID-1-like F-box protein, MAPKKK, Ethylene-responsive TFs (ERF) 110 and 112, zinc finger proteins, and an ABC transporter G family member (Tables 14 and 20). Several proteins that belong to these protein families have been shown to have a role in drought tolerance in rice (Kumar et al., 2017).

Down-regulated genes in response to drought stress were enriched for GO terms involving membranes, heme and tetrapyrrole binding, catalytic activity, ion binding, nucleic acid binding, oxidoreductase activity, response to stimulus, oxidation-reduction, response to stress, programmed cell death, defense response, and small molecule metabolic process (Table 24 and Figure 29). Down-regulated genes encode aquaporins, iron-phytosiderophore transporter, Fe^{2+} transport, silicon efflux transporter, a jacalin-related salt stress-induced protein, glycolipid transfer protein, membrane-associated DUF588 domain-containing protein, solute carriers, WRKY107, and CAMK, a Ca^{2+}/CaM dependent protein kinase (Tables 14 and 20).

The induction of expression of genes encoding proteins involved in membrane-related processes, ion and solute shuttles, kinase signaling cascades, ERF TFs, and down-regulation of aquaporins agree with current canonical and non-canonical drought stress-responsive pathways in rice (Dash et al., 2018). Such studies will hopefully lead to molecular breeding strategies for the development of rice varieties that are drought tolerant, as is being done in *Arabidopsis* (Kudo et al., 2019; Umezawa et al., 2006).

With copious data from drought stress responses in the model plant *Arabidopsis*, it is important to understand the difference in drought stress responses between rice and *Arabidopsis*. There is considerable overlap in drought-regulated genes and their functional groups in these two model organisms. For example, in both plants, drought modulates ABA-dependent and independent signaling pathways as well as DREB and ERF TFs (Nakashima et al., 2009). However, there is more to be understood here. Rice, a monocot, and *Arabidopsis*, a dicot, diverged 130-200 million years ago and display clear structural and functional differences (Krom and Ramakrishna, 2008). For example, rice roots develop aerenchyma, specialized structures to store gas, which aid the plant to withstand flooding. *Arabidopsis* becomes nutrient deficient and chlorotic in flooded, anaerobic conditions. While transcriptomic gene regulatory network comparisons have been made between these two model species, further analyses to understand the similarities and differences need to be done (Krom and Ramakrishna, 2008; Lijavetzky et al., 2003; Yadav et al., 2019; Izawa et al., 2003). The RNA-seq data presented here can be compared to transcriptomic profiles of *Arabidopsis* seedlings exposed to PEG, and this comparison can be broadened by looking at the expression of transcript isoforms or chromatin remodeling (Colaneri and Jones, 2013; Filichkin et al., 2010). Ultimately, this dataset can help identify novel features of drought stress response mechanisms in this important crop plant.

Action of SR1 in rice

In the comparison of WT with *Ossr1* mutant under control conditions, there are 142/69 (Pipeline1/Pipeline 2) DE genes at 1 hour, 124/87 DE genes at 3 hours, and 146/204 DE genes when comparing across timepoints (Table 10). The calmodulin-binding transcription activator, *SR1*, appears as the most significant down-regulated gene in the mutant in both pipelines, confirming the full knockout nature of the mutant (Tables 11 and 17). A serine/threonine-protein kinase receptor precursor (LOC_Os04g01320) is down-regulated in the mutant, highly significant in both pipelines and appears significant in all comparisons: 1hr, 3hr, and combined timepoints (1hr and 3hr DE genes are not shown). Additionally, a number of kinases appear down-regulated in the mutant as well as auxin and cytokinin-related genes and a pathogenesis-related protein (PRB1). The most up-regulated gene in *Ossr1* in Pipeline 1 is a ferredoxin-nitrite reductase (LOC_Os2g52730), which also appears in Pipeline 2. Aquaporin (LOC_Os03g64330) appears in the top 20 DE gene lists of both pipelines as well. GO analysis returned down-regulation of lipid-biosynthesis and oxidoreductase activity and up-regulation of transferase activity (of glycosyl and hexosyl groups) in *Ossr1* in both pipelines (Table 21 and Figure 26).

Our transcriptomic study has provided some interesting insight into the action of *Os*SR1. As a serine threonine-protein kinase receptor precursor (SRK-R) appeared as a top 3 DE gene in all WT and *Ossr1* comparisons in both pipelines, there is potential for interaction between *Os*SR1 and the promoter of this receptor kinase gene (Tables 12 and 18). Further analysis can look for the CG-1 DNA-binding sequence of *Os*SR1 in the *SRK-R* promoter. Additionally, *Os*SR1 may inhibit the expression of genes encoding aquaporin, ferredoxin-nitrite reductase, LRR-receptor-like protein kinase, and sucrose synthase. Further insight into the rice-specific action of SR1 can be gleaned from comparing the DE genes in this study to that done by Prasad

et al. (2016). From DE gene counts, it seems that *Os*SR1 does not regulate nearly as many genes in rice as was seen in *Arabidopsis* when WT was compared to a full knock-out *Atsr1*. In that study, over 3,000 genes were mis-regulated in the mutant (Prasad et al., 2016). Genes involved in drought, salt, and cold stress response were over-represented in the GO analysis. Comparing orthologs that are differentially expressed in both species may identify an overlapping or distinct role for SR1.

Action of *Os*SR1 in response to osmotic stress

Looking at the transcriptome of WT and *Ossr1* in PEG-treated conditions, there are 53/25 (Pipeline1/Pipeline 2) DE genes at 1hour, 62/111 at 3 hours, and 67/315 when averaging across timepoints (Table 10). Again, *SR1* was the most significant down-regulated gene in the mutant in both comparisons, and the serine/threonine-protein kinase gene appears again in the top most significant down-regulated genes in the mutant (Tables 12 and 18). The same ferredoxin-nitrite reductase is up-regulated in the mutant in the treated condition in both pipelines, as well as the aquaporin, but only in Pipeline 2 in treated condition. A sucrose synthase (Os04t0249500, LOC_Os04g17650) appears up-regulated in the mutant in Pipeline 1 in both treated and control conditions. Lipid biosynthesis GO term as well as membrane-related terms were associated with down-regulated genes, while transferase activity was associated with up-regulated genes in *Ossr1* in treated conditions, as it was in control conditions (Table 22 and Figure 27).

The most significant up-regulated DE-gene in the mutant (Pipeline 1) is a BURP-domain containing protein 4 (Table 12). *Arabidopsis* RD22 is a BURP domain family member and involved in the ABA-dependent drought signaling pathway (Harshavardhan et al., 2014). Proteins with BURP domains are secreted and can be involved in cell wall enlargement (Park et

al., 2015). This same BURP-domain containing protein 4 is a top DE gene in WT in control conditions, thus it is not only mis-regulated due to drought but may be regulated by *Os*SR1 independent of osmotic conditions (Table 11).

The beauty of this factorial experiment is that we can use multiple comparisons to guide us when asking a single question. We evaluated WT in control and treated conditions (WT_C vs WT_T) and evaluated *Ossr1* in control and treated conditions (Mu_C vs Mu_T). We can look at genes found in the WT comparison which are not found in the mutant comparison to further identify genes potentially influenced by SR1 in drought stress response.

Looking at top annotated DE genes from both pipelines, 17/40 of the up-regulated, DE genes found in WT are not found in the mutant when exposed to drought stress (Tables 13, 14, 19, and 20). These genes may be regulated directly or indirectly by SR1 in stress response and encode HOX24 transcription factor, galactinol synthase, diydroflavonol-4-reductase, fumarylacetoacetase, and protease inhibitor seed storage (LTPL) 17. Three protein phosphatase 2C (PP2C) genes are up-regulated in WT in treated conditions and two of these are not seen in mutant conditions. The parasitic plant, *Striga,* maintains high transpiration rates in drought conditions. A mutation in PP2C leads to ABA insensitivity, and *Striga'*s stomata remain open, maintaining high levels of transpiration in drought conditions (Fujioka et al., 2019). Based on this observation in *Striga* and the fact that expression of PP2C is down-regulated the *Ossr1* mutant, it is likely the PP2C may be involved in drought stress response in rice, and *Os*SR1 may regulate drought response by modulating PP2C expression. The role of PP2C in drought responses was also reported in other studies (Kumar et al., 2017).

Fifty percent of the down-regulated DE genes found in WT are not found in the mutant when exposed to drought stress (Tables 13, 14, 19, and 20). The proteins these genes encode

include: GOS9, a jacalin-related salt stress-induced protein, zinc-induced facilitator-like 1, cytosolic sulfotransferase 5 and 15, CHIT17, GDSL-like lipase acylhydrolase, rhodanese-like domain-containing protein (a transferase involved in detoxifying cyanide), WRKY 107, and glycol lipid transfer domain-containing protein. WRKY and CHIT 17 genes were induced together in a resistant rice cultivar in response to *Magnaporthe oryzae* infection (Bagnaresi et al., 2012).

Drought Stress Response in the absence of *Os*SR1

In *Ossr1,* an aquaporin gene is a top down-regulated gene in treated conditions in both pipelines (Tables 13 and 19). Disease resistance proteins of different families appear in both pipeline's top 20 down-regulated DE gene lists: pathogenesis-related protein PRB1-2, disease resistance protein RPS2, and NB-ARC domain-containing protein. CAMK_KIN1 SNF1, a CAMK which is a Ca^{2+}/CaM-dependent protein kinase is down-regulated in treated conditions (Pipeline 2). Three peroxidases and two silicon efflux transporters are down-regulated in treated conditions (Pipeline 1).

In response to osmotic stress, the mutant up-regulates genes encoding homeobox leucine zipper TF and MAPKKKs (seen in both Pipelines). Pipeline 1 reports up-regulation of ethylene-responsive transcription factor (ERF) 111 and EID1-like F-box protein 3. Overexpression of an *OsERF* in rice led to the regulation of a calmodulin-like protein (CML) gene that is a positive regulator of drought stress (Jung et al., 2017). ERF and EID1-like F-box protein 3 are known to be involved in regulating ABA signaling (Koops et al., 2011). WRKY and DIVARICATA transcription factors as well as SENESCENCE-ASSOCIATED GENE 21 (SAG21) are up-

regulated in the mutant (Pipeline 1), and two nodulin related proteins are up-regulated in the mutant (Pipeline 2).

Transcriptomic studies paint a broad picture of the genetic state of an organism at a moment in time. Robust statistical methods can focus this broad picture down to the level of a gene. The next step in deciphering the action of *Os*SR1 will be to analyze the promoter sequences of candidate DE genes to identify if the *Os*SR1 DNA binding site (CG-1) is present and test the binding of SR1 to the promoter of one or more of these genes. From this, we will know if the gene of interest is a direct or indirect target of *Os*SR1.

Continued research of *Os*SR1 in stress response will need to be done to determine how this transcription factor impacts gene expression in various conditions and at various developmental stages in rice. The assortment of transgenic lines generated in this study and RNA-seq results will aid in this discovery.

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Appendix 1. Supplementary Tables

Supplementary Table 1. Pipeline 1: Top 100 DE genes in comparisons averaging over timepoints. Up and down refer to the expression of genes in the treated as compared to control group or the mutant as compared to WT. Gene IDs are in RAP-DB format.

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
1	WT_CvsMu_C	Down	Os10t0375600-01	0.00E+00	CMTA2_ARATH Calmodulin-binding transcription activator 2 OS
2	WT_CvsMu_C	Down	Os04t0103800-01	1.48E-108	
3	WT_CvsMu_C	Down	Os06t0329400-00	1.33E-70	
4	WT_CvsMu_C	Down	Os04t0103700-01	3.94E-64	Y2913_ARATH G-type lectin S-receptor-like serine/threonine-protein kinase At2g19130 OS
5	WT_CvsMu_C	Down	Os01t0847600-01	1.06E-26	AKRCA_ARATH Aldo-keto reductase family 4 member C10 OS
6	WT_CvsMu_C	Down	Os06t0665800-01	5.69E-14	HMA9_ORYSJ Cation-transporting ATPase HMA5 OS
7	WT_CvsMu_C	Down	Os09t0521300-00	4.59E-12	PCF2_ORYSJ Transcription factor PCF2 OS
8	WT_CvsMu_C	Down	Os04t0125700-01	1.08E-06	LRK91_ARATH L-type lectin-domain containing receptor kinase IX.1 OS
9	WT_CvsMu_C	Down	Os05t0160600-01	1.08E-06	DGP14_ARATH Putative protease Do-like 14 OS
10	WT_CvsMu_C	Down	Os06t0246200-00	1.18E-06	
11	WT_CvsMu_C	Down	Os11t0529900-00	3.63E-06	CUS_ORYSJ Bisdemethoxycurcumin synthase OS
12	WT_CvsMu_C	Down	Os04t0139100-00	9.04E-06	
13	WT_CvsMu_C	Down	Os08t0290700-01	3.41E-05	Y1103_ORYSJ Flavonoid O-methyltransferase-like protein Os11g0303600 OS
14	WT_CvsMu_C	Down	Os07t0453200-00	4.80E-05	
15	WT_CvsMu_C	Down	Os04t0357300-01	1.04E-04	PIF1_DANRE ATP-dependent DNA helicase PIF1 OS
16	WT_CvsMu_C	Down	Os04t0112200-00	1.74E-04	E2FB_ARATH Transcription factor E2FB OS
17	WT_CvsMu_C	Down	Os09t0538000-05	4.50E-04	
18	WT_CvsMu_C	Down	Os04t0116800-01	4.56E-04	KCS6_ARATH 3-ketoacyl-CoA synthase 6 OS
19	WT_CvsMu_C	Down	Os09t0403300-00	9.56E-04	C7351_ARATH Cytokinin hydroxylase OS
20	WT_CvsMu_C	Down	Os09t0505400-00	1.75E-03	PIN5B_ORYSJ Probable auxin efflux carrier component 5b OS
21	WT_CvsMu_C	Down	Os12t0290200-01	1.75E-03	
22	WT_CvsMu_C	Down	Os07t0127700-01	1.88E-03	PR13_HORVU Pathogenesis-related protein PRB1-3 OS
23	WT_CvsMu_C	Down	Os04t0445100-01	3.75E-03	HS232_ORYSJ 23.2 kDa heat shock protein OS
24	WT_CvsMu_C	Down	Os04t0415800-00	3.83E-03	

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
25	WT_CvsMu_C	Down	Os02t0548301-01	4.27E-03	
26	WT_CvsMu_C	Down	Os06t0230000-01	4.63E-03	
27	WT_CvsMu_C	Down	Os11t0641800-01	6.94E-03	LAC20_ORYSJ Laccase-20 OS
28	WT_CvsMu_C	Down	Os03t0562400-01	8.35E-03	
29	WT_CvsMu_C	Down	Os03t0369000-01	8.49E-03	PER2_MAIZE Peroxidase 2 OS
30	WT_CvsMu_C	Down	Os03t0667500-01	8.56E-03	IRT1_ORYSJ Fe(2+) transport protein 1 OS
31	WT_CvsMu_C	Down	Os09t0514500-00	1.11E-02	
32	WT_CvsMu_C	Down	Os06t0564700-01	1.23E-02	CYSKP_SPIOL Cysteine synthase, chloroplastic/chromoplastic OS
33	WT_CvsMu_C	Down	Os09t0431750-00	1.23E-02	ZFP2_ARATH Zinc finger protein 2 OS
34	WT_CvsMu_C	Down	Os12t0211900-01	1.23E-02	
35	WT_CvsMu_C	Down	Os12t0570700-01	1.49E-02	MT4A_ORYSJ Metallothionein-like protein 4A OS
36	WT_CvsMu_C	Down	Os03t0658800-01	1.61E-02	C87A3_ORYSJ Cytochrome P450 87A3 OS
37	WT_CvsMu_C	Down	Os04t0630300-01	1.64E-02	ANRCS_VITVI Anthocyanidin reductase ((2S)-flavan-3-ol-forming) OS
38	WT_CvsMu_C	Down	Os10t0528900-01	2.15E-02	GSTU6_ORYSJ Probable glutathione S-transferase GSTU6 OS
39	WT_CvsMu_C	Down	Os10t0352000-01	3.55E-02	
40	WT_CvsMu_C	Down	Os01t0916400-01	4.04E-02	SEBP1_ARATH Selenium-binding protein 1 OS
41	WT_CvsMu_C	Down	Os10t0335000-00	4.32E-02	DIR4_ARATH Dirigent protein 4 OS
42	WT_CvsMu_C	Down	Os05t0247100-02	4.52E-02	XIP2_ORYSJ Xylanase inhibitor protein 2 OS
43	WT_CvsMu_C	Down	Os03t0424550-00	4.71E-02	RAP_ORYSJ RAP domain-containing protein, chloroplastic OS
1	WT_CvsMu_C	Up	Os10t0207450-01	1.32E-257	
2	WT_CvsMu_C	Up	Os04t0565750-00	5.63E-119	
3	WT_CvsMu_C	Up	Os02t0772100-01	6.78E-92	
4	WT_CvsMu_C	Up	Os02t0765900-00	6.68E-75	NIR_ORYSJ Ferredoxin--nitrite reductase, chloroplastic OS
5	WT_CvsMu_C	Up	Os01t0262401-00	7.78E-72	
6	WT_CvsMu_C	Up	Os04t0510000-01	1.60E-69	Y3643_ORYSJ B3 domain-containing protein Os03g0164300 OS
7	WT_CvsMu_C	Up	Os12t0117700-01	4.04E-60	
8	WT_CvsMu_C	Up	Os07t0511400-01	6.50E-54	

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
9	WT_CvsMu_C	Up	Os03t0629800-01	9.92E-52	
10	WT_CvsMu_C	Up	Os09t0358000-00	8.00E-46	Y5189_ARATH Probable LRR receptor-like protein kinase At1g51890 OS
11	WT_CvsMu_C	Up	Os01t0262300-00	6.61E-45	
12	WT_CvsMu_C	Up	Os01t0382450-01	1.92E-38	
13	WT_CvsMu_C	Up	Os10t0381601-01	5.12E-38	
14	WT_CvsMu_C	Up	Os02t0288600-01	8.37E-37	BURP4_ORYSJ BURP domain-containing protein 4 OS
15	WT_CvsMu_C	Up	Os04t0115700-02	4.98E-33	
16	WT_CvsMu_C	Up	Os12t0228500-01	1.64E-30	GSTT3_ARATH Glutathione S-transferase T3 OS
17	WT_CvsMu_C	Up	Os03t0115800-01	9.91E-30	
18	WT_CvsMu_C	Up	Os12t0169300-00	1.34E-29	
19	WT_CvsMu_C	Up	Os02t0727200-00	2.81E-23	
20	WT_CvsMu_C	Up	Os02t0258300-01	3.76E-23	XLG1_ARATH Extra-large guanine nucleotide-binding protein 1 OS
21	WT_CvsMu_C	Up	Os01t0960500-01	2.41E-22	RGLG3_ARATH E3 ubiquitin-protein ligase RGLG3 OS
22	WT_CvsMu_C	Up	Os12t0584700-01	1.32E-21	
23	WT_CvsMu_C	Up	Os10t0494100-00	4.37E-21	
24	WT_CvsMu_C	Up	Os12t0508400-00	4.49E-19	
25	WT_CvsMu_C	Up	Os05t0580200-01	5.74E-19	
26	WT_CvsMu_C	Up	Os04t0116600-01	4.27E-18	KCR1_ARATH Very-long-chain 3-oxoacyl-CoA reductase 1 OS
27	WT_CvsMu_C	Up	Os10t0142900-01	4.45E-18	
28	WT_CvsMu_C	Up	Os04t0223600-01	6.06E-18	
29	WT_CvsMu_C	Up	Os11t0491600-00	8.00E-17	
30	WT_CvsMu_C	Up	Os05t0501001-01	1.40E-16	
31	WT_CvsMu_C	Up	Os07t0636600-01	5.71E-14	DIR5_ARATH Dirigent protein 5 OS
32	WT_CvsMu_C	Up	Os07t0457300-01	1.34E-12	
33	WT_CvsMu_C	Up	Os11t0213000-01	1.80E-12	CRK29_ARATH Cysteine-rich receptor-like protein kinase 29 OS
34	WT_CvsMu_C	Up	Os04t0618900-00	1.96E-12	
35	WT_CvsMu_C	Up	Os04t0115700-01	2.11E-12	

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
36	WT_CvsMu_C	Up	Os07t0580700-01	4.59E-12	
37	WT_CvsMu_C	Up	Os03t0861300-01	6.34E-12	PIP28_ORYSJ Probable aquaporin PIP2-8 OS
38	WT_CvsMu_C	Up	Os04t0249500-00	9.94E-11	SUS7_ORYSJ Sucrose synthase 7 OS
39	WT_CvsMu_C	Up	Os03t0426350-00	1.65E-10	
40	WT_CvsMu_C	Up	Os04t0116200-01	1.67E-10	
41	WT_CvsMu_C	Up	Os04t0204000-00	1.71E-10	UGT13_HORVV UDP-glucosyltransferase UGT13248 OS
42	WT_CvsMu_C	Up	Os04t0677000-01	2.69E-10	
43	WT_CvsMu_C	Up	Os10t0104000-01	2.95E-10	
44	WT_CvsMu_C	Up	Os05t0472400-00	8.16E-10	ZIP9_ORYSJ Zinc transporter 9 OS
45	WT_CvsMu_C	Up	Os04t0474900-01	1.74E-09	BGL13_ORYSJ Beta-glucosidase 13 OS
46	WT_CvsMu_C	Up	Os10t0429400-00	1.87E-09	BPM1_ARATH BTB/POZ and MATH domain-containing protein 1 OS
47	WT_CvsMu_C	Up	Os03t0105500-01	2.11E-09	
48	WT_CvsMu_C	Up	Os05t0209500-01	1.43E-08	
49	WT_CvsMu_C	Up	Os07t0511100-00	1.75E-08	
50	WT_CvsMu_C	Up	Os07t0110000-01	2.81E-08	
51	WT_CvsMu_C	Up	Os05t0175450-00	3.30E-08	Y3643_ORYSJ B3 domain-containing protein Os03g0164300 OS
52	WT_CvsMu_C	Up	Os12t0554100-01	3.85E-08	TBL16_ARATH Protein trichome birefringence-like 16 OS
53	WT_CvsMu_C	Up	Os10t0159166-01	5.99E-08	
54	WT_CvsMu_C	Up	Os11t0638700-00	7.21E-07	
55	WT_CvsMu_C	Up	Os04t0117900-01	7.62E-07	AMI1_ORYSJ Amidase 1 OS
56	WT_CvsMu_C	Up	Os04t0117600-01	1.36E-06	DUS3L_ORYSJ tRNA-dihydrouridine(47) synthase [NAD(P)(+)]-like OS
57	WT_CvsMu_C	Up	Os01t0165800-01	7.51E-06	
58	WT_CvsMu_C	Up	Os04t0397800-02	7.51E-06	
59	WT_CvsMu_C	Up	Os09t0349901-00	8.07E-06	RLK6_ARATH Receptor-like protein kinase At3g21340 OS
60	WT_CvsMu_C	Up	Os10t0139700-01	8.14E-06	C89A2_ARATH Cytochrome P450 89A2 OS
61	WT_CvsMu_C	Up	Os06t0141400-01	8.33E-06	NO93_SOYBN Early nodulin-93 OS
62	WT_CvsMu_C	Up	Os01t0692400-01	9.81E-06	

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
63	WT_CvsMu_C	Up	Os04t0114801-00	9.86E-06	
64	WT_CvsMu_C	Up	Os02t0252400-01	2.70E-05	DOF3_ORYSJ Dof zinc finger protein 3 OS
65	WT_CvsMu_C	Up	Os04t0249600-01	2.97E-05	STR18_ARATH Thiosulfate sulfurtransferase 18 OS
66	WT_CvsMu_C	Up	Os02t0734400-00	4.64E-05	PXG4_ARATH Probable peroxygenase 4 OS
67	WT_CvsMu_C	Up	Os08t0247700-00	4.94E-05	Y3475_ARATH Probable LRR receptor-like serine/threonine-protein kinase At3g47570 OS
68	WT_CvsMu_C	Up	Os09t0436000-01	5.27E-05	
69	WT_CvsMu_C	Up	Os04t0379933-01	8.84E-05	
70	WT_CvsMu_C	Up	Os05t0166600-00	1.74E-04	Y2913_ARATH G-type lectin S-receptor-like serine/threonine-protein kinase At2g19130 OS
71	WT_CvsMu_C	Up	Os07t0457200-01	1.81E-04	AGM1_ORYSJ Phosphoacetylglucosamine mutase OS
72	WT_CvsMu_C	Up	Os05t0121750-00	1.97E-04	
73	WT_CvsMu_C	Up	Os01t0672632-01	2.31E-04	
74	WT_CvsMu_C	Up	Os04t0115600-01	2.74E-04	
75	WT_CvsMu_C	Up	Os11t0632200-01	2.74E-04	
76	WT_CvsMu_C	Up	Os04t0319900-00	3.54E-04	
77	WT_CvsMu_C	Up	Os10t0469300-00	3.54E-04	Y1571_ARATH Probable leucine-rich repeat receptor-like protein kinase At1g35710 OS
78	WT_CvsMu_C	Up	Os06t0325900-00	4.09E-04	C71C4_MAIZE indole-2-monooxygenase OS
79	WT_CvsMu_C	Up	Os04t0606601-00	5.54E-04	
80	WT_CvsMu_C	Up	Os01t0749800-00	6.41E-04	OFP2_ARATH Transcription repressor OFP2 OS
81	WT_CvsMu_C	Up	Os01t0858900-01	6.60E-04	STLP1_ORYSJ Sialyltransferase-like protein 1 OS
82	WT_CvsMu_C	Up	Os01t0564300-01	6.91E-04	FKB65_ARATH Peptidyl-prolyl cis-trans isomerase FKBP65 OS
83	WT_CvsMu_C	Up	Os01t0267300-00	7.40E-04	SPZ12_ORYSJ Putative serpin-Z12 OS
84	WT_CvsMu_C	Up	Os05t0477900-01	9.35E-04	NLTP_MALDO Non-specific lipid-transfer protein OS
85	WT_CvsMu_C	Up	Os12t0576700-01	1.01E-03	PPA1_ARATH Probable inactive purple acid phosphatase 1 OS
86	WT_CvsMu_C	Up	Os03t0309400-01	1.14E-03	PME67_ARATH Probable pectinesterase 67 OS
87	WT_CvsMu_C	Up	Os09t0554101-00	1.17E-03	
88	WT_CvsMu_C	Up	Os02t0297200-01	1.36E-03	
89	WT_CvsMu_C	Up	Os10t0469100-02	2.61E-03	

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
90	WT_CvsMu_C	Up	Os07t0132500-01	4.37E-03	LRK44_ARATH L-type lectin-domain containing receptor kinase IV.4 OS
91	WT_CvsMu_C	Up	Os01t0510200-01	6.24E-03	
92	WT_CvsMu_C	Up	Os07t0122000-01	7.84E-03	
93	WT_CvsMu_C	Up	Os12t0528801-01	8.49E-03	
94	WT_CvsMu_C	Up	Os05t0161100-01	1.60E-02	
95	WT_CvsMu_C	Up	Os05t0189900-01	2.08E-02	PPA1_SOLLC Acid phosphatase 1 OS
96	WT_CvsMu_C	Up	Os10t0472900-01	2.13E-02	KCS12_ARATH 3-ketoacyl-CoA synthase 12 OS
97	WT_CvsMu_C	Up	Os01t0533900-01	2.68E-02	AB11B_ARATH ABC transporter B family member 11 OS
98	WT_CvsMu_C	Up	Os02t0484600-00	2.78E-02	PMAT2_ARATH Phenolic glucoside malonyltransferase 2 OS
99	WT_CvsMu_C	Up	Os08t0358050-00	3.18E-02	
100	WT_CvsMu_C	Up	Os01t0341300-01	4.57E-02	
1	WT_TvsMu_T	Down	Os10t0375600-01	2.98E-71	CMTA2_ARATH Calmodulin-binding transcription activator 2 OS
2	WT_TvsMu_T	Down	Os06t0329400-00	1.76E-37	
3	WT_TvsMu_T	Down	Os04t0103800-01	3.36E-27	
4	WT_TvsMu_T	Down	Os04t0103700-01	6.99E-13	Y2913_ARATH G-type lectin S-receptor-like serine/threonine-protein kinase At2g19130 OS
5	WT_TvsMu_T	Down	Os01t0847600-01	3.41E-07	AKRCA_ARATH Aldo-keto reductase family 4 member C10 OS
6	WT_TvsMu_T	Down	Os06t0246200-00	2.06E-03	
7	WT_TvsMu_T	Down	Os04t0125700-01	6.43E-03	LRK91_ARATH L-type lectin-domain containing receptor kinase IX.1 OS
8	WT_TvsMu_T	Down	Os03t0562400-01	8.78E-03	
9	WT_TvsMu_T	Down	Os11t0529900-00	1.48E-02	CUS_ORYSJ Bisdemethoxycurcumin synthase OS
10	WT_TvsMu_T	Down	Os09t0521300-00	2.00E-02	PCF2_ORYSJ Transcription factor PCF2 OS
11	WT_TvsMu_T	Down	Os09t0360500-01	2.37E-02	KCS17_ARATH 3-ketoacyl-CoA synthase 17 OS
1	WT_TvsMu_T	Up	Os01t0262401-00	1.00E-21	
2	WT_TvsMu_T	Up	Os01t0382450-01	7.03E-19	
3	WT_TvsMu_T	Up	Os04t0565750-00	1.05E-18	
4	WT_TvsMu_T	Up	Os02t0288600-01	4.84E-18	BURP4_ORYSJ BURP domain-containing protein 4 OS
5	WT_TvsMu_T	Up	Os09t0358000-00	7.36E-17	Y5189_ARATH Probable LRR receptor-like protein kinase At1g51890 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
6	WT_TvsMu_T	Up	Os05t0580200-01	3.08E-15	
7	WT_TvsMu_T	Up	Os02t0765900-00	2.98E-14	NIR_ORYSJ Ferredoxin--nitrite reductase, chloroplastic OS
8	WT_TvsMu_T	Up	Os02t0772100-01	7.84E-14	
9	WT_TvsMu_T	Up	Os10t0381601-01	9.93E-12	
10	WT_TvsMu_T	Up	Os01t0262300-00	1.18E-11	
11	WT_TvsMu_T	Up	Os07t0511400-01	1.85E-11	
12	WT_TvsMu_T	Up	Os04t0510000-01	2.75E-09	Y3643_ORYSJ B3 domain-containing protein Os03g0164300 OS
13	WT_TvsMu_T	Up	Os03t0629800-01	8.74E-09	
14	WT_TvsMu_T	Up	Os04t0115700-01	1.16E-08	
15	WT_TvsMu_T	Up	Os05t0501001-01	5.94E-08	
16	WT_TvsMu_T	Up	Os10t0207450-01	1.13E-06	
17	WT_TvsMu_T	Up	Os12t0228500-01	1.99E-06	GSTT3_ARATH Glutathione S-transferase T3 OS
18	WT_TvsMu_T	Up	Os05t0175450-00	4.38E-06	Y3643_ORYSJ B3 domain-containing protein Os03g0164300 OS
19	WT_TvsMu_T	Up	Os07t0511100-00	2.72E-05	
20	WT_TvsMu_T	Up	Os05t0472400-00	4.02E-05	ZIP9_ORYSJ Zinc transporter 9 OS
21	WT_TvsMu_T	Up	Os12t0169300-00	4.67E-05	
22	WT_TvsMu_T	Up	Os04t0618900-00	7.83E-05	
23	WT_TvsMu_T	Up	Os12t0117700-01	7.83E-05	
24	WT_TvsMu_T	Up	Os12t0584700-01	1.11E-04	
25	WT_TvsMu_T	Up	Os03t0115800-01	2.86E-04	
26	WT_TvsMu_T	Up	Os06t0141400-01	4.69E-04	NO93_SOYBN Early nodulin-93 OS
27	WT_TvsMu_T	Up	Os10t0159166-01	4.69E-04	
28	WT_TvsMu_T	Up	Os02t0216200-01	5.43E-04	
29	WT_TvsMu_T	Up	Os10t0142900-01	5.43E-04	
30	WT_TvsMu_T	Up	Os01t0672632-01	5.82E-04	
31	WT_TvsMu_T	Up	Os05t0166600-00	5.82E-04	Y2913_ARATH G-type lectin S-receptor-like serine/threonine-protein kinase At2g19130 OS
32	WT_TvsMu_T	Up	Os03t0426350-00	6.93E-04	

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
33	WT_TvsMu_T	Up	Os01t0692400-01	1.22E-03	
34	WT_TvsMu_T	Up	Os10t0139700-01	1.66E-03	C89A2_ARATH Cytochrome P450 89A2 OS
35	WT_TvsMu_T	Up	Os12t0508400-00	2.04E-03	
36	WT_TvsMu_T	Up	Os10t0429400-00	2.06E-03	BPM1_ARATH BTB/POZ and MATH domain-containing protein 1 OS
37	WT_TvsMu_T	Up	Os02t0727200-00	2.60E-03	
38	WT_TvsMu_T	Up	Os04t0115700-02	4.37E-03	
39	WT_TvsMu_T	Up	Os04t0249500-00	4.78E-03	SUS7_ORYSJ Sucrose synthase 7 OS
40	WT_TvsMu_T	Up	Os04t0204000-00	5.31E-03	UGT13_HORVV UDP-glucosyltransferase UGT13248 OS
41	WT_TvsMu_T	Up	Os04t0112100-01	5.40E-03	
42	WT_TvsMu_T	Up	Os04t0223600-01	5.40E-03	
43	WT_TvsMu_T	Up	Os11t0491600-00	6.43E-03	
44	WT_TvsMu_T	Up	Os04t0606601-00	8.11E-03	
45	WT_TvsMu_T	Up	Os01t0267300-00	1.88E-02	SPZ12_ORYSJ Putative serpin-Z12 OS
46	WT_TvsMu_T	Up	Os11t0620300-01	2.00E-02	NLTP2_MAIZE Probable non-specific lipid-transfer protein 2 OS
47	WT_TvsMu_T	Up	Os08t0317101-00	2.18E-02	
48	WT_TvsMu_T	Up	Os03t0371000-01	2.41E-02	C70B2_ARATH Cytochrome P450 709B2 OS
49	WT_TvsMu_T	Up	Os02t0240100-01	2.49E-02	PER70_MAIZE Peroxidase 70 OS
50	WT_TvsMu_T	Up	Os11t0213000-01	2.65E-02	CRK29_ARATH Cysteine-rich receptor-like protein kinase 29 OS
51	WT_TvsMu_T	Up	Os10t0469300-00	2.69E-02	Y1571_ARATH Probable leucine-rich repeat receptor-like protein kinase At1g35710 OS
52	WT_TvsMu_T	Up	Os10t0469100-02	3.34E-02	
53	WT_TvsMu_T	Up	Os04t0319900-00	3.40E-02	
54	WT_TvsMu_T	Up	Os01t0960500-01	4.10E-02	RGLG3_ARATH E3 ubiquitin-protein ligase RGLG3 OS
55	WT_TvsMu_T	Up	Os04t0114801-00	4.10E-02	
56	WT_TvsMu_T	Up	Os07t0636600-01	4.56E-02	DIR5_ARATH Dirigent protein 5 OS
1	Mu_CvsMu_T	Down	Os07t0677300-01	2.40E-44	PER2_ORYSJ Peroxidase 2 OS
2	Mu_CvsMu_T	Down	Os03t0107300-01	7.56E-42	LSI2_ORYSJ Silicon efflux transporter LSI2 OS
3	Mu_CvsMu_T	Down	Os02t0745100-01	3.27E-34	NIP21_ORYSJ Aquaporin NIP2-1 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
4	Mu_CvsMu_T	Down	Os11t0179700-00	1.63E-33	DIR2_ARATH Dirigent protein 2 OS
5	Mu_CvsMu_T	Down	Os01t0550800-01	1.90E-33	
6	Mu_CvsMu_T	Down	Os07t0442900-01	1.65E-32	CSPL7_ORYSJ CASP-like protein 1D1 OS
7	Mu_CvsMu_T	Down	Os07t0635200-00	4.39E-30	C70B2_ARATH Cytochrome P450 709B2 OS
8	Mu_CvsMu_T	Down	Os02t0684100-00	6.04E-30	SOT15_ARATH Cytosolic sulfotransferase 15 OS
9	Mu_CvsMu_T	Down	Os05t0503650-01	1.10E-29	
10	Mu_CvsMu_T	Down	Os01t0347600-01	1.13E-28	BROM1_ANACO Fruit bromelain OS
11	Mu_CvsMu_T	Down	Os01t0347800-00	1.25E-28	BROM1_ANACO Fruit bromelain OS
12	Mu_CvsMu_T	Down	Os07t0645300-01	3.53E-28	DMP1_ARATH Protein DMP1 OS
13	Mu_CvsMu_T	Down	Os06t0641500-00	3.39E-27	C7D55_HYOMU Premnaspirodiene oxygenase OS
14	Mu_CvsMu_T	Down	Os01t0915900-01	3.85E-27	
15	Mu_CvsMu_T	Down	Os02t0218800-01	5.98E-27	C74A4_ORYSJ Allene oxide synthase 4 OS
16	Mu_CvsMu_T	Down	Os07t0104500-01	1.36E-26	PER1_ORYSJ Peroxidase 1 OS
17	Mu_CvsMu_T	Down	Os12t0259800-00	1.61E-26	LAC25_ORYSJ Laccase-25 OS
18	Mu_CvsMu_T	Down	Os05t0134800-00	1.14E-25	PER2_MAIZE Peroxidase 2 OS
19	Mu_CvsMu_T	Down	Os07t0648000-01	2.68E-25	
20	Mu_CvsMu_T	Down	Os02t0650300-01	2.97E-25	YSL15_ORYSJ Iron-phytosiderophore transporter YSL15 OS
21	Mu_CvsMu_T	Down	Os03t0667500-01	4.89E-25	IRT1_ORYSJ Fe(2+) transport protein 1 OS
22	Mu_CvsMu_T	Down	Os10t0191300-01	8.30E-25	PR12_HORVU Pathogenesis-related protein PRB1-2 OS
23	Mu_CvsMu_T	Down	Os09t0311600-01	2.53E-24	RPS2_ARATH Disease resistance protein RPS2 OS
24	Mu_CvsMu_T	Down	Os10t0547500-01	3.29E-24	LSI3_ORYSJ Silicon efflux transporter LSI3 OS
25	Mu_CvsMu_T	Down	Os06t0692100-01	3.85E-23	PSYR1_ARATH Tyrosine-sulfated glycopeptide receptor 1 OS
26	Mu_CvsMu_T	Down	Os01t0916000-01	6.80E-23	
27	Mu_CvsMu_T	Down	Os11t0307300-01	9.49E-23	OMT2_SORBI Probable O-methyltransferase 2 OS
28	Mu_CvsMu_T	Down	Os01t0916100-01	9.77E-23	
29	Mu_CvsMu_T	Down	Os03t0307200-01	1.22E-22	NAS2_ORYSJ Nicotianamine synthase 2 OS
30	Mu_CvsMu_T	Down	Os03t0185700-01	1.33E-21	TBT2_ORYSJ Tryptamine benzoyltransferase 2 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
31	Mu_CvsMu_T	Down	Os04t0659200-01	1.33E-21	
32	Mu_CvsMu_T	Down	Os11t0593000-01	1.70E-21	NPC4_ARATH Non-specific phospholipase C4 OS
33	Mu_CvsMu_T	Down	Os04t0438200-01	1.72E-21	
34	Mu_CvsMu_T	Down	Os09t0513200-00	2.05E-21	S35F1_HUMAN Solute carrier family 35 member F1 OS
35	Mu_CvsMu_T	Down	Os03t0297600-01	2.78E-21	PYL4_ARATH Abscisic acid receptor PYL4 OS
36	Mu_CvsMu_T	Down	Os05t0424300-01	2.98E-21	C71A1_PERAE Cytochrome P450 71A1 OS
37	Mu_CvsMu_T	Down	Os08t0189300-01	3.06E-21	GL84_ORYSJ Germin-like protein 8-4 OS
38	Mu_CvsMu_T	Down	Os08t0189200-01	6.99E-21	GL83_ORYSJ Germin-like protein 8-3 OS
39	Mu_CvsMu_T	Down	Os02t0131800-01	7.45E-21	NRAT1_ORYSJ Metal transporter NRAT1 OS
40	Mu_CvsMu_T	Down	Os03t0103100-01	1.02E-20	CCDP_MAIZE Cortical cell-delineating protein OS
41	Mu_CvsMu_T	Down	Os06t0692600-01	1.14E-20	PSYR1_ARATH Tyrosine-sulfated glycopeptide receptor 1 OS
42	Mu_CvsMu_T	Down	Os07t0532800-00	2.81E-20	NEP1_NEPGR Aspartic proteinase nepenthesin-1 OS
43	Mu_CvsMu_T	Down	Os12t0137700-01	3.49E-20	SOT5_ARATH Cytosolic sulfotransferase 5 OS
44	Mu_CvsMu_T	Down	Os06t0542300-01	5.50E-20	HIP29_ARATH Heavy metal-associated isoprenylated plant protein 29 OS
45	Mu_CvsMu_T	Down	Os10t0150300-01	5.78E-20	
46	Mu_CvsMu_T	Down	Os02t0620600-01	5.84E-20	AMT12_ORYSJ Ammonium transporter 1 member 2 OS
47	Mu_CvsMu_T	Down	Os05t0399300-01	1.35E-19	CHI2_ORYSJ Chitinase 2 OS
48	Mu_CvsMu_T	Down	Os05t0162000-01	1.80E-19	PER5_VITVI Peroxidase 5 OS
49	Mu_CvsMu_T	Down	Os01t0810800-01	2.36E-19	SIRK_ARATH Senescence-induced receptor-like serine/threonine-protein kinase OS
50	Mu_CvsMu_T	Down	Os04t0381700-00	3.34E-19	FIT_ARATH Transcription factor FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR OS
51	Mu_CvsMu_T	Down	Os11t0241200-01	4.23E-19	
52	Mu_CvsMu_T	Down	Os01t0952800-01	4.26E-19	IRO2_ORYSJ Protein IRON-RELATED TRANSCRIPTION FACTOR 2 OS
53	Mu_CvsMu_T	Down	Os12t0260500-01	5.21E-19	SDR5_ARATH Short-chain dehydrogenase reductase 5 OS
54	Mu_CvsMu_T	Down	Os10t0323500-01	6.25E-19	BGL34_ORYSJ Beta-glucosidase 34 OS
55	Mu_CvsMu_T	Down	Os07t0519100-01	6.40E-19	C16B1_PICSI Cytochrome P450 716B1 OS
56	Mu_CvsMu_T	Down	Os10t0105700-01	8.81E-19	SDC2_ORYSJ Serine decarboxylase 2 OS
57	Mu_CvsMu_T	Down	Os05t0415800-01	2.10E-18	CP51_SORBI Obtusifoliol 14-alpha demethylase OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
58	Mu_CvsMu_T	Down	Os02t0118600-00	2.26E-18	RGA3_SOLBU Putative disease resistance protein RGA3 OS
59	Mu_CvsMu_T	Down	Os04t0635600-00	5.62E-18	
60	Mu_CvsMu_T	Down	Os03t0307300-01	7.17E-18	NAS1_ORYSJ Nicotianamine synthase 1 OS
61	Mu_CvsMu_T	Down	Os05t0596500-01	8.65E-18	ATL12_ARATH Putative RING-H2 finger protein ATL12 OS
62	Mu_CvsMu_T	Down	Os04t0521100-01	9.06E-18	PIP23_ORYSJ Probable aquaporin PIP2-3 OS
63	Mu_CvsMu_T	Down	Os03t0201500-01	1.02E-17	RSGI6_CLOTH Anti-sigma-I factor RsgI6 OS
64	Mu_CvsMu_T	Down	Os07t0630400-01	1.42E-17	RNS1_ARATH Ribonuclease 1 OS
65	Mu_CvsMu_T	Down	Os03t0368600-00	1.77E-17	PER2_MAIZE Peroxidase 2 OS
66	Mu_CvsMu_T	Down	Os05t0503500-00	1.77E-17	
67	Mu_CvsMu_T	Down	Os04t0125700-01	2.59E-17	LRK91_ARATH L-type lectin-domain containing receptor kinase IX.1 OS
68	Mu_CvsMu_T	Down	Os11t0134900-01	2.59E-17	ZIFL1_ARATH Protein ZINC INDUCED FACILITATOR-LIKE 1 OS
69	Mu_CvsMu_T	Down	Os11t0687050-00	2.82E-17	RGA4R_ORYSJ Disease resistance protein RGA4 OS
70	Mu_CvsMu_T	Down	Os02t0285300-01	5.20E-17	PCAP1_ARATH Plasma membrane-associated cation-binding protein 1 OS
71	Mu_CvsMu_T	Down	Os11t0555300-00	6.77E-17	DRL21_ARATH Putative disease resistance protein At3g14460 OS
72	Mu_CvsMu_T	Down	Os01t0347401-00	7.92E-17	BROM1_ANACO Fruit bromelain OS
73	Mu_CvsMu_T	Down	Os09t0272900-02	8.46E-17	RPS5_ARATH Disease resistance protein RPS5 OS
74	Mu_CvsMu_T	Down	Os02t0595900-00	9.86E-17	NAR21_ORYSJ High-affinity nitrate transporter-activating protein 2.1 OS
75	Mu_CvsMu_T	Down	Os06t0641400-01	1.53E-16	C71DC_CATRO Tabersonine 16-hydroxylase 1 OS
76	Mu_CvsMu_T	Down	Os04t0506500-00	2.16E-16	Y1137_ARATH G-type lectin S-receptor-like serine/threonine-protein kinase At1g61370 OS
77	Mu_CvsMu_T	Down	Os05t0213900-01	2.22E-16	
78	Mu_CvsMu_T	Down	Os05t0135000-01	2.26E-16	PER2_MAIZE Peroxidase 2 OS
79	Mu_CvsMu_T	Down	Os02t0157600-01	2.30E-16	STR17_ARATH Rhodanese-like domain-containing protein 17 OS
80	Mu_CvsMu_T	Down	Os04t0423800-01	3.30E-16	PER72_ARATH Peroxidase 72 OS
81	Mu_CvsMu_T	Down	Os07t0639400-01	3.94E-16	PER2_MAIZE Peroxidase 2 OS
82	Mu_CvsMu_T	Down	Os03t0368900-01	4.65E-16	PER2_MAIZE Peroxidase 2 OS
83	Mu_CvsMu_T	Down	Os06t0490400-00	6.85E-16	PER1_ORYSJ Peroxidase 1 OS
84	Mu_CvsMu_T	Down	Os08t0477500-01	7.25E-16	PLP2_ORYSJ Patatin-like protein 2 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
85	Mu_CvsMu_T	Down	Os02t0291400-00	7.63E-16	CNBL8_ORYSJ Calcineurin B-like protein 8 OS
86	Mu_CvsMu_T	Down	Os01t0237000-01	8.79E-16	CSPLB_ORYSJ CASP-like protein 1C1 OS
87	Mu_CvsMu_T	Down	Os04t0438101-00	1.14E-15	
88	Mu_CvsMu_T	Down	Os01t0521600-00	1.40E-15	
89	Mu_CvsMu_T	Down	Os09t0564200-01	1.42E-15	CYSP_HEMSP Thiol protease SEN102 OS
90	Mu_CvsMu_T	Down	Os07t0257200-01	2.60E-15	NRAM5_ORYSJ Metal transporter Nramp5 OS
91	Mu_CvsMu_T	Down	Os04t0567200-01	3.34E-15	ALMTA_ARATH Aluminum-activated malate transporter 10 OS
92	Mu_CvsMu_T	Down	Os10t0552600-01	3.52E-15	14KD_DAUCA 14 kDa proline-rich protein DC2.15 OS
93	Mu_CvsMu_T	Down	Os11t0700500-01	3.87E-15	MYBA1_ORYSJ Myb-related protein MYBAS1 OS
94	Mu_CvsMu_T	Down	Os02t0184900-01	4.09E-15	C71D8_SOYBN Cytochrome P450 71D8 OS
95	Mu_CvsMu_T	Down	Os01t0291500-01	4.47E-15	AT4_ORYSJ Acyl transferase 4 OS
96	Mu_CvsMu_T	Down	Os08t0398300-01	5.15E-15	AB7A_ARATH ABC transporter A family member 7 OS
97	Mu_CvsMu_T	Down	Os01t0803300-01	5.79E-15	WTR38_ARATH WAT1-related protein At5g07050 OS
98	Mu_CvsMu_T	Down	Os06t0136900-01	7.99E-15	AHL24_ARATH AT-hook motif nuclear-localized protein 24 OS
99	Mu_CvsMu_T	Down	Os11t0270000-01	9.35E-15	
100	Mu_CvsMu_T	Down	Os04t0452700-01	9.59E-15	MST1_ORYSJ Sugar transport protein MST1 OS
1	Mu_CvsMu_T	Up	Os04t0244800-01	2.07E-29	HIP26_ARATH Heavy metal-associated isoprenylated plant protein 26 OS
2	Mu_CvsMu_T	Up	Os03t0125100-01	5.06E-28	BCH_GENLU Beta-carotene 3-hydroxylase, chloroplastic OS
3	Mu_CvsMu_T	Up	Os03t0820300-01	2.71E-27	ZAT7_ARATH Zinc finger protein ZAT7 OS
4	Mu_CvsMu_T	Up	Os03t0386000-01	2.61E-26	YGI3_SCHPO Uncharacterized WD repeat-containing protein C2A9.03 OS
5	Mu_CvsMu_T	Up	Os12t0153200-01	3.07E-21	
6	Mu_CvsMu_T	Up	Os01t0868000-00	3.84E-21	EF110_ARATH Ethylene-responsive transcription factor ERF110 OS
7	Mu_CvsMu_T	Up	Os05t0494600-01	4.77E-21	EDL3_ARATH EID1-like F-box protein 3 OS
8	Mu_CvsMu_T	Up	Os02t0209300-01	6.99E-21	
9	Mu_CvsMu_T	Up	Os01t0901600-01	6.34E-20	4CLL6_ORYSJ 4-coumarate--CoA ligase-like 6 OS
10	Mu_CvsMu_T	Up	Os09t0325700-01	6.92E-20	P2C68_ORYSJ Probable protein phosphatase 2C 68 OS
11	Mu_CvsMu_T	Up	Os01t0314800-01	3.24E-18	SAG21_ARATH Protein SENESCENCE-ASSOCIATED GENE 21, mitochondrial OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
12	Mu_CvsMu_T	Up	Os05t0541100-01	3.96E-18	IQD1_ARATH Protein IQ-DOMAIN 1 OS
13	Mu_CvsMu_T	Up	Os03t0268600-01	5.73E-18	P2C30_ORYSJ Probable protein phosphatase 2C 30 OS
14	Mu_CvsMu_T	Up	Os11t0182100-01	6.80E-18	
15	Mu_CvsMu_T	Up	Os01t0863300-01	3.06E-17	DIV_ANTMA Transcription factor DIVARICATA OS
16	Mu_CvsMu_T	Up	Os07t0418500-01	4.84E-17	C70B2_ARATH Cytochrome P450 709B2 OS
17	Mu_CvsMu_T	Up	Os05t0583000-01	8.86E-17	WRK28_ARATH WRKY transcription factor 28 OS
18	Mu_CvsMu_T	Up	Os01t0609200-00	3.14E-16	AB35G_ORYSJ ABC transporter G family member 35 OS
19	Mu_CvsMu_T	Up	Os04t0541700-01	1.00E-15	HOX22_ORYSJ Homeobox-leucine zipper protein HOX22 OS
20	Mu_CvsMu_T	Up	Os08t0249000-01	3.17E-15	
21	Mu_CvsMu_T	Up	Os01t0699100-01	4.65E-15	M3K17_ARATH Mitogen-activated protein kinase kinase kinase 17 OS
22	Mu_CvsMu_T	Up	Os10t0173000-01	5.94E-15	
23	Mu_CvsMu_T	Up	Os12t0594000-00	1.05E-14	JGB_ARATH Protein JINGUBANG OS
24	Mu_CvsMu_T	Up	Os06t0652300-00	1.19E-14	FCL2_ORYSJ Putative GDP-L-fucose synthase 2 OS
25	Mu_CvsMu_T	Up	Os01t0128300-00	1.82E-14	MHZ4_ORYSJ Protein MAO HUZI 4, chloroplastic OS
26	Mu_CvsMu_T	Up	Os04t0345500-00	2.95E-14	
27	Mu_CvsMu_T	Up	Os06t0728700-01	4.30E-14	RVE2_ARATH Protein REVEILLE 2 OS
28	Mu_CvsMu_T	Up	Os02t0462800-01	8.43E-14	WRK11_ARATH Probable WRKY transcription factor 11 OS
29	Mu_CvsMu_T	Up	Os07t0622700-01	8.43E-14	MHPC_PARXL 2-hydroxy-6-oxononadienedioate/2-hydroxy-6-oxononatrienedioate hydrolase OS
30	Mu_CvsMu_T	Up	Os07t0592600-01	9.99E-14	GH38_ORYSJ Probable indole-3-acetic acid-amido synthetase GH3.8 OS
31	Mu_CvsMu_T	Up	Os07t0614300-01	3.34E-13	Y2740_DICDI von Willebrand factor A domain-containing protein DDB_G0292740 OS
32	Mu_CvsMu_T	Up	Os09t0555500-01	4.16E-13	PSY3_ORYSJ Phytoene synthase 3, chloroplastic OS
33	Mu_CvsMu_T	Up	Os10t0580400-01	7.94E-13	DUR3_ORYSJ Urea-proton symporter DUR3 OS
34	Mu_CvsMu_T	Up	Os06t0661200-00	1.37E-12	
35	Mu_CvsMu_T	Up	Os01t0802600-00	1.51E-12	EDL3_ARATH EID1-like F-box protein 3 OS
36	Mu_CvsMu_T	Up	Os05t0588900-01	1.81E-12	HSR4_ARATH Protein HYPER-SENSITIVITY-RELATED 4 OS
37	Mu_CvsMu_T	Up	Os07t0115500-01	1.96E-12	
38	Mu_CvsMu_T	Up	Os03t0820400-01	2.28E-12	ZAT8_ARATH Zinc finger protein ZAT8 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
39	Mu_CvsMu_T	Up	Os11t0429000-01	2.44E-12	
40	Mu_CvsMu_T	Up	Os09t0457900-01	4.00E-12	EF109_ARATH Ethylene-responsive transcription factor ERF109 OS
41	Mu_CvsMu_T	Up	Os03t0141200-01	4.50E-12	BAMY2_ORYSJ Beta-amylase 2, chloroplastic OS
42	Mu_CvsMu_T	Up	Os02t0205500-01	8.86E-12	KCS11_ARATH 3-ketoacyl-CoA synthase 11 OS
43	Mu_CvsMu_T	Up	Os10t0205700-01	9.84E-12	
44	Mu_CvsMu_T	Up	Os10t0392400-01	1.09E-11	TI11D_ORYSJ Protein TIFY 11d OS
45	Mu_CvsMu_T	Up	Os01t0209700-01	1.12E-11	G2OX3_ORYSJ Gibberellin 2-beta-dioxygenase 3 OS
46	Mu_CvsMu_T	Up	Os07t0154100-01	1.28E-11	NCED4_ORYSJ 9-cis-epoxycarotenoid dioxygenase NCED4, chloroplastic OS
47	Mu_CvsMu_T	Up	Os03t0792800-01	1.31E-11	E138_ARATH Glucan endo-1,3-beta-glucosidase 8 OS
48	Mu_CvsMu_T	Up	Os12t0633600-01	1.70E-11	CSCLA_ARATH CSC1-like protein RXW8 OS
49	Mu_CvsMu_T	Up	Os06t0127100-01	2.53E-11	DRE1C_ORYSJ Dehydration-responsive element-binding protein 1C OS
50	Mu_CvsMu_T	Up	Os04t0671200-01	2.53E-11	PAO2_ARATH Probable polyamine oxidase 2 OS
51	Mu_CvsMu_T	Up	Os03t0296200-00	2.63E-11	
52	Mu_CvsMu_T	Up	Os03t0844700-01	3.05E-11	ALB4_ARATH ALBINO3-like protein 1, chloroplastic OS
53	Mu_CvsMu_T	Up	Os08t0374600-00	6.09E-11	ACCR3_ARATH Putative serine/threonine-protein kinase-like protein CCR3 OS
54	Mu_CvsMu_T	Up	Os03t0214200-01	6.93E-11	NNJA1_ORYSJ Ninja-family protein MODD OS
55	Mu_CvsMu_T	Up	Os05t0457200-01	7.93E-11	P2C49_ORYSJ Probable protein phosphatase 2C 49 OS
56	Mu_CvsMu_T	Up	Os10t0553300-01	8.05E-11	TPP2_ORYSJ Probable trehalose-phosphate phosphatase 2 OS
57	Mu_CvsMu_T	Up	Os02t0766700-01	9.98E-11	BZP23_ORYSJ bZIP transcription factor 23 OS
58	Mu_CvsMu_T	Up	Os05t0211100-01	1.02E-10	CP51_SORBI Obtusifoliol 14-alpha demethylase OS
59	Mu_CvsMu_T	Up	Os01t0846300-01	1.14E-10	P2C09_ORYSJ Probable protein phosphatase 2C 9 OS
60	Mu_CvsMu_T	Up	Os05t0206000-01	1.20E-10	
61	Mu_CvsMu_T	Up	Os07t0688600-00	1.80E-10	BH149_ARATH Transcription factor bHLH149 OS
62	Mu_CvsMu_T	Up	Os06t0213300-00	2.05E-10	
63	Mu_CvsMu_T	Up	Os06t0216350-00	2.77E-10	OPR1_ORYSJ 12-oxophytodienoate reductase 1 OS
64	Mu_CvsMu_T	Up	Os02t0201300-01	3.05E-10	
65	Mu_CvsMu_T	Up	Os01t0135700-01	3.08E-10	CML16_ORYSJ Probable calcium-binding protein CML16 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
66	Mu_CvsMu_T	Up	Os03t0250000-01	3.21E-10	
67	Mu_CvsMu_T	Up	Os02t0620400-01	3.23E-10	
68	Mu_CvsMu_T	Up	Os02t0605900-01	3.89E-10	CHI6_ORYSJ Chitinase 6 OS
69	Mu_CvsMu_T	Up	Os11t0151400-01	4.00E-10	C94C1_ARATH Cytochrome P450 94C1 OS
70	Mu_CvsMu_T	Up	Os02t0649300-01	4.68E-10	HOX24_ORYSJ Homeobox-leucine zipper protein HOX24 OS
71	Mu_CvsMu_T	Up	Os05t0324700-01	4.90E-10	
72	Mu_CvsMu_T	Up	Os01t0955100-01	5.10E-10	CML31_ORYSJ Probable calcium-binding protein CML31 OS
73	Mu_CvsMu_T	Up	Os01t0859100-01	8.77E-10	ZWIP4_ARATH Zinc finger protein WIP4 OS
74	Mu_CvsMu_T	Up	Os11t0490100-01	1.87E-09	MT127_ARATH Probable methyltransferase At1g27930 OS
75	Mu_CvsMu_T	Up	Os03t0191900-01	1.91E-09	RAP24_ARATH Ethylene-responsive transcription factor RAP2-4 OS
76	Mu_CvsMu_T	Up	Os03t0250000-02	2.17E-09	
77	Mu_CvsMu_T	Up	Os09t0478733-00	2.18E-09	XERIC_ARATH Probable E3 ubiquitin-protein ligase XERICO OS
78	Mu_CvsMu_T	Up	Os02t0601000-01	2.93E-09	
79	Mu_CvsMu_T	Up	Os01t0858350-01	3.48E-09	C94C1_ARATH Cytochrome P450 94C1 OS
80	Mu_CvsMu_T	Up	Os01t0954000-01	3.77E-09	NQR2_ORYSJ Probable NADPH:quinone oxidoreductase 2 OS
81	Mu_CvsMu_T	Up	Os07t0687900-01	4.52E-09	GOLS2_SOLLC Galactinol synthase 2 OS
82	Mu_CvsMu_T	Up	Os03t0316200-01	6.08E-09	GOLS2_SOLLC Galactinol synthase 2 OS
83	Mu_CvsMu_T	Up	Os01t0121600-01	6.25E-09	
84	Mu_CvsMu_T	Up	Os08t0114200-01	6.37E-09	GUN19_ORYSJ Endoglucanase 19 OS
85	Mu_CvsMu_T	Up	Os07t0438700-01	8.86E-09	OXR1_HUMAN Oxidation resistance protein 1 OS
86	Mu_CvsMu_T	Up	Os11t0536000-01	1.11E-08	
87	Mu_CvsMu_T	Up	Os05t0457300-00	1.23E-08	P2C49_ORYSJ Probable protein phosphatase 2C 49 OS
88	Mu_CvsMu_T	Up	Os03t0341300-01	1.30E-08	SWT16_ORYSJ Bidirectional sugar transporter SWEET16 OS
89	Mu_CvsMu_T	Up	Os05t0497200-01	1.32E-08	ERF81_ARATH Ethylene-responsive transcription factor 12 OS
90	Mu_CvsMu_T	Up	Os05t0461600-01	1.68E-08	
91	Mu_CvsMu_T	Up	Os03t0645900-00	2.00E-08	NCED3_ORYSJ 9-cis-epoxycarotenoid dioxygenase NCED3, chloroplastic OS
92	Mu_CvsMu_T	Up	Os08t0520600-00	2.85E-08	

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
93	Mu_CvsMu_T	Up	Os08t0536800-01	2.85E-08	BH137_ARATH Transcription factor bHLH137 OS
94	Mu_CvsMu_T	Up	Os09t0272000-01	2.85E-08	PIK1_ORYSJ Disease resistance protein Pik-1 OS
95	Mu_CvsMu_T	Up	Os01t0298400-01	3.06E-08	MYB2_ORYSJ Transcription factor MYB2 OS
96	Mu_CvsMu_T	Up	Os04t0448950-01	3.54E-08	ZEP_ARATH Zeaxanthin epoxidase, chloroplastic OS
97	Mu_CvsMu_T	Up	Os04t0603601-00	3.69E-08	
98	Mu_CvsMu_T	Up	Os09t0442100-01	3.79E-08	RIPK_ARATH Serine/threonine-protein kinase RIPK OS
99	Mu_CvsMu_T	Up	Os06t0318800-01	3.86E-08	
100	Mu_CvsMu_T	Up	Os02t0625300-00	4.84E-08	PPCK1_ARATH Phosphoenolpyruvate carboxylase kinase 1 OS
1	WT_CvsWT_T	Down	Os02t0650300-01	1.64E-54	YSL15_ORYSJ Iron-phytosiderophore transporter YSL15 OS
2	WT_CvsWT_T	Down	Os06t0169001-01	8.63E-48	GOS9_ORYSJ Protein GOS9 OS
3	WT_CvsWT_T	Down	Os01t0550800-01	8.93E-44	
4	WT_CvsWT_T	Down	Os09t0564200-01	4.44E-43	CYSP_HEMSP Thiol protease SEN102 OS
5	WT_CvsWT_T	Down	Os11t0134900-01	2.47E-39	ZIFL1_ARATH Protein ZINC INDUCED FACILITATOR-LIKE 1 OS
6	WT_CvsWT_T	Down	Os03t0107300-01	6.19E-34	LSI2_ORYSJ Silicon efflux transporter LSI2 OS
7	WT_CvsWT_T	Down	Os02t0745100-01	2.41E-33	NIP21_ORYSJ Aquaporin NIP2-1 OS
8	WT_CvsWT_T	Down	Os05t0503650-01	1.42E-28	
9	WT_CvsWT_T	Down	Os10t0323500-01	1.52E-26	BGL34_ORYSJ Beta-glucosidase 34 OS
10	WT_CvsWT_T	Down	Os01t0355250-00	1.27E-25	SALT_ORYSJ Salt stress-induced protein OS
11	WT_CvsWT_T	Down	Os07t0442900-01	6.26E-25	CSPL7_ORYSJ CASP-like protein 1D1 OS
12	WT_CvsWT_T	Down	Os12t0137700-01	6.86E-25	SOT5_ARATH Cytosolic sulfotransferase 5 OS
13	WT_CvsWT_T	Down	Os02t0218800-01	1.42E-24	C74A4_ORYSJ Allene oxide synthase 4 OS
14	WT_CvsWT_T	Down	Os03t0667500-01	1.42E-24	IRT1_ORYSJ Fe(2+) transport protein 1 OS
15	WT_CvsWT_T	Down	Os12t0260500-01	3.21E-24	SDR5_ARATH Short-chain dehydrogenase reductase 5 OS
16	WT_CvsWT_T	Down	Os01t0915900-01	3.62E-24	
17	WT_CvsWT_T	Down	Os11t0593000-01	1.68E-23	NPC4_ARATH Non-specific phospholipase C4 OS
18	WT_CvsWT_T	Down	Os01t0220100-01	2.00E-23	GUN2_ORYSJ Endoglucanase 2 OS
19	WT_CvsWT_T	Down	Os02t0684100-00	1.83E-22	SOT15_ARATH Cytosolic sulfotransferase 15 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
20	WT_CvsWT_T	Down	Os03t0809000-01	2.35E-22	DIR15_ARATH Dirigent protein 15 OS
21	WT_CvsWT_T	Down	Os02t0779400-00	8.78E-22	
22	WT_CvsWT_T	Down	Os02t0131800-01	1.03E-21	NRAT1_ORYSJ Metal transporter NRAT1 OS
23	WT_CvsWT_T	Down	Os06t0641400-01	1.17E-21	C71DC_CATRO Tabersonine 16-hydroxylase 1 OS
24	WT_CvsWT_T	Down	Os07t0677300-01	1.81E-21	PER2_ORYSJ Peroxidase 2 OS
25	WT_CvsWT_T	Down	Os02t0306401-00	2.57E-21	NAATA_HORVU Nicotianamine aminotransferase A OS
26	WT_CvsWT_T	Down	Os12t0571100-01	3.15E-21	MT4C_ORYSJ Metallothionein-like protein 4C OS
27	WT_CvsWT_T	Down	Os07t0635200-00	5.16E-21	C70B2_ARATH Cytochrome P450 709B2 OS
28	WT_CvsWT_T	Down	Os12t0567800-01	5.39E-21	MT4C_ORYSJ Metallothionein-like protein 4C OS
29	WT_CvsWT_T	Down	Os04t0288100-00	5.87E-21	GL814_ORYSJ Germin-like protein 8-14 OS
30	WT_CvsWT_T	Down	Os12t0259800-00	6.71E-21	LAC25_ORYSJ Laccase-25 OS
31	WT_CvsWT_T	Down	Os10t0191300-01	9.29E-21	PR12_HORVU Pathogenesis-related protein PRB1-2 OS
32	WT_CvsWT_T	Down	Os08t0189300-01	1.39E-20	GL84_ORYSJ Germin-like protein 8-4 OS
33	WT_CvsWT_T	Down	Os08t0302000-01	4.07E-20	PER40_ARATH Peroxidase 40 OS
34	WT_CvsWT_T	Down	Os01t0916000-01	5.08E-20	
35	WT_CvsWT_T	Down	Os01t0247700-01	5.84E-20	SLAH1_ARATH S-type anion channel SLAH1 OS
36	WT_CvsWT_T	Down	Os07t0519100-01	1.06E-19	C16B1_PICSI Cytochrome P450 716B1 OS
37	WT_CvsWT_T	Down	Os03t0185700-01	1.67E-19	TBT2_ORYSJ Tryptamine benzoyltransferase 2 OS
38	WT_CvsWT_T	Down	Os09t0311600-01	1.70E-19	RPS2_ARATH Disease resistance protein RPS2 OS
39	WT_CvsWT_T	Down	Os04t0659200-01	2.46E-19	
40	WT_CvsWT_T	Down	Os03t0307300-01	4.53E-19	NAS1_ORYSJ Nicotianamine synthase 1 OS
41	WT_CvsWT_T	Down	Os04t0438200-01	6.39E-19	
42	WT_CvsWT_T	Down	Os10t0105700-01	7.06E-19	SDC2_ORYSJ Serine decarboxylase 2 OS
43	WT_CvsWT_T	Down	Os07t0257200-01	8.96E-19	NRAM5_ORYSJ Metal transporter Nramp5 OS
44	WT_CvsWT_T	Down	Os07t0421300-01	9.71E-19	AGL2_BACTQ Alpha-glucosidase 2 OS
45	WT_CvsWT_T	Down	Os09t0513200-00	9.71E-19	S35F1_HUMAN Solute carrier family 35 member F1 OS
46	WT_CvsWT_T	Down	Os01t0810800-01	1.05E-18	SIRK_ARATH Senescence-induced receptor-like serine/threonine-protein kinase OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
47	WT_CvsWT_T	Down	Os05t0424300-01	1.05E-18	C71A1_PERAE Cytochrome P450 71A1 OS
48	WT_CvsWT_T	Down	Os06t0641500-00	1.05E-18	C7D55_HYOMU Premnaspirodiene oxygenase OS
49	WT_CvsWT_T	Down	Os07t0645300-01	1.05E-18	DMP1_ARATH Protein DMP1 OS
50	WT_CvsWT_T	Down	Os11t0687050-00	1.66E-18	RGA4R_ORYSJ Disease resistance protein RGA4 OS
51	WT_CvsWT_T	Down	Os07t0630400-01	2.21E-18	RNS1_ARATH Ribonuclease 1 OS
52	WT_CvsWT_T	Down	Os04t0381700-00	3.76E-18	FIT_ARATH Transcription factor FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR OS
53	WT_CvsWT_T	Down	Os05t0399300-01	5.10E-18	CHI2_ORYSJ Chitinase 2 OS
54	WT_CvsWT_T	Down	Os08t0189200-01	1.07E-17	GL83_ORYSJ Germin-like protein 8-3 OS
55	WT_CvsWT_T	Down	Os03t0368600-00	1.73E-17	PER2_MAIZE Peroxidase 2 OS
56	WT_CvsWT_T	Down	Os06t0158500-01	2.85E-17	PIKS2_ORYSJ Disease resistance protein Piks-2 OS
57	WT_CvsWT_T	Down	Os10t0547500-01	2.91E-17	LSI3_ORYSJ Silicon efflux transporter LSI3 OS
58	WT_CvsWT_T	Down	Os03t0297600-01	3.10E-17	PYL4_ARATH Abscisic acid receptor PYL4 OS
59	WT_CvsWT_T	Down	Os10t0552600-01	3.44E-17	14KD_DAUCA 14 kDa proline-rich protein DC2.15 OS
60	WT_CvsWT_T	Down	Os01t0237000-01	4.15E-17	CSPLB_ORYSJ CASP-like protein 1C1 OS
61	WT_CvsWT_T	Down	Os08t0556300-01	4.53E-17	ACD11_ARATH Accelerated cell death 11 OS
62	WT_CvsWT_T	Down	Os01t0952800-01	5.31E-17	IRO2_ORYSJ Protein IRON-RELATED TRANSCRIPTION FACTOR 2 OS
63	WT_CvsWT_T	Down	Os01t0347600-01	7.04E-17	BROM1_ANACO Fruit bromelain OS
64	WT_CvsWT_T	Down	Os06t0692600-01	1.07E-16	PSYR1_ARATH Tyrosine-sulfated glycopeptide receptor 1 OS
65	WT_CvsWT_T	Down	Os02t0157600-01	1.29E-16	STR17_ARATH Rhodanese-like domain-containing protein 17 OS
66	WT_CvsWT_T	Down	Os05t0162000-01	1.34E-16	PER5_VITVI Peroxidase 5 OS
67	WT_CvsWT_T	Down	Os03t0307200-01	1.39E-16	NAS2_ORYSJ Nicotianamine synthase 2 OS
68	WT_CvsWT_T	Down	Os03t0237100-01	1.53E-16	DMAS1_ORYSJ Deoxymugineic acid synthase 1 OS
69	WT_CvsWT_T	Down	Os07t0104500-01	1.74E-16	PER1_ORYSJ Peroxidase 1 OS
70	WT_CvsWT_T	Down	Os07t0193000-01	5.03E-16	HIPL1_ARATH HIPL1 protein OS
71	WT_CvsWT_T	Down	Os02t0285300-01	5.28E-16	PCAP1_ARATH Plasma membrane-associated cation-binding protein 1 OS
72	WT_CvsWT_T	Down	Os11t0241200-01	5.28E-16	
73	WT_CvsWT_T	Down	Os05t0596500-01	5.41E-16	ATL12_ARATH Putative RING-H2 finger protein ATL12 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
74	WT_CvsWT_T	Down	Os04t0506500-00	8.80E-16	Y1137_ARATH G-type lectin S-receptor-like serine/threonine-protein kinase At1g61370 OS
75	WT_CvsWT_T	Down	Os12t0203200-01	1.06E-15	
76	WT_CvsWT_T	Down	Os11t0555300-00	1.71E-15	DRL21_ARATH Putative disease resistance protein At3g14460 OS
77	WT_CvsWT_T	Down	Os01t0591300-01	1.76E-15	AL2C4_ARATH Aldehyde dehydrogenase family 2 member C4 OS
78	WT_CvsWT_T	Down	Os03t0201500-01	2.20E-15	RSGI6_CLOTH Anti-sigma-I factor RsgI6 OS
79	WT_CvsWT_T	Down	Os08t0424100-00	2.34E-15	ATCA7_ARATH Alpha carbonic anhydrase 7 OS
80	WT_CvsWT_T	Down	Os01t0850550-00	2.45E-15	LAC6_ORYSJ Laccase-6 OS
81	WT_CvsWT_T	Down	Os08t0398300-01	2.45E-15	AB7A_ARATH ABC transporter A family member 7 OS
82	WT_CvsWT_T	Down	Os07t0532800-00	2.59E-15	NEP1_NEPGR Aspartic proteinase nepenthesin-1 OS
83	WT_CvsWT_T	Down	Os02t0102300-01	2.74E-15	HARC1_ARATH Protein HIGH ARSENIC CONTENT 1, mitochondrial OS
84	WT_CvsWT_T	Down	Os02t0118600-00	3.29E-15	RGA3_SOLBU Putative disease resistance protein RGA3 OS
85	WT_CvsWT_T	Down	Os01t0941200-01	3.32E-15	E13B_HORVU Glucan endo-1,3-beta-glucosidase GII OS
86	WT_CvsWT_T	Down	Os09t0431300-00	3.32E-15	MYB36_ARATH Transcription factor MYB36 OS
87	WT_CvsWT_T	Down	Os11t0676500-00	3.32E-15	
88	WT_CvsWT_T	Down	Os09t0561000-01	4.49E-15	WAK2_ARATH Wall-associated receptor kinase 2 OS
89	WT_CvsWT_T	Down	Os03t0365800-01	5.68E-15	SCE3_BRANA Sinapine esterase OS
90	WT_CvsWT_T	Down	Os02t0512000-01	9.09E-15	SAU40_ARATH Auxin-responsive protein SAUR40 OS
91	WT_CvsWT_T	Down	Os01t0185900-01	9.79E-15	WRK72_ARATH Probable WRKY transcription factor 72 OS
92	WT_CvsWT_T	Down	Os06t0306300-01	1.11E-14	PER1_ORYSJ Peroxidase 1 OS
93	WT_CvsWT_T	Down	Os08t0477500-01	1.19E-14	PLP2_ORYSJ Patatin-like protein 2 OS
94	WT_CvsWT_T	Down	Os08t0345500-00	1.32E-14	CSLD3_ORYSJ Cellulose synthase-like protein D3 OS
95	WT_CvsWT_T	Down	Os12t0568500-01	1.52E-14	MT4B_ORYSJ Metallothionein-like protein 4B OS
96	WT_CvsWT_T	Down	Os04t0452600-00	1.89E-14	MST1_ORYSJ Sugar transport protein MST1 OS
97	WT_CvsWT_T	Down	Os03t0619875-01	2.27E-14	
98	WT_CvsWT_T	Down	Os01t0347401-00	2.96E-14	BROM1_ANACO Fruit bromelain OS
99	WT_CvsWT_T	Down	Os06t0692100-01	2.98E-14	PSYR1_ARATH Tyrosine-sulfated glycopeptide receptor 1 OS
100	WT_CvsWT_T	Down	Os02t0595900-00	3.48E-14	NAR21_ORYSJ High-affinity nitrate transporter-activating protein 2.1 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
1	WT_CvsWT_T	Up	Os01t0858350-01	2.78E-31	C94C1_ARATH Cytochrome P450 94C1 OS
2	WT_CvsWT_T	Up	Os10t0542700-00	2.72E-26	
3	WT_CvsWT_T	Up	Os09t0325700-01	2.79E-25	P2C68_ORYSJ Probable protein phosphatase 2C 68 OS
4	WT_CvsWT_T	Up	Os05t0494600-01	5.09E-24	EDL3_ARATH EID1-like F-box protein 3 OS
5	WT_CvsWT_T	Up	Os01t0699500-01	1.40E-22	M3K17_ARATH Mitogen-activated protein kinase kinase kinase 17 OS
6	WT_CvsWT_T	Up	Os12t0168100-01	9.14E-22	EF110_ARATH Ethylene-responsive transcription factor ERF110 OS
7	WT_CvsWT_T	Up	Os03t0125100-01	9.91E-22	BCH_GENLU Beta-carotene 3-hydroxylase, chloroplastic OS
8	WT_CvsWT_T	Up	Os03t0820300-01	1.89E-20	ZAT7_ARATH Zinc finger protein ZAT7 OS
9	WT_CvsWT_T	Up	Os04t0541700-01	2.77E-20	HOX22_ORYSJ Homeobox-leucine zipper protein HOX22 OS
10	WT_CvsWT_T	Up	Os07t0418500-01	4.31E-20	C70B2_ARATH Cytochrome P450 709B2 OS
11	WT_CvsWT_T	Up	Os04t0244800-01	4.26E-19	HIP26_ARATH Heavy metal-associated isoprenylated plant protein 26 OS
12	WT_CvsWT_T	Up	Os07t0687900-01	7.46E-18	GOLS2_SOLLC Galactinol synthase 2 OS
13	WT_CvsWT_T	Up	Os01t0802600-00	1.13E-17	EDL3_ARATH EID1-like F-box protein 3 OS
14	WT_CvsWT_T	Up	Os01t0901600-01	1.17E-17	4CLL6_ORYSJ 4-coumarate--CoA ligase-like 6 OS
15	WT_CvsWT_T	Up	Os03t0820400-01	1.35E-17	ZAT8_ARATH Zinc finger protein ZAT8 OS
16	WT_CvsWT_T	Up	Os02t0649300-01	1.73E-17	HOX24_ORYSJ Homeobox-leucine zipper protein HOX24 OS
17	WT_CvsWT_T	Up	Os06t0216350-00	1.97E-17	OPR1_ORYSJ 12-oxophytodienoate reductase 1 OS
18	WT_CvsWT_T	Up	Os09t0555500-01	8.91E-17	PSY3_ORYSJ Phytoene synthase 3, chloroplastic OS
19	WT_CvsWT_T	Up	Os01t0846300-01	1.38E-16	P2C09_ORYSJ Probable protein phosphatase 2C 9 OS
20	WT_CvsWT_T	Up	Os11t0177400-01	2.08E-16	AB25G_ARATH ABC transporter G family member 25 OS
21	WT_CvsWT_T	Up	Os10t0173000-01	2.72E-16	
22	WT_CvsWT_T	Up	Os06t0133400-01	7.02E-16	
23	WT_CvsWT_T	Up	Os02t0764700-01	1.29E-15	EF112_ARATH Ethylene-responsive transcription factor ERF112 OS
24	WT_CvsWT_T	Up	Os10t0393800-01	1.41E-15	GDL9_ARATH GDSL esterase/lipase At1g28600 OS
25	WT_CvsWT_T	Up	Os03t0268600-01	5.23E-15	P2C30_ORYSJ Probable protein phosphatase 2C 30 OS
26	WT_CvsWT_T	Up	Os05t0477900-01	8.62E-15	NLTP_MALDO Non-specific lipid-transfer protein OS
27	WT_CvsWT_T	Up	Os06t0728700-01	8.97E-15	RVE2_ARATH Protein REVEILLE 2 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
28	WT_CvsWT_T	Up	Os03t0296200-00	1.66E-14	
29	WT_CvsWT_T	Up	Os07t0154100-01	2.02E-14	NCED4_ORYSJ 9-cis-epoxycarotenoid dioxygenase NCED4, chloroplastic OS
30	WT_CvsWT_T	Up	Os01t0583150-00	3.35E-14	
31	WT_CvsWT_T	Up	Os02t0669100-01	1.49E-13	DHN1_ORYSJ Dehydrin DHN1 OS
32	WT_CvsWT_T	Up	Os02t0766700-01	2.77E-13	BZP23_ORYSJ bZIP transcription factor 23 OS
33	WT_CvsWT_T	Up	Os06t0216300-01	3.22E-13	OPR1_ORYSJ 12-oxophytodienoate reductase 1 OS
34	WT_CvsWT_T	Up	Os03t0341300-01	4.81E-13	SWT16_ORYSJ Bidirectional sugar transporter SWEET16 OS
35	WT_CvsWT_T	Up	Os07t0592100-01	5.14E-13	TPRL1_ERYCB Tropinone reductase-like 1 OS
36	WT_CvsWT_T	Up	Os01t0121600-01	6.45E-13	
37	WT_CvsWT_T	Up	Os01t0583100-01	1.31E-12	P2C06_ORYSJ Probable protein phosphatase 2C 6 OS
38	WT_CvsWT_T	Up	Os01t0298400-01	1.62E-12	MYB2_ORYSJ Transcription factor MYB2 OS
39	WT_CvsWT_T	Up	Os12t0633600-01	2.27E-12	CSCLA_ARATH CSC1-like protein RXW8 OS
40	WT_CvsWT_T	Up	Os01t0128300-00	2.39E-12	MHZ4_ORYSJ Protein MAO HUZI 4, chloroplastic OS
41	WT_CvsWT_T	Up	Os01t0699400-01	3.22E-12	M3K17_ARATH Mitogen-activated protein kinase kinase kinase 17 OS
42	WT_CvsWT_T	Up	Os10t0524300-01	3.82E-12	
43	WT_CvsWT_T	Up	Os07t0550600-01	4.86E-12	BEBT_TOBAC Benzyl alcohol O-benzoyltransferase OS
44	WT_CvsWT_T	Up	Os05t0530400-01	4.98E-12	HFA4D_ORYSJ Heat stress transcription factor A-4d OS
45	WT_CvsWT_T	Up	Os03t0316200-01	5.01E-12	GOLS2_SOLLC Galactinol synthase 2 OS
46	WT_CvsWT_T	Up	Os12t0594000-00	9.17E-12	JGB_ARATH Protein JINGUBANG OS
47	WT_CvsWT_T	Up	Os05t0457200-01	1.18E-11	P2C49_ORYSJ Probable protein phosphatase 2C 49 OS
48	WT_CvsWT_T	Up	Os08t0538600-01	2.57E-11	
49	WT_CvsWT_T	Up	Os11t0177750-00	2.85E-11	AB21G_ARATH ABC transporter G family member 21 OS
50	WT_CvsWT_T	Up	Os03t0214200-01	3.40E-11	NNJA1_ORYSJ Ninja-family protein MODD OS
51	WT_CvsWT_T	Up	Os11t0582000-00	4.29E-11	
52	WT_CvsWT_T	Up	Os05t0126800-01	4.47E-11	Y4833_ARATH Uncharacterized protein At4g08330, chloroplastic OS
53	WT_CvsWT_T	Up	Os03t0386000-01	4.87E-11	YGI3_SCHPO Uncharacterized WD repeat-containing protein C2A9.03 OS
54	WT_CvsWT_T	Up	Os05t0583050-01	5.81E-11	

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
55	WT_CvsWT_T	Up	Os09t0400700-01	5.97E-11	
56	WT_CvsWT_T	Up	Os06t0318800-01	7.12E-11	
57	WT_CvsWT_T	Up	Os06t0652300-00	9.03E-11	FCL2_ORYSJ Putative GDP-L-fucose synthase 2 OS
58	WT_CvsWT_T	Up	Os08t0471401-00	1.60E-10	HEC1_ARATH Transcription factor HEC1 OS
59	WT_CvsWT_T	Up	Os01t0954000-01	2.61E-10	NQR2_ORYSJ Probable NADPH:quinone oxidoreductase 2 OS
60	WT_CvsWT_T	Up	Os03t0250000-02	3.03E-10	
61	WT_CvsWT_T	Up	Os02t0733900-01	3.27E-10	
62	WT_CvsWT_T	Up	Os04t0625200-00	3.66E-10	PLSC_COCNU 1-acyl-sn-glycerol-3-phosphate acyltransferase OS
63	WT_CvsWT_T	Up	Os08t0392100-00	3.74E-10	ACCH1_ARATH 1-aminocyclopropane-1-carboxylate oxidase homolog 1 OS
64	WT_CvsWT_T	Up	Os01t0859100-01	4.14E-10	ZWIP4_ARATH Zinc finger protein WIP4 OS
65	WT_CvsWT_T	Up	Os01t0863300-01	4.61E-10	DIV_ANTMA Transcription factor DIVARICATA OS
66	WT_CvsWT_T	Up	Os03t0640000-00	5.17E-10	PLP3_ORYSJ Patatin-like protein 3 OS
67	WT_CvsWT_T	Up	Os05t0583000-01	5.17E-10	WRK28_ARATH WRKY transcription factor 28 OS
68	WT_CvsWT_T	Up	Os08t0545500-00	6.35E-10	DRE1I_ORYSJ Dehydration-responsive element-binding protein 1I OS
69	WT_CvsWT_T	Up	Os03t0258200-01	6.95E-10	CXE17_ARATH Probable carboxylesterase 17 OS
70	WT_CvsWT_T	Up	Os03t0247900-01	7.14E-10	ACR8_ARATH ACT domain-containing protein ACR8 OS
71	WT_CvsWT_T	Up	Os11t0168500-00	7.63E-10	EF110_ARATH Ethylene-responsive transcription factor ERF110 OS
72	WT_CvsWT_T	Up	Os05t0421600-01	1.16E-09	NAC48_ORYSJ NAC domain-containing protein 48 OS
73	WT_CvsWT_T	Up	Os04t0372750-00	1.25E-09	QORH_SPIOL Quinone-oxidoreductase homolog, chloroplastic OS
74	WT_CvsWT_T	Up	Os03t0323600-01	1.46E-09	
75	WT_CvsWT_T	Up	Os04t0167900-00	1.46E-09	P2C37_ORYSJ Probable protein phosphatase 2C 37 OS
76	WT_CvsWT_T	Up	Os05t0541100-01	1.59E-09	IQD1_ARATH Protein IQ-DOMAIN 1 OS
77	WT_CvsWT_T	Up	Os09t0493000-01	1.65E-09	
78	WT_CvsWT_T	Up	Os08t0174300-00	1.72E-09	ANTA_GENTR Anthocyanin 5-aromatic acyltransferase OS
79	WT_CvsWT_T	Up	Os09t0378700-00	1.94E-09	PUB19_ARATH U-box domain-containing protein 19 OS
80	WT_CvsWT_T	Up	Os01t0699100-01	2.26E-09	M3K17_ARATH Mitogen-activated protein kinase kinase kinase 17 OS
81	WT_CvsWT_T	Up	Os05t0537400-00	2.32E-09	P2C50_ORYSJ Protein phosphatase 2C 50 OS

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
82	WT_CvsWT_T	Up	Os08t0441001-00	2.44E-09	
83	WT_CvsWT_T	Up	Os03t0351700-01	2.82E-09	
84	WT_CvsWT_T	Up	Os03t0815100-01	2.97E-09	NAC2_ORYSJ NAC domain-containing protein 2 OS
85	WT_CvsWT_T	Up	Os03t0718800-01	3.28E-09	CCDP_MAIZE Cortical cell-delineating protein OS
86	WT_CvsWT_T	Up	Os01t0795600-01	3.48E-09	
87	WT_CvsWT_T	Up	Os02t0206700-01	4.26E-09	U73C3_ARATH UDP-glycosyltransferase 73C3 OS
88	WT_CvsWT_T	Up	Os03t0792800-01	4.31E-09	E138_ARATH Glucan endo-1,3-beta-glucosidase 8 OS
89	WT_CvsWT_T	Up	Os06t0356800-01	4.58E-09	XIP1_WHEAT Xylanase inhibitor protein 1 OS
90	WT_CvsWT_T	Up	Os07t0124300-00	5.54E-09	
91	WT_CvsWT_T	Up	Os07t0688600-00	8.03E-09	BH149_ARATH Transcription factor bHLH149 OS
92	WT_CvsWT_T	Up	Os03t0844700-01	9.18E-09	ALB4_ARATH ALBINO3-like protein 1, chloroplastic OS
93	WT_CvsWT_T	Up	Os02t0232000-01	1.03E-08	HFC2A_ORYSJ Heat stress transcription factor C-2a OS
94	WT_CvsWT_T	Up	Os08t0428100-00	1.08E-08	SKI14_ARATH F-box protein SKIP14 OS
95	WT_CvsWT_T	Up	Os02t0483500-01	1.08E-08	PMAT1_ARATH Phenolic glucoside malonyltransferase 1 OS
96	WT_CvsWT_T	Up	Os05t0457300-00	1.19E-08	P2C49_ORYSJ Probable protein phosphatase 2C 49 OS
97	WT_CvsWT_T	Up	Os04t0564700-01	1.26E-08	STXB5_MOUSE Syntaxin-binding protein 5 OS
98	WT_CvsWT_T	Up	Os06t0604600-01	1.39E-08	
99	WT_CvsWT_T	Up	Os07t0192000-01	1.77E-08	AATP9_ARATH AAA-ATPase At3g28580 OS
100	WT_CvsWT_T	Up	Os12t0187800-00	1.83E-08	

Supplementary Table 2. Pipeline 2: Top 100 DE genes in comparisons averaging over timepoints. Up and down refer to the expression of genes in the treated as compared to control or the mutant as compared to WT groups. Gene IDs are in MSU format.

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
1	WT_CvsMu_C	Down	LOC_Os10g22950	4.37E-14	calmodulin-binding transcription activator, putative, expressed
2	WT_CvsMu_C	Down	LOC_Os04g01330	2.19E-13	expressed protein
3	WT_CvsMu_C	Down	LOC_Os04g01320	9.99E-11	serine threonine-protein kinase receptor precursor, putative, expressed
4	WT_CvsMu_C	Down	LOC_Os02g34190	9.58E-09	expressed protein
5	WT_CvsMu_C	Down	LOC_Os06g45500	4.29E-07	copper-transporting ATPase, putative, expressed
6	WT_CvsMu_C	Down	LOC_Os04g02640	5.07E-06	3-ketoacyl-CoA synthase 6, putative, expressed
7	WT_CvsMu_C	Down	LOC_Os01g62860	2.46E-05	oxidoreductase, aldo keto reductase family protein, putative, expressed
8	WT_CvsMu_C	Down	LOC_Os10g35330	2.46E-05	expressed protein
9	WT_CvsMu_C	Down	LOC_Os11g32610	3.21E-05	chalcone and stilbene synthases, putative, expressed
10	WT_CvsMu_C	Down	LOC_Os04g03579	7.14E-05	protein kinase, putative, expressed
11	WT_CvsMu_C	Down	LOC_Os07g23030	1.37E-04	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
12	WT_CvsMu_C	Down	LOC_Os05g06814	1.47E-04	expressed protein
13	WT_CvsMu_C	Down	LOC_Os09g34950	1.50E-04	TCP family transcription factor, putative, expressed
14	WT_CvsMu_C	Down	LOC_Os10g09990	3.26E-04	cytokinin-O-glucosyltransferase 3, putative, expressed
15	WT_CvsMu_C	Down	LOC_Os04g02140	5.27E-04	transposon protein, putative, CACTA, En Spm sub-class, expressed
16	WT_CvsMu_C	Down	LOC_Os11g25220	1.22E-03	oxidoreductase, short chain dehydrogenase reductase domain containing protein, expressed
17	WT_CvsMu_C	Down	LOC_Os03g36490	1.60E-03	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
18	WT_CvsMu_C	Down	LOC_Os03g20740	1.76E-03	expressed protein
19	WT_CvsMu_C	Down	LOC_Os02g34390	1.99E-03	expressed protein
20	WT_CvsMu_C	Down	LOC_Os04g28820	1.99E-03	retrotransposon protein, putative, unclassified, expressed
21	WT_CvsMu_C	Down	LOC_Os11g25210	2.38E-03	retrotransposon protein, putative, unclassified, expressed
22	WT_CvsMu_C	Down	LOC_Os08g19420	2.70E-03	O-methyltransferase, putative, expressed
23	WT_CvsMu_C	Down	LOC_Os04g02790	2.83E-03	expressed protein
24	WT_CvsMu_C	Down	LOC_Os09g36700	4.34E-03	ribonuclease T2 family domain containing protein, expressed
25	WT_CvsMu_C	Down	LOC_Os03g36480	4.73E-03	retrotransposon protein, putative, Ty3-gypsy subclass, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
26	WT_CvsMu_C	Down	LOC_Os07g20600	4.81E-03	retrotransposon, putative, centromere-specific
27	WT_CvsMu_C	Down	LOC_Os10g05160	5.74E-03	expressed protein
28	WT_CvsMu_C	Down	LOC_Os11g35920	5.91E-03	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
29	WT_CvsMu_C	Down	LOC_Os06g12455	5.91E-03	expressed protein
30	WT_CvsMu_C	Down	LOC_Os11g42490	6.80E-03	retrotransposon protein, putative, unclassified, expressed
31	WT_CvsMu_C	Down	LOC_Os02g34380	8.46E-03	retrotransposon protein, putative, LINE subclass, expressed
32	WT_CvsMu_C	Down	LOC_Os07g27030	9.28E-03	OsFBX236 - F-box domain containing protein, expressed
33	WT_CvsMu_C	Down	LOC_Os09g26200	1.01E-02	ZOS9-11 - C2H2 zinc finger protein, expressed
34	WT_CvsMu_C	Down	LOC_Os07g05450	1.45E-02	sulfotransferase domain containing protein, expressed
35	WT_CvsMu_C	Down	LOC_Os07g09760	1.45E-02	retrotransposon protein, putative, unclassified, expressed
36	WT_CvsMu_C	Down	LOC_Os07g12670	1.60E-02	expressed protein
37	WT_CvsMu_C	Down	LOC_Os09g20040	1.64E-02	resistance-like protein I2GA-SH194-2, putative, expressed
38	WT_CvsMu_C	Down	LOC_Os07g07770	1.73E-02	ribosomal protein S13p S18e, putative, expressed
39	WT_CvsMu_C	Down	LOC_Os09g36680	2.04E-02	ribonuclease T2 family domain containing protein, expressed
40	WT_CvsMu_C	Down	LOC_Os04g33920	2.14E-02	LTPL102 - Protease inhibitor seed storage LTP family protein precursor, expressed
41	WT_CvsMu_C	Down	LOC_Os03g43430	2.14E-02	mttA Hcf106 family protein, putative, expressed
42	WT_CvsMu_C	Down	LOC_Os01g37380	2.16E-02	transposon protein, putative, unclassified, expressed
43	WT_CvsMu_C	Down	LOC_Os01g39270	2.21E-02	NAD binding domain of 6-phosphogluconate dehydrogenase containing protein, expressed
44	WT_CvsMu_C	Down	LOC_Os03g40720	2.28E-02	UDP-glucose 6-dehydrogenase, putative, expressed
45	WT_CvsMu_C	Down	LOC_Os01g46790	2.35E-02	expressed protein
46	WT_CvsMu_C	Down	LOC_Os10g31240	2.36E-02	plant protein of unknown function domain containing protein, expressed
47	WT_CvsMu_C	Down	LOC_Os03g04760	2.40E-02	expressed protein
48	WT_CvsMu_C	Down	LOC_Os01g37350	2.44E-02	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
49	WT_CvsMu_C	Down	LOC_Os04g52130	2.70E-02	coproporphyrinogen III oxidase, chloroplast precursor, putative, expressed
50	WT_CvsMu_C	Down	LOC_Os06g15750	2.82E-02	NB-ARC domain containing protein, expressed
51	WT_CvsMu_C	Down	LOC_Os04g02970	2.94E-02	subtilisin-like protease precursor, putative, expressed
52	WT_CvsMu_C	Down	LOC_Os01g66600	2.94E-02	rhodanese-like, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
53	WT_CvsMu_C	Down	LOC_Os01g29804	2.94E-02	expressed protein
54	WT_CvsMu_C	Down	LOC_Os08g30014	3.18E-02	expressed protein
55	WT_CvsMu_C	Down	LOC_Os11g17530	3.19E-02	pentatricopeptide, putative, expressed
56	WT_CvsMu_C	Down	LOC_Os04g53800	3.20E-02	leucoanthocyanidin reductase, putative, expressed
57	WT_CvsMu_C	Down	LOC_Os10g21130	3.22E-02	expressed protein
58	WT_CvsMu_C	Down	LOC_Os04g26870	3.38E-02	oxidoreductase, aldo keto reductase family protein, putative, expressed
59	WT_CvsMu_C	Down	LOC_Os03g32470	3.52E-02	leucoanthocyanidin dioxygenase, putative, expressed
60	WT_CvsMu_C	Down	LOC_Os09g15284	3.59E-02	expressed protein
61	WT_CvsMu_C	Down	LOC_Os12g05600	3.59E-02	hydrolase, alpha beta fold family protein, putative, expressed
62	WT_CvsMu_C	Down	LOC_Os06g36880	3.59E-02	cysteine synthase, putative, expressed
63	WT_CvsMu_C	Down	LOC_Os05g34380	3.70E-02	cytochrome P450, putative, expressed
64	WT_CvsMu_C	Down	LOC_Os06g44790	4.04E-02	expressed protein
65	WT_CvsMu_C	Down	LOC_Os07g03600	4.04E-02	SCP-like extracellular protein, expressed
66	WT_CvsMu_C	Down	LOC_Os02g57010	4.12E-02	RNA recognition motif containing protein, putative, expressed
67	WT_CvsMu_C	Down	LOC_Os02g28334	4.45E-02	expressed protein
68	WT_CvsMu_C	Down	LOC_Os10g04600	4.46E-02	OsFBX359 - F-box domain containing protein, expressed
69	WT_CvsMu_C	Down	LOC_Os01g48500	4.46E-02	expressed protein
70	WT_CvsMu_C	Down	LOC_Os04g02960	4.47E-02	OsSub32 - Putative Subtilisin homologue, expressed
71	WT_CvsMu_C	Down	LOC_Os11g47130	4.52E-02	protein transporter, putative, expressed
72	WT_CvsMu_C	Down	LOC_Os07g07450	4.54E-02	versicolorin reductase, putative, expressed
1	WT_CvsMu_C	Up	LOC_Os07g01904	4.20E-08	expressed protein
2	WT_CvsMu_C	Up	LOC_Os07g10850	1.05E-07	retrotransposon protein, putative, unclassified, expressed
3	WT_CvsMu_C	Up	LOC_Os01g73000	1.74E-07	copine, putative, expressed
4	WT_CvsMu_C	Up	LOC_Os07g32710	4.29E-07	retrotransposon protein, putative, unclassified, expressed
5	WT_CvsMu_C	Up	LOC_Os07g44250	6.57E-07	dirigent, putative, expressed
6	WT_CvsMu_C	Up	LOC_Os03g64330	2.57E-06	aquaporin protein, putative, expressed
7	WT_CvsMu_C	Up	LOC_Os03g51600	4.04E-06	tubulin FtsZ domain containing protein, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
8	WT_CvsMu_C	Up	LOC_Os04g14690	5.07E-06	flavin-containing monooxygenase family protein, putative, expressed
9	WT_CvsMu_C	Up	LOC_Os07g27350	6.21E-06	atuA, putative, expressed
10	WT_CvsMu_C	Up	LOC_Os02g52730	6.21E-06	ferredoxin--nitrite reductase, putative, expressed
11	WT_CvsMu_C	Up	LOC_Os02g53240	6.48E-06	expressed protein
12	WT_CvsMu_C	Up	LOC_Os04g18770	7.40E-06	retrotransposon protein, putative, unclassified, expressed
13	WT_CvsMu_C	Up	LOC_Os02g15820	8.17E-06	extra-large G-protein-related, putative, expressed
14	WT_CvsMu_C	Up	LOC_Os04g01850	9.26E-06	hypothetical protein
15	WT_CvsMu_C	Up	LOC_Os04g02530	9.26E-06	expressed protein
16	WT_CvsMu_C	Up	LOC_Os02g50140	1.07E-05	caleosin related protein, putative, expressed
17	WT_CvsMu_C	Up	LOC_Os04g02754	1.67E-05	amidase family protein, putative, expressed
18	WT_CvsMu_C	Up	LOC_Os05g23255	1.76E-05	expressed protein
19	WT_CvsMu_C	Up	LOC_Os05g50390	1.89E-05	expressed protein
20	WT_CvsMu_C	Up	LOC_Os03g43100	2.01E-05	expressed protein
21	WT_CvsMu_C	Up	LOC_Os06g49350	2.01E-05	retrotransposon protein, putative, unclassified, expressed
22	WT_CvsMu_C	Up	LOC_Os05g39540	2.46E-05	metal cation transporter, putative, expressed
23	WT_CvsMu_C	Up	LOC_Os10g24004	2.46E-05	expressed protein
24	WT_CvsMu_C	Up	LOC_Os01g49750	2.46E-05	expressed protein
25	WT_CvsMu_C	Up	LOC_Os11g10670	2.46E-05	expressed protein
26	WT_CvsMu_C	Up	LOC_Os02g18690	2.86E-05	BURP domain containing protein, expressed
27	WT_CvsMu_C	Up	LOC_Os03g02470	3.80E-05	expressed protein
28	WT_CvsMu_C	Up	LOC_Os05g11900	4.32E-05	expressed protein
29	WT_CvsMu_C	Up	LOC_Os10g07160	4.61E-05	retrotransposon, putative, centromere-specific, expressed
30	WT_CvsMu_C	Up	LOC_Os06g40240	4.68E-05	retrotransposon protein, putative, unclassified, expressed
31	WT_CvsMu_C	Up	LOC_Os10g33104	5.30E-05	expressed protein
32	WT_CvsMu_C	Up	LOC_Os04g17650	6.87E-05	sucrose synthase, putative, expressed
33	WT_CvsMu_C	Up	LOC_Os09g15310	7.14E-05	expressed protein
34	WT_CvsMu_C	Up	LOC_Os01g15740	7.14E-05	expressed protein

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
35	WT_CvsMu_C	Up	LOC_Os04g02580	7.70E-05	expressed protein
36	WT_CvsMu_C	Up	LOC_Os05g25090	8.43E-05	expressed protein
37	WT_CvsMu_C	Up	LOC_Os02g49510	8.43E-05	amino acid transporter, putative, expressed
38	WT_CvsMu_C	Up	LOC_Os04g58050	9.84E-05	expressed protein
39	WT_CvsMu_C	Up	LOC_Os05g01840	1.02E-04	hypothetical protein
40	WT_CvsMu_C	Up	LOC_Os10g19910	1.19E-04	expressed protein
41	WT_CvsMu_C	Up	LOC_Os12g07180	1.31E-04	transposon protein, putative, Pong sub-class, expressed
42	WT_CvsMu_C	Up	LOC_Os11g29910	1.65E-04	plastocyanin-like domain containing protein, putative, expressed
43	WT_CvsMu_C	Up	LOC_Os04g43090	1.65E-04	expressed protein
44	WT_CvsMu_C	Up	LOC_Os04g02450	1.65E-04	rust-resistance protein Lr21, putative, expressed
45	WT_CvsMu_C	Up	LOC_Os05g34490	1.75E-04	expressed protein
46	WT_CvsMu_C	Up	LOC_Os01g71840	2.12E-04	retrotransposon protein, putative, unclassified, expressed
47	WT_CvsMu_C	Up	LOC_Os10g29400	2.28E-04	transposon protein, putative, CACTA, En Spm sub-class, expressed
48	WT_CvsMu_C	Up	LOC_Os04g39900	2.29E-04	Os4bglu13 - beta-glucosidase homologue, similar to Os4Bglu12 exoglucanase b-glucosidase, expressed
49	WT_CvsMu_C	Up	LOC_Os07g32680	3.04E-04	retrotransposon protein, putative, unclassified, expressed
50	WT_CvsMu_C	Up	LOC_Os05g48710	3.49E-04	expressed protein
51	WT_CvsMu_C	Up	LOC_Os10g05290	4.31E-04	tRNA rRNA methyltransferase, putative, expressed
52	WT_CvsMu_C	Up	LOC_Os10g05190	5.27E-04	transposon protein, putative, CACTA, En Spm sub-class, expressed
53	WT_CvsMu_C	Up	LOC_Os09g38104	5.38E-04	hypothetical protein
54	WT_CvsMu_C	Up	LOC_Os09g26530	5.67E-04	expressed protein
55	WT_CvsMu_C	Up	LOC_Os07g32700	6.14E-04	retrotransposon protein, putative, unclassified, expressed
56	WT_CvsMu_C	Up	LOC_Os07g09880	6.99E-04	expressed protein
57	WT_CvsMu_C	Up	LOC_Os04g52790	7.20E-04	expressed protein
58	WT_CvsMu_C	Up	LOC_Os03g14820	7.34E-04	expressed protein
59	WT_CvsMu_C	Up	LOC_Os04g02040	7.34E-04	NBS-LRR, putative, expressed
60	WT_CvsMu_C	Up	LOC_Os09g19350	8.73E-04	expressed protein
61	WT_CvsMu_C	Up	LOC_Os04g18780	8.73E-04	retrotransposon protein, putative, unclassified

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
62	WT_CvsMu_C	Up	LOC_Os05g42160	9.32E-04	expressed protein
63	WT_CvsMu_C	Up	LOC_Os04g12710	1.16E-03	indole-3-acetate beta-glucosyltransferase, putative, expressed
64	WT_CvsMu_C	Up	LOC_Os02g30480	1.19E-03	retrotransposon protein, putative, unclassified, expressed
65	WT_CvsMu_C	Up	LOC_Os06g04930	1.27E-03	expressed protein
66	WT_CvsMu_C	Up	LOC_Os11g31840	1.53E-03	retrotransposon protein, putative, Ty1-copia subclass, expressed
67	WT_CvsMu_C	Up	LOC_Os07g25710	1.68E-03	myb-like DNA-binding domain containing protein, expressed
68	WT_CvsMu_C	Up	LOC_Os07g31200	1.76E-03	expressed protein
69	WT_CvsMu_C	Up	LOC_Os01g63970	2.19E-03	sialyltransferase family domain containing protein, expressed
70	WT_CvsMu_C	Up	LOC_Os10g29390	2.62E-03	expressed protein
71	WT_CvsMu_C	Up	LOC_Os03g25510	3.12E-03	expressed protein
72	WT_CvsMu_C	Up	LOC_Os06g49000	3.12E-03	expressed protein
73	WT_CvsMu_C	Up	LOC_Os10g05020	3.22E-03	cytochrome P450, putative, expressed
74	WT_CvsMu_C	Up	LOC_Os09g01540	3.66E-03	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
75	WT_CvsMu_C	Up	LOC_Os11g41400	3.78E-03	expressed protein
76	WT_CvsMu_C	Up	LOC_Os09g23540	4.07E-03	dehydrogenase, putative, expressed
77	WT_CvsMu_C	Up	LOC_Os10g01500	4.11E-03	expressed protein
78	WT_CvsMu_C	Up	LOC_Os02g12470	4.81E-03	hypothetical protein
79	WT_CvsMu_C	Up	LOC_Os07g39240	4.81E-03	expressed protein
80	WT_CvsMu_C	Up	LOC_Os03g39920	4.92E-03	retrotransposon protein, putative, unclassified, expressed
81	WT_CvsMu_C	Up	LOC_Os10g07150	5.03E-03	transposon protein, putative, unclassified, expressed
82	WT_CvsMu_C	Up	LOC_Os01g23880	5.03E-03	expressed protein
83	WT_CvsMu_C	Up	LOC_Os04g33420	5.03E-03	DNA-binding protein S1FA, putative, expressed
84	WT_CvsMu_C	Up	LOC_Os01g07170	5.33E-03	HORMA domain containing protein, putative, expressed
85	WT_CvsMu_C	Up	LOC_Os04g02520	5.85E-03	Leucine Rich Repeat family protein, expressed
86	WT_CvsMu_C	Up	LOC_Os11g29900	6.02E-03	expressed protein
87	WT_CvsMu_C	Up	LOC_Os02g15350	6.07E-03	dof zinc finger domain containing protein, putative, expressed
88	WT_CvsMu_C	Up	LOC_Os07g14490	6.22E-03	OsWAK68 - OsWAK pseudogene, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
89	WT_CvsMu_C	Up	LOC_Os07g03040	6.56E-03	expressed protein
90	WT_CvsMu_C	Up	LOC_Os10g25400	7.89E-03	GDSL-like lipase acylhydrolase, putative, expressed
91	WT_CvsMu_C	Up	LOC_Os07g30760	8.36E-03	UDP-glucoronosyl and UDP-glucosyl transferase domain containing protein, expressed
92	WT_CvsMu_C	Up	LOC_Os08g10870	9.28E-03	hypothetical protein
93	WT_CvsMu_C	Up	LOC_Os05g40010	1.01E-02	LTPL17 - Protease inhibitor seed storage LTP family protein precursor, expressed
94	WT_CvsMu_C	Up	LOC_Os03g57900	1.04E-02	zinc finger A20 and AN1 domain-containing stress-associated protein, putative, expressed
95	WT_CvsMu_C	Up	LOC_Os10g35160	1.09E-02	expressed protein
96	WT_CvsMu_C	Up	LOC_Os01g38580	1.13E-02	beta,beta-carotene 9,10-dioxygenase, putative, expressed
97	WT_CvsMu_C	Up	LOC_Os05g48720	1.14E-02	transposon protein, putative, Mariner sub-class, expressed
98	WT_CvsMu_C	Up	LOC_Os05g45170	1.26E-02	glucosyl transferase, putative, expressed
99	WT_CvsMu_C	Up	LOC_Os04g46770	1.31E-02	transposon protein, putative, unclassified, expressed
100	WT_CvsMu_C	Up	LOC_Os02g06630	1.66E-02	peroxidase precursor, putative, expressed
1	WT_TvsMu_T	Down	LOC_Os10g22950	5.14E-14	calmodulin-binding transcription activator, putative, expressed
2	WT_TvsMu_T	Down	LOC_Os04g01330	2.33E-13	expressed protein
3	WT_TvsMu_T	Down	LOC_Os04g01320	3.46E-10	serine threonine-protein kinase receptor precursor, putative, expressed
4	WT_TvsMu_T	Down	LOC_Os02g34190	1.10E-08	expressed protein
5	WT_TvsMu_T	Down	LOC_Os11g32610	3.77E-05	chalcone and stilbene synthases, putative, expressed
6	WT_TvsMu_T	Down	LOC_Os08g19420	1.07E-04	O-methyltransferase, putative, expressed
7	WT_TvsMu_T	Down	LOC_Os09g36700	1.07E-04	ribonuclease T2 family domain containing protein, expressed
8	WT_TvsMu_T	Down	LOC_Os04g02640	1.19E-04	3-ketoacyl-CoA synthase 6, putative, expressed
9	WT_TvsMu_T	Down	LOC_Os05g06814	1.31E-04	expressed protein
10	WT_TvsMu_T	Down	LOC_Os10g09990	1.55E-04	cytokinin-O-glucosyltransferase 3, putative, expressed
11	WT_TvsMu_T	Down	LOC_Os04g03579	3.27E-04	protein kinase, putative, expressed
12	WT_TvsMu_T	Down	LOC_Os03g36490	3.35E-04	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
13	WT_TvsMu_T	Down	LOC_Os01g62860	3.35E-04	oxidoreductase, aldo keto reductase family protein, putative, expressed
14	WT_TvsMu_T	Down	LOC_Os03g36480	5.63E-04	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
15	WT_TvsMu_T	Down	LOC_Os10g35330	5.84E-04	expressed protein

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
16	WT_TvsMu_T	Down	LOC_Os10g04600	8.81E-04	OsFBX359 - F-box domain containing protein, expressed
17	WT_TvsMu_T	Down	LOC_Os09g36680	1.03E-03	ribonuclease T2 family domain containing protein, expressed
18	WT_TvsMu_T	Down	LOC_Os04g34610	1.37E-03	expressed protein
19	WT_TvsMu_T	Down	LOC_Os06g15170	1.80E-03	3-ketoacyl-CoA synthase, putative, expressed
20	WT_TvsMu_T	Down	LOC_Os07g32620	1.94E-03	anthocyanidin 5,3-O-glucosyltransferase, putative, expressed
21	WT_TvsMu_T	Down	LOC_Os01g48800	2.10E-03	purine permease, putative, expressed
22	WT_TvsMu_T	Down	LOC_Os03g20740	2.21E-03	expressed protein
23	WT_TvsMu_T	Down	LOC_Os11g42490	2.21E-03	retrotransposon protein, putative, unclassified, expressed
24	WT_TvsMu_T	Down	LOC_Os09g19650	2.45E-03	3-ketoacyl-CoA synthase precursor, putative, expressed
25	WT_TvsMu_T	Down	LOC_Os06g06780	2.45E-03	harpin-induced protein, putative, expressed
26	WT_TvsMu_T	Down	LOC_Os02g40100	2.93E-03	plant protein of unknown function DUF869 domain containing protein, expressed
27	WT_TvsMu_T	Down	LOC_Os05g26840	3.20E-03	permease domain containing protein, putative, expressed
28	WT_TvsMu_T	Down	LOC_Os07g23030	3.41E-03	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
29	WT_TvsMu_T	Down	LOC_Os08g14000	3.69E-03	retrotransposon protein, putative, Ty1-copia subclass, expressed
30	WT_TvsMu_T	Down	LOC_Os09g25490	4.32E-03	CESA9 - cellulose synthase, expressed
31	WT_TvsMu_T	Down	LOC_Os04g02790	4.37E-03	expressed protein
32	WT_TvsMu_T	Down	LOC_Os09g26200	4.41E-03	ZOS9-11 - C2H2 zinc finger protein, expressed
33	WT_TvsMu_T	Down	LOC_Os01g54620	4.91E-03	CESA4 - cellulose synthase, expressed
34	WT_TvsMu_T	Down	LOC_Os02g41630	4.95E-03	phenylalanine ammonia-lyase, putative, expressed
35	WT_TvsMu_T	Down	LOC_Os04g47930	5.09E-03	aluminum-activated malate transporter, putative, expressed
36	WT_TvsMu_T	Down	LOC_Os07g02440	5.68E-03	peroxidase precursor, putative, expressed
37	WT_TvsMu_T	Down	LOC_Os10g32980	5.85E-03	CESA7 - cellulose synthase, expressed
38	WT_TvsMu_T	Down	LOC_Os11g17530	6.02E-03	pentatricopeptide, putative, expressed
39	WT_TvsMu_T	Down	LOC_Os04g02140	6.72E-03	transposon protein, putative, CACTA, En Spm sub-class, expressed
40	WT_TvsMu_T	Down	LOC_Os01g67090	6.82E-03	IQ calmodulin-binding motif domain containing protein, expressed
41	WT_TvsMu_T	Down	LOC_Os04g02960	6.85E-03	OsSub32 - Putative Subtilisin homologue, expressed
42	WT_TvsMu_T	Down	LOC_Os10g21130	6.87E-03	expressed protein

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
43	WT_TvsMu_T	Down	LOC_Os05g30220	7.21E-03	disease resistance RPP13-like protein 1, putative, expressed
44	WT_TvsMu_T	Down	LOC_Os11g14560	7.44E-03	transposon protein, putative, CACTA, En Spm sub-class, expressed
45	WT_TvsMu_T	Down	LOC_Os04g43760	8.52E-03	phenylalanine ammonia-lyase, putative, expressed
46	WT_TvsMu_T	Down	LOC_Os05g32820	8.87E-03	peptide-N4-asparagine amidase A, putative, expressed
47	WT_TvsMu_T	Down	LOC_Os03g32470	8.98E-03	leucoanthocyanidin dioxygenase, putative, expressed
48	WT_TvsMu_T	Down	LOC_Os10g40620	9.29E-03	expressed protein
49	WT_TvsMu_T	Down	LOC_Os08g05690	9.38E-03	ABC transporter, ATP-binding protein, putative, expressed
50	WT_TvsMu_T	Down	LOC_Os06g45500	9.38E-03	copper-transporting ATPase, putative, expressed
51	WT_TvsMu_T	Down	LOC_Os05g27304	9.44E-03	peptide transporter PTR2, putative, expressed
52	WT_TvsMu_T	Down	LOC_Os10g33420	9.50E-03	non-lysosomal glucosylceramidase, putative, expressed
53	WT_TvsMu_T	Down	LOC_Os03g61720	9.62E-03	glycerol-3-phosphate acyltransferase, putative, expressed
54	WT_TvsMu_T	Down	LOC_Os11g26910	1.00E-02	SKP1-like protein 1B, putative, expressed
55	WT_TvsMu_T	Down	LOC_Os05g42060	1.09E-02	UDP-glucoronosyl UDP-glucosyl transferase, putative, expressed
56	WT_TvsMu_T	Down	LOC_Os05g24650	1.15E-02	DUF567 domain containing protein, putative, expressed
57	WT_TvsMu_T	Down	LOC_Os02g34380	1.21E-02	retrotransposon protein, putative, LINE subclass, expressed
58	WT_TvsMu_T	Down	LOC_Os01g65210	1.21E-02	proton-dependent oligopeptide transport, putative, expressed
59	WT_TvsMu_T	Down	LOC_Os08g06100	1.22E-02	O-methyltransferase, putative, expressed
60	WT_TvsMu_T	Down	LOC_Os01g67360	1.26E-02	methyltransferase, putative, expressed
61	WT_TvsMu_T	Down	LOC_Os11g42540	1.36E-02	oxidoreductase, aldo keto reductase family protein, putative, expressed
62	WT_TvsMu_T	Down	LOC_Os01g37400	1.41E-02	retrotransposon protein, putative, Ty1-copia subclass
63	WT_TvsMu_T	Down	LOC_Os02g34390	1.41E-02	expressed protein
64	WT_TvsMu_T	Down	LOC_Os04g08828	1.41E-02	cytochrome P450, putative, expressed
65	WT_TvsMu_T	Down	LOC_Os06g12455	1.42E-02	expressed protein
66	WT_TvsMu_T	Down	LOC_Os06g10870	1.52E-02	retrotransposon protein, putative, unclassified, expressed
67	WT_TvsMu_T	Down	LOC_Os04g02970	1.55E-02	subtilisin-like protease precursor, putative, expressed
68	WT_TvsMu_T	Down	LOC_Os04g26870	1.64E-02	oxidoreductase, aldo keto reductase family protein, putative, expressed
69	WT_TvsMu_T	Down	LOC_Os07g49110	1.67E-02	D-alanine--D-alanine ligase family, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
70	WT_TvsMu_T	Down	LOC_Os10g11270	1.73E-02	sulfotransferase domain containing protein, expressed
71	WT_TvsMu_T	Down	LOC_Os07g09340	1.74E-02	plasma membrane ATPase, putative, expressed
72	WT_TvsMu_T	Down	LOC_Os11g35920	1.76E-02	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
73	WT_TvsMu_T	Down	LOC_Os07g23120	1.82E-02	expressed protein
74	WT_TvsMu_T	Down	LOC_Os11g25210	1.93E-02	retrotransposon protein, putative, unclassified, expressed
75	WT_TvsMu_T	Down	LOC_Os10g25090	1.97E-02	STRUBBELIG-RECEPTOR FAMILY 6 precursor, putative, expressed
76	WT_TvsMu_T	Down	LOC_Os05g12410	1.99E-02	BURP domain containing protein, expressed
77	WT_TvsMu_T	Down	LOC_Os03g15530	2.00E-02	expressed protein
78	WT_TvsMu_T	Down	LOC_Os09g19780	2.08E-02	expressed protein
79	WT_TvsMu_T	Down	LOC_Os07g34390	2.08E-02	ankyrin repeat family protein, putative, expressed
80	WT_TvsMu_T	Down	LOC_Os11g25220	2.11E-02	oxidoreductase, short chain dehydrogenase%2Freductase domain containing protein, expressed
81	WT_TvsMu_T	Down	LOC_Os04g59330	2.13E-02	expressed protein
82	WT_TvsMu_T	Down	LOC_Os01g48130	2.15E-02	no apical meristem protein, putative, expressed
83	WT_TvsMu_T	Down	LOC_Os03g22634	2.21E-02	terpene synthase, putative, expressed
84	WT_TvsMu_T	Down	LOC_Os11g45990	2.22E-02	von Willebrand factor type A domain containing protein, putative, expressed
85	WT_TvsMu_T	Down	LOC_Os07g05450	2.46E-02	sulfotransferase domain containing protein, expressed
86	WT_TvsMu_T	Down	LOC_Os01g37410	2.47E-02	retrotransposon protein, putative, Ty1-copia subclass, expressed
87	WT_TvsMu_T	Down	LOC_Os04g14220	2.47E-02	disease resistance protein RPM1, putative, expressed
88	WT_TvsMu_T	Down	LOC_Os11g08550	2.53E-02	retrotransposon protein, putative, unclassified, expressed
89	WT_TvsMu_T	Down	LOC_Os07g23150	2.55E-02	transferase family protein, putative, expressed
90	WT_TvsMu_T	Down	LOC_Os04g28820	2.57E-02	retrotransposon protein, putative, unclassified, expressed
91	WT_TvsMu_T	Down	LOC_Os07g44090	2.67E-02	myb-related protein Hv33, putative, expressed
92	WT_TvsMu_T	Down	LOC_Os02g36150	2.69E-02	cytochrome P450, putative, expressed
93	WT_TvsMu_T	Down	LOC_Os09g34320	2.70E-02	expressed protein
94	WT_TvsMu_T	Down	LOC_Os10g10175	2.74E-02	expressed protein
95	WT_TvsMu_T	Down	LOC_Os09g25290	2.74E-02	methyladenine glycosylase, putative, expressed
96	WT_TvsMu_T	Down	LOC_Os04g29210	2.81E-02	FAD-binding and arabino-lactone oxidase domains containing protein, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
97	WT_TvsMu_T	Down	LOC_Os08g12800	2.81E-02	glucan endo-1,3-beta-glucosidase precursor, putative, expressed
98	WT_TvsMu_T	Down	LOC_Os04g55800	2.96E-02	sulfate transporter, putative, expressed
99	WT_TvsMu_T	Down	LOC_Os04g13140	3.03E-02	vignain precursor, putative, expressed
100	WT_TvsMu_T	Down	LOC_Os03g36080	3.03E-02	expressed protein
1	WT_TvsMu_T	Up	LOC_Os07g01904	4.79E-07	expressed protein
2	WT_TvsMu_T	Up	LOC_Os03g64330	1.20E-06	aquaporin protein, putative, expressed
3	WT_TvsMu_T	Up	LOC_Os07g10850	3.18E-06	retrotransposon protein, putative, unclassified, expressed
4	WT_TvsMu_T	Up	LOC_Os01g73000	4.75E-06	copine, putative, expressed
5	WT_TvsMu_T	Up	LOC_Os07g44250	7.37E-06	dirigent, putative, expressed
6	WT_TvsMu_T	Up	LOC_Os10g05020	1.02E-05	cytochrome P450, putative, expressed
7	WT_TvsMu_T	Up	LOC_Os02g14720	1.02E-05	expressed protein
8	WT_TvsMu_T	Up	LOC_Os03g25490	2.27E-05	cytochrome P450 72A1, putative, expressed
9	WT_TvsMu_T	Up	LOC_Os09g19350	2.27E-05	expressed protein
10	WT_TvsMu_T	Up	LOC_Os07g32710	5.61E-05	retrotransposon protein, putative, unclassified, expressed
11	WT_TvsMu_T	Up	LOC_Os05g50390	7.08E-05	expressed protein
12	WT_TvsMu_T	Up	LOC_Os05g11900	8.88E-05	expressed protein
13	WT_TvsMu_T	Up	LOC_Os07g27350	9.72E-05	atuA, putative, expressed
14	WT_TvsMu_T	Up	LOC_Os04g02530	1.06E-04	expressed protein
15	WT_TvsMu_T	Up	LOC_Os10g33104	1.14E-04	expressed protein
16	WT_TvsMu_T	Up	LOC_Os02g52730	1.25E-04	ferredoxin--nitrite reductase, putative, expressed
17	WT_TvsMu_T	Up	LOC_Os02g53240	1.28E-04	expressed protein
18	WT_TvsMu_T	Up	LOC_Os04g02754	1.28E-04	amidase family protein, putative, expressed
19	WT_TvsMu_T	Up	LOC_Os04g18770	1.31E-04	retrotransposon protein, putative, unclassified, expressed
20	WT_TvsMu_T	Up	LOC_Os04g12710	1.55E-04	indole-3-acetate beta-glucosyltransferase, putative, expressed
21	WT_TvsMu_T	Up	LOC_Os02g49510	1.67E-04	amino acid transporter, putative, expressed
22	WT_TvsMu_T	Up	LOC_Os02g18690	2.16E-04	BURP domain containing protein, expressed
23	WT_TvsMu_T	Up	LOC_Os04g01850	2.33E-04	hypothetical protein

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
24	WT_TvsMu_T	Up	LOC_Os02g30480	2.85E-04	retrotransposon protein, putative, unclassified, expressed
25	WT_TvsMu_T	Up	LOC_Os03g51600	3.21E-04	tubulin FtsZ domain containing protein, putative, expressed
26	WT_TvsMu_T	Up	LOC_Os10g19910	4.35E-04	expressed protein
27	WT_TvsMu_T	Up	LOC_Os01g15740	4.56E-04	expressed protein
28	WT_TvsMu_T	Up	LOC_Os04g02450	5.18E-04	rust-resistance protein Lr21, putative, expressed
29	WT_TvsMu_T	Up	LOC_Os10g24004	5.24E-04	expressed protein
30	WT_TvsMu_T	Up	LOC_Os04g17650	5.36E-04	sucrose synthase, putative, expressed
31	WT_TvsMu_T	Up	LOC_Os11g10670	5.47E-04	expressed protein
32	WT_TvsMu_T	Up	LOC_Os06g40240	5.70E-04	retrotransposon protein, putative, unclassified, expressed
33	WT_TvsMu_T	Up	LOC_Os04g14690	6.21E-04	flavin-containing monooxygenase family protein, putative, expressed
34	WT_TvsMu_T	Up	LOC_Os09g15310	7.10E-04	expressed protein
35	WT_TvsMu_T	Up	LOC_Os05g25090	7.10E-04	expressed protein
36	WT_TvsMu_T	Up	LOC_Os03g43100	7.33E-04	expressed protein
37	WT_TvsMu_T	Up	LOC_Os01g55590	7.33E-04	AMP-binding enzyme, putative, expressed
38	WT_TvsMu_T	Up	LOC_Os11g41400	7.57E-04	expressed protein
39	WT_TvsMu_T	Up	LOC_Os04g39900	7.73E-04	Os4bglu13 - beta-glucosidase homologue, similar to Os4Bglu12 exoglucanase b-glucosidase, expressed
40	WT_TvsMu_T	Up	LOC_Os05g39540	8.42E-04	metal cation transporter, putative, expressed
41	WT_TvsMu_T	Up	LOC_Os04g02040	8.81E-04	NBS-LRR, putative, expressed
42	WT_TvsMu_T	Up	LOC_Os07g32680	8.81E-04	retrotransposon protein, putative, unclassified, expressed
43	WT_TvsMu_T	Up	LOC_Os10g07160	8.81E-04	retrotransposon, putative, centromere-specific, expressed
44	WT_TvsMu_T	Up	LOC_Os06g49350	1.02E-03	retrotransposon protein, putative, unclassified, expressed
45	WT_TvsMu_T	Up	LOC_Os05g23255	1.03E-03	expressed protein
46	WT_TvsMu_T	Up	LOC_Os09g38104	1.19E-03	hypothetical protein
47	WT_TvsMu_T	Up	LOC_Os05g07450	1.47E-03	S-domain receptor-like protein kinase, putative, expressed
48	WT_TvsMu_T	Up	LOC_Os04g30330	1.47E-03	OsWAK59 - OsWAK receptor-like cytoplasmic kinase OsWAK-RLCK, expressed
49	WT_TvsMu_T	Up	LOC_Os10g29400	1.49E-03	transposon protein, putative, CACTA, En Spm sub-class, expressed
50	WT_TvsMu_T	Up	LOC_Os01g49750	1.76E-03	expressed protein

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
51	WT_TvsMu_T	Up	LOC_Os07g31200	1.94E-03	expressed protein
52	WT_TvsMu_T	Up	LOC_Os03g02470	2.45E-03	expressed protein
53	WT_TvsMu_T	Up	LOC_Os04g58050	2.53E-03	expressed protein
54	WT_TvsMu_T	Up	LOC_Os05g01840	3.75E-03	hypothetical protein
55	WT_TvsMu_T	Up	LOC_Os06g04930	3.75E-03	expressed protein
56	WT_TvsMu_T	Up	LOC_Os04g02050	3.75E-03	bifunctional 3-phosphoadenosine 5-phosphosulfate synthetase, putative, expressed
57	WT_TvsMu_T	Up	LOC_Os04g02520	3.81E-03	Leucine Rich Repeat family protein, expressed
58	WT_TvsMu_T	Up	LOC_Os04g01990	3.81E-03	pentatricopeptide domain containing protein, putative, expressed
59	WT_TvsMu_T	Up	LOC_Os04g43090	3.89E-03	expressed protein
60	WT_TvsMu_T	Up	LOC_Os11g07440	3.92E-03	plant neutral invertase domain containing protein, expressed
61	WT_TvsMu_T	Up	LOC_Os04g32920	4.14E-03	potassium transporter, putative, expressed
62	WT_TvsMu_T	Up	LOC_Os07g09880	4.40E-03	expressed protein
63	WT_TvsMu_T	Up	LOC_Os12g07180	4.40E-03	transposon protein, putative, Pong sub-class, expressed
64	WT_TvsMu_T	Up	LOC_Os05g42160	4.41E-03	expressed protein
65	WT_TvsMu_T	Up	LOC_Os08g03020	4.53E-03	lectin-like receptor kinase 1, putative, expressed
66	WT_TvsMu_T	Up	LOC_Os07g32700	5.52E-03	retrotransposon protein, putative, unclassified, expressed
67	WT_TvsMu_T	Up	LOC_Os07g25710	5.85E-03	myb-like DNA-binding domain containing protein, expressed
68	WT_TvsMu_T	Up	LOC_Os02g50140	6.82E-03	caleosin related protein, putative, expressed
69	WT_TvsMu_T	Up	LOC_Os02g49480	6.86E-03	helix-loop-helix DNA-binding domain containing protein, expressed
70	WT_TvsMu_T	Up	LOC_Os09g26530	6.87E-03	expressed protein
71	WT_TvsMu_T	Up	LOC_Os10g05290	7.21E-03	tRNA rRNA methyltransferase, putative, expressed
72	WT_TvsMu_T	Up	LOC_Os03g51610	7.21E-03	Inositol 1, 3, 4-trisphosphate 5 6-kinase, putative, expressed
73	WT_TvsMu_T	Up	LOC_Os04g52790	7.62E-03	expressed protein
74	WT_TvsMu_T	Up	LOC_Os11g31840	9.29E-03	retrotransposon protein, putative, Ty1-copia subclass, expressed
75	WT_TvsMu_T	Up	LOC_Os11g40470	9.29E-03	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
76	WT_TvsMu_T	Up	LOC_Os06g13560	9.29E-03	SAM dependent carboxyl methyltransferase, putative, expressed
77	WT_TvsMu_T	Up	LOC_Os05g23880	9.29E-03	lipoxygenase, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
78	WT_TvsMu_T	Up	LOC_Os04g20810	9.44E-03	receptor protein kinase, putative, expressed
79	WT_TvsMu_T	Up	LOC_Os04g12690	9.59E-03	N-hydroxythioamide S-beta-glucosyltransferase, putative, expressed
80	WT_TvsMu_T	Up	LOC_Os02g15820	9.73E-03	extra-large G-protein-related, putative, expressed
81	WT_TvsMu_T	Up	LOC_Os01g10240	9.77E-03	urate anion exchanger, putative, expressed
82	WT_TvsMu_T	Up	LOC_Os03g20600	9.79E-03	expressed protein
83	WT_TvsMu_T	Up	LOC_Os04g02120	1.05E-02	expressed protein
84	WT_TvsMu_T	Up	LOC_Os08g13440	1.06E-02	cupin domain containing protein, expressed
85	WT_TvsMu_T	Up	LOC_Os09g01540	1.09E-02	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
86	WT_TvsMu_T	Up	LOC_Os03g02230	1.09E-02	expressed protein
87	WT_TvsMu_T	Up	LOC_Os02g12470	1.10E-02	hypothetical protein
88	WT_TvsMu_T	Up	LOC_Os02g08330	1.22E-02	gp176, putative, expressed
89	WT_TvsMu_T	Up	LOC_Os09g11460	1.24E-02	AP2 domain containing protein, expressed
90	WT_TvsMu_T	Up	LOC_Os10g07150	1.33E-02	transposon protein, putative, unclassified, expressed
91	WT_TvsMu_T	Up	LOC_Os04g36670	1.36E-02	expressed protein
92	WT_TvsMu_T	Up	LOC_Os05g34490	1.37E-02	expressed protein
93	WT_TvsMu_T	Up	LOC_Os06g13080	1.41E-02	spotted leaf 11, putative, expressed
94	WT_TvsMu_T	Up	LOC_Os11g45320	1.41E-02	expressed protein
95	WT_TvsMu_T	Up	LOC_Os10g05250	1.42E-02	protein kinase domain containing protein, expressed
96	WT_TvsMu_T	Up	LOC_Os04g43680	1.50E-02	MYB family transcription factor, putative, expressed
97	WT_TvsMu_T	Up	LOC_Os04g18780	1.54E-02	retrotransposon protein, putative, unclassified
98	WT_TvsMu_T	Up	LOC_Os03g25510	1.64E-02	expressed protein
99	WT_TvsMu_T	Up	LOC_Os01g07170	1.65E-02	HORMA domain containing protein, putative, expressed
100	WT_TvsMu_T	Up	LOC_Os04g39150	1.68E-02	pathogenesis-related Bet v I family protein, putative, expressed
1	Mu_CvsMu_T	Down	LOC_Os07g26110	1.55E-12	membrane associated DUF588 domain containing protein, putative, expressed
2	Mu_CvsMu_T	Down	LOC_Os02g51110	1.30E-11	aquaporin protein, putative, expressed
3	Mu_CvsMu_T	Down	LOC_Os09g33830	1.88E-11	solute carrier family 35 member F1, putative, expressed
4	Mu_CvsMu_T	Down	LOC_Os10g39980	2.03E-11	expressed protein

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
5	Mu_CvsMu_T	Down	LOC_Os06g47700	4.88E-11	serine threonine-protein kinase BRI1-like 2 precursor, putative, expressed
6	Mu_CvsMu_T	Down	LOC_Os07g26100	6.47E-11	expressed protein
7	Mu_CvsMu_T	Down	LOC_Os06g43410	6.89E-11	cytochrome P450, putative, expressed
8	Mu_CvsMu_T	Down	LOC_Os07g45080	7.02E-11	expressed protein
9	Mu_CvsMu_T	Down	LOC_Os01g68720	7.02E-11	keratin, type I cytoskeletal 9, putative, expressed
10	Mu_CvsMu_T	Down	LOC_Os02g36414	8.40E-11	transporter family protein, putative, expressed
11	Mu_CvsMu_T	Down	LOC_Os04g12010	8.48E-11	glycosyltransferase, putative, expressed
12	Mu_CvsMu_T	Down	LOC_Os03g22050	8.66E-11	CAMK_KIN1 SNF1 Nim1_like.16 - CAMK includes calcium calmodulin depedent protein kinases, expressed
13	Mu_CvsMu_T	Down	LOC_Os03g20420	8.66E-11	alpha-N-arabinofuranosidase A, putative, expressed
14	Mu_CvsMu_T	Down	LOC_Os03g01700	1.05E-10	expressed protein
15	Mu_CvsMu_T	Down	LOC_Os01g72360	1.08E-10	expressed protein
16	Mu_CvsMu_T	Down	LOC_Os07g12900	1.08E-10	cadmium zinc-transporting ATPase, putative, expressed
17	Mu_CvsMu_T	Down	LOC_Os10g33440	1.60E-10	NB-ARC domain containing protein, expressed
18	Mu_CvsMu_T	Down	LOC_Os01g48800	1.84E-10	purine permease, putative, expressed
19	Mu_CvsMu_T	Down	LOC_Os01g18744	1.87E-10	transferase family protein, putative, expressed
20	Mu_CvsMu_T	Down	LOC_Os05g35010	2.37E-10	cytochrome P450, putative, expressed
21	Mu_CvsMu_T	Down	LOC_Os03g08720	2.45E-10	transferase family protein, putative, expressed
22	Mu_CvsMu_T	Down	LOC_Os05g33130	2.48E-10	CHIT17 - Chitinase family protein precursor, expressed
23	Mu_CvsMu_T	Down	LOC_Os02g12690	2.59E-10	cytochrome P450, putative, expressed
24	Mu_CvsMu_T	Down	LOC_Os03g25040	2.60E-10	GDSL-like lipase acylhydrolase, putative, expressed
25	Mu_CvsMu_T	Down	LOC_Os01g48710	2.68E-10	heavy metal-associated domain containing protein, expressed
26	Mu_CvsMu_T	Down	LOC_Os06g04540	2.93E-10	DNA binding protein, putative, expressed
27	Mu_CvsMu_T	Down	LOC_Os05g51780	3.56E-10	zinc finger, C3HC4 type domain containing protein, expressed
28	Mu_CvsMu_T	Down	LOC_Os02g32009	6.64E-10	expressed protein
29	Mu_CvsMu_T	Down	LOC_Os02g01220	6.98E-10	rhodanese-like domain containing protein, putative, expressed
30	Mu_CvsMu_T	Down	LOC_Os02g02640	7.08E-10	NBS-LRR disease resistance protein, putative, expressed
31	Mu_CvsMu_T	Down	LOC_Os03g46470	7.97E-10	metal cation transporter, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
32	Mu_CvsMu_T	Down	LOC_Os04g37990	8.78E-10	transporter family protein, putative, expressed
33	Mu_CvsMu_T	Down	LOC_Os04g46750	8.91E-10	glycosyl transferase 8 domain containing protein, putative, expressed
34	Mu_CvsMu_T	Down	LOC_Os07g45370	9.27E-10	expressed protein
35	Mu_CvsMu_T	Down	LOC_Os02g42220	9.29E-10	transposon protein, putative, unclassified, expressed
36	Mu_CvsMu_T	Down	LOC_Os03g02040	9.42E-10	remorin, putative, expressed
37	Mu_CvsMu_T	Down	LOC_Os01g68730	1.09E-09	RNA-binding protein FUS, putative, expressed
38	Mu_CvsMu_T	Down	LOC_Os04g52640	1.15E-09	SHR5-receptor-like kinase, putative, expressed
39	Mu_CvsMu_T	Down	LOC_Os05g06970	1.26E-09	peroxidase precursor, putative, expressed
40	Mu_CvsMu_T	Down	LOC_Os03g25330	1.26E-09	peroxidase precursor, putative, expressed
41	Mu_CvsMu_T	Down	LOC_Os10g01640	1.42E-09	decarboxylase, putative, expressed
42	Mu_CvsMu_T	Down	LOC_Os01g11910	1.47E-09	basic helix-loop-helix, putative, expressed
43	Mu_CvsMu_T	Down	LOC_Os02g54180	1.60E-09	expressed protein
44	Mu_CvsMu_T	Down	LOC_Os01g09080	1.67E-09	WRKY107, expressed
45	Mu_CvsMu_T	Down	LOC_Os01g14520	1.71E-09	C4-dicarboxylate transporter malic acid transport protein domain containing protein, expressed
46	Mu_CvsMu_T	Down	LOC_Os01g40870	1.82E-09	aldehyde dehydrogenase, putative, expressed
47	Mu_CvsMu_T	Down	LOC_Os08g01710	1.85E-09	GLTP domain containing protein, putative, expressed
48	Mu_CvsMu_T	Down	LOC_Os11g05480	1.85E-09	transcription factor, putative, expressed
49	Mu_CvsMu_T	Down	LOC_Os02g03900	1.90E-09	metal transporter Nramp6, putative, expressed
50	Mu_CvsMu_T	Down	LOC_Os08g44910	1.93E-09	DNA binding protein, putative, expressed
51	Mu_CvsMu_T	Down	LOC_Os10g38090	2.03E-09	cytochrome P450, putative, expressed
52	Mu_CvsMu_T	Down	LOC_Os03g45920	2.03E-09	tubulin FtsZ domain containing protein, putative, expressed
53	Mu_CvsMu_T	Down	LOC_Os07g01410	2.17E-09	peroxidase precursor, putative, expressed
54	Mu_CvsMu_T	Down	LOC_Os05g19910	2.49E-09	transferase family protein, putative, expressed
55	Mu_CvsMu_T	Down	LOC_Os04g37980	2.59E-09	transporter family protein, putative, expressed
56	Mu_CvsMu_T	Down	LOC_Os03g63900	2.85E-09	1-aminocyclopropane-1-carboxylate oxidase 2, putative, expressed
57	Mu_CvsMu_T	Down	LOC_Os04g47930	2.93E-09	aluminum-activated malate transporter, putative, expressed
58	Mu_CvsMu_T	Down	LOC_Os08g36040	2.93E-09	plant viral response family protein, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
59	Mu_CvsMu_T	Down	LOC_Os07g36210	2.95E-09	expressed protein
60	Mu_CvsMu_T	Down	LOC_Os06g06000	2.98E-09	expressed protein
61	Mu_CvsMu_T	Down	LOC_Os10g11500	3.04E-09	SCP-like extracellular protein, expressed
62	Mu_CvsMu_T	Down	LOC_Os01g12070	3.09E-09	endoglucanase precursor, putative, expressed
63	Mu_CvsMu_T	Down	LOC_Os02g45890	3.51E-09	sulfotransferase domain containing protein, expressed
64	Mu_CvsMu_T	Down	LOC_Os06g05990	3.61E-09	zinc finger family protein, putative, expressed
65	Mu_CvsMu_T	Down	LOC_Os09g14100	3.61E-09	disease resistance protein RPS2, putative, expressed
66	Mu_CvsMu_T	Down	LOC_Os01g24560	3.93E-09	fruit bromelain precursor, putative, expressed
67	Mu_CvsMu_T	Down	LOC_Os03g42259	3.97E-09	hypervariable Bacillus group-specific protein, putative, expressed
68	Mu_CvsMu_T	Down	LOC_Os07g06490	3.97E-09	DNA binding protein, putative, expressed
69	Mu_CvsMu_T	Down	LOC_Os07g33610	4.02E-09	cytochrome P450, putative, expressed
70	Mu_CvsMu_T	Down	LOC_Os05g30300	4.17E-09	Os5bglu21 - beta-glucosidase homologue, similar to G. max isohydroxyurate hydrolase, expressed
71	Mu_CvsMu_T	Down	LOC_Os11g46860	4.18E-09	wall-associated receptor kinase-like 4 precursor, putative, expressed
72	Mu_CvsMu_T	Down	LOC_Os11g42950	4.26E-09	expressed protein
73	Mu_CvsMu_T	Down	LOC_Os02g02490	4.37E-09	phytosulfokine receptor precursor, putative, expressed
74	Mu_CvsMu_T	Down	LOC_Os02g01890	4.44E-09	cytochrome P450, putative, expressed
75	Mu_CvsMu_T	Down	LOC_Os03g25320	4.61E-09	peroxidase precursor, putative, expressed
76	Mu_CvsMu_T	Down	LOC_Os02g43410	4.88E-09	transposon protein, putative, unclassified, expressed
77	Mu_CvsMu_T	Down	LOC_Os03g18710	4.91E-09	expressed protein
78	Mu_CvsMu_T	Down	LOC_Os05g41990	5.20E-09	peroxidase precursor, putative, expressed
79	Mu_CvsMu_T	Down	LOC_Os04g31290	5.47E-09	helix-loop-helix DNA-binding domain containing protein, expressed
80	Mu_CvsMu_T	Down	LOC_Os04g42740	5.47E-09	serine threonine-protein kinase receptor precursor, putative, expressed
81	Mu_CvsMu_T	Down	LOC_Os06g36330	5.47E-09	MATE domain containing protein, expressed
82	Mu_CvsMu_T	Down	LOC_Os04g59020	5.47E-09	integral membrane protein, putative, expressed
83	Mu_CvsMu_T	Down	LOC_Os01g33810	5.48E-09	disease resistance protein RPM1, putative, expressed
84	Mu_CvsMu_T	Down	LOC_Os05g49840	5.62E-09	phospholipase, putative, expressed
85	Mu_CvsMu_T	Down	LOC_Os10g17650	5.67E-09	Os10bglu34 - beta-glucosidase homologue, similar to Os3bglu6, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
86	Mu_CvsMu_T	Down	LOC_Os08g26820	5.67E-09	plant protein of unknown function domain containing protein, expressed
87	Mu_CvsMu_T	Down	LOC_Os04g10750	5.67E-09	inorganic phosphate transporter, putative, expressed
88	Mu_CvsMu_T	Down	LOC_Os01g13560	5.86E-09	membrane associated DUF588 domain containing protein, putative, expressed
89	Mu_CvsMu_T	Down	LOC_Os02g09490	6.30E-09	dehydrogenase, putative, expressed
90	Mu_CvsMu_T	Down	LOC_Os04g45860	6.33E-09	transposon protein, putative, unclassified, expressed
91	Mu_CvsMu_T	Down	LOC_Os01g58910	7.30E-09	auxin-induced protein 5NG4, putative, expressed
92	Mu_CvsMu_T	Down	LOC_Os11g32160	7.31E-09	expressed protein
93	Mu_CvsMu_T	Down	LOC_Os01g68740	8.04E-09	keratin, type I cytoskeletal 9, putative, expressed
94	Mu_CvsMu_T	Down	LOC_Os09g15365	8.04E-09	hydrophobic protein, putative, expressed
95	Mu_CvsMu_T	Down	LOC_Os06g12310	8.04E-09	aquaporin protein, putative, expressed
96	Mu_CvsMu_T	Down	LOC_Os04g35730	8.47E-09	transposon protein, putative, unclassified, expressed
97	Mu_CvsMu_T	Down	LOC_Os02g43370	8.95E-09	transposon protein, putative, unclassified, expressed
98	Mu_CvsMu_T	Down	LOC_Os08g30770	9.07E-09	ABC transporter, ATP-binding protein, putative, expressed
99	Mu_CvsMu_T	Down	LOC_Os11g01830	9.25E-09	staphylococcal nuclease homologue, putative, expressed
100	Mu_CvsMu_T	Down	LOC_Os08g08970	9.68E-09	Cupin domain containing protein, expressed
1	Mu_CvsMu_T	Up	LOC_Os02g43330	1.55E-12	homeobox associated leucine zipper, putative, expressed
2	Mu_CvsMu_T	Up	LOC_Os09g15670	1.55E-12	protein phosphatase 2C, putative, expressed
3	Mu_CvsMu_T	Up	LOC_Os03g20680	5.87E-12	late embryogenesis abundant protein 1, putative, expressed
4	Mu_CvsMu_T	Up	LOC_Os09g38320	8.05E-12	phytoene synthase, chloroplast precursor, putative, expressed
5	Mu_CvsMu_T	Up	LOC_Os03g44380	1.25E-11	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed
6	Mu_CvsMu_T	Up	LOC_Os02g30910	2.03E-11	nodulin MtN3 family protein, putative, expressed
7	Mu_CvsMu_T	Up	LOC_Os04g45810	2.03E-11	homeobox associated leucine zipper, putative, expressed
8	Mu_CvsMu_T	Up	LOC_Os01g62760	2.07E-11	protein phosphatase 2C, putative, expressed
9	Mu_CvsMu_T	Up	LOC_Os03g03370	2.22E-11	fatty acid hydroxylase, putative, expressed
10	Mu_CvsMu_T	Up	LOC_Os06g03930	5.46E-11	cytochrome P450 86A1, putative, expressed
11	Mu_CvsMu_T	Up	LOC_Os09g21120	6.29E-11	armadillo beta-catenin repeat family protein, putative, expressed
12	Mu_CvsMu_T	Up	LOC_Os07g05940	6.47E-11	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
13	Mu_CvsMu_T	Up	LOC_Os05g38290	6.47E-11	protein phosphatase 2C, putative, expressed
14	Mu_CvsMu_T	Up	LOC_Os03g60580	6.89E-11	actin-depolymerizing factor, putative, expressed
15	Mu_CvsMu_T	Up	LOC_Os06g44190	6.89E-11	expressed protein
16	Mu_CvsMu_T	Up	LOC_Os03g14370	6.89E-11	ACT domain containing protein, expressed
17	Mu_CvsMu_T	Up	LOC_Os06g46740	7.02E-11	early nodulin 20 precursor, putative, expressed
18	Mu_CvsMu_T	Up	LOC_Os07g29750	9.69E-11	glycosyl hydrolases family 16, putative, expressed
19	Mu_CvsMu_T	Up	LOC_Os01g37832	9.94E-11	thioredoxin, putative, expressed
20	Mu_CvsMu_T	Up	LOC_Os01g50400	1.05E-10	STE_MEKK_ste11_MAP3K.5 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed
21	Mu_CvsMu_T	Up	LOC_Os06g46920	1.05E-10	dihydroflavonol-4-reductase, putative, expressed
22	Mu_CvsMu_T	Up	LOC_Os04g17100	1.08E-10	heavy metal-associated domain containing protein, expressed
23	Mu_CvsMu_T	Up	LOC_Os03g18490	1.10E-10	RPGR, putative, expressed
24	Mu_CvsMu_T	Up	LOC_Os05g46480	1.13E-10	late embryogenesis abundant protein, group 3, putative, expressed
25	Mu_CvsMu_T	Up	LOC_Os01g40094	1.16E-10	protein phosphatase 2C, putative, expressed
26	Mu_CvsMu_T	Up	LOC_Os03g45280	1.56E-10	dehydrin, putative, expressed
27	Mu_CvsMu_T	Up	LOC_Os03g51350	1.66E-10	expressed protein
28	Mu_CvsMu_T	Up	LOC_Os02g51040	1.66E-10	expansin precursor, putative, expressed
29	Mu_CvsMu_T	Up	LOC_Os01g50910	1.76E-10	late embryogenesis abundant protein, group 3, putative, expressed
30	Mu_CvsMu_T	Up	LOC_Os10g39660	1.77E-10	expressed protein
31	Mu_CvsMu_T	Up	LOC_Os08g01910	1.84E-10	expressed protein
32	Mu_CvsMu_T	Up	LOC_Os01g63930	1.89E-10	cytochrome P450, putative, expressed
33	Mu_CvsMu_T	Up	LOC_Os10g09240	2.07E-10	expressed protein
34	Mu_CvsMu_T	Up	LOC_Os06g10820	2.20E-10	helix-loop-helix DNA-binding domain containing protein, expressed
35	Mu_CvsMu_T	Up	LOC_Os07g23570	2.42E-10	cytochrome P450 72A1, putative, expressed
36	Mu_CvsMu_T	Up	LOC_Os01g32780	3.22E-10	universal stress protein domain containing protein, putative, expressed
37	Mu_CvsMu_T	Up	LOC_Os03g16170	3.24E-10	protein phosphatase 2C, putative, expressed
38	Mu_CvsMu_T	Up	LOC_Os11g26560	3.56E-10	expressed protein
39	Mu_CvsMu_T	Up	LOC_Os03g51920	3.71E-10	peptidase, M50 family, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
40	Mu_CvsMu_T	Up	LOC_Os11g07910	3.78E-10	transmembrane 9 superfamily member, putative, expressed
41	Mu_CvsMu_T	Up	LOC_Os02g44870	3.78E-10	dehydrin, putative, expressed
42	Mu_CvsMu_T	Up	LOC_Os02g10310	3.78E-10	fumarylacetoacetase, putative, expressed
43	Mu_CvsMu_T	Up	LOC_Os04g01590	3.78E-10	arginase, putative, expressed
44	Mu_CvsMu_T	Up	LOC_Os01g47490	3.78E-10	Lg106, putative, expressed
45	Mu_CvsMu_T	Up	LOC_Os03g33580	5.09E-10	mitotic checkpoint protein, putative, expressed
46	Mu_CvsMu_T	Up	LOC_Os03g24040	5.50E-10	seven in absentia protein family domain containing protein, expressed
47	Mu_CvsMu_T	Up	LOC_Os07g48830	5.69E-10	glycosyl transferase 8 domain containing protein, putative, expressed
48	Mu_CvsMu_T	Up	LOC_Os02g47840	6.28E-10	universal stress protein domain containing protein, putative, expressed
49	Mu_CvsMu_T	Up	LOC_Os01g67540	6.32E-10	AMP-binding domain containing protein, expressed
50	Mu_CvsMu_T	Up	LOC_Os02g44090	6.64E-10	zinc finger protein, putative, expressed
51	Mu_CvsMu_T	Up	LOC_Os05g33960	7.08E-10	peptide transporter PTR2, putative, expressed
52	Mu_CvsMu_T	Up	LOC_Os05g46040	7.08E-10	protein phosphatase 2C, putative, expressed
53	Mu_CvsMu_T	Up	LOC_Os02g52780	7.97E-10	bZIP transcription factor, putative, expressed
54	Mu_CvsMu_T	Up	LOC_Os03g09170	8.50E-10	ethylene-responsive transcription factor, putative, expressed
55	Mu_CvsMu_T	Up	LOC_Os06g11290	9.29E-10	12-oxophytodienoate reductase, putative, expressed
56	Mu_CvsMu_T	Up	LOC_Os05g40010	9.42E-10	LTPL17 - Protease inhibitor seed storage LTP family protein precursor, expressed
57	Mu_CvsMu_T	Up	LOC_Os03g60570	1.03E-09	ZOS3-22 - C2H2 zinc finger protein, expressed
58	Mu_CvsMu_T	Up	LOC_Os03g60370	1.06E-09	histidine acid phosphatase, putative, expressed
59	Mu_CvsMu_T	Up	LOC_Os10g36180	1.08E-09	expressed protein
60	Mu_CvsMu_T	Up	LOC_Os05g49730	1.10E-09	protein phosphatase 2C, putative, expressed
61	Mu_CvsMu_T	Up	LOC_Os05g41490	1.23E-09	circadian clock coupling factor ZGT, putative, expressed
62	Mu_CvsMu_T	Up	LOC_Os11g07600	1.26E-09	ABC-2 type transporter domain containing protein, expressed
63	Mu_CvsMu_T	Up	LOC_Os01g04590	1.31E-09	expressed protein
64	Mu_CvsMu_T	Up	LOC_Os06g35960	1.56E-09	HSF-type DNA-binding domain containing protein, expressed
65	Mu_CvsMu_T	Up	LOC_Os03g60560	1.67E-09	ZOS3-21 - C2H2 zinc finger protein, expressed
66	Mu_CvsMu_T	Up	LOC_Os05g10670	1.85E-09	zinc finger CCCH type family protein, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
67	Mu_CvsMu_T	Up	LOC_Os01g64360	1.88E-09	MYB family transcription factor, putative, expressed
68	Mu_CvsMu_T	Up	LOC_Os08g44340	1.90E-09	monodehydroascorbate reductase, putative, expressed
69	Mu_CvsMu_T	Up	LOC_Os12g05260	1.90E-09	phytosulfokines precursor, putative, expressed
70	Mu_CvsMu_T	Up	LOC_Os05g51670	1.98E-09	NAD dependent epimerase dehydratase family protein, putative, expressed
71	Mu_CvsMu_T	Up	LOC_Os01g09640	2.01E-09	Myb transcription factor, putative, expressed
72	Mu_CvsMu_T	Up	LOC_Os01g50410	2.03E-09	STE_MEKK_ste11_MAP3K.6 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed
73	Mu_CvsMu_T	Up	LOC_Os08g42590	2.03E-09	mtN19, putative, expressed
74	Mu_CvsMu_T	Up	LOC_Os07g40290	2.03E-09	OsGH3.8 - Probable indole-3-acetic acid-amido synthetase, expressed
75	Mu_CvsMu_T	Up	LOC_Os02g33380	2.12E-09	pectinesterase inhibitor domain containing protein, putative, expressed
76	Mu_CvsMu_T	Up	LOC_Os05g38660	2.12E-09	expressed protein
77	Mu_CvsMu_T	Up	LOC_Os03g26870	2.17E-09	WD-40 repeat family protein, putative, expressed
78	Mu_CvsMu_T	Up	LOC_Os12g07030	2.22E-09	expressed protein
79	Mu_CvsMu_T	Up	LOC_Os05g07890	2.49E-09	embryo-specific 3, putative, expressed
80	Mu_CvsMu_T	Up	LOC_Os01g46760	2.49E-09	protein phosphatase 2C, putative, expressed
81	Mu_CvsMu_T	Up	LOC_Os11g26770	2.63E-09	transposon protein, putative, unclassified, expressed
82	Mu_CvsMu_T	Up	LOC_Os01g03750	2.63E-09	expressed protein
83	Mu_CvsMu_T	Up	LOC_Os03g42520	2.71E-09	expressed protein
84	Mu_CvsMu_T	Up	LOC_Os09g28210	2.89E-09	bHelix-loop-helix transcription factor, putative, expressed
85	Mu_CvsMu_T	Up	LOC_Os09g21180	2.89E-09	homeobox associated leucine zipper, putative, expressed
86	Mu_CvsMu_T	Up	LOC_Os04g47700	2.89E-09	expressed protein
87	Mu_CvsMu_T	Up	LOC_Os02g26720	2.93E-09	Inositol 1, 3, 4-trisphosphate 5 6-kinase, putative, expressed
88	Mu_CvsMu_T	Up	LOC_Os03g41060	2.93E-09	GASR2 - Gibberellin-regulated GASA GAST Snakin family protein precursor, putative, expressed
89	Mu_CvsMu_T	Up	LOC_Os09g33690	2.93E-09	Os9bglu32 - beta-glucosidase homologue, similar to G. max hydroxyisourate hydrolase, expressed
90	Mu_CvsMu_T	Up	LOC_Os03g17790	3.02E-09	OsRCI2-5 - Putative low temperature and salt responsive protein, expressed
91	Mu_CvsMu_T	Up	LOC_Os05g15530	3.04E-09	aminotransferase domain containing protein, putative, expressed
92	Mu_CvsMu_T	Up	LOC_Os05g49770	3.08E-09	CTP synthase, putative, expressed
93	Mu_CvsMu_T	Up	LOC_Os09g30160	3.14E-09	zinc finger, C3HC4 type domain containing protein, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
94	Mu_CvsMu_T	Up	LOC_Os03g20120	3.25E-09	glycosyl transferase 8 domain containing protein, putative, expressed
95	Mu_CvsMu_T	Up	LOC_Os08g10500	3.25E-09	expressed protein
96	Mu_CvsMu_T	Up	LOC_Os01g01620	3.25E-09	kinase, pfkB family, putative, expressed
97	Mu_CvsMu_T	Up	LOC_Os11g30484	3.58E-09	ZOS11-03 - C2H2 zinc finger protein, expressed
98	Mu_CvsMu_T	Up	LOC_Os02g57840	3.60E-09	remorin C-terminal domain containing protein, putative, expressed
99	Mu_CvsMu_T	Up	LOC_Os05g46350	3.61E-09	IQ calmodulin-binding motif domain containing protein, expressed
100	Mu_CvsMu_T	Up	LOC_Os03g19290	3.64E-09	mitochondrial import inner membrane translocase subunit Tim17, putative, expressed
1	WT_CvsWT_T	Down	LOC_Os07g26110	2.85E-12	membrane associated DUF588 domain containing protein, putative, expressed
2	WT_CvsWT_T	Down	LOC_Os02g51110	1.75E-11	aquaporin protein, putative, expressed
3	WT_CvsWT_T	Down	LOC_Os09g33830	1.75E-11	solute carrier family 35 member F1, putative, expressed
4	WT_CvsWT_T	Down	LOC_Os03g22050	3.18E-11	CAMK_KIN1 SNF1 Nim1_like.16 - CAMK includes calcium calmodulin depedent protein kinases, expressed
5	WT_CvsWT_T	Down	LOC_Os04g12010	3.62E-11	glycosyltransferase, putative, expressed
6	WT_CvsWT_T	Down	LOC_Os01g68720	5.81E-11	keratin, type I cytoskeletal 9, putative, expressed
7	WT_CvsWT_T	Down	LOC_Os03g20420	7.23E-11	alpha-N-arabinofuranosidase A, putative, expressed
8	WT_CvsWT_T	Down	LOC_Os10g39980	8.72E-11	expressed protein
9	WT_CvsWT_T	Down	LOC_Os07g45080	9.19E-11	expressed protein
10	WT_CvsWT_T	Down	LOC_Os06g43410	9.42E-11	cytochrome P450, putative, expressed
11	WT_CvsWT_T	Down	LOC_Os07g45370	1.01E-10	expressed protein
12	WT_CvsWT_T	Down	LOC_Os05g33130	1.08E-10	CHIT17 - Chitinase family protein precursor, expressed
13	WT_CvsWT_T	Down	LOC_Os03g25040	1.19E-10	GDSL-like lipase acylhydrolase, putative, expressed
14	WT_CvsWT_T	Down	LOC_Os02g01220	1.32E-10	rhodanese-like domain containing protein, putative, expressed
15	WT_CvsWT_T	Down	LOC_Os01g09080	1.63E-10	WRKY107, expressed
16	WT_CvsWT_T	Down	LOC_Os10g33440	1.77E-10	NB-ARC domain containing protein, expressed
17	WT_CvsWT_T	Down	LOC_Os02g36414	1.85E-10	transporter family protein, putative, expressed
18	WT_CvsWT_T	Down	LOC_Os07g26100	1.92E-10	expressed protein
19	WT_CvsWT_T	Down	LOC_Os08g01710	2.05E-10	GLTP domain containing protein, putative, expressed
20	WT_CvsWT_T	Down	LOC_Os03g08720	2.05E-10	transferase family protein, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
21	WT_CvsWT_T	Down	LOC_Os05g51780	2.05E-10	zinc finger, C3HC4 type domain containing protein, expressed
22	WT_CvsWT_T	Down	LOC_Os02g12690	2.13E-10	cytochrome P450, putative, expressed
23	WT_CvsWT_T	Down	LOC_Os05g35010	2.13E-10	cytochrome P450, putative, expressed
24	WT_CvsWT_T	Down	LOC_Os01g72360	2.31E-10	expressed protein
25	WT_CvsWT_T	Down	LOC_Os01g48710	2.53E-10	heavy metal-associated domain containing protein, expressed
26	WT_CvsWT_T	Down	LOC_Os06g47700	3.06E-10	serine threonine-protein kinase BRI1-like 2 precursor, putative, expressed
27	WT_CvsWT_T	Down	LOC_Os06g06400	3.50E-10	NBS-LRR type disease resistance protein, putative, expressed
28	WT_CvsWT_T	Down	LOC_Os03g46470	3.58E-10	metal cation transporter, putative, expressed
29	WT_CvsWT_T	Down	LOC_Os01g11910	3.64E-10	basic helix-loop-helix, putative, expressed
30	WT_CvsWT_T	Down	LOC_Os04g45860	3.80E-10	transposon protein, putative, unclassified, expressed
31	WT_CvsWT_T	Down	LOC_Os01g40870	4.13E-10	aldehyde dehydrogenase, putative, expressed
32	WT_CvsWT_T	Down	LOC_Os03g01700	4.13E-10	expressed protein
33	WT_CvsWT_T	Down	LOC_Os03g02040	4.71E-10	remorin, putative, expressed
34	WT_CvsWT_T	Down	LOC_Os01g18744	4.87E-10	transferase family protein, putative, expressed
35	WT_CvsWT_T	Down	LOC_Os11g05480	6.44E-10	transcription factor, putative, expressed
36	WT_CvsWT_T	Down	LOC_Os05g06970	6.78E-10	peroxidase precursor, putative, expressed
37	WT_CvsWT_T	Down	LOC_Os10g01640	7.32E-10	decarboxylase, putative, expressed
38	WT_CvsWT_T	Down	LOC_Os03g08470	7.39E-10	AP2 domain containing protein, expressed
39	WT_CvsWT_T	Down	LOC_Os01g24560	7.66E-10	fruit bromelain precursor, putative, expressed
40	WT_CvsWT_T	Down	LOC_Os05g19910	7.68E-10	transferase family protein, putative, expressed
41	WT_CvsWT_T	Down	LOC_Os10g17650	7.88E-10	Os10bglu34 - beta-glucosidase homologue, similar to Os3bglu6, expressed
42	WT_CvsWT_T	Down	LOC_Os04g59020	8.13E-10	integral membrane protein, putative, expressed
43	WT_CvsWT_T	Down	LOC_Os06g22290	8.15E-10	legume lectins beta domain containing protein, expressed
44	WT_CvsWT_T	Down	LOC_Os08g30770	8.58E-10	ABC transporter, ATP-binding protein, putative, expressed
45	WT_CvsWT_T	Down	LOC_Os11g42950	9.56E-10	expressed protein
46	WT_CvsWT_T	Down	LOC_Os02g43410	1.02E-09	transposon protein, putative, unclassified, expressed
47	WT_CvsWT_T	Down	LOC_Os02g03900	1.08E-09	metal transporter Nramp6, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
48	WT_CvsWT_T	Down	LOC_Os09g38800	1.10E-09	OsWAK88 - OsWAK pseudogene, expressed
49	WT_CvsWT_T	Down	LOC_Os02g54180	1.15E-09	expressed protein
50	WT_CvsWT_T	Down	LOC_Os02g02640	1.15E-09	NBS-LRR disease resistance protein, putative, expressed
51	WT_CvsWT_T	Down	LOC_Os02g32009	1.15E-09	expressed protein
52	WT_CvsWT_T	Down	LOC_Os01g68730	1.17E-09	RNA-binding protein FUS, putative, expressed
53	WT_CvsWT_T	Down	LOC_Os03g42259	1.25E-09	hypervariable Bacillus group-specific protein, putative, expressed
54	WT_CvsWT_T	Down	LOC_Os04g46750	1.25E-09	glycosyl transferase 8 domain containing protein, putative, expressed
55	WT_CvsWT_T	Down	LOC_Os11g45120	1.34E-09	conserved hypothetical protein
56	WT_CvsWT_T	Down	LOC_Os03g25330	1.35E-09	peroxidase precursor, putative, expressed
57	WT_CvsWT_T	Down	LOC_Os02g43370	1.46E-09	transposon protein, putative, unclassified, expressed
58	WT_CvsWT_T	Down	LOC_Os04g52640	1.57E-09	SHR5-receptor-like kinase, putative, expressed
59	WT_CvsWT_T	Down	LOC_Os05g49840	1.64E-09	phospholipase, putative, expressed
60	WT_CvsWT_T	Down	LOC_Os07g33610	1.76E-09	cytochrome P450, putative, expressed
61	WT_CvsWT_T	Down	LOC_Os03g09970	1.89E-09	sulfate transporter, putative, expressed
62	WT_CvsWT_T	Down	LOC_Os07g36210	1.92E-09	expressed protein
63	WT_CvsWT_T	Down	LOC_Os02g01890	2.03E-09	cytochrome P450, putative, expressed
64	WT_CvsWT_T	Down	LOC_Os01g59570	2.16E-09	senescence-induced receptor-like serine threonine-protein kinase precursor, putative, expressed
65	WT_CvsWT_T	Down	LOC_Os01g13560	2.27E-09	membrane associated DUF588 domain containing protein, putative, expressed
66	WT_CvsWT_T	Down	LOC_Os03g13390	2.29E-09	oxidoreductase, aldo keto reductase family protein, putative, expressed
67	WT_CvsWT_T	Down	LOC_Os05g41990	2.34E-09	peroxidase precursor, putative, expressed
68	WT_CvsWT_T	Down	LOC_Os10g38090	2.35E-09	cytochrome P450, putative, expressed
69	WT_CvsWT_T	Down	LOC_Os11g32160	2.70E-09	expressed protein
70	WT_CvsWT_T	Down	LOC_Os02g18410	2.78E-09	salt stress root protein RS1, putative, expressed
71	WT_CvsWT_T	Down	LOC_Os03g25320	2.94E-09	peroxidase precursor, putative, expressed
72	WT_CvsWT_T	Down	LOC_Os10g11500	2.94E-09	SCP-like extracellular protein, expressed
73	WT_CvsWT_T	Down	LOC_Os03g25030	3.35E-09	GDSL-like lipase acylhydrolase, putative, expressed
74	WT_CvsWT_T	Down	LOC_Os01g33810	3.48E-09	disease resistance protein RPM1, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
75	WT_CvsWT_T	Down	LOC_Os07g15370	3.62E-09	metal transporter Nramp6, putative, expressed
76	WT_CvsWT_T	Down	LOC_Os01g14520	3.67E-09	C4-dicarboxylate transporter malic acid transport protein domain containing protein, expressed
77	WT_CvsWT_T	Down	LOC_Os04g10750	3.67E-09	inorganic phosphate transporter, putative, expressed
78	WT_CvsWT_T	Down	LOC_Os04g37980	3.77E-09	transporter family protein, putative, expressed
79	WT_CvsWT_T	Down	LOC_Os01g24550	3.93E-09	fruit bromelain precursor, putative, expressed
80	WT_CvsWT_T	Down	LOC_Os12g10220	4.29E-09	expressed protein
81	WT_CvsWT_T	Down	LOC_Os06g04540	4.29E-09	DNA binding protein, putative, expressed
82	WT_CvsWT_T	Down	LOC_Os04g57410	4.39E-09	methylthioribose kinase, putative, expressed
83	WT_CvsWT_T	Down	LOC_Os02g45890	4.53E-09	sulfotransferase domain containing protein, expressed
84	WT_CvsWT_T	Down	LOC_Os06g06000	4.84E-09	expressed protein
85	WT_CvsWT_T	Down	LOC_Os08g08980	4.84E-09	cupin domain containing protein, expressed
86	WT_CvsWT_T	Down	LOC_Os11g04020	5.43E-09	major facilitator superfamily antiporter, putative, expressed
87	WT_CvsWT_T	Down	LOC_Os06g05990	5.43E-09	zinc finger family protein, putative, expressed
88	WT_CvsWT_T	Down	LOC_Os11g46860	5.64E-09	wall-associated receptor kinase-like 4 precursor, putative, expressed
89	WT_CvsWT_T	Down	LOC_Os08g07010	5.72E-09	ABC-2 type transporter domain containing protein, expressed
90	WT_CvsWT_T	Down	LOC_Os07g12900	5.72E-09	cadmium zinc-transporting ATPase, putative, expressed
91	WT_CvsWT_T	Down	LOC_Os04g31290	5.86E-09	helix-loop-helix DNA-binding domain containing protein, expressed
92	WT_CvsWT_T	Down	LOC_Os11g08370	5.86E-09	transporter, major facilitator family, putative, expressed
93	WT_CvsWT_T	Down	LOC_Os11g38040	6.00E-09	expressed protein
94	WT_CvsWT_T	Down	LOC_Os07g35480	6.00E-09	glucan endo-1,3-beta-glucosidase precursor, putative, expressed
95	WT_CvsWT_T	Down	LOC_Os11g01830	6.34E-09	staphylococcal nuclease homologue, putative, expressed
96	WT_CvsWT_T	Down	LOC_Os06g35650	6.55E-09	reticuline oxidase-like protein precursor, putative, expressed
97	WT_CvsWT_T	Down	LOC_Os04g03579	6.81E-09	protein kinase, putative, expressed
98	WT_CvsWT_T	Down	LOC_Os09g11460	6.87E-09	AP2 domain containing protein, expressed
99	WT_CvsWT_T	Down	LOC_Os02g02780	7.07E-09	protein kinase family protein, putative, expressed
100	WT_CvsWT_T	Down	LOC_Os09g39070	7.19E-09	thiol protease SEN102 precursor, putative, expressed
1	WT_CvsWT_T	Up	LOC_Os09g15670	6.38E-13	protein phosphatase 2C, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
2	WT_CvsWT_T	Up	LOC_Os02g43330	8.16E-13	homeobox associated leucine zipper, putative, expressed
3	WT_CvsWT_T	Up	LOC_Os03g20680	2.85E-12	late embryogenesis abundant protein 1, putative, expressed
4	WT_CvsWT_T	Up	LOC_Os09g38320	3.58E-12	phytoene synthase, chloroplast precursor, putative, expressed
5	WT_CvsWT_T	Up	LOC_Os03g44380	4.15E-12	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed
6	WT_CvsWT_T	Up	LOC_Os06g03930	4.15E-12	cytochrome P450 86A1, putative, expressed
7	WT_CvsWT_T	Up	LOC_Os03g03370	5.53E-12	fatty acid hydroxylase, putative, expressed
8	WT_CvsWT_T	Up	LOC_Os01g62760	6.81E-12	protein phosphatase 2C, putative, expressed
9	WT_CvsWT_T	Up	LOC_Os02g30910	7.65E-12	nodulin MtN3 family protein, putative, expressed
10	WT_CvsWT_T	Up	LOC_Os04g45810	8.30E-12	homeobox associated leucine zipper, putative, expressed
11	WT_CvsWT_T	Up	LOC_Os07g05940	9.41E-12	9-cis-epoxycarotenoid dioxygenase 1, chloroplast precursor, putative, expressed
12	WT_CvsWT_T	Up	LOC_Os05g40010	1.10E-11	LTPL17 - Protease inhibitor seed storage LTP family protein precursor, expressed
13	WT_CvsWT_T	Up	LOC_Os05g38290	1.17E-11	protein phosphatase 2C, putative, expressed
14	WT_CvsWT_T	Up	LOC_Os06g44190	1.17E-11	expressed protein
15	WT_CvsWT_T	Up	LOC_Os03g60580	1.42E-11	actin-depolymerizing factor, putative, expressed
16	WT_CvsWT_T	Up	LOC_Os09g21120	1.75E-11	armadillo beta-catenin repeat family protein, putative, expressed
17	WT_CvsWT_T	Up	LOC_Os06g46920	2.30E-11	dihydroflavonol-4-reductase, putative, expressed
18	WT_CvsWT_T	Up	LOC_Os06g46740	2.57E-11	early nodulin 20 precursor, putative, expressed
19	WT_CvsWT_T	Up	LOC_Os01g40094	3.18E-11	protein phosphatase 2C, putative, expressed
20	WT_CvsWT_T	Up	LOC_Os02g10310	3.18E-11	fumarylacetoacetase, putative, expressed
21	WT_CvsWT_T	Up	LOC_Os01g50400	3.57E-11	STE_MEKK_ste11_MAP3K.5 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed
22	WT_CvsWT_T	Up	LOC_Os03g18490	4.01E-11	RPGR, putative, expressed
23	WT_CvsWT_T	Up	LOC_Os08g42590	4.02E-11	mtN19, putative, expressed
24	WT_CvsWT_T	Up	LOC_Os04g17100	5.48E-11	heavy metal-associated domain containing protein, expressed
25	WT_CvsWT_T	Up	LOC_Os03g45280	6.13E-11	dehydrin, putative, expressed
26	WT_CvsWT_T	Up	LOC_Os02g44870	6.75E-11	dehydrin, putative, expressed
27	WT_CvsWT_T	Up	LOC_Os01g50910	7.23E-11	late embryogenesis abundant protein, group 3, putative, expressed
28	WT_CvsWT_T	Up	LOC_Os01g37832	7.86E-11	thioredoxin, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
29	WT_CvsWT_T	Up	LOC_Os02g51040	8.21E-11	expansin precursor, putative, expressed
30	WT_CvsWT_T	Up	LOC_Os01g32780	8.68E-11	universal stress protein domain containing protein, putative, expressed
31	WT_CvsWT_T	Up	LOC_Os01g47490	8.68E-11	Lg106, putative, expressed
32	WT_CvsWT_T	Up	LOC_Os08g01910	9.19E-11	expressed protein
33	WT_CvsWT_T	Up	LOC_Os01g63930	9.33E-11	cytochrome P450, putative, expressed
34	WT_CvsWT_T	Up	LOC_Os03g24040	9.88E-11	seven in absentia protein family domain containing protein, expressed
35	WT_CvsWT_T	Up	LOC_Os04g01590	9.95E-11	arginase, putative, expressed
36	WT_CvsWT_T	Up	LOC_Os03g60570	1.01E-10	ZOS3-22 - C2H2 zinc finger protein, expressed
37	WT_CvsWT_T	Up	LOC_Os10g09240	1.01E-10	expressed protein
38	WT_CvsWT_T	Up	LOC_Os07g48830	1.06E-10	glycosyl transferase 8 domain containing protein, putative, expressed
39	WT_CvsWT_T	Up	LOC_Os10g39660	1.07E-10	expressed protein
40	WT_CvsWT_T	Up	LOC_Os02g52780	1.08E-10	bZIP transcription factor, putative, expressed
41	WT_CvsWT_T	Up	LOC_Os05g46040	1.11E-10	protein phosphatase 2C, putative, expressed
42	WT_CvsWT_T	Up	LOC_Os03g51350	1.14E-10	expressed protein
43	WT_CvsWT_T	Up	LOC_Os03g09170	1.14E-10	ethylene-responsive transcription factor, putative, expressed
44	WT_CvsWT_T	Up	LOC_Os05g46480	1.18E-10	late embryogenesis abundant protein, group 3, putative, expressed
45	WT_CvsWT_T	Up	LOC_Os03g14370	1.20E-10	ACT domain containing protein, expressed
46	WT_CvsWT_T	Up	LOC_Os11g26560	1.32E-10	expressed protein
47	WT_CvsWT_T	Up	LOC_Os05g41490	1.36E-10	circadian clock coupling factor ZGT, putative, expressed
48	WT_CvsWT_T	Up	LOC_Os03g16170	1.63E-10	protein phosphatase 2C, putative, expressed
49	WT_CvsWT_T	Up	LOC_Os02g44090	1.63E-10	zinc finger protein, putative, expressed
50	WT_CvsWT_T	Up	LOC_Os03g51920	1.72E-10	peptidase, M50 family, putative, expressed
51	WT_CvsWT_T	Up	LOC_Os03g60370	1.72E-10	histidine acid phosphatase, putative, expressed
52	WT_CvsWT_T	Up	LOC_Os03g33580	1.76E-10	mitotic checkpoint protein, putative, expressed
53	WT_CvsWT_T	Up	LOC_Os07g23570	1.91E-10	cytochrome P450 72A1, putative, expressed
54	WT_CvsWT_T	Up	LOC_Os01g50410	2.05E-10	STE_MEKK_ste11_MAP3K.6 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed
55	WT_CvsWT_T	Up	LOC_Os06g35960	2.10E-10	HSF-type DNA-binding domain containing protein, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
56	WT_CvsWT_T	Up	LOC_Os01g09640	2.10E-10	Myb transcription factor, putative, expressed
57	WT_CvsWT_T	Up	LOC_Os06g45184	2.13E-10	retrotransposon protein, putative, Ty3-gypsy subclass, expressed
58	WT_CvsWT_T	Up	LOC_Os10g36180	2.27E-10	expressed protein
59	WT_CvsWT_T	Up	LOC_Os09g28210	2.29E-10	bHelix-loop-helix transcription factor, putative, expressed
60	WT_CvsWT_T	Up	LOC_Os11g07910	2.39E-10	transmembrane 9 superfamily member, putative, expressed
61	WT_CvsWT_T	Up	LOC_Os05g51670	2.49E-10	NAD dependent epimerase dehydratase family protein, putative, expressed
62	WT_CvsWT_T	Up	LOC_Os12g07030	2.53E-10	expressed protein
63	WT_CvsWT_T	Up	LOC_Os04g35540	3.06E-10	amino acid permease family protein, putative, expressed
64	WT_CvsWT_T	Up	LOC_Os01g67540	3.50E-10	AMP-binding domain containing protein, expressed
65	WT_CvsWT_T	Up	LOC_Os05g46350	3.64E-10	IQ calmodulin-binding motif domain containing protein, expressed
66	WT_CvsWT_T	Up	LOC_Os01g64360	3.90E-10	MYB family transcription factor, putative, expressed
67	WT_CvsWT_T	Up	LOC_Os10g25400	3.90E-10	GDSL-like lipase acylhydrolase, putative, expressed
68	WT_CvsWT_T	Up	LOC_Os08g10500	4.11E-10	expressed protein
69	WT_CvsWT_T	Up	LOC_Os01g04590	4.36E-10	expressed protein
70	WT_CvsWT_T	Up	LOC_Os02g43540	4.36E-10	retrotransposon protein, putative, unclassified, expressed
71	WT_CvsWT_T	Up	LOC_Os08g33710	4.45E-10	ribonuclease T2 family domain containing protein, expressed
72	WT_CvsWT_T	Up	LOC_Os05g49730	4.50E-10	protein phosphatase 2C, putative, expressed
73	WT_CvsWT_T	Up	LOC_Os07g42280	4.57E-10	von Willebrand factor type A domain containing protein, expressed
74	WT_CvsWT_T	Up	LOC_Os03g26870	5.38E-10	WD-40 repeat family protein, putative, expressed
75	WT_CvsWT_T	Up	LOC_Os03g60560	5.66E-10	ZOS3-21 - C2H2 zinc finger protein, expressed
76	WT_CvsWT_T	Up	LOC_Os05g43460	5.66E-10	DUF567 domain containing protein, putative, expressed
77	WT_CvsWT_T	Up	LOC_Os08g32980	5.66E-10	expressed protein
78	WT_CvsWT_T	Up	LOC_Os11g26770	6.12E-10	transposon protein, putative, unclassified, expressed
79	WT_CvsWT_T	Up	LOC_Os03g42520	6.95E-10	expressed protein
80	WT_CvsWT_T	Up	LOC_Os06g10820	7.10E-10	helix-loop-helix DNA-binding domain containing protein, expressed
81	WT_CvsWT_T	Up	LOC_Os09g25090	7.10E-10	CAMK_KIN1 SNF1 Nim1_like.34 - CAMK includes calcium calmodulin depedent protein kinases, expressed
82	WT_CvsWT_T	Up	LOC_Os10g25030	7.10E-10	red chlorophyll catabolite reductase, putative, expressed

	Comparison	Up- or Down-	Gene ID	Adjusted p-value	Annotation
83	WT_CvsWT_T	Up	LOC_Os02g32580	7.57E-10	expressed protein
84	WT_CvsWT_T	Up	LOC_Os03g17790	7.68E-10	OsRCI2-5 - Putative low temperature and salt responsive protein, expressed
85	WT_CvsWT_T	Up	LOC_Os02g47840	7.68E-10	universal stress protein domain containing protein, putative, expressed
86	WT_CvsWT_T	Up	LOC_Os01g74450	7.86E-10	aquaporin protein, putative, expressed
87	WT_CvsWT_T	Up	LOC_Os02g56900	7.88E-10	thioredoxin family protein, putative, expressed
88	WT_CvsWT_T	Up	LOC_Os05g49770	7.88E-10	CTP synthase, putative, expressed
89	WT_CvsWT_T	Up	LOC_Os11g04104	7.88E-10	major facilitator superfamily antiporter, putative, expressed
90	WT_CvsWT_T	Up	LOC_Os04g34610	7.88E-10	expressed protein
91	WT_CvsWT_T	Up	LOC_Os04g51410	8.05E-10	expressed protein
92	WT_CvsWT_T	Up	LOC_Os01g11600	8.19E-10	expressed protein
93	WT_CvsWT_T	Up	LOC_Os05g26840	8.31E-10	permease domain containing protein, putative, expressed
94	WT_CvsWT_T	Up	LOC_Os02g13800	8.72E-10	HSF-type DNA-binding domain containing protein, expressed
95	WT_CvsWT_T	Up	LOC_Os03g20120	9.61E-10	glycosyl transferase 8 domain containing protein, putative, expressed
96	WT_CvsWT_T	Up	LOC_Os03g59320	1.01E-09	expressed protein
97	WT_CvsWT_T	Up	LOC_Os07g24000	1.01E-09	AWPM-19-like membrane family protein, putative, expressed
98	WT_CvsWT_T	Up	LOC_Os07g38290	1.02E-09	plastocyanin-like domain containing protein, putative, expressed
99	WT_CvsWT_T	Up	LOC_Os04g57550	1.04E-09	amine oxidase, flavin-containing, domain containing protein, expressed
100	WT_CvsWT_T	Up	LOC_Os11g05530	1.05E-09	expressed protein

Appendix 2. Pipeline 2 script for RNA-seq analysis. Read cleaning, alignment, and counting was performed on the Summit supercomputing system. Read counts, alignment statistics, and count statistics were transferred from Summit for analysis in R.

```
1 #Analysis of RNA-seq data¬
2¬
3 #log in to supercomputer SUMMIT¬
4¬
5 ssh -l sere@colostate.edu login.rc.colorado.edu¬
6¬
7 #enter CSUpassword,push #answer DUO key on phone app¬ 8¬
9 #enter into scompile, a specific compute node on SUMMIT¬

10 ¬
11 ssh scompile¬
12 ¬
13 #load David King's account space (which includes STAR, trimmomatic, and

... other nice things), I've made an alias for his working bashrc environment:

... david¬ 14 ¬
15 david¬ 16 ¬

17 #load other programs from david's bashrc¬
18 ¬
19 STAR trimmomatic #the "t" is not capitalized¬
20 ¬
21 #check working directory and navigate into desired folder¬
22 ¬
23 cd /scratch/summit/sere@colostate.edu/rice¬
24 ¬
25 #Navigate to folder where raw reads are #Transfer them into supercomputer

... folder¬
26 ¬
27 Seres-MacBook-Pro:Rawdata serewilliams$ scp S_2_1.fq.gz S_2_2.fq.gz
```

```
... sere@colostate.edu@login.rc.colorado.edu:/scratch/summit/sere@colostate.edu/ ... rice Password:
S_2_1.fq.gz
... 26% 350MB 23.9MB/s 00:40 ETAa¬

28 ¬
29 #Unzip files #& makes it run in the background, job can be checked with jobs

... command¬
30 ¬
31 gunzip *.fq.gz & jobs¬
32 ¬
33 #create trimmomatic.sbatch script so that the two paired end files from each

... sample can be run together #more on trimmomatic can be found here:
... http://www.usadellab.org/cms/?page=trimmomatic #Here are some notes from
... Bolger at the above webpage: #Remove adapters
... (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10) #Remove leading low quality or N bases ... (below quality 3)
(LEADING:3) #Remove trailing low quality or N bases (below ... quality 3) (TRAILING:3) #Scan the read with
a 4-base wide sliding window,
... cutting when the average quality per base drops below 15
... (SLIDINGWINDOW:4:15) #Drop reads below the 36 bases long (MINLEN:36)¬

34 ¬
35 nano trimmomatic.sbatch¬

36 ¬
37 #!/usr/bin/env bash #SBATCH --nodes=1 #SBATCH --ntasks=8 #SBATCH

... --time=1:00:00 #SBATCH --qos=normal #SBATCH --partition=shas
... NTHREADS=${SLURM_NTASKS} # passes --ntasks set above echo "[$0]
... $SLURM_JOB_NAME $@" # log the command line export TMPDIR=$SLURM_SCRATCH ... export TMP=$TMPDIR date #
timestamp¬

38 ¬
39 # erinnishgrp@colostate.edu projects setup
```

```
... PROJ_DIR=/projects/dcking@colostate.edu source $PROJ_DIR/paths.bashrc¬
40 ¬
41 # This large chunk of params comes from the website demo, but... # notice

... the Trimmomatic-0.36/adapters/TruSeq3-PE.fa path given to ILLUMINACLIP ...
trim="ILLUMINACLIP:/projects/dcking@colostate.edu/src/Trimmomatic-0.36/ ... adapters/TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 ... MINLEN:36"¬

42 ¬
43 infile=$1 #like 01_input/SRR1234567_1.fastq¬
44 ¬
45 in1=$infile # make the mate pair filename by replacing _1 with _2

... in2=${infile/_1.fq/_2.fq}¬
46 ¬
47 # strip the input directory with 'basename' basename1=$(basename $in1)

... basename2=$(basename $in2) # make all the file output names
... out1paired=${basename1/.fq/.trimmed.fq} # makes:
... SRR1234567_1.trimmed.fastq out1unpaired=${basename1/.fq/.unpaired.fq} #
... makes: SRR1234567_1.unpaired.fastq out2paired=${basename2/.fq/.trimmed.fq} ... # makes:
SRR1234567_2.trimmed.fastq
... out2unpaired=${basename2/.fq/.unpaired.fq} # makes:
... SRR1234567_2.unpaired.fastq¬

48 ¬
49 trimmomatic PE -threads $SLURM_NTASKS -phred33

... $out1unpaired $out2paired $out2unpaired $trim¬ 50 ¬
51 ##end of script¬
52 ¬

53 #print the final script before running the job ... in the terminal output¬

$in1 $in2 $out1paired
so that the script is printed
```

```
54 ¬
55 cat trimmomatic.sbatch¬
56 ¬
57 #Call this script specifying the first file (and the file path to that file)¬ 58 ¬
59 sbatch trimmomatic.sbatch ../inputdir/S_1_1.fq sa ls¬
60 ¬
61 #sa checks to see if the job is failed, pending, running, or completed #ls

... into the directory to make sure all files are present # move files so as to

... organize the trimmed files¬
62 ¬
63 mv *.trimmed.fq ../trimmed/S_1¬ 64 ¬

65 #repeat for all samples (S_1_1.fq through S_29_1.fq) #tidy remaining files¬ 66 ¬
67 mv *.unpaired.fq ../trimmed/unpaired mv slurm* oldslurm¬
68 ¬

69 #Must find and download desired reference genome and annotations #Novogene ... used a genome from
SolGenomics, IRGSPb5.fa.masked with annotations from
... RAP_genes.gff3 (from "BI Davis..." file:
... /Users/serewilliams/Documents/Sere/Grad_School/Reddy_Lab/

... Effect_of_PEG_Treatment/Effect of PEG 7/Novogene) #I will use: #The Rice
... genome annotation project (http://rice.plantbiology.msu.edu/index.shtml)
... Oryza Sativa pseudomolecule version 7.0, found here:
... #http://rice.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/
... annotation_dbs/pseudomolecules/version_7.0/ #Download and move files into a ... genome directory
(genomedirRGAP7) and an annotation directory (RAP) #Must

... create an index using the reference genome for STAR: STAR_indexRGAP7.sbatch¬ 70 ¬
71 nano STAR_indexRGAP7.sbatch¬
72 ¬

73 #!/usr/bin/env bash #SBATCH --nodes=1 #SBATCH --ntasks=8 #SBATCH
... --time=1:00:00 #SBATCH --qos=normal #SBATCH --partition=shas
... NTHREADS=${SLURM_NTASKS} # passes --ntasks set above echo "[$0]
```

```
... $SLURM_JOB_NAME $@" # log the command line export TMPDIR=$SLURM_SCRATCH ... export TMP=$TMPDIR date #
timestamp¬

74 ¬
75 # erinnishgrp@colostate.edu projects setup

... PROJ_DIR=/projects/dcking@colostate.edu source $PROJ_DIR/paths.bashrc¬ 76 ¬
77 STAR --runThreadN $NTHREADS --runMode genomeGenerate --genomeDir

... ../genomedirRGAP7 --genomeFastaFiles ../RAP/RGAP7/RGAP7_genome.con

... --sjdbGTFtagExonParentTranscript ../RAP/RGAP7/RGAP7_annotations.gff3¬ 78 ¬
79 ##end of script¬
80 ¬

81 cat STAR_indexRGAP7.sbatch¬
82 ¬
83 sbatch STAR_indexRGAP7.sbatch¬
84 ¬
85 #Align trimmed reads to RGAP7 reference genome using STAR with an maximum

... intron limit of 10000bp¬
86 ¬
87 nano STAR_alignIntron.sbatch¬
88 ¬
89 #!/usr/bin/env bash #SBATCH --nodes=1 #SBATCH --ntasks=8 #SBATCH

... --time=1:00:00 #SBATCH --qos=normal #SBATCH --partition=shas
... NTHREADS=${SLURM_NTASKS} # passes --ntasks set above echo "[$0]
... $SLURM_JOB_NAME $@" # log the command line export TMPDIR=$SLURM_SCRATCH ... export TMP=$TMPDIR date #
timestamp¬

90 ¬
91 # erinnishgrp@colostate.edu projects setup

... PROJ_DIR=/projects/dcking@colostate.edu source $PROJ_DIR/paths.bashrc¬
```

```
92 ¬
93 STAR --runThreadN $NTHREADS --runMode alignReads --genomeDir

... ../genomedirRGAP7 --readFilesIn ../trimmed/S_1_1.trimmed.fq
... ../trimmed/S_1_2.trimmed.fq --alignIntronMax 10000 --outSAMtype BAM
... SortedByCoordinate --outFileNamePrefix S_1

STAR --runThreadN $NTHREADS
... --runMode alignReads --genomeDir ../genomedirRGAP7 --readFilesIn
... ../trimmed/S_2_1.trimmed.fq ../trimmed/S_2_2.trimmed.fq --alignIntronMax
... 10000 --outSAMtype BAM SortedByCoordinate --outFileNamePrefix S_2

STAR
... --runThreadN $NTHREADS --runMode alignReads --genomeDir ../genomedirRGAP7 ... --
readFilesIn ../trimmed/S_4_1.trimmed.fq ../trimmed/S_4_2.trimmed.fq
... --alignIntronMax 10000 --outSAMtype BAM SortedByCoordinate
... --outFileNamePrefix S_4

STAR --runThreadN $NTHREADS --runMode alignReads ... --genomeDir ../genomedirRGAP7 --
readFilesIn ../trimmed/S_5_1.trimmed.fq
... ../trimmed/S_5_2.trimmed.fq --alignIntronMax 10000 --outSAMtype BAM
... SortedByCoordinate --outFileNamePrefix S_5

STAR --runThreadN $NTHREADS
... --runMode alignReads --genomeDir ../genomedirRGAP7 --readFilesIn
... ../trimmed/S_6_1.trimmed.fq ../trimmed/S_6_2.trimmed.fq --alignIntronMax
... 10000 --outSAMtype BAM SortedByCoordinate --outFileNamePrefix S_6

STAR
... --runThreadN $NTHREADS --runMode alignReads --genomeDir ../genomedirRGAP7 ... --
readFilesIn ../trimmed/S_7_1.trimmed.fq ../trimmed/S_7_2.trimmed.fq
... --alignIntronMax 10000 --outSAMtype BAM SortedByCoordinate
... --outFileNamePrefix S_7

STAR --runThreadN $NTHREADS --runMode alignReads ... --genomeDir ../genomedirRGAP7 --
readFilesIn ../trimmed/S_8_1.trimmed.fq
... ../trimmed/S_8_2.trimmed.fq --alignIntronMax 10000 --outSAMtype BAM
... SortedByCoordinate --outFileNamePrefix S_8
```

```
STAR --runThreadN $NTHREADS
... --runMode alignReads --genomeDir ../genomedirRGAP7 --readFilesIn
... ../trimmed/S_9_1.trimmed.fq ../trimmed/S_9_2.trimmed.fq --alignIntronMax
... 10000 --outSAMtype BAM SortedByCoordinate --outFileNamePrefix S_9

STAR
... --runThreadN $NTHREADS --runMode alignReads --genomeDir ../genomedirRGAP7 ... --
readFilesIn ../trimmed/S_10_1.trimmed.fq ../trimmed/S_10_2.trimmed.fq
... --alignIntronMax 10000 --outSAMtype BAM SortedByCoordinate
... --outFileNamePrefix S_10

STAR --runThreadN $NTHREADS --runMode alignReads ... --genomeDir ../genomedirRGAP7 --
readFilesIn ../trimmed/S_11_1.trimmed.fq
... ../trimmed/S_11_2.trimmed.fq --alignIntronMax 10000 --outSAMtype BAM
... SortedByCoordinate --outFileNamePrefix S_11

STAR --runThreadN $NTHREADS
... --runMode alignReads --genomeDir ../genomedirRGAP7 --readFilesIn
... ../trimmed/S_12_1.trimmed.fq ../trimmed/S_12_2.trimmed.fq --alignIntronMax ... 10000 --outSAMtype BAM
SortedByCoordinate --outFileNamePrefix S_12

STAR
... --runThreadN $NTHREADS --runMode alignReads --genomeDir ../genomedirRGAP7 ... --
readFilesIn ../trimmed/S_13_1.trimmed.fq ../trimmed/S_13_2.trimmed.fq
... --alignIntronMax 10000 --outSAMtype BAM SortedByCoordinate
... --outFileNamePrefix S_13

STAR --runThreadN $NTHREADS --runMode alignReads ... --genomeDir ../genomedirRGAP7 --
readFilesIn ../trimmed/S_14_1.trimmed.fq
... ../trimmed/S_14_2.trimmed.fq --alignIntronMax 10000 --outSAMtype BAM
... SortedByCoordinate --outFileNamePrefix S_14

STAR --runThreadN $NTHREADS
... --runMode alignReads --genomeDir ../genomedirRGAP7 --readFilesIn
... ../trimmed/S_15_1.trimmed.fq ../trimmed/S_15_2.trimmed.fq --alignIntronMax ... 10000 --outSAMtype BAM
SortedByCoordinate --outFileNamePrefix S_15
```

```
STAR
... --runThreadN $NTHREADS --runMode alignReads --genomeDir ../genomedirRGAP7 ... --
readFilesIn ../trimmed/S_16_1.trimmed.fq ../trimmed/S_16_2.trimmed.fq

93... --alignIntronMax 10000 --outSAMtype BAM SortedByCoordinate
... --outFileNamePrefix S_16

STAR --runThreadN $NTHREADS --runMode alignReads ... --genomeDir ../genomedirRGAP7 --
readFilesIn ../trimmed/S_17_1.trimmed.fq
... ../trimmed/S_17_2.trimmed.fq --alignIntronMax 10000 --outSAMtype BAM
... SortedByCoordinate --outFileNamePrefix S_17 STAR --runThreadN $NTHREADS
... --runMode alignReads --genomeDir ../genomedi

rRGAP7 --readFilesIn
... ../trimmed/S_18_1.trimmed.fq ../trimmed/S_18_2.trimmed.fq --alignIntronMax ... 10000 --outSAMtype BAM
SortedByCoordinate --outFileNamePrefix S_18

STAR
... --runThreadN $NTHREADS --runMode alignReads --genomeDir ../genomedirRGAP7 ... --
readFilesIn ../trimmed/S_19_1.trimmed.fq ../trimmed/S_19_2.trimmed.fq
... --alignIntronMax 10000 --outSAMtype BAM SortedByCoordinate
... --outFileNamePrefix S_19

STAR --runThreadN $NTHREADS --runMode alignReads ... --genomeDir ../genomedirRGAP7 --
readFilesIn ../trimmed/S_20_1.trimmed.fq
... ../trimmed/S_20_2.trimmed.fq --alignIntronMax 10000 --outSAMtype BAM
... SortedByCoordinate --outFileNamePrefix S_20

STAR --runThreadN $NTHREADS
... --runMode alignReads --genomeDir ../genomedirRGAP7 --readFilesIn
... ../trimmed/S_21_1.trimmed.fq ../trimmed/S_21_2.trimmed.fq --alignIntronMax ... 10000 --outSAMtype BAM
SortedByCoordinate --outFileNamePrefix S_21

STAR
... --runThreadN $NTHREADS --runMode alignReads --genomeDir ../genomedirRGAP7 ... --
readFilesIn ../trimmed/S_22_1.trimmed.fq ../trimmed/S_22_2.trimmed.fq
... --alignIntronMax 10000 --outSAMtype BAM SortedByCoordinate
... --outFileNamePrefix S_22
```

```
STAR --runThreadN $NTHREADS --runMode alignReads ... --genomeDir ../genomedirRGAP7 --
readFilesIn ../trimmed/S_23_1.trimmed.fq
... ../trimmed/S_23_2.trimmed.fq --alignIntronMax 10000 --outSAMtype BAM
... SortedByCoordinate --outFileNamePrefix S_23

STAR --runThreadN $NTHREADS
... --runMode alignReads --genomeDir ../genomedirRGAP7 --readFilesIn
... ../trimmed/S_24_1.trimmed.fq ../trimmed/S_24_2.trimmed.fq --alignIntronMax ... 10000 --outSAMtype BAM
SortedByCoordinate --outFileNamePrefix S_24

STAR
... --runThreadN $NTHREADS --runMode alignReads --genomeDir ../genomedirRGAP7 ... --
readFilesIn ../trimmed/S_29_1.trimmed.fq ../trimmed/S_29_2.trimmed.fq
... --alignIntronMax 10000 --outSAMtype BAM SortedByCoordinate
... --outFileNamePrefix S_29¬

94 ¬
95 cat STAR_alignIntron.sbatch sbatch STAR_alignIntron.sbatch sa¬
96 ¬
97 #move files mv slurm* oldslurm mv *.bam ../alignoutdirRGAP7/BAM mv

... *final.out ../alignoutdirRGAP7/logs mv *.out ../alignoutdirRGAP7/other mv

... *.tab ../alignoutdirRGAP7/other¬
98 ¬
99 #decide how to count aligned reads (HTSeq, featureCounts, or Verse) ->

... choosing HTSeq because, while it's slow, it's widely accepted and accepts
... GFF3 annotation format files while the others only accept GTF format (I
... think) #choose options for HTSeq: -f bam, -s no, -i ID, -t gene (format,
... stranded, identifier, type) #identifier is in column 9 of GFF3 file #type is ... in column 3 of GFF3
file, along with exon, etc. Exon is not right and will

... return counts per exon, not counts per gene #HTSeq is not fast, one sample ... will take 1hr 40min ->
extending time to 12hrs for sbatch and doing four
... samples per job #using HTSeq v0.11.2 (I think), this is the latest version ... available and was
updated in February 2019¬
```

```
100 ¬

101 mkdir counts nano HTSeq.sbatch¬
102 ¬
103 GNU nano 2.3.1 File:

... HTSeq.sbatch¬ 104 ¬

105 #!/usr/bin/env bash #SBATCH --nodes=1 #SBATCH --ntasks=12 #SBATCH
... --time=12:00:00 #SBATCH --qos=normal #SBATCH --partition=shas
... NTHREADS=${SLURM_NTASKS} # passes --ntasks set above echo "[$0]
... $SLURM_JOB_NAME $@" # log the command line export TMPDIR=$SLURM_SCRATCH ... export TMP=$TMPDIR date #
timestamp¬

106 ¬
107 # erinnishgrp@colostate.edu projects setup

... PROJ_DIR=/projects/dcking@colostate.edu source $PROJ_DIR/paths.bashrc¬ 108 ¬

109 htseq-count -f bam -s no -i ID -t gene
... ../alignoutdirRGAP7/BAM/S_2Aligned.sortedByCoord.out.bam
... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./counts/S_2.counts htseq-count -f bam ... -s no -i ID -t
gene ../alignoutdirRGAP7/BAM/S_4Aligned.sortedByCoord.out.bam ... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./c
ounts/S_4.counts htseq-count -f bam ... -s no -i ID -t
gene ../alignoutdirRGAP7/BAM/S_5Aligned.sortedByCoord.out.bam ... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./c
ounts/S_5.counts htseq-count -f bam ... -s no -i ID -t
gene ../alignoutdirRGAP7/BAM/S_6Aligned.sortedByCoord.out.bam ... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./c
ounts/S_6.counts¬

110 ¬
111 cat HTSeq.sbatch sbatch HTSeq.sbatch sa¬
112 ¬
113 #make new HTseq1.sbatch for next four samples (nano into original

... HTSeq.sbatch and cntrl-O to write out and save as HTSeq1.sbatch)¬ 114 ¬
```

```
115 nano HTSeq1.sbatch¬
116 ¬
117 #!/usr/bin/env bash #SBATCH --nodes=1 #SBATCH --ntasks=12 #SBATCH

... --time=12:00:00 #SBATCH --qos=normal #SBATCH --partition=shas
... NTHREADS=${SLURM_NTASKS} # passes --ntasks set above echo "[$0]
... $SLURM_JOB_NAME $@" # log the command line export TMPDIR=$SLURM_SCRATCH ... export TMP=$TMPDIR date #
timestamp¬

118 ¬
119 # erinnishgrp@colostate.edu projects setup

... PROJ_DIR=/projects/dcking@colostate.edu source $PROJ_DIR/paths.bashrc¬ 120 ¬

121 htseq-count -f bam -s no -i ID -t gene
... ../alignoutdirRGAP7/BAM/S_7Aligned.sortedByCoord.out.bam
... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./counts/S_7.counts htseq-count -f bam ... -s no -i ID -t
gene ../alignoutdirRGAP7/BAM/S_8Aligned.sortedByCoord.out.bam ... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./c
ounts/S_8.counts htseq-count -f bam ... -s no -i ID -t
gene ../alignoutdirRGAP7/BAM/S_9Aligned.sortedByCoord.out.bam ... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./c
ounts/S_9.counts htseq-count -f bam ... -sno-iID-tgene
... ../alignoutdirRGAP7/BAM/S_10Aligned.sortedByCoord.out.bam
... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./counts/S_10.counts¬

122 ¬
123 cat HTSeq1.sbatch sbatch HTSeq1.sbatch sa¬ 124 ¬
125 #repeat for remaining samples¬
126 ¬
127 htseq-count -f bam -s no -i ID -t gene

... ../alignoutdirRGAP7/BAM/S_11Aligned.sortedByCoord.out.bam
... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./counts/S_11.counts htseq-count -f ... bam-sno-iID-tgene
... ../alignoutdirRGAP7/BAM/S_12Aligned.sortedByCoord.out.bam
... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./counts/S_12.counts htseq-count -f ... bam-sno-iID-tgene
... ../alignoutdirRGAP7/BAM/S_13Aligned.sortedByCoord.out.bam
... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./counts/S_13.counts htseq-count -f ... bam-sno-iID-tgene
... ../alignoutdirRGAP7/BAM/S_14Aligned.sortedByCoord.out.bam
... ../RAP/RGAP7/RGAP7_annotations.gff3 > ./counts/S_14.counts¬
```

```
128 ¬
129 #repeat for all remaining samples (not shown here)¬
130 ¬
131 #transfer bam, log, and count files from supercomputer to my computer (and

... backup with external hard drive) #in new terminal window on my computer:¬ 132 ¬

133 scp
... sere@colostate.edu@login.rc.colorado.edu:/scratch/summit/sere@colostate.edu/ ...
rice/alignoutdirRGAP7/logs/*.out
... /Users/serewilliams/Documents/Sere/Grad_School/Reddy_Lab/RNA-seq_Analysis_2/ ... My_Analysis/STAR¬

134 ¬
135 #enter password,push when prompted #repeat for bam files and count files¬ 136 ¬
137 scp

... sere@colostate.edu@login.rc.colorado.edu:/scratch/summit/sere@colostate.edu/ ...
rice/alignoutdirRGAP7/BAM/*.bam
... /Users/serewilliams/Documents/Sere/Grad_School/Reddy_Lab/RNA-seq_Analysis_2/ ... My_Analysis/STAR/BAM
scp

... sere@colostate.edu@login.rc.colorado.edu:/scratch/summit/sere@colostate.edu/ ...
rice/sbatch/counts/*.counts
... /Users/serewilliams/Documents/Sere/Grad_School/Reddy_Lab/RNA-seq_Analysis_2/ ... My_Analysis/HTSeq¬

138 ¬
139 #gather quality information from STAR and HTSeq for all samples into one

... excel file¬ 140 ¬

141 ###did not transfer BAM files to my comp.¬ 142 ¬
```

Appendix 3. Script for DE analysis with edgeR.

```
sessionInfo()

## R version 3.6.0 (2019-04-26)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Mojave 10.14
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## loaded via a namespace (and not attached):
##  [1] compiler_3.6.0  magrittr_1.5    tools_3.6.0     htmltools_0.3.6
##  [5] Rcpp_1.0.1      stringi_1.4.3   rmarkdown_1.14  highr_0.8
##  [9] knitr_1.23      stringr_1.4.0   xfun_0.8        digest_0.6.19
## [13] evaluate_0.14

setwd("/Users/serewilliams/Documents/Sere/Grad_School/Reddy_Lab/RNA-seq_Analysis_2/My_Analysis/") #set working directory
library(edgeR)

## Loading required package: limma

x <- read.csv("HTSeq/Counts1.csv", quote = "", row.names="Gene") #read in counts file
head(x)

##               WT_C_1.1 WT_T_1.1 Ossr1_C_1.1 Ossr1_T_1.1 WT_C_1.2 WT_T_1.2
## LOC_Os01g01010     1310      423        1175         902      787      975
## LOC_Os01g01019      142       86          89         152       57       68
## LOC_Os01g01030      557      268         619         709      367      620
## LOC_Os01g01040     2045      960        1892        1937     1164     1625
## LOC_Os01g01050      763      356         737         713      381      706
## LOC_Os01g01060     2979     1031        2933        3279     1820     3397
##               Ossr1_C_1.2 Ossr1_T_1.2 WT_C_1.3 WT_T_1.3 Ossr1_C_1.3
## LOC_Os01g01010         919         905     1361      599         646
## LOC_Os01g01019          68         128      124       93          33
## LOC_Os01g01030         312         511      617      493         315
## LOC_Os01g01040        1159        1654     1819     1656        1174
## LOC_Os01g01050         637         725      856      543         385
## LOC_Os01g01060        1464        2960     3236     2717        1746
##               Ossr1_T_1.3 WT_C_3.1 WT_T_3.1 Ossr1_C_3.1 Ossr1_T_3.1
```

```
## LOC_Os01g01010         878     1349      932        1318         877
## LOC_Os01g01019         111      144       54         109          61
## LOC_Os01g01030         740      567      313         538         377
## LOC_Os01g01040        1918     2118     1850        1844        1681
## LOC_Os01g01050         735      839      562         895         638
## LOC_Os01g01060        4682     2990     1019        2448        1435
##                 WT_C_3.2 WT_T_3.2 Ossr1_C_3.2 Ossr1_T_3.2 WT_C_3.3 WT_T_3.3
## LOC_Os01g01010     1190      653        1199         612     1166      790
## LOC_Os01g01019       92       36          93          18       85       66
## LOC_Os01g01030      544      334         507         215      359      302
## LOC_Os01g01040     1839     1498        1806        1117     1953     1419
## LOC_Os01g01050      742      532         804         429      919      668
## LOC_Os01g01060     3398     1327        2551         629     1274      693
##                 Ossr1_C_3.3 Ossr1_T_3.3
## LOC_Os01g01010        1305         587
## LOC_Os01g01019         119          58
## LOC_Os01g01030         594         179
## LOC_Os01g01040        1772        1152
## LOC_Os01g01050         755         522
## LOC_Os01g01060        2523         407
```

```
#read in sample matrix to prepare for group comparisons
targets <- read.csv("HTSeq/targets.csv", quote = "", row.names = "Sample") #r
ead in targets file. Targets are a matrix of the sample descriptors.
head(targets)
```

```
##      Variety Treatment Timepoint BioRep
## S_1       WT         C         1      1
## S_2       WT         T         1      1
## S_29   Ossr1         C         1      1
## S_4    Ossr1         T         1      1
## S_5       WT         C         1      2
## S_6       WT         T         1      2
```

```
Group <- factor(paste(targets$Variety, targets$Treatment, targets$Timepoint,
sep = "_")) #create new column of groups, combining sample infomation (exclud
ing bioreps)
group <- cbind(targets, Group)
Group
```

```
##  [1] WT_C_1    WT_T_1    Ossr1_C_1 Ossr1_T_1 WT_C_1    WT_T_1    Ossr1_C_1
##  [8] Ossr1_T_1 WT_C_1    WT_T_1    Ossr1_C_1 Ossr1_T_1 WT_C_3    WT_T_3
## [15] Ossr1_C_3 Ossr1_T_3 WT_C_3    WT_T_3    Ossr1_C_3 Ossr1_T_3 WT_C_3
## [22] WT_T_3    Ossr1_C_3 Ossr1_T_3
## 8 Levels: Ossr1_C_1 Ossr1_C_3 Ossr1_T_1 Ossr1_T_3 WT_C_1 ... WT_T_3
```

```
group
```

```
##      Variety Treatment Timepoint BioRep     Group
## S_1       WT         C         1      1    WT_C_1
## S_2       WT         T         1      1    WT_T_1
```

```
## S_29   Ossr1         C         1      1 Ossr1_C_1
## S_4    Ossr1         T         1      1 Ossr1_T_1
## S_5       WT         C         1      2    WT_C_1
## S_6       WT         T         1      2    WT_T_1
## S_7    Ossr1         C         1      2 Ossr1_C_1
## S_8    Ossr1         T         1      2 Ossr1_T_1
## S_9       WT         C         1      3    WT_C_1
## S_10      WT         T         1      3    WT_T_1
## S_11   Ossr1         C         1      3 Ossr1_C_1
## S_12   Ossr1         T         1      3 Ossr1_T_1
## S_13      WT         C         3      1    WT_C_3
## S_14      WT         T         3      1    WT_T_3
## S_15   Ossr1         C         3      1 Ossr1_C_3
## S_16   Ossr1         T         3      1 Ossr1_T_3
## S_17      WT         C         3      2    WT_C_3
## S_18      WT         T         3      2    WT_T_3
## S_19   Ossr1         C         3      2 Ossr1_C_3
## S_20   Ossr1         T         3      2 Ossr1_T_3
## S_21      WT         C         3      3    WT_C_3
## S_22      WT         T         3      3    WT_T_3
## S_23   Ossr1         C         3      3 Ossr1_C_3
## S_24   Ossr1         T         3      3 Ossr1_T_3
```

```
#create gene column of gene annotations downloaded from RGAP version 7, gff3 file
gene <- read.table("annotation/annotation.txt", sep = ';')
head(gene)
```

```
##               V1                                                 V2
## 1 LOC_Os01g01010      TBC domain containing protein, expressed
## 2 LOC_Os01g01019                                expressed protein
## 3 LOC_Os01g01030       monocopper oxidase, putative, expressed
## 4 LOC_Os01g01040                                expressed protein
## 5 LOC_Os01g01050      R3H domain containing protein, expressed
## 6 LOC_Os01g01060 40S ribosomal protein S5, putative, expressed
```

```
dim(gene) #dim gives table coordinates (rows x columns)
```

```
## [1] 57585     2
```

```
dim(x) #gives table coordinates for counts table
```

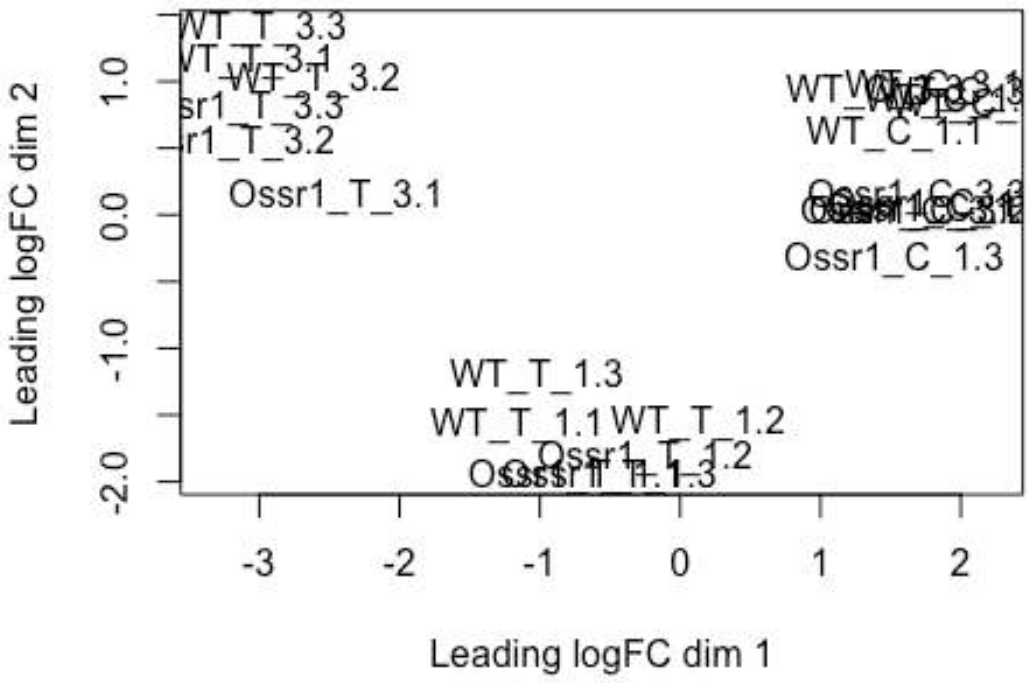
```
## [1] 52879    24
```

```
ix <- gene[[1]]%in%rownames(x) #%in% returns true/false for every entry in two objects: so as to compare genes in annotation file to genes in counts table.
 There are more (~5000) genes in annotation file than in counts table.
head(ix)
```

```
## [1] TRUE TRUE TRUE TRUE TRUE TRUE
```

```
length(ix)
```

```
## [1] 57585

gene <- gene[ix,]
dim(gene)

## [1] 52879     2

#now the edgeR analysis includes group and gene info
y <- DGEList(counts=x, group=Group, genes = gene)
y$samples

##                  group lib.size norm.factors
## WT_C_1.1       WT_C_1 58727456            1
## WT_T_1.1       WT_T_1 33287792            1
## Ossr1_C_1.1 Ossr1_C_1 49695630            1
## Ossr1_T_1.1 Ossr1_T_1 60126350            1
## WT_C_1.2       WT_C_1 32306704            1
## WT_T_1.2       WT_T_1 56335378            1
## Ossr1_C_1.2 Ossr1_C_1 34136728            1
## Ossr1_T_1.2 Ossr1_T_1 51612551            1
## WT_C_1.3       WT_C_1 55368229            1
## WT_T_1.3       WT_T_1 55544262            1
## Ossr1_C_1.3 Ossr1_C_1 32739114            1
## Ossr1_T_1.3 Ossr1_T_1 56337390            1
## WT_C_3.1       WT_C_3 62976185            1
## WT_T_3.1       WT_T_3 55039111            1
## Ossr1_C_3.1 Ossr1_C_3 55108430            1
## Ossr1_T_3.1 Ossr1_T_3 51744962            1
## WT_C_3.2       WT_C_3 49120794            1
## WT_T_3.2       WT_T_3 53322190            1
## Ossr1_C_3.2 Ossr1_C_3 50807943            1
## Ossr1_T_3.2 Ossr1_T_3 30583617            1
## WT_C_3.3       WT_C_3 52342886            1
## WT_T_3.3       WT_T_3 47140817            1
## Ossr1_C_3.3 Ossr1_C_3 48340707            1
## Ossr1_T_3.3 Ossr1_T_3 31424051            1

plotMDS(y)
```

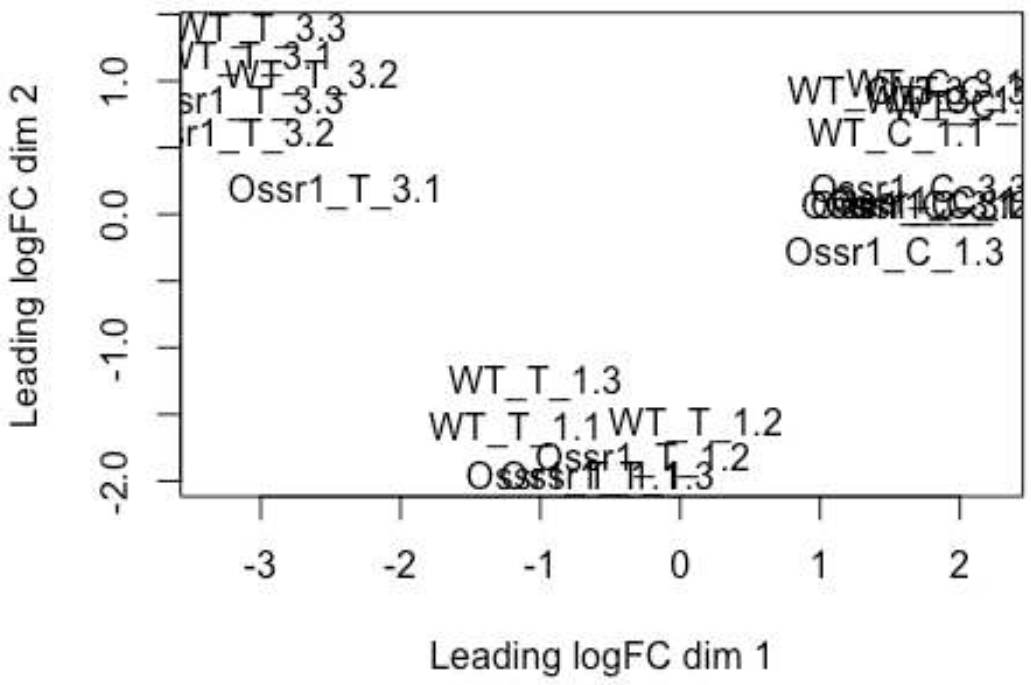



```
#create design matrix
design <- model.matrix(~0+group, data=y$samples, colnames("group$Group"))
colnames(design) <- gsub("group","",colnames(design)) #removes "group" from c
olumn name
colnames(design) <- levels(y$samples$group) #makes names of columns levels th
at can be used for contrasts later
design
```

```
##             Ossr1_C_1 Ossr1_C_3 Ossr1_T_1 Ossr1_T_3 WT_C_1 WT_C_3 WT_T_1
## WT_C_1.1            0         0         0         0      1      0      0
## WT_T_1.1            0         0         0         0      0      0      1
## Ossr1_C_1.1         1         0         0         0      0      0      0
## Ossr1_T_1.1         0         0         1         0      0      0      0
## WT_C_1.2            0         0         0         0      1      0      0
## WT_T_1.2            0         0         0         0      0      0      1
## Ossr1_C_1.2         1         0         0         0      0      0      0
## Ossr1_T_1.2         0         0         1         0      0      0      0
## WT_C_1.3            0         0         0         0      1      0      0
## WT_T_1.3            0         0         0         0      0      0      1
## Ossr1_C_1.3         1         0         0         0      0      0      0
## Ossr1_T_1.3         0         0         1         0      0      0      0
## WT_C_3.1            0         0         0         0      0      1      0
## WT_T_3.1            0         0         0         0      0      0      0
```

```
## Ossr1_C_3.1         0         1         0         0      0      0      0
## Ossr1_T_3.1         0         0         0         1      0      0      0
## WT_C_3.2            0         0         0         0      0      1      0
## WT_T_3.2            0         0         0         0      0      0      0
## Ossr1_C_3.2         0         1         0         0      0      0      0
## Ossr1_T_3.2         0         0         0         1      0      0      0
## WT_C_3.3            0         0         0         0      0      1      0
## WT_T_3.3            0         0         0         0      0      0      0
## Ossr1_C_3.3         0         1         0         0      0      0      0
## Ossr1_T_3.3         0         0         0         1      0      0      0
##             WT_T_3
## WT_C_1.1         0
## WT_T_1.1         0
## Ossr1_C_1.1      0
## Ossr1_T_1.1      0
## WT_C_1.2         0
## WT_T_1.2         0
## Ossr1_C_1.2      0
## Ossr1_T_1.2      0
## WT_C_1.3         0
## WT_T_1.3         0
## Ossr1_C_1.3      0
## Ossr1_T_1.3      0
## WT_C_3.1         0
## WT_T_3.1         1
## Ossr1_C_3.1      0
## Ossr1_T_3.1      0
## WT_C_3.2         0
## WT_T_3.2         1
## Ossr1_C_3.2      0
## Ossr1_T_3.2      0
## WT_C_3.3         0
## WT_T_3.3         1
## Ossr1_C_3.3      0
## Ossr1_T_3.3      0
## attr(,"assign")
## [1] 1 1 1 1 1 1 1 1
## attr(,"contrasts")
## attr(,"contrasts")$group
## [1] "contr.treatment"

#groups are alphabetized, NOT in the order of the counts table

#filter out genes that are lowly expressed in multiple samples
keep <- filterByExpr(y)
y <- y[keep, , keep.lib.sizes=FALSE]
y$samples

##                group lib.size norm.factors
## WT_C_1.1      WT_C_1 58718265            1
```

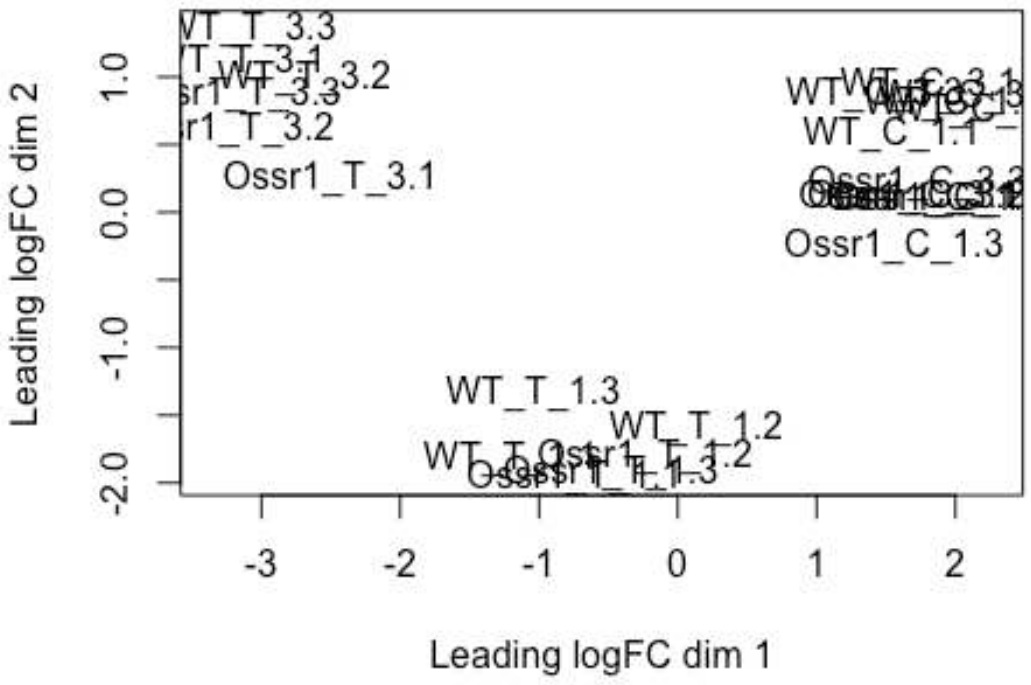
```
## WT_T_1.1       WT_T_1 33281827            1
## Ossr1_C_1.1 Ossr1_C_1 49687965            1
## Ossr1_T_1.1 Ossr1_T_1 60114639            1
## WT_C_1.2       WT_C_1 32302420            1
## WT_T_1.2       WT_T_1 56325550            1
## Ossr1_C_1.2 Ossr1_C_1 34130884            1
## Ossr1_T_1.2 Ossr1_T_1 51604154            1
## WT_C_1.3       WT_C_1 55359779            1
## WT_T_1.3       WT_T_1 55536219            1
## Ossr1_C_1.3 Ossr1_C_1 32734261            1
## Ossr1_T_1.3 Ossr1_T_1 56328983            1
## WT_C_3.1       WT_C_3 62966846            1
## WT_T_3.1       WT_T_3 55031528            1
## Ossr1_C_3.1 Ossr1_C_3 55100052            1
## Ossr1_T_3.1 Ossr1_T_3 51736076            1
## WT_C_3.2       WT_C_3 49110307            1
## WT_T_3.2       WT_T_3 53313326            1
## Ossr1_C_3.2 Ossr1_C_3 50800581            1
## Ossr1_T_3.2 Ossr1_T_3 30578860            1
## WT_C_3.3       WT_C_3 52333679            1
## WT_T_3.3       WT_T_3 47132025            1
## Ossr1_C_3.3 Ossr1_C_3 48332469            1
## Ossr1_T_3.3 Ossr1_T_3 31419281            1

plotMDS(y)
```



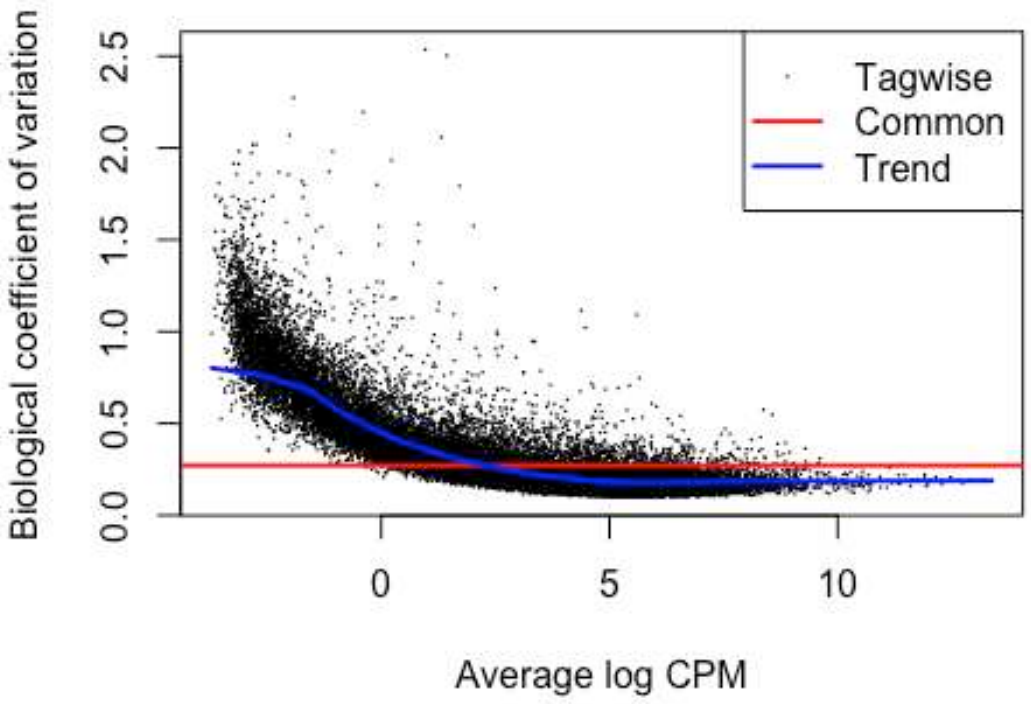

```
#normalize libraries
y <- calcNormFactors(y)
y$samples
```

```
##                 group lib.size norm.factors
## WT_C_1.1       WT_C_1 58718265    0.9805174
## WT_T_1.1       WT_T_1 33281827    0.8752161
## Ossr1_C_1.1 Ossr1_C_1 49687965    1.0895735
## Ossr1_T_1.1 Ossr1_T_1 60114639    1.0296289
## WT_C_1.2       WT_C_1 32302420    0.9878620
## WT_T_1.2       WT_T_1 56325550    1.0086337
## Ossr1_C_1.2 Ossr1_C_1 34130884    1.0694864
## Ossr1_T_1.2 Ossr1_T_1 51604154    1.0583309
## WT_C_1.3       WT_C_1 55359779    1.0499672
## WT_T_1.3       WT_T_1 55536219    0.9310220
## Ossr1_C_1.3 Ossr1_C_1 32734261    1.0242990
## Ossr1_T_1.3 Ossr1_T_1 56328983    1.0988311
## WT_C_3.1       WT_C_3 62966846    0.9737863
## WT_T_3.1       WT_T_3 55031528    0.9146178
## Ossr1_C_3.1 Ossr1_C_3 55100052    1.0593579
## Ossr1_T_3.1 Ossr1_T_3 51736076    0.9673183
## WT_C_3.2       WT_C_3 49110307    1.0925651
## WT_T_3.2       WT_T_3 53313326    0.8949883
```

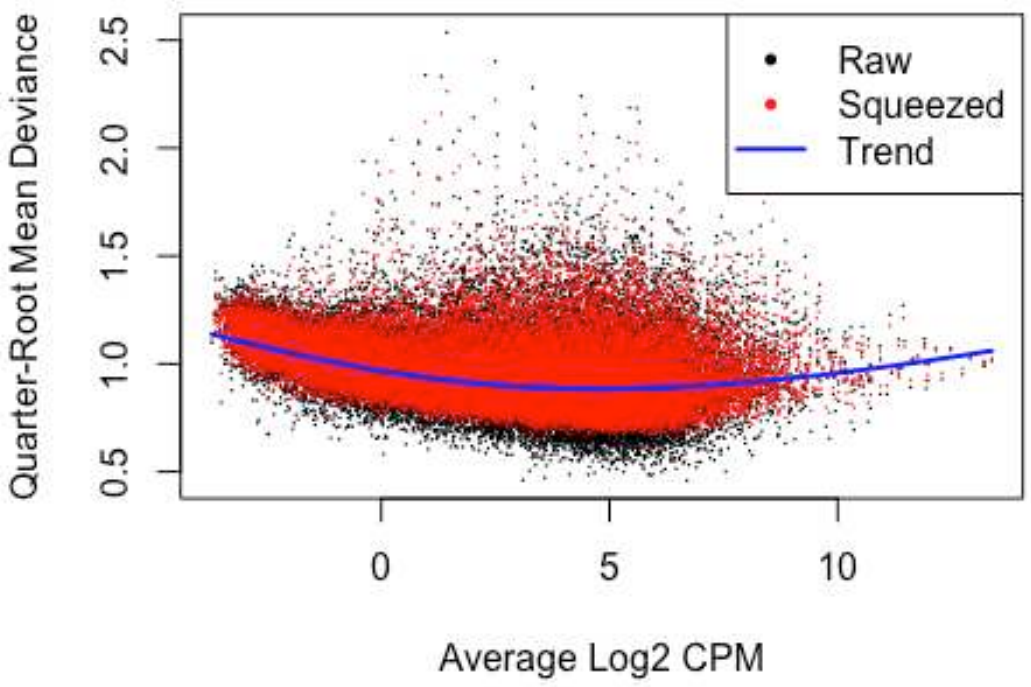
```
## Ossr1_C_3.2 Ossr1_C_3 50800581    1.0285747
## Ossr1_T_3.2 Ossr1_T_3 30578860    0.9722380
## WT_C_3.3       WT_C_3 52333679    1.0426199
## WT_T_3.3       WT_T_3 47132025    0.8871113
## Ossr1_C_3.3 Ossr1_C_3 48332469    1.1026372
## Ossr1_T_3.3 Ossr1_T_3 31419281    0.9197016

plotMDS(y)
```



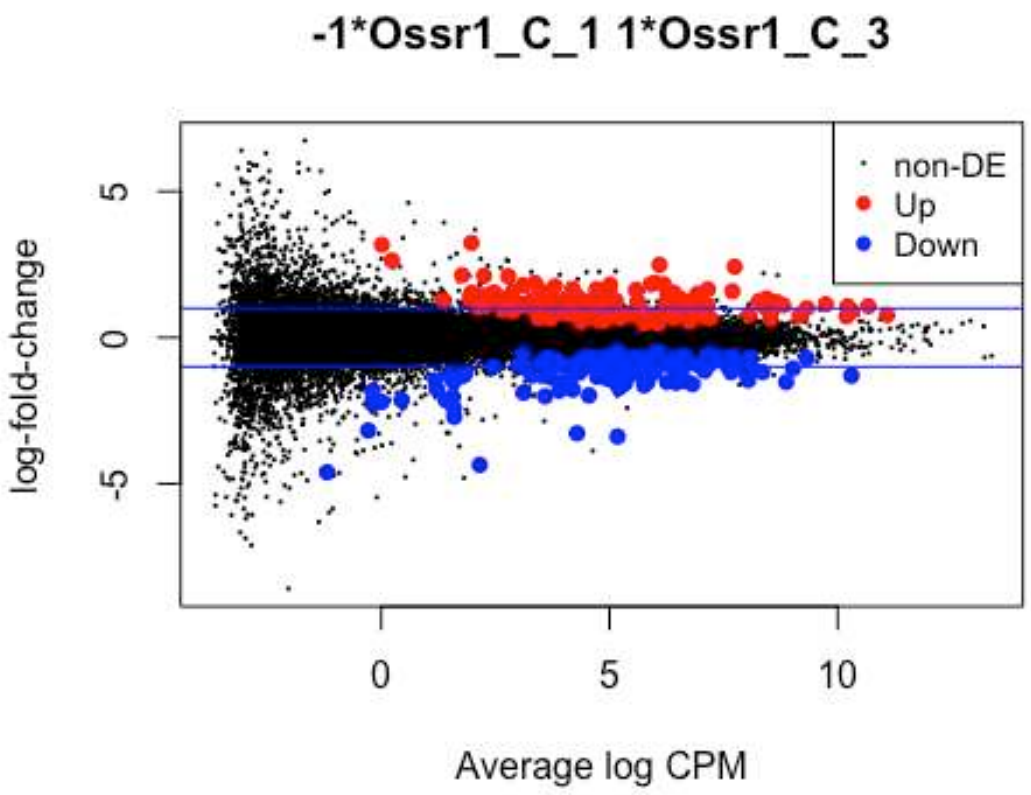

```
#estimate dispersion
y <- estimateDisp(y,design)
y$common.dispersion

## [1] 0.07365209

sqrt(y$common.dispersion) #square root of common dispersion gives the coeffic
ient of biological variation

## [1] 0.2713892

plotBCV(y)
```




```
#try fit with quasi-liklihood F-test
fit <- glmQLFit(y,design) #using quasi-likihood fit test, as is recommended f
or RNA-seq data,
plotQLDisp(fit)
```

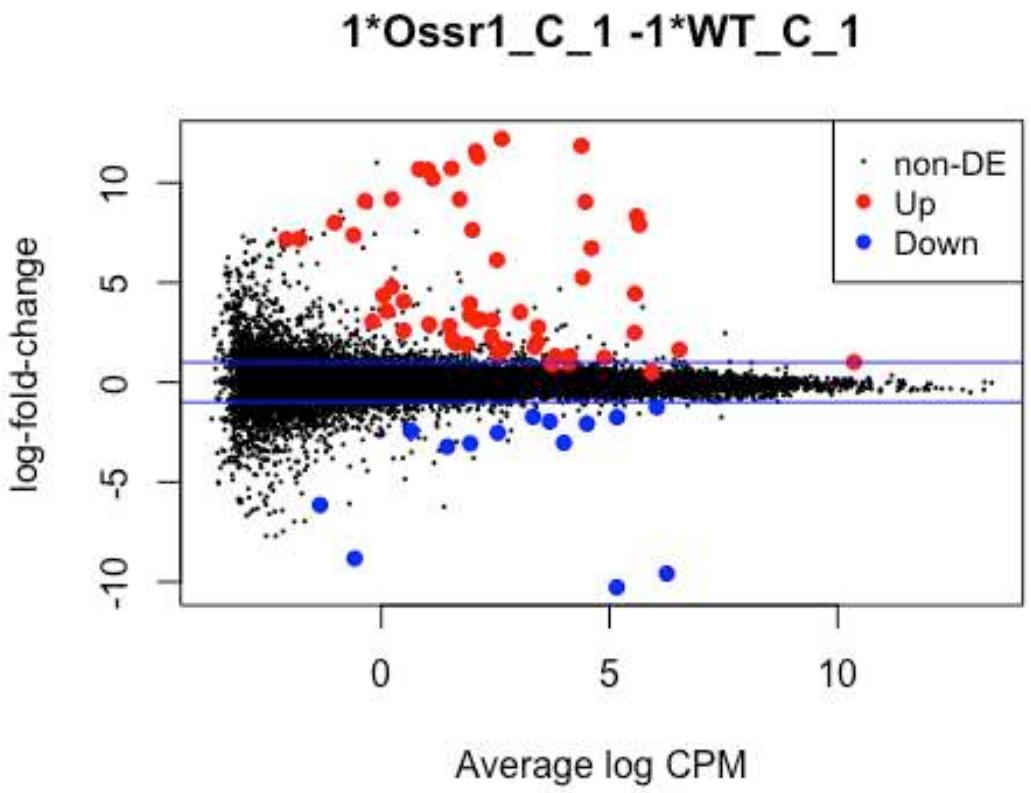



```
# try a comparison to see how test runs
qlf <- glmQLFTest(fit, contrast=c(-1,1,0,0,0,0,0,0)) #comparing Ossr1_C_1 vs.
 Ossr1_C_3 *remember, samples are now alphabetical by column
summary(decideTests(qlf))

##        -1*Ossr1_C_1 1*Ossr1_C_3
## Down                        133
## NotSig                    27308
## Up                          142

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```

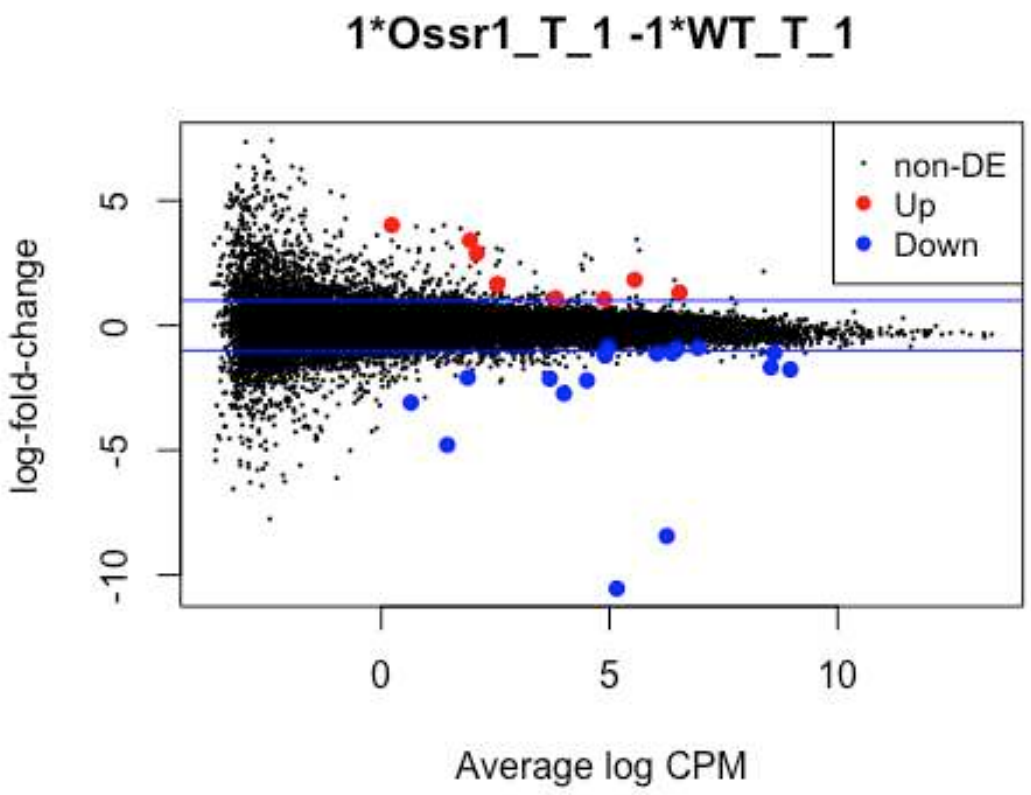



```
# test reverse comparison
qlf <- glmQLFTest(fit, contrast=c(1,-1,0,0,0,0,0,0)) #comparing Ossr1_C_3 vs
Ossr1_C_1
summary(decideTests(qlf))

##        1*Ossr1_C_1 -1*Ossr1_C_3
## Down                       142
## NotSig                   27308
## Up                         133

#now test desired questions using contrasts
colnames(fit) #contrasts refer to columns in this order

## [1] "Ossr1_C_1" "Ossr1_C_3" "Ossr1_T_1" "Ossr1_T_3" "WT_C_1"    "WT_C_3"

## [7] "WT_T_1"    "WT_T_3"

qlf <- glmQLFTest(fit, contrast=c(1,0,0,0,-1,0,0,0)) #comparing 1. Ossr1_C_1v
sWT_C_1
summary(decideTests(qlf))

##        1*Ossr1_C_1 -1*WT_C_1
## Down                      14
```

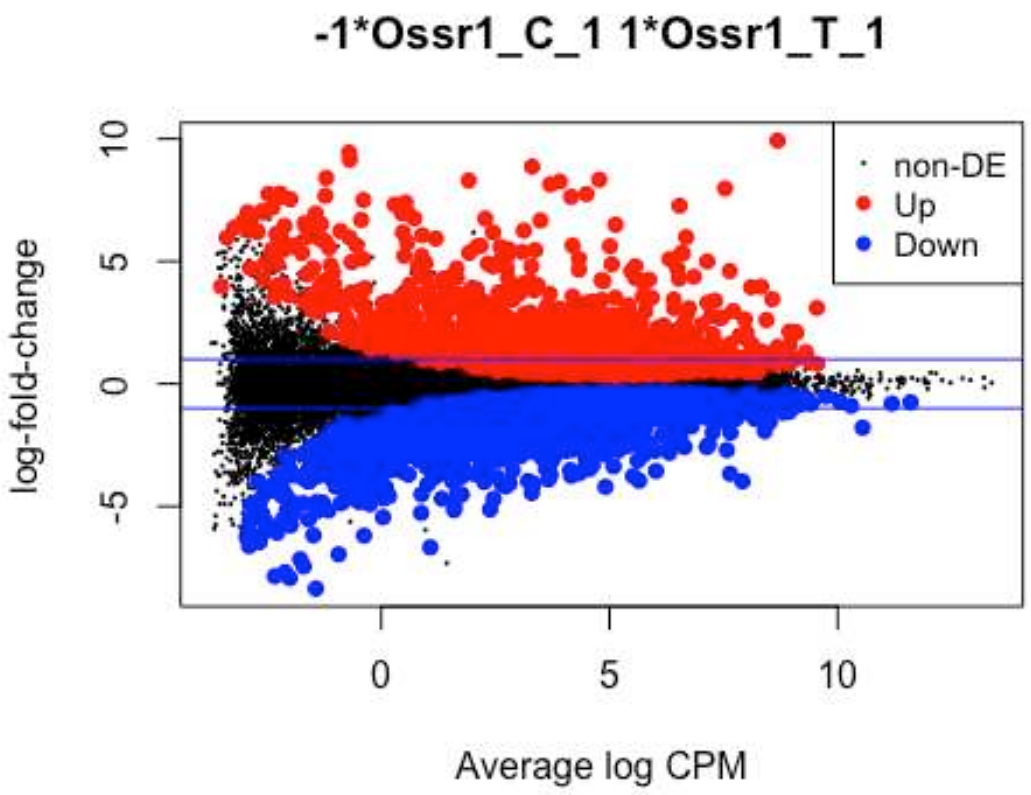
```
## NotSig                  27514
## Up                         55

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



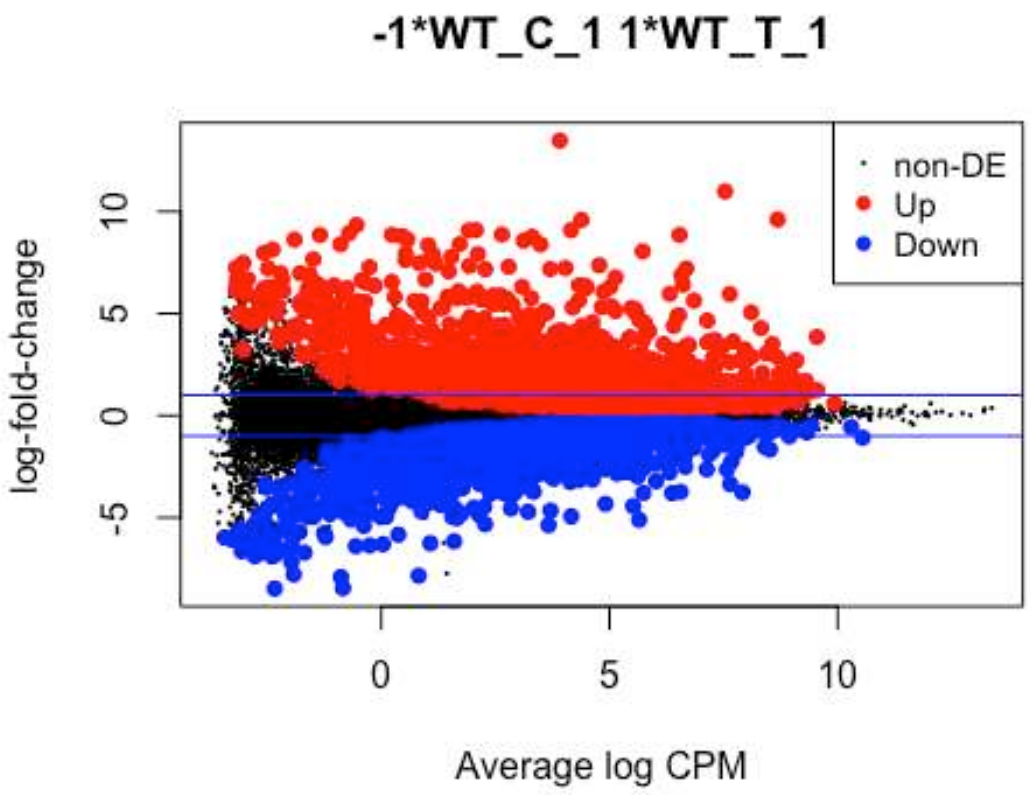

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_C_1vsWT_C_1.csv")

qlf <- glmQLFTest(fit, contrast=c(0,0,1,0,0,0,-1,0)) #comparing 2. Ossr1_T_1v
sWT_T_1
summary(decideTests(qlf))

##        1*Ossr1_T_1 -1*WT_T_1
## Down                      17
## NotSig                 27558
## Up                         8

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```




```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_T_1vsWT_T_1.csv")

qlf <- glmQLFTest(fit, contrast=c(-1,0,1,0,0,0,0,0)) #comparing 3. Ossr1_T_1v
sOssr1_C_1
summary(decideTests(qlf))

##        -1*Ossr1_C_1 1*Ossr1_T_1
## Down                       2171
## NotSig                    23715
## Up                         1697

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```

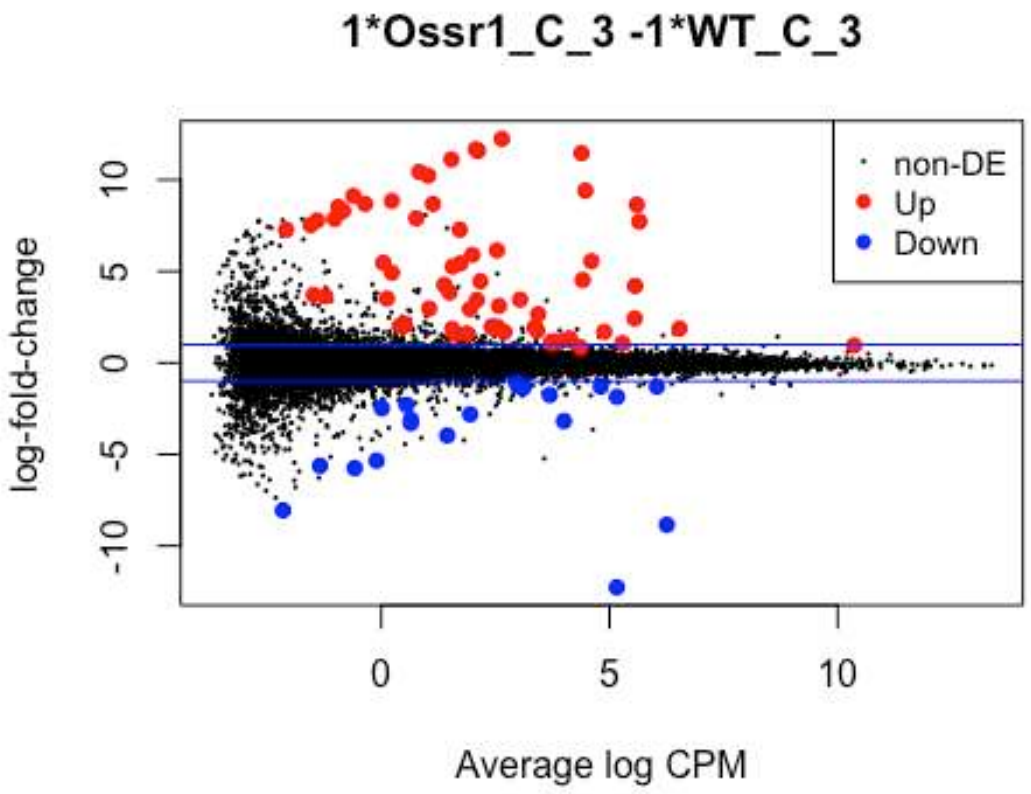



```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_T_1vsOssr1_C_1.csv")

qlf <- glmQLFTest(fit, contrast=c(0,0,0,0,-1,0,1,0)) #comparing 4. WT_T_1vsWT
_C_1
summary(decideTests(qlf))

##        -1*WT_C_1 1*WT_T_1
## Down                1973
## NotSig             23105
## Up                  2505

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



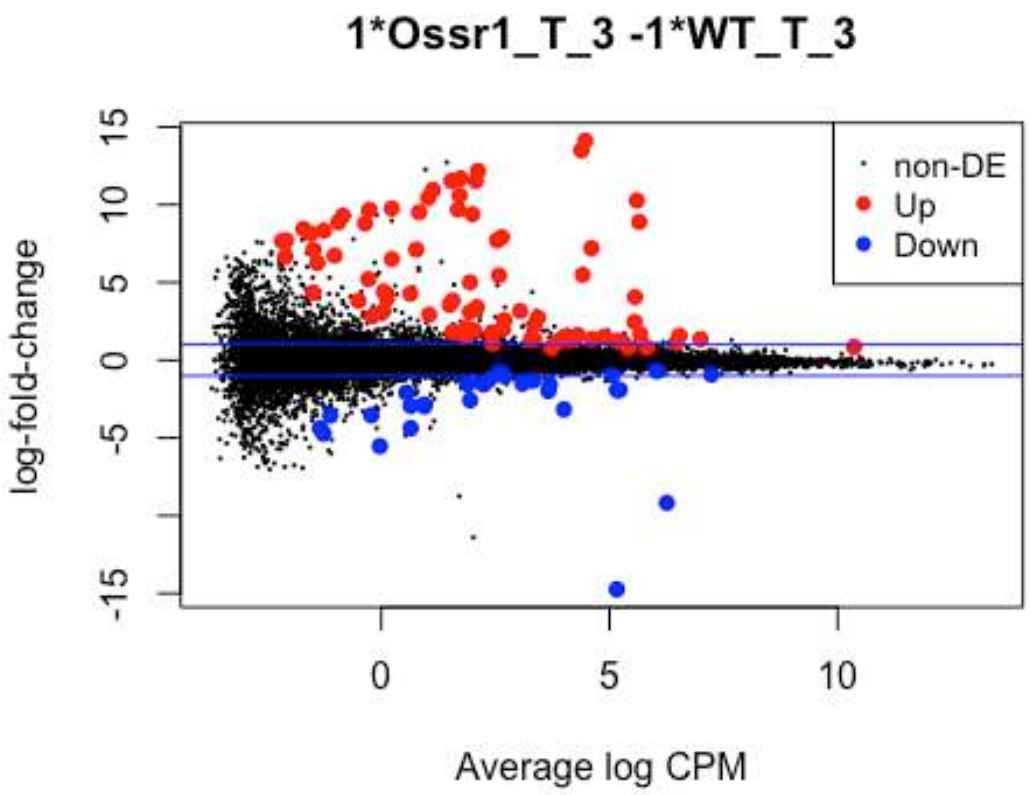

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
str(out)

## Formal class 'TopTags' [package "edgeR"] with 1 slot
##   ..@ .Data:List of 4
##   .. ..$ :'data.frame':  27583 obs. of  7 variables:
##   .. .. ..$ V1    : Factor w/ 57585 levels "ChrSy.fgenesh.CDS.1",..: 43818
 17442 12158 36081 13920 45724 12934 7233 14922 34326 ...
##   .. .. ..$ V2    : Factor w/ 8649 levels " ELMO%2FCED-12 family protein,
putative, expressed",..: 5961 188 2679 3356 2213 5671 2153 5961 239 188 ...
##   .. .. ..$ logFC : num [1:27583] 7.21 8.86 9.61 -3.21 3.58 ...
##   .. .. ..$ logCPM: num [1:27583] 6.68 6.54 8.69 6.02 4.75 ...
##   .. .. ..$ F     : num [1:27583] 286 254 235 229 203 ...
##   .. .. ..$ PValue: num [1:27583] 4.49e-14 1.54e-13 3.36e-13 4.43e-13 1.43
e-12 ...
##   .. .. ..$ FDR   : num [1:27583] 1.24e-09 2.12e-09 3.05e-09 3.05e-09 7.20
e-09 ...
##   .. ..$ : chr "BH"
##   .. ..$ : chr "-1*WT_C_1 1*WT_T_1"
##   .. ..$ : chr "glm"

write.csv(out, file = "edgeR/190625/WT_T_1vsWT_C_1.csv")
```

```
qlf <- glmQLFTest(fit, contrast=c(0,1,0,0,0,-1,0,0)) #comparing 5. Ossr1_C_3v
sWT_C_3
summary(decideTests(qlf))

##        1*Ossr1_C_3 -1*WT_C_3
## Down                      20
## NotSig                 27496
## Up                        67

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```

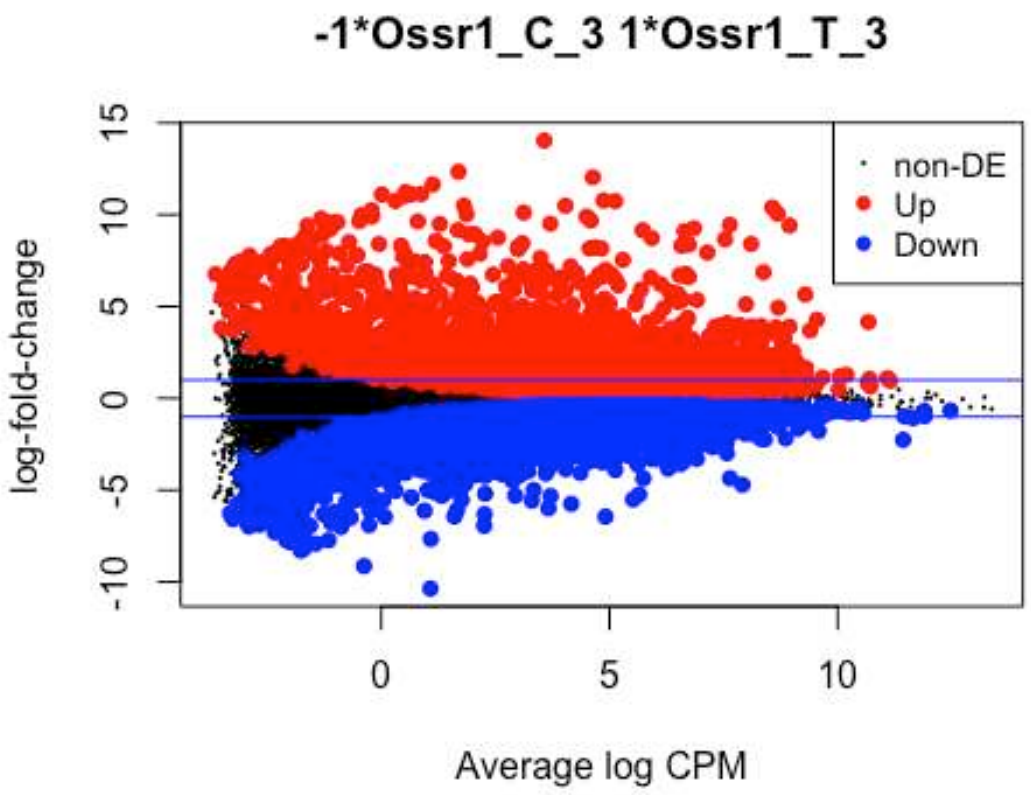



```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_C_3vsWT_C_3.csv")

qlf <- glmQLFTest(fit, contrast=c(0,0,0,1,0,0,0,-1)) #comparing 6. Ossr1_T_3v
sWT_T_3
summary(decideTests(qlf))

##        1*Ossr1_T_3 -1*WT_T_3
## Down                      28
## NotSig                 27472
## Up                        83
```

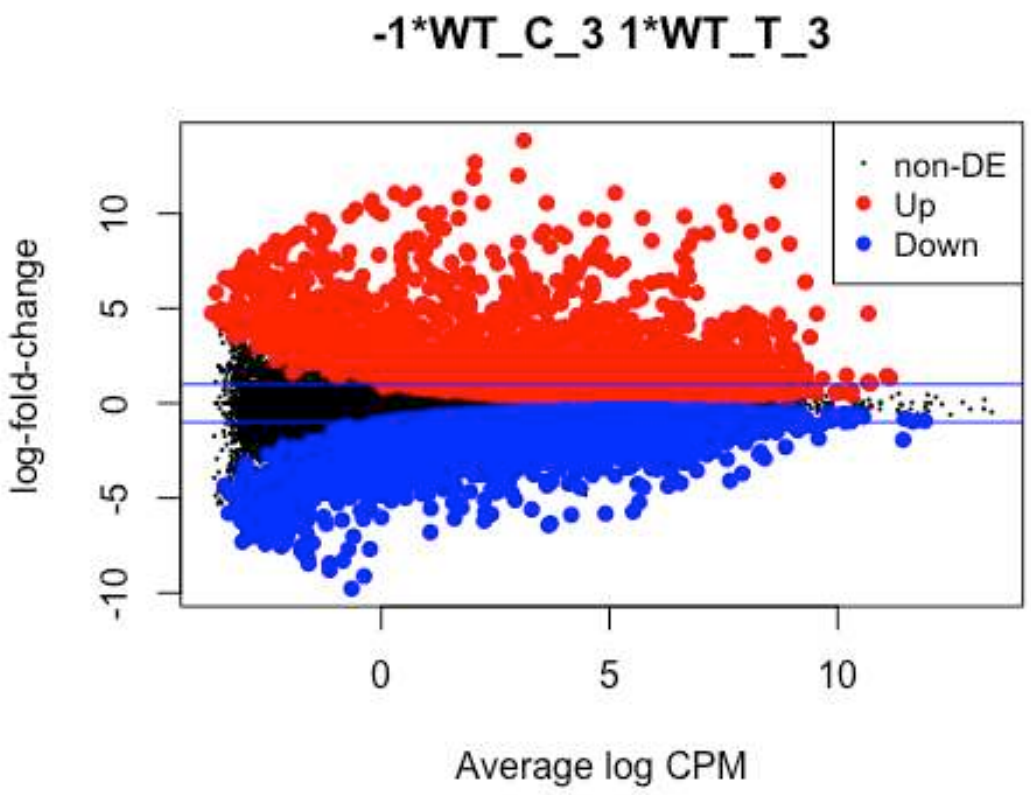
```
plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



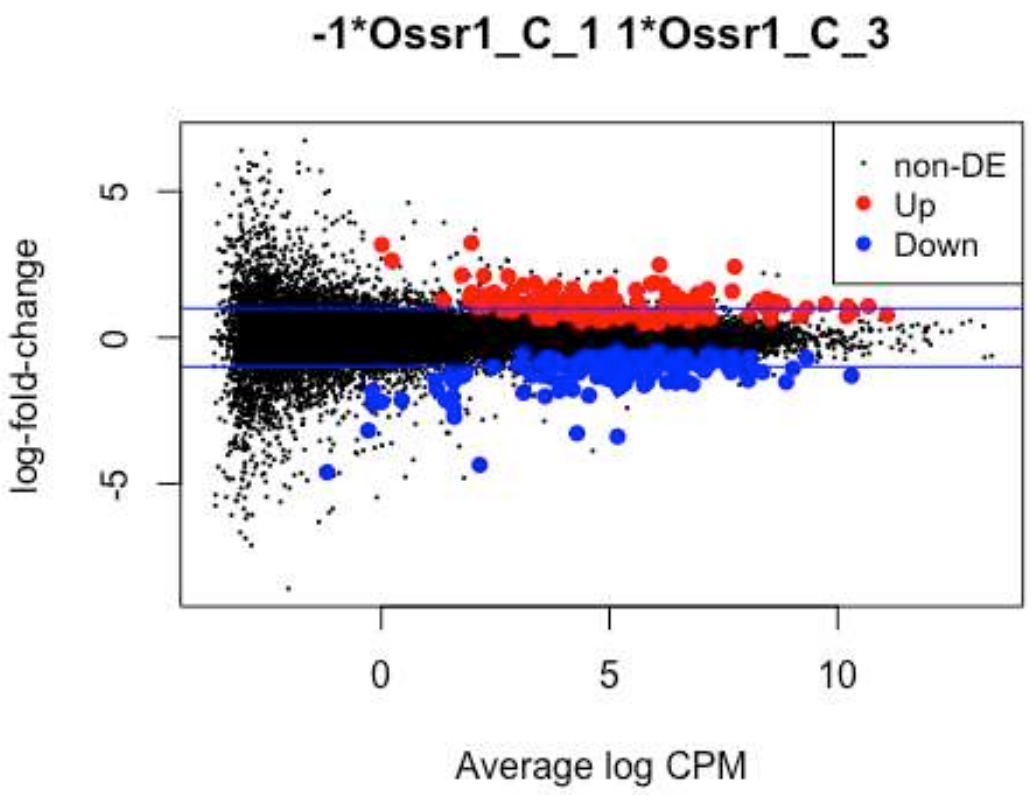

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_T_3vsWT_T_3.csv")

qlf <- glmQLFTest(fit, contrast=c(0,-1,0,1,0,0,0,0)) #comparing 7. Ossr1_T_3v
sOssr1_C_3
summary(decideTests(qlf))

##        -1*Ossr1_C_3 1*Ossr1_T_3
## Down                       4686
## NotSig                    17706
## Up                         5191

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



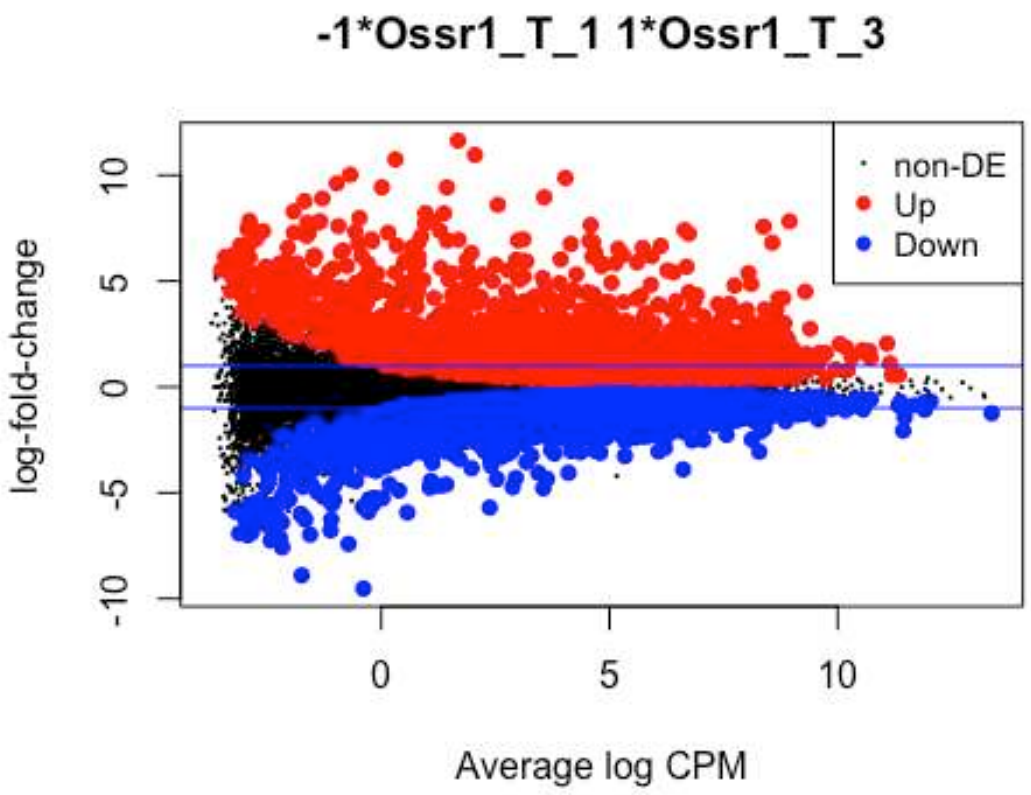

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_T_3vsOssr1_C_3.csv")

qlf <- glmQLFTest(fit, contrast=c(0,0,0,0,0,-1,0,1)) #comparing 8. WT_T_3vsWT
_C_3
summary(decideTests(qlf))

##        -1*WT_C_3 1*WT_T_3
## Down                5122
## NotSig             17025
## Up                  5436

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



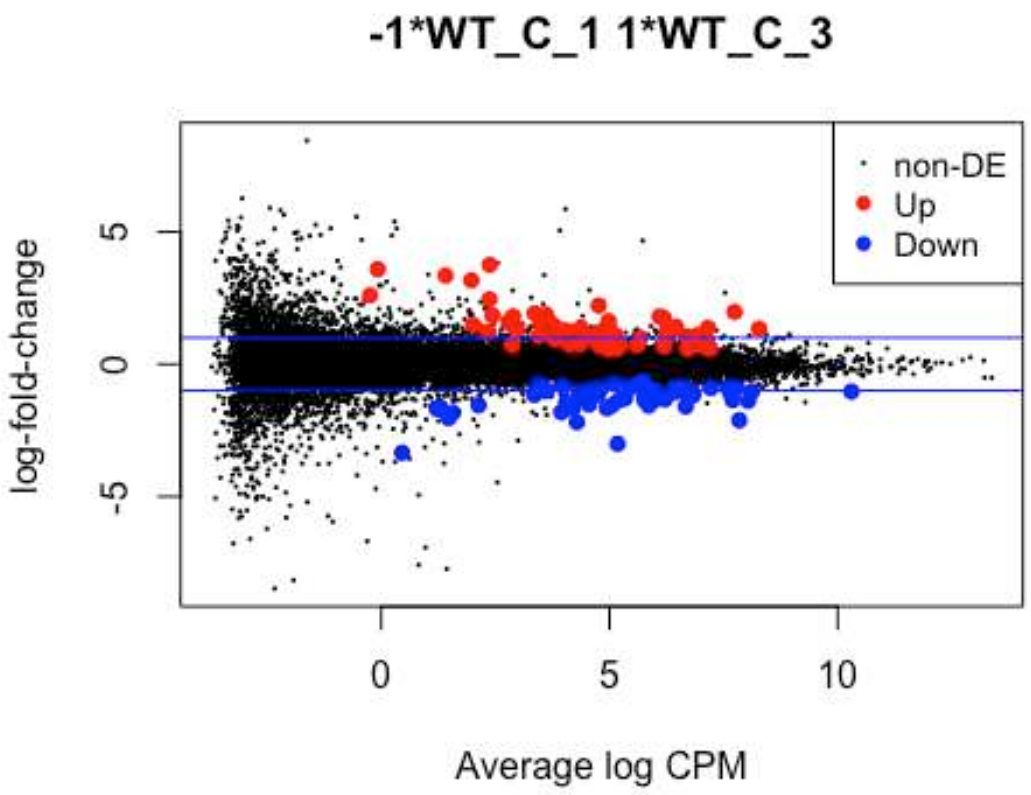

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/WT_T_3vsWT_C_3.csv")

qlf <- glmQLFTest(fit, contrast=c(-1,1,0,0,0,0,0,0)) #comparing 9. Ossr1_C_3v
sOssr1_C_1
summary(decideTests(qlf))

##        -1*Ossr1_C_1 1*Ossr1_C_3
## Down                        133
## NotSig                    27308
## Up                          142

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



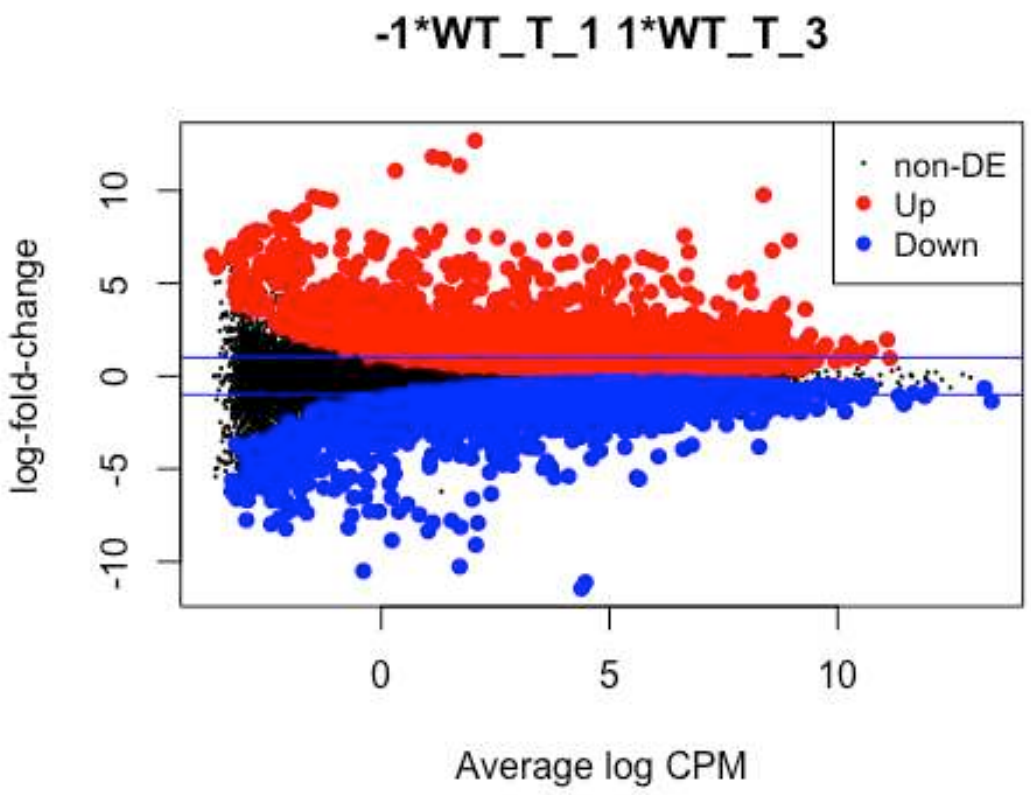

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_C_3vsOssr1_C_1.csv")

qlf <- glmQLFTest(fit, contrast=c(0,0,-1,1,0,0,0,0)) #comparing 10. Ossr1_T_3
vsOssr1_T_1
summary(decideTests(qlf))

##        -1*Ossr1_T_1 1*Ossr1_T_3
## Down                       3953
## NotSig                    18906
## Up                         4724

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



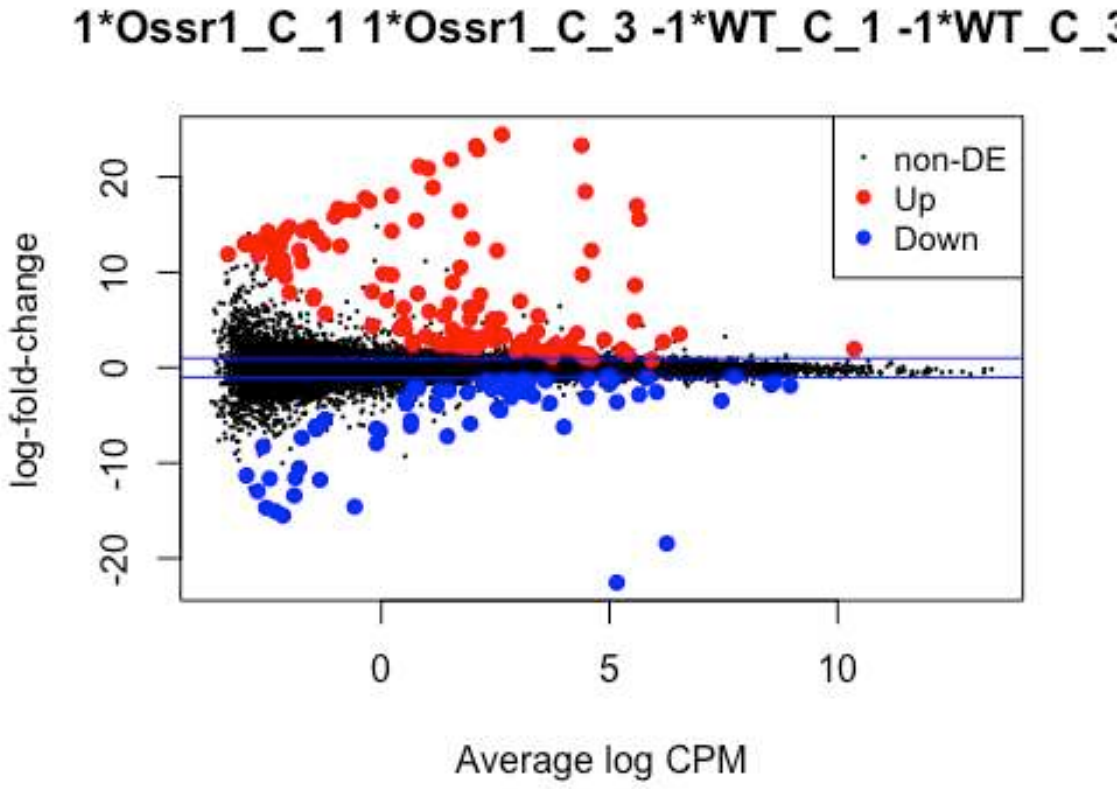

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_T_3vsOssr1_T_1.csv")

qlf <- glmQLFTest(fit, contrast=c(0,0,0,0,-1,1,0,0)) #comparing 11. WT_C_3vsW
T_C_1
summary(decideTests(qlf))

##        -1*WT_C_1 1*WT_C_3
## Down                   59
## NotSig              27463
## Up                     61

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



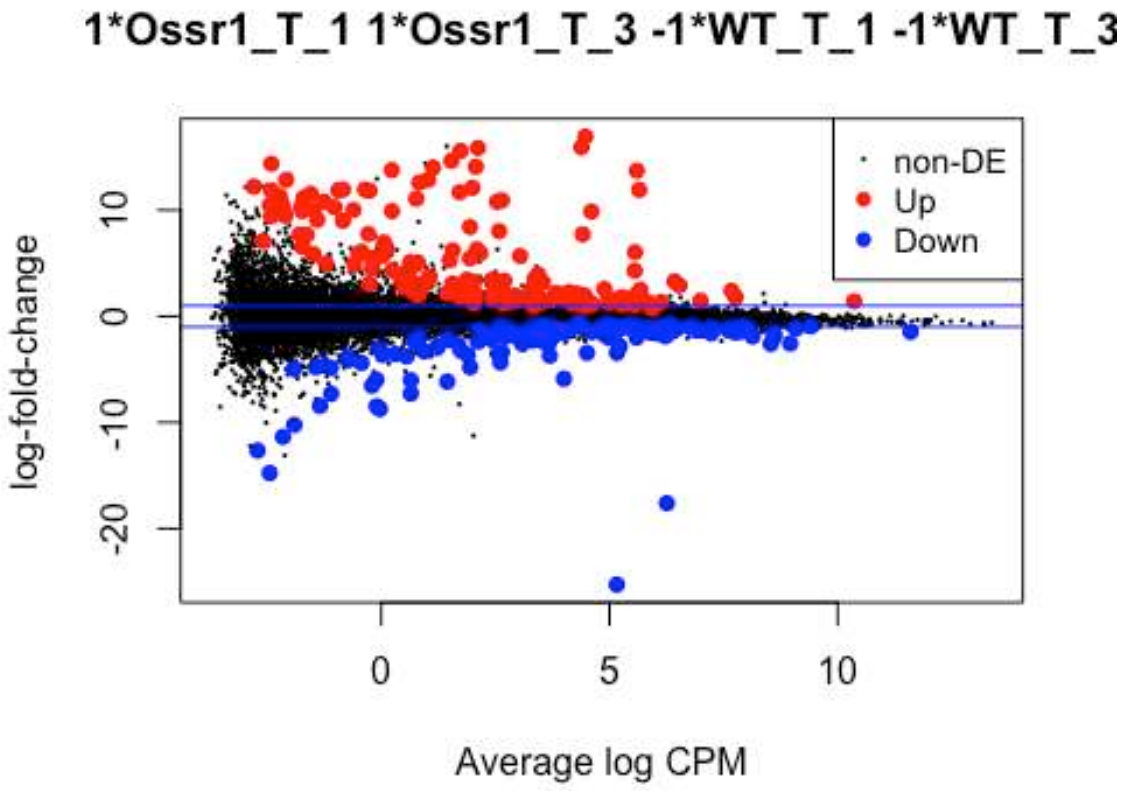

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/WT_C_3vsWT_C_1.csv")

qlf <- glmQLFTest(fit, contrast=c(0,0,0,0,0,0,-1,1)) #comparing 12. WT_T_3vsW
T_T_1
summary(decideTests(qlf))

##        -1*WT_T_1 1*WT_T_3
## Down                4272
## NotSig             18722
## Up                  4589

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



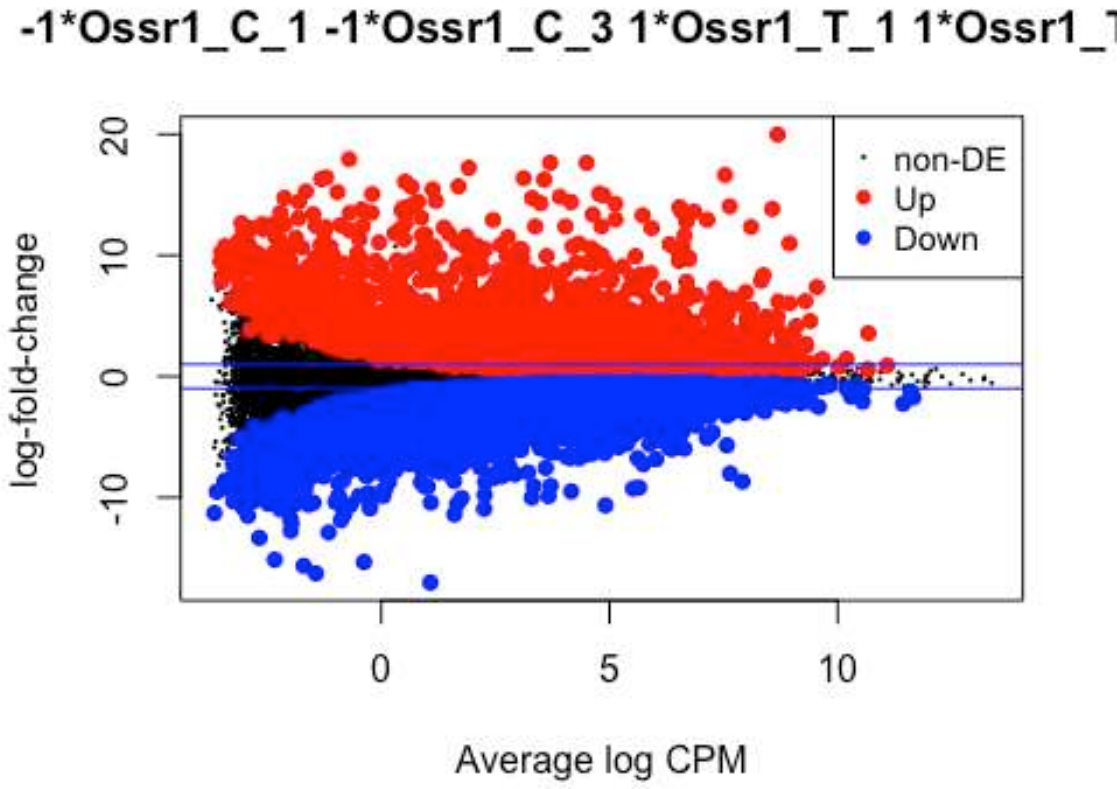

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/WT_T_3vsWT_T_1.csv")

qlf <- glmQLFTest(fit, contrast=c(1,1,0,0,-1,-1,0,0)) #comparing 13. Ossr1_Cv
sWT_C
summary(decideTests(qlf))

##        1*Ossr1_C_1 1*Ossr1_C_3 -1*WT_C_1 -1*WT_C_3
## Down                                          72
## NotSig                                     27379
## Up                                           132

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```



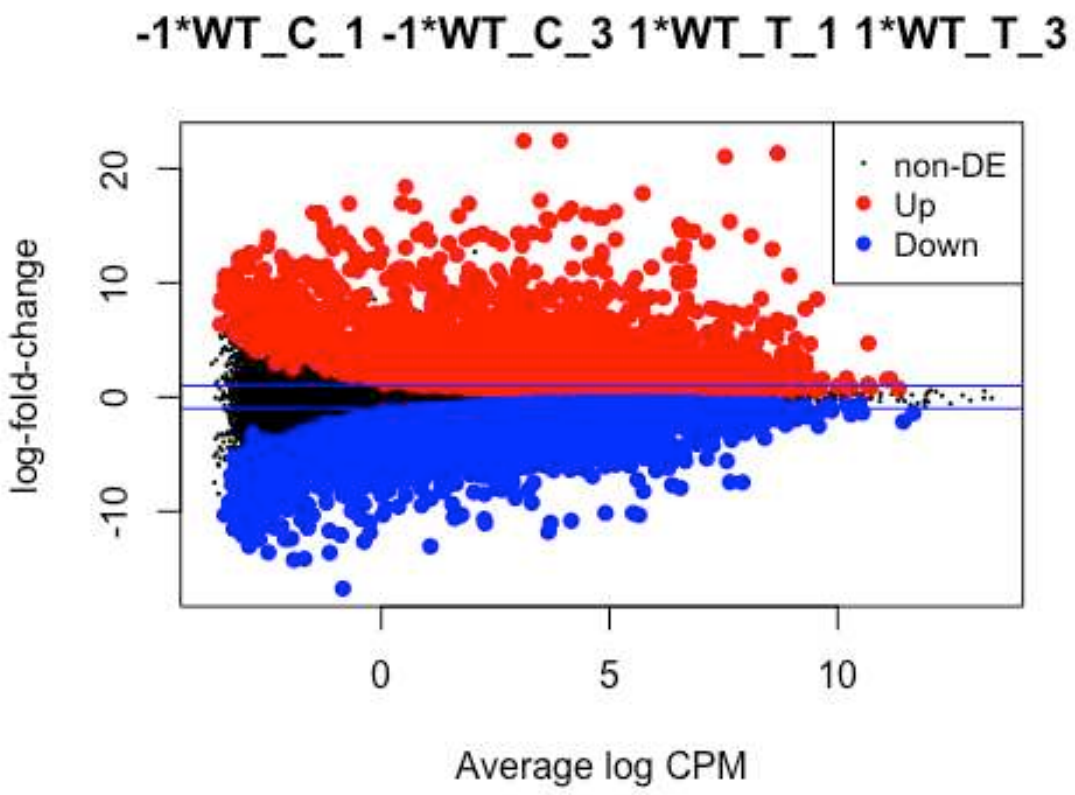

```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_CvsWT_C.csv")

qlf <- glmQLFTest(fit, contrast=c(0,0,1,1,0,0,-1,-1)) #comparing 14. Ossr1_Tv
sWT_T
summary(decideTests(qlf))

##        1*Ossr1_T_1 1*Ossr1_T_3 -1*WT_T_1 -1*WT_T_3
## Down                                          152
## NotSig                                      27268
## Up                                            163

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```




```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_TvsWT_T.csv")

qlf <- glmQLFTest(fit, contrast=c(-1,-1,1,1,0,0,0,0)) #comparing 15. Ossr1_Tv
sOssr1_C
summary(decideTests(qlf))

##        -1*Ossr1_C_1 -1*Ossr1_C_3 1*Ossr1_T_1 1*Ossr1_T_3
## Down                                                4729
## NotSig                                             17853
## Up                                                  5001

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```




```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/Ossr1_TvsOssr1_C.csv")

qlf <- glmQLFTest(fit, contrast=c(0,0,0,0,-1,-1,1,1)) #comparing 16. WT_TvsWT
_C
summary(decideTests(qlf))

##        -1*WT_C_1 -1*WT_C_3 1*WT_T_1 1*WT_T_3
## Down                                    5202
## NotSig                                 16896
## Up                                      5485

plotMD(qlf)
abline(h=c(-1,1), col="blue")
```




```
out <- topTags(qlf, n=nrow(x), sort.by="p.value")
write.csv(out, file = "edgeR/190625/WT_TvsWT_C.csv")
```