12-2020

Molecular Genetic Analysis of Drought Resistance and Productivity Traits of Rice Genotypes

Yheni Dwiningsih
University of Arkansas, Fayetteville

Molecular Genetic Analysis of Drought Resistance and Productivity Traits of Rice Genotypes

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy in Crop, Soil, and Environmental Sciences

by

Yheni Dwiningsih
Satya Wacana Christian University
Bachelor of Science in Biology, 2007
Satya Wacana Christian University
Master of Science in Biology, 2009

December 2020
University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

_____
Andy Pereira, Ph. D.
Dissertation Director

_____	_____
Richard Esten Mason, Ph.D.	Lisa Wood, Ph. D.
Committee Member	Committee Member
_____	_____
Paul A. Counce, Ph. D.	Ainong Shi, Ph. D.
Committee Member	Committee Member

Abstract

Rice (*Oryza sativa* L.) is the staple food for a majority of the world's population, and uses 30% of the global fresh water during its life cycle. Drought at the reproductive stage is the most important abiotic stress factor limiting grain yield. The United States is the third largest exporter of rice, and Arkansas is the top rice-producing state. The Arkansas rice-growing region in the Lower Mississippi belt is among the 10 areas with the highest risk of water scarcity. Adapted U.S. rice cultivars were screened for drought resistant (DR) traits to find sources for breeding U.S. rice cultivars for a water saving agricultural system. A recombinant inbred line (RIL) population, derived from varieties Kaybonnet (DR) and ZHE733 (drought sensitive), termed K/Z RILs was chosen for genetic analysis of DR traits. The objectives of this research were to 1) analyze the phenotypic and grain yield components of the K/Z RIL rice population for drought-resistance-related traits, 2) evaluate the Abscisic Acid (ABA) response of the K/Z RIL rice population on root architectural traits in relation to drought stress resistance, 3) screen polymorphic molecular markers to identify genes linked to productivity traits of grain yield under drought stress, measured by number of filled grain per panicle using bulk segregant analysis (BSA), and 4) identify QTLs and candidate genes in the K/Z RIL population for drought resistance associated with vegetative morphological traits, grain yield components under drought stress and well-watered conditions, and root architectural traits related to ABA response. The RIL population was screened in the field at Fayetteville (AR) by controlled drought stress (DS) treatment at the reproductive stage, and the effect of DS quantified by measuring drought-related traits. ABA sensitivity was quantified by measuring root architectural traits at the V3 stage. Based on the filled grain per panicle number, 13.13% of K/Z RIL population and parent Kaybonnet were highly drought resistant, while 75.75% of RILs and parent ZHE733 were

drought sensitive. Under ABA conditions, Kaybonnet and 48 drought resistant lines exhibit ABA sensitivity, implying regulation of osmotic stress tolerance via ABA-mediated cell signaling. Based on BSA screening, 13 polymorphic markers potentially linked to DR traits were identified. QTL analysis was performed with 4133 SNPs markers by using QTL IciMapping. A total of 213 QTLs and 628 candidate genes within the QTL regions were identified for drought-related traits. The RT-qPCR analysis of the candidate genes revealed that a high number of drought resistance genes were up-regulated in Kaybonnet as the drought-resistant parent. Information from this research will serve an important step towards improvement of adapted Arkansas rice cultivars for higher grain production under DS conditions.

Acknowledgments

I would like to thank my adviser, Dr. Andy Pereira for giving me the opportunity to study under his supervision and for his valuable guidance. I will always be grateful for his unconditional support from the very beginning of my graduate school journey up to the end.

To my Committee Members, Dr. Richard Esten Mason, Dr. Paul A. Counce, Dr. Lisa Wood, and Dr. Ainong Shi for their valuable advice and constructive suggestions during my research and courses.

To the Rice Genomics staffs and students at the University of Arkansas, Dr. Julie Thomas, Dr. Anuj Kumar, Dr. Chirag Gupta, Dr. Niranjan Baisakh, Dr. Supratim Basu, Dr. Ramegawda Venkategawda, Dr. Jawaher Alkahtani, Dr. Bhuvan Pathak, Dr. Marcos da Silva, Miranti Rahmaningsih, Sara Yingling, Charles Ruiz, Elizabeth Crowley, and Cintia Rodriguez for their help in conducting my experiments, constructive suggestions, skillful help, and meticulous supervision.

To Fulbright Scholarship Program for the administrative support, necessary facilities, international experiences, and for generating all kind of resources supporting the program.

To my family, mom, dad, sister, brother in law, nephew, niece, grandparents, aunts, and uncles. Thank you for the unconditional love, prayers, and support.

Dedication

To My Mom, My Dad, and My Sister

Table of Contents

Page

List of Tables

Chapter 1 **Page**

Chapter 2

Chapter 3

Chapter 4

List of Tables (continued)

Chapter 5

List of Figures

Chapter 1 **Page**

Chapter 2

List of Figures (continued)

List of Figures (continued)

List of Figures (continued)

List of Figures (continued)

Chapter 4

List of Figures (continued)

Chapter 5

List of Figures (continued)

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Introduction

Rice is a major world food crop and staple food for nearly half the world's population, including 17 countries in Asia and the Pacific, eight countries in Africa, seven countries in Latin America and the Caribbean, and one country in the Near East (Maclean et al., 2013). Rice is grown in tropical, subtropical, and temperate regions (Vaughan, 1989) with more than 90% of total rice in the world produced and consumed by Asian countries (Riveros, 2000). The world-wide rice production reaches 650 million tons per annum, grown in 117 countries on over 156 million hectares (ha) of land, most of which is developed for irrigated rice cultivation. In 2016, China and India produced 350 million tons of rice, which is more than half of the total rice production in the world (FAOSTAT, 2015).

There are four primary cultivation systems for growing rice based on a land area: irrigated (55%), rainfed lowland (25%), upland (12%), and flood prone (8%) (Khush, 1997). Around 35 million ha of land is developed for rainfed lowland rice production in South and Southeast Asia, while around 19 million ha of upland rice and more than 14 million ha of rainfed lowland rice are affected by water insecurity (Centritto et al., 2009). Unpredictability of the water supply is the primary problem in rice production, with severe droughts and damaging floods often coming in the same season (Kundzewicz, 2007). According to Rejesus et al. (2012), it takes 1,432 litres of water to produce 1 kg of rice. Because of the high need for water, the primary limitation for rice production is drought. Therefore, drought resistant varieties of rice are required for increasing rice production under rainfed or deficit-irrigated conditions.

Rice belongs to the genus *Oryza* and comprises approximately 21 wild species and two cultivated species: *O. sativa*, grown in Asian countries; and *O. glaberrima*, grown in Africa. Furthermore, *O. sativa* can be classified into two major subspecies: *indica* and *japonica*. While

indica can be futher sub-classified into *indica* and *aus*, while *japonica* sub-classified into *tropical japonica*, *temperate japonica*, and *aromatic* (Matsuo, 1952; Glaszmann, 1987; Ni et al., 2002; Garris et al., 2005; Ebana et al., 2010). *Tropical japonica* is commonly grown in the U.S. (Mae, 1997), while *temperate japonica* with short, wide grains is adapted to cooler seasons and longer days in the higher latitudes, and is grown in Japan, Korea, and northern China. The long grain *indica* is preferred in South Asia and parts of South East Asia.

Genetic improvements, in diverse rice genotypes that increase rice production, will be necessary to meet the food demands of the world's population by 2050, when the global population is projected to reach up to six billion (Godfray, 2010; Tester and Langridge, 2010). Modern genomics and genetic approaches, associated with phenotyping and breeding methodologies, are needed to exploit the genes and metabolic pathways of diverse rice germplasm for improving rice drought resistance (Mir et al., 2012). As defined by Levitt (1980), drought resistance mechanisms can be classified into three categories: avoidance, tolerance, and escape. Drought avoidance of plants can be achieved by improving water uptake and decreasing water loss; for example by developing more efficient root systems, reducing stomatal conductance for lowering water loss, leaf rolling or folding for reducing the absorption of radiation and evaporation surface, and increased signaling machanisms for abscisic acid (ABA) biosynthesis for reducing water loss. Rice genotypes that employ drought avoidance mechanisms usually have deep and thick roots, robust root branching, a higher root-to-shoot ratio, early stomatal closure, and leaf rolling elasticity (Blum et al., 1989; Samson et al., 2002; Wang et al., 2006). Moreover, drought tolerance can also be achieved by osmotic regulation, antioxidant activity, and smaller cell size, and drought escape by a short life cycle.

Three stages of rice development have important effects on rice production: these are the vegetative, reproductive, and terminal stages. Drought stress in the vegetative stage results in reduced production of new tillers and leaf elongation, rolling of existing leaves, and higher rates of leaf death (Cutlter et al., 1980; O'Toole and Cruz, 1980; Hsiao et al., 1984; Turner et al., 1986). Moreover, drought conditions during the reproductive stage can reduce the grain yield because of more spikelet sterility leading to unfilled grains (Ndjiondjop et al., 2010). Mechanisms of drought resistance in different rice genotypes can be identified through screening for drought resistance at every stage of rice development. Furthermore, drought resistance genes can be identified by understanding drought resistance mechanisms.

Plant biotechnologists and breeders can be helped by the evaluation of rice genotypes to effectively find phenotypic and physiological traits for drought resistance. Both physiological and molecular information will be useful in determining strategies for selecting and improving drought resistance in high-yielding commercial cultivars. Consequently, screening the natural variation for drought-resistance-related traits, will provide the genetic resources for improving resistance to drought among commercial rice varieties. Furthermore, quantitative trait locus (QTL) mapping can be used to construct high-density molecular genetic maps based on several traits related to drought resistance (Lilley et al., 1996; Zhang et al., 1999, 2001; Robin et al., 2003). Therefore, high-resolution mapping of genes responsible for diverse phenotypic traits will help breeders use molecular markers for tagging these traits based on their selection, which will improve breeding efficiency for drought resistance.

U.S. is the third largest exporter of rice with an export value of US$ 1.8 billion. Maintaining rice production in the U.S. is therefore very crucial. The first trial conducted on rice in the U.S. was established in Virginia in 1609, and commercial cultivation was started in South

Carolina. Today, rice is grown in seven U.S. states: Arkansas, California, Louisiana, Mississippi, Missouri, Texas, and Florida (Kulp et al., 2000). Arkansas, the largest rice producer, accounts for more than 40% of U.S. rice production of long and medium grain varieties (Quick Stats, 2016). In 2003, 36% of Arkansas's land was used for rice production with an economic value of US$ 995,217,000, substantially lower than soybeans (US$ 1,435,145,000), but more than twice the value of corn (US$ 471,362,000). The rice was grown on 1,546,000 acres with the average yield being 164.4 bushels/acre (3356.58 kg/acre). Rice is grown in 40 counties including Poinsett (134,944 harvested acreage), Arkansas (117,675 harvested acreage), Cross (106,254 harvested acreage), Jackson (101,762 harvested acreage), and Lawrence (99,480 harvested acreage). Stuttgart in Arkansas County (AR) is known as the rice capital of the U.S.

Rice also uses two to three times as much water as other food crops, which totals 30% of the world's freshwater resources world-wide. Stability of rice production is facilitated by economical use of water, which is most essential during flowering and grain formation. Rice production in Arkansas is dependent on ground water for stable irrigation (Henry et al., 2016). Managing this resource judiciously and growing water-use-efficient (WUE) rice will help insure a steady supply of rice grain in the world market. Efficient rice production methods are especially critical in the Arkansas rice-growing region in the Lower Mississippi belt, for it is among the 10 areas with the highest risk of water scarcity in the country as are the agricultural areas in California, Nebraska, Ohio, Dakotas, N. Texas, and Minnesota (Shi et al., 2013; http://www.businessinsider.com/us-drought-water-scarcity-2013-5).

Literature Review

Rice

Rice is the most important economic crop on Earth and is produced on more than 500 million ha, which is 11% of the Earth's total arable land. Rice is produced in a wide range of locations and under a variety of climatic conditions, from the wettest areas to the driest deserts. In 2016, total rice production reached 472.39 million tons, with almost 90% of rice being produced and consumed in Asia. The largest rice-producing countries are China, India, Indonesia, Bangladesh, Vietnam, and Thailand which account for more than three quarters of world rice production.

Rice belongs to a tropical C3 photosynthetic type of semi-aquatic annual grass and grows in wetland soils and low-radiation habitats. It produces new tillers from nodes, and each productive tiller has a terminal flowering head or panicle. A rice plant's height can range from approximately 0.4 m to more than 5 m, depending on the variety and environmental conditions. The optimal temperature for maximum rice photosynthesis is 25–30^{o}C. Rice is grown optimally in a medium loam containing 50% clay due to minerals availability and water holding capability (Maclean et al., 2013).

The ovary of a self-pollinating rice plant is fertilized by the pollen grain usually fertilization completed within five to six hours after pollination. Both pollen (male) and ovary (female) are in the same flowers, allowing each flower to pollinate itself. Furthermore, after ripening, fertilization occurs and can be classified into four stages: milky, dough, yellow-ripe, and maturity. Generally, the growth duration of the rice plant is three to six months, depending on the variety and the environment. Rice grain yield is based on the amount of starch that fills the spikelets and determined after heading. As the size and weight of the grain increases, starch

and sugars are translocated from the culms and leaf sheaths. Moreover, the color of the grain changes from green to gold, and the leaves begin to senesce. There are three yield components in rice: (i) the number of panicles per unit of land area, (ii) the average number of grains produced per panicle, and (iii) the average weight of individual grains. The rice grain, generally called a seed, consists of the true fruit, or caryopsis, and the hull, which encloses the caryopsis. Furthermore, the caryopsis consists of the embryo and endosperm. The surface of the caryopsis contains several thin layers of differentiated tissues that enclose the embryo and endosperm. In *indica* rice, the hull usually consists of the palea, lemmas, and rachilla; whereas the hull in *japonica* rice generally includes rudimentary glumes and a portion of the pedicel. The hull weight is usually about 20% of the grain's total weight. A single rice grain weighs about 10-45 mg at 0% moisture content. Among rice varieties, the grain length, width, and thickness vary widely (Maclean et al., 2013).

Rice germination is the stage when the radicle or coleoptile (embryonic shoot) emerges from the ruptured seed coat. This germination process starts when seed dormancy has been broken, and the seed absorbs adequate water and is exposed to a temperature between 10 and 40^{o}C. In aerated conditions, the radicle is the first to emerge from the embryo, followed by the coleoptile. In contrast, under anaerobic conditions, the coleoptile is the first to emerge. Every stem of rice consists of a series of nodes and internodes. The number of nodes varies from 13 to 16, and they are separated by long internodes. Generally, the length of internodes increase from the lower to the upper part of the stem and vary depending on the rice variety and environmental conditions. The tillering stage begins as soon as the seedling is self-supporting and usually ends at panicle initiation. Tillers growing from the main stem, called primary tillers, generate secondary tillers, which may in turn generate tertiary tillers. Tillering capacity differs depending

on variety and environmental conditions such as spacing, light, nutrient supply, and cultural practices (Maclean et al., 2013).

The leaf blade is attached at the node by the leaf sheath, which encircles the stem. The leaf blade and the leaf sheath meet at what is called the auricles which is covered by coarse hair. The upright membrane above the auricles is called the ligule. Furthermore, the rice root system consists of crown roots (including mat roots) and nodal roots. Crown roots develop from nodes below the soil surface, while nodal roots develop from nodes above the soil surface. The major structures of the panicle are the base, axis, primary and secondary branches, pedicel, rudimentary glumes, and spikelets. The rice flower is enclosed in the lemma and palea, which may be either awned or awnless. Moreover, the flower consists of the pistil (including the stigmas, styles, and ovary) and stamens.

Rice plant growth can be classified into three agronomic phases of development: the vegetative phase (including germination, seedling, and tillering stages, usually 60 days), the reproductive phase (including panicle initiation, flowering, booting, and heading stages, usually 30 days), and the ripening period, (the time after heading, usually 30 days) (Table 1.1.). Growth during these phases influence the grain yield. The characteristics of the vegetative growth phase are active tillering, a gradual increase in plant height, and leaf emergence at regular intervals. The vegetative stage ends when a flag leaf emerges from a culm. Furthermore, the reproductive phase is characterized by culm elongation, a decline in tiller number, booting, emergence of the flag leaf, heading, and flowering.

Rice grains are classified into three categories: short-grain, medium-grain, and long-grain. The short grain is less than twice as long as it is wide, the medium grain is about three times as long as it is wide, and the long grain is about four to five times as long as it is wide.

Rice provides carbohydrates, proteins, minerals, vitamins, and fibers. Most rice is consumed in a polished state and prepared/cooked by boiling or steaming. It is the main item in most people's diets in some cultures and frequently the basic ingredient of every meal. In Asia, rice is commonly eaten with bean curd, fish, vegetables, meat, and spices, depending on local availability and economic access. Glutinous rice is the staple food in Lao PDR and northeast Thailand. In Japan and China, rice is used to make alcoholic beverages, such as sake and wang-tsiu. In Tanzania, however, rice is used for making bread (Maclean et al., 2013). In the U.S., rice is used as directly cooked food (58%), processed food and beer (16%), and pet food (10%). Rice is sold in many forms, such as frozen, canned, quick-cook rice, and boil-in-the-bag rice.

Based on FAO, other parts of rice besides the grain, such as bran, hulls, and straw, are also very useful. Generally, milled rice is marketed precooked, canned, dried, puffed for breakfast cereals, or used as rice flour for extrusion-cooked foods, puddings and breads, cakes and crackers, noodles and rice paper, fermented foods and vinegars, rice starch, and syrups. About five to eight percent of grain weight is contained in the rice bran, which is usually used for livestock feed, a pickling medium, a medium for growing mushrooms, and as a growing medium for some enzymes, as well as for flours, concentrates, oils, and dietary fiber. Moreover, the hulls and husks are used for fuel. Meanwhile, the rice straw is sometimes used for livestock feed (Maclean et al., 2013).

Rice plants are often attacked by diseases, insects, nematodes, rodents, and weeds. The major diseases that affect rice are caused by fungal, bacteria, and viruses including blast, sheath blight, sheath-rot, bacterial blight, and rice tungro (Gnanamanickam, 2019) (Table 1.2.). Insect pests attack rice plants in all rice growth stages and can also damage rice grains in storage. The most dangerous insects are Pyralidae (stem borrers), rice water weevils, termites (root feeders),

leafhoppers, planthoppers, and stink bugs (Ane & Hussain, 2015). Rice production can also be negatively affected by weeds competing for nutrients, sunlight, and water. Based on Smith (1988), the weeds most dangerous to rice are red rice, barnyard grass, bearded sprangletop, amazon sprangletop, broadleaf signalgrass, annual sedge, ducksalad, hemp sesbania, spreading dayflower, northern jointvetch, and eclipta (Smith, 1988).

Genetically modified rice varieties have been developed to tolerate herbicides (Gunther, 2007), resist insects (Fujimoto et al., 1993), enhance vitamin A concentrations (Christensen, 2000), produce human protein (Boyle, 2011), generate nutrients, increase grain size, and accelerate photosynthesis (C4 photosynthesis) (Bullis, 2015). In 2009, pest resistance rice was approved in China. These genetically modified rice varieties are still not available for commercial production and consumption because of controversies including environmental impact, food safety, and ethics.

Rice germplasm collection

In 1800s, the United States Department of Agriculture (USDA) started collecting rice germplasm from all over the world and catalogued 17,359 accessions (Bockelman et al., 2002). These accessions are being stored and evaluated for agronomic traits, morphological characteristics, grain quality, abiotic and biotic stress resistance, and DNA analysis at the USDA-ARS, Dale Bumpers National Rice Research Center (Stuttgart, Arkansas). The evaluation of agronomic traits showed the diversity in the collection for days from emergence to heading ranging from 37 to 183 days, with an average of 103 ± 20 days. Plant height ranged from 41 to 208 cm, with an average of 118 ± 26 cm. About 62% were awnless, 26% had short and partial awns, 5% had long and partial awns, and 7% had long and full awns. Around 14% of the

collection were of the erect plant type, 32% of the intermediate type, 49% of the open type, and 5% of the spreading type. Regarding panicle type, 3% were erect, 89% open, and 8% spreading.

Drought stress

Drought is the most important abiotic stressor that affects rice production worldwide. Drought is estimated to frequently affect 19-23 million ha of rice lands. For example, drought conditions in some parts of India caused grain yield reduction of up to 40% and losses around US$ 800 million. Rainfed lowlands, which comprise almost 30% of the world's total rice-producing area, are affected by severe and regular droughts (IRRI, 2011). The effects of drought on many aspects of rice production have long been recognized. Drought conditions affect morphological, physiological, and grain yield characteristics. The morphological impacts of drought can include leaf rolling (Sobarado, 1987), and reduction in productive tiller numbers (Mostajeran and Rahimi-Eichi, 2009; Bunnag and Pongthai, 2013), plant biomass (Farooq et al., 2009), and plant height (Sarvestani et al., 2008; Bunnag and Pongthai, 2013; Sokoto and Muhammad, 2014). Drought stress also affects the physiological processes of the rice plant, reducing photosynthesis activity (Ji et al., 2012; Lauteri et al., 2014; Yang et al., 2014), transpiration rate (Siddique et al., 2003), stomatal conductance (Dingkuhn et al., 1991), osmotic adjustment (Steponkus et al., 1982; Turner et al., 1986; Fukai and Cooper, 1995), relative water content (Teulat et al., 2003), chlorophyll content (Sairam et al., 1996), and chlorophyll fluorescence (Lichteuthaler & Miehe, 1997). Moreover, rice is sensitive to drought stress at the reproductive stage, that affects grain yield components by interfering with pollination and grain filling (Hsiao, 1982; Venuprasad et al., 2008). Grain yield components affected by drought stress include the number of panicles per plant, panicle length, spikelet per panicle number, filled grain per panicle, and hundred grain weight.

Drought impacts are usually first apparent in agriculture through decreases in soil moisture and high evapotranspiration. Because all nutrients are taken up with water, water scarcity decrease the nutrient uptake of rice plants and limit their root growth. This condition causes large yearly fluctuations in rice production. The average of global yield reduction due to drought stress is 18 million tons annually (O'Toole, 2004; Lakshmi et al., 2012). Environments with water deficits are often characterized by relatively low and irregular rainfall. In addition, drought conditions also interferes with the rice plant's ability to absorb nitrogen and other nutrients, which in turn inhibits kernel development and reduces its nutritional value (Tuberosa et al., 2003). Therefore, it is very important to develop and commercialize productive drought resistant varieties of rice.

Rice plants mainly respond to drought stress conditions by leaf rolling, stomatal closure, and increased the ability to respond appropriately to ABA signals to minimize the overall water deficit (Price et al., 2002). A complex network of stress signaling and regulation of gene expression occurs in rice plants responding and adapting to stressors. The stress signals are perceived through diverse known and unknown sensors and transduced by various signaling components in the plant to various physiological and metabolic responses to the stress conditions (Zhu, 2002; Matsukura et al., 2010; Lata and Prasad, 2011).

New methods to more effectively increase the long-term sustainability of rice production are needed. Severe drought is an abiotic stress that can lead to plant death. Different mechanisms allow rice to avoid, tolerate, and escape the effects of drought. One method for developing new varieties resistant to drought stress is the "gene-by-gene" approach (Tuberosa et al., 2003).

Based on Levitt (1980), the plants' drought resistance mechanisms can be classified into three categories: avoidance, tolerance, and escape. Drought avoidance involves maintaining cell

turgor through an increase in water uptake and/or reduction in water loss, allowing the plant to maintain a relatively high water status and thereby avoiding the deleterious effects of dehydration. This strategy implies a higher capacity to absorb water from the soil by means of a deep root system and/or a lower water loss from the canopy with leaf rolling. In rainfed rice, a deep root system is important, which is not as critical to a well-watered or flooded rice cropping system. Furthermore, drought tolerance harnesses biochemical mechanisms allowing cells to tolerate water loss. This mechanism depends on osmotic adjustment or "stay-green" traits. Rice varieties capable of high osmotic adjustment produce longer roots and more root biomass. During drought stress, scavenging of free radicals plays an important role in reducing the damage to the biochemical machinery of the cell resulting from an excessive redox potential. Drought escape is characterized by early flowering in rice where "stored" water is exploited before the onset of seasonal drought.

An induction in ABA concentration is a universal response from plants subjected to drought and other abiotic stresses. ABA regulates the expression of genes whose products may protect the cell from the harmful effects of excessive dehydration. An increased ABA concentration improves the root-to-shoot ratio, an adaptive change that, at a later stage, can be beneficial for avoiding dehydration when water is available in deeper layers of the soil profile. Ethylene production modifies the role of ABA in sustaining root cell elongation under drought conditions (Singh et al., 1996).

Breeding and the development of better agronomic practices have been central to improving rice grain yield during the past century. A higher resistance to drought contributed to remarkable improvements in yield stability in environments with different degrees of access to water. The purpose of breeding is to identify and enhance resistance of crops to drought stress.

Conventional breeding is often based on empirical selection for yield (Atlin and Lafitte, 2002), which is far from optimal since yield is a quantitative trait and characterized by low heritability and high genotype × environment (G × E) interactions (Babu et al., 2003). Heterogeneous environments and genotypic adaptation are the main reasons for the slow progress in developing drought resistant rice varieties (Fukai and Cooper, 1995).

Genetic improvements for drought resistance has been achieved using a conventional approach that involves selecting for yield and secondary traits (Farooq et al., 2009). Molecular markers linked to drought resistance in rice are important tools for screening and selection of drought resistant genotypes for use in future breeding programs (Muhammad et al., 2014). Furthermore, molecular breeding based on the screening of drought resistant genotypes from all over the world and an understanding of the drought resistance mechanisms will help in identifying genes from drought-adaptive germplasm (Yu et al., 2012). In recent years, several collaborative research programs have been initiated to screen rice germplasm collections for drought resistant traits so that drought stress problems can be addressed world-wide. Scientists from different disciplines, including plant physiology, agronomy, molecular biology, and plant breeding, are analyzing drought stress response mechanisms in plants and using this knowledge for applications in breeding for the improvement of traditional cultivars that are high-yielding but drought-sensitive (Kamoshita et al., 2008).

The International Rice Research Institute (IRRI) developed drought resistant rice varieties, such as variety 5411 which is grown in the Philippines and Sookha in Nepal. Meanwhile, in 2013, the Japanese National Institute for Agrobiological Sciences developed a drought resistant rice variety by introgression of the *deeper rooting-1* (*dro-1*) gene from a deep-rooting upland rice variety, Kinandang Patong, into a drought-sensitive but popular commercial

rice variety, IR64 (Palmer, 2013). The introgression of *dro-1* resulted in a 10% grain reduction, a marked improvement compared to the 60% reduction of wild-type IR64 under moderate drought conditions. This drought resistant variety has a deeper root system that improves its ability to absorb water and nutrients from deeper layers of soil.

K/Z Recombinant Inbred Line (RIL) population

In 2003, the rice K/Z RIL population was developed in Stuttgart, AR by J. Neil Rutger and Thomas H. Tai using a cross between the low phytic acid mutant KBNT*lpa*1-1 of the *tropical japonica* cultivar "Kaybonnet" and the *indica* cultivar "ZHE733." A subset of 137 F_2 lines from the population was used to map the *lpa*1-1 mutation to a 2.2 cM interval on chromosome 2. The full population was continued through the F_{10} or F_{11} generations of recombinant inbred lines (RIL) to provide materials useful for additional genetic studies. An individual from the RIL population has more recombination events than an individual from an F_2 or Double Haploid population. The mapping population is identified as Genetic Stocks Oryza (GSOR) 100001 through 100355 in the Germplasm Resources Information Network (GRIN) Global. Six RILs (100051, 100052, 100054, 100101, 100157, and 100181) have been removed from the collection by USDA Dale Bumpers National Rice Research Center after recent analyses determined that the lines are not recombinants; they are either a self, or the heterogeneity is higher than expected. Moreover, 14 lines were identified to have alleles that are skewed towards one parent or the other: 100004, 100011, 100031, 100035, 100061, 100090, 100116, 100124, 100138, 100190, 100204, 100215, 100232, and 100307.

The KBNT*lpa*/ZHE733 RIL (K/Z RIL) $F_{10–11}$ population is the first mapping population deposited at the GSOR Collection (http://www.ars.usda.gov/Main/docs.htm?docid = 8318) for distribution (Rutger and Tai, 2005). A subset of this population has been used to map a gene

regulating phytic acid concentrations (Rutger and Tai, 2005; Andaya and Tai, 2005). A detailed evaluation of the K/Z RIL population based on Simple Sequence Repeat (SSR) markers would enhance the ability of diverse research groups to utilize this population for mapping genes involved in yield and diseases resistance.

Indica and *japonica* crosses result in linkage blocks (LBs) and recombination suppression on rice chromosome 12 (Jia et al., 2012). Recombination suppression is a complex biological phenomenon often resulting in large linkage blocks, where a set of genes is inherited together. The existence of sexual incompatibility between *indica* and *japonica* rice on chromosome 12 suggests that *japonica* and *indica* rice possess distinct morphological and molecular differences (Kovach et al., 2007). *Indica* is commonly grown in lowlands throughout tropical Asia, while *japonica* is cultivated in dry fields in temperate East Asia, upland areas of Southeast Asia, and high elevations in South Asia and the U.S. Kaybonnet and ZHE733 are known to have phenotypic differences in terms of plant height and heading dates.

Kaybonnet (KBNT*Ipa*1-1) is one of the parents in the K/Z population, which is a low phytic acid mutant induced by exposure to gamma rays (Liu et al., 2005). Kaybonnet was developed from crossing Katy and Newbonnet. Katy is a *tropical japonica* cultivar with large introgressions from *indica* landrace Tetep (Figure 1.1.) (Gravois and Helms., 1998). Morphologically, KBNT*Ipa*1-1 is tall, with glabrous leaves and hulls and an average yield compared to ZHE733. It is susceptible to rice blast (Lee et al., 2005), rice water weevils, and straighthead (Yan et al., 2005). KBNT*lpa* has a 55% reduction in phytic acid P compared to wild-type Kaybonnet (Larson et al., 2000) and contains approximately 1.39 mg/g of phytic acid. KBNT*lpa* posses a single recessive gene, *lpa1-1*, for low phytic acid (Rutger et al., 2004). The *lpa1* locus is mapped on chromosome 2 between SSR markers RM3542 and RM482 (Figure

1.2.). The *lpa1* gene regulates phytic acid biosynthesis in plants (Andaya and Tai, 2005). Phytic acid binds minerals (K, Mg, Mn, Fe, Ca, and Zn) and prevents the absorption of these important minerals from food (Torre et al., 1991), leading to micronutrient deficiencies. Low phytic acid rice could improve phosphate bioavailability in food and reduce phytic acid excretion (Raboy, 2001).

The second parent of the K/Z RIL population is ZHE733, an *indica* rice cultivar that originated in China that can be grown in the southern U.S. It is a semi-dwarf, and like the majority of semi-dwarf varieties contains *sd-1* (Spielmeyer et al., 2002) is pubescent, high-yielding, early-maturing, and resistant to rice blast (Yan and Cai, 1991) and straightheads. The semi-dwarf gene, *sd-1*, was found on chromosome 1 at the location of 38.7 Mb (Monna et al., 2002; Spielmeyer et al., 2002). The pedigree of ZHE733 contains IR29 (Figure 1.1.), which contains *sd-1* (Khush and Gomez, 1985; Monna et al. 2002), indicating that *sd-1* may be one of the factors controlling height variations in ZHE733. This variety contains approximately 2.56 mg/g of phytic acid. ZHE733 is also useful for studying the anatomical, physiological, and biochemical changes in chalkiness formation among early-maturing *indica* rice varieties (Shen and Cheng, 1999; Jiang et al., 2002).

The K/Z RIL population has proven to be an excellent mapping population. Molecular characterization of the KBNT*lpa*×ZHE733 RILs population (K/Z RILs) was found useful in the mapping of rice blast resistance genes, the genes associated with low phytic acid composition, and other agronomic traits (Rutger and Tai, 2005). Based on the research of Liu et al. (2005), the 255 K/Z RILs showed polymorphism for 109 markers, 172 K/Z RILs (67.5%) were homozygous, 42 K/Z RILs (16.4%) were heterozygous, 30 K/Z RILs (11.8%) had non-parental alleles, and 11 K/Z RILs (4.3%) were heterozygous and had non-parental alleles. The average

frequencies of heterozygosity and non-parental alleles per K/Z RIL were 1.3% and 0.4%, respectively; theoretically, these should be 0.2% and 0.1%.

The segregation of marker loci in the K/Z RIL populations was expected to be a 1:1 ratio of alleles from the KBNT*lpa* : ZHE733 parents. Segregation distortion can be caused by a number of physiological or genetic factors such as gametic or zygotic selection (Nakagahra, 1986; Peng et al., 2008), chromosome rearrangement (Tanksley, 1984), genetical incompatibility (Cryder et al., 1991; Liedl and Anderson, 1993), pollen competition (Mangelsdorf and Jones, 1926; Liedl and Anderson, 1993), and preferential fertilization (Schwemmle, 1968; Gadish and Zamir, 1986).

Liu et al. (2005) characterized 269 lines of the K/Z RIL population by using SSR markers. One hundred and seven markers were mapped on 12 rice chromosomes, representing a total of 1016.3 cM of genetic distance with an average of 9.3 cM between each pair of markers (Figure 1.3.). This is shorter than the genetic distance of 1565.9 cM for the same number of SSR markers from the Cornell2001 database in Gramene (http://www.gramene.org). Similarly, He et al. (2001) reported that the genetic distance of each chromosome in a RIL population was shorter than that in a double haploid population. The total genetic distance in the RIL population of ZYQ8/JX17 (*indica/japonica*) was 70.5% of that in the double haploid population derived from the same rice cross. The order of the markers on chromosomes 1, 2, and 4–11 agreed with the Cornell2001 data, but there were some discrepancies on chromosome 3 and 12. Furthermore, cluster analysis was applied to the 238 K/Z RILs using the UPGMA method, which showed a clear separation of the K/Z RILs into 10 sub-groups (Figure. 1.3. and 1.4.). This linkage map would facilitate the development of DNA markers to tag genes for agronomically important traits and also for marker-assisted selection.

The linkage map of the K/Z RIL population was previously constructed and used for mapping QTL for resistance to rice sheath blight and blast disease (Liu et al., 2008; Lee et al., 2009; Jia and Liu, 2011). Furthermore, Liu et al. (2016) reported QTL for heading date and plant height in the K/Z RIL population. The QTLs for heading date are qHD3.1 on chromosome 3, qHD7.1 and qHD7.2 on chromosome 7, and qHD8.1 on chromosome 8. Likewise, the QTLs for plant height detected on chromosome 1 and 3 were qPHT1.1 and qPHT3.1.

Quantitative trait loci (QTL) in rice

Quantitative trait loci (QTL) are chromosomal segments that contain genes governing quantitative traits. They are defined and mapped using molecular markers such as Single Nucleotide Polymorphisms (SNPs), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs), Restriction Fragment Length Polymorphisms (RFLPs), and Random Amplified Polymorphic DNAs (RAPDs), as well as quantitative traits controlled by two or more genes and affected by environmental variation, including plant height, grain yield, abiotic and biotic stress. This mapping method is very affordable to plant research programs because of developments in genomic technology and statistical analysis methods (Zhu et al., 2008). The aim of the QTL is to identify which regions in the genome are associated with the traits of interest by constructing QTL maps. Five criteria determine whether plant populations are suitable for QTL (Yu and Buclker, 2006; Yu et al., 2006): (i) ideal sample size, (ii) multi-family sample, (iii) presence of population structure in the sample, (iv) presence of both population structure and familial relationships in the sample, and (v) the presence of several population structure and familial relationships in the sample. SNPs are the markers of choice for quantitative trait dissection studies because of their higher genome density, lower mutation rate, more reliable detection system, higher mapping resolution, and lower cost.

Several QTL analyses studies have been conducted on rice. In 1996, QTLs for osmotic adjustment were mapped by Lilley et al. The QTL for leaf rolling in the double haploid population of IR64/Azucena under drought stress were identified by Courtois et al. (2000). Price et al. (1997 & 2002), mapped QTL for leaf rolling under drought stress on chromosome 1 by using an F2 population. QTLs for tiller number, plant height, total root number, root to shoot ratio, and root dry weight were identified by Kanbar et al. (2002) in the double haploid population of CT9993/IR62266 under well-watered conditions. QTLs linked to leaf length, tiller number and nitrogen content were identified by Xu et al. (2009) in the Backcross Inbred Line (BIL) population derived from a cross between *temperate japonica* and *aus.* In 2010, Gomez et al. also detected QTL for leaf rolling and leaf drying on chromosome 1 in the RIL population.

QTL analysis had been used to obtain genomic information about many agronomic traits (Table 1.3.). For example, *sd1* for plant height, *OsMADS13* for flowering time, *Pi-ta* for blast resistance, *GS3* and *qSW5* for grain length and width, *SSII-3* and *Waxy* for alkaline spreading value and amylose content, and *Rc* for pericarp color. Moreover, gene linkage, also known as linkage drag (LD) or pleiotropy, has been found for one SNP to be significantly associated with multiple traits. For instance, SNPs at 31 Mb on chromosome 4 were associated with rice blast disease resistance and flag leaf width, and SNPs at 4.2-4.6 Mb on chromosome 6 were found associated with rice blast disease resistance, amylose content, and flowering time. This LD can be either good or bad for plant breeders, depending on the breeding objectives.

Genomic information from QTL analysis is very useful for enhancing the plant breeding program through marker-assisted selection (MAS). Furthermore, drought resistant varieties can be developed by MAS-based introgression of specific alleles based on QTL information. MAS is

also useful for pyramiding several QTLs for drought resistant traits, thereby producing a source for developing drought resistant rice cultivars (Xu et al., 2005).

Objectives of the study

Keeping the above information in mind the present study on "Molecular Genetic Analysis of Drought Resistance and Productivity Traits of Rice Genotypes" has been initiated with the following objectives.

1. Phenotypic and grain yield components analysis of the K/Z RIL rice population for drought-resistance-related traits.
2. Evaluation of the Abscisic Acid (ABA) response on root architectural traits in relation to drought stress resistance in the K/Z RIL rice population.
3. Screening of the polymorphic molecular markers to identify genes linked to productivity traits of grain yield under drought stress, measured by number of filled grain per panicle using bulk segregant analysis.
4. Identification of QTLs and candidate genes in the K/Z RIL population for drought resistance associated with vegetative morphological traits, grain yield components under drought stress and well-watered conditions, and root architectural traits related to ABA response.

References

Andaya, C. B. & Tai, T. H. (2005). Fine mapping of the rice low phytic acid (*Lpa1*) locus. *Theor Appl Genet, 111,* 489-495. doi:10.1007/s00122-005-2038-0

Ane, N. U. & Hussain, M. (2015). Diversity of insect pests in major rice growing areas of the world. *Journal of Entomology and Zoology Studies, 4, 1,* 36-41.

Ashfaq, M., Haider, M. S., Ali, A., Ali, M., Hanif, S., & Mubashar, U. (2014). Screening of diverse germplasms for genetic studies of drought tolerance in rice (*Oryza sativa* L.). *Caryologia, 67, 4,* 296-304. doi:10.1080/00087114.2014.976091

Atlin, G. N. & Lafitte, H. R. (2002). Marker-assisted breeding versus direct selection for drought tolerance in rice. In: Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice. 208. Saxena, N. P. and O'Toole, J. C., Eds., Proc. Int. Workshop on Field Screening for Drought Tolerance in Rice, Patancheru, India. 11–14 Dec 2000. ICRISAT, Patancheru, India, and The Rockefeller Foundation, New York.

Babu, R. C., Nguyen, B. D., Chamarerk, V., Shanmugasundaram, P., Chezhian, P., Jeyaprakash, P., Ganesh, S. K., Palchamy, A., Sadasivam, S., Sarkarung, S., Wade, L. J., & Nguyen, H.T. (2003). Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. *Crop Sci., 43,* 1457-1469.

Blum, A., Shpiler, L., Golan, G., & Mayer, J. (1989). Yield stability and canopy temperature of wheat genotypes under drought stress. *Field Crop Research, 22,* 289-296.

Bockelman, H. E., Dilday, R. H., Yan, W., & Wesenberg, D. M. (2002). Germplasm collection, preservation, and utilization. 597–625. *In* C.W. Smith and R.H. Dilday (ed.) Rice: Origin, diversity in *Oryza sativa* L. *Genetics, 169,* 1631-1638.

Boyle, R. (2011, 1 November). Rice is genetically modified to produce human blood protein. *Popular Science*.

Bullis, K. (2015, December). Speeding plant growth to feed the world. *MIT Technology Review*.

Bunnag, S. & Pongthai, P. (2013). Selection of rice (*Oryza sativa* L.) cultivars tolerant to drought stress at the vegetative stage under field conditions. *American Journal of Plant Science. 4, 9,* 1701-1708.

Centritto, M., Lauteri, M., Monteverdi, M. C., & Serraj, R. (2009). Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. *Journal of Experimental Botany, 60, 8,* 2325-2339. doi:10.1093/jxb/erp123

Christensen, J. (2000, 10 November). Scientist at work: Ingo Potrykus; Golden Rice in a Grenade-Proof Greenhouse. *New York Times*.

Courtois, B., McLaren, G., Sinha, P. K., Prasad, K., Yadav, R. & Shen, L. (2000). Mapping QTL associated with drought avoidance in upland rice. *Molecular Breeding, 6,* 55-66.

Cryder, C. M., Corgan, J. N., Urquhart, N. S., & Clason, D. (1991). Isozyme analysis of progeny derived from *Allium fistulosum · Allium cepa*. *Theor Appl Genet., 82,* 337-345.

Cutler, J. M., Steponkus, P. L., Wach, M. J., & Shahan, K. W. (1980). Dynamic aspects and enhancement of leaf elongation in rice. *Plant Physiol*., *66,* 147-152.

Dingkuhn, M., de Vries, F. W. T. P., De Datta, S. K & Van Laar, H. H (1991). Concepts for a new plant type for direct seeded flooded tropical rice. Manila, Philippines: International Rice Research Institute.

Ebana, K., Yonemaru, J., Fukuoka, S., Iwata, H., Kanamori, H., Namiki, N. et al. (2010). Genetic structure revealed by a whole-genome single-nucleotide polymorphism survey of diverse accessions of cultivated Asian rice (*Oryza sativa* L.). *Breeding Science, 60*, 390-397.

FAOSTAT. (2015, 12 September). Top production rice-paddy 2012. Retrieved from http://faostat.fao.org/site/339/default.aspx

Farooq, M., Wahid, A., Lee, D. J., Ito, O., & Siddique, K. H. M. (2009). Advances in drought resistance of rice. *Critical Rev. Plant Sci., 28,* 199-217.

Fujimoto, H., Itoh, K., Yamamoto, M., Kyozuka, J., & Shimamoto, K. (1993). Insect resistant rice generated by introduction of a modified δ-endotoxin gene of *Bacillus thuringiensis*. *Bio/Technology, 11*(10), 1151-1155. doi:10.1038/nbt1093-1151

Fukai, S., & Cooper, M. (1995). Development of drought resistant cultivars using physio morphological traits in rice. *Field Crop Res., 40,* 67-86.

Gadish, I., & Zamir, D. (1986). Differential zygotic abortion in an interspecific Lycopersicon cross. *Genome, 29,* 156–159.

Garris, A. J., Tai, T. H., Coburn, J., Kresovich, S., & McCouch, S. (2005). Genetic structure and history, technology, and production. Crop Production, Ser. #6149. New York, NY: John Wiley & Sons.

Glaszmann, J. C. (1987). Isozymes and classification of Asian rice varieties. *Theoretical Applied Genetics, 74*, 21-30.

Gnanamanickam, S. S. (2019). Biological Control of Rice Diseases. Switzerland: Springer Nature.

Godfray, H. C. (2010). Food security: the challenge of feeding 9 billion people. *Science, 327,* 812-818.

Gomez, S. M., Boopathi, N. M., Kumar, S., Ramasubramanian, T., Chengsong, Z., Jeyaprakash, P., Senthil, A., & Babu, R. C. (2010). Molecular mapping and location of QTLs for drought-resistance traits in indica rice (*Oryza sativa* L.) lines adapted to target environments. *Acta Physiology Plant, 32*, 355-364.

Gravois, K. A., & Helms, R. S. (1998). Seeding date effects on rough rice yield and head rice and selection for stability. *Euphytica, 102*, 151-61.

Gunther, M. (2007). Genetically Engineered Rice gets into the U.S. Food Supply. CNN Money.

He, P., Li, J. Z., Zheng, X. W., Shen, L. S., Lu, C. F., Chen, Y., & Zhu, L. H. (2001). Comparison of molecular linkage maps and agronomic trait loci between DH and RIL populations derived from the same rice cross. *Crop Sci., 41*, 1240-1246.

Henry, C., Hirsh, S., Anders, M., Vories, E., Reba, M., Watkins, K., & Hardke, J. (2016). Annual Irrigation Water Use for Arkansas Rice Production. *J. Irrig. Drain Eng*., doi:10.1061/(ASCE)IR.1943-4774.0001068, 05016006.

Hsiao, T. C. (1982). The soil plant atmosphere continuum in relation to drought and crop production. In: Drought Resistance in Crops with Emphasis on Rice, IRRI, Los Banos, Philippines, p39-52.

Hsiao, T. C., O'Toole, J. C., Yambao, E. B., & Turner, N. C. (1984). Influence of osmotic adjustment on leaf rolling and tissue death in rice (*Oryza sativa* L.). *Plant Physiol., 75*, 338.

IRRI (International Rice Research Institute). (2011). Rough rice production by country and geographical region-USDA. Trend in the rice economy. In: World rice statistics. http://irri.org/index.php?option=com_k2&view=item&layout=item&id=9151&lang=en

Ji, K. X., Wang, Y. Y., Sun, W. N., Lou, Q. J., Mei, H. W., Shen, S. H., & Chen, H. (2012). Drought responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. *Journal of Plant Physiology, 169, 4*, 336-344.

Jia, Y., & Liu, G. (2011). Mapping quantitative trait loci for resistance to rice blast. *Phytopathology, 101*, 176-187.

Jia, Y., Jia, M. H., Wang, X., & Liu, G. (2012). Indica and Japonica Crosses Resulting in Linkage Block and Recombination Suppression on Rice Chromosome 12. *PLoS ONE, 7*(8), e43066. doi:10.1371/journal.pone.0043066

Jiang, W., Zhou, M., & Li, T. (2002). Anatomical study on the chalkiness formation of early indica rice. *J Zhejiang Univ (Sci ed), 29*, 459-463.

Kamoshita, A., Babu, R. C., Boopathi, N. M., & Fukai, S. (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crop Res., 109,* 1-23.

Kanbar, A., Shashidhar, H. E., & Hittalmani, S. (2002). Mapping of QTL associated with root and related traits in DH population of rice (*Oryza sativa* L). *Indian Journal of Genetics, 62*, *4*, 287-290.

Khush, G. S, & Gomez, K. A. (1985). Parentage of IRRI crosses IR1IR50000. The International Rice Research Institute, Manila.

Khush, G. S. (1997). Origin, dispersal, cultivation and variation of rice. *Plant Molecular Biology, 35*, 25-34.

Kovach, M. J., Sweeney, M. T., & McCouch, S.R. (2007). New insights into the history of rice domestication. *Trends Genet., 23*, 578–587.

Kulp, K., Ponte, Jr., & Joseph, G. (2000). Handbook of Cereal Science and Technology. Marcel Dekker. pp. 203. ISBN 978-0-8247-8294-8

Kundzewicz, Z. W., Mata, L. J., Arnel, N. W., Döll, P., Kabat, P., Jimenēz, B., Miller, K. A., Oki, T., Sen, Z., & Shiklomanov, I. A. (2007). Freshwater resources and their management. *Climate Change*, 173-210.

Lakshmi, P. M., Chen, X., Clarke, J., Salmeron, J., & Nguyen, H. T. (2012). RNAi-mediated disruption of squalene synthase improves drought tolerance and yield in rice. *J Exp. Bot., 63*, 163-75.

Larson, S. R., Rutger, J. N., Young, K. A., & Raboy, V. (2000). Isolation and genetic mapping of a non-lethal rice low phytic acid 1 mutation. *Crop Sci., 40*, 1397-1405.

Lata, C., & Prasad, M. (2011). Role of DREBs in regulation of abiotic stress responses in plants. *J. Expt. Bot., 62,* 4731-4748.

Lauteri, M., Haworth, M., Serraj, R., Monteverdi, M. C., & Centritto, M. (2014). Photosynthetic diffusional constraints affect yield in drought stressed rice cultivars during flowering. *PLoS One*, *9*(10), e109054.

Lee, F. N., Cartwright, R. D., Jia, Y., Correll, J. C., Moldenhauer, K. A. K., Gibbons, J. W., Boyett, V., Zhou, E., Boza, E., & Seyran, E. (2005). A preliminary characterization of the rice blast fungus on 'Bank' rice. http://www.uark.edu/depts/agripub/Publications/researchseries/529.3.pdf. Cited 23 Feb 2007

Lee, S., Wamishe, Y., Jia, Y., Liu, G., & Jia, M. H. (2009). Identification of two major resistance genes against race IE-1 k of *Magnaporthe oryzae* in the indica rice cultivar Zhe733. *Mol Breed., 24*, 127-134.

Levitt, J. (1980). Responses of plants to environmental stresses, Vol. 2. Water, radiation, salt and other stresses. *New York: Academic Press*, 93-128.

Lichteuthaler, H. K., & Miehe, J. A. (1997). Fluorescence imaging as a diagnostic tool for plant stress. *Trends Plant Sci., 2*, 316-320.

Liedl, B., & Anderson, N. O. (1993). Reproductive barriers: identification, uses and circumvention. *Plant Breed Rev., 11*, 11-154.

Lilley, J. M., Ludlow, M. M., McCouch, S. R., & O'Toole, J. C. (1996). Locating QTLs for osmotic adjustment and dehydration tolerance in rice. *J. Exp. Bot., 47*, 1427-1436.

Liu, E., Liu, Y., Wu1, G., Zeng, S., Thi, T. G. T., Liang, L., Liang, Y., Dong, Z., She, D., Wang, H., Zaid, I. U., & Hong, D. (2016). Identification of a candidate gene for panicle length in rice (*Oryza sativa* L.) in association and linkage analysis. Frontier in Plant Sciences. 7:00596.

Liu, G., Bernhardt, J. L., Jia, M. H., Wamishe, Y. A., & Jia, Y. (2005). Characterization of a recombinant inbred line population of rice using SSR markers. Breeding, Genetics and Physiology.

Liu, G., Bernhardt, J. L., Jia, M. H., Wamishe, Y. A., & Jia, Y. (2008). Molecular characterization of the recombinant inbred line population derived from a japonica-indica rice cross. *Euphytica, 159*, 73-82.

Maclean, J. L., Dawe, D. C., Hardy, B., & Hettel, G. P. (2013). *Rice Almanac*. Los Banos: International Rice Research Institute (IRRI).

Mae. (1997). Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. *Plant soil, 196*, 201-210.

Mangelsdorf, P. C., & Jones, D. F. (1926). The expression of mendelian factors in the gametophyte of maize. *Genetics, 11*, 423-455.

Matsukura, S., Mizoi, J., Yoshida, T., Todaka, D., Ito, Y., Maruyama, K., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2010). Comprehensive analysis of rice DREB2-type genes that encode transcription factors involved in the expression of abiotic stress-responsive genes**.** *Mol. Genet. Genomics, 283*, 185-196.

Matsuo, T. (1952). Gynecological studies on cultivated rice. Bulletin National Institute of Agriculture Science Series. D3: 1-111.

McCouch, S. R., Wright, M., Tung, C., Maron, L., McNally, K., Fitzgerald, M., et al. (2016) Open access resources for genome-wide association mapping in rice. *Nature Communication, 7,* 10532.

Mir, R. R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R., & Varshney, R. K. (2012). Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theor Appl Genet., 125*, 625-645.

Moldenhauer, K., Wilson, C. E., Counce, P., & Hardke, J. (2012). Rice Growth and Development. In Arkansas Rice Production Handbook. University of Arkansas Division of Agriculture.

Monna, L., Kitazawa, N., Yoshino, R., Suzuki, J., Masuda, H., Maehara, Y., Tanji, M., Sato, M., Nasu, S., & Minobe, Y. (2002). Positional cloning of rice semi-dwarfing gene, sd-1: rice ‘‘Green revolution gene’’ encodes a mutant enzyme involved in gibberellin synthesis. *DNA Res., 9*, 11-17.

Mostajeran, A., & Rahimi-Eichi, V. (2009). Effects of drought stress on growth and yield of rice (*Oryza sativa* L.) cultivars and accumulation of proline and soluble sugars in sheath and blades of their different ages leaves. *American-European Journal of Agricultural & Environ Sciences, 5, 2,* 264-272.

Nakagahra, M. (1986). Geographic distribution of gametophyte genes in wide crosses of rice cultivars. In: Khush GS (ed) Rice genetics. Proceedings of the international rice genetics symposium, IRRI, Manila, pp 73-82.

Ndjiondjop, M. N., Cisse, F., Futakuchi, K., Lorieux, M., Manneh, B., Bocco, R., & Fatondji, B. (2010). Effect of drought on rice (*Oryza* spp.) genotypes according to their drought tolerance level. In *Second Africa Rice Congress: Innovation and Partnerships to Realize Africa’s Rice Potential in Mali, 2010*.

Nei, M. (1972). Genetic distance between populations. *Am Nat., 106,* 283-292.

Ni, J., Colowit, P. M., & Mackill, D. J. (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science, 42*, 601-607.

O’Toole, J. C. (2004). Rice and water: the final frontier. In: The first international conference on rice for the future. Bangkok, Thailand: The Rockefeller Foundation.

O’Toole, J. C., & Cruz, R. T. (1980). Response of leaf water potential, stomatal resistance, and leaf rolling to water stress. *Plant Physiology, 65,* 428-432.

Palmer, N. (2013, 29 September). Newly-discovered rice gene goes to the root of drought resistance. *The Cornucopia Institute*. Retrieved from http://www.cornucopia.org

Peng, S., Khush, G. S., Virk, P., Tang, Q., & Zou, Y. (2008). Progress in ideotype breeding to increase rice yield potential. *Field Crop Research, 108,* 32-38.

Price, A. H., Young, E. M., & Tomos, A. D. (1997). Quantitative trait loci associated with stomatal conductance, leaf rolling and heading date mapped in upland rice (*Oryza sativa* L.). *New Phytology, 137,* 83-91.

Price, A., Steele, K., Moore, B., & Jones, R. (2002). Upland rice grown in soil-filled chambers and exposed to contrasting water-deficit regimes: II. Mapping quantitative trait loci for root morphology and distribution. *Field Crop Res., 76,* 25-43.

Quick Stats, USDA-NASS, State Agriculture Overview: Arkansas (2016). https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=ARKANS AS

Raboy, V., Young, K. A., Dorsch, J. A., & Cook, A. (2001). Genetics and breeding of seed phosphorus and phytic acid. *J Plant Physiol., 158,* 489-497.

Rejesus, R. M., Mohanty, S., & Balagtas, J. V. (2012). Forecasting Global Rice Consumption (Working Paper). Lafayette, In: Purdue University. Retrieved from http://www.agecon.purdue.edu/staff/balagtas/rice_timeseries_v6.pdf

Riveros, F. (2000). Keynote address of the 18th session of IRC. In J. S. Nanda (Eds.), Rice Breeding and Genetics, Research Priorities and Challenges (pp. 1–8). Enfield: Science Publishers.

Robin, S., Pathan, M. S., Courtois, B., Lafitte, R., Carandang, S., Lanceras, S., Amante, M., Nguyen, H. T., & Li, Z. (2003). Mapping osmotic adjustment in an advanced back-cross inbred population of rice. *Theor. Appl. Genet*., *107,* 96-1288.

Rutger, J. N., & Tai, T. (2005). Registration of K/Z mapping population of rice. *Crop Science, 45,* 2671-2672.

Rutger, J. N., Raboy, V., Moldenhauer, K. A. K., Bryant, R. J., Lee, F. N., & Gibbons, J. W. (2004). Registration of KBNT *lpa1-1* low phytic acid germplasm of rice. *Crop Science., 44,* 363.

Sairam, R. K., Shukla, D. S., & Deshmukh, P. S. (1996). Effect of homobrassinolide seed treatment on germination, β-amylase activity and yield of wheat under moisture stress conditions. *Indian Journal of Plant Physiology, 1,* 141-144.

Samson, B., Hasan, M., & Wade, L. (2002). Penetration of hardpans by rice lines in the rainfed lowlands. *Field Crop Research, 76,* 175-188.

Sarvestani, Z. T., Pirdashti, H., Sanavy, S. A., & Alouchi, H. B. (2008). Study of water stress effects in different growth stages on yield and yield components of different rice (*Oryza sativa* L.) cultivars. *Pakistan Journal of Biological Sciences, 11*, *10*, 1303-1309.

Schwemmle, J. (1968). Selective fertilization in Oenothera. *Adv Genet., 14*, 225-324.

Shen, B. & Cheng, N. (1999). Character of physiological and biochemical changes during chalkiness formation in early indica rice varieties. *Acta Bot Boreali-Occidentalia Sin.*, *19*, 290-295.

Shi, D., Devineni, N., Lall, U., & Piñero, E. (2013). America's water risk: water stress and climate variability. Earth Institute, Columbia University, New York.

Siddique, K. H. M., Loss, S. P., & Thomson, B. D. (2003). Cool season grain legumes in dryland Mediterranean environments of Western Australia: Significance of early flowering, in: Saxena N.P. (Ed.), management of Agricultural Drought. Science Publishers, Enfield (NH), pp151-161.

Singh, V. P., Singh, R. K., Singh, B. B., & Zeigler, R. S. (1996). Physiology of Stress Tolerance in Rice: Proceedings of the International Conference on Stress Physiology of Rice 28 February–5 March 1994 Lucknow U. P. India, 239.

Smith, R. J. (1988). Weed Technology. 2, 232-241.

Sobarado, M. A. (1987). Leaf rolling: a visual indication of water deficit in corn (*Zea mays* L.) Maydica. 32, 9-18.

Sokoto, M. B., & Muhammad, A. (2014). Response of rice varieties to water stress in Sokoto, Sudan Savannah, Nigeria. *Journal of Biosciences & Medicines, 2*, *1*, 68-74.

Spielmeyer, W., Ellis, M. H., & Chandler, P. M. (2002). Semidwarf (sd1), ''green revolution'' rice, contains a defective gibberellin 20 oxidase gene. *Proc Natl Acad Sci USA, 99*, 9043-9048.

Steponkus, P. L., Shahan, K. W., & Cutler, J. M. (1982). Osmotic adjustment in rice. In: Drought Resistance in Crops with Emphasis on Rice. IRRI, Los Banos, Laguna, Philippines 18: 1-194.

Tanksley, S. D. (1984). Linkage relationships and chromosomal locations of enzyme-coding genes in pepper, *Capsicum annum*. *Chromosoma, 89*, 352-360.

Tester, M. & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science, 327*, 818-822.

Teulat, B., Zoumarou-Wallis, N., Rotter, B., Ben Salem, M., Bahri, H., & This, D. (2003). QTL for relative water content in field-grown barley and their stability across Mediterranean environments. *Theor Appl Genet., 108,* 181-188.

Torre, M., Rodrigues, A. R., & Saura-Calixto, F. (1991). Effects of dietary fiber and phytic acid on mineral availability. *Crit Rev Food Sci Nutr., 1,* 1-22.

Tuberosa, R., Grillo, S., & Ellis, R. P. (2003). Unravelling the genetic basis of drought tolerance in crops. *Abiotic Stresses in Plants,* 71-122.

Turner, N. C., O'Toole, J. C., Cruz, R. T., Namuco, O. S., & Ahmad, S. (1986). Response of seven diverse rice cultivars to water deficits. I. Stress development, canopy temperature, leaf rolling and growth. *Field Crops Res., 13,* 257-271.

Vaughan, D. A. (1989). The genus Oryza L. Current status of taxonomy. IRRI Research Paper. Serial Number 138.

Venuprasad, R., Cruz, M. T., Amante, M., Magbanua, R., Kumar, A., & Atlin, G. N. (2008). Response to two cycles of divergent selection for grain yield under drought stress in four rice breeding populations. *Field Crop Res., 107,* 3, 232-244.

Wang, H., Inukai, Y., & Yamauchi, A. (2006). Root development and nutrient uptake. *Critical Review of Plant Sciences, 25,* 279-301.

Xu, Y., This, D., Pausch, R., Vonhof, W., Coburn, J., & Comstock, J. (2009). Leaf-level water use efficiency determined by carbon isotope discrimination in rice seedlings: genetic variation associated with population structure and QTL mapping. *Theor Appl Genet, 118,* 1065-1081.

Xu, Y., McCouch, S. R., & Zhang, Q. (2005). How can we use genomics to improve cereals with rice as a reference genome? *Plant Mol. Biol., 59,* 7-26.

Yan, W. & Cai, G. (1991). Zhe733, a high-yielding, blast (BI)-resistant, good quality indica rice for China. *Intern Rice Res Newsl., 16,* 14.

Yan, W., Dilday, R. H., Tai, T. H., Gibbons, J. W., McNew, R. W., & Rutger, J. N. (2005). Differential response of rice germplasm to straighthead induced by arsenic. *Crop Sci., 45,* 1223-1228.

Yang, P. M., Huang, Q. C., Qin, G. Y., Zhao, S. P., & Zhou, J. G. (2014). Different drought-stress responses in photosynthesis and reactive oxygen metabolism between autotetraploid and diploid rice. *Photosynthetica, 52, 2,* 193-202.

Yu, J. & Buckler, E. S. (2006). Genetic association mapping and genome organization of maize. *Curr. Opin. Biotechnol, 17,* 155-160.

Yu, J., Pressoir, G., Briggs, W. H., Bi, I. V., Yamasaki, M., Doebley, J. F., McMullen, M. D., Gaut, B. S., Nielsen, D. M., Holland, J. B., Kresovich, S., & Buckler, E. S. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat. Genet., 38*, 203-208.

Yu, S., Liao, F., Wang, F., Wen, W., Li, J., Mei, H., & Luo, L. (2012). Identification of rice transcription factors associated with drought tolerance using the Ecotilling method. *PLoS ONE, 7*, e30765.

Zhang, J., Babu, R. C., Pantuwan, G., Kamoshita, A., Blum, A., Sarkarung, S., O'Toole, J. C., & Nguyen, H. T. (1999). Molecular dissection of drought tolerance in rice: from physio-morphological traits to field performance. In*: Genetic improvement of rice for water-limited environments* (eds) O. Ito, J. C. O'Toole and B. Hardy. International Rice Research Institute, Manila, Philippines, 331-343.

Zhang, J., Zheng, H., Aarti, A., Pantuwan, G., Nguyen, T., Tripathy, J., Sarial, A., Robin, S., Babu, R., & Nguyen, B. D. (2001). Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. *Theor. Appl. Genet., 103*, 19-29.

Zhu, C., Gore, M., Buckler, E. S., & Yu, J. (2008). Status and Prospects of Association Mapping in Plants. *The Plant Genome, 1*, 5-20. doi:10.3835/plantgenome2008.02.0089

Zhu, J. K. (2002). Salt and drought stress signal transduction in plants. *Ann. Rev. Plant Biol., 53*, 247-273.

Tables

Table 1.1. Classification of rice plant growth (Moldenhauer et al., 2012).

Growth Phase	Level of growth stages
Vegetative	S0: Dry, unimbibed seed S1: Emergence of coleoptile S2: Emergence of radicle S3: Emergence of prophyll from coleoptile V1: The first complete leaf pushes through the prophyll and forms a collar V2: The second leaf is fully emerged and progresses accordingly V3: The development of the third leaf V4: The development of the fourth leaf V5: Collar formation on leaf 5 on main stem and usually the first tiller is emerging V6: Collar formation on leaf 6 on main stem V7: Collar formation on leaf 7 on main stem V8: Collar formation on leaf 8 on main stem V9: Collar formation on leaf 9 on main stem V10: Collar formation on leaf 10 on main stem V11: Collar formation on leaf 11 on main stem V12: Collar formation on leaf 12 on main stem V13: Collar formation on leaf 13 on main stem
Reproductive	R0: Panicle development has initiated R1: Panicle branches have formed R2: Collar formation on flag leaf R3: Panicle exertion from boot, tip of panicle is above collar of flag leaf R4: One or more florets on the main stem panicle has reached anthesis R5: At least one caryopsis on the main stem panicle is elongating to the end of the hull R6: At least one caryopsis on the main stem panicle has elongated to the end of the hull R7: At least one grain on the main stem panicle has a yellow hull R8: At least one grain on the main stem has a brown hull R9: All grains which reached R6 have a brown hull

Table 1.2. List of rice diseases (Gnanamanickam, 2019).

Fungal diseases	
Blast (leaf, neck, nodal and collar)	*Magnaporthe grisea*
Sheath blight	*Rhizoctonia solani*
Sheath-rot	*Sarocladium oryzae*
Aggregate sheath	*Ceratobasidium oryzae-sativae*
Black horse riding	*Curvularia lunata*
Brown spot	*Cochliobolus miyabeanus*
Crown sheath rot	*Gaeumannomyces graminis*
Downy mildew	*Sclerophthora macrospora*
Eyespot	*Drechslera gigantea*
False smut	*Ustilaginoidea virens*
Kernel smut	*Tilletia barclayana*
Leaf smut	*Entyloma oryzae*
Leaf scald	*Microdochum oryzae*
Narrow brown leaf spot	*Cercospora oryzae*
Pecky rice (kernel spotting)	*Fusarium spp.*
Root rots	*Pythium spp.*
Seedling blight	*Curvularia spp.*
Sheath spot	*Rhizoctonia oryzae*
Alternaria leaf spot	*Alternaria padwickii*
Stem rot	*Sclerotium oryzae*
Seed-rot and seedling disease	*Phytium spinosum*
Bacterial diseases	
Bacterial blight	*Xanthomonas oryzae* pv. *oryzae*
Bacterial leaf streak	*Xanthomonas oryzae* pv. *oryzicola*
Foot rot	*Erwinia chrysanthemi*
Grain rot	*Burkholderia glumae*
Sheath brown rot	*Pseudomonas fuscovaginae*
Viral Disease	
Rice tungro	Complex virus transmitted by green leafhopper *Nephotettix spp.*

Table 1.3. Candidate genes for agronomic traits in rice (McCouch et al., 2016).

Candidate genes	Traits	Chr	Genes	Biological Pathway	Position (bp)
SD1	Plant height	1	LOC_Os01g66100	Gibberellin enzyme	38,418,739
EP3/LP	Panicle length	2	LOC_Os02g15950	F-box transcription factor; cytokinin homeostasis	9,109,565
OsMADS47	Panicle branch number	3	LOC_Os03g08754	MADS-Box transcription factor	4,468,547
OsKs1	Panicle internode length	4	LOC_Os04g52230	Gibberellin enzyme	31,029,056
CYP90D3	Panicle branch length, panicle internode length	5	LOC_Os05g11130	Brassinosteroid enzyme	6,264,833
GID1	Booting	6	LOC_Os05g33730	Soluble gibberellin receptor	19,891,242
OsGA2 oxidase-5	Shoot biomass	7	LOC_Os07g01340	Gibberellin enzyme	216,325
OsBZR1	Panicle internode length	7	LOC_Os07g39220	Transcription factor; brassinosteroid homeostasis	23,477,027
FZP	Secondary panicle branching	7	LOC_Os07g47330	AP2 domain transcription factor	28,297,303
WRKY2	Panicle branch length traits	10	LOC_Os10g42850	WRKY transcription factor	23,095,323

Figures

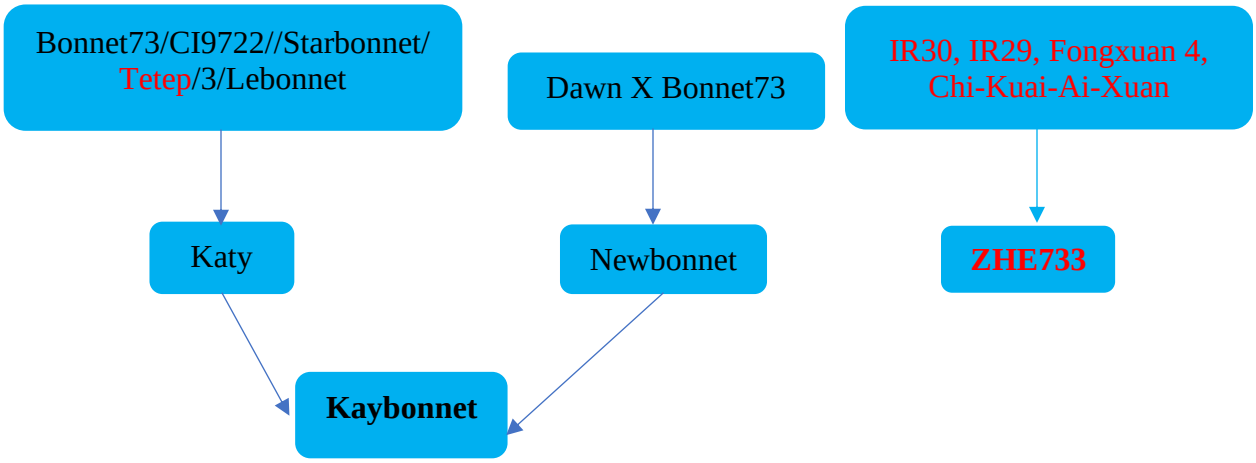

Figure 1.1. The pedigree of Kaybonnet and ZHE733 (http://www.gramene.org), Black: *japonica*, Red: *indica*.

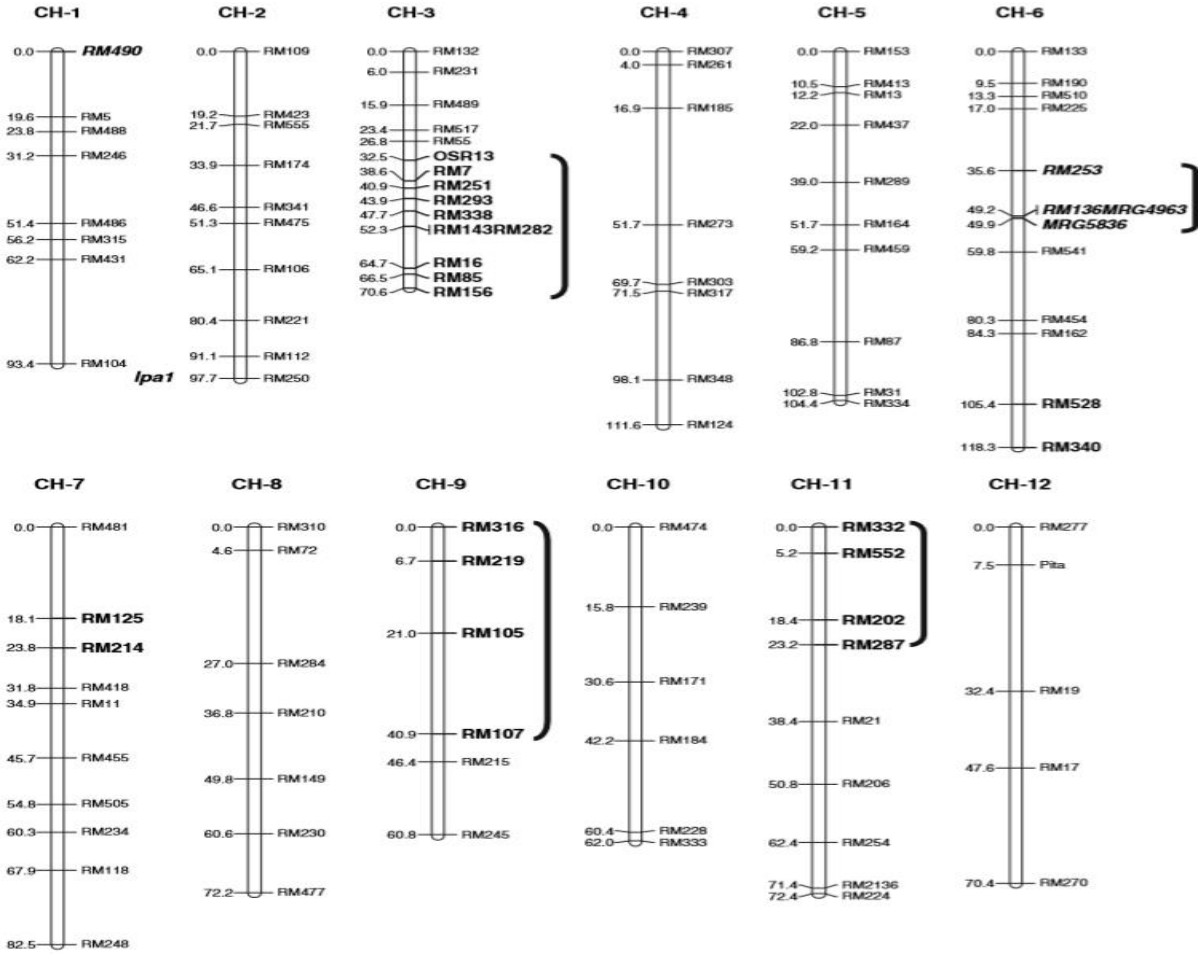

Figure 1.2. A genetic linkage map of 107 SSR markers on 12 rice chromosomes based on 269 RILs of the K/Z RIL population (Liu et al., 2005).

Figure 1.3. Clustering of 238 RILs of the K/Z RIL population using UPGMA method based on Nei's (1972) genetic distance (Liu et al., 2005).

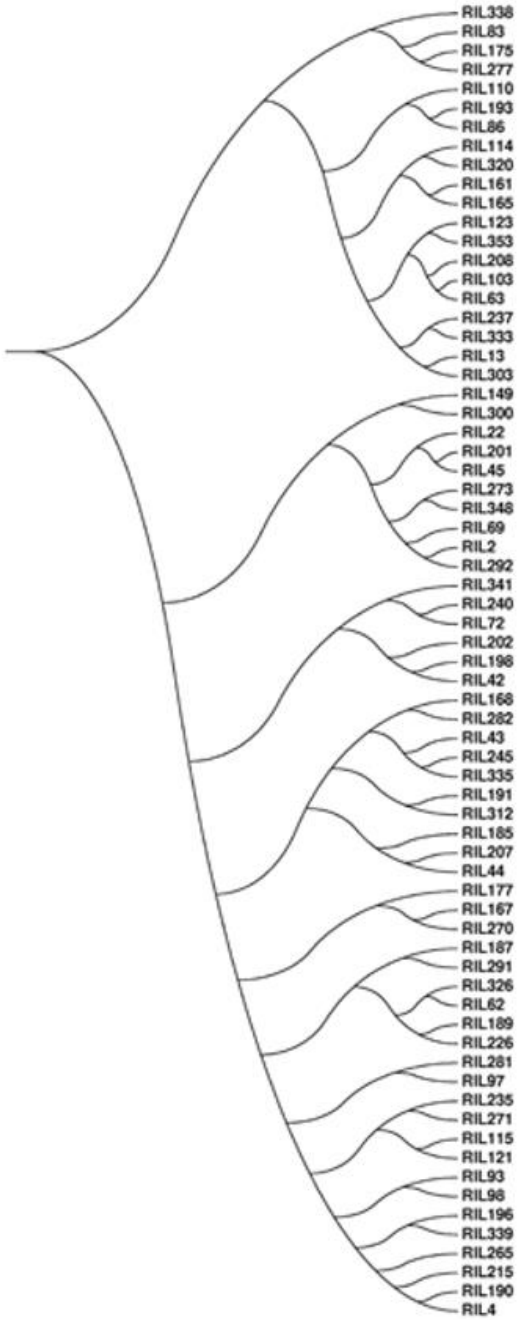

Figure 1.4. Clustering of the selected representative RILs in the K/Z RIL population using the UPGMA method (Liu et al., 2005).

CHAPTER 2. ANALYSIS OF PHENOTYPIC AND GRAIN YIELD COMPONENTS OF THE RICE KAYBONNET/ZHE733 RIL POPULATION FOR DROUGHT RESISTANCE RELATED TRAITS

Abstract

Rice (*Oryza sativa* L.) is the principal food for half of the world population with U.S. as the third largest exporter of rice. Arkansas is the largest rice-producing state in the U.S. Rice production in Arkansas is dependent on the ground water for stable irrigation. The Arkansas rice-growing region in the Lower Mississippi belt is among the 10 areas with the highest risk of water scarcity in the country. In our research, we screened adapted U.S. rice cultivars, comprising *tropical japonica* rice genotypes, for drought resistant (DR) traits to search sources for breeding U.S. rice cultivars for a water saving agricultural system. A RIL population derived from varieties Kaybonnet (DR) and ZHE733 (drought sensitive), termed K/Z RILs, were available from the USDA Dale Bumpers National Rice Research Center and chosen for genetic analysis of DR traits. The objective of this research is to analyze the phenotypic and grain yield components of the K/Z RIL rice population for drought-resistance-related traits. The RIL population was screened at Fayetteville (AR) by controlled drought stress (DS) treatment at the reproductive stage, and the effect of DS was quantified by measuring plant height, productive tiller number, leaf rolling score, flag leaf width, flag leaf length, chlorophyll content (SPAD), biological yield, spikelet per panicle number, filled grain per panicle number, panicle length, primary panicle branch number, hundred grain weight, spikelet number per plant, and filled grain number per plant. DS treatment at the reproductive stage of the K/Z RIL population revealed that water deficit negatively affects all DR traits. Based on the filled grain per panicle number, 13.13% of K/Z RIL population and parent Kaybonnet were highly drought resistant. However, 75.75% and parent ZHE733 were drought sensitive. The majority of the phenotypic and grain yield components of the K/Z RIL population showing a continuous distribution, with additive and complementary gene action underpinning the phenotypes. Information from this study will serve as a valuable resource for developing drought resistant rice varieties, and would be an

important step to improve Arkansas rice genotypes/varieties adapted for higher grain production under DS and water deficit conditions.

Introduction

Rice is the staple food for the majority of the population in the world since thousands of years. Rice cultivation in North America began in 1690 in South Carolina. Meanwhile, rice was tried initially in Arkansas in the Mississippi Delta in the 1890s with commercial production only beginning in Arkansas in the Grand Prairie around 1908 (Spicer, 1964). Right now, Arkansas is the largest rice-producing state in the U.S. because 50 percent of rice (9 billion pounds) in this country is produced by Arkansas in more than 1.6 million acres with long-grain *tropical japonica* varieties (Baldwin, 2011). Most of the rice cultivation in Arkansas is under flood irrigated conditions (USDA, 1989). Rice production in Arkansas is dependent on ground water for stable irrigation (Henry et al., 2016). Rice also uses 2-3 times the amount of water as other food crops, which totals 30% of the world's freshwater resources world-wide. The Arkansas rice-growing region in the Lower Mississippi belt is among the 10 areas with the highest risk of water scarcity in the country, as are the agricultural areas in California, Nebraska, Ohio, Dakotas, Texas, and Minnesota (Shi et al., 2013). Moreover, rice production in the U.S. is about 2% of the world rice production and accounts for 10% of the total rice export, making the U.S. as the third largest exporter of rice, mostly to Canada, Haiti, Mexico, Japan, the Middle East, Sub-Saharan Africa, and the European Union. The exported rice variety is free from genetically modified rice. The form of rice exported are rough rice, milled rice, brown rice, and parboiled rice (Arkansas Cooperative Extension Service, 1982).

Total rice production world-wide is about 600 million tons annually cultivated in 149 million ha (Bernier et al., 2007). Nevertheless, the annual improvement of the rice production has declined from 2.4% (in 1980s) to 0.9% because of the several reasons, including drought conditions (DS) (Hossain, 2007). IRRI (2007) reported that 75% of the total rice production is under irrigation systems. Meanwhile, about 45% of the global rice area is predicted to have

unstable irrigation input (Crosson, 1995). Furthermore, rice production from the rainfed lowland rice area that accounts for about 25% of the total rice area, has decreased because of insufficient and unpredictable rainfall during the growing season. Thus, rice is more sensitive to DS conditions compared to other crops. DS conditions become a critical threat to food sustainability because this abiotic stress limits the crop development and yield (Howell, 2001). Based on Qing et al. (2001), DS conditions affect morphological, physiological, and biochemical process in rice, resulting in the grain yield reduction. Jaleel et al. (2008) identified that DS conditions cause stomatal closure, leading to the limitation of gas exchange and reduces photosynthetic activity; reduction in water potential, water content, and turgor pressure; reduction in cell growth; disturbance in metabolism and the death of the plant. Moreover, Farooq et al. (2008) and Razmjoo et al. (2008) also characterized that DS conditions decrease plant growth by changing physiological and biochemical activities, such as respiration, photosynthesis, ion uptake, and nutrient absorption. Therefore, development of drought resistant rice varieties that survive and produce better yield under DS conditions is required, that could reduce water deficit through water-saving irrigation (Impa et al., 2005; Zhao et al., 2008).

Drought resistant rice varieties can be developed based on the drought resistance mechanisms found by survey of natural variants in rice germplasm. Based on analysis and description by Levitt (1980), drought resistance mechanisms are classified into three categories including a) ‘drought avoidance’ via increasing water uptake and decreasing water loss, b) ‘drought tolerance’ via osmotic regulation and antioxidant capacity, and c) ‘drought escape’ via completing the life cycle in a shorter time. A better understanding of the morphological and grain yield responses of rice under DS conditions is very useful for breeders to distinguish the genetic mechanisms of drought resistance, towards the development of drought resistant rice varieties

(Nam et al., 2001; Martinez, et al., 2007). The reactions of the rice plants to the DS conditions depend on the rice varieties, growth stages (vegetative and reproductive), intensity (mild or severe), and duration of the stress (Chaves et al., 2002), providing a multi-factorial scheme for breeding and selection.

During the reproductive stage, rice plants are more sensitive to the DS conditions with any intensity, because it effects pollination and reduces assimilate translocation to the reproductive organs that leads to flower abortion and/or finally increase in unfilled grain per panicle (Hsiao et al., 1976). Moreover, Kumar et al. (2006) and Davatgar et al. (2009) also reported that DS conditions at the reproductive stage significantly increase unfilled grains per panicle. Based on Sarkarung et al. (1995), water deficit at the reproductive period reduces the grain yield significantly by affecting panicle growth and finally decreases the filled grain number and grain weight. In a previous study, Dikshit et al. (1987) found a correlation between grain yield reduction and DS conditions with the severe intensity at the maturity stage.

Many studies have been done to identify morphological, physiological, and grain yield characteristics of rice plants under DS conditions, in order to develop drought resistant rice varieties. Manickavelu et al. (2006) reported that DS conditions significantly affect morphological, physiological, and grain yield parameters, such as relative water content, leaf rolling, leaf drying, panicle length, grains per panicle, biomass yield, harvest index, root/shoot ratio, and root length in the recombinant inbred lines (RIL) population IR58821/IR52561. Based on Swain et al. (2010), DS conditions reduce panicle number by 72% and grain yield by 12% in a study of eighteen rice genotypes. Audebert (2000) found that plant height, leaf area, biomass, productive tiller number, rooting pattern, and plant development are disturbed under DS conditions. Singh et al. (2013) also observed that DS conditions decreased plant height by

13.9%, productive tiller number by 28.6%, panicle length by 12.13%, filled grain per panicle number by 37.14%, spikelet per panicle number by 15.4%, spikelet fertility by 22.24%, grain yield per plant by 55.35%, biological yield per plant by 43.28%, and harvest index by 9.05% evaluated over six generations (P1, P2, B1, B2, F1, and F2) of six crosses of rice.

Pantuwan et al. (2000) identified an association between delay in flowering time with the reduction in grain yield, harvest index, and filled grain per panicle number by conducting four sets of experiments with different drought duration and intensity in the lowland conditions. In 1969, Kramer reported DS conditions decreased leaf area, cell size, and cell volume. Several studies found correlation between drought resistant rice varieties and osmotic adjustment, stomatal conductance, leaf rolling, leaf senescence, and early maturity (Singh, 1993); thicker and deeper roots (Yadav et al., 1997); greater root penetration (Clark et al., 2000); leaf relative water content (Courtois et al., 2000); and membrane stability (Tripathy et al., 2000). Thus, screening for morphological, physiological, biochemical, and grain yield characteristics should be applied for selecting drought resistant rice varieties.

The objective of this research is to analyse phenotypic and grain yield components of the K/Z RIL rice population for drought-resistance-related traits. Information from this study of U.S. adapted genotypes will serve as a valuable resource for developing drought resistant rice varieties.

Materials and Methods

Screening of diverse rice genotypes for grain yield components in the greenhouse and field

Plant material

A set of rice varieties Bengal (*Tropical japonica*), Kaybonnet (*Tropical japonica*), Vandana (*aus*), Nagina-22 (N22) (*Indica*), Nipponbare (NB) (*Temperate japonica*), and Aochiu (*Indica*) were obtained from the United States Department of Agriculture (USDA) Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA.

Screening of diverse rice genotypes for grain yield components in the greenhouse

Drought stress treatment at the vegetative stage

Rice seeds were germinated by imbibing with deionized water in an incubator in the dark conditions at 37^{o}C until S3 stage. The experimental design was a randomized complete block design with five replications and two treatments (well-watered (WW) and drought stress (DS) conditions). Each seedling was planted in a PVC pot of size 12.7 cm x 12.7 cm filled with a known weight of Redi-earth potting mix (Sun Gro Horticulture). DS treatment was performed at the V6 stage by withholding water from pots until the soil moisture level reduced to 50% of field capacity (FC) and maintained for continuous 10-days by weighing them daily and replenishing the water lost through evapo-transpiration (Batlang et al., 2013). For the control (WW), the soil moisture was maintained at 100% FC. The DS response was quantified by counting the filled grain per panicle number at the maturity stage. All of the rice genotypes were grown in the greenhouse at Altheimer laboratory location, University of Arkansas, Fayetteville in the growing season (May-November) in 2015. The temperature in the greenhouse was maintained between 28 to 30^{o}C during the day and 22 to 23^{o}C at night, and the light was set at a light/dark 14/10 hours cycle with the average of light intensity 580 µmol m-^{2}s-^{1} and 65% relative humidity.

Drought stress treatment at the reproductive stage

Rice seeds were germinated by imbibing with deionized water in an incubator in the dark conditions at 37^{o}C until S3 stage. The experimental design was a randomized complete block design with five replications and two treatments (WW and DS conditions). Each seedling was planted in a PVC pot of size 12.7 cm x 12.7 cm filled with a known weight of Redi-earth potting mix (Sun Gro Horticulture). DS treatment was performed at R3 stage by withholding the water until the soil moisture level reduced to 50% of FC and maintained for continuous 10-days by weighing them daily and replenishing water lost through evapo-transpiration (Batlang et al., 2013). For the control (WW), the soil moisture was maintained at 100% FC. The DS response was measured by counting the filled grain per panicle number at the maturity stage. All of the rice genotypes were grown in the greenhouse at Altheimer laboratory location, University of Arkansas, Fayetteville in the growing season (May-November) of 2015. The temperature in the greenhouse was maintained between 28 to 30^{o}C during the day and 22 to 23^{o}C at night, and the light was set at a light/dark 13/11 hours cycle with the average light intensity 580 µmol m-^{2}s-^{1} and 65% relative humidity.

Screening of diverse rice genotypes for grain yield components in the field

The set of diverse rice genotypes were evaluated in the field at Fayetteville, AR, USA. in the growing season (May-November) in 2015. The experimental design was a randomized complete block design with five replications and two treatments (WW and DS conditions) in single-row plots of 5 m length with a spacing of 0.3 m between plants. The rice plants were planted in the control plot with normal irrigation (WW conditions) and in the drought plot, where water could be drained. The rice plants in the vegetative stage were maintained with the normal irrigation for at least 30 days, and DS treatment was then initiated at the reproductive stage (R3).

DS conditions were monitored with three tensiometers that were installed at three positions in the DS plot just after draining, the first was at the beginning of the plot, the second in the middle, and the third at the end of the plot. The DS conditions was maintained continuously up to -70 kPa (severe stress). Once the soil tension reduced to -70 kPa at 30 cm soil depth, life-saving irrigation was provided thereafter through flash flooding in the DS plot and water was drained after 24h to impose the next cycle of DS till maturity. Moreover, to fertilize the field (WW and DS plots), Urea was applied in three applications at the rate of 20 g per square meter. The first application after 10 days of transplanting, the second at maximum tillering stage, and the third at panicle initiation. The weeds were controlled by manual removal. The effect of drought stress was quantified by counting the number of filled grain per panicle, spikelet per panicle, and panicle length at the maturity stage.

Phenotypic analysis of the K/Z RIL population for drought resistance related traits and grain yield components

Plant material

A RIL population derived from varieties Kaybonnet and ZHE733, termed K/Z RILs, of 198 lines available from the USDA Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA. The RIL population was originally developed to map a *low phytic acid (lpa)* mutant locus in Kaybonnet (Larson et al., 2000) out of the cross to the *indica* parent ZHE733 and use of SSR markers in the RIL population. The *japonica x indica* population is polymorphic for many markers, thus facilitating fine mapping of a variety of genes. Since the Kaybonnet and ZHE733 parents differ in grain quality under normal and high night temperature (HNT), and are polymorphic for many markers, the population is useful for genetic studies of multiple simple and complex traits.

Drought stress treatment at the reproductive stage

Seed from the K/Z RIL population of 198 lines and the two parents (Kaybonnet and ZHE733), were germinated and grown in the greenhouse under controlled conditions of 28 to 30^{o}C day and 22 to 23^{o}C night and 14h light/10h dark cycle; at average light intensity of 580 µmol $m^{-2}s^{-1}$ and 65% relative humidity, in sterilized field soil for 20 days (until V3 stage) (Figure 2.1.). From the greenhouse, uniform plants (seedling at V3 stage) were selected and transplanted to the field (Figure 2.2.), divided into 6 batches (of 7-day intervals) based on their heading day data from USDA to synchronize drought treatment at reproductive stage. The latest heading day lines were thus seeded and transplanted early, and the earliest heading day lines seeded last.

The K/Z RIL population was evaluated in the field at Fayetteville, AR, USA over three growing seasons (May-November) in 2016, 2017, and 2018. The population was grown in a randomized complete block design with five replications and two treatments (WW and DS conditions) in single-row plots of 5 m length with a spacing of 0.3 m between plants (Figure 2.3.). The rice seedlings were transplanted in the control plot with normal irrigation (WW conditions), and in the drought plot for stress treatment. At this stage, rice plants were in the vegetative stage where all plants were maintained with the normal irrigation for at least 30 days. Then, DS treatment was initiated at the R3 reproductive stage (Figure 2.4.). DS conditions were monitored with three tensiometers that were installed at three separated spots in the DS plot just after draining, the first was in the beginning of the plot, the second in the middle, and the third at the end of the plot (Figure 2.5.). The DS condition was maintained continuously up to -70 kPa (severe stress). Once the soil tension reduced to -70 kPa at 30 cm soil depth, life-saving irrigation was provided thereafter through flash flooding in the DS plot, and water was drained after 24h to impose the next cycle of DS till maturity. Moreover, to fertilize the field (WW and DS plots),

Urea was applied in three applications at the rate of 20 g per square meter. The first application was given after 10 days of transplanting, the second at maximum tillering stage, and the third at panicle initiation. The weeds were controlled by manual removal.

The phenotypic effects of stress were quantified by measuring plant height, productive tiller number, leaf rolling score, flag leaf width, flag leaf length, estimated chlorophyll content (SPAD), biological yield, spikelet per panicle number, filled grain per panicle number, panicle length, primary panicle branch number, hundred grain weight, spikelet number per plant, and filled grain number per plant with five replications (plants) per line.

Measurement of morphological and physiological traits under drought stress

Heading date or flowering time was recorded as the period from the germinating date to the time when 50% of the panicles have exerted. Plant height was measured from the ground surface to the tallest panicle tip before harvesting with a ruler. The total of the productive tiller number per plant was recorded after 10-days of DS treatment. Productive tiller number means number of tillers bearing panicle at the reproductive stage.

The leaf rolling score on the first five leaves on the tallest tiller of each plant was identified after 10 days of DS treatment based on the standard evaluation system for rice (IRRI, 2013). The range in score is from 1 to 5, 1 indicating unrolled leaves and fully turgid, 2 indicating leaves are folded (Deep-V-shaped), 3 indicating leaves are fully cupped (U-shaped), 4 indicating leaves margins touching (O-shaped), and 5 indicating completely rolled leaves. Flag leaf width was measured in the widest area of the leaf on the tallest culm of each plant by using a ruler after 10 days of the DS treatment. Flag leaf length was measured from the beginning of the ligula to the end of the tip of the leaf on the tallest tiller of each plant by using a ruler after 10 days of the DS treatment. The chlorophyll content of the fully expanded leaves on the tallest

culm of each plant in the WW and DS conditions was measured by using Soil and Plant Analyzer Development (SPAD)-502 Plus Chlorophyll Meter (Spectrum Technologies, USA). Each leaf was inserted into the sample slot of the SPAD in such a way to avoid the midrib and three reading was captured for each leaf.

Measurement of grain yield component traits under drought stress

Biological yield, the total above ground parts of the rice plant (panicles, stems, and leaves) at the maturity stage were oven-dried at 80^{o}C for 72 hours and then weighed. The spikelet per panicle number was counted manually for each panicle. Manual counting was used to determine filled grain per panicle number. Manual examination and measurement was used to determine unfilled grain per panicle number. Panicle length, the length per panicle was measured from the panicle neck to the panicle tip. Primary panicle branch number was determined by counting the branches that directly come out from the peduncle. The hundred grain weight was calculated based on the weight of hundred filled grain of each plant on a 14% grain moisture content. Spikelet number per plant, the total number of spikelets per plant was counted manually. The total number of filled grain per plant was counted manually.

Statistical analysis

This experiment was conducted in a randomized complete block design with five replications and two treatments (WW and DS). Blocks represent a random effect and treatments (WW and DS) represent a fixed effect. The data from three years growing seasons (2016, 2017, and 2018) of K/Z RIL population and 2 parents both under WW and DS conditions for morphological traits and grain yield components were analysed by analysis of variance (ANOVA) using JMP version 12.0. The Tukey's HSD was performed to compare the means of the two treatments (WW and DS) among all of the rice lines in the K/Z RIL population for

significant effects (Tukey's HSD, $P < 0.05$) using JMP version 12. Moreover, the correlation analysis was achieved by using JMP version 12.0 to correlate morphological traits and grain yield components under WW and DS conditions of the K/Z RIL population. Shapiro-Wilk test was used to test a normal distribution for each trait by using SAS 9.4.

Results and Discussion

The type of rice generally grown in the U.S. is *tropical japonica* (Mae, 1997). Furthermore, Kaybonnet (Arkansas) and Bengal (Louisiana) are adapted varieties in the Southern U.S. and belong to *tropical japonica* background. In the DS conditions at the reproductive stage (R3), they exhibited the highest filled grain per panicle number and the least reduction in biomass (Figure 2.6.), comparable to the internationally recognized varieties Nagina-22 (N22) and Vandana. Bengal, Kaybonnet, Vandana, and N22 are drought resistance because they have less reduction of filled grain per panicle number under DS conditions whether in the vegetative or reproductive stage, while Nipponbare (NB) and Aochiu are drought sensitive (Figure 2.6). Dingkuhn et al. (1989) reported that in general *tropical japonica* performed the best with highest water use efficiency (WUE), *indica* showed medium, and *aus* has the lowest level of WUE. For further studies on drought resistance, we chose the adapted Arkansas variety Kaybonnet which has a background of introgression from *indica/aus* varieties into *japonica* (Figure 2.7.). In addition, Kaybonnet showed more drought resistance than the well-known drought resistant varieties, Vandana and N22. We received a K/Z RIL population from USDA Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA, which was selfed and completely inbred for F10-11 generations, derived from Kaybonnet and ZHE733 as the parents. This RIL population has been previously studied for biotic stress. Based on the filled grain per panicle number, Kaybonnet and ZHE733 showed contrasting characteristics under DS conditions (Figure 2.8. and 2.9.). Therefore, this K/Z RIL population was chosen as an excellent segregating population for molecular genetic studies on abiotic stress resistance.

Screening and identification of K/Z RIL population for morphological traits under DS

Morphological traits such as plant height, productive tiller number, leaf rolling score, flag leaf width, flag leaf length, and chlorophyll content (SPAD) exhibited significant responses to the DS conditions at the reproductive stage in the K/Z RIL population.

Heading date is one of the most important agronomic traits which accompanies the transition from vegetative to reproductive stage and contributes to grain yield production and the commercial potential of rice (Weng et al., 2014; Liang et al., 2013). Furthermore, Jing (2018) reported that the heading date is controlled by pleiotropic genes. This study also showed that the heading date in the K/Z RIL population has a continuous distribution, indicating that multiple genes control this trait. The range of the heading days in the K/Z RIL population is from 65 days to 110 days, with most lines having 85-90 days to heading/flowering (Figure 2.10.). According to Jung and Müller (2009), heading date is regulated by multiple factors such as transcription factors, phytohormones, enzymes, photoreceptors, and environmental conditions (day length and temperature). Moreover, DS conditions during vegetative stage might delay the heading date. A number of studies indicated that heading date has a positive correlation with the grain yield (Xiao et al., 1996; Yue et al., 1997; Lu et al., 1997; Xing et al., 2002; Guo et al., 2003; Mei et al., 2005; Xue et al., 2008; Zhang et al., 2008; Cao et al., 2010; Wei et al., 2010; Yan et al., 2011; Liang et al., 2013). This study also showed that the heading date has a positive correlation with productive tiller number, flag leaf length, chlorophyll content, leaf rolling score, primary panicle branch number, spikelet per panicle number, filled grain per panicle number, unfilled grain per panicle number, spikelet number per plant, and filled grain number plant. On the other hand, heading date exhibited negative correlation with plant height, flag leaf width, biological yield, panicle length, and hundred grain weight (Figure 2.26).

Drought response of K/Z RIL population for plant height and productive tiller number

Plant height and productive tiller number determine grain yield in the rice plants (Moldenhauer and Nathan, 2004; Sakamoto and Matsuoko, 2008; Huang et al., 2013). Plant height is one of the important factors that determines plant architecture and contributes to grain production (Weng et al., 2014; Venuprasad et al., 2009). The dwarf plants maintain high grain yield due to less lodging, but if the plants are too short or too tall, it will affect the grain production because of the insufficient growth. According to Setter et al. (1997), an overly tall plant will accommodate many leaves and increase photosynthesis activity, but it can also cause lodging. In contrast, excessive short plant height will reduce the light intensity available, cause crowded leaves, and lead to decreased photosynthesis activity (Peng et al., 1994). Therefore, it is important to select for the appropriate plant height in rice breeding. The number or length of the internode cells are related to plant height. Cell elongation is correlated to the cell wall plasticity that is influenced by environmental conditions and plant hormones. The cell wall plasticity is regulated by transcription factors such as the MYB family genes (Feller et al., 2011). According to Swain and Singh (2005), dwarf or semi-dwarf plants that reduced their endogeneous gibberellin levels that regulates cell elongation and controls plant height. Plant height in the K/Z RIL population showed continuous distribution (Figure 2.11.), indicating that this trait is quantitative with a range of 26-98 cm in WW, and 10-74 cm in DS conditions (Table 2.1.). DS conditions during the reproductive stage reduced the plant height 51% in K/Z RIL population. According to Zhang et al. (2018), DS conditions decreased plant height 20% during reproductive stage and 3.2% during vegetative stage. In another study on rice, Ahmadikhah & Marufinia (2016) reported that severe drought stress conditions reduced plant height 8 cm. Furthermore, Guan et al. (2010) stated that plant height was reduced 17% , 25%, and 33 % under mild,

moderate, and severe DS conditions, respectively. Zhen et al. (2019) also found that plant height under severe drought decreased 14.8 cm and in mild drought decreased 13.2 cm. Many studies also indicated that DS conditions significantly reduced the plant height (Sarvestani et al., 2008; Ashfaq et al., 2012; Bunnag and Pongthai, 2013; Sokoto and Muhammad, 2014). With increasing plant age, plant height increase quadratically with maximum height in the K/Z RIL population 98 cm in WW and 74 cm in DS conditions.

At the reproductive stage, plant height is affected by DS conditions. Most of the lines (56.60%) in the K/Z RIL population are drought sensitive with more than 50% reduction of the plant height under DS conditions. Meanwhile, 23.70% lines belong to the moderately drought resistant class with the reduction of 30-49% and 19.70% lines are highly drought resistance with 0-29% reduction in plant height (Table 2.2.). This criterion was used for each trait and follows classification of De Freitas et al. (2016). According to the previous studies (Zhuang et al., 1997; Babu et al., 2003; Lanceras et al., 2004; Vikram et al., 2011), plant height is a trait influenced by the environment, DS conditions limits plant height development, as a result affecting yield. Moreover, plant height of the K/Z RIL population is positively correlated with productivity traits such as estimated chlorophyll content, panicle length, primary panicle branch number, spikelet per panicle number, filled grain per panicle number, hundred grain weight, spikelet number per plant, and filled grain number per plant. However, plant height of the K/Z RIL population is negatively correlated with flag leaf width, flag leaf length, leaf rolling score, and biological yield (Figure 2.26.). Therefore, maintaining an appropriate plant height is important to produce optimum grain yield under WW and DS conditions.

‘Productive tiller’ number or tillers with a grain bearing panicle in rice plants plays an important role in plant productivity, which primarily determining the panicle number per plant

(Li et al., 2003). The higher number of productive tillers in rice plants are considered to contribute to higher grain yield. Productive tiller number in the K/Z RIL population exhibited a normal distribution (Figure 2.12.) indicating that productive tiller number is polygenic in nature with the range 2-22 in WW and 2-20.70 in DS conditions (Table 2.1.). With the advancement of plant age, the productive tiller number increases quadratically, usually the maximum tiller number is attained at 80 days after germination (Murayama, 1995). The maximum productive tiller number in K/Z RIL population is 22 in WW and 20.70 in DS conditions, reduced 13% in the DS. Furthermore, previous studies have also reported that productive tiller number was reduced in the DS conditions at the reproductive stage due to nutrient deficiencies and high competition for assimilates distribution between young tillers and developing panicles, and consequently the young tillers will die (Black and Siddoway, 1977; Power and Alessi, 1978; Masle, 1985). Several other studies also reported that DS conditions reduced productive tiller number until 2 tillers (Mostajeran and Rahimi-Eichi, 2009; Ashfaq et al., 2012; Bunnag and Pongthai, 2013; Ahmadikhah and Marufinia, 2016).

Based on the data analysis of productive tiller number from DS screening of the K/Z RIL population, 72.20% lines were identified as highly drought resistance (0-29% reduction), 16.60% lines classified as moderately drought resistance (30-49% reduction), and 11.10% lines as drought sensitive with more than 50% reduction (Table 2.2.). The rice RILs exhibited differential responses to DS conditions due to the complexity of interactions between molecular, biochemical, and physiological processes that affect plant growth (Wadhwa et al., 2010). The productive tiller number trait is generally positively correlated with flag leaf width, biological yield, primary panicle branch number, spikelet per panicle number, filled grain per panicle number, unfilled grain per panicle number, spikelet number per plant, and filled grain number

per plant. However, the productive tiller number of the K/Z RIL population is negatively correlated with plant height, flag leaf length, estimated chlorophyll content, leaf rolling score, panicle length, and hundred grain weight (Figure 2.26.). Spielmeyer et al. (2002) reported a reduction in plant height under DS conditions followed by increase in number of tillers. Xing and Zhang (2010) also reported that productive tiller number was negatively correlated with plant height. Several studies also suggest that increasing productive tiller number will increase the panicle number per plant, spikelet per panicle number, and also plant biomass (Hanada, 1995; Hayashi, 1995; Murata and Matsushima, 1975; Yoshida, 1981; Jennings et al., 1979; Deng et al., 2015). Previous studies also confirmed that under DS conditions, productive tiller number decreased, while leaf rolling increased (Mukamuhirwa et al., 2019).

Drought response of K/Z RIL population for leaf rolling score, flag leaf width, flag leaf length, and estimation of chlorophyll content (SPAD)

Leaf morphology contributes to the grain yield due to its relationship to the cell number, chlorophyll content, and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) per unit area that influences the photosynthetic rate (Zhen et al., 2019). In the rice plant, flag leaf is an important photosynthetic organ and influencing the grain yield because this leaf is the main source of the carbohydrates resulting from photosynthetic activity which gets accumulated in the grains (Ghosh et al., 1990; Jebbouj and Yousfi, 2009; Li et al., 1998; Monyo, et al., 1973). Previous studies have reported that more than 50% of the carbohydrate in the rice grains are produced by the flag leaf (Tomoshiro et al., 1983; Gladun et al., 1993). In addition, the flag leaf plays an important role in the grain filling process and photo-assimilates transportation. Previous studies have reported that flag leaf traits such as flag leaf area, chlorophyll content, and flag leaf dry weight showed positive correlation with the grain yield under DS conditions (Biswal and

Kohli, 2013; Yue et al., 2006; Li et al., 1998). Therefore, various flag leaf parameters have been used for selecting drought resistant rice.

Leaf rolling is an important adaptive response to DS conditions as a symptom of moisture stress and visual evaluation for scoring drought resistance of rice plants (Kadioglu and Terzi, 2007). When plants experience DS conditions, the leaves start rolling to maintain internal water status and metabolic activities thereby preventing water loss via transpiration, and shows the drought escape mechanism of the rice plants. Moreover, leaf rolling is induced by the decrease in turgor and lack of osmotic adjustment (Hsiao et al., 1984). Thus, delayed leaf rolling has been investigated as a favorable character in rice. In the screen of the K/Z RIL population under DS conditions, the leaf rolling score showed normal distribution within the range of 1-4 with most lines having a score around 2.85 (Figure 2.13.). The lowest leaf rolling score (1) exhibited drought resistance and the highest leaf rolling score (5) showed drought sensitivity. Subashri et al. (2009) and Salunke et al. (2011), reported that variation in leaf rolling score among rice lines is a genetic trait. According to Cal et al. (2018), leaf rolling score under DS conditions is more affected by leaf morphology, than by leaf water status and stomatal conductance. Henson (1982) characterized that stomata are closed before leaf rolling occurred. Moreover, Pandey and Shukla (2015) indicated that drought resistant rice lines have the leaf rolling character to limit transpiration and also have the ability to recover faster; although, some rice genotypes can continue transpiration while rolled.

In the correlation analysis of K/Z RIL population, the leaf rolling score is positively correlated with heading date, plant height, flag leaf width, flag leaf length, estimated chlorophyll content, panicle length, primary panicle branch number, spikelet per panicle number, filled grain per panicle number, hundred grain weight, and filled grain number per plant. On the other hand,

leaf rolling score of K/Z RIL population showed negative correlation with productive tiller number, biological yield, unfilled grain per panicle number, and spikelet number per plant (Figure 2.26.). A number of studies have characterized that leaf rolling score was not well correlated with the grain yield per plant and biological yield under DS conditions (Lafitte et al., 2003; Turner et al., 1986). According to Fen et al. (2015), leaf rolling score also has negative correlation with chlorophyll content and productive tiller number.

Flag leaf width has a strong correlation with grain yield, and is known to be related to panicle length and spikelet per panicle number, because flag leaf width affects photosynthetic activity in the rice plants (Zhang et al., 2015; Dingkuhn et al., 2015). Wider flag leaves may enhance photosynthetic area, so that the source supply is improved, and as a result grain yield increases. Flag leaf width of the K/Z RIL population showed high variation ranging from 0.8 cm to 2.1 cm (Figure 2.14.) with the average 1.35 cm. The variation of the flag leaf width are correlated with longitudinal cell division, cell elongation, cell arrangement, and changes in vascular bundles which are influenced by genetic and environmental factors (He et al., 2018). Under DS conditions, flag leaf width is reduced due to insufficient water and nutrients. Based on Cho et al. (2013), narrow flag leaves are caused by reduction of lateral-axis cells and fewer longitudinal veins. In the K/Z RIL population, this flag leaf parameter showed a positive correlation with leaf rolling score, biological yield, spikelet per panicle number, filled grain per panicle number, hundred grain weight, productive tiller number, and spikelet number per plant. Meanwhile, the flag leaf width in this RIL population exhibits a negative correlation with heading date, plant height, flag leaf length, estimated chlorophyll content, panicle length, primary panicle branch number, unfilled grain per panicle, and filled grain number per plant (Figure 2.26.). However, previous studies have confirmed that flag leaf width showed significant

positive correlations with grain yield per plant, panicle number, and spikelet per panicle number (Zhang et al., 2015; Zhou et al., 2012).

Flag leaf length is also an important parameter that controls rice grain yield. The length of the flag leaf correlated with cell division of the mesophyll cells specially the longitudinal cells (Yuan, 1997). In the K/Z RIL population, flag leaf length exhibited a normal distribution ranging from 14.17 cm to 54 cm, and most lines have a flag leaf length of 30.47 cm (Figure 2.15.). In the screen of the K/Z RIL population under DS condititons, flag leaf length shows a positive correlation with heading date, productive tiller number, plant height, estimated chlorophyll content, leaf rolling score, spikelet per panicle number, filled grain per panicle number, hundred grain weight, spikelet number per plant, and filled grain number per plant (Figure 2.26.). Nevertheless, the flag leaf length in the K/Z RIL population showed negative correlations with flag leaf width, biological yield, panicle length, primary panicle branch number, and unfilled grain per panicle (Figure 2.26.). Vangahun (2012) reported that plants with a long flag leaf usually have a wide leaf angle because of leaf drooping and have a reduced light capture, which consequently decreased photosynthetic activity and finally reduced grain yield. Thus, short flag leaves are more favorable than longer ones.

Besides flag leaf criteria that determines the rice plant productivity, chlorophyll content also has a significant correlation with the grain yield. Chlorophyll is a pigment contributing to photosynthetic activity and plays an important role in carbohydrate supply to the rice grain. Therefore, high chlorophyll content in the leaf is a desired characteristic. A number of studies have characterized that DS conditions reduced chlorophyll content in the rice plant due to drought stress changing the metabolic functions (Sairam et al., 1996; Ranjbarfordoei et al., 2000; Pirdashti et al., 2009; Cha-um et al., 2010; Sikuku et al., 2012; Ha, 2014; Maisura et al., 2014).

Under DS conditions, rice plants produce reactive oxygen species (ROS), such as O_2^- and H_2O_2 that lead to lipid peroxidation, impairing the chlorophyll biosynthetic pathway, with destruction of the chloroplast membrane, and as a result, chlorophyll damage (Mirnoff, 1993; Foyer et al., 1994; Hirt and Shinozaki, 2004). Destruction of the chlorophyll causes a change in green color of the leaf into yellow, decreasing light harvesting and induces decline in energy for dark reactions of photosynthesis and consequently reducing grain yield and plant biomass (Jaleel et al., 2009). Thus, chlorophyll stability is a favorable parameter for screening of drought resistant rice varieties.

In this study, the K/Z RIL population displayed a continuous distribution in the chlorophyll content with the range from 22.5 to 59.4 with the average 39.7 (Figure 2.16.). Zhen et al. (2019) also demonstrated that chlorophyll content in various rice genotypes varied. Moreover, chlorophyll content in this rice population exhibited a positive correlation with heading date, plant height, flag leaf length, leaf rolling score, primary panicle branch number, spikelet per panicle number, filled grain per panicle number, and filled grain number per plant (Figure 2.26.). Several other studies have also shown that chlorophyll content showed significant positive correlation with the grain yield, due to maintaining higher chlorophyll content under DS conditions, that were associated with transpiration efficiency under DS conditions in rice, sorghum, wheat, maize, and barley (Benbella and Paulsen, 1998; Borrell et al., 2000; Haussmann et al., 2002; Verma et al., 2004; Cha-um et al., 2010). On the other hand, chlorophyll content of the K/Z RIL population showed a negative correlation with flag leaf width, productive tiller number, biological yield, panicle length, unfilled grain number, hundred grain weight, and spikelet number per plant (Figure 2.26.).

Screening and identification of K/Z RIL population for grain yield components under DS

Rice grain yield is a quantitative trait that is affected by genetics and environmental factors (Wang et al., 2012). Many studies indicate that grain yield in rice is affected by several traits such as plant height, productive tiller number, growth period, panicle length, primary panicle branch number, spikelet per panicle number, filled grain per panicle, and hundred grain weight (Moldenhauer and Nathan, 2004; Sakamoto and Matsuoka, 2008; Huang et al., 2013). DS conditions generate numerous responses in the rice plants such as morphological, molecular, biochemical, and physiological changes which significantly affect grain yield. Drought decreased assimilate translocation, while increasing spikelet sterility; and also reducing grain filling rate, grain size, and weight.

The biological yield of the K/Z RIL population showed a continuous distribution (Figure 2.17.) ranging from 3.5 to 49 g under WW conditions, while under DS conditions showed a range from 1.7 to 47 g (Table 2.1.). Under DS conditions, 67.70% of the K/Z RIL population lines were highly drought resistance, 24.70% lines showed moderate drought resistance, and 7.60% showed drought sensitivity (Table 2.2.). According to Pandey et al. (2015), the important component responsible for biological yield was reduction in grain yield, followed by plant height, productive tiller number, and leaf morphology reduction under drought. The highly drought resistant lines showed longer panicle length, higher primary panicle branch number, higher spikelet per panicle number, higher filled grain per panicle number, and higher productive tiller number. Furthermore, a number of previous studies indicated that under drought, the fresh and dry weight of shoots and roots were reduced (Centritto et al., 2009; Mostajeran and Rahimi-Eichi, 2009; Ji et al., 2012), leading from reduction in photosynthetic rate and that of biochemical processes (Usman et al., 2013). Farooq et al. (2009) characterized that the reduction

of the biological yield under DS conditions depends on the severity of the drought. Under mild, moderate, and severe drought, the reduction of the biological yield was 12.5%, 18.0%, and 35.5%, respectively. Biological yield is a primary trait contributing to plant productivity, and the biological yield of the K/Z RIL population exhibited a significant positive correlation with productive tiller number, flag leaf width, primary panicle branch number, and unfilled grain per panicle (Figure 2.26.). Meanwhile, a clear negative relationship has been observed between biological yield of the K/Z RIL population with heading date, plant height, flag leaf length, estimated chlorophyll content, leaf rolling score, panicle length, spikelet number per panicle, filled grain number per panicle, hundred grain weight, spikelet number per plant, and filled grain number per plant (Figure 2.26.). Rice genotypes containing higher biological yield are crucial to meet the increasing demands of food, fodder, and bio-fuel. Therefore, the use of natural genetic variation among the rice lines of the K/Z RIL population is important for screening and identification of high biological yield in the rice plants.

In this study, spikelet per panicle number of K/Z RIL population displayed a normal distribution (Figure 2.18.) with a range of 25-328 under WW conditions and 18-239 under DS conditions (Table 2.1.). Based on the screening under DS conditions, the K/Z RIL population showed different responses to drought. Most of the lines exhibited high drought resistance (39.90%) followed by moderate drought resistance (35.30%) and drought sensitive response (24.70%) (Table 2.2.). Ahmadikhah and Marufinia (2016) indicated that under severe drought, the spikelet per panicle number reduced to 18 spikelets. A positive correlation was found between ‘spikelet per panicle number’ of the K/Z RIL population with heading date, productive tiller number, plant height, flag leaf width, flag leaf length, estimated chlorophyll content, leaf rolling score, panicle length, primary panicle branch number, filled grain per panicle number,

unfilled grain per panicle number, spikelet number per plant, and filled grain number per plant. On the other hand, spikelet per panicle number of the K/Z RIL population has a negative correlation with biological yield and hundred grain weight (Figure 2.26.). Several other studies also found that under DS conditions at the reproductive stage, grain yield per plant exhibited a positive correlation with spikelet fertility (Raman et al., 2012; Kumar et al., 2014). In addition, Konate et al. (2016) indicated that spikelet per panicle number also determines grain yield besides productive tiller number, panicle length, and hundred grain weight. The pollination process in rice plants is very sensitive to DS conditions. Consequently, DS conditions during flowering time induce spikelet sterility due to drought, leading to pollen sterility and zygotic abortion, and slow grain filling resulting in the reduction of grain yield.

Filled grain per panicle number is one of the yield components that affect the productivity of rice plants. A continuous distribution exhibited in the filled grain per panicle number of the K/Z RIL population under WW and DS conditions (Figure 2.19.). The range in the filled grain per panicle number is 2-179 under WW conditions and 0-97 under DS conditions (Table 2.1.). The difference between the filled grain per panicle number of different lines is caused by the genetic variation between lines and the response of RILs to environmental factors (Liu et al., 2010). Based on the filled grain per panicle number in the DS conditions, most of the lines showed sensitivity to drought (75.75%), followed by highly drought resistant lines (13.13%), and moderately drought resistant lines (11.11%) (Table 2.2.). DS at the reproductive stage causes slow grain filling and shortening of the grain filling period due to drought-interrupted phloem loading and assimilate translocation (Farooq et al., 2009; Shahryari et al., 2008; Kamoshita et al., 2004; Botwright Acuña et al., 2008). Based on the correlation analysis, filled grain per panicle number of the K/Z RIL population showed a positive relationship with heading date, productive

tiller number, plant height, flag leaf width, flag leaf length, estimated chlorophyll content, leaf rolling score, panicle length, primary panicle branch number, spikelet number per panicle, hundred grain weight, spikelet number per plant, and filled grain number per plant. Nevertheless, filled grain per panicle number showed a negative correlation with biological yield and unfilled grain per panicle number (Figure 2.26.).

Under WW and DS conditions, panicle length of the K/Z RIL population has a normal distribution (Figure 2.20.), ranging from 10.61 to 33.30 cm under WW conditions and 9.61 to 27.51 cm under DS conditions (Table 2.1.). Based on the level of panicle length reduction under drought, 84.30% lines of the K/Z RIL population showed highly drought resistant phenotype, 13.10% moderately drought resistance, and 2.50% drought sensitive phenotype (Table 2.2.). Cha-um et al. (2010) also identified that panicle length was significantly reduced under DS conditions. The panicle length of the K/Z RIL population exhibited a positive correlation with the other traits such as plant height, flag leaf width, leaf rolling score, primary panicle branch number, spikelet per panicle number, filled grain per panicle number, unfilled grain per panicle number, hundred grain weight, spikelet number per plant, and filled grain number per plant. Furthermore, the panicle length of the K/Z RIL population showed a negative correlation with heading date, productive tiller number, flag leaf length, estimated chlorophyll content, and biological yield (Figure 2.26.). According to Kumar et al. (2014), panicle length is an important component that affects grain yield per plant by determining panicle architecture like spikelet per panicle number, and also panicle length shows higher heritability than grain yield. Various studies have shown that the rice plants with longer panicle length potentially have higher spikelet per panicle number (IRRI, 1994; Farooq et al., 2009; Shahryari et al., 2008; Kamoshita et al., 2004). Liu et al. (2010) reported that panicles use assimilates for their growth and development.

The rice subspecies have differences in panicle length, for example, *aromatic* has the longest panicle (30 cm) followed by *aus* and *indica*, then *temperate japonica* which has the shortest panicle length (21 cm), while *tropical japonica* shows the largest range for panicle length. Thus, panicle length can be used as a selection criterion in a rice breeding program.

Another grain yield component of the K/Z RIL population, primary panicle branch number also showed a continuous distribution (Figure 2.21.) with a range of 2-23 under WW conditions and 2-17 under DS conditions (Table 2.1.). Based on the primary panicle branch number responses to drought, most of the lines exhibited highly drought resistance (72.70%) followed by moderately drought resistance (20.20%), and drought sensitive (7.10%) (Table 2.2.). The primary panicle branch number of the K/Z RIL population has a strong correlation with heading date, productive tiller number, plant height, flag leaf width, estimated chlorophyll content, leaf rolling score, biological yield, panicle length, spikelet per panicle number, filled grain per panicle number, unfilled grain per panicle number, hundred grain weight, spikelet number per plant, and filled grain number per plant. However, primary panicle branch number showed a negative correlation with flag leaf length (Figure 2.26.), and is also an important factor determining grain yield, with the higher primary panicle branch number showing higher grain yield (Sakamoto and Matsuoka, 2008; Kovi et al., 2011). Therefore, the higher primary panicle branch number is a favorable trait in the rice breeding program.

In the K/Z RIL population, hundred grain weight also exhibited a normal distribution under WW and DS conditions (Figure 2.22.), with a range between 0.01 to 3.33 g under WW conditions and from 0.01 to 3.21 g under DS conditions (Table 2.1.). Variation of the grain weight is mainly controlled by genetic factors. Based on the hundred grain weight reduction under drought, the K/Z RIL population could be classified into highly drought resistance,

moderately drought resistance, and sensitive to drought with corresponding reduction of 54.04%, 11.11%, and 34.84%, respectively (Table 2.2.). Several other studies also suggest that reduction in the grain size commonly occurred under DS conditions (Castillo et al., 2006; Venuprasad et al., 2007; Mostajeran and Rahimi-Eichi, 2009; Ji et al., 2012). Moreover, Kamoshita et al. (2004) confirmed that DS conditions impaired floret initiation, inducing spikelet sterility, slowed down the grain filling process, interrupted leaf gas exchange components, decreasing the size of the source and sink tissues, disrupting assimilate distribution and consequently causing reduction in the grain weight. Hundred grain weight showed a positive correlation with plant height, flag leaf width, flag leaf length, leaf rolling score, panicle length, primary panicle branch number, and filled grain per panicle number. On the other hand, hundred grain weight showed a negative correlation with heading date, productive tiller number, estimated chlorophyll content, biological yield, spikelet per panicle number, unfilled grain per panicle number, spikelet number per plant, and filled grain number per plant (Figure 2.26.).

A continuous distribution is also observed in the spikelet number per plant under WW and DS conditions (Figure 2.23.), ranging from 225 to 5390 under WW and 71.92 to 3321.20 under DS conditions (Table 2.1.). Most lines in the K/Z RIL population showed a response phenotype of drought sensitive (43.40%) followed by moderately drought resistance (28.80%), and highly drought resistance (27.80%) (Table 2.2.). A number of previous studies confirmed that rice grain yield decreased under DS conditions (Bouman et al., 2005; Centritto et al., 2009; Pirdashti et al., 2009; Venuprasad et al., 2011; Ahadiyat et al., 2014; Maisura et al., 2014). The primary component contributing the most to grain yield reduction under DS conditions at the reproductive stage are correlated with pollen sterility and leaf morphology variation (Pandey el at., 2015). Spikelet number per plant of the K/Z RIL population exhibited a positive correlation

with the morphological and yield traits including heading date, productive tiller number, flag leaf width, flag leaf length, panicle length, primary panicle branch number, spikelet per panicle number, filled grain per panicle number, unfilled grain per panicle number, and filled grain number per plant. Based on this correlation analysis, the rice lines that have earlier heading date showed higher grain yield which indicates that lines with a longer life span are correlated with the higher yield due to higher assimilates accumulation. A negative correlation was also found between spikelet number per plant with plant height, estimated chlorophyll content, leaf rolling score, biological yield, and hundred grain weight (Figure 2.26.).

Frequency distribution of the filled grain number per plant also exhibited a normal distribution (Figure 2.24.) with the range from 15 to 1957 under WW and from 0 to 1231 under DS conditions. According to the reduction of the filled grain number per plant under DS conditions, most of the lines in the K/Z RIL population could be classified as drought sensitive (73.30%), followed by highly drought resistance (14.20%), and moderately drought resistance (12.50%) (Table 2.2.). A previous study reported that several rice varieties showed a decrease in grain yield up to 81% under DS conditions, and this reduction was influenced by duration and severity of the drought (Pantuwan et al., 2000). Filled grain number per plant shows a positive association with heading date, productive tiller number, plant height, flag leaf length, estimated chlorophyll content, leaf rolling score, panicle length, primary panicle branch number, spikelet per panicle number, filled grain per panicle number, hundred grain weight, and spikelet number per plant. Filled grain number per plant also showed a negative correlation with flag leaf width, biological yield, and unfilled grain per panicle number (Figure 2.26.).

The reduction in the grain yield under DS conditions due to drought was caused by a decline in photosynthetic rate, interruption in stomatal conductance, reduced chlorophyll content,

decreased leaf size, impaired stem elongation, disturbed activities of sucrose and starch synthesis enzymes, reduced assimilate distribution, all resulting in the reduction of plant productivity (Anjum et al., 2011). Furthermore, this grain yield reduction also depends on the severity and the duration of drought stress (Kumar et al., 2014). For example, DS conditions at the vegetative stage declined the grain yield by 21-50.6%, while severe drought at the flowering stage declined the grain yield by 42-83.7%, and moderate to severe drought during the whole reproductive stage declined grain yield by 51-90.6%. Many studies indicated that rice plants are more sensitive to DS conditions at the reproductive stage than during the vegetative stage, since rice plants hardly recovered from the damage caused by reproductive stage drought (Dixit et al., 2014; Guan et al., 2010; Swamy et al., 2017). During the vegetative stage, drought induced stomatal closure that consequently impaired carbohydrate synthesis for cell division of tillers, panicles, and grains. These damages were however considered reparable (Anjum et al., 2011). Furthermore, DS at the reproductive stage causes flowering abortion and finally reduction in the panicle length, primary panicle branch number, spikelet per panicle number, and filled grain per panicle number (Hsiao et al., 1976).

'Heritability' has a predictive function in plant breeding since it measures the phenotypic variance that is associated with the genotype (Songsri et al., 2008). Furthermore, heritability facilitates the selection of genotypes based on their phenotypic performance (Bitew, 2016). Heritability was categorized as low (<50%), medium (>50 – 60%), and high (>60%) (Babu et al., 2012; Ashok et al., 2013). Broad sense heritability of the K/Z RIL population ranged from 77% (panicle length in the DS condition) to 91% (plant height in the WW condition). High heritability (> 60%) was found for all of the phenotypic and grain yield related traits in the WW and DS conditions (Table 2.1.). This high heritability suggests that additive gene action had a primary

role. Moreover, heritability of the traits is essential in determining the response to selection. The results indicated that plant height, productive tiller number, biological yield, spikelet per panicle number, filled grain per panicle number, panicle length, primary panicle branch number, hundred grain weight, spikelet number per plant, and filled grain number per plant exhibited high heritability, and could be considered as the most applicable traits for selection and improvement of the traits to obtain stable and higher yield under DS condition. Several studies also exhibited that grain yield under DS conditions had moderate to high heritability (Kumar et al., 2008; Bernier et al., 2007; Venuprasad et al., 2007). Heritability of the most traits showed no significant different in WW and DS conditions. According to Bernier et al. (2007), this demonstrates that the drought screening methodology was highly repeatable.

Conclusions

The K/Z RIL population was screened and used for the identification of natural variation for phenotypic and grain yield components under drought at the reproductive stage. We screened the K/Z RIL population in the field for phenotypic traits (plant height, productive tiller number, leaf rolling score, flag leaf width, flag leaf length, and estimated chlorophyll content (SPAD)) and grain yield components (biological yield, spikelet per panicle number, filled grain per panicle number, panicle length, primary panicle branch number, hundred grain weight, spikelet number per plant, and filled grain number per plant). Based on the analysis of variance, there was a significant variation between WW and DS treatments and also the interaction between treatments and the rice lines of the K/Z RIL population. DS conditions at the reproductive stage of K/Z RIL population revealed that water deficit negatively affects all morphological, physiological, and grain yield components. A number of previous studies also characterized that phenotypic and grain yield components were severely affected by drought stress at the reproductive stage. Our focus was to identify the rice lines maintaining higher phenotypic and grain yield components under drought stress. By compiling the results, 25 lines out of 198 lines of K/Z RIL population and parent Kaybonnet were drought resistant showing higher plant height, more productive tiller number, and higher values for all grain yield components with ≤ 29% reduction in the phenotypic and grain yield components, as compared to WW plants in field conditions. However, 16 rice lines and parent ZHE733 were drought sensitive with ≥ 50% reduction in all phenotypic and grain yield components, compared to WW plants in field conditions, exhibiting shorter panicle length, smaller productive tiller number, and lower values of all grain yield components. The majority of the plant phenotypic and grain yield components of the K/Z RIL population showing a continuous distribution under WW and DS conditions, confirming that these components are controlled by many genes and influenced by

environmental factors. In the correlation analysis, most of the grain yield components showed better correlation with major phenotypic traits. High heritability (> 60%) was found for all of the phenotypic and grain yield related traits in the WW and DS conditions. These results suggest that this information is useful to dissect the genetic architecture of phenotypic and grain yield components of drought resistance in K/Z RIL population, and to use this valuable resources for developing drought resistant rice varieties, that would be an important step forward to improve adapted Arkansas and U.S. rice genotypes for higher grain production under DS conditions.

References

Ahadiyat, Y. R., Hidayat, P., & Susanto, U. (2014). Drought tolerance, phosphorus efficiency and yield characters of upland rice lines. *Emir J Food Agric.*, *26*, *1*, 25-34.

Ahmadikhah, A. & Marufinia, A. (2016). Effect of reduced plant height on drought tolerance in rice. *3 Biotech*, *6*, 221. doi:10.1007/s13205-016-0542-3

Anjum, S. A., Xie, X., Wang, L., Saleem, M. F., Man, C., & Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *Afr J Agric Res.*, *6*, *9*, 2026-2032.

Arkansas Cooperative Extension Service. (1982). Rice production handbook. MP 19207. 5M-488RV. Cooperative Extension Service, University of Arkansas, Fayetteville. Pp 60.

Ashfaq, M., Haider, M. S., Khan, A. S., & Allah, S. U. (2012). Breeding potential of the basmati rice germplasm under water stress conditions. *Afr J Biotechnol.*, *11*, *25*, 6647-6657.

Ashok, K. T., Singh, S. K., Amita, S., & Bhati, P. K. (2013). Appraisal of Genetic Variability for Yield and Its Component Characters in Rice (*Oryza sativa* L.). *Biolife*, *1*, 84-89.

Audebert, A., Asch, F., & Dingkuhn, M. (2000). Morpho-Physiological Research on Drought Tolerance in Rice at WARDA. In: Proceedings of an International Workshop on Field Screening for Drought Tolerance in Rice 11-14 Dec 2000. ICRISAT, Patancheru, India. 59-60.

Babu, R. C., Nguyen, B. D., & Chamarerk, V. (2003). Genetic analysis of drought resistance in rice by molecular markers. *Crop Science*, *43*, 1457-1469.

Babu, V. R., Shreya, K., Dangi, K. S., Usharani, G., & Shankar, A. S. (2012). Correlation and path analysis studies in popular rice hybrids of India. *Int. J. Sci. Res. Pub.*, *2*, 1-5.

Baldwin, K. (2011). Consolidation and Structural Change in the U.S. Rice Sector. DIANE Publishing. ISBN 978-1-4379-8478-1.

Batlang, U., Baisakh, N., Ambavaram, M. M., & Pereira, A. (2013). Phenotypic and physiological evaluation for drought and salinity stress responses in rice. *Methods in Molecular Biology*, *956*, 209-25. doi:10.1007/978-1-62703-194-3_15

Benbella, M. & Paulsen, G. M. (1998). Efficacy of treatments for delaying senescence of wheat leaves: I Senescence under controlled condition. *Agron. J.*, *90*, 329-332.

Bernier, J., Kumar, A., Venuprasad R., Spaner, D., & Gary A. (2007). A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Science, 47*, 507-518.

Biswal, A. K. & Kohli, A. (2013). Cereal flag leaf adaptations for grain yield under drought: knowledge status and gaps. *Mol Breeding, 31, 4,* 749-766.

Bitew, J. M. (2016). Estimation of genetic parameters, heritability and genetic advance for yield related traits in upland rice (*Oryza sativa* L. and *Oryza glaberrima* Steud.) genotypes in northwestern Ethiopia. *World Scientific News, 47,* 340-350.

Black, A. L. & Siddoway, F. H. (1977). Hard red and durum spring wheat responses to seeding date and NP-fertilization on fallow. *Agronomy Journal, 69,* 885-888.

Borrell, A. K., Hammer, G. L., & Henzell, R. G. (2000). Does maintaining green leaf area in sorghum improve yield under drought?. *Crop Sci., 40,* 1037-1048.

Botwright, A. T. L., Latte, H. R., & Wade, L. J. (2008). Genotype and environment interactions for grain yield of upland rice backcross lines in diverse hydrological environments. *Field Crops Res., 108, 2,* 117-125.

Bouman, B. A. M., Peng, S., Castañeda, A. R., & Visperas, R. M. (2005). Yield and water use of irrigated tropical aerobic rice systems. *Agric Water Manag., 74, 2,* 87-105.

Bunnag, S., & Pongthai, P. (2013). Selection of rice (*Oryza sativa* L.) cultivars tolerant to drought stress at the vegetative stage under field conditions. *Am J Plant Sci., 4, 9,* 1701-1708.

Cal, A. J., Sanciangco, M., Rebolledo, M. C., Luquet, D., Torres, R. O., McNally, K. L., & Henry, A. (2018). Leaf morphology, rather than plant water status, underlies genetic variation of rice leaf rolling under drought. *Plant, Cell & Environment, 42,* 1532-1544. doi:10.1111/pce.13514

Cao, L. Y., Wu, J. L., Fan, Y. Y., Cheng, S. H., & Zhuang, J. Y. (2010). QTL analysis for heading date and yield traits using recombinant inbred lines of indica rice grown in different cropping seasons. *Plant Breeding, 129,* 676-682.

Castillo, E. G., Tuong, T. P., Singh, U., Inubushi, K., & Padilla, J. (2006). Drought response of dry-seeded rice to water stress timing and N-fertilizer rates and sources. *Soil Sci Plant Nutr., 52, 4,* 496-508.

Centritto, M., Lauteri, M., Monteverdi, M. C., & Serraj, R. (2009). Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. *J Exp Bot., 60, 8,* 2325-2339.

Cha-um, S., Yooyongwech, S., & Supaibulwatana, K. (2010). Water deficit stress in the reproductive stage of four indica rice (*Oryza sativa* L.) genotypes. *Pak J Bot., 42, 5,* 3387-3398.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodriques, M. L., Ricardo, C. P. P., Osorio, M. L., Carvatho, I., Faria T., & Pinheiro, C. (2002). How plants cope with water stress in the field photosynthesis and growth? *Ann. Bot., 89,* 907-916.

Cho, S. H., Yoo, S. C., Zhang, H. T., Pandeya, D., Koh, H. J, & Hwang, J. Y. (2013). The rice narrow leaf2 and narrow leaf3 loci encode WUSCHEL-related homeobox 3A (OsWOX3A) and function in leaf, spikelet, tiller and lateral root development. *New Phytol., 198, 4,* 1071-1084.

Clark, L. J., Aphale, S. L., & Barraclough, P. B. (2000). Screening the ability of rice roots to overcome the mechanical impedance of wax layers: importance of test conditions and measurement criteria. *Plant Soil, 219,* 187-196.

Courtois, B., McLaren, G., Sinha, P. K., Prasad, K., Yadav, R., & Shen, L. (2000). Mapping QTLs associated with drought avoidance in upland rice. *Mol Breed, 6,* 55-66.

Crosson, P. (1995). Natural resource and environmental consequences of rice production. In: Fragile lives in fragile ecosystems, Proceedings of the international rice research conference, IRRI, Los Baños, Manila, Philippines, pp 83-100.

Davatgar, N., Neishabouri, M. R., Sepaskhah, A. R., & Soltani, A. (2009). Physiological and morphological responses of rice (*Oryza sativa* L.) to varying water stress management strategies. *Int. J. Plant Prod., 3, 4,* 19-32.

De Freitas, G. P. M., Basu, S., Ramegowda, V., Braga, E. B., & Pereira, A. (2016). Comparative analysis of gene expression in response to cold stress in diverse rice genotypes. *Biochemical and Biophysical Research Communications, 471, 1,* 253-259. doi:10.1016/j.bbrc.2016.02.004

Deng, F., Wang, L., Ren, W. J., Mei, X. F., & Li, S. X. (2015). Optimized nitrogen managements and poly-aspartic acid urea improved dry matter production and yield of indica hybrid rice. *Soil & Tillage Research, 145,* 1-9.

Dikshit, U. N., Parida, D., & Satpathy, D. (1987). Response of short duration rice cultivars to drought stress. *IRRI Newsl., 12, 6,* 14-15.

Dingkuhn, M., Cruz, R. T., O'Toole, J. C., & Doerffling. (1989). Net photosynthesis, water uses efficiency, leaf water potential, and leaf rolling as affected by water deficit in tropical upland rice. *Aust. J. Agric. Res., 40,* 1171-1181.

Dingkuhn, M., Laza, M. R. C., Kumar, U., Mendez, K. S., Collard, B., & Jagadish, K.S.V. (2015). Improving yield potential of tropical rice: achieved levels and perspectives through improve dideo types. *Field Crops Res., 182,* 43-59. doi:10.1016/j.fcr.2015.05.025

Dixit, S., Singh, A., Ma, T. S. C., Maturan, P. T., Amante, M., & Kumar, A. (2014). Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC Genet., 15, 16*.

Farooq, H., Basra, S. M. A., Wahid, H., & Rehman, H. (2009). Exogenously applied nitric oxide enhances the drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). *J Agron Crop Sci., 195, 4*, 254-261.

Farooq, M., Basra, S. M. A., Wahid, A., Cheema, Z. A., Cheema, M. A., & Khaliq, A. (2008). Physiological role of exogenously applied glycine betaine in improving drought tolerance of fine grain aromatic rice (*Oryza sativa* L.). *J. Agron. Crop Sci., 194*, 325-333.

Feller, A., Machemer, K., Braun, E. L., & Grotewold, E. (2011). Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. *Plant J., 66*, 94-116. doi:10.1111/j.1365-313X.2010. 04459.x PMID: 21443626

Fen, L. L., Ismail, M. R., Zulkarami, B., Rahmani, M. S. A., & Islam, M. R. I. (2015). Physiological and molecular characterization of drought responses and screening of drought tolerant rice varieties. *Biosci. J., Uberlândia, 31, 3,* 709-718.

Foyer, C. H., Descourvieres, P., & Kunert, K. J. (1994). Photo oxidative stress in plants. *Plant Physiol., 92*, 696-717.

Ghosh, S., Sahai, V. N., & Saran, S. (1990). Role of flag leaf on grain yield and spikelet sterility in rice cultivar. *Oryza, 27*, 87-89.

Gladun, I. V. & Karpov, E. A. (1993). Distribution of assimilates from the flag leaf of rice during the reproductive period of development. *Russ J Plant Physiol., 40*, 215-219.

Guan, Y. S., Serraj, R., Liu, S. H., Xu, J. L., Ali, J., Wang, W. S., Venus, E., Zhu, L. H., & Li, Z. K. (2010). Simultaneously improving yield under drought stress and non-stress conditions: A case study of rice (*Oryza sativa* L.). *J. Exp. Bot., 61*, 4145-4156.

Guo, L. B., Luo, L. J., Xing, Y. Z., Xu, C. G., Mei, H. W., Wang, Y. P., Zhong, D. B., Qian, Q., Ying, C. S., & Shi, C. H. (2003). Dissection of QTLs in two years for important agronomic traits in rice (*Oryza sativa* L.). *Chin J Rice Sci., 17, 3*, 211-218. (in Chinese with English abstract)

Ha, P. T. T. (2014). Physiological responses of rice seedlings under drought stress. *J Sci Devel., 12, 5*, 635-640.

Hanada, K. (1995). Differentiation and development of tiller buds. In: Science of the rice plant: Physiology, Vol. 2, T. Matsuo, K. Kumazawa, R. Ishii, K. Ishihara and H. Hirata, eds. 546-5655. Tokyo: Food and Agriculture Policy Research Center.

Haussmann, B. I. G., Mahalakshmi, V., Reddy, B. V. S., Seetharama, N., Hash, C. T., Geiger, H. H. (2002). QTL mapping of stay-green in two sorghum recombinant inbred populations. *Theor Appl Genet., 106*, 133-142.

Hayashi, H. (1995). Translocation, storage and partitioning of photosynthetic products. In: Science of the rice plant: Physiology, Vol. 2, T. Matsuo, K. Kumazawa, R. Ishii, K. Ishihara and H. Hirata, eds. 546-5655. Tokyo: Food and Agriculture Policy Research Center.

He, P., Wang, X., Zhang, X., Jiang, Y., Tian, W., Zhang, X., Li, Y., Sun, Y., Xie, J., Ni, J., He, G., & Sang, X. (2018). Short and narrow flag leaf1, a GATA zinc finger domain-containing protein, regulates flag leaf size in rice (*Oryza sativa*). *BMC Plant Biology, 18*, 273. doi:10.1186/s12870-018-1452-9

Henry, C., Hirsh, S., Anders, M., Vories, E., Reba, M., Watkins, K., & Hardke, J. (2016). Annual Irrigation Water Use for Arkansas Rice Production. *J. Irrig. Drain Eng*., doi:10.1061/(ASCE)IR.1943-4774.0001068, 05016006

Henson, I. E. (1982). Abscisic acid and water relations of rice (*Oryza sativa* L.): Sequencial responses to water stress in the leaf. *Annals of Botany, 50*, 9-24. doi:10.1093/oxfordjournals.aob.a086349

Hirt, H., & Shinozaki, K. (2004). Plant responses to abiotic stress. Springer, Berlin.

Hossain, M. (2007). Rice facts: a balancing act. *Rice Today, 6, 37*.

Howell, T. A. (2001). Enhancing water use efficiency in irrigated agriculture. *Agronomy Journal, 93*, 281-289.

Hsiao, T. C., O'Toole, J. C., Yambao, E. B., & Turner, N. C. (1984). Influence of osmotic adjustment on leaf rolling and tissue death in rice. *Plant Physiol., 75*, 328.

Hsiao, T. C., Fereres, E., Acvedo, E., & Henderson, W. (1976). Water stress and dynamics of growth and yield of crop plants. pp: 281-305. In: (eds. Lang, O.L., Kappan, L., and Schulz, E.D.). Water and plant life. Problems and modern approaches. Springer-verlog.

Huang, M., Jiang, L., Xia, B., Zou, Y., Jiang, P., & Ao, H. (2013). Yield gap analysis of super hybrid rice between two subtropical environments. *Australian Journal of Crop Science, 7*, 600-608.

Huang, R., Jiang, L., Zheng, J., Wang, T., Wang, H., & Huang, Y. (2013). Genetic bases of rice grain shape: so many genes, so little known. *Trends Plant Sci., 18*, 218-226. doi:10.1016/j.tplants.2012.11.001

Impa, S. M., Nadaradjan, S., & Boominathan, P. (2005). Carbon isotope discrimination accurately reflects variability in WUE measured at a whole plant level in rice. *Crop Science, 45,* 2517-2522.

IRRI (International Rice Research Institute). (1994). Rice almanac (2nd Eds). pp 181. IRRI, Los Baños, Philippines.

IRRI. (2013). SES (Standard Evaluation System for rice). International Network for Genetic Evaluation of Rice, Genetic Resource Center, Los Baños, Philippines.

IRRI. (2007). The rice environments or ecosystems. http://www.knowledgebank.irri.org/ericeproduction/0.3._Rice_environments.htm.

Jaleel, C. A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H. J., Somasundaram, R., & Panneerselvam, R. (2009). Drought stress in plants: A review on morphological characteristics and pigments composition. *Int J Agric Biol., 11,* 100-105.

Jaleel, C. A., Manivannan, P., Lakshmanan, G. M. A., Gomathinayagam, M., & Panneerselvam, R. (2008). Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. *Colloids Surf. B: Biointerfaces, 61,* 298-303.

Jebbouj, R. & Yousfi, B. E. (2009). Barley yield losses due to defoliation of upper three leaves either healthy or infected at boot stage by *Pyrenophora teres f. teres. Eur J. Plant Pathol., 125, 2,* 303-315.

Jennings, P. R., Coffman, W. R., & Kauffman, H. E. (1979). Rice improvement. Los Baños, Philippines: International Rice Research Institute.

Ji, K. X., Wang, Y. Y., Sun, W. N., Lou, Q. J., Mei, H. W., Shen, S. H., & Chen, H. (2012). Drought-responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. *J Plant Physiol., 169, 4,* 336-344.

Jing, L., Rui, X., Chunchao, W., Lan, Q., Xiaoming, Z., Wensheng, W., Yingbin, D., Lizhen, Z., Yanyan, W., Yunlian, C., Lifang, Z., Weihua, Q., & Qingwen, Y. (2018). A heading date QTL, qHD7.2, from wild rice (*Oryza rufipogon*) delays flowering and shortens panicle length under long-day conditions. *Scientific Reports, 8,* 2928. doi:10.1038/s41598-018-21330-z

Jung, C. & Muller, A. E. (2009). Flowering time control and applications in plant breeding. *Trends Plant Sci., 14,* 563-573.

Kadioglu, A. & Terz, R. (2007). A dehydration avoidance mechanism: Leaf rolling. *Bot Rev., 73, 4,* 290-302.

Kamoshita, A., Rofriguez, R., Yamauchi, A., & Wade, L. J. (2004). Genotypic variation in response of rainfed lowland to prolonged drought and re-watering. *Plant Prod Sci., 7, 4,* 406-420.

Konate, A. K., Zongo, A., Kam, H., Sanni, A., & Audebert, A. (2016). Genetic variability and correlation analysis of rice (*Oryza sativa* L.) inbred lines based on agro-morphological traits. *African Journal of Agricultural Research, 11, 35,* 3340-3346.

Kovi, M. R., Bai, X. F., Mao, D. H., & Xing, Y. Z. (2011). Impact of seasonal changes on spikelets per panicle, panicle length and plant height in rice (*Oryza sativa* L.). *Euphytica, 179,* 319-331. doi:10.1007/s10681-0100332-7

Kramer, P. J. (1969). Plant and soil water relationship. TATA McGrawHill, Bombey New Delhi, pp. 360.

Kumar, A., Bernier, J., Verulkar, S., Lafitte, H. R., & Atlin, G. N. (2008). Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. *Field Crops Res, 107,* 221-231.

Kumar, A., Dixit, S., Ram, T., Yadaw, R. B., Mishra, K. K., & Mandal, N. P. (2014). Breeding high-yielding drought-tolerant rice: Genetic variations and conventional and molecular approaches. *J Exp Bot., 65, 21,* 6265-6278.

Kumar, R., Sarawgi, A. K., Ramos, C., Amarante, S. T., Ismail, A. M., & Wade, W. J. (2006). Partitioning of dry matter during drought stress in rainfed lowland rice. *Field Crops Res., 96,* 455-465.

Lafitte, R., Blum, A., & Atlin, G. (2003). Using secondary traits to help identify drought-tolerant genotypes. In K. S. Fischer, R. Lafitte, S. Fukai, G. Atlin, & B. Hardy (Eds.), *Breeding rice for drought prone environments.* Los Baños, Philippines: International Rice Research Institute.

Lanceras, J. C., Pantuwan, G., & Jongdee, B. (2004). Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant Physiology, 135,* 384-399.

Levitt, J. (1980). Responses of plants to environmental stresses, Vol. 2. Water, radiation, salt and other stresses. *New York: Academic Press,* 93-128.

Li, X., Qian, Q., Fu, Z., Wang, Y., Xiong, G., Zeng, D., Wang, X., Liu, X., Teng, S., Hiroshi, F., Yuan, M., Luok, D., Han, B., & Li, J. (2003). Control of tillering in rice. *Nature, 422,* 618-621.

Li, Z. K., Pinson, S. R. M., Stansel, J. W., & Paterson, A. H. (1998). Genetic dissection of the source-sink relationship affecting fecundity and yield in rice (*Oryza sativa* L.). *Mol Breed., 4, 5,* 415-426.

Liang, G., Zhen-hua Z., & Jie-yun, Z. (2013). Quantitative Trait Loci for Heading Date and Their Relationship with Genetic Control of Yield Traits in Rice (*Oryza sativa*). *Rice Science, 20, 1,* 1-12.

Liu, G., Mei, H., Zou, G., & Luo, L. (2010). Sensitivities of rice grain yield and other panicle characters to late-stage drought stress revealed by phenotypic correlation. *Molecular Breeding, 25*, 603-613.

Lu, C. F., Shen, L. S., Tan, Z. B., Xu, Y. B., He, P., Chen, Y., & Zhu, L. H. (1997). Comparative mapping of QTLs for agronomic traits of rice across environments by using a doubled-haploid population. *Theor Appl Genet., 94*, 145-150.

Mae. (1997). Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. *Plant soil, 196,* 201-210.

Maisura, C. M. A., Lubis, I., Junaedinand, A., & Ehara, H. (2014). Some physiological character responses of rice under drought conditions in a paddy system. *J Int Soc Southeast Asian Agric Sci., 20, 1*, 104-114.

Manickavelu, A., Nadarajan, N., Ganesh, S. K., Gnanamalar, R. P., & Babu, R. C. (2006). Drought tolerance in rice: morphological and molecular genetic consideration. *Plant Growth Regul., 50,* 121-138. doi:10.1007/s10725-006-9109-3

Martinez, J. P., Silva, H., Ledent J. F., & Pinto, M. (2007). Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.). *European J. Agron., 26,* 30-38.

Masle, J. (1985). Competition among tillers in winter wheat: Consequences for growth and development of the crop. In Wheat growth and modelling, W. Day and R. K. Atkin, eds, 33–54. New York: Plenum Press.

Mei, H. W., Li, Z. K., Shu, Q. Y., Guo, L. B., Wang, Y. P., Yu, X. Q., Ying, C. S., & Luo, L. J. (2005). Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two backcross populations. *Theor Appl Genet., 110*, 649-659.

Mirnoff, N. (1993). The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol., 125*, 27-58.

Moldenhauer, K., & Nathan, S. (2004). *1-Rice growth and development. In: Slaton, N.(Ed.), Rice Production Handbook.* Arkansas: University of Arkansas.

Monyo, J. H. & Whittington, W. J. (1973). Genotypic difference in flag leaf area and their contribution to grain yield in wheat. *Euphytica, 22, 3,* 600-606.

Mostajeran A. & Rahimi-Eichi, V. (2009). Effects of drought stress on growth and yield of rice (*Oryza sativa* L.) cultivars and accumulation of proline and soluble sugars in sheath and blades of their different ages leaves. *Am-Eur J Agric Environ Sci., 5, 2,* 264-272.

Mukamuhirwa, A., Hovmalm, H. P., Bolinsson, H., Ortiz, R., Nyamangyoku, D., & Johansson, E. (2019). Concurrent Drought and Temperature Stress in Rice—A Possible Result of the Predicted Climate Change: Effects on Yield Attributes, Eating Characteristics, and Health Promoting Compounds. *Int. J. Environ. Res. Public Health, 16*, 1043. doi:10.3390/ijerph16061043

Murata, Y. & Matsushima, S. (1975). Rice. In Crop physiology: Some case histories, L. T. Evans, ed. 73-99. London: Cambridge University Press.

Murayama, N. (1995). Development and senescence of an individual plant. In Science of the rice plant: Physiology, Vol 2, T. Matsuo, K. Kumazawa, R. Ishii, K. Ishihara and H. Hirata, eds.119-178. Tokyo: Food and Agriculture Policy Research Center.

Nam, N. H., Chauhan, Y. S., & Johansen, C. (2001). Effect of timing of drought stress on growth and grain yield of extra-short-duration pigeon pea lines. *J. Agric. Sci., 136,* 179-189.

Pandey, V. & Shukla, A. (2015). Acclimation and Tolerance Strategies of Rice under Drought Stress. *Rice Science, 22, 4,* 147-161.

Pantuwan, G., Fukai, S., Cooper, M., Rajatasereekul, S. & O'Toole, J. C. (2000). Yield Responses of Rice (*Oryza Sativa* L.) Genotypes to Water Deficit in Rainfed Lowlands. In: Proceedings of an International Workshop on Field Screening for Drought Tolerance in Rice 11–14 Dec 2000. ICRISAT, Patancheru, India. pp. 54-58.

Peng, S., Khush, G . S., & Cassman, K. G. (1994). Evolution of the new plant ideotype for increased yield potential breaking the yield barrier. In: Proceedings of a Workshop on Rice Yield Potential in Favourable Environments. International Rice Research Institute, Philippines. pp. 5–20.

Pirdashti, H., Sarvestani, Z. T., & Bahmanyar, M. A. (2009). Comparison of physiological responses among four contrast rice cultivars under drought stress conditions. *Proc World Acad Sci Engin Technol., 49,* 52-53.

Power, J. F. & J. Alessi. (1978). Tiller development and yield of standard and semi dwarf spring wheat varieties as affected by nitrogen fertilizer. *Journal of Agriculture Science, 90*, 97-108.

Qing, Z. M., Jing, L. G., & Kai, C. R. (2001). Photosynthesis characteristics in eleven cultivars of sugarcane and their responses to water stress during the elongation stage. Proceedings of International Society of Sugarcane Technologist. 24, 642-643.

Raman, A., Verulkar, S. B., Mandal, N. P., Variar, M., Shukla, V. D., Dwivedi, J. L., Singh, B. N., Singh, O. N., Swain, P., Mall, A. K., Robin, S., Chandrababu, R., Jain, A., Ram, T., Hittalmani, S., Haefele, S., Piepho, H. P., & Kumar, A. (2012). Drought yield index to select high yielding rice lines under different drought stress severities. *Rice, 5, 31*, 1-12.

Ramegowda, V., Basu, S., Krishnan, A., & Pereira, A. (2014). Rice growth under drought kinase is required for drought tolerance and grain yield under normal and drought stress conditions. *Plant Physiology, 166, 3*, 1634-1645. doi:10.1104/pp.114.248203.

Ranjbarfordoei, A., Samson, R., Damne, P. V., & Lemeur, R. (2000). Effects of drought stress induced by polyethylene glycol on pigment content and photosynthetic gas exchange of *Pistacia khinjuk* and *P. mutica*. *Photosynthetica, 38, 3*, 443-447.

Razmjoo, K., Heydarizadeh, P., & Sabzalian, M. R. (2008). Effect of salinity and drought stresses on growth parameters and essential oil content of *Matricaria chamomile*. *Int. J. Agric. Biol., 10*, 451-454.

Sairam, R. K., Shukla, D. S., & Deshmukh, P. S. (1996). Effect of *Homo brassinolide* seed treatment on germination, β-amylase activity and yield of wheat under moisture stress conditions. *Indian Journal of Plant Physiology, 1*, 141-144.

Sakamoto, T. & Matsuoka, M. (2008). Identifying and exploiting grain yield genes in rice. *Current Opinion in Plant Biology, 11, 2,* 209-214. doi:10.1016/j.pbi.2008.01.009

Salunkhe, A. S., Poornima, R., Prince, K. S., Kanagaraj, P., Sheeba, J. A., Amudha, K., Suji, K. K., Senthil, A., & Babu, R. C. (2011). Fine mapping QTL for drought resistant traits in rice (*Oryza sativa* L.) using bulk segregant analysis. *Mol Biotechnol., 49,1*, 90-95.

Sarkarung, S., Singh, O. N., Roy, J. K., Vanavichit, A., & Bhekasut, P. (1995). Breeding strategies for rainfed lowland ecosystem. In: Fragile Lives in Fragile Ecosystem. Proc. Intl. Rice Research Conf. 13-17 Feb., IRRI. Los Baños, Laguna, Philippines, pp. 709-720.

Sarvestani, Z. T., Pirdashti, H., Sanavy, S. A., & Balouchi, H. (2008). Study of water stress effects in different growth stages on yield and yield components of different rice (*Oryza sativa* L.) cultivars. *Pak J Biol Sci., 11, 10,* 1303-1309.

Setter, T. L., Laureles, E. V., & Mazaredo, A. M. (1997). Lodging reduces yield of rice by self-shading and reductions in canopy photosynthesis. *Field Crops Research, 49*, 95-106.

Shahryari, R., Gurbanov, E., Gadimov, A., & Hassanpanah, D. (2008). Tolerance of 42 bread wheat genotypes to drought stress after anthesis. *Pak J Biol Sci., 11, 10*, 1330-1335.

Shi, D., Devineni, N., Lall, U., & Piñero, E. (2013). America's water risk: water stress and climate variability. Earth Institute, Columbia University, New York.

Sikuku, P. A., Onyango, J. C., & Netondo, G. W. (2012). Physiological and biochemical responses of five NERICA rice varieties (*Oryza sativa* L.) to water deficit at vegetative and reproductive stage. *Agric Biol J North Am., 3, 3*, 93-104.

Singh, A., Sengar, K., & Sengar, R. S. (2013). Gene regulation and biotechnology of drought tolerance in rice. *Int J Biotechnol Bioeng Res., 4, 6,* 547-552.

Singh, K. B. (1993). Problems and prospects of stress resistance breeding in chickpea. In: Breeding for Stress Tolerance in Cool-Season Food Legumes, (eds. Singh, K.B., Saxena M.C.), John Wiley, U.K. pp. 17-35.

Sokoto, M. B. & Muhammad, A. (2014). Response of rice varieties to water stress in Sokoto, Sudan Savannah, Nigeria. *J Biosci Med., 2, 1,* 68-74.

Songsri, P., Jogloy, S., Kesmala, T., Vorasoot, N., Akkasaeng, C., Patanothai, A., & Holbrook, C. C. (2008). Heritability of drought resistant traits and correlation of drought resistant and agronomic traits in peanut. *Crop Science, 48, 6,* 2245-2253.

Spicer, J. M. (1964). Beginnings of the Rice Industry in Arkansas. Arkansas Rice Promotion Association and Rice Council.

Spielmeyer, W., Ellis, M. H., & Chandler, P. M. (2002). Semi dwarf (sd-1), "green revolution" rice, contains a defective gibberellin 20-oxidase gene. *Proc. Natl. Acad. Sci. USA, 99,* 9043-9048

Subashri, M., Robin, S., Vinod, K. K., Rajeswari, S., Mohanasundaram, K. & Raveendran, T. S. (2009). Trait identification and QTL validation for reproductive stage drought resistance in rice using selective genotyping of near flowering RILs. *Euphytica, 166, 2,* 291-305.

Swain, S. M. & Singh, D. P. (2005). Tall tales from sly dwarves: novel functions of gibberellins in plant development. *Trends Plant Sci., 10,* 123-129.

Swain, P., Mall, A. K., Bose, L. K., Baig, M. J., & Singh, D. N. (2010). Drought susceptibility index as a parameter to identify drought tolerant rice genotypes for rainfed uplands. In: Abs. National Symposium on Sustainable Rice Production System Under Changed Climate. CRRI, Cuttack, Orissa, India. 27-29 Nov. pp. 42.

Swamy, B. P. M., Shamsudin, N. A. A., Rahman, S. N. A., Mauleon, R., Ratnam, W., Sta Cruz, M. T., & Kumar, A. (2017). Association Mapping of Yield and Yield-related Traits Under Reproductive Stage Drought Stress in Rice (*Oryza sativa* L.). *Rice, 10, 21*.

Tomoshiro, T., Mitsunori, O. & Waichi, A. (1983). Comparison of the formation of dry substance by the old and new type of rice cultivars. *Japan J Crop Sci., 52*, 299-305.

Tripathy, J. N., Zhang, J., Robin, S., Nguyen, T. T., & Nguyen, H. T. (2000). QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theor Appl Genet., 100,* 1197-1202.

Turner, N., C., O'Toole, J. C., Cruz, R. T., Namuco, O. S., & Ahmad, S. (1986). Responses of seven diverse rice cultivars to water deficits. I. Stress development, canopy temperature, leaf rolling and growth. *Field Crops Research, 13,* 257-271. doi:10.1016/0378-4290(86)90027-4

USDA. (1989). Rice situation and outlook yearbook. United States Department of Agriculture, Economic Research Service. Pp 45.

Usman, M., Raheem, Z. F., Ahsan, T., Iqbal, A., Sarfaraz, Z. N., & Haq, Z. (2013). Morphological, physiological and biochemical attributes as indicators for drought tolerance in rice (*Oryza sativa* L.). *Eur J Biol Sci., 5, 1,* 23-28.

Vangahun, J. M. (2012). Inheritance of flag leaf angle in two rice (*Oryza sativa* L.) cultivars. A thesis submitted to the school of graduate studies, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Venuprasad, R., Dalid, C. O., Del Valle, M., Zhao, D., Espiritu, M., Cruz, M. S., & Atlin, G. N. (2009). Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoretical and Applied Genetics, 120,* 177-190.

Venuprasad, R., Impa, S. M., Vowda, G. R. P., Atlin, G. N., & Serraj, R. (2011). Rice near-isogenic-lines (NILs) contrasting for grain yield under lowland drought stress. *Field Crops Res., 123, 1,* 38-46.

Venuprasad, R., Lafitte, H. R., & Atlin, G. N. (2007). Response to direct selection for grain yield under drought stress in rice. *Crop Sci., 47, 1,* 285-293.

Verma, V., Foullces, M. J., Worland, A. J., Sylvester-Bradley, R., Caligari, P. D. S., & Snape, J. W. (2004). Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. *Euphytica, 135,* 255-263

Vikram, P., Swamy, B. P. M., & Dixit, S. (2011). qDTY1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genetics, 12, 89*.

Wadhwa, R., Kumari, N., & Sharma, V. (2010). Varying light regimes in naturally growing *Jatropha curcus*: pigment, proline and photosynthetic performance. *J Stress Physiol Biochem., 6, 4,* 67-80.

Wang, S., Wu, K., Yuan, Q., Liu, X., Liu, Z., Lin, X., et al. (2012). Control of grain size, shape and quality by OsSPL16 in rice. *Nat. Genet., 44*, 950-954. doi:10.1038/ng.2327

Wei, X. J., Xu, J. F., Guo, H. N., Jiang, L., Chen, S. H., Yu, C. Y., Zhou, Z. L., Hu, P. S., Zhai, H. Q., & Wan, J. M. (2010). DTH8 suppresses flowering in rice, influencing plant height and yield potential simultaneously. *Plant Physiol., 153*, 1747-1758.

Weng, X., Wang, L., Wang, J., Hu, Y., Du, H., Xu, C., Xing, Y., Li, X., Xiao, J., & Zhang, Q. (2014). Grain Number, Plant Height, and Heading Date7 Is a Central Regulator of Growth, Development, and Stress Response. *Plant Physiology, 164*, 735-747.

Xiao, J., Li, J., Yuan, L., & Tanksley, S. D. (1996). Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. *Theor Appl Genet., 92*, 230-244.

Xing, Y. Z., Tan, Y. F., Hua, J. P., Sun, X. L., Xu, C. G., & Zhang, Q. (2002). Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theor Appl Genet., 105*, 248-257.

Xing, Y. & Zhang, Q. (2010). Genetic and molecular bases of rice yield. *Annu. Rev. Plant Biol., 61*, 421-442.

Xue, W. Y., Xing, Y. Z., Weng, X. Y., Zhao, Y., Tang, W. J., Wang, L., Zhou, H. J., Yu, S. B., Xu, C. G., Li, X. H., & Zhang, Q. F. (2008). Natural variation in Ghd7 is an important regulator of heading date and yield potential in rice. *Nat Genet., 40*, 761-767.

Yadav, R., Courtois, B., Huang, N., & McLaren, G. (1997). Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. *Theor Appl Genet., 94*, 619-632.

Yan, W. H., Wang, P., Chen, H. X., Zhou, H. J., Li, Q. P., Wang, C. R., Ding, Z. H., Zhang, Y. S., Yu, S. B., Xing, Y. Z., & Zhang, Q. F. (2011). A major QTL, Ghd8, plays pleiotropic roles in regulating grain productivity, plant height, and heading date in rice. *Mol Plant., 4*, 319-330.

Yoshida, S. (1981). Fundamentals of rice crop science. Los Baños, Philippines: International Rice Research Institute. Los Baños, Philippines: International Rice Research Institute.

Yuan, L. P. (1997). Hybrid rice breeding for super high yield. *Hybrid Rice, 12, 6*, 1-6.

Yue, B., Xue, W., Luo, L., & Xing, Y. (2006). QTL analysis for flag leaf characteristics and their relationships with yield and yield traits in rice. *Acta Genetica Sinica, 33*, 824-832.

Yue, S. B., Li, J. X., Tan, Y. F., Gao, Y. J., Li, X. H., Zhang, Q., & Saghai, M. M. A. (1997). Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proc Natl Acad Sci USA., 94*, 9226-9231.

Zhang, Y. S., Wu, W., & Xu, C. G. (2008). Two pleiotropic intervals of rice were assessed using NILs constructed by two methods. *Hereditas, 30, 6,* 781-787. (in Chinese with English abstract)

Zhang, B., Ye, W., Ren, D., Tian, P., Peng, Y., Gao, Y., Ruan, B., Wang, L., Zhang, G., Guo, L., Qian, Q., & Gao, Z. (2015). Genetic analysis of flag leaf size and candidate genes determination of a major QTL for flag leaf width in rice. *Rice, 8, 2*. doi:10.1186/s12284-014-0039-9s

Zhang, J., Zhang, S., Cheng, M., Jiang, H., Zhang, X., Peng, C., Lu, X., Zhang, M., & Jin, J. (2018). Effect of drought on agronomic traits of rice and wheat: A meta-analysis. *Int. J. Environ. Res. Public Health, 15, 839*. doi:10.3390/ijerph15050839

Zhao, X., Xu, J., Zhao, M., Lafitte, R., Zhu, L., Fu, B., Gao, Y., & Li, Z. (2008). QTLs affecting morphophysiological traits related to drought tolerance detected in overlapping introgression lines of rice (*Oryza sativa* L.). *Plant Science, 174,* 618-625.

Zhen, B., Guo, X., Zhou, X., Lu, H., & Wang, Z. (2019). Effect of the alternating stresses of drought and waterlogging on the growth, chlorophyll content, and yield of rice (*Oryza sativa* L.). *J. Irrig. Drain Eng., 145, 5*.

Zhou, L. H., Xie, Y. C., Chen, T., Zhang, Y. D., Zhu, Z., & Zhao, Q. Y. (2012). Relations between flag leaf morphology and yield and QTL analysis of related traits. *Jiangsu J Agri Sci., 28,* 207-2011.

Zhuang, J. Y., Lin, H. X., & Lu, J. (1997). Analysis of QTL×environment interaction for yield components and plant height in rice. *Theoretical and Applied Genetics, 95,* 799-808.

Figures

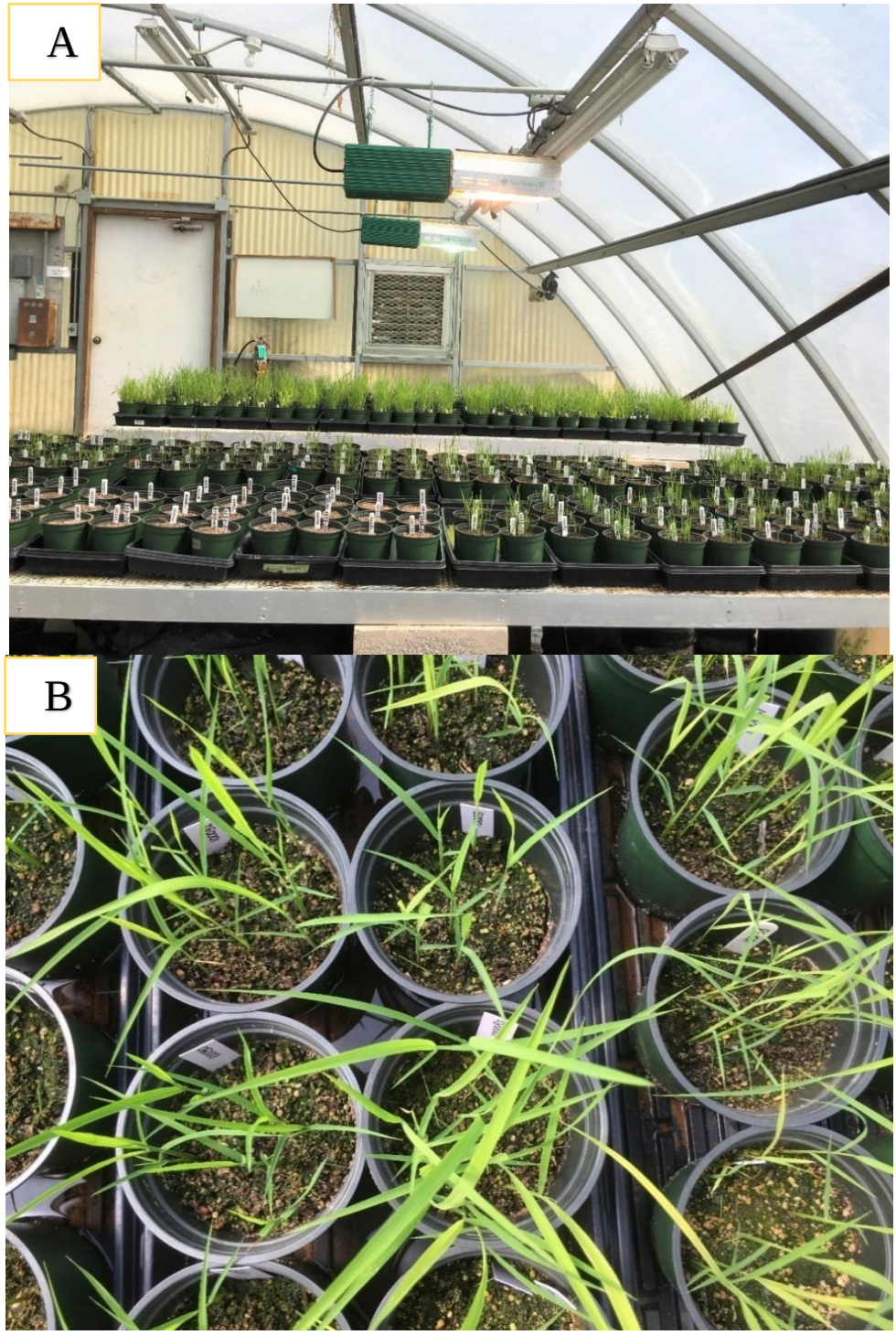

Figure 2.1. K/Z RIL population seeds and two parents (Kaybonnet and ZHE733) were germinated and grown in the greenhouse at controlled conditions (28 to 30ºC day and 22 to 23ºC night and 14h light/10h dark cycle and average light intensity 580 µmol $m^{-2}s^{-1}$ and 65% relative humidity) with sterilized field soil (A) for 20 days until V3 stage (B).

Figure 2.2. V3 stage plants per line were transplanted to the field divided into 6 batches (7-day intervals) based on their heading day data from USDA to synchronize drought treatment at reproductive stage.

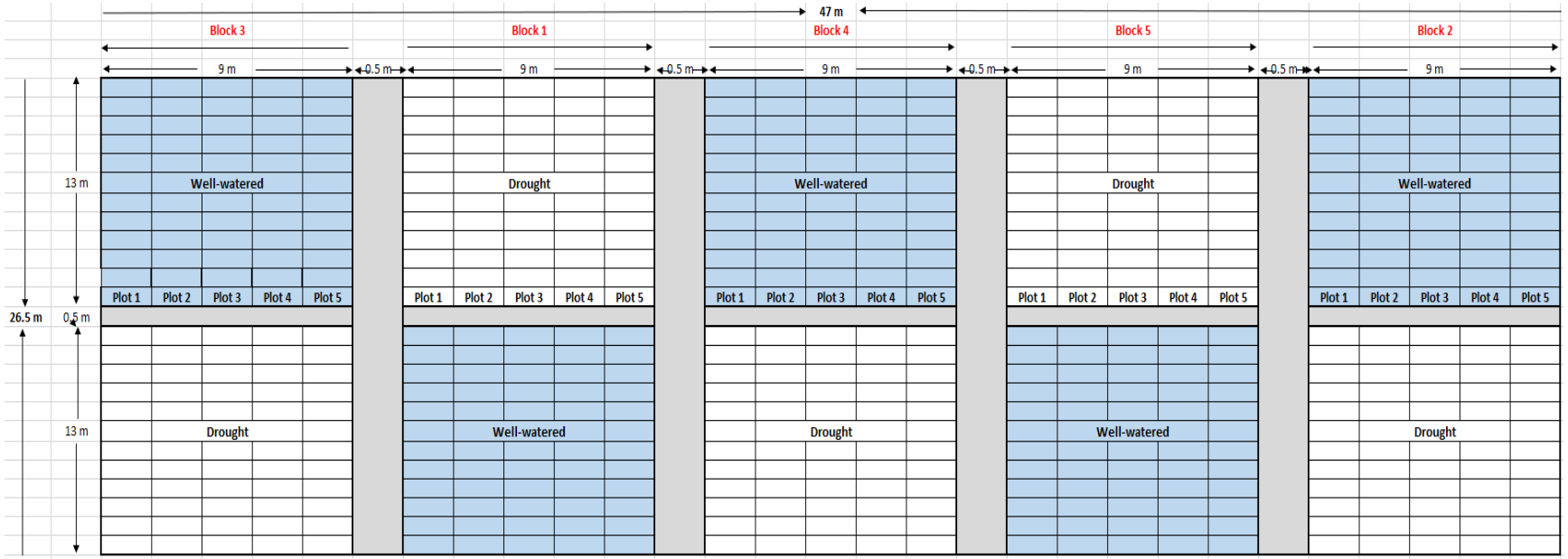

Figure 2.3. Field design.

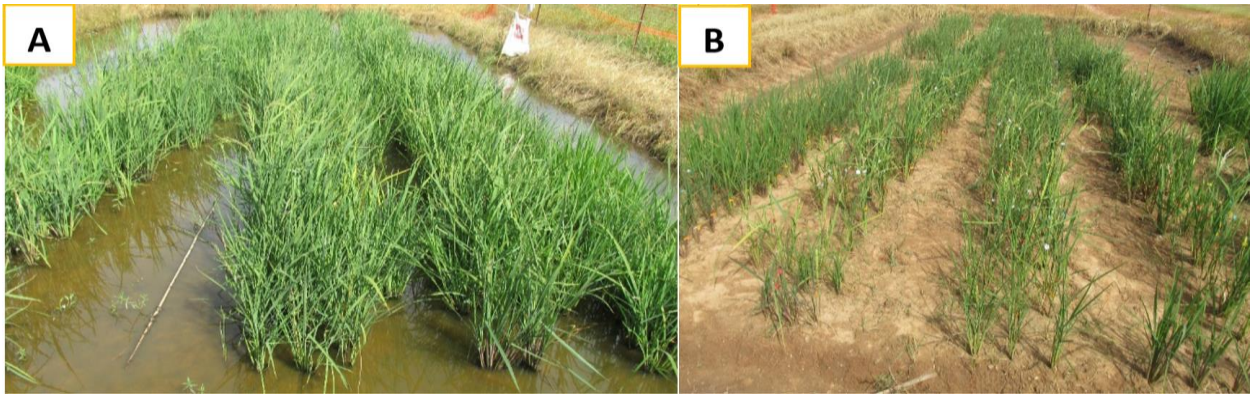

Figure 2.4. Field conditions for well-watered (A) and drought stress treatments (B) at the reproductive stage (R3 stage) of K/Z RIL population.

Figure 2.5. Tensiometer to monitor drought stress conditions that were installed at three spots in the drought stress plot just after draining, the first was in the beginning of the plot, the second in the middle, and the third at the end of the plot. The DS condition was maintained continuously up to -70 kPa soil water potential at the reproductive stage exhibited the severe DS conditions.

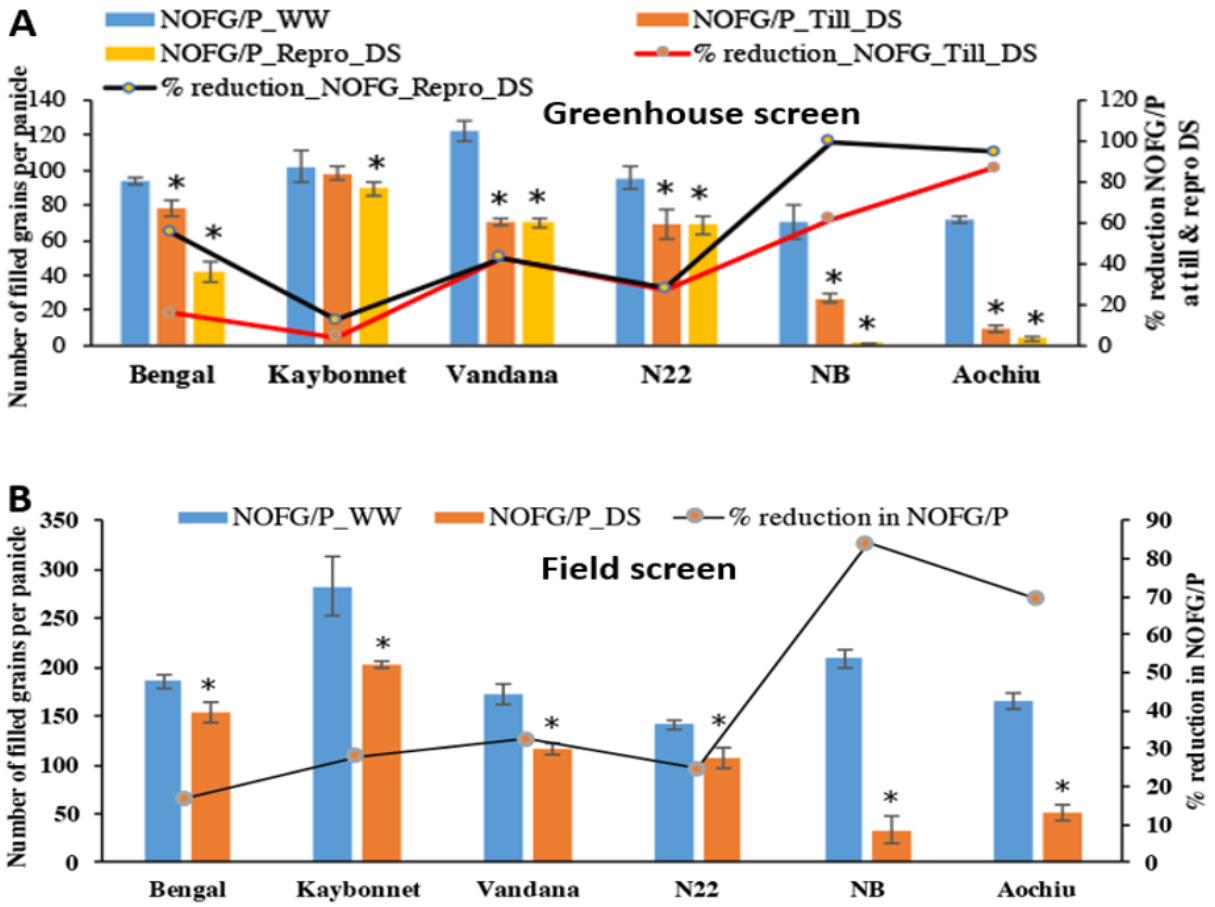

Figure 2.6. Screening of six diverse rice genotypes for filled grains per panicle number (NOFG). Varieties Kaybonnet (Arkansas) and Bengal (Louisiana) exhibit a high filled grains per panicle number under drought stress conditions at vegetative and reproductive stages in greenhouse and field conditions.

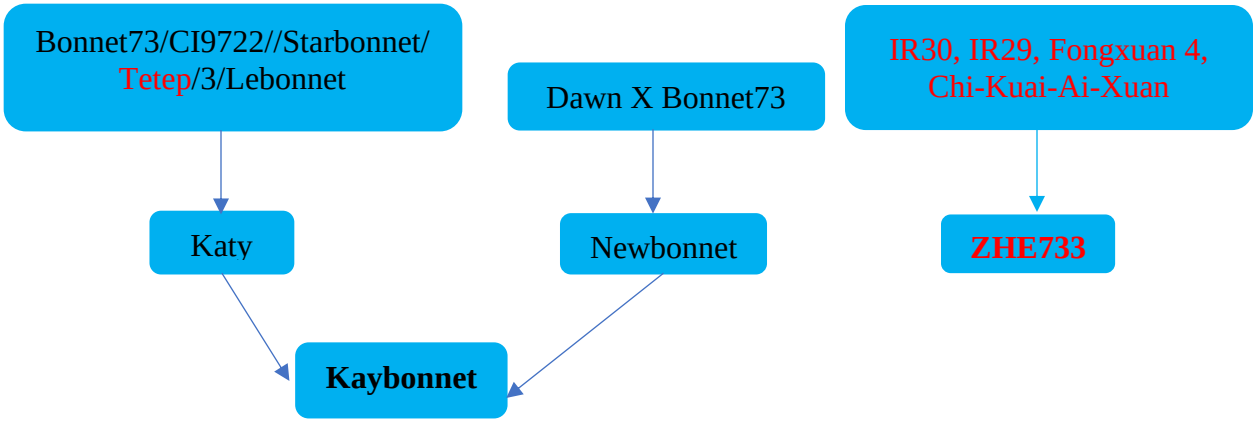

Figure 2.7. Pedigree of Kaybonnet and ZHE733. Kaybonnet is a cross between Katy and Newbonnet, Katy is a *tropical japonica* cultivar with large introgressions from *indica* landrace Tetep. ZHE733 was developed from a multiple cross of IR30, IR29, Fongxuan 4, Chi-Kuai-Ai-Xuan. Black: *japonica* and Red: *indica*.

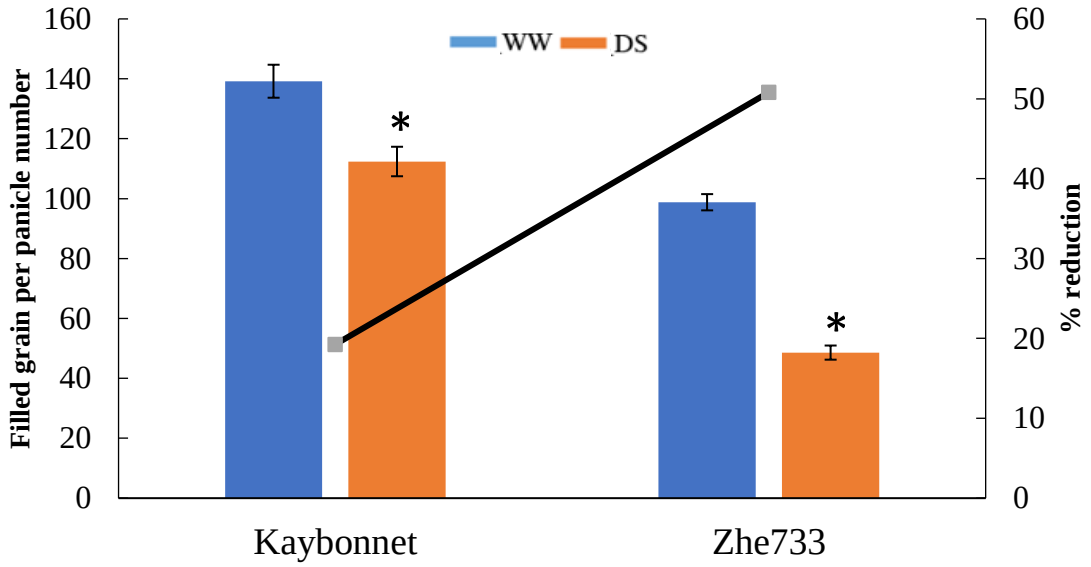

Figure 2.8. Filled grain per panicle number in Kaybonnet and ZHE733. Kaybonnet maintained high filled grain per panicle number under DS than ZHE733. Furthermore, the distribution of water use efficiency traits has been shown to be highest in *tropical japonica* (Kaybonnet) and medium in *indica* (ZHE733).

Figure 2.9. Panicle phenotypes of Kaybonnet (KB) as drought resistant parent under WW and DS, compared to drought sensitive parent ZHE733 in WW and DS conditions. KB shows higher seed set under drought than ZHE733.

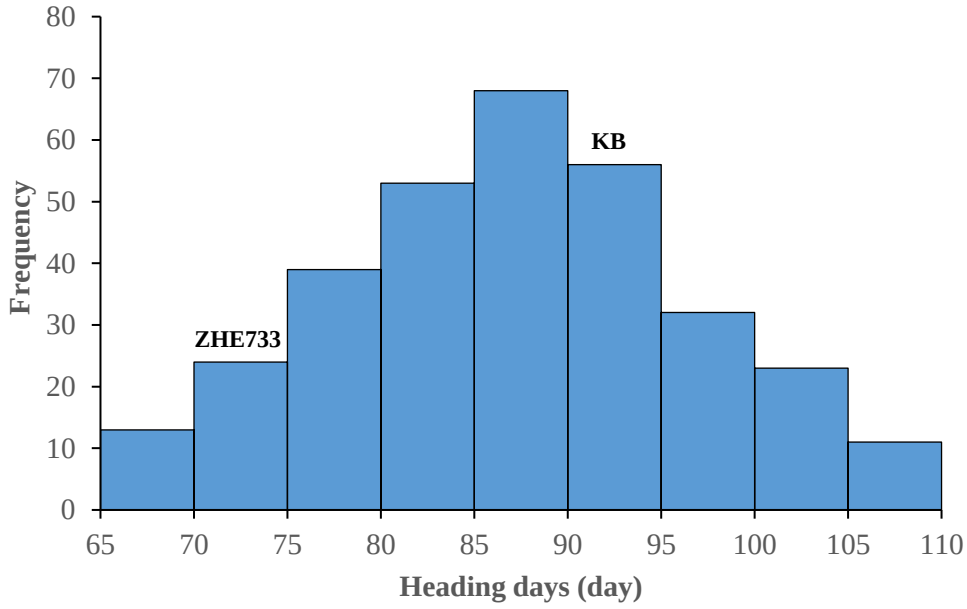

Figure 2.10. Frequency distribution of heading date in the K/Z RIL population.

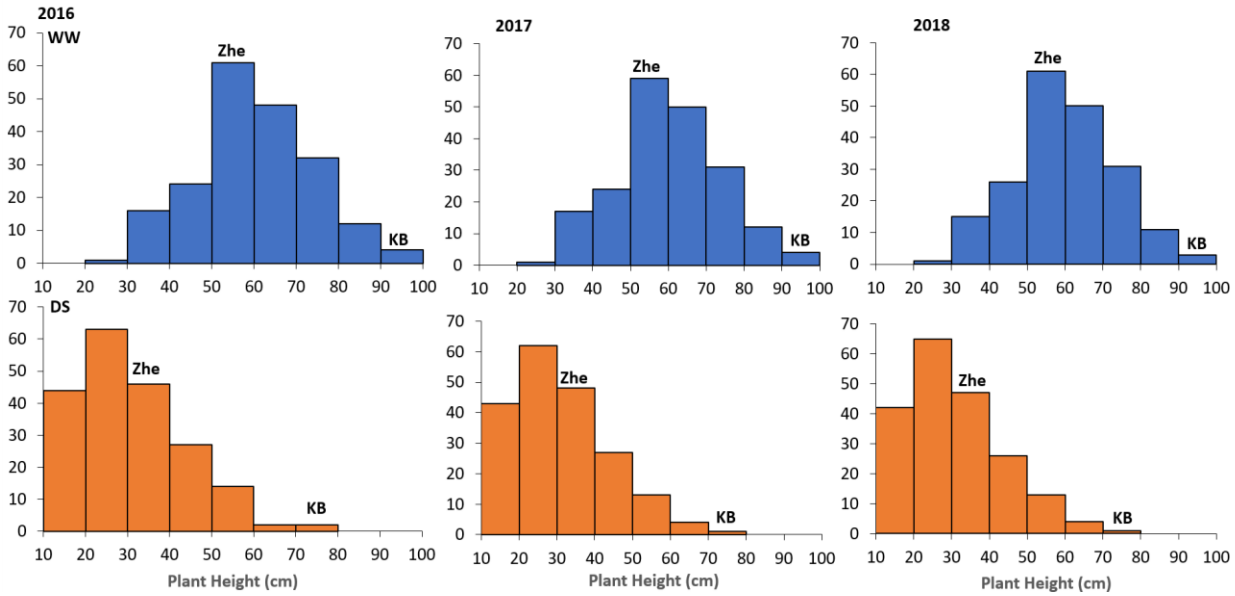

Figure 2.11. Frequency distribution of plant height in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

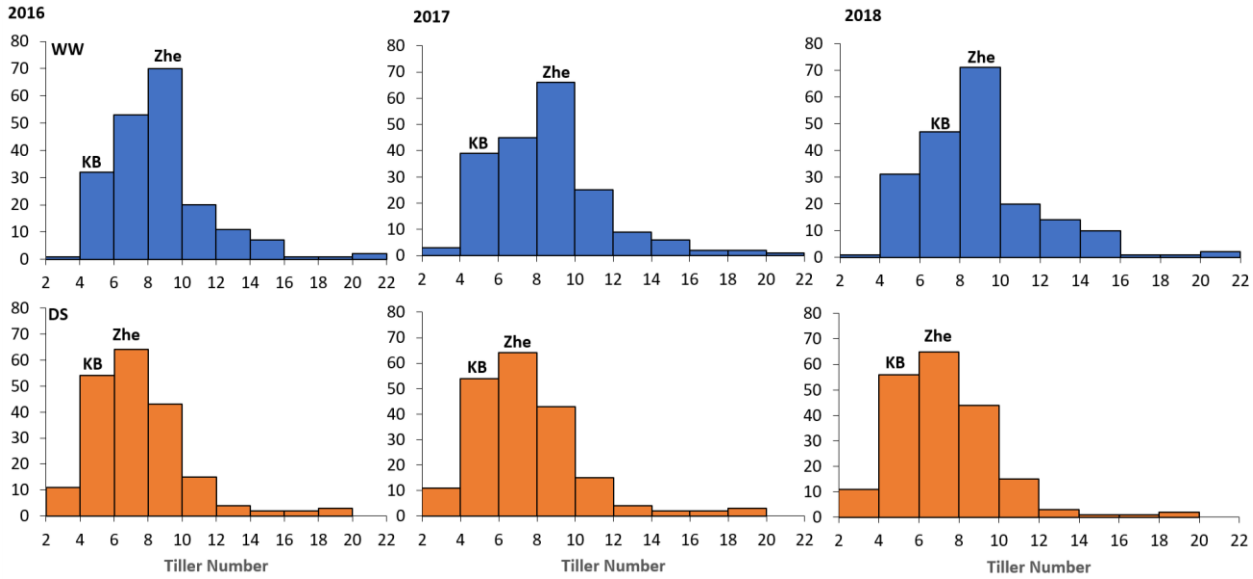

Figure 2.12. Frequency distribution of productive tiller number in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

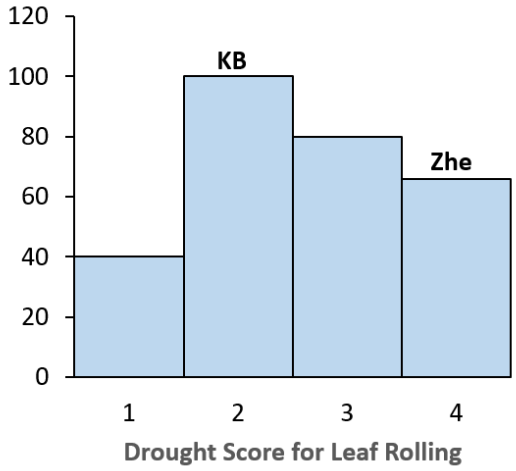

Figure 2.13. Frequency distribution of leaf rolling score in the K/Z RIL population under DS conditions.

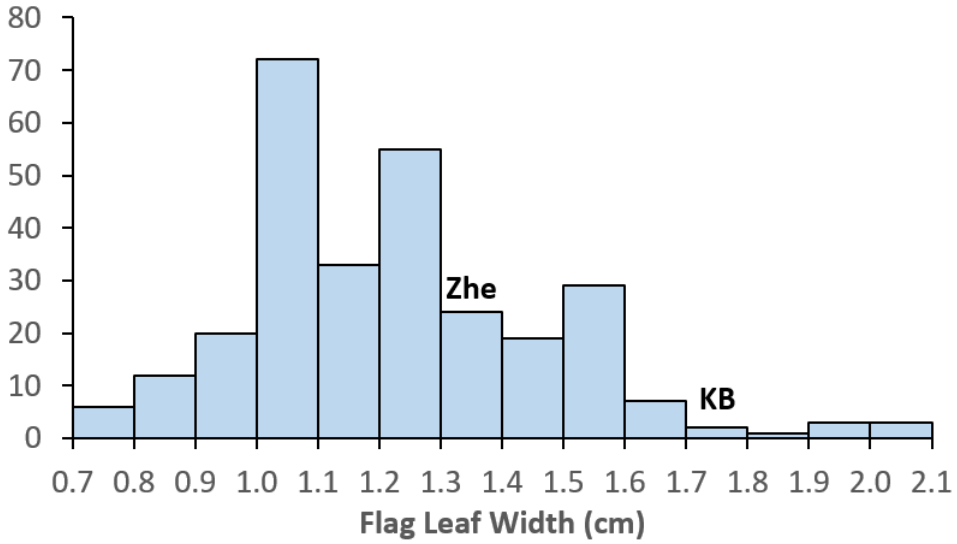

Figure 2.14. Frequency distribution of flag leaf width in the K/Z RIL population under DS conditions.

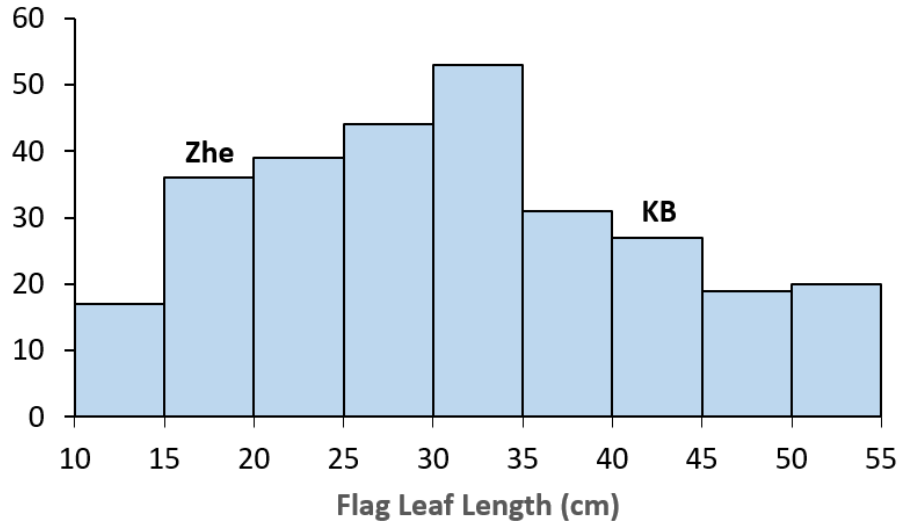

Figure 2.15. Frequency distribution of flag leaf length in the K/Z RIL population under DS conditions.

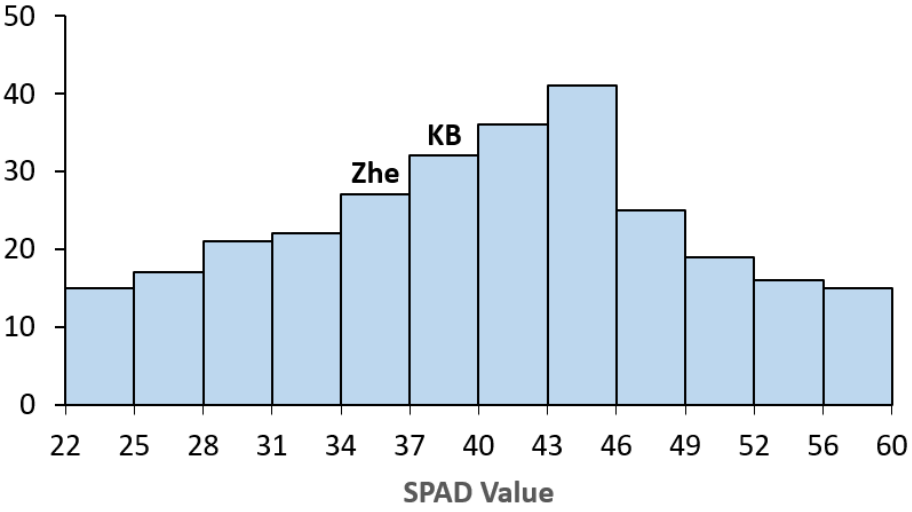

Figure 2.16. Frequency distribution of estimated chlorophyll content (SPAD) in the K/Z RIL population under DS conditions.

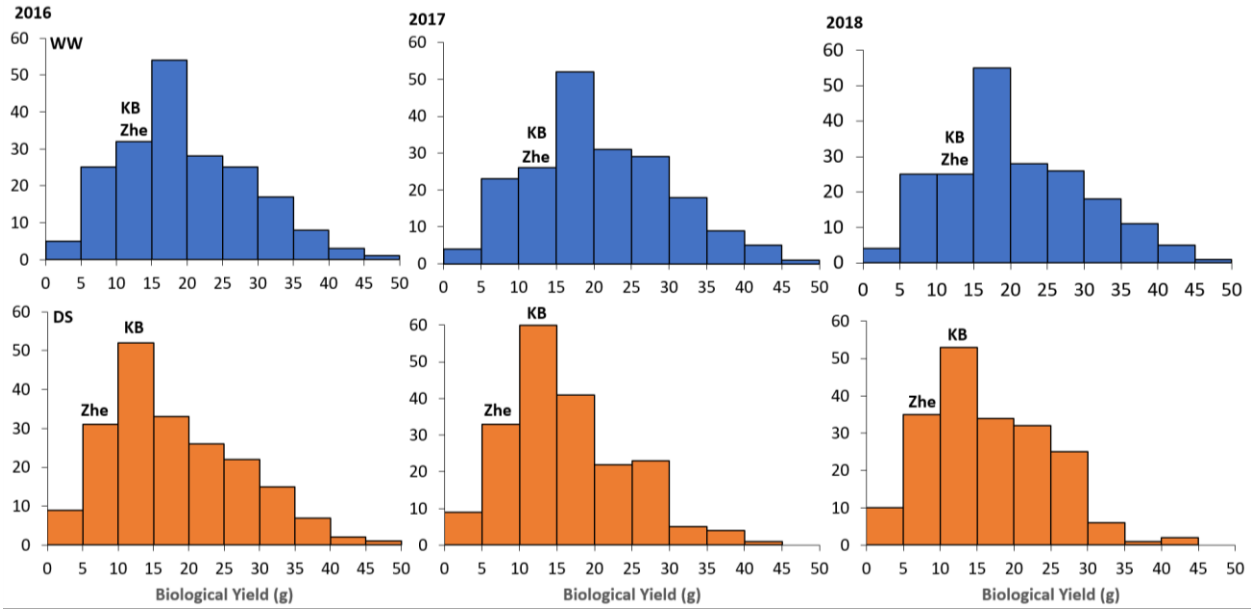

Figure 2.17. Frequency distribution of biological yield in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

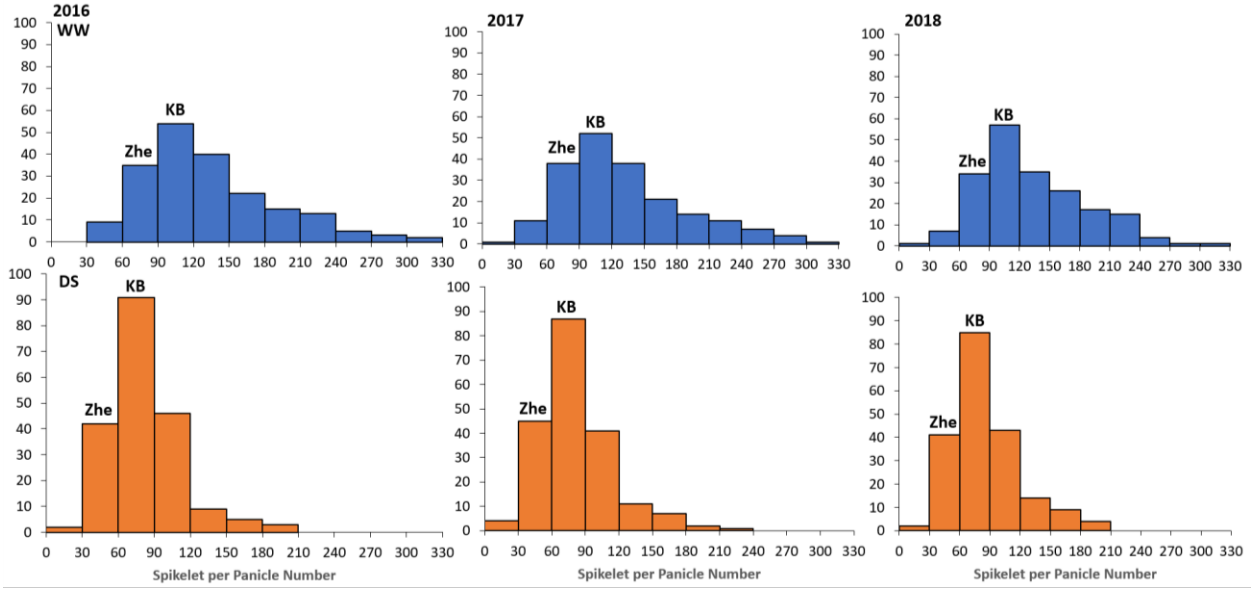

Figure 2.18. Frequency distribution of spikelet per panicle number in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

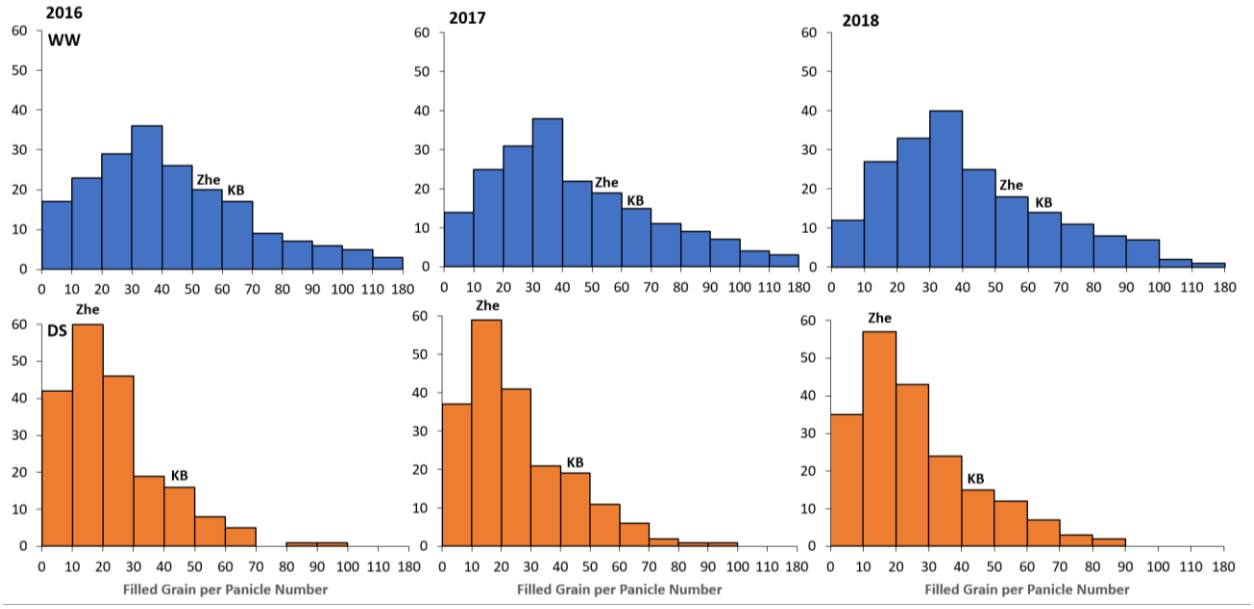

Figure 2.19. Frequency distribution of filled grain per panicle number in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

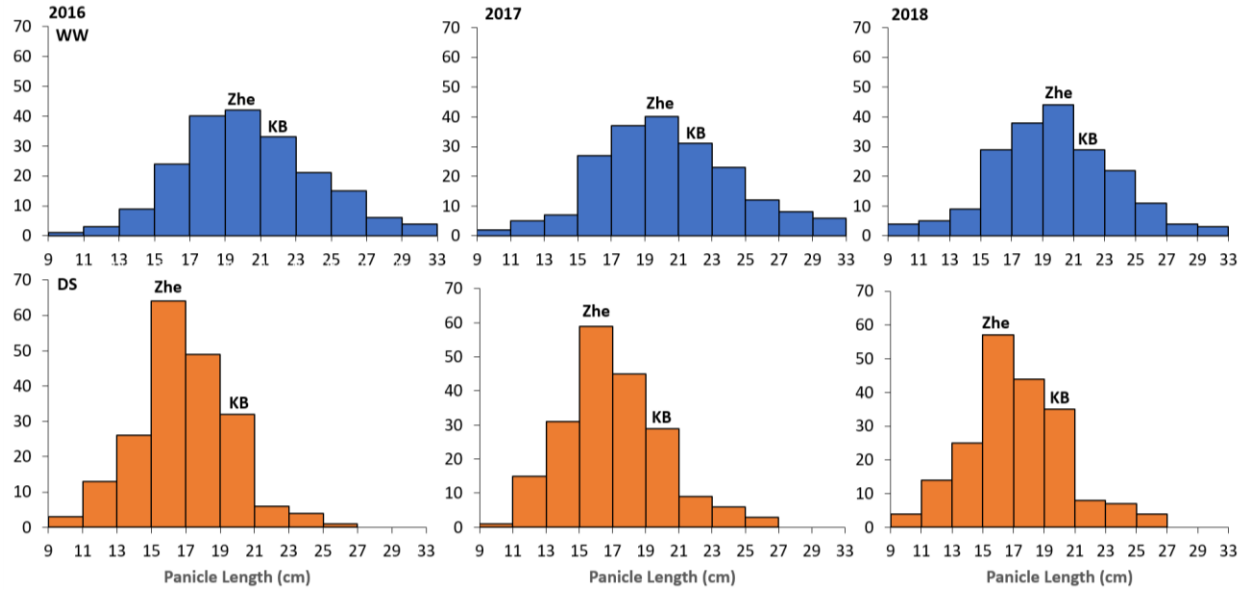

Figure 2.20. Frequency distribution of panicle length in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

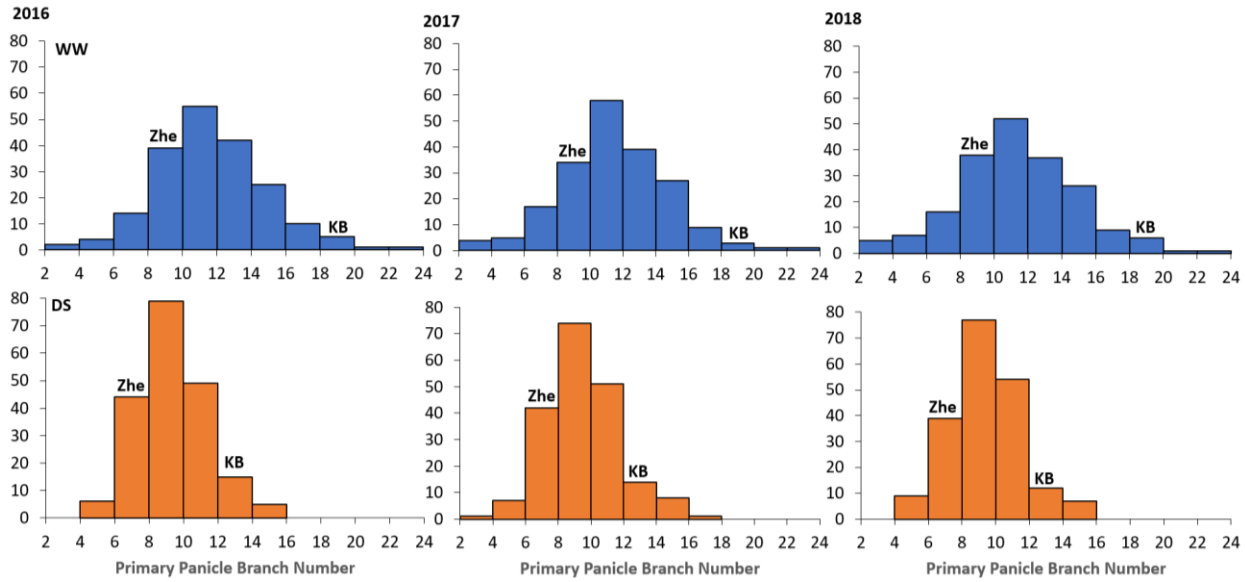

Figure 2.21. Frequency distribution of primary panicle branch number in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

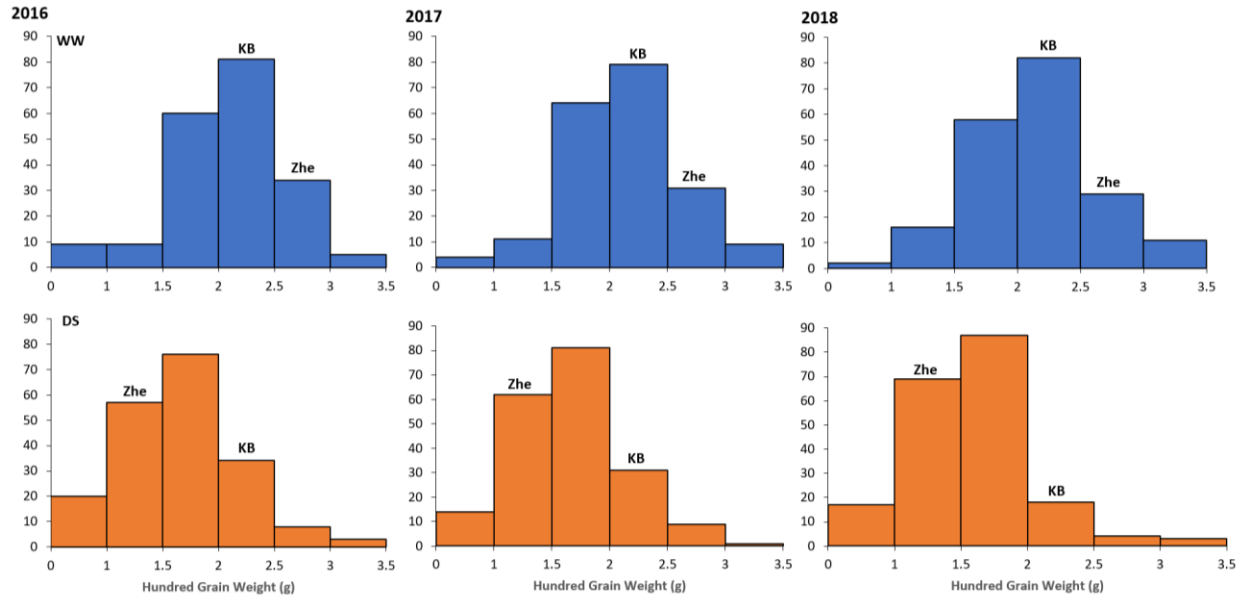

Figure 2.22. Frequency distribution of hundred grain weight in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

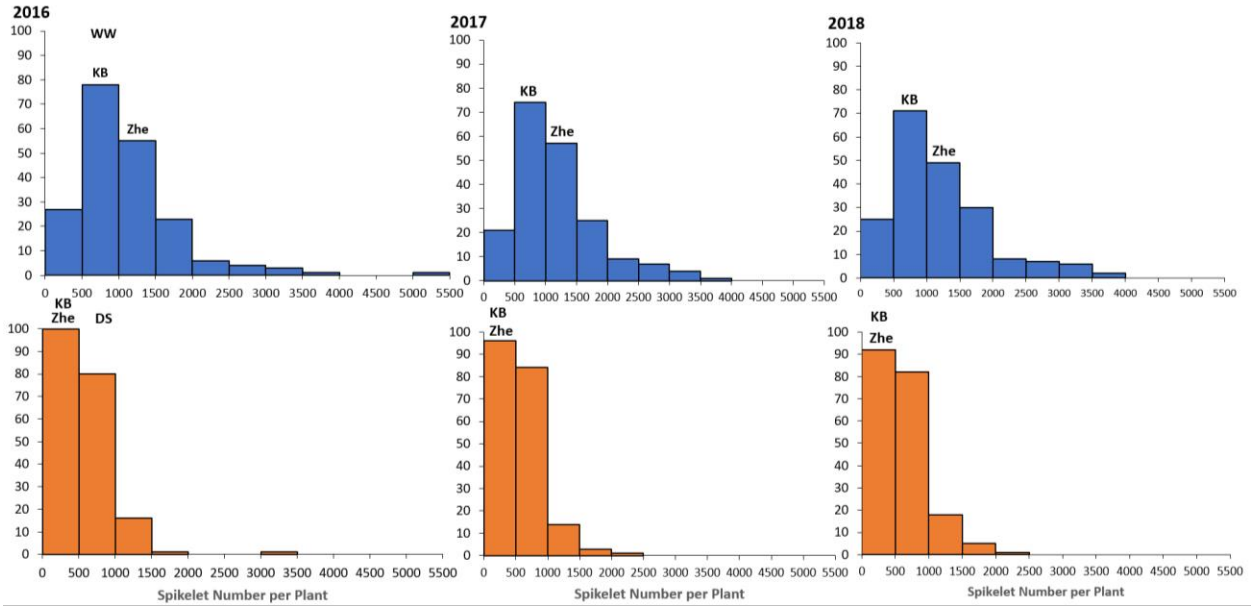

Figure 2.23. Frequency distribution of spikelet number per plant in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

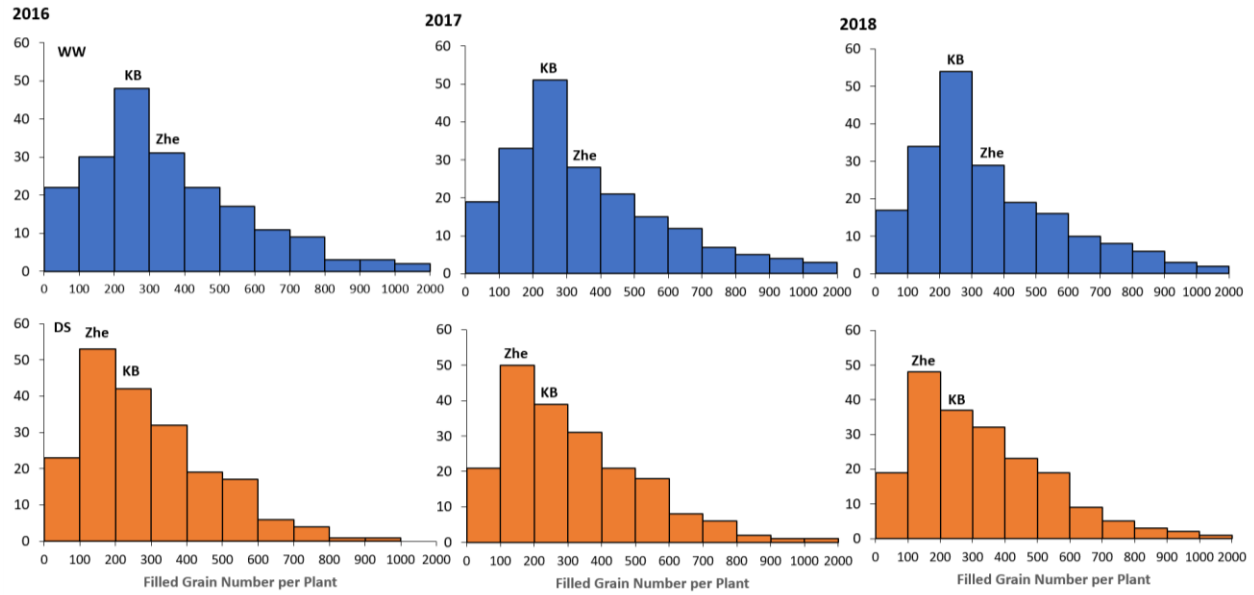

Figure 2.24. Frequency distribution of filled grain number per plant in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

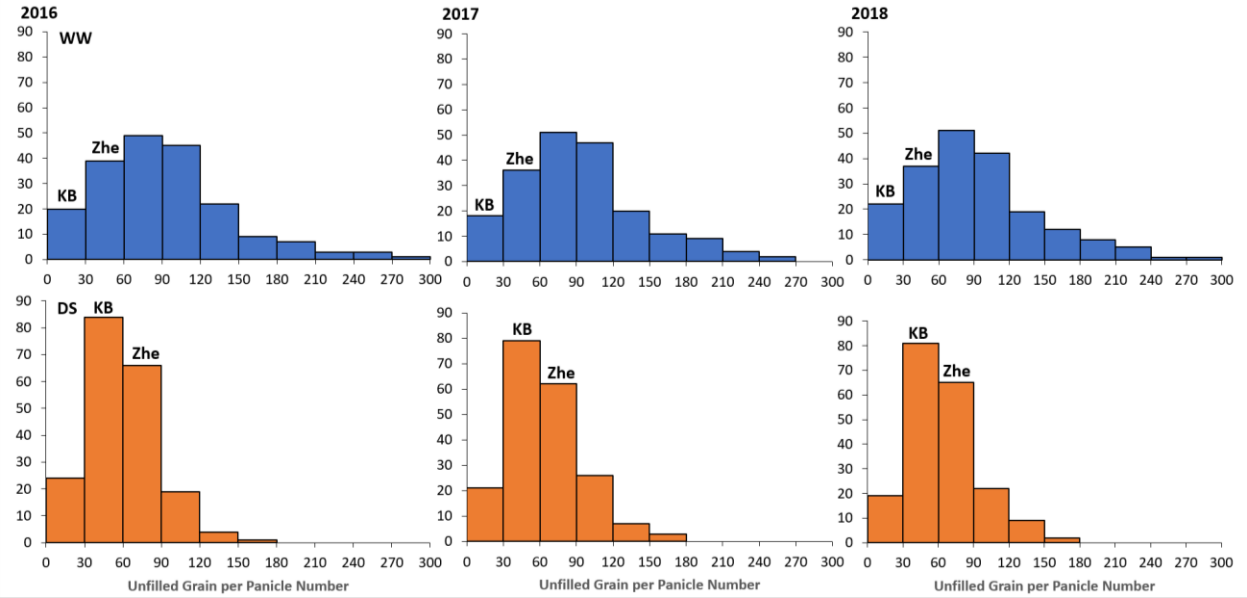

Figure 2.25. Frequency distribution of unfilled grain per panicle number in the K/Z RIL population under WW and DS conditions in 2016, 2017, and 2018 growing seasons.

Figure 2.26. Pearson's correlation coefficients shown in correlation matrix heat map between grain yield components and morphological traits of the K/Z RIL population under WW & DS conditions. The correlations (-1 to + 1) are colored either in blue (positive correlation) or in red (negative correlation). HD, Heading day under WW and DS; TN-WW, Productive tiller number under WW; TN-DS, Productive tiller number under DS; PH-WW, Plant height (cm) under WW; PH-DS, Plant height (cm) under DS; FLW-DS, Flag leaf width (cm) under DS; FLL-DS, Flag leaf length (cm) under DS; SV-DS, Estimated chlorophyll content (SPAD value) under DS; DLR-DS, Drought leaf rolling under DS; BY-WW, Biological yield (g) under WW; BY-DS, Biological yield (g) under DS; PL-WW, Panicle length (cm) under WW; PL-DS, Panicle length (cm) under DS; PPB-WW, Primary panicle branch number under WW; PPB-DS, Primary panicle branch number under DS; SP-WW, Spikelet per panicle number under WW; SP-DS, Spikelet per panicle number under DS; FG-WW, Filled grain per panicle number under WW; FG-DS, Filled grain per panicle number under DS; UFG-WW, Unfilled grain per panicle number under WW; UFG-DS, Unfilled grain per panicle number under DS; HGW-WW, Hundred grain weight (g) under WW; HGW-DS, Hundred grain weight (g) under DS; SPP-WW, Spikelet number per plant under WW; SPP-DS, Spikelet number per plant under DS; FGP-WW; Filled grain number per plant under WW; FGP-DS, Filled grain number per plant under DS.

Tables

Table 2.1. The average and range values of morphological traits and grain yield components of the K/Z RIL population under WW and DS conditions.

Traits	Treat-ments	Kaybonnet			ZHE733			K/Z RIL Population						H^2 (%)
		2016	2017	2018	2016	2017	2018	Average			Range			
								2016	2017	2018	2016	2017	2018	
Plant height (cm)	WW	98a	96a	100a	51a	53a	55a	60.03a	63.51a	65.89a	28-98	26-97	29-98	91
	DS	84b	81b	85b	31b	34b	32b	29.99b	31.72b	34.95b	10-73	11-74	14-70	89
Tiller number	WW	5a	4a	5a	8a	10a	9a	8.11a	8.40a	8.74a	3-22	2-21.60	3.60-21.4	90
	DS	4b	4b	4b	7a	8a	7a	6.98a	7.10a	7.08a	2-19	2-20.70	2.10-19.2	87
Biological yield	WW	14.80a	15.63a	15.25a	13.4a	14.72a	15.91a	20a	21.4a	21.5a	4-49	3.5-48	4.6-48	86
	DS	14.10a	13.52a	13.76a	6.50b	8.01b	8.72b	17.50a	16.3a	16.7a	2-47	1.7-40	2.1-40.2	81
Spikelet per panicle number	WW	104a	117a	125a	90a	101a	103a	133.35a	141.21a	148.14a	45-328	25-321	27-317	84
	DS	74b	81b	88b	55b	63b	71b	81.68b	89.71b	97.02b	26.8-188.4	18-239	22-209	79
Filled grain per panicle number	WW	66a	63a	69.7a	43.2a	40.1a	41.5a	43.07a	47.81a	49.74a	2-176	5-171	7-179	82
	DS	50a	47a	53a	16b	14b	12b	22.56b	25.72b	29.67b	0-97	0-91	0-86	79
Panicle length (cm)	WW	21.7a	22.9a	19.5a	18.6a	16.9a	16.2a	21.24a	23.47a	22.01a	11.90-33.30	11.50-32.70	10.61-30.72	80
	DS	19.3a	18.5a	19.1a	15.6a	13.1a	13.6a	17.88a	18.07a	17.08a	11.64-26.62	10.12-27.51	9.61-26.21	77
Primary panicle branch number	WW	19a	21a	18a	8a	9a	11a	11.17a	12.57a	11.85a	3-22	2-23	3-23	79
	DS	13b	14b	12b	7a	8a	9a	9.28a	8.69a	9.61a	5.4-15.6	2-17	4-15	77
Hundred grain weight (g)	WW	2.55a	2.87a	2.37a	2.63a	2.98a	2.59a	2.05a	2.28a	2.42a	0-3.19	0-3.27	0-3.33	85
	DS	2.33a	2.64a	2.19a	1.31b	1.68b	1.29b	1.17b	1.09b	1.38b	0-3.08	0-3.14	0-3.21	82
Spikelet number per plant	WW	520a	547a	562a	1016a	1089a	1117a	1098.14a	1124a	1091a	225-5390	281-3995	257-3972	85
	DS	296b	302b	316b	382.2b	427b	442b	574.21b	592b	561b	98.40-3321.20	71.92-2491	87.29-2489	81
Filled grain number per plant	WW	264a	281a	247a	302a	341a	327a	353.15a	389.27a	365.22a	18-1936	25-1832	15-1957	81
	DS	201b	228b	189b	111b	152b	141b	272.81b	291.42b	276.72b	0-950	0-1115	0-1231	78

Table 2.2. Effects of drought stress on morphological traits and grain yield components exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity in the K/Z RIL population of 198 lines in 2016, 2017, and 2018 growing seasons.

RILs	Plant Height			Tiller Number			Biological Yield			Spikelet per Panicle Number			Filled Grain per Panicle Number			Panicle Length			Primary Panicle Branch Number			Hundred Grain Weight			Spikelet Number per Plant			Filled Grain Number per Plant			Leaf Rolling Score		
	H*	M*	S*	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	2**	3**	4**
Kay-bonnet	V			V			V			V			V			V			V			V			V			V			V		
ZHE733		V		V			V					V			V	V				V				V			V			V			V
100001		V			V		V			V					V	V			V					V			V	V				V	
100002		V		V	V		V			V			V			V			V			V			V			V					V
100005	V			V			V				V		V			V			V			V				V			V			V	
100006	V			V			V					V	V			V				V				V			V	V				V	
100007	V			V			V			V			V			V			V			V			V				V				V
100008	V			V			V			V					V	V			V					V		V		V				V	
100009		V		V				V		V			V			V			V			V			V			V				V	
100010	V					V			V		V				V	V					V	V					V	V				V	
100012	V				V		V			V					V	V			V			V				V		V				V	
100014	V				V		V				V		V			V			V			V					V	V				V	
100015	V				V		V				V				V	V			V			V					V	V					V
100016			V	V			V			V			V			V			V			V				V		V				V	
100017	V			V			V				V				V	V			V			V					V	V			V		
100018		V		V			V				V		V			V			V			V				V		V				V	
100019		V			V			V			V				V	V			V			V					V	V					V
100020			V	V				V		V					V	V				V		V			V			V				V	
100021			V	V				V		V			V			V			V			V			V			V			V		
100022		V		V			V				V				V	V			V				V		V			V			V		
100023			V	V			V					V	V			V			V			V					V	V			V		
100024			V	V			V			V					V	V			V				V			V		V				V	
100025		V		V			V				V		V			V			V			V				V		V					V

Table 2.2. Effects of drought stress on morphological traits and grain yield components exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity in the K/Z RIL population of 198 lines in 2016, 2017, and 2018 growing seasons (Continued).

RILs	Plant Height			Tiller Number			Biological Yield			Spikelet per Panicle Number			Filled Grain per Panicle Number			Panicle Length			Primary Panicle Branch Number			Hundred Grain Weight			Spikelet Number per Plant			Filled Grain Number per Plant			Leaf Rolling Score		
	H*	M*	S*	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	2**	3**	4**
100026			V	V				V		V				V		V			V			V				V			V			V	
100027	V			V			V			V					V	V			V			V				V		V				V	
100028	V			V				V			V			V		V				V		V					V		V			V	
100029			V	V			V					V			V	V			V			V				V				V		V	
100030		V		V			V			V					V	V			V					V		V				V			V
100032			V	V				V		V				V		V			V				V		V				V			V	
100033		V				V	V			V					V	V			V			V					V	V				V	
100034		V				V		V				V		V		V				V		V					V		V			V	
100036		V		V			V			V			V			V			V			V				V		V				V	
100038	V				V		V			V					V	V			V			V				V		V				V	
100039		V		V				V		V					V	V			V			V			V			V				V	
100040	V				V			V			V			V		V				V		V					V		V			V	
100042	V			V			V			V					V	V			V					V		V			V			V	
100043				V					V	V					V	V			V			V			V					V			V
100046			V			V		V			V				V	V			V			V					V	V					V
100048		V		V			V				V				V	V			V			V					V			V		V	
100049			V	V			V				V				V	V			V					V			V	V			V		
100050			V	V				V			V			V		V			V			V				V			V			V	
100053	V					V	V			V			V			V			V			V					V	V				V	
100055			V	V				V			V				V		V		V			V				V		V				V	
100056	V				V		V					V			V	V				V		V					V	V				V	
100057	V			V			V			V					V	V			V			V			V			V					V
100058		V		V				V		V				V		V			V			V			V				V			V	
100062	V			V				V		V					V	V			V			V			V					V		V	
100064	V			V				V				V		V		V			V			V					V		V			V	
100065			V		V		V					V			V		V			V			V				V	V					V
100066			V			V		V				V		V		V			V			V					V		V		V		
100067	V				V				V			V			V	V			V			V					V	V			V		
100086			V	V			V					V			V		V			V				V			V			V			V

Table 2.2. Effects of drought stress on morphological traits and grain yield components exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity in the K/Z RIL population of 198 lines in 2016, 2017, and 2018 growing seasons (Continued).

RILs	Plant Height			Tiller Number			Biological Yield			Spikelet per Panicle Number			Filled Grain per Panicle Number			Panicle Length			Primary Panicle Branch Number			Hundred Grain Weight			Spikelet Number per Plant			Filled Grain Number per Plant			Leaf Rolling Score		
	H*	M*	S*	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	2**	3**	4**
100092			V	V					V			V			V	V			V			V				V				V	V		
100096			V	V				V				V		V		V			V			V					V		V			V	
100097		V		V			V			V			V			V			V			V				V		V				V	
100098	V			V				V		V					V	V			V			V				V		V				V	
100102	V			V					V		V				V	V			V			V				V		V				V	
100106		V			V				V		V				V	V			V			V					V	V				V	
100107	V			V			V					V			V	V			V			V					V	V				V	
100108			V	V				V			V			V		V			V				V		V				V		V		
100114	V			V			V				V				V	V			V					V			V		V			V	
100115		V		V				V			V			V		V			V			V				V			V				V
100118			V		V				V	V					V	V			V					V			V		V			V	
100119	V				V			V			V				V	V			V				V				V			V		V	
100120			V	V			V			V					V	V			V			V			V			V					V
100121		V		V			V			V			V			V			V				V			V				V			V
100122			V	V			V			V					V	V			V					V	V			V					V
100123	V			V				V			V				V	V			V			V					V			V			V
100126			V	V				V			V				V	V			V				V			V				V			V
100129	V			V				V			V			V		V			V			V					V		V		V		
100130		V			V		V			V					V	V			V			V					V	V				V	
100131	V			V			V			V					V	V			V				V		V			V			V		
100133			V		V		V					V	V			V			V			V					V	V					V
100134		V		V			V				V				V	V			V			V			V			V			V		
100135	V			V			V			V			V			V			V			V				V		V				V	
100137	V			V				V		V					V	V			V			V			V			V					V
100139			V	V			V					V	V				V			V		V					V	V			V		
100141			V	V					V		V				V	V				V				V		V			V				V
100142		V			V				V		V				V	V			V			V					V	V					V
100144			V	V				V			V			V		V			V			V			V				V		V		

Table 2.2. Effects of drought stress on morphological traits and grain yield components exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity in the K/Z RIL population of 198 lines in 2016, 2017, and 2018 growing seasons (Continued).

RILs	Plant Height			Tiller Number			Biological Yield			Spikelet per Panicle Number			Filled Grain per Panicle Number			Panicle Length			Primary Panicle Branch Number			Hundred Grain Weight			Spikelet Number per Plant			Filled Grain Number per Plant			Leaf Rolling Score		
	H*	M*	S*	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	2**	3**	4**
100145			V	V			V					V			V	V				V			V				V	V				V
100146			V			V	V			V					V	V			V			V				V		V				V	
100149			V	V				V			V				V	V			V			V				V		V			V		
100150			V	V			V				V				V		V		V			V					V			V	V		
100151	V			V			V				V				V	V			V			V				V		V			V		
100153		V		V			V				V				V	V			V			V				V				V		V	
100154	V				V		V			V					V	V			V					V	V			V				V	
100155			V	V			V			V					V	V				V		V			V			V			V		
100156			V	V			V			V					V		V		V					V		V		V			V		
100158			V			V		V		V					V	V			V			V					V	V			V		
100160			V	V			V					V			V		V			V					V			V				V	
100162			V	V			V				V		V			V				V		V			V			V			V		
100163		V		V			V			V			V			V					V	V				V		V					V
100164			V		V		V					V			V			V			V				V			V				V	
100169			V	V				V		V				V		V			V			V			V				V		V		
100170			V	V			V					V			V		V			V					V			V				V	
100171			V	V			V					V			V	V				V		V						V	V			V	
100172			V	V			V					V			V		V				V				V			V		V		V	
100175		V		V			V					V			V	V				V					V			V	V			V	
100176	V			V			V			V					V			V			V	V			V			V					V
100178			V	V					V	V					V	V			V						V	V			V					V
100179			V		V				V		V				V	V				V		V					V	V				V	
100180			V	V			V					V			V		V			V					V			V			V		
100182			V	V			V			V					V	V			V						V	V			V				V	
100185			V	V			V			V					V	V			V						V	V				V			V	
100188			V	V			V				V				V	V			V			V				V				V	V		
100191			V	V				V				V			V	V				V			V				V	V					V
100193			V	V			V					V			V		V			V					V	V			V				V	

Table 2.2. Effects of drought stress on morphological traits and grain yield components exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity in the K/Z RIL population of 198 lines in 2016, 2017, and 2018 growing seasons (Continued).

RILs	Plant Height			Tiller Number			Biological Yield			Spikelet per Panicle Number			Filled Grain per Panicle Number			Panicle Length			Primary Panicle Branch Number			Hundred Grain Weight			Spikelet Number per Plant			Filled Grain Number per Plant			Leaf Rolling Score		
	H*	M*	S*	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	2**	3**	4**
100196			V	V			V			V					V	V				V				V	V			V			V		
100197			V	V			V			V					V	V				V		V			V				V			V	
100198			V	V			V			V			V				V			V		V			V			V				V	
100200			V	V			V			V					V	V			V				V		V			V				V	
100201			V	V			V					V			V		V		V					V			V	V				V	
100202		V		V			V				V				V	V			V					V			V			V		V	
100203		V			V		V				V				V	V				V			V				V	V				V	
100208			V	V			V			V					V	V			V			V			V			V				V	
100209	V				V		V					V			V	V					V	V					V			V		V	
100210	V			V				V		V					V	V			V			V			V			V				V	
100211			V	V			V				V				V		V		V					V			V	V					V
100212		V		V				V				V		V		V				V		V					V		V				V
100213		V		V			V			V					V	V			V					V		V				V		V	
100214			V	V			V					V			V	V				V				V			V			V		V	
100217			V	V			V				V				V	V			V				V			V				V		V	
100220			V	V			V					V			V			V			V			V			V	V				V	
100222			V	V			V				V				V	V			V			V				V		V				V	
100223			V	V			V			V					V	V			V				V		V			V				V	
100224	V			V				V		V					V	V			V					V	V			V				V	
100225			V	V			V				V				V	V				V			V			V				V		V	
100228			V	V			V			V					V	V			V			V			V			V				V	
100230			V		V			V			V				V	V			V			V					V			V		V	
100231			V			V			V	V					V	V			V					V			V			V		V	
100233		V			V		V			V			V			V				V		V				V		V				V	
100234			V	V			V			V					V	V			V			V			V				V			V	
100237			V	V			V			V					V	V			V				V		V					V		V	
100238		V				V	V			V					V	V			V			V					V			V	V		
100239		V		V					V	V					V	V			V					V		V		V				V	

Table 2.2. Effects of drought stress on morphological traits and grain yield components exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity in the K/Z RIL population of 198 lines in 2016, 2017, and 2018 growing seasons (Continued).

RILs	Plant Height			Tiller Number			Biological Yield			Spikelet per Panicle Number			Filled Grain per Panicle Number			Panicle Length			Primary Panicle Branch Number			Hundred Grain Weight			Spikelet Number per Plant			Filled Grain Number per Plant			Leaf Rolling Score		
	H*	M*	S*	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	2**	3**	4**
100240		V		V				V			V				V	V				V				V	V			V			V	
100241			V	V			V				V				V	V			V					V	V					V		V	
100242	V			V				V				V		V		V				V		V				V			V			V	
100245		V		V				V			V			V		V			V				V				V			V			V	
100246		V				V	V					V			V			V			V		V				V	V				V	
100249			V			V	V			V					V	V			V					V			V	V				V	
100250			V	V			V					V			V	V			V					V		V		V				V	
100251		V		V			V			V					V	V			V				V		V			V			V		
100253		V		V			V			V					V	V			V			V			V			V				V	
100254	V			V			V				V				V	V			V			V					V	V				V	
100255			V	V			V				V				V	V				V		V					V			V			V
100256			V	V			V			V					V	V			V					V		V			V			V	
100259		V		V			V				V				V	V			V					V	V			V			V		
100263			V	V			V				V				V	V			V			V					V	V				V	
100265			V	V				V			V			V			V			V		V				V			V			V	
100266			V		V		V					V			V	V				V				V			V	V				V	
100272		V			V		V			V					V	V			V					V		V		V				V	
100273		V			V			V		V					V	V			V					V		V		V				V	
100277		V			V		V					V			V		V			V					V			V	V			V		
100280			V	V			V			V					V	V			V					V	V			V					V
100281			V	V				V			V				V	V			V					V	V				V			V	
100282			V			V			V	V					V	V			V					V			V			V		V	
100283			V	V			V				V				V	V			V					V		V		V				V	
100284			V			V	V			V					V	V			V					V			V	V				V	
100285			V	V			V					V			V		V		V			V				V				V			V	
100288			V	V				V				V			V		V				V			V			V			V			V	
100292			V	V			V			V					V	V			V			V				V			V			V	
100293			V		V		V					V			V	V			V			V					V	V				V	

Table 2.2. Effects of drought stress on morphological traits and grain yield components exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity in the K/Z RIL population of 198 lines in 2016, 2017, and 2018 growing seasons (Continued).

RILs	Plant Height			Tiller Number			Biological Yield			Spikelet per Panicle Number			Filled Grain per Panicle Number			Panicle Length			Primary Panicle Branch Number			Hundred Grain Weight			Spikelet Number per Plant			Filled Grain Number per Plant			Leaf Rolling Score		
	H*	M*	S*	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	2**	3**	4**
100295			V			V		V				V		V		V			V					V			V		V		V	
100298			V	V			V				V				V		V		V			V			V			V				V	
100299			V	V			V				V				V	V			V					V		V		V				V	
100300			V	V			V				V				V	V			V				V				V		V			V	
100302		V		V			V				V				V	V			V			V			V			V				V	
100303			V	V			V			V					V	V			V					V	V			V				V	
100308	V				V		V					V			V	V			V			V					V	V				V	
100310		V			V		V					V	V			V			V			V					V	V				V	
100311		V		V			V				V				V		V			V			V				V			V			V	
100313			V			V	V			V					V	V			V					V			V	V				V	
100315			V			V		V				V			V		V				V			V			V		V			V	
100319			V	V				V			V				V	V			V					V			V	V				V	
100321			V	V			V			V			V			V			V			V			V			V				V	
100322			V	V			V				V				V	V			V					V		V				V		V	
100323			V	V			V				V				V	V			V					V		V		V				V	
100324			V			V		V			V				V	V				V				V			V		V			V	
100325			V	V			V					V			V			V			V			V			V	V				V	
100327			V		V		V				V				V	V			V					V			V	V				V	
100328			V	V			V					V			V		V			V				V		V		V				V	
100329			V	V			V				V				V	V			V					V		V		V					V
100330			V		V		V				V		V			V				V		V					V	V				V	
100333			V	V			V				V				V	V			V			V			V			V					V
100334		V		V				V				V		V			V				V	V					V		V			V	
100335		V				V	V					V			V		V				V	V					V			V		V	
100336			V	V			V					V			V	V			V					V	V			V			V		
100337			V	V			V				V		V			V			V					V		V		V					V
100338			V			V	V			V					V		V		V					V			V	V				V	
100339			V	V			V			V					V	V			V			V			V					V		V	

Table 2.2. Effects of drought stress on morphological traits and grain yield components exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity in the K/Z RIL population of 198 lines in 2016, 2017, and 2018 growing seasons (Continued).

RILs	Plant Height			Tiller Number			Biological Yield			Spikelet per Panicle Number			Filled Grain per Panicle Number			Panicle Length			Primary Panicle Branch Number			Hundred Grain Weight			Spikelet Number per Plant			Filled Grain Number per Plant			Leaf Rolling Score		
	H*	M*	S*	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	H	M	S	2**	3**	4**
100340			V	V			V				V				V	V			V			V					V	V			V	
100341			V	V					V			V			V	V					V			V		V		V			V	
100342			V	V			V					V			V	V				V				V			V	V			V	
100344			V		V		V			V					V	V			V					V	V				V		V	
100345			V			V	V				V				V		V		V			V					V			V		V
100348			V	V				V		V					V	V			V			V			V					V	V	
100351		V		V				V			V			V		V			V					V		V			V		V	
100352			V			V	V			V					V	V			V				V			V		V			V	

(*) H = Highly drought resistant lines (0-29% reduction), M = Moderately drought resistant lines (30-49% reduction), S = Drought sensitive lines (≥50% reduction)

(**) 2 = Score 2.0 - 2.9, 3 = Score 3.0 - 3.9, 4 = Score 4.0 - 4.9

Table 2.3. Percentage of the K/Z RIL population exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity of 198 lines in 2016, 2017, and 2018 growing seasons.

Drought Resistant Category	Percentage Highly drought resistant lines (0-29% reduction)	Percentage Moderately drought resistant lines (30-49% reduction)	Percentage Drought sensitive lines (≥50% reduction)
Plant height	19.7	23.7	56.6
Tiller number	72.2	16.6	11.1
Biological yield	67.7	24.7	7.6
Spikelet per panicle number	39.9	35.3	24.7
Filled grain per panicle number	13.13	11.11	75.75
Panicle length	84.3	13.1	2.5
Primary panicle branch number	72.7	20.2	7.1
Hundred grain weight	54.04	11.11	34.84
Spikelet number per plant	27.8	28.8	43.4
Filled grain number per plant	14.2	12.5	73.3

CHAPTER 3. PHYSIOLOGICAL RESPONSE OF THE K/Z RIL RICE POPULATION TO ABSCISIC ACID TREATMENTS

Abstract

Rice is a major source of food for more than half the world's population, with almost 90% of total rice in the world produced and consumed by Asian countries. Drought due to water shortage in soil causes osmotic stress and is one of the major constraints to extending rice production. Polyethylene glycol (PEG) induces osmotic stress on rice plants, which simulates some aspects of the drought stress conditions. The plant hormone abscisic acid (ABA) plays an important role in signaling responses to environmental stress, including drought. Under reduced water conditions, ABA triggers stomatal closure in order to lessen transpiration and enhance water conservation leading to drought resistance (Lim et al., 2015). Roots can be screened for their sensitivity to ABA, which reflects their stress response (Park et al., 2016), where ABA hypersensitive lines show induction of stomatal closure by exposure to ABA (e.g. 3 µM). The objective is to evaluate the ABA response of the K/Z RIL population on root architectural traits in relation to drought stress resistance. The 'K/Z' RIL population of 198 lines, derived from varieties Kaybonnet (Drought Resistant) and ZHE733 (Drought Sensitive), were used for molecular genetic analysis of the drought response pathways. This K/Z RIL population was screened for drought stress in the field under drought stress (DS) and well-watered (WW) conditions, on PEG media (0 MPa, -0.5 MPa, and -1.2 MPa), and for ABA sensitivity (0 µM, 3 µM, and 5 µM). The effect of drought stress in the field was quantified by calculating the filled grains per panicle number (FG). Drought stress effect in PEG media and ABA sensitivity were quantified by measuring root architectural traits: root length (RL), root to shoot ratio (RSR), total root number (TRN), number of roots with a shallow angle (SRN), number of roots with a deep angle (DRN), and root fresh weight (RFW). Kaybonnet and 48 drought resistant lines examined under control conditions display more FG, longer RL, higher RSR, more DRN, and heavier RFW

compared to ZHE733 and 150 drought sensitive lines. Under exogeneous ABA treatment in root media, Kaybonnet and 48 drought resistant lines exhibited an ABA-sensitive phenotype. In the presence of ABA; the RL, RSR, TRN, SRN, DRN, and RFW were significantly reduced, implying that Kaybonnet and the 48 drought resistant lines regulate osmotic stress tolerance via ABA-mediated cell signaling. This study provides the necessary genetic information and phenotype details that can be used to develop drought resistant rice plants from the populations studied

Introduction

Rice (*Oryza sativa* L.) is the primary dietary staple food for most of the population in the world that provides 20% calories and 15% protein of the human diet (IRRI, 2002). This crop is an annual plant and belongs to the family Poaceae and genus *Oryza*, and species *sativa* which is further classified into two major subspecies, *indica* and *japonica*. Rice is cultivated in a wide range of latitude from 53^{o} North to 40^{o} South (Mae et al., 1997). In 2018, the world-wide rice production was 782 million tons (FAOSTAT, 2020), Asia producing and consuming about 92% of the world's rice, with China, India, and Indonesia producing and consuming the most (FAO, 2001). U.S. is the third largest exporter of rice after Thailand and Vietnam, and produces about 1.5% of the world's rice crop, and Arkansas the largest rice-producer, accounting for more than 40% of U.S. rice production of long and medium grain varieties (Quick Stats, 2016). According to Chopra and Prakash (2002), based on the acreages of the irrigation systems, most of the rice world-wide (57%) is growing under flooded irrigation, 25% on rainfed lowland, 10% on the uplands, 6% in deep-water, and 2% in tidal wetlands, with drought being a major constraint in rice production. The rice plant uses 30% of the world's freshwater resources world-wide, using 2-3 times more water than the other crops. However, drought threat is increased due to the increasing demands of water for urban and industrial uses (Venuprasad et al., 2008). At present, the world-wide's rice production needs to be increased 40% to feed the world population by 2025 (FAOSTAT, 2002).

Drought conditions reduce the rice yields by more than 30%, that is equivalent to a loss of US$ 800 million annually. Carlos et al. (2008) reported that the water deficit conditions also decrease the grain quality. The reduction of grain yield under drought conditions depends on the growth stage of the rice plants, with the most sensitive being the reproductive stage, due to drought induced pollen sterility and decrease in photosynthetic rate, resulting in the reduction of

the filled grain per panicle number and the grain weight (O'Toole, 1982). Photosynthetic rate reduction under drought conditions is influenced by stomatal and non-stomatal factors (Flexas and Medrano, 2002). Previous studies indicate that drought stress causes stomatal closure, which subsequently decreases the transpiration rate and carbon dioxide uptake (de Souza et al., 2013).

Rice plants are mostly grown in flooded areas under anaerobic soil conditions. However, root cells still need oxygen for growing, by developing aerenchyma in the roots and shoot in order to diffuse oxygen from the photosynthetic tissue to the roots and also into the rhizosphere (Ando et al., 1983). Roots are also the first plant organ in contact with water stress in the soil, responsible to absorb water and nutrients (Comas et al., 2013), and consequently are important in drought adaptation of the rice plants (Baldoni et al., 2015). Under drought stress conditions, most of the rice genotypes reduce their root growth. Gornall et al. (2010) suggested that semi-dwarf rice varieties showed sensitivity to drought stress conditions due to their shallow rooting system.

Levitt (1980) proposed that root system architecture is one of the primary factors of drought resistance mechanisms, especially in the drought avoidance category. Moreover, rice plants with a deeper and thicker root system help to avoid drought by their ability to penetrate better the hard soil pans under drought stress conditions, in order to absorb water and nutrients in the deeper layers of the soil (Babu et al., 2001; Comas et al., 2013; Smith and De Smet, 2012; Rich and Watt, 2013; Uga et al., 2013). Kitomi et al. (2015) proposed that genetic manipulation of the root system is important for increasing drought avoidance ability in rice plants. A quantitative trait locus (QTL) for depth and root angle, namely *dro-1* was mapped by Uga et al. (2013). Root architectural traits that are significantly important in drought avoidance mechanisms are root length, root angle, root diameter, number of crown roots, and lateral root density. Under drought conditions, a deeper and larger root system helps the rice plants to absorb

water from greater depths in the soil profile. Root diameter plays an important role in exploring water in a compact hard soil under drought conditions (Pagès et al., 2010). A steeper root angle allows the rice plants to take up water and nutrients from deeper in the soil under drought conditions. Crown roots are correlated with lodging resistance and increased surface area to take up water. Under water deficit conditions, a greater number of lateral roots will be able to absorb more water. In addition, root system architecture is an important character for screening drought resistant rice lines or genotypes. Amongst rice genotypes that exhibit different root system architecture, can also be found genetic variation for their response to drought.

Drought avoidance mechanisms allow several rice genotypes with deeper and larger root systems to survive under water deficit conditions. Upland rice having root system architecture with larger, thicker, and more lateral roots showed high water uptake capacity and performed better than lowland or flooded rice under water deficit (Ingram et al., 1994; Nguyen et al., 1997). Lowland rice varieties have shorter roots of about 5-10 cm due to their adaptation to growth under flooded conditions. Development of lowland rice varieties with deeper root systems, similar to the roots of the upland rice varieties, are needed to stabilize yield under unstable rainfall environments. Roots take up water and nutrients from the soils, and are an essential organ for optimal growth and development of plants. Root growth and development are therefore influenced by genetic and environmental factors (Fageria and Moreira, 2011; Fageria, 2013).

Under drought conditions, the root system architecture influences the rice plant function. For example, under mild drought conditions, the root growth is maintained, but shoot growth is reduced. Additionally, Fageria (2013) indicated that root growth and development exhibited a positive correlation with grain yield. Moreover, the high level and stability of grain yield under drought conditions is influenced by a well-developed root system, such as longer and thicker

roots. These longer and thicker roots contain more xylem vessels, and show high penetration ability to take up water and nutrients from the soil to the shoots (Nguyen et al., 1997; Clark et al., 2008). According to Lynch (2013) and Uga et al. (2013), the rooting depth is influenced by several components including total area of the root cross-section, stele area, cortex, the number of aerenchyma, cortical-cells, and xylem vessels. The stele area consisting of xylem and phloem vessels, determines the ability of the roots to retain water in the vascular tissues, due to the xylem which is responsible for water and nutrient transportation from the root to the shoot (Kondo et al., 2000; Fukai & Cooper, 1995). However, rice has limited water absorption below 60 cm. A number of previous studies characterized many root system traits that influenced drought resistance mechanisms (Fageria and Moreira, 2011; Feng et al., 2012). Eghball and Maranville (1993) identified that drought conditions affected the root structure by reduction in the root length and development of more lateral roots. Zhang et al. (2018) also stated that under drought conditions, the lateral roots of rice have more development. Several studies also reported that under drought conditions, some rice genotypes show a drought avoidance mechanism of developing deeper and thicker root systems with more branches and higher root to shoot ratio (Samson et al., 2002; Wang and Yamauchi, 2006; Gowda et al., 2011). Therefore, an understanding of the root physiology under drought conditions is important to develop drought resistant rice varieties.

Under drought conditions, roots deliver chemical signals to the shoot by producing Abscisic acid (ABA), cytokinin, malate, and other solutes (Schachtman & Goodger, 2008). These chemical signals, particularly ABA, mediate stomatal closure and transpiration (Dry et al., 1999). Drought in the soil causes osmotic stress to plants, followed by two pathways leading to stress adaptation of the plants; the ABA-dependent & ABA-independent pathways. ABA is a

plant hormone that is important in the response to environmental stresses, including drought. Under drought conditions, the endogenous content of ABA in rice plants increases, demonstrating the involvement of ABA in the drought resistance mechanisms (Hamayun et al., 2010). Besides ABA, other phytohormones that respond to the drought conditions are jasmonic acid, salicylic acid, gibberellins, auxins, and cytokinins, but their roles are somewhat less clear (Narusaka et al., 2003). A number of previous studies identified that ABA is an important component in the drought tolerance strategy by regulating stomatal closure (Ahmad et al., 2014), improving antioxidant enzymes (Latif, 2014; Li et al., 2014), increasing carbon metabolism and protein transport (Wang et al., 2007; Ye et al., 2011; Kumar et al., 2013). Under reduced water conditions, ABA triggers stomatal closure in order to lessen transpiration and enhance drought resistance (Lim et al., 2015). Teng et al. (2014) reported that application of exogenous ABA in the drought conditions increases the recovery of stomatal conductance, transpiration rate, and finally photosynthetic activity by inducing the expression of drought responsive genes. One of the drought responsive genes that is induced by ABA is PYR/PYL/RCARs (pyrabactin resistance/PYR1-LIKE/ regulatory components of ABA receptors) (Park et al., 2009) which is important in the regulation of SnRK2 kinase (sucrose nonfermenting1-related protein kinase 2) activity (Gonzalez-Guzman et al., 2012). This SnRK2 controlled stomatal closure is done by regulating the guard cell movement (Kim et al., 2010). According to Park et al. (2016), roots can be screened for sensitivity to ABA, distiguishing the stress response phenotypes. Furthermore, ABA sensitivity of roots can be an indicator of drought stress resistance in the rice plants.

Polyethylene glycol (PEG) induces rice osmotic stress which can simulate drought stress conditions in the culture medium (Handa et al., 1982; Bhasakaran et al., 1985; Newton et al., 1986; Newton et al., 1989; Dodd and Donovan, 1999; Sidari et al., 2008). Additionally, PEG is a

polyether compound, non-ionic, non-plasmolysing, water-soluble polymer, with structure H-(O-CH_2-CH_2)$_n$-OH, and has different molecular weights that show different viscosity because of the chain length effects (Kahovec et al., 2002). Generally, PEG has high molecular weights such as PEG 6000 and PEG 8000. These characteristics make PEG become one of the most useful molecules to create osmotic pressure that simulates drought conditions in biochemical-based experiments. A number of previous studies used PEG to simulate drought stress conditions in several crops such as rice (Agrawal et al., 2016; Xiong et al., 2014; Teng et al., 2014; Kano et al., 2011; Basu et al., 2010; Shehab et al., 2010; Sato & Yokoya, 2008; Pandey et al., 2004; Al-Bahrany et al., 2002), soybean (Hamayun et al., 2010), wheat (Pei et al., 2010), corn (Khodarahmpour et al., 2011), sorghum (Tsago et al., 2014), sugarcane (Patade et al., 2009), chickpea (Saglam et al., 2014), pigeonpea (Kumar et al., 2011), common bean (Büyük et al., 2017), ground nut (Venkateswarlu & Ramesh, 1993), Brassica species (Alam et al., 2014), and *Stevia rebaudiana* (Hajihashemi et al., 2016). Drought stress conditions induced by PEG influence serious physiological, biochemical, and molecular changes in the plants. Khodarahmpour et al. (2011) characterized that PEG stress reduced root length (60%) and shoot length (89.8%). In addition, Pei et al. (2010) indicated that PEG stress decreased yield, chlorophyll content, and leaf water potential.

PEG in the culture media induced dehydration by decrease in the water potential gradient between the plant cells and the culture media, declining moisture content, and leading to the reduction in turgidity of plant cells (Heyser & Nabors, 1981). Additionally, under drought stress conditions induced by PEG in the culture media, plant cells accumulate several solutes including hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and proline (Ushimaru et al., 1994; Al-Bahrany et al., 2002; Porcel and Ruiz-Lozano, 2004; Niedzwiedz-Siegien et al., 2004; Pei et al.,

2010; Khodarahmpour et al., 2011). According to Gigon et al. (2004), drought stress induced membrane lipid peroxidation that impairs cell membrane stability. This cell membrane stability is one of the indicators of drought stress resistance, with higher cell membrane stability associated with drought stress resistance. Furthermore, MDA is used to estimate membrane damage because this solute is derived from polyunsaturated fatty acids produced from the decomposition of membranes (Jiang and Zhang, 2001). The accumulation of proline is a symptom of stress due to drought conditions (Pei et al., 2010). In PEG stress, plants cells also show a defence mechanism by accumulating antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and peroxidases like ascorbate peroxidase (APX) to deal with free radicals (Ouvrard et al., 1996). On the other hand, under PEG stress, glutathione and soluble sugar concentration in the leaves are decreased. Thus, alteration of the concentration of these solutes under drought conditions can be used as a biochemical markers to select drought-resistant rice genotypes.

In rice improvement programs under drought stress conditions, deep rooting is a primary target trait (Gowda et al., 2011). Previous study reported that Japanese National Institute for Agrobiological Sciences developed a drought-resistant rice variety that has a deeper root system that improves its ability to absorb water and nutrients from deeper layers of soil (Palmer, 2013). This drought-resistant rice variety was developed by inserting the *deeper rooting-1* (*dro-1*) gene from a deep-rooting upland rice variety, Kinandang Patong, into a drought-prone but popular commercial rice variety, IR64. The resulting rice variety has more than twice as deep roots as the wild-type IR64. The *dro*-1 gene only alters the angle of the root growth, and does not increase the overall root density, consequently the excessive investment in more root mass is avoided in the *dro-1* lines. Under moderate drought conditions, this variety showed 10% grain reduction,

compared to the 60% reduction of wild-type IR64. In addition, under severe drought, wild-type IR64 totally failed to produce grain yield, but this modified variety only exhibited 30% yield reduction compared to the wild-type IR64 growing under control conditions.

The objective of this research is to evaluate the ABA response of the K/Z RIL population on root architectural traits in relation to drought stress resistance. This research is part of the genetic improvement in drought resistance for rice.

Materials and Methods

Plant Material

The RIL population derived from varieties Kaybonnet (drought resistant) and ZHE733 (drought sensitive), termed K/Z RILs, of 198 lines (Table 3.1.) were made available from the USDA Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA.

Drought Stress Treatment in the Field

The K/Z RIL population seeds of 198 lines and two parents (Kaybonnet and ZHE733) were germinated and grown in the greenhouse under controlled conditions (28 - 30^{o}C day and 22 - 23^{o}C night, 14h light/10h dark cycle, with average light intensity 580 µmol $m^{-2}s^{-1}$ and 65% relative humidity) with sterilized field soil for 20 days (until V3 stage) and uniform plants were selected and transplanted to the field, divided into 6 batches (7-day intervals) based on their heading day data from USDA, to synchronize drought treatment at reproductive stage. Thus, the latest heading day lines were seeded and transplanted early and the earliest heading day lines seeded last.

This RIL population was evaluated in the field at Fayetteville, AR, USA over three growing seasons (May-November) in 2016, 2017, and 2018. The population was grown in a randomized complete block design with five replications and two treatments, well-watered (WW) and drought stress (DS) conditions, in single-row plots of 5 m length with a spacing of 0.3 m between plants. Blocks represent a random effect and treatments (WW and DS) represent a fixed effect. The rice plants were planted in a control plot with normal irrigation (WW conditions) and a drought plot for stress treatment. In this stage, rice plants were in the vegetative stage where all plants were maintained with the normal irrigation for at least 30 days. Then, DS treatment was given at the reproductive stage (R3). DS conditions were monitored with three tensiometers that were installed at three spots in the DS plot just after draining, the first was in

the beginning of the plot, the second in the middle, and the third at the end of the plot. The DS condition was maintained continuously up to -70 kPa (severe stress). Once the soil tension reduced to -70 kPa at 30 cm soil depth, life-saving irrigation was provided thereafter through flash flooding in the DS plot and water was drained after 24h to impose the next cycle of DS till maturity. Moreover, to fertilize the field (WW and DS plots), Urea was applied in three applications at the rate of 20 g per square meter. The first application after 10 days of transplanting, the second at maximum tillering stage, and the third at panicle initiation. The weeds were controlled by manual removal. The effect of drought stress was quantified by calculating the filled grains per panicle number (FG) with five replications (plants) per line.

Drought Stress Treatment in PEG Media and Screening for ABA Sensitivity

Seed Sterilization

Seeds were washed with 70% ethanol for 60 seconds then rinsed 3 times with sterilized water, washed with 30% bleach solution (60 ml bleach + 1 ml 20% SDS + 139 ml sterilized water) for 45 minutes and rinsed two times with sterilized water.

Germination

Sterilized seeds (S0 stage) were germinated in 2 ml tubes containing germination media (Chu's N-6 Basal Salts with Vitamins, Macronutrients, Micronutrients) (Figure 3.1.A) until S3 stage (Figure 3.1.B) in the growth chamber (maintained at temperature: 28/22^{o}C day/night, light intensity: 600umol/m^{2}/s, relative humidity: 60%).

Drought Stress Treatment in PEG Media for the Parents

PEG induces rice osmotic stress which can simulate drought stress conditions in the culture medium. The experiment of drought stress treatment for PEG stress media was setup for hydroponic treatment. The S3 stage of Kaybonnet and ZHE733 plants were transplanted into the

PEG media treatment set up for different osmotic potentials: 0 MPa (control), -0.5 MPa (moderate drought), and -1.2 MPa (severe drought) induced by polyethylene glycol (PEG 8000) and grown in the growth chamber (temperature: 28/22^{o}C day/night, light intensity: 600umol/m^{2}/s, relative humidity: 60%) until V3 stage (Figure 3.1.C.). The effect of drought stress was quantified by measuring the root architectural traits (Figure 3.1.D): maximum root length (RL), root to shoot ratio (RSR), total root number (TRN), number of roots with a shallow angle (0-45^{o}) (SRN), number of roots with a deep angle (45-90^{o}) (DRN), and root fresh weight (RFW) with five replications per line.

Screening for ABA Sensitivity of K/Z RIL Population

The experiment of screening ABA sensitivity in the K/Z RIL population setup in hydroponic treatment. S3 stage of Kaybonnet, ZHE733, and 198 lines were transplanted into ABA media at different concentration levels: 0 µM (control), 3 µM, and 5 µM; and then grown in the growth chamber (temperature: 28/22^{o}C day/night, light intensity: 600umol/m^{2}/s, relative humidity: 60%) until V3 stage. The effect of ABA sensitivity was quantified by measuring the root architectural traits (Figure 3.1.D): maximum root length (RL), root to shoot ratio (RSR), total root number (TRN), number of roots with a shallow angle (0-45^{o}) (SRN), number of roots with a deep angle (45-90^{o}) (DRN), and root fresh weight (RFW) with five replications per line.

Statistical Analysis

The data of drought stress treatments in the field, from growing seasons of three years (2016, 2017, and 2018) for 198 lines and 2 parents, both under WW and DS conditions for filled grain per panicle number, were analysed by analysis of variance (ANOVA) using JMP version 12.0. The effect of rice lines, treatments (PEG and ABA); and interaction of rice lines and treatments were included in the statistical model. The Fisher's LSD test was performed to

compare the means of the two treatments (PEG and ABA) among all of the rice lines in the K/Z RIL population for significant effects (Fisher's LSD, $P < 0.05$) using JMP version 12. The correlation analysis was achieved by using JMP version 12.0 to correlate root architectural traits under ABA and filled grain per panicle under WW and DS conditions of the K/Z RIL population.

Results and Discussion

Root growth and development vary among the rice genotypes studied here. Greub (2015) identified that the root length of Kaybonnet showed the longest root compared to most other diverse rice genotypes, such as Bengal, Sipirasikkam (GSOR 310428), *O. glaberrima*, IR64, Nagina-22 (N22), and Vandana (Figure 3.2.). Root length is probably the most important architectural trait for drought avoidance, by enabling the roots to reach deeper water levels in the ground. Additionally, Greub (2015) also determined that Kaybonnet had larger xylem area than Bengal, *O. glaberrima*, IR64, Nagina-22 (N22), Vandana, and parental line ZHE733 (Figure 3.3. and 3.4.). The number and size of the xylem vessels are associated with their conductivity of water; a larger xylem size is correlated with larger axial conductance and increased water uptake. Among these lines, Bengal and Nagina-22 (N22) are examples of drought-resistant rice genotypes, while IR64 and Nippobare as the reference for drought-sensitive genotypes.

Drought stress is one of the major abiotic stress factors that causes significant reduction in growth, development, and grain yield production, due to this stress changing morphological, physiological, and molecular characteristics of the rice plants. In this research, drought resistance responses phenotypes were quantified by the filled grain per panicle number under field DS conditions (Figure 3.6.), and response phenotypes under PEG media for RL (Figure 3.5.A., 3.5.B., & 3.7.), RSR (Figure 3.8.), TRN (Figure 3.9.), SRN (Figure 3.10.), DRN (Figure 3.11.), and RFW (Figure 3.12.). The phenotypic differences between Kaybonnet (drought resistant), ZHE733 (drought sensitive), and 198 lines of K/Z RIL population (48 drought resistant and 150 drought sensitive lines) (Table 3.1.), lead us to investigate the ABA sensitivity by measuring root architectural traits. These response traits may be the most significant factors characterizing the drought avoidance mechanisms.

ABA can be obtained from endogenous or exogenous sources. Knowledge of the roles of ABA in the drought resistance mechanism has been gained from experiments with endogenous or exogenous ABA sources. Drought stress regulates endogenous ABA biosynthesis by up-regulating ABA biosynthetic genes that include *ZEP*, *NCED, AtAAO3*, *MCSU,* and *AtSDR1* (Seo et al., 2000; Iuchi et al., 2001; Xiong et al., 2002). The higher concentration of endogenous ABA is induced primarily from increased ABA biosynthesis. Under drought stress treatment, endogenous ABA concentration in the roots increase. Moreover, drought resistant rice plants produce more ABA than drought sensitive plants. However, under non-stress conditions, a high content of exogenous ABA reduces the plant growth of the drought resistant rice plants due to ABA sensitivity. Under drought stress conditions, a higher level of exogenous ABA is useful for plants due to the role of ABA in inducing stomatal closure to minimize water loss by limiting transpiration. Additionally, ABA decreases stress damage in the plant cells through the expression of stress-responsive genes. In ABA sensitive rice plants, exogenous ABA increases the expression of the ABA biosynthetic genes. Thereby, ABA is involved in drought resistance protective mechanisms (Hasegawa et al., 2000; Bray, 2002; Finkelstein et al., 2002).

In this study, Kaybonnet and 48 drought resistant lines of the K/Z RIL population grown under control conditions in the culture media, display longer RL (Figure 3.13.), higher RSR (Figure 3.14.), more DRN (Figure 3.17.), and heavier RFW (Figure 3.18.) compared to ZHE733 and 150 drought sensitive lines. Moreover, the RL, RSR, DRN, and RFW phenotypes in the control culture media have a positive correlation to FG under DS conditions in the field (Figure 3.19.). This is because of the deeper, thicker, and larger root system that also re-allocate assimilates from shoot to root under the DS conditions. According to Park et al. (2009), the increase of RSR under DS conditions is correlated with the increasing of leaf sucrose-phosphate

synthase and root invertase activity that influence the availability of the sucrose in leaves and transport them to the roots, thus producing more dry matter and soluble sugar in roots. In addition, RL shows a positive correlation with RFW (Figure 3.19.) that probably relate to higher absorption of water and nutrients. In a previous study also drought resistant rice genotypes Swarnaprabha and Kattamodan displayed a positive correlation with the root and shoot length under DS conditions (Swapna and Shylaraj, 2017). Kano et al. (2011) also indicated that root length of Nipponbare displayed a positive correlation with shoot dry matter that leads to higher root distribution in the soil under DS conditions.

Drought treatment in the soil causes osmotic stress to plants, in response to which there are two pathways for stress adaptation: the ABA-dependent & ABA-independent pathways. ABA-mediated cell signaling acts as a mechanism for osmotic stress tolerance. The ABA-dependent pathway of cellular defense under osmotic stress, influences the water use efficiency of the plant by reducing transpiration and growth (Lim et al., 2015). In exogeneous ABA treatments of 3 and 5 µM, Kaybonnet and the 48 drought resistant lines exhibited an ABA-sensitive phenotype. In the presence of exogenous ABA; the responses of RL (Figure 3.13.), RSR (Figure 3.14.), TRN (Figure 3.15.), SRN (Figure 3.16.), DRN (Figure 3.17.), and RFW (Figure 3.18.) were significantly reduced during the V3 stage. Lim et al. (2015) indicated that exogenous ABA repressed the expression of the genes associated with growth in the ABA sensitive plants. Additionally, these Kaybonnet and 48 drought resistant lines exhibited drought resistance at the R3 stage (Figure 3.6.) by showing higher filled grain per panicle number, implying that Kaybonnet and 48 drought resistant lines regulate osmotic stress tolerance via ABA-mediated cell signaling. Drought resistance response at the reproductive stage in the field is characterized by a higher number of filled grain per panicle, and better root architectural traits

under PEG treatment; as a consequence of ABA sensitivity. The bZIP transcription factors have been shown to control ABA sensitivity and drought stress resistance in rice plants (Park et al., 2016). In drought resistant genotypes, exogenous ABA significantly enhanced the expression of ABA biosynthetic genes. Furthermore, ABA sensitivity is associated with drought stress resistance through its effect on the stomatal movement (Lim et al., 2015; Duan et al., 2008; Todorov et al., 1998). According to Park et al. (2016), the drought resistant plants that showed ABA sensitivity were characterized by higher leaf temperature, smaller apertures of stomata that associated with transpiration activity, and showed resistance to drought by limiting water loss by osmotic adjustment, and increased reactive oxygen species to protect the plant cells from oxidative damage. A large number of genes associated with the defense response to osmotic stress are regulated by ABA. Lim et al. (2015) showed that the rice plants that grew in media with 2 or 5 µM ABA had significantly longer roots and shoots compared to the plants grown in control media. These data suggested that the rice plants were insensitive to ABA and exhibited the ABA-dependent pathway in response to drought stress.

In general, under DS conditions, the level of endogenous ABA in plant cells increases. ABA transport in a diffusive process due to its permeable property at the cell membrane. When rice plants are treated with exogenous ABA (3 and 5 µM) on their roots, an increase in ABA content of leaves can be detected quickly, indicating an efficient transport system for ABA in plants. Furthermore, ABA regulates a large number of genes correlated with drought resistance mechanisms. ABA induces the expression of stress-resistance genes, including *NCED3, KIN2, COR15A,* and *RD29B* (Park et al., 2016). However, exogenous ABA repressed the expression of the genes associated with growth in ABA sensitive plants. Under DS treatment (Zhao et al., 2001), reactive oxygen species and nitric oxide induced ABA biosynthesis, with reactive oxygen

species and nitric oxide accumulating after 20 minutes of treatment, while ABA increased after 60 minutes. Thus, ABA prevents plants from stress damage at a later stage and increases plant stress resistance by regulating stomatal closure (Figure 3.20.).

According to the physiological response of Kaybonnet and ZHE733 to ABA and PEG treatments, Kaybonnet exhibited ABA sensitivity that induced stomatal closure under DS conditions, then maintain photosynthesis and water use efficiency, and Kaybonnet also performed PEG insensitive that influenced increasing of the root length, and finally maintain the filled grain per panicle under drought. Meanwhile, ZHE733 showed ABA insensitivity and under DS conditions the stomata remain open, then showing reduction in photosynthesis and water use efficiency, and in the PEG treatment, ZHE733 exhibited PEG sensitive that influenced the reduction of root length, thus reduce the filled grain under drought. Therefore, Kaybonnet showed drought avoidance mechanisms, while ZHE733 identified as drought sensitive (Figure 3.20).

Conclusions

The rice genotype Kaybonnet and 48 RILs out of the population of 198 RILs exhibit drought resistance with an ABA-sensitive phenotype during seedling and vegetative growth stage, implying that these genotypes regulates osmotic stress tolerance via ABA-mediated cell signaling. In the presence of exogenous ABA, the root architectural traits of these drought resistant rice plants such as RL, RSR, TRN, SRN, DRN, and RFW were significantly reduced during the V3 stage. Additionally, these Kaybonnet and 48 drought resistant lines exhibited drought resistance at the R3 stage showing higher number of filled grain per panicle. This study identifies drought resistance RILs along with physiological response to ABA that can be used to develop drought-resistant rice plants.

References

Agrawal, L., Gupta, S., Mishra, S. K., Pandey, G., Kumar, S., Chauhan, P. S., Chakrabarty, D., & Nautiyal, C. S. (2016). Elucidation of Complex Nature of PEG Induced Drought-Stress Response in Rice Root Using Comparative Proteomics Approach. *Front. Plant Sci., 7, 1466*. doi:10.3389/fpls.2016.01466

Ahmad, M., Zaffar, G., Razvi, S. M., Dar, Z. A., Mir, S. D., Bukhari, S. A., & Habib, M. (2014). Resilience of cereal crops to abiotic stress: A review. *Afr J Biotechnol, 13, 29,* 2908-2921.

Alam, M. M., Nahar, K., Hasanuzzaman, M., & Fujita, M. (2014). Trehalose-induced drought stress tolerance: A comparative study among different Brassica species. *Plant Omics, 7, 4*.

Al-Bahrany, A. (2002). Callus growth and proline accumulation in response to polyethylene glycol-induced osmotic stress in Rice, *Oryza sativa* L. *Pakistan Journal of Biological sciences, 5, 12*, 1294-1296.

Ando, T., Yoshida, S., & Nishiyama, I. (1983). Nature of oxidizing power of rice roots. *Plant Soil, 72*, 57-71.

Babu, R. C., Shashidhar, H. E., Lilley, J. M., Thanh, N. D., Ray, J. D., Sadasivam, S., et al. (2001). Variation in root penetration ability, osmotic adjustment and dehydration tolerance among accessions of rice adapted to rainfed lowland and upland ecosystems. *Plant Breeding, 120*, 233-238.

Baldoni, E., Genga, A., & Cominelli, E. (2015). Plant MYB transcription factors: their role in drought response mechanisms. *International Journal of Molecular Sciences, 16*, 15811-15851

Basu, S., Roychoudhury, A., Saha, P. P., & Sengupta, D. N. (2010). Differential antioxidative responses of indica rice cultivars to drought stress. *Plant Growth Regulation, 60, 51*.

Bhasakaran, S., Smith, R. H., & Newton, R. J. (1985). Physiological changes in cultured sorghum cells in response to induced water stress I. Free Proline. *PI. Physiol., 79,* 239-248.

Bray, E. A. (2002). Abscisic acid regulation of gene expression during water deficit stress in the era of the Arabidopsis genome. *Plant Cell Environ., 25*, 153-161. doi:10.1046/j.13653040.2002.00746.x

Büyük, İ. & Aras, S. (2017). Genome-wide in silico identification, characterization and transcriptional analysis of the family of growth-regulating factors in common bean (*Phaseolus vulgaris* L.) subjected to polyethylene glycol induced drought stress. *Arch Biol Sci., 69, 1,* 5-14.

Carlos, A. C. C., Orivaldo, A., Rogério, P. S., & Gustavo, P. M. (2008). Grain quality of upland rice cultivars in response to cropping systems in the Brazilian tropical savanna. *Sci. Agric., (Piracicaba, Braz.), 65, 5*, 468-473.

Chopra, V. L. & Prakash, S. (2002). Evolution and Adaptation of Cereal Crops. Science Publishers Inc, NH, USA.

Clark, L. J., Price, A. H., Steele, K. A., & Whalley, W. R. (2008). Evidence from near-isogenic lines that root penetration increases with root diameter and bending stiffness in rice. *Funct Plant Biol., 35, 11*, 1163-1171.

Comas, L. H., Becker, S. R., Von Mark, V. C., Byrne, P. F., & Dierig, D. A. (2013). Root traits contributing to plant productivity under drought. *Frontiers in Plant Science*. doi:10.3389/fpls. 2013.00442

de Souza, T. C., de Castro, E. M., Magalha ˜es, P. C., Lino, L. D. O., Alves, E. T., & de Albuquerque, P. E. P. (2013). Morphophysiology, morphoanatomy, and grain yield under field conditions for two maize hybrids with contrasting response to drought stress. *Acta Physiol Plant, 35*, 3201-3211.

Dodd, G. L. & Donovan, L. A. (1999). Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. *Am. J. Bot. PMID:10449394. 86*, 1146-1153.

Dry, P. R. & Loveys, B. R. (1999). Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. *Vitis, 38*, 151-156.

Duan, J., Li, J., Guo, S. & Kang, Y. (2008). Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. *Journal of Plant Physiology, 165,* 1620-1635.

Eghball, B. & Maranville, J. W. (1993). Root development and nitrogen influx of corn genotypes grown under combined drought and nitrogen stresses. *Agron., J., 85*, 147-152.

Fageria, N. K. & Moreira, A. (2011). The role of mineral nutrition on root crop growth of crop plants. *Adv Agron., 110,* 251-331.

Fageria, N. K. (2013). The Role of Plant Roots in Crop Production. Boca Raton, FL: CRC Press.

FAO. (2001). Food balance sheets. FAOSTAT-Agriculture Data.

http://faostat.org

FAOSTAT. (2020). Top production rice-paddy 2018.

http://faostat.fao.org/site/339/default.aspx

FAOSTAT. (2002). FAO Statistical Databases.

http://apps.fao.org/

Feng, F. J., Xu, X. Y., Du, X. B., Tong, H. H., Luo, L. J., & Mei, H. W. (2012). Assessment of drought resistance among wild rice accessions using a protocol based on single-tiller propagation and PVC-tube cultivation. *Aust J Crop Sci., 6,* 1205-1211.

Finkelstein, R., Gampala, S., & Rock, C. (2002). Abscisic acid signaling in seeds and seedlings. *Plant Cell, 14,* S15-S45.

Flexas, J. & Medrano, H. (2002). Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Ann Bot., 89,* 183-189.

Fukai, S. & Cooper, M. (1995). Development of drought-resistant cultivars using physiomorphological traits in rice. *Field Crops Res., 40,* 67-86.

Gigon, A., Matos, A. R., Laffray, D., Zuily-Fodil, Y., & Pham-Thi, A. T. (2004). Effect of drought stress on lipid metabolism in the leaves of *Arabidopsis thaliana* (ecotype Columbia). *Ann. Bot., 94,* 345-351.

Gonzalez-Guzman, M., Pizzio, G. A., Antoni, R., Vera-Sirera, F., Merilo, E., Bassel, G. W., Fernandez, M. A., Holdsworth, M. J., Perez-Amador, M. A., Kollist, H., & Rodriguez, P. L. (2012). Arabidopsis PYR/PYL/ RCAR receptors play a major role in quantitative regulation of stomatal aperture and transcriptional response to abscisic acid. *Plant Cell, 24, 6,* 2483-2496.

Gornall, J., Betts, R., Burke, E., Clark, R., Camp, J., Willett, K., et al. (2010). Implications of climate change for agricultural productivity in the early twenty-first century. Philosophical Transactions of the Royal Society of London B: *Biological Sciences, 365,* 2973-2989.

Gowda, V. R. P., Henry, A., Yamauchi, A., Shashidhar, H. E., & Serraj, R. (2011). Root biology and genetic improvement for drought avoidance in rice. *Field Crops Res, 122, 1,* 1-13.

Greub, C. P. (2015). A Morpho-physiological Analysis of Diverse Drought Resistant and Sensitive Rice Genotypes to Identify Distinguishing Anatomical Root Phenes. Theses and Dissertations. University of Arkansas. 1281.

Hajihashemi, A. & Geuns. (2016). Gene transcription and steviol glycoside accumulation in *Stevia rebaudiana* under polyethylene glycol-induced drought stress in greenhouse cultivation. *FEBS Open Bio, 6,* 937-944. doi:10.1002/2211-5463.12099

Hamayun , M., Khan, S. A., Shinwari, Z. K., Khan, A. L., Ahmad, N., & Lee, I. (2010). Effect of polyethylene glycol induced drought stress on physio-hormonal attributes of soybean. *Pak. J. Bot., 42, 2,* 977-986.

Handa, A. K., Bressan, R. A., Handa, S., & Hasegawa, P. M. (1982). Characteristics of cultured tomato cells after prolonged exposure medium containing polyethylene glycol. *PI. Physiol., 69,* 514-521.

Hasegawa, P. M., Bressan, R. A., Zhu, J. K., & Bohnert, H. J. (2000). Plant cellular and molecular responses to high salinity. *Annu Rev Plant Physiol Plant Mol Biol., 51,* 463-499.

Heyser, J. W. & Nabors, M. W. (1981). Osmotic adjustment of cultures tobacco cells (*Nicotiana tabacum* var. Samsum) grown on sodium chloride. PI. *Physiol., 67,* 720-727.

Ingram, K. T., Bueno, F. D., Namuco, O. S., Yambao, E. B., & Beyrouty, C. A. (1994). Rice root traits for drought resistance and their genetic variation. In G.J.D. Kirk ed., Rice Roots: Nutrient and water use. International Rice Research Institute, Philippines. 67-77.

IRRI. (2002). A Proposal for IRRI to Establish a Grain Quality and Nutrition Research Centre. Metro Manila, Philippines.

Iuchi, S., Kobayashi, M., Taji, T., Naramoto, M., Seki, M., Kato, T., Tabata, S., Kakubari, Y., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2001). Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. *Plant J., 27,* 325-333.

Jiang, M. Y. & Zhang, J. H. (2001). Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. *Plant Cell Physiol., 42,* 1265-1273.

Kahovec, J., Fox, R. B., & Hatada, K. (2002). Nomenclature of regular single-strand organic polymers. *Pure and Applied Chemistry, 74, 10,* 1921-1956. doi:10.1351/pac200274101921

Kano, M., Inukai, Y., Kitano, H., & Yamauchi, A. (2011). Root plasticity as the key root trait for adaptation to various intensities of drought stress in rice. *Plant Soil, 342,* 117-128. doi:10.1007/s11104-010-0675-9

Khodarahmpour, Z. (2011). Effect of drought stress induced by polyethylene glycol (PEG) on germination indices in corn (*Zea mays* L.) hybrids. *African Journal of Biotechnology, 10, 79.* doi:10.5897/AJB11.2639

Kim, T. H., Bohmer, M., Hu, H., Nishimura, N., & Schroeder, J. I. (2010). Guard cell signal transduction network: Advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. *Annu Rev Plant Biol., 61,* 561-591.

Kitomi, Y., Kanno, N., Kawai, S., Mizubayashi, T., Fukuoka, S., & Uga, Y. (2015). QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of DEEPER ROOTING 1. *Rice, 8, 16*

Kondo, M., Murty, M. V. R., & Aragones, D. V. (2000). Characteristics of root growth and water uptake from soil in upland rice and maize under water stress. *Soil Sci. Plant Nutr., 46,* 721-732.

Kumar, A. K, Suresh Kumar, M., Sudha, M., Vijayalakshmi, D., Vellaikumar, S., Senthil, N., & Raveendran, M. (2013). Identification of genes controlling ABA accumulation in rice during drought stress and seed maturation. *Int J Adv Biotechnol Res., 4, 4,* 481-487.

Kumar, R. R., Karajol, K., & Naik, G. R. (2011). Effect of Polyethylene Glycol Induced Water Stress on Physiological and Biochemical Responses in Pigeonpea (*Cajanus cajan* L. Millsp.). *Recent Research in Science and Technology, 3, 1,* 148-152.

Latif, H. H. (2014). Physiological responses of *Pisum sativum* plant to exogenous ABA application under drought conditions. *Pak J Bot., 46, 3,* 973-982.

Levitt, J. (1980). Responses of plants to environmental stresses, Vol. 2. Water, radiation, salt and other stresses. *New York: Academic Press*, 93-128.

Li, C. N., Yang, L. T., Srivastava, M. K., & Li, Y. R. (2014). Foliar application of abscisic acid improves drought tolerance of sugarcane plant under severe water stress. *Int J Agric Innov Res., 3, 1*, 101-107.

Lim, C. W., Baek, W., Jung, J., Kim, J. H., & Lee, S. C. (2015). Function of ABA in stomatal defense against biotic and drought stresses. *Int. J. Mol. Sci. 16,* 15251-15270. doi:10.3390/ijms160715251

Lynch, J. P. (2013). Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Ann Bot., 112*, 347-357.

Mae. (1997). Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. *Plant soil, 196*, 201-210.

Narusaka, Y., Nakashima, K., Shinwari, Z. K., Sakuma, Y., Furihata, T., Abe, H., Narusaka, M., Shinozaki, K., & Shinozaki, K. Y. (2003). Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of Arabidopsis rd29A gene in response to dehydration and high salinity stresses. *Plant J., 34*, 137-14.

Newton, R. J., Bhasakaran, S., Puryear, J. D., & Smith, R. H. (1986). Physiological changes in cultured sorghum cells in response to induced water stress II. Soluble carbohydrates and organic acids. PI. *Physiol., 81*, 626-629.

Newton, R. J., Sen, S., & Puryear, J. D. (1989). Solute contribution to osmotic potential loblolly pine (*Pinus taeda* L.) callus. J. PI. *Physiol., 134*, 746-750.

Nguyen, H. T., Babu, R. C., & Blum, A. (1997). Breeding for drought resistance in rice: Physiological and molecular genetics considerations. *Crop Sci., 37, 5*, 1426-1434.

Niedzwiedz-Siegien, I., Bogatek-Leszczynska, R., Come, D., & Corbineau, F. (2004). Effects of drying rate on dehydration sensitivity of excised wheat seedling shoots as related to sucrose metabolism and antioxidant enzyme activities. *Plant Sci., 167*, 879-888.

O'Toole, J. C. (1982). Adaptation of rice to drought prone environments. Drought Resistance in Crops with Emphasis on Rice. IRRI, Los Baños, Philippines.

Ouvrard, O., Cellier, F., Ferrare, K., Tousch, D., Lamaze, T., Dupuis, J. M., & Casse-Delbart, F. (1996). Identification and expression of water stress- and abscisic acid-regulated genes in a drought-tolerant sunflower genotype. *Plant Mol. Biol., 31*, 819-829.

Pagès, L., Serra, V., Draye, X., Doussan, C., & Pierret, A. (2010). Estimating root elongation rates from morphological measurements of the root tip. *Plant Soil, 328*, 35-44.

Palmer, N. (2013). Newly-discovered rice gene goes to the root of drought resistance. *The Cornucopia Institute*. http://www.cornucopia.org

Pandey, R., Agarwal, R. M., Jeevaratnam, K., & Sharma, G. L. (2004). Osmotic stress-induced alterations in rice (*Oryza sativa* L.) and recovery on stress release. *Plant Growth Regulation*, 79-87.

Park, S. Y., Fung, P., Nishimura, N., Jensen, D. R., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T. F., Alfred, S. E., Bonetta, D., Finkelstein, R., Provart, N. J., Desveaux, D., Rodriguez, P. L., McCourt, P., Zhu, J. K., Schroeder, J. I., Volkman, B. F., & Cutler, S. R. (2009). Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science, 324*, 1068-1071.

Park, C., Lim, C. W., & Lee, S. C. (2016). The pepper CaOSR1 protein regulates the osmotic stress response via abscisic acid signaling. *Front. Plant Sci. 7*, 890. doi:10.3389/fpls.2016.00890

Patade, V. Y., Bhargava, S., & Suprasanna. (2009). Halopriming imparts tolerance to salt and PEG induced drought stress in sugarcane. *Agriculture, Ecosystems and Environment, 134*, 24-28. doi:10.1016/j.agee.2009.07.003

Pei, Z. F., Ming, D. F., Liu, D., Wan, G. L., Geng, X. X., Gong, H. J., & Zhou, W. J. (2010). Silicon Improves the Tolerance to Water-Deficit Stress Induced by Polyethylene Glycol in Wheat (*Triticum aestivum* L.) Seedlings. *J Plant Growth Regul., 29*, 106-115. doi:10.1007/s00344-009-9120-9

Porcel, R. & Ruiz-Lozano, J. M. (2004). *Arbuscular mycorrhizal* influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. *J. Exp. Bot. 55,* 1743-1750.

Quick Stats, USDA-NASS, State Agriculture Overview: Arkansas. (2016). https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=ARKANSAS

Rich, S. M. & Watt, M. (2013). Soil conditions and cereal root system architecture: Review and considerations for linking Darwin and Weaver. *Journal of Experimental Botany, 64*, 1193-1208.

Saglam, A., Terzi, R., & Demiralay, M. (2014). Effect of polyethylene glycol induced drought stress on photosynthesis in two chickpea genotypes with different drought tolerance. *Acta Biologica Hungarica, 65, 2*. doi:10.1556/ABiol.65.2014.2.6

Samson, B. K., Hasan, H., & Wade, L. J. (2002). Penetration of hardpans by rice lines in the rainfed lowlands. *Field Crops Res., 76, 2/3*, 175-188.

Sato, Y. & Yokoya, S. (2008). Enhanced tolerance to drought stress in transgenic rice plants overexpressing a small heat-shock protein, sHSP17.7. *Plant Cell Rep*., *27*, 329-334. doi:10.1007/s00299-007-0470-

Schachtman, D. P. & Goodger, J. Q. D. (2008). Chemical root to shoot signaling under drought. *Trends in Plant Science, 13, 6,* 281-287. doi:10.1016/j.tplats.2008.04.003

Seo, M., Peeters, A. J., Koiwai, H., Oritani, T., Marion-Poll, A., Zeevaart, J. A., Koornneef, M., Kamiya, Y., & Koshiba, T. (2000). The Arabidopsis aldehyde oxidase 3 (AAO3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. *Proc Natl Acad Sci USA, 97,* 12908-12913.

Shehab, G. G., Ahmed, O. K., & El-Beltagi, H. S. (2010). Effects of Various Chemical Agents for Alleviation of Drought Stress in Rice Plants (*Oryza sativa* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 38, 1,* 139-148. doi:10.15835/nbha3813627

Sidari, M., Mallamaci, C., & Muscolo, A. (2008). Drought, salinity and heat differently affect seed germination of *Pinus pinea*. *J. For. Res., 13,* 326-330. doi:10.1007/s10310-008-0086-4.

Smith, S., & De Smet, I. (2012). Root system architecture: Insights from Arabidopsis and cereal crops. Philosophical Transactions of the Royal Society of London. Series B, *Biological Sciences, 367, 1595,* 1441-1452.

Swapna, S. & Shylaraj, K. S. (2017). Screening for osmotic stress responses in rice varieties under drought condition. *Rice Science, 24, 5,* 253-263. doi:10.1016/j.rsci.2017.04.004

Teng, K. Q., Li, J. Z., Liu, L., Han, Y. C., Du, Y. X., Zhang, J., Sun, H. Z., & Zhao, Q. Z. (2014). Exogenous ABA induces drought tolerance in upland rice: The role of chloroplast and ABA biosynthesis related gene expression on photosystem II during PEG stress. *Acta Physiol Plant, 36, 8,* 2219-2227. doi:10.1007/s11738-014-1599-4

Todorov, D., Alexieva, V.V., & Karanov, E. (1998). Effect of putrescine, 4-PU-30, and abscisic acid on maize plants grown under normal, drought, and re-watering conditions. *J. Plant Growth Regul., 17,* 197-203. doi:10.1007/PL00007035

Tsago, Y., Andargie, M., & Takele, A. (2014). *In vitro* selection of sorghum (*Sorghum bicolor* (L) Moench) for polyethylene glycol (PEG) induced drought stress. ***Plant Science Today****, 1, 2,* 62-68. ISSN 2348-1900. doi:10.14719/pst.2014.1.2.14

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., Kitomi, Y., Inukai, Y., Ono, K., Kanno, N., Inoue, H., Takehisa, H., Motoyama, R., Nagamura, Y., Wu, J., Matsumoto, T., Takai, T., Okuno, K., & Yano, M. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics, 45,* 1097-1102.

Ushimaru, T., Shibasaka, M., & Tsuji, H. (1994). Resistance to oxidative injury in submerged rice seedlings after exposure to air. *Plant Cell Physiol., 35,* 211-218.

Venkateswarlu , B. & Ramesh, K. (1993). Cell membrane stability and biochemical response of cultured cells of groundnut under polyethylene glycol-induced water stress. *Plant Science, 90, 2,* 179-185. doi:10.1016/0168-9452(93)90238-U

Venuprasad, R., Cruz, M. T., Amante, M., Magbanua, R., Kumar, A., & Atlin, G. N. (2008). Response to two cycles of divergent selection for grain yield under drought stress in four rice breeding populations. *Field Crop Res., 107*, 3, 232-244.

Wang, H. & Yamauchi, A. (2006). Growth and function of roots under abiotic stress in soil. In: Huang B R. Plant-Environment Interactions. 3rd edn. New York: CRC Press: 271-320.

Wang, S. X., Xia, S. T., Peng, K. Q., Kuang, F. C., Yong, C., & Xiao, L. T. (2007). Effects of formulated fertilizer synergist on abscisic acid accumulation, proline content and photosynthetic characteristics of rice under drought. *Rice Sci., 14, 1*, 42-48.

Xiong, H., Li, J., Liu, P., Duan, J., Zhao, Y., et al. (2014). Overexpression of OsMYB48-1, a Novel MYB-Related Transcription Factor, Enhances Drought and Salinity Tolerance in Rice. *PLoS ONE, 9, 3*, e92913. doi:10.1371/journal.pone.0092913

Xiong, L., Shumaker, K. S., & Zhu, J. K. (2002). Cell signaling during cold, drought and salt stresses. *Plant Cell, 14*, 165-183.

Ye, N. H., Zhu, G. H., Liu, Y. G., Li, Y. X., & Zhang, J. H. (2011). ABA controls H_2O_2 accumulation through the induction of OsCATB in rice leaves under water stress. *Plant Cell Physiol., 52, 4*, 689-698.

Zhang, J., Zhang, S., Cheng, M., Jiang, H., Zhang, X., Peng, C., Lu, X., Zhang, M., & Jin, J. (2018). Effect of drought on agronomic traits of rice and wheat: A meta-analysis. *Int. J. Environ. Res. Public Health, 15, 839*. doi:10.3390/ijerph15050839

Zhao, Z., Chen, G., & Zhang, C. (2001). Interaction between reactive oxygen species and nitric oxide in drought-induced abscisic acid synthesis in root tips of wheat seedlings. *Australian Journal of Plant Physiology, 28, 10,* 1055-1061.

Figures

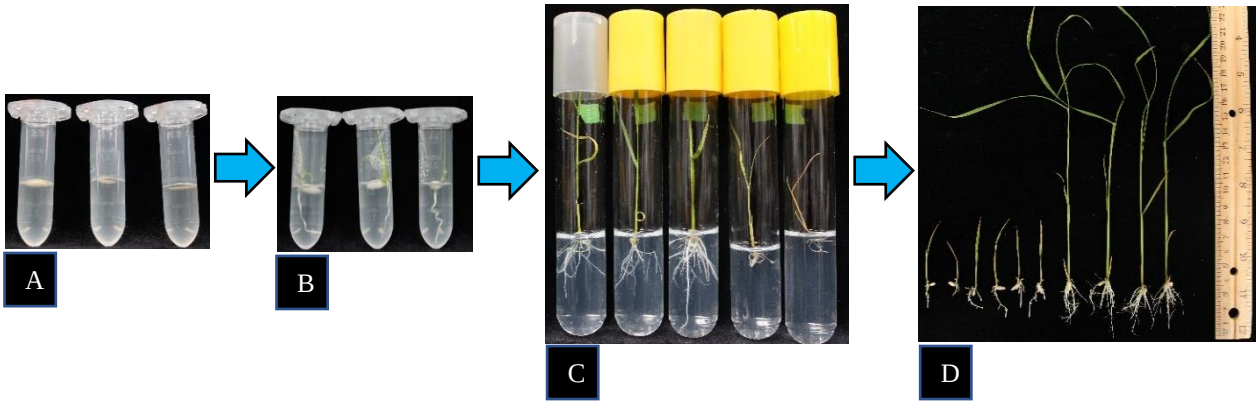

Figure 3.1. Germination media (A), S3 stage of rice (B), V3 stage of rice (C), root architecture measurement (D).

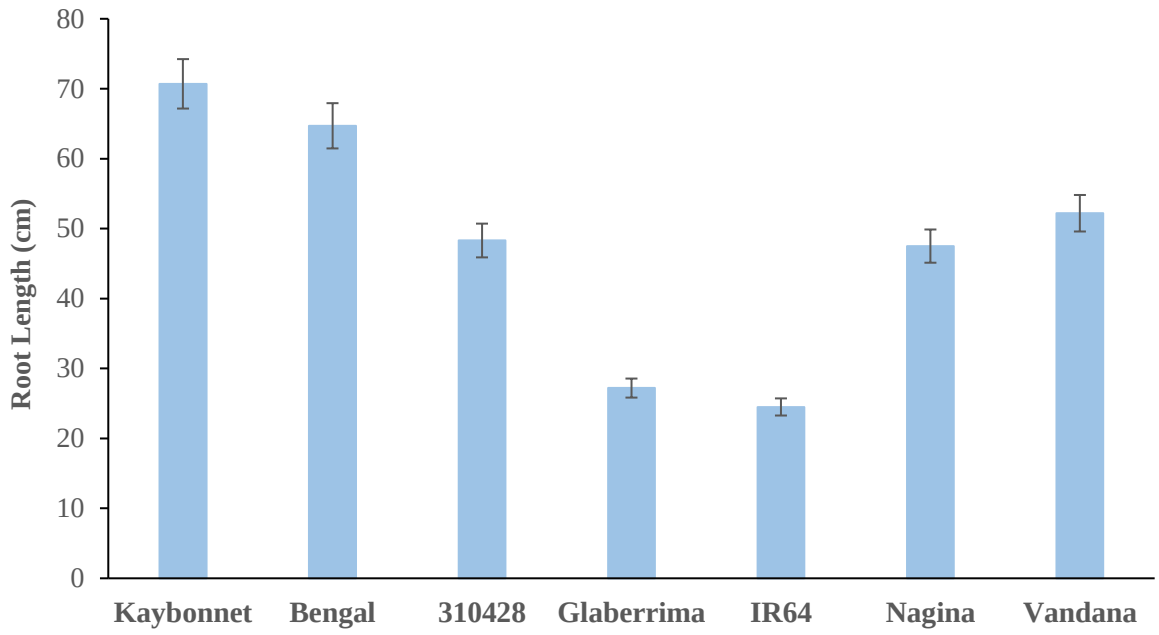

Figure 3.2. Root length of diverse rice genotypes (Greub, 2015).

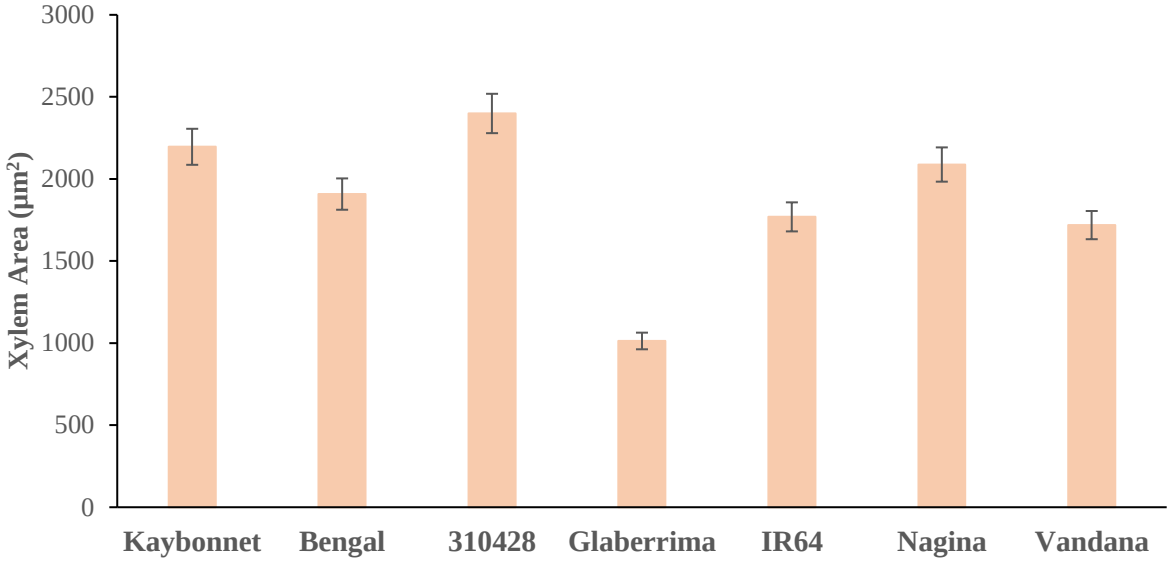

Figure 3.3. Xylem area of diverse rice genotypes (Greub, 2015).

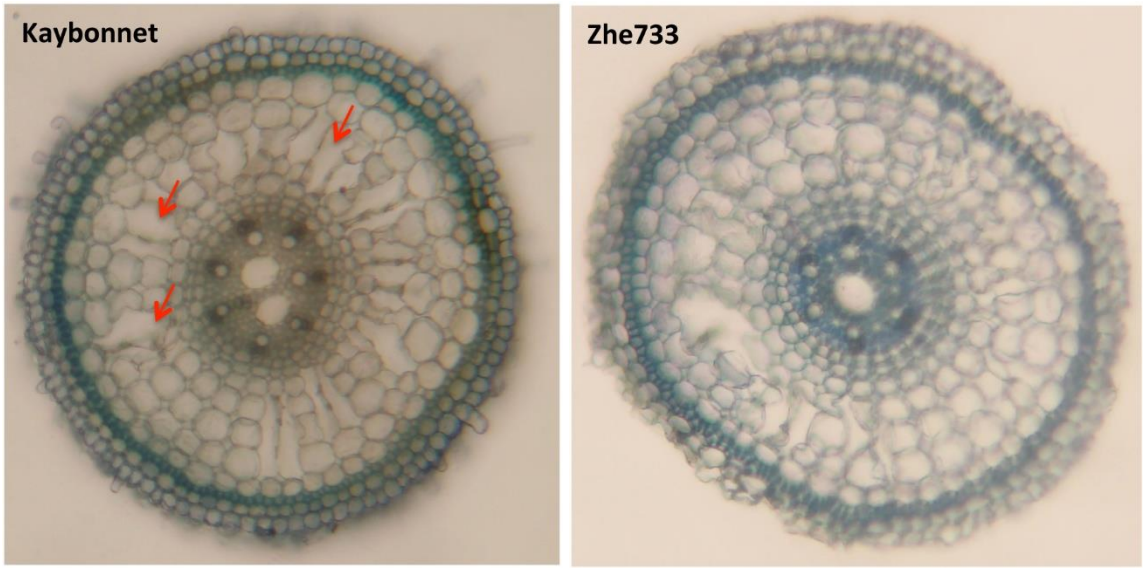

Figure 3.4. Root architecture and anatomy of Kaybonnet and ZHE733 (Greub, 2015)

Figure 3.5. Differences in root length of the K/Z RIL population parent lines Kaybonnet and ZHE733 in response to PEG and ABA, (A) Kaybonnet as PEG resistant, (B) ZHE733 as PEG sensitive, (C) Kaybonnet as ABA sensitive, (D) ZHE733 as ABA insensitive plant.

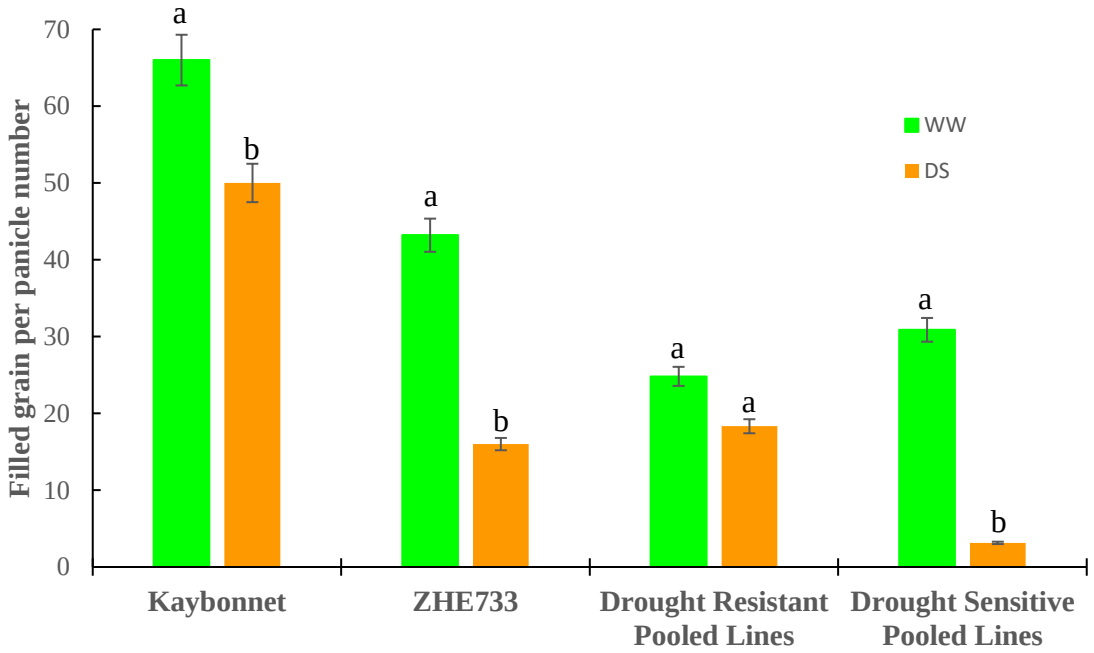

Figure 3.6. Response of the K/Z RIL population to drought stress in the field. Different letters indicate significant difference between WW and DS ($P < 0.05$; ANOVA followed by Fisher's LSD test).

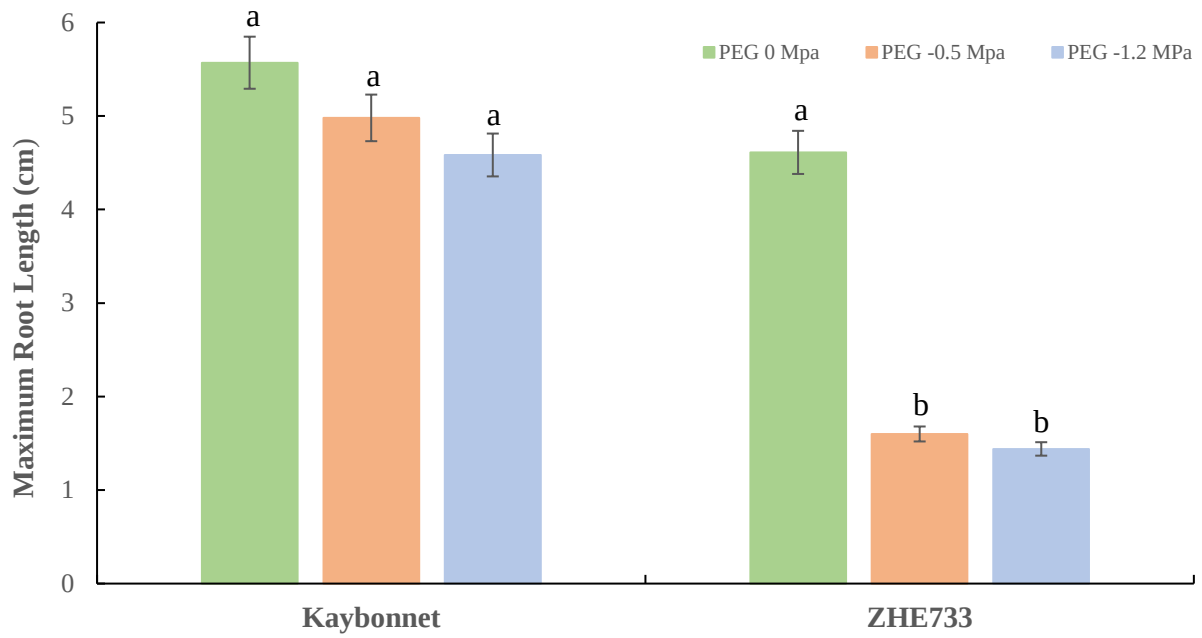

Figure 3.7. Maximum root length in parental genotypes of the K/Z RIL population (Kaybonnet and ZHE733) treated with PEG 0 MPa, -0.5 MPa, and -1.2 MPa. Different letters indicate significant difference between PEG 0 MPa, -0.5 MPa, -1.2 MPa ($P < 0.05$; ANOVA followed by Fisher's LSD test).

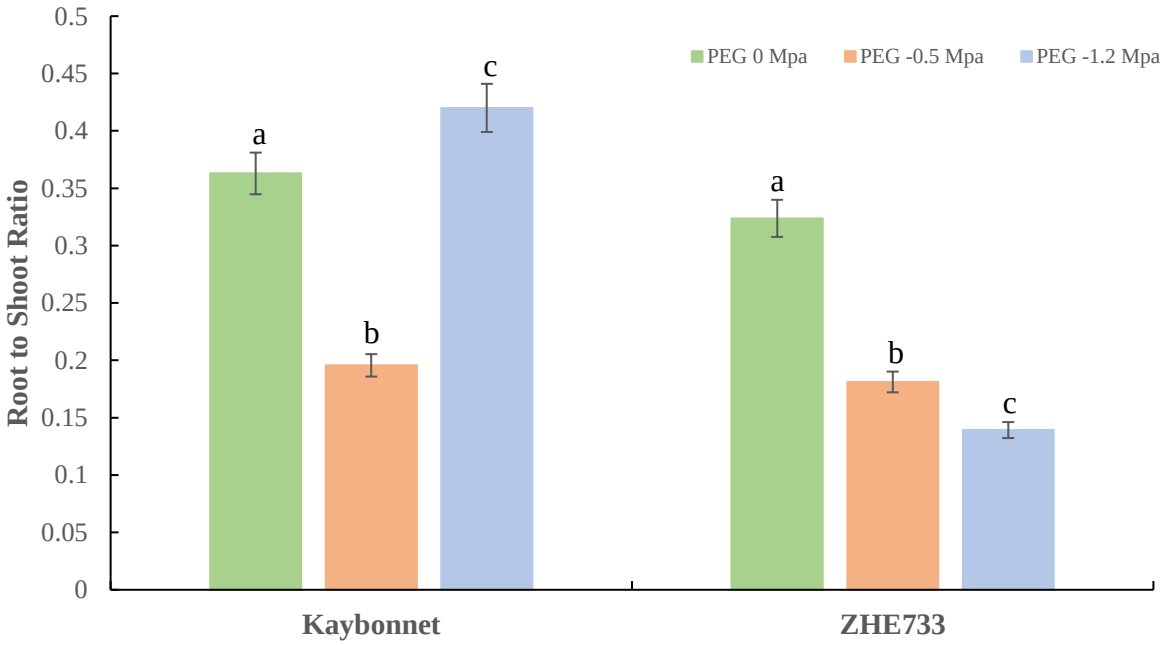

Figure 3.8. Root to shoot ratio in parental genotypes of the K/Z RIL population (Kaybonnet and ZHE733) treated with PEG 0 MPa, -0.5 MPa, and -1.2 MPa. Different letters indicate significant difference between PEG 0 MPa, -0.5 MPa, -1.2 MPa ($P < 0.05$; ANOVA followed by Fisher's LSD test).

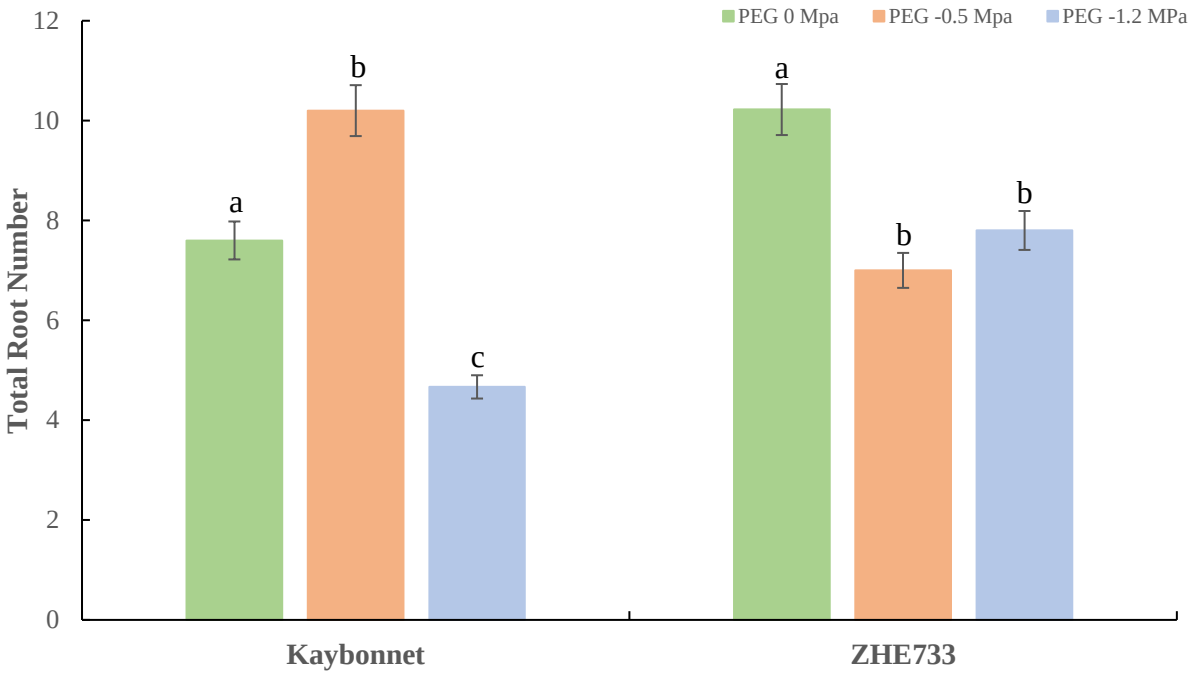

Figure 3.9. Total root number in parental genotypes of the K/Z RIL population (Kaybonnet and ZHE733) treated with PEG 0 MPa, -0.5 MPa, and -1.2 MPa. Different letters indicate significant difference between PEG 0 MPa, -0.5 MPa, -1.2 MPa ($P < 0.05$; ANOVA followed by Fisher's LSD test).

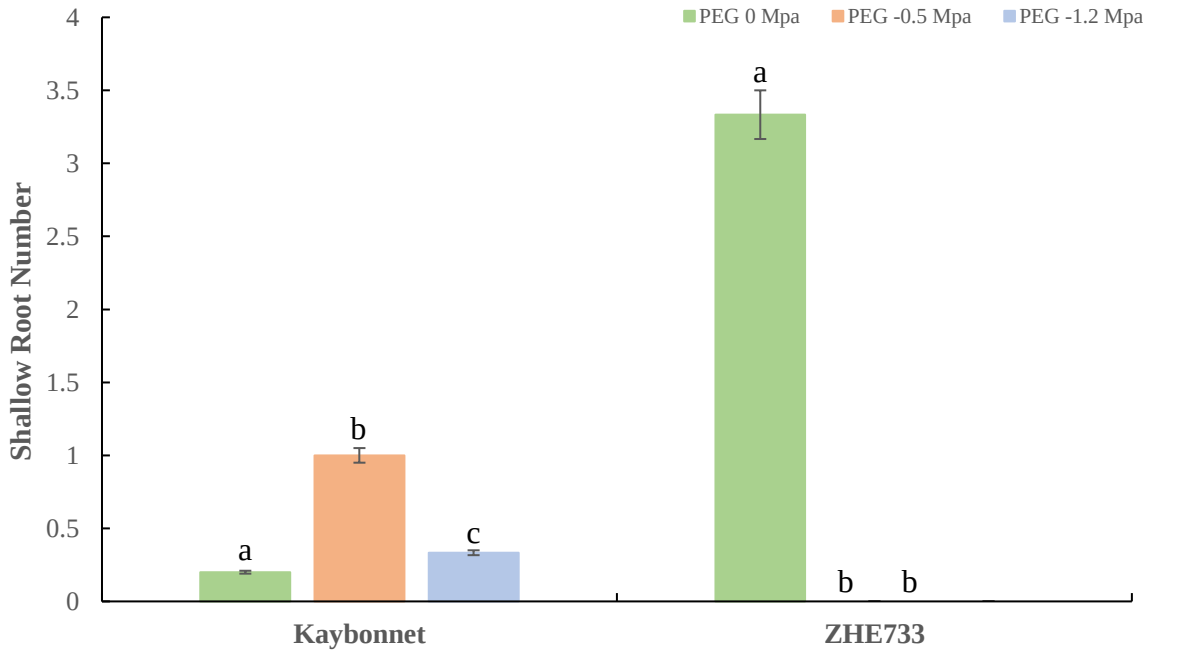

Figure 3.10. Shallow root number in parental genotypes of the K/Z RIL population (Kaybonnet and ZHE733) treated with PEG 0 MPa, -0.5 MPa, and -1.2 MPa. Different letters indicate significant difference between PEG 0 MPa, -0.5 MPa, -1.2 MPa ($P < 0.05$; ANOVA followed by Fisher's LSD test).

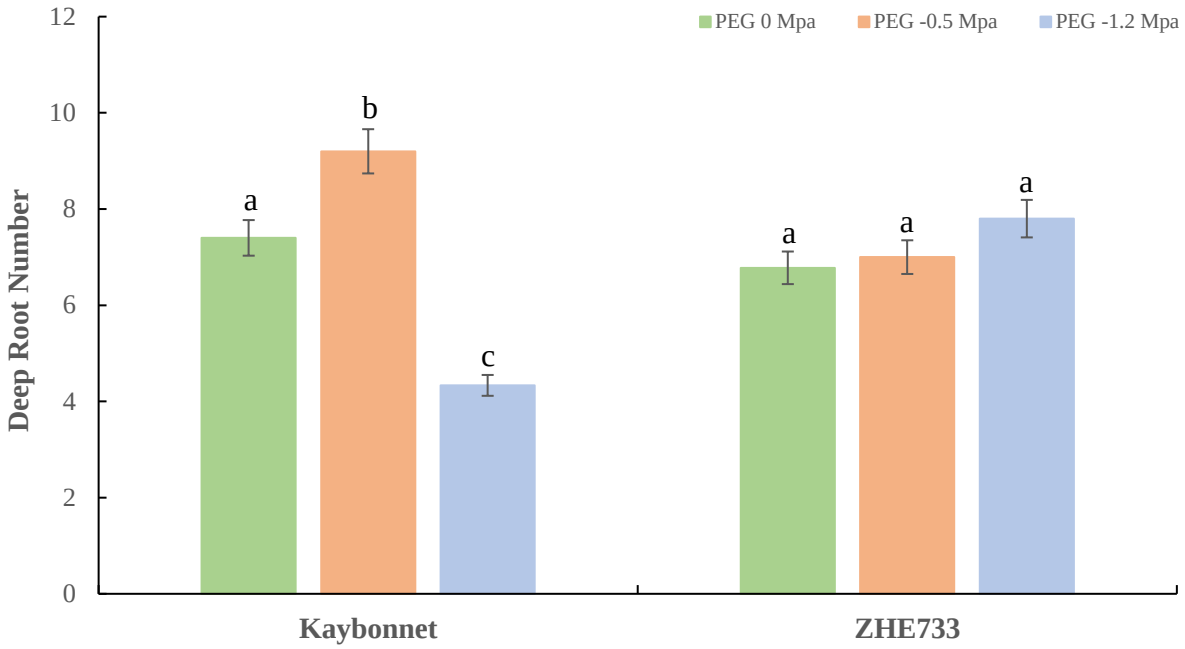

Figure 3.11. Deep root number in parental genotypes of the K/Z RIL population (Kaybonnet and ZHE733) treated with PEG 0 MPa, -0.5 MPa, and -1.2 MPa. Different letters indicate significant difference between PEG 0 MPa, -0.5 MPa, -1.2 MPa ($P < 0.05$; ANOVA followed by Fisher's LSD test).

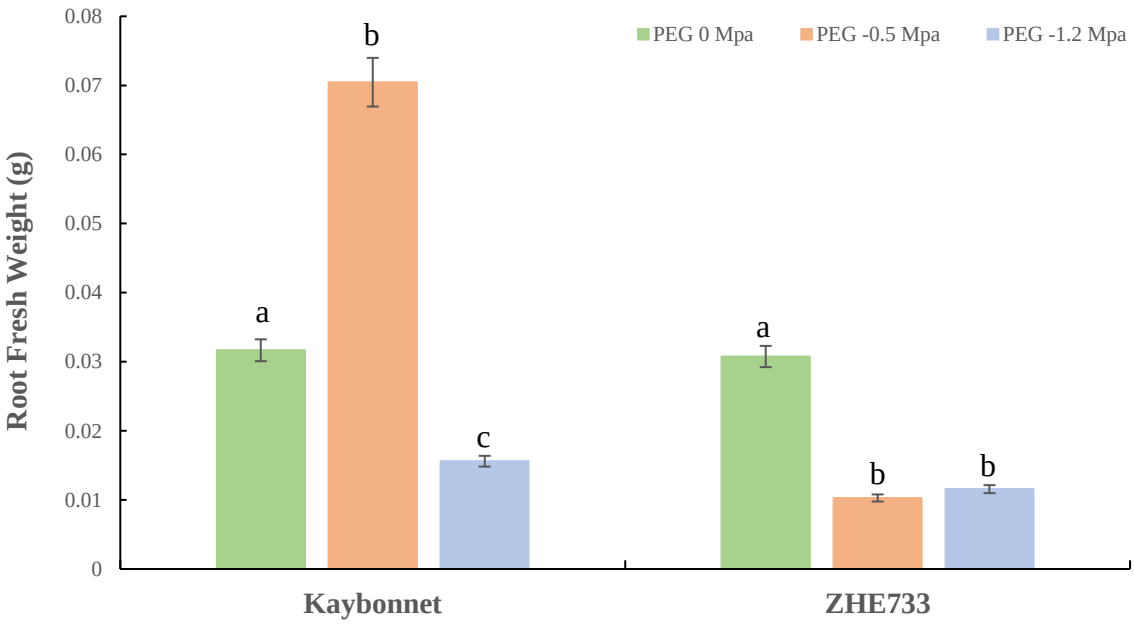

Figure 3.12. Root fresh weight in parental genotypes of the K/Z RIL population (Kaybonnet and ZHE733) treated with PEG 0 MPa, -0.5 MPa, and -1.2 MPa. Different letters indicate significant difference between PEG 0 MPa, -0.5 MPa, -1.2 MPa ($P < 0.05$; ANOVA followed by Fisher's LSD test).

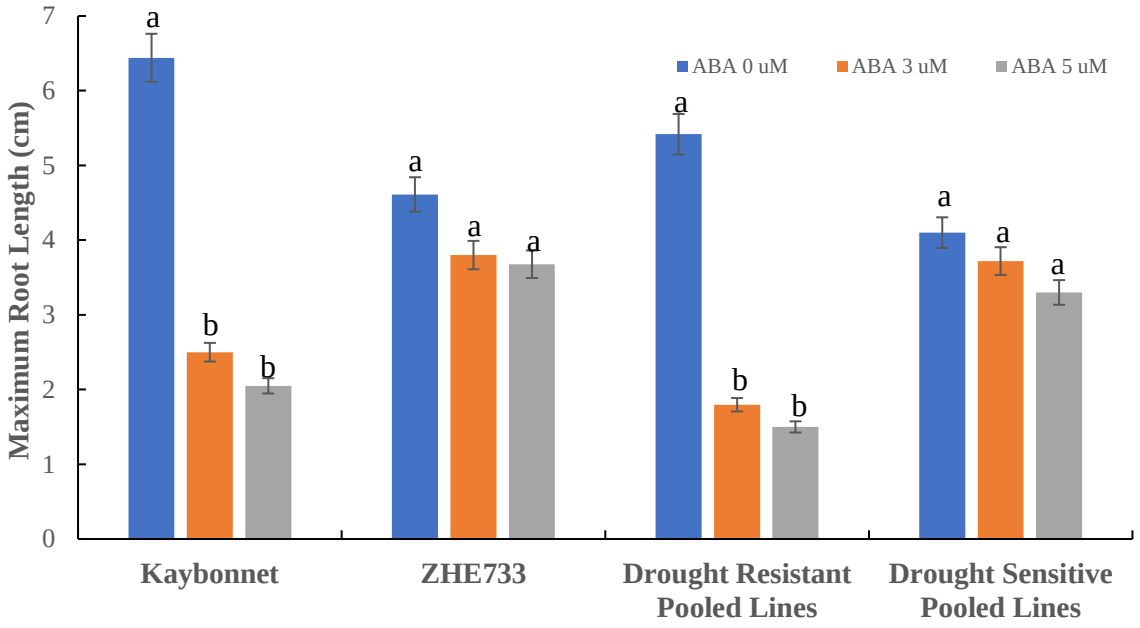

Figure 3.13. Maximum root length in the K/Z RIL population treated with ABA 0 µM, ABA 3 µM, and ABA 5 µM. Different letters indicate significant difference between ABA 0 µM, ABA 3 µM, and ABA 5 µM (*P* < 0.05; ANOVA followed by Fisher's LSD test).

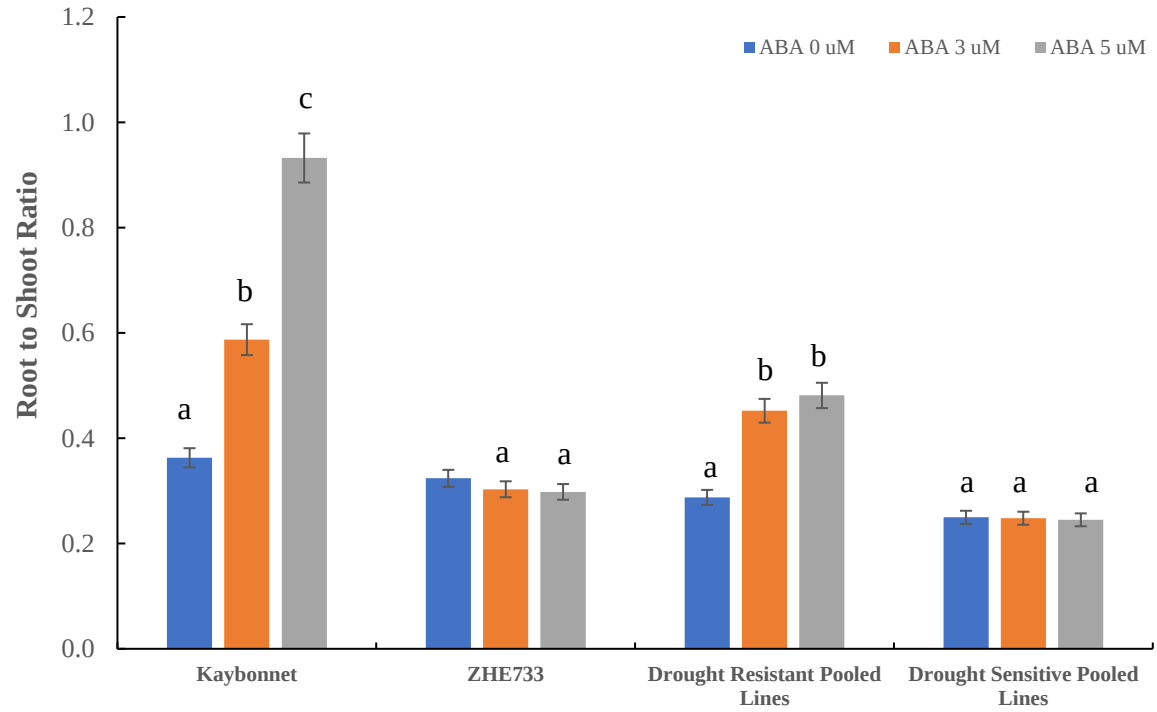

Figure 3.14. Root to shoot ratio in the K/Z RIL population treated with ABA 0 µM, ABA 3 µM, and ABA 5 µM. Different letters indicate significant difference between ABA 0 µM, ABA 3 µM, and ABA 5 µM (*P* < 0.05; ANOVA followed by Fisher's LSD test)

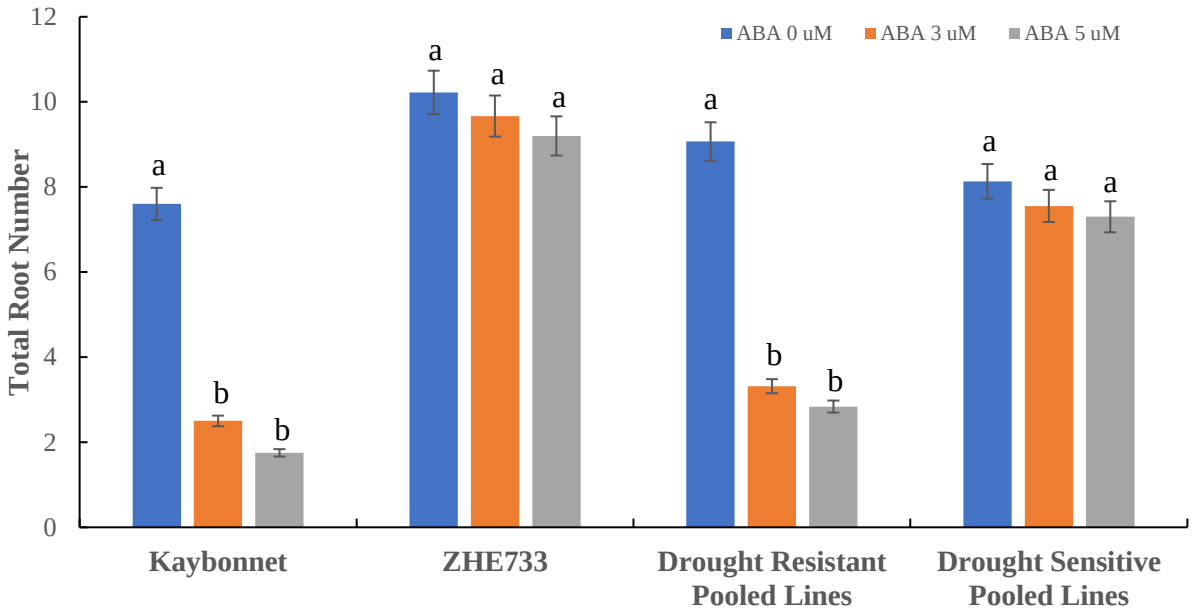

Figure 3.15. Total root number in the K/Z RIL population treated with ABA 0 µM, ABA 3 µM, and ABA 5 µM. Different letters indicate significant difference between ABA 0 µM, ABA 3 µM, and ABA 5 µM (*P* < 0.05; ANOVA followed by Fisher's LSD test).

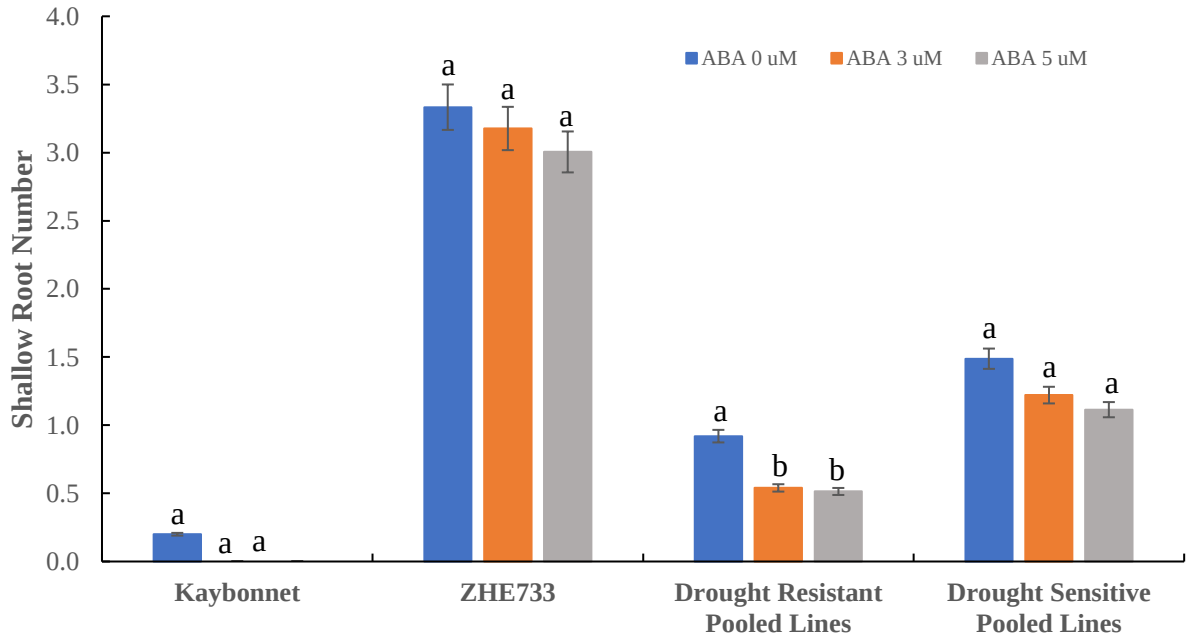

Figure 3.16. Shallow root number in the K/Z RIL population treated with ABA 0 µM, ABA 3 µM, and ABA 5 µM. Different letters indicate significant difference between ABA 0 µM, ABA 3 µM, and ABA 5 µM (*P* < 0.05; ANOVA followed by Fisher's LSD test).

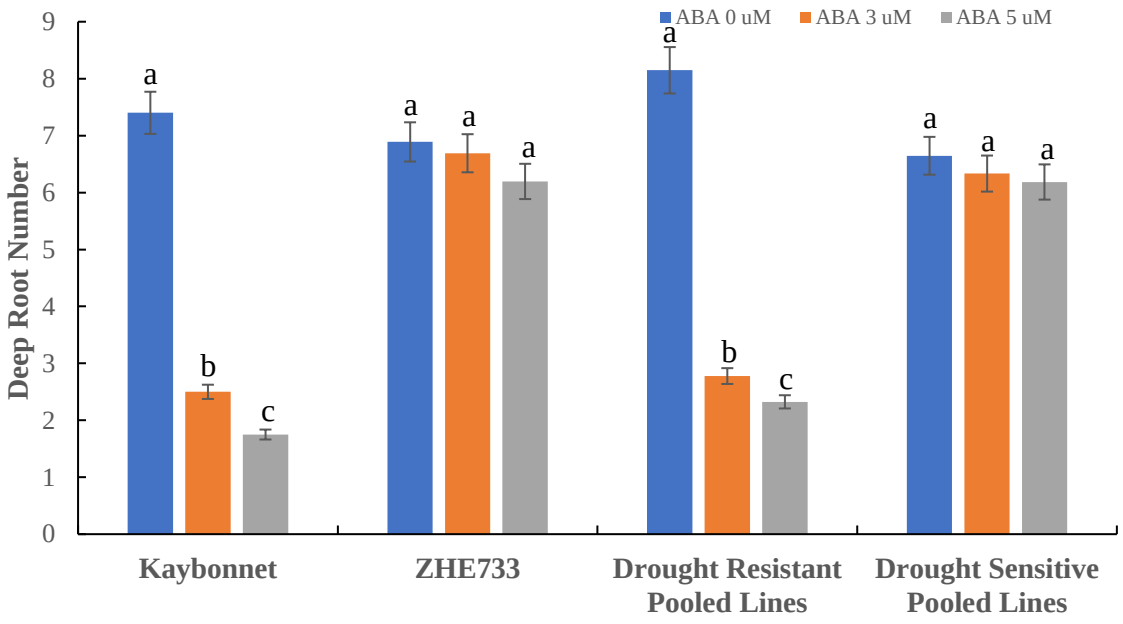

Figure 3.17. Deep root number in the K/Z RIL population treated with ABA 0 µM, ABA 3 µM, and ABA 5 µM. Different letters indicate significant difference between ABA 0 µM, ABA 3 µM, and ABA 5 µM ($P < 0.05$; ANOVA followed by Fisher's LSD test).

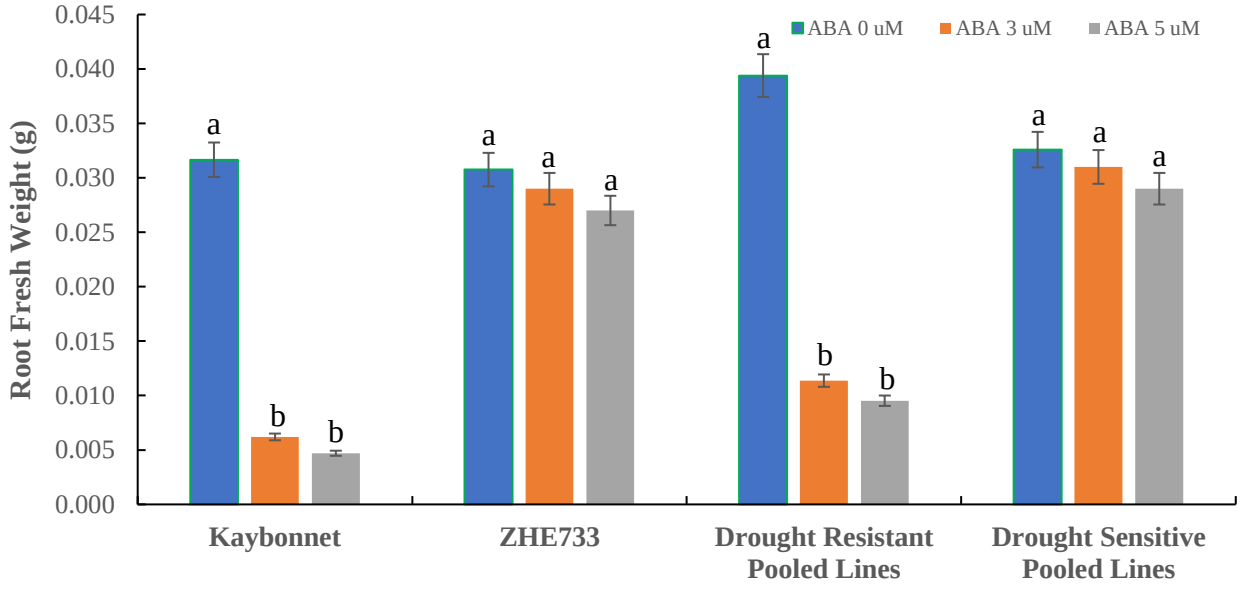

Figure 3.18. Root fresh weight in the K/Z RIL population treated with ABA 0 µM, ABA 3 µM, and ABA 5 µM. Different letters indicate significant difference between ABA 0 µM, ABA 3 µM, and ABA 5 µM ($P < 0.05$; ANOVA followed by Fisher's LSD test).

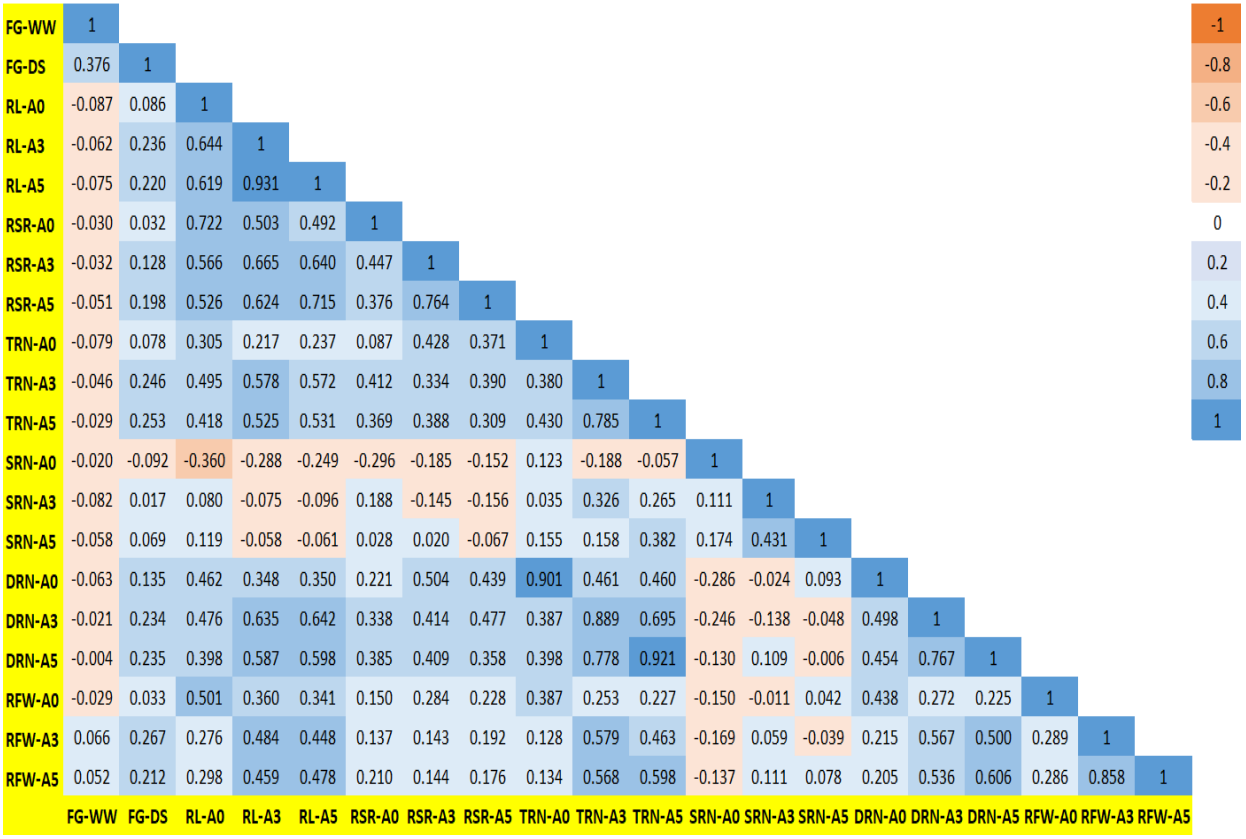

Figure 3.19. Heat map showing Pearson's correlation coefficient for filled grain per panicle number screened under WW and DS, and 6 root architectural traits screened under ABA 0 µM, ABA 3 µM, and ABA 5 µM of the K/Z RILs. The correlation (-1 to +1) are colored either in blue (positive correlation) or orange (negative correlation)

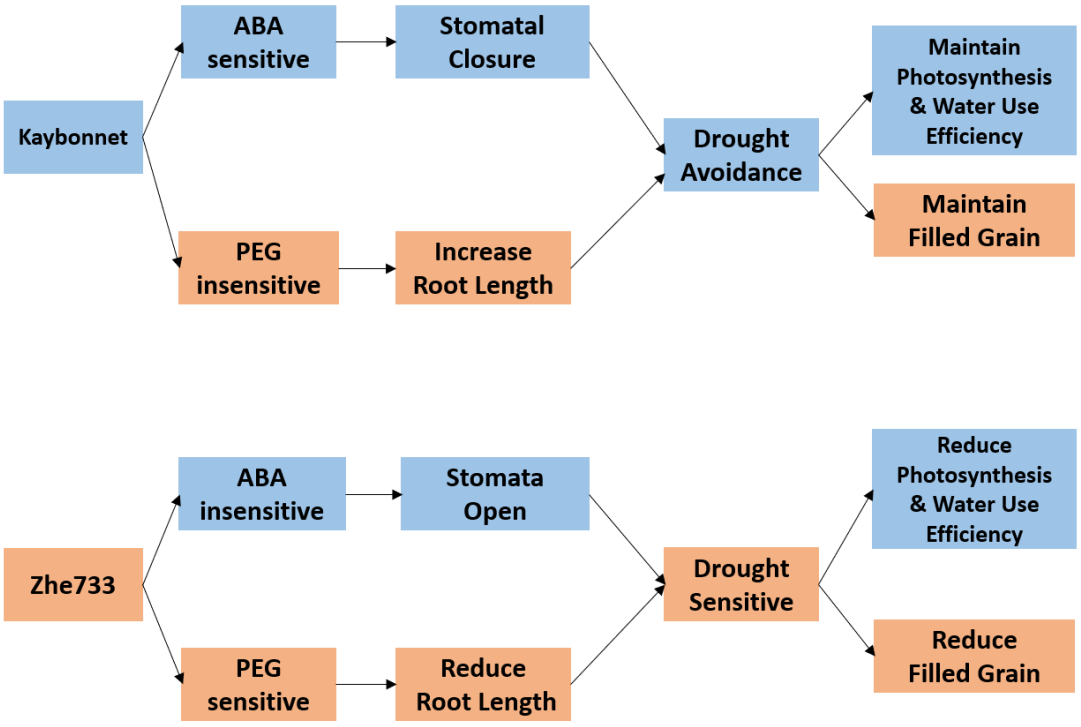

Figure 3.20. Physiological response of Kaybonnet and ZHE733 to ABA and PEG treatments.

Tables

Table 3.1. Effects of drought stress on filled grain per panicle number under field conditions, exhibiting drought resistance and drought sensitivity in K/Z RIL population of 198 lines.

Drought resistant lines	Drought sensitive lines
Kaybonnet, 100002, 100005, 100006, 100007, 100009, 100014, 100016, 100018, 100021, 100023, 100025, 100026, 100028, 100032, 100034, 100036, 100040, 100050, 100053, 100058, 100064, 100066, 100096, 100097, 100108, 100115, 100121, 100129, 100133, 100135, 100139, 100144, 100162, 100163, 100169, 100198, 100212, 100233, 100242, 100245, 100265, 100295, 100310, 100321, 100330, 100334, 100337, 100351	ZHE733, 100001, 100008, 100010, 100012, 100015, 100017, 100019, 100020, 100022, 100024, 100027, 100029, 100030, 100033, 100038, 100039, 100042, 100043, 100046, 100048, 100049, 100055, 100056, 100057, 100062, 100065, 100067, 100086, 100092, 100098, 100102, 100106, 100107, 100114, 100118, 100119, 100120, 100122, 100123, 100126, 100130, 100131, 100134, 100137, 100141, 100142, 100145, 100146, 100149, 100150, 100151, 100153, 100154, 100155, 100156, 100158, 100160, 100164, 100170, 100171, 100172, 100175, 100176, 100178, 100179, 100180, 100182, 100185, 100188, 100191, 100193, 100196, 100197, 100200, 100201, 100202, 100203, 100208, 100209, 100210, 100211, 100213, 100214, 100217, 100220, 100222, 100223, 100224, 100225, 100228, 100230, 100231, 100234, 100237, 100238, 100239, 100240, 100241, 100246, 100249, 100250, 100251, 100253, 100254, 100255, 100256, 100259, 100263, 100266, 100272, 100273, 100277, 100280, 100281, 100282, 100283, 100284, 100285, 100288, 100292, 100293, 100298, 100299, 100300, 100302, 100303, 100308, 100311, 100313, 100315, 100319, 100322, 100323, 100324, 100325, 100327, 100328, 100329, 100333, 100335, 100336, 100338, 100339, 100340, 100341, 100342, 100344, 100345, 100348, 100352

CHAPTER 4. IDENTIFICATION OF MARKERS LINKED TO DROUGHT RESISTANT TRAITS OF K/Z RIL RICE POPULATION BY BULKED SEGREGANT ANALYSIS

Abstract

Rice is the dominant staple food for more than 50% of the global population, with U.S. as the third largest exporter of rice, with an export value of US$ 1.8 billion. Rice also uses 2-3 times more water as other food crops, which totals 30% of the world's freshwater resources world-wide. Stability of rice production is facilitated by economic use of water, which is most critical during flowering and grain formation. In our research, we screened adapted U.S. rice cultivars, comprising *tropical japonica* rice genotypes, for drought resistant (DR) traits to search sources for breeding U.S. rice cultivars for a water saving agricultural system. A RIL population derived from varieties Kaybonnet (DR) and ZHE733 (drought sensitive), termed K/Z RILs, were available from the USDA Dale Bumpers National Rice Research Center and chosen for genetic analysis of DR traits. The RIL population was screened in the field at Fayetteville (AR) by giving a controlled drought stress treatment at the reproductive stage, and the effect of stress was quantified by counting number of filled grains per panicle. After a DR scoring of 198 lines of the K/Z RIL population, based on the number of filled grain per panicle, a subset of 40 lines were selected from the two extreme phenotypic tails and used for the bulked segregant analysis (BSA). The parents of K/Z RIL population and a subset of the 20 drought resistant lines (bulk resistant) and 20 drought sensitive lines (bulk sensitive) were screened by using 278 SSR markers to find polymorphisms linked to the yield-related trait number of filled grains per panicle under drought. From this BSA screen, a total of 13 polymorphic markers were identified. The SSR markers with potential linkage to DR traits are RM9, RM109, RM114, RM131, RM139, RM236, RM34, RM133, RM135, RM137, RM152, RM154, and RM155 on chromosome 1, 2, 3, 4, 6, 8, 11, and 12. Five genes, LOC_Os01g04930 (near RM9), LOC_Os01g12700 (near RM34), LOC_Os02g04640 (near RM236), LOC_Os06g08290 (near

RM133), and LOC_Os06g08250 (linked to RM133) associated to drought stress response traits were identified. These markers and genes can be used for drought resistance breeding using the resistance lines for introgression and improvement of U.S. rice.

Introduction

Rice is one of the oldest food crops and has been cultivated for 8,200-13,500 years (Molina et al., 2011). U.S. is the third largest exporter of rice, and Arkansas the largest U.S. rice-producer, which accounts for more than 40% of the total U.S. rice production of long and medium grain varieties (Quick Stats, 2016), contributing an important agriculture commodity for the state. Rice production in Arkansas has an economic value of US$ 995,217,000 grown on 1,546,000 acres (half of soybean acreage), compared to soybean (US$ 1,435,145,000), and corn (US$ 471,362,000). However, rice production in Arkansas is dependent on ground water for stable irrigation (Henry et al., 2016). Managing this resource of water by judicious use during the crop season with water-use-efficient (WUE) rice will provide a steady supply of rice grain of export quality in the world market. The Arkansas rice-growing region in the Lower Mississippi belt is among the 10 areas with the highest risk of water scarcity in the country, as are the agricultural areas (Shi et al., 2013; http://www.businessinsider.com/us-drought-water-scarcity-2013-5) in California, Nebraska, Ohio, Dakotas, N. Texas, and Minnesota. Interestingly, the distribution of WUE traits has been found to be highest in *tropical japonica*, medium in *indica*, and lowest in *aus* rice (Dingkuhn et al., 1989).

The natural drought conditions influence morphological, physiological, biochemical, and molecular changes to the rice plants and consequently cause a significant reduction in grain yield production world-wide, a threat to food security, and responsible for the great famines (Farooq et al., 2009). A number of previous studies reported that grain yield losses due to drought conditions depend on the level of the drought conditions. The grain losses under mild drought conditions (soils dried beyond -20 kPa) is 22.6% (Carrijo et al., 2017), while in the medium

drought conditions (~40% soil water deficit) is 53-92% (Lafitte et al., 2007), and in severe drought (soil water content decreased below saturation) is >50% (Daryanto et al., 2017).

Drought resistance is a quantitative trait and the regions in the genomes that consist of genes associated with a specific quantitative trait are termed as quantitative trait loci (QTL). Furthermore, genetic studies to locate genes or QTLs using molecular markers is termed as quantitative trait locus (QTL) mapping (Manikavelu et al., 2006). According to Kumar (2017), 911 QTLs correlated with 109 drought resistant traits have been mapped by using 39 mapping rice populations. Yue et al. (2008) reported QTLs linked to drought resistant traits at the reproductive stage. QTLs associated with grain yield under drought conditions also mapped (Chandra Babu, 2010; Zhang et al., 2009a; Dixit et al., 2014; Saikumar et al., 2014).

Simple Sequence Repeats (SSRs) or microsatellites are the most convenient and popular molecular markers used to search for QTLs linked to drought resistant traits in rice genotypes often screened in segregating populations made between *indica* and *japonica* subspecies due to technical simplicity, low cost, and being highly polymorphic. Many researchers have used SSR markers to identify polymorphisms between rice varieties (Sow et al., 2014; Das et al., 2013; Jin et al., 2010). Besides SSR, the others molecular markers used are Single Nucleotide Polymorphisms (SNPs), Amplified Fragment Length Polymorphisms (AFLPs), Restriction Fragment Length Polymorphisms (RFLPs), and Random Amplified Polymorphic DNAs (RAPDs). SSRs are molecular markers with short nucleotide repeats from 1 to 5 bases per unit, are polymerase chain reaction (PCR) based markers with annealing temperatures between 50 – 55 ^{o}C, co-dominant, multi-allelic with mono-, di-, tri-, tetra-, penta-, or hexa-nucleotide repeat motifs, non-redundant SSR primer pairs, and distributed throughout the genome of 12 rice chromosomes (Temnykh et al., 2000). A total of 2414 SSR primer pairs representing 2240

marker loci have been developed and validated for rice (McCouch et al., 2002). SSR markers in rice have been well identified and annotated because of the completion of the rice genome reference sequence from Nipponbare (MSU7) and the availability of many additional genomics resources, with a distribution of one SSR every 157 kb. About 92% of these primer pairs are ≥24 bp in length and consist of several motifs that contribute to variable repeats such as (GA) motifs (36%), (AT) motifs (15%), (CCG) motifs (8%), (AAG) motifs (8%), (ATT) motifs (7%), (AC) motifs (6%), (CCT) motifs (5%), (ATAG) motifs (5%), (CCA) motifs (3%), (CTG) motifs (3%), (CGT) motifs (2%), and (AAAG) motifs (2%). The most prevalent of the SSR motifs is (GA) which is abundantly represented in rice. Furthermore, the SSR loci present on the molecular genetic map of rice were mapped with every primer pair that was annotated with RM (Rice Microsatellite) followed by a unique number identifier. The position of the SSR markers on the genetic map of the 12 chromosomes of rice is presented in the Gramene database (http://www.gramene.org). These positions were identified by PCR using the primer sequences specified for each SSR marker from 3284 publicly sequenced BAC/PAC clones representing 460 MB estimated to be 83% of the rice genome. Chromosomes 1, 3, and 5 have the highest density of SSR markers with 5.6, 5.8, and 5.6 SSRs per Mb, respectively. Meanwhile, chromosomes 8, 9, 10, 11, and 12 have the lowest density with 3.8, 3.5, 3.5, 3.7. and 3.6 SSRs per Mb, respectively. These SSR markers are very important to localize the genes linked to drought stress resistance on the rice chromosomes. However, the disadvantages of SSR markers are related to the gel electrophoresis requirement and limitation of distribution in the genomic regions.

SSR markers have been identified linked to many genes in rice. Bligh et al. (1995) and Ayres et al. (1997) found an SSR marker correlated with the *waxy* gene on chromosome 6 that was important in regulating starch quality in rice. Two SSR markers also characterized are

associated with the *ges* gene on chromosome 7 which controls embryo size, vitamin, protein, and oil in the rice grain (Panaud et al., 1996; Koh et al., 1996). In addition, Xiao et al. (1996) identified 2 SSR markers on chromosome 1 linked to rice yield.

Bulk segregant analysis is a rapid and simple genotyping method to identify molecular markers linked to genomic regions or to a specific gene by using only the parents and two DNA bulks (pools) sampled from a segregating population of a single cross which is cost-effective and time-efficient (Michelmore et al., 1991). Two DNA bulks with contrasting traits (e.g., resistant and sensitive to drought conditions) are analysed to find molecular markers that differentiate them. In the BSA approach, one needs to only genotype a few number of plants to identify molecular markers near a gene of interest. Furthermore, molecular markers that are polymorphic between the bulks will be linked to the specific gene that regulates or is responsible for the phenotypic difference of the trait (Chesnokov & Artemyeva, 2015). The BSA method only focuses on the genomic region of interest of target loci. The frequency of false positives will increase when smaller bulks are used. According to Lee et al. (2010), this BSA technique can be applied to analyze simple and complex traits. Vikram et al. (2012) stated that BSA is the most efficient genotyping technique compared to the other genotyping techniques like whole population genotyping (WPG) and selective genotyping (SG), BSA requires 92.1% fewer data points.

A number of previous studies have been carried out using BSA method to identify molecular markers linked to specific loci. Michelmore et al. (1991) identified 3 RAPD markers linked to a gene for resistance to downy mildew in lettuce by BSA method. Likewise, Barakat et al. (2012) used BSA to characterize 12 SSR markers related to QTL of heat tolerance at grain filling rate in wheat. BSA analysis with SNP markers was also used by Becker et al. (2011) on an

F2 population derived from a cross between *Arabidopsis* Ler-0 high Sulfur and Selenium ionomics mutant to map the mutant allele. Vikram et al. (2012) used the BSA approach for detecting two QTLs linked to grain yield under drought conditions in rice by using two recombinant inbred line populations, Basmati 334/Swarna and N22/MTU1010. BSA analysis was also used to identify two SSR markers RM205 and RM336 associated with sheath blight resistance by Yadav et al. (2015) in the BPT-5204/ARC10531 $F_{2:3}$ rice population. Zhang et al. (2009b) identified two SSR markers RM3735 and RM3586 linked to heat tolerance in rice at the flowering stage, by using BSA method with an F2 population crossed between 996, a heat tolerant variety and 4628, a heat-sensitive variety. The BSA strategy was also used by Shashidar et al. (2005) to identify 2 RAPD markers (OPAE-09 and OPAE-14) linked to grain yield in 89 doubled haploid rice lines from an IR64/Azucena cross, with IR64 as high yielding parent and Azucena as low yielding parent. Four SSR markers (RM8085, RM212, RM302, and RM3825) were found on chromosome 1 correlated with leaf rolling and leaf drying in rice analysed by BSA method with 343 SSR markers in 250 RILs from a IR20/Nootripathu cross (Salunke et al., 2011).

The objective of this study is to screen polymorphic molecular markers to identify genes linked to productivity traits of grain yield under drought stress, measured by number of filled grains per panicle using bulk segregant analysis.

Materials and Methods

Plant material

A RIL population derived from rice varieties Kaybonnet and ZHE733, termed K/Z RILs, of 198 lines were available from the USDA Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA, and were requested for our drought resistant studies. This RIL population was derived from an F2 population by single seed descent (SSD) method. A total of forty K/Z RILs and two parental lines (Kaybonnet and ZHE733) were selected based on the filled grain per panicle number under drought stress condition to be used in this study, after screening for grain yield of the 198 lines of the K/Z RIL population under drought at the reproductive stage (R3). These forty lines consist of twenty drought resistant lines (100018, 100198, 100233, 100321, 100330, 100135, 100023, 100036, 100005, 100310, 100006, 100025, 100139, 100009, 100002, 100014, 100162, 100163, 100007, 100097); and twenty drought sensitive lines (100282, 100324, 100224, 100170, 100042, 100327, 100220, 100029, 100086, 100201, 100213, 100285, 100182, 100342, 100180, 100030, 100322, 100191, 100280, 100237) (Table 4.1.).

Drought stress treatment at the reproductive stage

The K/Z RIL population seeds of the 198 lines and two parental lines (Kaybonnet and ZHE733) were germinated and grown in the greenhouse at controlled conditions (28 to 30 ^{o}C day and 22 to 23 ^{o}C night, with a 14h light/10h dark cycle and average light intensity 580 µmol $m^{-2}s^{-1}$, 65% relative humidity) in sterilized field soil for 20 days (until V3 stage). Uniform plants were selected and transplanted to the field, divided into 6 batches (at 7-day intervals) based on their heading day data from USDA, to synchronize drought treatment at the reproductive stage. Thus, the latest heading day lines were seeded and transplanted early and the earliest heading day lines seeded last.

This RIL population was evaluated in the field at Fayetteville, AR, USA in the growing seasons (May-November) in 2016. The population was grown in a randomized complete block design with five replications and two treatments, well-watered (WW) and drought stress (DS) conditions in single-row plots of 5 m length with a spacing of 0.3 m between plants. The rice plants were planted in the control plot with normal irrigation (WW conditions) and drought plot separately at a higher ground level. At this stage, rice plants were in the vegetative stage where all plants were maintained with normal irrigation for at least 30 days. The DS treatment was then given at the reproductive stage (R3), and DS conditions were monitored with three tensiometers that were installed at three spots in the DS plot just after draining, the first in the front end of the plot, the second in the middle, and the third at the end of the plot. The DS condition was maintained continuously up to -70 kPa (severe stress). Once the soil tension reduced to -70 kPa at 30 cm soil depth, life-saving irrigation was provided thereafter through flash flooding in the DS plot and water was drained after 24h to impose the next cycle of DS till maturity. Moreover, to fertilize the field (WW and DS plots), Urea was applied in three applications at the rate of 20 g per square meter. The first application after 10 days of transplanting, the second at maximum tillering stage, and the third at panicle initiation. The weeds were controlled by manual removal. The effect of stress was quantified by measuring the filled grain per panicle number with five replications per line.

Bulk Segregant Analysis

Based on the number of filled grains per panicle from data under drought from the field experiment (Figure 4.4.), the RILs were classified into subsets of the population and were used to make pools for Bulked Segregant Analysis (BSA) (Figure 4.5.), and mapping with candidate gene markers. Young leaves of 2-week old plants of the selected lines and their parents

(Kaybonnet and ZHE733) were sampled. Their genomic DNA were extracted by using cetyl tri-methyl ammonium bromide (CTAB) method described by Murray and Thompson (1980) and tested with 278 rice SSR markers (Appendix 1) that were randomly distributed across all the 12 rice chromosomes. The primer sequences and chromosomal location of the SSR markers were obtained from the Gramene Version 21 database (http://www.gramene.org) and from the Nipponbare rice genome reference (IRGSP, 2005). The genomic DNA of the two parents was initially screened using the 278 SSR primers (Appendix 1). For BSA, equal amounts and the same concentration (10 ng/µl) of genomic DNA from each 20 drought resistant lines and 20 drought sensitive lines were used to construct two bulks, drought resistant bulk and drought sensitive bulk, respectively. DNA amplifications were performed with Thermocycler (BIO-RAD, MA, USA) under the following PCR conditions: an initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 1 min; annealing at 50, 55 or 60°C for 1 min; and extension at 72°C for 2 min followed by a 17 min final extension at 72°C. The amplification products were electrophoresed in 2 to 3% agarose gels (0.5 TBE). The run was performed with the constant current for 3.5 hours at 80 volt. The gel pictures were recorded by the gel documentation system (Alpha Imager®, USA). The size of amplified band for each SSR marker was determined based on the 1 kb plus DNA ladder (10787-018, Life Technologies). Each SSR band was scored as 1 for presence or 0 for absence or missing observation, for each genotype. The polymorphic primers were then used to screen the resistance and sensitive bulks (Figure 4.6.).

Statistical analysis of drought stress treatment at the reproductive stage

This experiment was conducted in a randomized complete block design with five replications and two treatments (WW and DS). Blocks represent a random effect and treatments

(WW and DS) represent a fixed effect. The data from 198 lines and 2 parents both under WW and DS conditions for grain yield components were analysed by analysis of variance (ANOVA) using JMP version 12.0. The Tukey's HSD (Honestly Significant Difference) was performed to compare the means of the two treatments (WW and DS) among all of the rice lines in the K/Z RIL population for significant effects (Tukey's HSD, $P < 0.05$) using JMP version 12.0.

SSR marker analysis

Polymorphic information content (PIC) values were calculated for each SSR locus as described by Anderson et al. (1993). PIC values identifying polymorphisms within a population depend on the number of detectable alleles and their frequency. Amplified fragments of different sizes were considered as different alleles. DNA bands that were amplified by a given primer were scored as presence (1) or absence (0) for all the samples under study. The PIC values of individual primers were calculated based on the formula $PIC_i = 1 - \Sigma(P_{ij})^2$. P_{ij} as the frequency of *j*th allele for *i* marker and the summation extends up to the total number of allele for the given marker. Based on Botstein et al. (1980), PIC is classified into three categories: SSR markers with PIC>0.50 are highly informative markers, 0.50<PIC<0.25 are informative markers, and PIC<0.25 are slightly informative markers.

Identification of candidate genes in polymorphic SSR markers

The position of polymorphic SSR markers were identified based on the Gramene (http://archive.gramene.org/ qtl/) database (Temnykh et al., 2000). In future analysis, the genes present within 25 Kb of the polymorphic SSR marker positions were identified using the MSU rice reference genome annotation release 7.0 (http://rice.plantbiology.msu.edu/) (Kawahara et al., 2013).

Results and Discussion

Among *tropical japonica* varieties from Southern U.S., the U.S. adapted varieties (Agrama et al., 2007) Kaybonnet (Arkansas) and Bengal (Louisiana) show the least reduction in biomass under drought, and exhibit a high number of filled grains under drought at the reproductive stage (Figure 4.1.), quite comparable to the internationally recognized varieties N22 and Vandana. For further molecular genetic analysis of drought resistant traits, we chose to continue studies with the adapted Arkansas cultivar Kaybonnet (KB) (Figure 4.3.) which has a genetic background of similar origin as Dawn (http://archive.gramene.org/newsletters/varieties/Dawn.html) developed by Dr. Beachell from Century Patna 231 (introgression from *indica/aus* cultivars into *japonica*) (Figure 4.3.).

In a screen of U.S. cultivars and well-known stress resistance rice genotypes, for the drought resistant trait number of filled grains per panicle, Kaybonnet (Arkansas) and Bengal (Louisiana) exhibit a high number of filled grains per panicle under drought at the reproductive stage in greenhouse and field conditions (Figure 4.1.). This has been consistent over the years, probably a result of adaptation to the U.S. growing conditions and selection for hardiness, with drought resilience comparable to the previously identified drought resistant genotypes from *indica* and *aus* ssp. such as N22 and Vandana. For further molecular genetic analysis of drought response, we chose to continue studies with the adapted Arkansas cultivar Kaybonnet.

The filled grain per panicle number of Kaybonnet and ZHE733, parents of the K/Z RIL population, under drought stress treatment at flowering are 50 and 16, respectively (Table 4.1.). Based on the filled grain per panicle number under drought stress, Kaybonnet is the drought resistant parent, while ZHE733 the drought sensitive parent. Kaybonnet shows higher seed set under drought stress conditions than ZHE733 (Figure 4.2.). The distribution of filled grain per panicle number from 198 lines of the K/Z RIL population is continuous, ranging from 0 to 97,

with the average 22.56 under drought stress conditions (Figure 4.4.). Furthermore, using the data on filled grain per panicle under drought, a bulk segregant analysis (BSA) strategy was carried out with 278 SSR markers (Appendix 1) to identify markers linked to the drought resistant (DR) trait. Two bulks with 20 drought resistant lines and 20 drought sensitive lines were selected based on the filled grain per panicle number from 198 lines of K/Z RIL population (Figure 4.5.). The filled grain per panicle number of the drought resistant bulk was 13.20-97.00, while for the drought sensitive bulk was 0.00-1.80 (Table 4.1.). The level of polymorphisms between the parents (Kaybonnet and ZHE733), the drought resistant bulk, and the drought sensitive bulk was evaluated by calculating polymorphism information content (PIC) values for each of the 278 SSR markers (Appendix 1). Out of the SSR markers tested, 3.23% were highly informative (PIC>0.50), 54.84% informative markers (0.50<PIC<0.25), and 41.94% markers classified as slightly informative markers (PIC<0.25), based on Botstein et al. (1980). The 12 chromosomes of rice have different sizes, so each chromosome has a different number of polymorphic SSR markers. Table 4.2. displays the variety of polymorphisms of SSR markers from each chromosome, chromosome 1 shows the most polymorphic with 23 polymorphic SSR markers (14.2%), while chromosome 12 is the least polymorphic with 7 polymorphic SSR markers (4.3%). Most of the SSRs displays a single allele fragment of 100-5000 bp size. Out of the 278 SSR markers tested, 162 (58.3%) primers were found polymorphic in the Kaybonnet & ZHE733 parents, with the other 116 (41.7%) primer pairs monomorphic. Each of these polymorphic markers was used to screen the DNA bulks of 20 resistance and the 20 sensitive lines of the K/Z RIL population.

The PIC value is a reflection of the allele diversity frequency among the parents (Kaybonnet and ZHE733), the drought resistant bulk, and drought sensitive bulk. A total of 449

alleles were amplified with an average of 1.62 alleles per locus of 278 SSR markers across 12 chromosomes with ranged PIC value from 0.00 to 0.63 and the average of PIC value of 0.24 (Appendix 1). From 278 SSR markers, 116 (41.73%) SSR markers have PIC values 0.00, 119 (42.81%) SSR markers have PIC values 0.38, 34 (12.23%) SSR markers have PIC values 0.50, and 9 (3.24%) SSR markers have PIC values 0.63. Among the polymorphic markers, 153 SSR markers produced two alleles and 9 SSR markers generated three alleles (Appendix 1). Thirteen SSR markers were identified linked to the DR trait that showed polymorphisms between two bulks, with 2 markers on chromosome 1 (RM9 and RM34), 3 markers on chromosome 2 (RM109, RM236, and RM154), 2 markers on chromosome 3 (RM114 and RM135), 1 marker on chromosome 4 (RM131), 1 marker on chromosome 6 (RM133), 2 markers on chromosome 8 (RM137 and RM152), 1 marker on chromosome 11 (RM139), and 1 marker on chromosome 12 (RM155) (Table 4.3.). The PIC of these markers range from 0.50 to 0.63 (Table 4.3.). According to Botstein et al. (1980), a PIC value more than 0.50 mean that these markers are highly informative DNA markers for detecting polymorphism. Meanwhile, PIC value less than 0.50 are not effective for estimating polymorphism.

As shown in the Figure 4.6., from one of the examples of an SSR marker linked to the DR trait RM109 on chromosome 1 shows that the drought resistant bulk had homozygous band pattern of resistance parent Kaybonnet, while the drought sensitive bulk had homozygous band pattern of the sensitive parent ZHE733. These results suggest that the drought resistant phenotype during reproductive stage was controlled by multiple genes. The results showed that RM9, RM34, RM109, RM236, RM154, RM114, RM135, RM131, RM133, RM137, RM152, RM139, and RM155 are possibly linked to DR trait. Most of those SSR markers are dinucleotide repeats (69.23%) that are similar to the observation of Cho et al. (2000) and Jain et al. (2004).

Similar results were obtained by Bool (2010), who also identified RM131 on chromosome 4 linked to the grain yield under drought stress conditions at reproductive stage using the BSA method with an F3 population of IR78910-34-B-2-2 (drought resistant parent)/IR72 (drought sensitive parent). The markers identified here can be useful for marker-assisted selection (MAS) for drought resistant breeding of U.S. rice cultivars.

Comparative mapping analysis was done using the QTL Annotation Rice Online database (Yonemaru et al., 2010; http://qtaro.abr.affrc.go.jp/cgi-bin/gbrowse/Oryza_sativa) and the Gramene QTL database (Gupta et al., 2016; https://archive.gramene.org/qtl/) for each of the thirteen polymorphic marker-linked regions to other QTLs. These include the marker RM9 (2.3 Mb) on chromosome 1 which has been found linked to QTL for panicle number. RM34 (7.0 Mb) region on chromosome 1 was mapped close to a region with QTLs for root characteristics, panicle number, salinity tolerance, and lodging resistance. The marker RM109 (2.2 Mb) linked segment on chromosome 2 is linked to QTLs for seed characteristics and grain quality; RM236 (2.1 Mb) region on chromosome 2 was associated with QTL for seed characteristics; RM154 (1.1 Mb) region on chromosome 2 has been correlated with QTL for panicle number; RM114 (18.2 Mb) region on chromosome 2 was found linked to QTL for panicle number, shoot/seedling characteristics, grain quality, and abiotic stress tolerance. The RM135 (3.3 Mb) linked segment on chromosome 3 was found correlated with QTLs for traits involved in root, shoot/seedling, flowering, grain quality, and drought tolerance. The RM131 (2.0 Mb) chromosomal segment on chromosome 4 was found linked to QTLs for panicle/flower characteristics, insect resistance, and cold tolerance. The RM133 (4.0 Mb) linked segment on chromosome 6 mapped close to the region for QTLs in panicle/flower characteristics, seed characteristics, culm/leaf, dwarf, flowering, grain quality, insect resistance, and soil stress tolerance to drought. RM137 (1.6 Mb)

on chromosome 8 was found associated with QTLs for panicle/flower characteristics, flowering, soil stress tolerance, and drought tolerance. The RM152 (0.6 Mb) segment on chromosome 8 was found linked to QTLs involved in panicle/flower characteristics, flowering, and drought tolerance. The RM139 (1.2 Mb) segment on chromosome 11 was reported to have correlation with QTLs for panicle/flower characteristics and soil stress tolerance. The RM155 (3.0 Mb) segment on chromosome 12 was found linked with QTLs for seed characteristics, grain quality, blast resistance, and soil stress tolerance.

A number of previous studies have also used BSA approach to identify SSR markers linked to drought resistance in rice. Kanagaraj et al. (2010) identified 3 SSR markers (RM212, RM302, and RM3825) on chromosome 1 associated with drought resistance in rice by using BSA strategy with 23 recombinant inbred lines of IR20/Nootripathu with extreme drought response. IR20 is the drought sensitive parent with shallow root system and Nootripathu as drought resistant parent with thicker and deep root system. The SSR markers localize to a region between 135.8 and 143.7 cM. This region on chromosome 1 has been found to be correlated with many DR traits such as grain yield, panicle length, biomass, plant height, tiller number, deep root mass, relative water content, and deep root to shoot ratio. Furthermore, Venuprasad et al. (2009) were able to characterize two SSR markers, RM324 on chromosome 2 and RM416 on chromosome 3, linked to grain yield under drought by using BSA approach with a 490 RIL rice population from the cross Apo/^{2*}Swarna. Kumar et al. (2005) reported that two SSR markers, RM223 on chromosome 8 and RM263 on chromosome 2 were associated with DR traits such as leaf rolling, leaf drying, relative water content, canopy temperature, stress recovery, plant height, and relative biomass. These SSR markers identified by the BSA method included 174 SSR markers in 38 diverse rice genotypes. The BSA strategy was also used by Bool (2010) to

characterize two SSR markers, RM113 on chromosome 1 and RM131 on chromosome 4 linked to grain yield under drought stress conditions at the reproductive stage with 510 SSR markers in an F3 population of IR78910-34-B-2-2/IR72. Two SSR markers on chromosome 1, RM431 and RM12091 identified by Ghimire et al. (2012), were correlated with grain yield under drought conditions in two RIL populations of Swarna (drought sensitive parent) and Dhagaddeshi (drought resistant parent) and also IR64 (drought sensitive parent), and Dhagaddeshi by using BSA approach.

Bharathkumar et al. (2014) characterized the SSR marker RM324 associated with drought tolerance using 122 diverse rice genotypes. The SSR marker RM27933 was found linked to grain yield under drought stress conditions identified by Boopathi et al. (2013) using BSA method in an $F_{2:3}$ rice population. Palanog et al. (2014) found six SSR markers (RM246, RM450, RM250, RM232, RM518, and RM19) in the Kali Aus/IR64 population and characterized eight SSR markers (RM495, RM572, RM246, RM211, RM250, RM231, RM3, and RM340) in the Kali Aus/MTU1010 population that correlated with grain yield under drought stress conditions at the reproductive stage using BSA-mapping with 600 SSR markers. RM231 on chromosome 3 was found linked to grain yield under drought stress conditions at the reproductive stage by Yadaw et al. (2013) using BSA method in the BC_1 population of IR77298-5-6-18/2*Sabitri. Venuprasad et al. (2011) identified RM486 and RM472 on chromosome 1 at 162.8 cM linked to DR traits that strongly associated with plant height; probably because of the presence of *sd1* locus in this region. The BSA approach was also used by Rajendra et al. (2016) to identify three SSR markers, RM1092, RM129, and RM157B that correlated with the DR trait using 36 diverse rice genotypes. Four SSR markers, RM20A, RM302, RM212, and RM286 identified by Freeg et al. (2016) could be useful for screening drought resistant lines by BSA method. Similarly,

Awasthi and Lal (2014) characterized four SSR markers, RM263, RM3825, RM212, and RM22 associated with DR traits in rice.

The total numbers of genes identified by thirteen polymorphic SSR markers using the MSU rice reference genome annotation release 7.0. were 93 with an average of 7 genes per polymorphic SSR markers (Table 4.3.). These genes were annotated into three important functional groups including biological process, molecular function, and cellular component. Furthermore, several genes identified linked to polymorphic markers were associated with abiotic stress responses including drought. For example, LOC_Os01g04930 (near RM9), LOC_Os01g12700 (near RM34), LOC_Os02g04640 (near RM236), and LOC_Os06g08290 (near RM133), detected a MYB family transcription factor that has been associated with drought response function (Nakashima et al., 2014; Lv et al., 2017; Wang et al., 2017; Wei et al., 2017). Additionally, LOC_Os06g08250 (linked to RM133) identified a zinc-finger family protein (ZFP) that was also linked to drought stress response (Liu et al., 2007; Huang et al., 2012; Luo et al., 2012). All of these genes were found involved in various biological processes, including physiological processes within the rice plants to reduce the effect of the drought stress, thus increasing the plants ability to tolerate drought stress and maintain their grain yield productivity under drought stress conditions. Therefore, identification of genes linked to polymorphic markers is an important method for determining major genes responsible for drought stress resistance in rice plants. These genes can be used for rice breeding to improve drought resistance.

Conclusions

To study the molecular genetics of drought resistant traits in a *tropical japonica* rice variety Kaybonnet adapted to U.S./Arkansas growing conditions, a RIL population developed by crossing Kaybonnet to a diverse *indica* variety ZHE733 was evaluated for drought resistant parameters. Based on the filled grain per panicle number under drought stress conditions, Kaybonnet was classified as drought resistant parent, and ZHE733 as the drought sensitive parent. Bulk segregant analysis (BSA) of the RILs was carried out with 278 characterized SSR markers to identify markers linked to the drought resistant traits. Two bulks of 20 drought resistant lines and 20 drought sensitive lines each, were selected based on the filled grain per panicle number from 198 lines of the KZ RIL population. Chromosome 1 showed the most polymorphic SSR markers with 23 polymorphic markers (14.2%), while chromosome 12 was the least polymorphic with 7 polymorphic markers (4.3%). Out of the 278 SSR markers tested, 162 (58.3%) primer pairs were polymorphic between the Kaybonnet and ZHE733 parents. A total of 449 alleles were amplified with an average of 1.62 alleles per locus, of 278 SSR markers across 12 chromosomes which ranged in PIC value from 0.00 to 0.63 and an average PIC value of 0.24. The SSR markers with potential linkage to DR traits are RM9, RM109, RM114, RM131, RM139, RM236, RM34, RM133, RM135, RM137, RM152, RM154, and RM155 on chromosomes 1, 2, 3, 4, 6, 8, 11, and 12. The total numbers of potential genes identified with these polymorphic SSR markers were 93, annotated into three important functional groups of biological process, molecular function, and cellular component. Among these, 5 genes were identified linked to 13 polymorphic markers to genes for drought stress response. These markers and genes can be effectively used for molecular breeding of drought resistance in U.S. rice.

References

Agrama, H.A., Eizenga, G.C., & Yan, W. (2007). Association mapping of yield and its components in rice cultivars. *Mol. Breed, 19,* 341-356.

Anderson, J. A., Churchill, G. A., Autrique, J. E., Tanksley, S. D., & Sorrells, M. E. (1993). Optimizing parental selection for genetic linkage maps. *Genome, 36, 1,* 181-186. doi:10.1139/g93-024

Awasthi, S. I. & Lal, J. P. (2014). Validation of SSR markers associated with drought tolerant QTLs in rice (*Oryza sativa* L.). *International Journal of Scientific Footprints, 2, 3,* 99-113.

Ayres, N. M., McClung, A. M., Larkin, P. D., Bligh, H. F. J., Jones, C. A., Park, W. D. (1997). Microsatellites and a single nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of U.S. rice germplasm. *Theor Appl Genet., 94,* 773-781.

Barakat, M. N., Al-Doss, A. A., Elshafel, A. A., & Moustafa, K. A. (2012). Bulked segregant analysis to detect quantitative trait loci (QTL) related to heat tolerance at grain filling rate in wheat using simple sequence repeat (SSR) markers. *African Journal of Biotechnology, 11, 61,* 12436-12442.

Becker, A., Chao, D. Y., Zhang, X., Salt, D. E., & Baxter, I. (2011). Bulk segregant analysis using single nucleotide polymorphism microarrays. *PLoS ONE, 6, 1,* 15993. doi:10.1371/journal.pone.0015993

Bharathkumar, S., Jitendra, K., Pragnya, P. J., Sai, K. R., Chaitanya, K. G., & Reddy, J. N. (2014). A molecular survey in rice germplasms for multiple abiotic stress tolerance to changing climate using microsatellite (SSR) markers. *Eco. Env. & Cons., 20,* 277-282.

Bligh, H. F. J., Till, R. I., & Jones, C. A. (1995). A microsatellite sequence closely linked to the waxy gene of *Oryza sativa*. *Euphytica, 86,* 83-85.

Bool, M. E. L. (2010). Detection of putative QTL quantitative trait loci for drought tolerance in rice (*Oryza sativa* L.) through bulk segregant analysis. *Agris.*

Boopathi, N. M., Swapnashri, G., Kavitha, P., Sathish, S., Nithya, R., Ratnam, W., & Kumar, A. (2013). Evaluation and bulked segregant analysis of major yield QTL *qtl12.1* introgressed into indigenous elite line for low water availability under water stress. *Rice Science, 20, 1,* 25-30. doi:10.1016/S1672-6308(13)60104-3

Botstein, D., White, R. L., Skalnick, M. H., & Davies, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. *Am. J. Hum. Genet., 32,* 314-331.

Carrijo, D. R., Lundy, M. E., & Linquist, B. A. (2017). Rice yields and water use under alternate wetting and drying irrigation: A meta-analysis. *Field Crop Res., 203*, 173-180.

Chandra, B. R. (2010). Breeding for drought resistance in rice: An integrated view from physiology to genomics. *Electron J Plant Breeding, 1, 4*, 1133-1141.

Chesnokov, Y. V. & Artemyeva, A. M. (2015). Evaluation of the measure of polymorphism information of genetic diversity. *Agricultural biology, 50, 5*, 571-578. doi:10.15389/agrobiology.2015.5.571eng

Cho, Y. G., Ishii, T., Temnykh, S., Chen, X., Lipovich, L., & McCouch S. R. (2000). Diversity of microsatellites derived from genomic libraries and Gene bank sequences in rice (*Oryza sativa* L.). *Theor. Appl. Genet., 100*, 713-722.

Daryanto, S., Wang, L., & Jacinthe, P.A. (2017). Global synthesis of drought effects on cereal, legume, tuber and root crops production. *A review. Agric. Water Manag., 179*, 18-33.

Das, B., Sengupta, S., Parida, S. K., Roy, B., Ghosh, M., Prasad, M., et al. (2013). Genetic diversity and population structure of rice landraces from Eastern and North Eastern States of India. *BMC Genet., 14, 1*, 71. doi:10.1186/ 1471-2156-14-71

Dingkuhn, M., Cruz, R. T., O'Toole, J. C., & Dorffling, K. (1989). Net photosynthesis, water use efficiency, leaf water potential and leaf rolling as affected by water deficit in tropical upland rice. *Aust. J. Agric., 40,* 1171-1181.

Dixit, S., Singh, A., Sta Cruz, M. T., Maturan, P. T., Amante, M., & Kumar, A. (2014). Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC Genet., 15, 16*.

Farooq, H., Basra, S. M. A., Wahid, H., & Rehman, H. (2009). Exogenously applied nitric oxide enhances the drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). *J Agron Crop Sci., 195, 4*, 254-261.

Freeg, H. A., Anis, G. B., Abo-Shousha, A. A., El-Banna, A. N., & El-Sabagh, A. (2016). Genetic diversity among some rice genotypes with different drought tolerance based on SSR markers. *Cercetari Agronomice in Moldova, 3, 167,* 39-50. doi:10.1515/cerce-2016-0024

Ghimire, K. H., Quiatchon, L. A., Vikram, P., Swamy, B. P. M., Dixit, S., Ahmed, H., Hernandez, J. E., Borromeo, T. H., & Kumar, A. (2012). Identification and mapping of a QTL (*qDTY$_{1.1}$*) with a consistent effect on grain yield under drought. *Field Crops Research, 131,* 88-96. doi:10.1016/j.fcr.2012.02.028

Gupta, P., Naithani, S., Tello-Ruiz, M. K., Chougule, K., D'Eustachio, P., Fabregat, A., Jiao, Y., Keays, M., Lee, Y. K., Kumari, S., Mulvaney, J., Olson, A., Preece, J., Stein, J., Wei, S., Weiser, J., Huerta, L., Petryszak, R., Kersey, P., Stein, L. D., Ware, D., & Jaiswal, P.

(2016). Gramene Database: Navigating Plant Comparative Genomics Resources. *Curr Plant Biol.*, 7-15. PMID: 28713666. doi:10.1016/j.cpb.2016.12.005.

Henry, C., Hirsh, S., Anders, M., Vories, E., Reba, M., Watkins, K., & Hardke, J. (2016). Annual irrigation water use for Arkansas rice production. *J. Irrig. Drain Eng*., doi:10.1061/(ASCE)IR.1943-4774.0001068 , 05016006.

Huang, J., Sun, S., Xu, D., et al. (2012). A TFIIIA-type zinc finger protein confers multiple abiotic stress tolerances in transgenic rice (*Oryza sativa* L.). *Plant Molecular Biology*, *80, 3*, 337-350.

http://archive.gramene.org/newsletters/varieties/Dawn.html

http://qtaro.abr.affrc.go.jp/cgi-bin/gbrowse/Oryza_sativa

http://www.gramene.org

https://archive.gramene.org/qtl/

IRGSP. (2005). The map-based sequence of the rice genome. *Nature, 436,* 793-800.

Jain, S., Jain, R. K., & McCouch, S. R. (2004). Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. *Theor. Appl. Genet., 109*, 965-977.

Jin, L., Lu, Y., Xiao, P., Sun, M., Corke, H., & Bao, J. (2010). Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. *Theor Appl Genet., 121, 3,* 475-87.

Kamoshita, A., Babu, R. C., Boopathi, N., & Fukai, S. (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crops Res, 109*, 1-23.

Kanagaraj, P., Silvas, J. P., Sheeba, J. A., Biji, K. R., Paul, S. B., Senthil, A., & Babu, R. C. (2010). Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). *Current Science, 98, 6.*

Kawahara, Y., de la Bastide, M., Hamilton J. P., Kanamori, H., McCombie, W. R., Ouyang, S., Schwartz, D. C., Tanaka, T., Wu, J., Zhou, S., Childs, K. L., Davidson, R. M., Lin, H., Quesada-Ocampo, L., Vaillancourt, B., Sakai, H., Lee, S. S., Kim, J., Numa, H., Itoh, T., Buell, C. R., Matsumoto, T. (2013). Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. *Rice*, *6, 4.*

Koh, H. J., Heu, M. H., & McCouch, S. R. (1996). Molecular mapping of the *ge* gene controlling the super-giant embryo character in rice (*Oryza sativa* L.). *Theor Appl Genet., 93*, 257-261.

Kumar, A. (2017). Development and Characterization of Rice Genotypes for Water Use Efficiency and Drought Resistance. Theses and Dissertations. University of Arkansas.

Kumar, B., Gomez, S. M., Boopathi, N. M., Kumar, S. S., Kumaresan, D., Biji, K. R., Babu, B. K., Prasad, N. S. R., Shanmugasundaram, P., & Babu, R. C. (2005). Identification of Microsatellite Markers Associated with Drought Tolerance in Rice (*Oryza sativa* L.) using Bulked Line Analysis. *Tropical Agricultural Research, 17,* 39-47.

Lafitte, H. R., Yongsheng, G., Yan, S., & Li, Z. K. (2007). Whole plant responses, key processes, and adaptation to drought stress: The case of rice. *J. Exp. Bot., 58,* 169-175.

Lee, H. S., Ahn, S. N., Sasaki, K., Chun, N. J., Choi, K. S., & Sato, T. (2010). Identification of molecular markers for photoblastism in weedy rice. *Korean J. Breed. Sci., 42,* 144-150.

Liu, K., Wang, L., Xu, Y., et al. (2007). Overexpression of OsCOIN, a putative cold inducible zinc finger protein, increased tolerance to chilling, salt and drought, and enhanced proline level in rice. *Planta, 226, 4,* 1007-1016.

Luo, X., Bai, X., Zhu, D., et al. (2012). GsZFP1, a new Cys2/His2-type zinc-finger protein, is a positive regulator of plant tolerance to cold and drought stress. Planta, 235, 6, 1141-1155.

Lv, Y., Yang, M., Hu, D., Yang, Z., Ma, S., Li, X., et al. (2017). The OsMYB30 transcription factor suppresses cold tolerance by interacting with a JAZ Protein and suppressing β-Amylase expression. *Plant Physiol. 173,* 1475-1491. doi:10.1104/pp.16.01725

Manikavelu, A., Nadarajan, N., Ganesh, S. K., Gnanamalar, R. P., & Chandra, B. R. (2006). Drought tolerance in rice: Morphological and molecular genetic consideration. *Plant Growth Regul., 50, 2/3,* 121-138.

McCouch, S. R., Teytelman, L., Xu, Y., Lobos, K. B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., Xing, Y., Zhang, Q., Kono, I., Yano, M., Fjellstrom, R., DeClerck, G., Schneider, D., Cartinhour, S., Ware, D., & Stein, L. (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research, 9,* 257-279.

Michelmore, R. W., Paran, I., & Kesseli, R. V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregation populations. *Proceedings of the National Academy of Sciences, 88,* 9828-9832.

Molina, J., Sikora, M., Garud, N., Flowers, J. M., Rubinstein, S., Reynolds, A., et al. (2011). Molecular evidence for a single evolutionary origin of domesticated rice. *Proc. Natl. Acad. Sci. USA., 108,* 8351-8356. doi:10.1073/pnas.1104686108

Murray, M. G. & Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research, 8, 19*.

Nakashima, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2014). The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. *Front. Plant Sci. 5, 170*. doi:10.3389/fpls.2014.00170

Palanog, A. D., Swami, B. P. M., Shamsudin, N. A. A., Dixit, S., Hernandez, J. E., Boromeo, T. H., Cruz, P. C. S., & Kumar, A. (2014). Grain yield QTLs with consistent-effect under reproductive-stage drought stress in rice. *Field Crops Research, 161,* 46-54. doi:10.1016/j.fcr.2014.01.004

Panaud, O., Chen, X., & McCouch, S. R. (1996). Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Mol Gen Genet., 252,* 597-607.

Quick Stats, USDA-NASS, State Agriculture Overview: Arkansas. (2016). https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=ARKANSAS

Rajendra, P. N. S., Suresh, R., Michael, G. S., Chandra, B. R., & Shanmugasundaram, P. (2016). Identification of microsatellite markers linked to drought tolerance in rice (*Oryza sativa* L.) through bulked line analysis. *Electronic Journal of Plant Breeding, 7, 4,* 895-903. doi:10.5958/0975-928X.2016.00122.8

Saikumar, S., Gouda, P. K., Saiharini, A., Varma, C. M. K., Vineesha, O., Padmavathi, G., & Shenoy, V. V. (2014). Major QTL for enhancing rice grain yield under lowland reproductive drought stress identified using an *O. sativa/O. glaberrima* introgression line. *Field Crops Res., 163,* 119-131.

Salunkhe, A. S., Poornima, R., Prince, K. S. J., Kanagaraj, P., Sheeba, J. A., Amudha, K., Suji, K. K., Senthil, A., & Babu, R. C. (2011). Fine mapping QTL for drought resistant traits in rice (*Oryza sativa* L.) using bulk segregant analysis. *Mol Biotechnol., 49,* 90-95. doi:10.1007/s12033-011-9382-x

Shashidhar, H. E., Vonod, M. S., Sudhir, N., Sharma, G. V., & Krishnamurthy, K. (2005). Markers linked to grain yield using bulked segregant analysis approach in rice (*Oryza sativa* L.). *Rice Genet. Newsl., 22,* 69-71.

Shi, D., Devineni, N., Lall, U., & Piñero, E. (2013). America's water risk: water stress and climate variability. Earth Institute, Columbia University, New York http://www.businessinsider.com/us-drought-water-scarcity-2013-5

Sow, M., Ndjiondjop, M. N., Sido, A., Mariac, C., Laing, M., & Bezançon, G. (2014). Genetic diversity, population structure and differentiation of rice species from Niger and their potential for rice genetic resources conservation and enhancement. *Genet Resour Crop Evol., 61, 1,* 199-13.

Temnykh, S., Park, W. D., Ayers, N., Cartinhour, S., Hauck, N., Lipovich, L., Cho, Y. G., Ishii, T., & McCouch, S. R. (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics, 100*, 697-712.

Venuprasad, R., Bool, M.E., Quiatchon, L., Cruz, M. T. S., Amante, M., & Atlin, G. N. (2011). A large-effect QTL for rice grain yield under upland drought stress on chromosome 1. *Mol Breeding, 30*, 535-547. doi:10.1007/s11032-011-9642-2

Venuprasad, R., Dalid, C. O., Valle, M. D., Zhao, D., Espiritu, M., Cruz, M. T. S., Amante, M., Kumar, A., & Atlin, G. N. (2009). Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theor Appl Genet., 120*, 177-190. doi:10.1007/s00122-009-1168-1

Vikram, P., Swamy, B. P. M., Dixit, S., Ahmed, H., Cruz, M. T. S., Singh, A. K., Ye, G., & Kumar, A. (2012). Bulk segregant analysis: "An effective approach for mapping consistent-effect drought grain yield QTLs in rice". *Field Crops Research, 134*, 185-192.

Wang, F. Z., Chen, M. X., Yu, L. J., Xie, L. J., Yuan, L. B., Qi, H., et al. (2017). OsARM1, an R2R3 MYB transcription factor, is involved in regulation of the response to arsenic stress in rice. Front. Plant Sci. 8, 1868. doi:10.3389/fpls.2017. 01868

Wei, Q., Luo, Q., Wang, R., Zhang, F., He, Y., Zhang, Y., et al. (2017). A wheat R2R3-type MYB transcription factor TaODORANT1 positively regulates drought and salt stress responses in transgenic tobacco plants. *Front. Plant Sci. 8*, 1374. doi:10.3389/fpls.2017.01374

Xiao, J., Grandillo, Ahn. S. N., McCouch, S. R., Tanksley, S. D., Li, J., & Yuan, L. (1996). Genes from wild rice improve rice yield. *Nature, 384*, 223-224.

Yadav, S., Anuradha, G., Kumar, R. R., Vemireddy, L. R., Sudhakar, R., et al. (2015). Bulked segregant analysis to detect main effect of QTL associated with sheath blight resistance in BPT-5204/ARC10531 rice (*Oryza sativa* L). *J Rice Res., 3, 149*. doi:10.4172/2375-4338.1000149

Yadaw, R. B., Dixit, S., Raman, A., Mishra, K. K., Vikram, P., Swamy, B. P. M., Cruz, M. T. S., Maturan, P. T., Pandey, M., & Kumar, A. (2013). A QTL for high grain yield under lowland drought in the background of popular rice variety Sabitri from Nepal. *Field Crops Research, 144*, 281-287. doi:10.1016/j.fcr.2013.01.019

Yonemaru, J., Yamamoto, T., Fukuoka, S., Uga, Y., Hori, K., & Yano, M. (2010). Q-TARO:QTL Annotation Rice Online Database. *Rice, 3, 194*. doi:10.1007/s12284-010-9041-z

Yue, B., Xue, W. Y., Luo, L. J., & Xing, Y. Z. (2008). Identification of quantitative trait loci for four morphologic traits under water stress in rice (*Oryza sativa* L.). *J Genet Genom., 35, 9*, 569-575.

Zhang, Y. S., Luo, L. J., Liu, T. M., Xu, C. G., & Xing, Y. Z. (2009a). Four rice QTL controlling number of spikelets per panicle expressed the characteristics of single mendelian gene in near isogenic backgrounds. *Theor Appl Genet., 118, 6,* 1035-1044.

Zhang, G., Chen, L., Xiao, G., Xiao, Y., Chen, X., & Zhang, S. (2009b). Bulked segregant analysis to detect QTL related to heat tolerance in rice (*Oryza sativa* L.) using SSR markers. *Agricultural science in China, 8, 4,* 482-487. doi:10.1016/S1671-2927(08)60235-7

Figures

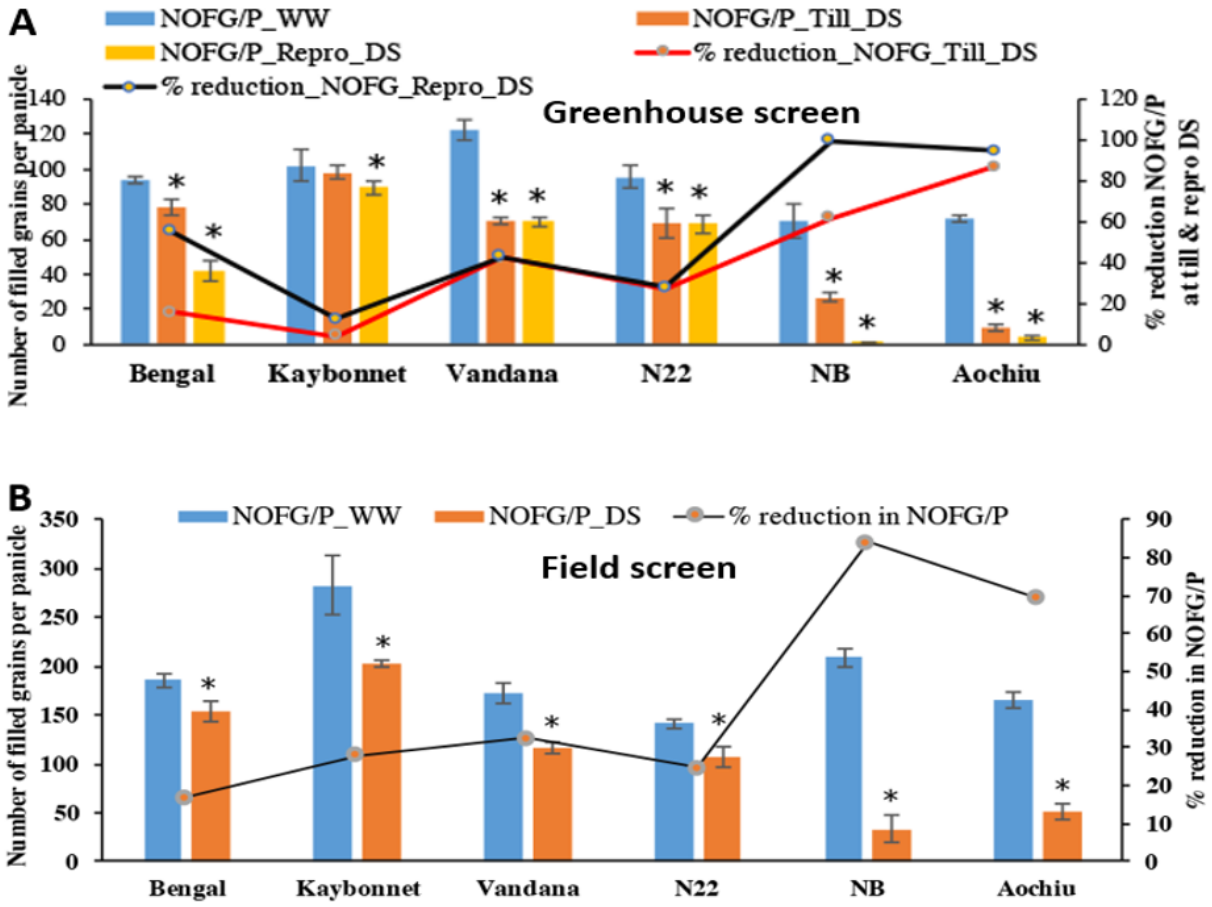

Figure 4.1. Drought screen of six diverse rice genotypes for number of filled grains per panicle (NOFG/P) under controlled drought at reproductive stages in greenhouse (A) and field conditions (B). In the greenhouse, the effect of drought stress was compared for initiation at the early tillering stage and at the reproductive stage of panicle initiation.

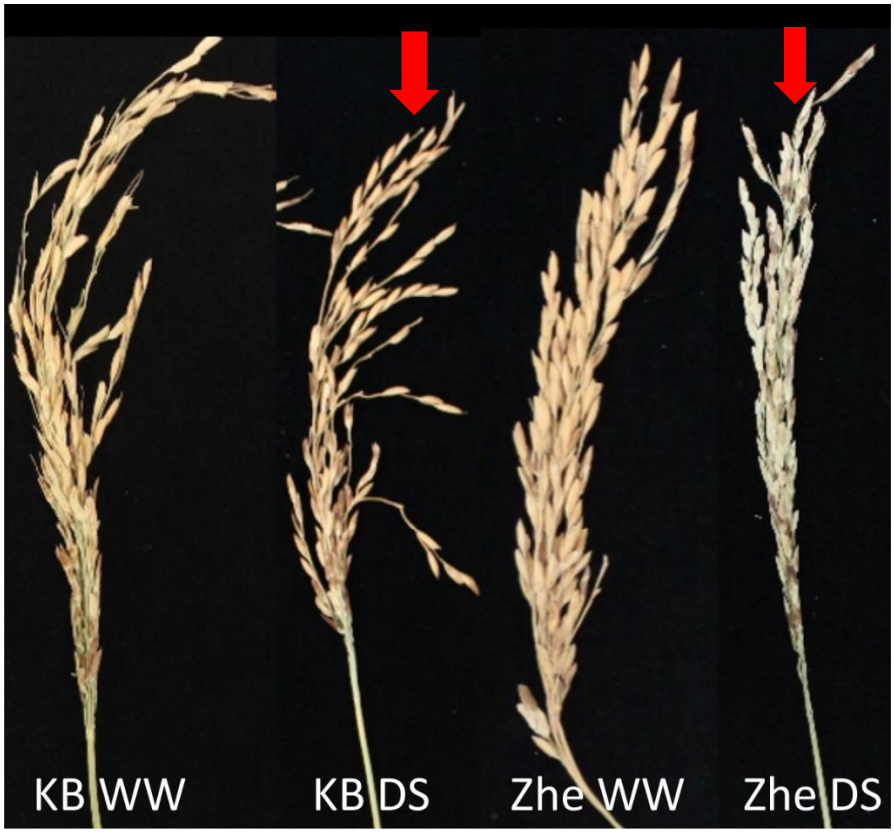

Figure 4.2. Panicle phenotypes of Kaybonnet (KB) as drought resistant parent under WW and DS, compared to drought sensitive parent ZHE733 under WW and DS treatments. KB shows higher seed set under drought than ZHE733.

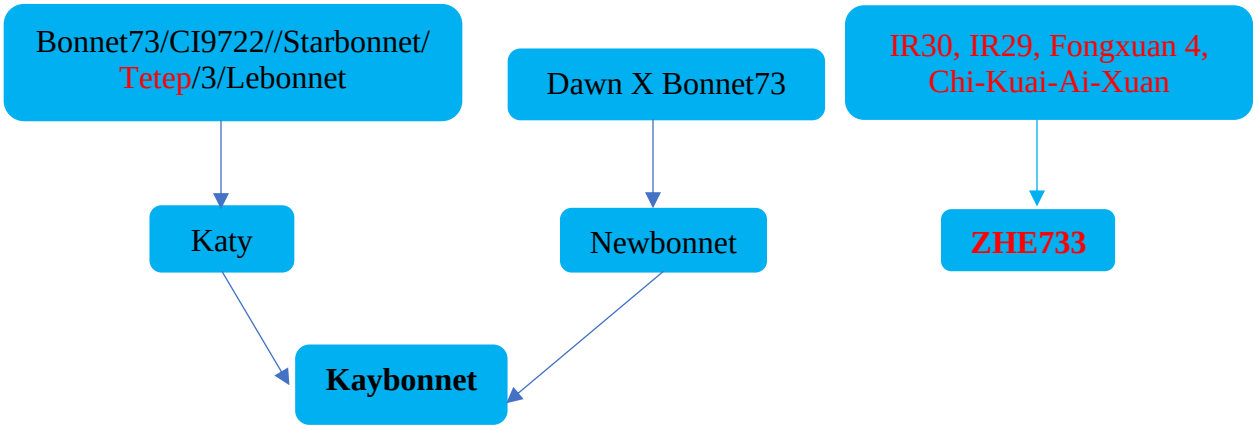

Figure 4.3. Pedigree of the K/Z RIL parents. Kaybonnet is a cross between Katy and Newbonnet, Katy is a *tropical japonica* cultivar with large introgressions from *indica* landrace Tetep. ZHE733 was developed from a multiple cross of IR30, IR29, Fongxuan 4, Chi-Kuai-Ai-Xuan. Black: *Japonica*, Red: *Indica*

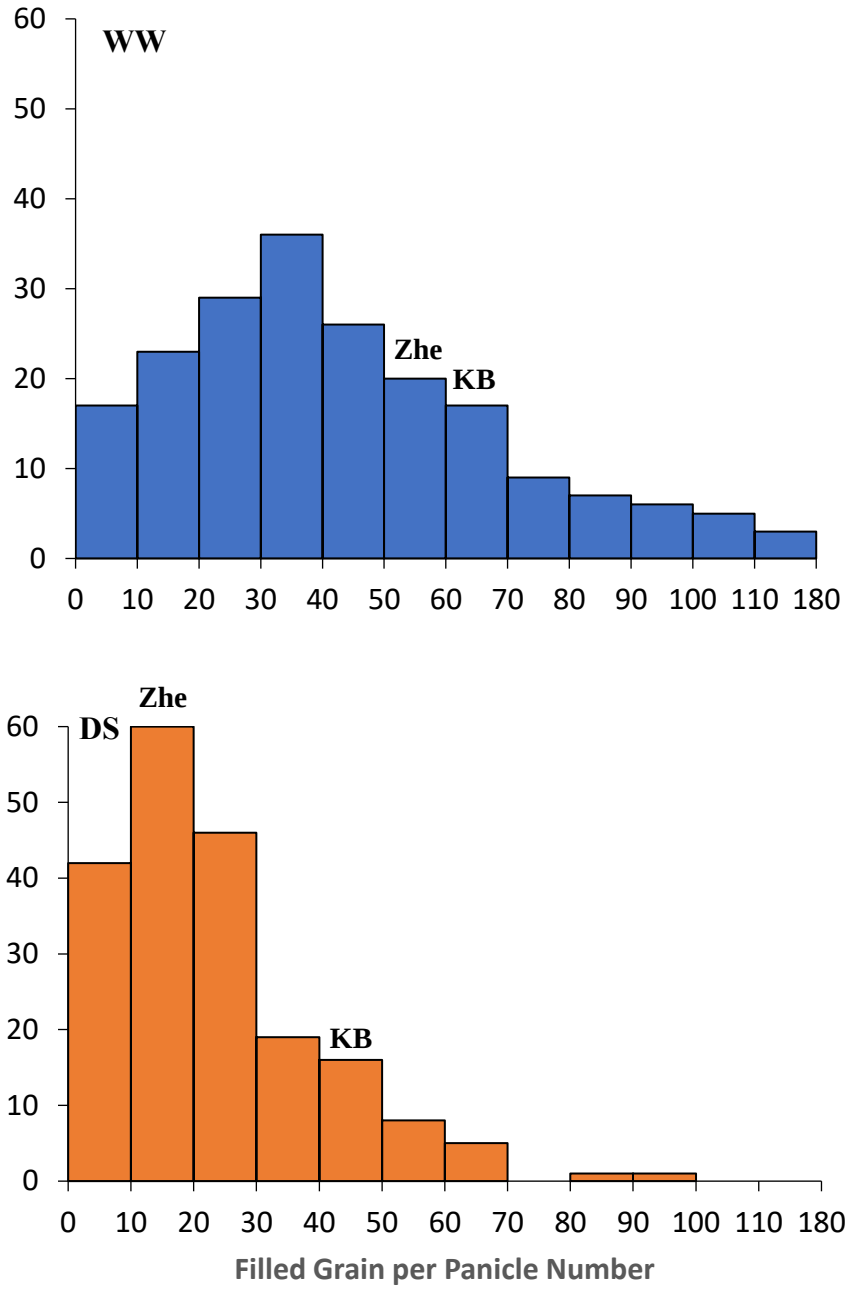

Figure 4.4. Frequency distribution of the K/Z RIL population for number of filled grains per panicle. This continuous frequency distribution across different classes, suggests that multiple genes control the number of filled grains per panicle.

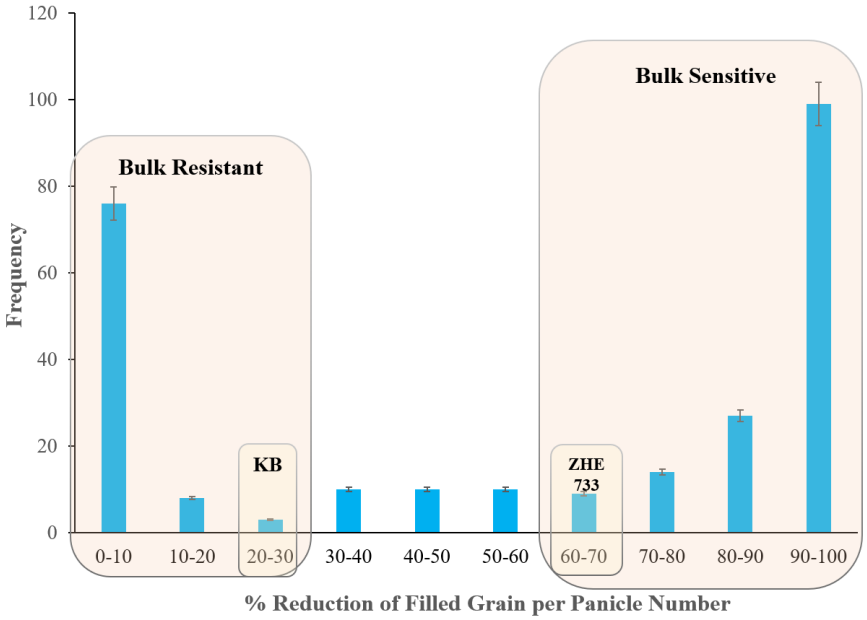

Figure 4.5. Frequency distribution of the K/Z RIL population for the reduction in the number of filled grain per panicle (NOFG/P class intervals) under drought stress. We defined 20 RILs (bulk resistant) on the basis of consistently showing high drought resistance (≤25% reduction) as drought resistant progeny, and an additional set of 20 RILs (bulk sensitive) consistently showing high sensitivity (≥ 60% reduction) as drought sensitive progeny.

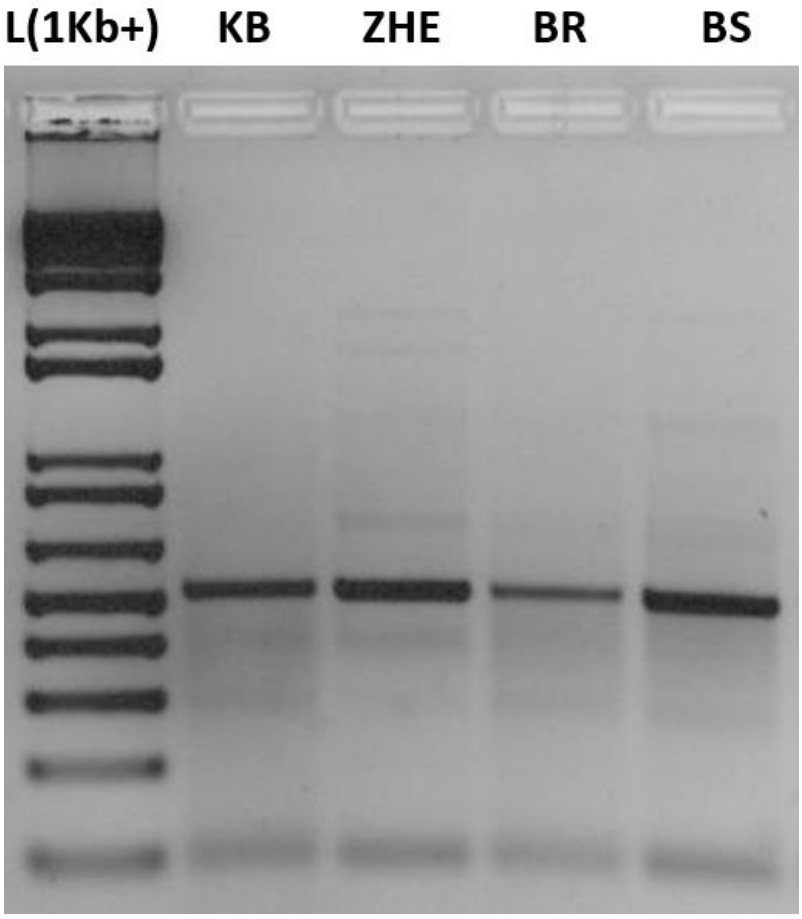

Figure 4.6. Example of banding pattern from BSA of an SSR marker RM 109 in K/Z RIL population, with KB (Kaybonnet, drought resistant parent), ZHE (ZHE733, drought sensitive parent), BR (Bulk Resistant), and BS (Bulk Sensitive). Most of the SSRs displayed a single allele fragment of 100-5000 bp size, with polymorphisms of a few nucleotides. Out of the 278 SSR markers, 162 (58.3%) primers were polymorphic on the Kaybonnet & ZHE733. The other 116 (41.7%) primer pairs were monomorphic. Out of 162 SSR polymorphic primers between parents, thirteen primer pairs for RM9 & RM34 (Chr 1), RM109, RM236, & RM154 (Chr 2), RM114 & RM135 (Chr 3), RM131 (Chr 4), RM133 (Chr 6), RM137 & RM152 (Chr 8), RM139 (Chr 11), and RM155 (Chr 12) showed polymorphism between bulks and were tested for co-segregation among the individual genotypes constituting the bulks. The drought resistant lines had majority of alleles from the resistance parent Kaybonnet, and the sensitive lines had most alleles similar to ZHE733, the sensitive parent.

Tables

Table 4.1. Drought resistant and sensitive lines of K/Z RIL population based on the filled grain per panicle number in the drought stress conditions used in this study.

No	Lines	Filled grain per panicle number			Type
		Well-watered	Drought Stress	Reduction (%)	
1	Kaybonnet	66.00	50.00	24.24	Drought resistant
2	ZHE733	43.20	16.00	62.96	Drought sensitive
3	100018	102.60	97.00	5.46	Drought resistant
4	100198	51.40	51.00	0.78	Drought resistant
5	100233	54.60	54.60	0.00	Drought resistant
6	100321	67.00	66.20	1.19	Drought resistant
7	100330	69.00	69.00	0.00	Drought resistant
8	100135	23.00	22.60	1.74	Drought resistant
9	100023	20.00	19.80	1.00	Drought resistant
10	100036	46.60	42.00	9.87	Drought resistant
11	100005	33.60	29.40	12.50	Drought resistant
12	100310	22.40	22.40	0.00	Drought resistant
13	100006	31.60	28.00	11.39	Drought resistant
14	100025	52.20	45.00	13.79	Drought resistant
15	100139	24.80	24.20	2.42	Drought resistant
16	100009	64.60	54.00	16.41	Drought resistant
17	100002	72.00	65.00	9.72	Drought resistant
18	100014	56.80	47.40	16.55	Drought resistant
19	100162	16.00	13.20	17.50	Drought resistant
20	100163	33.00	27.00	18.18	Drought resistant
21	100007	43.60	37.40	14.22	Drought resistant
22	100097	37.20	30.40	18.28	Drought resistant
23	100282	6.00	0.40	93.33	Drought sensitive
24	100324	6.00	0.00	100.00	Drought sensitive
25	100224	16.00	0.00	100.00	Drought sensitive
26	100170	17.00	0.00	100.00	Drought sensitive
27	100042	13.00	0.20	98.46	Drought sensitive
28	100327	18.00	0.60	96.67	Drought sensitive
29	100220	19.00	0.00	100.00	Drought sensitive
30	100029	12.00	0.60	95.00	Drought sensitive
31	100086	6.00	0.00	100.00	Drought sensitive
32	100201	16.00	0.40	97.50	Drought sensitive
33	100213	8.00	0.00	100.00	Drought sensitive
34	100285	6.00	0.00	100.00	Drought sensitive
35	100182	15.00	0.00	100.00	Drought sensitive
36	100342	18.00	0.00	100.00	Drought sensitive
37	100180	6.00	0.00	100.00	Drought sensitive
38	100030	11.00	0.00	100.00	Drought sensitive
39	100322	11.00	0.00	100.00	Drought sensitive
40	100191	24.00	1.80	92.50	Drought sensitive
41	100280	12.00	0.00	100.00	Drought sensitive
42	100237	17.00	0.60	96.47	Drought sensitive

Table 4.2. Polymorphic markers of Kaybonnet and ZHE733 as K/Z RIL population parents.

Chromosome	Number of polymorphic SSR markers	Number of total SSR markers
1	23	41
2	22	34
3	16	28
4	9	19
5	13	21
6	14	23
7	12	21
8	15	28
9	12	19
10	6	15
11	13	17
12	7	12
Total	**162**	**278**

Table 4.3. Polymorphic markers of Kaybonnet, ZHE733, bulked drought resistant, and bulked drought sensitive.

Chromosome	Polymorphic SSR markers	PIC value	Physical map position (Mb) (Temnykh et al., 2000)	QTL Annotation Rice Online Database	Genes within 25 Kb of the polymorphic SSR markers position	MSU gene annotation (Kawahara et al., 2013)
1	RM9	0.50	2300000	Panicle number	LOC_Os01g04920	Glycosyl transferase
					LOC_Os01g04930	MYB family transcription factor
					LOC_Os01g04950	Peptide transporter (PTR2)
					LOC_Os01g04960	Transposon protein
1	RM34	0.50	7000000	Root characteristics, panicle number, salinity tolerance, and lodging resistance	LOC_Os01g12660	AAA-type ATPase family protein
					LOC_Os01g12670	Expressed protein
					LOC_Os01g12680	C4-dicarboxylate transporter/malic acid transport protein
					LOC_Os01g12690	Plant-specific domain TIGR01568 family protein
					LOC_Os01g12700	MYB family transcription factor
					LOC_Os01g12710	Oxidoreductase, short chain dehydrogenase/reductase family domain containing protein
2	RM109	0.50	2200000	Seed characteristics and grain quality	LOC_Os02g04760	Cycloartenol synthase
					LOC_Os02g04770	Expressed protein
					LOC_Os02g04780	Expressed protein
					LOC_Os02g04790	Expressed protein
					LOC_Os02g04800	AIG2-like family domain containing protein
					LOC_Os02g04810	Auxin response factor 5
					LOC_Os02g04820	Retrotransposon protein

Table 4.3. Polymorphic markers of Kaybonnet, ZHE733, bulked drought resistant, and bulked drought sensitive (Continued).

Chromosome	Polymorphic SSR markers	PIC value	Physical map position (Mb) (Temnykh et al., 2000)	QTL Annotation Rice Online Database	Genes within 25 Kb of the polymorphic SSR markers position	MSU gene annotation (Kawahara et al., 2013)
2	RM236	0.50	2100000	Seed characteristics	LOC_Os02g04630	Sodium/calcium exchanger protein
					LOC_Os02g04640	Myb-like DNA-binding domain containing protein
					LOC_Os02g04650	Activator of 90 kDa heat shock protein ATPase homolog
					LOC_Os02g04660	Arginine N-methyltransferase 5
					LOC_Os02g04670	Glucan endo-1,3-beta-glucosidase precursor
					LOC_Os02g04680	OsSPL3-SBP-box gene family member
					LOC_Os02g04690	Cycloartenol synthase
					LOC_Os02g04700	tRNA synthetases class II domain containing protein
2	RM154	0.63	1100000	Panicle number	LOC_Os02g02820	Helix-loop-helix DNA-binding domain containing protein
					LOC_Os02g02830	Ubiquitin-conjugating enzyme
					LOC_Os02g02840	Ras-related protein
					LOC_Os02g02850	Bifunctional protein folD
					LOC_Os02g02860	Glutamyl-tRNA synthetase
					LOC_Os02g02870	Glycine-rich protein 2
					LOC_Os02g02880	Expressed protein
					LOC_Os02g02890	Peptidyl-prolyl cis-trans isomerase
					LOC_Os02g02900	Expressed protein

Table 4.3. Polymorphic markers of Kaybonnet, ZHE733, bulked drought resistant, and bulked drought sensitive (Continued).

Chromosome	Polymorphic SSR markers	PIC value	Physical map position (Mb) (Temnykh et al., 2000)	QTL Annotation Rice Online Database	Genes within 25 Kb of the polymorphic SSR markers position	MSU gene annotation (Kawahara et al., 2013)
3	RM114	0.50	18200000	Panicle number, shoot/seedling characteristics, grain quality, and abiotic stress tolerance	LOC_Os03g31770	Transposon protein
					LOC_Os03g31790	Expressed protein
					LOC_Os03g31839	Transposon protein
					LOC_Os03g31870	Hypothetical protein
3	RM135	0.63	3300000	Root characteristics, shoot/seedling characteristics, flowering, grain quality, and drought tolerance	LOC_Os03g06520	Sulfate transporter
					LOC_Os03g06530	Expressed protein
					LOC_Os03g06540	Retrotransposon protein
					LOC_Os03g06550	Retrotransposon protein
					LOC_Os03g06560	Retrotransposon protein
					LOC_Os03g06570	IQ calmodulin-binding motif family protein
					LOC_Os03g06580	MTN26L2-MtN26 family protein
					LOC_Os03g06600	Expressed protein
					LOC_Os03g06610	Expressed protein
4	RM131	0.50	2000000	Panicle/flower characteristics, insect resistance, and cold tolerance	LOC_Os04g04240	Sterol 3-beta-glucosyltransferase
					LOC_Os04g04254	Sterol 3-beta-glucosyltransferase
					LOC_Os04g04270	Retrotransposon protein
					LOC_Os04g04280	Retrotransposon protein
					LOC_Os04g04290	Retrotransposon protein
					LOC_Os04g04300	Retrotransposon protein
					LOC_Os04g04310	Hypothetical protein
					LOC_Os04g04320	Expressed protein

Table 4.3. Polymorphic markers of Kaybonnet, ZHE733, bulked drought resistant, and bulked drought sensitive (Continued).

Chromosome	Polymorphic SSR markers	PIC value	Physical map position (Mb) (Temnykh et al., 2000)	QTL Annotation Rice Online Database	Genes within 25 Kb of the polymorphic SSR markers position	MSU gene annotation (Kawahara et al., 2013)
6	RM133	0.63	4000000	Panicle/flower characteristics, seed characteristics, culm/leaf, dwarf, flowering, grain quality, insect resistance, and soil stress tolerance	LOC_Os06g08200	Expressed protein
					LOC_Os06g08210	Expressed protein
					LOC_Os06g08220	Expressed protein
					LOC_Os06g08230	Expressed protein
					LOC_Os06g08240	Expressed protein
					LOC_Os06g08250	Zinc finger family protein
					LOC_Os06g08270	Expressed protein
					LOC_Os06g08280	Protein kinase domain containing protein
					LOC_Os06g08290	MYB family transcription factor
					LOC_Os06g08300	FAD dependent oxidoreductase domain containing protein
8	RM137	0.63	1600000	Panicle/flower characteristics, flowering, soil stress tolerance, and drought tolerance	LOC_Os08g03370	Divergent PAP2 family domain containing protein
					LOC_Os08g03380	Heat shock protein DnaJ
					LOC_Os08g03390	Pre-mRNA-splicing factor SLU7
					LOC_Os08g03400	Expressed protein
					LOC_Os08g03410	Glutelin
					LOC_Os08g03420	Kelch repeat protein
					LOC_Os08g03430	Extracellular ligand-gated ion channel
					LOC_Os08g03440	Actin
					LOC_Os08g03450	Ribosomal protein L37
8	RM152	0.50	600000	Panicle/flower characteristics, flowering, and drought tolerance	LOC_Os08g01930	KH domain-containing protein
					LOC_Os08g01940	Non-lysosomal glucosylceramidase
					LOC_Os08g01950	Transferase family protein
					LOC_Os08g01960	Transferase family protein
					LOC_Os08g01970	Retrotransposon protein

Table 4.3. Polymorphic markers of Kaybonnet, ZHE733, bulked drought resistant, and bulked drought sensitive (Continued).

Chromosome	Polymorphic SSR markers	PIC value	Physical map position (Mb) (Temnykh et al., 2000)	QTL Annotation Rice Online Database	Genes within 25 Kb of the polymorphic SSR markers position	MSU gene annotation (Kawahara et al., 2013)
11	RM139	0.50	1200000	Panicle/flower characteristics and soil stress tolerance	LOC_Os11g03230	Nucleoside-triphosphatase
					LOC_Os11g03240	MATE efflux family protein
					LOC_Os11g03250	Retrotransposon protein, Ty3-gypsy subclass
					LOC_Os11g03260	ligA
					LOC_Os11g03270	Nucleoside-triphosphatase
					LOC_Os11g03280	Transposon protein
					LOC_Os11g03290	Nucleoside-triphosphatase
12	RM155	0.63	3000000	Seed characteristics, grain quality, blast resistance, and soil stress tolerance	LOC_Os12g06260	Harpin-induced protein 1 domain containing protein
					LOC_Os12g06270	Retrotransposon protein
					LOC_Os12g06280	Expressed protein
					LOC_Os12g06290	Expressed protein
					LOC_Os12g06300	Expressed protein
					LOC_Os12g06330	CPuORF6 - conserved peptide uORF-containing transcript
					LOC_Os12g06335	Expressed protein

CHAPTER 5. IDENTIFICATION OF QTLS AND CANDIDATE GENES ASSOCIATED WITH DROUGHT-RELATED TRAITS OF THE K/Z RIL RICE POPULATION

Abstract

Rice is the main staple food for over 60% of the global population and has played an important role in human nutrition for the past 10,000 years. Rice also uses 2-3 times the amount of water as other food crops, which totals 30% of the world's freshwater resources world-wide. Stability of rice production is facilitated by the economic use of water, which is most essential during the period of flowering and grain formation. In this research, progeny of a cross between an adapted U.S. rice cultivars with a *tropical japonica* genome, and an *indica* rice genotype, were screened for drought resistant (DR) traits to identify DR genes, that would be useful for breeding U.S. rice cultivars for a water saving agricultural system. A recombinat inbred line (RIL) population, generated from selfed progeny of the cross between the drought resistant *tropical japonica* U.S. cultivar Kaybonnet and an *indica* drought sensitive cultivar ZHE733, was chosen for Quantitative Trait Locus (QTLs) analysis of drought-resistance related traits. Quantification of the DR traits were carried out for spikelet per panicle number (SP), panicle length (PL), primary panicle branch number (PPB), filled grain per panicle number (FG), biological yield (BY), heading day (HD), plant height (PH), productive tiller number (TN), flag leaf width (FLW), leaf rolling score (LR), root length (RL), root to shoot ratio (RSR), total root number (TRN), shallow root number (SRN), deep root number (DRN), and root fresh weight (RFW). The K/Z RIL population was screened in the field at Fayetteville (AR), by controlled drought stress treatment at the reproductive stage, and abscisic acid (ABA) sensitivity screen at the V3 stage in culture media, and the effects of stress quantified by measuring the drought-response traits. QTL analysis was performed with a set of 4133 Single Nucleotide Polymorphism (SNP) markers by using QTL IciMapping software version 4.2.53. A total of 213 QTLs and 628 candidate genes within the DR-QTL regions were identified for the drought-related traits. The RT-qPCR results of candidate DR genes revealed that the gene expression of seven known DR-

related genes, 15 candidate DR genes with known annotations, and two candidate DR genes with unknown annotations within the DR-QTL regions were up-regulated in the drought resistant parent (Kaybonnet) compared to the drought sensitive parent (ZHE733) under DS conditions. The findings of this research provide important information to understand the specific roles of the set of candidate genes with altered expression under drought in enhancing drought stress resistance, and to develop drought-resistant rice varieties with greater productivity under DS conditions.

Introduction

Rice (*Oryza sativa*) is one of the nutritional and commercially productive cereal crops providing the principal food for approximately 2.5 billion people world-wide, and a model species for monocot and cereal plants with a compact diploid genome size of around 500 Mb, with 12 chromosomes (Edwards and Batley, 2010). Rice has the smallest genome size among the major cereals like maize, wheat, and barley. This crop belongs to the family Gramineae and is grown across a wide range of topography from 53º North in North-Eastern China to 35º South in New South Wales, Australia (Mae, 1997; Santos et al., 2003). The primary rice-producing countries are China, India, Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil, Japan, U.S., Pakistan, and the Republic of Korea (FAOSTAT, 2018). Furthermore, U.S. is the third largest exporter of rice with Arkansas as the biggest rice producer of long and medium grain varieties (Quick Stats, 2016).

The three major cereal crops: rice (23%), wheat (17%), and maize (10%) are consumed by the global population to full fill 50% of the calorie intake (Khush, 2003). Based on several statistical estimates and surveys, the next 20 years have been predicted to be important for the global rice production to be increased by 30% to meet the food demands from the increasing world population and economic development (Khush, 2001). Based on FAO (2004), around 852 million people were under starvation in 2000-2002. Currently, the rate of global population growth exceeds the rate of the increase in food production. In addition, drought has become the most crucial constraint in rice production due to global climate change, and the competition with urban and industrial users of the limited water available (Tuong and Bouman, 2003; Farooq et al., 2009). Around 50% of the total rice area globally is affected by drought. Around 130 million ha of rice field in Asia are annually affected by drought and predicted to become more frequent

in many areas in the future (Rahimi et al., 2013). When compared to the current drought levels, future drought could reduce rice production even more (Zhao et al., 2017). Drought stress mainly affects physiological, morphological, and molecular-level factors of the rice crop plants (Ito et al., 1999). All of the rice growth stages; seedling, vegetative, and reproductive are affected by drought, with the reproductive stage being the most sensitive to drought stress conditions (Bunnag and Pongthai, 2013; Hsiao, 1982; O'Toole, 1982). Moreover, drought stress conditions at the reproductive stage cause a significant reduction in the grain yield components such as spikelet per panicle number, panicle length, primary panicle branch number, filled grain per panicle number, and hundred grain weight, all leading to a decrease in grain yield per plant (Sadeghi and Danesh, 2011).

A previous study reported that drought stress conditions at the reproductive stage might reduce the grain yield up to 77% (Ito et al., 1999). On the other hand, the production of 1 kg of rice needs 3,000-5,000 liters of water, which is 3 times more than other cereal crops like maize and wheat (Bouman, 2002). Annually, the total rice yield loss due to drought stress conditions is around 18 million tons. However, the use of advanced genomics technologies with high-quality rice genome sequence information is very useful to develop drought-resistant rice genotypes that perform better under drought stress conditions. The identification of quantitative trait loci (DR-QTL) regulating grain yield under drought stress conditions, can be done by employing genome information of several drought-resistant rice genotypes such as Vandana, Nagina-22 (N22), Bengal, and Kaybonnet in a drought molecular-breeding program (Dixit et al., 2014; Venuprasad et al., 2009). Development of a drought-resistant rice genotype can help to increase yield production and stability for ensuring food security.

QTLs are chromosomal segments that encode genes for quantitative traits, whose effects are determined by making quantitative measurements. These quantitative traits are controlled by one or more genes and affected by environmental variation, with phenotypes such as plant height, grain yield, abiotic and biotic stress. QTLs are defined and mapped using molecular markers. This mapping method is very affordable to plant research programs because of the developments in genomic technology and statistical analysis methods (Zhu et al., 2008). Single nucleotide polymorphisms (SNPs) are widely used DNA markers to identify QTLs for important traits that can effectively speed up plant breeding. These SNPs are characterized as the most abundant variation in rice genomes that are very useful for high-resolution genotyping and to produce the highest resolution maps (McCouch et al., 2010). Additionally, the use of SNPs are more efficient and cost effective (Edwards and Batley, 2010). SNPs become the most popular DNA markers in the 21st century due to the development of genotyping by sequencing (GBS) techniques (Thomson, 2014). QTL analysis has been used to obtain genomic information about many agronomic traits. Genomic information from QTL analysis is therefore very useful for enhancing plant breeding programs through marker-assisted selection (MAS). In the past few decades, QTL mapping for agronomic and physiological traits under abiotic stress conditions, has resolved several problems. A number of QTLs associated with drought resistant (DR) traits have been identified in *Oryza sativa* (Mardani et al., 2013). Furthermore, DR-QTL mapping has been very useful to identify the genes and chromosomal segments associated with complex DR traits.

The development of drought-resistant rice genotypes has been slow because of the genetic complexity that controls grain yield traits under drought and also the high genotype-environment (GXE) interaction associated with these traits (Barnabás et al., 2008). However,

several studies at IRRI have reported that development of mapping populations derived from a drought-resistant variety and a high-yielding variety has proven effective in combining drought resistance with high yield potential. These mapping populations have also shown transgressive segregants with higher yield compared to the parents under drought and normal conditions.

GBS is the most popular next generation sequencing method which is a cost effective, rapid, and accurate genotyping technique to discover high-throughput SNPs for implementation and acceleration of the QTL mapping process, and gene discovery in rice breeding programs. To reduce the complexity of the genome, the GBS technique use restriction enzyme digestion followed by adapter ligation, PCR, and sequencing. The choice of the restriction enzyme influences the sequencing results. Rice has been genotyped by this GBS technique and the resulting high-quality SNPs used to construct high density linkage maps, to identify consistent grain yield component traits under drought stress conditions. A number of previous studies have been reported for QTL mapping with GBS to discover a large number of SNPs and the construction of high density linkage maps, to identify QTL for complex traits such as grain weight and grain length (Bhatia et al., 2018), drought resistance in chickpea (Jaganathan et al., 2015), rust resistant and flag leaf traits in wheat (Hussain et al., 2017), and plant architecture and yield traits in maize (Su et al., 2017).

Transcription factors (TFs) are important factors functioning in the abiotic stress signal transduction pathway, and the control of downstream gene expression (Nakashima et al., 2014). There are several large TF families in plants (Umezawa et al., 2006), such as myeloblastosis (MYB), myelocytomatosis (MYC), APETALA type 2/ethylene responsive factors (AP2/ERF), NAM/ATAF/ CUC transcription factor (NAC), basic region/leucine zipper motif (bZIP), WRKY amino-acid domain containing transcription factors (WRKY), and Cys2His2 zinc-finger proteins

(ZFP) among others. Furthermore, over-expression of TFs such as AREB1 (Oh et al., 2005) and DREB/CBF (Datta et al., 2012) significantly enhance resistance to drought stress conditions in the rice plants. Studies from our lab. (Ambavaram et al., 2014), showed that over-expression of the TF HYR (transcription factor **H**igher **Y**ield **R**ice) in rice plants improves photosynthesis activity leading to higher grain yield under normal and drought stress conditions. Moreover, the information about drought related TFs are important to develop drought-resistant rice varieties.

Many genes related to drought stress resistance in rice have been identified by using several methods such as Expressed Sequence Tag (EST) profiling, transcript profiling via massively parallel signature sequencing (MPSS), microarrays and quantitative real time PCR, and RNA gel blot analysis (Rabello, et al., 2008), and also comparative proteome analysis (Xiong et al., 2010). On the other hand, only few genes have been functionally validated for their drought resistance ability in rice (Sahoo et al., 2013). Important examples include the stress-responsive rice NAC genes like SNAC1, OsNAC6/SNAC2, and OsNAC5 which enhance drought resistance when over-expressed (Nakashima et al., 2014).

The plant hormone abscisic acid (ABA) plays an important role in adaptive responses to drought stress conditions, by regulating stomatal closure to limit water loss through transpiration (Schroeder et al., 2001 & Finkelstein et al., 2002). Endogenous ABA concentration increase under drought stress conditions, for helping plants adapt to the water deficit (Xiong et al., 2002). In addition, ABA controls the expression of many drought-responsive genes involved in a protective response (Shinozaki and Yamaguchi-Shinozaki, 2007). Several transcription factors involved in the regulation of ABA-responsive gene expression include ABFs/AREBs (Kim, 2006), CBF/DREB, MYB, NAC, and WRKY (Berri et al., 2009; Yamaguchi-Shinozaki and Shinozaki, 2005).

The objective of this research is to identify of QTLs and candidate genes in the K/Z RIL population for drought resistance associated with vegetative morphological traits, grain yield components under drought stress and well-watered conditions, and root architectural traits related to ABA response. Identification of QTLs from the two contrasting cultivars for drought-related traits will contribute to our understanding of the genetic control of rice productivity at the sensitive reproductive stage under drought stress conditions, and lead to accelerating the development of drought-resistant rice varieties with improved grain yield under drought stress conditions.

Materials and Methods

Mapping population

A RIL population derived from the varieties Kaybonnet (*Oryza sativa*, an upland *japonica* type) and ZHE733 (*Oryza sativa*, an *indica* type), termed K/Z RILs, was available and requested from the USDA Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA. This RIL population was derived from an F2 population by single seed descent (SSD), after selfing for **X** generations. In the current study, the available 198 RIL lines and parents were used for phenotypic evaluation of the morphological traits and grain yield components under well-watered (WW) and drought stress (DS) conditions, and also the response of root architectural traits to ABA treatments.

Drought stress treatment at the reproductive stage

Seed of the K/Z RIL population and two parents (Kaybonnet and ZHE733) were germinated and grown in the greenhouse in sterilized field soil for 20 days (until V3 stage), under controlled conditions of 28 to 30^{o}C day, and 22 to 23^{o}C night, in a 14h light/10h dark cycle; with average light intensity of 580 µmol $m^{-2}s^{-1}$, and 65% relative humidity. Uniform plants were selected and transplanted to the field separetely in 6 batches (at 7-day intervals) based on their heading day data from USDA to synchronize the drought treatment at the reproductive stage. The latest heading day lines were thus seeded and transplanted early and the earliest heading day lines seeded last.

This RIL population was evaluated in the field at Fayetteville, AR, USA of the growing seasons (May-November) in 2016 with annual rainfall 849.63 mm (7^{th} driest year for Fayetteville, AR). The population was grown in a randomized complete block design with five replications and two treatments of well-watered (WW) and drought stress (DS) conditions, in

single-row plots of 5 m length with a spacing of 0.3 m between plants. Blocks represent a random effect and treatments (WW and DS) represent a fixed effect. The rice plants were planted in the control plot with normal irrigation (WW conditions) for the whole growth period, and the drought stress (DS) plot with treatment as described below. The rice plants in the vegetative stage were all maintained with the normal irrigation for at least 30 days. The DS treatment was given at the reproductive stage (R3). DS conditions were monitored with three tensiometers that were installed at three separate positions in the DS plot just after draining, the first was in the beginning of the plot, the second in the middle, and the third at the end of the plot. The DS conditions were maintained continuously up to -70 kPa (severe stress). Once the soil tension reduced to -70 kPa at 30 cm soil depth, life-saving irrigation was provided thereafter through flash flooding in the DS plot and water drained after 24h to impose the next cycle of DS till maturity. No rainfall occurred during this drought period at the study site. To fertilize the field (WW and DS plots), Urea was applied in three applications at the rate of 20 g per square meter. The first application after 10 days of transplanting, the second at maximum tillering stage, and the third at panicle initiation. The weeds were controlled by manual removal when needed. In total 2 parental genotypes and 198 recombinant inbred lines were used in this field study.
The effect of drought stress was quantified by measuring morphological traits and grain yield components such as heading day (HD), plant height (PH), productive tiller number (TN), flag leaf width (FLW), leaf rolling score (LR), spikelet per panicle number (SP), panicle length (PL), primary panicle branch number (PPB), filled grain per panicle number (FG), hundred grain weight (HGW), and biological yield per plant (BY) with five replications per line.

The data under WW and DS conditions for morphological traits and grain yield components were analyzed by analysis of variance (ANOVA) using JMP version 12.0. The

Tukey's HSD was performed to compare the means of the two treatments (WW and DS) among all of the rice lines in the K/Z RIL population for significant effects (Tukey's HSD, $P < 0.05$) using JMP version 12. Shapiro-Wilk test was used to test a normal distribution for each trait by using SAS 9.4.

Screening for ABA sensitivity

A total of 2 parental genotypes and 198 recombinant inbred lines seeds were washed with 70% ethanol for 60 seconds then rinsed 3 times with sterilized water, washed with 30% bleach solution (60 ml bleach + 1 ml 20% SDS + 139 ml sterilized water) for 45 minutes and rinsed two times with sterilized water. Sterilized seeds (S0 stage) were germinated in 2 ml tubes contained germination media (Chu's N-6 Basal Salts with Vitamins, Macronutrients, Micronutrients) until S3 stage in the growth chamber (temperature: 28/22^{o}C day/night, light intensity: 600umol/m^2/s, relative humidity: 60%).

The experiment of screening for ABA sensitivity of the K/Z RIL population was setup in hydroponic treatment. The seedlings at S3 stage of Kaybonnet, ZHE733, and 198 lines were transplanted into ABA media at different concentration levels: 0 µM (control), 3 µM, and 5 µM, then grown in the growth chamber (temperature: 28/22^{o}C day/night, light intensity: 600umol/m^2/s, relative humidity: 60%) until V3 stage. The effect of ABA sensitivity was quantified by measuring the root architectural traits: maximum root length (RL), root to shoot ratio (RSR), total root number (TRN), number of roots with a shallow angle (0-45^{o}) (SRN), number of roots with a deep angle (45-90^{o}) (DRN), and root fresh weight (RFW) with five replications per line/treatment.

Genotyping

Young leaves of 2 week old plants of the 198 selected lines and their parents (Kaybonnet and ZHE733) were sampled. Their genomic DNA were extracted by using cetyl tri-methyl ammonium bromide (CTAB) method as described (Murray and Thompson, 1980). DNA concentration from each plant was measured with NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and the DNA quality was checked by electrophoresis in 1.5% agarose gels (0.5 TBE). The run was performed with the constant supply for 3.5 hours at 80 volt. The gel pictures was recorded with gel documentation system (Alpha Imager®, USA). High-quality DNA in range from 25.00 – 144.89 ng/µl was used for SNP genotyping. The DNA of 2 parental genotypes and 198 lines were sent to University of Minnesota Genomics Center for Genotyping-by-Sequencing (GBS). Two hundred GBS Libraries were created by using single-end library type with enzyme combination *Pstl* and *Mspl* for DNA digestion, and the digested DNAs ligated to the adapter. Then, all libraries were sequenced in 1 lane of a NextSeq 1x150-bp run with mean reads per sample of two millions base pairs (bp) (Figure 5.1.). Mean quality scores above Q30 for all libraries was chosen (Figure 5.2.).

SNP identification

SNP identification was analyzed from the sequence reads, and were generated in a FASTQ file. De-multiplexed FASTQ files were generated using Illumina BCL2FASTQ software. FASTQ files with more than the targeted number of reads (2,000,000) were subsampled down to 2,000,000 number reads. The first 12 bases were removed from the beginning of each read in order to remove adapter sequences, using Trimmomatic to remove adapter sequences at the 3’ ends of reads. The FASTQ files were aligned to the reference genome of Nipponbare, *Oryza sativa* spp. Japonica version MSU7 by using Burrows-Wheeler

Alignment (BWA) software. The sequences that perfectly matched and aligned were processed for SNP calling. Freebayes was used to jointly call variants across all samples simultaneously. The raw Variant Call Format (VCF) file generated by Freebayes was filtered using VCF tools to remove variants with minor allele frequency less than 1%, variants with genotype rates less than 95%, samples with genotype rates less than 50%, variants with 100% missing data, variants with monomorphic markers between parents, and variants with more than 50% heterozigosity. The filtered data file with final set of SNPs in nucleotide-based hap map format was converted to an ABH-based format, where "A" represents donor allele, "B" represents recipient allele, and "H" represents heterozygous allele.

Linkage map construction and QTL mapping

The genotypic data for 198 lines of the K/Z RIL population with filtered SNP markers, were used for linkage map construction by using the linkage mapping function in the QTL IciMapping software version 4.2.53 (Meng et al., 2015) with a recombination frequency (r) set at 0.45. The Kosambi mapping function was used to convert recombination frequencies to map distance (cM) (Kosambi, 1943). Furthermore, the markers were ordered with a threshold logarithm of odd (LOD) set at 2.5.

The morphological traits and grain yield components used to conduct QTL analysis were spikelet per panicle number (SP), panicle length (PL), primary panicle branch number (PPB), filled grain per panicle number (FG), biological yield (BY), plant height (PH), productive tiller number (TN); each trait or yield component under WW and DS conditions. In addition data for heading date (HD), flag leaf width (FLW), and leaf rolling score (DLR) under DS conditions were collected. The root architectural traits for ABA sensitivity screening, include root length (RL), root to shoot ratio (RSR), total root number (TRN), shallow root number (SRN), deep root

number (DRN), and root fresh weight (RFW) at different level of ABA concentrations: 0 µM (control), 3 µM, and 5 µM were also measured for QTL mapping.

QTL analysis was done with 4133 Single Nucleotide Polymorphism (SNP) markers by using QTL IciMapping software version 4.2.53 with inclusive composite interval mapping (ICIM) function (Meng et al., 2015). QTLs explaining ≥ 2% phenotypic variance (PVE) with LOD ≥ 2.5 were declared significant. QTL nomenclature used is based on the trait name, chromosome number, and their physical map position on the genome (Solis et al., 2018; McCouch, 2008). The left and the right markers flanking the QTLs were determined. Genotypic frequency was calculated according to the closest marker to the QTL peak.

Identification of candidate genes within the QTL regions

The candidate genes present within the QTL regions were identified based on the position of the SNP markers flanking the QTL regions, and the nearest predicted/annotated gene in the region, using the MSU rice *japonica* reference genome annotation release 7.0 as the reference. All the genes within 25 Kb of the identified QTLs position were classified into three major functional categories, including biological process, molecular function, and cellular component. The key functional genes regulating drought-related traits and ABA sensitivity were further analyzed by extracting RNA from the drought responsive line and used for analysis of their gene expression under DS conditions.

RT-qPCR validation of the key functional genes identified within the QTL regions regulating drought-related traits and ABA sensitivity

The leaf samples for RNA extraction and quantification of gene expression were collected from the two parental genotypes of the K/Z RIL population; Kaybonnet as drought resistant parent and ZHE733 as drought-sensitive parent under DS (at 30% field capacity) and

WW conditions with three biological replicates. RNA was extracted using the RNeasy® Plant Mini Kit (Qiagen Inc, Hilden, Germany), the quality and concentration of each extracted RNA sample determined by using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and RNA quality checked by gel electrophoresis in 1.5% agarose gels (0.5 TBE). The RNA samples that met the criteria of having a 260/280 ratio of 1.8–2.1, or 260/230 ratio ≥2.0, were used for complementary DNA (cDNA) synthesis, with one microgram of each RNA sample reverse transcribed to complementary DNA (cDNA) using GoScript® Reverse Transcription System (Promega, Madison, Wisconsin, USA). For individual gene expression analysis, real-time quantitative polymerase chain reaction (RT-qPCR) analysis were conducted using GoTaq® qPCR Master Mix (Promega, Madison, Wisconsin, USA) with gene-specific primers at melting temperatures of 55–60°C, for primer lengths of 18–25bp, and amplicon lengths of 101– 221bp and Ubiquitin primers as standard. Seven known drought resistance genes and 26 candidate drought resistance genes identified within the QTL regions were used as primers for generating the gene expression data (Table 5.7.). These 26 candidate genes include 15 candidate genes with known annotations to drought response and 11 candidate genes from the mapped traits with high LOD and PVE with unknown annotations of drought response. The RT-qPCR reaction samples were prepared in a total volume of 20µL, containing 10µL of SYBR green master mix, 1µL of cDNA template, 8µL of ddH_2O, and 1µL of each primer. RT-qPCR was performed with a BIO-RAD CFX-96 instrument (Bio-Rad Laboratories, Inc., Hercules, California, USA). Increasing temperature (0.5°C/5 s) from 65°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-ΔΔCt. The average of three biological replicates and three technical replicates for each sample was used to obtain each expression value (Ramegowda et al., 2014; Bevilacqua et al., 2015; De Freitas et al., 2016). The standard error was used to separate means for significant effects.

Result and Discussion

The K/Z RIL population was developed at the USDA Dale Bumpers National Rice Research Center, Stuttgart, Arkansas, USA from crossing between diverse parental genotypes from different subspecies, Kaybonnet (*tropical japonica*) and ZHE733 (*indica*) by SSD method to create segregating progenies with high genetic variability for selection desirable genes. Furthermore, based on the screening of grain yield under DS conditions at reproductive stage (R3), among the two parental genotypes, Kaybonnet is drought resistant while ZHE733 displays a drought sensitive phenotype (Figure 5.3.). The progeny of a cross between drought resistant and sensitive genotypes are useful to study the inheritance of drought resistance, and identify important QTLs for variation in grain yield under DS conditions (Islam et al., 2012). The K/Z RIL population was studied for filled grain per panicle number, and out of 198 lines, 13.13% were found to be highly drought resistant lines, 11.11% moderately drought resistant lines, and 75.75% drought sensitive lines (Table 5.1.), suggesting there are multiple factors involved in inheritance and expression of drought resistant phenotypes.

Variation in morphological traits of RILs under drought stress conditions

Rice is more sensitive to DS conditions compared to the other cereal crops such as wheat, rye, and barley (Huang et al., 2014). Significant differences were observed among the parental lines (Kaybonnet and ZHE733) and the RIL population for morphological traits (Table 5.2.). In the RIL population, the morphological traits measured showed normal frequency distribution, for

PH under WW and DS conditions (Figure 5.4.), TN under WW and DS conditions (Figure 5.5.), HD (Figure 5.6.), flag leaf width (Figure 5.7.), and DLR (Figure 5.8.) revealing quantitative inheritance, and thus the morphological traits were suitable for QTL analysis (Fang et al., 2019).

The two parental lines are diverse in the morphological traits. Kaybonnet, the donor parent, has high stature, low tiller number, late heading day, wide flag leaf width, and low leaf rolling score (Table 5.2.) while ZHE733, the recurrent parent, has short stature, higher tiller number, early heading day, narrow flag leaf width, and high leaf rolling score (Table 5.2.). In addition to the two parental lines having variation for morphological traits, when subjected to DS conditions, Kaybonnet showed superior performance to ZHE733. Kaybonnet showed more drought resistance while ZHE733 is sensitive to DS conditions, thus exhibiting less resistance. Within the RIL population, there was a wide range of morphological traits showing variation across WW and DS conditions: PH and TN. All the morphological traits in the RIL population exhibited a typical segregation pattern, with normal distribution. Moreover, within the RIL population, a contrast was observed under DS conditions; all the morphological traits under DS showed a significant reduction compared with the traits measured under WW conditions (Table 5.2.). These results are in agreement with previous studies showing that water deficit conditions have a negative effect on plant development and growth due to a loss of turgor (Hsiao et al., 1970; Specht et al., 2001; Farooq et al., 2011; Todaka et al., 2015). The reduction of the plant height and productive tiller number under DS conditions are associated with the reduction of the cell cycle processes, cell expansion and elongation (Mantovani and Iglesias, 2008).

Genetic variation for grain yield components under reproductive stage drought stress

The effect of DS conditions on rice are different at every growth stage. Rice is very sensitive to DS conditions, especially at the reproductive stage, and grain yield is dramatically

reduced even under slight DS conditions (Kamoshita et al., 2008; Palanog et al., 2014). The effective way to reduce grain yield loss under DS conditions is to develop drought-resistant rice varieties, that are however very challenging due to the complexity of the drought resistant trait. The identification of QTLs associated with the grain yield components under DS conditions, and their utilization in molecular breeding, is an alternative method of increasing breeding effectiveness. Moreover, identified QTLs are very useful for incorporation in a marker assisted breeding (MAB) strategy (Venuprasad et al., 2011).

The mean values of the grain yield components showed a significant difference in performance between the parents in WW and DS conditions (Table 5.3.). Mean values for all the grain yield components were higher in Kaybonnet compared with ZHE733 and the frequency distributions of these components were normal under WW and DS conditions, indicating polygenic control of the traits as shown: BY (Figure 5.9.), SP (Figure 5.10.), FG (Figure 5.11.), PL (Figure 5.12.), and PPB (Figure 5.13.). Transgressive segregation was observed for the grain yield components under WW and DS conditions. Both parental lines experienced a reduction in all grain yield components under DS conditions. ZHE733 showed a greater reduction in all grain yield components compared with Kaybonnet. Moreover, there were significant differences among the RILs for all the grain yield components under WW and DS conditions.

Variation in root architectural traits under ABA conditions

The K/Z RIL population showed variation in their root architectural traits in response to ABA (0 µM, 3 µM, and 5 µM), such as reduction in RL (Figure 5.14.), increase in RSR (Figure 5.15.), reduction in TRN (Figure 5.16), decrease of SRN (Figure 5.17.), shortening in DRN (Figure 5.18.), and lessening RFW (Figure 5.19.). Furthermore, the distribution of root architectural traits studied under ABA conditions showed a near normal distribution. The range

of RL, RSR, TRN, SRN, DRN, and RFW of the K/Z RIL population under ABA conditions also exhibited a wide variation (Table 5.4.). Additionally, the average of root architectural traits of the RILs lie between the parents in ABA conditions.

The parents, Kaybonnet and ZHE733 had contrasting responses under ABA conditions, where Kaybonnet as drought-resistant parent showed more sensitivity to ABA compared to ZHE733 as the drought sensitive parent. Both parents experienced a reduction in RL, TRN, SRN, DRN, and RFW under ABA conditions compared to control conditions. Kaybonnet showed a greater reduction in RL, TRN, SRN, DRN, and RFW compared to ZHE733. Although both parents and the RIL population exhibited a reduction in RL, TRN, SRN, DRN, and RFW under ABA conditions, the reduction was greater in Kaybonnet. RSR increased for Kaybonnet and RIL population under ABA conditions.

Previous studies have demonstrated that ABA sensitivity is associated with drought stress resistance through its effect on the stomatal movement (Lim et al., 2015; Duan et al., 2008; Todorov et al., 1998). Lim et al. (2015) also indicated that drought-sensitive rice plants grown in media with 2 or 5 μM ABA had significantly longer roots and shoots compared to the plants in control media. These data suggested that the drought-sensitive rice plants were insensitive to ABA and exhibited the ABA-dependent pathway in response to drought stress.

Correlation of morphological traits and grain yield components under WW and DS conditions with root architectural traits under ABA conditions

Correlation analysis increases an understanding of the overall contribution of various rice plant traits to each other (Gibert et al., 2016). A Pearson's correlation coefficient analysis was carried out on morphological traits and grain yield components under WW and DS conditions, and also on root architectural traits under ABA conditions, to analyze the correlations among

them. Significant correlations were observed among all of the traits studied (Figure 5.20.). Furthermore, “FG-DS as the major trait among the grain yield components under DS conditions showed significant positive correlations with most of the morphological traits, including HD, TN-WW, PH-WW, PH-DS, FLW, and DLR”. FG-DS also has positive correlations with other grain yield components, such as PL-WW, PL-DS, PPB-WW, SP-WW, SP-DS, and FG-WW. Additionally, FG-DS exhibited significant positive correlations with most of the root architectural traits under ABA conditions such as RL-ABA3, RL-ABA5, RSR-ABA3, RSR-ABA5, TRN-ABA3, TRN-ABA5, DRN-ABA0, DRN-ABA3, DRN-ABA5, RFW-ABA0, RFW-ABA3, and RFW-ABA5. However, significant negative correlations were also observed between FG-DS and the morphological trait like TN-DS, and also with the grain yield components such as BY-WW, BY-DS, and PPB-DS. Several root architectural traits also showed significant negative correlations with FG-DS, including RL-ABA0, RSR-ABA0, TRN-ABA0, SRN-ABA0, SRN-ABA3, and SRN-ABA5.

In this study, a positive correlation found between FG-DS with most of the morphological traits, the other grain yield components, and the major root architectural traits under ABA conditions, indicate that the rice drought-resistant plants maintain their grain yield under DS conditions through development of cell elongation, maintenance of cellular membrane integrity, and regulation of osmotic stress tolerance via ABA-mediated cell signaling (Kanbar et al., 2009; Ramegowda et al., 2015; Ding et al., 2016; Basu et al., 2016; Catalos et al., 2017; Nada et al., 2018; Hassaoni et al., 2018; Li et al., 2019; Kim et al., 2020). Furthermore, the negative correlation between FG-DS with BY under WW and DS conditions, and also with root architectural traits under ABA 0 uM indicate that there is more assimilate distribution into the grains compared to the other plant components, under DS conditions. Moreover, ABA sensitivity

was found correlated with the drought resistance of rice plants (Lim et al., 2015; Duan et al., 2008; Todorov et al., 1998).

High-density genetic linkage map with GBS markers

A genetic linkage map is an important tool to explore the plant genome, and to obtain information of allele introgression during plant breeding efforts (De Soursa et al., 2015). By using high-density genetic linkage map leads to narrow down the location of QTLs into a specific region and predict more accurate candidate genes for gene cloning, then validation with reverse genetics approaches (Hattori et al., 2009). Based on the GBS analysis, 28,598 SNP markers were obtained from 200 samples (2 parental lines and 198 RILs) (Figure 5.21.) with heterozygosity level of 1.3% and non-parental alleles at 0.4%. The filtering process of the SNP markers was done based on the missing data (≤90%), minor allele frequency (MAF < 1%), polymorphic markers between parents, recombinant frequency, and percentage of heterozygosity. Furthermore, 4133 filtered SNP markers were obtained, and were used in the development of the high-density genetic linkage map by using QTL IciMapping software version 4.2.53 with the Kosambi mapping function.

The number of SNP markers mapped to each chromosome varied from 182 SNP markers found on chromosome 12 to 562 SNP markers on chromosome 1, with an average of 344.42 SNP markers per chromosome. Chromosome 1 is the longest and chromosome 12 the smallest. Moreover, the total length of the genetic linkage map was 6063.12 cM (varied from 343.72 cM on chromosome 10 to 676.52 cM on chromosome 2), with the average of 505.26 cM per chromosome. A calculated average genetic distance between two SNP markers across the chromosome was 1.58 cM (ranged from 0.92 cM on chromosome 6 to 2.89 cM on chromosome 12). The density of the genetic linkage map was 0.69 SNP markers per cM (Table 5.5.) or an

average of 1 SNP marker every 1.5 cM. This high-density genetic linkage map covered 373 Mb of the rice genome and can be used to identify QTLs with higher resolution and reliability for application in rice breeding under DS conditions. Moreover, the high-density genetic linkage map led to identification and selection of candidate genes within the QTL regions that are involved in improving drought resistance in the rice plants, and towards developing drought-resistant rice varieties. Many previous studies have also used high-density linkage maps for QTL mapping (Bhattarai & Subudhi, 2018; Sabar et al., 2019; Barik et al., 2019; Melandri et al., 2020; Barik et al., 2020).

QTL mapping of morphological and yield traits under reproductive stage drought stress conditions and root architectural traits under ABA conditions

The identification of QTLs for morphological traits, grain yield components, and root architectural traits is important to understand the genetic complexity of the drought-related traits. The genetic variation of drought-related traits is regulated by numerous genes that have a large effect on the traits (Baisakh et al., 2020). In this research, 213 QTLs were identified for morphological traits and grain yield components under WW and DS treatments, and also root architectural traits under ABA treatments (Table 5.6. and Figure 5.22.). The identified QTLs varied under WW, DS, and ABA treatments. Additionally, the number of QTLs varied for morphological traits, grain yield components, and root architectural traits. Among the studied traits, root architectural traits had the highest number of QTLs with 147 QTLs. A total of 16 OTLs were identified for morphological traits. Moreover, 50 QTLs were detected for grain yield component traits. For root architectural traits, 147 QTLs were identified. Furthermore, the identified QTL LODs ranged from 2.506 to a maximum value of 28.849, indicating that the identified QTLs were not in the noise regions and contained important genes with large influence

on the performance of rice under DS conditions. Moreover, the identified QTLs explained phenotypic variation of 0.189 to 13.809% (Table 5.6.).

The QTLs were scattered unevenly on different chromosomes. The identified QTLs were distributed in the following chromosome: chromosome 1 (6 QTLs), chromosome 2 (18 QTLs), chromosome 3 (21 QTLs), chromosome 4 (20 QTLs), chromosome 5 (28 QTLs), chromosome 6 (24 QTLs), chromosome 7 (14 QTLs), chromosome 8 (21 QTLs), chromosome 9 (13 QTLs), chromosome 10 (28 QTLs), chromosome 11 (4 QTLs), and chromosome 12 (16 QTLs). Chromosome 5 and 10 harbored the largest number of QTLs with 28 QTLs, respectively. However, chromosome 11 had the lowest number of QTLs with 4 QTLs. Furthermore, 47 QTL clusters or QTL hot spots were detected, including chromosome 1 (1 QTL cluster), chromosome 2 (5 QTL clusters), chromosome 3 (2 QTL clusters), chromosome 4 (5 QTL clusters), chromosome 5 (7 QTL clusters), chromosome 6 (4 QTL clusters), chromosome 7 (5 QTL clusters), chromosome 8 (4 QTL clusters), chromosome 9 (2 QTL clusters), chromosome 10 (7 QTL clusters), chromosome 11 (1 QTL cluster), and chromosome 12 (4 QTL clusters). QTL clusters are regions in the genome in which several QTLs are co-localized (Singh et al., 2017). The co-localization of QTLs for morphological traits, grain yield components, and root architectural traits because of the pleiotropic action of a single gene or multiple linked genes (Struder and Doebley, 2011). These QTL clusters contained 2 to 12 QTLs. Furthermore, QTL clusters for morphological traits, grain yield components, and root architectural traits were identified on chromosome 3, 6, and 8. These regions are potential targets for grain yield improvement under DS conditions.

Most of the QTL identified in this study were mapped to approximately the same locations as previous reports (Mu et al., 2003; Courtois et al., 2003; Lanceras et al., 2004;

Bernier et al., 2007; Bernier et al., 2009; Venuprasad et al., 2009; Mishra et al., 2013; Yadaw et al., 2013; Wang et al., 2014; Palanog et al., 2014; Saikumar et al., 2014; Prince et al., 2015). Chromosome 1 harbored the highest number of QTLs for PH (Monna et al., 2000; Lanceras et al., 2004; Zhou et al., 2016; Jiang-xu et al., 2016; Yadav et al., 2019a; Zeng et al., 2019; Xu et al., 2020). The most QTLs associated with grain yield were located on chromosome 5 and 6 (Solis et al., 2018; Yadav et al., 2019b; Baisakh et al., 2020), whereas QTLs of HD were mainly located on chromosome 3 (Takahashi et al., 2001; Bernier et al., 2007; Xu et al., 2020). Moreover, chromosome 10 had the highest number of QTLs for root architectural traits with 20 QTLs (Xu et al., 2001; Lou et al., 2015; Kitomi et al., 2015; Gimhani et al., 2018).

Candidate genes underlying QTL regions

Identification of candidate genes within a QTL region is useful for marker-assisted pyramiding to develop drought-resistant rice varieties (Bhattarai et al., 2018), and is also important for developing transgenic rice with enhanced drought resistance (Varshney et al., 2011). In this research, we identified candidate genes involved in many biological processes, molecular functions, cell components, and drought response (Table 5.6.). The QTL clusters contain candidate genes with large pleiotropic effects. The total number of candidate genes identified in 213 QTLs for morphological traits and grain yield components under WW and DS conditions, and also root architectural traits under ABA conditions, were 628 with an average of 3 genes per QTL (Table 5.6.). These candidate genes were distributed unevenly on different chromosome, including chromosome 1 (51 genes), chromosome 2 (98 genes), chromosome 3 (49 genes), chromosome 4 (49 genes), chromosome 5 (79 genes), chromosome 6 (47 genes), chromosome 7 (49 genes), chromosome 8 (52 genes), chromosome 9 (31 genes), chromosome 10 (73 genes), chromosome 11 (14 genes), and chromosome 12 (36 genes). Among these,

chromosome 2 contained the highest number of candidate genes, while chromosome 11 has the lowest candidate genes. Many known genes present within these QTL regions include genes with homology to APETALA2 (AP2)/ETHYLENE RESPONSE FACTOR (ERF) transcription factor, malate dehydrogenase protein, photosystem II oxygen evolving complex protein PsbQ family protein, WRKY transcription factor, MYB transcription factor, Zinc Finger (ZFN) protein, endoplasmic reticulum protein, DEAD-box RNA helicase, glycosyl transferase protein, Late Embryogenesis Abundant (LEA) protein, and no apical meristem protein (NAC).

A transcription factor family well-known for drought response like APETALA2 (AP2)/ETHYLENE RESPONSE FACTOR (ERF) (Lata and Prasad, 2011; Mizoi et al., 2012; Licausi et al., 2013; Phukan et al., 2017), has family members present in several of the QTL regions such as QTLs for PH-WW on chromosome 1 (LOC_Os01g66270); RL-ABA3, RL-ABA5, RSR-ABA3, and RFW-C on chromosome 2 (LOC_Os02g54160); and FG-WW, FG-DS, RL-ABA3, and RL-ABA5 on chromosome 5 (LOC_Os05g49010). Furthermore, the QTL regions for PH-WW on chromosome 1 is adjacent to the semi-dwarfing gene *sd1* locus (38.3 Mb). A strong linkage has been found previously between *sd1* and QTL for drought-related traits (Vikram et al., 2015). The *sd1* locus is also associated with underground and above ground traits in rice, such as plant height and root architectural traits (Yadav et al., 1997). Reduction in plant height under DS conditions, is the adaptation of rice plants to DS (Table 5.2.). An important QTL for grain yield components under DS, FG located on chromosome 5, overlaps with 12 candidate genes. There is also an overlap between QTL for root architectural traits, RL under ABA conditions and FG under DS, suggesting that ABA is involved in the drought stress resistance mechanism. Under DS conditions, ethylene biosynthesis is increased and interacts with AP2/ERF, and finally a response to water deficit (Abiri et al., 2017; Nakano et al., 2006;

Ma et al., 2014). Additionally, AP2/ERF responds to ABA in order to help activate ABA dependent and independent stress responsive genes. Transgenic rice with over-expression of an AP2/ERF showed an increase in drought resistance (Pan et al., 2012). An understanding of the AP2/ERF gene functions in the drought resistance mechanisms in rice, may provide valuable information to facilitate the improved adaptation of rice to DS conditions.

An important candidate gene, LOC_Os03g56280, known to regulate malate dehydrogenase in response to drought stress was found in the QTL regions for HD, BY-DS, TN-DS, RL-ABA3, RL-ABA5, RSR-WW, RSR-ABA3, TRN-ABA3, SRN-ABA3, DRN-ABA3, DRN-ABA5, RFW-ABA3, and RFW-ABA5 on chromosome 3. Another candidate gene that is responsible for carbohydrate metabolism was detected on chromosome 9 (LOC_Os09g08120) in the QTL regions for BY-DS, RL-ABA5, RFW-ABA5, RSR-C, and SRN-ABA3. Malate dehydrogenase is an enzyme that catalyzes the oxidation of malate to oxaloacetate by using NAD(H)/NADP(H) as a cofactor. Additionally, this enzyme can be expressed in different parts of the rice plants, such as root, leaf, panicle, and stem and was induced in the presence of water deficit (Nan et al., 2020). Transgenic plants over-expressing malate dehydrogenase exhibited increasing drought-resistance compared to wild-type. Malate dehydrogenase was also identified as a drought responsive protein by Agrawal et al. (2016). Under DS conditions, drought-resistant genotypes accumulate a higher level of malate dehydrogenase, that protects membranes from damage by Reactive Oxygen Species (ROS) (Guo et al., 2018). By elucidating the function of malate dehydrogenase, the drought response in rice can be better understood.

A genes encoding photosynthesis function (LOC_Os04g44190) is present in the QTL regions for BY-WW, RL-ABA3, and RL-ABA5 on chromosome 4. This gene is involved in the light reaction of photosystem II (PSII) and is known to control stomatal closure, and protect

plants from dehydration (Sasi et al., 2018). Under DS conditions, the photosynthetic rate is decreased due to reduction in photosynthetic electron transport and carbon assimilation, resulting in reduction of grain yield. Moreover, PSII is a pigment-protein complex in thylakoid membranes that is responsible for oxygen evolution, water splitting, and plastoquinone reduction (Lu, 2016). LOC_Os04g44190 encoding a PsbQ family protein that belongs to the class of PSII extrinsic proteins, and under DS conditions this protein changes expression due to change in PSII efficiency (Sasi et al., 2018). Therefore, PsbQ protein plays an important role in drought stress resistance.

A WRKY transcription factor that is involved in drought stress response and plant development was detected on chromosome 5 (LOC_Os05g49210) of the QTL regions for TN-DS, RSR-ABA0, SRN-ABA3, SRN-ABA5, RFW-ABA3, and RFW-ABA5. Shen et al. (2012) reported that transgenic rice with over-expression of OsWRKY30 showed improved drought resistance. Likewise, silencing WRKY genes in transgenic rice demonstrated increased drought sensitivity. In addition, expression of the WRKY transcription factor induced ABA accumulation under DS conditions, leading to stomatal closure and reduction in water loss (Chen et al., 2010; Schroeder et al., 2001). Yan et al. (2015) also reported that the expression of a WRKY transcription factor was increased by ABA treatment.

Genes present on chromosome 5 incude LOC_Os05g49240 in the QTL regions for TN-WW, RSR-ABA0, SRN-ABA3, SRN-ABA5, RFW-ABA3, and RFW-ABA5; and also on chromosome 10 (LOC_Os10g41460) in the QTL regions for SRN-ABA3, RFW-ABA3, and RFW-ABA5, were identified MYB transcription factor, a known transcription factor in drought response (Tang et al., 2019; Baldoni et al., 2015; Li et al., 2015; Dai et al., 2007; Ma et al., 2009; Yang et al., 2012; Xiong et al., 2014; Quan et al., 2010). Transgenic rice with over-expression of

OsMYB6 exhibited increased resistance to drought compared to wild-type and contained higher proline catalase (CAT) and superoxide dismutase (SOD) activity. Additionally, OsMYB6 transgenic rice plants also showed higher expression of abiotic stress-responsive genes under DS conditions (Tang et al., 2019). Katiyar et al. (2012) also reported that the expression of MYB genes is controlled by drought. Increasing drought resistance correlated with over-expression of MYB genes and ABA accumulation (Xiong et al., 2014).

A stress-responsive transcription factor, Zinc Finger (ZFN) protein (LOC_Os06g49080), is located on chromosome 6 in the QTL regions for FG-WW, BY-WW, RL-ABA3, RL-ABA5, RFW-ABA0, and RFW-ABA5. The ZFN protein was reported to improve drought resistance in plants, suggesting that the ZFN protein contribute to the higher yield under DS conditions via control of stomatal closure (Huang et al., 2009; Ciftci-Yilmaz et al., 2000; Mukhopadhyay et al., 2004; Sakamoto et al., 2004).

A gene involved in sugar metabolism, OsSAC1 was found on chromosome 7 (LOC_Os07g02520) in the QTL regions for SP-DS and SRN-ABA5. OsSAC1 regulates sugar partitioning in the carbon metabolism of young leaves and developing leaf sheaths (Zhu et al., 2018). OsSAC1 encodes an endoplasmic reticulum protein that causes sugar accumulation in the rice leaves and can be used to produce energy and construct carbon skeletons.

The genomic region on chromosome 8 (LOC_Os08g06344) in the QTLs for FG-DS, TN-WW, TN-DS, RL-ABA3, RL-ABA5, SRN-ABA3, RFW-ABA3, and RFW-ABA5 encodes a DEAD-box RNA helicase which was reported to improve drought resistance in rice (Nawaz & Kang, 2019; Vashisht et al., 2006; Macovi et al., 2012). Over-expression of OsRH58, a chloroplast DEAD-box RNA helicase, in transgenic rice showed improved drought resistance,

displayed by better survival rate than the wild-type under DS conditions (Nawaz & Kang, 2019). Furthermore, gene expression of the OsRH58 was increased under drought.

A glycosyl transferase protein, is encoded by the gene LOC_Os11g30760 on chromosome 11 within the QTL regions for SRN-ABA5, RFW-ABA3, and RFW-ABA5. Moreover, a glycosyl transferase protein was also shown to be involved in drought stress adaptation in plants (Lam et al. 2007; Keppler and Showalter 2010). Glycosylation, a process of glycosyltransferases that transfer sugar moieties from activated donor sugar molecules to acceptor molecules (Jones and Vogt, 2001; Lairson et al., 2008; Li et al., 2015), is crucial in the biosynthesis of plant cell walls (Cao et al., 2008).

A gene regulating late embryogenesis abundant (LEA) protein was detected on chromosome 12 (LOC_Os12g02700) underlying the QTL regions for BY-DS, RSR-ABA5, SRN-ABA0, and SRN-ABA5. LEA protein has a major role in drought resistance in plants (Xiao et al., 2007; Duan & Cai, 2012; Magwanga et al., 2018; Liang et al., 2019; Chen et al., 2019; Kamarudin et al., 2019). Under DS conditions, LEA genes showed higher expression in the drought resistant plants compared to drought sensitive. In support of LEA functions, Xiao et al. (2007) reported that transgenic rice with over-expression a LEA protein gene OsLEA3-1 exhibited higher grain yield compared to wild-type under DS conditions.

Within the QTL region for TRN-ABA0 and TRN-ABA3 on chromosome 12, LOC_Os12g29330, (OsNAC139) was identified. OsNAC139 is a member of the NAC transcription factor family that is known to control plant response to drought (Kikuchi et al., 2003; Nakashima et al., 2007; Takasaki et al., 2010; Shim et al., 2018) by producing no apical meristem (NAM)/NAC protein. Rice contains 151 NAC genes (Puranik et al., 2013), from which several studies have reported that over-expression of OsNAC genes leads to improved drought

resistance (Nakashima et al., 2007; Hu et al., 2008; Yokotani et al., 2009; Zheng et al., 2009; Jeong et al., 2010).

Among all the candidate genes identified within the QTL regions, various transcriptomes correlated with drought stress resistance were detected. Drought resistance of the rice plants can be either associated with metabolic regulation or osmoregulation. In conclusion, this study has revealed a number of potential candidate genes for developing drought-resistant rice varieties.

RT-qPCR validation of the key functional genes identified within the QTL regions regulating drought-related traits and ABA sensitivity

Information about the differences in the expression of drought resistance genes between drought-resistant and sensitive genotypes and their relationship to morphological characterisctics, grain yield components, and root architectural traits under DS conditions has been limited. However, the study of the differences in gene expression between resistance and sensitive genotypes could improve the efficiency and opportunities of developing drought resistant varieties.

The identified candidate genes within the QTL regions regulate morphological traits and grain yield components under WW and DS, and also root architectural traits under ABA treatments, were further analyzed to exemplify their roles in increasing drought stress resistance in rice by identifying their expression under different conditions. In this research, QTLs controlling morphological and yield traits under reproductive stage drought stress conditions were identified and compared with those identified under WW conditions. The QTLs under DS conditions were different from those identified under WW conditions. This might be due to the difference in expression of the genes for morphological and grain yield traits under DS and WW conditions. Plant respond and adapt to the drought stress through several processes, such as

physiological, biochemical, and molecular processes that are regulated by transcriptional regulators. When rice plants are exposed to drought stress, certain genes are activated or repressed. Proteins, as the products of the activated genes, will protect the plants from the damage of drought stress (Dai et al., 2007). The known genes and transcription factor families that have been proven to regulate the plant response to drought stress are AP2/ERF, WRKY, MYB, NAC, NAP, and bZIP (Wu et al., 2017; Mao et al., 2017; Tang et al., 2017; Butt et al., 2017; Zhu et al., 2018; Sun et al., 2018). Understanding the regulation of gene expression in response to drought stress is important to develop drought-resistant rice varieties. Seven known drought resistance genes and 26 out of 628 candidate genes obtained from the QTL regions were selected for identifying their expression between drought-resistant parent (Kaybonnet) and drought sensitive parent (ZHE733) under DS conditions. These 26 candidate drought resistance genes comprise of 15 candidate genes with known annotations to be responsive to drought stress, and 11 candidate loci comprising of genes from the traits with high LOD and PVE with unknown annotations (Table 5.7.).

RT-qPCR confirmed that seven known drought resistance genes showed differential gene expression patterns in the drought resistant (Kaybonnet) and drought sensitive (ZHE733) parents. The RT-qPCR results revealed that 6 out of 7 known drought resistance genes were up-regulated in Kaybonnet relative to ZHE733 under DS conditions when compared to WW conditions (Figure 5.25.). One of the drought gene OsNAC9 showed down-regulation in Kaybonnet relative to ZHE733 when the relative expression values was compared between DS and WW conditions for each genotype. But the OsNAC9 relative gene expression under DS (Figure 5.24.) and WW conditions (Figure 5.25.) showed higher expression in Kaybonnet than ZHE733. This can be explained, eventhough the relative reduction in gene expression for OsNAC9 is high in

Kaybonnet than ZHE733 when comparisons are made between DS vs WW conditions for each genotype but the overall expression value for this gene is higher in Kaybonnet than ZHE733 under both WW and DS conditions. These results suggested that known drought resistance genes, including OsMYB109, OsNAP, OsNAC5, OsNAC9, OsNAC10, OsZIP23, and OsZIP46 also played an important role in regulating the drought resistance of Kaybonnet.

Out of 15 annotated candidate genes, LOC_Os01g66270 (ERF/Ethylene Response Factor) and LOC_Os10g41460 (MYB protein) showed up-regulation in Kaybonnet relative to ZHE733 under DS conditions when compared to WW conditions (Figure 5.25.). All other 13 candidate genes, LOC_Os02g54160 (APETALA2/ERF transcription factor), LOC_Os03g56280 (Malate dehydrogenase), LOC_Os04g44190 (Light reaction photosystem II), LOC_Os05g49010 (APETALA2/ERF transcription factor), LOC_Os05g49210 (WRKY transcription factor), LOC_Os05g49240 (MYB protein), LOC_Os06g49080 (Brassinosteroid/BR signaling), LOC_Os07g02520 (Regulation of sugar partitioning in carbon-demanding young leaves and developing leaf sheaths), LOC_Os08g06344 (DEAD-box RNA helicase), LOC_Os09g08120 (Carbohydrate metabolic process), LOC_Os11g30760 (Galactosyltransferase activity), LOC_Os12g02700 (Late embryogenesis abundant/LEA protein), and LOC_Os12g29330 (No apical meristem/NAM protein domain) eventhough showed down-regulation in Kaybonnet relative to ZHE733 under DS conditions when compared to WW conditions do not support the phenotypic traits associated with each loci. These genes are inherently up-regulated in WW (Figure 5.23.) and DS conditions (Figure 5.24.) compared to ZHE733, so eventhough the relative amount was down-regulated in DS compared to WW (Figure 5.25.), the intrinsic gene expression values of that genes are higher in Kaybonnet compared to ZHE733 suggesting a cause for better DS phenotype of Kaybonnet compared to ZHE733.

In order to verify the results from QTL mapping for two candidate regions for the traits, the loci high LOD and PVE score were studied for gene-expression between Kaybonnet and ZHE733. One of the region for the trait PHC has LOD score 24.42 and PVE 13.81%. The window region of 10 Kb upstream and downstream was selected where the polymorphic SNP was detected on chromosome 2. This region has 6 candidate genes, LOC_Os02g44590, LOC_Os02g44599, LOC_Os02g44610, LOC_Os02g44620, LOC_Os02g44630, and LOC_Os02g44642 (Table 5.7.) with unknown annotations. The second region selected for gene-expression study also had high LOD score 21.82 and PVE 8.49% and is associated with traits BYD, TNC, and TND; and the region spanning the polymorphic marker selected included 5 genes: LOC_Os10g07030, LOC_Os10g07040, LOC_Os10g07050, LOC_Os10g07060, and LOC_Os10g07080 with unknown annotations on chromosome 10.

Four candidate genes (LOC_Os02g44590, LOC_Os02g44610, LOC_Os10g07040, and LOC_Os10g07080) out of 11 candidate genes with unknown annotations showed up-regulated in Kaybonnet relative to ZHE733 under DS conditions when compared to WW conditions (Figure 5.24). Seven other candidate genes (LOC_Os02g44599, LOC_Os02g44620, LOC_Os02g44630, LOC_Os02g44642, LOC_Os10g07030, LOC_Os10g07050, and LOC_Os10g07060) even though showed down-regulation in Kaybonnet relative to ZHE733 under DS conditions when compared to WW conditions (Figure 5.25.), but the relative gene expression under DS (Figure 5.24.) and WW conditions (Figure 5.23.) showed higher expression in Kaybonnet and ZHE733, suggesting that higher intrinsic values of candidate genes in Kaybonnet compared to ZHE733 are probably enough for the traits. LOC_Os10g07040 and LOC_Os10g07080 up-regulated in Kaybonnet relative to ZHE733 under DS conditions when compared to WW conditions (Figure

5.25.) and also showed higher relative gene expression under DS (Figure 5.24.) and WW conditions (Figure 5.23.) in Kaybonnet.

Among the candidate drought resistance genes not annotated to drought stress function, LOC_Os10g07040 showed high up-regulation in Kaybonnet compared to ZHE733 under DS and WW conditions (Figure 5.23., 5.24., and 5.25.), correlated with chalcone synthase, according to the MSU rice reference genome annotation release 7.0 (http://rice.plantbiology.msu.edu) that is involved in the drought stress response in rice (Hu et al., 2017), Arabidopsis (Nakabayashi et al., 2013), and tobacco (Hu et al., 2019). The other candidate drought resistance gene that also showed high up-regulated in Kaybonnet compared to ZHE733 under DS and WW conditions (Figure 5.23., 5.24., and 5.25.) was LOC_Os10g07080 is related to myosin (Jiang et al., 2004) and transposon protein (Cho et al., 2017) that regulates cell growth and developmental processes in rice. These candidate genes could be functioning in a cumulative manner in order to show a measurable positive effect on improving drought resistance in rice and the effect of genes can further be exploited to develop drought resistant cultivar.

A large number of genes were up-regulated in Kaybonnet (drought resistant parent), indicating that the drought resistant cultivar had higher capability to modulate drought resistance genes when exposed to DS conditions, thereby enhancing its resistance level compared to drought sensitive parent (ZHE733). Modulation of a higher number of up-regulated expressed genes with different transcription factor gene families is a crucial characteristics of drought resistant genotypes. Similar results were also obtained by Hayano-Kanashiro et al. (2009) who showed that drought-resistant maize genotypes inducted more genes compared to the sensitive genotypes under DS conditions. Seven of the known drought resistance genes, including OsMYB109, OsNAP, OsNAC5, OsNAC9, OsNAC10, OsZIP23, and OsZIP46, and also 15

candidate drought resistance genes identified within QTL regions have been strongly associated with direct roles in drought stress resistance. For example, transcription factors MYB, NAP, NAC, ZIP, and APETALA2/ERF are responsive to dehydration induced by water deficit conditions (Tang et al., 2019; Baldoni et al., 2015; Li et al., 2015; Dai et al., 2007; Ma et al., 2009; Yang et al., 2012; Xiong et al., 2014; Quan et al., 2010; Lata and Prasad, 2011; Mizoi et al., 2012; Licausi et al., 2013; Phukan et al., 2017; Nakashima et al., 2007; Hu et al., 2008; Yokotani et al., 2009; Zheng et al., 2009; Jeong et al., 2010). These results provide strong evidence for genes expressed under DS conditions being involved in various physiological, biochemical, and molecular processes within the rice, in order to reduce the effects of drought stress, thereby enhancing their ability to resist the drought stress and maintain their grain yield production under DS conditions. Therefore, the up-regulation of the drought genes in Kaybonnet compared to ZHE733 provide important information to characterize the function of candidate drought resistance genes and to understand the drought stress mechanisms in rice.

The known drought resistance genes and candidate genes within QTL regions involved in regulatory response to drought include a large family of genes expressed under DS conditions. Proteins expressed by known and candidate drought resistance genes played important roles in (1) cellular protection, including structural adaptation and osmotic adjustment, and (2) drought responses by interaction with other proteins and transcription factors, such as MYB, NAP, NAC, bZIP, and APETALA2/ERF. Under DS conditions, in drought resistant genotype Kaybonnet, exogenous ABA significantly improved the expression of ABA biosynthetic genes suggesting Kaybonnet genotype must be maintaining the water potential and cellular activity of the cell by closing the stomata.

Based on the RT-qPCR results, it may also be suggested that there is a correlation between gene expression, transcriptional regulation, and resistance to drought across resistance and sensitive genotypes. Therefore, the up-regulation of the drought genes and novel candidate genes in Kaybonnet compared to ZHE733 provided an important information to characterize the function of candidate drought resistance genes. All in all, these results enhance our understanding of the role of candidate drought resistance genes in the regulation of drought stress response, and this research has also revealed a number of potential candidate drought resistance genes that could be used to develop rice cultivars with greater drought resistance.

Conclusions

In the RIL population, all the morphological traits, grain yield components, and root architectural traits showed normal frequency distribution, revealing quantitative inheritance, thus these traits were suitable for QTL analysis. Furthermore, a positive correlation between FG-DS with most of the morphological traits, the other grain yield components, and the major root architectural traits under ABA conditions indicate that the rice drought-resistant plants maintain their grain yield under DS conditions by developing cell elongation, maintaining cellular membrane integrity, and regulation of osmotic stress tolerance via ABA-mediated cell signaling. QTL analysis was performed with 4133 SNPs markers by using QTL IciMapping. A total of 213 QTLs and 628 candidate genes within the QTL regions were identified for drought-related traits. The RT-qPCR results revealed that high number of genes were up-regulated in Kaybonnet as the drought-resistant parent, including seven known drought resistance genes, 15 candidate drought resistance genes within QTL regions with known annotations showed higher instrinsic values in Kaybonnet, and two candidate genes with unknown annotations. Candidate genes identified within the QTL regions contribute to drought resistant traits and an understanding of the regulation of gene expression in response to drought stress, which is important to develop drought-resistant rice varieties.

References

Abiri, R., Shaharuddin, N. A., Maziah, M., Norhana, Z., Yusof, B., Atabaki, N., Sahebi, M., Valdiani, A. Kalhori, N., Azizi, P., & Hanafi, M. M. (2017). Role of ethylene and the APETALA 2/ethylene response factor superfamily in rice under various abiotic and biotic stress conditions. *Environmental and Experimental Botany, 134*, 33-34.

Agrawal, L., Gupta, S., Mishra, S. K., Pandey, G., Kumar, S., Chauhan, P. S., Chakrabarty, D. & Nautiyal, C. S. (2016). Elucidation of complex nature of PEG induced drought-stress response in rice root using comparative proteomics approach. *Front. Plant Sci. 7*, 1466. doi:10.3389/fpls.2016.01466

Ahmad, B., Zhang, S., Yao, J., Rahman, M. U., Hanif, M., Zhu, Y., & Wang, X. (2019). Genomic organization of the B3-domain transcription factor family in grapevine (*Vitis vinifera* L.) and expression during seed development in seedless and seeded cultivars. *International Journal of Molecular Sciences*.

Ambavaram, M. M. R., Basu, S., Krishnan, A., Ramegowda, V., Batlang, U., Rahman, L., Baisakh, N., & Pereira, A. (2014). Coordinated regulation of photosynthesis in rice increases yield and tolerance to environmental stress. *Nature Communications, 5, 5302*. doi:10.1038/ncomms6302

Azevedo, C., Santos-Rosa, M. J., & Shirasu, K. (2001). The U-box protein family in plants. *Trends in Plant Science, 6, 8*, 354-358. doi:10.1016/S1360-1385(01)01960-4

Baisakh, N., Yabes, J., Gutierrez, A., Mangu, V., Ma, P., Famoso, A., & Pereira, A. (2020). Genetic mapping identifies consistent quantitative trait loci for yield traits of rice under greenhouse drought conditions. *Genes, 11, 62*. doi:10.3390/genes11010062

Baldoni, E., Genga, A., & Cominelli, E. (2015). Plant MYB transcription factors: their role in drought response mechanisms. *Int. J. Mol. Sci., 16*, 15811-15851. doi:10.3390/ijms160715811

Barik, S. R., Pandit, E., Mohanty, S. P., Nayak, D. K., & Pradhan, S. K. (2020). Genetic mapping of physiological traits associated with terminal stage drought tolerance in rice. *BMC Genetics, 21, 76*. doi:10.1186/s12863-020-00883-x

Barik, S. R., Pandit, E., Pradhan, S. K., Mohanty, S. P., & Mohapatra, T. (2019). Genetic mapping of morpho-physiological traits involved during reproductive stage drought tolerance in rice. *PLoS ONE, 14, 12*, e0214979. doi:10.1371/ journal.pone.0214979

Barnabás, B., Jager, K., & Feher, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ, 31*, 11-38.

Basu, S., Ramegowda, V., & Pereira, A. (2016). Plant adaptation to drought stress. *F1000Research, 5.*

Bernier, J., Kumar, A., Ramaiah, V., Spaner, D., & Atlin, G. (2007). A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop* Science, 47, 2, 507-518.

Bernier, J., Serraj, R., & Kumar, A. (2015). The large-effect drought resistance QTL qtl12.1 increases water uptake in upland rice. *Field Crops Research, 110, 2,* 139-146.

Berri, S., Abbruscato, P., Faivre-Rampant, O., Brasileiro, A. C., Fumasoni, I., Satoh, K., Kikuchi, S., Mizzi, L., Morandini, P., & Pe, M. E. (2009). Characterization of WRKY co-regulatory networks in rice and Arabidopsis. *BMC Plant Biol., 9, 120.*

Bevilacqua, C. B., Basu, S., Pereira, A., Tseng, T. M., Zimmer, P. D., & Burgos, N. R. (2015). Analysis of stress-responsive gene expression in cultivated and weedy rice differing in cold stress tolerance. *PLoS ONE, 10, 7,* 1-22. doi:10.1371/journal.pone.0132100

Bhatia, D., Wing, R. A., Yu, Y., Chougule, K., Kudrna, D., Lee, S., Rang, A., & Singh, K. (2018). Genotyping by sequencing of rice interspecific backcross inbred lines identifies QTLs for grain weight and grain length. *Euphytica, 214, 41*.

Bhattarai, U. & Subudhi, P. K. (2018). Identification of drought responsive QTLs during vegetative growth stage of rice using a saturated GBS-based SNP linkage map. *Euphytica 214, 38*. doi:10.1007/s10681-018-2117-3

Bouman, B. A. M., Hengsdijk, H., Hardy, B., Bindraban, P. S., Tuong, T. P., & Ladha, J. K. (2002). Water-wise rice production. Proceedings of the International Workshop on Water-wise Rice Production, 8-11 April 2002, Los Baños, Philippines. Los Baños (Philippines): International Rice Research Institute. 356 p.

Bunnag, S. & Pongthai, P. (2013). Selection of rice (*Oryza sativa* L.) cultivars tolerant to drought stress at the vegetative stage under field conditions. *American Journal of Plant Sciences, 4,* 1701-1708. doi:10.4236/ajps.2013.49207

Byun, M. Y., Cui, L. H., Oh, T. K., Jung, Y., Lee, A., Park, K. Y., Kang, B. G., & Kim, W. T. (2017). Homologous U-box E3 Ubiquitin ligases OsPUB2 and OsPUB3 are involved in the positive regulation of low temperature stress response in rice (*Oryza sativa* L.). *Front. Plant Sci.* doi:10.3389/fpls.2017.00016

Cao, P., Bartley, L. E., Jung, K., & Ronald, P. C. (2008). Construction of a Rice glycosyltransferase phylogenomic database and identification of rice-diverged glycosyltransferases. *Molecular Plant, 1, 5,* 858-877.

Catolos, M., Sandhu, N., Dixit, S., Shamsudin, N. A. A., Naredo, M. E. B., McNally, K. L., Henry, A., Diaz, M. G., & Kumar, A. (2017). Genetic loci governing grain yield and root development under variable rice cultivation conditions. Front. *Plant Sci., 8,* 1763. doi:10.3389/fpls.2017.01763

Chen, H., Lai, Z., Shi, J., Xiao, Y., Chen, Z., & Xu, X. (2010). Roles of Arabidopsis WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. *BMC Plant Biol., 10,* 281.

Chen, Y., Li, C., Zhang, B., Yi, J., Yang, Y., Kong, C., Lei, C., & Gong, M. (2019). The role of the Late Embryogenesis-Abundant (LEA) protein family in development and the abiotic stress response: a comprehensive expression analysis of Potato (*Solanum Tuberosum*). *Genes, 10, 148,* doi:10.3390/genes10020148

Cho, J. & Paszkowski, J. (2017). Regulation of rice root development by a retrotransposon acting as a microRNA sponge. *eLife, 6, e30038*. doi:10.7554/eLife.30038

Ciftci-Yilmaz, S., Morsy, M. R., Song, L., Coutu, A., Krizek, B. A., Lewis. M. W., & Warren, D. (2000). The WRKY superfamily of plant transcription factors. *Trends Plant Sci., 5,* 199-206.

Courtois, B., Shen, L., Petalcorin, W., Carandang, S., Mauleon, R., & Li, Z. (2003). Locating QTLs controlling constitutive root traits in the rice population IAC 165 x Co39. *Euphytica, 134, 3,* 335-345.

Cutler, S. R., Rodriguez, P. L., Finkelstein, R. R., & Abrams, S.R. (2010). Abscisic acid: emergence of a core signaling network. *Annu. Rev. Plant Biol*., 61, 651-679.

Dai, X., Xu, Y., Ma, Q., Xu, W., Wang, T., Xue, Y., & Chong, K. (2007). Overexpression of an R1R2R3 MYB gene, OsMYB3R-2, increases tolerance to freezing, drought, and salt stress in transgenic Arabidopsis. *Plant Physiol., 143,* 1739-1751.

Datta, K., Baisakh, N., Ganguly, M., Krishnan, S., Shinozaki, K. Y., & Datta, S. K. (2012). Overexpression of Arabidopsis and rice stress genes' inducible transcription factor confers drought and salinity tolerance to rice. *Plant Biotechnology, 10,* 579-586. doi:10.1111/j.1467-7652.2012.00688.x

De Freitas, G. P. M., Basu, S., Ramegowda, V., Braga, E. B., & Pereira, A. (2016). Comparative analysis of gene expression in response to cold stress in diverse rice genotypes. *Biochemical and Biophysical Research Communications, 471,1,* 253-259. doi:10.1016/j.bbrc.2016.02.004

De Souza, L. M., Le Guen, V., Cerqueira-Silva, C. B. M., Silva, C. C., Mantello, C. C., & Conson, A. R. O. (2015). Genetic diversity strategy for the management and use of

rubber genetic resources: more than 1,000 wild and cultivated accessions in a 100-genotype core collection. *PLoS One, 10,* e0134607. doi:10.1371/journal.pone.0134607

Ding, L., Li, Y., Wang, Y., Gao, L., Wang, M., Chaumont, F., Shen, Q., & Guo, S. (2016). Root ABA accumulation enhances rice seedling drought tolerance under ammonium supply: interaction with aquaporins. *Front. Plant Sci., 7,* 1206. doi:10.3389/fpls.2016.01206

Dixit, S., Singh, A., Cruz, M., Maturan, P. T., Amante, M., & Kumar, A. (2014). Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. *BMC Genetics, 15, 16.*

Duan, J. & Cai, W. (2012). OsLEA3-2, an abiotic stress induced gene of rice plays a key role in salt and drought tolerance. *PLoS ONE, 7, 9,* e45117. doi:10.1371/journal.pone.0045117

Duan, J., Li, J., Guo, S. & Kang, Y. (2008). Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. *Journal of Plant Physiology, 165,* 1620-1635.

Edwards, D. & Batley, J. (2010). Plant genome sequencing: applications for crop improvement. *Plant Biotech J., 8,* 2-9.

Fang, N., Wei, X., Shen, L., Yu, Y., Li, M., Yin, C., He, W., Guan, C., Chen, H., Zhang, H., & Bao, Y. (2019). Fine mapping of a panicle blast resistance gene Pb-bd1 in Japonica landrace Bodao and its application in rice breeding. *Rice, 12, 18.* doi:10.1186/s12284-019-0275-0

FAO. (2004). Human energy requirements report of a joint FAO/WHO/ UNU expert consultation. FAO Food and Nutrition Technical Report Series No.1 Food and Agriculture Organization of the United Nations: Rome.

Farooq, M., Basra, S. M. A., Wahid, A., Ahmad, N. & Saleem, B. A. (2009). Improving the drought tolerance in rice (*Oryza sativa* L.) by exogenous application of salicylic acid. *J. Agronomy & Crop Science, 195,* 237-246. doi:10.1111/j.1439-037X.2009.00365.x

Farooq, M., Siddique, K. H. M., Rehman, H., Aziz, T., Lee, D. J., & Wahid, A. (2011). Rice direct seeding: experiences, challenges and opportunities. *Soil Till. Res., 111,* 87-98.

Finkelstein, R. R., Gampala, S. S. & Rock, C. D. (2002). Abscisic acid signaling in seeds and seedlings. *Plant Cell (Suppl), 14,* 15-45.

Food and Agriculture Organization of the UN (FAO). (2018). FAOSTAT Database. http://www.fao.org/faostat/en/#data

Gibert, A., Gray, E. F., Westoby, M., Wright, I. & Falster, D.S. (2016) Data from: on the link between functional traits and growth rate: meta-analysis shows effects change with plant size, as predicted. *Dryad Digital Repository*. doi:10.5061/dryad.701q8.

Gimhani, D. R., Abhayawickrama, B. P., Saliha, M. S. F., & Kottearachchi, N. S. (2018). Mapping and validation of quantitative trait loci for root architectural traits in rice (*Oryza sativa*) under non-stress and salinity stress conditions. *Tropical Agricultural Research, 29, 3,* 241-254.

Guo, R., Shi, L. X., Jiao, Y., Li, M. X., Zhong, X. L., Gu, F. X., Liu, Q., Xia, X., & Li, H. R. (2018). Metabolic responses to drought stress in the tissues of drought-tolerant and drought-sensitive wheat genotype seedlings. *AoB PLANTS, 10,* ply016. doi:10.1093/aobpla/ply016

Hassouni, K. E., Alahmad, S., Belkadi, B., Filali-Maltouf, A., Hickey, L. T., & Bassi, F. M. (2018). Root system architecture and its association with yield under different water regimes in durum wheat. *Crop Science, 58,* 2331-2346. doi:10.2135/cropsci2018.01.0076

Hattori, Y., Nagai, K., Furukawa, S., Song, X. J., Kawano, R., Sakakibara, H., Wu, J., Matsumoto, T., Yoshimura, A., Kitano, H., Matsuoka, M., Mori, H., & Ashikari, M. (2009). The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. *Nature, 460,* 1026-1030. doi:10.1038/ nature08258

Hayano-Kanashiro, C., Calderon-Vazquez, Ibarra-Laclette, E., Herrera-Estrella, L., & Simpson, J. (2009). Analysis of gene expression and physiological responses in three Mexican maize landraces under drought stress and recovery irrigation. *Plos One, 4, 10*. doi:10.1371/journal.pone.0007531

Hsiao, T. C. (1970). Rapid changes in levels of polyribosomes in *Zea mays* in response to water stress. *PI. Physiol., 46,* 281-285.

Hsiao, T. C. (1982). The soil-plant-atmosphere continuum in relation to drought and crop production. In: Drought resistance in crops, with emphasis on rice. International Rice Research Institute, Los Baños, Philippines, pp 39-52.

Hu, B., Yao, H., Peng, X., Wang, R., Li, F., Wang, Z., Zhao, M., & Jin, L. (2019). Overexpression of Chalcone synthase improves flavonoid accumulation and drought tolerance in Tobacco. *Preprints, 2019060103*. doi:10.20944/preprints201906.0103.v1

Hu, H., Dong, C., Sun, D., Hu, Y., & Xie, J. (2018). Genome-wide identification and analysis of U-Box E3 Ubiquitin-protein ligase gene family in banana. *Int J Mol Sci., 19, 12,* 3874. doi:10.3390/ijms19123874

Hu, H., You, J., Fang, Y., Zhu, X., Qi, Z., & Xiong, L. (2008). Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice. *Plant Mol Biol., 67,* 169-81.

Hu, L., He, H., Zhu, C., Peng, X., Fu, J., He, X., Chen, X., Ouyang, L., Bian, J., & Liu, S. (2017). Genome-wide identification and phylogenetic analysis of the chalcone synthase gene family in rice. *J Plant Res., 130, 1,* 95-105. doi:10.1007/s10265-016-0871-7

Huang, L., Zhang, F., Zhang, F., Wang, W., Zhou, Y., & Fu, B. (2014). Comparative transcriptome sequencing of tolerant rice introgression line and its parents in response to drought stress. *BMC Genomics, 15, 1026*. doi:10.1186/1471-2164-15-1026

Huang, X., Chao, D., Gao, J., Zhu, M., Shi, M., & Lin, H. (2009). A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. *Genes & Development, 23,* 1805-1817. doi:10.1101/gad.1812409

Hussain, W., Baenziger, P. S. & Belamkar, V. (2017). Genotyping-by-sequencing derived high-density linkage map and its application to QTL mapping of flag leaf traits in bread wheat. *Sci. Rep. 7, 16394*.

Islam, A. S. F., Ali, M. R., Gregorio, G. B., & Islam, M. R. (2012). Genetic diversity analysis of stress tolerant rice (*Oryza sativa* L.). *African J. Biotech., 11, 85*, 15123-15129.

Ito, O., O'Toole, J. C., & Hardy, B. (1999). Genetic improvement of rice for water-limited environments. International Rice Research Institute, Los Banos, Philippines 353 p.

Jaganathan, D. (2015). Genotyping-by-sequencing based intra-specific genetic map refines a "QTL-hotspot" region for drought tolerance in chickpea. *Mol. Genet. Genom., 290,* 559-571.

Jeong, J. S., Kim, Y. S., Baek, K. H., Jung, H., Ha, S., Choi, Y. D., Kim, M., Reuzeau, C., & Kim, J. (2010). Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions. *Plant Physiology, 153*, 185-197.

Jiang, S. & Ramachandran, S. (2004). Identification and molecular characterization of myosin gene family in *Oryza sativa* genome. *Plant and Cell Physiology, 45, 5,* 590-599. doi:10.1093/pcp/pch061

Jiang-xu, W., Jian, S., Cheng-xin, L., Hua-long, L., Jing-guo, W., Hong-wei, Z., & De-tang, Z. (2016). Genetic dissection of the developmental behavior of plant height in rice under different water supply conditions. *Journal of Integrative Agriculture, 15, 12*, 2688-2702.

Jones, P. & Vogt, T. (2001). Glycosyltransferases in secondary plant metabolism: tranquilizers and stimulant controllers. *Planta, 213*, 164-174. doi:10.1007/s004250000492

Kamarudin, Z. S., Yusop, M. R., Ismail, M. R., Mohamed, M., Harun, A. R., Yusuff, O., Magaji, U., & Fatai, A. (2019). LEA gene expression assessment in advanced mutant rice genotypes under drought stress. *International Journal of Genomics*. doi:10.1155/2019/8406036

Kamoshita, A., Babu, R. C., Boopathi, N. M., & Fukai, S. (2008). Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. *Field Crop Res., 109,* 1-23. doi:10.1016/j.fcr.2008.06.010

Kanbar, A., Toorchi, M., & Shashidhar, H. E. (2009). Relationship between root and yield morphological characters in rainfed low land rice (*Oryza sativa* L.). *Cereal Research Communication, 37, 2,* 261-268. doi:10.1556/CRC.37.2009.2.14

Katiyar, A., Smita, S., Lenka, S. K., Rajwanshi, R., Chinnusamy, V., & Bansal, K. C. (2012). Genome-wide classification and expression analysis of MYB transcription factor families in rice and Arabidopsis. *BMC Genomics, 13, 544.*

Keppler, B. D. & Showalter, A. M. (2010). IRX14 and IRX14-LIKE, two glycosyl transferases involved in glucuronoxylan biosynthesis and drought tolerance in Arabidopsis. *Molecular Plant, 3, 5,* 834-841. doi:10.1093/mp/ssq028

Khush, G. (2003). Productivity improvements in rice. *Nutr. Rev., 61,* 114-116. doi:10.1301/nr.2003.jun.S114-S116

Khush, G. S. (2001). Green revolution: the way forward. *Nat. Rev.* Genet., 2, 815-822. doi:10.1038/35093585

Kikuchi, S., Satoh, K., Nagata, T., Kawagashira, N., Doi, K., & Kishimoto, N. (2003). Collection, mapping, and annotation of over 28,000 cDNA clones from japonica rice. *Science, 301,* 376-379.

Kim, S. Y. (2006). The role of ABF family bZIP class transcription factors in stress response. *Physiol. Plant., 126,* 519-527.

Kim, Y., Chung, Y. S., Lee, E., Tripathi, P., Heo, S., & Kim, K. (2020). Root response to drought stress in rice (*Oryza sativa* L.). *International Journal of Molecular Sciences, 21, 1513*. doi:10.3390/ijms21041513

Kitomi, Y., Kanno, N., Kawai, S., Mizubayashi, T., Fukuoka, S., & Uga, Y. (2015). QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of DEEPER ROOTING 1. *Rice, 8, 16*. doi:10.1186/s12284-015-0049-2

Kosambi, D. D. (1943). The estimation of map distances from recombination values. *Ann Eugenics, 2,* 172-175. doi:10.1111/j.1469-1809.1943.tb02321.x.

Lairson, L. L., Henrissat, B., Davies, G. J., & Withers, S. G. (2008). Glycosyltransferases: structures, functions, and mechanisms. *Annu. Rev. Biochem., 77,* 521-555. doi:10.1146/annurev.biochem.76.061005.092322

Lam, K. C., Ibrahim, R. K., Behdad, B., & Dayanandan, S. (2007). Structure, function, and evolution of plant O-methyltransferases. Genome, 50, 1001-1013.

Lanceras, J. C., Pantuwan, G., Jongdee, B., & Toojinda, T. (2004). Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant Physiology, 135,* 384-399.

Lata, C. & Prasad, M. (2011). Role of DREBs in regulation of abiotic stress responses in plants. *J. Exp. Bot., 62,* 4731-4748. doi:10.1093/jxb/err210

Li, C., Ng, C. K. Y., & Fan, L. M. (2015). MYB transcription factors, active players in abiotic stress signaling. *Environ. Exp. Bot., 114,* 80-91.

Li, R., Li, M., Ashraf, U., Liu, S., & Zhang, J. (2019). Exploring the relationships between yield and yield-related traits for rice varieties released in China from 1978 to 2017. *Front. Plant Sci., 10,* 543. doi:10.3389/fpls.2019.00543

Liang, Y., Kang, K., Gan, L., Ning, S., Xiong, J., Song, S., Xi, L., Lai, S., Yin, Y., Gu, J., Xiang, J., Li, S., Wang, B., & Li, M. (2019). Drought-responsive genes, late embryogenesis abundant group3 (LEA3) and vicinal oxygen chelate, function in lipid accumulation in *Brassica napus* and Arabidopsis mainly via enhancing photosynthetic efficiency and reducing ROS. *Plant Biotechnology Journal,* 17, 2123-2142. doi:10.1111/pbi.13127

Licausi, F., Ohme-Takagi, M., & Perata, P. (2013). APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. *New Phytol., 199,* 639-649. doi:10.1111/nph.12291

Lim, C. W., Baek, W., Jung, J., Kim, J. H., & Lee, S. C. (2015). Function of ABA in stomatal defense against biotic and drought stresses. *Int. J. Mol. Sci. 16,* 15251-15270. doi:10.3390/ijms160715251

Lou, Q., Chen, L., Mei, H., Wei, H., Feng, F., Wang, P., Xia, H., Li, T., & Luo, L. (2015). Quantitative trait locus mapping of deep rooting by linkage and association analysis in rice. *Journal of Experimental Botany, 66, 15,* 4749-4757. doi:10.1093/jxb/erv246

Lu, Y. (2016). Identification and roles of photosystem II assembly, stability, and repair factors in Arabidopsis. *Front. Plant Sci., 7, 168*. doi:10.3389/fpls.2016.00168

Ma, B., Chen, H., Chen, S. Y., & Zhang, J. S. (2014). Role of ethylene in plant growth and responses to stresses, phytohormones: A window to metabolism signaling and biotechnological applications. *Springer and Biotechnological Applications*, *Springer*, 81-118.

Ma, Q., Dai, X., Xu, Y., Guo, J., Liu, Y., Chen, N., Xiao, J., Zhang, D., Xu, Z., & Zhang, X. (2009). Enhanced tolerance to chilling stress in OsMYB3R-2 transgenic rice is mediated

by alteration in cell cycle and ectopic expression of stress genes. *Plant Physiol., 150*, 244-256.

Macovi, A., Vaid, N., Tula, S., & Tuteja, N. (2012). A new DEAD-box helicase ATP-binding protein (OsABP) from rice is responsive to abiotic stress. *Plant Signal Behav., 7*, 1138-1143.

Mae. (1997). Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. *Plant soil, 196*, 201-210.

Magwanga, R. O., Lu, P., Kirungu, J. N., Lu, H., Wang, X. Cai, X., Zhou, Z., Zhang, Z., Salih, H., Wang, K., & Liu, F. (2018). Characterization of the late embryogenesis abundant (LEA) proteins family and their role in drought stress tolerance in upland cotton. *BMC Genetics, 19, 6*. doi:10.1186/s12863-017-0596-1

Mantovani, A. & Iglesias, R. R. (2008). Factors limiting seed germination of terrestrial bromeliads in the sandy coastal plains (Restinga) of Maricá, Rio de Janeiro, Brazil. *Rodriguésia, 59*, 135-50.

Mardani, Z., Rabiei, B., Sabouri, H., & Sabouri, A. (2013). Mapping of QTLs for germination characteristics under non-stress and drought stress in rice. *Rice Sci., 20*, 391-399. doi:10.1016/S1672-6308(13)60150-X

Mauri, N., Fernandez-Marcos, M., Costas, C., Desvoyes, B., Pichel, A., Caro, E., & Gutirrez, C. (2016). GEM, a member of the GRAM domain family of proteins, is part of the ABA signaling pathway. *Scientific Reports, 6, 22660*.

McCarty, D. R., Carson, C. B., Stinard, P. S., & Robertson, D. S. (1989). Molecular analysis of viviparous-1: An abscisic acid-insensitive mutant of maize. *Plant Cell, 1*, 523-532.

McCouch, S. R. (2008). Gene nomenclature system for rice. *Rice, 1*, 72-84. doi:10.1007/s12284-008-9004-9

McCouch, S. R., Zhao, K., Wright, M., Tung, C. W., Ebana, K., Thomson, M., Reynolds, A., Wang, D., Declerck, G., Ali, M. L., McClung, A., Eizenga, G., & Bustamante, C. (2010). Development of genome-wide SNP assays for rice. *Breed Sci., 60*, 524-535.

Melandri, G., Prashar, A., McCouch, S. R., Linden, G., Jones, H. G., Kadam, N., Jagadish, K., Bouwmeester, H., & Ruyter-Spira, C. (2020). Association mapping and genetic dissection of drought-induced canopy temperature differences in rice. *Journal of Experimental Botany, 71, 4*, 1614-1627. doi:10.1093/jxb/erz527

Meng, L., Li, H., Zhang, L., & Wang, J. (2015). QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in bi-parental populations. *The Crop Journal, 3*, 269-283.

Mishra, K. K., Vikram, P., & Yadaw, R. B. (2013). QDTY12.1: A locus with a consistent effect on grain yield under drought in rice. *BMC Genetics, 14, 12*.

Mizoi, J., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2012). AP2/ERF family transcription factors in plant abiotic stress responses. *Biochim. Biophys. Acta., 1819*, 86-96. doi:10.1016/j.bbagrm.2011.08.004

Mu, P., Li, Z., & Li, C. (2003). QTL mapping of the root traits and their correlation analysis with drought resistance using DH lines from paddy and upland rice cross. *Chinese Science Bulletin, 48, 24*, 2718-2724.

Mukhopadhyay, A., Vij, S., & Tyagi, A. K. (2004). Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. *Proc. Natl. Acad. Sci. USA., 101*, 6309-6314.

Nada, R. M. & Abogadallah, G. M. (2018). Root-favoured biomass allocation improves growth and yield of field-grown rice (*Oryza sativa* L.) plants only when the shoot sink is expandable. *Acta Physiol Plant, 40*, 123. doi:10.1007/s11738-018-2697-5

Nakabayashi, R., Yonekura-Sakakibara, K., Urano, K., Suzuki, M., Yamada, Y., Nishizawa, T., Matsuda, F., Kojima, M., Sakakibara, H., & Shinozaki, K. (2013). Enhancement of oxidative and drought tolerance in Arabidopsis by overaccumulation of antioxidant flavonoids. *The Plant Journal*. doi:10.1111/tpj.12388

Nakano, T., Suzuki, K., Fujimura, T., & Shinshi, H. (2006). Genome-wide analysis of the ERF gene family in Arabidopsis and rice. *Plant Physiol., 140, 2*, 411-432.

Nakashima, K., Tran, L. S., Van Nguyen, D., Fujita, M., Maruyama, K., Todaka, D., Ito, Y., Hayashi, N., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2007). Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress responsive gene expression in rice. *Plant J., 51*, 617-630.

Nakashima, K., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2014). The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. *Front Plant Sci., 5*, 1-7.

Nan, N., Wang, J., Shi, Y., Qian, Y., Jiang, L., Huang, S., Liu, Y., Wu., Y., Liu, B., & Xu, Z. (2020). Rice plastidial NAD-dependent malate dehydrogenase 1 negatively regulates salt stress response by reducing the vitamin B6 content. *Plant Biotechnology Journal, 18*, 172-184. doi:10.1111/pbi.13184

Nawaz, G. & Kang, H. (2019). Rice OsRH58, a chloroplast DEAD-box RNA helicase, improves salt or drought stress tolerance in Arabidopsis by affecting chloroplast translation. *BMC Plant Biology, 19, 17.* doi:10.1186/s12870-018-1623-8

Oh, S. J., Song, S. I., Kim, Y. S., Jang, H. J., Kim, S. Y., Kim, M., Kim, Y. K., Nahm, B. H., & Kim, J. K. (2005). Arabidopsis CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiol., 138, 1,* 341-351.

O'Toole, J. C. (1982). Adaptation of rice to drought-prone environments. In: Drought Resistance in Crops with Emphasis on Rice. IRRI, Los Banos, Philippines, pp. 195-213.

Palanog, A. D., Swamy, B. P. M., & Shamsudin, N. A. A. (2014). Grain yield QTLs with consistent-effect under reproductive stage drought stress in rice. *Field Crops Research, 161*, 46-54.

Pan, Y., Seymour, G. B., & Lu, C. (2012). An ethylene response factor (ERF5) promoting adaptation to drought and salt tolerance in tomato. *Plant Cell Rep., 31,* 349-360. doi:10.1007/s00299-011-1170-3

Phukan, U. J., Jeena, G. S., Tripathi, V., & Shukla, R. K. (2017). Regulation of Apetala2/ethylene response factors in plants. *Front. Plant Sci., 8, 150*. doi:10.3389/fpls.2017.00150

Prince, S. J., Beena, R., Gomez, S. M., Senthivel, S., & Babu, R. C. (2015). Mapping consistent rice (*Oryza sativa* L.) yield QTLs under drought stress in target rainfed environments. *Rice, 8, 1*.

Puranik, S., Sahu, P. P., Mandal, S. N., Parida, S. K., & Prasad, M. (2013). Comprehensive genome-wide survey, genomic constitution and expression profiling of the NAC transcription factor family in foxtail millet (*Setaria italica* L.). *PLoS One., 8, 64594*. doi:10.1371/journal.pone.0064594

Quan, R., Hu, S., Zhang, Z., Zhang, H., Zhang, Z., & Huang, R. (2010). Overexpression of an ERF transcription factor TSRF1 improves rice drought tolerance. *Plant Biotechnol. J., 8,* 476-488.

Quick Stats, USDA-NASS, State Agriculture Overview: Arkansas (2016). https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=ARKANS AS

Rabello, A. R., Guimarães, C. M., Rangel, P. H. N., da Silva, F. R., Seixas, D., de Souza, E., Brasileiro, A. C. M., Spehar, C. R., Ferreira, M. E., & Mehta, Â. (2008). Identification of drought-responsive genes in roots of upland rice (*Oryza sativa* L.). *BMC Genom., 9, 485*.

Rahimi, J., Ebrahimpour, M., & Khalili, A. (2013). Spatial changes of extended De Martonne climatic zones affected by climate change in Iran. *Theor. Appl. Climatol., 112, 3-4*, 409-418.

Ramegowda, V., Basu, S., Gupta, C., & Pereira, A. (2015). Regulation of grain yield in rice under well-watered and drought stress conditions by GUDK. *Plant Signaling & Behavior, 10, 11.* doi:10.1080/15592324.2015.1034421

Ramegowda, V., Basu, S., Krishnan, A., & Pereira, A. (2014). Rice growth under drought kinase is required for drought tolerance and grain yield under normal and drought stress conditions. *Plant Physiology, 166, 3,* 1634-1645. doi:10.1104/pp.114.248203

Ryu, M. Y., Cho, S. K., Hong, Y., Kim, J., Kim, J. H., Kim, G. M., Chen, Y., Knoch, E., Moller, B. L., Kim, W. T., Lyngkjaer, M. F., & Yang, S. W. (2019). Classification of barley U-box E3 ligases and their expression patterns in response to drought and pathogen stresses. *BMC Genomics, 20, 326.* doi:10.1186/s12864-019-5696-z

Sabar, M., Shabir, G., Shah, S. M., Aslam, K., Naveed, S. A., & Arif, M. (2019). Identification and mapping of QTLs associated with drought tolerance traits in rice by a cross between Super Basmati and IR55419-04. *Breeding Science, 69,* 169-178. doi:10.1270/jsbbs.18068

Sadeghi, S. M. & Danesh, R. K. (2011). Effects of water deficit role at different stages of reproductive growth on yield components of rice. *World Applied Sciences Journal, 13, 9,* 2021-2026.

Sahoo, K. K., Tripathi, A. K., Pareek, A., & Singla-Pareek, S. L. (2013). Taming drought stress in rice through genetic engineering of transcription factors and protein kinases. *Plant Stress, 7, 1,* 60-72.

Saikumar, S., Gouda, P. K., & Saiharini, A. (2014). Major QTL for enhancing rice grain yield under lowland reproductive drought stress identified using an *O. sativa/O. glaberrima* introgression line. *Field Crops Research, 163,* 119-131.

Sakamoto, H., Maruyama, K., Sakuma, Y., Meshi, T., Iwabuchi, M., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2004). Arabidopsis Cys2/His2-type zinc-finger proteins function as transcription repressors under drought, cold, and high-salinity stress conditions. *Plant Physiol., 136,* 2734-2746.

Santos, A. B., Fageria, N. K., & Prabhu, A. S. (2003). Rice ratooning management practices for higher yields. *Communication of Soil Sciences and Plant Analysis, 34,* 881-918.

Sasi, S., Venkatesh, J., Daneshi, R. F., & Gururani, M. A. (2018). Photosystem II extrinsic proteins and their putative role in abiotic stress tolerance in higher plants. *Plants, 7, 100.* doi:10.3390/plants7040100

Schroeder, J. I., Kwak, J. M., & Allen, G. J. (2001). Guard cell abscisic acid signaling and engineering drought hardiness in plants. *Nature, 410,* 327-330.

Shen, H., Liu, C., Zhang, Y., & Meng, X. (2012). OsWRKY30 is activated by MAP kinases to confer drought tolerance in rice. *Plant Mol Biol., 80,* 241-253.

Shim, J. S., Oh, N., Chung, P. J., Kim, Y. S., Choi, Y. D., & Kim, J. K. (2018). Overexpression of OsNAC14 improves drought tolerance in rice. *Front. Plant Sci., 9, 310.* doi:10.3389/fpls.2018.00310

Shinozaki, K. & Yamaguchi-Shinozaki, K. (2007). Gene networks involved in drought stress response and tolerance. *J. Exp. Bot., 58,* 221-227.

Singh, U. M., Yadav, S., Dixit, S., Ramayya, P. J., Devi, M. N., Raman, K. A., & Kumar, A. (2017). QTL hotspots for early vigor and related traits under dry direct-seeded system in rice (*Oryza sativa* L.). *Frontiers in Plant Science, 8, 286*.

Solis, J., Gutierrez, A., Mangu, V., Sanchez, E., Bedre, R., Linscombe, S., & Baisakh, N. (2018). Genetic mapping of quantitative trait loci for grain yield under drought in rice under controlled greenhouse conditions. *Front. Chem., 5, 129*. doi:10.3389/fchem.2017.00129

Specht, J. E., Chase, K., Macrander, M., Graef, G. L., Chung, J., Markwell, J. P., Germann, M., Orf, J. H., & Lark, K. G. (2001). Soybean response to water: a QTL analysis of drought tolerance. *Crop Science, 41,* 493-509. doi:10.2135/cropsci2001.412493x

Studer, A. J. & Doebley, J. F. (2011). Do large effect QTL fractionate? A case study at the maize domestication QTL teosinte branched1. Genetics, 188, 3, 673-681. doi:10.1534/genetics.111.126508

Su, C., Wang W., Gong, S., Zuo, J., Li, S., & Xu, S. (2017). High density linkage map construction and mapping of yield trait QTLs in maize (*Zea mays*) using the genotyping-by sequencing (GBS) technology. *Front.Pl. Sci., 8, 706.*

Takahashi, Y., Shomura, A., Sasaki, T., & Yano, M. (2001). Hd6, a rice quantitative trait locus involved in photoperiod sensitivity, encodes the α subunit of protein kinase CK2. *Proc Natl. Acad. Sci., 98,* 7922-7927.

Takasaki, H., Maruyama, K., Kidokoro, S., Ito, Y., Fujita, Y., & Shinozaki, K. (2010). The abiotic stress responsive NAC-type transcription factor OsNAC5 regulates stress inducible genes and stress tolerance in rice. *Mol. Genet. Genomics, 284, 173-183*. doi:10.1007/s00438-010- 0557-0

Tang, Y., Bao, X., Zhi, Y., Wu, Q., Guo, Y., Yin, X., Zeng, L., Li, J., Zhang, J., He, W., Liu, W., Wang, Q., Jia, C., Li, Z., & Liu, K. (2019). Overexpression of a MYB family gene, OsMYB6, increases drought and salinity stress tolerance in transgenic rice. *Front. Plant Sci., 10, 168*. doi:10.3389/fpls.2019.00168

Thomson, M. J. (2014). High-throughput SNP genotyping to accelerate crop improvement. *Plant Breed. Biotech., 2, 3,* 195-212. doi:10.9787/PBB.2014.2.3.195

Todaka, D., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2015). Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants. *Front. Plant Sci., 18, 684*. doi:10.3389/fpls.2015.00084

Todorov, D., Alexieva, V.V., & Karanov, E. (1998). Effect of putrescine, 4-PU-30, and abscisic acid on maize plants grown under normal, drought, and re-watering conditions. *J. Plant Growth Regul., 17*, 197-203. doi:10.1007/PL00007035

Tuong, T. P. & Bouman, B. A. M. (2003). Rice production in water-scarce environments. In: Kijne, J. W., Barker, R., Molden, D. (eds) Water productivity in agriculture: limits and opportunities for improvement. CABI Publishing, UK, pp 53-67

Umezawa, T., Fujita, M., Fujita, Y., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2006). Engineering drought tolerance in plants: Discovering and tailoring genes to unlock the future. *Curr Opin Biotechnol., 17, 2*, 113-122.

Varshney, R. K., Bansal, K. C., Aggarwal, P. K., Datta, S. K., & Craufurd, P. Q. (2011). Agricultural biotechnology for crop improvement in a variable climate: Hope or hype?. *Trends in Plant Science, 16, 7*, 363-371.

Vashisht, A. A. & Tuteja, N. (2006). Stress responsive DEAD-box helicases: a new pathway to engineer plant stress tolerance. *J Photochem Photobiol., 84*, 150-60.

Venuprasad, R., Dalid, C. O., Del Valle, M., Zhao, D., Espiritu, M., Sta Cruz, M. T., Amante, M., Kumar, A., & Atlin, G. N. (2009). Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theoretical and Applied Genetics, 120, 1*, 177-190.

Venuprasad, R., Impa, S. M., Veeresh Gowda, R. P., Atlin, G. N., & Serraj, R. (2011). Rice near-isogenic-lines (NILs) contrasting for grain yield under lowland drought stress. *Field Crops Research*, 123, 38-46.

Vikram, P., Swamy, B. P. M., Dixit, S., Singh, R., Singh, B. P., Miro, B., Kohli, A., Henry, A., Singh, N. K. & Kumar, A. (2015). Drought susceptibility of modern rice varieties: an effect of linkage of drought tolerance with undesirable traits. *Scientific Reports, 5, 14799*. doi:10.1038/srep14799

Wang, N., Liu, Y., Cong, Y., Wang, T., Zhong, X., Yang, S., Li, Y., & Gai, J. (2016). Genome-wide identification of soybean U-Box E3 ubiquitin ligases and roles of GmPUB8 in negative regulation of drought stress response in Arabidopsis. *Plant Cell Physiol., 57*, 1189-1209. doi:10.1093/pcp/pcw068

Wang, X., Pang, Y., Zhang, J., Zhang, Q., Tao, Y., Feng, B., Zheng, T., Xu, J., & Li, Z. (2014). Genetic background effects on QTL and QTL × environment interaction for yield and its

component traits as revealed by reciprocal introgression lines in rice. *Crop Journal, 2, 6,* 345-357.

Xiao, B., Huang, Y., Tang, N., & Xiong, L. (2007). Over-expression of a LEA gene in rice improves drought resistance under the field conditions. *Theor Appl Genet., 115, 1,* 35-46. doi:10.1007/s00122-007-0538-9

Xiong, H., Li, J., Liu, P., Duan, J., Zhao, Y., Guo, X., Li, Y., Zhang, H., Ali, J., & Li, Z. (2014). Overexpression of OsMYB48-1, a novel MYB-related transcription factor, enhances drought and salinity tolerance in rice. *PLoS ONE, 9, 92913*.

Xiong, H., Li, J., Liu, P., Duan, J., Zhao, Y., Guo, X., Li, Y., Zhang, H., Ali, J., & Li, Z. (2014). Overexpression of OsMYB48-1, a novel MYB-related transcription factor, enhances drought and salinity tolerance in rice. *PLoS ONE, 9, 92913*.

Xiong, J. H., Fu, B. Y., Xu, H. X., & Li, Y. S. (2010). Proteomic analysis of PEG-simulated drought stress responsive proteins of rice leaves using a pyramiding rice line at the seedling stage. *Bot Stud., 51, 2,* 137-145.

Xiong, L., Schumaker, K.S., and Zhu, J.-K. (2002). Cell signaling during cold, drought, and salt stress. *Plant Cell, 14,* 165-183.

Xu, J., Li, J. Z., Zheng, X. W., & Zou, L. X. (2001). QTL mapping of the root traits in rice. *Acta Genetica Sinica, 28, 5,* 433-438.

Xu, P., Yang, J., Ma, Z., Yu, D., Zhou, J., Tao, D., & Li, Z. (2020). Identification and validation of aerobic adaptation QTLs in upland rice. *Life, 10, 65.* doi:10.3390/life10050065

Yadav, R., Courtois, B., Huang, N., & McLaren, G. (1997). Mapping genes controlling root morphology and root distribution in a double-haploid population of rice. *Theor. Appl. Genet., 94,* 619-632.

Yadav, S., Sandhu, N., Majumder, R. R., Dixit, S., Kumar, S., Singh, S. P., Mandal, N. P., Das, S. P., Yadaw, R. B., Singh, V. K., Sinha, P., Varshey, R. K., & Kumar, A. (2019b). Epistatic interactions of major effect drought QTLs with genetic background loci determine grain yield of rice under drought stress. *Scientific Reports, 9,* 2616. doi:10.1038/s41598-019-39084-7

Yadav, S., Sandhu, N., Singh, V. K., Catolos, M., & Kumar, A. (2019a). Genotyping-by-sequencing based QTL mapping for rice grain yield under reproductive stage drought stress tolerance. *Scientific Reports, 9,* 14326. doi:10.1038/s41598-019-50880-z

Yadaw, R. B., Dixit, S., Raman, A., Mishra, K. K., Vikram, P., Swamy, B. P. M., Sta Cruz, M. T., Maturan, P. T., Pandey, M., & Kumar, A. (2013). A QTL for high grain yield under lowland drought in the background of popular rice variety Sabitri from Nepal. *Field Crops Research, 144,* 281-287.

Yan, Y., Jia, H., Wang, F., Wang, C., Shuchang, L., & Xingqi, G. (2015). Overexpression of GhWRKY27a reduces tolerance to drought stress and resistance to *Rhizoctonia solani* infection in transgenic *Nicotiana benthamiana*. *Front Physiol., 6, 265*.

Yang, A., Dai, X., & Zhang, W. (2012). A R2R3-type MYB gene, OsMYB2, is involved in salt, cold, and dehydration tolerance in rice. *J. Exp. Bot., 63*, 2541-2556.

Yokotani, N., Ichikawa, T., Kondou, Y., Matsui, M., Hirochika, H., Iwabuchi, M., & Oda, K. (2009). Tolerance to various environmental stresses conferred by the salt-responsive rice gene ONAC063 in transgenic Arabidopsis. *Planta, 229*, 1065-1075.

Zeng, Y., Chen, Y., Ji, Z., Liang, Y., Zheng, A., Wen, Z., & Yang, C. (2019). Control of plant height by 24 alleles at 12 quantitative trait loci in rice. *Crop Breeding and Applied Biotechnology, 19,* 200-207. doi:10.1590/1984-70332019v19n2a28

Zhao, T. & Dai, A. (2017). Uncertainties in historical changes and future projections of drought. Part II: Model-simulated historical and future drought changes. *Clim. Chang., 144*, 535-548.

Zheng, X., Chen, B., Lu, G., & Han, B. (2009). Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. *Biochem Bioph Res Co., 379*, 985-989.

Zhou, L., Lie, S., Wu, W., Chen, D., Zhan, X., Zhu, A., Zhang, Y., Cheng, S., Cao, L., Lou, X., & Xu, H. (2016). Dissection of genetic architecture of rice plant height and heading date by multiple-strategy-based association studies. *Scientific Reports, 6, 29718.* doi:10.1038/srep29718

Zhu, X., Shen, W., Huang, J., Zhang, T., Zhang, X., Cui, Y., Sang, X., Ling, Y., Li, Y., Wang, N., Zhao, F., Zhang, C., & He, G. (2018). Mutation of the OsSAC1 gene, which encodes an endoplasmic reticulum protein with an unknown function, causes sugar accumulation in rice leaves. *Plant Cell Physiol, 59, 3,* 487-499. doi:10.1093/pcp/pcx203.

Figures

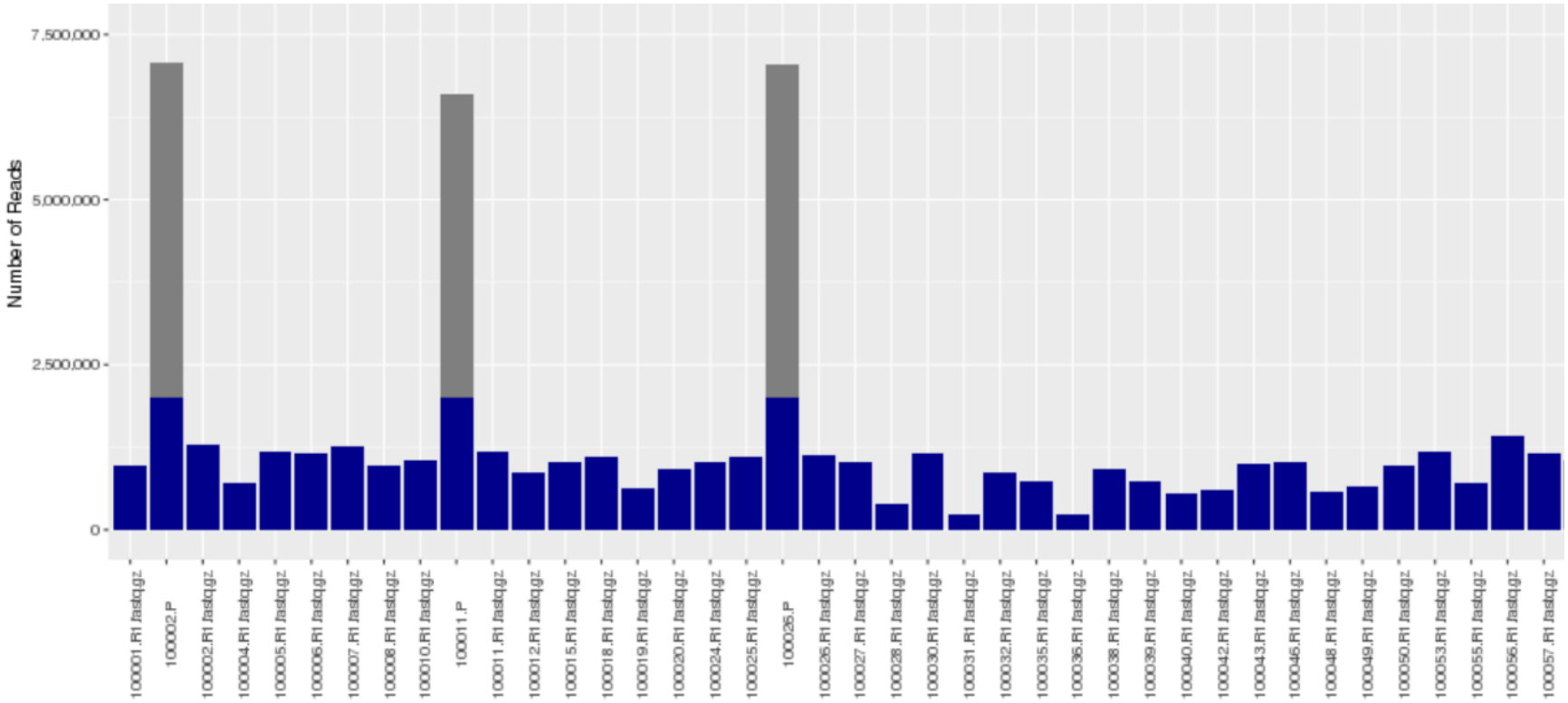

Figure 5.1. Number of reads per sample, two millions base pairs (bp). FASTQ files with more than the targeted number of reads (2,000,000) were subsampled down to 2,000,000 number reads. The first 12 bases were removed from the beginning of each read in order to remove adapter sequences.

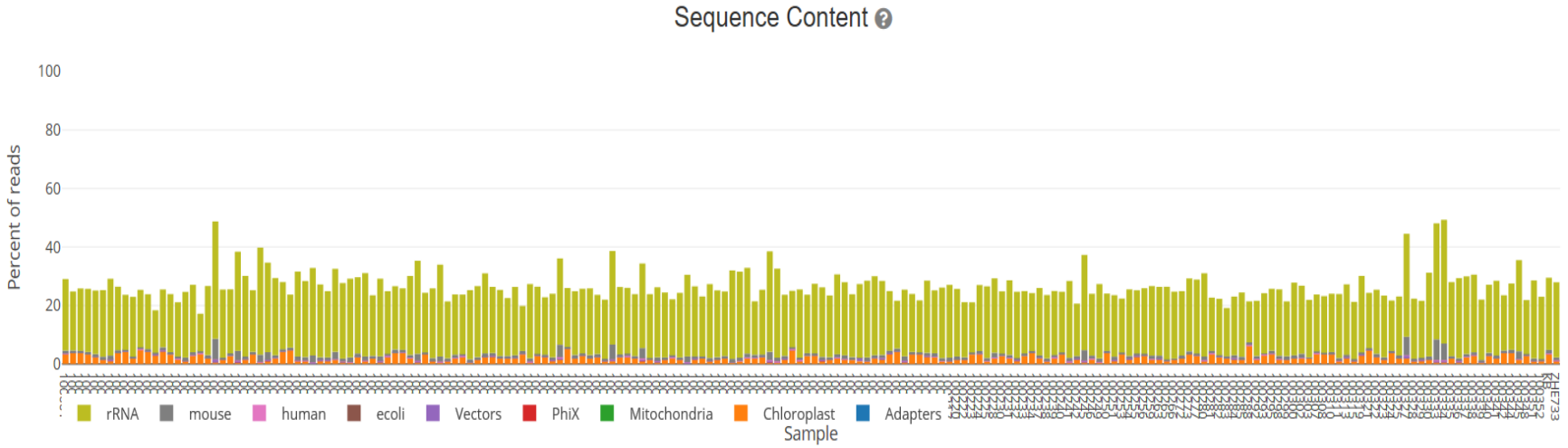

Figure 5.2. Mean quality scores above Q30 for all two hundred GBS libraries was chosen. All libraries were created by using single-end library type with enzyme combination *PstI* and *MspI* for DNA digestion, and the digested DNAs ligated to the adapter. Then, all libraries were sequenced in 1 lane of a NextSeq 1x150-bp run with mean reads per sample of two millions base pairs (bp).

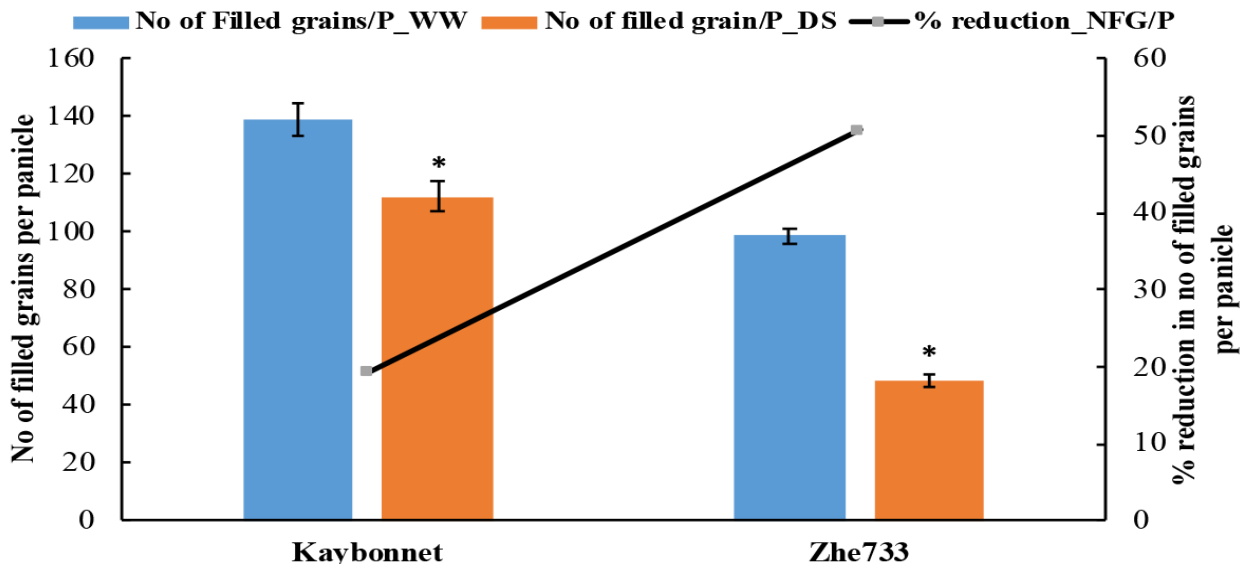

Figure 5.3. Number of filled grains per panicle in Kaybonnet and ZHE733. Kaybonnet maintained higher number of filled grains under DS than ZHE733. Furthermore, the distribution of water use efficiency (WUE) traits has been shown to be highest in *tropical japonica* (KB) and medium in *indica* (ZHE733).

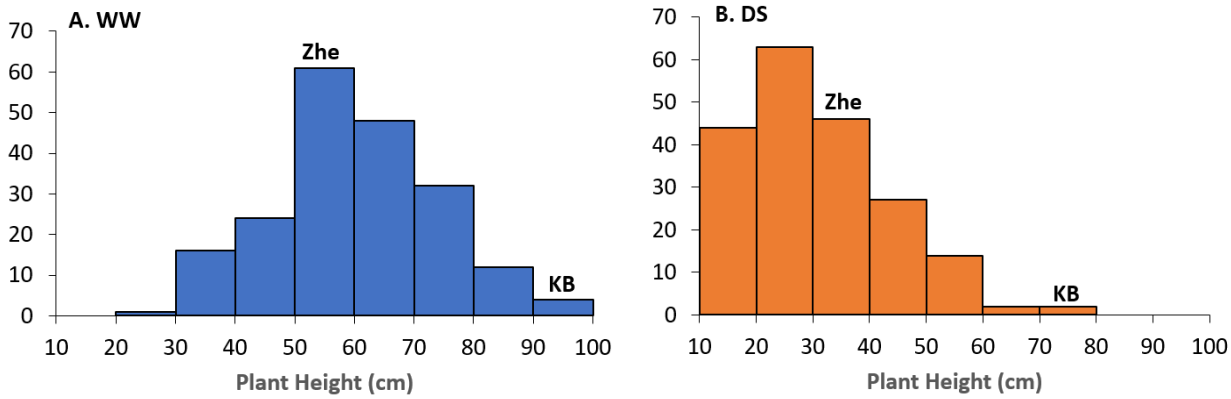

Figure 5.4. Frequency distribution of plant height under WW (A) and DS (B) conditions.

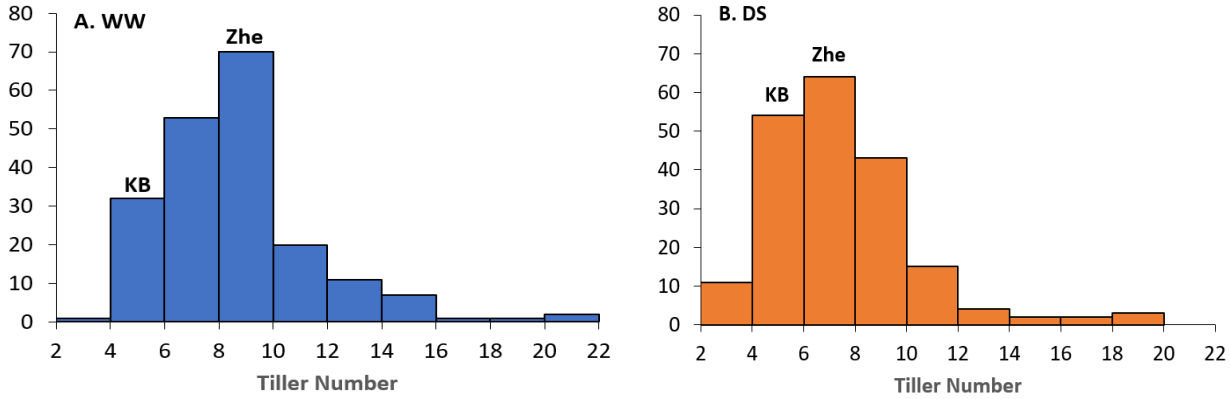

Figure 5.5. Frequency distribution of productive tiller number under WW (A) and DS (B) conditions.

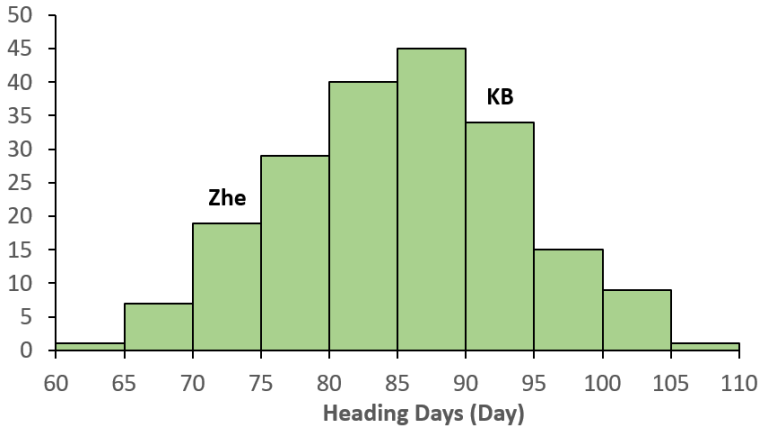

Figure 5.6. Frequency distribution of heading days.

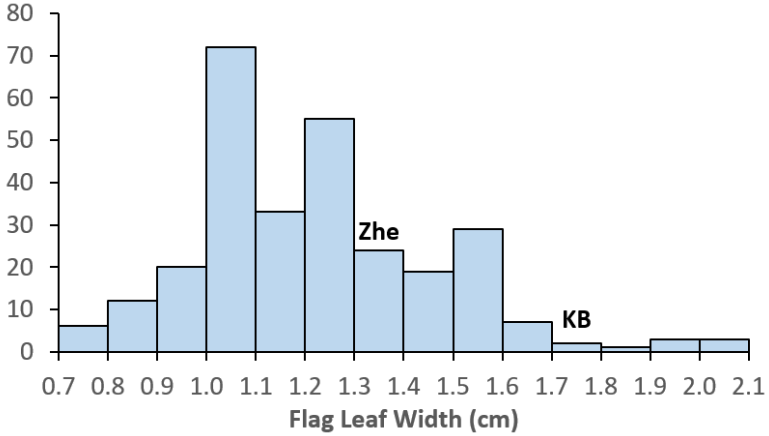

Figure 5.7. Frequency distribution of flag leaf width.

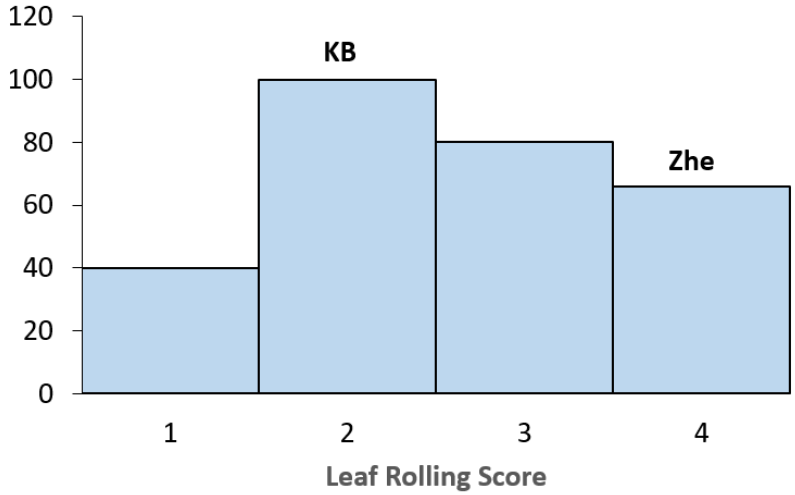

Figure 5.8. Frequency distribution of leaf rolling score.

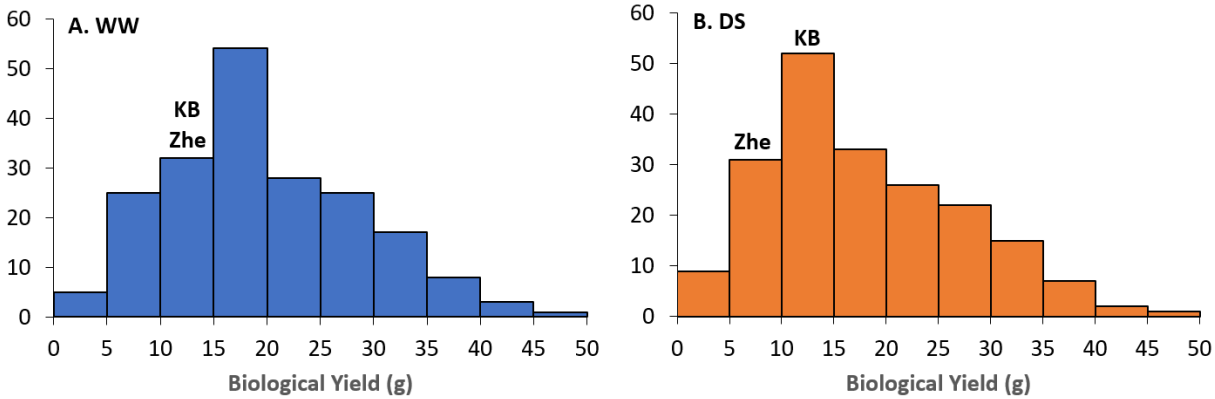

Figure 5.9. Frequency distribution of biological yield under WW (A) and DS (B) conditions.

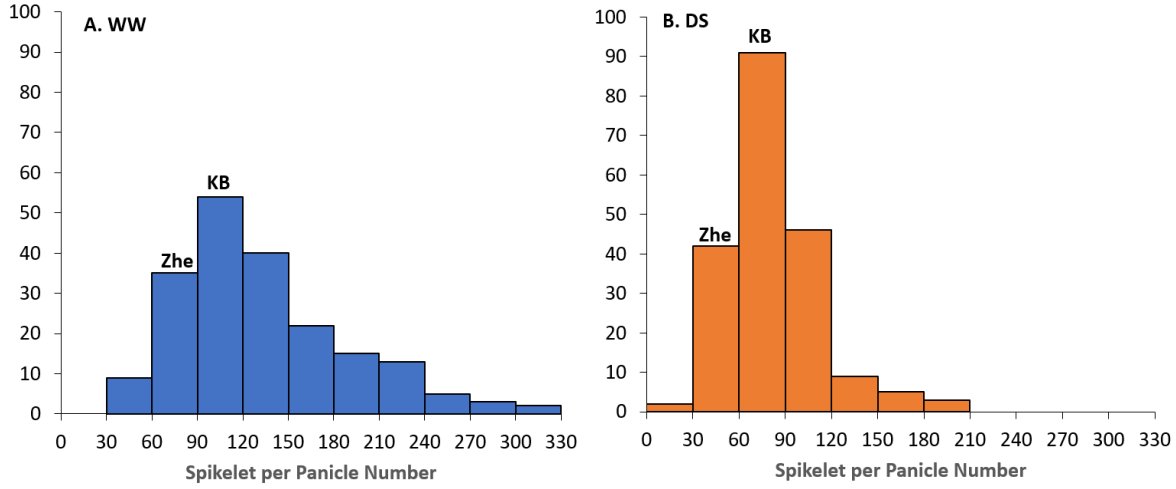

Figure 5.10. Frequency distribution of spikelet per panicle number under WW (A) and DS (B) conditions.

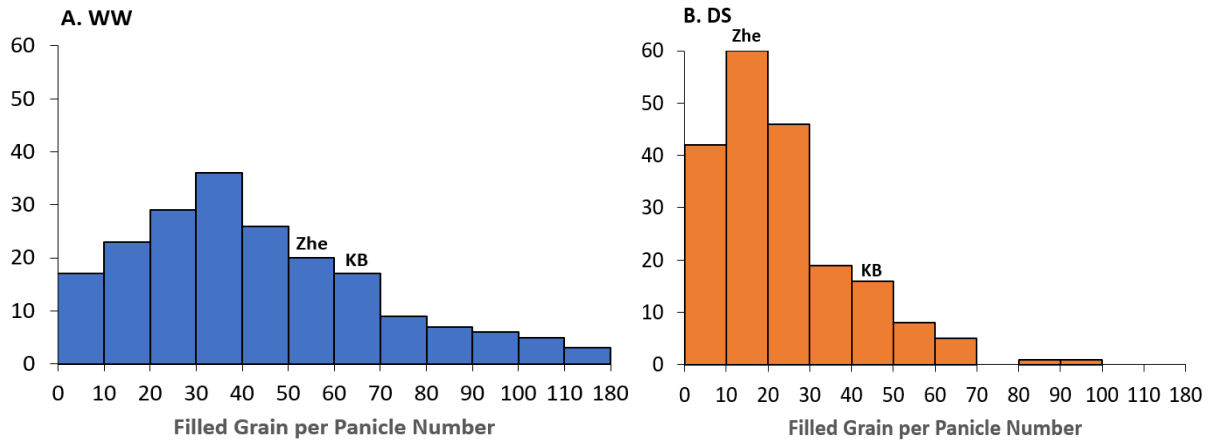

Figure 5.11. Frequency distribution of filled grain per panicle number under WW (A) and DS (B) conditions.

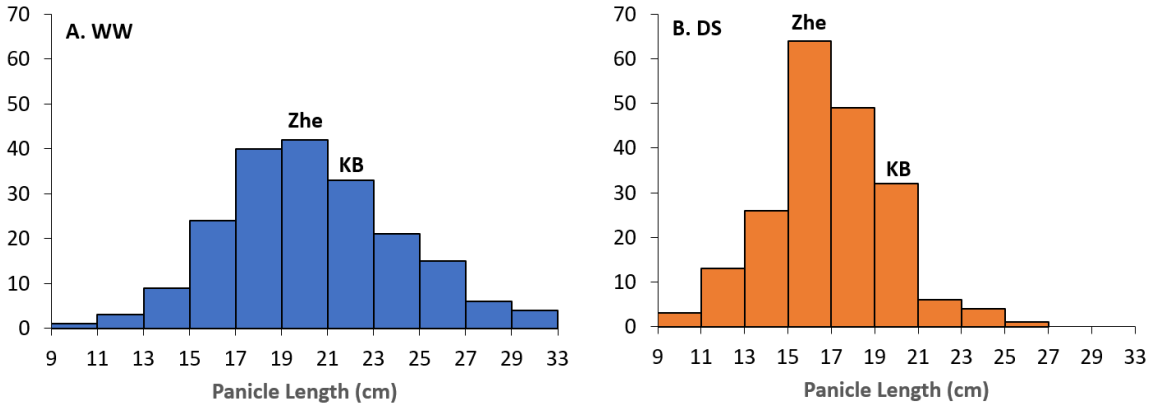

Figure 5.12. Frequency distribution of panicle length under WW (A) and DS (B) conditions.

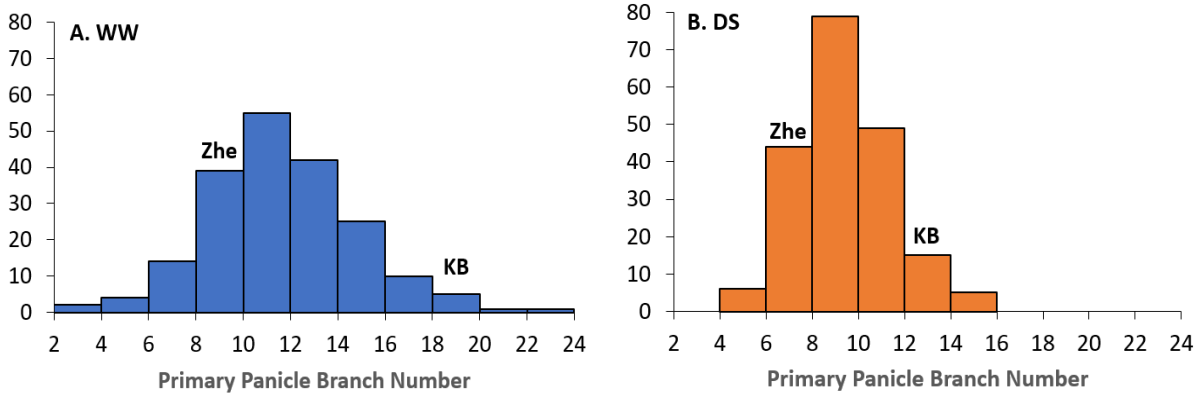

Figure 5.13. Frequency distribution of primary panicle branch number under WW (A) and DS (B) conditions.

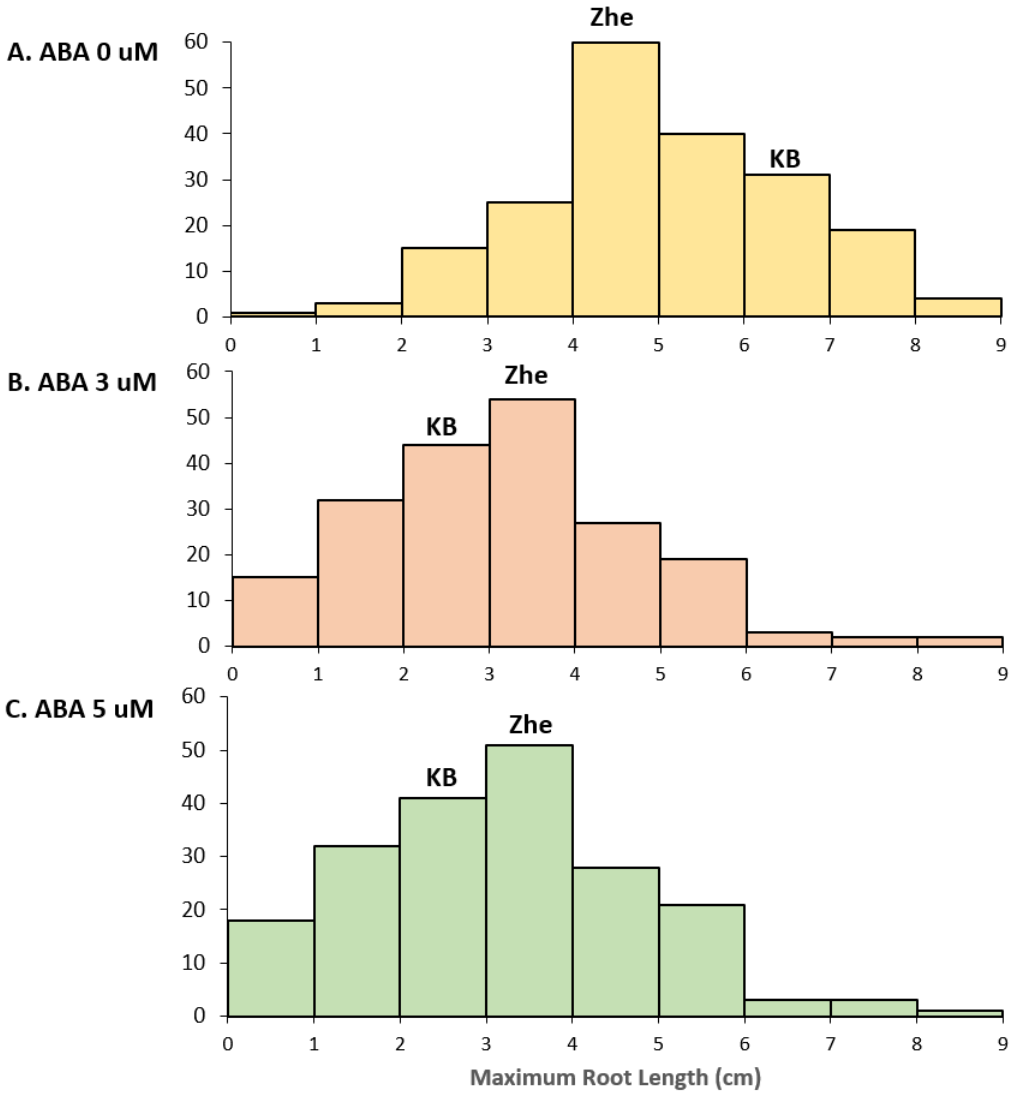

Figure 5.14. Frequency distribution of root length under control (ABA 0 µM) (A), ABA 3 µM (B), and ABA 5 µM (C).

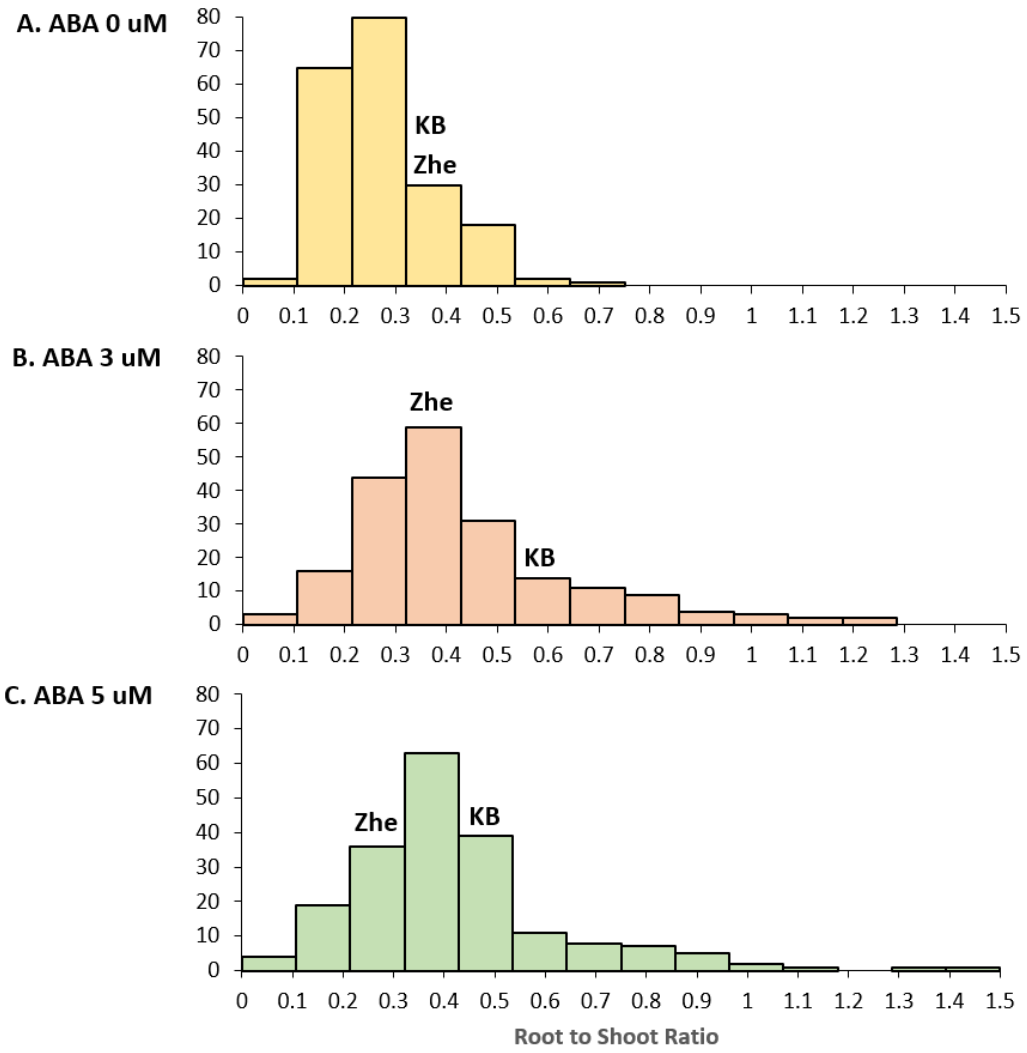

Figure 5.15. Frequency distribution of root to shoot ratio under control (ABA 0 µM) (A), ABA 3 µM (B), and ABA 5 µM (C).

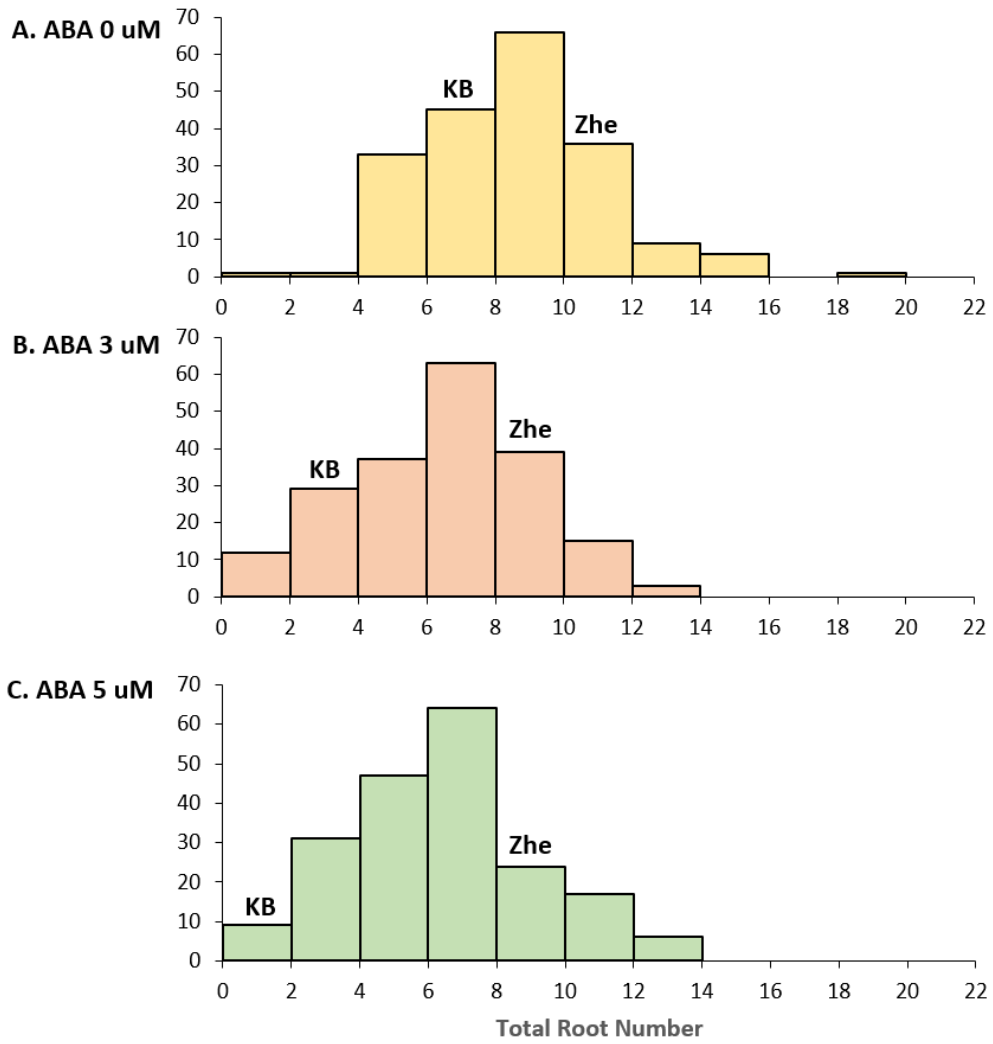

Figure 5.16. Frequency distribution of total root number under control (ABA 0 µM) (A), ABA 3 µM (B), and ABA 5 µM (C).

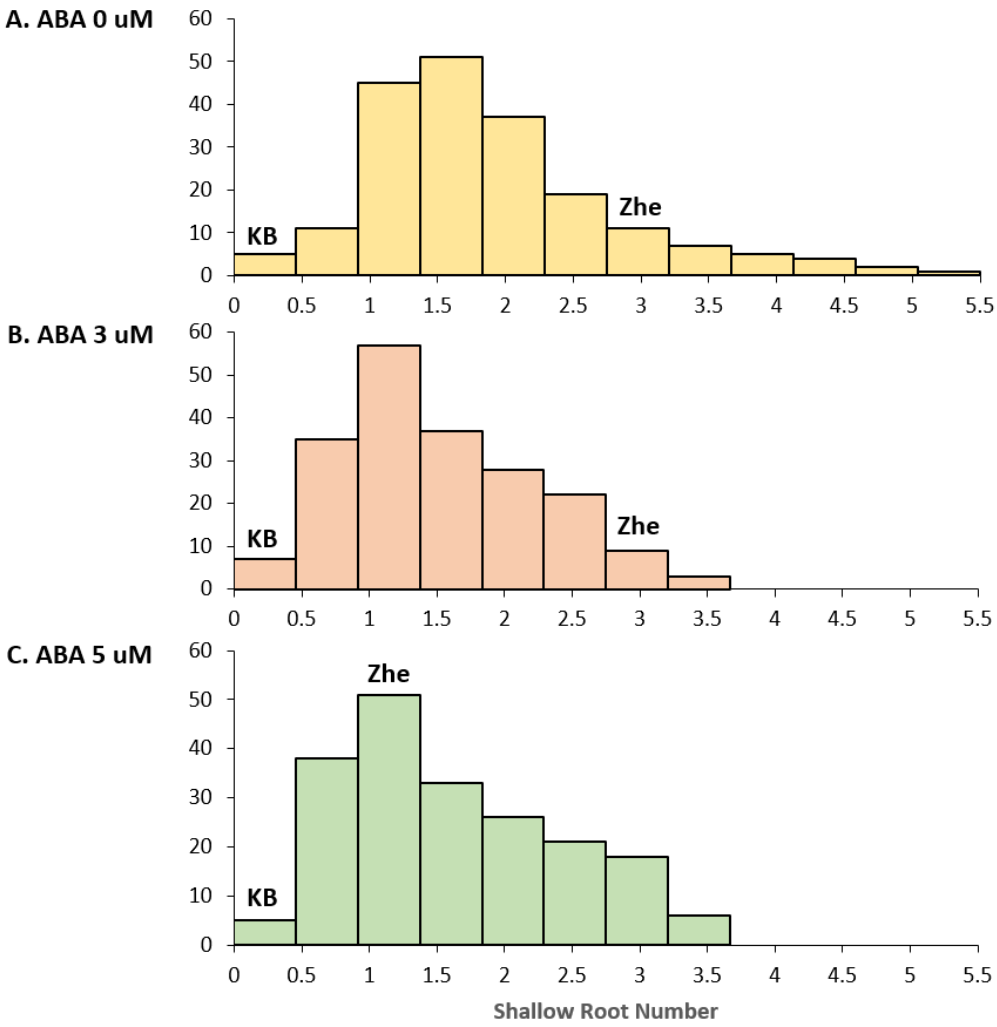

Figure 5.17. Frequency distribution of shallow root number under control (ABA 0 µM) (A), ABA 3 µM (B), and ABA 5 µM (C).

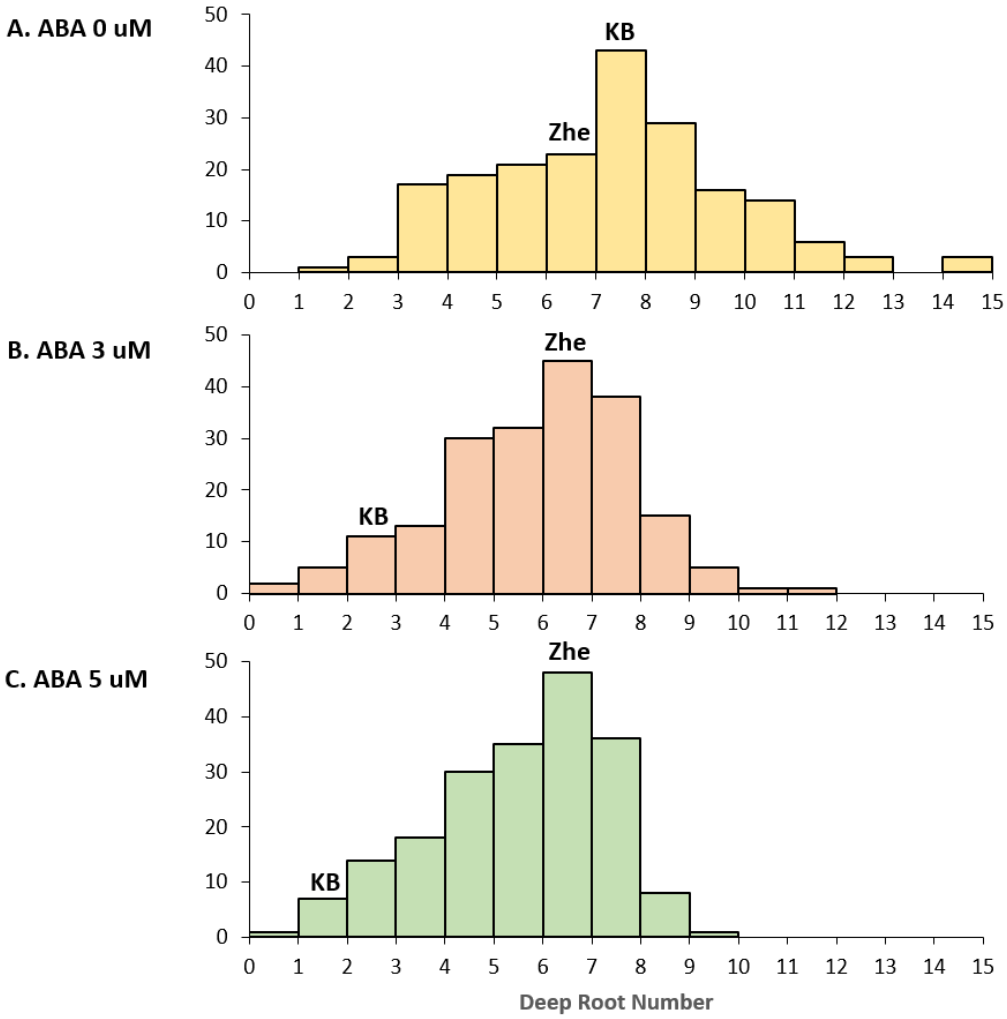

Figure 5.18. Frequency distribution of deep root number under control (ABA 0 µM) (A), ABA 3 µM (B), and ABA 5 µM (C).

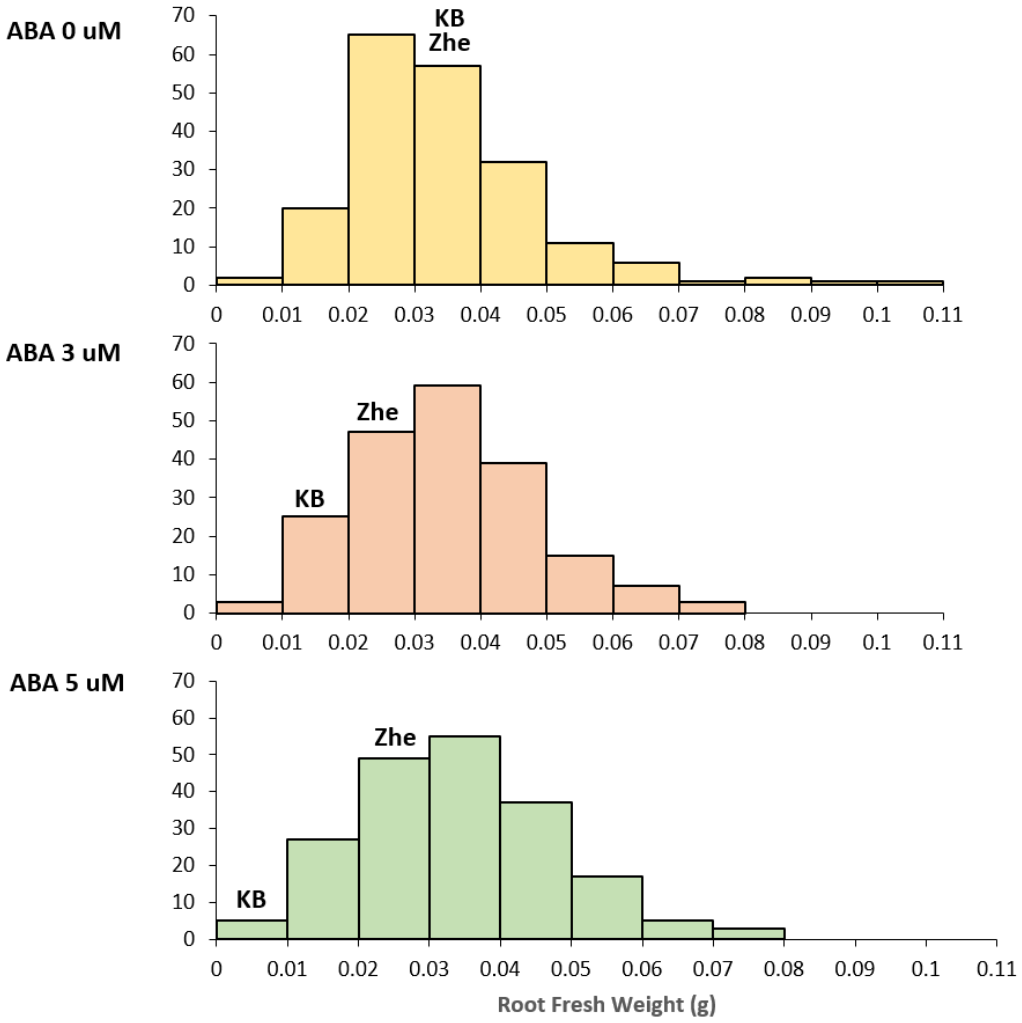

Figure 5.19. Frequency distribution of root fresh weight under control (ABA 0 µM) (A), ABA 3 µM (B), and ABA 5 µM (C).

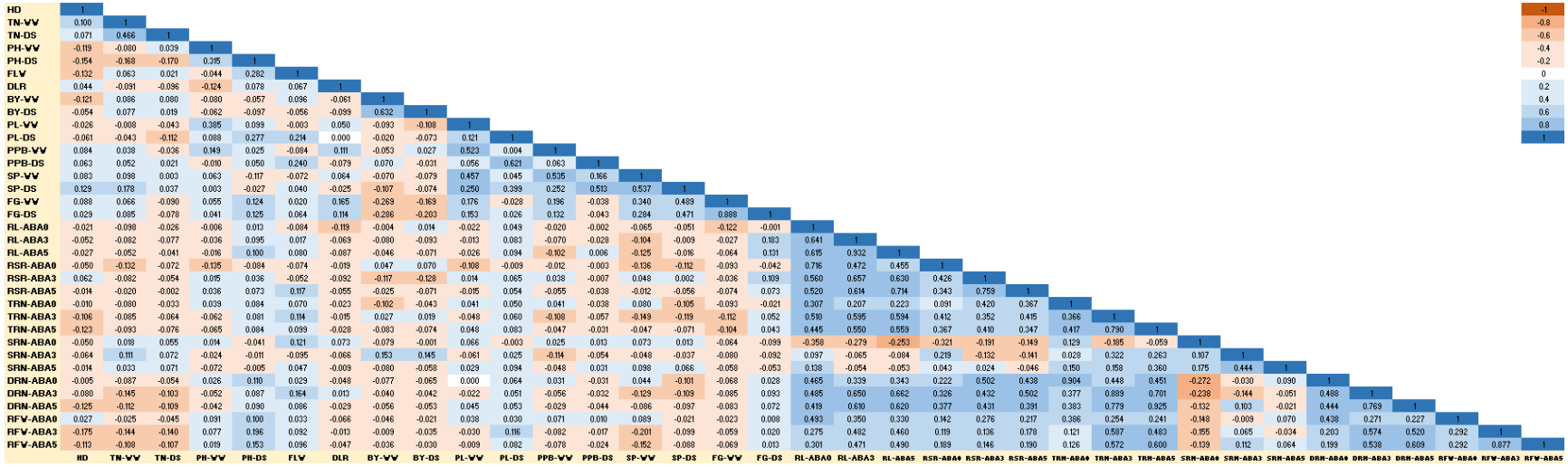

Figure 5.20. Correlation of morphological traits and grain yield components under WW and DS conditions with root architectural traits under ABA conditions. The correlations (-1 to + 1) are colored either in blue (positive correlation) or in red (negative correlation). HD, Heading day; TN-WW, Productive tiller number under WW; TN-DS, Productive tiller number under DS; PH-WW, Plant height (cm) under WW; PH-DS, Plant height (cm) under DS; FLW-DS, Flag leaf width (cm) under DS; DLR-DS, Drought leaf rolling under DS; BY-WW, Biological yield (g) under WW; BY-DS, Biological yield (g) under DS; PL-WW, Panicle length (cm) under WW; PL-DS, Panicle length (cm) under DS; PPB-WW, Primary panicle branch number under WW; PPB-DS, Primary panicle branch number under DS; SP-WW, Spikelet per panicle number under WW; SP-DS, Spikelet per panicle number under DS; FG-WW, Filled grain per panicle number under WW; FG-DS, Filled grain per panicle number under DS; RL-ABA0, Root length under ABA 0 µM; RL-ABA3, Root length under ABA 3 µM; RL-ABA5, Root length under ABA 5 µM; RSR-ABA0, Root shoot ratio under ABA 0 µM; RSR-ABA3, Root shoot ratio under ABA 3 µM; RSR-ABA5, Root shoot ratio under ABA 5 µM; TRN-ABA0, Total root number under ABA 0 µM; TRN-ABA3, Total root number under ABA 3 µM; TRN-ABA5, Total root number under ABA 5 µM; SRN-ABA0, Shallow root number under ABA 0 µM; SRN-ABA3, Shallow root number under ABA 3 µM; SRN-ABA5, Shallow root number under ABA 5 µM; DRN-ABA0, Deep root number under ABA 0 µM; DRN-ABA3, Deep root number under ABA 3 µM; DRN-ABA5, Deep root number under ABA 5 µM; RFW-ABA0, Root fresh weight under ABA 0 µM; RFW-ABA3, Root fresh weight under ABA 3 µM; RFW-ABA5, Root fresh weight under ABA 5 µM.

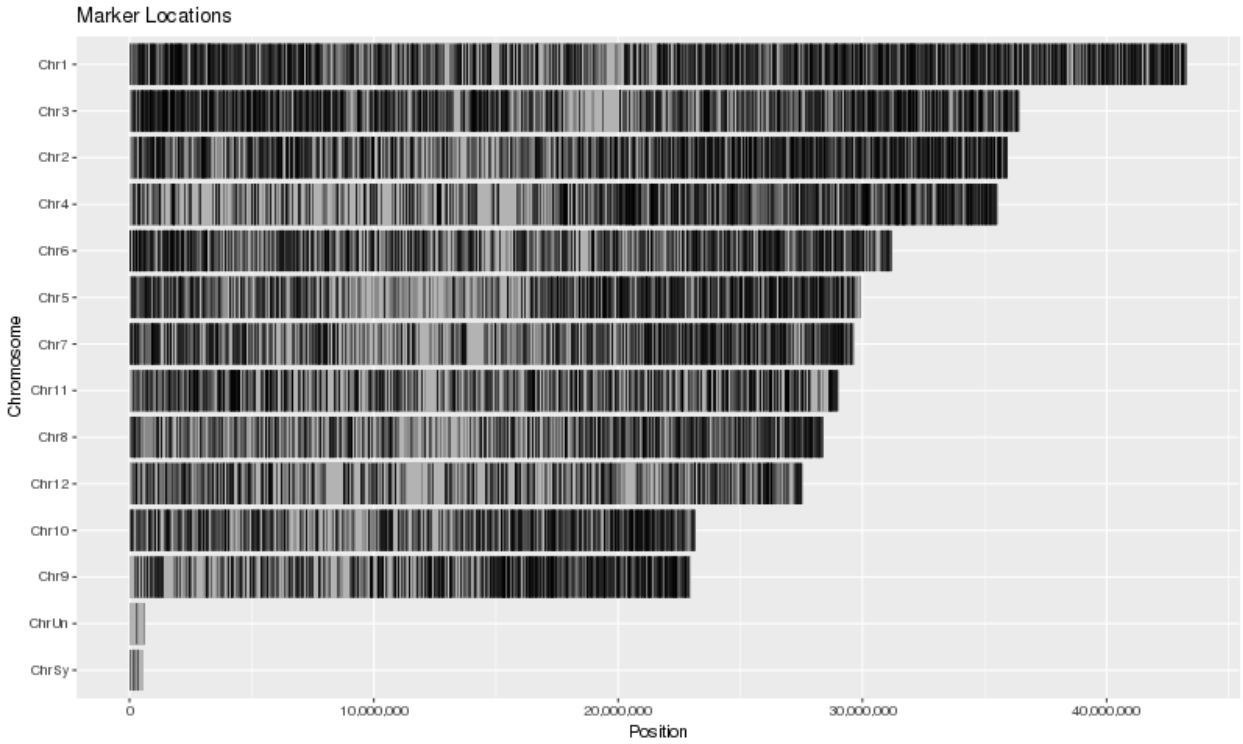

Figure 5.21. Chromosome length coverage (bp) on each chromosome from GBS analysis.

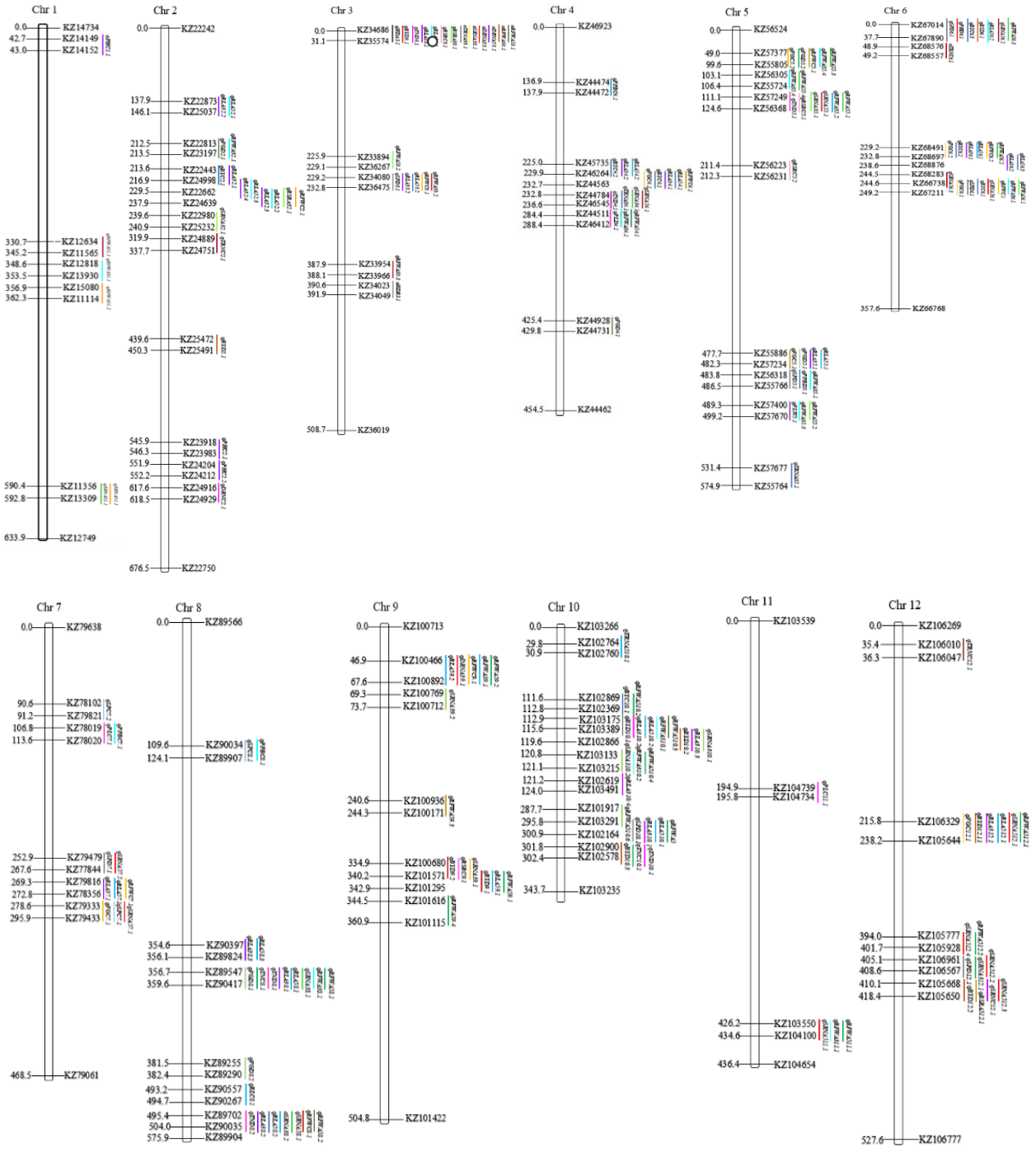

Figure 5.22. QTLs location of morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions on the 12 rice chromosomes. The representative markers and the detected QTLs are shown on the right, and genetic distance (cM) are shown on the left of the chromosome (chr).

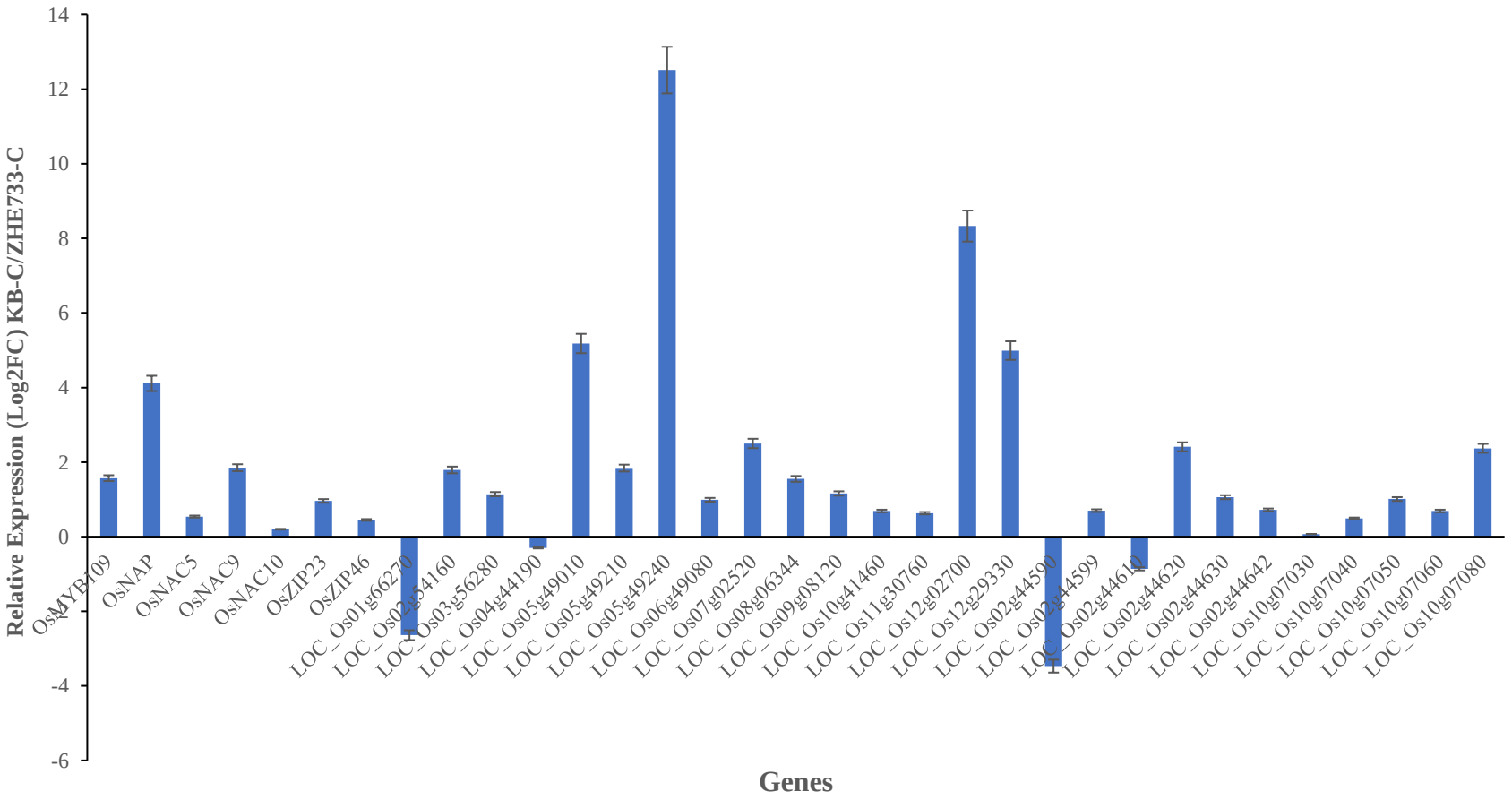

Figure 5.23. Expression profile of seven known drought resistance genes and 26 candidate drought resistance genes within QTL regions in leaf tissues of the parental K/Z RIL population, Kaybonnet under WW conditions (KB-C) relative to ZHE733 under WW conditions (ZHE733-C)

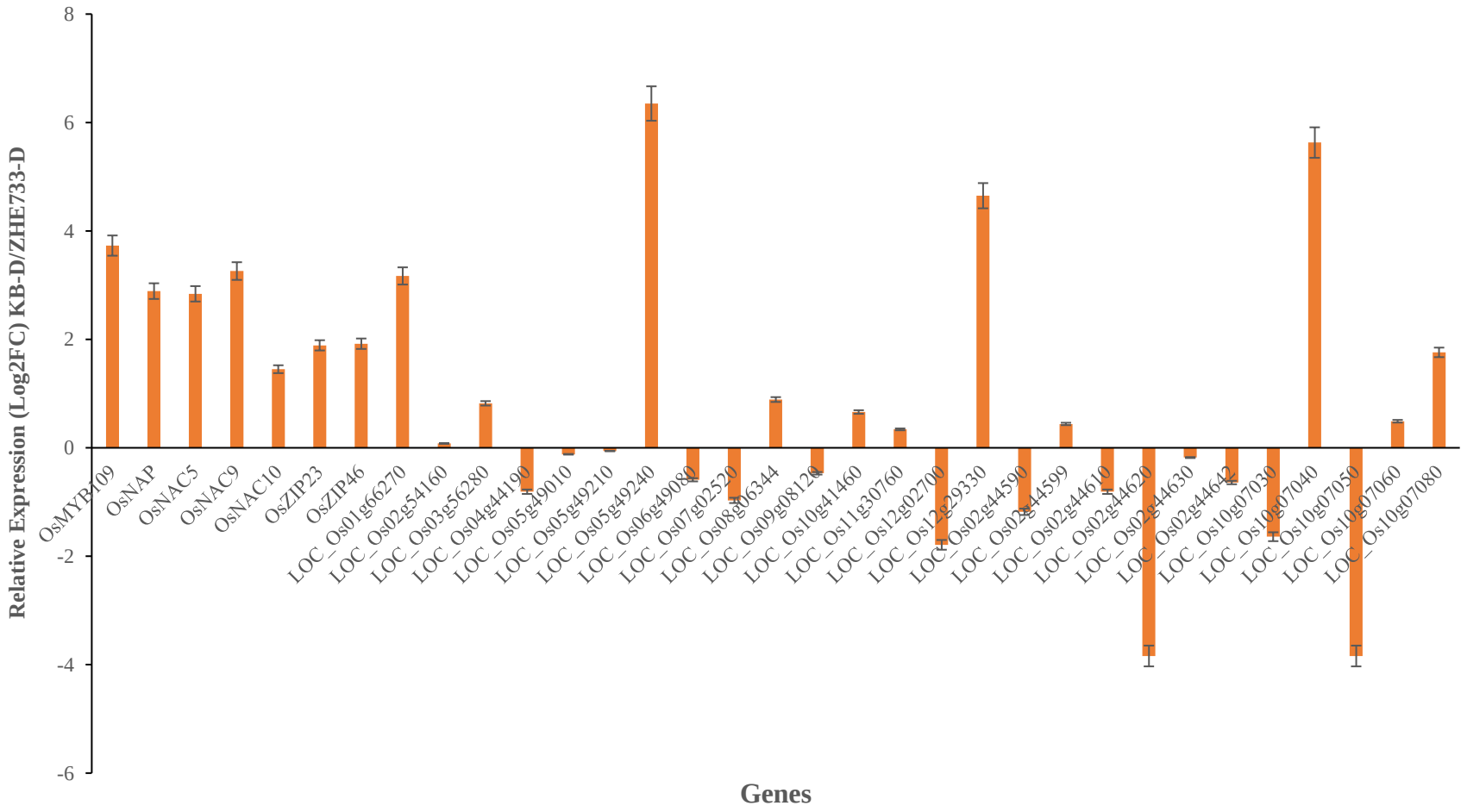

Figure 5.24. Expression profile of seven known drought resistance genes and 26 candidate drought resistance genes within QTL regions in leaf tissues of the parental K/Z RIL population, Kaybonnet under DS conditions (KB-D) relative to ZHE733 under DS conditions (ZHE733-D)

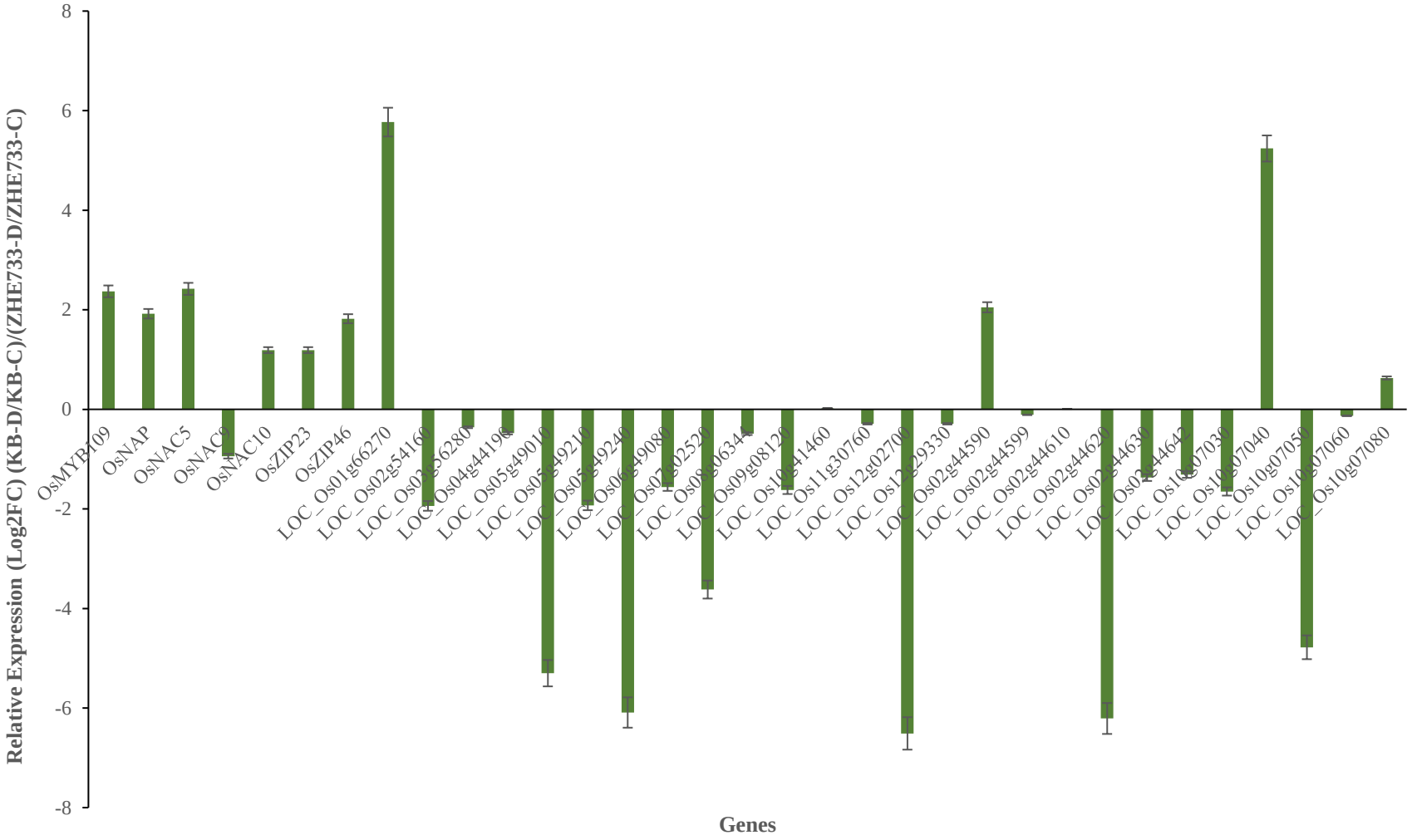

Figure 5.25. Expression profile of seven known drought resistance genes and 26 candidate drought resistance genes within QTL regions in leaf tissues of the parental K/Z RIL population, Kaybonnet relative to ZHE733 under DS conditions when compared to WW conditions.

Tables

Table 5.1. Effects of drought stress on filled grain per panicle number exhibiting highly drought resistance, moderately drought resistance, and drought sensitivity in K/Z RIL population of 198 lines.

Highly drought resistant lines (0-29% reduction)	Moderately drought resistant lines (30-49% reduction)	Drought sensitive lines (≥50% reduction)
Kaybonnet, 100002, 100005, 100006, 100007, 100009, 100014, 100016, 100018, 100021, 100023, 100025, 100036, 100053, 100097, 100121, 100133, 100135, 100139, 100162, 100163, 100198, 100233, 100310, 100321, 100330, 100337	100026, 100028, 100032, 100034, 100040, 100050, 100058, 100064, 100066, 100096, 100108, 100115, 100129, 100144, 100169, 100212, 100242, 100245, 100265, 100295, 100334, 100351	ZHE733, 100001, 100008, 100010, 100012, 100015, 100017, 100019, 100020, 100022, 100024, 100027, 100029, 100030, 100033, 100038, 100039, 100042, 100043, 100046, 100048, 100049, 100055, 100056, 100057, 100062, 100065, 100067, 100086, 100092, 100098, 100102, 100106, 100107, 100114, 100118, 100119, 100120, 100122, 100123, 100126, 100130, 100131, 100134, 100137, 100141, 100142, 100145, 100146, 100149, 100150, 100151, 100153, 100154, 100155, 100156, 100158, 100160, 100164, 100170, 100171, 100172, 100175, 100176, 100178, 100179, 100180, 100182, 100185, 100188, 100191, 100193, 100196, 100197, 100200, 100201, 100202, 100203, 100208, 100209, 100210, 100211, 100213, 100214, 100217, 100220, 100222, 100223, 100224, 100225, 100228, 100230, 100231, 100234, 100237, 100238, 100239, 100240, 100241, 100246, 100249, 100250, 100251, 100253, 100254, 100255, 100256, 100259, 100263, 100266, 100272, 100273, 100277, 100280, 100281, 100282, 100283, 100284, 100285, 100288, 100292, 100293, 100298, 100299, 100300, 100302, 100303, 100308, 100311, 100313, 100315, 100319, 100322, 100323, 100324, 100325, 100327, 100328, 100329, 100333, 100335, 100336, 100338, 100339, 100340, 100341, 100342, 100344, 100345, 100348, 100352

Table 5.2. The average and range values of morphological traits of K/Z RIL population.

Traits	Treatments	Kaybonnet	ZHE733	K/Z RIL Population	
				Average	Range
Plant height (cm)	WW	98	51	60.03	28 - 98
	DS	84	31	29.99	10 - 73
Tiller number	WW	5	8	8.11	3 - 22
	DS	4	7	6.98	2 - 19
Heading day (day)	-	91	71	87	65 - 109
Flag leaf width (cm)	DS	1.7	1.3	1.2	0.7 - 2
Leaf rolling score	DS	2	4	2.8	1 - 4

Table 5.3. The average and range values of grain yield components of K/Z RIL population under WW and DS conditions.

Traits	Treatments	Kaybonnet	ZHE733	K/Z RIL Population	
				Average	Range
Biological yield	WW	14.80	13.40	20	4 - 49
	DS	14.10	6.50	17.50	2 - 47
Spikelet per panicle number	WW	104	90	133.35	45 - 328
	DS	74	55	81.68	26.8 - 188.4
Filled grain per panicle number	WW	66	43.2	43.07	2 - 176
	DS	50	16	22.56	0 - 97
Panicle length (cm)	WW	21.7	18.6	21.24	11.90 - 33.30
	DS	19.3	15.6	17.88	11.64 - 26.62
Primary panicle branch number	WW	19	8	11.17	3 - 22
	DS	13	7	9.28	5.4 - 15.6

Table 5.4. The average and range values of root architectural traits of K/Z RIL population under ABA conditions.

Traits	Treatments	Kaybonnet	ZHE733	K/Z RIL Population	
				Average	Range
Root length (cm)	ABA 0 uM	6.570	4.611	4.428	0.500 - 8.933
	ABA 3 uM	2.500	3.800	3.600	0.094 - 8.651
	ABA 5 uM	2.050	3.678	3.491	0.100 - 8.367
Root to shoot ratio	ABA 0 uM	0.363	0.324	0.259	0.079 - 0.669
	ABA 3 uM	0.587	0.303	0.336	0.013 - 1.216
	ABA 5 uM	0.432	0.298	0.335	0.014 - 1.411
Total root number	ABA 0 uM	6.600	10.222	8.362	1.667 - 18.667
	ABA 3 uM	2.500	9.667	6.986	1.000 - 11.333
	ABA 5 uM	1.750	9.200	6.600	1.000 - 12.333
Shallow root number	ABA 0 uM	0.200	3.333	1.649	0.000 - 5.667
	ABA 3 uM	0.000	3.177	1.092	0.000 - 3.540
	ABA 5 uM	0.000	3.005	1.047	0.000 - 3.667
Deep root number	ABA 0 uM	7.400	6.889	7.043	1.667 - 14.667
	ABA 3 uM	2.500	6.490	6.391	0.000 - 11.000
	ABA 5 uM	1.750	6.195	6.155	0.000 - 9.333
Root fresh weight	ABA 0 uM	0.032	0.031	0.034	0.001 - 0.114
	ABA 3 uM	0.056	0.029	0.033	0.000 - 0.083
	ABA 5 uM	0.005	0.027	0.026	0.000 - 0.078

Table 5.5. Summary of the SNP markers distribution and genome coverage in the linkage map of the K/Z RIL population.

Chromo-some	Number of SNP markers	Chromosome length (cM)	Number of SNP markers/cM	Minimum interval (cM)	Maximum interval (cM)	Average interval (cM)	Number of gaps > 5 cM
1	562	633.97	0.89	0.26	49.56	1.13	17
2	499	676.52	0.74	0.26	52.91	1.36	19
3	428	508.73	0.84	0.26	31.13	1.19	12
4	385	454.47	0.85	0.26	20.91	1.18	10
5	287	574.97	0.50	0.26	54.46	2.00	12
6	387	357.61	1.08	0.26	37.71	0.92	7
7	320	468.49	0.68	0.26	55.84	1.46	17
8	258	575.94	0.45	0.27	47.52	2.23	23
9	283	504.78	0.56	0.27	36.95	1.78	18
10	246	343.72	0.72	0.26	22.27	1.39	10
11	296	436.37	0.68	0.26	57.14	1.47	12
12	182	527.55	0.34	0.27	54.65	2.89	20
Total	**4133.00**	**6063.12**	**8.32**	**3.15**	**521.05**	**19.00**	**177.00**
Average	**344.42**	**505.26**	**0.69**	**0.26**	**43.42**	**1.58**	**14.75**

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping.

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
PHC	*qPHC1.1*	1	10.57	4.85	KZ14152	43	38480787	9	Protein phosphorylation
SRN_ABA3	*qSRNA31.1*	1	12.73	0.55	KZ13309	592	30730895	10	Flavone 7-O-glucosyltransferase, UDP-dependent glucosyltransferase, UV-B tolerance (Os01t0736300-01)
SRN_ABA5	*qSRNA51.1*	1	4.01	0.36					
SRN_ABA5	*qSRNA51.2*	1	3.84	0.61	KZ11114	360	65294	13	Lipid_metabolism.exotics_(steroids,_squalene_etc).sphingolipids, Disease resistance response (Os01t0100900-01)
RFW_ABA3	*qRFWA31.1*	1	6.66	2.18	KZ13930	352	36249837	8	RNA.regulation_of_transcription.unclassified_Post-transcriptional regulation of abiotic stress response (Os01t0844300-01)
DLR	*qDLR1.1*	1	13.78	0.93	KZ11565	341	13168487	11	Phosphoesterase family protein. (Os01t0102000-01)
PHC	*qPHC2.1*	2	24.42	13.81	KZ23918	546	27023516	7	Transport.Major_Intrinsic_Proteins.PIP
PHC	*qPHC2.2*	2	17.19	8.63	KZ24204	552	29443430	11	Protein phosphorylation
BYC	*qBYC2.1*	2	4.71	2.46	KZ22443	214	10716904	9	Protein phosphorylation_intracellular component
RL_ABA3	*qRLA32.1*	2	24.97	0.61					
BYD	*qBYD2.1*	2	3.76	1.38	KZ25491	448	9672804	6	Subtilisin 13
RL_ABA3	*qRLA32.2*	2	24.99	0.61	KZ25037	145	4594675	6	Misc.cytochrome_P450_oxidation-reduction process
RL_ABA5	*qRLA52.1*	2	17.11	0.59					

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
RL_ABA3	*qRLA32.3*	2	28.85	0.61	KZ24639	236	33179587	5	Stress.abiotic.heat
RL_ABA5	*qRLA52.2*	2	20.72	0.59					
RSR_ABA3	*qRSRA32.1*	2	3.04	0.60					
RFW_C	*qRFWC2.1*	2	4.11	0.67					
RL_ABA3	*qRLA32.4*	2	21.93	0.61	KZ24998	221	4009517	8	Misc.cytochrome_P450_oxidation-reduction process
RL_ABA5	*qRLA52.3*	2	13.79	0.59					
TRN_C	*qTRNC2.1*	2	3.91	8.48	KZ24889	322	35261285	12	Protein.targeting.secretory pathway.golgi_ETHYLENE RESPONSE 3
SRN_ABA3	*qSRNA32.1*	2	9.46	0.54	KZ22980	240	19798387	10	Nucleotide binding
DRN_C	*qDRNC2.1*	2	2.59	6.32	KZ24916	618	35424339	13	RNA.regulation_of_transcription.putative transcription_regulator
FGD	*qFGD2.1*	2	6.44	1.24	KZ22813	213	17731845	11	Conserved hypothetical protein
RFW_ABA3	*qRFWA32.1*	2	6.44	1.24					
HDR	*qHDR3.1*	3	2.69	6.73	KZ34023	391	14343272	8	Heat stress transcription factor

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
HDA	*qHDA3.1*	3	14.22	1.50	KZ35574	19	32079708	6	Signaling.receptor_kinases. leucine_rich_repeat_XI
BYD	*qBYD3.1*	3	10.57	6.48					
TND	*qTND3.1*	3	7.12	2.85					
RL_ABA3	*qRLA33.1*	3	32.94	0.61					
RL_ABA5	*qRLA53.1*	3	24.79	0.59					
RSR_C	*qRSRC3.1*	3	3.77	8.62					
RSR_ABA3	*qRSRA33.1*	3	3.75	0.85					
TRN_ABA3	*qTRNA33.1*	3	3.42	3.21					
SRN_ABA3	*qSRNA33.1*	3	14.99	0.59					
DRN_ABA3	*qDRNA33.1*	3	5.04	1.77					
DRN_ABA5	*qDRNA53.1*	3	5.21	2.75					
RFW_ABA3	*qRFWA33.1*	3	15.50	2.33					
RFW_ABA5	*qRFWA53.1*	3	26.08	0.94					
SPD	*qSPD3.1*	3	3.38	1.44	KZ34080	230	14854849	9	Signaling.receptor_kinases. leucine_rich_repeat_XI
RL_ABA3	*qRLA33.2*	3	22.33	0.61					
RL_ABA5	*qRLA53.2*	3	14.22	0.59					
RFW_C	*qRFWC3.1*	3	4.86	0.63					
RFW_ABA3	*qRFWA33.2*	3	8.74	2.09					
RFW_ABA3	*qRFWA3.3*	3	3.45	0.40	KZ33954	388	13996593	11	Transferase activity, transferring glycosyl groups
RFW_ABA5	*qRFWA53.2*	3	13.74	0.85	KZ36267	228	6052052	15	MADS-box transcription factor 1, Protein LEAFY HULL STERILE 1, Protein SEPALLATA-like

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
FGC	*qFGC4.1*	4	3.04	0.93	KZ44563	232	1858209	10	UDP-glucuronosyl/UDP-glucosyltransferase domain containing protein
BYC	*qBYC4.1*	4	4.38	2.63					
RL_ABA3	*qRLA34.1*	4	26.85	0.61					
RL_ABA5	*RLA54.1*	4	18.72	0.59					
RFW_C	*qRFWC4.1*	4	4.41	0.69					
FGD	*qFGD4.1*	4	2.68	2.22	KZ44928	427	18142843	4	WALL-ASSOCIATED KINASE GENE 49
PLD	*qPLD4.1*	4	2.70	6.08	KZ46412	287	33077542	8	Serine-type endopeptidase activity
RFW_ABA3	*qRFWA34.1*	4	4.05	2.29					
RFW_ABA5	*qRFWA54.1*	4	13.73	0.89					
PPBC	*qPPBC4.1*	4	4.73	10.34	KZ44474	137	436771	7	PS.photorespiration.hydroxypyruvate_reductase
BYC	*qBYC4.2*	4	3.58	2.78	KZ45735	226	26174168	7	PS.lightreaction.photosystem_II.PSII_polypeptide_subunits
RL_ABA3	*qRLA34.2*	4	22.34	0.61					
RL_ABA5	*qRLA54.2*	4	14.21	0.59					
TND	*qTND4.1*	4	4.51	1.82	KZ44784	233	15908254	6	Hormone_metabolism.auxin.induced-regulated-responsive-activated
TRN_ABA3	*qTRNA34.1*	4	3.28	1.25					
SRN_ABA3	*qSRNA34.1*	4	16.65	0.55					
SRN_ABA5	*qSRNA54.1*	4	3.89	0.38					

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
PLD	*qPLD4.2*	4	2.70	6.08	KZ44511	286	1233282	7	ERF domain containing protein. (Os04t0649100-02);APETALA2 transcription factor, Seed shattering through abscission zone (AZ) development (Os04t0649100-03)
RFW_ABA3	*qRFWA34.2*	4	4.05	2.29					
RFW_ABA5	*qRFWA54.2*	4	13.72	0.89					
FGC	*qFGC5.1*	5	5.74	1.12	KZ57234	481	28101481	12	Drought-responsive ethylene response factor 10, drought-responsive ERF 10, ethylene response factor 84, APETALA2/ethylene-responsive element binding protein 97
FGD	*qFGD5.1*	5	2.53	4.74					
RL_ABA3	*qRLA35.1*	5	25.08	0.61					
RL_ABA5	*qRLA55.1*	5	16.95	0.59					
SPD	*qSPD5.1*	5	4.81	1.34	KZ55766	485	12898523	13	Chlorophyll a/b binding protein domain (IPR023329)
PPBD	*qPPBD5.1*	5	2.75	6.28					
RFW_ABA3	*qRFWA35.1*	5	7.30	1.18					
TND	*qTND5.1*	5	4.34	2.38	KZ57249	112	28239630	10	Similar to WRKY transcription factor 43_Similar to SANT/MYB protein. (Os05t0567600-00)
RSR_C	*qRSRC5.1*	5	4.14	7.09					
SRN_ABA3	*qSRNA35.1*	5	12.19	0.58					
SRN_ABA5	*qSRNA55.1*	5	12.92	0.71					
RFW_ABA3	*qRFWA35.2*	5	8.33	2.28					
RFW_ABA5	*qRFWA55.1*	5	18.98	0.89					

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
FLW	*qFLW5.1*	5	2.58	1.42	KZ57400	492	29630193	6	Uridine-diphospho-(UDP)-glucose 4-epimerase, Cell wall carbohydrate partitioning during nitrogen (N) limitation (Os05t0595100-01)
RFW_ABA3	*qRFWA35.3*	5	4.59	2.24					
RFW_ABA5	*qRFWA55.2*	5	11.29	0.90					
RSR_C	*qRSRC5.2*	5	2.56	1.17	KZ56231	212	19929746	5	Transcriptional regulation of miR528, Antiviral response, Defense to rice stripe virus (RSV) (Os05t0408200-01)
TRN_ABA3	*qTRNA35.1*	5	2.62	1.44	KZ57677	550	8159670	7	None
FGC	*qFGC5.2*	5	3.43	2.76	KZ55805	93	14042825	8	None
FGD	*qFGD5.2*	5	11.14	1.41					
RFW_C	*qRFWC5.1*	5	3.99	0.79					
RFW_ABA3	*qRFWA35.4*	5	4.85	2.62					
RFW_ABA5	*qRFWA5.3*	5	13.31	0.95					
RFW_ABA3	*qRFWA35.4*	5	2.82	1.22	KZ55724	105	10471559	8	None
RFW_ABA5	*qRFWA55.4*	5	9.86	0.86					
SPD	*qSPD5.2*	5	4.81	1.34	KZ56318	484	2737642	10	Serine-type endopeptidase activity
PPBD	*qPPBD5.2*	5	2.75	6.28					
RFW_ABA3	*qRFWA35.5*	5	7.30	1.18					
FGC	*qFGC6.1*	6	3.09	0.83	KZ67211	248	15499358	6	Zinc finger, PHD-type domain containing protein. (Os06t0468400-01)
SPD	*qSPD6.1*	6	3.38	1.65					
BYC	*qBYC6.1*	6	4.35	3.77					
SRN_ABA5	*qSRNA56.1*	6	7.53	0.52					
RFW_C	*qRFWC6.1*	6	4.76	0.65					
RFW_ABA3	*qRFWA36.1*	6	10.60	2.14					
RFW_ABA5	*qRFWA56.1*	6	17.85	0.86					

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
FGC	*qFGC6.2*	6	3.71	0.87	KZ68491	230	29755505	9	Control of rice architecture via BR signaling (Os06t0704300-01)_Tetrapyrrole biosynthetic process
BYC	*qBYC6.2*	6	3.01	2.66					
RL_ABA3	*qRLA36.1*	6	26.24	0.61					
RL_ABA5	*qRLA56.1*	6	18.12	0.59					
RFW_C	*qRFWC6.2*	6	7.32	0.68					
RFW_ABA5	*qRFWA56.2*	6	14.09	0.90					
SPD	*qSPD6.2*	6	4.09	2.27	KZ67890	34	23243264	9	Oxygen transport (GO:0015671)
PHD	*qPHD6.1*	6	5.22	1.78					
BYC	*qBYC6.3*	6	4.79	5.86					
BYD	*qBYD6.1*	6	7.73	6.72					
RL_ABA5	*qRLA56.2*	6	16.19	0.59					
SRN_ABA5	*qSRNA56.2*	6	16.99	0.79					
RFW_ABA5	*qRFWA56.3*	6	19.39	0.94					
RL_ABA3	*qRLA36.2*	6	22.40	0.61	KZ68876	235	8158597	8	Glycolysis.plastid_branch.glucose-6-phosphate_isomerase_Similar to Calmodulin-binding heat-shock protein. (Os06t0256300-01)
RL_ABA5	*qRLA56.3*	6	14.28	0.59					
TRN_C	*qTRNC6.1*	6	3.05	5.49	KZ68576	49	30975227	8	Similar to Protein kinase APK1A, chloroplast precursor (EC 2.7.1.-). (Os06t0727400-01)
SRN_ABA5	*qSRNA56.3*	6	5.84	0.57	KZ68283	241	27639890	7	MADS-box transcription factor, Vegetative development (Os06t0667200-01)

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
FGC	*qFGC7.1*	7	3.06	1.27	KZ79333	283	28219138	10	Lipid_metabolism.lipid_degradation.lipases
SPC	*qSPC7.1*	7	2.58	9.88					
SRN_ABA5	*qSRNA57.1*	7	4.92	0.71					
SPC	*qSPC7.2*	7	4.62	6.37	KZ79821	91	9822937	7	Protein of unknown function DUF594 domain containing protein. (Os07t0269400-00)
SPD	*qSPD7.1*	7	4.85	2.12	KZ77844	265	912849	9	Stress biotic_Endoplasmic reticulum protein, Regulation of sugar partitioning in carbon-demanding young leaves and developing leaf sheaths (Os07t0116300-01)
SRN_ABA5	*qSRNA57.2*	7	11.32	0.75					
PLC	*qPLC7.1*	7	5.05	9.62	KZ78019	107	11608339	13	Regulation of nutrient metabolism and endosperm development (Os07t0296900-01)
PPBC	*qPPBC7.1*	7	2.91	6.27					
RL_ABA3	*qRLA37.1*	7	21.40	0.61	KZ78356	272	19009172	6	UDP-glucuronosyl/UDP-glucosyltransferase family protein. (Os07t0502900-00)
RL_ABA5	*qRLA57.1*	7	13.28	0.59					
RFW_C	*qRFWC7.1*	7	3.58	0.69					
RL_ABA3	*qRLA37.2*	7	21.40	0.61	KZ79816	272	9592225	4	OsFbox360, Os_F0235
RL_ABA5	*qRLA57.2*	7	13.28	0.59					
RFW_C	*qRFWC7.2*	7	3.58	0.69					

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
SPC	*qSPC8.1*	8	3.40	5.75	KZ90034	115	26147631	11	Embryo and endosperm development (Os08t0525500-01)_carbohydrate metabolic process_UDP-glucuronic acid 4-epimerase 2
PPBC	*qPPBC8.1*	8	2.66	7.25					
TND	*qTND8.2*	8	3.76	2.74	KZ89702	497	21549809	8	Aromatic amino acid family biosynthetic process (GO:0009073)
RL_ABA3	*qRLA38.2*	8	29.31	0.61					
RL_ABA5	*qRLA58.2*	8	20.68	0.59					
SRN_ABA3	*qSRNA38.2*	8	10.96	0.55					
SRN_ABA5	*qSRNA58.1*	8	8.04	0.65					
RFW_C	*qRFWC8.1*	8	3.53	0.72					
RFW_ABA5	*qRFWA58.2*	8	17.11	0.88					
RL_C	*qRLC8.1*	8	2.51	3.85	KZ90267	494	4011572	10	Misc.UDP_glucosyl_and_glucoronyl_transferases
RL_ABA3	*qRLA38.3*	8	20.77	0.61	KZ90397	355	6664289	9	None
RL_ABA5	*qRLA58.3*	8	12.65	0.59					
FGD	*qFGD8.1*	8	2.78	3.79	KZ89547	357	3507836	6	RNA.processing.RNA_helicase_DEAD-box RNA helicase, Pre-mRNA splicing under cold stress (Os08t0159900-01)
TNC	*qTNC8.1*	8	4.75	5.74					
TND	*qTND8.1*	8	4.72	1.77					
RL_ABA3	*qRLA38.1*	8	24.48	0.61					
RL_ABA5	*qRLA58.1*	8	16.35	0.59					
SRN_ABA3	*qSRNA38.1*	8	14.53	0.55					
RFW_ABA3	*qRFWA38.1*	8	10.88	1.18					
RFW_ABA5	*qRFWA58.1*	8	13.59	0.85					

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
FGD	*qFGD8.2*	8	2.59	1.93	KZ89290	382	17435478	8	Defense response
RL_ABA5	*qRLA59.2*	9	18.60	0.60	KZ100892	66	18009087	8	Adaptation to high temperature (Os09t0471900-01)
DRN_ABA3	*qDRNA39.1*	9	2.61	1.37					
RFW_C	*qRFWC9.1*	9	4.67	0.77					
RFW_ABA3	*qRFWA39.1*	9	10.52	2.26					
RFW_ABA5	*qRFWA59.2*	9	18.88	0.93					
SRN_ABA3	*qSRNA39.2*	9	6.32	0.54	KZ100769	71	17110249	5	Heat shock factor (HSF)-type, DNA-binding domain containing protein. (Os09t0455200-01)
RFW_ABA5	*qRFWA59.3*	9	13.26	0.84	KZ100936	241	18722840	5	Transmembrane transport (GO:0055085)
BYD	*qBYD9.1*	9	3.37	2.24	KZ101571	341	4220164	7	Carbohydrate metabolic process (GO:0005975)
RL_ABA5	*qRLA59.1*	9	13.57	0.59					
RFW_ABA5	*qRFWA59.1*	9	13.79	0.91					
RSR_C	*qRSRC9.1*	9	4.16	2.16					
SRN_ABA3	*qSRNA39.1*	9	14.82	0.66					
RFW_ABA5	*qRFWA59.4*	9	9.43	0.92	KZ101616	348	4991542	6	Intracellular protein transport (GO:0006886)

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
SPD	*qSPD10.1*	10	3.03	0.98	KZ102164	300	12048509	6	Intracellular protein transport (GO:0006886)
RL_ABA3	*qRLA310.1*	10	20.11	0.61					
RL_ABA5	*qRLA510.1*	10	11.11	0.59					
RFW_ABA5	*qRFWA510.1*	10	10.89	0.89					
BYC	*qBYC10.1*	10	7.25	5.09	KZ102869	112	3205210	10	RNA.transcription
RFW_ABA5	*qRFWA510.2*	10	12.82	0.89					
BYD	*qBYD10.1*	10	3.16	2.71	KZ103389	114	5746729	6	Similar to F-box domain containing protein. (Os10t0183800-00)
RL_ABA3	*qRLA310.2*	10	22.17	0.61					
RL_ABA5	*qRLA510.2*	10	14.40	0.58					
RFW_ABA3	*qRFWA310.1*	10	4.53	0.94					
RFW_ABA5	*qRFWA510.3*	10	12.53	0.88					
BYD	*qBYD10.2*	10	6.71	4.31	KZ102866	118	3156402	5	None
RL_ABA3	*qRLA310.3*	10	23.86	0.61					
SRN_ABA3	*qSRNA310.1*	10	9.44	0.55					
BYD	*qBYD10.3*	10	21.82	8.49	KZ102900	302	3719254	10	Similar to Chalcone and stilbene synthases, N-terminal domain containing protein. (Os10t0158400-00)
TNC	*qTNC10.1*	10	8.07	9.56					
TND	*qTND10.1*	10	6.36	2.62					
RL_ABA3	*qRLA310.4*	10	23.34	0.61	KZ102619	122	18141893	6	Similar to Zinc finger, C3HC4 type family protein, expressed. (Os10t0481400-01)

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
TRN_ABA5	*qTRNA510.1*	10	2.63	6.03	KZ102764	30	19970851	4	Regulation of disease resistance response and programmed cell death (PCD) (Os10t0516800-01)
SRN_ABA3	*qSRNA310.2*	10	13.00	0.55	KZ103215	121	22263987	11	Protein.postranslational_modification_MYB-like (IPR017877)
RFW_ABA3	*qRFWA310.2*	10	16.01	2.28					
RFW_ABA5	*qRFWA510.4*	10	9.34	0.49					
BYD	*qBYD10.4*	10	3.16	2.71	KZ103175	113	21860264	6	Sexual reproduction_cell_wall.modification
RL_ABA3	*qRLA310.5*	10	22.17	0.61					
RL_ABA5	*qRLA510.3*	10	14.40	0.58					
RFW_ABA3	*qRFWA310.3*	10	4.53	0.94					
RFW_ABA5	*qRFWA510.5*	10	12.53	0.88					
RFW_ABA5	*qRFWA510.6*	10	2.63	0.19	KZ103291	294	4450748	9	Oxidation-reduction process (GO:0055114)
PLC	*qPLC11.1*	11	2.77	5.09	KZ104739	195	24272046	6	MATH-BTB protein 52, MDC protein with a BTB domain 52, MDC protein having BTB domain 52

Table 5.6. QTLs identified for morphological and yield traits under DS and WW conditions and root architectural traits under ABA conditions with IciMapping (Continued).

Trait name	QTLs	Chromo-some	LOD	PVE (%)	Peak Marker	Position (cM)	Candidate gene position (bp)	Number of genes within 25 kb of the marker closest to the QTL peak	Gene annotation
SRN_ABA5	*qSRNA511.1*	11	6.68	0.72	KZ104100	433	17929674	8	Protein glycosylation (GO:0006486)
RFW_ABA3	*qRFWA311.1*	11	7.31	2.12					
RFW_ABA5	*qRFWA511.1*	11	13.01	0.87					
FGC	*qFGC12.1*	12	6.71	2.29	KZ106329	218	3871218	10	Cell redox homeostasis (GO:0045454)
BYD	*qBYD12.1*	12	8.33	6.57					
RL_ABA3	*qRLA312.1*	12	26.59	0.61					
RL_ABA5	*qRLA512.1*	12	18.34	0.59					
SRN_ABA5	*qSRNA512.1*	12	13.66	0.78					
RFW_ABA5	*qRFWA512.1*	12	21.40	0.91					
SPD	*qSPD12.1*	12	3.38	1.74	KZ106567	408	24389885	4	None
SRN_ABA3	*qSRNA312.1*	12	15.26	0.59					
SRN_ABA5	*qSRNA512.2*	12	7.11	0.49					
BYD	*qBYD12.2*	12	3.19	6.34	KZ105668	412	953389	10	Late embryogenesis abundant protein, LEA-14 (IPR004864)
RSR_ABA5	*qRSRA512.1*	12	2.58	0.55					
SRN_C	*qSRNC12.1*	12	3.39	1.08					
SRN_ABA5	*qSRNA512.3*	12	6.37	0.65					
TRN_C	*qTRNC12.1*	12	3.81	7.00	KZ106047	36	17401152	4	No apical meristem (NAM) protein domain containing protein. (Os12t0477400-01)_NAC DOMAIN-CONTAINING PROTEIN 139
SRN_ABA5	*qSRNA512.4*	12	4.57	0.59	KZ105928	398	15104353	8	Autophagy-related protein 1010 (IPR012445)
RFW_ABA5	*qRFWA512.2*	12	9.65	0.94					

Table 5.7. Known drought resistance genes and candidate drought resistance genes within QTL regions for generating gene expression with RT-qPCR.

No.	Genes	Forward	Reverse
Internal control gene			
1	Ubiquitin	5'-CGCAAGTACAACCAGGACAA-3'	5'-GCTGTGACCACACTTCTTCTT-3'
Known drought resistance genes			
1	OsMYB109	5'-ACGTCATATGATGTCGTCCGAAACCGCCG-3'	5'-ACGTGAATTCTTAAGCTGGTGGAACAGACCG-3’
2	OsNAP	5'-CACCAAGACCAACTGGATCA-3'	5'-GTGGCTGCTCTTCTTGTAGAT-3'
3	OsNAC5	5'-CGTCAAGACCAACTGGATCA-3'	5'-CGATCACTCCCTTCTTGTTGTA-3'
4	OsNAC9	5'-GGGTCAAGACTGATTGGATCAT-3'	5'-CATCTTCTCCCACTCGTTCTTC-3'
5	OsNAC10	5'-CCAAGAACCCGAGGAATCAA-3'	5'-ATGGGCTGGAAATCTGACTG-3'
6	OsZIP23	5'-GAAGGTTGTCGAGAGAAGACAG-3'	5'-TGCTACCTCAGCTTCCAATTC-3'
7	OsZIP46	5'-GGATGATCAAGAACAGGGAGTC-3'	5'-AGCCTTCTGTTCCTTCAGTTT-3'
Candidate drought resistance genes within QTL regions with known annotations to be responsive to drought stress			
1	LOC_Os01g66270	5'-GGATGTACGGCGCTCAC-3'	5'-ACATCAGGATCTCCATGTCATC-3'
2	LOC_Os02g54160	5'-GCTCCTATTGAGGACCCTATCA-3'	5'-GGTATCATTCTCCCAGCCAAAG-3'
3	LOC_Os03g56280	5'-TGCTGGCGTTGTGGAAT-3'	5'-GCACGACCTAACCTCACTTT-3'
4	LOC_Os04g44190	5'-GAGGAGGACTTGGTGATTGATG-3'	5'-GCCGAGGTCAATGTACAAGAA-3'
5	LOC_Os05g49010	5'-CCGCGACGAACTGTGAT-3'	5'-GATCACTCCTGCGTCACTTT-3'
6	LOC_Os05g49210	5'-CTTCAAGACGGCGGAGAAG-3'	5'-CCGACGAGCAATCCCATATT-3'
7	LOC_Os05g49240	5'-GCCACTACGAAGACCTCAC-3'	5'-TCCAGTATCCCTGCGTCT-3'
8	LOC_Os06g49080	5'-GTCAACCATTTGGGAGCTTTC-3'	5'-GGAGGAGTTACCATCCCATTAAG-3'
9	LOC_Os07g02520	5'-CACAAGGTACTGGTGGTTCTC-3'	5'-CGTTGCTGCTGTAGATGTAGT-3'
10	LOC_Os08g06344	5'-CTGTTCCAGAGGACCTGAAAG-3'	5'-CGCTTCCACCATATCCTGTT-3'
11	LOC_Os09g08120	5'-GGGAGCGACGACGTTAAG-3'	5'-GTCGGCTCCAACTCCAAC-3'
12	LOC_Os10g41460	5'-CTACCGCGATAGGTGGGA-3'	5'-ATCTTGTGGCGGCACTG-3'
13	LOC_Os11g30760	5'-CCGTCACGTTCCGCTAC-3'	5'-TGGACGATCGATGATGGTTG-3'
14	LOC_Os12g02700	5'-CACCCTTCAGAGCCCATAA-3'	5'-GATGGCAAGGACGAGGAG-3'
15	LOC_Os12g29330	5'-GCTTGTGGTGCACTACCTC-3'	5'-TGAAGAAGTACCTCTCCTGCTC-3'
Candidate drought resistance genes from the traits with high LOD and PVE with unknown annotations			
1	LOC_Os02g44590	5'-GACGAGCACCTAATGAAGGAG-3'	5'-GCCGGCATCGTGTAGATAG-3'
2	LOC_Os02g44599	5'-CAACCTCGGCGATCTTCTG-3'	5'-GGACCTTCGCGTACATGG-3'
3	LOC_Os02g44610	5'-CATGAGAGAGACACTGCCATAG-3'	5'-AAAGAGAAGTCGGCGATCTG-3'
4	LOC_Os02g44620	5'-TGATCTGTTCTTCGGTGGAAAG-3'	5'-CCTAAGCAGGGCATGATTGT-3'
5	LOC_Os02g44630	5'-GGTCTTGGTGCTGAGATTGT-3'	5'-GTCCCTGGCATTCCTCTTG-3'
6	LOC_Os02g44642	5'-GACCTGAAACTACCGAACCTG-3'	5'-GTCCCTGAAGTCGCAAATCT-3'
7	LOC_Os10g07030	5'-ACAAGCACGCAAGCAGT-3'	5'-CCTCGTCAAGTCCTCCCT-3'
8	LOC_Os10g07040	5'-CAACGATCCTCGCCATCG-3'	5'-CGGTAAGGTGCTCGCTTT-3'
9	LOC_Os10g07050	5'-CAGCTGCCCATGCATCC-3'	5'-CGTCCTCTCTGTCGCATTT-3'
10	LOC_Os10g07060	5'-GGTCCCTTAGTCCATCAGATCA-3'	5'-TACTGCCTGTTCCGTTCCT-3'
11	LOC_Os10g07080	5'-CCCGCACCTAGCATCTTG-3'	5'-GCGGGATGTCGACGTATG-3'

CONCLUSIONS

Over-all conclusions

Molecular genetic analysis of drought resistance and productivity traits of rice genotypes were conducted. Grain yield of the Arkansas cultivar Kaybonnet was higher under drought stress compared to known drought resistant cultivars (Vandana and Nagina-22). The K/Z RIL population with 13.13% highly drought resistant, 11.11% moderately drought and 75.75% sensitive lines based on the filled grain per panicle number is used for mapping multiple drought response parameters. Drought resistance mechanism in K/Z RIL population shows ABA-dependent pathway, with high correlation between root architecture and drought response. The SSR markers with potential linkage to drought resistance are RM9 (Chr 1), RM34 (Chr 1), RM109 (Chr 2), RM236 (Chr 2), RM154 (Chr 2), RM114 (Chr 3), RM135 (Chr 3), RM131 (Chr 4), RM133 (Chr 6), RM137 (Chr 8), RM152 (Chr 8), RM139 (Chr 11), and RM155 (Chr 12). There are 213 QTLs and 628 candidate genes related to drought resistant traits. The RT-qPCR results revealed that high number of genes were up-regulated in Kaybonnet as the drought-resistant parent, including seven known drought resistance genes, 15 candidate drought resistance genes within QTL regions with known annotations showed higher instrinsic values in Kaybonnet, and two candidate genes with unknown annotations. Results of this research will serve an important step to improve adapted Arkansas rice cultivars for higher grain production under DS conditions.

APPENDICES

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location.

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
1	RM9	ggtgccattgtcgtcctc	acggccctcatcaccttc	Polymorphic	190, 140, 190, 140	2	0.5	1
2	RM109	gccgccggagagggagagagag	ccccgacgggatctccatcgtc	Polymorphic	400, 390, 400, 390	2	0.5	2
3	RM114	cagggacgaatcgtcgccggag	ttggccccttgaggttgtcgg	Polymorphic	150, 160, 150, 160	2	0.5	3
4	RM131	tcctccctcccttcgcccactg	cgatgttcgccatggctgctcc	Polymorphic	220, 350, 220, 350	2	0.5	4
5	RM139	gagagggaggaagggaggcggc	ctgccatggcagagaaggggcc	Polymorphic	130, 150, 130, 150	2	0.5	11
6	RM236	gcgctggtggaaaatgag	ggcatccctctttgattcctc	Polymorphic	140, 150, 140, 150	2	0.5	2
7	RM2	acgtgtcaccgcttcctc	atgtccgggatctcatcg	Polymorphic	180, 170, 170, 170	2	0.38	7
8	RM3	acactgtagcggccactg	cctccactgctccacatctt	Monomorphic	1000	1	0.00	6
9	RM4	ttgacgaggtcagcactgac	agggtgtatccgactgatcg	Polymorphic	110, 120, 120, 120	2	0.38	12
10	RM5	tgcaacttctagctgctcga	gcatccgatcttgatggg	Polymorphic	110, 120, 110, 110	2	0.38	1
11	RM8	cacgtggcgtaaatacacgt	ggccaaaccctaaccctg	Polymorphic	225, 300, 225, 225	2	0.38	2
12	RM10	ttgtcaagaggaggcatcg	cagaatgggaaatgggtcc	Polymorphic	150, 140, 140, 140	2	0.38	7
13	RM16	cgctagggcagcatctaaa	aacacagcaggtacgcgc	Polymorphic	160, 210, 210, 210	2	0.38	3
14	RM18	ttccctctcatgagctccat	gagtgcctggcgctgtac	Polymorphic	160, 150, 150, 150	2	0.38	3

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
15	RM19	caaaaacagagcagatgac	ctcaagatggacgccaaga	Polymorphic	2000, 2100, 2100, 2100	2	0.38	12
16	RM22	ggtttgggagcccataatct	ctgggcttctttcactcgtc	Monomorphic	160	1	0.00	3
17	RM23	cattggagtggaggctgg	gtcaggcttctgccattctc	Monomorphic	2000	1	0.00	1
18	RM24	gaagtgtgatcactgtaacc	tacagtggacggcgaagtcg	Monomorphic	2000	1	0.00	1
19	RM25	ggaaagaatgatcttttcatgg	ctaccatcaaaaccaatgttc	Polymorphic	140, 150, 150, 150	2	0.38	8
20	RM26	gagtcgacgagcggcaga	ctgcgagcgacggtaaca	Polymorphic	300, 500, 500, 500	2	0.38	5
21	RM27	ttttccttctcacccacttca	tctttgacaagaggaaagaggc	Monomorphic	140	1	0.00	2
22	RM29	cagggacccacctgtcatac	aacgttggtcatatcggtgg	Polymorphic	140, 160, 160, 160	2	0.38	2
23	RM32	agtctacgtggtgtacacgtgg	tgcggcctgccgtttgtgag	Polymorphic	125, 150, 150, 150	2	0.38	8
24	RM34	gaaatggcaatgtgtgcg	gccggagaaccctagctc	Polymorphic	180, 170, 180, 170	2	0.50	1
25	RM36	caactatgcaccattgtcgc	gtactccacaagaccgtacc	Monomorphic	1650	1	0.00	3
26	RM38	acgagctctcgatcagccta	tcggtctccatgtcccac	Polymorphic	260, 250, 250, 260	2	0.50	8
27	RM39	gcctctctcgtctccttcct	aattcaaactgcggtggc	Polymorphic	125, 130, 125, 125	2	0.38	5
28	RM41	aagtctagtttgcctccc	aatttctacgtcgtcgggc	Polymorphic	400, 410, 410, 400	2	0.50	9

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
29	RM44	acgggcaatccgaacaacc	tcgggaaaacctaccctacc	Monomorphic	1650	1	0.00	8
30	RM47	actccactccactccccac	gtcagcaggtcggacgtc	Polymorphic	250, 210, 210, 210	2	0.38	7
31	RM48	tgtcccactgctttcaagc	cgagaatgagggacaaataacc	Polymorphic	190, 200, 190, 190	2	0.38	2
32	RM49	ttcggaagttggttactgatca	ttggagcggattcggagg	Monomorphic	1650	1	0.00	3
33	RM50	actgtaccggtcgaagacg	aaattccacgtcagcctcc	Polymorphic	200, 210, 210, 210	2	0.38	6
34	RM51	tctcgattcaatgtcctcgg	ctacgtcatcatcgtcttccc	Monomorphic	125	1	0.00	7
35	RM52	ctactcgcgcgtggagtt	tgtcttactggtgaagctgg	Polymorphic	120, 130, 130, 130	2	0.38	8
36	RM53	acgtctcgacgcatcaatgg	cacaagaacttcctcggtac	Polymorphic	200, 210, 200, 200	2	0.38	2
37	RM55	ccgtcgccgtagtagagaag	tcccggttattttaaggcg	Monomorphic	225	1	0.00	3
38	RM60	agtcccatgttccacttccg	atggctactgcctgtactac	Polymorphic	170, 180, 180, 180	2	0.38	3
39	RM70	gtggacttcatttcaactcg	gatgtataagatagtccc	Monomorphic	170	1	0.00	7
40	RM71	ctagaggcgaaaacgagatg	gggtgggcgaggtaataatg	Polymorphic	130, 120, 120, 120	2	0.38	2
41	RM72	ccggcgataaaacaatgag	gcatcggtcctaactaaggg	Polymorphic	200, 175, 200, 200	2	0.38	8
42	RM80	ttgaaggcgctgaaggag	catcaacctcgtcttcaccg	Monomorphic	1650	1	0.00	8
43	RM81A	gagtgcttgtgcaagatcca	cttcttcactcatgcagttc	Polymorphic	110, 100, 100, 100	2	0.38	1

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
44	RM81B	gagtgcttgtgcaagatcca	cttcttcactcatgcagttc	Polymorphic	110, 100, 100, 100	2	0.38	1
45	RM82	tgcttcttgtcaattcgcc	cgactcgtggaggtacgg	Polymorphic	110, 120, 120, 120	2	0.38	7
46	RM83	actcgatgacaagttgagg	cacctagacacgatcgag	Polymorphic	550, 560, 560, 560	2	0.38	12
47	RM84	taagggtccatccacaagatg	ttgcaaatgcagctagagtac	Polymorphic	110, 120, 120, 120	2	0.38	1
48	RM86	tacacctcatcgatcaatcg	ctttcgaatctgaagatc	Polymorphic	170, 190, 190, 190	2	0.38	1
49	RM87	cctctccgatacaccgtatg	gcgaaggtacgaaaggaaag	Polymorphic	180, 190, 190, 190	2	0.38	5
50	RM88	actcatcagcatggccttgctc	taatgctccaccttcaccac	Polymorphic	1200, 1300, 1300, 1300	2	0.38	8
51	RM101	gtgaatggtcaagtgacttaggtggc	acacaacatgttccctcccatgc	Polymorphic	290, 300, 300, 300	2	0.38	12
52	RM102	aactttcccaccaccaccgcgg	agcagcagcaagccagcaagcg	Polymorphic	600, 550, 550, 550	2	0.38	1
53	RM103	cttccaattcaggccggctggc	cgccacagctgaccatgcatgc	Monomorphic	100	1	0.00	6
54	RM104	ggaagaggagagaaagatgtgtgtcg	tcaacagacacaccgccaccgc	Monomorphic	100	1	0.00	1
55	RM105	gtcgtcgacccatcggagccac	tggtcgaggtggggatcgggtc	Polymorphic	5000, 4500, 5000, 5000	2	0.38	9
56	RM108	tctcttgcgcgcacactggcac	cgtgcaccaccaccaccacca c	Monomorphic	100	1	0.00	9
57	RM110	tcgaagccatccaccaacgaag	tccgtacgccgacgaggtcgag	Polymorphic	110, 130, 130, 110	2	0.50	2

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
58	RM111	cacaacctttgagcaccgggtc	acgcctgcagcttgatcaccgg	Monomorphic	100	1	0.00	6
59	RM112	gggaggagaggcaagcggagag	agccggtgcagtggacggtgac	Monomorphic	110	1	0.00	2
60	RM113	caccattgcccatcagcacaac	tcgccctctgctgcttgatggc	Monomorphic	130	1	0.00	1
61	RM115	ttgccgcagtggccgttaccac	aggaggcggcggaaatggaagg	Polymorphic	140, 150, 150, 150	2	0.38	6
62	RM117	cgatccattcctgctgctcgcg	cgcccccatgcatgagaagacg	Polymorphic	180, 190, 190, 190	2	0.38	12
63	RM118	ccaatcggagccaccggagagc	cacatcctccagcgacgccgag	Polymorphic	140, 130, 140, 140	2	0.38	7
64	RM119	catccccctgctgctgctgctg	cgccggatgtgtgggactagcg	Polymorphic	140, 130, 130, 130	2	0.38	4
65	RM120	cacacaagccctgtctcacgacc	cgctgcgtcatgagtatgta	Monomorphic	110	1	0.00	11
66	RM121	accgtcgccttccactttcccc	ttcggggttgccggtgatgttg	Polymorphic	210, 220, 210, 210	2	0.38	6
67	RM122	gagtcgatgtaatgtcatcagtgc	gaaggaggtatcgctttgttggac	Polymorphic	210, 200, 200, 210	2	0.50	5
68	RM124	atcgtctgcgttgcggctgctg	catggatcaccgagctcccccc	Monomorphic	200	1	0.00	4
69	RM125	atcagcagccatggcagcgacc	aggggatcatgtgccgaaggcc	Polymorphic	100, 110, 100, 100	2	0.38	7
70	RM126	cgcgtccgcgataaacacaggg	tcgcacaggtgaggccatgtcg	Polymorphic	150, 160, 160, 160	2	0.38	8
71	RM127	gtgggatagctgcgtcgcgtcg	aggccagggtgttggcatgctg	Polymorphic	200, 180, 180, 200	2	0.50	4
72	RM128	agcttgggtgatttcttggaagcg	acgacgaggagtcgccgtgcag	Monomorphic	1000	1	0.00	1

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
73	RM129	tctctccggagccaaggcgagg	cgagccacgacgcgatgtaccc	Polymorphic	1000, 1250, 1250, 1000	2	0.50	1
74	RM130	tgttgcttgccctcacgcgaag	ggtcgcgtgcttggtttggttc	Polymorphic	400, 380, 380, 400	2	0.50	3
75	RM132	atcttgttgtttcggcggcggc	catggcgagaatgcccacgtcc	Polymorphic	220, 210, 220, 220	2	0.38	3
76	RM133	ttggattgttttgctggctcgc	ggaacacggggtcggaagcgac	Polymorphic	250, 275, 250, 265	3	0.625	6
77	RM134	acaaggccgcgagaggattccg	gctctccggtggctccgattgg	Monomorphic	100	1	0.00	7
78	RM135	ctctgtctcctcccccgcgtcg	tcagcttctggccggcctcctc	Polymorphic	500, 490, 500, 480	3	0.625	3
79	RM136	gagagctcagctgctgcctctagc	gaggagcgccacggtgtacgcc	Monomorphic	850	1	0.00	6
80	RM137	gacatcgccaccagcccaccac	cgggtggtccccgaggatcttg	Polymorphic	700, 710, 710, 690	3	0.625	8
81	RM138	agcgcaacaaccaatccatccg	aagaagctgcctttgacgctatgg	Polymorphic	210, 200, 210, 210	2	0.38	2
82	RM140	tgcctcttccctggctcccctg	ggcatgccgaatgaaatgcatg	Monomorphic	230	1	0.00	1
83	RM141	caccaccaccaccacgcctctc	tcttggagaggaggaggcgcgg	Monomorphic	200	1	0.00	6
84	RM142	ctcgctatcgccatcgccatcg	tcgagccatcgctggatggagg	Polymorphic	180, 190, 190, 180	2	0.50	4
85	RM143	gtcccgaaccctagcccgaggg	agaggccctccacatggcgacc	Polymorphic	150, 160, 150, 150	2	0.38	3
86	RM144	tgccctggcgcaaatttgatcc	gctagaggagatcagatggtagtgcatg	Polymorphic	240, 200, 200, 240	2	0.50	11

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
87	RM145	ccggtaggcgccctgcagtttc	caaggaccccatcctcggcgtc	Polymorphic	180, 170, 180, 180	2	0.38	2
88	RM146	ctattattccctaacccccatacccctcc	agagccactgcctgcaaggccc	Monomorphic	280	1	0.00	5
89	RM147	tacggcttcggcggctgattcc	cccccgaatcccatcgaaaccc	Polymorphic	100, 110, 110, 110	2	0.38	10
90	RM148	atacaacattagggatgaggctgg	tccttaaaggtggtgcaatgcgag	Monomorphic	125	1	0.00	3
91	RM149	gctgaccaacgaacctaggccg	gttggaagcctttcctcgtaacacg	Polymorphic	190, 200, 200, 190	2	0.50	8
92	RM150A	cacgacgacgacgagcagcagc	gctcgagggagagcgacctgcc	Polymorphic	150, 160, 150, 150	2	0.38	1
93	RM151	ggctgctcatcagctgcatgcg	tcggcagtggtagagtttgatctgc	Monomorphic	400	1	0.00	1
94	RM152	gaaaccaccacacctcaccg	ccgtagaccttcttgaagtag	Polymorphic	2000, 2100, 2000, 2100	2	0.50	8
95	RM153	gcctcgagcatcatcatcag	atcaacctgcacttgcctgg	Polymorphic	150, 140, 140, 140	2	0.38	5
96	RM154	accctctccgcctcgcctcctc	ctcctcctcctgcgaccgctcc	Polymorphic	150, 140, 160, 140	3	0.625	2
97	RM155	gagatggccccctccgtgatgg	ctgccctcaatcggccacacct	Polymorphic	100, 120, 110, 120	3	0.625	12
98	RM156	gccgcaccctcactccctcctc	tcttgccggagcgcttgaggtg	Polymorphic	100, 110, 100, 100	2	0.38	3
99	RM157A	cctcctcctcacgaatcccgcc	gggcttcttctccgccggcttc	Polymorphic	110, 100, 110, 110	2	0.38	3
100	RM158	atggtgagagttgctgccgccg	gatgacgcagaacggcatcgcc	Polymorphic	220, 210, 210, 200	3	0.625	1

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
101	RM160	agctagcagctatagcttagctggagatcg	tctcatcgccatgcgaggcctc	Monomorphic	110	1	0.00	9
102	RM161	tgcagatgagaagcggcgcctc	tgtgtcatcagacggcgctccg	Polymorphic	130, 120, 120, 120	2	0.38	5
103	RM162	gccagcaaaaccagggatccgg	caaggtcttgtgcggcttgcgg	Polymorphic	200, 150, 200, 200	2	0.38	6
104	RM164	tcttgcccgtcactgcagatatcc	gcagccctaatgctacaattcttc	Polymorphic	280, 220, 220, 220	2	0.38	5
105	RM165	ccgaacgcctagaagcgcgtcc	cggcgaggtttgctaatggcgg	Polymorphic	130, 120, 130, 130	2	0.38	1
106	RM166	ggtcctgggtcaataattgggttacc	ttgctgcatgatcctaaaccgg	Polymorphic	300, 270, 270, 300	2	0.50	3
107	RM167	gatccagcgtgaggaacacgt	agtccgaccacaaggtgcgttgtc	Polymorphic	100, 110, 100, 100	2	0.38	11
108	RM168	tgctgcttgcctgcttccttt	gaaacgaatcaatccacggc	Monomorphic	100	1	0.00	3
109	RM169	tggctggctccgtgggtagctg	tcccgttgccgttcatccctcc	Polymorphic	700, 710, 710, 710	2	0.38	5
110	RM170	tcgcgcttcttcctcgtcgacg	cccgcttgcagaggaagcagcc	Polymorphic	400, 390, 390, 380	3	0.625	6
111	RM171	aacgcgaggacacgtacttac	acgagatacgtacgcctttg	Polymorphic	300, 280, 280, 280	2	0.38	10
112	RM172	tgcagctgcgccacagccatag	caaccacgacaccgccgtgttg	Polymorphic	140, 150, 150, 150	2	0.38	7
113	RM173	cctacctcgcgatccccccctc	ccatgaggaggaggcggcgatc	Monomorphic	100	1	0.00	5

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
114	RM174	agcgacgccaagacaagtcggg	tccacgtcgatcgacacgacgg	Monomorphic	160	1	0.00	2
115	RM175	cttcggcgccgtcatcaaggtg	cgttgagcagcgcgacgttgac	Polymorphic	120, 130, 120, 120	2	0.38	3
116	RM177	ccctcttagacagaggccagagg	gtagccgaagatgaggccgccg	Monomorphic	300	1	0.00	4
117	RM178	tcgcgtgaaagataagcggcgc	gatcaccgttccctccgcctgc	Polymorphic	100, 110, 110, 110	2	0.38	5
118	RM180	ctacatcggcttaggtgtagcaacacg	acttgctctacttgtggtgaggggactg	Monomorphic	110	1	0.00	7
119	RM181	acgggagcttctccgacagcgc	tatgcttttgccgtgtgccgcg	Monomorphic	190	1	0.00	11
120	RM182	tgggatgcagagtgcagttggc	cgcaggcacggtgccttgtaag	Polymorphic	230, 250, 230, 230	2	0.38	7
121	RM184	atcccattcgccaaaaccggcc	tgacacttggagagcggtgtgg	Monomorphic	180	1	0.00	10
122	RM185	agttgttgggagggagaaaggcc	aggaggcgacggcgatgtcctc	Polymorphic	120, 150, 120, 120	2	0.38	4
123	RM186	tcctccatctcctccgctcccg	gggcgtggtggccttcttcgtc	Monomorphic	100	1	0.00	3
124	RM187	ccaagggaaagatgcgacaattg	gtggacgctttatattatggg	Monomorphic	150	1	0.00	11
125	RM188	tccgcctctcctctcgcttccc	gcaacgcacaaccgaaccgagc	Polymorphic	140, 150, 140, 140	2	0.38	5
126	RM190	ctttgtctatctcaagacac	ttgcagatgttcttcctgatg	Polymorphic	110, 100, 100, 100	2	0.38	6

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
127	RM191	cccatcctcaccgatctctctaaac	gtgcgcacggaggaggaaaggg	Monomorphic	190	1	0.00	2
129	RM194	gccctgcttcttgcccaccacc	tccagggagggcaaggctgagc	Polymorphic	190, 200, 200, 200	2	0.38	5
130	RM195	agaaagagaggccgtcggcggc	gggctcacccccaaacctgcag	Monomorphic	200	1	0.00	8
131	RM197	gatccgtttttgctgtgccc	cctcctctccgccgatcctg	Polymorphic	150, 160, 150, 150	2	0.38	6
132	RM200	cgctagggaatttggattga	cgatgagcaggtatcgatgagaag	Polymorphic	100, 110, 110, 100	2	0.50	1
133	RM201	ctcgtttattacctacagtacc	ctacctcctttctagaccgata	Polymorphic	140, 150, 150, 150	2	0.38	9
134	RM202	cagattggagatgaagtcctcc	ccagcaagcatgtcaatgta	Polymorphic	125, 150, 150, 150	2	0.38	11
135	RM203	cctatcccattagccaaacattgc	gatttacctcgacgccaacctg	Monomorphic	150	1	0.00	3
136	RM204	gtgactgacttggtcataggg	gctagccatgctctcgtacc	Polymorphic	120, 100, 100, 100	2	0.38	6
137	RM205	ctggttctgtatgggagcag	ctggcccttcacgtttcagtg	Polymorphic	130, 100, 100, 130	2	0.50	9
138	RM206	cccatgcgtttaactattct	cgttccatcgatccgtatgg	Polymorphic	100, 110, 110, 110	2	0.38	11
139	RM207	ccattcgtgagaagatctga	cacctcatcctcgtaacgcc	Polymorphic	110, 120, 120, 120	2	0.38	2
140	RM208	tctgcaagccttgtctgatg	taagtcgatcattgtgtggacc	Polymorphic	150, 160, 160, 160	2	0.38	11
141	RM209	atatgagttgctgtcgtgcg	caacttgcatcctcccctcc	Monomorphic	125	1	0.00	11

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
142	RM210	tcacattcggtggcattg	cgaggatggttgttcacttg	Polymorphic	120, 150, 120, 120	2	0.38	8
143	RM211	ccgatctcatcaaccaactg	cttcacgaggatctcaaagg	Polymorphic	110, 120, 120, 120	2	0.38	2
144	RM212	ccactttcagctactaccag	cacccatttgtctctcattatg	Polymorphic	100, 110, 100, 100	2	0.38	1
145	RM213	atctgtttgcaggggacaag	aggtctagacgatgtcgtga	Polymorphic	120, 110, 110, 110	2	0.38	2
146	RM214	ctgatgatagaaacctcttctc	aagaacagctgacttcacaa	Polymorphic	140, 100, 100, 100	2	0.38	7
147	RM215	caaaatggagcagcaagagc	tgagcacctccttctctgtag	Polymorphic	150, 140, 150, 150	2	0.38	9
148	RM216	gcatggccgatggtaaag	tgtataaaaccacacggcca	Monomorphic	100	1	0.00	10
149	RM217	atcgcagcaatgcctcgt	gggtgtgaacaaagacac	Polymorphic	110, 150, 150, 150	2	0.38	6
150	RM218	tggtcaaaccaaggtccttc	gacatacattctacccccgg	Polymorphic	130, 120, 120, 130	2	0.50	3
151	RM219	cgtcggatgatgtaaagcct	catatcggcattcgcctg	Polymorphic	180, 190, 190, 190	2	0.38	9
152	RM220	ggaaggtaactgtttccaac	gaaatgcttcccacatgtct	Polymorphic	100, 110, 110, 100	2	0.50	1
153	RM221	acatgtcagcatgccacatc	tgcaagaatctgacccgg	Polymorphic	180, 190, 190, 190	2	0.38	2
154	RM222	cttaaatgggccacatgcg	caaagcttccggccaaaag	Polymorphic	200, 210, 200, 200	2	0.38	10
155	RM223	gagtgagcttgggctgaaac	gaaggcaagtcttggcactg	Polymorphic	100, 110, 100, 100	2	0.38	8

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp) KB, ZHE733, BR*, BS*	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer					
156	RM224	atcgatcgatcttcacgagg	tgctataaaaggcattcggg	Polymorphic	110, 100, 100, 110	2	0.50	11
157	RM225	tgcccatatggtctggatg	gaaagtggatcaggaaggc	Polymorphic	300, 290, 290, 290	2	0.38	6
158	RM226	agctaaggtctgggagaaacc	aagtaggatggggcacaagctc	Polymorphic	100, 120, 100, 100	2	0.38	1
159	RM227	acctttcgtcataaagacgag	gattggagagaaaagaagcc	Monomorphic	100	1	0.00	3
160	RM228	ctggccattagtccttgg	gcttgcggctctgcttac	Polymorphic	110, 100, 100, 100	2	0.00	10
161	RM229	cactcacacgaacgactgac	cgcaggttcttgtgaaatgt	Polymorphic	110, 100, 110, 110	2	0.38	11
162	RM230	gccagaccgtggatgttc	caccgcagtcacttttcaag	Monomorphic	225	1	0.00	8
163	RM231	ccagattatttcctgaggtc	cacttgcatagttctgcattg	Monomorphic	100	1	0.00	3
164	RM232	ccggtatccttcgatattgc	ccgacttttcctcctgacg	Monomorphic	100	1	0.00	3
165	RM233A	ccaaatgaacctacatgttg	gcattgcagacagctattga	Monomorphic	100	1	0.00	2
166	RM233B	ccaaatgaacctacatgttg	gcattgcagacagctattga	Monomorphic	100	1	0.00	5
167	RM234	acagtatccaaggccctgg	cacgtgagacaaagacggag	Monomorphic	120	1	0.00	7
168	RM235	agaagctagggctaacgaac	tcacctggtcagcctctttc	Monomorphic	100	1	0.00	12
169	RM237	caaatcccgactgctgtcc	tgggaagagagcactacagc	Polymorphic	110, 100, 110, 110	2	0.38	1
170	RM238A	gatggaaagcacgtgcacta	acaggcaatccgtagactcg	Monomorphic	100	1	0.00	6

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
171	RM238B	gatggaaagcacgtgcacta	acaggcaatccgtagactcg	Monomorphic	100	1	0.00	1
172	RM239	tacaaaatgctgggtacccc	acatatgggacccacctgtc	Monomorphic	150	1	0.00	10
173	RM240	ccttaatgggtagtgtgcac	tgtaaccattccttccatcc	Monomorphic	110	1	0.00	2
174	RM241	gagccaaataagatcgctga	tgcaagcagcagatttagtg	Monomorphic	110	1	0.00	4
175	RM242	ggccaacgtgtgtatgtctc	tatatgccaagacggatggg	Monomorphic	160	1	0.00	9
176	RM243	gatctgcagactgcagttgc	agctgcaacgatgttgtcc	Monomorphic	100	1	0.00	1
177	RM244	ccgactgttcgtccttatca	ctgctctcgggtgaacgt	Monomorphic	100	1	0.00	10
178	RM245	atgccgccagtgaatagc	ctgagaatccaattatctgggg	Monomorphic	100	1	0.00	9
179	RM246	gagctccatcagccattcag	ctgagtgctgctgcgact	Monomorphic	1000	1	0.00	1
180	RM247	tagtgccgatcgatgtaacg	catatggttttgacaaagcg	Polymorphic	110, 100, 100, 100	2	0.38	12
181	RM248	tccttgtgaaatctggtccc	gtagcctagcatggtgcatg	Polymorphic	100, 110, 110, 110	2	0.38	7
182	RM249	ggcgtaaaggttttgcatgt	atgatgccatgaaggtcagc	Monomorphic	120	1	0.00	5
183	RM250	ggttcaaaccaagctgatca	gatgaaggccttccacgcag	Polymorphic	160, 140, 160, 160	2	0.38	2
184	RM251	gaatggcaatggcgctag	atgcggttcaagattcgatc	Polymorphic	110, 140, 110, 110	2	0.38	3
185	RM252	ttcgctgacgtgataggttg	atgacttgatcccgagaacg	Polymorphic	160, 170, 170, 160	2	0.50	4

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
186	RM253	tccttcaagagtgcaaaacc	gcattgtcatgtcgaagcc	Monomorphic	100	1	0.00	6
187	RM254	agccccgaataaatccacct	ctggaggagcatttggtagc	Polymorphic	150, 140, 140, 140	2	0.38	11
188	RM255	tgttgcgtgtggagatgtg	cgaaaccgctcagttcaac	Monomorphic	130	1	0.00	4
189	RM256	gacagggagtgattgaaggc	gttgatttcgccaagggc	Monomorphic	100	1	0.00	8
190	RM257	cagttccgagcaagagtactc	ggatcggacgtggcatatg	Polymorphic	160, 140, 140, 160	2	0.50	9
191	RM258	tgctgtatgtagctcgcacc	tggcctttaaagctgtcgc	Monomorphic	700	1	0.00	10
192	RM259	tggagtttgagaggaggg	cttgttgcatggtgccatgt	Polymorphic	160, 150, 160, 160	2	0.38	1
193	RM260	actccactatgacccagag	gaacaatcccttctacgatcg	Monomorphic	100	1	0.00	12
194	RM261	ctacttctccccttgtgtcg	tgtaccatcgccaaatctcc	Monomorphic	110	1	0.00	4
195	RM262	cattccgtctcggctcaact	cagagcaaggtggcttgc	Polymorphic	130, 150, 130, 130	2	0.38	2
196	RM263	cccaggctagctcatgaacc	gctacgtttgagctaccacg	Polymorphic	130, 150, 150, 130	2	0.50	2
197	RM264	gttgcgtcctactgctacttc	gatccgtgtcgatgattagc	Polymorphic	160, 170, 160, 160	2	0.38	8
198	RM265	cgagttcgtccaagtgagc	catccaccattccaccaatc	Monomorphic	100	1	0.00	1
199	RM266	tagtttaaccaagactctc	ggttgaacccaaatctgca	Monomorphic	110	1	0.00	2
200	RM267	tgcagacatagagaaggaagtg	agcaacagcacaacttgatg	Monomorphic	110	1	0.00	5

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
201	RM268	gtgctatgcacgatccatagca	cgtttctttggaagcggaggga	Polymorphic	210, 200, 200, 200	2	0.38	11
202	RM269	gaaagcgatcgaaccagc	gcaaatgcgcctcgtgtc	Polymorphic	100, 110, 110, 100	2	0.50	10
203	RM270	ggccgttggttctaaaatc	tgcgcagtatcatcggcgag	Monomorphic	130	1	0.00	12
204	RM271	tcagatctacaattccatcc	tcggtgagacctagagagcc	Monomorphic	100	1	0.00	10
205	RM272	aattggtagagaggggagag	acatgccattagagtcaggc	Monomorphic	120	1	0.00	1
206	RM273	gaagccgtcgtgaagttacc	gtttcctacctgatcgcgac	Polymorphic	300, 310, 300, 300	2	0.38	4
207	RM274	cctcgcttatgagagcttcg	cttctccatcactcccatgg	Monomorphic	200	1	0.00	5
208	RM275	gcattgatgtgccaatcg	cattgcaacatcttcaacatcc	Monomorphic	100	1	0.00	6
209	RM278	gtagtgagcctaacaataatc	tcaactcagcatctctgtcc	Polymorphic	110, 100, 100, 100	2	0.38	9
210	RM279	gcgggagagggatctcct	ggctaggagttaacctcgcg	Monomorphic	100	1	0.00	2
211	RM280	acacgatccactttgcgc	tgtgtcttgagcagccagg	Monomorphic	100	1	0.00	4
212	RM281	accaagcatccagtgaccag	gttcttcatacagtccacatg	Monomorphic	100	1	0.00	8
213	RM283	gtctacatgtacccttgttggg	cggcatgagagtctgtgatg	Monomorphic	100	1	0.00	1
214	RM284	atctctgatactccatccatcc	cctgtacgttgatccgaagc	Monomorphic	100	1	0.00	8
215	RM285	ctgtgggcccaatatgtcac	ggcggtgacatggagaaag	Polymorphic	100, 110, 110, 100	2	0.50	9

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
216	RM286	ggcttcatctttggcgac	ccggattcacgagataaactc	Polymorphic	200, 210, 210, 210	2	0.38	11
217	RM287	ttccctgttaagagagaaatc	gtgtatttggtgaaagcaac	Polymorphic	220, 230, 230, 220	2	0.50	11
218	RM288	ccggtcagttcaagctctg	acgtacggacgtgacgac	Monomorphic	100	1	0.00	9
219	RM289	ttccatggcacacaagcc	ctgtgcacgaacttccaaag	Monomorphic	100	1	0.00	5
220	RM290	acccttattcctgctctcctc	gtgctgtagatggaagggag	Monomorphic	140	1	0.00	2
221	RM291	gttgcactacgtattctgag	gatccagataaatgaggcac	Monomorphic	180	1	0.00	5
222	RM292	actgctgttgcgaaacgc	tgcagcaaatcaagctggaa	Polymorphic	300, 310, 310, 300	2	0.50	1
223	RM293	tcgttgggaggtatggtacc	ctttatctgatccttgggaagg	Polymorphic	200, 210, 210, 200	2	0.50	3
224	RM294A	ttggcctagtgcctccaatc	gagggtacaacttaggacgca	Monomorphic	100	1	0	1
225	RM295	cgagacgagcatcggataag	gatctggtggaggggagg	Polymorphic	200, 210, 210, 210	2	0.38	7
226	RM296	cacatggcaccaacctcc	gccaagtcattcactactctgg	Polymorphic	100, 110, 110, 110	2	0.38	9
227	RM297	tctttggaggcgagctgag	cgaagggtacatctgcttag	Polymorphic	120, 110, 110, 110	2	0.38	1
228	RM298	ctgatcactggatcgatcatg	catgccaagatgcaacag	Monomorphic	200	1	0.00	7
229	RM300	gcttaaggacttctgcgaacc	caacagcgatccacatcatc	Polymorphic	110, 100, 100, 100	2	0.38	2
230	RM301	ttactctttgtgtgtgtgtgag	ctacgacacgtcatagatgacc	Polymorphic	210, 200, 210, 210	2	0.38	2

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
231	RM302	tcatgtcatctaccatcacac	atggagaagatggaatacttgc	Polymorphic	150, 200, 150, 150	2	0.38	1
232	RM303	gcatggccaaatattaaagg	ggttggaaatagaagttcggt	Monomorphic	100	1	0.00	4
233	RM304	tcaaaccggcacatataagac	gatagggagctgaaggagatg	Monomorphic	100	1	0.00	10
234	RM305	tactgccaaaggcgagcttc	gtgagaggctacagctaacc	Polymorphic	310, 300, 300, 310	2	0.50	5
235	RM306	caaggtcaagaatgcaatgg	gccactttaatcattgcatc	Monomorphic	100	1	0.00	1
236	RM307	gtactaccgacctaccgttcac	ctgctatgcatgaactgctc	Monomorphic	100	1	0.00	4
237	RM308	ggctgcacacgcacactata	ttacgcatatggtgagtaggc	Monomorphic	100	1	0.00	8
238	RM309	gtagatcacgcacctttctgg	agaaggcctccggtgaag	Monomorphic	100	1	0.00	12
239	RM310	ccaaaacatttaaaatatcatg	gcttgttggtcattaccattc	Polymorphic	150, 200, 150, 150	2	0.38	8
240	RM311	tggtagtataggtactaaacat	tcctatacacatacaaacatac	Monomorphic	200	1	0.00	10
241	RM312	gtatgcatatttgataagag	aagtcaccgagtttaccttc	Monomorphic	100	1	0.00	1
242	RM313	tgctacaagtgttcttcaggac	gctcacctttgtgttccac	Monomorphic	150	1	0.00	12
243	RM314	ctagcaggaactcctttcagg	aacattccacacacacacgc	Monomorphic	100	1	0.00	6
244	RM315	gaggtacttcctccgtttcac	agtcagctcactgtgcagtg	Polymorphic	200, 190, 190, 190	2	0.38	1
245	RM316	ctagttgggcatacgatggc	acgcttatatgttacgtcaac	Monomorphic	300	1	0.00	9

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
246	RM317	catacttaccagttcaccgcc	ctggagagtgtcagctagttga	Polymorphic	200, 190, 200, 200	2	0.38	4
247	RM318	gtacggaaaacatggtaggaag	tcgagggaaggatctggtc	Polymorphic	160, 180, 180, 180	2	0.38	2
248	RM319	atcaaggtacctagaccaccac	tcctggtgcagctatgtctg	Monomorphic	100	1	0.00	1
249	RM320	caacgtgatcgaggatagatc	ggatttgcttaccacagctc	Monomorphic	200	1	0.00	7
250	RM321	ccaacactgccactctgttc	gaggatggacaccttgatcg	Monomorphic	200	1	0.00	9
251	RM322	caagcgaaaatcccagcag	gatgaaactggcattgcctg	Monomorphic	100	1	0.00	2
252	RM323	caacgagcaaatcaggtcag	gttttgatcctaaggctgctg	Polymorphic	410, 400, 410, 410	2	0.38	1
253	RM324	ctgattccacacacttgtgc	gattccacgtcaggatcttc	Polymorphic	100, 150, 150, 150	2	0.38	2
254	RM325A	gacgatgaatcaggagaacg	ggcatgcatctgagtaatgg	Monomorphic	150	1	0.00	8
255	RM327	ctactcctctgtccctcctctc	ccagctagacacaatcgagc	Monomorphic	150	1	0.00	2
256	RM328	catagtggagtatgcagctgc	ccttctcccagtcgtatctg	Polymorphic	180, 160, 180, 180	2	0.38	9
257	RM329	cattcggctgctgctattc	gcttgtcacatcttgcacag	Monomorphic	130	1	0.00	1
258	RM330A	caatgaagtggatctcggag	catcaatcagcgaaggtcc	Polymorphic	140, 120, 120, 120	2	0.38	10
259	RM331	gaaccagaggacaaaaatgc	catcatacatttgcagccag	Monomorphic	100	1	0.00	8
260	RM332	gcgaaggcgaaggtgaag	catgagtgatctcactcaccc	Polymorphic	120, 100, 100, 120	2	0.50	11

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
261	RM333	gtacgactacgagtgtcaccaa	gtcttcgcgatcactcgc	Monomorphic	120	1	0.00	10
262	RM334	gttcagtgttcagtgccacc	gactttgatctttggtggacg	Polymorphic	120, 100, 120, 120	2	0.38	5
263	RM335	gtacacacccacatcgagaag	gctctatgcgagtatccatgg	Monomorphic	250	1	0.00	4
264	RM336	cttacagagaaacggcatcg	gctggtttgtttcaggttcg	Polymorphic	210, 200, 200, 200	2	0.38	7
265	RM337	gtaggaaaggaagggcagag	cgatagatagctagatgtggcc	Monomorphic	200	1	0.00	8
266	RM338	cacaggagcaggagaagagc	ggcaaaccgatcactcagtc	Polymorphic	200, 210, 200, 200	2	0.38	3
267	RM339	gtaatcgatgctgtgggaag	gagtcatgtgatagccgatatg	Polymorphic	160, 150, 160, 160	2	0.38	8
268	RM340	ggtaaatggacaatcctatggc	gacaaatataagggcagtgtgc	Polymorphic	100, 150, 150, 150	2	0.38	6
269	RM341	caagaaacctcaatccgagc	ctcctcccgatcccaatc	Monomorphic	200	1	0.00	2
270	RM342A	ccatcctcctacttcaatgaag	actatgcagtggtgtcaccc	Polymorphic	110, 120, 100, 100	3	0.625	9
271	RM343	ccacgaacccttgcatc	gtgatgatgcgtcggttg	Polymorphic	120, 100, 100, 100	2	0.38	6
272	RM344	cagagacaatagtccctgcac	gtaggaggagatggatgatgg	Monomorphic	120	1	0.00	8
273	RM345	attggtagctcaatgcaagc	gtgcaacaaccccacatg	Polymorphic	250, 260, 260, 260	2	0.38	6
274	RM347	cacctcaaacttttaaccgcac	tccggcaagggatacggcgg	Monomorphic	300	1	0.00	3
275	RM348	ccgctactaatagcagagag	ggagctttgttcttgcgaac	Monomorphic	100	1	0.00	4

Appendix 1. List of 278 SSR Primers used for genotyping of parental and selected lines of K/Z RIL population along with their product size, number of allele amplified, and chromosomal location (Continued).

No.	Markers	Primer sequence (www.gramene.org)		Polymorphism between KB and ZHE733	Size (bp)	Number of allele	PIC Value	Chromosomal location (www.gramene.org)
		Forward primer	Reverse primer		KB, ZHE733, BR*, BS*			
276	RM349	ttgccattcgcgtggaggcg	gtccatcatccctatggtcg	Polymorphic	100, 110, 120, 120	3	0.625	4
277	RM350	tgatcgtcgcgattcccggc	ccccaccctgcgcctctccc	Monomorphic	100	1	0.00	8
278	RM351	ccatcctccaccgcctctcg	tggaggaaggaaaggggacg	Monomorphic	100	1	0.00	7

(*) BR = Bulk Resistant, BS = Bulk Sensitive