Phenotypic and Gene Expression Analysis of Diverse Rice Genotypes in Response to Drought

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Phenotypic and Gene Expression Analysis of Diverse Rice Genotypes in Response to Drought

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Crop, Soil, and Environmental Sciences

by

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Abstract

Drought is one of the most limiting factors for rice (*Oryza sativa* L.) growth and development with vegetative and reproductive stages the most sensitive and distinct phases. During the vegetative stage, drought can cause reduction in growth and biomass accumulation. Moreover, water stress at reproductive stage can reduce yield significantly. Plants are protected against drought by three different mechanisms: drought avoidance, drought tolerance, and drought escape. An integrated approach combining physiology, breeding, and genomics could be an effective way to characterize and mitigate this problem. The objectives of this research were to (1) screen a diverse set of rice genotypes at both vegetative and reproductive stages for drought response; (2) characterize the genetic differences in mechanisms of drought response conferring drought stress resistance; and (3) study the expression patterns of genes that contribute to yield under water stress conditions. At the vegetative stage, drought was applied by withholding water at 50% of the field capacity for ten days, while in the reproductive stage drought was given at pre-anthesis for three to four days. Results from the first study showed that the diverse genotypes exhibit different drought resistance mechanisms. Padi Tarab Arab and N22 exhibit drought avoidance and tolerance mechanisms while GPNO 25912 exhibits a tolerance mechanism. Gene expression analysis using RNA from plants early after drought stress identified clear differences between resistant and sensitive genotypes. The resistant genotypes showed a high induction in the relative expression of drought stress genes under drought compared to control, while the three sensitive genotypes showed low, no, late, or inconsistent induction in expression. Results from the second study demonstrated that between the two types of samples for gene expression analysis in four different genotypes, the inflorescence gives a higher correlation with phenotypic measurements than the flag leaf during reproductive stage. Meanwhile, both invertase genes and
transcription factors confer positive effects to drought resistance particularly in relation to number of grain per panicle and panicle length.
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I dedicated this thesis to my husband, my parents, and my sister.
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Chapter 1

Introduction and Literature Review
INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important of staple crops, feeding nearly half the world’s population (Maclean et al., 2013). In fifty years (1960 to 2010), rice production has increased by more than 500 million tons (Rejesus et al., 2012; Maclean et al., 2013). The greatest producers and consumers of this grass plant are Asian countries accounting for about 90% of total rice production and consumption worldwide (Riveros, 2000; USITC, 2015), predominately by China and India. This crop is also important for the State of Arkansas. According to the United States Department of Agriculture (USDA) crop report, Arkansas is the largest producer of rice in the U.S., with an estimated production area of almost one million hectares in 2013 and producing around 5.7 million metric tons in 2014 (Hardke and Wilson, 2014; USDA, 2015).

There are two main systems of rice cultivation: the upland or dry system and the lowland or wet system (Grist, 1986). Upland rice grows in rainfed, well-drained soil without any water accumulation or flooding period for more than 80% of the rice’s duration (Bouman et al., 2007). Although most upland rice fields are located in Asia, this system is the dominant rice culture in Africa and South America (Gupta and O’Toole, 1986). In contrast, lowland rice fields need to be flooded from the time of planting until harvesting approaches (Grist, 1986). Most of the rice growing countries cultivate rice using the wet system, which was likely developed in China and only can be adapted to a small number of crops (Bhagat, 2003; Bouman et al., 2007; Maclean et al., 2013). The purposes of wet cultivation or puddling are to reduce nutrient loss and to control weeds and pests (Kirchhof et al., 2000; Bouman et al., 2007). Because of the high water needs of the crop, drought condition is one of the most important limiting factors for rice production. The problem of water deficit is becoming more severe due to the needs of the growing human population and global climate change (Ndjiondjop et al., 2010).
Establishing an irrigation system is one approach to mitigate water deficit through crop management (Blum, 2016). This system is able to maintain ponded water for at least 80% of the crop’s duration (Bouman et al., 2007). Approximately 75% of the total rice fields in the world are using an irrigation system for their water supply (Bouman and Tuong, 2001). In most irrigated areas, this system is only supplementary during wet or rainy season. However, irrigation becomes the main water source for the entire dry season (Bouman et al., 2007; Blum, 2016). This supplemental irrigation is efficient and applicable for places with water deficit problem (Fereres and Soriano, 2007).

Another approach to overcome the problem of water deficit stress is by developing drought resistant rice cultivars. The resistance refers to genotypes that can grow and produce a normal yield under drought conditions (Ito et al., 2000). Drought resistant genotypes have been shown to reduce yield loss in some crops by minimizing the gap between yield potential and actual yield under drought conditions (Cattivelli et al., 2008). Because drought is a multi-component morpho-physiological interaction, the response of plants to drought are also complex (Ndjiondjop et al., 2010). There are three phases during rice development that have an impact on grain yield: vegetative, reproductive, and ripening stages. Drought conditions at these phases can cause spikelet sterility and unfilled grains (Ndjiondjop et al., 2010). Sarvestani et al. (2008) reported that water deficit could reduce mean rice grain yield by 21, 50, and 21% respectively in these three phases. One objective of this research is to identify the drought resistant genotypes by conducting screening at these phases, particularly in vegetative and reproductive phases, as well as to help understand the diverse mechanisms of drought resistance in the different genotypes.
LITERATURE REVIEW

Rice taxonomy, morphology, and importance of the crop

Rice belongs to the Poaceae or Gramineae family, genus *Oryza*. The genus *Oryza* consists of twenty-two or twenty-five grass species. However, there are only two species that are important for human cereal needs: *Oryza sativa* Linn. and *Oryza glaberrima* Steud (Grist, 1986; OECD, 1999; Maclean et al., 2013). *O. sativa* is grown worldwide while *O. glaberrima* is mostly grown in West Africa. Hybrids from crossing of *O. sativa* and *O. glaberrima* replace *O. glaberrima* in many parts of Africa due to higher yield (Linares, 2002). Based on ecological diversification, *O. sativa* is divided into two major subspecies: *indica* which is adapted to the tropical regions and *japonica* that is adapted to temperate or subtropical regions and tropical upland (high latitude) (Maclean et al., 2013). Both *O. sativa* and *O. glaberrima* are diploid with 12 pairs of chromosome (Sanchez et al., 2013).

Rice is a monocot forming a fibrous root system (OECD, 1999). The development of the roots is started from the nodes of the stem below ground level (Grist, 1986). However, some rice varieties also develop a root system above the soil surface, which is known as nodal or adventitious roots. These roots will function when the rice plants grow in water depth above 80 cm (Morita and Yamazaki, 1993; Maclean et al., 2013). The above ground organs or shoots of rice consist of stem, leaves, tillers, and reproductive organs. The length and thickness of internodes and nodes vary depending on the varieties and environmental factors. The culm is hollow in the internodes but solid in the nodes, cylindrical, smooth and more or less erect. The rice leaf consists of two parts, leaf sheath and leaf blade or lamina (Grist, 1986; Maclean et al., 2013). From the axis of each leaf, a tiller arises. Rice tillers are basically composed of four organs: stem, leaves, a panicle, and roots (Hanada, 1993). The reproductive organs consist of the
panicle and spikelet. Each spikelet has six stamens and an ovary with a two-branched stigma (Grist, 1986; OECD, 1999).

There are three leading cereal crops in the world: wheat, rice, and corn. Among these three cereal crops, rice is the most consumed by humans (Maclean et al., 2013). As a cereal crop, rice is primarily utilized for its grain. Rice grain comprises of carbohydrate (more than 85%), protein, ash, fat, and fiber (Grist, 1986). Biologically, rice protein is the richest by virtue of its high true digestibility (88%) among cereal proteins (Government of India, 2011). The vast majority of rice farming is in developing countries, particularly in low and low-middle income Asian countries (East, South, and Southeast). Therefore, rice is very substantial for Asian economies, and also their culture and diet (Maclean et al., 2013; USITC, 2015). Although rice is mostly grown and consumed in Asia, the development of rice as staple food in North America and Europe is increasing. Meanwhile in West Africa, rice has been the indigenous’ staple food for more than 50 years (Maclean et al., 2013).

United States of America is one of the countries in North America where rice is becoming more important. Total rice consumption increased about 634,000 metric tons in four years from 2011 to 2015 (Statista, 2016). Although the country is not included among the greatest rice producers, U.S. is one of the major rice exporters (Vegas, 2016). From 2007 to 2014, U.S. produced on average 40% more than national consumption. Most importers are countries in the Western Hemisphere, including Mexico and Central America. Because of the particular requirements of rice growth, U.S. rice production is predominantly concentrated in the southern states (Arkansas, Louisiana, Mississippi, Texas, and Missouri) and northern California (USITC, 2015). Long grain, non-aromatic rice is the most variety grown in this country. This type of rice was planted about 70% of the total harvested area in 2000 (Snyder and Slaton, 2001).
Arkansas is the leading rice producer in the U.S. In 2013, the state accounted for around 43 to 48 percent of total nation rice production (Hardke and Wilson, 2014; USITC, 2015). Rice is the second highest commodity in Arkansas after soybean. However, the rice industry in the U.S. is currently facing increased competition in global markets, particularly from lower-priced exporter countries. In addition to the complex regulation from the government and consumer expectations and concerns, water deficit is one of the obstacles for rice production in the U.S. (USITC, 2015).

**Rice growth and development**

The life cycle of rice is around 90–180 days. The growth duration depends on the variety and the environment where the rice is grown (Maclean et al., 2013). The growth duration for Arkansas cultivars is 105 to 145 days (Moldenhauer et al., 2014). Maclean et al. (2013) and Moldenhauer et al. (2014) divided rice growth into three developmental phases: vegetative, reproductive, and ripening. However, Grist (1986) added one more phase by dividing the vegetative phase into active-vegetative and vegetative-lag.

Seed germination to emergence, seedling, and active tillering to maximum tiller or stem elongation are the easiest characteristics to be observed during the vegetative phase. Other characteristics are a gradual increase in plant height and leaf emergence at regular intervals. The duration of this phase is about 44 to 87 days. IR64, a widely grown productive variety developed at IRRI, takes about 45 to 55 days for completing this phase (Grist, 1986; Government of India, 2011; Moldenhauer et al., 2014). At the end of this phase, initiation in tillering decreases and panicle initiation occurs (Grist, 1986).

The reproductive phase begins with panicle initiation, continues with heading, and ends with flowering stage. The duration for this phase is around 19 to 25 days for subtropical regions and 30 to 35 for tropical regions (Government of India, 2011; Moldenhauer et al., 2014). Under
drought, this phase is characterized by a decline in tiller number, booting, emergence of the flag leaf, heading and flowering (Moldenhauer et al., 2014). Some environmental issues like water shortage or drought stress during the previous phase (vegetative) can delay the occurrence of flowering (Ndjiondjop et al., 2010).

The final phase of the rice growing stage is the ripening phase. This phase is characterized by grain growth and development. The ripening phase starts at the flowering stage, followed by grain filling with a milky material until the individual is mature. The length of this period varies from 15 to 40 days for subtropical regions and about 30 days for tropical regions. (Grist, 1986; Government of India, 2011; Maclean et al., 2013; Moldenhauer et al., 2014). Although it is a final phase, the stability of the environment should be maintained.

Drought stress

The most limiting factor to plant growth is the lack of water. This constraint is often caused by deficient precipitation. However, human population and consumption are also responsible for water shortages in agriculture (Van Lanen et al., 2007; Farooq et al., 2009). Drought is classified into three major categories: (i) meteorological drought; (ii) hydrological drought; and (iii) agricultural drought. The meteorological perspective is mostly used to describe the level of water scarcity. However, among these three categories, in agricultural context drought is used to describe water shortage for crops. The severity of drought is determined by evaluating the level of precipitation, evapotranspiration, crop production, and plant growth (Dai, 2011; Manavalan and Nguyen, 2012).

This occurrence of drought has become more frequent and intense worldwide. FAO reported that in each part of the world, the areas that are suffering from water deficit are increasing, including agricultural areas. This is also due to the significant reduction in
precipitation, almost in every region in the world. Karim and Rahman (2015) reported that almost 47% of the terrestrial land surface in the world is categorized as dry, and 70% of these areas are utilized for agricultural activities. Southern Africa, the Sahel region of Africa, and the Horn of Africa are some places in this continent that have been affected greatly by water scarcity. In the Horn of Africa particularly, drought has severely affected severely this region for almost 12 years. In 2009, the production of wheat in Kenya, one country located in the Horn of Africa, dropped by to 45% after the worst drought happened in that country. The Mediterranean (southern Europe and northern Africa) is also a region that has been affected by drought. Australia has suffered from drought since 2002. In 2010, Russia experienced a very and intensive drought all over the country. One year before this scarcity happened in Russia, Yunan, a province in China, began entering a period of a devastating drought that affected 6.3 million people. Another region in Asia that also suffers a water deficit is southern Asia. In the U.S., drought mostly occurred in the southern states and California. An extreme drought in 2012 which covered the area of U.S. Great Grain Belt, pushed up world food prices (Union of Concerned Scientists, 2011; FAO, 2013; Nakashima et al., 2014).

**Drought stress in rice growth, development, and production**

Drought affects every phase of rice growth. Some studies conducted by Rahman and Yoshida (1985), Farooq et al. (2009), and Ndjiondjop et al. (2010) found that drought stress impaired seed germination and early seedling growth, reduced plant growth and development in the vegetative phase, delayed flowering at the reproductive phase, and decreased the rate of grain filling. Moreover, drought reduces plant turgor pressure (Farooq et al., 2009), which is important for cell elongation, cell enlargement, and in maintaining water potential (Gardner et al., 1995). Photosynthesis is also inhibited by drought because stomata will close when the water is deficit;
therefore, reducing the plant’s capacity to absorb CO\textsubscript{2}. The closing of stomata will also decrease the transpiration rate of the plant (Manavalan and Nguyen, 2012).

The reproductive phase, particularly at flowering stage, is the most affected by drought during rice growth and development (Farooq et al., 2009; Bunnag and Pongthai, 2013). Previous reports have shown a yield reduction up to 90% when drought stress occurs in this phase (Basnayake et al., 2006). During the reproductive phase, sterility will occur when rice experiences drought. This sterility is caused by imperfect ovary formation and poorly-developed anthers formed during the drought stress condition (Takeoka et al., 1992). The vegetative phase is another critical stadium for the success of rice growth and development (Bunnag and Pongthai, 2013). Drought in the vegetative phase can delay flowering up to 3 to 4 weeks, particularly when drought occurs in the beginning of this phase (Bouman and Tuong, 2001). Therefore, environmental stress can affect the final production of rice when drought attacks this phase.

Drought significantly affects rice production, particularly in the rainfed rice field. In Asia, where most rice is produced, this environmental uncertainty of rain affects about 23 million ha of rice growing area (Pandey and Bhandari, 2009). The regions that are most affected by drought are eastern India, Thailand, and Lao PDR (Maclean et al., 2013). In some states in eastern India (Jharkhand, Orissa, and Chhattisgarh) drought reduced rice production by up to 40% of total production (Serraj et al., 2011). A survey that was also conducted from these areas for 30 years, demonstrated increases of the incidence of poverty in rice farmers due to the effect of drought on rice production (Pandey and Bhandari, 2009). An extreme era of drought that has persisted in California for several years had caused many rice farmers to quit from this business (Summer, 2015a). Based on USDA report data, the quantity of rice supply from California in 2013 declined 33% from the previous year. The reduction caused an increase in rice prices up to
10% (Summer, 2015b). Last year, almost 25% of the $5 billion rice crop in California was lost due to this water scarcity (Koba, 2014; Associated Press, 2016). In addition, according to the U.S. Drought Monitor in summer 2015, Arkansas is one of the states that is severely affected by this phenomenon. This condition resulted in lower yield than last year (Hickey, 2015).

**Drought resistance in rice**

There are three adaptive mechanisms of plants in response to drought stress conditions: (i) drought avoidance; (ii) drought tolerance; and (iii) drought escape. Drought avoidance mechanism mitigates drought by maintaining the high water status during water shortage period. Both plant constitutive and adaptive traits control the avoidance mechanism. This includes osmotic adjustment (OA) as well as maintenance of high water status by increasing the osmotic force in the plant cell (Ito et al., 2000; Blum, 2011). The second mechanism maintains some or all components to function normally under drought condition. Drought tolerance mechanism includes both cellular and molecular adaptation activities (Blum, 2016). The drought escape mechanism enables plants to complete their life cycle before water stress occurs (Manavalan and Nguyen, 2012). Photoperiodic sensitivity, a short growth period, and flexibility of the cropping season are some of the characteristics that can contribute to this mechanism. In actual plants, these three mechanisms appear to not always work independently (Kobata, 1995).

The African rice *O. glaberrima*, is known for its resistance to drought (Ndjiondjop et al., 2010). Nagina 22 is also resistant to drought, although they both have some undesirable traits. These genotypes are frequently used as a drought-tolerant donor in breeding programs (Kumar et al., 2014). There are also some rice cultivars in West Africa and northern Thailand that possess suitable photoperiodicity. Other cultivars in Japan from the local upland variety are not photosensitive or thermosensitive. With all these special characteristics, those cultivars are
considered to have the ability to resist drought by using drought escape mechanism (Kobata, 1995).

**Gene Expression in Response to Drought Stress**

Drought stress can impose specific growth conditions on plants that may induce plants to defend against this environmental stress condition. Drought defense mechanisms in plants are primarily induced by the stress signals. Abscisic acid (ABA) is known for its critical role in mediating response to various stress signals in plants (Tuteja, 2007). ABA is one of the five main endogenous phytohormones. However, instead of being recognized as a plant growth activator, ABA is initially acknowledged as a plant growth inhibitor. When ABA was first discovered, it was shown to inhibit the growth of the plants by involvement in an abscission mechanism (Finkelstein, 2013).

As a stress signaling hormone, ABA biosynthesis is triggered by dehydration in the roots caused by drought stress, following which ABA moves towards the leaves leading to stomatal closure, which mediates control of stomatal conductance (Neméskéri et al., 2012). Stomatal conductance is dependent on changes in leaf water potential, leaf temperature, and carboxylation (CO₂ fixation) that can affect the assimilation rate (Farquhar and Sharkey, 1982). Stomatal closure reduces transpiration that can be correlated to the increase in water use efficiency (WUE) (Farooq et al., 2009). This ABA stress-signaling pathway is transmitted by ABA-dependent and ABA-independent mechanisms (Tuteja, 2007). The difference between these pathways lies on the involvement of the genes that control ABA biosynthesis and subsequent ABA signaling (Ciarmiello et al., 2011).

Another component that also can be enhanced by drought stress condition is reactive oxygen species (ROS). ROS accumulation is the result of the partial reduction of atmospheric
O₂. Although normally ROS can be removed rapidly by antioxidative mechanisms, drought stress can impair these mechanisms (Cruz de Carvalho, 2008; Ciarmiello et al., 2011). Similar to increased ABA synthesis, ROS also functions as an alarm signal that triggers defense responses by specific signal transduction pathways that involve H₂O₂ as a secondary messenger (Cruz de Carvalho, 2008). Therefore, both ABA and ROS can function in drought stress signaling and also as a defense mechanism. Both ABA biosynthesis pathway and ROS production are controlled by changes in gene expression (Tuteja, 2007; Cruz de Carvalho, 2008). The changes in expression of the genes or protein turnover, show that there are many alterations in the abundance of transcripts and proteins. This condition indicates that transcriptional and post-transcriptional regulation plays an essential role in the adaptation of cellular functions to the environmental changes (Ciarmiello et al., 2011).

In order to identify genes from the ABA biosynthesis pathway and ROS production that are responsible for response under drought conditions, the changes in gene expression of these response pathways should be analyzed (Pe’er, 2003; Cruz de Carvalho, 2008; Blum, 2011). Recent advances in molecular biology and genomics have provided an insight into plant gene regulatory networks involved in drought stress response. An understanding of the changes in gene regulatory patterns and the underlying mechanisms in different drought resistant genotypes, will help classify, breed, and select drought resistant rice cultivars expressing different mechanisms for stability under drought stress (Ciarmiello et al., 2011).
REFERENCES


Chapter 2

Screening for Drought Resistance in Diverse Rice Genotypes at Vegetative Stage
ABSTRACT

Drought is the most prominent constraint to rice (*Oryza sativa* L.) growth and development. Among the growth phases of rice, the vegetative stage is one of the most vulnerable phases that will give great effects towards productivity of the crop. Therefore, application of drought during the vegetative phase followed by physiological and molecular analysis of the plant will contribute knowledge about different pathways and genetic mechanisms conferring drought stress resistance in diverse rice genotypes. Eighteen rice genotypes from the USDA mini-core collection, with unknown status of drought resistance were randomly selected for the study. In addition, N22, a well-known drought resistant line was used as a positive control genotype. Ten days drought stress treatment of fifty percent field water capacity was applied to each genotype. The drought resistance levels were categorized on the basis of reduction in growth measured in plant biomass. The screening identified three resistant genotypes and ten moderately-resistant genotypes. One resistant genotype (*O. sativa* cv. Padi Tarab Arab), two moderately-resistant genotypes (*O. glaberrima* accession GPNO 25912, and *O. sativa* cv. N22) and three sensitive *O. sativa* genotypes (Pakkali, LA PLATA GENA F.A., and E B Gopher) were used further for measurement of physiological parameters (photosynthesis rate, stomatal conductance, and water use efficiency) and gene expression analysis. Leaf samples were taken for gene expression analysis at five and ten days after drought stress was applied. Insignificant differences were observed between control and stress plants for almost all the resistant and moderately-resistant genotypes in all physiological parameters. In contrast, sensitive genotypes demonstrated significant differences for these parameters. Gene expression analysis identified clear differences between resistant (resistant and moderately-resistant) and sensitive genotypes. A high and consistent induction in the relative expression of drought stress genes in drought compared to
control was observed in Padi Tarab Arab, GPNO 25912, and N22, while the three sensitive genotypes showed low, no, late, or inconsistent induction for the expression. This analysis identified Padi Tarab Arab and N22 to exhibit drought avoidance and tolerance mechanisms while GPNO 25912 exhibited only tolerance mechanism as a defense under drought stress condition.
INTRODUCTION

Drought is the most serious constraint to rice growth and development. This is a consequence of water being the major component of plants, comprising more than 80% of plant fresh weight (Ferguson, 1960; Chavarria and Dos Santos, 2012). Drought is mainly caused by low precipitation (Van Lanen et al., 2007). The problems of water deficit have become more severe due to competition of agriculture and human uses of water which is become a dwindling resource. Global climate change may be one of the main causes (Ndjiondjop et al., 2010), affecting many aspects such as precipitation, temperature, and potential evapotranspiration (Van Lanen et al., 2007). However, environmental factors are not the only source of drought. The increase of the human population and consumption are also having important roles in causing drought (Farooq et al., 2009). It is predicted that land surface with extreme drought will increase from 1 to 3% nowadays to 30% by the 2090s (IPCC, 2007).

Rice growth and development begins with the germination of the seed and ends with the formation of the grain (Dunand and Saichuk, 2015). The first phase of all these stages is the vegetative phase. Vegetative phase is one of the most important periods in rice growth and development. The vegetative parts of the rice plant consist of roots, stems, and leaves. This phase is characterized by seed germination, seedling emergence, leaf emergence, tillering, and plant height growth (Beighley, 2010). Furthermore, Vergara and Chang (1985) divided this phase into two phases based on the sensitivity on photoperiod: (i) basic vegetative phase (BVP) is the phase where the plant is insensitive to photoperiod, and (ii) photoperiod-sensitive phase (PSP) which is the phase just after BVP is completed and is affected by photoperiod.

Every phase of plant growth can be affected by drought, including vegetative phase. During this period, most of the crops cannot withstand a water deficit less than 85% of relative
humidity (Manavalan and Nguyen, 2012). The most affected process during vegetative phase is the growth of the cell. Plant growth depends on both cell expansion and cell division. However, drought affects cell expansion more than the cell division (Blum, 2016). The broader effects of the interference of cell expansion and cell division are: (i) inhibition of leaf production and decline in leaf area (LAI); (ii) reduction of stem elongation that affects the plant height; (iii) reduction of tillering; and (iv) reduction of root growth (Bouman and Tuong, 2001; Blum, 2011; Aslam et al., 2013). Although drought reduces root growth, root-shoot ratio increases during drought. The reason is because shoot is less sensitive to drought than root (Aslam et al., 2013). Therefore, roots will grow faster than shoots. In addition, drought impairs the photosynthesis rate by stomatal closure which relies on the water status of the guard cells. Subsequently, stomatal closure reduces the absorption of carbon dioxide (CO$_2$) assimilation (Farooq et al., 2012; Manavalan and Nguyen, 2012). Stomatal closure also reduces transpiration which causes an increase in water use efficiency (WUE) (Blum, 2016). Ultimately, all these interferences by drought will reduce the total biomass of the crop (Sarvestani et al., 2008). Another significant impact when drought occurs during vegetative phase is a delayed flowering time. A study in rice demonstrated a 50 days of delay in rice flowering when drought occurred at the pre-reproductive phase (Ndjiondjop et al., 2010; Blum, 2016). Consequently, flowering delay will have a great impact in rice production.

Plants respond to drought stress during their life cycle in a number of ways, involved in perception and programmed response leading towards protection under drought, which is often determined by the genetic constitution of the host plant. A variety of drought response mechanisms have been identified, each with specific physiological and gene expression programmed responses. ‘Drought avoidance’ is the ability of plants to maintain relatively high
tissue water potential despite a shortage of soil moisture. Drought tolerance refers to the relative capacity to sustain or conserve plant function in a dehydrated state (Blum, 2005), and is the second line of defense after dehydration avoidance. The physiological response of drought resistance has been broadly defined as being determined by drought avoidance and/or drought tolerance (Levitt, 1972). The production and accumulation of phytohormone abscisic acid, toxic, solutes, and oxidative damage in every phase of crop growth and development, including vegetative stage are also part of drought stress (Blum, 2011; Demidchik, 2012; Manavalan and Nguyen, 2012). When plants experience any dehydration or desiccation, they accumulate abscisic acid (ABA), a plant hormone that regulates and inhibits many aspects of plant growth and development. ABA later is described as a “stress hormone” because this hormone plays a critical role in evoking response to various environmental stresses, including drought, particularly in the vegetative stage (Blum, 2011; Finkelstein, 2013; Basu et al., 2014). Moreover, ABA recognizes multiple signal transduction pathways under osmotic stress conditions, thus, this hormone can regulate the expression of genes and transcription factors which are responsible for responding and adapting to environmental stress (Tuteja, 2007; Nakashima et al. 2014).

Zeaxanthin epoxidase (ZEP) and 9-cis-epoxycarotenoid dioxygenase (NCED) are some of the genes that are responsible for stress response and ABA biosynthesis regulation. The overexpression of both genes has been shown to increase drought resistance in some plants like Arabidopsis (Arabidopsis thaliana) and Petunia hybrida (Park et al., 2008; Ahrazem et al., 2012; Estrada-Melo et al., 2015). As described in Chapter 1, there are two mechanisms of ABA pathway, dependent and independent. Both pathways are regulated by several transcription factors, such as dehydration responsive element binding protein 2C (DREB2C) (Tuteja, 2007; Yoshida et al., 2014). DREB2C plays important role particularly in ABA-independent pathway.
Moreover, this gene induces the expression of various stress-responsive genes in plants (Lata and Prasad, 2011). DREB2C is a member of APETALA2 (AP2)/Ethylene Response Factor (ERF) subfamily. A study by Todaka et al. (2015) exhibited an enhancement of tolerance to drought, cold, and high salinity due to the overexpressing of AP2/ERF transcription factor.

Another significant effect of abiotic stress on plants is oxidative damage. Oxidative damage or stress is a ubiquitous phenomenon, leading to damage caused by the formed reactive oxygen species (ROS). ROS is the result of the partial reduction of atmospheric oxygen (O₂). There are basically four forms of cellular ROS, singlet oxygen (¹O₂), superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂) and hydroxyl radical (HO·) (Wu et al., 1999; Cruz de Carvalho, 2008; Demidchik, 2012). ROS is produced both in normal and stress conditions, and most plants already have well-developed defense systems against ROS. However, ROS and the defense systems increase greatly when the plant experiences stress (Wu et al., 1999; Alscher et al., 2002). The defense systems have evolved both non-enzymatic and enzymatic antioxidant mechanisms. The enzymatic mechanism comprises of two main constituents: superoxide dismutase (SOD), enzymes and metabolites from the ascorbate-glutathione cycle, and Catalase (CAT) (Scandalios, 2005; Cruz de Carvalho, 2008). Previous studies demonstrated that the overexpression of both antioxidants could increase the tolerance to any specific stress (Wu et al., 1999; Alscher et al., 2002). In addition to that, SOD and CAT can act to modulate the expression of other ROS responsive genes (Scandalios, 2005).

ROS is well-known for producing free-radicals that can accumulate antioxidant. However, there are also other processes that enhance antioxidant production such as photorespiration, as this process is able to regulate ROS as a consequence of the ability to increase H₂O₂ (Wingler et al., 2000; Merewitz et al., 2011). There are two key enzymes of
photorespiration, glycolate oxidase 1 (GLO1) and glycolate oxidase 4 (GLO4). The expression levels of both enzymes are used to estimate photorespiration due to the water stress (Peterhansel et al., 2010; Zhang et al., 2012). In addition to that, other enzymes Rad50 (Radiation sensitive50) and CDC (Cell Division Control) also work to confer water stress resistance through the antioxidant pathway. Both enzymes are involved in DNA damage repair mechanisms (Kitsios and Doonan, 2011; Gatei et al., 2014). The antioxidant pathway is also positively correlated to other processes such as osmolytes and flavonoid biosynthesis. An osmolyte, polyamine, is important to any biotic and abiotic stress by tolerating the exposure of stress factors (Pietta, 1999; Minocha et al., 2014). S-adenosylmethionine decarboxylase (SamDC) is a protein that regulates polyamine biosynthesis and positively affects signaling pathway and defense mechanisms to biotic and abiotic stresses (Basu et al., 2014; Minocha et al., 2014). Antioxidant production can also be detected by the accumulation of flavonoids. Flavonoids are secondary metabolites with an ability to scavenge and reduce the free radicals (Pietta, 1999). Phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) are two enzymes that have redundant role in flavonoid biosynthesis (Huang et al., 2010; Dao et al., 2011).

Besides those two components, an increase in water deficit is also responsible for the intracellular accumulation of solutes. The mechanism of plants to respond to this change is defined as osmotic adjustment. Osmotic adjustment maintains the plant’s high water status by controlling cellular turgor when tissue water potential declines. There are some plant processes that can be controlled by osmotic adjustment under drought stress condition: stomatal conductance and photosynthesis, delayed leaf senescence and death, reduced flower abortion, root growth, and water extraction from soil. For these reasons, osmotic adjustment is considered a significant mechanism of drought resistance, particularly for dehydration avoidance (Blum,
The accumulation of solutes can induce expression of some transcription factors and other genes. Slow anion channel associated 1 (SLAC1), plasma membrane intrinsic proteins (PIP2;3 and PIP2;6) are some of these genes. SLAC1, PIP2;3, and PIP2;6 are responsible for stomatal conductance which is one of the components of osmotic adjustment and also can be induced by the synthesis of ABA (Negi et al., 2008; Manavalan and Nguyen, 2012; Yooyongwech et al., 2013). SLAC1 is specifically expressed in guard cell that results in restoration of stomatal response (Negi et al., 2008; Vahisalu et al., 2008). The other two genes, PIP2;3 and PIP2;6, are also recognized as aquaporins. This is a result of the water channel activity that is frequently observed. Moreover, these genes also putatively involve in water relations (Da Ines, 2008; Huang et al., 2012). PIP2 genes that are highly homologous to each other are positively correlated with WUE and drought tolerance (Yooyongwech et al., 2013). Another process that is also controlled by osmotic adjustment under water stress condition is photosynthesis (Shangguan et al., 1999). There are two genes that correlate with photosynthesis process, light-harvesting chlorophyll a/b-binding protein (LHCP) and oxygen evolving enhancer proteins (OEP). Some previous studies identified an enhancement in expression of these genes in response to stress induced by drought, salinity, and ABA (Xu et al., 2011; Cheng et al., 2015).

There are two main objectives of this study. The first objective is to screen a diverse set of rice genotypes at the vegetative growth stage for drought resistance (growth under drought). The second objective is to characterize the genetic difference of mechanisms of drought response conferring drought stress resistance in different genotypes during the vegetative phase of rice growth and development. As described above, different mechanisms of drought resistance are expressed to give protection against drought stress in plants. The expression of genes that are
responsible for the different drought resistance mechanisms will provide information on the employment of these pathways in drought stress response. Drought resistance is a complicated trait which includes some aspects from physiology, breeding, and genomics (Blum, 2011). Therefore, recognizing the importance of different pathways of drought resistance in different germplasm sources will be useful towards employment of an integrated plant breeding program for development of drought tolerant or resistant plant varieties.

MATERIALS AND METHODS

Plant materials

A subset of eighteen diverse rice genotypes from the United States Department of Agriculture (USDA) mini-core collection were randomly selected for this experiment (Table 2-1) (Agrama et al., 2010). Drought resistant genotype Nagina 22 (N22) was used as a resistant-reference genotype (Kumar et al., 2014; Vikram et al., 2015).

Drought stress treatment at vegetative stage

Rice seeds were germinated by imbibing the seeds with deionized water in an incubator in the dark at 27°C for seven days. Each emerged seedling was placed in single pots filled with a Redi-earth potting mix (Sun Gro Horticulture Distribution). Thirty days after germination, drought stress was applied by withholding the water until the moisture level dropped to 50% of field capacity (FC) maintained for ten days (Batlang et al., 2013). Fifty percent of FC was calculated by weighing the saturated pots (100% of FC) for each pot. In this stress period, each pot was weighed daily at a fixed time of the day, and water lost was replenished to maintain the required FC (Ramegowda et al., 2014). Control plants were maintained under well-watered conditions (100% of FC). All the rice genotypes were grown in the greenhouse at Altheimer
Laboratory, University of Arkansas, Fayetteville. The temperature was maintained between 28 to 30°C (Ghadirnezhad and Fallah, 2014). The experimental design was a completely randomized design (CRD) with five replications.

In order to determine growth response to drought stress, we measured photosynthesis rate using a portable photosynthesis meter LI-COR 6400XT at a CO₂ concentration of 370 μmol/mol, light intensity of 1,000 μmol/m²/s, and 55% to 60% relative humidity at the tenth day of drought stress application (Ramegowda et al., 2014; De Freitas et al., 2016). The 2nd fully expanded leaf from the top of the plants was used to as a sample for measuring these parameters. The parameters observed are: photosynthesis rate, stomatal conductance, and instantaneous water use efficiency (iWUE) (Farquhar and Sharkey, 1982; Krause, 1991; Blum, 2011). The iWUE was calculated by dividing photosynthesis rate to transpiration rate. Being one of the components that will be reduced due to drought stress, total biomass were measured by weighing dry above-ground biomass production at the end of drought stress (Sarvestani et al., 2008; Ramegowda et al., 2014). Analysis of variance was performed to assess the drought stress among genotypes. Tukey’s HSD was used to compare means for significant effects (P≤0.05) using JMP version 12.

**Gene expression analysis**

RNA isolation and quantification of gene expression were conducted for leaf samples from two biological replicates, of six selected genotypes taken on the fifth and tenth days of stress, both for treated and control plants. The genotypes consist of one resistant-reference genotype (N22), two putative-resistant genotypes (GPNO 25912 and Padi Tarab Arab), and three sensitive-putative genotypes (Pakkali, E B Gopher, and LA PLATA GENA F.A.). RNA was isolated using TRIzol reagent, and complementary DNA (cDNA) synthesis from the mRNA was conducted using 2μg total DNAse-treated RNA by GoScript® Reverse Transcription System
(Promega). The qRT PCR experiments were conducted using GoTaq® qPCR Master Mix (Promega), with gene-specific primers and Ubiquitin primers as standard. Nineteen genes, including transcription factors and other genes, which are accountable for drought stress response and adaptation were used as primers for generating the gene expression data (Table 2–2). Increasing temperature (0.5°C/10 s) from 55°C to 95°C was used to perform the melting curve analysis, with un-transcribed RNA run as negative control. The relative difference in expression for each sample in individual experiments was determined by normalizing the threshold cycle (Ct) value for each gene against the Ct value of Ubiquitin and calculated relative to the respective control samples as a calibrator using the equation $2^{-\Delta\Delta Ct}$. The average of two biological replicates was used to obtain each expression value (Ramegowda et al., 2014; Bevilacqua et al., 2015; De Freitas et al., 2016). Standard error was used to separate means for significant effects.

RESULTS AND DISCUSSION

Screening of rice genotypes for drought resistance at vegetative stage

Biomass is defined as a product of green plants converting sunlight into plant material through the process of photosynthesis (McKendry, 2002). Therefore, biomass is an important factor in growth analysis as it is the basis for the calculation of net primary production and growth rate (Golzarian et al., 2011). Vegetative biomass was measured by weighing all above-ground dry matter for both control and stress plants at the end of drought stress. Based on the analysis on variance, there are significant differences between control and stress plants and among the nineteen genotypes from the USDA mini-core collection in terms of percentage reduction of plant biomass. There is also a specific interaction between treatment and genotypes.
(Table 2-3). This interaction shows the difference in reduction between control and stress plants. Based on this parameter, rice genotypes were divided into three classes: (i) resistant (0-29% of reduction), (ii) moderately-resistant (30-49% of reduction), and (iii) sensitive (≥50% of reduction). This categorization follows the method conducted by De Freitas et al. (2016).

Figure 2-1 illustrates plant biomass of nineteen diverse genotypes both in control and stress plants as well as percentage reduction of plant biomass. The percentage reduction and the categorization of resistance is also shown in Table 2-1. Two genotypes from the species (sp.) *O. sativa* (Manga Kely 694 and Padi Tarab Arab) and one genotype from *O. glaberrima* sp. (TOg 7025) are categorized as resistant genotypes with lower than 30% reduction in plant biomass. Subsequently, ten genotypes (nine are *O. sativa* sp., including N22 and one is *O. glaberrima* sp.) are classified as moderately-resistant, with no reduction in biomass above 50%, while the rest of genotypes (all genotypes are from *O. sativa* sp.) with reduction in biomass above 50% are classified as sensitive. The two genotypes from *O. glaberrima* sp. (TOg 7025 and GPNO 25912) are categorized as resistant and moderately-resistant to drought stress, respectively. The original African rice, *O. glaberrima* sp., is well-known for its resistance to abiotic stresses, such as water depth, iron toxicity, infertile soils, severe climate, and human neglect. A previous study from De Freitas et al. (2016) also showed that TOg 7025 is a cold resistant. However, African rice farmers nowadays prefer planting the Asian rice (*O. sativa* sp.) to the African rice because of the higher yield (Linares, 2002). Another resistant genotype, Manga Kely 694, is a hybrid from *O. sativa* subspecies (ssp.) *indica* and *aus*. The *aus* spp. is also reputed for its resistance to drought stress and early maturity. This subspecies is originally distributed along the coasts of India and China. In Bangladesh, *aus* spp. is grown during the summer season from March to June (Garris et al., 2005).
The cluster dendrogram by the percentage reduction of plant biomass reveals two major clusters (Figure 2-2). The dendrogram analysis was conducted by JMP version 12 analysis program. In the present study, there are four genotypes of pure indica ssp. and seven genotypes of pure japonica ssp. Three out of four genotypes of indica ssp. are identified as resistant to drought stress and included in the first cluster, while four among seven genotypes of japonica ssp. are identified as resistant and added in the second cluster. Based on a previous study from Lilley et al. (1996), indica ssp. tends to have a high osmotic adjustment and in the opposite, japonica ssp. has low osmotic adjustment. Osmotic adjustment is one of the most important mechanisms in responsible for drought resistance in plants. Osmotic adjustment is needed to maintain turgor pressure of the plant under stress condition (Fischer and Fukai, 2003).

**Response of drought stress on physiological processes in rice genotypes**

Water is the most abundant and crucial resource in plants and is involved in many physiological, cellular, and molecular processes (Ferguson, 1959). Apparently, water shortage will give great negative effects for plant growth and development. In this study, we measured three physiological parameters which are known to be significantly affected by water deficit: photosynthesis, stomatal conductance, and iWUE. The results given in this study strongly present that drought stress significantly affects to these physiological processes. There are significant differences between control and stress plants and among the genotypes in these parameters (P≤0.05) (Table 2-4). There is also a specific interaction between treatment and genotypes (P≤0.05), although the interaction occurs only for the differences in reduction for the stressed plants among the genotypes (Figure 2-3, Figure 2-4, and Figure 2-5).

In order to determine whether these three parameters are related to each other and to the plant biomass measurement, we calculated the correlation between the percentage reduction of
the stress response parameters (plant biomass, photosynthesis, stomatal conductance, and iWUE). A positive and significant correlation between percentage reduction of photosynthesis and percentage reduction of plant biomass ($R^2=0.51877; P\leq0.05$) reveals that the alteration of photosynthesis affects the biomass of the plant (Figure 2-6) as it is also shown previously (Ramegowda et al., 2014). A similar bilinear positive correlation is also observed between percentage reductions in photosynthesis to the percentage reduction in stomatal conductance. Data obtained in this study shows a positive and significant correlation between these two reductions ($R^2=0.44029; P\leq0.05$) (Figure 2-7). In an earlier study, Flexas and Medrano (2002) suggested that stomatal closure is one of the dominant limitation to photosynthesis under drought conditions particularly when the plant is experiencing mild or moderate water deficit. Stomatal closure predominantly will alter the accumulation of one of the main photosynthesis constituents, $CO_2$, inside the leaf and accordingly reduce photosynthesis.

Another significant parameter that is correlated to photosynthesis is WUE. Based on an agronomic perspective, WUE can be referred as a ratio between dry matter production (yield) of the plants and the water used to produce the yield, while physiologically, it is a ratio between photosynthesis and evapotranspiration (Gardner et al., 1995; Blum, 2005). Figure 2-8 illustrates a positive and significant correlation between percentage reduction of iWUE and percentage reduction of photosynthesis ($R^2=0.42992; P\leq0.05$). Similarly, this phenomenon was demonstrated in previous studies (Nguyen et al., 2004; Blum, 2005; Ramegowda et al., 2014), suggesting that one of the key factors in determining drought resistance is WUE. High WUE will help plant maintaining the osmotic adjustment and reducing water loss.
Drought stress responsive expression patterns of genes

To study the relationship of drought responses to gene expression changes, six genotypes were randomly selected from the different resistance categories for further analysis by gene expression. One of the resistant genotypes (Padi Tarab Arab), two moderately-resistant genotypes (GPNO 25912 and N22), and three sensitive genotypes (Pakkali, LA PLATA GENA F.A., and E B Gopher) were used for a time course analysis of gene expression. Samples were taken at day 5 and day 10 of drought stress treatment and control well-watered plants to determine the differential plant responses during early and late drought treatment. The relative expression of gene presented in the graphs is the relative induction value of the genes in the drought stressed plants compared to the control plants.

The synthesis of osmolytes and antioxidants is one of the pathways that confers drought resistance for the plants (Alscher et al., 2002; Basu et al., 2014). This pathway is strongly correlated with the drought stress tolerance mechanism. In response to water stress, plants accumulate protective proteins such as organic osmolytes. Polyamines, one of these organic osmolytes, can also act as an antioxidant (Burg and Ferraris, 2008). Data obtained in a previous study indicated that in a water stress-resistant cultivar, the accumulation of polyamines increased, whereas the sensitive genotype showed no rise in polyamines accumulation (Montesinos-Pereira et al., 2014). In the present study, we screened for the expression of SamDC to study the variation of polyamine accumulation. As illustrated in Figure 2-9, all resistant and moderately-resistant genotypes demonstrate high induction of the relative expression, while none of the sensitive genotypes have increased expression of SamDC. Polyamines are known for their roles in many metabolic processes in plants such as cell division and organogenesis because this polycation stimulates DNA replication, transcription and translation (Kaur-Sawhney et al.,
Accordingly, polyamine biosynthesis can protect plants from stress damage. As ubiquitous molecules, polyamines are distributed in all the parts of the plant cell (Basu et al., 2014).

One of the consequences of drought stress is the increase in ROS accumulation which damages lipids, proteins, and DNA. As outlined in the introduction, ROS enhancement can also trigger the defense responses of the plant to the stress. One of the responses is the production of antioxidants (Cruz de Carvalho, 2008). The relative expression of two antioxidant enzymes, SOD and CAT, were used to determine the variation in antioxidant production in response to the accumulation of ROS. This pathway is referred to as enzymatic antioxidant defense response (Scandalios, 2005). As shown in Figure 2-10, the resistant and moderately-resistant genotypes show induction of these antioxidants. Among the sensitive genotypes, only E B Gopher upregulates the expression of SOD, but at a later time period. Both antioxidants can reduce the damage due to drought stress by converting free radicals into less reactive species. Accordingly, SOD catalyzes $\text{O}_2^-$ to $\text{H}_2\text{O}_2$, and then CAT reduces $\text{H}_2\text{O}_2$ to $2\text{H}_2\text{O}$ (Scandalios, 2005).

As described in the introduction that photorespiration can also increase antioxidant production with the GLO enzymes as the key components of this process. Photorespiration produces $\text{H}_2\text{O}_2$ free radicals as their final product (Wingler et al., 2000; Peterhansel et al., 2010; Zhang et al., 2012). Figure 2-11 details the relative expression of GLO1 and GLO4 in resistant and sensitive genotypes. Padi Tarab Arab, GPNO25912, and N22 reveal the induction of both enzymes compared to control non-stressed plants. Amongst the sensitive genotypes, E B Gopher again shows its late induction during drought. Other sensitive genotypes (Pakkali and LA PLATA GENA F.A.) also show late induction in GLO4. However, this late induction does not confer the resistance to these genotypes in our screen.
The expression of some genes involve in DNA repair pathway, Rad50 and CDC were also quantified to assess their role in drought response. A previous study analyzing the role of Rad50 in mammalian cells suggested that this gene is important in detecting DNA double-strand breaks and DNA replication restart (Gatei et al., 2014). Early and late inductions of the expression of Rad50 are demonstrated by Padi Tarab Arab, GPNO 25912, and N22 genotypes, while Pakkali and E B Gopher (sensitive genotypes) show late induction of Rad50 observed at day 10 (Figure 2-12 A). Another DNA repair gene (CDC) is upregulated in early and late drought stress by all resistant genotypes, while one sensitive genotypes (E B Gopher) exhibits late induction of CDC at Day 10 (Figure 2-12 B). CDC is an enzyme that is part of large a CDK (Cyclin Dependent Kinases) family (Kitsios and Doonan, 2011). Schuppler et al. (1998) suggested that drought stress increases the activity of this enzyme by inducing a phosphorylation signal. Both enzymes form a pathway of DNA damage repair for plants in response to water stress.

Further antioxidant quantification can be identified by the accumulation of flavonoids. To estimate the accumulation of flavonoids, we calculated the relative expression of PAL and CHS. As reported in a previous study, PAL has an important role in flavonoid biosynthesis, with lack of flavonoids leading to increased sensitivity of plants to abiotic stress (Huang et al., 2010). Figure 2-13 A illustrates that PAL is upregulated in both early and late drought stress in Padi Tarab Arab and N22 genotypes, while GPNO 25912 only shows early induction in PAL expression. PAL is downregulated in all sensitive genotypes. Similar behavior is also observed in the expression of CHS, a key enzyme in flavonoid biosynthesis which is also involved in the salicylic acid defense pathway. All resistant genotypes and one sensitive genotype (E B Gopher) show induction of the gene during drought (Figure 2-13 B). Expression of the CHS gene is
known to be affected by UV and wounding by pathogen attack (Dao et al., 2011), and helps plants produce more flavonoids in conferring resistance to biotic and abiotic stresses.

We calculated the expression of two genes related to the photosynthesis process, LHCP and OEP, in order to identify the responses due to water stress conditions. Both LHCP and OEP are important for photosynthesis particularly for the activity of enzyme photosystem II (PSII) (Mayfield, 1991; Xu et al., 2011). PSII is an enzyme inside the chloroplast that basically oxidizes water and transfers electrons to enzyme photosystem I (PSI). Furthermore, PSI generates NADPH up to energy is produced (Nelson and Yocum, 2006). The resistant and moderately-resistant genotypes exhibit high induction in the relative expression of these genes. In reverse, most of the sensitive genotypes show low or no induction (Figure 2-14). The accumulation of these genes helps plants to maintain photosynthesis under stress condition, which is part of the drought stress tolerance mechanism. Early induction at day 5 occurs in both moderately-resistant genotypes (GPNO 25912 and N22), while late induction at day 10 is shown by the resistant genotype (Padi Tarab Arab) and one sensitive genotype (E B Gopher). However, despite the induction of these genes in E B Gopher, there is still significant reduction in photosynthesis from 8.66 µmol CO$_2$/m$^2$/s$^1$ to 1.96 µmol CO$_2$/m$^2$/s$^1$ (Figure 2-3).

The alteration of photosynthesis is partially affected by the changes in stomatal behavior. Through stomata, plant leaves take up CO$_2$ to produce energy for the plant (Farquhar and Sharkey, 1982; Ishii, 1995). To monitor this process, three stomatal conductance related genes were used in this study, encoding the SLAC1, PIP2;3 and PIP2;6. A previous study by Imai et al. (2015) concluded that SLAC1, a protein associated with stomatal closure, showed high transcript level in a drought resistant genotype. The resistant and moderately-resistant genotypes and one sensitive genotype (E B Gopher) exhibit induction of SLAC1 in both early and late drought.
stress (Figure 2-15 A). Another sensitive genotype (Pakkali) also shows an induction only in early drought. A similar pattern as SLAC1 is also evident for the aquaporin proteins (PIP2;3 and PIP2;6). As shown in Figure 2-15 B and C, the resistant genotypes constantly give induction for the expression of drought stress induced genes. Meanwhile, E B Gopher shows low and late induction for both genes. PIP2;3 and PIP2;6 are highly homologous, therefore the expression of these genes in the genotypes are similar. These genes are correlated with many processes in plants, such as transport process, photosynthesis, cell wall-related processes, hormone metabolism, and stress responses (Da Ines, 2008). Stomatal conductance plays a role in the drought avoidance pathway, maintaining water supply, sustaining leaf hydration and turgidity so as to delay stomatal closure (Blum, 2011). Among the resistant genotypes, GPNO 25912 demonstrates significant differences in stomatal conductance between control and stress plants from 0.1857 mmol H₂O/m²/s to 0.1267 mmol H₂O/m²/s (Figure 2-4). GPNO 25912 also has the highest percentage reduction in photosynthesis among these three genotypes (17.07%) (Figure 2-3). This suggests that GPNO 25912 exhibits a drought tolerance mechanism rather than drought avoidance mechanism.

Stomatal closure is a process that is induced by the accumulation of ABA. As described in the introduction, drought stress can increase ABA concentration. ABA levels is fluctuate depending on the physiological and environmental changes in the plant (Tuteja, 2007; Finkelstein, 2013). NCED and ZEP, two important enzymes of ABA biosynthesis were used to estimate the accumulation of this phytohormone. In a previous study by Estrada-Melo et al. (2015), transgenic plants that overexpressed NCED under water stress condition showed a significant increase in drought resistance compared to the control plants. The transgenic plants also showed an increase in ABA and proline concentration. N22 exhibits high induction in the
relative expression of NCED, while other resistant genotypes show low or no induction, particularly for GPNO 25912 (Figure 2-16 A). Two sensitive genotypes (Pakkali and E B Gopher) exhibit an induction only at day 10. Almost a similar pattern is shown in the expression of ZEP (Figure 2-16 B). The resistant genotypes demonstrate high induction, whereas the sensitive genotypes Pakkali and E B Gopher show a late induction at day 10. The late induction for Pakkali and E B Gopher do not support resistance under water stress condition. As reported previously, ZEP-overexpressing plants increased drought resistance by reducing water loss, losing only 44% of water compared with 52% in the wild type plants (Park et al., 2008). This indicates that the plants exhibit a drought avoidance mechanism by withholding water content.

In engineering for drought stress resistance, transcription factors have often been used to enhance stress resistance in plants. Transcription factors are known for their role in regulating gene expression, including in response to environmental stress factors (Xiong et al., 2005; Hussain et al., 2011). DREB2C, a member of the subfamily of AP2/ERF domain transcription factors, is a transcription factor that regulates the expression and binds to the stress-responsive promoter element DRE/CRT (dehydration-responsive element/C-repeat) (Lee et al., 2010), which is strongly correlated with the ABA-independent stress signaling pathway (Tuteja, 2007; Yoshida et al., 2014). Figure 2-17 A shows the relative expression of DREB2C. N22 demonstrates high induction compared to other resistant genotypes, while the sensitive genotypes downregulate the expression of DREB2C. Two previous studies in rice and Arabidopsis concluded that the overexpression of this gene exhibits response to different abiotic stresses (Lee et al., 2010; Hwang et al., 2012). Other genes that also belong to the AP2/ERF transcription factor family are ERF genes. In our study, we used two ERF genes, EFR#68 and ERF#71. Both genes are upregulated in all resistant genotypes, while sensitive genotypes demonstrate no
induction except for E B Gopher in ERF#68 at day 10 (Figure 2-17 B and C). In the study by Wang et al. (2014) these genes were shown to be highly induced by the increase of ABA levels in stressed plants. Consequently, the induction of these transcription factors leads to enhancement of plant stress resistance or tolerance.

CONCLUSIONS

There has been much research on the significance of genes for the enhancement of water stress resistance or tolerance in drought sensitive plant genotypes. However, in this study, the focus is on how the induction in expression of drought stress related genes during vegetative stage is correlated with the response and drought stress adaptation in a diverse set of resistant and sensitive genotypes. Three resistant genotypes (Padi Tarab Arab, GPNO 25912, and N22) demonstrate induction for almost all drought stress related genes both in early and late drought stress, while drought sensitive genotypes show low, no, late, or inconsistent induction in expression of drought stress related genes. Partial and late inductions in sensitive genotypes (Pakkali and E B Gopher) do not enhance the drought resistance. All resistant genotypes exhibit a drought tolerance mechanism, particularly by maintaining photosynthesis and antioxidant activity. Among these three genotypes, only GPNO 25912 does not utilize drought avoidance mechanism in responding to water stress condition. The resistant-reference genotype, N22, strongly exhibits both drought tolerance and avoidance mechanisms, underpinning a drought resistance mechanism (Blum, 2005).
REFERENCES


Table 2-1. List of diverse rice genotypes and their phenotypic categorization based on the reduction of plant biomass

<table>
<thead>
<tr>
<th>% Reduction of Plant Biomass</th>
<th>Resistance Category (*)</th>
<th>Name (**)</th>
<th>Code of Genotypes (GSOR)</th>
<th>Origin</th>
<th>Species/Subspecies (****)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.77</td>
<td>R</td>
<td>Padi Tarab Arab</td>
<td>311643</td>
<td>Malaysia</td>
<td>O. sativa/TRJ</td>
</tr>
<tr>
<td>23.43</td>
<td>R</td>
<td>TOg 7025</td>
<td>311603</td>
<td>Sierra Leone</td>
<td>O. glaberrima</td>
</tr>
<tr>
<td>25.34</td>
<td>R</td>
<td>Manga Kely 694</td>
<td>310503</td>
<td>Madagascar</td>
<td>O. sativa/IND-AUS</td>
</tr>
<tr>
<td>30.02</td>
<td>M-R</td>
<td>GPNO 25912</td>
<td>311688</td>
<td>El Salvador</td>
<td>O. glaberrima</td>
</tr>
<tr>
<td>30.09</td>
<td>M-R</td>
<td>Somewake</td>
<td>310883</td>
<td>Japan</td>
<td>O. sativa/TEJ</td>
</tr>
<tr>
<td>30.81</td>
<td>M-R</td>
<td>ARC 10633</td>
<td>311734</td>
<td>India</td>
<td>O. sativa/IND</td>
</tr>
<tr>
<td>31.16</td>
<td>M-R</td>
<td>4595</td>
<td>311484</td>
<td>China</td>
<td>O. sativa/IND</td>
</tr>
<tr>
<td>32.86</td>
<td>M-R</td>
<td>N22 (reference)</td>
<td>310747</td>
<td>India</td>
<td>O. sativa/AUS</td>
</tr>
<tr>
<td>36.13</td>
<td>M-R</td>
<td>BHIM DHAN</td>
<td>310016</td>
<td>Indonesia</td>
<td>O. sativa/TRJ</td>
</tr>
<tr>
<td>37.38</td>
<td>M-R</td>
<td>Indonesia Seln</td>
<td>310219</td>
<td>Afghanistan</td>
<td>O. sativa/ARO</td>
</tr>
<tr>
<td>43.14</td>
<td>M-R</td>
<td>Red Khosha Cerma</td>
<td>311685</td>
<td>Russian Federation</td>
<td>O. sativa/TEJ</td>
</tr>
<tr>
<td>46.90</td>
<td>M-R</td>
<td>WIR 911</td>
<td>310220</td>
<td>Afghanistan</td>
<td>O. sativa/AUS</td>
</tr>
<tr>
<td>48.08</td>
<td>M-R</td>
<td>Safut Khosha</td>
<td>310015</td>
<td>Indonesia</td>
<td>O. sativa/IND</td>
</tr>
<tr>
<td>65.85</td>
<td>S</td>
<td>Mayang Khang</td>
<td>310052</td>
<td>The Philippines</td>
<td>O. sativa/TRJ</td>
</tr>
<tr>
<td>67.42</td>
<td>S</td>
<td>Quinimpol</td>
<td>311787</td>
<td>Russian Federation</td>
<td>O. sativa/TEJ</td>
</tr>
<tr>
<td>73.21</td>
<td>S</td>
<td>Krasnodarski 3352</td>
<td>311769</td>
<td>The Philippines</td>
<td>O. sativa/ARO</td>
</tr>
<tr>
<td>76.10</td>
<td>S</td>
<td>Pakkali</td>
<td>311284</td>
<td>Argentina</td>
<td>O. sativa/AUS</td>
</tr>
<tr>
<td>80.92</td>
<td>S</td>
<td>E B Gopher</td>
<td>310020</td>
<td>USA</td>
<td>O. sativa/TRJ</td>
</tr>
</tbody>
</table>

(*) Resistance categorization follows the method by De Freitas et al. (2016). R (resistant)=0-29%; M-R (moderately-resistant)=30-49%; S (sensitive)<49%.

(**) Genotype code of the USDA mini-core collection (Agrama et al., 2010).

(***) Subspecies code: aus=AUS; indica=IND; temperate japonica=TEJ; tropical japonica=TRJ; aromatic=ARO.
Table 2-2. Summary of genes tested indicating evidence and role in stress response

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene ID</th>
<th>Trigger</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT</td>
<td>Os02g0115700</td>
<td>Drought, Heavy metal stress</td>
<td>Increases abiotic stress resistance</td>
<td>Scandalios, 2005</td>
</tr>
<tr>
<td>CDC</td>
<td>Os01g0856000</td>
<td>Drought</td>
<td>Transient inhibition of cell cycle progression or even cell cycle exit</td>
<td>Kitsios and Doonan, 2011</td>
</tr>
<tr>
<td>CHS</td>
<td>Os04g0103900</td>
<td>UV, Wounding, Pathogen</td>
<td>Involving in the salicylic acid defense pathway</td>
<td>Dao et al., 2011</td>
</tr>
<tr>
<td>DREB2C</td>
<td>Os08g0521600</td>
<td>Drought, Heat, Cold</td>
<td>Conferring altered stress response</td>
<td>Lee et al., 2010</td>
</tr>
<tr>
<td>ERF68</td>
<td>Os01g0313300</td>
<td>Anoxia, Drought, Salt</td>
<td>Responding to drought, wounding, and pathogen infection</td>
<td>Xu et al., 1999</td>
</tr>
<tr>
<td>ERF71</td>
<td>Os06g0194000</td>
<td>Hypoxia</td>
<td>Improving hypoxic stress survival</td>
<td>Hess et al., 2011</td>
</tr>
<tr>
<td>GLO1</td>
<td>Os03g0786100</td>
<td>Drought, Pathogen</td>
<td>Adapting to stress condition</td>
<td>Fahnenstich et al., 2008</td>
</tr>
<tr>
<td>GLO4</td>
<td>Os07g0616500</td>
<td>Drought, Pathogen</td>
<td>Adapting to stress condition</td>
<td>Fahnenstich et al., 2008</td>
</tr>
<tr>
<td>LHCP</td>
<td>Os09g0346500</td>
<td>Drought</td>
<td>Increasing drought stress resistance</td>
<td>Xu et al., 2011</td>
</tr>
<tr>
<td>NCED</td>
<td>Os02g0704000</td>
<td>Drought, Salt</td>
<td>Increasing drought stress resistance</td>
<td>Estrada-Melo et al., 2015</td>
</tr>
<tr>
<td>OEP</td>
<td>Os07g0544800</td>
<td>Drought, Salt</td>
<td>Increasing drought stress resistance</td>
<td>Cheng et al., 2015</td>
</tr>
<tr>
<td>PAL</td>
<td>Os02g0626100</td>
<td>Drought, Pathogen</td>
<td>Involving in growth, development, and responses to environmental stresses</td>
<td>Huang et al., 2010</td>
</tr>
<tr>
<td>PIP2;3</td>
<td>Os04g0521100</td>
<td>Drought, Salt</td>
<td>Increasing salt stress resistance</td>
<td>Da Ines, 2008</td>
</tr>
<tr>
<td>PIP2;6</td>
<td>Os04g0233400</td>
<td>Drought</td>
<td>Increasing drought stress resistance</td>
<td>Huang et al., 2012</td>
</tr>
<tr>
<td>Rad50</td>
<td>Os02g0497500</td>
<td>DNA damage</td>
<td>DNA replication restart</td>
<td>Gatei et al., 2014</td>
</tr>
<tr>
<td>SamDC</td>
<td>Os04g0498600</td>
<td>Salt, Drought, Cold, Light, ABA</td>
<td>Polyamine biosynthesis during periods of abiotic stress in rice</td>
<td>Basu et al., 2014</td>
</tr>
<tr>
<td>SLAC1</td>
<td>Os04g0574700</td>
<td>Drought</td>
<td>Involving in stomatal conductance</td>
<td>Inai et al., 2015</td>
</tr>
<tr>
<td>SOD</td>
<td>Os03g0351500</td>
<td>Drought, Salt, Mn toxicity, Cold</td>
<td>Increasing abiotic stress resistance</td>
<td>Alscher et al., 2002</td>
</tr>
<tr>
<td>ZEP</td>
<td>Os04g0448900</td>
<td>Drought, Salt</td>
<td>Increasing osmotic stress resistance</td>
<td>Park et al., 2008</td>
</tr>
</tbody>
</table>
Table 2-3. Analysis of variance for plant biomass

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Plant Biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment*Genotype</td>
<td>18</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 2-4. Analysis of variance for photosynthesis, stomatal conductance, and instantaneous water use efficiency (iWUE)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Photosynthesis</th>
<th>Stomatal Conductance</th>
<th>iWUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>18</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment*Genotype</td>
<td>18</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Figure 2-1. Response of drought stress on plant biomass of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.
Figure 2-2. Cluster analysis of nineteen rice genotypes represented in the dendrogram, based on the percentage reduction of plant biomass. Drought resistance categories: R=resistant; M-R=moderately-resistant; S=sensitive.
Figure 2-3. Response of drought stress on photosynthesis of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.

Figure 2-4. Response of drought stress on stomatal conductance of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.
Figure 2-5. Response of drought stress on instantaneous water use efficiency (iWUE) of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.
Figure 2-6. Correlation between % reduction of photosynthesis and % reduction of plant biomass in diverse rice genotypes. Regressions were significant at P≤0.05.

Figure 2-7. Correlation between % reduction of stomatal conductance and % reduction of photosynthesis in diverse rice genotypes. Regressions were significant at P≤0.05.
Figure 2-8. Correlation between % reduction of photosynthesis and % reduction of instantaneous water use efficiency (iWUE) in diverse rice genotypes. Regressions were significant at $P \leq 0.05$.

\[ y = 0.8842x + 13.724 \]

\[ R^2 = 0.4299 \]
Figure 2-9. Gene expression analysis by qRT-PCR to characterize mechanisms of drought response in relation to polyamine biosynthesis: S-adenosylmethionine decarboxylase (SamDC). The values are mean of two replicates ±SE.
Figure 2-10. Gene expression analysis by qRT-PCR to characterize mechanisms of drought response in relation to ROS scavenging enzymes: A) Superoxide Dismutase (SOD) and B) Catalase (CAT). The values are mean of two replicates ±SE.
Figure 2-11. Gene expression analysis by qRT-PCR to characterize mechanisms of drought response in relation to photorespiration: A) Glycolate oxidase 1 (GLO1) and B) Glycolate oxidase 4 (GLO4). The values are mean of two replicates ±SE.
Figure 2-12. Gene expression analysis by qRT-PCR to characterize mechanisms of drought response in relation to DNA damage repair: A) Radiation sensitive (Rad50) and B) Cell division control (CDC). The values are mean of two replicates ±SE.
Figure 2-13. Gene expression analysis by qRT-PCR to characterize mechanisms of drought response in relation to flavonoid biosynthesis: A) Phenylalanine ammonia lyase (PAL) and B) Chalcone synthase (CHS). The values are mean of two replicates ±SE.
Figure 2.14. Gene expression analysis by qRT-PCR to characterize mechanisms of drought response in relation to photosynthesis: A) Chlorophyll A-B binding protein LHCP and B) Oxygen evolving enhancer protein (OEP). The values are mean of two replicates ±SE.
Figure 2-15. Gene expression analysis by qRT-PCR to characterize mechanisms of drought response in relation to stomatal conductance: A) Slow anion channel associated 1 (SLAC-1), B) Plasma membrane intrinsic proteins (PIP2;3), and C) PIP2;6. The values are mean of two replicates ±SE.
Figure 2-16. Gene expression analysis by qRT-PCR to characterize mechanisms of drought response in relation to ABA biosynthesis: A) 9-cis-epoxycarotenoid dioxygenase (NCED) and B) Zeaxanthin epoxidase (ZEP). The values are mean of two replicates ±SE.
Figure 2-17. Gene expression analysis by qRT-PCR to characterize mechanisms of drought response in relation to transcription factor genes: A) Dehydration responsive element binding protein 2C (DREB2C), B) Ethylene response factor (ERF#68), and C) ERF#71. The values are mean of two replicates ±SE.
Chapter 3

Evaluation of Diverse Rice Genotypes for Drought Resistance at the Reproductive Stage
ABSTRACT

One of the key requirements for rice (*Oryza sativa* L.) production is the availability of water. Water deficit therefore strongly affects rice production. Among the major stages of rice growth and development, the reproductive phase or anthesis is the most affected stage by water stress. Water stress imposed during this stage can reduce grain yield by up to 77% and longer periods of drought can reduce 90% of grain yield. To evaluate the genetic variation among rice accessions for drought tolerance, fifteen rice genotypes from the USDA mini-core collection were randomly selected for the study. Additionally, two drought-resistant reference genotypes (N22 and Vandana) and two sensitive reference genotypes (Cypress and Nipponbare) were used as the positive and negative control, respectively. Water stress was applied 3-4 days before anthesis stage and the drought response was measured by analysis of the reduction in yield parameters. Drought resistance levels were categorized by comparing genotypes under drought and well-watered control for measurements of panicle length, number of grain per panicle, number of filled grain per panicle, 100-grain weight, and total grain weight per panicle. Fourteen out of fifteen genotypes were identified with various levels of resistance to drought stress. One genotype, WIR 3039 (*O. sativa* sp. *temperate japonica* spp.) showed sensitivity to drought. Four genotypes, AMANE (*O. sativa* sp. *indica* spp.), 2 (*O. sativa* sp. *aromatic-tropical japonica-temperate japonica* spp.), HKG 98 (*O. sativa* sp. *aus* spp.), and resistant reference genotype N22 were used further for gene expression analysis with samples taken after drought was applied to the plants. Gene expression analysis revealed that inflorescence tissue gives positive and higher correlation with phenotypic measurements than flag leaf during reproductive stage drought. Expression levels of invertase genes and transcription factors exhibit positive effects to drought resistance particularly in relation to number of grain per panicle and panicle length.
INTRODUCTION

One of the key necessities of rice (*Oryza sativa* L.) cultivation is the availability of water. Water is needed almost the entire time of rice growth, particularly in lowland rice fields where 95% rice in the world is cultivated (Bouman et al., 2007). According to Bouman (2009), on average, it is estimated that 1,432 liters of water are consumed to produce 1 kg of rice in a lowland rice field. Among the major stages of rice growth and development, reproductive stage or anthesis is the most affected by water stress condition (Garrity and O’Toole, 1994; Ito et al., 2000; Farooq et al., 2009; Bunnag and Pongthai, 2013). Ito et al. (2000) reported that water stress imposed during this stage can cause grain yield reduction up to 77% and longer period of drought can reduce 90% of grain yield. Another report from a study in Thailand counted the reduction of rice production reaching 55-68% (Polthanee et al., 2014). Drought especially affects the pollination process. The decrease in photosynthesis reduces sugar delivery to reproductive tissue resulting in failure of male gametophyte development. This will cause male flower sterility which is also called *hideriaodachi* (Takeoka et al., 1992; Garrity and O’Toole, 1994; Brancher et al., 1996; Ito et al., 2000).

As a member of the Gramineae family, rice inflorescence as a reproductive organ is a panicle that bears single-flowered spikelets (Sleper and Poehlman, 2006). As described in Chapter 1, each single-flowered spikelet has two stigmas and six stamens and it is surrounded by lemma and palea. The rice flower normally blooms between 10 in the morning and 2 in the afternoon, over a period of 3-7 days. The blooming varies between cultivars and it is affected by environmental factors such as temperature, humidity, light, and drought (Grist, 1986; Takeoka et al., 1993; Sleper and Poehlman, 2006). Heading and anthesis starts almost at the same time. Heading is a stage when the panicle is fully visible, while anthesis is the process where the rice
spikelet opens and is ready to start fertilization (Hoshikawa, 1993). Rice is normally self-pollinated, although cross-pollination is also possible to some extent depending on the varieties and environmental effects (Grist, 1986).

Due to the significant effects of drought during the rice reproductive stage, understanding the genetics, physiology, and molecular basis of yield components in response to drought will help in the development of resilient cultivars. In order to determine the genetic variation in terms of drought resistance at reproductive stage, previous studies used some parameters such as yield and its components under stress and the correlation between them, the ratio between yield under stress and yield under non-stress conditions, and a “stress index” (Blum, 1988; Garrity and O’Toole, 1994; Yue et al., 2006; Sellamuthu et al., 2015). In the study by Garrity and O’Toole (1994), spikelet fertility was shown to be highly correlated with grain yield when water stress was applied at the reproductive stage. The correlation value for three years’ data reached up to more than 50%. The time of application of water stress to plants has great influence on the success of drought tolerance screening at a reproductive stage. This timing is strongly correlated to the flowering date (Garrity and O’Toole, 1994; Sellamuthu et al., 2015). Drought resistance is a complex quantitative trait, therefore mapping the quantitative trait loci (QTLs) is one way to build an understanding on the genetics of drought resistance and development of more drought resistant cultivars (Lanceras et al., 2004; Mir et al., 2012; Sellamuthu et al., 2015).

The future goal is the identification of genes associated with drought resistance. Xiong (2013) assumed that approximately 8-10% of rice genes are responsive to drought stress. Potential candidate genes can be involved in cell protection through osmotic adjustment, detoxification/antioxidant activity, repairs, and or involved in regulation of other genes involved in drought response (transcription factors). There are some approaches to identify potential genes
that are correlated to drought resistance such as by integration of the genes with QTL maps, association mapping, expression analysis using qRT-PCR, allele mining, transformation, and TILLING (Mir et al., 2012). Many genes correlated to water stress response have been identified. Based on genome-wide gene expression, the expression of several drought-induced genes were shown to be enhanced in the resistance genotype N22, and down-regulated in the sensitive genotype IR64 (Xiong, 2013; Hu and Xiong, 2014).

Drought resistance is a complex trait that uses multiple strategies to respond to drought stress and enhance adaptation. The response can be by inducing a shorter life cycle, reducing water loss, improving water uptake, accumulation of osmoprotectants, antioxidants, and reactive oxygen species (ROS) scavengers, and many other approaches (Hu and Xiong, 2014). As described earlier during the reproductive stage, drought inhibits photosynthesis and consequently will reduce nutrient supply to the generative organs. Several genes have been identified that alter their expression due to the change in sugar status (Barnabas et al., 2008). Therefore, the genes that are responsive to sugar signals could be among those that can control flower fertility during water stress conditions.

Among proteins that are responsive to sugar changes, sucrose (Suc) synthase (SS) and invertase, are two enzymes that are also able to enhance the drought resistance of the plants (Ricard et al., 1991; Kim et al., 2000; Ji et al., 2005). Both proteins catalyze Suc as a source of carbon and energy (Sturm, 1999). SS catalyzes the reversible conversion of Suc and a nucleoside diphosphate into the corresponding nucleoside diphosphate-glucose and fructose (Baroja-Fernández et al., 2012). Previous studies demonstrated that the activity and content of SS declined due to water stress application for a few days, however the SS transcript level was upregulated by a decrease of water potential of the plants (Gonzalez et al., 1995; Dejardin et al.,
In addition, they also suggested that the expression of SS is due to the increase of abscisic acid (ABA) level. Another important protein besides SS is invertase. Plants have several different invertases based on their subcellular localization: cell wall (CIN), vacuolar (VIN), and cytosolic (NIN) invertases. CIN and VIN share some biochemical properties such as having the same optimum pH, while NIN has higher optimum neutral or alkaline pH (7.0-7.8). That is why cytosolic invertase is also called neutral/alkaline invertase (Sturm, 1999; Fotopoulos, 2005). Several previous studies in different crops concluded that invertases responded rapidly toward water deficit by up-regulation in the expression of the proteins. They assumed that the fast responses are important for plant growth and development of stress resistance (Kim et al., 2000; Ji et al., 2005; Xiang et al., 2011).

Among the drought responsive genes, transcription factors also have a great influence in abiotic stress protection. In the previous chapter, some transcription factors have been identified to have responses on water stress conditions on the plants. Some transcription factor families such as NAC, bZIP, GRAS, MYB, WRKY, and zinc finger are drought-responsive (Huang et al., 2009; Liu et al., 2014; Xiong et al., 2014; Banerjee and Roychoudhury, 2015; Xu et al., 2015; Zhang et al., 2016). Transcription factors are defined as DNA-binding proteins that regulate gene expression at the level of mRNA transcription (Xiong et al., 2005). These regulatory proteins are important for environmental stress responses because they control multiple pathways and can be used to regulate complex metabolic pathways in plants (Hussain et al., 2011). Transcription factors comprise a large portion of a plant’s genome. In Arabidopsis, around 1,717 genes are transcription factors, while rice has around 1,859 transcription factor genes (PlantTFDB, 2016). These genes account for ~6% and ~2.6% respectively of their genome (Shiu et al., 2005; Hussain et al., 2011).
Basic leucine zipper (bZIP) is a transcription factor family, comprising 94 genes in rice (Xiong et al., 2005). These proteins are well-known for having crucial roles in plant development, physiological process, and biotic/abiotic stress responses (Wang et al., 2015). Among these 94 genes, OsbZIP23 and OsbZIP46 are recognized for their ability to improve drought resistance in rice. These two genes have high similarity and are correlated to abscisic acid (ABA) pathways (Xiang et al., 2008; Tang et al., 2012). GRAS is another rice transcription factor family that has at least 57 genes with the respective homologs found in several plants such Arabidopsis, tomato, petunia, and barley. The expression of the GRAS family is induced by osmotic stress and can enhance drought resistance in transgenic rice plants (Xiong et al., 2005; Xu et al., 2015). Among the transcription factors in rice, NAC is one of the largest families comprising about 149 genes (Xiong et al., 2005). The name NAC is originally taken from the first three proteins identified comprising NAM (no apical meristem), ATAF1-2, and CUC2 (cup-shaped cotyledon). These three proteins contain a similar DNA-binding domain (Liu et al., 2014). Several genes from this family are known to enhance abiotic stress resistance, especially drought (Nuruzzaman et al., 2013). OsNAC5, OsNAC9, and OsNAC10 are some of the genes that enhancing drought resistance and increase grain yield under drought stress conditions (Jeong et al., 2010; Redillas et al., 2012; Jeong et al., 2013).

In addition to these large transcription factor gene families, there are several other gene families that can enhance water stress resistance in rice. CBL (Calcineurin B-Like) interacting protein kinase (CIPK), lipid transfer protein (LTP), stress associated protein (SAP), Ski-interacting protein (SKIP), CYP707A, and BURP are some of the families (Xiang et al., 2007; Kanneganti and Gupta, 2008; Ding et al., 2009; Huo et al., 2009; Zheng et al., 2012; Guo et al., 2013). CIPK is one of the protein sensors for an increase in cytosolic free Ca$^{2+}$ concentration.
This process is known to be important for plant development and signaling processes such as for light, hormone, sugar, and stress responses. In rice there are 30 genes in this family. OsCIPK23, one member of this family, is identified as a multi-stress induced gene during reproductive stage (Xiang et al., 2007; Yang et al., 2008). LTP is another gene family that also plays a role in stress resistance during reproductive stage, and is known to be responsive to several environmental changes including drought (Vignols et al., 1997). A study from Guo et al. (2013) identified the alteration in expression of OsDIL, a member of the LTP family, in rice that gave drought resistance in both vegetative and reproductive stages.

One of the most important gene families for plant stress studies is SAP. There are 18 genes that code for SAP related proteins. Overexpression of some SAP genes such as OsiSAP1 and OsiSAP8 is detected under several abiotic stresses and enhances resistance (Kanneganti and Gupta, 2008). Induction in expression of SKIP genes, a rice gene family homologous with the human gene family, also gives positive modulation of stress resistance (Huo et al., 2009). A study by Zhang et al. (2015) tested several drought-induced genes including a SKIP gene, OsSKIPA, and identified a significant change in expression of genes in response to drought stress conditions.

As described in the previous chapters, ABA is one of most important components that is induced during environmental stress conditions and regulates the responses (Tuteja, 2007). ABA level is controlled by the balance between ABA biosynthesis and catabolism. ABA biosynthesis is controlled by Zeaxanthin epoxidase (ZEP) and 9-cis-epoxycarotenoid dioxygenase (NCED), while the key enzyme for ABA catabolism is 8’-hydroxylation that is controlled by the CYP707A gene family (Cai et al., 2015). In previous studies, CYP707A was identified to maintain ABA at a lower level after drought stress conditions and enhance drought resistance in
rice (Umezawa et al., 2006; Cai et al., 2015). The last gene family described previously is BURP, which is named after the proteins BNM2, USP, RD22, and PG1β. OsRUBP16, is one gene from this family identified to be able to increase abiotic stress sensitivity in rice (Ding et al., 2009; Liu et., 2014).

All these different genes and gene families regulate and express several pathways that can be used to overcome the damage caused by drought conditions. This chapter has two objectives. The first objective is to screen a diverse set of rice genotypes at the reproductive stage for grain yield under drought conditions. The second objective is to study the expression patterns of genes that contribute to yield under water stress conditions.

MATERIALS AND METHODS

Plant material

Seventeen diverse rice genotypes from the United States Department of Agriculture (USDA) mini-core collection were randomly selected for this experiment (Table 3-1) (Agrama et al., 2010). In addition, two drought resistant genotypes (Nagina 22 (N22) and Vandana) were included as resistant-reference genotypes (Mutum et al., 2013), and two drought sensitive genotypes (Cypress and Nipponbare) as the sensitive-reference genotypes (Baker, 2004; Degenkolbe et al., 2009).

Drought stress treatment at reproductive stage

The reproductive stress experiment was also conducted at the Altherimer greenhouse, University of Arkansas, Fayetteville. The method of seed germination was similar to the first experiment (Chapter 2). The seeds were imbibed with deionized water in a dark chamber at 27°C for seven days. Each emerged seedling was placed in single pots filling with a Redi-earth potting
mix (Sun Gro Horticulture Distribution). Drought stress was applied by withholding water at the pre-anthesis stage for 3 to 4 days, followed by rewatering, while control plants were kept maintained under well-watered conditions (Ramegowda et al., 2014). The temperature was maintained between 28 to 30°C (Ghadirnezhad and Fallah, 2014). The experimental design was a completely randomized design (CRD) with five replications.

On the last day of drought stress treatment, physiological parameters were measured such as photosynthesis rate, stomatal conductance, and instantaneous water use efficiency (iWUE) using a portable photosynthesis meter LI-COR 6400XT at a CO₂ concentration of 370 μmol/mol, light intensity of 1,000 μmol/m²/s, and 55% to 60% relative humidity at the tenth day of drought stress application (Ramegowda et al., 2014; De Freitas et al., 2016). Samples used for physiological analysis were from the 2nd fully expanded leaf from the top of each plant (Farquhar and Sharkey, 1982; Krause, 1991; Blum, 2011). The response of plants to drought during reproductive stage, were measured for several yield components. The components included panicle length, number of grain per panicle, number of filled grain per panicle, 100-grain weight, and total grain weight per panicle (Ramegowda et al., 2014). These parameters were measured after harvesting and drying. Analysis of variance was performed to assess the drought stress among genotypes. Tukey’s HSD was used to separate means for significant effects (P≤0.05) using JMP version 12.

**Gene expression analysis**

We quantified the expression of genes related to drought responses in reproductive stage by isolating RNA from two parts of the plants: flag leaf and inflorescence (Ji et al., 2005). Four genotypes were randomly selected for gene expression analysis, with samples taken after drought stress was applied to the plants. The genotypes used are: AMANE, 2, HKG 98, and the resistant-
reference genotype N22. RNA was isolated using TRIzol reagent, complementary DNA (cDNA) synthesis from the mRNA was conducted using 2μg total DNase-treated RNA by GoScript® Reverse Transcription System (Promega). The qRT PCR experiments were conducted using GoTaq® qPCR Master Mix (Promega), with gene-specific primers and Ubiquitin as standard.

A total of eighteen genes related to drought stress response were used as primers for generating the gene expression data (Table 3-2 and Table 3-3). Melting curve analysis was performed by increasing the temperature (0.5°C/10 s) from 55°C to 95°C, with un-transcribed RNA run as negative control. The relative difference in expression for each sample in individual experiments was determined by normalizing the threshold cycle (Ct) value for each gene against the Ct value of Ubiquitin and calculated relative to the respective control samples as a calibrator using the equation 2-ΔΔCt. The average of two biological replicates was used to obtain each expression value (Ramegowda et al., 2014; Bevilacqua et al., 2015; De Freitas et al., 2016). Standard error was used to separate means for significant effects.

**RESULTS AND DISCUSSION**

**Screening of rice genotypes for drought resistance at reproductive stage**

In general, there are three objectives for selection in plant breeding (Blum, 1988): (i) uniform superiority in all environments, (ii) relatively better in poor environments, and (iii) relatively better in the favored environment. In broader context, Sleper and Poehlman (2006) proposed that in rice breeding, the main objectives are having high-yield potential, yield stability, and grain quality. Sellamuthu et al. (2015) emphasized more on the yield stability if we are conducting stress tolerance rice breeding. In this study, measurements were made on number of grain per panicle and number of filled grain per panicle to determine the high-yield potential.
Both parameters were also compared in control and stress conditions to evaluate which genotypes have acceptable yield stability and weighed the rice grain to determine the quality of grain in terms of grain filling. In addition to this, the panicle length of the plants was measured to evaluate whether water stress has an effect on panicle development. Ji et al. (2005) assumed that in order to determine the rice reproductive ability during drought stress, it is not only the spikelet fertility but also panicle exertion that has to be studied. Table 3-4 shows that water stress has significant effects on all yield components among genotypes and within treatments (control and stress) ($P \leq 0.05$), showing suitability of experimental conditions for giving stress. There is also a specific interaction between genotype and treatments ($P \leq 0.05$). This interaction occurs because there are differences in the reduction of the parameters measured in the stressed plants among the genotypes (Figure 3-1, Figure 3-2, Figure 3-3, Figure 3-4, and Figure 3-5).

The availability of water is one of the key agronomic resources for rice production. This also means that the high yield productivity of rice can be achieved when water supply is not limited (Sleper and Poehlman, 2006). Figure 3-1 illustrates the number of grain per panicle among genotypes in both control and stress plants, and the percentage reduction of the number of grain per panicle in stress plants. In term of the reduction, we come again using the categorization by De Freitas et al (2016). The reduction between 0-29% is categorized as resistant, 30-49% is moderately-resistant, and higher than 49% is sensitive. The categorization is also presented in Table 3-1. Almost all genotypes are having lower percentage reduction than 50%, including one sensitive-reference genotype (Cypress) with 46.45% of reduction. Two resistant-reference genotypes, N22 and Vandana demonstrate low reduction with only having 17.83% and 19.71% of reduction, respectively. In this study, we have one genotype from Oryza glaberrima species (sp.), TOg 7025. Based on the measurement of number of grain per panicle,
TOg 7025 is categorized as moderately-resistant. In a previous chapter, this genotype is categorized as resistant based on vegetative drought response. As discussed in Chapter 2, *O. glaberrima* sp. is known for its resistance to several abiotic stresses including drought (Linares, 2002). The reduction of grain number in drought stressed plants is a consequence of the sensitivity of the pollen, because of water stress that causes grain abortion following fertilization (Barnabas et al., 2008).

Another parameter that is correlated with yield-potential is the number of filled grain per panicle. Grain filling is the final growth stage in cereal crops including rice, where fertilized ovaries develop into caryopses (Barnabas et al., 2008). Results are given in Figure 3-2 showing the number of filled grain per panicle in both control and stressed plants among seventeen genotypes. This figure also illustrates the percentage reduction in number of filled grain per panicle in each genotype. Both sensitive-reference genotypes, Cypress and Nipponbare, have high percentage reduction of 85.65% and 85.86%, respectively. In contrast, both resistant-reference genotypes, N22 and Vandana, have lower reduction than 50%. TOg 7025, the *O. glaberrima* sp. accession, is categorized as resistant genotype with only 24.03% reduction. Among the genotypes, Red, a Pakistan variety, shows a low percentage of reduction (11.21%) and has the highest number of filled grain per panicle in stressed plants (117). Red was derived as a cross between the subspecies (spp.) of *aus, indica*, and *temperate japonica*. It is also shown in the previous chapter that the *aus* spp., a subspecies of *O. sativa*, is drought resistant (Garris et al., 2005; Bin Rahman and Zhang, 2016). WIR 3039, a *temperate japonica* spp., shows high reduction by having 80.76% of reduction. In the previous parameter (number grain per panicle), this genotype also shows reduction higher than 50% (59.26%). Both parameters, number of grain per panicle and number of filled grain per panicle are correlated to each other. As evident in the
Figure 3-7, considering this sample of genotypes, there is a positive and significant correlation between these parameters ($R^2=0.71935; P\leq 0.05$). The success of plants avoiding male flower sterility will have the effect of having less reduction in number of filled grain (Barnabas et al., 2008).

As a parameter to assess the grain quality, we calculated the weight of 100 seeds/husk grain and total grain weight per panicle. Figure 3-3 represents the 100-grain weight of control and drought stressed plants, and the percentage reduction for all seventeen genotypes. Cypress and Nipponbare (sensitive-reference genotypes) show a percentage reduction higher than 50% (66.38% and 55.89% respectively). The resistant-reference genotype N22, also exhibits high percentage of reduction in 100-grain weight (65.89%). Another *aus* spp. genotype, HKG 98, exhibits good resistance to drought with a low reduction (12.93%) and has the highest 100-grain weight in stressed plants (4.35 g). HKG 98 also demonstrates low reduction in number of grain per panicle and number of filled grain per panicle (28.41% and 18.40%, respectively). Barnabas et al. (2008) proposed that the reduction in grain weight in response to drought might be accounted for by the lower number of endosperm cells or the results of the impairment in starch synthesis.

Figure 3-4 illustrates another grain quality parameter, total grain weight per panicle. Total grain weight per panicle was calculated by multiplying the weight of one grain to the number of filled grain per panicle. Both sensitive reference genotypes (Cypress and Nipponbare) show their low resistance by having high percentage reduction almost 100% (95.18 and 95.72%, respectively). High reduction is also experienced by WIR 3039. As described in previous parameter (number of grain per panicle and number of filled grain per panicle), WIR 3039 also shows low resistance to drought stress. HKG 98 shows low reduction (28.92%) and the highest
total grain weight per panicle under drought stress conditions (3.56 g). Red genotype also presents a low reduction (26.10%) and a high total grain weight grain per panicle in stressed plants (2.65 g). A strong, positive, and significant correlation is shown in the correlation between percentage reduction of number of filled grain per panicle and percentage reduction of total grain weight per panicle (R²=0.72693; P≤0.05) (Figure 3-8). Both number of filled grain and grain weight have significant effects for the total of rice production.

The yield component parameter panicle length was also measured, after the final harvesting. Sellamuthu et al. (2015) concluded that under drought in reproductive stage, grain yield is highly-positive correlated with panicle length. In Figure 3-5 we present the data of panicle length for all genotypes in both condition (well-watered and stress) together with the percentage reduction from control to stress plants. All genotypes in this study display low reduction to panicle length in consequence of drought stress including the sensitive-reference genotypes, although the sensitive-reference genotypes are among the genotypes that having highest reduction in other parameters. To compare to previous reports suggesting high and positive correlation between grain yield components and panicle length, we calculated the correlation of panicle length with number of grain per panicle, and number of filled grain per panicle. Figures 3-9 and 3-10 show the correlation between panicle length and number of grain per panicle and number of filled grain per panicle, respectively. In both estimates, the correlations are positive and significant (P≤0.05). However, these correlations are not relatively high with the R² values for both are 0.30902 and 0.4182, respectively.

The differential response of the seventeen rice genotypes to drought at the reproductive stage was calculated based on the percentage reduction of all phenotypic parameters (number of grain per panicle, number of filled grain per panicle, 100-grain weight, total grain weight per
panicle, and panicle length) and represented by a dendrogram (Figure 3-6). The cluster analysis showed two main clusters as shown in the dendrogram. The first cluster is comprised of fourteen genotypes that tend to be more resistant and include both the resistant-reference genotypes, N22 and Vandana. Another cluster represents three genotypes that have a high percentage reduction for almost all phenotypic parameters. Both the sensitive-reference genotypes (Cypress and Nipponbare) and WIR 3039 are included in the second cluster. Among all the tested genotypes, only WIR 3039 is not included in the first cluster.

**Effects of drought stress on physiological processes in rice genotypes**

One of the most significant among the processes that can be affected by drought is photosynthesis. Water deficit conditions reduce photosynthesis rate that can result in a reduction of grain yield (Barnabas et al., 2008; Ji et al., 2012). In order to determine the effects of drought on physiological processes, the photosynthesis rate, stomatal conductance, and iWUE were measured. Table 3-5 presents the analysis of variance for these physiological parameters in two conditions and various genotypes. Water stress conditions give significant effects to all parameters (P≤0.05). There is also a specific interaction between genotype and treatment. This interaction occurs because there are differences in the level of reduction in the stressed plants among the genotypes (Figure 3-11, Figure 3-12, and Figure 3-13).

We calculated the correlation between the physiological and phenotypic parameters in order to determine the significance of the reduction in physiological processes to the phenotypic parameters. Figures 3-14, 3-15, and 3-16 illustrate the correlation between the percentage reduction of photosynthesis rate and the percentage reduction in three phenotypic parameters: number of grain per panicle, number of filled grain per panicle, and total grain weight per panicle. All correlations are positive although they are not relatively high (R²=0.02376, 0.15244,
and 0.09809, respectively). The low correlation might be due to the low reduction of photosynthesis. A bilinear positive and significant correlation is demonstrated between percentage reduction of stomatal conduction and percentage reduction of photosynthesis (R²=0.47013; P≤0.05) (Figure 3-17). The same result is also observed in Chapter 2. This case further supports that stomatal conductance is one of the causes of photosynthesis limitations (Perez-Martin et al., 2014). Another bilinear positive correlation is also demonstrated between percentage reduction of photosynthesis and percentage of iWUE (R²=0.15001) (Figure 3-18). The relationship is not relatively high due to low percentage reduction in iWUE.

Drought stress sugar-changed responsive expression patterns of genes

The productivity of rice or any plant greatly depends on the supply of photosynthesis product, that is carbohydrate (Suc, starch) (Ji et al., 2007; Barnabas et al., 2008). As mentioned previously, during water deficit photosynthesis may be inhibited and results in low amount of sugar produced. The low amount is not only because of the inhibition of photosynthesis, but also due to the utilization of the sugar as a consequence of the continuation of respiration (Barnabas et al., 2008). Suc, the main product of photosynthesis, controls many aspects of the plants’ growth and development. As a soluble sugar, Suc has a major role in the primary transport of sugar, as a nutrient, and a potential signal molecule due to the high sensitivity to environmental stresses (Winter and Huber, 2000; Rosa et al., 2009). This potential signal molecule of Suc can be utilized as a regulator of gene expression. The regulation of gene expression is affected by the change in sugar inside the plants. There are several genes that are regulated by alteration in sugar concentration such as invertases, Suc synthase (SS), sucrose-phosphate synthase (Winter and Huber, 2009).
In the present study, we analyzed the gene expression of several invertases and SS. Table 3-6 shows the correlation between the values of phenotypic parameters and gene expression values for invertases and SS genes. The gene expression analysis presented in this table is taken from flag leaf samples, while the phenotypic values are taken from the stressed plants. Four genotypes (AMANE, 2, HKG 98, and resistant-reference genotype N22) were selected randomly to conduct gene expression analysis. In general, the correlation between yield parameters and sugar-responsive gene expression of flag leaf is not relatively high. Among 30 correlations, only four are positively high ($R^2$≥0.5). Low correlation is also shown by the correlation between invertases and SS genes (Table 3-7). From 15 correlations, there is only one that is positively high ($R^2$≥0.5). Flag leaf is the most important leaf for cereal crops including rice, as the last leaf on each tiller for photosynthesis (GRDC, 2005; Zhang et al., 2015). It is estimated that flag leaf can increase grain weight for about 41 to 43% (Al-Tahir, 2014). Meanwhile, the correlation between these two analyses using inflorescence is relatively higher than the flag leaf (Table 3-8). Both panicle length and number of grain per panicle have positive correlation with the invertases and SS genes. However, number of filled grain per panicle, 100-grain weight, and total grain weight per panicle give negative correlation with the invertases and SS genes. This also happens to the correlation within the genes (Table 3-9). All correlations are positive and 11 out of 15 correlations are high ($R^2$≥0.5). Similar results were demonstrated by a study from Sherson et al. (2002). There is no expression of CIN2 and CIN4 in leaf of Arabidopsis while both genes were induced in flower. Biochemical assays from Nguyen et al. (2010) also showed that Suc, glucose, and fructose contents as stress signals were found to be significantly increased in anthers under water stress conditions. The less drought responsiveness in the genes in flag leaf samples than inflorescence could be due to lack of stress in flag leaf.

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Rice has nine CIN genes (CIN1-9) and two VIN genes (VIN1-2). Among the CIN genes, transcripts of CIN2, CIN3, and CIN4 are readily detected in anthers that are part of inflorescence (Ji et al., 2005). Meanwhile, transcripts for VIN1 and VIN2 are expressed in both flag leaf and anthers. However, the transcript is expressed higher in anthers than in flag leaf (Ji et al., 2005; Ji et al., 2007). Both acid invertases are strongly correlated with phloem unloading and source/sink regulation (Murayama and Handa, 2007). Other invertases different from CIN and VIN, according to pH optimum, are neutral/alkaline invertases (NIN). In contrast to acid invertases, NIN shows neutral or slightly alkaline pH optimum (Murayama and Handa, 2007; Dahro et al., 2016). There are eight genes that encoding NIN (NIN1-8) (Ji et al., 2005). It has been accepted that NIN is accumulated in cytoplasm although a new study from Dahro et al. (2016) suggested that NIN is also localized in mitochondria and chloroplast. The study also confirmed that NIN can enhance multiple abiotic stresses resistance including drought in *Poncirus trifoliate*. In addition to invertases, another enzyme that is also responsible for Suc metabolism is Suc synthase (SS). While invertase hydrolyzes Suc into glucose and fructose irreversibly, SS does it reversibly (Murayama and Handa, 2007). A study by Zhou et al. (2014) identified an SS gene (SS2) was upregulated by drought stress conditions in bermudagrass. The up-regulation of SS2 is associated with solute accumulation under drought stress and involved in osmotic adjustment.

Several transcription factors and their importance in drought resistance during vegetative stage have been discussed in the previous chapter. As we know the significance of these enzymes, several transcription factors were used during analysis of reproductive stage drought. The correlation between phenotypic parameters and transcription factors by flag leaf samples is again relatively not high (Table 3-10). Amongst 60 correlation, only nine are positive and high (R^2≥0.5). The low correlation also occurs between transcription factors in the flag leaf tissue.
There are 66 correlations within transcription factors and only ten of them are positive and high ($R^2 \geq 0.5$). Meanwhile, the correlation using inflorescence samples is relatively higher than the correlation using flag leaf samples. As detailed in Table 3-12, almost all correlation of transcription factors with panicle length and number of grain per panicle are positively high ($R^2 \geq 0.5$). However, the opposite results occur in the correlation between transcription factors and number of filled grain, 100-grain weight, and total grain weight per panicle. In the correlation within the transcription factors, only positive interactions occur and amongst 138 correlations only 11 that are not high ($R^2 < 0.5$) (Table 3-13).

The correlation between phenotypic parameters (panicle length, number of grain per panicle, number of filled grain per panicle, 100-grain weight, and total grain weight per panicle) and both group of genes (invertase genes and transcription factors) are relatively higher in the inflorescence samples than the flag leaf samples. As mentioned previously, during reproductive stage, the most affected process is pollination that can cause pollen sterility (Garrity and O’Toole, 1994; Ito et al., 2000). In addition to pollen, drought also causes abnormalities in structural and functional of ovary and female gametophyte development (Barnabas et al., 2008). Consequently, drought gives higher stress effects to reproductive part than to vegetative parts (flag leaf). Amongst the phenotypic parameters, number of filled grain, 100-grain weight, and total grain weight per panicle show negative correlations with invertase genes and transcription factors during inflorescence stage drought. As grain filling is the final stage in rice after ovaries have been fertilized, drought before pre-anthesis has more effects to the success of fertilization than the number of filled grain per panicle and grain weight (Bouman and Tuong, 2001).
CONCLUSIONS

Drought occurring during reproductive stage has a major effect on production. Water deficit interferes particularly at the meiosis stage during flower development, for both male and female parts. The aim of the experiments was to identify how different groups of drought stress related genes are correlated with the response and adaptation during reproductive stage. Almost all genotypes demonstrated drought resistance except for WIR 3039, a temperate japonica spp. The putative drought-resistant genotypes are significantly different from the sensitive-reference genotypes in almost all reduction measurements. Based on gene expression analysis in four genotypes (AMANE, 2, HKG 98, and N22), the inflorescence gives higher correlation with grain yield phenotypic parameters than flag leaf during reproductive stage. In addition to this, both invertase genes and transcription factors confer positive effects to drought resistance particularly in related with number of grain per panicle and panicle length.
REFERENCES


| Panicle Length | No. of Grain/Panicle | No. of Filled Grain/Panicle | 100-Grain Weight | Total Grain Weight/Panicle | Name (**) | Code of Genotypes (GSOR) | Origin | Species/Subspecies (***)
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(*) Resistance categorization (Cat.) follows the method by De Freitas et al. (2016). R (resistant)=0-29%; M-R (moderately-resistant)=30-49%; S (sensitive)<49%.

(**) Genotype code of the USDA mini-core collection (Agrama et al., 2010).

(***) Subspecies code: aus=AUS; indica=IND; temperate japonica=TEJ; tropical japonica=TRJ; aromatic=ARO.
Table 3-2. Summary of genes tested indicating evidence and role in stress response caused by sugar-changes

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<td>Drought, Cold</td>
<td>Correlated to cell division and elongation in peduncles</td>
<td>Ji et al., 2005</td>
</tr>
<tr>
<td>CIN4</td>
<td>Os03g0735800</td>
<td>Drought, Cold</td>
<td>Involved in the apoplastic unloading sugar</td>
<td>Nguyen et al., 2010</td>
</tr>
<tr>
<td>VIN1</td>
<td>Os04g0535600</td>
<td>Drought, Cold</td>
<td>The transcripts are absent from peduncles of well-watered and drought-stressed</td>
<td>Ji et al., 2007</td>
</tr>
<tr>
<td>VIN2</td>
<td>Os02g0106100</td>
<td>Drought, Cold</td>
<td>Increasing osmotic potential of the vacuoles to maintain turgor</td>
<td>Ji et al., 2007</td>
</tr>
<tr>
<td>NIN2</td>
<td>Os01g0332100</td>
<td>Drought, Cold, Pathogen</td>
<td>Correlated to stress response through antioxidant system</td>
<td>Xiang et al., 2011</td>
</tr>
<tr>
<td>SS2</td>
<td>Os03g0401300</td>
<td>Drought, Cold</td>
<td>Important for O₂ shortage stress response</td>
<td>Dejardin et al., 1999</td>
</tr>
</tbody>
</table>
Table 3-3. Summary of transcription factors tested indication evidence and role in stress response

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene ID</th>
<th>Trigger</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABAOX3</td>
<td>Os02g0703600</td>
<td>ABA degradation</td>
<td>Determining threshold levels of ABA during dehydration and after rehydration and enhancing drought tolerance.</td>
<td>Umezawa et al., 2006</td>
</tr>
<tr>
<td>OsBURP16</td>
<td>Os10g04094000</td>
<td>Drought, Cold, Salt, ABA</td>
<td>Increasing abiotic stress sensitivity</td>
<td>Liu et al., 2014</td>
</tr>
<tr>
<td>OsbZIP23</td>
<td>Os02g0766700</td>
<td>Drought, Salt, ABA</td>
<td>Conferring ABA sensitivity and salt and drought tolerance</td>
<td>Xiang et al., 2008</td>
</tr>
<tr>
<td>OsbZIP46</td>
<td>Os06g0211200</td>
<td>Drought, Heat, ABA</td>
<td>Improving drought tolerance</td>
<td>Tang et al., 2012</td>
</tr>
<tr>
<td>OsCIPK23</td>
<td>Os07g0150700</td>
<td>Drought</td>
<td>Conferring a hypersensitive response to drought stress</td>
<td>Yang et al., 2008</td>
</tr>
<tr>
<td>OsDIL</td>
<td>Os10g0148000</td>
<td>Drought</td>
<td>Conferring tolerance to drought stress during vegetative and reproductive stages</td>
<td>Guo et al., 2013</td>
</tr>
<tr>
<td>OsGRAS23</td>
<td>Os04g05904000</td>
<td>Drought</td>
<td>Modulating rice drought tolerance</td>
<td>Xu et al., 2015</td>
</tr>
<tr>
<td>OsiSAP8</td>
<td>Os06g0612800</td>
<td>Drought, Cold, Salt, Heat, Wounding, ABA, Heavy metal</td>
<td>Conferring tolerance to salt, drought and cold stress in vegetative and reproductive stages</td>
<td>Kanneganti and Gupta, 2008</td>
</tr>
<tr>
<td>OsNAC5</td>
<td>Os11g0184900</td>
<td>Drought, Salt, Cold</td>
<td>Enhancing drought tolerance and increases grain yield</td>
<td>Jeong et al., 2013</td>
</tr>
<tr>
<td>OsNAC9</td>
<td>Os03g08151000</td>
<td>Drought</td>
<td>Enhancing drought resistance and grain yield</td>
<td>Redillas et al., 2012</td>
</tr>
<tr>
<td>OsNAC10</td>
<td>Os11g0126900</td>
<td>Drought, Salt, ABA</td>
<td>Improving drought tolerance and grain yield</td>
<td>Jeong et al., 2010</td>
</tr>
<tr>
<td>OsSKIPa</td>
<td>Os02g0759800</td>
<td>Drought, Salt, ABA</td>
<td>Modulating cell viability and stress tolerance</td>
<td>Hou et al., 2009</td>
</tr>
</tbody>
</table>
Table 3-4. Analysis of variance for panicle length, no. of grain per panicle, no. of filled grain per panicle, 100-grain weight, and total grain weight per panicle

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Panicle Length</th>
<th>No. of Grain per Panicle</th>
<th>No. of Filled Grain per Panicle</th>
<th>100-grain weight</th>
<th>Total Grain Weight per Panicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>16</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype*Treatment</td>
<td>16</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 3-5. Analysis of variance for photosynthesis, stomatal conductance, and instantaneous water use efficiency (iWUE)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Photosynthesis</th>
<th>Stomatal Conductance</th>
<th>iWUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
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<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype*Treatment</td>
<td>16</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
### Table 3-6. Correlation between grain yield parameters and expression of invertase genes in the flag leaf

<table>
<thead>
<tr>
<th></th>
<th>Panicle Length</th>
<th>No. of Grain per Panicle</th>
<th>No. of Filled Grain</th>
<th>100-Grain Weight</th>
<th>Total Grain Weight/Panicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN2</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)*</td>
<td>(+)*</td>
</tr>
<tr>
<td>CIN4</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>VIN1</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>VIN2</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>NIN2</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>SS2</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

The positive and negative correlations were measured by Pearson Correlation

(-) = Negative correlation

(+)= Positive correlation

(+) * = Positive correlation with R^2>0.5

### Table 3-7. Correlation in expression between invertase genes in the flag leaf

<table>
<thead>
<tr>
<th></th>
<th>CIN2</th>
<th>CIN4</th>
<th>VIN1</th>
<th>VIN2</th>
<th>NIN2</th>
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</thead>
<tbody>
<tr>
<td>CIN4</td>
<td>(+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIN1</td>
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<td>(-)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIN2</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIN2</td>
<td>(+)</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>SS2</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

The positive and negative correlations were measured by Pearson Correlation

(-) = Negative correlation

(+)= Positive correlation

(+) * = Positive correlation with R^2>0.5
Table 3-8. Correlation between grain yield parameters and expression of invertase genes in the inflorescence

<table>
<thead>
<tr>
<th>Panicle Length</th>
<th>No. of Grain per Panicle</th>
<th>No. of Filled Grain</th>
<th>100-Grain Weight</th>
<th>Total Grain Weight/Panicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN2</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>CIN4</td>
<td>(+)</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>VIN1</td>
<td>(+)</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>VIN2</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>NIN2</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>SS2</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
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</tbody>
</table>

The positive and negative correlations were measured by Pearson Correlation

(-) = Negative correlation
(+)= Positive correlation
(+)* = Positive correlation with $R^2>0.5$

Table 3-9. Correlation in expression between invertase genes in the inflorescence

<table>
<thead>
<tr>
<th></th>
<th>CIN2</th>
<th>CIN4</th>
<th>VIN1</th>
<th>VIN2</th>
<th>NIN2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN4</td>
<td>(+)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIN1</td>
<td>(+)*</td>
<td>(+)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIN2</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(+)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIN2</td>
<td>(+)*</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)*</td>
<td></td>
</tr>
<tr>
<td>SS2</td>
<td>(+)*</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)*</td>
<td>(+)*</td>
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</tbody>
</table>

The positive and negative correlations were measured by Pearson Correlation

(-) = Negative correlation
(+)= Positive correlation
(+)* = Positive correlation with $R^2>0.5$
Table 3-10. Correlation between grain yield parameters and transcription factor expression in the flag leaf

<table>
<thead>
<tr>
<th>Factor</th>
<th>Panicle Length</th>
<th>No. of Grain per Panicle</th>
<th>No. of Filled Grain</th>
<th>100-Grain Weight</th>
<th>Total Grain Weight/Panicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>OsbZIP23</td>
<td>(-)</td>
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<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>OsbZIP46</td>
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<td>(+)</td>
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<td>(+)</td>
</tr>
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<td>OsGRAS23</td>
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<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
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<td>OsNAC5</td>
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<td>(-)</td>
<td>(+)*</td>
<td>(+)</td>
<td>(+)</td>
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<td>OsNAC9</td>
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<td>(-)</td>
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<td>(+)</td>
</tr>
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<td>OsNAC10</td>
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<td>(-)</td>
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<tr>
<td>OsCIPK23</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)*</td>
<td>(+)*</td>
</tr>
<tr>
<td>OsDIL</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)*</td>
<td>(+)</td>
</tr>
<tr>
<td>OsiSAP8</td>
<td>(-)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)*</td>
<td>(+)*</td>
</tr>
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<td>OsSKIPa</td>
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<td>(+)</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
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</table>

The positive and negative correlations were measured by Pearson Correlation
(-) = Negative correlation
(+)= Positive correlation
(+)* = Positive correlation with R²>0.5
<table>
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<th>Transcription Factor</th>
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<th>OsGRAS23</th>
<th>OsNAC5</th>
<th>OsNAC9</th>
<th>OsNAC10</th>
<th>OsCIPK23</th>
<th>OsDIL8</th>
<th>OsSAP8</th>
<th>OsSKIPa</th>
<th>ABA OX3</th>
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<td>(+)</td>
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<td>(+)*</td>
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<td>(+)*</td>
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<td>(+)*</td>
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<td>(+)*</td>
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<tr>
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<td>(+)</td>
<td>(+)*</td>
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<td>ABA OX3</td>
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<td>(+)</td>
<td>(+)</td>
<td>(+)*</td>
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<td>(+)</td>
</tr>
</tbody>
</table>

The positive and negative correlations were measured by Pearson Correlation

(-) = Negative correlation
(+)* = Positive correlation with \( R^2 > 0.5 \)

(+) = Positive correlation
Table 3-12. Correlation between grain yield parameters and transcription factor expression in the inflorescence

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>Panicle Length</th>
<th>No. of Grain per Panicle</th>
<th>No. of Filled Grain</th>
<th>100-Grain Weight</th>
<th>Total Grain Weight/Panicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>OsbZIP23</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
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<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
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<tr>
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<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>OsNAC5</td>
<td>(+)</td>
<td>(+)*</td>
<td>(-)</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>OsNAC9</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>OsNAC10</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>OsCIPK23</td>
<td>(+)*</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>OsDIL</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>OsiSAP8</td>
<td>(+)*</td>
<td>(+)*</td>
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<td>(-)</td>
<td>(-)</td>
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<td>OsSKIPa</td>
<td>(+)*</td>
<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>ABAOX3</td>
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<td>(+)*</td>
<td>(-)</td>
<td>(-)</td>
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The positive and negative correlations were measured by Pearson Correlation
(-) = Negative correlation
(+) = Positive correlation
(+) = Positive correlation with $R^2 > 0.5$
Table 3-13. Correlation in expression between transcription factors in the inflorescence

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<tr>
<th></th>
<th>OsbZIP 23</th>
<th>OsbZIP 46</th>
<th>OsGRAS 23</th>
<th>OsNAC 5</th>
<th>OsNAC 9</th>
<th>OsNAC 10</th>
<th>OsCIPK 23</th>
<th>OsDIL 8</th>
<th>OsiSAP a</th>
<th>OsSKIP</th>
<th>ABA OX3</th>
</tr>
</thead>
<tbody>
<tr>
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The positive and negative correlations were measured by Pearson Correlation

(-) = Negative correlation
(+)* = Positive correlation with $R^2 > 0.5$
Figure 3-1. Response of drought stress on number of grain per panicle of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.

Figure 3-2. Response of drought stress on number of filled grain per panicle of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.
Figure 3-3. Response of drought stress on 100-grain weight of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.

Figure 3-4. Response of drought stress on total grain weight per panicle of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.
Figure 3-5. Response of drought stress on panicle length of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.
Figure 3-6. Cluster analysis of seventeen rice genotypes represented in the dendrogram, based on the percentage reduction of yield components: no. of grain/panicle, no. of filled grain/panicle, 100-grain weight, total grain weight/panicle, and panicle length. Drought resistance categories: R=resistant; M-R=moderately-resistant; S=sensitive.
Figure 3-7. Correlation between % reduction of number of grain per panicle and % reduction of number of filled grain per panicle in diverse rice genotypes. Regressions were significant at P≤0.05.

\[ y = 1.4073x - 10.911 \]
\[ R^2 = 0.7193 \]

Figure 3-8. Correlation between % reduction of number of filled grain per panicle and % reduction of total grain weight per panicle in diverse rice genotypes. Regressions were significant at P≤0.05.

\[ y = 0.8096x + 23.627 \]
\[ R^2 = 0.7269 \]
Figure 3-9. Correlation between % reduction of panicle length and % reduction of number of grain per panicle in diverse rice genotypes. Regressions were significant at $P \leq 0.05$.

Figure 3-10. Correlation between % reduction of panicle length and % reduction of number of filled grain per panicle in diverse rice genotypes. Regressions were significant at $P \leq 0.05$. 
Figure 3-11. Response of drought stress on photosynthesis of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.

Figure 3-12. Response of drought stress on stomatal conductance of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.
Figure 3-13. Response of drought stress on instantaneous water use efficiency (iWUE) of diverse rice genotypes. All the data are average of five replicates. Means were separated using Tukey’s HSD at 5% probability level. The thin bars showing 95% confidence interval.
Figure 3-14. Correlation between % reduction of photosynthesis and % reduction of number of grain per panicle in diverse rice genotypes.

\[ y = 0.1433x + 26.199 \]
\[ R^2 = 0.0238 \]

Figure 3-15. Correlation between % reduction of photosynthesis and % reduction of number of filled grain per panicle in diverse rice genotypes.

\[ y = 0.6021x + 14.743 \]
\[ R^2 = 0.1524 \]
Figure 3-16. Correlation between % reduction of photosynthesis and % reduction of number of total grain weight per panicle in diverse rice genotypes.
Figure 3-17. Correlation between % reduction of stomatal conductance and % reduction of photosynthesis in diverse rice genotypes. Regressions were significant at P≤0.05.

\[ y = 0.5615x + 15.44 \]
\[ R^2 = 0.4701 \]

Figure 3-18. Correlation between % reduction of photosynthesis and % reduction of instantaneous water use efficiency (iWUE) in diverse rice genotypes.

\[ y = 0.2763x + 15.474 \]
\[ R^2 = 0.15 \]
GENERAL CONCLUSIONS

Drought occurring during vegetative and reproductive stages give great reductions in rice biomass and yield. Identification of mechanisms on how plants can survive under these conditions provides knowledge to improve the drought resistance of rice. Analysis of drought stress responses combining physiology, genomics, and breeding methodologies is an integrated approach to dissect and understand the challenge of drought stress on the rice crop. Due to the different effects and importance of drought on rice during vegetative and reproductive stages, screening for drought in these stages enables the identification of different mechanisms conferring drought resistance to the plants. The objectives this study were to (1) screen a diverse set of rice genotypes in both vegetative and reproductive stages; (2) characterize the genetic differences in mechanisms of drought response conferring drought stress resistance; and (3) study the expression patterns of genes contributing to yield under water stress conditions.

The results of the combined analysis show that the diverse genotypes conferred different drought resistance mechanisms to respond and adapt to the drought stress. In the first study, three putative-resistant genotypes exhibit different drought resistant mechanisms at the vegetative stage. Padi Tarab Arab and N22 exhibited drought avoidance and tolerance mechanisms while GPNO 25912 exhibited only tolerance mechanism in response to drought stress. Based on gene expression analysis, significant differences between resistant and sensitive genotypes were observed. A high and consistent induction in the relative expression of drought stress genes in drought compared to control was observed in Padi Tarab Arab, GPNO 25912, and N22, while the three sensitive genotypes showed low, no, late, or inconsistent induction in the expression of the genes. In the reproductive stage study, almost all genotypes demonstrated their drought resistance except for WIR 3039, a temperate japonica spp, in addition to two sensitive controls.
The putative-resistant genotypes are significantly different from the sensitive-reference genotypes in almost all response measurements. Based on gene expression analysis in four genotypes (AMANE, 2, HKG 98, and N22), the inflorescence shows higher correlation with phenotypic measurements of grain yield parameters than flag leaf during reproductive stage. Moreover, both sugar metabolism genes and transcription factors confer positive effects to drought resistance particularly in relation to yield parameters related to number of grain per panicle and panicle length.