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Stress Tolerance Enhancement of Rice by Genetic Manipulation of a bHLH-Myc2 Transcription Factor

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STRESS TOLERANCE ENHANCEMENT OF RICE BY GENETIC MANIPULATION OF A *BHLH-MYC2* TRANSCRIPTION FACTOR

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This dissertation is dedicated to my parents Luis Eduardo Sánchez Macias and Grace Mónica Timm Duque, who brought me to this world and gave me the best gift that a parent can give to a son: love, health, values and education. I also dedicate this work to my fiancée, Tatiana Paola Chavez Navarrete, for all her love and support throughout my doctoral education.

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LIST OF ABBREVIATIONS

µM	Micro molar
ABA	Abscisic acid
ABRE	ABA responsive elements
AQP	Aquaporin
bHLH	Basic helix hoop helix
BLAST	Basic local alignment search tool
bp	Base pair
CaMV35S	Cauliflower mosaic virus 35S promoter
cm	Centimeter
CoiI	Coronatine insensitive I
DMSO	Dimethyl sulfoxide
dNTPs	Deoxyribonucleotide triphosphates
DRE	Dehydration responsive element
dsDNA	Double stranded deoxyribonucleic acid
DW	Dry weight
E coli	*Escherichia coli*
ERD	Early responsive to dehydration
GA	Gibberellic acid
GFP	Green fluorescence protein
H	Hour/hours
hptII	Hygromycin phosphotransferase
HSP	Heat shock protein
JA	Jasmonic acid
JA-iLe	Jasmonyl isoleucine
JAZ	Jasmonate zim
Kbp	Kilo base pair
KD	Knock down
LB	Luria-Bertani broth
LF	Left border
LOX	Lipoxygenase
M	Molar
Mbp	Mega base pair
meJA	Methyl jasmonate
Min	Minute
ml	Milliliter
mM	Millimolar
NaCl	Sodium chloride
NCBI	National center for biotechnology information
NCED3	9-cis-epoxycarotenoid dioxygenase 3
ng	Nanogram

OD	Optical density
OE	Overexpresser
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PR	Pathogenesis related
RAP-DB	Rice annotation project database
RB	Right border
RE	Restriction endonuclease
RNA	Ribonucleic acid
RNAi	RNA interference
ROS	Reactive oxygen species
RPM	Rotation per minute
RT	Room temperature
RWC	Relative water content
SA	Salicylic acid
Sec	Second/seconds
Taq	*Thermus aquaticus*
TAT	Tyrosine aminotransferase
TF	Transcription factor
TW	Turgid weight
U	Unit
V	Volt
VSP	Vegetative storage protein
WT	Wild type

ABSTRACT

Rice yield is adversely affected by various abiotic and biotic stresses. Jasmonic acid (JA) signaling has been implicated in stress response of plants. The nuclear localized basic helix loop helix (bHLH) *Myc2* transcription factor is known to be a master regulator of genes involved in the response of the JA-mediated signaling pathway during stress and plant development. *Myc2* is also induced by wounding and mechanical damage, and is associated with resistance against herbivore insects. In order to understand the mode of action of *Myc2* in stress response of rice, overexpresser (OE) and knock-down (KD) mutants for *OsMyc2* were generated in rice. After 7 d of withholding water, *OsMyc2* OE plants showed better stress tolerance with respect to their growth and development, and physiological traits such as relative water content, membrane stability, chlorophyll fluorescence, etc. in comparison with the wild type (WT) and KD plants. Similar results were obtained for response to salinity stress (150 mM NaCl in hydroponics) where OE seedlings showed less chlorosis and better shoot and root growth as compared to the WT and KD lines. Furthermore, non-choice feeding assay of the transgenic rice plants with a specialist herbivore *Spodoptera frugiperda* showed that the life cycle of the insect was affected when the larvae were fed with tissues of the *OsMyc2* OE lines. Bioassay with blast fungus, *Magnaporthe oryzae*, did not show obvious difference with the number of lesions, but the size of lesions was smaller in OE lines relative to that in WT and KD lines. *OsMyc2*, in addition to its overexpression under various stresses, modulated the expression of genes in JA signaling and associated networks. These results suggested that the *OsMyc2* transcription factor is involved in multiple stress responses and can be manipulated to enhance stress tolerance in rice.

CHAPTER 1: INTRODUCTION

Rice (*Oryza sativa* L.), one of the most important cereal crops in the world (CGRFA, 2012; FAO, 2013), is very sensitive to abiotic stresses; drought and salt together can cause significant yield losses to the extent of ~40% (IRRI, 2014). The current and future global climate change scenario is likely to worsen the situation with increase in temperature, rise in sea level and dry spells. Furthermore, these environmental conditions will make crop plants more vulnerable to biotic stresses.

Natural genetic variations for abiotic stress tolerance extant in rice gene pool are being exploited in breeding to develop stress-resilient crops. Conventional breeding has been slow due to the complexity of the stress tolerance traits and low selection efficiency of the quantitatively inherited traits. Molecular interventions, such as marker-assisted selection and precision breeding through genetic engineering would complement traditional breeding to hasten the development of drought and salt tolerant rice. Several quantitative trait loci (QTL) and genes have been identified in the recent past using the primary and secondary gene pool of rice.

At the molecular level, plant's response to stress might involve a cascade of different stress responsive/tolerance genes, and most of them are known to be associated with the phytohormone abscisic acid (ABA; Madhava et al, 2006). Transcription factors (TFs), which constitute about 7% of the plant genome coding sequences, are known to participate in plant's early responses to biotic and/or abiotic stresses (Lindemose et al., 2013).

MYC (myelocytomatosis) proteins are coded by an important TF family involved in many biological processes, including stress responses and plant development. *Myc2* encodes a basic helix-loop-helix type TF that regulates jasmonic acid (JA) responsive genes from a Coronatine Insensitive 1 gene (COI1)-dependent pathway by the degradation of a Jasmonate

Zim-domain (JAZ), an important *Myc2* repressor protein through the ubiquitin proteasome pathway (Lorenzo et al., 2004; Santner and Estelle, 2007). *Myc2* is allelic to jasmonate insensitive 1 (*JIN1*), and contains a basic helix-loop-helix (bHLH) and a leucine zipper motif, which determines its specificity and affinity for specific DNA (Ji et al., 2012). Studies have shown that Myc2 is nuclear localized and may be involved in different biological processes, including pathogen defense, wound response, water deficit tolerance and root growth (Kazam et al., 2008; Woldemariam et al., 2013). Many studies have described Myc2 family genes role in abiotic stress responses and related them to the regulation of ABA responsive genes, signal transduction pathways, and to light regulated promoters (Yadav et al., 2005). Unlike other genes that are constitutively expressed, *bHLH-Myc2* has the capability to self-regulate its expression by feedback inhibition through the induction of a JAZ protein that interacts directly with Myc2. Different genes are known to be JA pathway dependent, and manipulation of *Myc2* has been shown to alter the expression of different genes, such as vegetative storage protein (*VSP2*) and tyrosine transaminase (*TAT1*) involved in wound response, lipoxygenase-3 (*LOX3*) related to oxidative stress, and pathogenesis related (PR) genes (Lorenzo et al., 2004; Shoji and Hashimoto, 2011; Domenico et al., 2012; Withers et al., 2012).

Jasmonate (Jas) signaling molecules are known to be involved in the activation of stress responsive genes providing the plant with tolerance to insects attack (Dombrecht et al., 2007). Most of these studies have characterized *AtMyc2* TF from the dicot model plant *Arabidopsis thaliana,* which shares low similarity with rice *OsMyc2* at both DNA and protein levels. The present study is unique in characterizing the role of *OsMyc2* TF from rice, an important food crop of global importance, in the plant's response to various stresses.

1.1 Research Objectives

With the long term goal of improving stress tolerance in rice, the present study was envisaged with the following objectives:

1) To determine the expression pattern of the *bHLH-Myc2* TF (*OsMyc2*) in different tissues and under drought stress in rice; and

2) To understand the role of *OsMyc2* in multiple stress responses of rice overexpresser and knock down mutants.

1.2 Origin and importance of rice

Rice (*Oryza spp.*), a cereal from the grass (Poaceae) family, has an unknown exact origin, but it is believed to be originated from South and East Asia, due to the abundance of wild species within these areas. Domesticated around the year 5000 B.C., rice has a genome size of ~430 Mbp with 12 chromosomes and six genome groups (A, B, C D, E, and F) in its gene pool. The cultivated rice (*Oryza sativa* L.) is the main source of food and energy for more than half of the world population, and is the second most produced cereal after wheat and the main staple food after corn (Acquaah, 2007; Gnanamanickam, 2009; Goff et al., 2002).

The International Rice Genebank, located in the International Rice Research Institute (IRRI), has the largest germplasm collection of rice with around 124,000 different accessions that represent the most important resource for genetic diversity and variety development (IRRI, 2015). Rice world production is dominated by China and India (FAO, 2015) in the amount of rice produced. Asia consumes around 90% of the total rice produced in the world. Of the total rice production, the U.S. produces less than 2%, but is one of the major rice exporters providing around 10% of the rice produced worldwide to markets, such as Central America, South America, Caribbean and the Middle East (http://www.ers.usda.gov/topics/crops/rice/trade.aspx).

The U.S. has six major rice producing states – California, Arkansas, Louisiana, Mississippi, Missouri and Texas. In 2014, the U.S. planted around 1,007,667 hectares of rice and had a production of 221,035,000 cwt. Rice is one of the most important commodities of Louisiana, where it was planted on 185,346 hectares with a production of 32,658,000 cwt in 2014 (http://www.usda.gov/nass/PUBS/TODAYRPT/cropan15.pdf).

Like any other crop, rice production is affected by two kinds of stresses: biotic stress caused by living organisms (insect attack, fungal/bacterial/viral infestations, etc.); and abiotic stress, caused by non-living organisms (lack/too much of water, high salt concentrations, extreme temperatures, etc.). These stresses can seriously affect plant growth, development and yield, and result in increased production expenses incurred in controlling a specific type of stress.

1.3 Drought stress tolerance

Drought is one of the major natural disasters in the U.S., overcome only by tropical cyclones. In 2012, drought caused an economical loss of $210.1 billion (Smith and Katz, 2013). Rice uses a significant amount of water (about 45% of the irrigation water for all crops) to complete its life cycle. Water deprivation can severely affect plant growth and yield. The effect is dependent on the severity of the drought and the growth stage of the rice plant; drought during the reproductive stage of rice causes the most reduction in yield. Drought affects seed germination greatly and leads to a poor crop establishment. It also stops plant growth by interfering with cell multiplication, enlargement and differentiation due to the decrease of cell turgor pressure, which is translated into mitosis interruption. Water deficit impairs nutrient uptake, photosynthesis, CO_2 uptake, and respiration (Lichtfouse, 2009).

At the molecular level, complex interactions among different networks are activated under stress, which are controlled by different phytohormones that are key regulators of different

plant metabolic pathways. Under drought stress, the plant activates a cascade of genes, and induces production of a high level of ABA. When exogenous ABA is applied to the plant, several genes related to drought stress are upregulated, which are known as ABA-dependent genes. On the other hand, there are some genes that are known to be activated during stress but are not affected by exogenous presence of ABA. These genes are called ABA-independent or *cis*-acting dehydration-responsive elements (DRE), and many of these genes are known to be also involved in cold and salt stress tolerance in plants (Shinozaki and Yamaguchi-Shinozaki, 2000).

Water stress reduces plant water potential by stomata closing, which affects CO_2 intake and malfunctioning of Rubisco and a reduced expression of photosynthesis related genes. Many studies have shown that ABA, together with ion transport elements and some transcription factors, such as 9-cis-epoxycarotenoid dioxygenase 3 (NCED3) responsible for stomatal closure, are highly upregulated during drought stress. ABA is then passively diffused to guard cells in response to pH changes and by specific transporters such as the ABC transporter family members (ABCG25 and ABCG40) and a member of a nitrate transporter family (AIT1/NRT1.2/NPF4.6; Osakabe et al., 2014). Kanno et al. (2012) demonstrated that ABCG25 and AIT1/NRT1.2/NPF4.6 export ABA and are localized in vascular tissue, in contrast to ABCG40, which is localized in the guard cell and is involved in ABA import. The increase of endogenous ABA enhances the production of signaling pathways operational in the assembly of reactive oxygen species (ROS), which stimulate an increase of cytosolic Ca^{2+}. This activates two anion channels – slow-activating sustained (S-type) and rapid-transient (R-type). These channels depolarize the plasma membrane and cause a reduction in inward K^+ channels (KAT1/KAT2) and H^+-ATPase related to stomatal opening and the activation of outward K^+ channels, such as

the Guard Cell Outward Rectifying K^+ Channel (GORK), important in K^+ efflux, which in the guard cells results in a cell turgor reduction leading to stomatal closure (Osakabe et al., 2014; Negi et al., 2008). Mutation of the *LENC1* gene, a positive regulator of *NCED3*, reduced *A. thaliana* capability to produce ABA, increasing its sensitivity to osmotic stress due to an increased water loss (Woo et al., 2011). In contrast, the upregulation of *NCED3* in both *A. thaliana* and *O. sativa* promoted ABA accumulation, which increased drought tolerance by reduced water loss due to stomatal closure, demonstrating the importance of this phytohormone and associated gene networks in plant stress tolerance (Hwang et al., 2010).

Several stress-related genes are highly expressed during water stress in the absence of ABA. Therefore, the existence of an ABA-independent response to stress is also involved in plant stress tolerance. DRE cis-elements have a specific core motif (TACCGACAT), which binds to DRE-binding proteins (RD29A), and ABRE cis elements (ACGTGG/TC), which binds to ABRE-binding proteins (RD22A and RD29B). Deletion and base substitution analyses and gel mobility shift assays demonstrated that these two mechanisms are independent of each other but can act coordinately (Narusaka et al., 2003). Plants with constitutive expression of the transcription factor *DREB1A* under the CaMV35S promoter have been shown to upregulate the expression of *RD29A*, *RD17*, *COR6.6, COR15a, ERD10* and *KIN1*, which are involved in stress tolerance, and the late embryogenesis abundant (LEA) proteins, which are involved in protection mechanisms (Smirnoff and Bryant, 1999).

After the activation of these early inducible stress regulatory proteins, the synthesis of functional proteins is an important step in plant's defense response to drought. The production of water channel transporters, known as aquaporins (AQPs), helps in plant water relations by increasing membrane permeability to water and other solutes, such as glycerol. In plants, AQPs

have four known subfamilies; plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic proteins (NIPs) and small basic intrinsic proteins (SIPs). Knock down of some PIP isoforms has shown a decrease in osmotic water permeability of protoplast, decreased hydraulic conductivity in root cortex cells, and susceptibility to drought and osmotic stress, demonstrating the importance of these proteins in plant stress tolerance (Alexandersson et al., 2005).

During stress, plants produce ROS, which at minimum concentrations are useful to manage stress, but at higher concentrations ROS are toxic to the plant, resulting in oxidative stress, which can ultimately lead to cell death. There are four forms of cellular ROS; singlet oxygen (O_2), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^\cdot$), all of them capable of oxidizing different cellular components like proteins, DNA, and RNA. Plants adapt to oxidative stress through the generation of detoxifying antioxidant enzymes, such as the superoxide dismutase (SOD), catalase (CAT), and the ascorbate peroxidase (APX; Cruz, 2008). Many reports have shown the evidence of the role of these antioxidant enzymes in plant's adaptation to drought and oxidative stress (Fu and Huang, 2001). An increase in the levels of the antioxidant enzymes through the overexpression of a zinc finger protein (*ZFP245*) in rice has been reported to enhance plant's tolerance to cold, drought and oxidative stress (Huang et al., 2009). Chloroplast transformation of rice with a manganese superoxide dismutase (*MnSOD*) from pea (*Pisum sativum*), under an oxidative stress-inducible SWPA2 promoter, showed reduced electrolyte leakage compared with wild type leaf discs under polyethylene glycol (PEG) 6000 simulated drought. The results suggest an important role of SOD in ROS scavenging and drought tolerance (Wang et al., 2005).

Drought stress tolerance is a complex trait orchestrated by several metabolic, physiological, biochemical, and molecular responses. Several studies have elucidated many components of this multi-genic trait, thus making possible to understand and exploit the information as tools to develop drought tolerant cultivars.

1.4 Salt stress tolerance

Salinity stress is a major problem in agriculture, affecting 20% of world's irrigated area, and causing ~$27.3 billion losses per year (Qadir et al., 2014). Rice is very sensitive to salt content in the soil, especially in the seedling stage, and can be severely affected by concentrations as low as 20-50 mM NaCl (Greenway and Munns, 1980; Saichuk et al., 2014). The complexity of salt stress tolerance traits has slowed down the progress of the development of salt tolerant crops. Nevertheless, some advances in the development of salt tolerant crops have been reported using phenotypic information of salt tolerant gene pools in crops like rice, barley and maize, but with little understanding of the tolerance mechanisms (Ashraf, 1994).

Many studies have helped to provide a better understanding of high salinity tolerance in plants. Transcriptome analysis has shown that more than 50% of the overexpressed genes during drought stress are also upregulated during salt stress, and more than 98% of salt inducible genes are also upregulated during drought stress (Shinozaki and Yamaguchi-Shinozaki, 2007). The cross-talk between the two stresses is because of the fact that high salt concentrations in the soil causes a physiological drought stress by limiting water uptake due to a negative osmotic potential between the outside and the inside of the plant root (Lee and Iersel, 2008).

By definition there are two mechanisms of salt stress tolerance: (1) by reducing salt intake by the plants; and (2) by decreasing salt concentrations in the cytoplasm (Munns, 2002). While natural variations for salt tolerance within the primary and secondary gene pool of rice

have been exploited for development of salt tolerant rice (Ashraf, 1994), recent studies have hinted at the exploitation of the halophyte resources for development of salt tolerant crops. Halophytes, such as smooth cordgrass can complete their life cycle in high salt concentrations (~200 mM) where more than 99% of other plants would die (Flowers and Colmer, 2008). Halophytes have been used as important models in the elucidation of salt stress tolerance in both dicots and monocots (Joshi et al., 2015). Using salt stress-responsive genes of smooth cordgrass, transgenic rice lines with enhanced salt tolerance have been developed (Baisakh et al., 2006, 2008, 2012; Joshi et al., 2013, 2015).

High salinity inhibits K^+ intake because K^+ transporters, such as HKT1 and LCT1 are nonselective cation channels (NSCs), which do not discriminate between K^+ and Na^+ and import toxic amounts of salt into the cell (Zhu, 2001). Intracellular homeostasis is vital for the proper functioning of the plant during stress. Plasma membrane Na^+/H^+ antiporters, such as the Salt Overly Sensitive1 (SOS1), have an important role in Na^+ exclusion from the cell cytoplasm by exchange and transport activity of H^+-ATPases and H^+ pyrophosphatases that create a proton reactive force to pump Na^+ out of the cell (Zhu, 2003). Expression of a *S. alterniflora* vacuolar ATPase subunit c1 (*SaVHAC1*) enhanced salt stress tolerance of transgenic rice plants, showing increased K^+/Na^+ ratios in leaf and root tissues and stomatal closure in comparison with the wild types (Baisakh et al., 2012).

Osmolytes and osmoproctectants are found in different forms – as sugars (fructose or glucose), sugar alcohols (glycerol, inositol), quaternary amino acid derivatives (betaine, proline) and sulfonium compounds (dimethyl sulfonium propironate; Yokoi et al., 2002; Joshi et al., 2015). These organic compounds are important in salt stress tolerance due to their function to adjust osmotic potential, and preserve enzyme integrity and protein stability in the presence of

salt ions without affecting cell internal pH. Moreover, some of them have shown to have a biochemical function as ROS scavengers with the help of antioxidant enzymes as shown by the accumulation of proline and SOD during salt stress (Serrano et al., 1999; Kartashov et al., 2008). *A. thaliana* plants, constitutively expressing a *Spartina alterniflora* myo-inositol 1-phosphate synthase gene (*SaINO1*), have shown greater tolerance to salt stress with reduced root growth inhibition under salt. Transgenic plants also showed reduced stress symptoms like leaf chlorosis, and proline accumulation, demonstrating that the *SaINO1* gene might be involved in salt stress tolerance due to accumulation of myo-inositol and other related derivative products (Joshi et al., 2013).

1.5 Biotic stresses and some tolerance approaches

Under edapho-climatic conditions favorable for rice production, biotic stresses can be a problem affecting rice production and productivity. In addition to diseases caused by fungi, bacteria, and viruses, insects are harmful to cultivated rice varieties, reducing yield and grain quality. Insects, such as the water weevil (*Lissorhoptrus oryzophilus* Kuschel), stink bug (*Oebalus pugnax*), or stem borers, such as the sugarcane borer (*Diatrea saccharalis*) represent serious problems to rice producers when not controlled properly. Cultural and chemical controls are very important to control infestations of water weevil and stem borers in the absence of resistant varieties due to the polygenic complexity of resistance traits (Stout and Reagan, 2014).

Fall armyworm (*Spodoptera frugiperda*), is an opportunist chewing insect that affects various crops like maize, cotton, rice and other grasses (Meagher and Nagoshi, 2004). Since rice is not the primary host, fall armyworm is considered an occasional (but an important) pest that feeds on the leaves of young plants, causing great damage when present in large numbers (Stout and Reagan, 2014). Fall armyworm management is primarily based on cultural, chemical and

biological controls, which consist of seasonal scouts followed by insecticide applications, weed elimination, and the use of germplasm capable to produce volatile compounds that attract Fall armyworm parasitoids (Yuan et al., 2008; Stout and Reagan, 2014).

Induced resistance studies have demonstrated the importance of phytohormones, such as Salicylic acid (SA) or Jasmonic acid (JA) in plant defense systems. Furthermore, hormonal cross-talk has been reported in plant defense-specific reactions, relating SA in response to sucking insects and JA in response to chewing insects, and both SA and JA work antagonistically to each other (Stam et al., 2013; Stout, 2014).

Transgenic approach has been used to develop rice plants expressing insecticidal crystal proteins (ICP) of *Bacillus thuringiensis* (*Bt*) to confer resistance against stem borers (Ho et al., 2006), but no transgenic rice has been commercially released to date. Many efforts have been dedicated to study induced resistance to understand the complicated phytohormone interaction networks and the development of elicitors that can enhance plant defense mechanisms (Stout and Reagan, 2014). Lack of resistance germplasm against many herbivore insects may change public perception against transgenic rice, and therefore genetic engineering could be a useful tool to develop insect resistant varieties to enhance rice production.

1.6 Jasmonic acid interaction with the basic helix-loop-helix (bHLH)-*Myc2* transcription factor

Jasmonic acid [JA; 3-oxo-2-(2'-pentenyl)-cyclopentaneeacetic acid], is derived from linoleic acid by the action of lipoxygenase (octadecanoid pathway), which catalyzes oxygenation of polyunsaturated fatty acids (Vick and Zimmerman, 1983). JA and its derivative methyl jasmonate (MeJA) were first identified as plant growth inhibitors known to stimulate plant senescence (Vick and Zimmerman, 1984; Hodson and Bryant, 2012). JA and MeJA upregulate

the expression of *Jar1* gene, a JA-amino synthetase, which is essential for the production of the bioactive form of JA, jasmonyl isoleucine (JA-Ile; Starswick and Tiryaki, 2004). JA-Ile induces the expression of Coronatine Insensitive1 (COI1), a protein containing a leucine-rich repeat (LRR) and an N-terminal F-box, which interacts with proteins targeting them for degradation through ubiquitination. COI1 interacts with the Jasmonate-Zim-Domain (JAZ), a repressor of the JA signaling, promoting its degradation (Devoto et al., 2002). JAZ family physically interacts with a basic helix-loop-helix (bHLH) *Myc2* TF, a positive regulator of the JA signaling pathway, to repress its activity. JAZ also works as a JA signaling feedback regulator by the production of a COI1 insensitive splice variant after the stimulation of JA-Ile (Chung and Howe, 2009; Narusaka et al., 2003). The nuclear localized *Myc2* TF, referred to as the master regulator of the JA signaling pathway, contains a G-box motif (5'-CACGTG-3') for DNA binding specificity, and is known to upregulate different genes involved in plant defense and JA biosynthesis, such as *VSP2*, *PDF1.2*, *TAT*, *LOX2* and *PR1* in *Arabidopsis thaliana* (Lorenzo et al., 2004). JA is known to accumulate during insect attack and wound damage. Plants with silenced JA acid signaling pathway by the downregulation of genes, such as Myc2 itself or upstream lipoxygenase, showed increased susceptibility to herbivore insect populations, suggesting that Myc2 is involved in plant defense mechanisms (Kessler et al., 2004; Lorenzo et al., 2004).

Overexpression of the *bHLH-Myc2* TF results in ABA sensitive plants, suggesting that ABA stimulates *Myc2* expression in a cross-talk with JA. Thus *Myc2* is expressed during drought and oxidative stress, and is known to upregulate the ABA responsive gene *RD22* during stress (Abe et al., 2003). *Myc2* is believed to participate in the regulation of the circadian clock, light signaling, and many studies have reported a Myc2 and VSP2 expression reduction under dark (Verhage et al., 2001; Kazan and Manners, 2013). In rice, *Myc2* have been reported to be

involved in spikelet development by the upregulation of genes like the *OsMADS1*/*LHS1*, which are involved in floral meristem initiation and specification (Cai et al., 2014). Thus, *Myc2* plays an active role in many plant development and stress response mechanisms, which makes it an important target to elucidate its active involvement in multiple stress responses of rice.

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CHAPTER 2: MATERIALS AND METHODS

2.1 Plant material and growth conditions

Dehusked seeds of transgenic (described below in 2.4) and wild type (WT) rice cultivar 'Nipponbare' were pre-sterilized with 70% ethanol by manual shaking for 1 min. Then the seeds were rinsed twice with autoclaved distilled water (ADW). Surface sterilization was done with 50% Clorox with a drop of tween-20 under constant agitation for 15 min. After that, seeds were rinsed 5-6 times with ADW, excess of water was dried with sterile filter paper and seeds were placed on petri dishes with MS + 2, 4-D (2.0 mg/L) for callus induction or ½ MS basal media (MS_0; Murashige and Skoog, 1962), supplemented with Hygromycin B (50 µg⁄ml) for germination of transgenic seeds. Seeds for callus induction were maintained in a growth chamber at 26±1 °C under continuous dark. Hygromycin-positive 7-day-old seedlings were planted in 1 gallon pots and maintained in the greenhouse at 29⁄21 °C day⁄night temperature regime under natural day light condition. WT seeds were germinated on MS basal media without Hygromycin.

2.2 MYC2 alignment and phylogeny

The protein sequence of *OsMyc2* TF (LOC_Os10g42430; Appendix I) was retrieved from the Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/). Myc2 orthologs (Appendix II) were obtained from the plant genomic resource Phytozome 10.3 (http://phytozome.jgi.doe.gov/pz/portal.html). All the sequences were aligned for phylogeny studies using the multiple sequence alignment tool ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

2.3 Cloning of *OsMyc2* and construction of plant transformation vector

OsMyc2 (2100 bp), was cloned from the first strand cDNA prepared from Nipponbare RNA, and it was then amplified using the following primers. OsMYC2-F: 5'-

GGCCAGATCTATGAACCTTTGGACGGACGACAACG containing the *Bgl* II restriction site (underlined) and OsMYC2-R: 5'-GAACGCTAGCTTACCGGGCGGCGGTG containing the *Nhe* I restriction site (underlined). The PCR recipe and conditions were same as described earlier (Baisakh et al., 2012). A master mix formed by approximately 100 ng of template DNA were used, 50 ng of forward and reverse primers, 200 µM dNTPs, 2 mM $MgCl_2$, 1 U *Taq* DNA polymerase and 1x PCR buffer in a total reaction volume of 25µl. Thermal profile was as follows: Initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 45 sec, annealing at 60 °C for 45 sec and extension at 72 °C for 1 min. A final cycle of primer extension was carried out at 72 °C for 10 min. The PCR product was partially double-digested for 10 min with a mixture of *Bgl* II and *Nhe* I at 37 °C. The digested product was run in a 1 % agarose gel and the 2100 bp fragment was excised from the gel, and was eluted using the Qiaquick gel extraction kit (Qiagen Inc, Valencia, CA). The fragment was then ligated to the pCAMBIA1301 vector (CAMBIA, Canberra, Australia) digested with the same restriction enzymes) using T4 DNA ligase kit (Invitrogen, Carlsbad, CA) as per the manufacturer's instructions. The recombinant plasmid was transformed to *Escherichia coli* using the heat shock method (Sambrook and Russell, 2001). Briefly, the ligation product was mixed with 100 µl chemically competent *E. coli* cells and kept on ice for 30 min, and then the mixture was incubated at 42 °C for 60 sec in a water bath followed by a cold treatment on ice for 2 min. Then 1 ml of Luria-Bertani (LB) liquid medium was added to the mixture and cells were grown at 37 °C for 1 h with constant shaking at 200 RPM in a shaker incubator. The cells were precipitated by centrifuging at 4000 RPM for 5 min and the pellet was re-suspended in 100 µl of LB liquid medium. The putatively transformed bacteria were streaked on plates containing LB solid medium and kanamycin (50 µg/ml) for selection. The plates were kept overnight inside an

incubator maintained at 37 °C. The next day, a few colonies were individually grown in LB liquid medium supplemented with kanamycin (50 µg/ml) at 37 °C overnight in an incubator shaker. The plasmids were extracted using the JenJet plasmid extraction kit (Fermentas, Amherst, NY). Plasmids were subjected to PCR analysis using *OsMyc2* cloning primers to identify plasmids containing the 2100 bp *OsMyc2* insert. The integrity and orientation of the insert in the recombinant plasmid (pCAMBIA1301/*OsMyc2*; Figure 2.1) were checked by restriction enzyme digestion and further verified by sequencing at the Gene Lab of LSU School of Veterinary Medicine.

The RNAi plasmid, used for the generation of knock down rice mutants, was kindly provided by Dr. Yinong Yang, Pennsylvania State University. KD mutants used in the present study were previously generated in Baisakh lab (Mangu et al., unpublished).

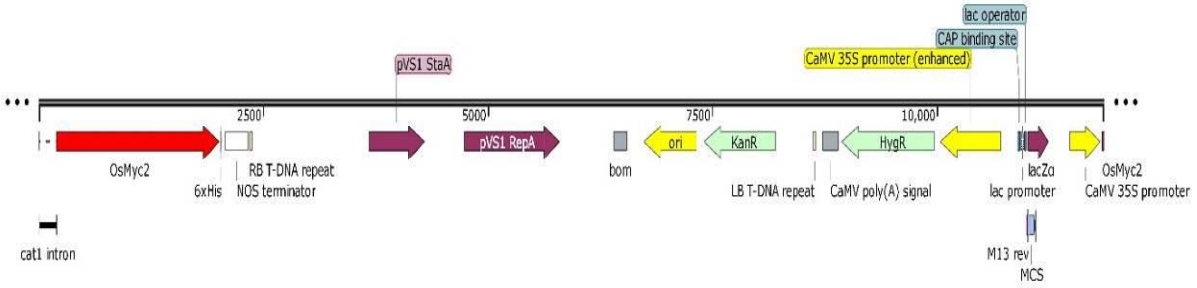

Figure 2.1. Linear vectors pCAMBIA1301/*OsMyc2*

2.4 *Agrobacterium tumefaciens*-mediated transformation

The recombinant plasmid (pCAMBIA1301/*OsMyc2*) was mobilized into the *Agrobacterium tumefaciens* strain LBA4404 by freeze-thaw method as described earlier (An et al., 1988). Ten µg of plasmid were mixed with 50 µl of competent cells and kept on ice for 30 min. The cells were then frozen in liquid nitrogen and immediately given heat shock at 37 °C for 4 min. Then the cells were cooled down on ice for 1 min, 1 ml of YEP media was added, and the

cells were incubated at 28 °C 4 h in shaker at 200 rpm. The cells were then centrifuged at 5000 RPM for 5 min and re-suspended in 100 µl of YEP medium. The bacterial cells were plated on YEP-agar plates containing of Rifampicin (20 µg/ml), tetracycline (5 µg⁄ml) and kanamycin (50 µg⁄ml). Individual colonies were multiplied on YEP liquid media and storage at -80 °C.

Embryogenic callus produced from mature (dehusked) seeds (described in 2.1) were genetically transformed as described earlier (Rao et al., 2009). LBA4404/pCAMBIA1301/OsMyc2 was pre-cultured for 48 h at 28 °C in YEP solid media with antibiotics, rifampicin (20 µg⁄ml), tetracycline (5 µg⁄ml) and kanamycin (50 µg⁄ml). The pre-cultured bacteria was sub-cultured in fresh AB liquid media with the same antibiotics and grown for 24 h. Bacteria cells were re-suspended in liquid MS medium containing 2 mg⁄L 2,4-D and 100 µM acetosyringone (AS) to a final concentration of A_{600} = 1.0 for transformation.

Three to four-week-old seed-derived rice embryogenic callus were vacuum-infiltrated (0.4–0.6 atm) with the engineered *Agrobacterium* suspension for 15 min and co-cultivated for 3 days on solid N6 (Chu et al., 1975) co-cultivation media at 25 °C under dark. Following co-cultivation, the calli were washed thrice in sterile distilled water and finally in liquid MS medium containing cefotaxime (250 µg/ml) and carbenicillin (250 µg/ml). The calli were then plated on solid MS medium containing the cefotaxime, carbenicillin and hygromycin (50 µg/ml) as the selection agent. Selection and regeneration of the putative transgenic callus was performed following the method described by Baisakh et al. (2001). The OsMyc2 RNAi transgenic rice lines used in this study were previously generated in Dr. Baisakh's laboratory. Henceforth, wild type (WT), overexpresser (OE), and knock down (KD) have been referred to as genotypes, and independent events within a genotype have been referred to as lines. All OE and KD lines were subjected to drought stress in T_1 generation, and five independent OE lines showing less drought

symptom and three KD lines were advanced in the greenhouse to achieve homozygosity in T_2 generation.

2.5 Subcellular localization of OsMyc2

Green fluorescence protein (GFP) was used as the reporter marker to detect the subcellular localization of OsMyc2. *OsMyc2* gene without the stop codon was isolated from rice cDNA with OsMYC2-fus-F 5'-GGCCAGATCTATGAACCTTTGGACGGAC and OsMYC2-fus-R 5'- CTAGACTAGTCCGGGCGGCGGTGCC primers containing the restriction sites for *Bgl* II and *Spe* I, respectively using the Phusion High-Fidelity PCR kit (New England Biolab, UK). The purified *OsMyc2* was cloned into pCAMBIA1304 vector digested with same restriction enzymes and before gfp in frame. The resulting pCAMBIA 1304/*OsMyc2-gfp* (Figure 2.2) and the pCAMBIA 1304 (as control) were bombarded onto onion epidermal cells using a PDS1000He particle gun (Bio-rad, Hercules, CA) as described in Joshi et al (2013). The GFP expression was visualized using a fluorescent microscope.

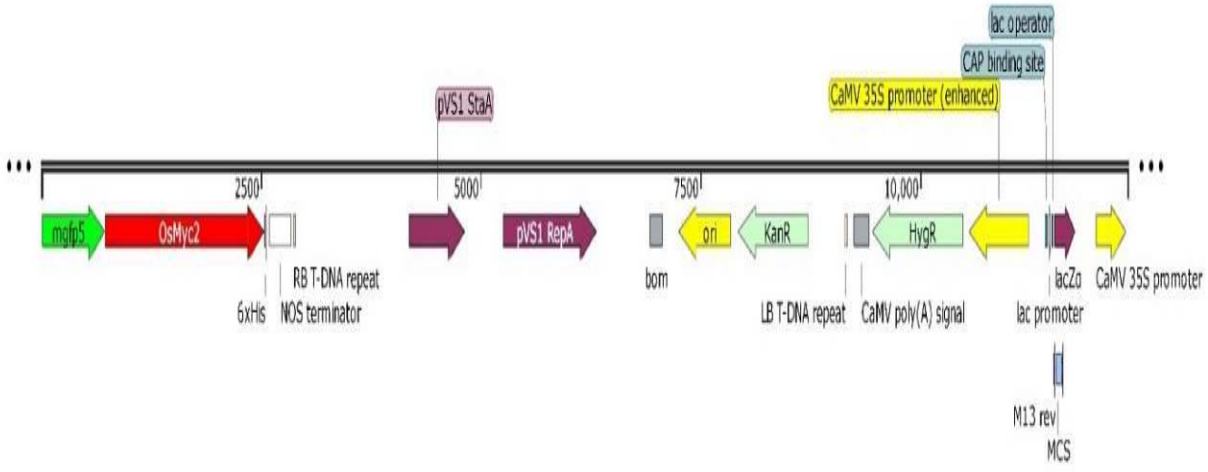

Figure 2.2. Linear vectors pCAMBIA1304/*OsMyc2-gfp*

2.6 Stress treatments

Non-transformed wild-type (WT), transgenic *OsMyc2* overexpresser (OE) and RNAi (KD) lines of rice cultivar 'Nipponbare' were germinated on ½ MS_0 media at 26 °C under 12 h/12 h light/dark regime inside a growth chamber. Ten one-week-old seedlings per genotype were placed on Styrofoam seedling float on Yoshida solution (Yoshida et al., 1976). Four-weeks-old rice seedlings were subjected to salt stress (150 mM NaCl) under hydroponics following the method described earlier (Baisakh et al., 2012). Floating leaf assay was prepared using leaf pieces (~2cm long), and placing them on Hoagland solution (Hoagland, 1950) with NaCl in concentrations of 0 (control), 100 mM and 150 mM.

One-week-old seedlings of WT and transgenic rice lines (6 plants/genotype) were planted in pots filled with garden soil:potting mix (3:1) inside the greenhouse maintained at 29⁄21 °C day⁄night temperature regime under natural day light condition during Spring 2014 and Fall 2014. Drought stress was imposed on 45-day-old plants by withholding water for 14 days following which water was resumed until maturity as described by Joshi et al. (2014).

2.7 RNA isolation, cDNA synthesis and expression of *OsMyc2* under drought stress

Leaf tissue was collected from unstressed control and drought-stressed plants at 7 and 14 days after stress treatment. Total RNA was isolated from ~100 mg leaf tissues of control and stressed plants using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's manual. Quality of total RNA was checked in a 1.2% formamide-denaturing agarose gel and quantification was done using a ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). First strand cDNA synthesis was carried out using iScript™ cDNA synthesis kit (Bio-Rad, Hercules, CA).

Semi-quantitative PCR was performed using cDNA as described by Baisakh et al. (2012) using OsMyc2-RT-F 5'- AAGCTCAACCAGCGCTTCTA and OsMyc2-RT-R 5'- CCTTCTTGAGCGACTCCATC specific primers. The rice Actin 1 gene (*OsAct1*) was used as the internal control for template validation. For qRT-PCR same 1st strand cDNA was used. PCR was performed with three biological replications using SYBR green master mix (Bio-Rad, Hercules, CA) in a MyiQ Real-Time PCR detection system (Bio-Rad, Hercules, CA). The rice elongation factor gene (*OsElf1a*) was used as the reference gene for normalization of gene expression difference, and expression values relative to WT under control were calculated as described by Joshi et al. (2013).

2.8 Physiological analysis of drought stressed plants

Physiological parameters such as chlorophyll fluorescence, relative water content (RWC), and membrane stability index (MSI) were taken on greenhouse-grown WT, OsMyc2 OE and KD lines at 0 (control), 3 and 7 days after withholding water. All physiological data were collected from four plants (biological replicates) of WT, and four independent lines of OE and three independent lines of KD.

2.8.1 Estimation of photosynthetic yield

Chlorophyll fluorescence was measured in dark adapted plants with a portable fluorometer (PAM-2100; Walz, Germany). The minimal fluorescence level (Fo) with all photosystem (PS) II reaction centers open was determined by measuring the modulated light, which was sufficiently low. Maximal fluorescence level (Fm) with all PSII reaction centers closed was determined by a 0.8-s saturating pulse in dark-adapted leaves. Chlorophyll fluorescence was measured as Fv/Fm where Fv = Fm - Fo.

2.8.2 Relative water content (RWC)

The RWC of the leaves was determined following the procedure of Slatyer (1967). Middle sections of second-youngest fully expanded leaves were collected and weighed [fresh weight (FW)]. The leaf pieces were immersed in dH_2O placed in dark at 4 °C overnight and weighed after brief blot-drying to remove excess water [turgid weight (TW)]. Then, the pieces were dried at 60 °C for 48 h and weighed [dry weight (DW)]. RWC was estimated in percentage of the water content at a given time and tissue as related to the water content at full turgor using the formula:

$$RWC\ (\%) = [(FW - DW)/ (TW – DW)] \text{ X } 100$$

2.8.3 Membrane stability index (MSI)

Membrane stability index (MSI) was determined as described by Sairam et al. (2002). Leaf samples (~0.1 g) were placed in 10 ml of ddH_20 and heated at 40 ºC for 30 min in a water bath. Then the electrical conductivity of the solution was recorded (C_1) using a hand-held pH/conductivity/TDS tester (Hann Instruments, Woonsocket, RI). Again samples were boiled on a water bath for 10 min, and conductivity of each sample was measured (C_2). The membrane stability index (MSI) was calculated as:

$$MSI = [1– (C1/C2)] \times 100$$

2.9 Phytohormone treatments

Seeds of five plants (biological replicates) of WT, OE, and KD each were germinated in ½ MS_0 media, and five 5-days-old seedlings , were placed in petri dishes containing MS_0 media with either jasmonic acid (100 and 50 µM), methyl jasmonate (50 µM), abscisic acid (50 µM), or gibberellic acid (50 µM). After 7 days of treatment with hormones, length of the shoots and roots was measured, and tissue samples were taken for RNA extraction. RT-PCR was conducted using

the *OsMyc2* primers as described in section 2.7. An ABA sensitivity assay was performed with seeds where 10 seeds (per plate) of WT, OE and KD lines were placed on ½ MS_0 media with 8 or 10 µM ABA. Germination percentage was taken after 7 days.

2.10 Fall armyworm culture and feeding assays

Fall armyworm culture and feeding assays were conducted according to Stout et al. (2009). The insect that was used in the present experiment came from a colony originated from the larvae collected in Bermuda grass pastures in Baton Rouge in 1997. Leaf pieces (~2 cm) of 10 plants (biological replicates) of each of four independent lines of OE, three independent lines of KD, and WT rice were put inside petri dishes layered with moist cotton. First instar-larvae were placed into the petri dishes with enough leaf (~4 per week) in order to never limit their feed. After 7 days, larvae were taken out of the petri dishes and weighed. Larvae were returned to the plate to complete their life cycle. The time that the larvae took to reach the pupae stage and their weight were taken.

2.11 Agronomic traits

Flowering time was measured as the time taken from seed germination until the first panicle emerged. Above ground plant tissues without panicles were dried at 50 °C for 48 h and weighed for determining shoot dry biomass. Grain yield (gram) was estimated by weighing all the seeds harvested from each plant. Other agronomic traits, such as plant height, number of tillers per plant, and percentage of fertility were taken for all genotypes. For all agronomic traits 10 plants (biological replicates) of each of five independent lines of OE, three independent lines of KD, and WT were used.

2.12 Statistical analyses

All physiological and agronomic data were analyzed by a one way ANOVA using PROC-GLM. Fisher's least significant difference (LSD) was used for a post-ANOVA analysis on mean observations. The level of significance was tested at 5% using 'F' test. All statistical analyses were performed using SAS version 9.4 (Copyright 2002-2012, SAS Institute, Cary, NC).

2.13 References

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CHAPTER 3: RESULTS

3.1 Alignment and phylogeny analysis of rice *Myc2* transcription factor

The cDNA sequence (2.1 Kb) of rice *Myc2* (LOC_Os10g42430) transcription factor was retrieved from the rice genome annotation project database (http://rice.plantbiology.msu.edu). Located on the 10th chromosome, OsMyc2 contains a basic helix loop helix structural motif and a G-box element (5'-CACGTG-3'), which provides DNA binding specificity (Figure 3.1).

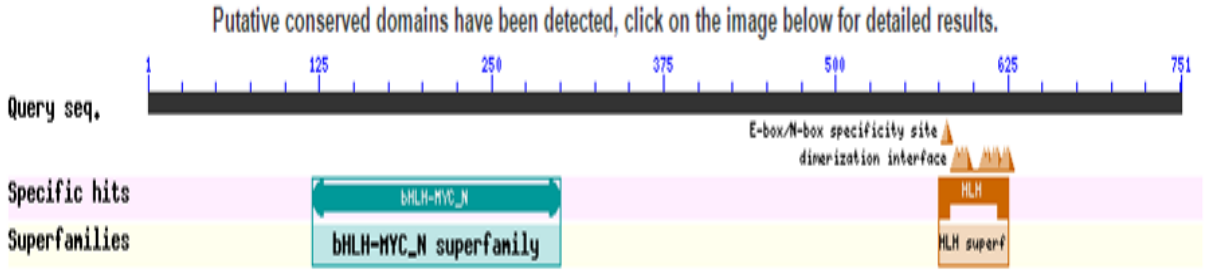


Figure 3.1. Motif and structure analysis of the OsMyc2 protein sequence

The *OsMyc2* used in this study is homologous to the *Arabidopsis thaliana Myc2* (AT1G32640.1) and to 47 other Myc2 homologs from different species (Appendix II). Multiple alignment of Myc2 protein sequences showed highly conserved regions among different species (Appendix III). Inter-species identity matrix indicated that the OsMyc2 was most similar to the homolog from *Sorghum bicolor* (81.80%) and was most distant from *Eutrema salsugineum* (47.84%). It shared 54.5% similarity with *Arabidopsis thaliana*. The phylogenetic tree constructed with alignment-based similarity matrix showed a cluster representing Myc2 members of the graminae family (Figure 3.2).

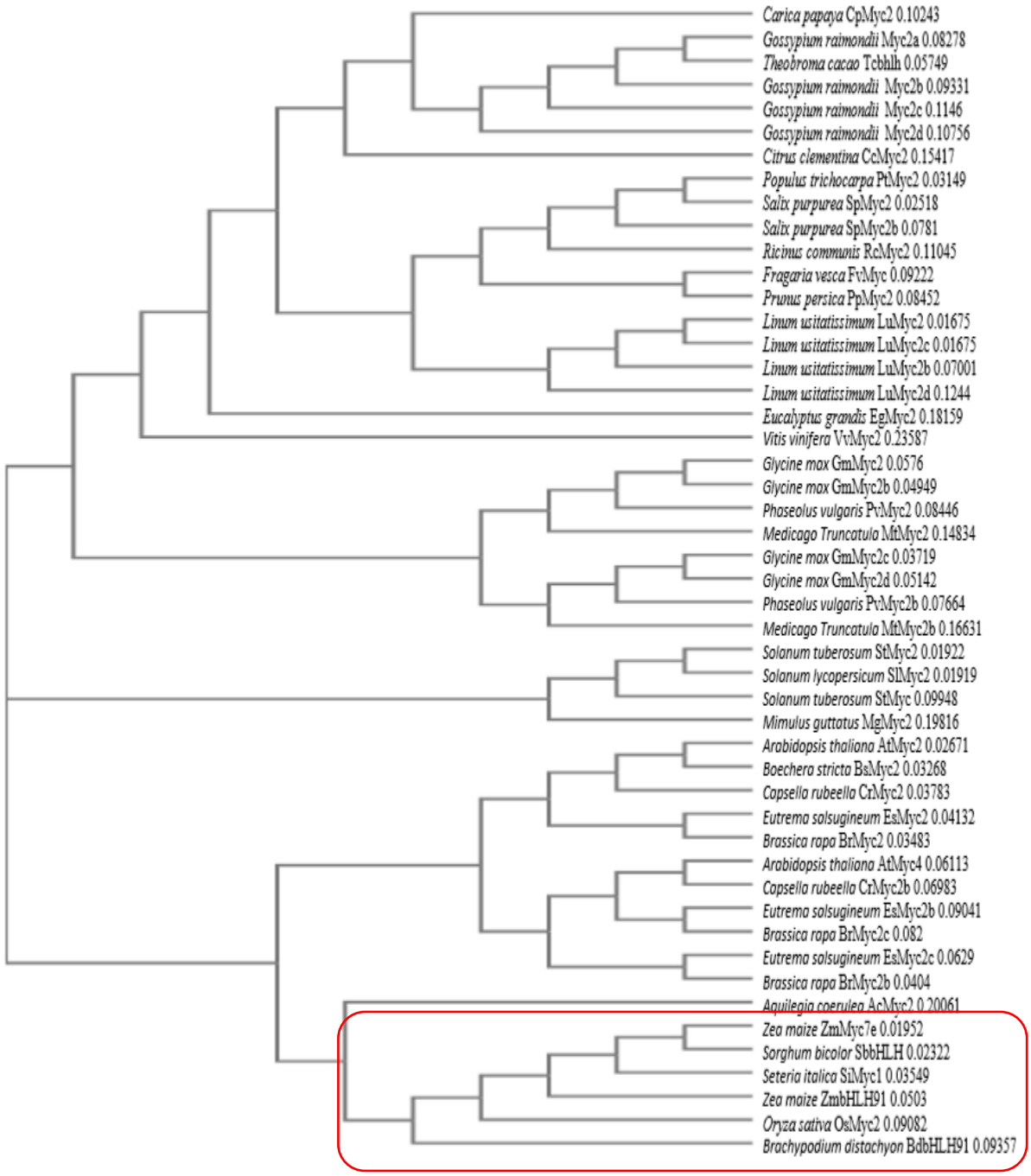


Figure 3.2. Phylogenetic tree constructed using the identity matrices of 48 Myc2 homologous sequences from different plants (Details provided in Appendix II)

3.2 Development of transgenics

A total of 40 independent transgenic events were obtained through *Agrobacterium tumefaciens*-mediated transformation. *OsMyc2* gene integration was confirmed by the amplification of a 760 bp fragment of selectable marker gene *hpt* (hygromycin phosphotransferase) in transgenics (Figure 3.3).

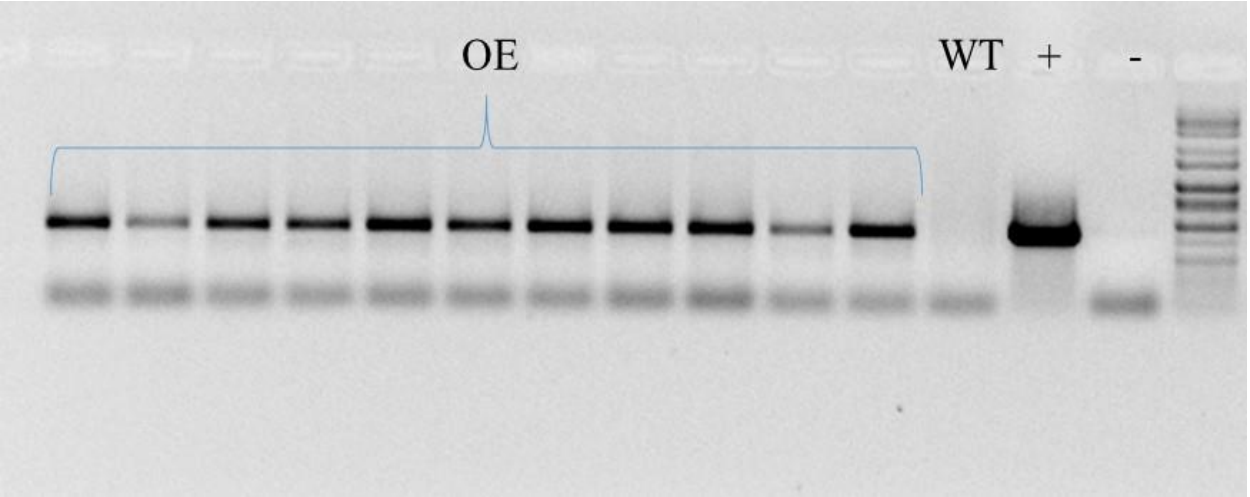


Figure 3.3. A representative gel showing the amplification of the 760 bp *hpt* gene fragment demonstrating T-DNA insertion in the genome of transgenic plants, but not in non-transformed wild type (WT). Water (-) was included as the no template control, and the plasmid pCAMBIA1301 was used as the positive (+) control

3.3 Subcellular localization of OsMyc2

Fluorescence microscopy of onion epidermal cells bombarded with the fusion plasmid pCAMBIA1304/*OsMyc2*:*gfp* and the non-modified plasmid pCAMBIA1304 (control) showed that OsMyc2 expression was localized in the nucleus (Figure 3.4a), whereas the GFP protein expressed under CaMV 35S promoter was expressed in the whole cell (Figure 3.4b).

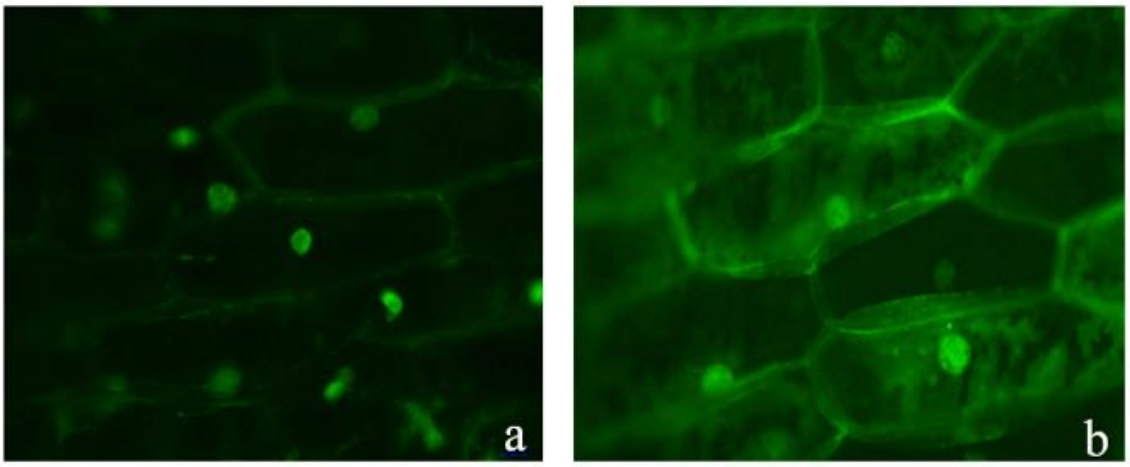


Figure 3.4. Subcellular localization of the rice *bHLH-Myc2* transcription factor using the reporter gene *gfp* and visualized in onion epidermal cells after particle bombardment, a) pCAMBIA1304/*OsMyc2*:*gfp* fusion vector, and b) pCAMBIA1304 empty vector

3.4 *OsMyc2* overexpression enhanced plant abiotic stress tolerance

Drought stress was imposed on 45-day-old plants by withholding water for a period of 14 days. *OsMyc2* OE lines showed reduced stress symptoms in comparison with the WT and KD mutants which started to show dehydration symptoms, such as leaf rolling and drying from day 7 onwards (Figure 3.5a). After 14 days of water deprivation, OE lines started showing drought symptoms, but the WT and some KD plants were almost dead. Upon resuming watering, the OE lines showed signs of recovery whereas the WT and KD plants were either dead or were unable to recover (Figure 3.5b). The stressed OE plants had higher biomass (with an increase of 58.6% to 248.3%) and longer roots (with an increase of 26.8% to 43.4%) as compared to the stressed WT. On the other hand, stressed KD55 and KD67 showed 8.6% and 19.0% reduction of biomass, respectively as compared to stressed WT (Appendix IV).

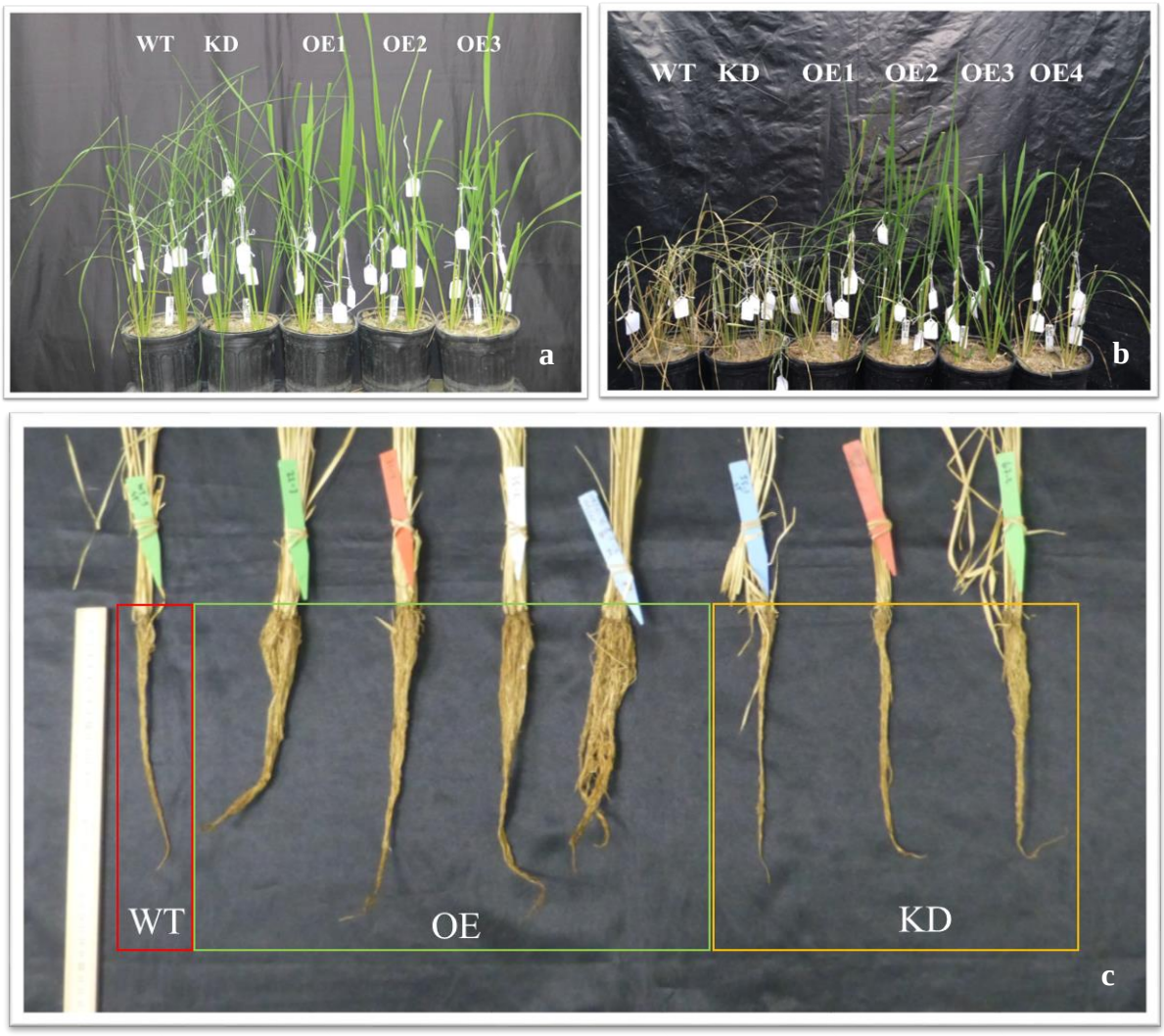


Figure 3.5. Wild type (WT), overexpresser (OE), and knock down (KD) rice plants at (a) 7 days and (b) 14 days after water withholding; (c) Root development of stressed plants

To determine if *OsMyc2* is involved in salt stress response of plants, a floating cut-leaf assay was performed with leaf pieces of WT, OE and KD plants at different salt (NaCl) concentrations (0 – control, 100 mM and 150 mM). Leaves of WT and KD lines showed higher chlorosis (chlorophyll bleaching) symptoms after 3 days as compared to leaves from OE lines (Figure 3.6). This result suggested a possible involvement of *OsMyc2* in salt stress tolerance mechanism, but seedling screening in hydroponic condition under 150 mM NaCl concentration did not show any difference in chlorosis and leaf drying among the genotypes.

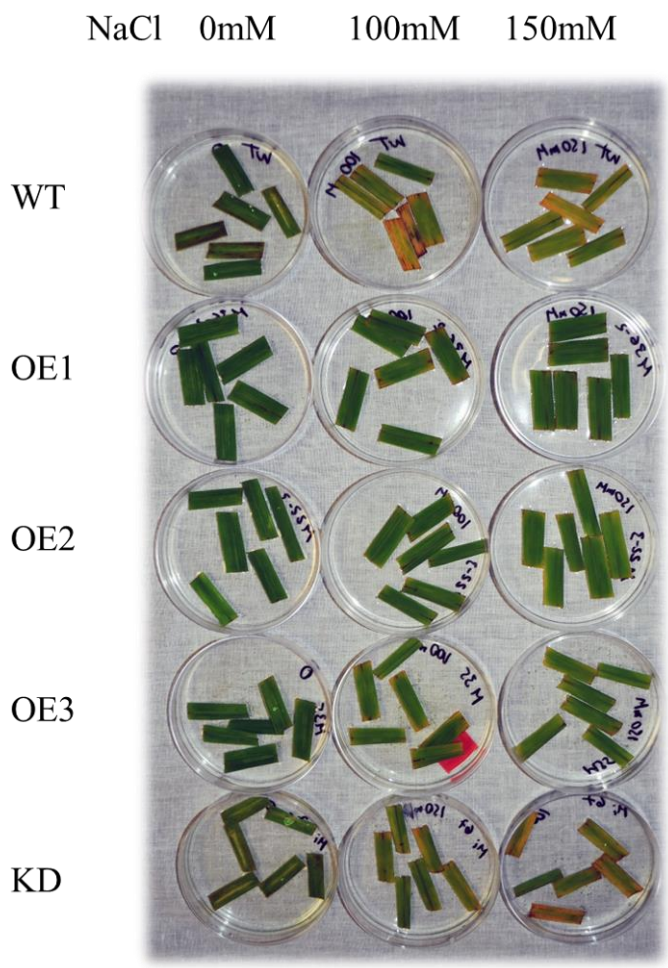


Figure 3.6. Salt tolerance screening by floating cut-leaf assay of wild type (WT), overexpresser (OE), and knock down (KD) rice genotypes on Hoagland solution under control (0 mM NaCl) and salt (100 mM and 150 mM NaCl) stress

3.5 Physiological response of drought stressed plants

The stomatal conductance did not show statistically significant difference (P = 0.82) among the WT, OE and KD lines under non-stressed control condition (Figure 3.7). But on the third day of stress, although drought symptoms were not apparent, stomatal conductance reduction was observed in all the lines and differences were evident between genotypes (P < 0.05). By day 7, WT and KD plants started to show severe stress symptoms as indicated in Figure 3.6a, where one-way ANOVA analysis indicated significant differences (P< 0.001)

among different genotypes. Interestingly, all of the OE and a few plants of KD67 showed a high reduction in stomatal conductance in comparison with WT plants.

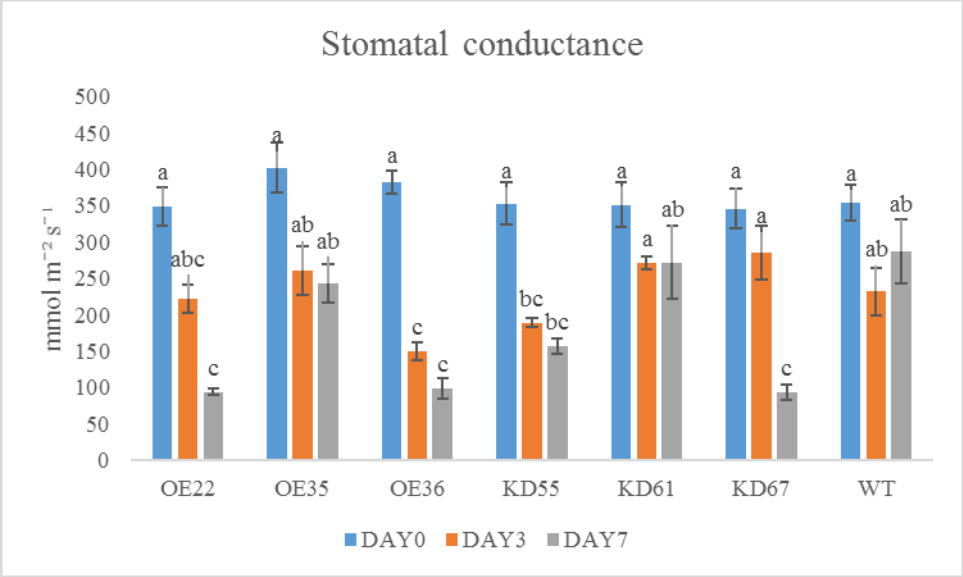


Figure 3.7. Stomatal conductance measured from the leaf samples from WT, OE and KD plants at 0 (control), 3 d and 7 d after drought stress was imposed. Values represent means ± SE of four independent replicates. Different letters represent statistical significance at 5% level based on Fisher's least significant difference (LSD) test across lines

Relative water content (RWC) was >80% in all genotypes until the third day of stress. Leaf rolling and drying with a significant ($P < 0.001$) reduction in RWC was observed at day 7 in WT (<20%) and KD (<40%) plants in comparison with all OE lines, which maintained a higher percentage of RWC (>80%; Figure 3.8a).

Membrane stability index (MSI) didn't show significant differences among genotypes at control (day 0) and at day 3 of withholding water, where the plants maintained >80% MSI (Figure 3.8b). However, at the seventh day, when stress symptoms were visible, a significant statistical difference ($P < 0.001$) was found among genotypes. OE lines maintained higher membrane stability and cellular integrity in contrast to the WT and KD plants.

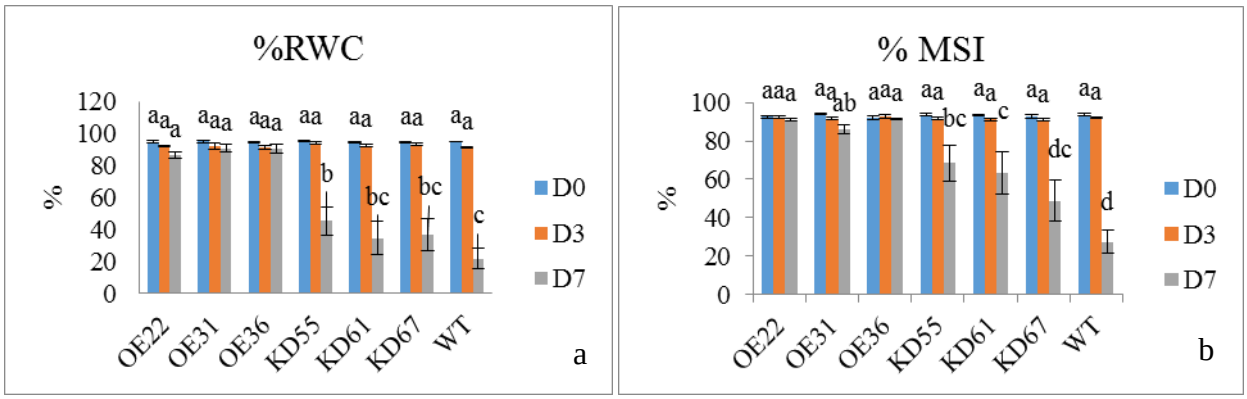


Figure 3.8. a) Percentage of relative water content and b) percentage of membrane stability index of leaf samples of overexpresser (OE), knock down (KD), and wild type (WT) plants during drought stress. Values represent means ± SE of four independent replicates. Different letter represent statistical significance at 5% level based on Fisher's least significant difference (LSD) test across lines

Photosynthetic efficiency of the PSII was determined by calculating the quantum yield of dark-adapted leaf tissues (Fv:Fm). Minimal differences were found among genotypes under non-stressed conditions, and at day 3 under stress, all genotypes recorded an Fv:Fm ratio between 0.6 – 0.7. However, clear differences were seen at day 7, where OE lines showed higher Fv:Fm ratio in comparison with the WT and majority of the KD lines(Figure 3.9a). Soil moisture content of the pots at 0 d, 3 d and 7d after stress imposition did not show significant differences among different genotypes (Figure 3.9b).

The organic compound 3, 3'-Diaminobenzidine (DAB), forms a brown precipitate after oxidation in the presence of H_2O_2. DAB assay with the leaves of WT, OE and KD lines collected from control (day 0) and stressed (day 7) plants showed dark brown coloration in WT and KD plants, indicating increased H_2O_2 accumulation under stress (day 7) in comparison with the OE plants (Figure 3.10).

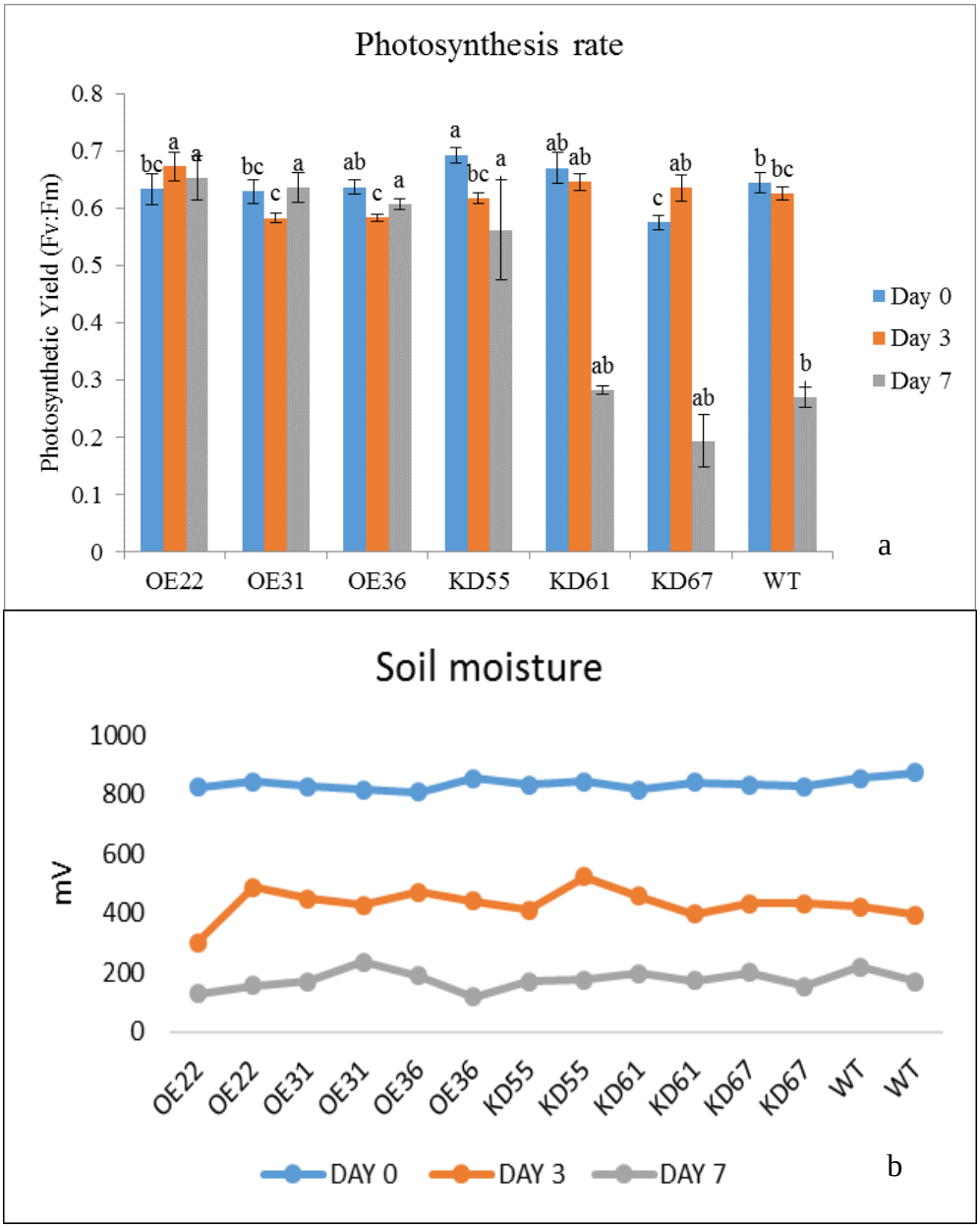


Figure 3.9. a) Photosynthesis efficiency of the PSII represented by the ratio Fv:Fm, measured from the dark adapted leaves of wild type (WT), overexpresser (OE), and knock down (KD) plants at 0 d, 3 d, and 7 d after drought stress imposition. Values represent means ± SE of four independent replicates. Different letters represent statistical significance at 5% level based on Fisher's least significant difference (LSD) test. b) Soil moisture content measured in each pot throughout the drought experiment

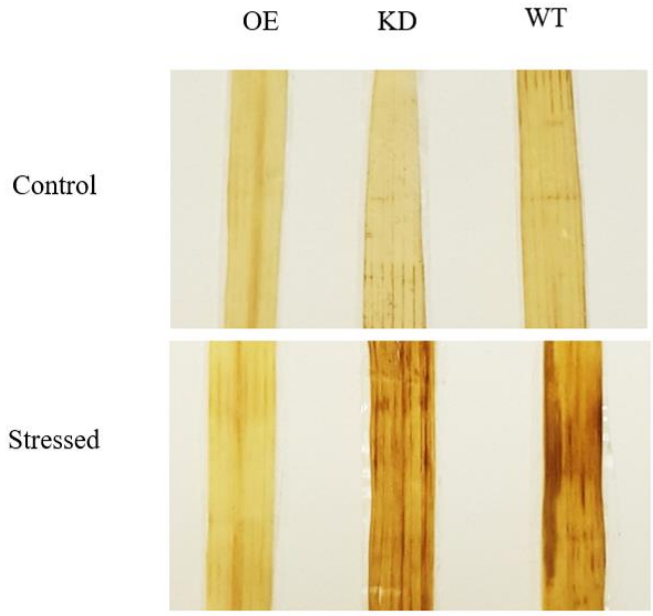


Figure 3.10. 3, 3'-Diaminobenzidine (DAB) assay of leaves from the control and drought stressed (Day 7) plants of overexpresser (OE), knock down (KD), and wild type (WT)

3.6 Gene expression analysis

The *OsMyc2* gene showed tissue-dependent variation in its expression pattern (Figure 3.11). Higher expression of the *OsMyc2* was observed in stem, immature panicle, lemma-palea and ovary. Its expression was relatively low in pollen, seed and stigma, while it was moderate in root and leaf tissues. Except for leaf, the expression seemed to be more in green tissues.

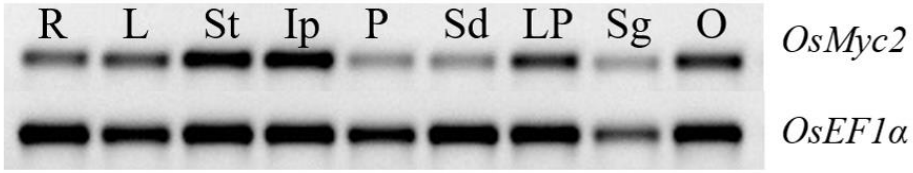


Figure 3.11. Semi-quantitative RT-PCR of the *OsMyc2* in different plant tissues: R = root, L = leaf, St = stem, Ip = immature panicle, P = pollen, Sd = seed, LP = lemma-palea, Sg = stigma, O = ovary. *OsEF1a* was used as an internal control, which showed similar expression pattern in different tissues

To demonstrate the involvement of *OsMyc2* in stress tolerance, its expression was monitored in OE and KD plants with respect to WT. The results showed that OE maintained a higher basal expression of *OsMyc2* compared to WT and KD lines under control condition (Figure 3.12a). There was an increase in its transcript accumulation in all genotypes under drought stress (Figure 3.12 b).

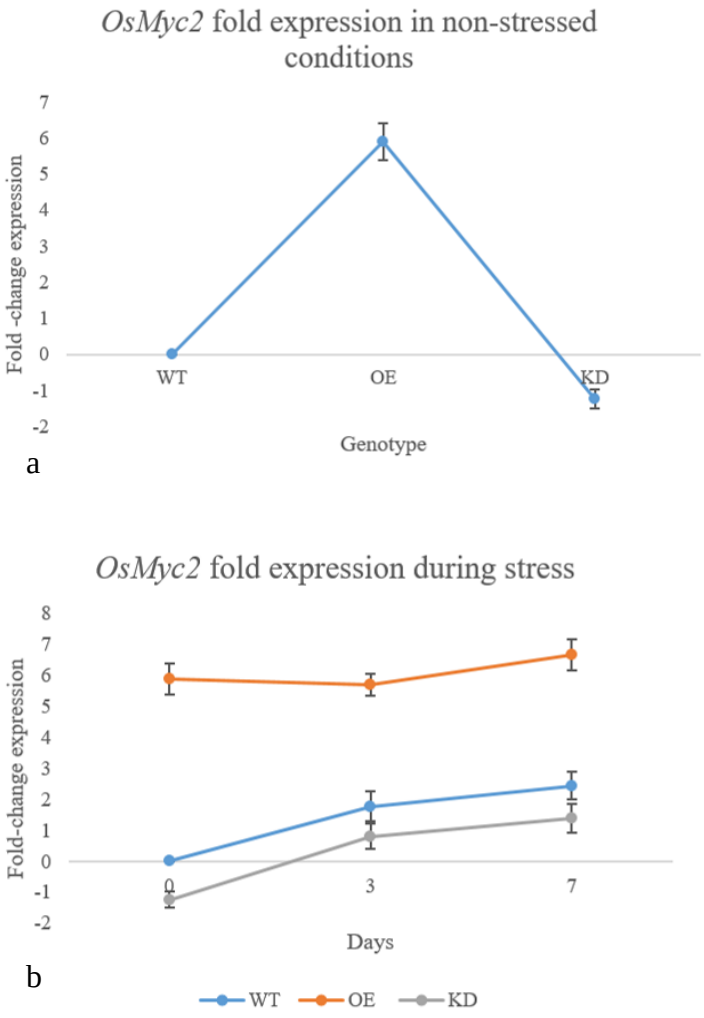


Figure 3.12. RT-PCR of the *OsMyc2* transcription factor under non-stressed control condition (a) and drought stress (b) in wild type (WT), overexpresser (OE), and knock down (KD) lines. Error bars represent standard error calculated using three independent biological replicates

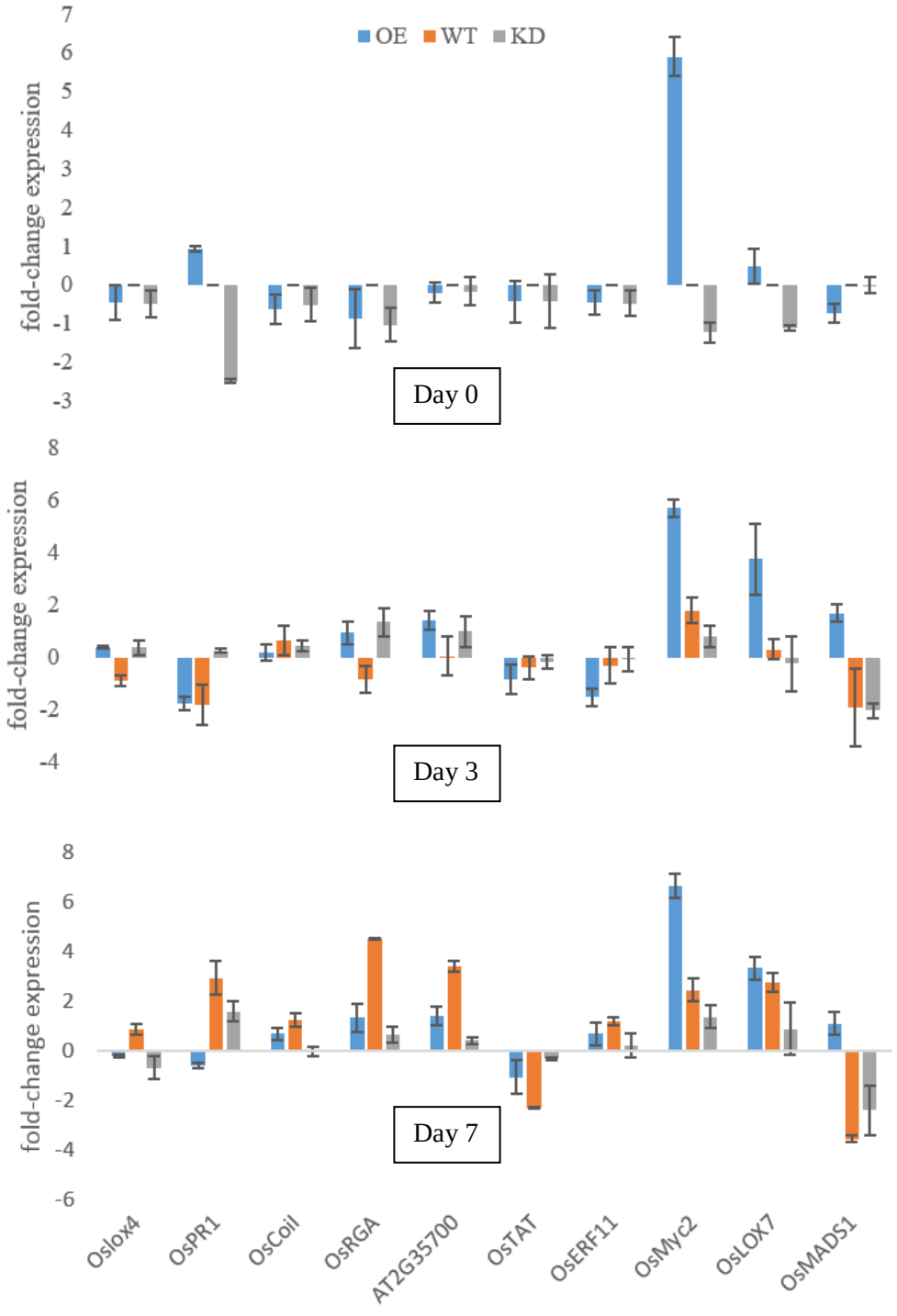


Figure 3.13. qRT-PCR different *Myc2* and stress responsive *Myc2*-related genes in wild type (WT), overexpresser (OE), and knock down (KD) lines. Error bars represent standard error calculated using three independent biological replicates

Expression of 10 different genes that have previously been suggested to be modulated by*Myc2* was analyzed in WT, OE and KD plants under stress. Under non-stressed control, most of the genes had very little endogenous expression in OE and KD lines as compared with the WT. Pathogenesis related protein 1 (*OsPR1, Os01g28500*) showed high basal expression in OE lines. But, at day 3 of stress, up-regulation of gibberellin responsive modulator (*OsRGA, Os01g45860*), lipoxygenase 4 (*OsLOX4, Os03g08220*), lipoxygenase 7 (*OsLOX7, Os08g39840*), a DREB subfamily gene with an AP2 domain from *Arabidopsis thaliana* (AT2G35700, *Os02g43970*) and *OsMADS1* (*Os03g0215400*) was observed in OE lines when compared with WT. At day 7, most of the stress-related genes were upregulated in all lines including the WT plants (Figure 3.13).

3.7 Phytohormone treatment and gene expression changes

In order to analyze the response of the *OsMyc2* transcription factor with different phytohormones and identify possible hormone cross-talk, 5 day old WT, OE and KD seedlings were germinated and treated with JA, MeJA, ABA and GA (Figure 3.14).

Seedlings placed on ½ MS media containing JA (100 µM) suffered shoot length reduction. WT and OE lines showed a shoot length reduction of 67.2% and 71.0%, respectively, whereas KD seedlings had a lower shoot length reduction (43.0%) demonstrating lower sensitivity in response to JA as a result of the downregulation of the *OsMyc2*. Similar results (but with higher sensitivity in OE lines) were observed for root growth. OE lines showed an increased sensitivity (49.0% reduction in length) in comparison with WT (26.2% reduction) and KD (27.5%) plants. Similar trend was observed in their response to MeJA (50 µM; Figure 3.15b), where a reduction of 60.6% and 62.0% of shoot growth was observed in WT and OE seedlings, respectively, and KD seedlings had a reduction of 41.3%. WT, OE, and KD plants resulted in

root length reduction of 16.4 %, 53.1% and 5.0%, respectively. For both hormones, OE lines showed an enhanced sensitivity in comparison with WT and KD lines, especially in root growth. In contrast, KD lines with downregulation of the *OsMyc2* showed reduced sensitivity to JA.

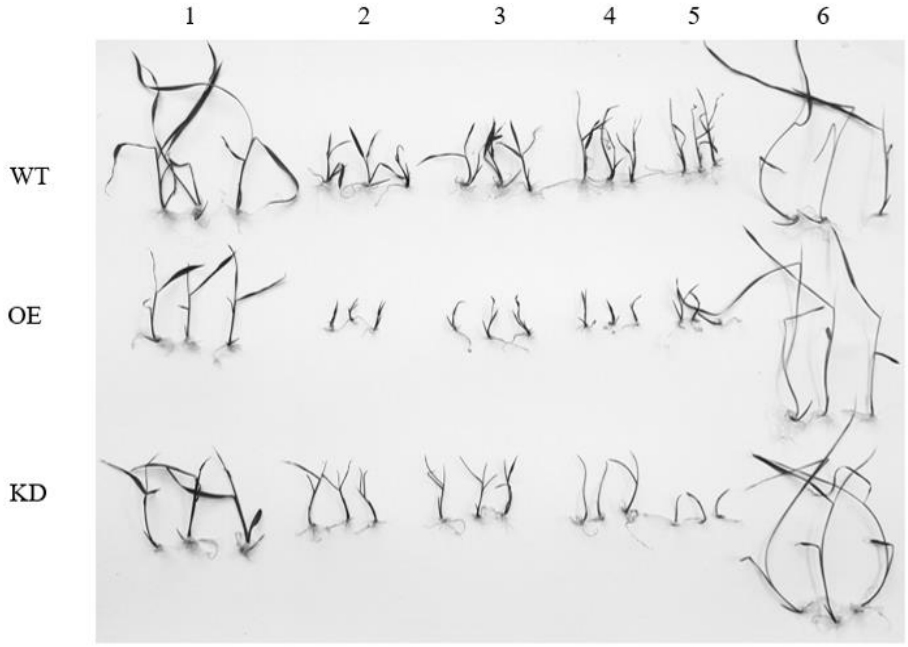
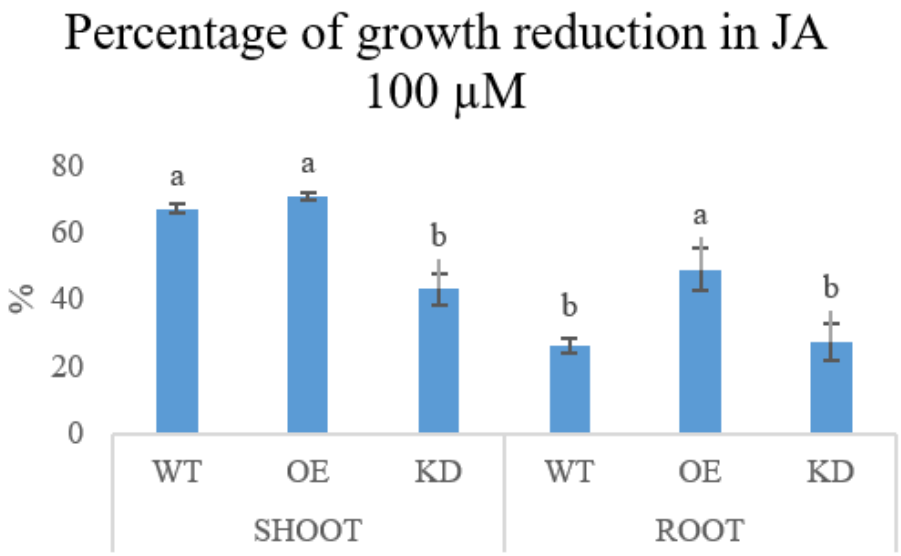
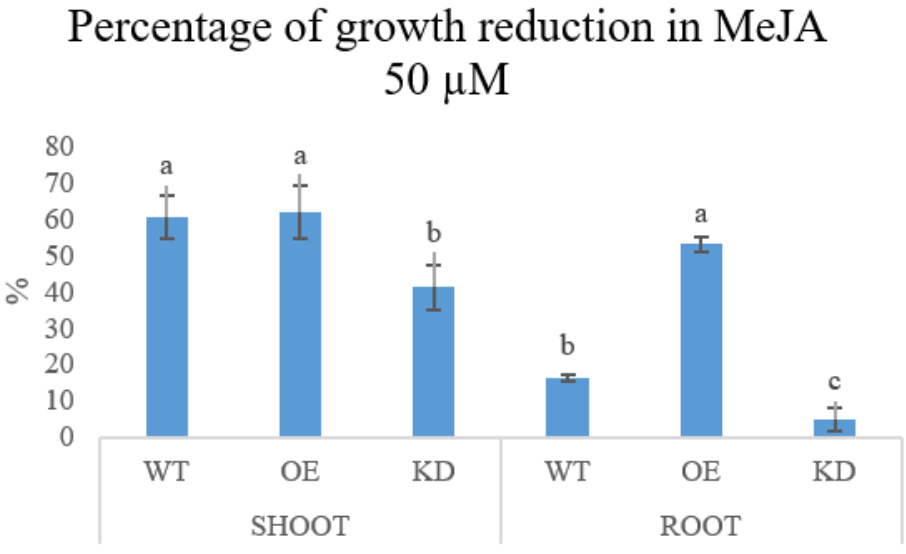


Figure 3.14. Wild type (WT), overexpresser (OE), and knock down (KD) seedlings treated with: 1 = Control, 2 = 100 µM JA, 3 = 50 µM JA, 4 = 50 µM MeJA, 5 = 50 µM ABA, 6 = 50 µM GA

Myc2 was shown to be induced by ABA. Higher growth reduction was observed in WT (69.9%, 16.0%) and KD (72.3%, 42.8%) as compared to OE (55.6%, 0.7%) for both shoot and root, respectively (Figure 3.16a). Growth enhancement was observed in all the genotypes when treated with 50 µM GA (Figure 3.17b). WT, OE and KD recorded a growth increase of 131.6%, 208.1%, and 184.5% for shoots, and 136.5%, 146.9%, and 130.6% for the roots. GA treatment exerted more influence on the shoot growth compared to the root growth, but all lines showed better growth of shoot under GA.

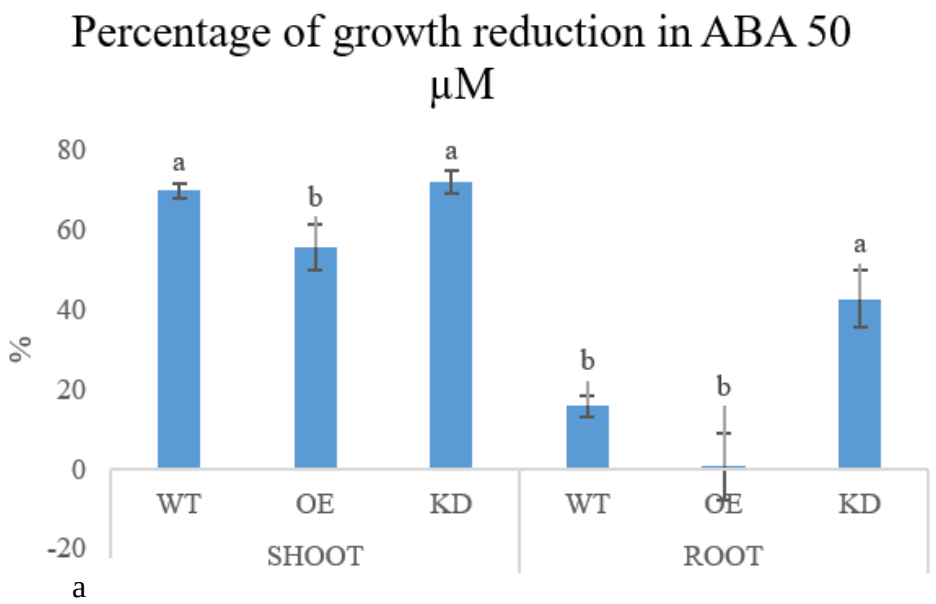


a

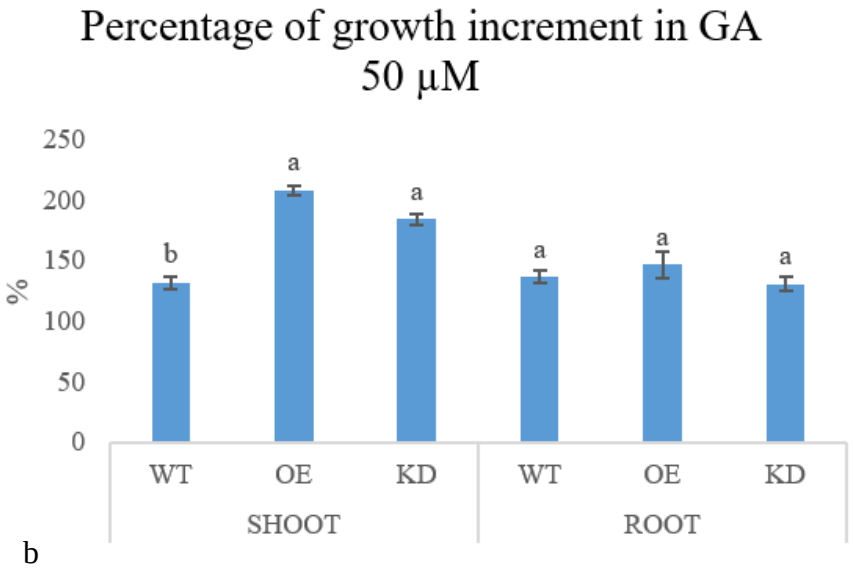


b

Figure 3.15. Percentage shoot/root growth reduction of wild type (WT), overexpresser (OE), and knock down (KD) seedlings placed on ½ MS media containing a) jasmonic acid (100 µM) and b) methyl jasmonate (100 µM)



a



b

Figure 3.16. Percentage of shoot and root growth reduction of wild type (WT), overexpreseer (OE), knock down (KD) seedlings placed on ½ MS media containing a) abscisic acid (50 µM) and b) gibberellic acid (50 µM)

Expression analysis showed that genes, such as *OsVSP2*, *OsLOX7*, *OsMADS1*, and *OsJAZ1* were upregulated in OE lines in comparison with WT and KD lines under control conditions (Figure 3.17). *OsMyc2* transcript accumulation was reduced in WT plants when treated with 50 µM GA. *OsJAZ1* was upregulated in OE plants under control and MeJA treatment, but was almost undetectable in WT and KD plants. On the other hand, it was upregulated by the application of GA in both WT and KD lines.

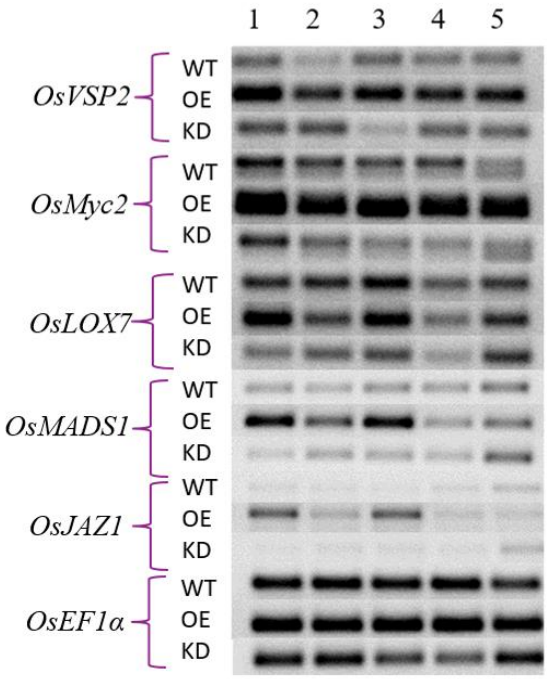


Figure 3.17. Semi-quantitative RT-PCR of six genes, *OsVSP2*, *OsMyc2*, *OsLOX7*, *OsMADS1*, and *OsJAZ1* in wild type (WT), overexpresser (OE) and knock down (KD) plants under 1) control conditions; 2) 100 µM JA; 3) 50 µM MeJA; 4) 50 µM ABA; 5) 50 µM GA; *OsEF1α* was used as an internal control

3.8 Effect of *OsMyc2* overexpression on *Spodoptera frugiperda*

Fall armyworm (*Spodoptera frugiperda*), is an opportunist herbivore that attacks rice and other crops. To establish if the genetic manipulation of the *Myc2* transcription factor can confer resistance against fall armyworm, a feeding assay was conducted by placing newly hatched

neonates on cut rice leaves (~2cm). After 7 days of feeding, no statistical differences were found for larvae weight among the genotypes. Larvae were then placed back into the petri dishes containing leaves of each respective genotype. The time each larva needed to reach the pupal stage and the pupae weight showed some significant differences ($P < 0.05$). Larvae fed with the OE36 line showed an increase in the time (>33 days) needed for pupae establishment (Figure 3.18a). Similarly, pupae from the same line (OE36) showed a reduction in weight (118.3 mg) as compared to the WT (153.4 mg) (Figure 3.18b).

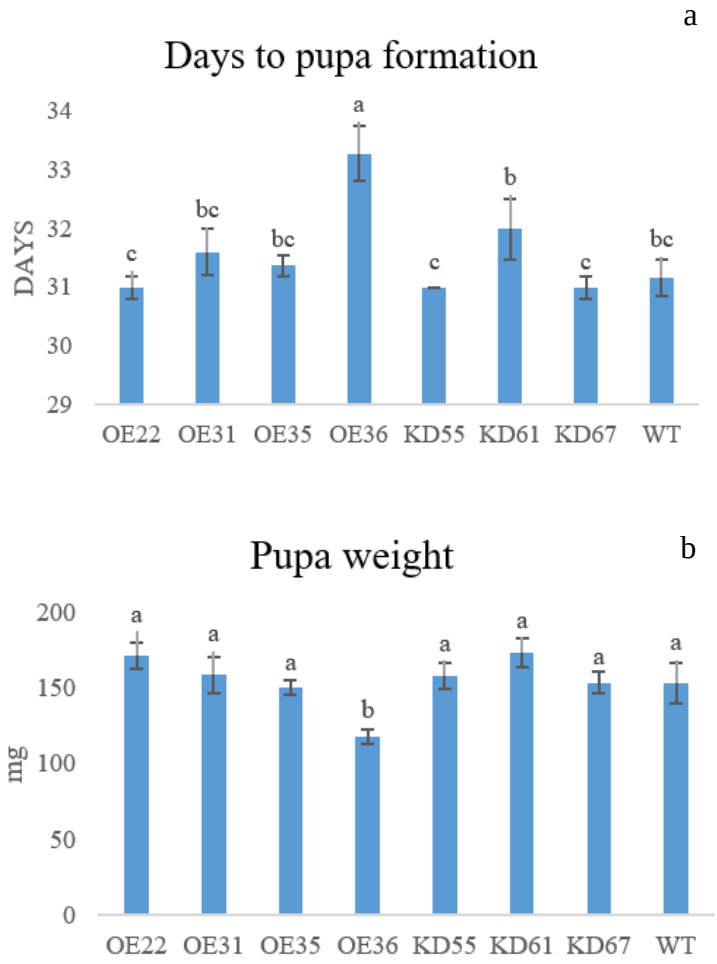


Figure 3.18**.** Average time needed for larvae fed from wild type (WT), overexpresser (OE), and knock down (KD) plants to reach the pupae stage (a) and pupae weights from each genotype (b). Error bars represent standard errors; different letters represent statistically different groups after LSD analysis ($P \leq 0.05$) across lines

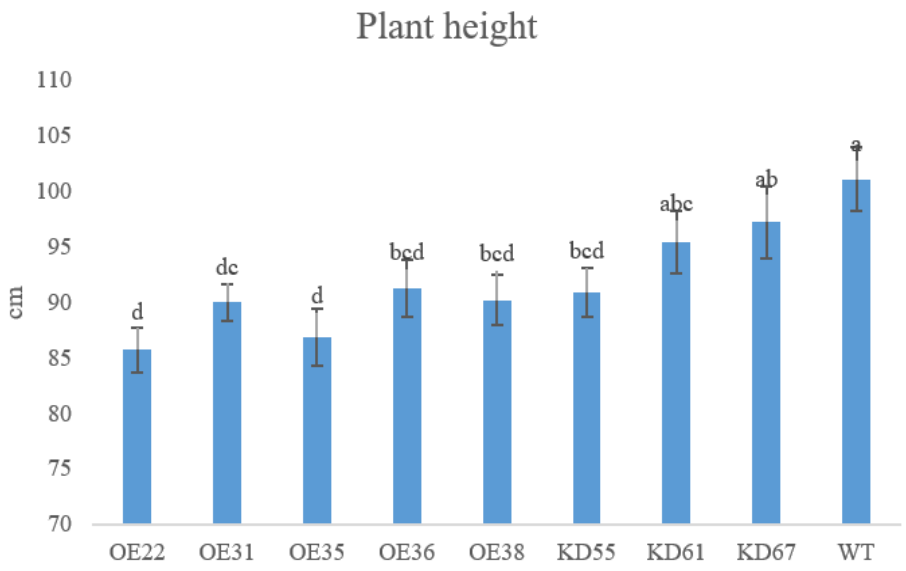
3.9 Growth and yield data analysis

A small but statistically significant ($P \leq 0.05$) difference was recorded in plant height, with a reduction observed in all OE lines and one KD line when compared to WT (Figure 3.19a). Percentage biomass was also reduced in OE35 and KD55 when compared to WT (Figure 3.19b).

All the genotypes had an average of ~4 tillers per plant, with the exception of OE35, which had an average of 3 tillers per plant (Figure 3.20a). Similar results were observed for spikelet fertility ($P = 0.01$). OE lines had a small reduction in the percentage of spikelet fertility, but all of the genotypes presented a fertility range from 82% to 95% (Figure 3.20b).

Apparently, *OsMyc2* manipulation resulted in flowering time alteration. All OE and KD lines had delayed flowering by an average of 7 days and 2-3 days, respectively, in comparison with the WT (Figure 3.21a). Under non-stressed conditions, OE35, OE36, and KD55 lines showed a reduction in yield in comparison with the WT genotype (Figure 3.21b). However, upon recovery following drought, stressed OE22 plants had higher average yield when compared to KD, whereas the WT plants were not able to recover after stress.

a



b

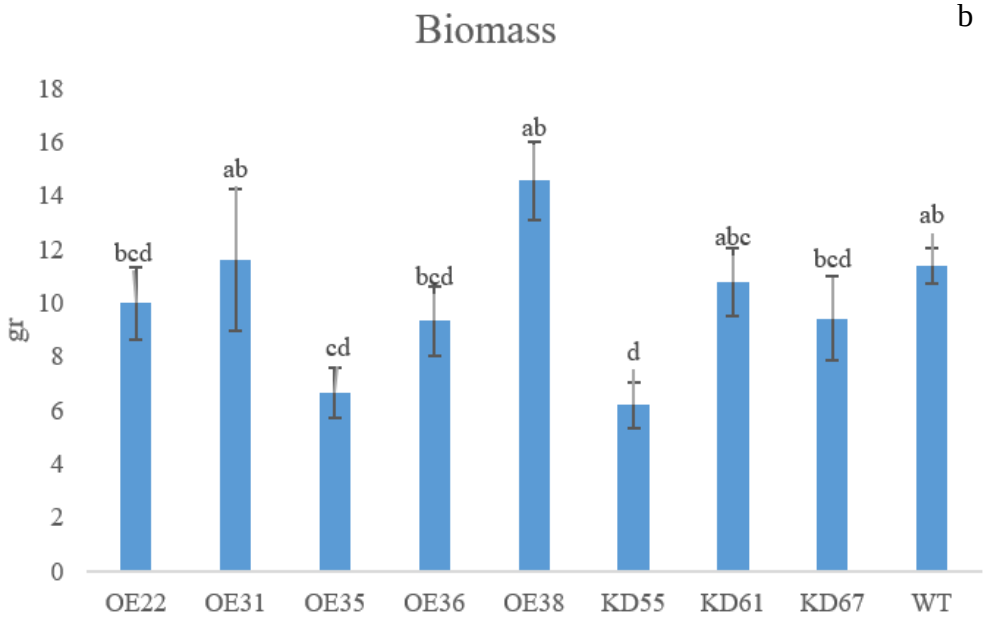


Figure 3.19. Plant heights measured from 10 plants each of wild type (WT), five overexpresser (OE), and three knock down (KD) lines (a) and biomass measured from 4 plants each of WT, five OE and three KD lines under non-stressed conditions (b). Values represent means ± SE of four independent replicates. Different letters represent statistical significance at 5% level based on Fisher's least significant difference (LSD) test across lines

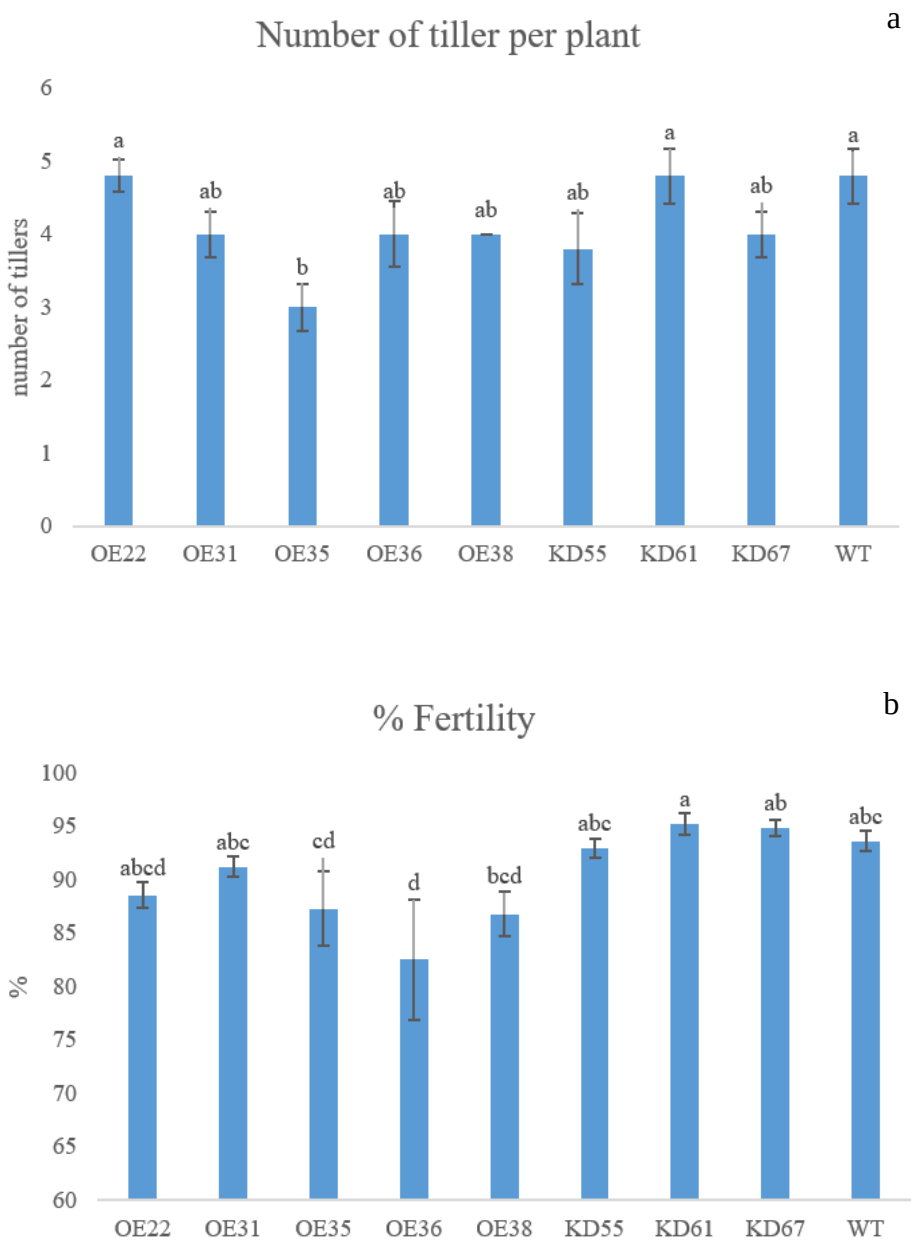


Figure 3.20. a) Number of tillers per plant; and b) percentage of fertility of wild type (WT), overexpresser (OE), and knock down (KD) lines under non-stressed conditions. Values represent means ± SE of four independent replicates. Different letter represent statistical significance at 5% level based on Fisher's least significant difference (LSD) test across lines

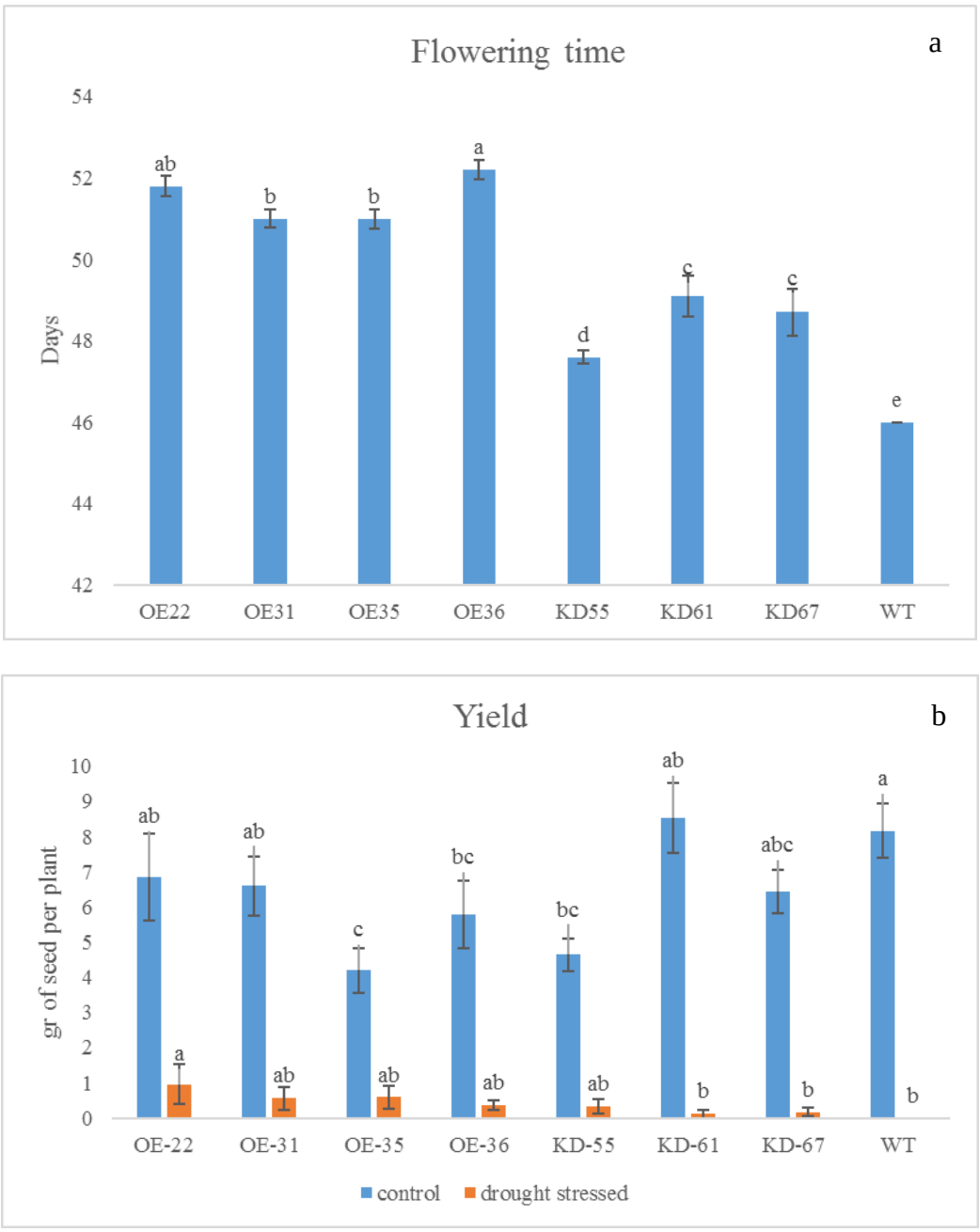


Figure 3.21. a) Flowering time; and b) Yield measured in grams per plant of non-stressed versus drought stressed plants of wild type (WT), overexpresser (OE), and knock down (KD) lines. Values represent means ± SE of four independent replicates. Different letters represent statistical significance at 5% level based on Fisher's least significant difference (LSD) test across lines

CHAPTER 4: DISCUSSION

4.1 OsMyc2: phylogeny, localization and expression

Studies in *Arabidopsis thaliana* have shown that about 5% of the plant genome codes for TFs, which are involved in gene regulation (Riechmann and Ratcliffe, 2000). *Myc2* is a TF that contains a G-box motif and a basic helix-loop-helix (bHLH) DNA binding domain involved in homo- and heterodimerization (Pattanaik et al., 2008). As expected, OsMyc2 shared high similarity with other poaceae family members, and highest identity was observed with *Sorghum bicolor*, which is an important drought tolerant crop (Paterson et al., 2009). On the other hand, the dicot model *Arabidopsis thaliana* Myc2 (AtMyc2) shared only 54.5% of identity with OsMyc2. Consistent with the role of a regulatory protein, OsMyc2 was found to be nuclear localized. Nuclear localization of Myc2 was also reported in tobacco (Lorenzo et al., 2004) and *Arabidopsis* (Chini et al., 2009).

OsMyc2 showed constitutive but differential expression in various tissues. Higher transcript accumulation was observed in stem, immature panicle, lemma-palea and in the ovary compared to leaf, root, pollen, seed and stigma. *Myc2* was expressed in all tissues of *Arabidopsis* plants, but, unlike rice, with higher expression in root tissue (Fernandez et al., 2011).

4.2 *OsMyc2* overexpression enhances stress tolerance

Although *Myc2* is known to be involved in plant defense, many reports have shown its implications in abiotic stresses. ABA is directly linked to plant abiotic stress (drought, salt and cold) tolerance, and *Myc2* has been reported to be positively regulated by ABA accumulation during drought stress (Osakabe et al., 2014). Rice plants overexpressing *OsMyc2* had a better shoot tissue tolerance, recovery and root development in comparison with WT and KD lines, which showed severe stress symptoms and mortality after 2 weeks of water deficit.

Lower stomatal conductance was observed in OE and some KD lines when compared with WT plants, which suggests that *Myc2* manipulation may have promoted stomatal closure during stress. Stomatal conductance was reduced under water deficit to prevent water loss (Miyashita et al., 2005). Mutation of a zinc finger protein, DTS (drought and salt tolerance), promoted stomatal closure by the modulation of genes involved in H_2O_2 homeostasis, enhancing drought tolerance and relative water content (RWC) in the plant (Huang et al., 2009). Furthermore, OE lines were capable to maintain an elevated percentage of relative water content. Drought-induced ABA accumulation is also known to trigger stomatal closure in order to prevent water loss by evapotranspiration, resulting in an increased percentage of relative water content in the OE plants to cope with stress. Increased RWC in OE led to increased membrane stability index and photosynthesis efficiency as compared to WT and KD plants. H_2O_2, as a secondary messenger, accumulates in the leaf tissue under stress. Enhanced reactive oxygen species (ROS) production under drought leads to increased ROS accumulation, which triggers plant stress response by manipulating the ABA-dependent signaling pathway and Ca^+ flux. High ROS accumulation, as observed by the dark brown coloration following H_2O_2 mediated oxidation of DAB (Thordal-Christensen et al., 1997), was observed in the leaves of WT and KD plants under drought stress, suggesting increased stress symptoms in comparison with the OE lines. Equal soil moisture content of the pots during the period of drought stress suggested that OE lines, indeed, performed better over WT and KD lines under similar moisture (dry) regime. Thus, the present results suggested that *Myc2* overexpression led to the protection of the photosynthesis machinery, and an increased cellular integrity and plasticity due to high RWC and ROS protection during stress.

Myc2, reported to be upregulated in response to water deficit, regulates the expression of different stress responsive genes, such as responsive to desiccation 22 (*RD22*), alcohol dehydrogenase I (*ADH1*) and many other genes involved in plant defense, and stress tolerance and adaptation (Abe et al., 2003; Shinozaki et al., 2007). Exogenous application of JA in *A. thaliana* has been shown to enhance the production of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPX; Yastreb et al., 2015). On the other hand, JA biosynthesis pathway silencing reduced the production of APX (Hazman et al., 2015). In rice, *Myc2* has been shown to have binding sites in genes related to the ascorbic acid (AsA) and tocochromanol biosynthesis pathways that are known to play an important role in the production of plant antioxidants (Jo and Hyun, 2011). The involvement of *Myc2* in ROS production is related to its role in lipid peroxidation (Elhiti and Stasolla, 2014). In *Arabidopsis*, *Myc2* is associated with the metabolic pathway of NADPH oxidases (*ATrbohD* and *ATrbhF*), associated with the production of ROS in the guard and mesophyll cells required for stomatal closure. Similar results were observed with exogenous applications of MeJa, which enhanced H_2O_2 production in the guard cells, triggering stomatal closure (Miller et al., 2010; Maruta et al., 2011).

Floating cut-leaf assay showed higher salt (NaCl) sensitivity of KD and WT plants in terms of chlorophyll bleaching in comparison to OE lines. In *A. thaliana*, JA induction was shown to provide plants with moderate salt tolerance, and *Myc2* mutants resulted in decreased antioxidant enzymes (SOD, CAT, and GPX) activity (Yastreb et al., 2015). However, at the seedling stage, no difference was found among the genotypes. Further investigations are needed in rice to elucidate the involvement of *Myc2* in salt stress response.

4.3. *OsMyc2* is drought stress-induced and modulates the expression of other downstream genes

Myc2, a key JA regulator, works in a *COI1*-dependent manner, and is upregulated after the degradation of the JAZ repressor by the 26S proteasome pathway as a target of the E3 ligase (Nakata et al., 2013). Shinozaki and Yamaguchi (2000) reported the induction of Myc2 expression under ABA stimuli by late drought response. In the present study, qRT-PCR data showed that *OsMyc2* is, in fact, positively induced by drought stress, with increased transcript accumulation under stress.

The expression of *OsCOI1*, which is upstream of *OsMyc2*, was not affected in the genotypes under control conditions, but under water deficit condition, its expression was upregulated in WT and OE plants, and remained unchanged in KD lines. Constitutive expression of *Myc2* enhanced the production of the *OsLOX7*, an ortholog of the *AtLOX2*. Transcript accumulation was also observed in WT and KD plants, but at lower levels. Thus, downregulation of *OsMyc2* might affect the production of compounds involved in JA generation, such as lipoxygenase, affecting the whole cycle as reported by Paschold et al. (2008). Biosynthesis of JA requires chloroplastidic linolenic acid synthesized by lipoxygenases in the allene oxide synthase branch (Porta and Rocha, 2002). Lipoxygenase accumulation in *A. thaliana* in response to desiccation stress was reported by Matos et al. (2008). Studies have shown that lipoxygenase is involved in the degradation of monogalactosyldiacylglycerol (MGDG), a highly desiccation-sensitive polar lipid in the cell membrane. MGDG forms cylindrical inverted hexagonal structure in water-lipid mixtures, instead of bilayers as digalactosyldiacylglycerols (DGDG). Reduction of MGDG increases the DGDG:MGDG ratio, which enhances membrane stability under water deficit, keeping enough fluidity to maintain biological processes (Gigon et al., 2004).

Furthermore lipoxygenase silencing has shown increased sensitivity to drought stress in rice cultivars (Liu et al., 2008).

OsMADS1 is an E-class gene involved in the determination of floral meristem initiation and specification. It contains five G-box motifs (G1, G2, G3, G4, and G5), G2 being a direct target of *OsMyc2* (Cai et al., 2014). *OsMADS1* is believed to control the differentiation of specific cell types in lemma and palea (Prasad et al., 2005). Furthermore, *OsMADS1* targets an auxin-responsive *OsMGH3*, involved in pollen viability (Yadav et al., 2011). *OsMADS1* was upregulated in OE lines, confirming its downstream localization in the *OsMyc2* pathway.

Under non-stressed control conditions, increased expression of the *OsJAZ* repressor was observed in OE plants, but not in WT or KD plants, which suggested a self-feedback regulation of *Myc2*. In *A. thaliana*, *Myc2* is known to directly trigger *JAZ* expression. Generation of stable JAZ proteins through alternative splicing to reduce JA sensitivity in cells with a high JA-Ile concentration has also been reported (Chung et al., 2009).

4.4 Hormonal regulation of the expression of *OsMyc2* and related genes

Myc2 is known to be responsive to ABA, JA and MeJA (Yadav et al., 2005). JA, first isolated as a growth inhibitor, triggers the expression of *Myc2* transcription factor (Lorenzo et al., 2004). JA insensitivity in *Myc2*-mutant plants further demonstrated the importance of *Myc2* as a downstream key regulator in the plant JA cascade response. The involvement of the OsMyc2 in the JA pathway was evident in the present study, where exogenous application of JA and MeJA had greater impact on root growth reduction in *Myc2* OE lines. On the other hand, KD lines exhibited reduced hormone sensitivity with lower percentage of root and shoot growth reduction in comparison with OE and WT plants. JA/MeJA treatments reduced the expression of the *OsJAZ1* repressor. This could be due to an increased interaction with the *OsCOI1*, which

enhances the expression of *OsMyc2* (Chini et al., 2007). The *Myc2* downstream target gene *OsVSP2* was overexpressed in WT plants by application of MeJA, but lower transcript accumulation was observed by JA treatment. Similar expression patterns were observed for *OsLOX7* and *OsMADS1* in WT plants. But, all these genes were upregulated in *OsMyc2* OE plants and downregulated in the KD plants. These results corroborate the previous report that *Myc2* plays a key role as a master regulator in the JA metabolic pathway (Nakata et al., 2013).

Exogenous ABA application reduced the shoot growth of WT and KD plants more than the OE lines. Similar results were observed at root level, where some OE lines didn't show any reduction at all. WT plants also showed an increased expression of *OsMyc2* under ABA stimulus, which suggests a positive cross-talk between ABA and JA (Abe et al., 2003; Shinozaki and Yamaguchi- Shinozaki, 2007). Interestingly, *OsLOX7* and *OsMADS1* were downregulated by exogenous ABA application, indicating a negative regulation of ABA on the downstream target genes of *Myc2*. Negative regulation of ABA inducible genes by DWA-associated proteins was reported in the Myc2 pathway, but yeast 2H studies showed that no direct interaction existed between DWA and Myc2 (Lee et al., 2010).

OsMyc2 OE plants showed slower growth in comparison with WT genotypes. Exogenous application of GA induced shoot elongation in all the genotypes, but the phenotype was more prominent in the OE and KD lines. However, the increase in root growth was similar in all genotypes. The cross-talk between GA and JA is not conclusive due to the evidence showing both positive and negative interaction between the two hormones (Kazan and Manners, 2013). In the present study, a negative regulation of the *OsMyc2* was observed by GA application, which was in agreement with the model presented in *A. thaliana* (Wild et al., 2012) where DELLA RGA-LIKE3 proteins negative regulate JAZ sequester enhancing *Myc2*

expression. Nevertheless, RGA proteins are degraded by GA, so JAZ repressor can freely bind to Myc2 restricting its activity. Similar to this finding, a slight upregulation of the *OsJAZ1* was observed by GA application in WT plants in the present study.

4.5 Effect of Myc2 overexpression on fall armyworm

Studies on the molecular mechanisms underlying plant's response to insect attack have shown that JA regulates plant's defense reaction against the attack of chewing insects, necrotrophic pathogens, and cell content feeders like spider mites or thrips (Stam et al., 2014). Overexpression or downregulation of *OsMyc2* did not have significant effect on the growth of 7-day-old fall armyworm (*Spodoptera frugiperda*). However, an increase of the time needed for the larvae to reach the pupa state and reduced pupae weight was observed in one of the OE lines. Such antibiosis effects might be due to the upregulation of *Myc2* target genes, such as *VSP* or *LOX*, and the production of associated secondary metabolites, alkaloids, terpenoids, phenylpropanoids, anti-nutritional proteins, etc. (Schweizer et al., 2013; Campos et al., 2014). Antixenosis has an important role in JA-triggered defense. In *A. thaliana*, it was shown that *Myc2*- branch of the JA pathway regulates the defense responses in plant that in turn affect the feeding preference of the insects (Verhage et al., 2011). Additional experiments with multiple choice feeding essays are needed to establish the role of *OsMyc2* TF in herbivore defense.

4.6 Myc2 expression and agronomic traits

No significant differences were found among the genotypes with respect to the number of tillers per plant or percentage spikelet fertility, but a reduction was observed in plant height, biomass and yield. Such characteristics have been observed in plants constitutively expressing transcription factors (Kasuga et al., 1999). Thus, utilization of stress-inducible promoters has been proposed to circumvent this problem (Smirnoff and Bryant, 1999). A significantly more

delay in days to flowering was observed in all *OsMyc2* OE lines than in the KD lines in comparison to WT, which implies that alteration in *Myc2* expression directly affected flowering. As has been discussed earlier, *Myc2* directly interacts with MADS box genes, which are involved in flowering and lemma-palea cell differentiation (Prasad et al., 2005). An interaction of *Myc2* and *SPA1* genes was observed in *A. thaliana*, where *Myc2*-mutants showed late flowering under long day conditions (Gangappa and Chattopadhyay, 2010).

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CHAPTER 5: SUMMARY AND CONCLUSIONS

5.1 Summary and conclusions

The role of a rice *bHLH-Myc2* transcription factor (*OsMyc2*) in (a)biotic stress response of rice was analyzed through the development of rice lines over/underexpressing *Myc2*. OsMYC2, a regulatory protein, was found to be nuclear localized. It was demonstrated that *OsMyc2* overexpression enhanced drought stress tolerance, providing OE plants with an enhanced capacity to maintain cell fluidity and plasticity, and stability to perform vital biological processes to survive under drought stress. A reduction of reactive oxygen species in the leaf tissue of OE lines under stress was also confirmed, which suggested a more efficient production of antioxidants under stress. Lipoxygenase, a protein involved in drought response and JA production was found to be upregulated under drought stress by the overexpression of *OsMyc2*.

Hormones are known to regulate plant responses to different stresses and development. *Myc2* is referred as a master regulator in the pathway of the JA biosynthesis. The upregulation of the *Myc2* repressor *JAZ1*, demonstrated a feedback regulation when *Myc2* is overexpressed. KD plants with reduced *Myc2* expression showed reduced sensitivity in the presence of JA or its derivate MeJA. In contrast, OE plants with ~6-fold more expression than WT, exhibited extreme sensitivity, demonstrating the participation of *OsMyc2* in JA stimuli. OE and KD plants had a slower seedling growth than the WT. However, GA treatment increased the growth in all genotypes, but OE lines showed higher growth, which may suggest a positive cross-talk between JA and GA in plant growth. Results with ABA treatment was inconclusive where all genotypes were sensitive, especially KD lines exhibited higher sensitivity. Downregulation of *Myc2*-related genes under ABA treatment suggested a negative regulation of genes located downstream of *Myc2* by ABA.

The observation that *OsMyc2* directly induced the expression of *MADS1*, a gene involved in spikelet development and flowering, corroborates to the finding that the OE lines exhibited delayed flowering as compared to the WT and KD. Constitutive expression of *OsMyc2* in the JA pathway might have a phenotypic cost associated with it. This was evident from the short height, and low grain and biomass yield of OE lines compared to WT and KD lines.

Although OE lines showed enhanced salt tolerance with less chlorophyll bleaching than WT and KD lines in floating cut-leaf assay in salt solution, the role of *OsMyc2* in salt stress tolerance could not be established as there was no difference among the genotypes with respect to the salt sensitivity/tolerance at the seedling stage under hydroponics conditions.

Although JA is directly linked with plant's response to chewing herbivore and wound, no significant difference in the weight of fall army worm first instars was observed when fed with leaf tissues from all the genotypes. However, an antibiosis effect as revealed by the reduction of pupae weight and an increase of the time needed to complete its life cycle was apparent in one of the OE lines.

5.2 Future perspectives

1) Detailed gene expression involving all the downstream interacting partners of *Myc2* will increase our understanding of its central role in stress response network of rice. After validating feedback regulation by *JAZ1*, further analysis is needed to comprehend the mechanism of *Myc2* self-regulation.

2) Quantification of the antioxidative enzymes will provide an answer to the question about the involvement of *Myc2* in the oxidative stress management and ROS production in rice.

3) Comparative lipidomics studies between OE and WT lines will establish the mechanism of *Myc2* in maintaining high membrane stability in OE plants under drought stress.

This information could be used as a tool in conventional breeding for assessment of drought tolerance/sensitivity of varieties.

5) Development of transgenic rice plants expressing *OsMyc2* under the control of a stress-inducible promoter will circumvent the problem of phenotypic/energy cost associated with its constitutive expression and achieve plants with normal agronomical traits.

6) Further experiments such as multiple-choice feeding assays are needed to find if plants overexpressing *Myc2* exhibit any antixenosis effect by modifying insect feeding preferences. Further, gene expression analysis under insect attack could help to understand the mechanisms of action of *Myc2* in plant's response to chewing insects.

7) An extensive screening of a large number of independent transgenic events is needed to determine the role of *Myc2* in salt stress tolerance response of OE lines.

8) This dissertation opens up an opportunity for international collaboration between LSU and the Biotechnology Research Center of Ecuador (CIBE) towards scientific research, projects, and human resources development.

APPENDIX I: OSMYC7E PROTEIN SEQUENCE

MWVLLSPLLTTKNPFHPIPIPTFPLLLFSSSLVGVLFQIKSNLEEEEIEIKSMNLWTDDNAS
MMEAFMASADLPAFPWGAASTPPPPPPPPHHHHQQQQQQVLPPPAAAPAAAAFNQDTL
QQRLQSIIEGSRETWTYAIFWQSSIDVSTGASLLGWGDGYYKGCDDDKRKQRSSTPAAA
AEQEHRKRVLRELNSLIAGAGAAPDEAVEEEVTDTEWFFLVSMTQSFPNGLGLPGQALF
AAQPTWIATGLSSAPCDRARQAYTFGLRTMVCLPLATGVLELGSTDVIFQTGDSIPRIRA
LFNLSAAAASSWPPHPDAASADPSVLWLADAPPMDMKDSISAADISVSKPPPPPPHQIQH
FENGSTSTLTENPSPSVHAPTPSQPAAPPQRQQQQQQSSQAQQGPFRRELNFSDFASNGG
AAAPPFFKPETGEILNFGNDSSSGRRNPSPAPPAATASLTTAPGSLFSQHTPTLTAAANDA
KSNNQKRSMEATSRASNTNNHPAATANEGMLSFSSAPTTRPSTGTGAPAKSESDHSDLE
ASVREVESSRVVAPPPEAEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRA
VVPNVSKMDKASLLGDAISYINELRGKLTALETDKETLQSQMESLKKERDARPPAPSGG
GGDGGARCHAVEIEAKILGLEAMIRVQCHKRNHPAARLMTALRELDLDVYHASVSVVK
DLMIQQVAVKMASRVYSQDQLNAALYTRIAEPGTAAR*

APPENDIX II: MYC2 ORTHOLOG SEQUENCES OBTAINED FROM THE PLANT GENOMIC RESOURCE PHYTOZOME 10.3

Phytozome Gene accession	Plant Species	Code (as in Figure 3.2)
evm.TU.supercontig_71.72	*Carica papaya*	Cp
29827.t000001	*Ricinus communis*	Rc
ppa002404m.g	*Prunus persica*	Pp
GSVIVG01013156001	*Vitis vinifera*	Vv
AT1G32640	*Arabidopsis thaliana*	At
AT4G17880	*Arabidopsis thaliana*	At
Thhalv10007075m.g	*Eutrema salsugineum*	Es
Thhalv10024688m.g	*Eutrema salsugineum*	Es
Thhalv10000808m.g	*Eutrema salsugineum*	Es
Ciclev10011214m.g	*Citrus clementina*	Cc
Carubv10008586m.g	*Capsella rubeella*	Cr
Carubv10004392m.g	*Capsella rubeella*	Cr
Aquca_026_00421	*Aquilegia coerulea*	Ac
Lus10004574.g	*Linum usitatissimum*	Lu
Lus10000484.g	*Linum usitatissimum*	Lu
Lus10004575.g	*Linum usitatissimum*	Lu
Lus10030970.g	*Linum usitatissimum*	Lu
Eucgr.E00277	*Eucalyptus grandis*	Eg
Si039973m.g	*Seteria italica*	Si
GRMZM2G001930	*Zea maize*	Zm
GRMZM2G049229	*Zea maize*	Zm
PGSC0003DMG400017535	*Solanum tuberosum*	St
PGSC0003DMG400001161	*Solanum tuberosum*	St
Potri.003G092200	*Populus trichocarpa*	Pt
Potri.001G142200	*Populus trichocarpa*	Pt
Phvul.002G141500	*Phaseolus vulgaris*	Pv
Phvul.003G285700	*Phaseolus vulgaris*	Pv
Gorai.004G184800	*Gossypium raimondii*	Gr
Gorai.006G216700	*Gossypium raimondii*	Gr
Gorai.008G226300	*Gossypium raimondii*	Gr
Gorai.003G182100	*Gossypium raimondii*	Gr
Solyc08g076930.1	*Solanum lycopersicum*	Sl
gene10501-v1.0-hybrid	*Fragaria vesca*	Fv

Phytozome Gene accession	Plant Species	Code (as in Figure 3.2)
Sobic.001G287600	*Sorghum bicolor*	Sb
Thecc1EG015714	*Theobroma cacao*	Tc
Migut.E00934	*Mimulus guttatus*	Mg
Glyma.09G204500	*Glycine max*	Gm
Glyma.01G018400	*Glycine max*	Gm
Glyma.01G096600	*Glycine max*	Gm
Glyma.08G271900	*Glycine max*	Gm
Brara.E01770	*Brassica rapa*	Br
Brara.F03601	*Brassica rapa*	Br
Brara.A00912	*Brassica rapa*	Br
Bostr.3359s0090	*Boechera stricta*	Bs
Bradi3g34200	*Brachypodium distachyon*	Bd
Medtr5g030430	*Medicago Truncatula*	Mt
Medtr8g067280	*Medicago Truncatula*	Mt
SapurV1A.0151s0080	*Salix purpurea*	Sp
SapurV1A.0741s0050	*Salix purpurea*	Sp

APPENDIX III: ALIGNMENT OF PROTEIN SEQUENCES OF MYC2 SHOWED HIGHLY CONSERVED REGIONS AMONG DIFFERENT SPECIES

```
CpMyc2          ------------------------------------------------------------
CcMyc2          ------------------------------------------------------------
GrMyc2          ------------------------------------------------------------
TcbHLH          ------------------------------------------------------------
GrMyc2b         ------------------------------------------------------------
GrMyc2c         ------------------------------------------------------------
GrMyc2d         ------------------------------------------------------------
EgMyc2          ------------------------------------------------------------
PtMyc2          ------------------------------------------------------------
SpMyc2          ------------------------------------------------------------
SpMyc2b         ------------------------------------------------------------
RcMyc2          ------------------------------------------------------------
FvMyc           ------------------------------------------------------------
PpMyc2          ------------------------------------------------------------
LuMyc2          ------------------------------------------------------------
LuMyc2c         ------------------------------------------------------------
LuMyc2b         ------------------------------------------------------------
LuMyc2d         ------------------------------------------------------------
StMyc2          ------------------------------------------------------------
SlMyc           ------------------------------------------------------------
StMyc           ------------------------------------------------------------
MgMyc2          ------------------------------------------------------------
GmMyc2          -----MVRIRTPCLRKSGRFAEGSHSLSLVLSLKLKFTLNALQINPKLEYLLILSLPNLN 55
GmMyc2b         ------------------------------------------------------------
PvMyc2          MVTPGRVLTKNSPWGIWVSRKARTCSLSLALRVYPLFSFFFSPVLPSKPNQFQFPKP-QS 59
MtMyc2          ------------------------------------------------------------
GmMyc2c         ------------------------------------------------------------
GmMyc2d         ------------------------------------------------------------
PvMyc2b         ------------------------------------------------------------
MtMyc2b         ------------------------------------------------------------
AtMyc2          ------------------------------------------------------------
BsMyc2          ------------------------------------------------------------
CrMyc2          ------------------------------------------------------------
EsMyc2          ------------------------------------------------------------
BrMyc2          ------------------------------------------------------------
AtMyc4          ------------------------------------------------------------
CrMyc2b         ------------------------------------------------------------
EsMyc2b         ------------------------------------------------------------
BrMyc2c         ------------------------------------------------------------
EsMyc2c         ----------MAVGGDFQIAPRTPLSPIATFPLSIFLQHVTSLCLSLQTGKVFNFHRKYS 50
BrMyc2b         ------------------------------------------------------------
VvMyc2          ------------------------------------------------------------
AcMyc2          ------------------------------------------------------------
ZmMyc7e         ------------------------------------------------------------
SbbHLH          ------------------------------------------------------------
SiMyc2          ------------------------------------------------------------
ZmbHLH91        ------------------------------------------------------------
OsMyc2          -------------------------MWVLLSPLLTTKNPFHPIPIPTFPLLLFSSSLVG 34
BdbHLH91        ------------------------------------------------------------
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```
CpMyc2          ------------------MNLWT------DDNASVMEAFMS--SDLSALW-P-------- 25
CcMyc2          -------MTDYRLPS--TMNLWT------DDNGSVMEAFMS--SDLTGIW-P-------- 34
GrMyc2          -------MTDYQLAP--TMNLWT------DDNASVMEAFMT--SDLSSIW-P-------- 34
TcbHLH          -------MTDYRLAT--AINLWT------DDNASVMEAFMS--SDLSALW-P-------- 34
GrMyc2b         -------MKDYGLAP--TMNLWT------DDNAPVMEAFMS--SDLSSLW-P-------- 34
GrMyc2c         -------MTDYRFAS--TMNLWT------DDNASVMEAFMS--SDLSALWQP-------- 35
GrMyc2d         ------------------MNLWS------DDNTSVMESFMS--SDISALWPP-------- 26
EgMyc2          -------MSDYRLTP--SMNLWS------DDNASMMEAFMS--SDLSSFWPP-------- 35
PtMyc2          -------MTDYRLPP--TMNLWT------DDNGSVMEAFMNS-SDLSSLWAP-------- 36
SpMyc2          -------MTDYRLPP--TMNLWT------EENGSVMEAFMNS-ADLSSLWAP-------- 36
SpMyc2b         -------MADSRLPT--TMNLWT------DDNATVMEAFMNS-SDLFSPWAP-------- 36
RcMyc2          -------MTDYRVAP--TMNLWS------DDNASVMEAFMN--TDLSALWQP-------- 35
FvMyc           -------MTDYRIPP--TMNLWT------DDNASLMEAFMSN-SDLTSFWAAQPAQP--- 41
PpMyc2          -------MTDYRIPP--TMNLWT------DDNASLMEAFMSS-SDLTSFWAAPSAQPTPQ 44
LuMyc2          ------------------MNLWT------DDNASVMEAFMN--SDLSSLWPPPPPTPLLH 34
LuMyc2c         ------------------------------------------------------------
LuMyc2b         ------------------MNLWT------DDNASVMEAFMN--SDLSSLWPPPPPTPLLH 34
LuMyc2d         -------MTDYRLQSPATMNLWT------DDNASVMEAFMN--SDLTSLWPPPPLP---- 41
StMyc2          ------------------MNLWN------NSTSDDNVSMMEA-FMSSDLSFWATTNSTTT 35
SlMyc           ----------MTEYSLPTMNLWN------NSTSDDNVSMMEA-FMSSDLSFWATN----- 38
StMyc           ------------------------------------------------------------
MgMyc2          ------------------MNIWT------PHSSAAAVTSAAE-GDP-------------T 22
GmMyc2          PSECFLSVTNPNHQLSQRMNLWT------DENSSVMEAFMPS-SDLSSIWPPP------- 101
GmMyc2b         ------------------MNLWT------DENSSVMEAFMSS-SDLSSIWPSP------- 28
PvMyc2          PITTHQSLTS-TTSVSEWMNLWT------DDNSSVMEAFMSS-PDLSSIWPPP------- 104
MtMyc2          ------------------MNLWS------DDNSSVMEAFMTS-SDLSTLWPPQ------- 28
GmMyc2c         ---------MTEYR----MNLWT------DDNSSVMEAFMSS-SDLSSLWLPTPQSA--- 37
GmMyc2d         ---------MTEYR----MNLWT------DDNSSVMEAFMSS-SDLSSLWLATPQSA--- 37
PvMyc2b         ---------MTEYRSPPTMNLWT------DDNASVMEAFMSS-SDFSSLWLPTPQSA--- 41
MtMyc2b         ------------------MNNIW------DDNSSVMEAFMTT-SDISSFWLPTPHSA--- 32
AtMyc2          ---------MTDYRLQPTMNLWT-----TDDNASMMEAFMSSS-DISTLWPPAS------ 39
BsMyc2          ---------MTDYRLQQPMNLWT-----TDDDASMMEAFMSSS-DISTLWPSAT------ 39
CrMyc2          ---------MTDYRLQPTMNLWT-----TDDNASMMEAFMSSS-DISNLWTPAA------ 39
EsMyc2          ---------MTDYRLQPTMNLWT-----ADDNASMMEAFMSSS-DISALWPPAT------ 39
BrMyc2          ---------MT----EPTMNLWT-----TDDNASMMEAFMSSSSDISALWQPAT------ 36
AtMyc4          --MSPTNVQVTDYHLNQSKTDTTNLWS-TDDDASVMEAFIGGGSDHSSLFPP-------- 49
CrMyc2b         --MSPTSVQITDYHLNQSTNGTTNLWS-NDEDASVMEAFIGG-SDQSSLFPPPS------ 50
EsMyc2b         --MSPPDVQLTDCHLNQSTTG-TNLWS-TDDDASVMEAFIGS--EHSSLWPLP------- 47
BrMyc2c         --MSSTNVQLTDHHLNQSTNG-TNLWSTTEDNASVMEPLIGS--EHSSLWPQP------- 48
EsMyc2c         SILSPSYAHMNDYFLNQSTAT-------DDNASAPMEAFIGT--NHSTLWPQ-------- 93
BrMyc2b         ------------------------------MEAFIGT--NHSSLWPQ-------- 15
VvMyc2          -------MTEYRVP---TMNLWT------DDNASMMEAFISS--DLSSFSWG-------- 34
AcMyc2          ---------MTDYRLPSTMNLWS------DDNASMMDAFMQS--DISPFNWQPS------ 37
ZmMyc7e         ------------------MNLWT------DDNASMMEAFMAS-ADLPTFPWGAP------ 29
SbbHLH          ------------------MNLWT------DDNASMMEAFMAS-ADLPTFPWGAT------ 29
SiMyc2          ------------------MNLWT------DDNASMMEAFMAS-ADLPAFPWGAP------ 29
ZmbHLH91        ------------------MNLWT------DDNASMMEAFMAS-ADLPAYPWGAP------ 29
OsMyc2          VLFQIKSNLEEEEIEIKSMNLWT------DDNASMMEAFMAS-ADLPAFPWGAA------ 81
BdbHLH91        ------------------MNLWT------DDNASMMEAFMASAADLPTFPWGAA------ 30
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CpMyc2          ------PPQSSASTSTPAPDAAK---------------------SLSQTQLSSVSVFNQE 58
CcMyc2          ------PSQSSASTADPMKTHIS---------------------SSSQQQQQQQQFFNQE 67
GrMyc2          ------PPQSSASTSTPVVAAAPPPPP-------PPAGLDPSKSFLP-HSQPSVSLLNQE 80
TcbHLH          ------PPQSSGSTSAPAAAAGP----------------DPSKSSLA-QSQPSVSLLNQE 71
GrMyc2b         ------PPLSSASTSTPAASAAGGGG----------GGHDLSVSFLA-QPQPSVSLLNQE 77
GrMyc2c         ------PPQSSASTSTPAVVASSAAAA-------ASGAPDLLKSSVAPQSHPSVALFNQE 82
GrMyc2d         ------PPPPPPPQ-----------------------------------QSQPSVP-LNQD 45
EgMyc2          ------PPPPISTPPLPLPHHQQPPPQQPHPQPPPPSSSATSSAAAAAAAAAFAAAFNQD 89
PtMyc2          ------PPQTSASFSTPAAAA------------------------AAQPSDKTMLNQE 64
SpMyc2          ------PPQSSASTSTPAAAAAV----------------------AAQPSDKTMLNQE 66
SpMyc2b         ------PPQSSTSTSTPAAAA------------------------AAEPSEKTMLSQE 64
RcMyc2          ------QQSSAASTSTPPLPNSTDPNR-----------------AAIINQSQQPLFNQE 71
FvMyc           AAHPLHQPQSSASTSDYPRPP----------------------AQAP-APVSAPFNQE 76
PpMyc2          PAHPQAQPQSSASTSDYPKAA----------------------AVAPSQPSITPFNQE 80
LuMyc2          HHHQPSSSSAVSTSTPPPDPIRP--------------------SSAPAGVAAQSQSLNQE 74
LuMyc2c         ------------------------------------------------------------
LuMyc2b         HHQ-PSSSSAVSTSTPPPDPIRP--------------------SSAPAGVASQSQSLNQE 73
LuMyc2d         ---------------PPPPPPPH--------------------SSASTSAGGGGATVNQD 66
StMyc2          NSASAAVVGVNSNLLHTNNNNNNNNNSPSVFPLSSSTSVS---AAAAVDASKSMPFFNQE 92
SlMyc           NSTSAAVVGVNSNLPHASSN------TPSVFAPSSSTSASTLSAAATVDASKSMPFFNQE 92
StMyc           ------------------------------------------------MPFFNQE 7
MgMyc2          TTMMDAFMASASDLTSFWPASGLGQHTPFVLTPSP-------PPPPAAAAAASSQFFNQE 75
GmMyc2          -----------------APPQP-------------------------QSTAVFNQD 115
GmMyc2b         -----------------APPQ---------------------------STAVFNQD 40
PvMyc2          -----------------APPQ---------------------------SAAVFNQD 116
MtMyc2          -----------------PPSQPP-------------------------QTTTGFNQD 43
GmMyc2c         ----ASTTTPGLETTRAPPP-----------------------------QSHSLLNQE 62
GmMyc2d         ----TSTTTPGTAKAPPPPPPPPPP------------------------AQSQSLLNQE 69
PvMyc2b         ----ASTTTPGADTARALPPPPP--------------------------SQSQSLFNQE 70
MtMyc2b         ----TSTT-----AAPVPPPP----------------------------QQSLFNQE 52
AtMyc2          ------TTTTTATTETTPTPAME---------------------------IPAQAGFNQE 66
BsMyc2          ------TTTTRTATTSTPTTAMD---------------------------IPAPAGFNQE 66
CrMyc2          ------TTTTTTTTTSAPTTAMD---------------------------IPVPAGFNQE 66
EsMyc2          ------AT----ASATAPATEME---------------------------IPAPAGFSQE 62
BrMyc2          ------TT----ATASTTA-------------------------------PAPAGFNEE 54
AtMyc4          -------------LPPPPLP-----------------------------------QVNED 61
CrMyc2b         ------PP-----LPPPAQS-----------------------------------QFNED 64
EsMyc2b         ------PT-----LPPPPPSQS---------------------------------QAGED 63
BrMyc2c         ------PL-----TPPPP-------------------------------------HVTED 60
EsMyc2c         ------PS-----LPPPPPLS----------------------------------QFNED 108
BrMyc2b         ------PP-----VP-TPSLS----------------------------------QFNED 29
VvMyc2          ------------------------------------------------------------
AcMyc2          ------SSASTTITTERERE REP---------------------NSSSKTLNQQPFNQD 69
ZmMyc7e         ----AGGGNSSAAAASPPPP-----------------------QMP-AATAPG---FNQD 58
SbbHLH          ----AGGGNSSAAAATPPPPP----------------------QMPAAAMAPG---FNQD 60
SiMyc2          ----AGGG-ASSAAATPPPP-----------------------QMP-AAMAPG---FNQD 57
ZmbHLH91        ----AGGG-------NPPPPQ----------------------MPPAMAMAPG---FNQD 53
OsMyc2          ----STPP------PPPPPPHHHHQQQQ-------------QQVLPPPAAAPAAAAFNQD 118
BdbHLH91        ----AATP---------PPP---------------------------AAVMPQQPAFNQD 50
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CpMyc2          TLQQRLQALIEGA-RESWTYAIFWQSSYD-YSGAS--------------VLGWGDGYYKG 102
CcMyc2          TLQQRLQQLIEGS-REGWTYAIFWQSSCD-YSGSS--------------MLGWGDGYYKG 111
GrMyc2          SLQQRLQALIEGA-RESWTYAIFWQSSYD-CSATT--------------VLGWGDGYYKG 124
TcbHLH          TLQQRLQALIEGA-RENWTYAIFWQSSYD-YSGTA--------------VLGWGDGYYKG 115
GrMyc2b         TLQQRLQALIEGA-RDCWTYAIFWQSSYD-YSGAT--------------VLGWGDGYYKG 121
GrMyc2c         TLQQRLQALIEGA-HECWTYAIFWQSSYD-YSGPA--------------VLGWGDGYYKG 126
GrMyc2d         SLQQRLQALLEGV-RNCWTYAIFWQSSYD-YAGAA--------------VLGWGDGYYKG 89
EgMyc2          TLQHRLQTLIDSTSRYPWTYAIFWQSSFDGYPGPAAAPPAASSASPPVPVLGWGDGYYKG 149
PtMyc2          TLQQRLQALIEGA-RESWTYAIFWQSSYD-CSGAS--------------VLGWGDGYYIG 108
SpMyc2          TLQQRLQALIEGA-RESWTYAIFWQSSYD-YSGAS--------------VLGWGDGYYKG 110
SpMyc2b         TLQQRLQTLIEGA-CESWTYAIFWQTSYD-YSGAS--------------VLGWGDGYYKG 108
RcMyc2          TLQQRLQALIEGA-RESWTYAIFWQSSYD-YSGAS--------------VLGWGDGYYKG 115
FvMyc           TLMQRLQALIEGA-RESWTYAIFWQSSYD-MSGAS--------------VLGWGEGFYKD 120
PpMyc2          TLMQRLQALIEGA-RESWTYAIFWQSSYD-YSGGT--------------VLGWG------ 118
LuMyc2          TLQQRLQALIDGA-RENWTYAIFWQSSYD-FSGAS--------------VLGWGDGYYKG 118
LuMyc2c         ------------------------------------------------------------
LuMyc2b         TLQQRLQALIDGA-RENWTYAIFWQSSYD-FSGAS--------------VLGWGDGYYKG 117
LuMyc2d         SLQQRLQALIDGA-RENWTYAIFWQSSYD-FSGASSSSSSST-------VLAWGDGYYKG 117
StMyc2          TLQQRLQALIDGA-RETWTYAIFWQSS-VVDFSSPS-------------VLGWGDGYYKG 137
SlMyc           TLQQRLQALIDGA-RETWTYAIFWQSS-VVDFSSPS-------------VLGWGDGYYKG 137
StMyc           SLQQRLQALIDGA-RESWAYAIFWQSSSTSDFATPS-------------VLGWGDGYYKG 53
MgMyc2          TLQQRLLALIEGA-RESWTYAIFWQSS-AAEYGAPA-------------ALTWGDGYYKG 120
GmMyc2          TLQHRLQALIEGA-RETWTYAIFWQSSYDYS-GST--------------LLGWGDGYYKG 159
GmMyc2b         TLQHRLQALIEGA-RETWTYAIFWQSSYDYS-GST--------------LLGWGDGYYKG 84
PvMyc2          TLQHRLQALIEGA-RESWTYAIFWQHSYDYS-GSA--------------LLGWGDGYYKG 160
MtMyc2          TLQQRLQALIEGA-KEIWTYAIFWQPSYDYS-GSS--------------LLGWGDGYYKG 87
GmMyc2c         TLQQRLQTLIEGA-RESWTYAIFWQSSYDYSSGTS--------------LLGWGDGYYKG 107
GmMyc2d         TLQQRLQTLIEGA-CESWTYAIFWQSSYDYSSGTS--------------LLGWGDGYYKG 114
PvMyc2b         TLQQRLQTLIEGA-EESWTYAIFWQSSYDYSSSTS--------------LLGWGDGYYKG 115
MtMyc2b         TLQHRLQALIEGA-KESWTYAIFWQSSYDYTMATP--------------LLGWGDGYYKG 97
AtMyc2          TLQQRLQALIEGT-HEGWTYAIFWQPSYDFSG---------------ASVLGWGDGYYKG 110
BsMyc2          SLQQRLQALIEGT-HEGWTYAIFWQPSYDFSG---------------ASVLGWGDGYYKG 110
CrMyc2          TLQQRLQALIEGT-HEGWTYAIFWQPSYDFSG---------------ASVLGWGDGYYKG 110
EsMyc2          TLQQRLQALIEGT-HEGWTYAIFWQPSYDFSG---------------ASVLGWGDGYYKG 106
BrMyc2          TLQQRLQALIEGT-NEGWTYAIFWQPSYDFSG---------------ASVLGWGDGYYKG 98
AtMyc4          NLQQRLQALIEGA-NENWTYAVFWQSSHGFAGEDN--------NNNNTVLLGWGDGYYKG 112
CrMyc2b         TLQQRLQALIEGA-NESWTYAVFWQSSYDFAGEDDGGG-----ESRNTAVLGWGDGYYKG 118
EsMyc2b         TLQQRLQALIEGA-RESWTYAVFWQLSYDFAGEDDGGGGG---GSINTPLLGWSDGYYKG 119
BrMyc2c         TLQQRLQALIEGA-RESWTYAVFWQLSHDFAGEDISN---------TAALLSWGDGYYKG 110
EsMyc2c         TLQQRLQALIESA-EENWTYAIFWQISHDFDSPTG----------DNTLILGWGDGYYRG 157
BrMyc2b         TLQQRLQALIESA-GEKWTYAIFWQISHDFESPAG----------DNAVVLGWGDGYYKG 78
VvMyc2          ----------PSSAASTWTYAIFWQSSVDFSGAS---------------LLGWGDGYYKG 69
AcMyc2          SLQQRLQAIIEGT-RESWTYAIFWQYSVDVSGAS---------------LLGWGDGYYKG 113
ZmMyc7e         TLQQRLQAMIEGS-RETWTYAIFWQSSLDSATGAS--------------LLGWGDGYYKG 103
SbbHLH          TLQQRLQAMIEGS-SETWTYAIFWQSSLDAATGAS--------------LLGWGDGYYKG 105
SiMyc2          TLQQRLQAMIEGS-RETWTYAIFWQSSVDAATGAS--------------LLGWGDGYYKG 102
ZmbHLH91        TLQQRLQAMIEGS-RETWTYAIFWQSSLDAATGAS--------------LLGWGDGYYKG 98
OsMyc2          TLQQRLQSIIEGS-RETWTYAIFWQSSIDVSTGAS--------------LLGWGDGYYKG 163
BdbHLH91        TLQQRLQAIIEGS-RETWTYAIFWQSSTDAGAGAS--------------LLGWGDGYYKG 95
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CpMyc2          EEDKAKSKSKSSSTP-SSLAEQEHRKKVLRELNSLISG----------PAATSDDAVDE 150
CcMyc2          EGEKGKS---SKIKT-SSAAEQEHRKKVLRELNSLISGS----------TSSPTDDAVDE 157
GrMyc2          EEDKGKA--KLKAPS-SSVAEQEHRKKVLRELNSLISG----------SAAPTDDAVDE 170
TcbHLH          EEDKGKG--KLKASS-STAAEQEHRKKVLRELNSLISG----------STSPTDDAVDE 161
GrMyc2b         EEDKGKG--ESKACS-SSVAEQEHRKKVLRELNSLISG----------STATADDAVDE 167
GrMyc2c         EEDKGKR--KLKT-S-SAVAEQEHRKKVLRELNSLISG----------STAPTDDAVDE 171
GrMyc2d         EEDKEKA--KSKASL-STIAEQQHRRKVLRELNSLISG----------STATTDDAVDE 135
EgMyc2          EEDKSKG--KAKISA-SSAAEQEHRKRVLRELNSLIAGPS-SA------AAAAPDDAVDE 199
PtMyc2          EEDKGKG--RMKNSA-SSAAEQEHRKKVLRELNSLIAGP----------SSVTDDAVDE 154
SpMyc2          EEDKGKG--RKKNSA-SSAAEQEHRKKVLRELNSLIAGP----------NSVTDDAVDE 156
SpMyc2b         EEDKGKA--IMKNSA-SSAAEQEHRKTVLRKLNSLIAGP----------NSVTDDAIDE 154
RcMyc2          EEDKGKG--KSKSTS-SSIAEQEHRKKVLRELNSLISGP----------TAITDDAVDE 161
FvMyc           ERDKVKT--KPKTTT-S-LVEQEYRKKVLRDLNSLISGAD----------TSADDAVVDQ 166
PpMyc2          -----KA--KAKTTT-S-AADQEYRKKVLRELNSLISGAD----------TSADDAVVDQ 159
LuMyc2          EDK-----VKSIKRN-FSPAEQEHRKKVLRELNSLISGP----------NSASDDVVDE 161
LuMyc2c         ------------------------------------------------------------
LuMyc2b         EDK-----VKSVKRN-FSPAEQEHRKKVLRELNSLISGP----------NSASDDVVDG 160
LuMyc2d         DEQKGNTTTKSSTRN-YTPAEQQHRKKVLRELNSLISGP----------NSASDDAVDE 165
StMyc2          EEDKAKR-KLAVSSP-AYIAEQEHRKKVLRELNSLIS-----G------APAGTDDAVDE 184
SlMyc           EEDKAKR-KLSVSSP-AYIAEQEHRKKVLRELNSLIS-----G------APPGTDDAVDE 184
StMyc           EENKNKR-RASSSSA-NFVAEQEHRKKVLRELNSLISGVQAAG------AGSGGDDAVDE 105
MgMyc2          EDDKGNR-KSASSP-----AEQEHRKKVLRELNSLISG---TQ------STTAADEPVDE 165
GmMyc2          DDD--KAKAKAKSKA-TSAAEQDHRKKVLRELNSLISGSSS----------ASASDDVDE 206
GmMyc2b         DDDKAKAKAKAKVKV-TSAAEQDHRKKVLRELNSLISGSSSS----------SAASDDVDE 134
PvMyc2          DDD--KAKAKAKAKA-TSAAEQDHRKKVLRELNSLISGSSA----------ASSDDVDE 206
MtMyc2          EED--KTKAK-KSKV-TSPAEQEHRRKVLRELNSLISGNPV----------TDESPVDE 132
GmMyc2c         EEDKVKAKGKTPKTT-S-SAEQDHRKKVLRELNSLISG-PS----------ASVDDVDE 153
GmMyc2d         EEDKDKVKTKAPKTR-S-SAEQDHRKKVLRELNSLISG-PS----------ASADDIDE 160
PvMyc2b         EEDKG--KGKAPKEM-S-SAEQDHRKKVLRELNSLISG-PS----------ASADDVDE 159
MtMyc2b         EDDKVKLKRVTPPE------EQAHRRKILRELNTLISGGSS----------VSDDAVEE 140
AtMyc2          EEDKANPRRRSSSPPFSTPADQEYRKKVLRELNSLISGG----------VAPSDDAVDE 159
BsMyc2          EEDKANPRRRSSSPPFSTPADQEYRKKVLRELNSLISGA----------VAPSDDAVDE 159
CrMyc2          DEDKAKPRQRSSSPPYSTPADQEYRKKVLRELNSLISGG----------VAPSDEAVDE 159
EsMyc2          EEDKGKPRQKSSSPPFSTPADQEYRKKVLRELNSLISGG----------AGPADDAVDE 155
BrMyc2          EEDKAKPRQRTSPPPFSTPADQEYRKKVLRELNSLISGG----------CGPTDDAVDE 147
AtMyc4          EEE--KSRKKKSNP--ASAAEQEHRKRVIRELNSLISGG----------VGGGDEAGDE 157
CrMyc2b         EEE--NSRKKKSNP--ASAAEQEHRRRVIRELNALISGGGGVV-----NNGGGSDEAGDE 169
EsMyc2b         EEEK-KSRKKKPNP--ASAADQEHRKRVIQELNSLISGGGGGG---TVNGGGNSDEAGDE 173
BrMyc2c         EEER-KSRKRKPNP--VSAAEQEHRKRVIRELNSLISGGGGGGGTVSSSGGGSSDEAGDE 167
EsMyc2c         EED--KDKKKKSSS--SNPAEQEHRKRVIRELNSLISGG----------IGVSDEANDE 202
BrMyc2b         EED--KEKKKKSSN--SNPAEQEHRKRVIRELNSLISGGGGGG-------VGVSDESNDE 127
VvMyc2          EEDKGKRKMTPSSVS-----EQEHRKKVLRELNSLISGT----------ASSSDDAVDE 113
AcMyc2          GEEDKLNKRKTTPTS---VAEQEHRKKVLRELNSLISGG----------VSSTDDAIEE 159
ZmMyc7e         CDE--DKRKQKP-LTPSAQAEQEHRKRVLRELNSLISG----------AAAAPDEAVEE 149
SbbHLH          CDD--DKRKQRP-LTPAAQAEQEHRKRVLRELNSLISG----------AAAAPDEAVEE 151
SiMyc2          CDE--DKRKQKP-LTPAAQAEQEHRKRVLRELNSLISG----------AAAAPDEAVEE 148
ZmbHLH91        CDD--DKRRHRPPLTPAAQAEQEHRKRVLRELNSLISGGASAA------PAPAPDEAVEE 150
OsMyc2          CDD--DKRKQRS-STPAAAAEQEHRKRVLRELNSLIAG----------AGAAPDEAVEE 209
BdbHLH91        CDD--ADKRARQQPTPASAAEQEHRKRVLRELNSLIAGG----------GAAAPDEAVEE 143
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CpMyc2          EVTDTEWFFLVSMTQSFVN-----GSGLPGQALFNSQPVWVAGSERLATSGCERARQGQV 205
CcMyc2          EVTDTEWFFLISMTQSFYVTGGGGGGGLPGQAYFGNSPVWVSGAERLANSGCDRARQGQV 217
GrMyc2          EVTDTEWFFLVSMTQSFVD-----GSGLPGQAFFNSSPVWVAGPDRLESSMCERAKQAQV 225
TcbHLH          EVTDTEWFFLVSMTQSFVN-----GGGLPGQAFFNSSPVWVAGSDRLATSICERARQGQV 216
GrMyc2b         EVTDTEWFFLVSMTQSFVT-----GSGLPGQALFNSSPVWVAGSDRLASSMCERARQGQL 222
GrMyc2c         EVTDTEWFFLVSMTQSFVN-----GGGLPGQALFNSTPVWVVGSERLASSTCERVRQGQV 226
GrMyc2d         EVTDTEWFFLVSMTQSFVN-----GNGLPGQAFFNSCPVWVAGSDRLANSTCERAKQGRV 190
EgMyc2          EVTDTEWFFLVSMTQSFGN-----DGSLPGQALYGSTPLWVSGGDRLADCGCERAKQARI 254
PtMyc2          EVTDTEWFFLVSMTQSFVN-----GSGLPGQALFNGSPVWVAGSERLGTSPCERARQGQV 209
SpMyc2          EVTDTEWFFLVSMTQSFVN-----GSGLPGQALFNGSPVWVAGSERLGTSPCERARQGQV 211
SpMyc2b         EVTDTEWFFLVSMTQSFVN-----GSGLPGQALFDGSPVWVAGSERLGASPCERARQGQV 209
RcMyc2          EVTDTEWFFLVSMTQSFVN-----GGGLPGQAFFNGSPVWVAGLERLASSSCERARQGQI 216
FvMyc           EVTDTEWFFLVSMTQNFVN-----GGGLPGQAFFHSNPVWVAGPDRLAASSCERARQGQV 221
PpMyc2          EVTDTEWFFLVSMTQSFVP-----GGGLPGQAFFHSTPVWVAG-DRLAASPCERARQGQL 213
LuMyc2          EVTDTEWFFLVSMTQSFVN-----GVGLPGQAFFNGSPVWLVGSDRMASAPCDRAKQGQV 216
LuMyc2c         ------------MTQSFVN-----GVGLPGQAFFNGSPVWLVGSDRMASAPCDRAKQGQV 43
LuMyc2b         EVTDTEWFFLVSMTQSFVN-----GVGLPGQAFFNGSPVWLVGSDRMASAPCDRAKQGQV 215
LuMyc2d         EVTDTEWFFLVSMTQSFVN-----GVGLPGQAFFNGFPAWLVGSDRMAAASCERAKQGQV 220
StMyc2          EVTDTEWFFLISMTQSFVN-----GSGLPGQALYSSSPIWVAGTEKLAASHCERVRQAQG 239
SlMyc           EVTDTEWFFLISMTQSFVN-----GSGLPGQALYSSSPIWVAGTEKLAASHCERVRQAQG 239
StMyc           EVTDTEWFFLISMTQSFAN-----GNGLPGLAMYSSSPIWVTGTEKLAGSQCERARQAQG 160
MgMyc2          EVTDTEWFFLISMTQSFAN-----GSGIPGQALYSSSPVWVTGPDKLAAYRCVRAHEAQR 220
GmMyc2          EVTDTEWFFLVSMTQSFVN-----GGGLPGQAFFNSTPVWVTGSDRLSASPCERARQGHM 261
GmMyc2b         EVTDTEWFFLVSMTQSFVN-----GGGLPGQAFFNSAPVWVTGGDRLSASACERARQGHV 189
PvMyc2          EVTDTEWFFLVSMTQSFVN-----GAGLPGQAFFNSNPVWVIGGDRLSTSPCERARQGQV 261
MtMyc2          EVTDTEWFFLVSMTQSFVN-----GTGLPGQAYYNSAPVWLTGAENLALSACERARQGQE 187
GmMyc2c         EVTDTEWFFLVSMTQSFVN-----GSGLPGQAFFNSSPVWVAGPDRLSESVCERAHQGQM 208
GmMyc2d         EVTDTEWFFLVSMTQSFVN-----GSGLPGQAFFNSSPVWVAGPERLSESACERARQGQL 215
PvMyc2b         EVSDTEWFFLVSMTQSFLS-----GSGLPGQAFLNSSPVWVAGADRLSDSTCERARQGQV 214
MtMyc2b         DVTDTEWFFLTSMTQSFVN-----GTGSLSQAYFNSTPVWITGAERLSGSPCERAREARV 195
AtMyc2          EVTDTEWFFLVSMTQSFAC-----GAGLAGKAFATGNAVWVSGSDQLSGSGCERAKQGGV 214
BsMyc2          EVTDTEWFFLVSMTQSFAC-----GAGLAGKAFSTGNAVWVSGSDQLSGSGCERAKQGGI 214
CrMyc2          EVTDTEWFFLVSMTQSFAC-----GAGLAGRAFSTGNAVWVSGSDQLSGSGCERAKQGGV 214
EsMyc2          EVTDTEWFFLVSMTQSFAC-----GSGLAGKAFSTANAVWVSGSDQLSGSGCERAKQGGI 210
BrMyc2          EVTDTEWFFLVSMTQSFAC-----GSGLAGKAFSTGNAVWVYGSDQLTGSGCERAKQGGV 202
AtMyc4          EVTDTEWFFLVSMTQSFVK-----GTGLPGQAFSNSDTIWLSGSNALAGSSCERARQGQI 212
CrMyc2b         EVTDTEWFFLVSMTQSFVS-----GTGLPGQAFSNSNTIWLSGSNALAGSSCERARQGQI 224
EsMyc2b         EVTDTEWFFLVSMTQSFIN-----GSGLPGQAFSDSQTIWLSGSNALAGSSCERARQGQI 228
BrMyc2c         DVSDTEWFFLVSMTQSFAN-----GSGLPGRAFSSSRTIWLSGSNALAGSSCERARQGQV 222
EsMyc2c         EVTDTEWFFLVSMTQSFAN-----GVGLPGESLLNSRVIWLSGSGALTGSGCERAHQGQI 257
BrMyc2b         EVTDTEWFFLVSMTQSFAN-----GVGLPGESFLNSRVIWLSGSGALTGSGCERANQGQI 182
VvMyc2          -----------------------------EALFNSSPVWVVGTERLMSSPCERARQAQF 143
AcMyc2          EVTDTEWFFLVSMTQSFIN-----GGGLPGQAFYSSVPVWIAGHDRLAASPCVRAKQAQE 214
ZmMyc7e         EVTDTEWFFLVSMTQSFLN-----GSGLPGQALFAGQPTWIAS--GLSSAPCERARQAYN 202
SbbHLH          EVTDTEWFFLVSMTQSFLN-----GSGLPGQALFAGQPTWIAS--GLSSAPCERARQAYN 204
SiMyc2          EVTDTEWFFLVSMTQSFLN-----GSGLPGQALFAGQPTWIAS--GLSSAPCERARQAYN 201
ZmbHLH91        EVTDTEWFFLVSMTQSFLN-----GSGLPGQALFAGHHTWIAA--GLSSAPCDRARQAYN 203
OsMyc2          EVTDTEWFFLVSMTQSFPN-----GLGLPGQALFAAQPTWIAT--GLSSAPCDRARQAYT 262
BdbHLH91        EVTDTEWFFLVSMTQSFPN-----GMGLPGQALYTRQPTWIAS--GLASAPCERARQAYT 196
                                          :      *:    :    * *..:.
```

```
CpMyc2          FGLQTMVCIPSAN-GVVELGSTELIIQSSDLMNKVRVLFNFSG-VDAGP-------WSMS 256
CcMyc2          FGLQTLVCIPSAN-GVVELGSTEVIIQNSDLMNKVRFLFNFNGSMEIGT-------WPSA 269
GrMyc2          FGLQTLVCIPSAN-GVVELGSTELITQSSDIMNKVRVLFNFNIEIEAGS-------WCMS 277
TcbHLH          FGLQTMVCIPSAN-GVVELGSTELITQSSDLMNKVRVLFNFNNGIEAGS-------WSMS 268
GrMyc2b         FGLQTIVCIPSVN-GVVELGSTELITQSSDLMNKVRILFNFNNGIEAGS-------WSVS 274
GrMyc2c         FGLQTMVCIPSAN-GVVELGSTELITQSSGLMNKVRVLFNFNNGIEAGY-------LSMC 278
GrMyc2d         FGLQTIVCIPLAN-GVVELGSSEFIIQSSDLVNKVRALFN---GIEAET-------WSMS 239
EgMyc2          FGLNTMVCVPVIG-GVVELGSTEPIYQSPDLLNKVRNLFNFTGGMELG----------FG 303
PtMyc2          FGLQTLVCIPSAN-GVVELGSTELIFQSSDLMNKVKVLFNFN-SLEVGS-------WPIG 260
SpMyc2          FGLQTLVCIPSAN-GVVELGSTELIFQSSDLMNKVRVLFNFN-SLEVGS-------WPVG 262
SpMyc2b         FGLQTLVCIPSAS-GVVELGSTELIFQSSDLMNKVRVLFDFN-SFEVGS-------WPIG 260
RcMyc2          FGLQTLVCIPSAN-GVVELGSTELIYQSIDLMNKVRVLFNFN-SLEAGS-------WPMG 267
FvMyc           FGLQTMVCVPTAN-GVVELGSTELIFQSSDLMNKVRVLFDFN-NLEVGS-------WPMG 272
PpMyc2          FGLQTMVCVPTAN-GVVELGSTELIYQSSDLTNKVRVLFNFN-NLEVGS-------WPMG 264
LuMyc2          FGLQTIVCIPSAN-GVVELGSTDSIFHSSDLMNKVRILFNFNSLESLGGGGAGSSWPLPP 275
LuMyc2c         FGLQTIVCIPSAN-GVVELGSTDSIFHSSDLMNKVRILFNFNSLESLGGGGAGSSWPPPP 102
LuMyc2b         FGLQTIVCIPSEN-GVVELGSTDSIFHSSDLMNKPEAELVHPGRRRLLR----ITTRERT 270
LuMyc2d         FGLQTMVCIPSQN-GVVELGSSELIPQSSDLMNKVRVLFNFSTV---------DVSTVWP 270
StMyc2          FGLQTIVCIPSAN-GVVELGSTELIVQSSDLMNKVRVLFNFSNDFG-------------S 285
SlMyc           FGLQTIVCIPSAN-GVVELGSTELIVQSSDLMNKVRVLFNFSNDLG-------------S 285
StMyc           FGLQTIVCIPSAN-GVVELGSTELIFESSDLMNKVKYLFNFNIDMGSVT-------GSGS 212
MgMyc2          FGLQTIVCIPSSN-GVVELGSTEVIFQSSDLMKKVRVLFNFNNGAETGS-------GSGS 272
GmMyc2          FGLQTLVCIPSAN-GVVELGSTELIFQNSDLMNKVKVLFNFSNNN---F--DMGSSWPAT 315
GmMyc2b         FGLQTLVCIPSAN-GVVELGSTELIFQNPDLMNKVKVLFNFSNNN---F--DMGSSWPAT 243
PvMyc2          FGLQTLVCIPSAN-GVVELGSTELIYQNPDLMNKVKVLFNFSNNN---F--DMGSSWPAT 315
MtMyc2          HGIQTLACIRSAD-GVLELGSTELIYQNNDLMNKVKMLFNFNNN----F--DFGSSWQLG 240
GmMyc2c         FGLQTLVCIPSAN-GVVELASTEVIFQNPDLMNKVRDLFNFNNN-------PETGSWALN 260
GmMyc2d         FGLQTLVCIPSAN-GVVELASAEVIFQNPDLMNKVRDLFNFNNNNNNNN--PETCSWALN 272
PvMyc2b         FGLQTLVCIPSAN-GVVELASTEVIFQNSDLMKKVRDLFNFNNP--------DAGFWPLN 265
MtMyc2b         HGFQTLVCIPTSSSGVVELASTEMIPYNADLMEKIRVLFNFNNP--------ETGSWPLN 247
AtMyc2          FGMHTIACIPSAN-GVVEVGSTEPIRQSSDLINKVRILFNFDGGAGD------LSGLNWN 267
BsMyc2          FGMQTIACIPSAN-GVVEVGSTEQIRQSSDLINKVRILFNFDGGAGD------LSGLNWN 267
CrMyc2          FGMQTIACIPSAN-GVVEVGSTERIRQTSDLVNKVRVLFNFDGGAGD------LSGLNWN 267
EsMyc2          FGMQTIACIPSAN-GVVELGSTEQIRQSSDLMNKVRVLFNFDGGAGD------LSGLNWN 263
BrMyc2          FGMQTIACIPSAN-GVVELGSTEQIRQSSDLMNKVRVLFNFNGGAGD------LSGLNWN 255
AtMyc4          YGLQTMVCVATEN-GVVELGSSEIIHQSSDLVDKVDTFFNFNNGGGE------FGSWAFN 265
CrMyc2b         YGLQTMVCVPCEN-GVVELGSSEIIHQSSDLVDKVDTFFNFNNGGGE------SGSWAFN 277
EsMyc2b         YGLETMVCIPAEN-GVVELGSSEIIHQSSDLIGKVRSFFNFNNGGGG-E----SGSWAFN 282
BrMyc2c         YGLETMVCIPTQN-GVVELGSLEIIHQSSELVDKVNSFFSFNGGGGGGE----SGSWAFN 277
EsMyc2c         YGLQTMVCIAAEN-GVVELGSSEVISQSSDLMDKVNSLFNFNNGNGG-E----ACSWGLD 311
BrMyc2b         YGLQTMVCIAAEN-GVVELGSSEAISQSSDLMDKVNSLFNSSNGNGG-E----ASSWGFG 236
VvMyc2          CSISITLKLDSVN--ATATGASNPIGNQQ---NSKSIQFE---------------------- 178
AcMyc2          LGLQTVVCIPLSD-GVVELGSTDLIFQSSDLMNKVRVLFNFNNNEIG-------SWLPSQ 266
ZmMyc7e         FGLRTMVCFPVGT-GVLELGSTDVVFKTAESMAKIRSLFGGG--AGGGSWPPVQPQAPSS 259
SbbHLH          FGLRTMVCFPVGT-GVLELGSTDVVFQTAESMAKIRSLFGGG--AGGGSWPPVQPQAPSH 261
SiMyc2          FGLRTMVCVPVGT-GVLELGSTDVVFQTAESMAKIRSLFGGGGGAGGGSWPPVQPPAPPP 260
ZmbHLH91        FGLRTMVCFPVGT-GVLELGSTDVVFQTAETMAKIRSLFGGG--PGGGSWPPVQPQAAPQ 260
OsMyc2          FGLRTMVCLPLAT-GVLELGSTDVIFQTGDSIPRIRALFNLS-AAAASSWPP-HPDAAS- 318
BdbHLH91        FGLRTMVCIPVGT-GVLELGATEVIFQTADSLGRIRSLFNLNGGGGGGGAGSSWPPVAPH 255
                 .:     .      .   .: : :            :
```

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CpMyc2          S------NPDQGENDPS-LWISEP-------AGGIEIKDSLHGGNSNSSGPGN------- 295
CcMyc2          MQ-----NPDQGENDPS-SWINDPSPTPAPTAGFIEIKDSTAAAATTTTTTTTTTPVIG 323
GrMyc2          NN-----TADQGENDPSSLWISDPHA-------GVEFKESSNTTTTTT--------TNHT 317
TcbHLH          NN-----TADQGENDPSSLWINDPNN-------GIELKESNNNSN-----------NNNT 305
GrMyc2b         NN-----TADQGENDPSSLWISEPNN-------GVEPKD-----------------NNNN 305
GrMyc2c         NN-----IADEGENDPSSLWISDPNS-------GVEYKES------------------HN 308
GrMyc2d         NN-----TD-----DPSSFWISDPN---------------------------------NI 256
EgMyc2          GN-----GNDQGESDPSSLWLNDPAG-------TVEVKDSAVAGAAAVTG-------SSN 344
PtMyc2          TT-----NTDQGENDPSSLWLTDP-----------ETKDGNAGIPSTTPA-------HQT 297
SpMyc2          IA-----NTDQGENDPSSLWLTDP-----------ESKDGNTGIPSTTPT-------HQT 299
SpMyc2b         TT-----NTDQGENDPSSFWLTDP-----------ETKDGNGGIPSNLNG-------NNQ 297
RcMyc2          -A-----NPDQGENDPSSLWISDPSQ------SGIEIKDGNSTVPSSGVG-------GVN 308
FvMyc           G------AADQGESDPSSLWINDNPS------STIEVVKESVNIAPATSG-------PST 313
PpMyc2          GG-----GADQGENDPSSLWIND-PS------STTIEVKDPVNMAPVTSA-------PTS 305
LuMyc2          TL-----NHDQGENDPSSLWISDPAV---------------------------------- 296
LuMyc2c         TP-----NHDQGENDPSSLWISDPAV---------------------------------- 123
LuMyc2b         IP-----RHSGSAIPPS------------------------------------------- 282
LuMyc2d         QS-----NPDQGENDPSSLWIADPTR---------------------------------- 291
StMyc2          GS-----WAVQPENDPSALWLTEPSS------SGMEVRESLNTVQ------------TNS 322
SlMyc           GS-----WAVQPESDPSALWLTDPSS------SGMEVRESLNTVQ------------TNS 322
StMyc           GS-----CAVHPETDPSALWLTDPSS------SVVEAKDSL-------------------- 242
MgMyc2          GS-----WALPDNVDPAALWLTDPSS------STMD-KDSFNNINNNNTT-------TNS 313
GmMyc2          S-------ADQGENDPSSLWLSDP-----------EVRDSVN------TAAATP---SVM 348
GmMyc2b         S-------ADQGENDPSSLWLSDP-----------EVRDSIN------TVAATP---SVS 276
PvMyc2          S-------ADQGENDPSTLWLNDP-----------EVRDSIN------TAAATPS-VSVS 350
MtMyc2          NNSAATIGGNQGENDPSLNWINDP-----------EARDSVDNNSLVTTTTAATN-ASIS 288
GmMyc2c         C----VATTDQGENDPSSLWLNP------------EIRDSS-----------------TV 287
GmMyc2d         C----VATTDQGENDPSSLWLNP------------EIKDSS-----------------TV 299
PvMyc2b         ----------QGENDPSSLWLNPSSS--------IEIKDTSN--------------AVAL 293
MtMyc2b         -------SITTSENDPSSVWLNDLSAS-----AAIEIRESTVN------TAAVPA-MNAT 288
AtMyc2          LD------PDQGENDPS-MWINDPIGTPGSNEPGNGAPS---------------------- 299
BsMyc2          LD------PDQGENDPS-MWINDPIGAPGSNEPGNGAPS---------------------- 299
CrMyc2          LD------PDQGENDPS-MWINDPITAPGSNEPGNGAPS---------------------- 299
EsMyc2          LD------PDQGENDPS-MWINDPIGAPGSNEPGNGAPS---------------------- 295
BrMyc2          LD------PDQGENDPT-MWINDPIGVA---EQGNGAPS---------------------- 284
AtMyc4          LN------PDQGENDPG-LWISEPNGVDSGL-VAAPVMNNGG------------------- 299
CrMyc2b         LN------PDQGENDPG-LWIGEPDSVGVELGLVAPVMNNTG------------------- 312
EsMyc2b         LT------PDQGENDPA-MWISEPNVAGIESGLVAPAMN-TG------------------- 316
BrMyc2c         LN------PDQGENDPA-TWINEPNVTGIEPVLGAPAT----------------------- 308
EsMyc2c         LN------PDQGENDPA-LWISEPTTTGVESGQVTPAIHNSN------------------- 346
BrMyc2b         LN------PDQGENDPA-LWITEPAIEPVQSG----------------------------- 261
VvMyc2          ------------------------------------------------------------
AcMyc2          LN------ADQGENDP-MLWITDPSLMETKDLTPAIVTPTLP----------------PE 303
ZmMyc7e         QQPA-AGADH-AETDPSMLWLADAPV--------MDIKDSLSHP----------SAEISV 299
SbbHLH          QQPA-AGPDQ-AETD---LWLADAPV--------MDIKDSMSHP----------SAEISV 298
SiMyc2          QQPA-AGADQ-AETDPSVLWLADAPV--------MDIKESLSHP----------SAEISV 300
ZmbHLH91        QQHA-AEADQAAETDPSVLWLADAPV--------VDIKDSYSHPS---------AAEISV 302
OsMyc2          -------------ADPSVLWLADAPP--------MDMKDSISAA------------DISV 345
BdbHLH91        QQHG-GDQ---AETDPSVLWLTDAPV--------GDMKESPSVE-------------ISV 290
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CpMyc2          SNNHQQISKNIQFE-NPSSSSLTENPSAIHTQNH-----QPTQ----------------- 332
CcMyc2          SGSASNLSKGIHFE-LPSSVSLTES------VDL-----QHQQ----------------- 354
GrMyc2          SNQNQQTQKSIQFCDNRSSSSLTENPSSIPAGNH-----HQQQ----------------- 355
TcbHLH          SHQNQQIQKSIQFCDNPSSSSLTENPSSIHVGN------HQQQ----------------- 342
GrMyc2b         GNHNPRIQ-------DPSTSSLTENPSSIHGGN-------QQQ----------------- 334
GrMyc2c         NNQNQQIEKSIQFHDNPSSSSLTENPSSIQQRQ---------------------------- 341
GrMyc2d         NNQ------------NPSSSSLTENPSSIHG------------------------------ 275
EgMyc2          YNGSNHGSKSIQLENNHVLSSMGEKPTAIHRDNPRHNYPQSNQ----------------- 387
PtMyc2          ANNNN----------HHSSSSLTDHSGGIHHVQNHHSHQQQQQ----------------- 330
SpMyc2          GNNNN----------HHSPSSLTDHSGGIHHVQNHHSHQQQQ----------------- 331
SpMyc2b         NKNYHSS--------NPSSSSLTDHLGGIHHVQN---HQQQQ----------------- 328
RcMyc2          NNSQHG---------SKGIQSVNPNSSCVTDNPSGTHMQNQQ----------------- 341
FvMyc           SNHHISKNPIPFDNNHPSSSGLSDNPSAVLQVSHHQQQQPQQQ----------------- 356
PpMyc2          TSTQPVSKPIQFESHQPSSSSLSENPSAIQLQQSQQQQQVQQ----------------- 347
LuMyc2          --EDGPGTGIAKTAAPPSTSGLTENNNISSAAGIHGSG----------------------- 332
LuMyc2c         --EDGPVTGIAKTAAPPSTSGLTENNNISSAAGIHGSG----------------------- 159
LuMyc2b         --KTAPSPGSPKQLLHRRQAAWQR---TTAESTAHGSG----------------------- 315
LuMyc2d         --LDPPQNG----ASYPSSSSLTE-----TPAGIQN------------------------- 316
StMyc2          VPSSNSNKQIAYGNENNHQSGNGQSCYNQQQQQNN-------P----------------- 358
SlMyc           VPSSNSNKQIAYGNENNHPSGNGQSCYNQQQQKN--------P----------------- 357
StMyc           INSSSRDVQLVFVNEN---SENG----TQNQQHS---------------------------- 269
MgMyc2          VPCSITSKQVAFGNENPNPCSSTLTDNPHNQTTN---------------------------- 347
GmMyc2          VPAQTQG---ISISKTMQLESSIQTPGSSTLTETPSSIHA---------------------- 385
GmMyc2b         VPAQTQG---IRFPRPCSWKVLFKP------------------------------------- 298
PvMyc2          VPPHNST---HGISKTMQLESSIQTPGSSTLTETPSSIHA---------------------- 387
MtMyc2          VPSHQHHNNNQNLSVSVTKTMQFETHGSSTLTEVPSVVHVSS------------------ 330
GmMyc2c         APPNSTVNKTLQFETPGS-STLTDTP-SAAAVHVP--------------------------- 320
GmMyc2d         SPPNSTVNKTMHFETPGS-STLTETPSAAAAVHVP--------------------------- 333
PvMyc2b         VSANASLSKTMPFETPGS-STLTETPSAAAAAHVP--------------------------- 327
MtMyc2b         IPANATVGKTLPFETNGSTSTLTETTAVNFAQRQNQ-------------------------- 324
AtMyc2          -SSSQLFSKSIQFENG-SSSTITENPNLDPTPSPVHSQ----T------------------ 336
BsMyc2          -SSSQLISKSMPFENG-SSSTITENPNPDLTPSPVHSK----T------------------ 336
CrMyc2          -SSSQLFSKSIQFENG-SSSTITETPNPDPTPSPVHSQ----T------------------ 336
EsMyc2          -SSSQLFSKSIQFENGGSSSTITENPNPDPTPSPVHSQ----T------------------ 333
BrMyc2          -SSSQLFAKSIQFENGGSSSTIIENPNPDPAPSPVHSQ----T------------------ 322
AtMyc4          -NDSTSNSDSQPISKLCNG-SSVENPNP---------------------------------- 325
CrMyc2b         -NNSASNSDSQPISKLCNG-SSVEDPKP---------------------------------- 338
EsMyc2b         -NNSTSNSDSHPISKLCNG-SSVENPKIS-----SSGF----N------------------ 348
BrMyc2c         -----SNSDSQTASKLCNG-SSVEHPKQ--------------Q------------------ 331
EsMyc2c         -SNSNSKSDSHQISKLEKNESSIENPRQ---------------------------------- 373
BrMyc2b         ------------SHKLEKNESSVENPRK---------------------------------- 277
VvMyc2          ---------------NPSSSSLTENP------------------------------------ 189
AcMyc2          VNVQHIPLSKSYQFEKPSSSSLNENPSMIIQVGHQHQHQHQHQPH--------------H 349
ZmMyc7e         SKPPP--HPPQIHFENGSTSTLTENPSPSVHAPPPPP--APAAPQ-QRQH---------- 344
SbbHLH          SKPPPPPPPPQIHFENASTSTLTENPSPSVHAAPPQP--APAAAP-QRQH---------- 345
SiMyc2          SKPPP---PPQIHFENGSSSTLTENPSPSVHAPPPPP--APAAAPPQRQH---------- 345
ZmbHLH91        SKPPPPPPPPQIHFENGSTSTLTENPSPSVHAPPAPP--APPQRQ--------------- 345
OsMyc2          SKPPPPPPHQIQHFENGSTSTLTENPSPSVHAPTPSQ--PAAPPQRQQQQ---------- 393
BdbHLH91        SKPPP--PPQIHHFENGSTSTLTENAGPSLHAHQQPATLAPAAPPRQNQHPHQLQLQHQQ 348
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CpMyc2          ---Q-IQTQNYISRELNFSQGGY-------VGNGDSNMLRPESGEILNFGESKRSSSNAN 381
CcMyc2          ---I-PQTQSFFTRELNFSEYAYDHNS---VKNGSSRLFKPESGEILNFAESKRSSCTGN 407
GrMyc2          ---Q-SHQQ-GQSLCLNFSDYGFDESSSVRNGNSSSHLLKPESGEILNFGESK-----RS 405
TcbHLH          ---Q-NHQQ-GHSFCLNFSDYGFDGSSSVRNGNSSSHLLKPESGEILNFGESK-----RS 392
GrMyc2b         ---Q---PQ-GQSFRLNFSDYGFDGNSSVKNVKFSAHLLKPESGEILNFGESK-----KS 382
GrMyc2c         ----------SQNFGLNFSDYGFDGSYSVRNGN-SSHLFKPESEETLNFGESK-----RS 385
GrMyc2d         --------------SLHFNNYG----------NSFSHLLKPESGEILNFGESK-----GI 306
EgMyc2          ---Q-MQGQSFFTRELNFSEFGFDGSS---ARNGNSHPMKPESGEILSFGESKR--VSCN 438
PtMyc2          ---QQIHTQSLFTRELNFGEHSTYDGSTVRNGNS--HLMKPESGEILNFGESKRS-PSSA 384
SpMyc2          -----MHTQSLFTRELNFGEHSTYDESTVRNGNF--HLMKPESGEILNFGESKRS-ASSA 383
SpMyc2b         -----IHSQSLFTRELNFGECSTYDGRSVRNGNS--HLTKPESGERLNFGESKRT-ASSA 380
RcMyc2          --------QSFFTRELNFGEYNGFDG---RNGNT--NVLKPESGEILNFGESKRS-SYSA 387
FvMyc           ---V-TQTQSFFTRELNFSDYNGYDGSSVKNSNSNSHSMKPESGEILNFGESKRT-SYSA 411
PpMyc2          ------QTQSFFTRELNFSDY-GYDGSSGKNSNSNSHSLKPESGEILSFGESKRS-SYSA 399
LuMyc2          -----QNQNSFFTRELNFGNSSL----------------KPEAGEILSFADSKRS--SSS 369
LuMyc2c         -----QNQNSFFTRELNFGNSSL----------------KPEAGEILSFADSKRS--SSS 196
LuMyc2b         -----QNQNSFFTRELNFGNSSL----------------KPEAGEILSFADSKRS--SSS 352
LuMyc2d         ------QTQSFFTRELNFGNGSFGGNQ-----------PKPESGEILNFGDNNSK--RSS 357
StMyc2          ---PQQQTQGFFTRELNFSEFGFDGNS--NKNENASLSCKPESGEILNFGDSTKK-SASS 412
SlMyc           ---PQQQTQGFFTRELNFSEFGFDGSS--NRNGNSSVSCKPESGEILNFGDSTKK-SASS 411
StMyc           -----QQTQGFFTKELNFSGYGFDGSSTRNKNGNSSISCKPETREILNFGDSSKR-SGS- 322
MgMyc2          -------NPGYLNRELNFSEFGAHGSS----NVRNAGLCKRESGEILNFGESIKT-SPFG 395
GmMyc2          ----IPQNQSVFSRELNFSEYGFDPKS---GNNQNHHSLKPESGEILSFGESRRTSYGGV 438
GmMyc2b         ----LVPNQSVFSRELNFSEYGFDPKT---GNNQNHHSLKPESGEILSFGESKRTSYGGV 351
PvMyc2          ----VPQNQSVFSRELNFSEYGFDPKS---GNTHNQHSLKPESCEIFSFSDSKRTSYGGG 440
MtMyc2          ----KQNNQSFFSKEMNLSDYG--------GSNNQQRLLKPESGDILCFGESKKSSYVAN 378
GmMyc2c         ----KSNGQGFFSRELNFSNS----------------LKPESGEILSFGESKKSSY--- 356
GmMyc2d         ----NSKSQGFFPRELNFSNS----------------LKPESGEILSFGESKKSSY--- 369
PvMyc2b         ----NPKNQGFFPRELNFSNS----------------LKPESGEILSFGESKKSSY--- 363
MtMyc2b         ----NNQNHSFFLKELNFSGS----------------MKPESGEILSFGESKKSSYITG 363
AtMyc2          ---QNPKFNNTFSRELNFSTSSS-------------TLVKPRSGEILNFGDEGKRSSGNP 380
BsMyc2          ---QNPKFNNNFSRELNFSTSSS-------------TLVKPRSGEILNFGDEGKRSSGNP 380
CrMyc2          ---QNLKFNNNFSRELNFSTSSS-------------TLVKPRSGEILNFGDEGKRSSINP 380
EsMyc2          ---QNPKFNNGFSRELNFSTSST-------------TLVKPRSGDILSFGDEGKRGSGNP 377
BrMyc2          ---QNPKFSNNFSRELNFSTSST-------------TLVKPRPGEILSFGDEGKRSSVNP 366
AtMyc4          ---KVLKSCEMVNFKNGIE---------------------------------NGQE---E 346
CrMyc2b         ---QVTKSSEMVSFKNGTDE-----------------------------NGFSGQSRFME 366
EsMyc2b         ---NHPKSSEIVSFKNGIE-----------------------------NGFSGQSRFVE 375
BrMyc2c         ---QNPQIS-------------------------------------------SSGFVE 343
EsMyc2c         ----NPQNPSLVEQDLNFSSSGL-------------NQNGNFPDGSSRMMKSSETLSFMA 416
BrMyc2b         ----NPQNPFLVEQDFNFQA-----------------------GSSKMMKPSETLSFTA 309
VvMyc2          ------------------------------------------------------------
AcMyc2          QQQQQHSGQSFFSKELNFSEYDG---------------SSTRNGSLQSFVHDSNK----- 389
ZmMyc7e         -QHQNQAHQGPFRRELNFSDFAST-----PSLAATPPFFKPESGEILSFGADSNARR-NP 397
SbbHLH          -QHQNQAHQGPFRRELNFSDFASTNP---SSLAATPPFFKPESGEILSFGADSNARR-NP 400
SiMyc2          -QHN-QAHQGPFRRELNFSEFASN-----PSMAAAPPFFKP----------DPVGHE-HP 387
ZmbHLH91        -----QQNQGPFRRELNFSDFASN-----PSLAAAPPFFKPESGEILSFGVDSNAQR-NP 394
OsMyc2          -QQSSQAQQGPFRRELNFSDFASN------GGAAAPPFFKPETGEILNFGNDSSSGRRNP 446
BdbHLH91        SQQQQQQQQGPFRRELNFSDFATN-----ASVTVTPPFFKPESGEILNFGADSTSRR-NP 402
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CpMyc2          GN-----------LFSGQPS-VVTEE---------N-----KKKRSPTSRGSN------- 408
CcMyc2          GN---------NSLLSNHSQ-FVAEES--------N-----KKKRSPTSRGST------- 437
GrMyc2          GN---------GNLFTGNSP-FAVE----------------NKKRSPNSRGSN------- 432
TcbHLH          GN---------GNLFSGNSQ-IGVEE---------N-----KKKRSPTSRGSN------- 421
GrMyc2b         GN---------GNLFSANSQ-LVVEE---------N-----KKKRSPTSRGSN------- 411
GrMyc2c         GN--------------------VVEE---------N-----KKKTSPTSRGSH------- 404
GrMyc2d         RN---------GNLIS------------------------RKKRSP----SN------- 321
EgMyc2          GN---------GNLYSGQSQ-LTAVEE--------S-----KKRRSPTSRGSN------- 468
PtMyc2          N----------GNFYSG----LVTEES--------N-----KKKKSPASRGGN------- 410
SpMyc2          N----------GNFCSG----LVTEES--------N-----KKKRSPASRGGN------- 409
SpMyc2b         N----------GSLYSG----LVTEES--------N-----KKKRS----GGN------- 402
RcMyc2          N----------GNLFPGHSQ-FATEEK--------N-----TKKRSPTSRGSN------- 416
FvMyc           NN---------GKLFSAQSQ-IAAEDT--------N-----KKKRSPSSRGS-------- 440
PpMyc2          N----------GKLFSGHSQ-IAAAED--------NN---SKKKRSPTSRGSN------- 430
LuMyc2          PN---------GNMFAGGHP-PAAEES--------NKKKRSNPNP--TSRGSNK------ 403
LuMyc2c         PN---------GNMFAGQKP-PAAEES--------NKKKRSNPNPNQTSRGSNK------ 232
LuMyc2b         PN---------GNIFAGGHP-PAAEES--------NKKKRSNPNPNQTSRGSNK------ 388
LuMyc2d         SN---------PNLNHHNPN-PQLEDS--------NTNKNKKKKPSPTSRGSNN------ 393
StMyc2          AN---------VNLFTGQSQ-FGAVE---------ENNNNKNKKRSATSRGSN------- 446
SlMyc           AN---------VNLFTGQSQ-FGAGE---------ENNN-KNKKRSATSRGSN------- 444
StMyc           -------------LFSGQSQ-FGPGTGLGLMEENKNKNNNNNKKRSLASRGNN------- 361
MgMyc2          --------------AQGENN-NNNNS---------NNNNKNKKKTSPTSRGSN------- 424
GmMyc2          NGNTNTNTNSNSHFFSGQSP-FVAAVDENK----KNNMSNNGKKRSPNSRGSN------- 486
GmMyc2b         NG----NSNSNSHFFSGQSP-FVAAADENTN---KNNINNNGKTKSPNSRGSN------- 396
PvMyc2          GGGVNGNSNSNSNFFSGQSP-FVAVADENN-------NNNNGKRRSPNSRGSN------- 485
MtMyc2          NG------NSNSNFFSGQSQ-LVSVAEENN----NGNGNGNGKRRSPNSRGSNN------ 421
GmMyc2c         NG----------SFFPG----VVAIEENN-------------KKRSP-VSRSSI------ 382
GmMyc2d         NG----------AFFPG----VVAVEENNN-------NNKNKKKRSPVVSRSSI------ 402
PvMyc2b         NG----------SYFPG----VAAEETNK-------------KRRSP-ASRSSI------ 389
MtMyc2b         NG----------TFFSGQSQ-FVAGEENR-------------KRKSP-ISRSSI------ 392
AtMyc2          DPS-------------------------------------SYSGQTQFEN-KRK------ 396
BsMyc2          DPS-------------------------------------SYSGQTQFEN-KRK------ 396
CrMyc2          DPS-------------------------------------SYSGQTQFEN-KRK------ 396
EsMyc2          DPS-------------------------------------SYSGQTQFDN-KRK------ 393
BrMyc2          DPS-------------------------------------SYSGQTQFEN-KRK------ 382
AtMyc4          DSS----------------------------------------NKK-RSPVSN---N------ 359
CrMyc2b         EDS----------------------------------------NKK-RSPVSN---N------ 379
EsMyc2b         EDS----------------------------------------NKK-RCLVSD---N------ 388
BrMyc2c         GDS----------------------------------------NKKKRCLVSD---K------ 357
EsMyc2c         EES----------------------------------------NKR-RSPVSKGSNN------ 432
BrMyc2b         EEG----------------------------------------NKR-RSPVSKGSNN------ 325
VvMyc2          ------------------------------------------NSKRSHRLQ---------- 198
AcMyc2          ---------------------------------------NKRSATSRGSN------- 400
ZmMyc7e         SPVPPAATASLTTAPGSLFS--QHTATMT-AAAANDAKNNNKRSMEATSRASNTNHHPAA 454
SbbHLH          SPAPPAATASLTTAPGSLFS--QHTATMTQAAAANDAKNNNKRSMEATSRASNTNHHPAA 458
SiMyc2          SPAPPAATASLTTAPGSLFS--QHTATLTAAPANDTKNNNNKRSMEATSRASNTNHHPAA 445
ZmbHLH91        SPAPP---ASLTTAPGSLFSQSQHTATAAANDAKNN-NNNNKRSMEATSLASNTNHHPAA 450
OsMyc2          SPAPPAATASLTTAPGSLFS--QHTPTLT-AAANDAKSNNQKRSMEATSRASNTNNHPAA 503
BdbHLH91        SPAPPAAAASLTTAPGSLFS--QHTATVT--APTNEAKNNPKRSMEATSRASNTNHHPSA 458
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CpMyc2          --EEGMLSFTSGVILPSS-GVR-SSAGA-----G-DSDH--SDLEASVVKEADSGRV--- 453
CcMyc2          --EEGMLSFTSGVILPSS-GVVKSSGGA-----G-DSDH--SDLEASVVKDPDSSR---- 482
GrMyc2          --EEAMLSFTSGVILPSS-GVVKSSGGA-----G-DSDH--SDLEASVVKEADSSRV--- 478
TcbHLH          --EEGMLSFTSGVILPSS-GVVKSSGGA-----G-DSDH--SDLEASVVKEADSSRV--- 467
GrMyc2b         --EDGMISFTSGAVLPCS-GVAKPGGCA-----R-DSDN--SDIEASVVKEADSSRV--- 457
GrMyc2c         --EDGMLSFSSAVVLPSS-GMMKSSGGA-----G-DSDN--SDIEASVVKEAECVKP--- 450
GrMyc2d         --EEGMLSFTSDVMK---------SGGG-----G-DSDH--SDLEVSVIKEADSARVTIT 362
EgMyc2          --EEGMLSFTSGVVLPSS-GMVKSSGGA-----G-DSDH--SDLEASVVKEADSSRV--- 514
PtMyc2          --EEGMLSFTSGVILSSS-GLVKSSGGT-----GGDSDH--SDLEASVVKEADSSRV--- 457
SpMyc2          --EEGMLSFTSGVILPSS-GVVKSSGGT-----GGDSDH--SDLEASVVKEADSSRV--- 456
SpMyc2b         --EEGMLSFTSGAIVPSS-CVLKSSGAT-----GGDSDH--SDLEASVVKEADSSRV--- 449
RcMyc2          --EEGMLSFTSGVVLPSS-GGVKSSGGT-----G-DSDH--SDLEASVVRETESSRV--- 462
FvMyc           --EEGILSFTSGVILPSSSGVVKSSAG------PADSDH--SDLEASVAKEADSSRV--- 487
PpMyc2          --DEGILSFSSGVILPSS-GVVKSGGGG-----AADSDH--SDLEASVVRETDSSRV--- 477
LuMyc2          --DEGMLSFTSGVILPSS-GTVKSSAGG-----TADSDP--SDLEASMVREVESRVVE-- 451
LuMyc2c         --DEGMLSFTSGVILPSS-GKVKSSAGG-----TADSDP--SDLEASMVREVESRVVE-- 280
LuMyc2b         --DEGMLSFTSGVILPSS-GKVKSSAGG-----TADSDP--SDLEASMVREVESRVVE-- 436
LuMyc2d         --DEGMLSFTSG-VLPSS-GSVKSNGGG-----MVDSDVDQSDLEPSVIKEVV--VAE-- 440
StMyc2          --EEGMLSFVSGTVLPSS--GMKSGGGR-----GEDSEH--SDLEASVVKEADSSR---V 492
SlMyc           --EEGMLSFVSGTVLPSS--GMKSGGGG-----GEDSEH--SDLEASVVKEADSSR---V 490
StMyc           --EEGMLSFVSGVILPTS--TMGKSGGG-----G-DSDH--SDLEASVVKEA-------I 402
MgMyc2          --DEGMLSFTSGMVKN------GGGGGG-----VVDSDQ--SDLEASVVKEVESSR---V 466
GmMyc2          --DDGMLSFTSGVIIP--ATNLKSGG-------GGDSDH--SDLEASVVKDP-------V 526
GmMyc2b         --DDGMLSFTSGVILP--ASNLKSGG-------GGDSDH--SDLEASVVKDP-------V 436
PvMyc2          --DDGMLSFTSRAILP--ATNLKSAG-------GGDSDH--SDLEASVVKDP-------V 525
MtMyc2          --DDGMLSFTSGVIVPPATSNLKFSGGT----GGGDSDH--SDLEASVVKEVDSSR---V 470
GmMyc2c         --DDGMLSFTS---LP--AANIKSGSGG-AGAGGGDSDH--SDLEASMVKQADS-R---V 428
GmMyc2d         --DDGMLSFTS---LP--AANIKSVNG--ACVGAGDSDH--SDLEASVAKQ--------V 443
PvMyc2b         --DDGMLSFTSGVIIP--ASNIKSGAVAGGGASGGDSEN--SDLEASVVKEADS-R---V 439
MtMyc2b         --DDGMLSFTSGVVLP--SSNMKSSSRG----GGGDSDH--SDLDVSAVKEGESSR---V 439
AtMyc2          --RSMVLNEDKVLSFG------DKTAG--------ESDH--SDLEASVVKEVAV------ 432
BsMyc2          --KSTVLSEDKVLSFGGG----DKTTGG-------ESDH--SDLEASVVKEVSV------ 435
CrMyc2          --KSTLLNEDKVLSFG------DKTAG--------ESDH--SDVEASVVKEVAV------ 432
EsMyc2          --KSVGLCDDKVLSFGGG----DKTGGG-------ESDH--SDLEASVVKEVP------- 431
BrMyc2          --KSI---DDKVLTFG--------TGGG-------ESDH--SDLEASVVKEIP------- 413
AtMyc4          --EEGMLSFTSVLP-------------C-------DSNH--SDLEASVAKEAESNRVV-- 393
CrMyc2b         --DEGMLSFTSVLPRP--------AKSG-------DSNH--SDLEASVVKEAESNRTV-- 418
EsMyc2b         --EEGMLSFTSVLPRP--------TKSG-------DSNH--SDLDASVVKEAESNRTV-- 427
BrMyc2c         --EEEMLSFTSVLPLP--------TKSN-------DSNR--SDLEASVVKEAESGRIA-- 396
EsMyc2c         --DEGMLSFSTVVRSA--------AKSG-------DSDH--SDLEASVVKEA----IV-- 467
BrMyc2b         --EEGMLSFSTVVRST--------AKSG-------ESDH--SDLEASVVKEA----IV-- 360
VvMyc2          --EES------------------SGGGG-------DSDH--SDLEASVSGRG-------- 221
AcMyc2          --DDGMMSFTSGVVLPS--AVVKSSAGG------VDSDH--SDLEASVREAESSR----V 444
ZmMyc7e         TANEGMLSFSSAPTTRPSTGTGAPAKSE--------SDH--SDLDASVREVESSRVVAPP 504
SbbHLH          TANEGMLSFSSAPTTRPSTGTGAPAKSE--------SDH--SDLDASVREVESSRVVAPP 508
SiMyc2          TANEGMLSFSSAPTTRPSTGTGAPAKSE--------SDH--SDLDASVREVESSRVVAPP 495
ZmbHLH91        AANEGMLSFSSAPTARPSAGTGAPAKSE--------SDH--SDLDASVREVESSRVVAPP 500
OsMyc2          TANEGMLSFSSAPTTRPSTGTGAPAKSE--------SDH--SDLEASVREVESSRVVAPP 553
BdbHLH91        TANEGMLSFSSAPTTRPSTGTGAPAKSE--------SDH--SDLEASVREVESSRVVPPP 508
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CpMyc2          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 513
CcMyc2          VEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 542
GrMyc2          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQKFYALRAVVPNVSKMDKASLLGD 538
TcbHLH          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 527
GrMyc2b         VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQKFYALRAVVPNVSKMDKASLLGD 517
GrMyc2c         LEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 510
GrMyc2d         AEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 422
EgMyc2          IEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 574
PtMyc2          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 517
SpMyc2          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 516
SpMyc2b         VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 509
RcMyc2          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 522
FvMyc           VDPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 547
PpMyc2          VDPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 537
LuMyc2          --PEKRPKKRGRKPANGREEPLNHAEAERQRREKLNQKFYALRAVVPNVSKMDKASLLGD 509
LuMyc2c         --PEKRPKKRGRKPANGREEPLNHVEAERQRREKLNQKFYALRAVVPNVSKMDKASLLGD 338
LuMyc2b         --PEKRPKKRGRKPANGREEPLNHVEAERQRREKLNQKFYALRAVVPNVSKMDKASLLGD 494
LuMyc2d         --PEKRPKKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 498
StMyc2          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 552
SlMyc           VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 550
StMyc           VEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 462
MgMyc2          VDPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 526
GmMyc2          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 586
GmMyc2b         VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 496
PvMyc2          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 585
MtMyc2          VEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 530
GmMyc2c         MEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 488
GmMyc2d         VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 503
PvMyc2b         VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 499
MtMyc2b         VEPGKRPKKRGRKPANGREEPLNHVEAERQRREKLNQKFYALRAVVPNVSKMDKASLLGD 499
AtMyc2          ---EKRPKKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 489
BsMyc2          ---EKRPKKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 492
CrMyc2          ---EKRPKKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 489
EsMyc2          ---EKRPKKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 488
BrMyc2          ---EKRPKKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 470
AtMyc4          VEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNVSKMDKASLLGD 453
CrMyc2b         VEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNISKMDKASLLGD 478
EsMyc2b         VEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNVSKMDKASLLGD 487
BrMyc2c         AEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNVSKMDKASLLGD 456
EsMyc2c         VEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNVSKMDKASLLGD 527
BrMyc2b         VEPEKKPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNVSKMDKASLLGD 420
VvMyc2          ----GRSLTRGFMPS-----ELCSGQSYKVQNLKMSN---------------------- 249
AcMyc2          VEPEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 504
ZmMyc7e         PEAEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 564
SbbHLH          PEAEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 568
SiMyc2          PEAEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 555
ZmbHLH91        PEAEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 560
OsMyc2          PEAEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 613
BdbHLH91        --EEKRPRKRGRKPANGREEPLNHVEAERQRREKLNQRFYALRAVVPNVSKMDKASLLGD 566
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CpMyc2          AISYINELRTKLQSAESDKEDLQKQLEAIKKEFGN--KESRPCPPPGDQ------ELKMS 565
CcMyc2          AISYINELRTKLQSAESDKEDLQKELASVKKELAGGGKDSHSGPSTSDQ------DLKMS 596
GrMyc2          AISYINELKSKLQSADSEKEEMQSQLEALKKNLSSK-----A-PPPHDQ------DLKIS 586
TcbHLH          AISYINELRTKLQNADSEKEELQKELEAMKKELSSKD-SRSA-PPAPDQ------DLKMS 579
GrMyc2b         AISYINELRTKVQDADSEKEELQKQLDEMKKQLASKESCWTA-PPPPDE------DRNMS 570
GrMyc2c         AISYINELSTKVQDAESEKQELQKQLEAMKKELAKKD--------SSPQ------NPKTS 556
GrMyc2d         AISYINELKIKLQNADSKKEELHKQLEETKKEGQRGG-------------------LTTS 463
EgMyc2          AIAYIKELNSKLQTTESDKENLQKQMESLKKELTNKD--SRSALPQSDK------DLSIS 626
PtMyc2          AISYINELKTKLQSAESSKEELENQVESMKRELVSKDS-----SSPPNQ------ELKMS 566
SpMyc2          AISYINELKTKLQSAESSKEELENQVESLKKEVVSKDS-----SPPPNQ------ELKTS 565
SpMyc2b         AISYINELRMKLQSTESSKEELEKRVESMKRELVIKDS-----NPPPKE------ELKMS 558
RcMyc2          AISYIKELRTKLQTAESDKEELEKEVESMKKEFLSKDSR--PGSPPPDK------ELKMS 574
FvMyc           AISYITELKTKLQTTESDKEDMQKQVETLSKELQESRS-----CSGLDQ------ELKG- 595
PpMyc2          AISYINELKAKLQTTESDKEDLQKQLESMNQDLG-CKD-----SSSLSD------DLKMS 585
LuMyc2          AISYIKELRSKLQSTESEKEELEKQVESMVKKPPPSSPSESKMSNNNNN------SISSN 563
LuMyc2c         AISYIKELRSKLQSTESEKEELEKQVESMVKKPPPSSPSESKMSNNNNN------SISSN 392
LuMyc2b         AISYIKELRSKLQSTESEKEELEKQVESMIKKPLPSSPSESKMSNNNNN------SISSN 548
LuMyc2d         AISYIKELRSKLQSTESSKEELERQVESIRKQQPEHQEYNKKAGSNE----------FGG 548
StMyc2          AISYINELKSKLQNTESDKEDLKSQIEDLKKESRRPGPPPP------NQ------DLKMS 600
SlMyc           AISYINELKSKLQNTESDKEDLKSQIEDLKKESRRPGPPPPP-----NQ------DLKMS 599
StMyc           AIAYINELKSKVQNSDLDKEELRSQIESLRKELANKGSS--------NY------SSSPP 508
MgMyc2          AIAYINELKSKLQNVELDKDELRRQLESSSSSMQKKKDK---------E------YSSAK 571
GmMyc2          AISYITELKSKLQTLESDKDVLHKQLEGVKKELEKTTDNVSSNHACNN------NNNNKL 640
GmMyc2b         AISYITELKSKLQTLESDKDGMQKQLEGVKKELEKTTENVSSNHAGNSS--SC-NNNNKL 553
PvMyc2          AISYITELKSKLQNLESDKDGLQKQLEGVKKELEKSSDNVSSNHTKHG------GNSNIK 639
MtMyc2          AISYITELKTKLQKTESDKDGLEKQLDGMKNEIQKINENQSHQPPQQQQ--QQQPIPNKP 588
GmMyc2c         AISYINELKLKLNGLDSEKGELEKQLDSAKKELELAT-KNPPPPPPPPP--GLPPSNNEE 545
GmMyc2d         AILYINELKSKLNVLDSEKTELEKQLDSTKKELELAT-KNPPPPPPPPPPPGPPPSNSVE 562
PvMyc2b         AISYINELKSKLSELESEKGELEKQLELVKKELELAT-KSPSPPPGPPP-------SNKE 551
MtMyc2b         AISYINELKSKLQGLESSKGELEKQLGATKKELELVASKNQSQNPIPLD----KEKEKTT 555
AtMyc2          AIAYINELKSKVVKTESEKLQIKNQLEEVKLELAGRKASASG------------GDMSSS 537
BsMyc2          AIAYINELKSKVVKTESEKIQIRNQLEEVKLELAGRKASAGG------------GDMSSS 540
CrMyc2          AIAYINELKAKVVKTESEKVMIKNQLEEVKMELAGRKASAGC------------GDMSSS 537
EsMyc2          AIAYINELKSKVTKTESEKTQIKTQLEEVKHELAGRKASAGG------------GDLASS 536
BrMyc2          AIAYINELKSKVTKTESEKTQIKTQLEEVKMELAGRKASAGG-------------DLSSS 517
AtMyc4          AISYISELKSKLQKAESDKEELQKQIDVMNKEAG---NAKSS------------VKDRKC 498
CrMyc2b         AISYINELKSKLQKVESDKEELQKQIEGMSKEAA---NEKSY------------VKERKC 523
EsMyc2b         AISYINELKSKLQKVESDKEELQKQIDVMSNENG-----KCS------------GGDRKY 530
BrMyc2c         AISYINELKAKLQKAEADKEELQKQIDGMSKEVGD-GNVKSS------------VKDQKC 503
EsMyc2c         AISYINELKSKLQQAESDKEEIQKQLDGMSKEGNGKSGGSR-------------VKERKC 574
BrMyc2b         AISYINELKSKLQQAESEKEEIQKQLDGMSKEGNGKSGASRA------------VKERRS 468
VvMyc2          ------------------------------------------------------------
AcMyc2          AISYINELRTKLQTAESDKDGLEAEVDSLKKELASKEPRPVPLPQLQSD------RDLRT 558
ZmMyc7e         AISYINELRGKLTSLETDKETLQTQVEALKKERDARPPSH--------------SAGLGG 610
SbbHLH          AISYINELRGKLTSLESDKDTLQAQIEALKKERDARPPAH--------------AAGLGG 614
SiMyc2          AISYINELRGKLTSLESDKDTLHAQIEALKKERDARPAPH--------------AAGLGG 601
ZmbHLH91        AISYINELRGKLTSLESDRETLQAQVEALKKERDARPHPHP-------------AAGLGG 607
OsMyc2          AISYINELRGKLTALETDKETLQSQMESLKKERDARPPAP---------------SGGG 657
BdbHLH91        AISYINELRGKMTALESDKDTLHSQIEALKKERDARPVAP---------------LSGV 610
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CpMyc2          N-QAGTKSIEIDVDVKIIGWDAMIRIQCSKKNHPAARLMAALKELDLDVHHASVSVVNDL 624
CcMyc2          N-HA-SKLIDLDIEVKIIGWDAMIRIQSSKKNHPAAKLMEALKELDLEVNHASMSVVNDL 654
GrMyc2          N-HTGDKLIDLEIEVKIIGWDAMIQIQCSKKNHPAAKLMAALKELDLDVHHASVSVVKDL 645
TcbHLH          N-HLGNKLVELEIDVKIIGWDAMIRIQCNKKNHPAARLMAALKELDLDVHHASVSVVNDL 638
GrMyc2b         -----NKLIELDIDVKIIGLDAMIRIQCSKKNHPAARLMTALKELDLDVHHASVSVVNDL 625
GrMyc2c         N-HLGNRLIELETEVKVIGWDAMIRIQCKRKNHPAARLMAALKELNLDVQHASVTVVNDL 615
GrMyc2d         -----HKLLELDIDVKTIGLDAMIRIQSNKKNHPAARLMAALQELDLDVHHASVSVVNDL 518
EgMyc2          S-NHGAKLIELDVDVKIIGWDVMIRIQSSKKNHPAAKLMQALMELDLDVHHASVSVVNDL 685
PtMyc2          N-DHGGRLIDMDIDVKISGWDAMIRIQCCKMNHPAARLMSALKDLDLDVQYANVTVMNDL 625
SpMyc2          N-DHGGGLIDMDIDVKISGWDAMIRIQCCKKNHPAARLMSALKDLDLDVLYANVTVMNDL 624
SpMyc2b         N-NHGVRLIDMDIDVKISGWDAMIRIQCCKKSHPAARLMSALRDLDLDVQYANVSVMNDL 617
RcMyc2          N-NHGSKAIDMDIDVKIIGWDAMIRIQCSKKNHPAARLMAALKDLDLDVHHASVSVVNDL 633
FvMyc           ----STKLIDLDIDVKILGWDARIQIQCSKKNHPAARLMAALMELDLDVHHASVSVVNDL 651
PpMyc2          KHQASSKLIDLDIDVKIIGWDAMIRIQCCKKNHPAARLMASLKELDLDVHHASISVVNDL 645
LuMyc2          NQASSKPVIEMDIDVKIIGWDAMIRIQCSKRNHPAARLMAALKELDLDVHHASVSVVNDL 623
LuMyc2c         NQASSKPVIEMDIDVKIIGWDAMIRIQCSKRNHPAARLMAALKELDLDVHHASVSVVNDL 452
LuMyc2b         NQASSKPVIEMDIDVKIIGWDAMIRIQCSKRNHPAARLMAALKELDLDVHHASVSVVNDL 608
LuMyc2d         GRGGKTKAIEMDIDVKIIGWDAMIRIQCSKENHPAARLMAGLKELDLDVHHASVSVVNDL 608
StMyc2          S-HTGGKIVDVDIDVKIIGWDAMIRIQCNKKNHPAARLMAALMELDLDVHHASVSVVNDL 659
SlMyc           S-HTGGKIVDVDIDVKIIGWDAMIRIQCNKKNHPAARLMAALMELDLDVHHASVSVVNDL 658
StMyc           S-NQDLKIVDMDIDVKVIGWDAMIRIQCSKKNHPAARLMAALKDLDLDVHHASVSVVNDL 567
MgMyc2          E-ESSKGIVDMEIDVKIIGWDAMIRVQCSKKNHPAAKMMVALRELDLDVHHASVSVVNDL 630
GmMyc2          SSNQPALIDLVEMDVKIIGWDAMITITCSKKNHPAATLMTALMELDLDVHYATVTLVNDL 700
GmMyc2b         SNQK--LIDVLEMDVKILGWDAMIRIHCSKKNHPGARLLTALMELDLDVHHANVNLVNDM 611
PvMyc2          SSNQ--ALIDLDIDVKIIGWDAMIRIQCSKKNHPAARLMAALMELDLDVHHASVSVVNDL 697
MtMyc2          SSNQ--ALIDLDIDVKIIGWDAMIRVQCSKKNHPAARLMAALMELDLEVHHASVSVVNDL 646
GmMyc2c         AKKTTTKLADLEIEVKIIGWDAMIRIQCSKKNHPAARLMAALKDLDLEVHHASVSVVNDL 605
GmMyc2d         PKKTTSKLADLELEVKIIGWDAMVRIQCSKKNHPAARLMAALKDLDLEVHHASVSVVNDL 622
PvMyc2b         AKETTSKLIDLELEVKIIGWDAMIRIQCSKKNHPAARLMAALKELDLDVNHASVSVVNDL 611
MtMyc2b         SSTSSSKLIDLDIDVKIMGWDAMIRIQCSKKNHPAAKLMAALKELDLDVNHASVSVVNDL 615
AtMyc2          --CSSIKPVGMEIEVKIIGWDAMIRVESSKRNHPAARLMSALMDLELEVNHASMSVVNDL 595
BsMyc2          --CSSIKPVGMEIEVKIIGWDAMVRVESSKKNHPAARLMSALMDLELEVNHASMSVVNDL 598
CrMyc2          S-CSSIKPVGMEIEVKIIGWDAMIRVESSKRNHPAARLMSALMDLELEVNHASMSVVNDL 596
EsMyc2          SPMMGIKPVGMEIEVKIIGWDAMIRVESSKRNHPAARLMTALMDLELEVNHASMSVVNDL 596
BrMyc2          CSMTAIKPVGMEIEVKIIGWDAMIRVESSKRNHPAARLMSALMDLELEVNHASMSVVNDL 577
AtMyc4          LNQESSVLIEMEVDVKIIGWDAMIRIQCSKRNHPGAKFMEALKELDLEVNHASLSVVNDL 558
CrMyc2b         ANQESGVTIEMEVDVKIIGWDAMIRVQCSKRNHPGAKFMEALKELDLEVNHASLSVVNDL 583
EsMyc2b         LNQDSGVSIEMEIDVKIIGWDAMIRIQCSKRNHPGAKFMEALKDLDLEVNHASLSVVNDF 590
BrMyc2c         LDQDSGVSIEVEIDVKIIGWDAMIRIQCGKKNHPGAKFMEALKELELEVNHASLSVVNEF 563
EsMyc2c         SNQDSASSIEMEIDVKIIGWDVMIRVQCSKKNHPGARFMEALKELDLEVNHASLSVVNDL 634
BrMyc2b         SYQDSASSVEMEIDVKIIGWDVMIRVQCSKKNHPGSRFMDALKELDLEVNHASLSVVNDL 528
VvMyc2          --HHGSKLVEMDIDVKIIGWDAMIRIQCSKKNHPAAKLMGALKELDLDVNHASVSVVNDL 307
AcMyc2          IDQHGKKSAEAEIDVKIMGWEAMIRIQCNKNNHPAARIMAAMKDLDLEVIYATVSVVKDL 618
ZmMyc7e         HDGG-PRCHAVEIDAKILGLEAMIRVQCHKRNHPSARLMTALRELDLDVYHASVSVVKDL 669
SbbHLH          HDGG-PRCHAVEIDAKILGLEAMIRVQCHKRNHPSARLMTALRELDLDVYHASVSVVKDL 673
SiMyc2          HDAG-PRCHAVEIDAKILGLEAMIRVQCHKRNHPSARLMTALRELDLDVYHASVSVVKDL 660
ZmbHLH91        HDAGGPRCHAVEIDAKILGLEAMIRVQCHKRNHPSARLMTALRELDLDVYHASVSVVKDL 667
OsMyc2          GDGG-ARCHAVEIEAKILGLEAMIRVQCHKRNHPAARLMTALRELDLDVYHASVSVVKDL 716
BdbHLH91        HDSG-PRCHAVEIEAKILGLEAMIRVQCHKRNHPAAKLMTALRELDLDVYHASVSVVKDI 669
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CpMyc2          MIQQATVKMGSRFYTQEQLRIALTSKVG-EA------ 654
CcMyc2          MIQQATVKMGSRFYTQEQLKNVLAAKVG-DTQ----- 685
GrMyc2          MIQQANVKMGSRFFTQEQLKSALTTKLG-DAR----- 676
TcbHLH          MIQQATVKMGSRFYTQEQLRIALTSKFG-DAR----- 669
GrMyc2b         MIQQASVKMGSRFYTQEQLRIALASKVG-DAR----- 656
GrMyc2c         MIQQATVKMGNPFYTQEQLRLALISKIG-SEI----- 646
GrMyc2d         MIQQVNVKMGNQFYNQEQLRIALTSKVG-DPR----- 549
EgMyc2          MIQQATVKMSGRFYTQEQLRLALSSKIG--------- 713
PtMyc2          MIQQATVKMGNRYYTQEELKVAISTKVG-DAR----- 656
SpMyc2          MIQQATVKMGSRFYTQEELRVAISTKVG-DAR----- 655
SpMyc2b         MIQQATVKMGSRFYTQEELRVAISTKVG-GVR----- 648
RcMyc2          MIQQATVKMGSRIYTQEQLRLALSTKVG-ET------ 663
FvMyc           MIQQATVRMGSRIYTQEQLRLALSAKVG-DAR----- 682
PpMyc2          MIQQATVKMGSRIYTQDQLRLALLSKIG-DSR----- 676
LuMyc2          MIQQASVKMGSRFYTQEQLRLALSVKVG-DTR----- 654
LuMyc2c         MIQQASVKMASRFYTQEQLRLALSTKVG-DTR----- 483
LuMyc2b         MIQQASVKMGSRFYTQEQLRLALSVKVG-DTR----- 639
LuMyc2d         MIQQATVKMGSRFYTQEELRLALSNKVGGDTR----- 640
StMyc2          MIQQATVKMGSRHYTEEQLRVALTSKIAETPLESR-- 694
SlMyc           MIQQATVKMGSRHYTEEQLRVALTSKIAETH------ 689
StMyc           MIQQATVKMGSRLYAQEQLTIALTSKFAESR------ 598
MgMyc2          MIQQATVKMEGRFFSQDQLRAALISKLVS-------- 659
GmMyc2          MIQQATVKMGSRFYTQEQLRAALSAKVG-DVR----- 731
GmMyc2b         TMLQATVKMGSRFYTQEQLRAALAAKVG-DAR----- 642
PvMyc2          MIQQATVKMGSRFYTQEQLRSALSAKVG-DVR----- 728
MtMyc2          MIQQATVKMGSRFYTQEQLRAALSSKVG-DVQ----- 677
GmMyc2c         MIQQATVNMGNKFYTQEQLLSALSSKVG-DEQR---- 637
GmMyc2d         MIQQATVNMGNKFYTQEQLLSALSSKVG-DELR---- 654
PvMyc2b         MIQQATVNMGNRFYTQEQLLSALSSKIG-NAL----- 642
MtMyc2b         MIQQASVNMGSRFYTQEQLLSLLSSKIG-DAQGD--- 648
AtMyc2          MIQQATVKMGFRIYTQEQLRASLISKIG--------- 623
BsMyc2          MIQQATVKMGFRIYTQEQLRASLISKIG--------- 626
CrMyc2          MIQQATVKMGFRIYTQEQLRASLISKIS--------- 624
EsMyc2          MIQQATVKMGFRIYTQEQLRASLISKIG--------- 624
BrMyc2          MIQQATVKMGFRIYTQEQLRASLISKIG--------- 605
AtMyc4          MIQQATVKMGNQFFTQDQLKVALTEKVGECP------ 589
CrMyc2b         MIQQATVKMGKEFFTQDQLKVALMEKVGECL------ 614
EsMyc2b         MIQQATVKMGNQFFTQDQLKASLMEKVGECP------ 621
BrMyc2c         MIQQATVKMGNQFFTQDQLKAALMERV---------- 590
EsMyc2c         MIQQATVKMGSQFFNHDQLKLALMSKVGEDN------ 665
BrMyc2b         MIQQATVKMGSQFFNHDQLRAALMLKVGGDN------ 559
VvMyc2          MIQQATVKMGSRFYTQDQLRLALSSKFADSR------ 338
AcMyc2          MVQQTNVKMSSRIYTPEQLRAALASRIFETR------ 649
ZmMyc7e         MIQQVAVKMASRVYTQDQLSAALYSRLAEPGSAMGR- 705
SbbHLH          MIQQVAVKMASRIYSQDQLNAALYSRLAEPGSAMGR- 709
SiMyc2          MIQQVAVKMASRVYSQEQLNAALYSRLAEPGTAMGR- 696
ZmbHLH91        MIQQVAVKMASRMYSQDQLSAALYSRLAEPGSVMGR- 703
OsMyc2          MIQQVAVKMASRVYSQDQLNAALYTRIAEPGTAAR-- 751
BdbHLH91        MIQQVAVKMPNRVYSQDQLNAALYSRLAEPGAPVPIR 706
                 : *. *.*    :  ::*   :  :.
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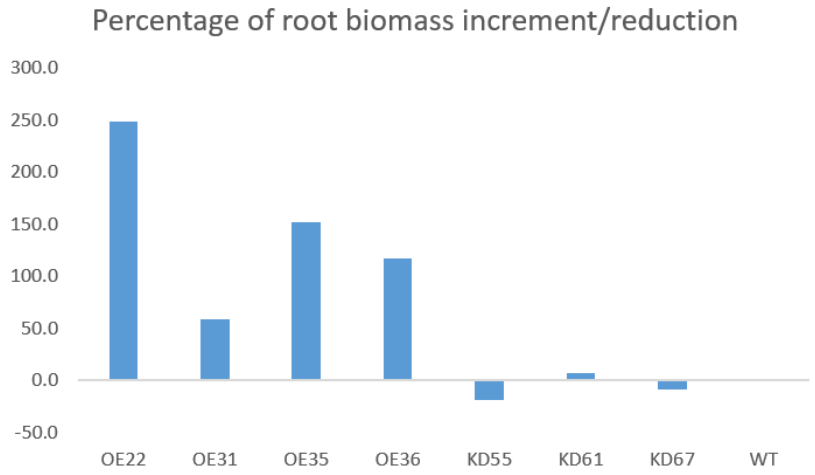
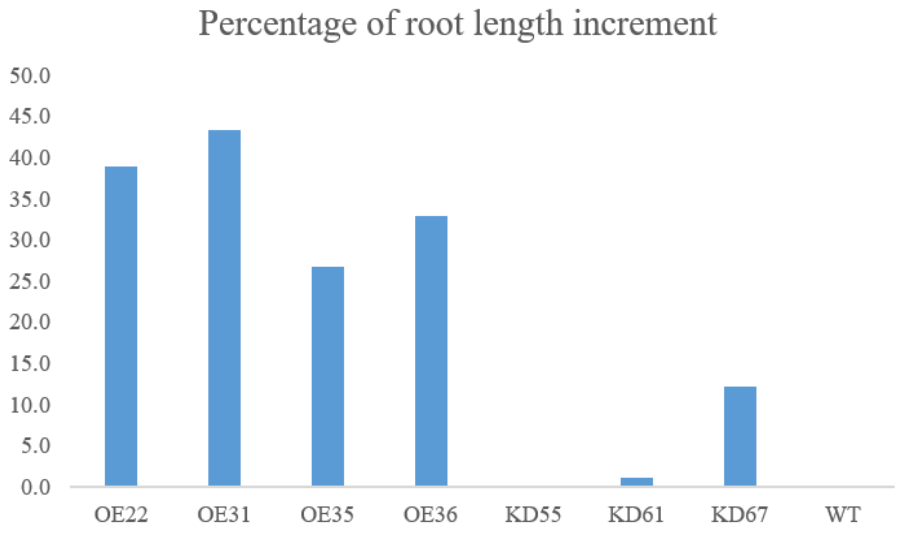
APPENDIX IV: ROOT BIOMASS AND LENGTH QUANTIFICATION OF DROUGHT STRESSED PLANTS AFTER RECOVERY



Percentage root biomass increment/reduction of wild type (WT), overexpresser (OE), and knock down (KD) lines



Percentage root length increment/reduction of wild type (WT), overexpresser (OE), and knock down (KD) lines

VITA

Luis Eduardo Sánchez Timm was born to Luis Eduardo Sánchez Macías and Grace Mónica Timm Duque in 1985 in Guayaquil, Ecuador's biggest city and located on the banks of the river Guayas. He has three brothers, Rafael, Guillermo and José Sánchez Timm. He finished his elementary school in "Escuela Espíritu Santo" followed by high school in the "Unidad Educativa Mariscal Sucre (UEMS)". In 2010, he obtained his degree as an Agronomist and Biologist Engineer from "Escuela Superior Politécnica del Litoral (ESPOL)", where he started to develop an interest in plant biotechnology. Then he worked in the "Centro de Investigaciones Biotecnológicas del Ecuador (CIBE)" under the mentorship of Dr. Efren Santos, who gave him the opportunity to participate in a project for the development of genetically engineered banana, and identification of putative resistance genes of banana variety "Calcutta IV" in response to the infection of *Mycosphaerella fijiensis*. In 2011, he was awarded the USDA-Borlaug scholarship for a scientific exchange program at LSU, where he worked with Dr. Niranjan Baisakh to identify and characterize stress-responsive genes using suppression subtractive hybridization (SSH) and other molecular tools. In 2012 he was granted an Ecuadorian government scholarship from SENESCYT to join laboratory for a Ph.D program under the supervision of Dr. Baisakh. He worked in a project to characterize the role of *Myc2* transcription factor in drought stress response of rice using contemporary molecular biology and biotechnology approaches. . Upon completion of his program at LSU, he will go back to Ecuador to join CIBE and participate in the development and implementation of new scientific projects.