Morpho-physiological and genetic characterizations of rice genotypes for abiotic stresses

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Holistic and growth stage-specific screening is needed for identifying tolerant genotypes and for formulating strategies to mitigate the negative effects of abiotic stresses on crops. The objectives of this study were to characterize the genetic variability of 100 rice lines for early-season vigor, growth and physiological plasticity, and drought and temperature tolerance. Five studies were conducted to accomplish these objectives. In study 1 and 2, 100 rice genotypes consisting of several cultivars and experimental breeding lines were characterized for early-season vigor using several shoot and root morphological, physiological, and yield related traits. In study 3, low- and high-temperature tolerance assessed on select rice cultivars/hybrids during early-season. In study 4, genotypic variability in response to drought stress tolerance using morpo-physiological traits including roots was assessed under pot-culture conditions in a mini-greenhouse conditions. In study 5, the 100 rice genotypes were used to identify and validate SNP markers, and genome-wide association study (GWAS) to generate genotypic and phenotypic data with the objective of identifying new genetic loci controlling drought stress traits. Significant variability was recorded among rice genotypes and treatments for

many traits measured. Early-season cumulative vigor response indices (CVRI) developed by summing individual responses indices for each trait varied among the rice genotypes, 21.36 (RU1404196) to 36.17 (N-22). Based on means and standard deviation of the CVRI, rice genotypes were classified as low- (43) and moderately low- (33), high- (16), and very high-vigor (5) groups. Total low-temperature response index values ranged from 18.48 to 23.15 whereas total high-temperature responses index values ranged from 42.01 to 48.82. Antonio, CLXL 745, and Mermentau were identified as sensitive to cold- and heat, and XL 753 was highly cold and heat tolerant genotypes tested. A cumulative drought stress response index (CDSRI) values varied between 14.7 (CHENIERE) and 27.9 (RU1402174) among the genotypes tested. This preliminary analysis of GWA indicated that substantial phenotypic and genotypic diversity exists in the 100 rice genotypes, despite their narrow genetic pool. The stress tolerant and high vigor rice genotypes will be valuable for rice breeders for developing new genotypes best suited under growing environments prone to early-season drought and temperature.

DEDICATION

I lovingly dedicate this dissertation manuscript to my parents, Mr. Hameed Jumaa, Mrs. Watfa Turkey, beloved wife Marwah Alnaisani, my sons Aiham and Aymen, for giving me love and their committed support for the success of all my life. Without their love, teachings, affection, encouragement, inspiration, and sacrifices this episode of my life would have never been possible. It is their hard work, infinite love, remarkable patience and silent prayers that improved the quality of this work and made this tough task easier for me. Finally, this dissertation is dedicated to my late and live teachers and all those believed in the value of education and the richness of learning.

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CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) production is essential to global food security. Rice provides 19 and 13% of the caloric intake and the protein for the world's population, with Southeast Asia receiving 48% of its the caloric and 38% of its protein intake from rice, respectively. Although rice is grown on five continents, it is primarily produced in a tropical climate, predominately in eastern, southern, and southeastern, southern Asia, and is generally consumed domestically. Around 80% of the world's rice production belongs to the tropical subspecies (Indica), with the 20% being the temperate subspecies (Japonica) that is produced in temperate and subtropical regions (Wailes and Chavez, 2011). U.S. rice production falls into the japonica group with plantings averaging 1.2 million hectares in 2017 (USDA NASS, 2017). Despite the United States accounts for less than 1% of the world's production and planting acreage of rice, but it is a fifth rice exporter. Rice has been grown in Arkansas, east Texas, and Louisiana since the mid-19th century. Recently, rice production has increased in the Mid-South of America, principally in the Mississippi River Delta areas around the states of Arkansas and Mississippi.

Early vigor plays a critical role under the directly seeded condition of rice increasing the plant's ability to compete against weeds and can be differentiated between cultivars, which could be taken as a selection criterion in upland rice breeding programmes. In rice, seedling vigor has been found to be associated with seed size and

density as well as other parameters of germinating seed (Pandey et al., 2000 and Shenoy et al., 1990). However, the associations among seed and seedling traits are not clearly understood, and there is a need to evaluate the available variability to identify genotypes with good appearance quality and early vigor for use in breeding. Several morpho-physiological quantitative traits such as germination rate and seedling growth are associated with early vigour in rice and performance is generally determined by genotype and modified by the environment (Perry, 1972). The seed vigour in rice also affects many of its agronomic traits and grain yield as well (Pandey et al., 1989). Vigour is under genetic control (Perry, 1972) and can be incorporated in the genetic background of high yielding varieties. There is a need to improve the early vigour of semi-dwarf rice cultivars. The increasing importance of direct seeding in many countries (Dingkuhn et al., 1992) has made it critical to improve the seedling vigour of rice cultivars. Using shoot and root trait obtained from such instruments, selection indices have been developed to rank genotypes into groups based on single and cumulative value indices (Singh et al., 2017b), as well as grouping genotypes using principal component analysis (PCA) (Asif et al., 2010). Application of these techniques in screening for roots parameters in diverse rice germplasm pools could help identify new donors and genotypes with early season vigor that is important for field establishment in the U.S. Mid-South rice production system.

Rice grain size is the main component of rice appearance quality and which also has a direct effect on the marketability or commercial success of improved cultivars (Redona and Mackill, 1998 and Rabiei et al., 2004). There is a considerable variability for increasing rice yield potential across cultivated subspecies and in their crossbreeds

(Peng et al., 2008). Several cultivar improvement programs around the world have primarily relied on the existing variation in Japonica, Indica, and Japonica x Indica subspecies combinations to realize grain yield and more magnificent environmental adaptation (Peng and Khush, 2003). The grain number of panicle, filled grain, and grain weight fraction of total spikelets have been used to estimate grain yield performance for various rice genotypes and environments (Yoshida, 1981). The grain number of panicle represents 60 to 80% of the variability in the rice grain yield (Ying et al., 1998). The 100-grain weight ranges from 2.47 to 2.77g (Fageria, 2007), but values as high as 2.9-3.0g have been reported in some elite lines and hybrids based interspecific cultivars (Zou et al., 2013; Somado et al., 2008). Estimate and comparison of growth, development, and physiological traits of rice genotypes using combined vigor response index (CVRI) may be done to examine the relationship among growth, physiological, and yield-related parameters. This approach is considered as one auxiliary way for breeders to better understand physiological changes during development of rice breeding lines and genotypes grown under conditions in Mid-south U.S.

Temperature is one of the primary environmental regulators of crop growth and development with significant effects on yield and quality. Several plant morpho-physiological traits have been used to identify temperature tolerance among crop genotypes (Singh et al., 2007). Crops have necessary requirements to complete their life cycle specific pheno-phases. Therefore, cardinal temperatures (minimum, optimum, and maximum) vary between crop species and among the physiological processes within a crop species. Changes in air temperature are mostly very sudden and plants cannot adjust to these changes to avoid damages. Temperature extremes (high and low) can have

detrimental effects on crop growth, development, and yield particularly at critical phenophases (Luo, 2011). Many plants suffer physiological injury when subjected to low temperatures (1-10 °C) and there is also some evidence that low temperature has an immediate effect on cellular metabolism in sensitive tissues (Zhang et al., 2010). High temperature negatively affects plant growth, survival, and crop yield. According to Peng et al., (2004) and Welch et al., (2010) high night temperatures have demonstrated negative effects on rice spikelet fertility and yields. Temperature tolerance is a multigenic trait and therefore simple, consistent, and applicable methods are required to assess genetic variability and cold tolerance in crops. Also, experimental facilities are needed to impose such stresses mimicking field environments including solar radiation (Reddy et al., 2001). Cold tolerance is also an important task that must be tackled vigilantly for the adaptation of rice to the early spring climate of central Mississippi where earliness is essential to avoid hot summer, little quantitative information is available on understanding actual changes of weather patterns on rice early season growth. Understanding physiological and photosynthesis responses on rice seedlings at early season cold temperature is essential to improve its management as a crop. Low-temperature stress is one of the significant abiotic factor that the inhibits seedling establishment, poor seed settings which results in low and agricultural productivity. In order to gain stable rice production cold tolerance at the seedling stage is a famous character (Xin and Browse, 2000; Lou et al., 2007; Law and Brandner, 2001). Most previous studies involving the impact of high temperature on rice production were designed to control the temperature over a small plant population size, while others analyzed the regression and correlation of historical data sets from long-term field

experiments. These strategies are inadequate because they include possible confounding effects from factors other than temperature. In addition, little information is available about the response of japonica cultivars to high temperature during the early seedling stage.

Early drought often results in delayed sowing or transplanting. Yield reductions from early droughts are often minimal and result mainly from a decrease in tiller numbers (Boonjung and Fukai, 1996 and Jongdee et al, 2006). This abiotic stress is therefore a major constraint to rice production in water-limited environments. There are three basic drought patterns affecting rice production: early, intermittent, and late drought stress. They are occurring during vegetative growth, maximum tillering, and after panicle initiation (United Nations 2011). Drought affects rice at morphological, physiological, and molecular levels such as delayed flowering, reduced dry matter accumulation and partitioning, and decreased photosynthetic capacity because of stomatal closure, metabolic limitations, and oxidative damage to chloroplasts. Small-statured rice plants with reduced leaf area and short growth duration are better able to tolerate drought stress (Dawe et al., 2011). Phenotyping for molecular breeding purposes allows developing molecular probes for marker-based selection. In this context, it is important that markers for component traits of a complex trait have proven physiological complementarities or synergies while being under distinct genetic control. However, very little is known on the extent of genetic variation for vigor-related traits under drought stress among tropical japonica varieties- the primary varietal group used in commercial production in the US Mid-south. Most earlier drought studies conducted on rice have primarily used genotypes

belonging to the indica rice subspecies that is the most predominant rice subspecies grown worldwide.

Root systems are challenging to study because their highly structured underground distribution, complexity of vigorous interactions with the environment, and their diversity of functions. Under controlled environments, moistened papers, hydroponics, or Petri dishes have been used for screening of root mass and architecture (Reynolds et al., 2012). Root scanning based on winRHIZO optical scanner is a simple, efficient methods, and accurate, under control environmental condition, which allow image analysis and examining the root morphological traits of roots like root length, root width, root density etc. This technique provides data that can easily be analyzed by established software protocols in a way of simple, rapid, labor and accurate screening of root characteristics. Root uses winRHIZO system to compare root characteristics of different early season rice cultivars for the variable condition (Wijewardana et al., 2015).

The rice crop is well diversed and has evolved into a tremendously broad base for genetic diversity as reflected by number of landraces existing today (Jackson, 2016). Assessment of genetic diversity is essential in plant breeding for improvement through selection. The evaluation of genetic variability in natural populations can give new insights into address issues of cultivar classification and domestication of crop plants (Vaughan, 1992). Therefore, it is essential to understand the variability for different morphological and grain characteristics, adaptability to harsh environments for nearly introduced genotypes (Bhat and Gowda, 2004). Knowledge of genetic diversity in crop species is fundamental for crop improvement and can be used a variety of morpho-physiological and molecular descriptors are used to characterize genetic diversity among

and within crop species. In rain-fed upland ecosystems, the characters like early vigor, drought tolerance, and other adaptive traits are considered in most of the breeding programmes leading to the identification of cultivars.

Substantial variations exist among the rice genotypes for various morphological, physiological and agronomic traits, but they are sensitive to the environment and have limited coverage in the genome, hindering their usage in the breeding programmed. Single Nucleotide Polymorphisms (SNPs), which are genome sites where DNA sequence differs by a single base when two or more individuals are compared, currently represent the most popular genetic markers. Not only SNPs are the most abundant form of genetic variation in eukaryotic organisms, being present in both coding and non-coding regions of nuclear and plastid DNA, but they are also stable, efficient, amenable to automation and increasingly cost-effective (Duran et al., 2009, Edwards and Batley 2010). Genome-Wide Association Study (GWAS) mapping, in contrast, can detect new regions associated with the trait of interest by testing the statistical associations between the variation of the trait and SNP variation at the whole genome level. The success of GWA studies relies on thorough phenotyping for the traits of interest coupled with a cost-effective high-throughput genotyping technology, enabling to rapidly scan the largest number of markers across the largest set of genotypes to yield high-density/quality haplotype maps. Evaluation of genotypes for phenotypic characters based on morphological variation is supplemented with DNA characterization, which helps in documentation and deployment of the available genetic variability. Study on the genetic polymorphism provides a scientific basis for the utilization of germplasm resources efficiently in crop improvement. Though a range of plant characters are currently

available for distinguishing closely related individuals, their sensitivity to the environment and little genome coverage hinders their further use in breeding programs.

Problem statement and objectives

The general objectives of this research were to evaluate the variability among the 100 elite rice lines for early-season vigor, physiological and growth, and yield variability through the grown season, drought tolerance mechanisms using morpho-physiological traits and genome-wide association mapping and identify markers for drought tolerance, and low- and high-temperature tolerance of selected rice cultivars. Also, develop a method to classify the 100 rice lines, based on variability for early- and whole-season vigor responses, into to various groups. Further to classify rice lines and cultivars into various draught-, low-, and high-temperature tolerant response groups, based on their relative scores of tolerance of the measured parameters. In the end, the study will provide a quantitative database and relative score for each rice lines/cultivar for vigor cultivar indices, and stress responses and associated SNP makers. The quantitative data specific to rice lines' vigor under optimum and stressful environments will be a valuable resource for rice breeders to develop new and efficient cultivars for changing environmental conditions for the US Midsouth and elsewhere.

CHAPTER II

ASSESSING EARLY-SEASON VIGOR IN A DIVERSE RICE PANELS USING MORPHO-PHYSIOLOGICAL TRAITS

Abstract

Early-season vigor is an important morphological determinant to assess the crop's growth rate and duration by using light interception, dry matter production and loss, and dry matter portioning. Formulating screening tools to assess early-season vigor via root and shoot characteristics would be useful for identifying genotypes with superior performance during the juvenile stage. A 2-year study was conducted using a sunlit pot-culture set-up to assess genetic variation among 100 rice genotypes for the shoot and root traits, and several physiological parameters at seedling growth stage, 25-30 days after seeding. Since there was no significant year or experimental time period x genotypes interaction for the trait and parameters measurements, the two year-data was combined for each genotype. An individual and cumulative response index (IVRI and CVRI) were estimated for each trait for all genotypes. Genotypes were classified into different categories using CVRI values and standard errors. Majority of the genotypes exhibited low vigor (43%) followed by moderate (33%), and very low 16%. However, 5 and 3 genotypes exhibited high and very high vigor, respectively. The CVRI values varied from 21.36 in genotype (RU1404196) considered as least vigorous to 36.17 in genotype (N-22) considered as the highest vigorous. The high vigor genotypes could be valuable genetic

resources for developing new varieties with early-season vigor as well as for physiological studies on canopy development for optimum light interception and weed competitiveness. Information gained from this study could be useful in identifying and formulating crop's growth rate and growth period as early and late season crop as well as avoiding photoperiod sensitive rice genotypes.

Introduction

Rice (*Oryza sativa* L.), originally grown in Southeast Asia 10,000 years ago, is cultivated in over one hundred countries globally and serves as a staple food for one half of the global population (Fageria, 2007). In 33 developing nations where rice is consumed as the primary staple food, 27 percent of dietary energy needs, 20 percent of dietary protein needs, and 3 percent of dietary far are provided from rice (Kennedy et al., 2002). Rice therefore bears an enormous pressure to keep productivity in check with population growth and dietary demand. In Asia, for example, it is predicted that demand for rice will increase by 69% over the next 30 years as a result of population growth (Hossain, 1997). Although the United States accounts for only less than 2% of world rice production, it is a major rice exporter and contributes 12 to 14% of the annualglobal rice exports (Childs and Livezey, 2006).

In recent years yields of improved inbred rice varieties grown in favorable conditions, such as those found in the US, have plateaued and even declined in some countries due to various challenges related to both biotic and abiotic stresses (Redoña, 2004). Already facing resource limitations on land and water availablility, enourmous pressure exists to keep rice productivity on pace with a continuously rising population. Technological advances in rice production must be made to meet future demand. One

approach to minimize the gap between rice production and future demand is to develop varieties with a broad range of adaptations to diverse growing conditions. For example, breeding programs may select varieties adapted to dry, direct seeding or aerobic cultivation to combat water scarcity (Cabangon et al., 2002). Another example would be selecting for longer, more expansive root systems that would increase water and nutrient uptake. This approach would require collaboration between breeders, physiologists, and other plant scientists to select genotypes adapted to variable environmental conditions (Dingkuhn et al., 2015). Consequently, there is limited research available related to rice root growth, as plant systems below ground are generally arduous to study and results can be complicated by strong interactions with unintended variables. Mississippi is one of five (5) rice-producing states in the US Mid-South. Producers generally plant from March to April, primarily through drill-seeding. According to studies conducted from 2007-2014, the optimal time to plant rice in Mississippi to avoid yield reduction is between the twentieth of March and the thirteenth of April (Golden et al., 2014) A major yield limiting factor in the US Midsouth is erractic rainfall during the summer season. Research conducted from 2006 to 2013 has shown that rice planted as early as the late March typically produces higher, more stable yields, thus, selection of rice genotypes well suited for early planting may help producers optimize their growing conditions throughout the growth of their crop (Walker, 2013).

Early season growth is a critical phase for rice that influences canopy development, tillering, and ultimately the overall crop stand. Early season vigor is a crucial trait that allows the plant to rapidly access resources, providing the ability to compete with weeds and pests (Namuco et al., 2009). However, early season vigor is a

complex trait manifested by the capacity of seedlings to rapidly accumulate shoot biomass. Early season vigor is a summation of the genotype's ability to germinate uniformly, synchronize emergence, and growth rapidly (Chen et al., 2015). As a complex trait, vigor can be simplified for genetic improvement by dissecting it further into component traits of less genetic complexity such as leaf area, leaf size, tiller number, leaf expansion rate, and leaf appearance (Rebetzke et al., 2007; Maydup et al., 2012). Improving early season vigor is considered the most relavent and useful strategy to mitigate poor, uneven crop stand establishment, combating one the major constraints in diect seeded rice systems. (Okami et al., 2015; Kumar et al., 2009; Singh et al., 2017a; Singh et al., 2017b).

Producing quantitative values and identifying important traits to screen and classify rice genotypes for early season vigor will be valuable to select current varieties and develop new genotypes better suited for the US Midsouth production system. In recent years the development of technologies such as optical scanners and analysis software have aided early vigor studies. One example, the WinRHIZO ocular scanner (Regent Instruments Inc., Canada) is a precise, quick, and simple method to analye root characteristics in cereals (Wijewardana et al., 2015; Singh et al., 2017b; Singh et al., 2018). Using shoot and root trait obtained from such instruments, selection indices have been developed to rank genotypes into groups based on single and cumulative value indices (Singh et al., 2017b), as well as grouping genotypes using principal component analysis (PCA) (Asif et al., 2010). Application of these techniques in screening for roots parameters in diverse rice germplasm pools could help identify new donors and genotypes with early season vigor that is important for field establishment in the U.S.

Mid-South rice production system. The objectives of this study were: (1) to evaluate root and shoot morphology and growth of 100 rice genotypes during the seedling growth stage (2) to develop a method to assess early season vigor variability; and (3) classify and rank the rice genotypes based on vigor response indices.

Materials and methods

Seed Materials and Facility

We selected one hundred genotypes from germplasm utilized by Mississippi State University's (MSU) rice breeding program in Stoneville, MS (Table 3). MSU's breeding program uses these varieties to develop new varieties adapted to the US Mid-South. About 95% of 100 these genotypes are tropical japonicas, the predominant subspecies grown in the US Mid-South, while 5% are indica types, the rice subspecies commonly grown in Asia. About 70% of the genotypes were breeding lines under development, and 30% were commercially released varieties. Of the released varieties, 25 are released for commercial use in the US Mid-South.

We conducted all experiments at the Environmental Plant Physiology laboratory at MSU's Rodney Foil Plant Science Research Facility located near Starkville, MS during the 2015 and 2016 growing seasons. In 2015, we conducted the experiment from June to July, growing the rice for 30 days. In 2016, we conducted the experiment August to September, again for 30 days. Environmental conditions for these two experiments showed in Table 1. In both studies, we sowed rice plants in 6-liter polyvinylchloride (PVC) pots 15.2 cm in diameter and 30.5 cm tall filled with a custom soil mixture of three parts sand to one-part topsoil and 500 g of gravel at the bottom of each pot. The mixture classified as a sandy loam (87% sand, 2% clay, and 11% silt). We sealed the

bottom of each pot with a plastic cap and drilled a hole 0.5 cm in diameter at the bottom for drainage. Initially, we sowed eight seeds at a depth of 3 cm in each pot. After emergence, we gradually thinned each pot to one single plant.. In both experiments, we organized pots using a randomized complete block design in three rows (replication) with 100 pots per row. Rice genotypes were assigned randomly to each of the 100 pots. Plants received exposure to natural sunlight and we irrigated three times per day (0800, 1300, and 1700 h) via an automated and computer-controlled drip system with a full-strength Hoagland's nutrient solution (Hewitt, 1952) designed for optimum plant growth. Plant growth and development as well as root and shoot morphological features were assessed 30 days after sowing (DAS).

Parameter Measurements

Shoot Growth and Development Traits

We measured the following morphological characteristics: leaf number (LN), plant height (PH), total number of tillers (TN), and dry biomass (leaves, stems, roots, and total dry weights, gm/ plant^{-1}), Biomass was oven dried at 75°C for 72 hours prior to being weighed. Shoot weight was calculated by summing leaf and stem weight for each genotype. The root/shoot ratio was calculated by dividing root weight (RW) by the sum of leaf weight and stem weight for each genotype. We determined leaf area (LA) using a leaf area meter (Li-3100 leaf area meter, Li-COR Inc., Lincoln, NE).

Table 2.1 Environmental detail including solar radiation, average relative humidity, and mean temperature during the experimental period for each year.

	Experiment 1			Experiment 2		
Environmental condition	Mean	Max	Min	Mean	Max	Min
Temperature C	27.27 ± 0.4	31.6	22.1	28.5 ± 0.2	31	26.3
Relative humidity	76.1 ± 1.2	88.5	67.0	75.9 ±1.5	90	54.6
Solar radiation	23.62 ± 0.8	29.0	12.6	21.3 ±0.7	26.7	13.4

Root Morphology, Architecture, and Root Parameters:

At 30 DAS, we terminated plant growth by separating the plant shoots from the roots at the soil surface level. We washed all soil media from the roots and placed them between moist paper towels until further analysis. To analyze the roots, we utilized the WinRHIZO Pro optical scanner (Version 2009, Regent Instruments, Inc.) adjusted to acquire root images at 800 by 800 dpi resolution. The WinRHIZO Pro software analyzed nine root parameters, including: root surface area (RSA), root average diameter (RAD), cumulative root length (RL), root volume (RV), number of forks (RNF), number of tips (RNT), and number of crossings (RNC). We measured the longest root length (LRL) with a ruler, and manually counted root number (RN). After scanning, we placed the roots in paper bags and dried them at 75 °C for three days before determining the dry weight.

Vigor Indices Utilized

For each experiment, we calculated the individual vigor index (I) for each parameter by dividing the value of each genotype (Vi) by the maximum value (V_x) among the genotypes for the given parameter (Eq. 1).

$$I = V_i/V_x \qquad \text{[Eq. 1]}$$

Then, we estimated the cumulative vigor response indices (CVRI) as the sum of all individual indices for all parameters for each genotype (Eq. 2).

$$CVRI\ (1) = (PH_i/PH_x) + (TN_i/TN_x) + (LN_i/LN_x) + (LA_i/LA_x) + (LW_i/LW_x) + (SW_i/SW_x) + (RW_i/RW_x) + (SHW_i/SHW_x) + (RS_i/RS_x) + (TW_i/TW_x) + (RL_i/RL_x) + (RSA_i/RSA_x) + (AD_i/AD_x) + (RV_i/RV_x) + (T_i/T_x) + (LRL_i/LRL_x) + (RN_i/RN_x) + (SPAD_i/SPAD_x) + (Fv/Fm_i/Fv/Fm_x). \quad [Eq.\ 2]$$

We determined the total vigor response index (TVRI) as the sum of all cumulative vigor response indices from each experiment (Eq. 3).

$$CVRI\ (1) + CVRI\ (2) = TVRI \quad [Eq.\ 3]$$

Finally, we used the total vigor response indices and standard deviation (SD) to classify genotypes into groups from very low-to-low, moderate, high, and very high vigor at early season growth and development stages.

Data Analysis

We performed an analysis of variance for root and shoot traits using the Proc GLM procedure in SAS 9.4(SAS Institute, 2011). The genotypes were considered as a fixed effect, while replications within an experiment were considered as a random effect. Separation of means was made using a least significant difference (LSD) test at P = 0.05. We calculated the standard error of each mean using SigmaPlot version 13 (Systat Software Inc., San Jose, CA). The correlation coefficient and regression of determination of the shoot and root parameters among rice genotypes were obtained using Pearson correlation (PROC CORR) and (PROC REG) within SAS. We completed a principal component analysis(PCA) on the correlation matrix of 100 genotypes and 19 response variables comprising PH, TN, LN, LA, LW, SW, RW, SHW, RS, TW, RL, RSA, AD, RV, T, LRL, RN, SPAD, and Fv/Fm using the PRINCOMP procedure of SAS (SAS

Institute, 2013). The results were abridged in biplots using SigmaPlot version 13 (Systat Software Inc., San Jose, CA).

Results and discussion

Morpho-Physiological Traits

Based on the joint variance analysis, the study observed significant variability among the genotype for all morpho-physiological parameters measured except leaf number of main stem (LN) and quantum efficiency of fluorescence (Fv/Fm) (Table 2). Selection for superior genotypes based on growth and yield per se at a single location or trial in a year may not be very effective (Shrestha et al., 2012, Annicchiarico, 1994). According to 90% of the genotype by year interaction of traits was not significant (table 2.2), the two year-data was combined for each genotype. Evaluation of genotypes for the stability of performance under varying environmental conditions including seasonal experiments is an essential part of any breeding program. Moreover, genetic variation is an essential factor that enhances plant's survival within its cultivated environment (Tariku et al., 2013; Diwan, 2006).

Growth Parameters

A crucial component for accelerating the expansion of newly amended crop genotypes including rice is rapid and precise phenotypic assessment of rice genotypes under different environmental conditions including planting them a year (season) after year (season). Despite technological innovations that describe genomes cheaply and rapidly, the ability to quickly and accurately measure plant performance in the

experiments remains a limiting factor in plant breeding and genetics. There was significant variation (P<0.001) among genotypes in the plant height, with a low of 15.3 cm (RU1404157 and RU1404196) to a high of 22cm (CL Jazzman and RU1401102), and with an average of 18.8cm (Table 3).

Simple traits such as seedling or plant height and dry weight have been identified as good indicators of seedling and early vigor (Regan et al., 1992). 126% reduction was recorded among genotypes between high 3.3 (CL 152) and low 2.6 (RU1404196) of leaf number. Maximum reduction of 216 per cent was observed in the genotype RU1404196 as compared with a general average 230.5 cm^2 of genotypes in leaf area. In rice, early vigor is attributed mostly to high leaf area index (LAI) during vegetative stage (Okami et al., 2011), in these experiments, genotype N-22 has the highest LA. The rate of early leaf area development (early vegetative vigor) is a determinant for resources colonization and yield competiveness of the rice seedling (Zhao et al., 2006) and yield potential (Dingkuhn et al., 1999).

Table 2.2 Analysis of variance across 100 rice genotypes and two year treatments and their interactions (genotypes by year) with rice morpho-physiological parameters measured 30 d after planting; plant height (PH), tiller number (TN), leaf number of main stem (LN), leaf area (LA), leaf dry weight (LW), stem dry weight (SW), root dry weight (RW), shoot dry weight (SHW), root/shoot ratio (RS), total dry weight (TW), root length (RL), root surface area (RSA), average root diameter (AD), root volume (RV), tips number (T), longest root length (LRL), root number (RN), SPAD, and quantum efficiency of fluorescence (Fv/Fm).

S.O.V	PH	TN	LN	LA	LW	SW	RW	SHW	RS	TW	RL	RSA	AD	RV	T	LRL	RN	SPAD	Fv/Fm
Genotypes	***	***	N.S	***	***	**	***	***	**	**	**	***	***	***	***	*	**	**	N.S
Year 1	**	***	*	***	**	**	**	***	**	***	**	***	**	**	N.S	**	**	**	N.S
Year 2	**	**	N.S	***	**	*	**	**	N.S	***	*	**	*	**	*	***	*	*	*
Years	N.S	N.S	N.S	N.S	*	N.S	**	N.S	*	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	*
G x Y	N.S	N.S	N.S	N.S	N.S	N.S	**	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	*	N.S

† *, **, *** represent significant differences at the 0.05, 0.01, and 0.001 P level, respectively.
†† NS represents nonsignificant differences at the 0.05 P level.

RU140196 has recorded the lowest PH, LN, LA, LW, and RW, which might be a reason to be the least vigor index among 100 genotypes. Also, INIA Tacuari and RU1404157 were recorded the lowest TN, SW, and SHW among genotypes, which caused to be in very low vigor index category. From another hand, N-22 was observed the highest LA, LW, SW, RW, SHW, and TW among genotypes, which indicated to be a high vigor index among 100 genotypes (Tables 2.3 and 2.6). 76% of the genotypes were below the grand average RW (0.7 g) and RS (0.2), respectively while 45% of genotypes were exceeded the general average in SHW (3.3 g) and TW (3.7 g), respectively. According to Fukai and Cooper 1995, seedling vigor is the plant's ability to emerge rapidly from soil or water and establish itself before its competitors (Bastiaans et al., 2011). Several parameters are closely associated with seedling vigor, were considered relevant in determining crop's vigor. Rapid emergence is a key trait for successful crop establishment (Namuco et al., 2009).

Table 2.3 Means of plant height (PH), tillers number (TN), leaf number of the main stem (LN), leaf area (LA), leaf dry weight (LW), stem dry weight (SW), root dry weight (RW), shoot dry weight (SHW), root/shoot ratio (RS), and total dry weight (TW) for 100 rice genotypes.

Serial No.	Genotype name	Measured growth parameters									
		PH	TN	LN	LA	LW	SW	RW	SHW	RS	TW
1	14CLPYT033	16.1	9	2.8	196.5	1.5	1.9	0.7	3.4	0.2	3.8
2	14CLPYT108	17.2	9.3	2.8	221.2	1.5	1.5	0.7	2.9	0.3	3.2
3	14CVPYT094	16.5	8.7	2.8	264.8	1.8	1.8	0.8	3.6	0.2	4
4	14CVPYT144	19.7	9.3	2.9	196.7	1.8	1.9	0.7	3.7	0.2	4
5	COLORADO	17.3	10	2.9	215.2	1.7	1.9	0.6	3.5	0.2	3.8
6	Bowman	16.3	8	2.9	210.4	1.4	1.3	0.7	2.7	0.3	3.2
7	CAFFEY	19.4	9.7	2.9	238.1	2	2	0.7	4	0.2	4.3
8	CHENIERE	17.6	9.5	3	174.4	1.4	1.6	0.7	3	0.2	3.4
9	CL Jazzman	22	8.5	3	211.4	1.4	2.2	0.7	3.6	0.2	4
10	CL111	21.2	9	3	261.5	1.9	1.9	0.8	3.8	0.2	4.1
11	CL142-AR	20.9	8.3	3	174.5	1.4	1.5	0.7	2.9	0.2	3.3
12	CL151	19.5	10.7	3	233.1	1.7	1.9	0.6	3.6	0.2	3.9
13	CL152	19.4	10.3	3.3	248.2	1.8	1.9	0.7	3.7	0.2	4.1
14	CL163	16.3	7.7	3	204.9	1.2	1.4	0.7	2.6	0.3	2.9
15	CL172	19.4	10	3.2	240.4	1.7	1.9	0.7	3.6	0.2	4
16	CL271	20.4	8.5	3	305	2.1	2.2	0.8	4.3	0.2	4.8
17	Cocodrie	20.3	8.5	2.9	287.6	1.7	1.9	0.7	3.5	0.2	3.8
18	NIPONBARE	15.8	7.8	2.6	219.6	1.5	1.2	0.7	2.7	0.3	3
19	ANTONIO	18.9	8.8	3.1	266.8	1.6	1.9	0.7	3.4	0.2	3.8
20	El Paso 144	16.2	11.5	3	341	2	2	1	4.1	0.3	4.5
21	GSOR100390	20.7	10.3	3	251.4	2.2	2.4	0.8	4.6	0.2	5
22	GSOR100417	20.4	10	2.8	263.4	1.7	1.7	0.5	3.4	0.2	3.8
23	GSOR101758	15.3	11.3	2.7	268.5	1.4	1.3	0.7	2.7	0.3	3
24	RU1104122	18.6	7.3	2.7	188.2	1.4	1.4	0.7	2.8	0.3	3.2
25	CLJZMN	21.2	8.7	3	240.8	1.7	1.7	0.7	3.4	0.2	3.8
26	INIA Tacuari	17.4	5.7	3	123.6	1.2	1.1	0.7	2.3	0.3	2.6
27	IRGA409	20.9	12.7	3.1	354.8	1.9	2.2	0.7	4.1	0.2	4.5
28	JES	17.9	12.7	3	360.6	1.8	1.8	0.7	3.6	0.2	4
29	JUPITER	16.4	8.2	3	225.1	1.8	1.8	0.8	3.6	0.2	4
30	LA 2008	20.4	9.3	2.9	186.9	1.6	1.6	0.7	3.2	0.2	3.5
31	LA 2134	21.3	10.3	2.8	238.8	1.5	1.6	0.6	3.1	0.2	3.5
32	LAKAST	17.8	7.8	3	203.6	1.4	1.5	0.9	2.9	0.3	3.3
33	MERMENTAU	19	8.2	2.9	195.5	1.5	1.7	0.8	3.1	0.3	3.5
34	Presidio	18.3	7.7	3.1	211.9	1.4	1.3	0.7	2.7	0.3	2.9
35	Rex	17.2	9.8	2.9	318.4	2	2.3	0.9	4.3	0.2	4.8
36	RoyJ	18.6	8.3	2.9	231.8	1.6	1.7	0.6	3.3	0.2	3.6
37	RU0603075	18.5	17.3	3	490.5	2.4	2.4	0.9	4.8	0.2	5.3
38	RU1201024	18.9	7.7	3	227.9	1.7	1.6	0.8	3.2	0.2	3.6
39	RU1201047	20.2	8	2.8	189.1	1.5	1.7	0.8	3.2	0.2	3.5
40	RU1201136	17.3	7.3	3.1	208.6	1.4	2.3	0.6	3.7	0.2	4
41	RU1204156	17.4	9.3	2.9	237.1	1.5	1.9	0.7	3.3	0.2	3.6
42	RU1204197	19.5	9.2	2.9	220.4	1.6	1.9	0.8	3.5	0.2	3.9
43	RU1301084	17.9	7.5	3.1	215.5	1.6	2	0.8	3.7	0.2	4.2
44	RU1301093	18.8	7.2	3	208.8	1.5	1.6	0.7	3.1	0.3	3.5
45	RU1301102	18.2	9.7	2.9	179.6	1.4	1.8	0.7	3.2	0.2	3.6
46	RU1302192	20.4	10	2.6	262.3	1.8	1.9	0.8	3.7	0.2	4.1
47	RU1303138	16.3	15	2.7	497.8	2.4	2.2	1	4.6	0.2	5.2
48	RU1303181	19.3	8.2	3	210.7	1.5	1.6	0.6	3.1	0.2	3.4
49	RU1304114	17.9	9.8	2.8	262.6	1.7	1.6	0.7	3.3	0.2	3.6
50	RU1304122	19.4	8.3	3.2	218.2	1.7	1.7	0.6	3.4	0.2	3.7
51	RU1304154	19.9	8.3	3	262.3	1.7	1.7	0.8	3.4	0.3	3.8

Table 2.3 (Continued)

52	RU1304156	21.9	8	2.9	217.4	1.7	1.7	0.7	3.4	0.2	3.8
53	RU1305001	20.6	8.8	2.7	247.8	1.8	1.7	0.8	3.5	0.2	4
54	RU1401067	19.3	7	3.2	212	1.4	1.8	0.6	3.1	0.2	3.4
55	RU1401070	18.1	6.8	3.1	196.2	1.3	1.5	0.6	2.8	0.2	3.1
56	RU1401090	19.2	8.3	3	156.1	1.4	1.6	0.6	2.9	0.2	3.3
57	RU1401099	18.4	8.8	2.9	226.1	1.7	1.9	0.8	3.6	0.2	4
58	RU1401102	22	8.7	3	230.5	1.6	2.1	0.7	3.7	0.2	4
59	RU1401145	19.1	8.2	2.9	144.6	1.2	1.5	0.8	2.7	0.3	3.1
60	RU1401161	18.9	7.8	3.2	216.1	1.6	1.6	0.6	3.2	0.2	3.5
61	RU1401164	20.8	10.8	3	238.3	1.7	2	0.6	3.7	0.2	4.1
62	RU1402005	19.3	9.8	3	241.2	1.8	1.8	0.7	3.6	0.2	4
63	RU1402031	19.6	10.7	2.9	228.3	1.7	1.9	0.7	3.6	0.2	4
64	RU1402065	19.1	10.3	2.8	194.9	1.7	2	0.6	3.7	0.2	4.1
65	RU1402115	19.6	10.2	2.9	262.7	1.9	1.9	0.6	3.7	0.2	4.2
66	RU1402131	21.3	10.2	2.8	306	2.1	2.1	0.7	4.3	0.2	4.7
67	RU1402134	20.9	10	2.9	294.2	1.8	1.8	0.6	3.6	0.2	4
68	RU1402149	19.3	8	2.9	192.8	1.4	1.8	0.5	3.2	0.2	3.4
69	RU1402174	17.3	9	2.9	167.2	1.4	1.5	0.6	2.9	0.2	3.1
70	RU1402189	20.4	7.7	3.1	197.4	1.4	1.8	0.7	3.2	0.2	3.5
71	RU1402195	20.6	10.3	2.7	281.9	1.8	1.6	0.6	3.4	0.2	3.7
72	RU1403107	17.7	6.7	3	181.4	1.4	1.6	0.6	3	0.2	3.3
73	RU1403126	17.8	9	3	211	1.6	1.8	0.6	3.4	0.2	3.8
74	RU1404122	16.4	9	2.7	168.2	1.2	1.4	0.8	2.5	0.3	2.9
75	RU1404154	20.6	7.7	2.8	235.3	1.7	1.6	0.7	3.3	0.2	3.7
76	RU1404156	18.1	7.8	3	199.7	1.4	1.5	0.7	2.9	0.2	3.2
77	RU1404157	15.3	7.8	2.6	149.3	1	1.1	0.6	2.1	0.3	2.3
78	RU1404191	19.2	9.2	2.9	273.3	1.9	1.6	0.6	3.5	0.2	3.9
79	RU1404193	21.4	7.7	3	196.3	1.5	1.4	0.6	2.9	0.2	3.2
80	RU1404194	17.2	6.8	2.9	162.3	1.2	1.3	0.7	2.4	0.4	2.7
81	RU1404196	15.3	8	2.6	106.6	0.9	1.4	0.5	2.3	0.3	2.5
82	RU1404198	17.8	8.5	2.8	202.7	1.8	1.7	0.8	3.4	0.3	3.9
83	RU1504083	18.1	7.5	2.8	221.6	1.4	1.8	0.8	3.2	0.3	3.6
84	RU1504100	18.9	8	3.1	236.7	1.6	1.7	0.7	3.3	0.2	3.7
85	RU1504114	19.9	7.7	2.9	215.6	1.5	1.5	0.7	2.9	0.4	3.3
86	RU1504122	18.9	9.5	3	241.4	1.7	2	0.7	3.7	0.2	4.1
87	RU1504154	19.8	8.3	3.1	191	1.4	1.7	0.8	3.1	0.3	3.6
88	RU1504156	17.8	7.8	2.8	171.1	1.3	1.5	0.6	2.8	0.3	3.1
89	RU1504157	20.1	10	2.9	234	1.8	2	0.7	3.8	0.2	4.1
90	RU1504186	18.9	8.5	2.9	215	1.4	1.7	0.6	3.1	0.2	3.4
91	RU1504191	19.1	9.5	3.1	218.2	1.6	1.6	0.6	3.2	0.2	3.6
92	RU1504193	20.1	7.8	3	194.6	1.4	1.6	0.7	3	0.2	3.4
93	RU1504194	18.8	7.2	2.7	151.4	1.3	1.6	0.5	2.8	0.2	3.1
94	RU1504196	19.9	8.7	3	269.2	1.8	1.6	0.7	3.3	0.2	3.7
95	RU1504197	15.6	9.2	2.9	209.5	1.4	1.4	0.7	2.8	0.3	3.2
96	RU1504198	18.2	8	3	190.8	1.4	1.4	0.6	2.8	0.2	3.1
97	Sabine	17.4	9	2.8	166.4	1.3	1.7	0.7	3	0.3	3.4
98	Taggart	18.6	7.3	2.9	183.2	1.5	1.8	0.7	3.3	0.2	3.7
99	Thad	17.7	7.8	2.9	201	1.5	1.7	0.7	3.2	0.2	3.6
100	N-22	19.4	14.5	2.7	539.1	3.1	2.6	1.2	5.7	0.2	6.5
	Mean	18.8	9	2.9	230.5	1.6	1.7	0.7	3.3	0.2	3.7

Genetic variation among genotypes could be used as a tool during rice growth stages through vigor related traits in seedlings including plant height, tiller number,

canopy ground cover, and early crop biomass (Netnet 2012; Caton et al., 2003; Zhao et al., 2006). In a screening conducted by Cairns et al., (2009) measured the indicators of early vigor which include shoot length, shoot biomass, leaf area, number of roots, root biomass, partitioning coefficients, and growth rates. These results showed that the phenotypic correlation suggested that traits that were related and combined could be used to define early vigor. In agreement with Saito et al., (2010), this study presents that genotypes with high or above average mean value for the morphological or growth parameters can be identified as cultivars with qualitative and desirable vigor status. This gives a clue for the selection of variety for best survival and competition. (Table 2.3 and figs. 2.3, 2.4)

Root Parameters

The adjustment of root system architecture of currently cultivated rice genotypes could increase the chances of improving them to adapt to different abiotic stresses in order to increase yield. Also, in these days, the adaptive direction of the root systems of presently available genotypes is relative to their response of optimizing new tools of technology. The mean value of each root parameters exhibited among 100 rice genotypes during the two years experiment was shown in Table 4. Significant variation among the tested genotypes based on the root length was observed. The RL differed significantly among genotypes and ranged from 4276.6 cm (RU1303138) to 8445.7 cm (RU1303138), with an average of 6011.3 cm (Table 2.4). Like growth parameters, genotypes RU1404194 and GSOR101758 were recorded as the lowest averages in 62% of root growth and development parameters that including RL, RSA, RV, LRL, and RN, which indicated to be the least genotypes in vigor indices among 100 genotypes (Tables 4 and 6).

Table 2.4 Means of root length (RL), root surface area (RSA), average diameter (AD), root volume (RV), tips number (T), longest root length (LRL), root number (RN), SPAD, and quantum efficiency of fluorescence (Fv/Fm) for 100 rice genotypes.

Serial No.	Genotype name	Root parameters							Physiological parameters	
		RL	RSA	AD	RV	T	LRL	RN	SPAD	Fv/Fm
1	14CLPYT033	6011.4	753.4	0.4	8.3	31196.8	35.3	60.7	38	0.7
2	14CLPYT108	4897.3	683.2	0.5	7.6	32785.8	36.7	53.2	39.7	0.7
3	14CVPYT094	6591.4	925.7	0.5	10.5	38511.5	41.7	53.5	39.9	0.6
4	14CVPYT144	6412.8	881.6	0.4	9.8	38085.7	39.7	54	39.7	0.7
5	COLORADO	6672.3	813.1	0.4	9.2	37827.7	37.2	70.8	40.1	0.7
6	Bowman	6453.6	836.1	0.4	8.8	31820.2	41.2	50	41.8	0.7
7	CAFFEY	6713.7	994	0.5	11.9	34366.3	43.8	60	40.4	0.8
8	CHENIERE	5624	780.4	0.4	8.8	34283.8	42.5	53.7	36.8	0.8
9	CL Jazzman	5778.7	817	0.5	9.4	32443.3	40.2	59.8	40.8	0.7
10	CL111	6233.9	870.3	0.4	10	33523.5	42.7	57.5	39.5	0.7
11	CL142-AR	6190.9	823.6	0.4	8.9	37716.7	45.2	47.8	41.4	0.7
12	CL151	6093.2	784.3	0.4	8.2	40199.2	41.2	51.2	35.6	0.7
13	CL152	7148.8	949.6	0.4	10.1	39925.3	40.3	61.8	35.9	0.6
14	CL163	5469.2	605	0.4	6.4	33674.2	40.2	45.3	40.3	0.7
15	CL172	5884.7	872.5	0.5	10.4	30607.5	40.3	59.5	39.4	0.7
16	CL271	6812.7	993.2	0.5	11.9	33986.2	40.8	54.3	40.4	0.7
17	Cocodrie	6031.3	800.4	0.4	8.6	31780.3	38.5	52.7	41.3	0.6
18	NIPONBARE	6713.1	799.8	0.4	7.7	40761.2	38.5	69	37.2	0.7
19	ANTONIO	5697.3	773.1	0.4	8.6	32523.2	37.3	54.7	38.7	0.7
20	El Paso 144	7385.9	861.3	0.4	9.1	38545.7	41.8	69.8	37	0.7
21	GSOR100390	7663.6	1073.6	0.5	12.2	35136.5	42.2	63.7	38	0.7
22	GSOR100417	5584.2	854.1	0.5	10.6	27563.3	35	55	38.8	0.7
23	GSOR101758	4276.6	631.9	0.5	7.7	27493.5	33.8	53.7	37.4	0.8
24	RU1104122	5162.7	707.3	0.4	7.9	25522.2	38.8	49.3	39.3	0.8
25	CLJZMN	5920.4	842.9	0.5	9.7	35934.5	42	63	37.5	0.6
26	INIA Tacuari	5871.1	579.5	0.4	5.5	32523.7	38.2	48.2	39.9	0.8
27	IRGA409	6901.5	1094	0.5	14.2	35070.8	46.7	69.3	42.4	0.7
28	JES	7022.1	1018.3	0.5	12.1	43166.8	41.5	76	39.6	0.7
29	JUPITER	6233.3	889.4	0.5	10.1	31380	41.8	60.2	42.1	0.7
30	LA 2008	5355.4	699.2	0.5	8.4	30146.3	39.8	44	41.4	0.6
31	LA 2134	6238.7	807	0.4	8.5	43324.7	39.3	58.2	38.3	0.7
32	LAKAST	5675.9	807.8	0.5	9.3	29882.2	43	53.2	41.8	0.5
33	MERMENTAU	5759.3	806.1	0.4	9.1	34764	38.3	62.3	36.6	0.6
34	Presidio	5312.5	573	0.4	5.5	36649	39.5	49.5	37.1	0.8
35	Rex	6956	1042.6	0.5	12.9	37401.2	40.5	69.8	41.3	0.9
36	RoyJ	7124.6	817.7	0.4	8.6	35772	42.8	55.7	40.6	0.7
37	RU0603075	7419.8	1293.2	0.6	18.7	48164	42.3	69.5	38.7	0.6
38	RU1201024	5725.1	844.1	0.5	9.9	27257.3	40.3	58	43.2	0.7
39	RU1201047	5920.1	792.1	0.4	8.5	30102.7	44.2	45.2	39.7	0.7
40	RU1201136	6017.2	796.9	0.4	8.6	27555.8	41.8	53.5	40.3	0.7
41	RU1204156	5508.2	710.7	0.4	7.5	36427.3	37.2	55.2	34.9	0.7
42	RU1204197	6659.7	853.7	0.4	9	36835.8	40.5	59.7	39.1	0.7
43	RU1301084	5954.1	743.7	0.4	8.4	27439.5	41	59.8	43	0.7
44	RU1301093	5568.6	753.6	0.4	8.4	30143	39	54.5	44.3	0.7
45	RU1301102	7035.9	952.5	0.4	10.4	37464.8	42	65.5	40.1	0.7
46	RU1302192	5806.2	923.9	0.5	11.9	26201.3	42.3	66.8	41.9	0.7
47	RU1303138	8445.7	1352.8	0.5	18	47124.5	43.8	80.2	44.5	0.7
48	RU1303181	5390	714.4	0.4	7.6	31882	43	48.8	40.8	0.6
49	RU1304114	6135.3	803.7	0.4	8.5	34293	37	59.7	38.4	0.7
50	RU1304122	6195.4	846.7	0.4	9.5	33487.5	37.7	57	39.8	0.9
51	RU1304154	5716.6	807.3	0.4	9.3	33689.8	38.2	58	38	0.8

Table 2.4 (Continued)

52	RU1304156	6247.2	767.8	0.4	8.1	35902.8	40	52.2	41	0.8
53	RU1305001	7420.7	1059.5	0.5	12.2	36285.8	41.7	56.2	42	0.7
54	RU1401067	5132.9	715.1	0.5	8.3	27180	37.7	52.5	41.7	0.7
55	RU1401070	5446.1	725.9	0.4	7.8	27939.8	42.7	46.8	42.9	0.8
56	RU1401090	5426.2	744.1	0.4	8.2	30864.7	43.3	53	39.1	0.8
57	RU1401099	6610.8	917.2	0.5	10.3	36093.7	40.5	51.2	41.1	0.7
58	RU1401102	5730.7	803.8	0.5	9	28930.7	40	60.2	39.4	0.6
59	RU1401145	4824.9	645.7	0.4	7.6	24317.8	37	52.5	42.7	0.7
60	RU1401161	5863.4	767.6	0.4	8.1	33153.7	43	52.2	37.5	0.8
61	RU1401164	6377	939.2	0.5	11.5	33867.5	41.8	60.7	40.1	0.8
62	RU1402005	6005.6	781.6	0.4	8.3	35149.2	42.3	55.5	41.5	0.6
63	RU1402031	6640.8	917.9	0.4	10.2	36466.8	42.7	55.2	40	0.7
64	RU1402065	6073.6	902.9	0.5	10.8	35501.5	43.2	64.5	40.9	0.7
65	RU1402115	6987.5	975.7	0.5	11.3	42342.7	40.2	69.3	40.3	0.7
66	RU1402131	6930.3	1009.4	0.5	11.8	32744.7	43.2	68.5	39.7	0.7
67	RU1402134	6551.4	893.9	0.4	9.8	35335.8	38.7	68.2	38.2	0.7
68	RU1402149	6175.9	742.8	0.4	7.2	34012	39.3	48.8	39.6	0.7
69	RU1402174	4612.6	619.9	0.4	6.8	26159.3	41.3	57.5	37	0.9
70	RU1402189	5700.9	751.4	0.4	8	32759.8	43	51.7	39.4	0.7
71	RU1402195	5019.7	711.7	0.4	8.1	27102.8	38.8	59.3	40.7	0.7
72	RU1403107	6668	776.9	0.4	8.1	39361.3	38.2	54	39.2	0.6
73	RU1403126	5686.8	823.4	0.5	9.8	34991.3	43.5	48	40.4	0.7
74	RU1404122	4655.2	639.6	0.4	7.1	27679	38.8	44.8	43.5	0.7
75	RU1404154	5731.9	722.9	0.4	7.5	28985.2	39.3	46.5	39.2	0.7
76	RU1404156	5615.1	742.7	0.4	7.9	29668	39.2	58.5	38.6	0.7
77	RU1404157	4534.1	595.4	0.4	6.3	33050.8	38.8	46	35.4	0.6
78	RU1404191	6009.1	840	0.5	9.5	36309.2	39.3	54.8	42.2	0.7
79	RU1404193	5754.7	756.7	0.4	8	37619.5	39.3	53.7	40	0.7
80	RU1404194	5503.8	598.9	0.4	5.6	31776.3	41.5	45	36.5	0.6
81	RU1404196	4436.2	523.7	0.4	5	32676.5	36	40.8	35.7	0.7
82	RU1404198	5652.3	661.4	0.4	6.6	30430.3	41.3	61.2	38	0.8
83	RU1504083	5974.6	748	0.4	7.5	31388	40.7	44.8	39.1	0.7
84	RU1504100	5895.1	839.8	0.4	9.7	33118	41.3	57.5	42.1	0.5
85	RU1504114	5075.8	704.1	0.4	7.9	28998.2	39.7	50	39	0.7
86	RU1504122	6066.6	794.9	0.4	8.5	37411	38.3	58.3	40.1	0.6
87	RU1504154	6305	817.3	0.4	8.7	36307	39.8	57.2	39.7	0.7
88	RU1504156	4903.2	630.5	0.4	6.7	32340.5	37.5	56.2	40.2	0.8
89	RU1504157	6120.5	885.4	0.5	10.3	30177.3	41.5	57.5	38.5	0.6
90	RU1504186	5725.6	719.7	0.4	7.3	30346.3	41.3	56.7	40.7	0.7
91	RU1504191	5746.8	811.5	0.5	9.2	30356.7	40	50.2	40.3	0.8
92	RU1504193	5592.2	740.5	0.4	8.1	29395.8	41.3	51.5	40.2	0.7
93	RU1504194	5113.2	636.9	0.4	6.4	31182.5	39.3	55.3	40.7	0.8
94	RU1504196	6245.6	866.6	0.4	9.6	31805.8	39.7	57.8	41.4	0.8
95	RU1504197	5289.8	711.1	0.4	7.7	26089.2	44	47.8	43	0.6
96	RU1504198	5297.3	743.1	0.4	8.4	28851.8	42.5	44.2	42	0.6
97	Sabine	6188.5	775.4	0.4	7.9	35137.3	39.8	57.5	42.4	0.7
98	Taggart	6027.4	797.7	0.4	8.5	35199.5	41.7	58.5	42	0.7
99	Thad	6156.5	840.9	0.4	9.2	31051.5	39	56.7	41.6	0.7
100	N-22	8010.4	1444.8	0.6	21.3	45658.8	42.8	81.5	40	0.6
	Mean	6011.3	816.3	0.4	9.2	33538.4	40.4	56.5	39.9	0.7

However, genotypes N-22 and RU0603075 were recorded as the highest averages in 62% of root growth and development parameters that including RSA, AD, RV, and T, which indicated to be the highest genotypes vigor indices among 100 genotypes (Tables 2.4 and 2.6). 198 and 199% reduction of variability among genotypes in root number of tips and root number, which interpretation the strong positive correlation between them (Table 2.4 and 2.5). It would be worthwhile to mention that approximately, 52% of genotypes were exceeded a grand average in RL, T, and RN while 62% of genotypes were above a general average of RSA, RV, and LRL, respectively (Table 2.4). Genotypes N-22, RU1303138, and RU0603075 are a potentially productive genotypes while RU1404196, RU1404157, and INIA Tacuari were identified with least vigor and root morphological traits and, therefore can be classified as a poor cultivar due to the consistent low traits exhibited in both experimental years which should be avoided in terms of crop yield, competitiveness, and productivity. Genetic diversity is important in plant breeding which is used in assessing diverse genotypes for improvement of more desirable traits. Rice crop is well-diverse and has evolved into a tremendously broad base for genetic diversity as reflected by a number of landraces existing today (Sujay, 2007). Early emergence of a vigorous crop stand provides better root anchorage and improves nutrient absorptive capacity (Farooq et al., 2011), root hairs protect the water status of young root tissue (Tanaka et al., 2014). Multiple studies have identified links between root traits and crop productivity (Kell, 2011) and other studies used root length as representative indicators for seedling vigor (Redoña and Mackill, 1996). Longer or deeper root length is a viable root trait in selecting crop genotypes. Deeper root enables the plant to access stored water in the deep layers of the soil substratum (Wasson et al.,

2012). Hailing et al., (2013) confirmed that root tips with large diameters had improved root penetration to the soil. Root growth and development parameters like RL, RV, RSA and root thickness determine the root hydraulic conductance that can potentially increase water uptake by rice under water-limited conditions (Henry et al., 2012); Singh et al., 2017b). In this study, rice genotypes with higher values of RL, RV, RSA, and RD may be a desirable cultivar with potential productivity under water-limited areas. Under specific conditions, the differences in root growth (RSA and RL) among rice cultivars may be due to the genetic differences in their root hydraulic conductivity (Henry et al., 2012). Similar to this study, Jaleel et al (2009) identified variability for root growth and proliferation among rice lines during seedling stages. Genotypic variation in root length and some identified root traits in this study suggests that these traits could be selected in breeding programs through marker-aided selection as well as through direct phenotypic screening (Reynolds et al., 2012).

Physiological parameters

Knowledge of relationships between growth performance and physiological parameters of seedlings and respective genotypic differences could permit selection of genotypes at early growth stages. Rice genotypes have yet to be classified in a way that will allow identifying of these genotypic differences for physiological traits. The value of SPAD index indicates the relative greenness of leaves that is an indirect point of chlorophyll content. The variability per cent of ranged minimum and maximum values of SPAD was 127% among genotypes (Table 2.4). The value of SPAD index was lowest for genotype RU1204156 (34.9) and highest for RU1404194 (44.5), with general average 39.9. A significant difference (P<0.05) in the value of SPAD index was observed

between genotypes and years interaction, this indicates that genotypes differ in chlorophyll concentration and response genetically to the different seasons (Table 2.2). The results of this study showed that 80% of rice genotypes differing in fluorescence (Fv/Fm) were exceeded the general average (0.7), with minimum and maximum fluorescence in LAKAST (0.5) and RU1304122 (0.9), respectively.

Simple Correlation Matrix

If the correlation is strong between a set of desirable traits then if we make a selection for one character, the other character will automatically be taken care (Falconer, 1964). Correlation studies indicate the magnitude of the association between pairs of characters and are useful for selecting genotypes with desirable combinations of characters thereby aiding the plant breeder in crop improvement. Table 2.5 shows the estimated simple correlation matrix among the variable of parameters among 100 rice genotypes. There is a significant correlation among the root and growth parameters except for LN. Correlation between shoot dry weight and total dry weight showed the highest positive significant correlation (98%) among all shoot, root, and physiological parameters and followed by a correlation between root surface area and root volume with (92%). However, the lowest negative correlation was observed between root-shoot ratio and fluorescence (Fv/Fm) -45%). The results of this study are reliable with the previous reports on rice (Farooq et al., 2009). The differences observed for aboveground parts (LN, LA and TN), chlorophyll content (SPAD value), and root growth (RN, RL and RSA) might have contributed to the cultivar differences (Jaleel et al., 2009; Farooq et al., 2011).

Table 2.5 Estimates of simple correlation matrix among the variables of rice parameters, obtained of 100 rice genotypes.

	TN	LN	LA	LW	SW	RW	SHW	RS	TW	RL	RSA	AD	RV	T	LRL	RN	SPAD	Fv/Fm
Plant height (PH)	0.01 n.s	0.17**	0.13*	0.25**	0.23***	0.07 n.s	0.17***	0.19***	0.15**	0.19***	0.24***	0.13*	0.18**	0.05 n.s	0.18**	0.07n.s	-0.02n.s	-0.10 n.s
Tiller number (TN)		0.23**	0.64**	0.59**	0.47374	0.29***	0.58***	0.52***	0.56***	0.32***	0.53***	0.36***	0.61***	0.27***	0.14*	0.48***	-0.2***	-0.41***
Leaf number of main stem (LN)			0.04n.s	0.02n.s	0.02n.s	0.01n.s	0.01n.s	0.01n.s	0.01n.s	0.03n.s	0.02n.s	0.08n.s	0.01n.s	0.01n.s	0.10n.s	0.01n.s	-0.01n.s	-0.01n.s
Leaf area (LA)				0.80**	0.56***	0.43***	0.54***	0.57***	0.49***	0.41***	0.67***	0.49***	0.73***	0.24***	0.24***	0.52***	0.13***	-0.31***
Leaf dry weight (LW)					0.65***	0.48***	0.71***	0.69***	0.65***	0.54***	0.77***	0.50***	0.79***	0.30***	0.31***	0.59***	0.11***	-0.39***
Stem dry weight (SW)						0.37***	0.64***	0.62***	0.59***	0.47***	0.64***	0.39***	0.64***	0.25***	0.26***	0.54***	-0.12***	0.39***
Root dry weight (RW)							0.47***	0.24***	0.43***	0.32***	0.48***	0.30***	0.52***	0.14***	0.15***	0.36***	-0.03***	-0.05n.s
Shoot dry weight (SHW)								0.75***	0.98***	0.52***	0.65***	0.37***	0.65***	0.35***	0.23***	0.56***	-0.09n.s	-0.45***
Root shoot ratio (RS)									0.68***	0.47***	0.66***	0.45***	0.70***	0.31***	0.26***	0.50***	-0.06n.s	-0.41***
Total dry weight (TW)										0.48***	0.59***	0.32***	0.59***	0.33***	0.21***	0.53***	0.07***	0.42***
Root length (RL)											0.78***	0.04n.s	0.54***	0.71***	0.28***	0.45***	-0.08n.s	-0.26***
Root surface area (RSA)												0.46***	0.92***	0.48***	0.39***	0.62***	0.02n.s	0.38***
Average root diameter (AD)													0.69***	0.16**	0.25***	0.39***	-0.13*	-0.22***
Root volume (RV)														0.27***	0.38***	0.63***	-0.09n.s	-0.38***
Tips number (T)															0.09n.s	0.27***	-0.06n.s	-0.23***
Longest root length (LRL)																0.15**	-0.13*	-0.07n.s
Root number (RN)																	0.09n.s	-0.61n.s
Chlorophyll content (SPAD)																		0.27***

† *, **, *** represent significant differences at the 0.05, 0.01, and 0.001 P level, respectively.

†† n.s represents nonsignificant differences at the 0.05 P level.

Classification of Rice Genotypes

Cumulative Vigor Response Index and PCA

Rice genotype classification is a complex process. Morphological, ecological and population characteristics were studied in the wild and cultivated rice (Counts and Lee, 1987; Pang et al., 1995). Different qualitative and quantitative characteristics were also studied (Fatokun et al., 1986). Rice diversity panels to identify trait combinations that occur naturally might cover genetic makeup, geographic origin, the ecosystem of adaptation and genetic levels of improvements (Jahn et al., 2011). Understanding physiological changes to improve photosynthetic efficiency in rice is one of the key components in present physiological research (Reynolds et al., 2012).

The CVRI values for $year_1$ and $year_2$ for each rice genotype or cultivar were derived by summing individual vigor response indices for all root and shoot parameters among the 100 rice genotypes evaluated in this study (Table 2.6). The CVRI-based technique was used to identify genotype as very low, low, moderate, high and very high vigor response index. The 100 rice genotypes were classified into these five (5) different groups based on the combined mean values of the vigor response indices of morpho-physiological parameters and standard deviation. Sixteen genotypes were identified within the range 21.36- 23.75 were classified as very low vigor response. Genotypes classified as low vigor response were within the range 23.76-26.13; forty-three (43) out of 100 tested rice genotypes were identified with this group. Thirty-three (33) rice genotypes within the range of 26.14 – 28.51 were classified as moderate vigor response. High vigor response rice genotypes within 28.52-30.90 range were five (5) out of the 100 rice genotypes.

Table 2.6 Classification of 100 rice genotypes based on combined response indices for morpho-physiological features during seedling growth stage, 30 d after planting.

Very low		Low		Moderate		High		Very high	
21.36-23.75		23.75-26.13		26.13-28.51		28.52-30.90		30.91-33.28	
RU1404196	(21.36)	RU1401070	(23.78)	RU1304156	(26.19)	JUPITER	(28.77)	RU0603075	(33.94)
RU1404157	(21.59)	RU1404156	(24.21)	LA 2134	(26.20)	RU1402131	(29.07)	RU1303138	(34.08)
INIA Tacuari	(22.91)	RU1504114	(24.24)	RU1504154	(26.20)	El Paso 144	(29.39)	N-22	(36.17)
RU1404122	(23.03)	RU1303181	(24.29)	RU1304154	(26.23)	JES	(29.69)		
RU1504156	(23.03)	RU1401090	(24.38)	RU1404191	(26.25)	IRGA409	(30.71)		
RU1504194	(23.06)	RU1404193	(24.51)	RU1402005	(26.28)				
RU1104122	(23.13)	RU1504186	(24.57)	CL Jazzman	(26.35)				
CL163	(23.24)	RU1402149	(24.57)	CLJZMN	(26.38)				
GSOR101758	(23.25)	RU1401067	(24.58)	RU1504196	(26.50)				
RU1401145	(23.41)	RU1504193	(24.62)	RU1504157	(26.65)				
RU1404194	(23.43)	Bowman	(24.68)	14CVPYT144	(26.67)				
RU1504198	(23.44)	LA 2008	(24.70)	RU1504122	(26.80)				
RU1402174	(23.50)	RU1301093	(24.71)	CL172	(26.85)				
14CLPYT108	(23.65)	RU1404154	(24.75)	RU1402065	(27.04)				
Presidio	(23.65)	RU1402195	(24.81)	14CVPYT094	(27.09)				
RU1504197	(23.72)	RU1504083	(24.81)	RU1204197	(27.11)				
		14CLPYT033	(24.86)	RU1401099	(27.16)				
		RU1204156	(24.86)	COLORADO	(27.22)				
		RU1201047	(24.90)	RU1302192	(27.31)				
		Thad	(25.09)	Rex	(27.32)				
		RU1402189	(25.10)	RU1402134	(27.36)				
		ANTONIO	(25.12)	RU1401164	(27.41)				
		NIPONBARE	(25.12)	RU1402031	(27.59)				
		LAKAST	(25.13)	RU1304122	(27.79)				
		GSOR100417	(25.17)	RU1301102	(27.80)				
		CHENIERE	(25.18)	CL271	(27.82)				
		RU1403107	(25.19)	CL111	(27.85)				
		RU1201136	(25.20)	CL152	(27.87)				
		Taggart	(25.25)	RoyJ	(27.88)				
		RU1401161	(25.27)	GSOR100390	(27.90)				
		Sabine	(25.31)	RU1305001	(28.16)				
		MERMENTAU	(25.35)	RU1402115	(28.25)				
		RU1504100	(25.43)	CAFFEY	(28.38)				
		RU1403126	(25.49)						
		RU1404198	(25.53)						
		Cocodrie	(25.57)						
		CL142-AR	(25.61)						
		RU1504191	(25.69)						
		RU1201024	(25.79)						
		CL151	(25.79)						
		RU1304114	(25.86)						
		RU1401102	(25.87)						
		RU1301084	(25.95)						

Identified genotypes are JUPITER (28.77), RU1402131 (29.07), El Paso 144 (29.39), JES (29.69), and IRGA409 (30.71).

Very high vigor response ranges between 30.91- 33.28. 3 and genotypes; RU0603075 (33.94), RU1303138 (34.08), and N-22(36.17) were classified in this category. Genotype RU1404196 has portrait a very weak trait while genotype N-22 has displayed a high level of consistent vigor traits under these two experimental years ($year_1$ and $year_2$) (Table 2.6). Rice Genotypes with moderate, high and very high vigor response are genotypes with good productivity potential. The high correlation coefficient (r^2) was observed between experimental $year_1$ or $year_2$ and CVRI for 100 rice genotypes; ($r^2 = 0.77$, P = 0.0001) ($r^2 = 0.76$, P = 0.0001), respectively, indicates that response of genotypes to all parameters was almost the same for $year_1$ and $year_2$ as well (Fig. 2.1).

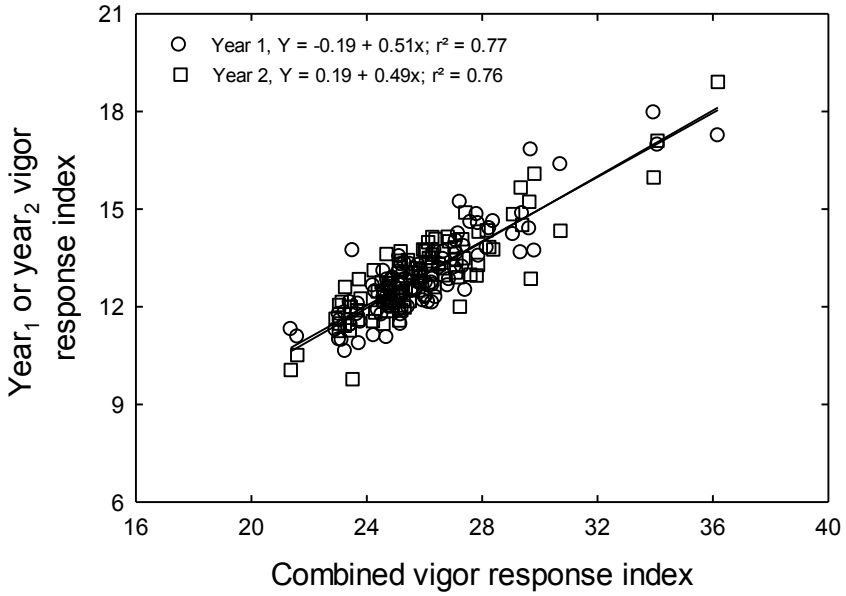

Figure 2.1 Relationship between combined vigor response index and year 1 or year 2 vigor response index of 100 rice genotypes.

The correlation coefficient between shoot or root vigor indices with combined vigor response index were almost equal value with (r^2 = 0.92, P = 0.0001) (r^2 = 0.93, P = 0.0001), respectively (Fig. 2.2).

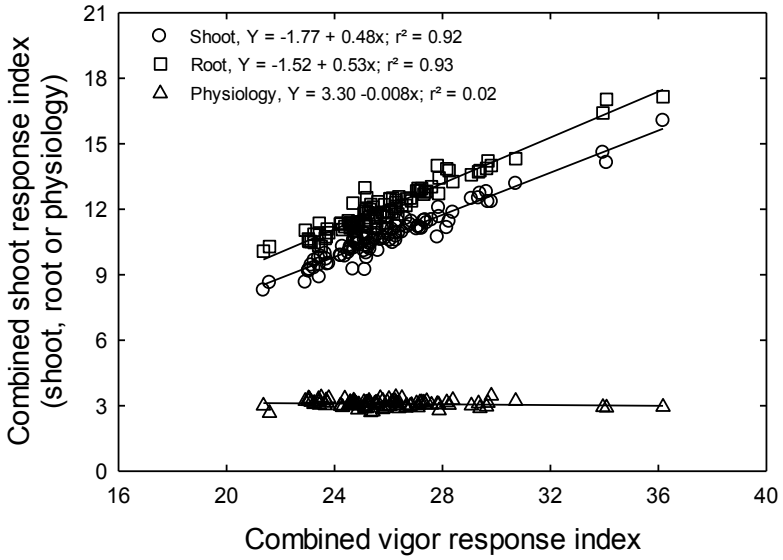

Figure 2.2 Relationship between combined vigor response index and shoot, root or physiological combined vigor response index of 100 rice genotypes.

This suggests that either root or shoot parameters might be sufficient in evaluating vigor during early season, and selection based on both shoot and root traits should be strong to enhance classification. There is no significant liner regression relationship between the physiological traits and combined vigor response index. This suggests that the effectiveness or significance of the two physiological parameters used in this study is being undermined by 19 morphological parameters.

Rice genotypes with strong early vigor are eligible for crop establishment in the direct-seeded systems, especially in upland growing environments (Namuco et al., 2009). Also,

the strength of relationships between growth performance and physiological parameters was analyzed using correlations in multivariate analysis including principal component analysis. PCA has been cast-off as a data density technique for preserving the total variance in the alteration and minimizing the mean square estimated errors (Ingebritsen and Lyon, 1985). In this study, PCA was performed to recognize the principal components of shoot, root and physiological parameters of 100 rice genotypes that best described the vigor response and to identify low, intermediate and high stability rice genotypes. For this analysis, only the intermediate and high stability were considered because our goal was to categorize the genotypes according to their vigor response and their consistency or stability. More than 70.1% of total variation among genotypes was explained by the first three PCs (Fig. 2.3 and 2.4).

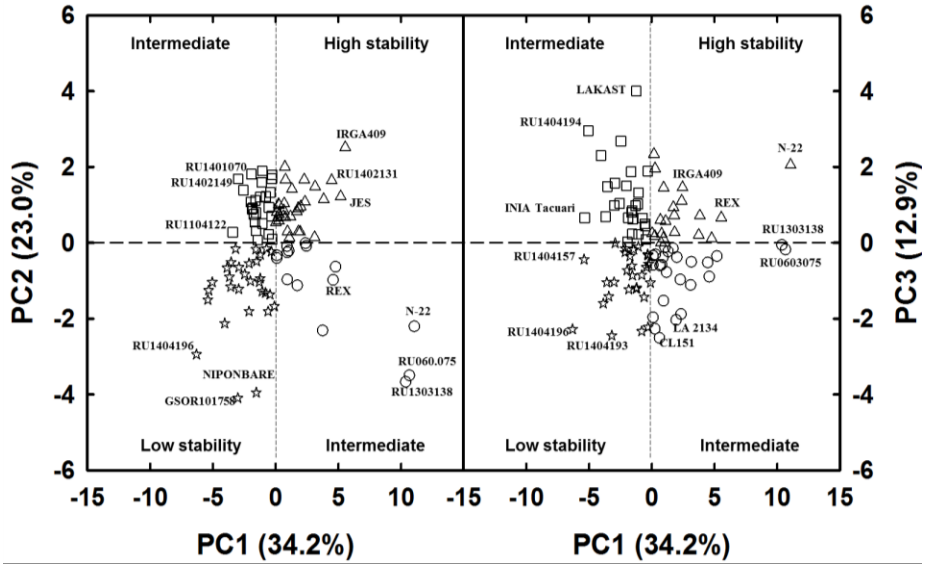

Figure 2.3 Principal component analysis (PCA) biplot for the first and second principal component (PC) scores (PC1 vs. PC2) and first and third principal component (PC1 vs. PC3), related to the classification of 100 rice genotypes for early-season vigor response index based on 19 morpho-physiological traits.

The first principal component (PC1) versus (PC2) can be interpreted as in lieu of positive values for LN, SPAD and Fv/Fm, and RS to a lesser extent. In other words, eigenvectors of PC1 had higher positive values for LW, LN, LA, RV, RN, TW, and SHW and to a lesser degree, RS, LN, Fv/Fm and SPAD (Fig. 2.4). Therefore, genotypes with high scores for PC1 tend to higher values for LW, LN, LA, RV, RN, TW, and SHW. This result matching by 95% with classification table indicates that using a vigor response index is a good method to classify genotypes along with PCA to get highly accurate results. For instance, the genotype N-22, with a higher score for PC1, had the higher values for LW, LN, LA, RV, RN, TW, and SHW (Fig. 2.3 and Table 2.4). Consequently, a biplot of PC1 vs. PC3 (Fig. 2.4) should separate the rice genotypes that have higher values for TW, RL, SW, RSA, RV, and TN with relatively low values for RS, LN, Fv/Fm and SPAD.

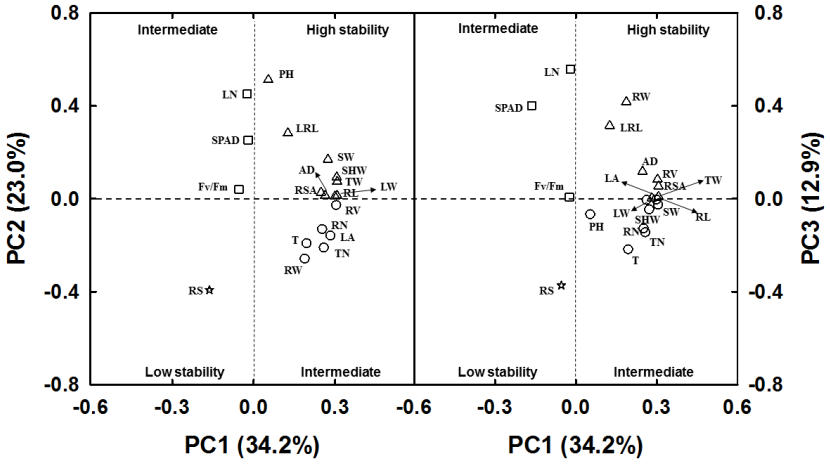

Figure 2.4 Principal component analysis (PCA) biplot for the first and second principal component (PC) scores (PC1 vs. PC2) and first and third principal component (PC1 vs. PC3), related to the classification of 100 rice genotypes for early-season vigor response index based on 100 rice genotypes.

Therefore, genotypes N-22, RU1303138, RU0603075, IRGA409, JES and REX are considered as a cultivar with high vigor stability because of its relatively higher scores for PC1 and PC2. In contrast, RU1404196, RU1404157, RU1404193, GSOR101758 and NIPONBARE can be classified as low stability as they have relatively small values for RS, LN, Fv/Fm and SPAD.

As a result, the biplot of PC1 vs. PC2 grouped the rice genotypes RU1401070, RU1402149, and RU1104122 are classified as a moderately stable variety or Intermediate on the left top corner of the graph. The CVRI analysis (Fig. 2.2) also identified Fv/Fm and SPAD is not an effective parameter in selecting vigor response index in classifying rice genotype. The scores of PC1, PC2, and PC3 collectively contributed greater importance in the rice genotype separation for vigor response stability. We used PCA to group the hybrids into the low and high stability of 100 rice genotypes. We discovered that TW, RL, LW, TN, RN, RSA, RN and SHW were the parameters that best described rice genotype stability. Evaluation of genotypes for the stability of performance under varying environmental conditions is an essential part of any breeding program (Tariku et al., 2013). Therefore, this experiment can be repeated under a varied environmental condition for high quality genotype selection.

CHAPTER III

PHYSIOLOGICAL AND YIELD VARIABILITY AMONG THE 100 ELITE RICE GERMPLASM

Abstract

Rice is one of the most important food crops mainly consumed as a stple food globally. But the global population is rapidly increasing which is projected to increase from seven to nine billion by 2050. Therefore, rice production also needs to be increased accordingly to sustain the global food security. Therefore, identifying genetic variability for yield related morpho-physiological traits and screening for early maturity rice lines are the two improtant traits to enhance rice production. In this study, 100 elite rice genotypes were screened in pot-culture under natural enviromental conditions for the identification best morpho-physiological descriptors for enhanced yield and early maturity. Different shoot morphogical and physiological parameters were measured at vegataitve, flowering and maturity stages to asses plant vigor, maturity and yield. Genotypyes were then evaluated and clustterd into different vigor, maturtiy and yield gropus based on the individual and cummulative vigor response indices. Cumulative vigor response index (CVRI) was calculated by summing up all indices of measured traits for each rice line. The CVRI of morpho-physiological traits varied from 8.43 (Sabine) and 8.72 (RU1404154) to 10.39 (CL JZMN) and 10.60 (GSOR100417), respectively of traits were taken at 55-60 and 105-115 DAS . However, the CVRI of

yield-related parameters varied from 6.48 (RU1404154) to 8.14 (RU0603075). Based on this method, five groups were identified; (42, 30, 20%) of genotypes were classified as high and very high vigor index, while (22, 28, and 36%) of genotypes were classified as low and very low vigor index, (36, 36, and 44%) falling under moderate vigor index at seedling growth, grain filling, and harvest stages, respectively. The high and significant correlation between the combined vigor response index (CVRI) and physiological vigor response index (r2 = 0.82 and 0.83) measured during seedling growth and grain filling stages, indicated that physiology parameters could be used in screening rice genotypes for vigor. The identified CVRI values for various lines will be useful in rice breeding programs to select and develop new genotypes with greater vigor and yield.

Introduction:

Rice (*Oryza sativa* L.) is an important global food crop that feeds more than half of the global population. It is used as a staple food particularly in rice producing areas of Asia, Africa and South America where people are getting their major energy requirements from rice and its derived products. Although, rice is grown and harvested from over 163 million ha in more than 100 countries with 481 million metric tons of production but this production is still not good enough to feed the increasing population and needs to be increased by another 38% within the next 25-30 years (Surridge, 2004; Joseph et al., 2010).

Global population is increasing rapidly and expected to reach up to 10 billion before 2100 (United Nation 2011). Rice production needs to be increased quantitatively as well as improved qualitatively in order to meet the demands of growing population of 21st century to maintain food safety. Although, green revolution and some research institutions

including International Rice Research Center (IRRI) helped to solve the world's demand for food to certain extent by developing improved rice verities and hybrids with improved quality and increased production, but this increase in production is not enough for the exploding population of 21st century. Worldwide rice production must reach 800 million metrics of rice to meet projected demand in 2025, which is 318 million metrics more than rice production in 2017. Ray et al., (2013) found global's agrarian production of four critical crops including rice may need to increase by 60-110% to meet increasing demands and provide food security. Mean yield of 9 Mg ha^{-1} is very close to the predestined climate-adjusted yield potential of sitting rice cultivars in the main rice-growing areas (Matthews et al., 1995).

In the United States, rice is mainly grown in two distinct regions including US Mid-south and the Sacramento Valley in California. These two U.S. rice production regions have developed different germplasm pools to address biotic and abiotic factors, particularly, traditional japonica varieties in Mid-south US are well adapted to the environmental situation as they have been improved by breeding programs in both distinct regions (McKenzie et al., 2014).

There are a number of problems limiting the increase in rice production including both biotic and abiotic factors. Global climate change is causing a major part of low crop yield due to the increased natural disasters mainly abiotic stress (Du et al., 2015) (drought, flooding, salinity etc., along with some other factors including poor quality seeds, low yielding cultivars, poor management practices, insect pests and diseases. Although, there are limited studies on the role of physiological tritas in relation to enhancing yield of rice cultivars during seedling and grain filling stages but some of the

physiological traits including photosynthesis, chlorophyll fluorescence, and air-canopy temperature differential, cellular membrane integrity (Sullivan, 1972) and specific leaf area (Murata ,1975) have been considered as selection tools for most of the abiotic stresses in relation to crop servival and yield. However, morphological yield related triats have been commonly used as a criterion to assess phenotypic variability and enhance yield. For example, rice grain yield is a complex parameter and is positively correlated with most of the quantitative yield related traits, including number of panicles, plant height, and grain weight, and dependent on the number of tillers, filled grain, and number of spikelets (Mohammadi et al., 2009; Xing and Zhang, 2010; Yoshida 1983). Similarly, total number of tillers and days to panicle initiation (Amirthadevarathinam, 1983), the number of panicles per plant and 1000-grain weight, spikelet number per panicle, grains per panicle have been found to have direct effect on enhancing grain yield per plant which ultimately enhances the total yield of the crop (Yang, 1986; Kumar, 1992; Ram, 1992; Sundaram and Palanisamy, 1994; Mehetre et al., 1994; Samonte et al., 1998; Sürek et al., 1998).

Phenotypic variability of any crop is dependent on the genetic variability present among the cultivars and is important for the comparison, identification and characterization of genetic variation at DNA or molecular level. Genetic variability among the genotypes can be estimated by measuring the physiological and morphological differences of economically important quantitative traits. Although, morpho-physiological and phenotypic assessment is labor intensive and time consuming but they are the ultimate responses of variability among the genotypes in the field, on which high yielding cultivars can be selected for breeding programs.

Rice is rich in genetic diversity and breeders have a wide option when they are looking for parental materials. However, the exploitation of high yielding rice genotypes requires elaborate knowledge of the genotypic variability presents in among the genotypes, the identification of yield enhancing traits, inputs requirements, and management practices (Dutta et al., 2013; Kishor et al., 2008). Therefore, screening for vigor and evaluation of genotypes for genetic variability are the two key factors for a successful breeding program. Also, a successful breeding program depends on the genetic diversity of a crop for achieving the goals of improving the plant vigor and producing high yielding varieties (Padulosi, 1993). Thus, the exploited genetic variability could be used to fill the significant gap between the maximum theoretical yield and the yield potential of the best available rice genotypes (Setter et al. 1995) or at least increase the mean farm yield of rice producers in order to enhance yield.

Screening for early vigor is considered as the most favorable and an effective approach to release any constraint (Okami et al., 2015). Early vigor the ability of young plant to attain rapid growth and development after emergence. Vigor indices provide information about the growth rate and uniform development of cultivars under variable environmental conditions (Powell and Matthews, 2005) which is very critical for crop growth and development when competing for limited resources of water, light, air etc. Vigorous plant usually competes successfully under limited resources and stress environmental conditions, influencing stand establishment and ultimately grain yield. Estimation and comparison of growth, development, and physiological traits of rice genotypes using combined vigor response index (CVRI) to examine the relationship among growth, physiological, and yield-related parameters is considered as one auxiliary

way for breeders to better understand physiological changes during development of rice breeding lines and genotypes grown under a sunlit condition in Mid-south U.S.

The objectives of this study were (1) to assess morpho-physiological traits during the seedling, grain filling and maturity stages and determine significant traits in relation to yield of 100 elite rice genotypes, (2) develop a method to identify vigor variability among the selected rice genotypes, and (3) classify and rank rice lines based on vigor response indices during reproductive stages.

Material and method

Planting material

An experiment, arranged in a randomized complete block design with four replications and comprising of 100 rice breeding lines and released varieties was conducted on 7 May 2015 at the Environmental Plant Physiology Laboratory, Mississippi State University, Mississippi State (lat. 33° 28′ N, long. 88° 47′ W). The soil medium used consisted of pure fine sand. 400 pots per line in 10 rows with 40 pots per row. Four pots per genotype are used. The PVC pots were 6" diameter by 24" high, with 12 litter capacity were arranged randomly in the lines. Six seed per pot were sowned in the morning at a depth of 1 inch. The plants were thinned to one after 11 days of emergence. A drip irrigation system using fresh water was extended from sowing to seedling emergence to all the pots after which Hoagland Nutrition Solution was used for irrigation three times daily:- (1) 8:00am, (2) 1:00pm, and (3) 6:00pm.

Growth and development

Plant height (PH, cm plant^{-1}) (measured from the base of the stem to tip of the collar -reaching leaf) and the tillers number (TN, no. plant^{-1}), leaf number of main stem (LN, no. plant^{-1}) were measured during vegetative and grain filling stages. Specific leaf area (SLA, cm^2 g^{-1} d wt.) (with two leaves taken randomly from the main stem after which leaf area was measured using a leaf area meter (LI-3100: Li-COR, Lincoln, NE, USA). Samples were put in the a dryer at 70°C for 72 h then after that specific leaf area was estimated by dividing the leaf area by dry leaf weight at vegetative (55-65 DAS) and grain filling stages (105-115 DAS).

Gas exchange parameters

Leaf net photosynthesis (Pn), transpiration rate (Tr), stomatal conductance (Cond), electron transport rate (ETR), and chlorophyll fluorescence (Fv'/Fm') were measured at vegetative stage 55- 65 DAS and at grain filling stage 105-115 DAS between 1000 and 1400 h on sunny days, using the third recently fully expanded leaf from the top using the penultimate leaves of the individual plant using an LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA). When measuring gas exchange parameters, the photosynthetic photon flux density (PPFD), provided by a 6400-02 LED light source, was set to 1500 µmol m^{-2} s $^{-1}$. The temperature and CO2 concentration in the leaf cuvette were set to 30°C and 350 ppm (ambient CO_2 level in the greenhouse), respectively. Leaf water use efficiency (WUE) was calculated as the ratio of photosynthesis (Pn) to transpiration rate (Tr).

Leaf total chlorophyll content

Chlorophyll was measured on the penultimate leaves at the vegetative stage and at grain-filling of the individual plant for all replications. Five leaf discs (2.0 cm^2 each) were acquired from mid-blade whereas avoiding the mid-vein and set in a vial (5-ml) with 5-ml dimethyl sulphoxide (DMSO) and incubated at room temperature for 24 h in darkness to allow for complete extraction of chlorophyll into the solution. The absorbance of the extract was measured in microtiter plates of polypropylene material using a Bio-Rad ultraviolet/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA) to estimate the concentrations of carotenoid content and total Chlorophyll, which was determined by summing up the values of chlorophyll a and chlorophyll b (Chapple et al., 1992). The concentrations of the total chlorophyll and carotenoid were calculated from the absorbance values at 470, 648, and 663 nm using equations described by Lichtenthaler (1987).

Leaf membrane thermal stability

Five leaves were harvested from all rice plants at vegetative stage 55-65 DAS and at grain-filling stage 105-115 DAS for the leaf membrane thermal stability assay using the procedure described by Martineau et al., (1979). Each sample was assayed to consist of two sets (control and treatment) of 5 leaf discs (1.3 cm^2 diameter punch) each, from the penultimate leaves. The samples were placed into two separate test tubes with 10-mL deionized water. Each sample was replicated four times. All leaf discs samples were thoroughly rinsed three times with deionized water then covered with aluminum foil to block evaporation of water. A treatment set of test tubes was submerged in a water bath for 20 minutes at 55 °C to a depth equal to the height of water in the tubes. A control set

of the test tube was left at room temperature (25 °C). Both sets of test tubes were then incubated at 10 °C for 24 h. Conductance was measured using an electrical conductivity meter (Corning Checkmate II; Corning Inc., Corning, NY) to determine a first measurement of both sets of test tubes (control, CEC1 and the treatment, TEC1). The two sets of test tubes were then autoclaved for 12 min at 0.1 MPa to release all the electrolytes and to eradicate tissues after which conductance was measured again on the control set test tubes (CEC2) and treatment set test tubes (TEC2). The leaf CMT was estimated using the following Equation [1].

$$\mathrm{CMT}\,(\%) = \frac{1-(\mathrm{TEC1}/\mathrm{TEC2})}{1-(\mathrm{CEC1}/\mathrm{CEC2})} \times 100 \qquad \text{[Eq. 1]}$$

The relative injury (RI) was calculated using following Equation [2]

$$\mathrm{RI} = 100 - \mathrm{CMT} \quad x100 \qquad \text{[Eq.2]}$$

Canopy temperature depression (CT)

Canopy temperature depression measurements were made of three randomly-fully expanded leaves from each rice genotypes for all replications via a handheld infrared thermometer (Model OS533E-OMEGASCOPE; OMEGA Engineering, Inc., Stamford, CT) at the vegetative 55-65 DAS and at grain filling stage (105-115) DAS. Thereafter, canopy temperature depression was estimated using Equation (3), in which Temperature (a) and Temperature (c) refer to air and canopy temperatures of the target leaf, respectively.

$$\mathrm{CTD} = \text{Temperature a} - \text{Temperature c} \qquad \text{[Eq. 3]}$$

Yield-related parameters

In this study, flag leaf area (FLA, cm^2) was determined by the average of three flag leaves were taken randomly of the individual plant for all replications at vegetative stage 55-65 DAS and at grain filling stage 105-115 DAS using leaf area meter (LI-3100: Li-COR, Lincoln, NE, USA). While shoot dry weight (SHW, g $plant^{-1}$) was calculated by summing up the values of the dry stem weight and the leaf dry weight of the individual plant at final harvest. Panicle emergency day (PED, d) was taken as the number of days counted from sowing to the first panicle initiation in each plant. Panicle length (PL, cm $plant^{-1}$) was measured using a ruler as the distance in centimetres from the base of the panicle neck node to the tip of the last spikelet of the panicle. Panicle number (PN, no. $plant^{-1}$), was, the number of mature panicles for each of the 100 rice genotypes cut from panicle neck node and counted for the individual plant in all replications. Five panicles were labeled, spread on paper, and allowed to air-dry for approximately two weeks in the laboratory from each plant to measure several traits including spikelet number per panicle (SPN), which was the sum from the primary branches of each panicle, 100-grain weight (100 Wt, g), and grain fililing (GF, %). Grain yield (GY, g $plant^{-1}$) was the sum of all grain panicles of individual plant in four replications while grain production efficiency (GPE, g kg^{-1} $plant^{-1}$) was determined as the ratio of grain yield and shoot dry weight of each plant.

Cumulative Vigour Response Index (CVI):

The individual vigor response index (I) was determined as ratio between the value of each rice genotype (V_s) and the maximum value (V_m) among all the rice genotypes Eq. 4.

$$I = Vs/Vm \quad \text{[Eq. 4]}$$

Then, the cumulative vigor response indices (CVRI) were summed from all the indices of responses parameters for each rice genotypes at vegetative, grain filling stages, and final harvest (Eq. 5).

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$$CVRI = \left(\frac{PHs}{PHm}\right) + \left(\frac{LNs}{LNm}\right) + \left(\frac{TNs}{TNm}\right) + \left(\frac{SLAs}{SLAm}\right) + \left(\frac{RIs}{RIm}\right) + \left(\frac{CTDs}{CTDm}\right) + \left(\frac{Chls}{Chlm}\right) + \left(\frac{Caros}{Carom}\right) + \left(\frac{Pns}{Pnm}\right) + \left(\frac{Conds}{Condm}\right) + \left(\frac{Trs}{Trm}\right) + \left(\frac{WUEs}{WUEm}\right) + \left(\frac{Fv/Fm\ s}{Fv/Fm\ m}\right) + \left(\frac{ETRs}{ETRm}\right) + \left(\frac{SHWs}{SHWm}\right) + \left(\frac{FLAs}{FLAm}\right) + \left(\frac{PEDs}{PEDm}\right) + \left(\frac{PNs}{PNm}\right) + \left(\frac{PLs}{PLm}\right) + \left(\frac{SPNs}{SPNm}\right) + \left(\frac{GF\%s}{GF\%m}\right) + \left(\frac{100\ Wts}{100\ Wtm}\right) + \left(\frac{GYs}{GYm}\right) + \left(\frac{GPEs}{GPEm}\right) \quad \text{[Eq. 5]}$$

The cumulative vigor response indices and standard deviation were used to classify rice genotypes into different groups such as Eq.6, 7, 8, and 9.

Low (less than min + 1SD). [Eq. 6]

Moderately low [greater than (min CVRI + 1SD) but less than (min CVRI + 2SD). [Eq. 7]

Moderately high [greater than (min CVRI + 2SD) but less than (min CVRI + 3SD). [Eq. 8]

High [greater than (min CVRI + 3SD). [Eq. 9]

Data analysis

Standard statistical protocols, ANOVA using general linear model "PROC GLM" procedure in SAS software (SAS Institute, Inc., Cary, NC), were employed to test the significance among the 100 genotypes for the morpho-physiological and yield-related parameters. Means were collected using Least Significant Difference (LSD; $P \geq 0.05$). Regression analysis was used to identify the relationships among the combined vigor

response indices and major growth and developmental traits. Sigma Plot 13 (Systat Software Inc., San Jose, CA) was used to plot the morpho-physiological and yield-related trait's relationships.

Results and discussion

A wide range of variation was observed among the 100 rice (*Oryza sativa* L.) genotypes for all the morpho-physiological traits measured during seedling growth and grain filling stages and the yield-related parameters. Genotypes differed significantly at $P \leq 0.001$ for most of the traits studied, which implies that the genotypes studied contain adequate genetic variability for potential exploitation through breeding. Plant growth and development is an extremely difficult physiological and biochemical process that includes the phases of seed germination, seedling development, young panicle formation, heading, flowering, pollination, fertilization, seed maturity, and aging. Each phase involves different metabolic changes and is affected by biotic and abiotic factors. Analysis of variance (ANOVA) results for the studied growth and physiological traits included the plant height, tiller number, specific leaf area, relative injury, chlorophyll content, net photosynthesis, water use efficiency, air-canopy temperature, carotene content, and stomatal conductance differed significantly among 100 genotypes at high levels of probability ($P \leq 0.001$), ($P \leq 0.01$), and ($P \leq 0.05$), respectively, at the vegetative (55-65 DAS) and grain filling stages (105-115 DAS) (Table 3.1). Also, shoot dry weight, flag leaf area, panicle initiation, panicle number, grain yield, and grain production efficiency showed highly significant differences among the genotypes at $P \leq$ 0.001 level ((Table 3.1).

Table 3.1 Analysis of variance across 100 rice genotypes and morpho-physiological parameters measured during vegetative, grain filling stages, and yield related paramers.

Morpho-physiological and yield related parameters	Source of variance		
	Genotypes at seedling growth (55-65) DAS†	Genotypes at grain filling (105-115) DAS	Genotypes at yield related parameters
Plant height, cm plant^{-1}	***††	***	-
Leaf number of main stem, no. plant^{-1}	NS †††	NS	-
Tiller number, no. plant^{-1}	***	***	-
Specific leaf area, cm^2 g^{-1} d wt.	***	***	-
Relative injury, %	***	*	-
Canopy-air temperature differential, °C	**	***	-
Chlorophyll, µg cm^{-2}	***	**	-
Carotenes, µg cm^{-2}	*	*	-
Net photosynthesis, µmol m^{-2} s^{-1}	***	*	-
Stomata conductance, mol m^{-2} s^{-1}	*	**	-
Transpiration rate, Tr, mmol H_2O m^{-2} s^{-1}	NS	NS	-
Water use efficiency, mmol CO_2 mol^{-1} H_2O	***	***	-
Fluorescence	NS	NS	-
Electron transport rate, µmol m^{-2} s^{-1}	NS	***	-
Shoot dry weight (SHW, g plant^{-1})	-	-	***
Flag leaf area (FLA, cm^2)	-	-	***
Panicle emergancy day (PED, d)	-	-	***
Panicle number (PN, no. plant^{-1})	-	-	***
Panicle length (PL, cm plant^{-1})	-	-	NS
Spiklet number per panicle (SPN	-	-	NS
Grain filied (GF, %),	-	-	NS
100-grain weight (100 Wt, g)	-	-	NS
Grain yield (GY, g plant^{-1}	-	-	***
Grain production efficiency (GPE, g kg^{-1} plant^{-1})	-	-	***

† Days after sowing
†† *, **, *** represent significant differences at the 0.05, 0.01, and 0.001 P level, respectively.
††† NS represents nonsignificant differences at the 0.05 P level.

Plant height, primary leaf length, and tillers number at given growth stage are heritable and stable varietal characters and have been used for describing rice genotypes (Shibuya and Oka, 1994). The growth and developmental traits of rice genotypes are presented in Table 3.2. Significant differences in plant height, which taken before

flowering at seedling growth stage and after flowering at grain filling stage, were found among the genotypes. About 55 and 48% of genotypes were higher than the general average for plant height in both growth stages, and only 6 and 15% exhibited significant differences among genotypes, respectively. Results were similar for number of tillers, which revealed only 9 and 10% showing significant differences of genotypes at both growths stages, respectively. There is a negative correlation between leaf number and tiller number (Table 3.9), which might have been caused by RU1301084 having the lowest leaf number (3.8) but the highest tillers number (96) at (55-65 DAS). Around 54 % of genotypes exhibited higher than the general average values for number of tillers, and N-22 showed the highest value with 114.5 among genotypes at vegetative stage (Table 3.2). The grand averages of leaf number and tiller number were 4.6±0.03 and 55.6±1.47, , respectively, at grain filling stage. Since the genotypes were grown under similar environmental conditions, the differences in morpho-physiological traits maybe assumed to be due to genotype. The variation in the growth cycle of a genotype is mainly due to variation in the vegetative growth stage. A coefficient of variation (C.V%) is useful in detecting the amount of variability present in the genotypes.

Table 3.2 Growth and developmental traits of 100 rice genotypes measured before and after flowering, plant height (PH, cm plant^{-1}), leaf number of the main stem (LN, no. plant^{-1}), and tillers number (TN, no. plant^{-1}).

Genotype number	Genotype name	PH		LN		TN	
		DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115
1	14CLPYT033	58.8	78.5	4.3	4.5	39.3	69.3
2	14CLPYT108	55.5	72.3	5	4.5	44.5	51.3
3	14CVPYT094	38.3	63.9	5	4.8	52.5	52.8
4	14CVPYT144	57.5	66.7	4.5	4.8	37.5	49.8
5	COLORADO	51.3	67.9	4.8	4.3	27	64.5
6	Bowman	44.5	83.8	4.3	4.3	32	44.5
7	CAFFEY	47.5	70	4.5	4.3	50.5	72.8
8	CHENIERE	53.3	68.3	4.3	4.8	40	45
9	CL Jazzman	49.5	77.9	5	5	35.8	71.8
10	CL111	54.8	83.1	5	5	41	51.5
11	CL142-AR	53.3	73	4.5	4.5	43	56.5
12	CL151	41.8	81.2	4.8	4.8	42.8	47.8
13	CL152	49.8	83.8	5	4.5	47	55
14	CL163	47.8	77.8	5	4.8	28.5	45.5
15	CL172	53	66.2	4.5	4.8	28	52
16	CL271	48.3	58.9	4.5	4	43	54
17	Cocodrie	46.8	72.3	4.5	4.8	59.5	65.3
18	NIPONBARE	39.8	66.7	4.5	4.5	20.6	34
19	ANTONIO	44	82.9	4.3	4.8	23	47
20	El Paso 144	45	62.6	4.8	4.5	76	88
21	GSOR100390	57	72.1	5.3	4.8	50	59.8
22	GSOR100417	52	73.5	4.3	4.8	44.8	72.5
23	GSOR101758	39.3	52.1	4	5	40.8	47.8
24	RU1104122	35.3	64.7	4.5	4	14.5	20.3
25	CLJZMN	47.5	73.7	4.3	4.5	35	59.8
26	INIA Tacuari	57.8	72	4.5	4.5	31.5	67.3
27	IRGA409	39	60.7	4.5	4.3	71.8	97
28	JES	43.5	49.3	4.3	4.3	76.8	104.5
29	JUPITER	51.3	65	4.3	4.8	51.8	62.3
30	LA 2008	45	63.3	4.5	4.5	45.3	64.5
31	LA 2134	51.8	64.8	5	4.8	35	55.5
32	LAKAST	53.8	69.7	4.5	4.5	31	42.5
33	MERMENTAU	54.5	69	5	4.8	37.5	62.3
34	Presidio	48.8	67	4.5	4.3	36	54
35	Rex	54.5	75.6	4.5	4.3	31.5	53.5
36	RoyJ	48.8	68.3	4.5	4.8	29.3	43.3
37	RU0603075	39	65.2	4.5	4.5	75.3	86.3
38	RU1201024	51	72.5	4.5	5	31	40.5
39	RU1201047	54.5	74.9	4.3	5	32	50.3
40	RU1201136	49	76.2	4.5	5	37.3	53
41	RU1204156	47	63.7	4.5	4.3	46	53
42	RU1204197	48.3	67	5	4.5	33.3	41.8
43	RU1301084	54.3	64.4	3.8	4.5	43.8	76.5
44	RU1301093	53.5	66.9	5	5.3	38	65
45	RU1301102	51.5	69.5	4.5	5	30	39.5
46	RU1302192	56.8	68.3	5	4.8	44.3	70.8
47	RU1303138	34.3	47.3	4	4.5	62.5	78.5
48	RU1303181	56	67.9	5	4.8	28.3	48.3
49	RU1304114	45	63.3	4.8	4	31.3	43.3
50	RU1304122	52	77	5	4.5	45.8	60
51	RU1304154	54.8	70.1	5	4.8	42	53

Table 3.2 (Continued)

52	RU1304156	51.8	76.1	4.5	5	28.3	43.5
53	RU1305001	49	61.9	4.3	4.3	35	64.5
54	RU1401067	49.5	70.2	5	4.8	29	53.8
55	RU1401070	57.5	69.7	4.8	5	23.8	40.8
56	RU1401090	49	78	4	5	28.8	43
57	RU1401099	54.3	79.2	4.8	4.8	48.5	49.8
58	RU1401102	52.3	78.2	4.5	5	42.5	56.5
59	RU1401145	53.5	63.3	4.8	4.8	23.3	52.8
60	RU1401161	42.8	63	4.5	4.3	34.8	39.5
61	RU1401164	51.3	65.6	4.5	4.3	47.8	59.5
62	RU1402005	48.3	62.5	4.8	4.8	38.3	48
63	RU1402031	50.5	63.3	5	4.8	34.3	48
64	RU1402065	47.8	64.3	4.8	4.5	55.5	63
65	RU1402115	49	67.6	4.8	4.8	38.3	54
66	RU1402131	45.5	65	4.5	5	50.5	70.5
67	RU1402134	50	64.3	4.8	5	33	45.3
68	RU1402149	50.8	67.6	4.5	4.3	55.3	67
69	RU1402174	49.3	70	5.3	4.3	33.8	45.8
70	RU1402189	51.5	74.3	4.3	4.5	32.5	56.3
71	RU1402195	47.8	70.9	4.5	5	50.5	76
72	RU1403107	52	71.7	4.5	4.5	51.8	62.8
73	RU1403126	50.8	64.3	4.5	4.3	43	54.8
74	RU1404122	48.5	73.6	4.8	4.3	29	42.5
75	RU1404154	44.8	66.3	4.8	4.3	32.5	51.3
76	RU1404156	42.8	67.4	4.8	4.3	32	57.8
77	RU1404157	52	69.5	4.5	4.5	36	40.3
78	RU1404191	48.3	65.6	4.3	4.5	54.5	69.3
79	RU1404193	52	68.4	4.8	4.8	45.8	68.3
80	RU1404194	50	73.3	4.8	5	33.8	61.8
81	RU1404196	47.5	62.8	4.3	4.5	34.8	58
82	RU1404198	43.5	69.2	4.5	4.8	42	66
83	RU1504083	39.5	70.1	4.5	4.8	42.5	55
84	RU1504100	52.8	67.4	5	4.8	34.5	40.8
85	RU1504114	56.3	77.1	4.5	4.8	41	54.8
86	RU1504122	57.5	70.5	5	4.8	34.3	47
87	RU1504154	50	75.5	4.8	4.8	51.3	56.3
88	RU1504156	49.8	70.4	4.8	4.5	36.3	49.5
89	RU1504157	59	78.6	5.3	4.3	45.3	66.8
90	RU1504186	52.5	69.7	5.3	5	33.8	55
91	RU1504191	55	65.5	4.8	4.8	37.3	63
92	RU1504193	52.5	78.5	4.8	5	49.3	50.5
93	RU1504194	49.5	83.3	4.3	4.5	40.3	56.5
94	RU1504196	56.5	74.7	4.5	4.5	29.5	43.8
95	RU1504197	54.8	66.1	4.8	4.5	42	56.8
96	RU1504198	52	79.4	4.5	4.8	40.3	43.3
97	Sabine	50.8	56.8	5	4	41.8	48.3
98	Taggart	49.5	69.2	4.3	4.3	31.5	45
99	Thad	50.5	70.5	4.8	4.5	36.8	53.5
100	N-22	44.3	82.6	4.8	5	23.5	114.5
	Mean	49.7±0.52	69.6±0.70	4.6±0.03	4.6±0.03	39.83±1.29	56.54±1.47
	LSD	7.75	7.06	0.75	0.75	12.49	15.09
	LSD + Mean	57.47	76.71	5.37	5.36	52.32	71.63
	C.V%	10.46	10.04	6.59	6.18	28.63	25.13

A coefficient of variation (C.V%) is useful in detecting the amount of variability present in the genotypes. The coefficient of variantion at seedling growth stage was slightly higher than that at grain filling stage for plant height, leaf number, and tillers number thus indicating the influences of an environmental factor on these traits along with growth stage (Table 3.2). Plant height, for seedlings or juvenile plants, is the distance from ground level to the tip of the tallest leaf. Along with lodging resistance, short plants give higher yields at closer plant spacing compared to taller cultivars. However, taller plants have an advantage in competing with weeds compared with short stature plant. Extremely dwarf height is also not goodecause grain yield increases quadratically with increasing plant height (Fageria et al., 2004). A slight increase in grain yield has been associated with reduced plant height (Evans et al., 1984). Jaballa (1995) reported the difference in the length trait among genotypes due togenetic variability. Murata (1975) reported that tillering begins at the four to five leaf stages and emergence is closely linked to the number of leaves. High tillering capacity basically is related to the maximum use of space and resources. Tillering has special importance under biotic and abiotic stresses due to compensation processes. High tillering compensates for missing plants at low densities, but cultivars with a limited tillering capacity lack this plasticity. Heavy tillering is not much advantageous in the direct seeded rice, which is a common practice in mechanized agriculture in South America and in the US midSouth. Under direct seeding, tillering capacity rarely affects grain yield within conventional seeding rates because the total panicle number per square meter depends more on the main culm than on tillers (Yoshida, 1981). The genotypes which produced higher number of effective tillers per plant showed higher grain yield in rice (Dutta et al., 2013). The lines

with higher number of total tillers also excelled in the number of productive tillers per plant. Light interception by the canopy is strongly influenced by leaf area, and is an important parameter to estimate yields for many crop growth models that use net photosynthesis, relative injury, canopy mass, and energy exchange (Fageria et al., 2006). Average and grand means of each of the physiological traits and coefficient variation (CV%) of 100 genotypes are presented in Table3.3. The results indicate that the rice genotypes varied significantly with respect to physiological traits. Expectedly, the coefficient variations of specific leaf area, relative injury, air-canopy temperature differential, chlorophyll, and carotenoids at seedling growth stage were generally higher than that at grain filling stage. The highest variabilities in all the characteristic considered were recorded in specific leaf area (19.08) followed by carotenoids (18.74). N-22 and RU1401164 exhibited the highest SLA (226.1cm^2 g^{-1} d wt), RI (66.5 %), CT (0.7 °C), Chl (49.2 µg cm^{-2}), and Caro (10.0 µg cm^{-2}) at seedling growth stage among genotypes. Genotypes 14CLPYT033, RU1402195, and GSOR100417 revealed the highest SLA (191.2 cm^2 g^{-1} d wt), RI (63.7 %), CT (0.1 °C), Chl (58.7 µg cm^{-2}), and Caro (12.3 µg cm^{-2}) at grain filling stage among genotypes. However, Sabine, RU1401067, RU1404154, and RU1304122 recorded the lowest values in physiological traits at both growth stages (Table 3.3). These genotypes with the highest or lowest values in physiological traits belong to the very high or very low vigor index classification (Table 3.6 and 3.7) indicating the importance of the physiological traits in the classification of genotypes. Genotypes were generally 52% higher than the grand mean of each physiological trait at seedling growth stage and 49% higher than grand mean at grain filling stage.

Table 3.3 Specific leaf area (SLA, $cm^2\ g^{-1}$ d wt), and biophysical, relative injury (RI, % $plant^{-1}$), canopy-air temperature differential (CDT, °C $plant^{-1}$), total chlorophyll (Chl, µg cm^{-2}), and carotenoids (Caro, µg cm^{-2}) of 100 rice genotypes measured before and after flowering.

Genotype number	Genotype name	SLA		RI%		CT		Chl		Caro	
		DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115
1	14CLPYT033	131.6	191.2	60.9	63.7	-0.6	-1	40.5	49.9	9.6	7.8
2	14CLPYT108	150.4	152.7	46.2	50.6	-1	-1.6	33.9	51.4	7.6	8.6
3	14CVPYT094	221	129.9	57.5	48	-1.7	-1.6	39.4	41.6	8.8	6.5
4	14CVPYT144	153.9	155.2	38.1	49.1	-2.1	-2.1	35.3	37.1	7.9	6.1
5	COLORADO	185.9	127.5	54.3	46.8	-2.4	-2.2	30.2	48.9	7.9	8
6	Bowman	166.9	152.6	45.2	47.6	-3.2	-1.3	36.6	53.3	9.9	7
7	CAFFEY	135.8	120.2	47.2	43.9	-1.8	-0.9	45.5	37	10.3	5.4
8	CHENIERE	176.8	159	51.6	49.7	-1.7	-1.6	35.8	37.5	7.8	5.7
9	CL Jazzman	160	142.5	57.1	47.5	-1.1	-1.1	35.4	43.8	8.1	7
10	CL111	138.7	142.8	46.5	39.2	-0.3	-0.8	33	34.1	6.9	8.9
11	CL142-AR	105.4	157	36.5	46.1	-4.2	-1.3	33.6	47.4	8.8	8.1
12	CL151	205.7	133.2	50.9	46.6	-1.8	-1.1	41.1	36.7	6.5	6.1
13	CL152	177.6	158.6	47	41.2	-2.8	-1.4	34	42	8.5	7.1
14	CL163	201.1	145.6	50.4	49.9	-0.9	-2.2	33.5	39.5	8.8	5.6
15	CL172	173	146.9	47.2	46.6	-3.4	-2.6	38	51.6	8	8.9
16	CL271	164.6	112.5	54	45.7	-3.2	-1.5	38.2	38.8	8.6	6
17	Cocodrie	171.2	159.7	59.7	45.1	-4.7	-2	41.3	44.3	9	7.2
18	NIPONBARE	202.5	152.8	52.3	39.1	-1.9	-1.1	32.6	43.5	4	7.4
19	ANTONIO	178.9	149.2	50.1	41.7	-3.8	-1.1	31.4	47.9	8.5	7.4
20	El Paso 144	170	148.8	51.5	43.1	-0.7	-1.6	27.2	49.5	6.8	7.7
21	GSOR100390	138	134.9	51.7	42.5	-0.6	-1.4	34.5	41.1	6.4	6.8
22	GSOR100417	191.4	146.3	36.9	48.2	-2.8	-1.8	38.9	38.1	9.2	12.3
23	GSOR101758	168.4	136.6	48.1	44.3	-3	-2	28.6	47.5	4.5	7.8
24	RU1104122	133.4	103.4	52.3	42.9	-2.8	-1.8	32.2	44.4	7.4	7.1
25	CLJZMN	135.8	139.1	49.1	43.9	-2.5	-1.7	37.4	47.6	8.1	7.2
26	INIA Tacuari	133.2	139.6	47.8	45.8	-2.1	-1.2	45.4	49.8	10.3	8.7
27	IRGA409	178.8	160.3	53.9	44	-2.3	-0.9	35.4	48.8	8.1	7.6
28	JES	183.4	157	41.3	37.4	-3.2	-0.8	30.7	44.8	7.3	6.9
29	JUPITER	109.8	126	48.4	48.4	-2.9	-2.2	47.5	40.9	8.7	6.5
30	LA 2008	95.6	142	50.5	49.8	-2.3	-1.5	40.5	46.2	9.3	7.1
31	LA 2134	202.2	160.7	39.1	47.1	0.4	-0.8	43.1	56.2	7.2	9
32	LAKAST	142.5	127.2	49.5	38.3	-3.1	-1.6	43.2	43.4	7.3	7.7
33	MERMENTAU	164.7	131.7	42.2	49.5	-0.8	-1.3	37.2	41.5	8	6
34	Presidio	194.6	135.2	53.7	53.5	-1.3	-0.7	40.9	56.5	6.7	9.5
35	Rex	129.3	125.3	42.5	42.8	-3.3	-1.5	38.7	45.3	8.1	10.2
36	RoyJ	154.1	133.4	58.5	42.1	-5	-1.8	38	36.5	5.8	5.7
37	RU0603075	192.8	123.3	53.4	44.8	-4.4	-0.8	33.9	49.6	5.4	8.1
38	RU1201024	107.1	116.2	47.1	49	-3.4	-2.3	38.7	45	8.7	6.9
39	RU1201047	138.9	127.4	45.5	45.7	-3.4	-0.8	39.4	41.7	5.8	7.3
40	RU1201136	172.3	108.1	47.5	43.6	-0.3	-0.8	37.1	40	6.6	6.8
41	RU1204156	161.8	121.6	49.3	54.5	-2.4	-1.7	38.9	50.5	7.5	7.7
42	RU1204197	156.1	125.7	43.2	50.6	-2.8	-1.1	29	46.7	7	7.5
43	RU1301084	115.8	138.6	41.2	47.3	-3	-1.2	28.1	50.3	5.3	8.1
44	RU1301093	126.8	125.8	47.3	39.3	-2	-1.4	37.9	37.6	6.9	6.2
45	RU1301102	145.7	126.5	53.3	40.3	-3.2	-2	39.4	43.9	5.1	7.2
46	RU1302192	118.8	123.1	54.9	42	-4.3	-0.9	30.4	47	5.9	7.5
47	RU1303138	143	164	53.4	44.3	-1.9	-1.5	37.9	49.1	4.9	8.3
48	RU1303181	128.7	147.3	42	54.6	-3.4	-2.1	34.6	38.2	8.1	5.9
49	RU1304114	176.6	170.7	42.7	43.1	-2.6	-1.4	37.2	46.9	7.1	8
50	RU1304122	147.6	134.4	40.4	48.8	-2.1	-2.8	35.6	33.3	9	4.1
51	RU1304154	164.7	126.1	47.1	46.8	-2.3	-1.5	31.9	42.6	7.9	5.3
52	RU1304156	159.7	148.5	54.6	42.4	-1.5	-1.8	31.3	33.2	6.2	4.9
53	RU1305001	205.7	135.7	51.3	37.4	-4.3	-1.9	36	42.8	6.5	6.7
54	RU1401067	98.6	144.8	45.9	49.6	-6.2	-1	24.7	38.8	8.5	6.7
55	RU1401070	108.7	118.3	46.5	50.3	-1.7	-2.2	32.4	53.9	8.7	9.9
56	RU1401090	137.4	119.4	49.7	46.8	-2	-1.3	30.2	42.3	8	7.4
57	RU1401099	145.8	129.5	45.8	39	-3.3	-0.9	35.9	47.1	10	7.8
58	RU1401102	137.8	126.4	44.6	42.2	-3.7	-1	30.3	44.4	6.1	6.7
59	RU1401145	132.5	156.4	52.9	37.5	-0.8	-1.4	40.5	40.5	9.6	6.6
60	RU1401161	190.4	114.6	41.8	47.3	-1.8	-1	49.2	53.4	10	8
61	RU1401164	130.6	148.2	44.6	51.1	-2.2	-1.3	30.3	48.5	8	8.7
62	RU1402005	147.4	114.8	58.7	44	-2.6	-1.2	34.9	49.2	6.7	8.4
63	RU1402031	202.9	138.1	46.1	46.3	-2.3	-0.3	32.9	45.2	6.5	7.2
64	RU1402065	89.6	124.6	48.2	43.3	-0.1	-2.1	39.9	50.6	8.7	8.5
65	RU1402115	141.7	126.7	51.7	46.6	-4.4	-2.3	33.9	43.8	7.6	6.5

Table 3.3 (Continued)

66	RU1402131	146	136.3	57.9	38.4	-2.1	-1.7	32.1	39.8	7.8	6.7
67	RU1402134	163.9	118.9	44.4	41	-2.8	-0.3	37	56.3	7.7	9.5
68	RU1402149	143.5	114.6	48.6	44.9	-3.1	-0.8	35.4	41.5	6.6	7.2
69	RU1402174	167.4	116.2	52.3	39	-3	-1.6	35.3	39.9	6.8	5.9
70	RU1402189	130.7	151.8	50.1	49.2	-2.5	-1.7	36.1	48.3	7.8	8.4
71	RU1402195	114.2	130.5	45.5	47.2	-4.3	0.1	34.4	58.8	6.4	7.6
72	RU1403107	158.5	136	53.6	49.9	-2.9	-1.5	35	43.6	7	6.7
73	RU1403126	167.2	133.5	52.4	44.2	-2	-2.4	35.3	45.1	7.1	7.2
74	RU1404122	113.6	117.2	46.1	41.6	-1.4	-0.6	44.4	39.6	9.8	5.8
75	RU1404154	146.1	94.5	38.1	34.7	-2	-1.7	36	46.1	8.5	7.5
76	RU1404156	153.5	149.9	62.2	44.5	-3.8	-1.6	41.3	52.8	10.2	9.3
77	RU1404157	152.5	129.4	51.9	58.2	-3.2	-1.4	34.1	46.5	8.3	7.5
78	RU1404191	151.7	132	41.3	37.7	-1.9	-1.8	41.9	40.8	9.8	7.4
79	RU1404193	148.2	131.1	49.9	47.4	-3.3	-1.5	33.7	48.9	5.5	8
80	RU1404194	165.8	128.2	44.1	55.2	-0.5	-0.5	39.1	42.6	7.8	7
81	RU1404196	136.4	140.6	45.1	52.2	-2.9	-1.7	37.4	48.3	8.2	8
82	RU1404198	171	117.1	49.9	41.5	-5.4	-1.2	31.9	46.9	4.2	8.7
83	RU1504083	182.7	151.6	51.8	56.3	-3.3	-0.4	38.6	33.2	8.9	5.2
84	RU1504100	168	121	55.9	43.4	-3.1	-0.9	35.1	41.9	6.9	6.6
85	RU1504114	141.5	141.7	53.1	42.3	-1.4	-1.3	35.9	41.4	7	6.8
86	RU1504122	156	147.5	60.9	42.5	-1.9	-1.1	30.9	40.9	6	6.4
87	RU1504154	170.8	142	56.3	41	-1.6	-2.3	41.5	53.6	7.5	8.4
88	RU1504156	207	133.7	39.3	43.7	-0.4	-1.5	38.2	45.6	7.4	7.4
89	RU1504157	153.8	146	51.5	45.8	-1	-1.8	31.5	47.8	6.6	7.7
90	RU1504186	151.6	131.5	57.1	42.9	-0.7	-1.7	37.7	34.9	7.7	5.6
91	RU1504191	217.9	130.2	45.2	49.8	-0.2	-2.3	37	51.1	8.9	8.5
92	RU1504193	149.1	153.9	50.1	47.1	-2.3	-1.9	39.8	41	5.5	6.5
93	RU1504194	172.3	125.6	55.7	49.7	-5.2	-1.7	40.7	39.5	7.6	5.9
94	RU1504196	149.7	130.4	52.6	36.3	-3.3	-0.9	43.1	45.3	7.1	6.9
95	RU1504197	131.6	132.8	63.5	45.5	-1.8	-1.1	35.4	39.5	9.3	6.9
96	RU1504198	128.6	127.5	51.7	41.6	-0.8	-2.6	32.6	43	7.1	7.5
97	Sabine	87.8	160.8	32.6	50.7	-3.4	-1.5	40	48.7	9.3	7.8
98	Taggart	125.6	135.2	46.1	50.3	-1.3	-2.2	45.8	49.4	9.6	8.3
99	Thad	156.4	139.1	53.7	53.9	-4.5	-1.9	39.9	48.8	9.5	7.6
100	N-22	226.1	131.1	66.5	52.8	0.7	-1.9	44.9	58.7	10.1	7.3
	Mean	154.6±2.9	136.2±1.5	49.4±0.6	45.7±0.5	2.4±0.1	1.5±0.06	36.5±0.5	44.9±0.6	7.7±0.1	7.3±0.1
	LSD	34.43	21.03	9.91	12.03	3.13	-1.24	7.66	11.71	2.95	2.77
	LSD +Mean	189.01	157.24	59.28	57.76	0.72	-0.21	44.16	56.62	10.64	10.1
	C.V%	19.08	11.49	63	11.1	-54.92	-37.83	12.71	12.04	18.74	16.6

The variability of the physiological parameters such as leaf chlorophyll content (Prasad and Djanaguiraman, 2011), relative injury and carotenoids (Zafar et al., 2017) has been studied previously. Rice grown under upland condition is often subjected to moisture stress and, in general, has less leaf area than rice grown under lowland conditions (Fageria et al., 2004). The rice plant at any point in time is composed of leaves of different ages physiologically (Ramasamy 2002). Thicker leaves usually have higher densities of chlorophyll per unit leaf area and, hence, have greater photosynthetic capacities than thinner leaves (Craufurd et al., 1999). Leaf size is directly associated with leaf angle, with short leaves tending to be more erect than longer ones. Further, short leaves are usually more evenly distributed throughout the canopy, which permits less

mutual shading of leaves and more efficient use of light for photosynthesis (Fageria et al., 2006). Canopy temperature and relative injury have to be given importance in the selection process for improvement in yield since most of the traits had a strong positive correlation with grain yield per plant. Hence, selection based on this character will be more useful for yield improvement in rice under normal condition. The correlation between leaf area and yield (Alluwar and Deotale 1991) suggests that chlorophyll and leaf area are essential in determining the yield (Raj and Tripathi 1999). The chlorophyll content in leaf tissues varies with the age of the plant and the species and the growing season (Yurkovskii et al., 1977). Rice breeding manipulation to increase crop yields require of rapid and efficient procedures to select the most appropriate and adapted genotypes, among numerous genotypes through the screening more of characters in rice breeding lines suited to the US mid-south growing environments. One of the keys to reaching high yield was photosynthesis in leaves that could meet the demand for grain filling and their concurrent process. The photosynthetic response to growth environment of the genotypes grown in this experiment was consistent with their relative responses in the controlled environment experiments. Indeed, some of the variation among cultivars in photosynthetic acclimation were also observed in several characteristics. One of the aims of the present work was to detect photosynthesis and fluorescences values of rice genotypes with contrasting performance under different growth stages, before flowering, at vegetative stage, and after flowering at grain filling stage. Our results are showing that the highest variability was in stomatal conductance (44.4%) at vegetative stage and (24.3%) at grain filling stage among all photosynthesis and fluorescences parameters (Table 3.4).

Table 3.4 Leaf gas exchange including net photosynthesis (Pn, µmol m^{-2} s^{-1}), stomatal conductance (Cond, mol m^{-2} s^{-1}), transpiration (Tr, mmol H_2O m^{-2} s^{-1}), water use efficiency (WUE, mmol CO_2 mol^{-1} H_2O), quantum efficiency of fluorescence (Fv'/Fm'), and electron transport rate (ETR, µmol m^{-2} s^{-1}) of 100 rice genotypes measured before and after flowering.

Gen. no.	Gen. name	Pn		Cond		Tr		WUE		Fv'/Fm'		ETR			
		DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115	DAS 55-65	DAS 105-115
1	14CLPYT033	41.1	33.4	4.7	1.6	11.3	12	3.1	2.7	0.6	0.5	183.6	173.6		
2	14CLPYT108	36.2	30.2	1.3	1.1	10.7	10.4	3.4	3	0.5	0.5	213	152.6		
3	14CVPYT094	34.2	27.8	1.5	1	10.9	10.7	3.6	2.5	0.6	0.4	207.3	149.8		
4	14CVPYT144	34.8	27.1	2.1	0.8	10.1	10	3.4	2.7	0.5	0.5	160.5	153.1		
5	COLORADO	29.1	31.7	1.6	0.9	9.4	11.3	3.1	2.8	0.5	0.5	176	141.5		
6	Bowman	32.2	28.6	1.4	0.9	10.7	10.2	3	2.9	0.6	0.5	167	134.5		
7	CAFFEY	33.3	33.1	2.7	1	9.7	11.4	3.4	2.9	0.5	0.5	160.4	172		
8	CHENIERE	34.6	32.4	1.9	1.2	10.2	11.1	3.4	3.1	0.5	0.6	147.2	171.3		
9	CL Jazzman	32.6	28.7	1.2	1.1	9.5	11.6	3.4	2.5	0.6	0.5	195.3	152.8		
10	CL111	30	27.8	1.3	1	9.4	9.8	3.2	2.8	0.5	0.4	131.1	152.9		
11	CL142-AR	33.4	30.1	1.7	1.1	10	9.9	3.4	3.1	0.5	0.5	148.1	173		
12	CL151	36.5	31.1	1.4	0.7	10.6	10.3	3.5	3.1	0.6	0.5	205.7	179.5		
13	CL152	31.9	28.3	1.2	1.2	9.2	10	3.5	2.9	0.5	0.5	135.8	147.1		
14	CL163	37.4	31.4	1.4	1.2	11.1	10.8	3.4	2.9	0.6	0.5	207.2	167.2		
15	CL172	33.6	32.4	1.4	1.5	9.4	10.7	3.6	3	0.5	0.5	148.2	173.7		
16	CL271	32.1	30.6	1.4	1.2	10.3	11.9	3.1	2.6	0.6	0.5	180.2	156.2		
17	Cocodrie	32.6	26.3	1.6	0.7	9.2	10.4	3.1	3.1	0.5	0.5	167.9	131.9		
18	NIPONBARE	32.7	31.4	1.7	1.1	10.4	10.9	3.2	3	0.6	0.6	178.3	159		
19	ANTONIO	35.9	34.2	3.3	1	10.6	9.2	3.4	3.8	0.5	0.5	155.3	169.4		
20	El Paso 144	32.1	30.1	1.2	0.9	9.6	11.7	3.3	2.6	0.5	0.5	134.1	136.9		
21	GSOR100390	33.3	31.1	1.5	1.4	10.3	11.4	3.2	2.7	0.6	0.5	202.1	156.4		
22	GSOR100417	36	34.4	1.7	1.6	9.5	11.5	3.8	3.1	0.5	0.6	164.2	170.1		
23	GSOR101758	28	23.4	0.9	1.3	10.7	10.9	3.1	2.7	0.6	0.5	198.1	132.7		
24	RU1104122	30.6	30.4	1.6	1.2	9.9	8.3	2.8	2.9	0.6	0.5	193	157.7		
25	CLJZMN	30.3	31.1	1	1.4	10.1	11.2	3	2.8	0.7	0.5	226.1	158.2		
26	INIA Tacuari	35.1	27.8	1.2	1.3	10.4	10.6	3.4	2.7	0.5	0.5	156.3	146.6		
27	IRGA409	32.4	30.1	1.4	1.3	9.1	11.4	3.6	2.8	0.5	0.6	156.2	143.1		
28	JES	32.9	32.6	1.6	1.6	10.2	11.8	3.2	2.8	0.5	0.6	206.7	166.1		
29	JUPITER	35.2	29.2	1.3	1	10.5	10.1	3.4	2.9	0.6	0.5	196.5	161.8		
30	LA 2008	33.9	33.8	2.6	1.2	9.8	11	3.4	3.2	0.6	0.5	204.6	156.3		
31	LA 2134	35.1	30.4	1.7	1.1	11	10.1	3.2	3	0.6	0.5	181.9	160.9		
32	LAKAST	33.8	29.9	1.7	0.8	9.9	9.9	3.4	3.1	0.5	0.6	144.4	161		
33	MERMENTAU	32.9	29	1.2	0.8	9.8	10.5	3.4	2.8	0.5	0.5	188.8	150.4		
34	Presidio	31.6	32.5	1.7	1.3	10.3	12.4	3.1	3.9	0.6	0.5	177.4	165.4		
35	Rex	33.8	30.3	2.2	0.9	10.2	10.6	3.3	3	0.6	0.5	192.6	153.2		
36	RoyJ	37.6	28.2	2.4	0.9	9.4	9.8	3.1	2.9	0.6	0.5	127	119		
37	RU0603075	32.6	30.7	1.6	1.1	9.3	10.4	3.5	3	0.5	0.5	196.3	156.4		
38	RU1201024	33.4	31.2	2.5	1.2	10.2	10.4	3.3	3.1	0.6	0.5	190.7	173.1		
39	RU1201047	36.1	26.8	1.7	0.8	9.9	10.1	3.6	2.8	0.5	0.5	156.5	144.1		
40	RU1201136	30.5	26.9	1	1.1	9.6	9.8	3.2	2.7	0.6	0.5	180.2	155.6		
41	RU1204156	37.2	30	1.8	0.9	9.9	11.6	3.7	2.6	0.6	0.5	225.2	130.2		
42	RU1204197	33.3	31.9	1.3	1.3	9.6	11.1	3.5	2.9	0.5	0.6	193.3	140.2		
43	RU1301084	29	27.3	1.1	0.9	8.7	10.7	3.4	2.5	0.5	0.5	192	132.3		
44	RU1301093	34.8	30.7	3	0.9	9.5	8.9	3.6	3.5	0.5	0.5	155.6	158.8		
45	RU1301102	33.2	29.7	1.4	0.7	9.5	9.7	3.5	3.1	0.6	0.5	197.6	159.6		
46	RU1302192	29.6	30.4	1.4	1.4	8.7	10.1	3.4	3.1	0.6	0.5	192.6	170		
47	RU1303138	33.2	27.6	1.5	0.6	9.5	8.8	3.5	3.1	0.6	0.5	196	164.4		
48	RU1303181	33.7	35.1	1.6	1.7	11.2	11.5	3	3.2	0.6	0.5	181.4	172.4		
49	RU1304114	36.2	29.3	1.7	0.9	10.3	10.6	3.5	2.8	0.6	0.6	215.9	157.5		
50	RU1304122	33.6	25.3	1.7	0.9	9.8	9.7	3.5	2.6	0.5	0.5	161.7	119.7		
51	RU1304154	32.5	30.3	1.6	1.1	11.4	10.7	2.9	2.9	0.7	0.5	210.7	169		
52	RU1304156	34.7	31.9	2.8	1.4	10.6	11.9	3.3	2.7	0.6	0.6	185	138.1		
53	RU1305001	34.4	28.6	1.6	1.1	9.8	10.1	3.5	3	0.6	0.5	202.8	122.4		
54	RU1401067	30.6	31.2	1	1	9.8	10.6	3.1	3	0.5	0.5	137	134.2		
55	RU1401070	30.2	31.8	1.2	1.4	10.9	11.3	3.4	2.8	0.6	0.5	173	178.3		
56	RU1401090	33.1	28.5	1.3	0.6	8.6	9.3	3.3	3.1	0.5	0.5	148.8	164.6		
57	RU1401099	34.2	29.3	1.4	0.9	10.6	10.9	3.3	2.7	0.5	0.5	127.5	150.1		
58	RU1401102	32.8	28.2	1.8	1.2	10.1	10.4	3.3	2.7	0.5	0.5	146.6	144.7		
59	RU1401145	32.8	27.3	1.3	1.3	9.8	10.9	3.4	2.5	0.5	0.5	142.3	148.8		

Table 3.4 (continued)

60	RU1401161	37.1	28.1	1.4	1.3	10.4	12	3.6	2.6	0.5	0.6	159.9	126.9
61	RU1401164	30.8	32.2	1.6	1.3	9.1	11.1	3.4	3.2	0.5	0.65	132.5	199.5
62	RU1402005	35.3	28.7	1.6	1	10.9	10.8	3.2	2.7	0.6	0.5	194.5	130.5
63	RU1402031	33.6	30.4	1.5	1	9.5	9.9	3.5	3.1	0.5	0.5	152.4	140.9
64	RU1402065	34.9	32.6	1.6	1.5	10.5	12	3.4	2.9	0.5	0.5	142.3	161.2
65	RU1402115	35.6	29	1.9	0.9	10.4	10.4	3.5	2.9	0.5	0.6	145.5	152.2
66	RU1402131	28.1	33.7	1.3	1.3	9.1	11.4	3.1	3	0.5	0.5	141.2	180.6
67	RU1402134	31.9	31.6	1.1	1.6	11.7	11.6	4	2.8	0.5	0.5	133	164.9
68	RU1402149	31.6	29.4	1.4	0.7	9.5	10.6	3.3	2.8	0.5	0.5	147.7	143.1
69	RU1402174	36	32.1	1.6	0.9	10.8	10.4	3.4	3.1	0.5	0.5	158.9	174.4
70	RU1402189	31.4	31.2	1.2	1.2	9.7	11.3	3.2	2.8	0.5	0.5	215	140.8
71	RU1402195	33.9	35	1.4	1.5	9.5	9.7	3.6	2.7	0.5	0.6	170.7	173.6
72	RU1403107	33.1	28.5	1.6	1.2	10.3	11.1	3.2	2.6	0.6	0.5	168.6	155.4
73	RU1403126	32.9	32.8	2.2	1.3	10.4	11.4	3.2	3	0.5	0.5	128.3	162.2
74	RU1404122	32.7	32.6	1.1	1.5	10.5	11.1	3.1	2.9	0.5	0.5	193.6	171.6
75	RU1404154	34.6	28.4	1.9	0.8	10.6	9.1	3.3	2.6	0.5	0.4	199.5	131.2
76	RU1404156	31.5	23.5	1.2	1	9.2	8.9	3.4	2.7	0.6	0.5	194.5	122.2
77	RU1404157	35.4	25.6	1.5	0.7	10.6	9.7	3.4	2.4	0.4	0.4	194.5	142
78	RU1404191	29.5	31.8	0.9	1.5	9.3	11.7	3.2	2.7	0.5	0.5	195.3	166.1
79	RU1404193	36.2	33.8	1.7	1.3	10.6	10.3	3.4	3.4	0.6	0.5	201.9	156
80	RU1404194	33.9	27.4	1.2	1	10.5	9.5	3.2	2.9	0.6	0.5	200.1	165.8
81	RU1404196	34.1	29.1	1.7	1.1	9.7	10.8	3.5	2.8	0.5	0.5	140.9	156.2
82	RU1404198	31.9	34.5	4.2	1.1	9.8	10.2	3.2	3.4	0.5	0.5	155.1	166
83	RU1504083	38	31.8	2.7	0.9	10.7	8.9	3.6	3.6	0.6	0.5	168.3	185.5
84	RU1504100	36.5	31.6	5.2	1.2	10.8	11.7	3.4	2.8	0.5	0.5	152.3	158.5
85	RU1504114	35.7	29.4	1.8	1.2	10.2	10.8	3.5	2.8	0.5	0.6	161.3	157
86	RU1504122	32.6	29.5	1.7	0.7	10.7	9.7	3.1	3	0.6	0.5	196.5	162.9
87	RU1504154	36.2	31.4	2.5	0.9	10.1	9.7	3.6	3.3	0.5	0.5	150.2	159.1
88	RU1504156	34.7	31	1.7	1.1	9.9	10.7	3.5	2.9	0.6	0.5	200.8	174.7
89	RU1504157	35.3	31.9	1.5	1.4	10.3	10.9	3.5	3	0.5	0.5	150.6	167.9
90	RU1504186	31	30.9	1.2	1.1	9.8	11.3	3.2	2.8	0.6	0.5	195.7	161.6
91	RU1504191	31.2	35.2	1.5	1.9	9.9	12.3	3.2	2.6	0.6	0.5	181.2	150.8
92	RU1504193	31	31.9	1	1.2	10.1	10.9	3.1	3	0.5	0.5	164.6	161.5
93	RU1504194	32.7	24.2	1.3	0.9	10.2	9.6	3.2	2.5	0.6	0.4	193.5	147
94	RU1504196	33.7	31.5	1.7	1.3	10.5	11.6	3.2	2.8	0.6	0.5	195	170.9
95	RU1504197	36	26.4	1.3	1	11.1	9.8	3.2	2.7	0.6	0.5	189.7	157.2
96	RU1504198	36.9	31.5	1.4	1.2	11.2	11.8	3.3	2.7	0.6	0.5	218.5	150.3
97	Sabine	36.1	28.8	1.9	0.8	10.5	10.1	3.4	2.9	0.6	0.4	186.9	152.7
98	Taggart	33.4	28.4	1.7	0.9	9.3	9.3	3.6	3	0.6	0.5	216.4	147.2
99	Thad	35.2	30	3	0.8	10.4	10.3	3.4	3	0.5	0.5	203	150.4
100	N-22	39.7	27.5	1.6	0.8	9.3	9.1	3.7	3	0.6	0.5	223.8	149.3
	Mean	33.6±0.2	30.1±0.3	1.7±0.08	1.1±0.03	10.1±0.06	10.6±0.09	3.3±0.02	2.9±0.03	0.5±0.005	0.5±0.004	176.4±2.65	155.4±1.53
	Range	28-41	23-35	0.9-5.7	0.6-1.9	12-Sep	12-Aug	2.8-4.0	2.4-3.9	0.4-0.7	0.4-0.6	127-226	119-199
	LSD	5.05	5.84	1.53	0.63	1.54	2.75	0.43	0.58	0.11	0.11	39.24	35.6
	LSD + Mean	38.66	35.98	3.24	1.73	11.63	13.33	3.77	3.48	0.65	0.62	215.64	190.99
	C.V%	7.12	8.12	44.4	24.3	6.01	8.1	6.02	8.86	9.05	8.25	15.03	9.87

However, the lowest variability was shown in transpiration (6.0 and 8.1%) at both growth stages, respectively. Genotypes 14CLPYT033, RU1402134, and CLJZMN exhibited the highest Pn (41.1 µmol m^{-2} s^{-1}), Cond (4.7 mol m^{-2} s^{-1}), Tr (11.7 mmol H_2O m^{-2} s^{-1}), WUE (4.0 mmol CO_2 mol^{-1} H_2O), Fv/Fm (0.7), and ETR (226.1 µmol m^{-2} s^{-1}) at seedling growth stage, among genotypes. Genotypes RU1504191, Presidio, and RU1401164 revealed the highest Pn (35.2 µmol m^{-2} s^{-1}), Cond (1.9 mol m^{-2} s^{-1}), Tr (12.4 mmol H_2O m^{-2} s^{-1}), WUE (3.90 mmol CO2 mol^{-1} H_2O), Fv/Fm (0.65), and ETR (199.5 µmol m^{-2} s_{-1}) at grain filling stage among genotypes (Table 3.4). However, GSOR101758, RU1401090, RU1104122, RU1404157, and RoyJ recorded the lowest values in physiological traits at both growth stages. Even though 65 percent of rice

genotypes showed higher values than the general mean for transpiration (10.1±0.06 mmol H_2O m^{-2} s^{-1}. only genotype RU1401164 was had significant difference among genotypes at both growth stages. In contrast, at both growth stages, all genotypes displayed FV/FM values falling under the optimum range (0.75–0.85, according to Bolhar-nordenkampf et al., 1989). The range of variation by mean values was more for the traits Pn, Cond, WUE, and ETR. In the present study, the variability among genotypes at seedling growth stage for all the characters were higher than that at grain filling stage. This relationship indicated that there was a small effect of the environment on these traits at grain filling stage. This was in agreement with the findings reported by Saxena et al. (2005) and Padmaja et al. (2008). The balance between photosynthesis and respiration determine plant growth. Plants adapt to climate stresses via multiple strategies, such as the adjustments of phenology and morphology (Wahid et al., 2007). The variability of the physiological parameters has been studied such as fluorescence parameters including photochemical efficiency of PSII (Fv/Fm), ETR, and stomatal conductance (Zhang et al., 2014; Farquhar and Sharkey, 1982). Rice yield comes from photosynthetic in leaves after flowering, which is influenced by the photosynthetic function decline in leaves and leading to the decrease in yield (Zhang et al., 2001). Rice yield potential is estimated to be about 16 tons ha^{-1}; however, average world yield is about 3.5 tons ha^{-1}. The low yield is associated with biotic and abiotic stresses and social and economic conditions of the farmers. Hence, if these stresses are reduced there is a great potential to improve rice yield. In this study, the mean value of each yield-related traits exhibited among the 100 rice genotypes are shown in Table 3.5. Shoot dry weight leaf area increased significantly in the vegetative and reproductive growth stages. The increase in shoot weight is mainly

associated with an increase in leaf area during these growth stages. Shoot weight is a genotype characteristic and is also influenced by environmental factors. Peng et al. (1999) described that yield improvement of lowland rice genotypes was due to increases in biomass production. The main yield components are the number of panicles, number of spikelet per panicle, the weight of grain, and grain filling. The variability in grain yield due to yield-related traits is in the order of SHW > PN > GPE > FLA > SPN > PL > 100 wt > PED > GF% (Table 3.5).

Table 3.5 Means of yield related parameters including: shoot dry weight (SHW, g plant^{-1}), flag leaf area (FLA, cm^{-2}), panicle emergency day (PED, d), panicle number (PN, no. plant^{-1}), panicle length (PL, cm plant^{-1}), spikelet number (SPN, no. panicle^{-1}), gra`in filled (GF, %), 100 grain weight (100 Wt. g), grain yield (GY, g plant^{-1}), and grain production efficiency (GPE, g kg^{-1}) of 100 rice genotypes measured during and after harvest (145 DAP).

Gen. number	Gen. name	Yield related traits									
		SHW	FLA	PED	PN	PL	SPN	GF%	100 Wt.	GY	GPE
1	14CLPYT033	153.6	45.4	86.8	47	26.5	18.2	95.2	2	71.7	492.6
2	14CLPYT108	112.8	36.4	89.5	32.3	25	15.4	93.7	1.9	65.8	588
3	14CVPYT094	112	40.9	107.3	25.4	24.8	15.5	76.1	2	44.2	402.3
4	14CVPYT144	129	32	91.3	34	28.7	14.2	83.6	1.9	57.3	459.2
5	COLORADO	78.2	36.2	90.3	26	23.3	21.4	81.2	2.2	40.3	445.5
6	Bowman	69.6	43	88	30.3	21.3	17	93	1.8	41.6	571.1
7	CAFFEY	113	32.7	86.5	42	20.7	14.7	95.3	1.5	66.9	601.1
8	CHENIERE	57.9	49.8	86.3	33.3	24.1	15.4	94.5	2.1	48	759.7
9	CL Jazzman	153.8	27.5	95	41.8	24.6	17	93.6	2	48.5	358.6
10	CL111	113.3	43.3	85.8	37.3	23	14.6	97.1	1.9	58.2	521.4
11	CL142-AR	134.1	28.2	92.5	39.1	26.2	17.8	96	2.2	66.7	494.9
12	CL151	85	36.9	91	28.5	26.6	15	91.5	2	38.9	462.8
13	CL152	136.3	44.2	93.8	34.8	22.2	13.6	90	1.9	48.1	400.8
14	CL163	57.8	56.7	87.3	30.5	30.2	17.3	93	2.1	42.5	634
15	CL172	122.6	47.9	88.8	28.3	24.5	15.9	96.9	2.2	70.5	593.1
16	CL271	89.8	25.2	86	41.5	23.4	12.3	96.2	1.9	74.8	741.6
17	Cocodrie	100.1	27.4	88.5	37	28.5	16	92.7	1.8	49	491.3
18	NIPONBARE	66.5	39.6	94	19.3	20.3	11.8	97.2	1.9	34.2	462.3
19	ANTONIO	81.3	47.1	97	24.1	23.8	16.3	85.9	2.2	42.8	453.2
20	El Paso 144	131	30.9	94.8	39.8	23.3	15	95.6	1.8	56.9	456.8
21	GSOR100390	137.1	50.3	88.8	34.3	23.3	16.2	87.9	1.9	63.6	521.7
22	GSOR100417	113.4	39.3	72	46.3	24.3	15.4	75.9	2	31.8	331.9
23	GSOR101758	97.5	38.6	90	32.9	23.2	13.1	87	2.1	42.2	430.5
24	RU1104122	29.3	23.8	97.3	48	27	14.8	88.9	2	39.1	436.7
25	CLJZMN	151	32.1	101.8	35.3	24.2	14.6	90.1	2	51.8	389
26	INIA Tacuari	73.6	39.1	88.5	39.5	27.1	16.4	91.1	2.1	36.6	509.2
27	IRGA409	114	44.1	106.8	45.1	23.7	14	93.5	2.1	47.3	418.2
28	JES	114.3	43.6	100.8	59.2	25.6	15	85.5	2.1	49.5	439.4
29	JUPITER	94.1	41.3	90	42.8	24.6	15.5	92.3	2.1	59.4	636.8
30	LA 2008	108.3	36.5	89	42.5	23.7	14.7	96.1	2.1	61.1	564.6
31	LA 2134	121.2	40.8	88.3	41.8	23.2	16.3	96.9	2.2	65.4	540.7
32	LAKAST	78.3	33.9	87.8	29.3	26.9	16	93.9	2.1	50	655.4

Table 3.5 (Continued)

33	MERMENTAU	101.4	32.1	90	41	24.2	14	93	2.1	54.2	534.7
34	Presidio	77.3	39.6	88.8	38.6	23	14.9	95.4	2.1	53.5	720.7
35	Rex	117.4	36.8	92.5	32.8	26.9	17.3	94.7	2.1	66.9	596.2
36	RoyJ	157.1	39.6	100.8	31.5	26.3	17.4	76.8	2	48.3	368.3
37	RU0603075	161.5	26.8	92.3	64.4	24.2	13.5	96.2	2.3	86.5	533.6
38	RU1201024	107.1	42.5	97.3	32.5	26.2	16.8	85	2.1	43.8	412
39	RU1201047	95.4	37.8	94.8	33	24.8	16.2	95.1	2.1	60.2	639.2
40	RU1201136	150.3	24.7	97.8	35.3	24.8	16.3	87.2	2.1	53.8	386.6
41	RU1204156	114.5	33.2	94	31.3	23.3	14.7	93.6	2	49.1	439.4
42	RU1204197	77.9	39.8	89	27.5	24.8	15.3	91.8	1.9	41.8	478
43	RU1301084	118.4	36.1	93.5	26.3	27.8	16.3	87.6	2.1	43.5	369.6
44	RU1301093	128.6	35.7	97.8	38.3	28	17.3	81	2	51.2	400.7
45	RU1301102	109	30.6	94.5	26.3	31.7	14	82.3	2	40.2	414
46	RU1302192	121	33.9	87	51.5	23.2	15.3	96	2.1	61.6	515.3
47	RU1303138	77.1	36.2	92.8	51	22.5	14.2	94.5	2	40.1	514.5
48	RU1303181	103.6	29.9	90.5	34.5	24.4	15.1	89.2	2.2	43	424
49	RU1304114	96	48	95.5	29	21.9	15.5	93.5	1.9	53.3	528.7
50	RU1304122	107.1	40	89.5	45.3	26.2	16.9	93.5	2	52.1	510.5
51	RU1304154	127.8	40.3	89	38.8	23.3	14.8	95.3	2.1	54.3	447.7
52	RU1304156	68	44.9	91.5	31.5	21.4	17.4	93	2.1	50.6	632.4
53	RU1305001	92.6	43.5	87.5	47.3	23.2	15	95.1	2.1	47.8	514.8
54	RU1401067	99.7	48.4	90.8	33	28.6	17	92.9	2.1	45.4	454.9
55	RU1401070	119.1	35.8	92	22.8	25.3	18.2	89.2	2	45.2	380.3
56	RU1401090	108.7	28.6	97	28.8	23.7	16.6	96.8	2	64.2	617.2
57	RU1401099	121.6	38	91.8	37.5	24.6	15.2	95.2	2	60.9	496.8
58	RU1401102	150.9	42.6	103	36	26.5	16.2	91.2	2.1	42.9	280.8
59	RU1401145	71.1	46.9	87.5	21	26.6	16.7	96.3	2	49	671.2
60	RU1401161	108.5	44.1	102	28.8	25.1	15.4	95.6	1.9	50.2	461
61	RU1401164	108	28.3	85.5	39.8	23.7	14.8	95.3	2	60.1	560.9
62	RU1402005	77.4	38.4	87.3	36.9	22.6	15.9	94.6	2	51.5	666.1
63	RU1402031	107.4	43.3	92.8	33	25.5	15.8	93.4	1.9	43	398.6
64	RU1402065	118.4	42.1	89	43.6	20.8	14.5	94.6	2	58	521.5
65	RU1402115	69.3	24	88.5	32.5	24	15.2	93.6	2.1	58.2	577.7
66	RU1402131	110.7	31.1	88.8	46.5	21.8	14.8	94.2	2	52.3	471.8
67	RU1402134	122	25	85.5	34.5	23	14.6	93.4	2	50.7	410.3
68	RU1402149	111.3	32.8	89.5	40.3	23.7	14.7	95.5	2	46.4	415.4
69	RU1402174	99.9	42.5	87	32.3	24.8	15.7	95.6	1.9	68.2	687.6
70	RU1402189	101.2	37.5	86	34.3	24.8	15	96.6	2.1	62.2	614.4
71	RU1402195	172.1	34.7	95.3	50.7	21	14.7	93.9	2.1	57.3	365.3
72	RU1403107	106.9	36.6	91.8	43.5	22.3	14.1	79.9	1.8	45.7	477.8
73	RU1403126	87.3	47.5	88.5	33.7	28	15.2	93.7	1.9	45.2	561.7
74	RU1404122	88	24.2	87.8	35.3	27.3	16.3	95.6	2.1	59.7	681
75	RU1404154	80.4	27.4	91	28	22.9	14.6	93.7	2	31.8	398.2
76	RU1404156	96.9	45.3	93	31	25	17.5	90	2.2	52.4	543.3
77	RU1404157	93.3	49.4	93	29	25.4	17	90.3	1.8	34	368.4
78	RU1404191	150.8	33.3	93	14.5	25.8	15.2	91.4	2	53.3	351.4
79	RU1404193	85.5	28.9	86	41.3	23.6	15.4	95.6	1.9	56.2	653.5
80	RU1404194	107.2	43	92.8	41.5	28.8	18.3	90.7	2.1	61.7	845.8
81	RU1404196	64	43.1	92.5	34	24.7	15.2	94.3	2	41.9	559.6
82	RU1404198	83.4	35.8	99.5	41.3	23.7	15.7	85	2	38.7	465.5
83	RU1504083	122.1	47.6	86.8	37.5	25.5	14.9	94.6	2	53.9	446.6
84	RU1504100	102.3	42	89	40.3	24.9	16.5	94.3	2	47.2	462.2
85	RU1504114	121.3	47.7	91.3	31	24.8	16.3	88.8	2	43.8	361.7
86	RU1504122	97.3	35.8	108	70	23.3	14.5	89.3	2.2	54.1	623.4
87	RU1504154	107.7	40.3	90.8	45	22.8	14.7	86.6	2.1	46.8	433.5
88	RU1504156	105.4	31.3	81.8	35.8	25.8	16.7	91.6	2.1	47	438
89	RU1504157	98.6	40	91.3	44.8	26	15.8	94.1	2	53.7	551.3
90	RU1504186	124.5	40.4	94.8	38.3	22.8	15.2	93.1	1.9	57.8	458.8
91	RU1504191	147.1	48.6	90.8	36.8	25.6	14.4	85.9	2.1	44.9	314.1
92	RU1504193	111.3	36.8	91	36.5	23.8	15.3	93.3	2.1	45	405.8
93	RU1504194	151.9	28.6	96.3	41.3	23.6	16.5	94.9	2	65.2	428.6
94	RU1504196	111.6	40.5	88.5	32	27.3	17.6	92.9	2.1	46.4	419.2
95	RU1504197	98.5	29	87.8	36	23.7	14.3	93.9	2.1	39.2	397.3
96	RU1504198	103.8	34.4	87.8	26.5	27.8	14.6	96.4	2	62.4	629.9
97	Sabine	95.7	47.1	87.3	35.5	23.6	14.8	91.5	2	62	636.2
98	Taggart	140.6	38.7	94.3	33.3	27.3	16.8	91.9	2.2	59.3	436.1
99	Thad	74	41.4	87.5	36.8	26.5	16.3	93.9	1.9	46.8	646.8
100	N-22	161.4	39.2	92.8	35	31	21	90.9	2	58.4	400.6
	Mean	107.3±2.7	38.1±0.7	91.5±0.5	36.6±0.9	25±0.2	15.7±0.1	91.8±0.5	2.0±0.01	51.8±1.0	500.9±10.9
	Range	29.3-172	23.8-56.7	72-108	14.5-70	20.3-31	11.8-21	75.9-97	1.5-2.3	31.8-86	280-845
	LSD	17.37	9.55	3.63	8.33	3.85	2.69	15.27	0.24	6.33	126.31
	LSD + Mean	124.72	47.65	95.13	44.93	28.65	18.39	107.07	2.24	58.13	627.21
	C.V%	24.69	18.32	5.88	23.49	8.86	9.37	5.17	5.91	19.34	21.82

In addition to these yield components, the yield is also positively and significantly associated with plant height, Pn, Chl, and number of tillers (Table 3.9). Days to panicle initiation showed almost the same trend with days to heading. Yaqoob et al. (2012) also observed that early heading lines matured earlier. Days to panicle initiation of genotype N-22, which belong to the indica species, was 95% lower than the other japonica genotypes and that result matched with Jennings et al. (1979), who reported the spikelet filling duration of japonicas as usually slightly longer than that of indicas. There is a negative relationship between days to panicle initiation and grain filling and that agrees with Yamamoto et al. (1991), who reported that in the grain filling of the spikelets, which were derived from secondary branches, is higher compared to spikelets on primary branches. The grand mean of grain filling for all genotypes was 91.8±0.5%, and that agrees with Yoshida, (1981) who reported the filled spikelet percentage is about 85% in rice. There must be a suitable number of panicles for achieving maximum yield. Differences have been spotted in grain yield among genotypes having the same amount of shoot dry matter, because differences exist in the utilization of photosynthesis among them (Yamamoto et al., 1991). Panicle size comprises panicle length or the number of spikelets per panicle. Panicles number, panicle size, and grain weight are mainly determined in the vegetative, reproductive, and the spikelet-filling growth stages, respectively. Hence, this means any biotic or abiotic stress during vegetative, reproductive, and spikelet filling growth stages can reduce rice yield. The 100-grain weight varied from 1.5 to 2.3 g, with an average value of 2.0 g. Therefore, there was a difference of about 8% in 100-grain weight between lowest and highest weight producing genotypes. The highest significance among genotypes for yield-related traits was

observed in grain yield (23%) and was followed by GPE (18%) while PL, SPN, GF, and 100-grain wt did not show any significant among genotypes (Table 3.1 and 3.5). Consistent with our hypothesis, based on the cumulative vigor response index (CVRI) and standard deviation, five categories were classified including very low, low, moderate, high, and very high vigor response indices for the morpho-physiological parameters during seedling growth and grain-filling stages and yield-related parameters at harvesting (Tables 3.6, 3.7, and 3.8).

Table 3.6 Classification of 100 rice genotypes into very low, low, moderate, high, and very high vigor index based on combined growth and physiological response indices through 55-65 DAP.

Very low 8.43-8.80	Low 8.81-9.17	Moderate 9.18-9.55	High 9.56-9.92	Very high 9.93-10.30
Sabine (8.43)	RU1504100 (8.82)	RU1404191 (9.19)	RU1404194 (9.56)	N-22 (9.96)
GSOR101758 (8.54)	LAKAST (8.85)	ANTONIO (9.21)	Presidio (9.57)	RU1104122 (9.99)
RU1401067 (8.64)	RU1204197 (8.86)	COLORADO (9.21)	RU1402131 (9.57)	RU1401070 (10.06)
RU1401090 (8.71)	NIPONBARE (8.87)	RU0603075 (9.22)	RU1402195 (9.57)	RU1402065 (10.08)
	RU1303138 (8.89)	RU1302192 (9.22)	RU1304156 (9.58)	14CLPYT033 (10.11)
	RU1404154 (9.02)	CL142-AR (9.22)	CL172 (9.60)	RU1401164 (10.27)
	RU1404157 (9.04)	Bowman (9.23)	RU1402189 (9.60)	RU1402134 (10.32)
	RU1301084 (9.08)	JES (9.27)	RU1301093 (9.60)	CL JZMN (10.39)
	RU1404196 (9.09)	14CVPYT144 (9.27)	RU1402005 (9.61)	
	RU1201136 (9.09)	RU1301102 (9.29)	Cocodrie (9.61)	
	14CVPYT094 (9.10)	RU1404198 (9.32)	RU1504194 (9.61)	
	CL111 (9.11)	Thad (9.32)	RU1504114 (9.63)	
	RU1401102 (9.11)	IRGA409 (9.34)	RU1204156 (9.63)	
	RU1504154 (9.13)	RU1402149 (9.35)	RU1504186 (9.64)	
	CAFFEY (9.14)	CL152 (9.36)	RU1504122 (9.64)	
	RU1304122 (9.15)	RU1401145 (9.37)	GSOR100417 (9.65)	
	RU1404122 (9.17)	RU1403126 (9.38)	RU1304114 (9.65)	
	RoyJ (9.17)	RU1401161 (9.40)	El Paso 144 (9.68)	
		CL Jazzman (9.42)	CL151 (9.69)	
		RU1402174 (9.43)	RU1401099 (9.71)	
		Taggart (9.43)	RU1403107 (9.76)	
		RU1404156 (9.45)	RU1504156 (9.78)	
		RU1402115 (9.45)	CL163 (9.78)	
		RU1402031 (9.45)	RU1504157 (9.82)	
		RU1504193 (9.46)	14CLPYT108 (9.84)	
		RU1504198 (9.48)	RU1504083 (9.85)	
		RU1303181 (9.50)	JUPITER (9.85)	
		RU1201047 (9.51)	CHENIERE (9.86)	
		GSOR100390 (9.51)	RU1304154 (9.87)	
		RU1504197 (9.51)	RU1504191 (9.88)	
		RU1201024 (9.51)	RU1504196 (9.88)	
		MERMENTAU (9.52)	INIA Tacuari (9.88)	
		RU1305001 (9.52)	LA 2134 (9.89)	
		RU1404193 (9.53)	Rex (9.92)	
		CL271 (9.54)		
		LA 2008 (9.54)		

Table 3.7 Classification of 100 rice genotypes into very low, low, moderate, high, and very high vigor index based on combined growth and physiological response indices through 105-115 DAP.

Very low 8.72-9.12	Low 9.13-9.52	Moderate 9.53-9.91	High 9.92-10.31	Very high 10.32-10.71
RU1404154 (8.72)	RU1504194 (9.13)	RU1301093 (9.53)	RU1402131 (9.92)	RU1402134 (10.32)
RU1104122 (9.00)	RU1301102 (9.14)	GSOR100390 (9.55)	CL Jazzman (9.93)	RU1504157 (10.35)
RU1404157 (9.03)	RU1402174 (9.18)	JUPITER (9.57)	Rex (9.94)	RU1402065 (10.37)
RU1304122 (9.03)	RU1504122 (9.20)	RU1404194 (9.58)	RU1402189 (9.95)	N-22 (10.39)
RoyJ (9.07)	Sabine (9.25)	RU1401067 (9.58)	RU0603075 (9.99)	RU1504191 (10.40)
RU1402149 (9.12)	RU1201047 (9.27)	RU1304154 (9.59)	RU1404122 (9.99)	Presidio (10.44)
	14CVPYT144 (9.29)	RU1504156 (9.59)	JES (10.00)	14CLPYT033 (10.52)
	CL111 (9.31)	Taggart (9.60)	14CLPYT108 (10.00)	RU1401164 (10.58)
	RU1301084 (9.31)	CL163 (9.63)	LA 2008 (10.00)	RU1402195 (10.59)
	Cocodrie (9.32)	RU1304114 (9.63)	INIA Tacuari (10.01)	GSOR100417 (10.60)
	CL271 (9.33)	RU1401145 (9.63)	RU1504193 (10.08)	
	MERMENTAU (9.34)	RU1504198 (9.64)	CL142-AR (10.09)	
	LAKAST (9.34)	RU1401099 (9.64)	RU1404198 (10.09)	
	RU1404156 (9.35)	RU1401102 (9.66)	CLJZMN (10.11)	
	RU1401090 (9.39)	RU1304156 (9.67)	IRGA409 (10.12)	
	RU1504197 (9.42)	RU1504196 (9.69)	ANTONIO (10.16)	
	RU1204156 (9.43)	Thad (9.70)	RU1303181 (10.17)	
	14CVPYT094 (9.43)	GSOR101758 (9.70)	RU1404193 (10.21)	
	CAFFEY (9.44)	Bowman (9.70)	LA 2134 (10.21)	
	CL151 (9.45)	El Paso 144 (9.72)	RU1401070 (10.24)	
	RU1201136 (9.45)	RU1403126 (9.72)		
	RU1402005 (9.45)	RU1404196 (9.74)		
	RU1504100 (9.46)	RU1204197 (9.77)		
	RU1303138 (9.46)	RU1504083 (9.78)		
	RU1402031 (9.47)	COLORADO (9.78)		
	RU1402115 (9.50)	CL172 (9.80)		
	RU1305001 (9.52)	RU1302192 (9.81)		
	RU1401161 (9.52)	CL152 (9.82)		
		RU1403107 (9.82)		
		NIPONBARE (9.83)		
		RU1504186 (9.83)		
		CHENIERE (9.84)		
		RU1404191 (9.87)		
		RU1201024 (9.89)		
		RU1504154 (9.90)		
		RU1504114 (9.90)		

The CVRI values ranged from 8.43,8.72, and 6.48 (highly lower index) for the genotypes Sabine, RU1404154, and RU1404154 to 10.39, 10.60, and 8.14 (highly vigor index) for the genotypes CLJZMN, GSOR100417, and RU0603075 at seedling growth, grain filling, and harvest stages, respectively. Also, 42, 30, and 20% of the genotypes were classified as having high and very high vigor index while 22, 28, and 36% of genotypes were classified as having low and very low vigor index, falling under moderate

vigor index (36, 36, and 44%) at seedling growth, grain filling, and harvest stages, respectively.

Table 3.8 Classification of 100 rice genotypes into very low, low, moderate, high, and very high vigor index based on cumulative yield and yield related response indices at harvest 145 DAP.

Very low 6.48-6.80	Low 6.81-7.11	Moderate 7.12-7.43	High 7.44-7.74	Very high 7.75<
RU1404154 (6.48)	COLORADO (6.82)	RU1201024 (7.11)	14CVPYT144 (7.44)	CL172 (7.76)
GSOR100417 (6.57)	RU1404157 (6.83)	MERMENTAU (7.12)	RU1504157 (7.44)	N-22 (7.77)
NIPONBARE (6.60)	RU1404198 (6.84)	RU1402115 (7.12)	RU1304122 (7.44)	RU1404194 (7.83)
RU1504197 (6.62)	GSOR101758 (6.86)	RU1402005 (7.13)	CL163 (7.45)	14CLPYT033 (8.12)
RU1204197 (6.67)	14CVPYT094 (6.86)	RU1401164 (7.13)	RU1504194 (7.46)	RU0603075 (8.14)
RU1104122 (6.68)	RU1204156 (6.91)	RU1201136 (7.13)	RU1302192 (7.50)	
RU1402134 (6.73)	RU1401070 (6.91)	El Paso 144 (7.15)	RU1402195 (7.52)	
CL151 (6.74)	RU1504156 (6.92)	RU1304114 (7.16)	JUPITER (7.53)	
RU1301102 (6.74)	Cocodrie (6.92)	CLJZMN (7.18)	RU1402174 (7.53)	
RU1403107 (6.80)	RU1404196 (6.94)	RU1401145 (7.19)	GSOR100390 (7.57)	
RU1303181 (6.80)	RU1402149 (6.94)	RU1304156 (7.19)	JES (7.61)	
	RU1303138 (6.95)	Thad (7.20)	Taggart (7.63)	
	RU1301084 (6.96)	RU1504196 (7.21)	CL142-AR (7.63)	
	INIA Tacuari (6.98)	RU1504100 (7.21)	LA 2134 (7.64)	
	RU1504193 (6.98)	RU1401161 (7.22)	Rex (7.68)	
	ANTONIO (6.98)	CL271 (7.23)		
	RU1402031 (7.00)	RU1403126 (7.23)		
	CL152 (7.05)	RU1504191 (7.23)		
	RU1504154 (7.07)	CL111 (7.25)		
	Bowman (7.07)	RU1404122 (7.25)		
	RU1402131 (7.07)	RU1504122 (7.26)		
	RU1504114 (7.08)	CL Jazzman (7.26)		
	CAFFEY (7.09)	RU1504186 (7.26)		
	LAKAST (7.09)	RU1504198 (7.28)		
	RU1404193 (7.10)	RU1305001 (7.28)		
		RoyJ (7.28)		
		Presidio (7.29)		
		RU1301093 (7.29)		
		RU1304154 (7.30)		
		CHENIERE (7.31)		
		RU1401090 (7.31)		
		14CLPYT108 (7.32)		
		RU1402065 (7.33)		
		RU1401067 (7.35)		
		RU1401102 (7.38)		
		RU1404156 (7.38)		
		RU1504083 (7.38)		
		RU1401099 (7.39)		
		RU1402189 (7.39)		
		IRGA409 (7.39)		
		RU1404191 (7.39)		
		LA 2008 (7.41)		
		Sabine (7.42)		
		RU1201047 (7.43)		

It is worth mentioning that some of the genotypes falling under the same category of classification at the seedling, grain filling, and harvest stages and that could be a measure of stability or adaptation of these genotypes to different growth stages. For example, genotypes N-22, 14CLPYT033, REX fell under the very high and high vigor response indices, respectively. Genotypes RU1401145, RU14031126, RU1504198, RU1201024, LAKAST, RU1303138, 14CVPYT094, and CAFFEY fell under moderate and low vigor response indices (Tables 3.6, 3.7, and 3.8). Similar methodologies were applied for successful screening of rice and corn hybrids for drought and cold tolerance (Singh et al., 2017;Wijewardana et al., 2015).

The correlation coefficient (r^2) between the CVRI and growth or physiology vigor response index is considered the same at both seedling growth stage as well as at grain filling. That is positively correlated (r^2 = 0.17 and 0.14) for growth at (P = 0.0001, n = 100) and (r^2 = 0.82 and 0.83 for physiology at P = 0.0001, n = 100) at seedling growth and grain filling stages, respectively (Figure 3.1 and 3.2). This implies the greater importance of physiological parameters than growth traits in identifying high vigor rice lines using these indices during seedling growth and grain-filling stages. Hence, the coefficient of determination (r^2) between the total vigor response index (TVRI) and cumulative vigor response index of morpho-physiology at seedling growth or grain filling flowering is exactly the same and are positively correlated (r^2 = 0.63 and 0.63) at P = 0.0001, n = 100).

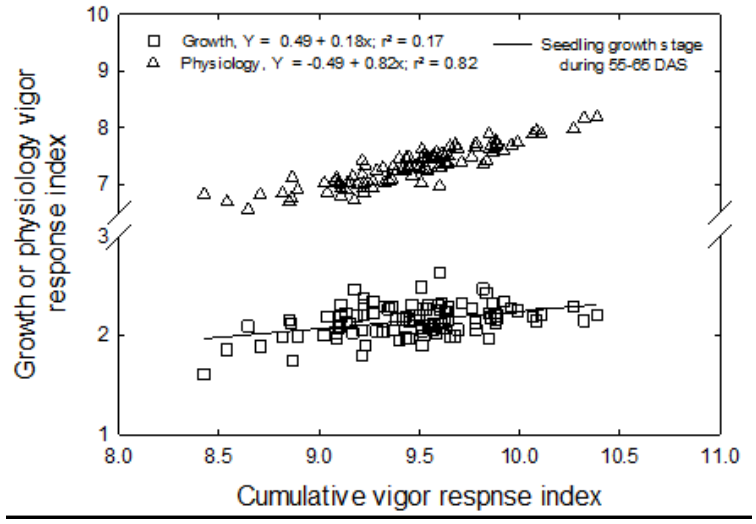

Figure 3.1 Relationship between combined vigor response index and growth or physiology vigor response index of 100 rice genotypes at vegetative stage during 55-65 DAS.

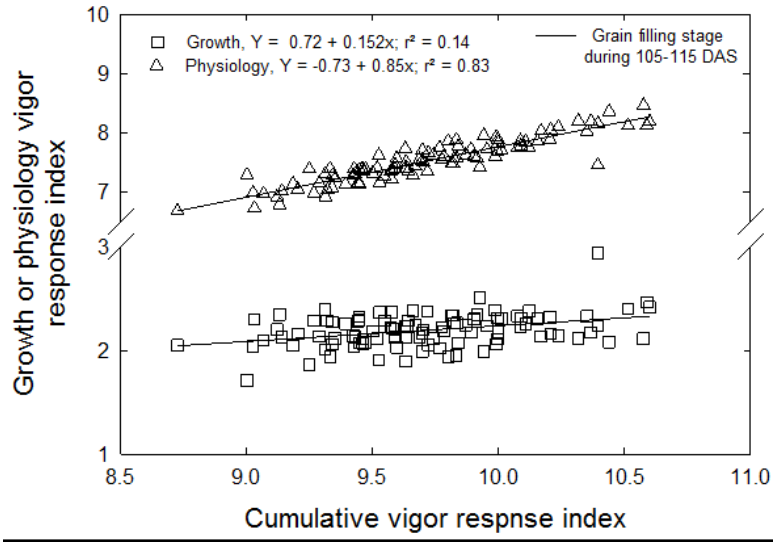

Figure 3.2 Relationship between combined vigor response index and growth or physiology vigor response index of 100 rice genotypes at grain filling stage during 105-115 DAS.

However, the relation between total vigor response index and yield-related vigor response index was weakly correlated at 0.36 (figure 3.3).

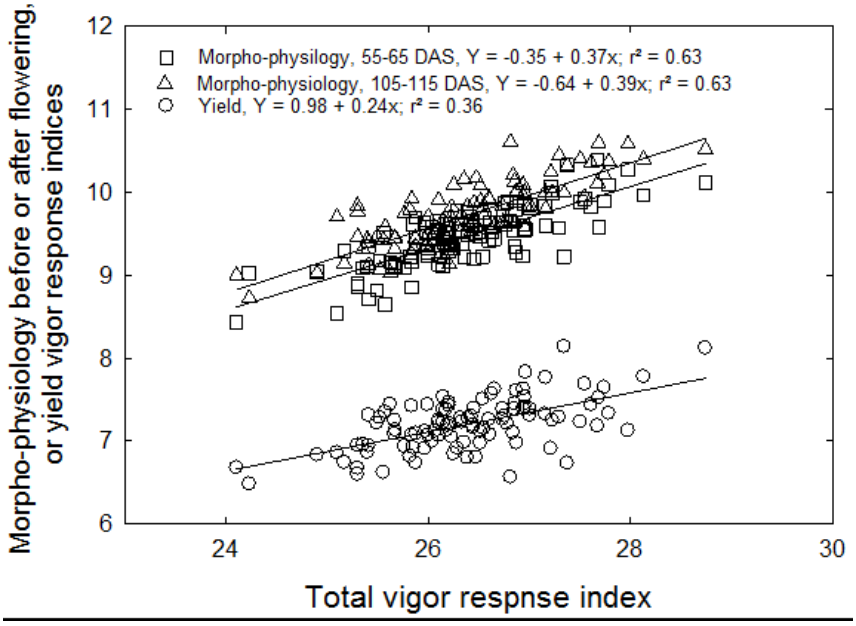

Figure 3.3 Relationship between combined vigor response index and morpho-physiology vigor index at vegetative stage or morpho-physiology vigor index at grain filling stage or yield related vigor index of 100 rice genotypes.

The degree of correlation among the traits is a key factor especially in a complex trait such as yield (Akinwale et al., 2011). The knowledge about the relationship between grain yield and its contributing characters is needed for an efficient selection strategy for the future breeding program. The correlation coefficients among growth, physiological, and yield-related studied traits are listed in Table 3.9. In the present study plant height, grain production efficiency, shoot dry weight, and panicle number showed highest significant positive correlation with grain yield (0.60**, 0.54***, 0.40**, and 0.26***) respectively.

Table 3.9 Correlation among various traits, growth, physiology, and yield related, of 100 rice lines and genotypes.

	PH	LN	TN	SLA	RI	CT	Chl	Caro	Pn	Cond	Tr	WUE	Fv'/Fm'	ETR	SHW	PED
PH		***0.34	**0.15	n.s0.09	n.s0.018	n.s0.06	n.s0.05	n.s0.02	n.s-0.02	n.s-0.01	n.s0.03	n.s0.01	n.s0.05	n.s0.03	***0.21	***-0.26
LN			n.s-0.09	n.s0.05	*0.12	**0.18	n.s-0.01	*-0.11	n.s0.06	n.s0.07	n.s0.03	n.s0.05	***0.21	***0.21	**0.13	n.s-0.06
TN				n.s0.08	n.s-0.02	n.s0.01	n.s-0.01	n.s0.04	n.s-0.04	n.s-0.07	n.s-0.05	n.s0.05	n.s-0.03	n.s-0.02	***0.34	n.s0.08
SLA					***0.11	n.s0.08	0.0041	n.s-0.06	n.s0.02	n.s-0.06	n.s-0.05	n.s0.01	n.s0.04	n.s0.03	n.s-0.04	n.s0.05
RI						*0.13	n.s0.03	n.s-0.02	*-0.13	n.s-0.08	n.s0.06	n.s-0.07	**0.13	*0.13	n.s-0.04	n.s0.04
CT							n.s0.02	n.s0.04	n.s0.03	n.s0.01	*0.11	n.s-0.05	***0.26	***0.19	**0.13	n.s-0.05
Chl								***0.65	n.s0.02	n.s0.01	n.s0.08	n.s-0.03	*0.13	**0.13	n.s0.05	n.s-0.05
Caro									n.s-0.02	***0.34	n.s0.02	n.s-0.01	n.s0.02	n.s-0.02	n.s-0.01	n.s-0.09
Pn											***0.28	***0.39	***0.24	***0.22	n.s-0.05	n.s-0.05
Cond											*0.13	***0.23	n.s0.05	n.s-0.02	n.s-0.01	n.s-0.03
Tr												***-0.49	***0.51	***0.28	n.s-0.01	n.s-0.03
WUE													**-0.15	n.s-0.05	n.s0.02	n.s-0.033
Fv'/Fm'														***0.60	n.s-0.01	n.s-0.06
ETR															n.s0.09	n.s-0.05
SHW																***0.20
PED																

Table 3.9 (Continued)

	PN	PL	SPN	GF	WT	GY	GPE
H	n.s-0.04	**0.14	***0.29	n.s0.01	n.s-0.03	**0.13	n.s-0.02
LN	n.s-0.05	n.s0.01	***0.21	n.s0.08	n.s-0.01	n.s0.01	*-0.11
TN	***0.70	**-0.15	**-0.15	n.s-0.01	n.s-0.02	***0.22	n.s-0.06
SLA	n.s-0.06	n.s0.02	n.s0.03	**0.17	n.s0.01	n.s-0.19	**-0.14
RI	n.s-0.01	n.s-0.05	n.s0.06	n.s0.01	n.s0.01	n.s-0.02	n.s0.01
CT	n.s0.028	n.s-0.09	*0.11	n.s0.01	n.s0.05	*0.10	n.s-0.05
Chl	n.s0.04	n.s-0.02	n.s0.02	n.s-0.06	n.s-0.02	*0.11	n.s0.06
Caro	n.s0.05	n.s0.01	n.s0.01	n.s-0.05	n.s-0.01	n.s0.06	n.s0.08
Pn	n.s-0.03	n.s-0.01	n.s0.04	n.s0.05	n.s0.06	n.s0.01	n.s0.05
Cond	n.s0.05	n.s-0.04	n.s0.03	n.s0.04	n.s0.06	n.s-0.02	n.s-0.03
Tr	**-0.13	n.s-0.05	n.s-0.05	n.s-0.03	n.s0.02	n.s0.04	n.s0.06
WUE	**0.17	n.s0.02	*0.10	n.s0.02	n.s0.06	n.s-0.01	n.s-0.03
Fv'/Fm'	n.s-0.08	n.s-0.01	*0.13	n.s0.06	n.s0.05	n.s0.04	n.s0.04
ETR	n.s-0.03	n.s-0.01	**0.15	n.s0.04	**0.14	*0.12	n.s-0.01
SHW	***0.35	n.s-0.07	n.s0.07	*0.11	n.s0.03	**0.40	***-0.43
PED	n.s-0.08	n.s-0.02	n.s-0.04	***0.23	n.s0.06	n.s-0.09	***-0.30
PN		***-0.19	***-0.17	n.s-0.07	n.s-0.01	***0.26	n.s0.01
PL			***0.36	n.s0.07	n.s-0.02	n.s-0.04	n.s0.02
SPN				*0.13	n.s0.02	n.s0.05	n.s-0.03
GF					n.s0.01	***-0.32	***-0.39
WT						n.s0.06	n.s0.01
GY							***0.54

These results did match the findings of Thirumeni and Subramanian (1999); Yogamenakshi and Ambularmathi (2004) who reported a significant correlation of grain yield per plant with plant height and shoot dry weight. That indicates the effect of genetic variability and environmental factors on grain yield in different genotypes. Grain yield recorded a highly significant positive correlation with panicle length (0.30), 100- grain weight (0.27), and leaf area (0.19), which that agree with (Ogunbayo et al., 2014). This suggests that selection directed towards these characters will be effective in ensuring high seed yield in rice. The observed positive correlation of grain yield with various parameters was supported by earlier researchers such as Basavaraja et al. (1997) for plant height; Rajeshwari and Nandrajan (2004) for the number of filled grains per panicle; Sharma and Dubey (1997) for panicle length; and Chakraborty et al. (2001) for 100-seed weight. There was a low but significant negative correlation between grain yield and panicle length (-0.36***) that did match the finding of Yolanda and Das (1995). Overall, the highest significant positive correlations found among 23 growths, physiological, related yield traits were between tillers number and number of panicle (0.70***) followed by between the total of chlorophyll and carotenes (0.65***). The lowest significant negative correlations were found between water use efficiency and transpiration rate (-0.49***) and followed by between shoot dry weight and grain production efficiency (-0.43***).

CHAPTER IV

DEVELOPING SCREENING TOOLS FOR EARLY-SEASON HIGH AND LOW TEMPERATURE STRESS TOLERANCE IN RICE

Abstract

Temperature is one of the key abiotic stress factors that affect various stages of plant growth and development. In the US Midsouth, rice plants get exposed to variable temperatures depending on the planting date. We hypothesize that rice cultivars vary in their response to temperature, and developing a method for low and high-temperature tolerance screening will help producers and breeders to select cultivars for management and breeding, respectively. Four rice cultivars, CL152, Bowman, Antonio, and Mermentau along with two hybrids XL 753 and CLXL 745 that were the most commonly grown in the US Midsouth were evaluated in this study for temperature tolerance. Five day/night temperature treatments, 20/12 (very low), 25/17 (low), 30/22 (optimum), 35/27 (high), and 40/32°C (very high) were imposed after the seedling establishment, 10 days after planting (DAP). Growth and developmental parameters including root and physiological parameters were recorded from plants harvested at 39 DAP. Rice cultivars and hybrids exhibited significant variability in their response to low and high temperatures. Based on total low- and high-temperature response indices, relative temperature response scores were derived. Total low-temperature response index values

ranged from 18.48 to 23.15 whereas total high-temperature responses index values ranged from 42.01 to 48.82. Antonio, CLXL 745, and Mermentau were identified as sensitive to cold- and heat, Bowman as sensitive to cold and moderately seneitive to heat, CL152 was moderately sensitive to cold- and heat, and XL 753 was highly cold/heat tolerant of the cultivars/hybrids tested. These results may be useful for breeders to develop new rice cultivars which could withstand low and high-temperature conditions during seedling stages. Further large-scale studies are needed to evaluate more cultivars or lines both in the controlled environments and field settings to come up with practical recommendations.

Introduction

Rice (*Oryza sativa* L.) is the staple food for about 50% of the world's population and plays an vital role in global food security. To meet the needs of the world's growing population, rice production has to be increased by several times despite the challenge of different biotic and abiotic stresses including variable temperature (Rathore et al., 2016). Global temperature increases, just one aspect of Climate Change, could play a significant role in future crop productivity and plant performance (Nagai and Makino, 2009). If surface air temperatures increase as some research expects, 1.4-5.8°C by 2100 due to global climate events, rice yields could be decreased as much as 41%. (IPCC 2007; Ceccarelli et al., 2010). Studies related to how climate change might affect crops have gained interest among researchers worldwide in recent years. Scientists have begun to study how temperature effects crop production of many staple crops such as rice, wheat, maize, rice, and cotton. Scientists are also looking at how plants can adapt to temperature change (Hoogenboom, 2000; Fahad et al., 2016, 2017;Gbetibouo and Hassan, 2005;

Reddy et al., 2017). Most plants have diverse defense mechanisms to reduce stress and minimize damage at the cellular and functional levels caused by temperature fluctuations. Maximal expression of traits under narrow cardinal temperature ranges vary among and within species. Plant processes can even vary within a particular variety. In the US Mid-South, rice planting and flowering generally coincide with low and high temperature in every year, respectively. Each season, however, is unique in timing and frequency of rains, temperature, radiation level, and other environmental yield determining factors.

Farmers and researchers need simple tools for selecting a cultivar suitable for a niche environment. Many crops are vulnerable to elevated temperatures caused by global warming. Previous studies have shown that yield of cereal crops including rice might significantly decrease due to global warming. Also, unlike other cereals such as barley and wheat, rice plants are susceptible to cold stress, further decreasing its productivity. Rice originated in tropical or subtropical areas and is considered as a sensitive crop to low-temperature. Rice growth and development are roughly limited below 15°C (Krishnan et al., 2011). Physiological processes and reproductive functions under high and low daytime temperatures have not been well documented (Fahad et al., 2016). In rice breeding programs, genetic variability for heat and cold tolerance has been estimated by researchers at germination, seedling, and reproductive stages (Singh et al., 2017a, b). Some studies have been conducted to understand the effects of high and low temperature on rice. According to Li et al., (1981) the amount of injury from temperature usually depends on the time of the occurrence (growth stage) and the duration of the stress (Li et al., 1981). Cold stress may have a direct effect on rice plants during early growth and development stages and lead to weak, stunted seedling growth, reduced tillers, and a

longer growth cycle (Lone et al., 2018; Shimono et al., 2002). Additionally, previous research has focused on the qualitative effects of temperature rise on rice productivity at the reproductive stage, but the quantitative impact of high/low temperature on seedling growth has been ignored.

Cold temperatures can damage rice plants furing all phases of growth from germination until grain filling (Ye et al., 2009). Cold tolerance at seedling emergence and during early vegetative stages are essential to establish an even plant population. According to Cruz and Milach (2013), excellent cold tolerance during the seedling stage is a substantial characteristic for regular rice production, especially in dry direct seeded rice. At vegetative stages, cold temperature damage is responsible for yellowing of the leaves and decreased tillering. Cold temperature stress typically has a negative effect on rice growth (Sanghera et al., 2011). Shoot and root biomass are used in the evaluation of genotypes for plant vigor at all developmental stages (Aghaee et al., 2011).

Chlorophyll content provides a good quantitative estimate of chlorosis in rice plants (Yoshida et al., 1981), giving a more detailed evaluation than visual analysis alone (Park et al., 2013). Another tool to measure photosynthetic activity in plants is chlorophyll fluorescence, which indicates the maximum photochemical efficiency of PS II. This can be used to assess cold sensibility or tolerance (Sikuku et al., 2010). Cold temperatures reduce the concentration of chlorophyll in sensitive rice genotypes (Dai et al., 1990; Aghaee et al., 2011). Chlorophyll content has been used as a tool to compare cold tolerance among distinct hybrid lines during grain filling (Wang et al., 2006), to observe plant revival after stress (Kuk et al., 2003), and to assess cold tolerance in transgenic plants (Tian et al., 2011).

Roots system architecture plays a vital role in plant growth, development, and ultimately crop yield. Rice is a model cereal plant that possesses a fibrous root system with crown roots that emerge post-embryonically from the stem nodes. The critical minimum temperature for root elongation is between 12 to 16 °C and between 7 to 16 °C for shoot elongation. (Nishiyama 1977). Kuwagata et al. (2004) reported that low root temperature reduces the ability of the roots to take up water and root hydraulic conductivity declines dramatically within several hours when roots are cooled below the critical temperature of 15 °C (Murai-Hatano et al., 2008).

Rice highly susceptible to heat stress during reproductive stages but has a higher tolerance at the vegetative stages (Jagadish et al., 2010). Shah et al. (2014) proved that a 2°C increase in temperature will lead to higher losses in rice productivity and qualitative attributes than previous simulations had projected in the indica and japonica ecotypes. At temperatures below the maximum for rice, the response of biomass production is one of the critical determinants of yield variations. By increasing temperature from 25 to 27 °C the biomass decreased by 16%, but a temperature increases from 25 to 28 °C increased biomass by 13-16% (Baker and Allen, 1993; Ohe et al., 2007). However, there is no significant difference in biomass when the temperature increased from 25 to 31°C (Kim et al., 1996). Yoshida (1981) reported that tiller number per plant determines panicle number, an essential component of grain yield. Generally, selection for heat tolerance can be performed by screening rice at temperatures higher than 38°C (Satake and Yoshida 1978). Indeed, rice is adversely affected by lower temperature below 20 °C in the temperate regions especially for indica subspecies and by elevated temperature above 30 °C in the tropics especially for the japonica subspecies. Grown in temperate regions, the

indica subspecies of rice is particularly affected by temperatures below 20 °C. Grown in tropical regions, the japonica subspecies of rice is particularly affected by temperatures above 30 °C (Krishnan et al., 2011). Limited data are available on the impact of high/low temperature on rice root morphology and root-related traits at early growth stages.

Early season vigor is an essential trait in rice development, playing a crucial role in canopy development and thus, light interception. Understanding how early season vigor is affected by temperature is essential. Breeding genotypes with cold and heat tolerance could be the best solution for minimizing the influence of low or high temperature on plants. Most studies related to rice and global warming have been conducted under controlled experimental conditions. For example, open-topped chambers and in closed greenhouses can be used to manipulate temperature (Lone et al., 2018; Amanullah et al.,2017; Chiba and Terao, 2015; Jagadish et al., 2007). Most previous studies involving the impact of high temperature on rice production were designed to control the temperature over a small plant population size, while others analyzed the regression and correlation of historical data sets from long-term field experiments. These strategies are inadequate because they include possible confounding effects from factors other than temperature. In addition, little information is available about the response of japonica cultivars to high temperature during the early seedling stage. The objectives of this experiment were: (1) to characterize cultivar responses to low and high temperatures, (2) develop a screening tool for cold and heat tolerance, and (3) determine early season vigor in rice canopy and tiller development, which could correlate with overall final yield.

Materials and methods

Experimental Condition

The experiment was conducted in five sunlit, controlled environment chambers known as Soil-Plant-Atmosphere-Research (SPAR) units located at the Rodney Foil Plant Science Research facility of Mississippi State University near Starkville, MS, USA. These chambers utilize natural sunlight while allowing complete control of many environmental factors including temperature, atmospheric gasses, plant nutrients, and moisture. Each chamber possesses a steel soil bin (2 m long by 0.5 m wide by 1 m deep) to house the root system and Plexiglas chamber (2 m long by 2.5 m tall by 1 m wide) to accommodate aerial plant parts. Zhao et al. 2006 reported that each growth chamber consists of a 1.27 cm thick Plexiglas dome, which allows 97% of the visible solar radiation to reach the plants (Zhao et al., 2006). In each SPAR chamber, the day temperature was adjusted at sunrise and returned to night temperature after sunset by 1h. The chamber CO_2 concentration was monitored and maintained at 400 μmol mol^{-1} using a dedicated LI-6250 CO_2 analyzer (Li-COR, Inc., Lincoln, NE). A chilled mixture of ethylene glycol and water were circulated through the cooling coils located outside the air handler to maintain a constant humidity and temperature inside each chamber via several parallel solenoid valves that closed or opened depending on the cooling requirement. Reddy et al. (2001) described more details of the operation and control of the SPAR facility. Using full-strength Hoagland nutrient solution via an automated drip irrigation system at the rate of 50 ml min^{-1} for 120 s per irrigation event, plants were fertigated three times a day at 0800, 1000, and 1700 h.

Plant materials and temperature treatments

Seeds from four rice cultivars, namely, CL152, Bowman, Antonio, and Mermentau along with two hybrids, namely, XL 753 and CLXL 745, were obtained from the Mississippi State University's Delta Research and Extension Center in Stoneville, MS (33° 42′ N, 90° 92′ W). Rice seeds were sown in PVC (polyvinyl chloride) pots (15 cm diameter by 30 cm high) filled with 600 g of gravel placed at the bottom of each pot to allow drainage, and a soil medium consisting of 75% sand and 25% topsoil. The soil mixture classified as a sandy loam (87% sand, 2% clay, and 11% silt). Each pot had a small hole at the bottom allow excess water to drain. Pots were organized in a completely randomized design with five replications per cultivar arranged in 10 rows with three pots per row. In total, one hundred and fifty pots were used for the experiment. Initially, eight seeds were sown per pot. Ten days after emergence, the plants were thinned to one per pot. Fungicide was sprayed at a rate of 68 mL/gallon after mixing 17 mL/32oz in a small pump up sprayer. Rice seeds were treated in a rice seed laboratory and examined to ensure they met the recommended seed quality standards before they were put in cold storage until use. The first and second extra pots, which used to test/check root growth, were harvested at 23 and 32 DAS, respectively. The treatments included five day/night temperature treatments: 20/12 (very low), 25/17 (low), 30/22 (optimum), 35/27 (high), and 40/32°C (very high). Treatments were imposed after seedling emergence and establishment, 10 days after sowing (DAS), and continued until harvest at 39 DAS. Each SPAR unit maintained its respective temperature stress treatment, and all plants were fertigated with the same water volume from sowing until

harvest. Plants were harvested 39 DAS and leaves, stems, and roots of all the plants were sampled for recording individual traits.

Measurements

Plant height (PH, cm plant^{-1}), tillers number (TN, no. plant^{-1}), the total number of leaves (LN, no. plant^{-1}) were measured by hand at the final harvest (39 DAS). Leaf area (LA, cm^2 plant^{-1}) was measured using leaf area meter (Li-3100 leaf area meter, Li-COR Inc., Lincoln, Nebraska, USA) Also, roots were cut from the stem, washed, separated for scanning by an optical scanner, and analyzed using WinRHIZO Pro software (Regent Instruments, Inc., Quebec, QC, Canada). Roots were untangled and cleaned for scanning to acquire root images of 800 by 800 dpi resolution, then analyzed to study root morphology with a computer linked to WinRHIZO optical scanner and software analysis system. The system provided the analyses of the following root growth and developmental parameters: cumulative root length (RL, cm plant^{-1}), root surface area (RSA, cm^2 plant^{-1}), average root diameter (RAD, mm plant^{-1}), root volume (RV, cm^3 plant^{-1}), number of tips (RNT, no. plant^{-1}), number of forks (RNF, no. plant^{-1}), and number of crossings (RNC, no. plant^{-1}). Then, leaf dry weight (LW, g plant^{-1}), stem dry weight (SW, g plant^{-1}), and root dry weight (RW, g plant^{-1}) were estimated after oven-drying all tissue samples at 75°C. Also, quantum efficiency (Fv'/Fm') which describes the photosynthetic capacity of leaves using Fluor-Pen (FP 100, FluorPen meter, Drasov, Czech Republic) and chlorophyll content using SPAD meter (SPAD-502, Minolta Camera Co. Ltd., Japan) were measured at 36 DAS.

Data analysis

An analysis of variance was performed to determine morpho-physiological parameters response to temperature stress using PROC MEANS and PROC GLM in SAS (SAS Institute, 2011, Cary, NC). The significance of differences among treatments was tested using LSD tests at P=0.05. Cultivars, temperature treatments, and their interactions were used as sources of variation for quantifying the effect of temperature treatments on early-season rice growth and development. Regression analysis was conducted using SigmaPlot version 13 (Systat Software Inc., San Jose, CA). The correlation of the morpho-physiological parameters to temperature stress was obtained using PROC CORR procedure in SAS (SAS Institute, 2011).

Total temperature response index

Total high and low-temperature response indices were calculated according to the procedure described by Singh et al. (2018) and Wijewardana et al. (2015). Initially, individual very low and low temperature response indices (IVLTRI) and (ILTRI) were calculated by dividing the value of parameter (Pvl) at very low temperature (20/12°C) or the value of parameter (Pl) at low (25/17°C) for a given cultivar by the value of the same parameter (Po) at optimum temperature (30/22°C) [Eq. 1 and 2]. Also, the individual high and very high-temperature response indices (IHTRI) and (IVHTRI) were calculated by dividing the value of parameter (Ph) at high temperature (35/27°C) or the value of parameter (Pvh) at very high (40/32°C) for a given cultivar by the value of the same parameter (Po) at optimum temperature (30/22°C) [Eq. 3 and 4]. Then, cumulative very low and low temperature response index (CVLRI) and (CLTRI) were calculated as sum of 19 IVLTRI or 19 ILTRI for each cultivar that includes PH , TN, LN , LA, LW , SW,

RW, AGW, TW, RS, RL, RSA, RAD, RV, RNT, RNF, RNC, SPAD, and Fv'/Fm' [Eq. 5 and 6]. Similarly, cumulative high and very high-temperature response index (CHTRI) and (CVHTRI) were calculated as the sum of 19 IHTRI or 19 IVHTRI for each cultivar [Eq. 7 and 8]. Finally, total low-temperature response index TLTRI was estimated by summing CVLTRI and CLTRI for each cultivar [Eq. 9]. Also, total high-temperature response index THTRI was generated by summing CHTRI and CVHTRI for each cultivar [Eq. 10].

$$\mathrm{IVLTRI} = \mathrm{Pvl/Po} \quad \text{[Eq. 1]}$$

$$\mathrm{ILTRI} = \mathrm{Pl/Po} \quad \text{[Eq. 2]}$$

$$\mathrm{IHTRI} = \mathrm{Ph/Po} \quad \text{[Eq. 3]}$$

$$\mathrm{IVHTRI} = \mathrm{Pvh/Po} \quad \text{[Eq. 4]}$$

$$\mathrm{CVLRI} = \left(\frac{\mathrm{PHvl}}{\mathrm{PHo}}\right) + \left(\frac{\mathrm{TNvl}}{\mathrm{TNo}}\right) + \left(\frac{\mathrm{LNvl}}{\mathrm{LNo}}\right) + \left(\frac{\mathrm{LAvl}}{\mathrm{LAo}}\right) + \left(\frac{\mathrm{LWvl}}{\mathrm{LWo}}\right) + \left(\frac{\mathrm{SWvl}}{\mathrm{SWo}}\right) + \left(\frac{\mathrm{RWvl}}{\mathrm{RWo}}\right) + \left(\frac{\mathrm{AGWvl}}{\mathrm{AGWo}}\right) + \left(\frac{\mathrm{TWvl}}{\mathrm{TWo}}\right) + \left(\frac{\mathrm{RSvl}}{\mathrm{RSo}}\right) + \left(\frac{\mathrm{RLvl}}{\mathrm{RLo}}\right) + \left(\frac{\mathrm{RSAvl}}{\mathrm{RSAo}}\right) + \left(\frac{\mathrm{RADvl}}{\mathrm{RADo}}\right) + \left(\frac{\mathrm{RVvl}}{\mathrm{RVo}}\right) + \left(\frac{\mathrm{RNTvl}}{\mathrm{RNTo}}\right) + \left(\frac{\mathrm{RNFvl}}{\mathrm{RNFo}}\right) + \left(\frac{\mathrm{RNCvl}}{\mathrm{RNCo}}\right) + \left(\frac{\mathrm{SAPADvl}}{\mathrm{SPADo}}\right) + \left(\frac{\mathrm{Fv'/Fm'vl}}{\mathrm{Fv'/Fm'o}}\right) \quad \text{[Eq. 5]}$$

$$\mathrm{CLRI} = \left(\frac{\mathrm{PHl}}{\mathrm{PHo}}\right) + \left(\frac{\mathrm{TNl}}{\mathrm{TNo}}\right) + \left(\frac{\mathrm{LNl}}{\mathrm{LNo}}\right) + \left(\frac{\mathrm{LAl}}{\mathrm{LAo}}\right) + \left(\frac{\mathrm{LWl}}{\mathrm{LWo}}\right) + \left(\frac{\mathrm{SWl}}{\mathrm{SWo}}\right) + \left(\frac{\mathrm{RWl}}{\mathrm{RWo}}\right) + \left(\frac{\mathrm{AGWl}}{\mathrm{AGWo}}\right) + \left(\frac{\mathrm{TWl}}{\mathrm{TWo}}\right) + \left(\frac{\mathrm{RSl}}{\mathrm{RSo}}\right) + \left(\frac{\mathrm{RLl}}{\mathrm{RLo}}\right) + \left(\frac{\mathrm{RSAl}}{\mathrm{RSAo}}\right) + \left(\frac{\mathrm{RADl}}{\mathrm{RADo}}\right) + \left(\frac{\mathrm{RVl}}{\mathrm{RVo}}\right) + \left(\frac{\mathrm{RNTl}}{\mathrm{RNTo}}\right) + \left(\frac{\mathrm{RNFl}}{\mathrm{RNFo}}\right) + \left(\frac{\mathrm{RNCl}}{\mathrm{RNCo}}\right) + \left(\frac{\mathrm{SAPADl}}{\mathrm{SPADo}}\right) + \left(\frac{\mathrm{Fv'/Fm'l}}{\mathrm{Fv'/Fm'o}}\right) \quad \text{[Eq. 6]}$$

$$\text{CHRI} = \left(\frac{\text{PHh}}{\text{PHo}}\right) + \left(\frac{\text{TNh}}{\text{TNo}}\right) + \left(\frac{\text{LNh}}{\text{LNo}}\right) + \left(\frac{\text{LAh}}{\text{LAo}}\right) + \left(\frac{\text{LWh}}{\text{LWo}}\right) + \left(\frac{\text{SWh}}{\text{SWo}}\right) + \left(\frac{\text{RWh}}{\text{RWo}}\right) + \left(\frac{\text{AGWh}}{\text{AGWo}}\right) + \left(\frac{\text{TWh}}{\text{TWo}}\right) + \left(\frac{\text{RSh}}{\text{RSo}}\right) + \left(\frac{\text{RLh}}{\text{RLo}}\right) + \left(\frac{\text{RSAh}}{\text{RSAo}}\right) + \left(\frac{\text{RADh}}{\text{RADo}}\right) + \left(\frac{\text{RVh}}{\text{RVo}}\right) + \left(\frac{\text{RNTh}}{\text{RNTo}}\right) + \left(\frac{\text{RNFh}}{\text{RNFo}}\right) + \left(\frac{\text{RNCh}}{\text{RNCo}}\right) + \left(\frac{\text{SAPADh}}{\text{SPADo}}\right) + \left(\frac{\text{Fv}'/\text{Fm}'\text{h}}{\text{Fv}'/\text{Fm}'\text{o}}\right) \quad \text{[Eq. 7]}$$

$$\text{CVHRI} = \left(\frac{\text{PHvh}}{\text{PHo}}\right) + \left(\frac{\text{TNvh}}{\text{TNo}}\right) + \left(\frac{\text{LNvh}}{\text{LNo}}\right) + \left(\frac{\text{LAvh}}{\text{LAo}}\right) + \left(\frac{\text{LWvh}}{\text{LWo}}\right) + \left(\frac{\text{SWvh}}{\text{SWo}}\right) + \left(\frac{\text{RWvh}}{\text{RWo}}\right) + \left(\frac{\text{AGWvh}}{\text{AGWo}}\right) + \left(\frac{\text{TWvh}}{\text{TWo}}\right) + \left(\frac{\text{RSvh}}{\text{RSo}}\right) + \left(\frac{\text{RLvh}}{\text{RLo}}\right) + \left(\frac{\text{RSAvh}}{\text{RSAo}}\right) + \left(\frac{\text{RADvh}}{\text{RADo}}\right) + \left(\frac{\text{RVvh}}{\text{RVo}}\right) + \left(\frac{\text{RNTvh}}{\text{RNTo}}\right) + \left(\frac{\text{RNFvh}}{\text{RNFo}}\right) + \left(\frac{\text{RNCvh}}{\text{RNCo}}\right) + \left(\frac{\text{SAPADvh}}{\text{SPADo}}\right) + \left(\frac{\text{Fv}'/\text{Fm}'\text{vh}}{\text{Fv}'/\text{Fm}'\text{o}}\right) \quad \text{[Eq. 8]}$$

$$\text{TLTRI} = \text{CVLTRI} + \text{CLTRI} \quad \text{[Eq. 9]}$$

$$\text{THTRI} = \text{CHTRI} + \text{CVHTRI} \quad \text{[Eq. 10]}$$

The mean value for each character (trait) at (very low and low) or at (high and very high) temperature treatments measured for each cultivar or hybrid were calculated and analyzed by principal components analysis (PCA). Similarities among shoot, root, and physiological parameters were measured by Pearson distance. The cold or heat tolerance of each cultivar or hybrids were determined according to the results from classification vigor index table and principal components analysis.

Results and Discussion

Rice productivity has increased throughout the past decades through combined advancement in genetics, breeding, and improved management practices.
The latter include, for example, selecting the optimum planting time and matching rice cultivars/hybrids with specific management recommendations such as irrigation and fertilization. One example of this combined effort would be selecting an optimum planting time for maximum potential productivity, selecting genotypes specified to those planting conditions, and outlining specific optimized management recommendations such as proper irrigation and fertilization. In the U.S. Mid-South, planting as early as productivity will allow can be a beneficial strategy for rice producers to avoid hot and drier summers, especially during flowering and the grain-filling period. This study utilized commercial cultivars and hybrids currently planted throughout the U.S. Mid-South to determine variability in cold and heat tolerance.

This experiment provides a potentially useful set of information for rice producers and breeders to potentially select the best-adapted genotypes which could withstand short periods of cold and heat stress, thus maximizing early season productivity. The analysis of variance shows that temperature has a significant ($P<0.001$) effect across all cultivars for all shoot and root morpho-physiological traits measured. (Table 4.1). However, there was also variability within the cultivar's response to temperature treatments.
Plant height, tiller number, total leaf number, and leaf area were highly significant ($P<0.001$). Root and physiological traits including root length, number of root tips, number of root crossings, and chlorophyll content also had significant variation

($P<0.01$). Root dry weight, total shoot dry weight, root surface area, and number of root forks had moderately significant ($P<0.05$) variability. Traits including stem dry weight, total dry weight, root-shoot ratio, root average diameter, and root volume showed no significant variability in response to temperature within genotypes. The temperature by genotype interaction was significant for 63% of the traits, and hybrid genotypes showed significant difference with genotypes in 52% of the traits indicating that genetic variation existed among genetic materials. However, temperature by genotypes interaction was not significant ($P > 0.05$) for stem dry weight, total shoot dry weight, root-shoot ratio, root average diameter, root volume, root number of forks, and fluorescence.

Table 4.1 Analysis of variance across the cultivars (Cul) and temperature (Temp.) treatments and their interaction (Cul X Temp.) for rice morphological parameters measured 24 days after treatment; plant height (PH), tillers number (TN), leaves number (LN), leaf area (LA), leaf weight (LW), stem weight (SW), root weight (RW), above ground weight (AGW), total weight (TW), root/shoot (RS), root length (RL), root surface area (RSA), average root diameter (RAD), root volume (RV), number of tips (RNT), number of forks (RNF), number of crossings (RNC), chlorophyll content (SPAD), and fluorescence (Fv'/Fm').

Source of Variance	PH	TN	LN	LA	LW	SW	RW	AGW	TW	RS	RL	RSA	RAD	RV	RNT	RNF	RNC	SPAD	Fv'/Fm'
Cul	***	***	**	***	***	ns	*	*	ns	ns	**	*	ns	ns	**	*	**	**	ns
Temp.	***	***	***	**	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
Cul x Temp.	**	*	*	**	**	ns	*	ns	*	ns	*	*	ns	ns	*	ns	*	*	ns
Cultivars†																			
CLXL 745	b	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
XL 753	c	a	a	a	ab	a	a	a	ab	a	a	a	a	a	a	a	ab	c	a
Mermentau	a	c	bc	b	bc	a	b	a	b	a	bc	bc	a	a	a	a	bc	bc	a
CL 152	a	b	b	b	bc	a	b	a	b	a	bc	bc	a	a	a	a	bc	d	a
Antonio	a	b	c	b	c	a	b	a	ab	a	c	c	a	a	b	a	bc	c	a
Bowman	b	c	c	b	c	a	b	a	b	a	c	c	a	a	b	a	c	b	a

The significance levels ***, **, *, and NS represent $P \leq 0.001$, $P \leq 0.01$, $P \leq 0.05$, and $P>0.05$ respectively.
† Cultivars with the same letter are not significantly different according to t test comparison at $P \leq 0.05$.

Shoot growth and development

The growth rate increases linearly between 22 and 31°C. A temperature of 22°C or below is considered subnormal for seedling growth. Temperatures above 22°C up to 35°C can be considered optimal for growth, but temperatures above 35°C can cause negative effects on rice. In this study, shoot growth (Fig. 4.1) and developmental parameters, measured 39 DAS, increased when temperature increased from very low (20/12°C) to high (35/27°C) then declined at very high (40/32°C) in all cultivars/hybrids. For instance, an increase of plant height varies among cultivars/hybrids, and the quadratic functions showed an increase and declinine of the plant height forgenotypes. CLXL 745 and Bowman increased by 4.58 cm and decreased -0.07cm 1 $°C^{-1}$ compared to Mermentau, CL 152, and Antonio with 3.0 cm and -0.05 cm 1°C, and XL 753 with 3.92 and -0.06cm 1 $°C^{-1}$ (Fig 4.1A). Kondo and Okamura (1931) and Osada et al. (1973) revealed that the plant height increased with the increase of temperature within the range of 30-35 °C. Although tiller number increased with temperature in all cultivars, the linear regression functions for XL 753, CLXL 745, CL 152, and Antonio and quadratic functions for Mermentau and Bowman, best described the effects of changing temperatures on tiller number respectively (Fig. 4.1B).

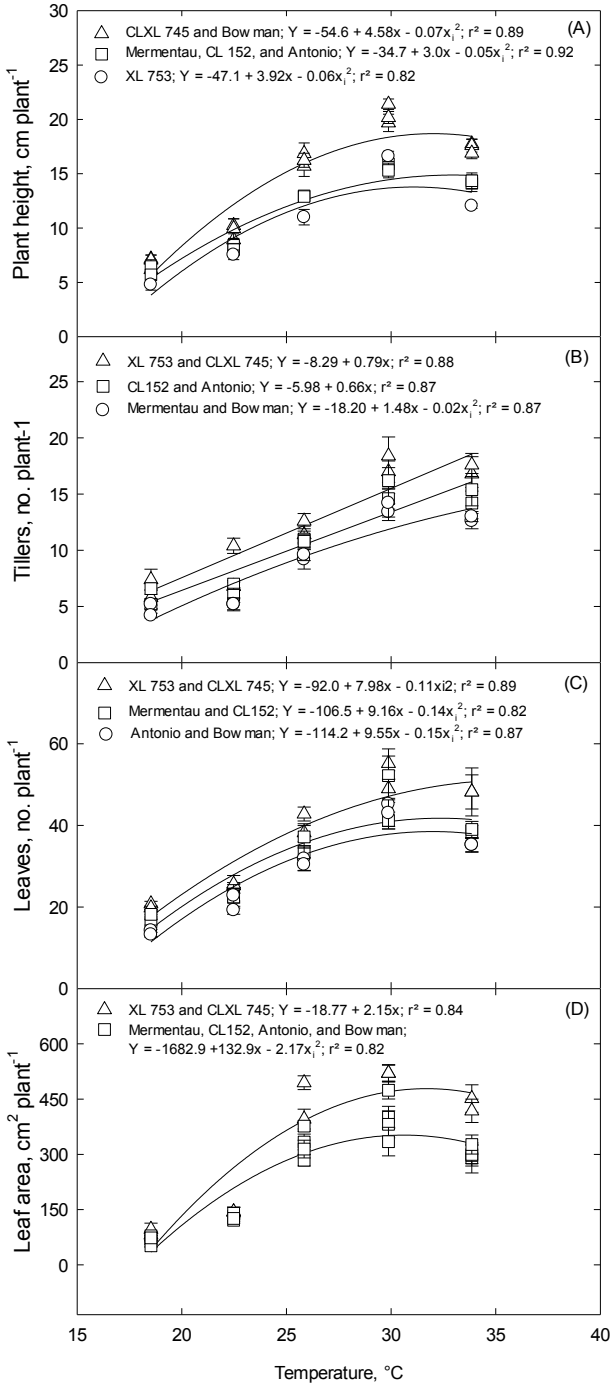

Figure 4.1 Temperature effect on (A) plant height, (B) tillers number, (C) leaves number, and (D) leaf area of rice cultivars. Measurements were taken at 39 days after sowing. Standard errors of the mean ± 5 observations are presented if the values are larger than the symbols.

Whole-plant leaf number and leaf area, on the other hand, increased quadratically with an increase in temperature in all cultivars/hybrids. Small differences were also recorded among genotypes (Table 4.1; Fig. 4.1C and D). Plant growth is negatively affected by cold stress (Sanghera et al., 2011). Tillering in rice is an important agronomic trait for grain production (Li et al., 2003) and profuse tillering is well known in weedy rice, increasing its competitiveness (Sanchez-Olguin et al., 2007). The temperature 40/33 °C affected leaf area, leaf number, and plant height positively while the temperature 28/21°C affected negatively on all these traits (Baker et al., 1992; Ohe et al., 2007). Yoshida (1981) reported that at 3–5 weeks after sowing, the temperature only slightly affected the relative growth rate and the tillering rate, except at the lowest temperature (22°C) tested. Tiller number per plant determines panicle number which is a critical component of grain yield.

Root growth and development

After seedling emergence, the root structures in young seedlings show higher weight proportions than shoot. Total root length, root surface area, root volume, and root diameters are indicators of root size and function (Costa et al., 2002), aidingnutrient uptake efficiency and performance under various stress conditions including temperature (Hammer et al., 2009; Rosolem et al., 1994) (Fig. 4.2). Root length increased linearly with an increase in temperature from (20/12 °C) to (35/27 °C) then declined at 40/32 °C for all genotypes. Quadratic response was observed in all cultivars/hybrids studied. CLXL 745 and XL 753 exhibited greater root length at the five temperatures tested (Fig. 4.2A).

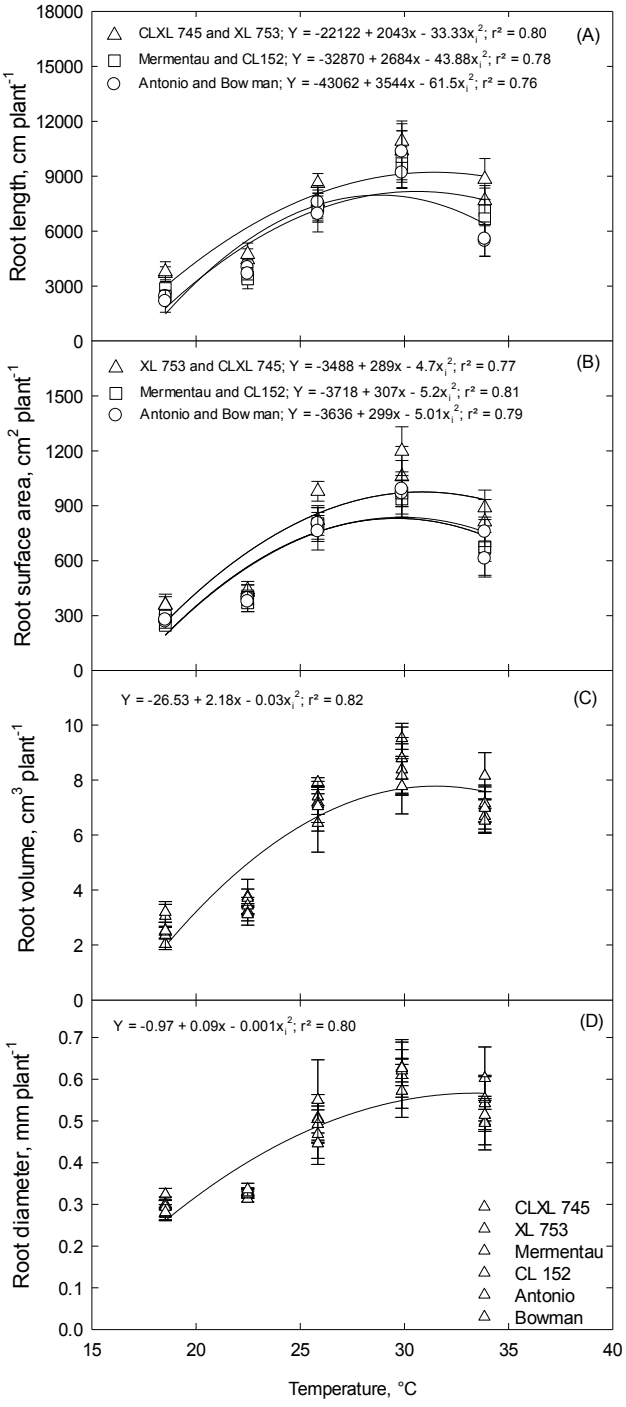

Figure 4.2 Temperature effect on (A) root length, (B) root surface area, (C) root volume, and (D) root diameter of rice cultivars. Measurements were taken at 39 days after sowing. Standard errors of the mean ± 5 observations are presented if the values are larger than the symbols.

Cumulative root length of 10,920 and 10,396 cm plant^{-1} was observed at 30°C for XL 753 and CLXL 745, respectively while minimum root length of 2,171 and 2,426 cm plant^{-1} was observed at 17°C for Bowman and Antonio, respectively. Similarly, root surface area showed quadratic response to temperature. The responses, however, were different among the cultivars and hybrids; hybrids XL 753 and CLXL 745 showed greater root surface area (1,199 and 1,060 cm^2 plant^{-1}), respectively, at the five temperatures tested compared to the other four cultivars, Mermentau, CL 152, Antonio, and Bowman (Fig. 4.2B and Fig. 4.5), with maximum values observed at 35/27 °C However, there were no differences in the root volume and root average diameter among the cultivars and hybrids tested, and they increased 2.18 cm^3 and 0.09 mm, respectively per 1°C^{-1} (Fig. 4.2C and D). Root forks, root crossing, and root tips increased quadratically across temperatures in all cultivars and hybrids (Fig.4. 3A, B, andC). The roots grown under low temperatures are smaller than the roots under high temperatures. Similar to the root observations in this study, Barber et al. (1988) showed that the root growth of selected rice cultivars decreased with decreasing environmental temperature and vice-versa. Nagasuga et al (2011) also noted that the reduction or increase in temperature depressed root growth. Root biomass and root dry weight were affected negatively when temperature increased above 35°C (Yoshida et al. 1981). Physiologically, roots are the most sensitive part of the plant to abiotic stresses including temperature. High-temperature influences the number of root branches and root volume (Barber et al., 1988).

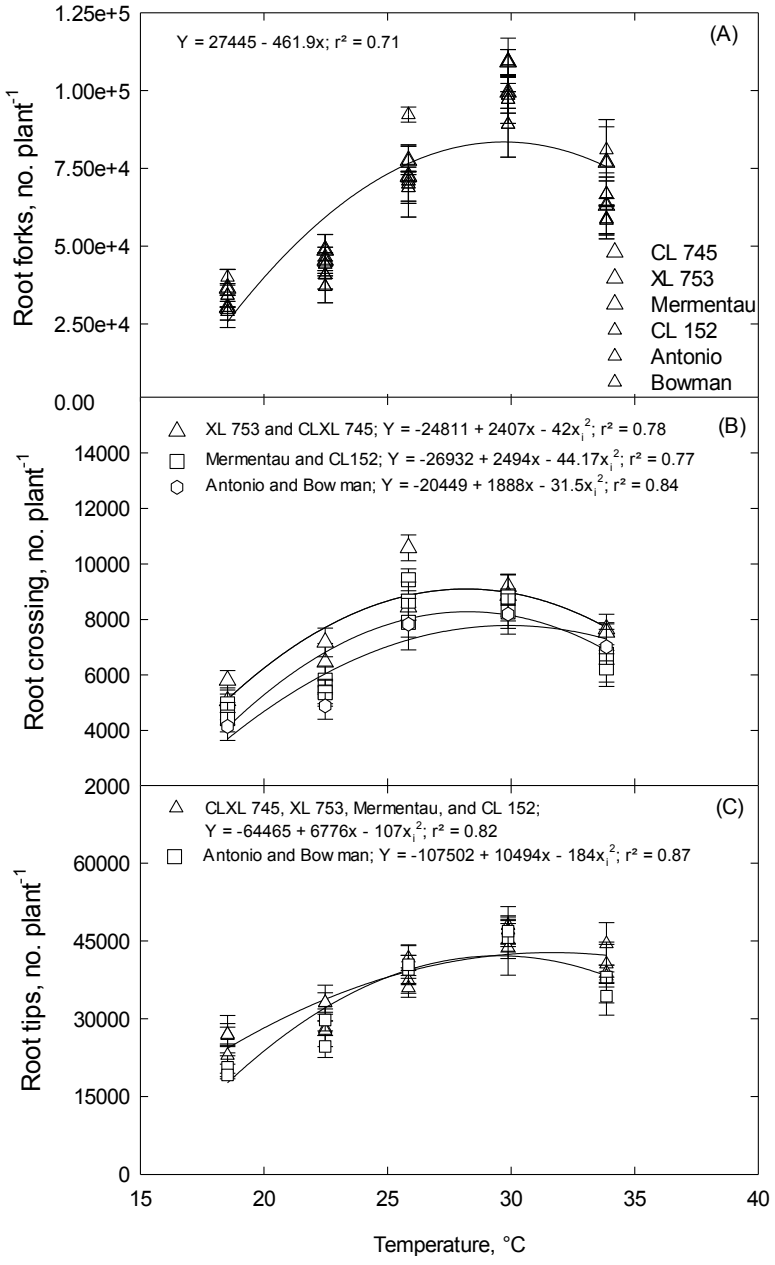

Figure 4.3 Temperature effect on (A) root forks, (B) root crossing, and (C) root tips of rice cultivars. Measurements were taken at 39 days after sowing. Standard errors of the mean ± 5 observations are presented if the values are larger than the symbols.

Physiological parameters

Chlorophyll content and maximal quantum yield of PSII photochemistry (Fv/Fm) are essential parameters for PSII activity. Anydecrease of chlorophyll content and Fv/Fm indicates a decrease of PSII activity. Cold stress significantly reduces the concentration of chlorophyll in susceptible rice genotypes (Aghaee et al., 2011). Chlorophyll content was used as a tool to evaluate the degree of cold tolerance of transgenic plants (Tian et al., 2011) to monitor plant recovery after stress (Kuk et al. 2003) and to compare chilling tolerance between distinct hybrid lines during grain filling (Wang et al., 2006). Chlorophyll content increased quadratically with increased temperature in all cultivars and hybrids (Fig. 4.4A). Variability of chlorophyll content ranged from 42.2 g cm^2 for CLXL 745 at 22.4°C to 30.3 g cm^2 for CL 152 at 18.5°C., indicates higher and lower chlorophyll content at the five temperatures tested, respectively (Fig. 4.4A). However, there were no differences in the fluorescence among the cultivars and hybrids tested, and fluorescence increased at 0.08 per $1°C^{-1}$ (Fig. 4.4B). Both negative response relationships between temperature and total chlorophyll content were reported by Nagi and Making (2009). On another hand, Yamada et al. (1996) suggested that Fv/Fm correlate with heat tolerance. Han et al. (2009) noted that Fv/Fm values decreased slightly with increased temperature, indicating the inhibition of PSII activity under high-temperature stress condition.

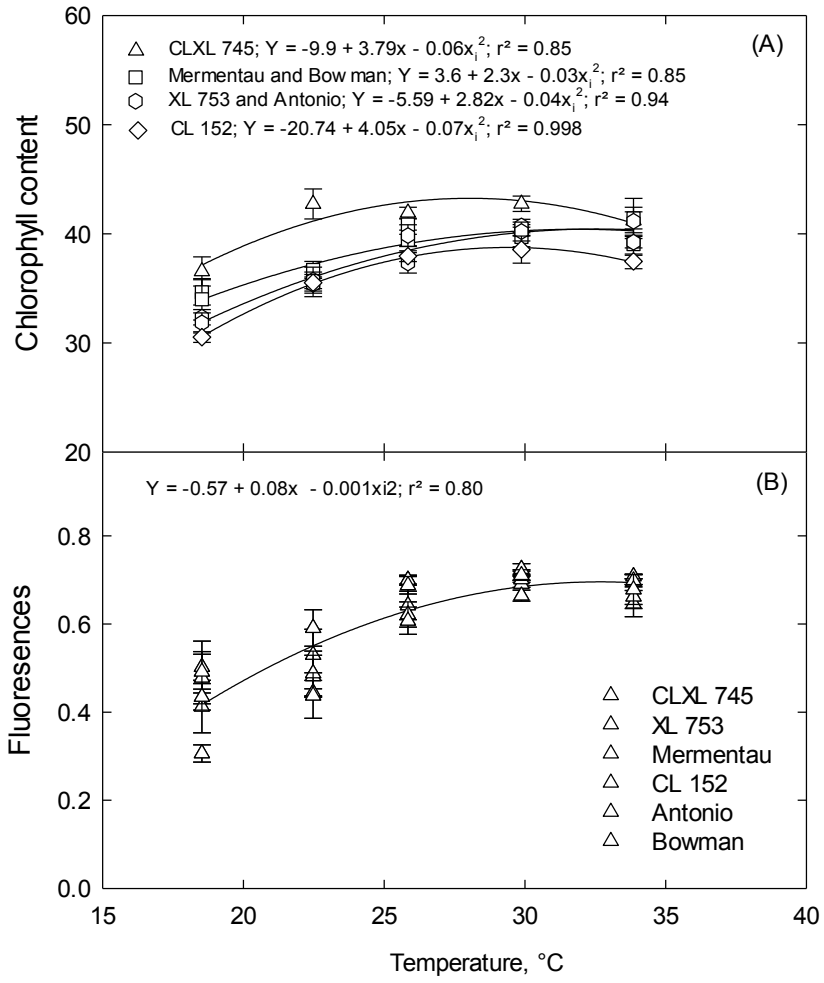

Figure 4.4 Temperature effect on (A) Chlorophyll content (SPAD) and (B) Fluorescence (Fv'/Fm') of rice cultivars. Measurements were taken at 36 days after sowing. Standard errors of the mean ± 5 observations are presented if the values are larger than the symbols.

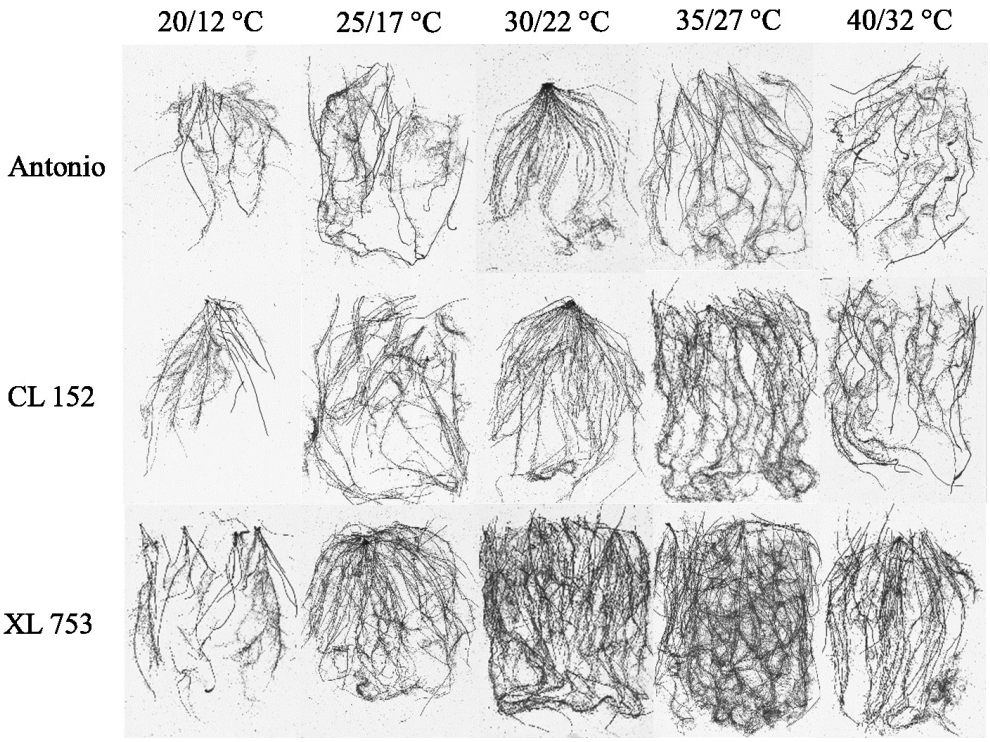

Figure 4.5 Root images for selected rice cultivars/hybrids grown at various temperatures, harvested 39 days after sowing.

Total dry weight

The production of percent root and shoots dry weight decreased in response to low or severely high temperature. Low temperature reduces the dry weight content of plants (Hnilickova et al., 2002; Singh et al., 2018). The genetic characteristics of the cultivars might be responsible for the variability in percent shoot dry weight obtained in a day. Rice cultivars having higher total dry weight may have higher temperature stress tolerance than other cultivars. As shown in Table 4.2, parameter, cultivar, and temperature effects on combined dry weight traits, the maximum leaf dry weight, stem

dry weight, and root dry weight were obtained at the high temperature treatment (35/27°C) in CLXL 45, XL 753, and Bowman, respectively.

The minimum values were recorded on Bowman, Antonio, and Mermentau at the very low temperature treatment (20/12 °C) with 0.33, 0.33, and 0.20 g plant^{-1}, respectively. The maximum shoot dry weight and maximum total dry weight was achieved at the high temperature treatment (35/27 °C) in CLXL 745 and XL 753 with 7.67 and 8.74 g plant^{-1}, respectively. The minimum value of shoot dry weight and total dry weight was obtained at the very low temperature treatment (20/12 °C) in Bowman cultivar with 0.67 and 0.90 g plant^{-1}, respectively. The maximum and minimum root-shoot ratio was related to Bowman (0.34) at very low 20/12 °C and XL 753 (0.13) at very high 40/32 °C temperature. Generally, all combined dry weight traits at the lower temperatures were smaller than the values at the higher temperatures, except for the root-shoot ratio. Plant dry weight was highest in the 35/27°C treatment than in the 30/22°C treatments and was lowest in the 40/32°C treatments, which was consistent with many previous reports (Ziska et al., 1997; Fukui, 2000). Thuy and Saitoh (2017) reported that under high-temperature conditions, the dry weight of shoots decreased drastically in most cultivars. Reduction of shoot dry weight and root biomass of rice genotypes have been reported earlier by Muhammad and Tarpley, (2009) and Mokhberdoran et al., (2009).

Table 4.2 Temperature effect on leaf dry weight, stem dry weight, root dry weight, above ground dry weight, total dry weight, and root shoot ratio of rice cultivars. Measurements were taken 39 DAS.

Parameters	Cultivar	Temperature, °C				
		20/12	25/17	30/22	35/27	40/32
				g plant^{-1}		
Leaf dry weight	CLXL 745	0.58	0.93	2.18	4.35	3.34
	XL 753	0.86	0.74	1.76	4.04	3.05
	Mermentau	0.48	0.69	1.88	3.77	2.14
	CL 152	0.43	0.55	1.72	3.02	2.10
	Antonio	0.37	0.70	2.05	3.78	2.25
	Bowman	0.33	0.75	1.61	3.62	1.94
Stem dry weight	CLXL 745	0.49	0.62	2.65	3.32	3.20
	XL 753	0.62	0.65	2.41	3.60	2.67
	Mermentau	0.35	0.53	2.04	3.07	2.04
	CL 152	0.30	0.50	1.71	3.56	1.99
	Antonio	0.33	0.43	2.09	3.52	2.01
	Bowman	0.34	0.47	1.88	3.09	2.13
Root dry weight	CLXL 745	0.30	0.37	0.95	1.02	0.94
	XL 753	0.33	0.39	0.72	1.10	0.73
	Mermentau	0.20	0.36	0.71	1.03	0.76
	CL 152	0.23	0.33	0.80	0.90	0.66
	Antonio	0.21	0.34	0.77	0.98	0.58
	Bowman	0.23	0.30	0.73	1.14	0.72
Above ground dry weight	CLXL 745	1.07	1.55	4.83	7.67	6.54
	XL 753	1.48	1.39	4.17	7.64	5.72
	Mermentau	0.83	1.22	3.92	6.84	4.18
	CL 152	0.73	1.05	3.43	6.58	4.09
	Antonio	0.70	1.13	4.14	7.30	4.26
	Bowman	0.67	1.22	3.49	6.71	4.07
Total plant dry weight	CLXL 745	1.37	1.92	5.78	8.69	7.48
	XL 753	1.81	1.78	4.89	8.74	6.45
	Mermentau	1.03	1.58	4.63	7.87	4.94
	CL 152	0.96	1.38	4.23	7.48	4.75
	Antonio	0.91	1.47	4.91	8.28	4.84
	Bowman	0.90	1.52	4.22	7.85	4.79
Root shoot ratio	CLXL 745	0.28	0.24	0.20	0.14	0.14
	XL 753	0.22	0.28	0.17	0.14	0.13
	Mermentau	0.24	0.30	0.18	0.15	0.18
	CL 152	0.32	0.31	0.23	0.14	0.16
	Antonio	0.30	0.30	0.19	0.14	0.14
	Bowman	0.34	0.25	0.21	0.17	0.18

Total low or high-temperature response index

The total low or high-temperature response indices (TLTRI or THTRI) were calculated to understand the coefficient of determination between shoot and root traits under suboptimal temperature conditions. TLTRI and THTRI values varied from 18.25 to 21.60 and from 39.65 to 46.21, respectively, and three temperature tolerant groups were identified based on TLTRI and THTRI values and their standard deviation or SD (Table 4.3)

Table 4.3 Classification of rice cultivars into various cold/heat tolerance groups based on total low temperature response index (TLTRI) and total high temperature response index (THTRI), respectively, along with individual scores in parenthesis.

Classification	Cultivar	TLTRI	Classification	Cultivar	THTRI
Cold sensitive TLTRI ≤ 20.06	Bowman Antonio CLXL 745 Mermentau	(18.48) (19.54) (19.80) (19.82)	Heat sensitive THTRI≤ 44.39	Antonio CLXL 745 Mermentau	(42.01) (43.59) (44.09)
Moderate 20.07 < TLTRI ≤ 21.63	CL152	(20.28)	Moderate 44.40 < THTRI ≤ 46.76	CL 152 Bowman	(44.54) (46.33)
Cold tolerant 21.64 < TLTRI ≤ 23.20	XL 753	(23.15)	Heat tolerant 46.77 < THTRI ≤ 49.14	XL 753	(48.82)
†SD = 1.57. Cold sensitive: TLTRI ≤ $TLTRI_{min}$ + 1.0 SD; Moderate: $TLTRI_{min}$ + 1.0 SD < TLTRI ≤ $TLTRI_{min}$ + 2.0 SD; Cold tolerant: $TLTRI_{min}$ + 2.0 SD < TLTRI ≤ $TLTRI_{min}$ + 3.0 SD.			‡ SD = 2.37. Heat sensitive: THTRI ≤ $THTRI_{min}$ + 1.0 SD; Moderate: $THTRI_{min}$ + 1.0 SD < THTRI ≤ $THTRI_{min}$ + 2.0 SD; Heat tolerant: $THTRI_{min}$ + 2.0 SD < THTRI ≤ $THTRI_{min}$ + 3.0 SD.		

Antonio, Bowman, Mermentau, and CL 152 were designated as the cold sensitive cultivars. The hybrid CLXL 745 was moderately cold sensitive, and the hybrid XL 753 was highly cold tolerant among 6 rice cultivars and hybrids tested. However, Antonio, CL 152, and Mermentau were designated as the heat sensitive, and CLXL 745 and Bowman as moderately heat tolerant.

The hybrid XL 753 was highly heat tolerant among the 6 rice cultivars and hybrids. Additionally, a strong, positive, and linear coefficient of determination (R^2 = 0.92 and 0.93; p = 0.0002) was obtained for the total low-temperature response index and total shoot and root low-temperature response index, respectively, using the six rice cultivars studied (Fig. 4.6).

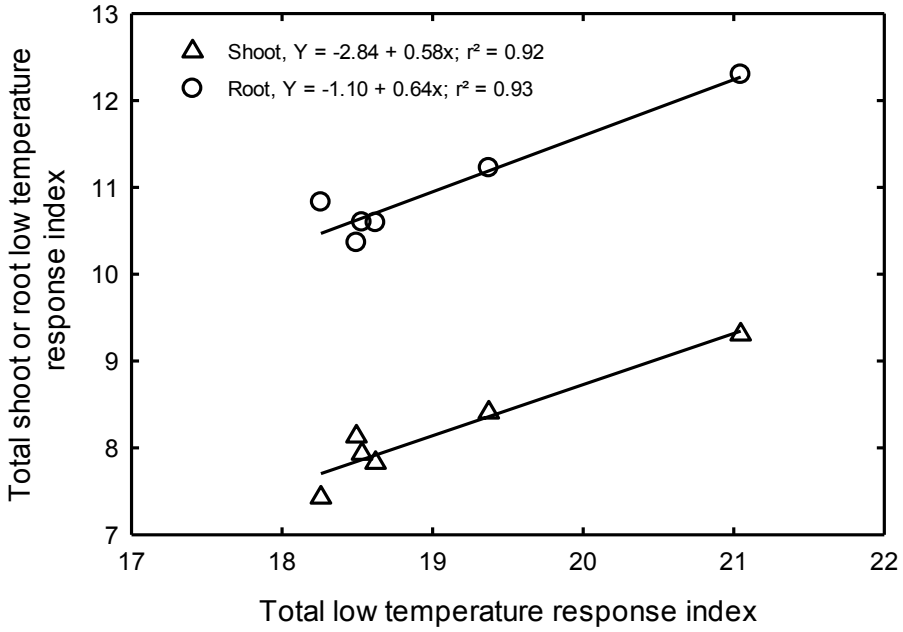

Figure 4.6 Correlation between total low temperature response index (TLTRI) and shoot or root traits of 6 rice cultivars, measured at 39 days after sowing.

Shoots and roots increased by 0.58 and 0.64 g 1 °C^{-1}, respectively, in TLTRI. Also, 97 or 0.94% of the total variation of the total high-temperature response index was explained by total shoot or root temperature response index, respectively, via linear regression analysis using the six rice cultivars studied (Fig. 4.7). Shoots and roots increased by 0.50 and 0.48 g 1 °C^{-1}, respectively, in THTRI.

These observations indicate that shoot and root traits are crucial for selecting cold and heat tolerance during the early establishment of rice cultivars.

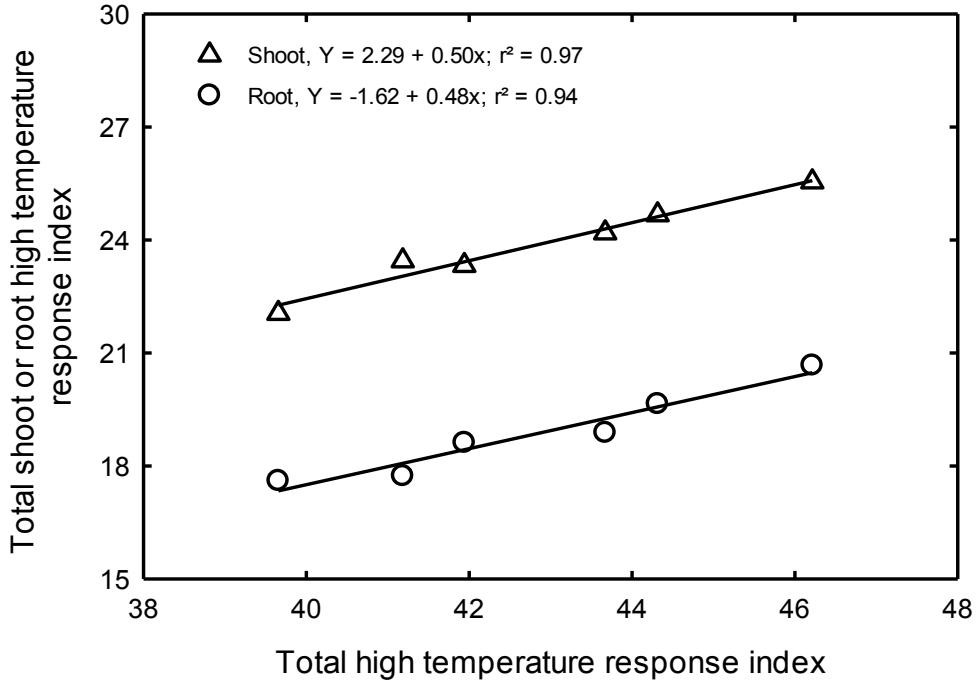

Figure 4.7 Correlation between total high temperature responses index (TLTRI) and shoot or root traits of 6 rice cultivars, measured at 39 days after sowing.

The regression coefficient between total low-temperature response index and the total high-temperature response index was 0.69 (Fig. 4.8). The TLTRI or THTRI methods provided a means for quantifying total variability, and thus, may be useful as selection criteria for screening rice cultivars for cold or heat tolerance. This information would be useful determining which traits are best suited among rice cultivars and hybrids for screening cold and heat tolerance in future environments.

Similar methodologies have been applied for successful screening of corn hybrids and rice cultivars for cold and drought tolerance (Wijewardana et al., 2015; Singh et al., 2017).

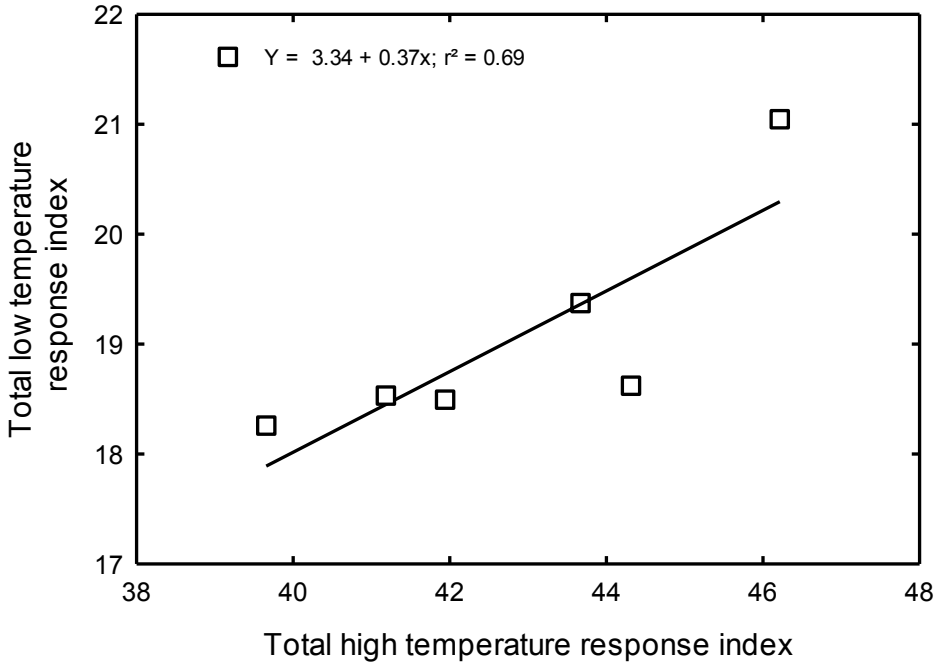

Figure 4.8 Correlation between total low temperature response index (TLTRI) and total high temperature response index (TLTRI) of 6 rice cultivars, measured at 39 days after sowing.

The identified heat or cold tolerant cultivars in this study could useful for breeders as genetic donors to develop new rice cultivars which could withstand low and high-temperature conditions during the early-season vegetative growth in dry direct seeding production practices. The hybrid XL 753, was identified to be the best cold and heat

tolerant cultivar. Therefore, it may be used along with other management practices for improving crop yields in commercial rice production.

The significant relationship of total low or high-temperature response index to total shoot or root temperature response index accentuates the significance of studying the shoot or root parameters, separately or in combination, for developing screening tools to determining cold or heat tolerance in rice. The variability among the cultivars and hybrids under optimum temperature conditions could be due to inherent genetic variation. The variability under cold or heat stress could be due to both genotypic and developed adaptive mechanisms to environmental signals. Hence, if a cultivar or hybrid shows an increased response towards high or low temperature, it confers a selective advantage and tolerance against the given abiotic stress. These improved performances may lead towards greater efficiency in the shoot and root production.

Principal component analysis

PCA model was used to elucidate relationships among rice cultivars and hybrids using the shoot, root, and physiological traits. They were classified into four categories. Consequently, this PCA model was used to develop cultivar-dependent temperature tolerant scores at low and high temperature conditions. Based on the PCA analysis, the first two principal components (PCs) accounted for 55% of the total variance at low and high temperature (Fig. 4.9). The hybrid XL 753 showed cold and heat tolerance in the low and high-temperature treatments. The cultivars Bowman and CLXL 745 showed moderate cold and heat tolerance under low and high temperatures. The cultivars Mermentau and Antonio showed cold and heat sensitivities in low and heat temperature

treatments. Finally, CL 152 was sensitive to cold and moderately heat tolerant in low and high-temperature treatments (Fig. 4.9).

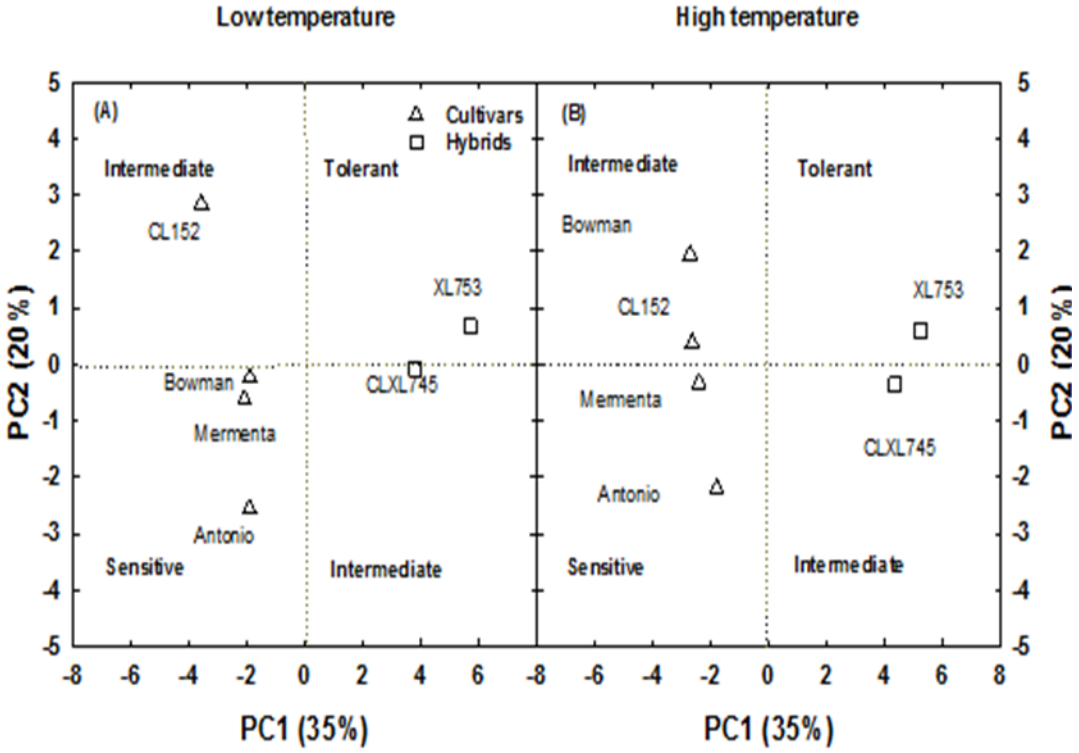

Figure 4.9 Principal component analysis for the first three principal component (PC) scores, PC1 and PC2 related to the classification of 6 rice cultivars and hybrids for low and high temperature tolerance.

CHAPTER V

DROUGHT STRESS TOLERANCE SCREENING OF ELITE RICE GENOTYPES USING LOW-COST PRE-FABRICATED MINI-HOOP MODULES

Absract

Drought is a major abiotic stress factor that affects growth and development of plants at all stages. Developing a screening tool to identify drought stress tolerance during seedling establishment is important in identifying genotypes for use in development and deployment of rice varieties suited to water-limited growing environments. Most drought studies have utilized mostly indica rice germplasm. An experiment was conducted to evaluate 100 rice genotypes, mostly belonging to the tropical japonica subspecies, for tolerance to drought stress using low-cost, pre-fabricated mini-hoop structures. The rice seedlings were subjected to two different soil moisture regimes- control pots managed at 100% and drought pots at 50% field capacity, from 12 to 30 days after sowing (DAS). Several morpho-physiological parameters including root traits were measured to assess the response of genotypes. Significant moisture stress X genotype interactions were found for most of the parameters measured. A cumulative drought stress response index (CDSRI) was developed by summing the individual response indices of all cultivars. The CDSRI varied between 14.7 and 27.9 among the genotypes tested. Based on CDSRI and standard deviation values, 5 and 28 genotypes

were identified as highly sensitive and sensitive to drought, respectively, and 45 as moderately sensitive.

On the other hand, 16 and six genotypes were classified as tolerant and highly tolerant to drought, respectively. Cheniere, a released cultivar, and RU1402174, an experimental breeding line, were identified as the least and most tolerant to drought among the 100 genotypes tested. Significant linear correlation coefficients were obtained between CDSRI and root growth parameters ($R^2 = 0.91$, n =100) and CDSRI with shoot growth parameters ($R^2 = 0.48$, n = 100), revealing root traits are important in studying and identifying drought tolerant lines during the seedling establishment stages in rice. The tolerant rice genotypes identified will be valuable for rice scientists in studying the mechanism for early season drought as well as for rice breeders for developing new genotypes best suited under growing environments prone to early-season drought.

Introduction

Rice (*Oryza sativa* L.) is one of the most widely consumed cereal crops across the globe, providing a staple diet for almost half of the human population (Song et al., 2003). As an annual C_3 crop of the Poaceae family, rice is diverse in adaptation, with rice-growing occupying large areas in the tropics, subtropics, semiarid tropics, and temperate regions of the world. Water is undoubtedly one of the most precious resource that rice requires to grow optimally during its entire life cycle. Rice needs water, not only for its growth and development but also to be able to produce good yields

With the onset of climate change-related challenges, the intensity and frequency of droughts are predicted to increase in most of the rice-growing areas. Droughts could extend further into water-limited irrigated areas with greater severity. For example, water scarcity already affects more than 23 million hectares of rainfed rice production area in South and Southeast Asia alone. In Africa, recurring drought affects nearly 80% of the potential 20 million hectares of rainfed lowland rice. Drought also affects rice production in Australia, China, USA, and many other countries. The world's irrigated area per capita has decreased from a peak of 48 ha/1000 people in late 1970 to about 42 ha/1000 people in 2002 (Gleick, 1993). Therefore, drought stress is a primary constraint to rice production and yield stability and, while it is generally avoided in irrigated rice production systems, it is a consistent feature across much of the 63.5 million ha of rainfed rice sown annually, mostly in tropical Asia, Africa, and Latin America (Narciso and Hossain, 2002).

Rice originated in semi-aquatic environments and is commonly considered as poorly-adapted to limited water conditions (Lafitte et al., 2007). Drought occurrence in

rice can be in both upland and non-irrigated lowland systems and can affect early juvenile, reproductive, and grain developmental stages of the crop. In rice, the early vigor of a plant *per se* and biomass accumulation are useful but complex traits for anticipating subsequent reproductive attributes. Early vigor is the ability of plants to rapidly accumulate biomass and leaf attributes until enhanced canopy development and closure are achieved. It is an emergent property resulting from many processes including resource acquisition and conversion, organ and morphogenetic dynamics, and plant and canopy architecture. Vigor favors rapid colonization of space and resources (Asch et al., 1999) and early vigor can thus contribute to improving yield stability, for example in drought-prone environments. By contributing to early canopy closure, it also reduces unproductive, non-transpirational water use and thus increases overall crop water use efficiency (Condon et al., 2004). Water-use efficiency (WUE) is indirectly enhanced by the improved weed competitiveness of the crop as conveyed by early vigor (Dingkuhn et al., 1999; Zhao et al., 2006). Therefore, the early vigor trait is an essential factor for enabling high yields particularly in short-duration varieties, and short duration in itself can directly translate into lower overall water consumption. Besides low water requirement, early maturing genotypes also provide an escape mechanism for avoiding diverse biotic and abiotic challenges effectively. For ensuring above ground shoot and vigor parameters, any genotype needs a proper root architecture to support it. However, root systems for any crop are difficult to study because of their highly structured underground distribution pattern, the complexity of vigorous interactions with the immediate environment, and their functional diversity. The root system of a rice plant, for example, consists of numerous nodal roots and their laterals. The growth direction

of these nodal roots affects the spatial distribution of the root system in the soil, which seems to relate to yield. Moreover, nodal roots that emerge from the most basal shoot unit of a tiller are usually thick and grow downwards.

Both agronomic and genetic options have been developed to manage rice in water-limited environments. One of the options to address this about affordability to resource-poor farmers is to develop drought-tolerant rice by exploiting its genetic variation. Identifying rice varieties and breeding lines with high levels of drought tolerance for use as donors in breeding and gene discovery is, therefore, one of the main challenges for rice research (Serraj and Atlin, 2008). However, little is known about how different traits express and respond to drought stress as well as on trade-offs of key traits with drought tolerance. During early vegetative growth, crop stand is established, tillers are formed, and organs for resource capture (leaf canopy and root system) are deployed. These processes also affect the resources available during later crop development phases (Finch-Savage et al., 2010), for example, through delays of flowering and maturity that can extend the growth cycle into the dry season (Wopereis et al., 1996). During the vegetative phase, the rapid ground cover achieved with early vigor (Shipley, 2006) can reduce soil evaporation, accelerate root access to soil water and nitrogen, and reduce competition with weeds (Zhao et al., 2006). Early vigor may also accelerate depletion of soil water reserves, making less water available for later crop stages (Zhang et al., 2005). Early vigor depends on both assimilate source (light capture and photosynthetic rate) and the sink constituted by structural growth (leaf appearance rate, potential size and tiller outgrowth). A recent study conducted under non-limiting resources (Luquet et al., 2012) identified organogenetic developmental rate

(DR = 1/phyllochron), together with tillering ability and leaf size, as major genotypic determinants of rice early vigor. The results suggested trade-offs between organ number and size. Across a large number of genotypes, DR was positively correlated with tillering and negatively correlated with leaf size and leaf starch concentration. Component traits of early vigor are thus in part physiologically linked regarding trade-offs, but may also be linked genetically (Granier and Tardieu, 2009). For example, earlier studies on seedling vigor related traits found associations between root and shoot traits that were contributing to early vigor (Redoña and Mackill, 1996). For breeders, component traits directly or indirectly contributing to yield are useful if they are easy to measure and correlated with yield while having greater genetic diversity than yield itself (Tuberosa et al., 2002). Phenotyping for molecular breeding purposes allows developing molecular probes for marker-based selection. In this context, it is important that markers for component traits of a complex trait have proven physiological complementarities or synergies while being under distinct genetic control. However, very little is known on the extent of genetic variation for vigor-related traits under drought stress among tropical japonica varieties- the primary varietal group used in commercial production in the US Midsouth. Most earlier drought studies conducted on rice have primarily used genotypes belonging to the indica rice subspecies that is the most predominant rice subspecies grown worldwide.

The overall objective of the present study, therefore, was to explore the morphogenetic plant features of rice related to early vigor and trait expression under limited water conditions among tropical japonicas. We hypothesized that the potential variability in the genotypes could serve as a pre-breeding resource for the U.S Midsouth and similar rice-growing areas under limited irrigation conditions. Specific objectives

were to (i) determine the variation in limited moisture stress tolerance among different tropical japonica rice genotypes; (ii) classify rice genotypes based on combined stress response to limited water conditions index, and (iii) study the interrelationships among different morphometric traits. We expect that this information will be useful for identifying promising genetic donors for tolerance to responsiveness to limited moisture stress that can be used for breeding promising rice varieties. The data could also be directly used by farmers and crop consultants as a decision-making tool for selecting released varieties best suited for water-limited rice production systems.

Materials and methods

Germplasm used and experimental setup

A total of 100 rice genotypes were evaluated for response to drought during the summer of 2016. Of these, 95 belonged to the tropical japonica varietal group, four were indicas (El Paso 144, Inia Tacuari, IRGA409, and N-22), and one was a temperate japonica (Niponbare). Seventy of these genotypes were breeding lines under development while 30 were commercially released varieties. Of the latter, 25 were released for commercial use in the US Midsouth. The experiment was conducted using pre-fabricated mini-hoop structures (Figure 1) located at the Rodney Foil Plant Science Research facility of Mississippi State University, Mississippi State, USA (33°28′ N, 88°47′ W), MS, USA. Each structure consisted of a PVC framework with 4 MIL polythene wrapping having the dimensions of 2m width x 1.5m height x 5m length. Space was enough to cover 300 pots in each structure and accommodate one experimental set of three replications.

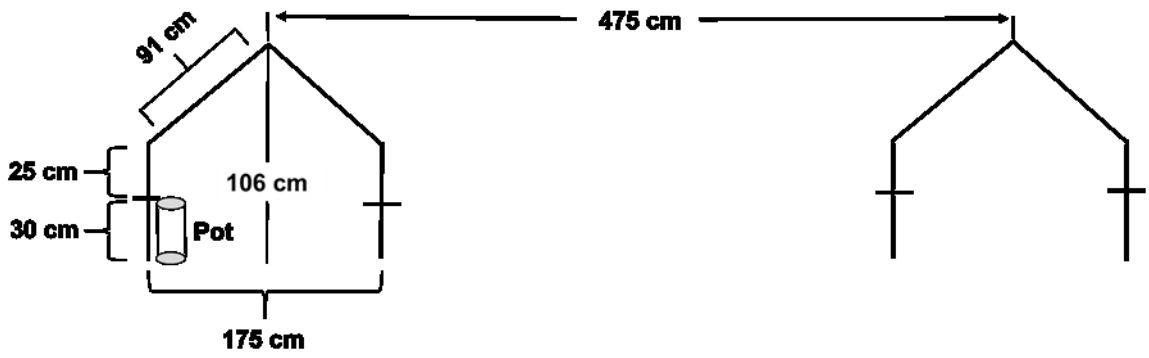

Figure 5.1 Pre-fabricated mini-hoop structures used in this study.

Fungicide-treated seeds were sown in 600 polyvinyl-chloride pots (15.2-cm diameter and 30.5-cm height) filled with the soil medium consisting of 3:1 sand/top soil classified as a sandy loam (87% sand, 2% clay, and 11% silt) with a 500 g of gravel at the bottom of each pot. Initially, five seeds were sown in each pot and, 7 d after emergence, the plants were thinned to one per pot. Plants were irrigated three times a day through an automated, computer-controlled drip system with full-strength Hoagland's nutrient solution (Hewitt, 1952), delivered at 0800,1200, and 1700 h until drought treatment was imposed on one set. After imposing drought treatment in the drought set, fertigation was managed through real-time determination of the soil moisture status of the pots.

Drought treatments

All test rice genotypes were laid out in three replications using a completely randomized design in both the control and drought stress treatment. Similar experiment to screen the rice genotypes at vegetative stage under drought conditions using rain out structures was previously carried out revealing the effectiveness of the experimental setup (Bunnag and Pongthai, 2013). After the establishment of seedlings (12 days after

sowing), the drought treatment was imposed by maintaining soil moisture at 50% until harvesting of the trial (30 days after sowing). The soil moisture status and the temperature in mini-hoop structures were monitored through real-time sensors throughout the experiment. A brief summary of the soil moisture status of the trial is shown in figure 2. The net solar radiation availability of approximately 97 percent under the mini-hoop structures was also monitored at various stages of the experiment using a light meter (Li-250A, LI-COR, Inc, Lincoln Nebraska, USA). Real-time temperature sensors were used to measure the diurnal temperature regimes and the average day temperature recorded was 33.22˚C while the night temperatures hovered around 22.66˚C.

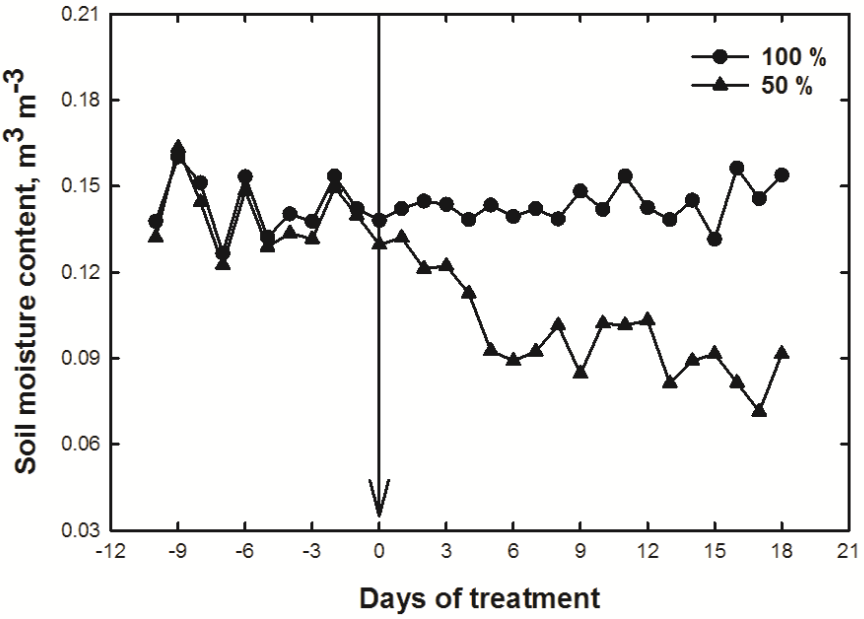

Figure 5.2 Real-time management of soil moisture status of the trial under the control and drought treatments. The arrow indicates the day when the treatments were imposed. Bars indicate standard errors of the mean of ± 3 replications.

Measurements

Developmental parameters

Plant height (PH), tiller number (TN), and leaf number (LN) were measured on the 28th day of sowing. Leaf area was measured using the LI-3100 leaf-area meter (LI-COR, Inc. Lincoln Nebraska, USA) on harvesting followed by measurement of plant components. Leaf dry weight (LDW), stem dry weight (SDW), shoot dry weight (SHDW) and total dry weights (TDW) were measured from all plants after oven drying at 75°C until constant weight was reached. From the shoot and root dry weight, root/shoot (RSR) was estimated in all rice lens and treatments. To account for genotypic differences, all comparisons were done concerning the control.

Physiological parameters

Instant chlorophyll measurements were recorded in all genotypes using a SPAD meter (SPAD 502 Minnolota Inc. Ontario, Canada) on the 25th day after sowing. Chlorophyll fluorescence was measured using the Fluropen 1000 (Photo System Instruments, Kolackova, Czech Republic) for OJIP Analysis. Application of chlorophyll fluorescence fast-transient analysis (OJIP) is a simple and non-invasive tool for monitoring chloroplast function. The OJIP analysis is used as a sensitive, reliable, and quick test for the functionality and vitality of the photosynthetic system. Minimal Fluorescence Intensity (F_o), Maximal Fluorescence Intensity (F_m), Maximal Variable Fluorescence (F_v) and F_v/F_m was measured to get the maximum potential quantum efficiency of Photosystem II to derive clues about the stress effect on the experimental rice lines.

Root image capture and analysis

Roots were cut and separated from the stems and washed thoroughly avoiding any disturbance to the root system. Longest root length (LRL) was determined using a metric ruler. The cleaned individual root systems were floated in 5 mm of water in a 0.3- by 0.2-m Plexiglas tray. Roots were untangled and separated with a plastic paintbrush to minimize root overlap. The tray was placed on top of a specialized dual-scan optical WinRHIZO scanner (Regent Instruments, Inc. Quebec, Canada, 2009), linked to a computer software system. Gray-scale root images were acquired according to the same procedure described (Brand et al., 2016: Reddy et al., 2017; Wijewardana et al., 2015) previously by setting the parameters to high accuracy (resolution 800 x 800 dpi). Acquired images were analyzed for the cumulative root length (CRL), root surface area (RSA), average root diameter (ARD), root volume (RV), number of roots (RN), number of root tips (NRT), number of root forks (NRF), and number of root crossings (NRC) using WinRHIZO optical scanner and associated software.

Data analysis, terminology and drought tolerance indices

The data from all measurements of root traits were recorded and compiled in Microsoft Excel 2016. Descriptive analysis including means, standard deviations (SD), coefficients of variation (CV), and analysis of variance (ANOVA), were calculated for the traits under control and drought treatments using SAS program (v 9.4, SAS Institute, Inc., Cary, NC, 2011) using a completely randomized design considering rice lines and drought as source of variance. Data were analyzed using a one-way ANOVA via PROC GLM in SAS to determine the effect of drought on the developmental, physiological and root parameters. The Fisher's projected least significant difference

test at P = 0.05 was employed to test the differences among the treatments for the measured parameters. The standard errors of the mean were calculated using Sigma Plot 13.0 (Systat Software, Inc, San Jose, CA, 2015) and presented in the figures as error bars.

Drought response characterization

All rice lines under study were classified into different response reaction groups based on their individual response to the drought stress and subsequent summation of individual index values for each trait. The Combined Drought Stress Response Indices (CDSRI) were calculated by adding Individual Drought Stress Response Indices (IDSRI) for all traits. Initially, IDSRI values for each parameter was calculated as the trait value of a parameter (Pvl) under drought for a given rice line divided by the trait value for same parameter (Po) under controlled conditions as follow:

IDSRI= Pvl /Po; and

CDSRI = (PH vl/ PH o) + (TN vl/ TN o) + (LN vl/LN o) + (LA vl/ LA o) + (LDW vl/ LDW o) (SDW vl/ SDW o) + (RDW vl/ RDW o) + (SHDW vl/ SHDW o) + (TDW vl/ TDW o) + (RS vl/ RS o) (LRL vl/ LRL o) + (F0 vl/ F0 o) + (FM vl/ FM o) + (FV vl/ FV o) + (Fv / Fm vl/ Fv / Fm o) (CRL vl/ CRL o) + (RSA vl/ RSA o) + (ARD vl/ ARD o) + (RV vl/ RV o) + (RN vl/ RN o) (NRT vl/ NRT o) + (NRF vl/ NRF o) + (NRC vl/ NRC o)

Based on the CDSRI values, rice genotypes were classified into five response groups- highly sensitive, sensitive, moderately sensitive, tolerant, and highly tolerant.

Results and Discussion

In the southern US, where rice is grown on more than two million acres (>809,000 hectares), almost all the varieties cultivated belong to the tropical japonica subspecies or varietal grouping. Tropical japonica rice, however, has not been well characterized for drought stress tolerance-related traits, in general, and using mini-hoop structures. This study is the first to screen a wide array of rice genotypes of diverse origin in a perfect or uniform phenotypic platform, with all traits closely monitored across treatments with desired automation. Therefore, assessing genetic variability for drought tolerance using this methodology in tropical japonicas could be important, not only for commercial cultivation under current drought-challenged conditions, but also for future development of tropical japonica varieties suited for water-limited environments and cropping systems requiring less water in the future. Drought is likely the most important environmental factor that adversely affects plant growth and development. Effects of drought on plants have been studied for a long time and changes induced by insufficient water supply have been examined from the whole plant/plant population level to biochemical and molecular level (Farooq et al., 2009). Many studies have been done on drought stress in rice but all these involved different methodologies, both in the field (Bunnag and Pongthai, 2013) and greenhouse (Luciano et al., 2012). Achieving early vigor quickly and accumulating biomass rapidly will be critical factors under both normal and stressed rice (Rebolledo et al., 2012). Recently, using mini-hoop structures has been found as an effective approach for studying the effect of drought conditions on a number of crops such as high value off-season vegetables like French bean and amaranth (Yadav et al., 2014). In Mississippi, tropical

japonica varieties were grown on almost 200,000 acres in 2016 (Redoña et al., 2017). Tropical japonica rice, however, has not been well characterized for drought stress tolerance-related traits, in general, and using mini-hoop structures, in particular. Moreover, physiological expression of rice genotypes and its suitable interpretation in rice breeding perspective can lead to more reliable control of water-stress severity and duration at the critical growth stages, and this will result in the development and utilization of effective selection measures on a longer term basis (O' Neill et al., 2006).

Performance of rice genotypes and interaction with drought

The analysis of variance for developmental traits revealed significant (P >0.001) differences among the rice genotypes, drought treatments, and genotype X drought interaction for all traits except LN and SDW (P >0.05) (Table 5.1). Among root traits, non-significant interaction was observed in RSA, ARD, NRT, LRL and RN while for physiological traits, non-significant interaction was observed in SPAD and F_v/F_m. Significant variation was observed for most traits among lines and even across experimental setups, indicating presence of genetic variation, which could be exploitable through breeding. Abiotic stresses like drought can affect the physiological status of an organism and have adverse effects on growth, development, and metabolism (Chutia and Borah, 2012). Negative effects of water deficit on mineral nutrition and metabolism drastically effects the plant developmental features like plant height and leaf area and alter assimilate partitioning among the plant organs (Zain et al., 2014).

Table 5.1 Analysis of variance across the genotype, treatments and their interaction for the morphological parameters measured viz plant height (PH, cm plant^{-1}), tiller number (TN, no. plant^{-1}), leaf number (LN, no. plant^{-1}), leaf area (LA, cm^2 plant^{-1}), leaf dry weight (LDW, g plant^{-1}), stem dry weight (SDW, g plant^{-1}), root dry weight (RDW, g plant^{-1}) shoot dry weight (SHDW, g plant^{-1}), root/shoot ratio (RSR), total dry weights (TDW, g plant^{-1}), longest root length (LRL, cm plant^{-1}), root surface area (RSA, cm^2 plant^{-1}), average root diameter (ARD, mm plant^{-1}), root volume (RV, cm^3 plant^{-1}), number of root tips (NRT, no. plant^{-1}), number of root forks (NRF, no. plant^{-1}), number of root crossings (NRC, no. plant^{-1}), cumulative root length (CRL, cm plant^{-1}), number of roots (RN, no. plant^{-1}), chlorophyll content (SPAD), minimal fluorescence intensity ($F_{o)}$, maximal fluorescence intensity ($F_{m)}$, maximal variable fluorescence ($F_{v),}$ and fluorescence (Fv/Fm). Measurements were taken at harvesting time, 28 days after sowing.

Source of Variation	PH	TN	LN	LA	LDW	SDW	RDW	SHDW	RSR	TDW	LRL	RSA	ARD	RV	NRT	NRF	NRC	CRL	RN	SPAD	F_o	F_m	F_v	F_v/F_m
Genotypes	***	***	NS	***	***	***	***	***	**	***	*	**	**	***	NS	***	***	***	***	***	**	***	***	*
Drought	***	*	NS	***	*	***	***	***	**	***	NS	NS	NS	**	NS	***	**	***	*	***	NS	*	***	NS
Control	***	***	NS	***	***	NS	***	**	NS	***	***	*	*	***	*	***	***	*	*	NS	***	***	***	*
Control x Drought	NS	*	NS	***	**	NS	*	*	*	*	NS	NS	NS	*	NS	***	***	*	NS		*	***	***	NS

Significant level ***, **, *, and N.S means P-value < 0.001, 0.01, 0.05, and not significant.

Developmental traits

All the developmental traits like PH, TN, LA, LDW, and SDW were affected by drought imposition except LN which more or less remained constant across treatments and even across genotypes (Table 5.2 and 5.3). Under drought conditions, PH ranged from 9.23 cm (RU0603075) to 25.50 cm (N-22), with an overall mean of 14.66 cm while the average PH under the control treatment was 21.02 cm. Maximum PH reduction of 11.50 cm was observed in RU1305001. Under drought conditions, 43 percent of the rice genotypes exceeded the average PH value of 14.66 cm. LA was also drastically reduced in all genotypes under drought conditions and ranged from 40.05 cm^2 (RU1504194) to 210.40 cm^2 (CL Jazzman), with an average of 104.54 cm^2 as compared to average LA of 294.30 cm^2 under the control treatment. Maximum reduction of 253 per cent was observed in the genotype RU1303138 Table 5.2). LA changes under drought treatment corresponded with the respective changes in LDW, with genotype RU1504194 having the lowest LDW of 0.41 g while maximum reduction of 2.633 g was observed in CL Jazzman. Mild to moderate water stress is sufficient to reduce leaf area in most crop species. Average decline of 2.09 g was observed with respect to TDW and maximum impact of drought treatment was observed on the genotype CL Jazzman (5.90 g). Thus, there was coherence of LA and TDW responses on the same genotype. Under drought conditions maximum TDW of 3.39 g was observed for N-22 and minimum TDW of 1.49 g was expressed by LA 2008 Tables 5.2 and 5.3.

Table 5.2 Drought stress effects on morphological parameters measured viz, plant height (PH, cm plant^{-1}), tiller number (TN, no. plant^{-1}), leaf number (LN, no. plant^{-1}), and leaf area (LA, cm^2 plant^{-1}). Measurements were made at harvesting time, 28 days after sowing.

Genotype No.	Genotype Name	Country of origion	Genotype class	PH		TN		LN		LA	
				C	D	C	D	C	D	C	D
1	14CLPYT033	USA	B.L	16.9	12.4	6.7	5.0	3.0	3.3	212.6	121.4
2	14CLPYT108	USA	B.L	19.0	15.3	7.3	5.7	3.0	3.0	272.5	109.5
3	14CVPYT094	USA	B.L	18.3	12.8	7.7	4.3	3.0	3.0	303.0	102.5
4	14CVPYT144	USA	B.L	22.7	16.0	7.3	4.7	3.0	3.0	251.0	70.7
5	COLORADO	USA	R.V	17.3	9.5	7.0	4.3	3.0	3.0	245.1	131.2
6	Bowman	USA	R.V	19.5	13.0	8.0	4.0	3.0	3.0	286.1	117.7
7	CAFFEY	USA	R.V	20.9	13.6	7.0	4.7	3.0	3.3	278.4	90.6
8	CHENIERE	USA	R.V	18.5	14.0	9.0	4.0	3.0	3.0	238.0	93.6
9	CL Jazzman	USA	R.V	22.5	14.5	13.0	6.3	3.0	3.0	722.1	343.7
10	CL111	USA	R.V	24.0	16.6	8.0	5.7	3.3	3.0	322.9	103.8
11	CL142-AR	USA	R.V	23.7	16.3	8.0	4.7	3.0	3.3	255.8	92.9
12	CL151	USA	R.V	22.6	13.7	8.7	5.7	3.3	3.0	298.8	95.1
13	CL152	USA	R.V.	22.0	13.3	9.3	4.3	3.7	3.0	346.0	119.9
14	CL163	USA	R.V.	17.9	14.0	5.0	5.3	3.0	3.0	229.7	115.8
15	CL172	USA	R.V.	20.2	16.9	7.7	5.0	3.3	3.3	292.4	82.3
16	CL271	USA	R.V.	22.2	13.9	8.7	5.7	3.3	3.0	332.9	102.8
17	Cocodrie	USA	R.V.	21.2	16.4	6.0	4.3	2.7	3.0	285.1	90.0
18	NIPONBARE	Japan	G.D.	18.4	12.7	7.7	5.0	3.0	3.0	359.4	94.1
19	ANTONIO	USA	R.V.	21.8	12.9	6.7	3.3	3.0	3.0	312.4	86.7
20	El Paso 144	USA	G.D.	18.1	13.3	13.0	5.3	3.0	3.0	395.3	108.2
21	GSOR100390	USA	G.D.	23.3	12.9	8.3	5.0	3.0	3.0	322.9	110.2
22	GSOR100417	USA	G.D.	22.4	17.3	10.0	5.3	3.0	3.0	443.3	108.6
23	GSOR101758	USA	G.D.	18.6	11.0	8.0	6.7	2.7	3.0	312.2	150.2
24	RU1104122	USA	R.V.	20.8	15.0	6.7	5.7	3.0	3.3	271.1	112.1
25	CLJZMN	USA	R.V.	24.5	18.5	7.7	6.0	3.3	3.3	297.0	143.2
26	INIA Tacuari	S.A	G.D.	19.8	15.5	6.0	4.3	3.0	3.3	171.2	91.2
27	IRGA409	Brazil	R.V.	22.0	16.5	10.7	5.3	3.3	2.7	362.0	116.9
28	JES	USA	R.V.	19.5	12.6	6.0	5.0	3.0	2.7	266.1	90.0
29	JUPITER	USA	R.V.	18.4	13.0	9.3	5.0	3.3	3.0	476.2	107.7
30	LA 2008	USA	B.L.	20.2	14.6	6.7	3.0	3.0	3.3	200.6	56.9
31	LA 2134	USA	B.L.	22.7	16.8	8.7	5.7	3.0	3.3	316.0	80.6
32	LAKAST	USA	R.V.	20.0	13.3	7.0	5.3	3.0	3.0	274.9	101.6
33	MERMENTAU	USA	R.V.	22.1	16.8	7.0	6.0	3.0	3.0	247.0	107.7
34	Presidio	USA	R.V.	22.2	14.2	7.0	5.3	3.0	3.0	290.8	106.5
35	Rex	USA	R.V.	19.5	13.7	6.3	4.3	3.0	3.3	299.1	115.0
36	RoyJ	USA	R.V.	22.8	14.3	9.0	4.7	3.0	3.3	361.0	90.3
37	RU0603075	USA	Has	16.3	9.2	12.7	8.0	3.0	3.0	520.6	188.3
38	RU1201024	USA	B.L.	20.2	13.5	6.7	6.0	3.0	2.7	302.6	130.4
39	RU1201047	USA	B.L.	23.7	15.1	5.0	4.7	3.0	3.0	246.1	103.4
40	RU1201136	USA	B.L.	19.8	13.8	7.0	3.3	3.3	3.0	249.3	71.5
41	RU1204156	USA	B.L.	19.2	13.8	7.7	6.3	3.3	3.0	282.6	89.5
42	RU1204197	USA	KM.	22.3	15.3	7.0	7.0	3.0	3.3	262.2	131.5
43	RU1301084	USA	B.L.	20.1	14.3	6.7	4.3	3.0	3.0	254.8	96.3
44	RU1301093	USA	B.L.	21.0	16.5	6.0	4.7	3.0	3.0	279.7	80.3
45	RU1301102	USA	B.L.	18.4	13.3	7.0	5.7	3.0	3.0	202.9	96.0
46	RU1302192	USA	B.L.	23.5	16.8	8.0	5.7	3.0	3.0	369.7	108.9
47	RU1303138	USA	B.L.	17.2	10.9	13.3	8.0	3.0	3.0	629.1	177.9
48	RU1303181	USA	B.L.	21.2	17.0	6.3	4.7	3.0	3.3	269.7	87.1
49	RU1304114	USA	B.L.	19.1	14.7	8.7	5.0	3.0	2.7	358.9	105.6
50	RU1304122	USA	B.L.	24.5	16.8	8.0	6.0	3.0	2.7	404.9	128.6
51	RU1304154	USA	B.L.	22.7	17.9	8.3	6.0	3.0	3.3	327.8	99.1
52	RU1304156	USA	B.L.	24.2	15.0	7.7	5.3	3.0	3.0	323.4	84.4
53	RU1305001	USA	B.L.	23.8	12.3	7.7	5.3	3.0	2.7	316.1	84.9
54	RU1401067	USA	B.L.	21.8	16.8	6.7	4.3	3.3	3.3	280.0	90.7

Table 5.2 (Continued)

55	RU1401070	USA	B.L.	21.5	14.7	5.7	5.3	3.0	3.3	186.7	68.1
56	RU1401090	USA	B.L.	21.7	14.1	8.7	4.7	3.0	3.0	191.6	95.1
57	RU1401099	USA	B.L.	20.4	15.9	8.0	5.3	3.3	3.0	296.9	91.5
58	RU1401102	USA	B.L.	24.7	18.0	7.3	4.7	3.0	3.3	287.0	177.3
59	RU1401145	USA	B.L.	20.5	17.4	6.7	5.7	3.0	3.3	156.0	92.1
60	RU1401161	USA	B.L.	21.9	13.7	6.3	6.0	3.3	3.0	295.9	91.7
61	RU1401164	USA	B.L.	24.2	15.0	10.7	5.7	3.0	3.0	356.3	106.4
62	RU1402005	USA	B.L.	20.9	13.2	8.7	5.0	3.0	3.0	340.9	109.7
63	RU1402031	USA	B.L.	23.1	13.9	6.7	4.7	3.0	3.0	277.1	90.5
64	RU1402065	USA	B.L.	21.1	14.3	8.7	5.3	3.0	3.0	216.0	106.8
65	RU1402115	USA	B.L.	21.8	15.5	8.0	5.3	3.0	3.0	345.8	101.8
66	RU1402131	USA	B.L .	23.0	15.1	9.0	7.7	3.0	3.3	408.4	142.7
67	RU1402134	USA	B.L.	23.8	17.4	8.3	5.7	3.0	3.0	391.2	98.6
68	RU1402149	USA	B.L .	21.3	13.1	6.7	6.3	3.0	3.0	195.3	88.0
69	RU1402174	USA	B.L.	17.2	14.0	7.0	5.0	3.0	3.0	243.1	97.3
70	RU1402189	USA	B.L .	23.1	18.2	6.3	6.0	3.3	3.0	253.8	115.7
71	RU1402195	USA	B.L.	23.6	19.9	8.0	6.7	3.0	3.3	396.8	140.1
72	RU1403107	USA	B.L .	18.1	13.8	6.3	4.7	3.0	3.3	229.2	77.8
73	RU1403126	USA	B.L.	16.6	11.9	10.3	5.7	3.3	3.0	434.6	119.0
74	RU1404122	USA	B.L .	17.5	14.5	6.3	5.3	3.0	3.3	209.3	98.8
75	RU1404154	USA	B.L.	22.5	17.0	6.7	4.3	2.7	3.0	313.0	83.0
76	RU1404156	USA	B.L .	19.8	13.8	6.3	3.7	3.0	2.7	260.9	90.1
77	RU1404157	USA	B.L.	19.1	12.4	6.0	3.7	3.0	2.7	169.5	100.6
78	RU1404191	USA	B.L .	20.7	12.8	8.0	4.7	3.0	2.7	369.2	72.1
79	RU1404193	USA	B.L.	25.8	17.1	7.0	6.0	3.0	3.0	259.8	118.9
80	RU1404194	USA	B.L .	21.1	16.4	6.7	4.3	3.0	3.0	189.3	115.3
81	RU1404196	USA	B.L.	14.8	13.3	6.7	5.7	3.0	3.0	85.8	86.9
82	RU1404198	USA	B.L .	20.3	17.2	7.3	5.3	3.0	3.3	273.2	123.5
83	RU1504083	USA	B.L.	19.8	14.3	5.3	5.7	2.7	3.0	261.2	118.5
84	RU1504100	USA	B.L .	21.7	13.5	7.0	5.7	3.0	3.0	330.4	85.4
85	RU1504114	USA	B.L.	24.1	14.2	8.3	3.3	3.0	3.3	303.4	83.8
86	RU1504122	USA	B.L .	21.1	15.6	9.0	5.7	3.0	3.0	291.8	106.6
87	RU1504154	USA	B.L.	22.9	16.7	8.3	5.7	3.0	3.0	242.7	115.7
88	RU1504156	USA	B.L .	19.2	15.7	6.0	4.7	3.0	3.0	240.4	93.0
89	RU1504157	USA	B.L.	23.5	15.3	8.0	4.7	3.0	3.0	290.8	88.6
90	RU1504186	USA	B.L .	21.4	15.3	7.3	5.7	3.0	3.0	261.1	119.6
91	RU1504191	USA	B.L.	22.2	14.6	8.3	5.7	3.3	2.7	277.3	93.5
92	RU1504193	USA	B.L .	22.9	16.1	6.3	5.3	3.0	3.0	266.5	98.5
93	RU1504194	USA	B.L.	20.6	12.5	5.7	3.7	2.7	3.0	174.6	40.1
94	RU1504196	USA	B.L .	22.8	14.5	8.0	5.0	3.0	3.3	377.9	106.3
95	RU1504197	USA	B.L.	18.4	12.5	7.3	6.0	3.0	3.0	290.4	109.7
96	RU1504198	USA	B.L .	20.7	12.7	7.3	3.7	3.0	3.3	255.3	69.8
97	Sabine	USA	R.V.	20.1	12.8	8.0	4.7	3.0	3.0	183.4	87.0
98	Taggart	USA	R.V.	20.5	13.5	6.3	4.7	3.0	3.0	226.6	106.0
99	Thad	USA	R.V	19.3	12.4	6.7	5.7	3.0	3.3	258.5	100.4
100	N-22	India	G.D	25.5	20.0	7.7	6.7	3.0	3.3	234.2	150.3
			Mean	21.0	14.7	7.7	5.2	3.0	3.1	294.3	105.9
			Genotypes	***	***	***	*	NS	NS	***	***
		Gen. X Treatments		NS		*		NS		**	

Under drought condition, RSR changed significantly with an average increment of 86 per cent. The minimum RSR under drought was expressed by 14CLPYT033 (0.129) and the maximum by RU1404196 (0.555), with an average value of 0.369 as compared to 0.198 under control conditions.

All the developmental traits like PH, TN, LA, LDW, and SDW were affected by drought imposition except LN which more or less remained constant across treatments and even across genotypes (Table 5.3).

Table 5.3 Drought stress effects on morphological parameters measured viz, leaf dry weight (LDW, g plant^{-1}), stem dry weight (SDW, g plant^{-1}), shoot dry weight (SHDW, g plant^{-1}), total dry weights (TDW, g plant^{-1}). Measurements were made at harvesting time, 28 days after sowing.

Genotype No.	Genotype name	LDW		SDW		SHDW		TDW	
		C	D	C	D	C	D	C	D
1	14CLPYT033	1.5	1.0	2.4	1.2	3.9	2.2	4.6	2.5
2	14CLPYT108	1.7	0.9	1.6	0.9	3.3	1.7	3.9	2.3
3	14CVPYT094	2.0	0.7	1.9	0.7	3.8	1.5	4.7	2.1
4	14CVPYT144	2.0	0.6	2.0	0.9	4.0	1.5	4.7	1.9
5	COLORADO	1.5	1.0	1.6	0.8	3.1	1.8	3.8	2.3
6	Bowman	2.0	0.8	1.9	1.0	3.9	1.9	4.8	2.5
7	CAFFEY	2.2	0.9	1.9	0.9	4.1	1.7	4.8	2.4
8	CHENIERE	1.7	0.5	1.9	0.9	3.6	1.4	4.3	1.7
9	CL Jazzman	3.9	1.2	3.3	0.8	7.1	2.1	8.8	2.9
10	CL111	2.0	0.9	1.7	0.9	3.8	1.8	4.5	2.5
11	CL142-AR	1.6	1.0	1.6	1.0	3.2	2.0	4.0	2.7
12	CL151	2.1	0.6	2.2	0.7	4.3	1.3	5.0	2.0
13	CL152	2.1	0.7	2.2	1.1	4.3	1.8	5.2	2.6
14	CL163	1.4	1.0	1.6	1.3	3.0	2.3	3.6	3.1
15	CL172	1.7	0.7	2.2	1.0	3.9	1.7	4.6	2.3
16	CL271	2.4	1.0	2.1	0.7	4.5	1.7	5.3	2.4
17	Cocodrie	2.1	0.6	1.8	1.1	3.9	1.7	4.4	2.5
18	NIPONBARE	2.1	0.8	2.0	0.6	4.1	1.4	4.9	1.8
19	ANTONIO	2.1	0.8	2.2	0.6	4.3	1.4	5.0	2.0
20	El Paso 144	2.5	1.0	2.8	1.1	5.2	2.2	6.2	2.9
21	GSOR100390	2.0	0.8	1.8	1.1	3.8	1.9	4.4	2.6
22	GSOR100417	2.1	1.0	1.9	0.3	4.0	1.3	4.7	1.9
23	GSOR101758	1.6	1.1	1.9	0.3	3.4	1.3	4.0	2.0
24	RU1104122	1.8	1.1	1.7	1.1	3.5	2.2	4.2	3.0
25	CLJZMN	2.1	1.0	1.8	1.0	3.9	2.1	4.6	2.8
26	INIA Tacuari	1.3	1.0	1.5	1.0	2.8	2.0	3.4	2.7
27	IRGA409	1.7	1.0	1.7	1.0	3.4	2.0	4.3	2.7
28	JES	1.7	0.9	2.2	1.1	3.9	2.0	4.7	2.9
29	JUPITER	2.7	0.9	3.0	1.0	5.6	1.9	6.6	2.5
30	LA 2008	1.8	0.5	1.6	0.7	3.4	1.2	4.1	1.5
31	LA 2134	1.7	1.0	1.9	1.1	3.7	2.1	4.3	2.8
32	LAKAST	1.6	0.9	1.8	1.1	3.4	2.0	4.2	2.7
33	MERMENTAU	1.7	0.9	1.9	0.9	3.6	1.7	4.3	2.4
34	Presidio	1.6	0.9	1.5	0.9	3.1	1.8	3.6	2.6
35	Rex	2.2	1.0	2.3	0.9	4.5	1.8	5.3	2.6
36	RoyJ	2.7	0.8	3.0	1.1	5.7	1.9	6.7	2.5
37	RU0603075	2.5	1.1	2.6	1.1	5.0	2.2	6.0	2.9
38	RU1201024	1.9	1.0	1.6	1.0	3.4	2.0	4.2	2.7
39	RU1201047	1.7	0.9	1.8	0.8	3.5	1.7	4.2	2.3
40	RU1201136	1.4	0.7	2.4	0.9	3.8	1.5	4.5	2.2
41	RU1204156	1.6	0.7	2.3	1.3	3.8	2.0	4.5	2.7
42	RU1204197	1.8	1.2	2.2	1.2	4.0	2.4	4.8	3.3
43	RU1301084	1.9	0.9	2.7	0.6	4.6	1.5	5.6	2.2
44	RU1301093	1.9	1.1	1.9	1.1	3.7	2.2	4.5	2.9
45	RU1301102	1.2	0.8	1.6	1.1	2.7	2.0	3.5	2.5
46	RU1302192	2.2	1.0	2.2	1.2	4.3	2.2	5.2	2.9
47	RU1303138	3.0	1.1	2.5	1.1	5.5	2.2	6.6	2.9

Table 5.3 (Continued)

48	RU1303181	1.6	0.9	1.8	0.9	3.4	1.8	4.0	2.5
49	RU1304114	1.9	0.9	1.8	1.2	3.7	2.1	4.5	2.7
50	RU1304122	2.4	1.0	2.6	0.8	5.1	1.9	6.0	2.7
51	RU1304154	2.2	1.0	2.2	0.9	4.3	1.9	5.2	2.7
52	RU1304156	2.1	1.0	2.1	0.9	4.2	1.9	5.0	2.6
53	RU1305001	2.1	1.1	1.8	1.0	3.9	2.1	4.8	2.9
54	RU1401067	1.5	0.9	2.1	0.8	3.5	1.7	4.1	2.4
55	RU1401070	1.5	0.7	1.9	0.8	3.4	1.5	4.0	2.1
56	RU1401090	1.7	0.7	1.9	0.8	3.6	1.5	4.2	2.0
57	RU1401099	1.9	0.8	2.1	1.2	4.0	2.0	4.7	2.7
58	RU1401102	1.9	1.2	2.5	1.4	4.3	2.6	5.0	3.2
59	RU1401145	1.3	0.9	1.7	1.2	3.0	2.1	3.7	2.9
60	RU1401161	1.6	0.8	1.8	0.9	3.4	1.7	4.0	2.4
61	RU1401164	2.2	0.9	2.4	0.9	4.7	1.7	5.4	2.3
62	RU1402005	2.2	1.1	2.2	1.1	4.4	2.2	5.2	2.9
63	RU1402031	1.8	0.7	1.9	1.4	3.7	2.1	4.4	2.6
64	RU1402065	1.8	1.0	2.3	0.9	4.1	1.9	4.9	2.5
65	RU1402115	2.2	0.9	1.9	0.9	4.1	1.9	4.9	2.6
66	RU1402131	2.4	1.3	2.4	0.8	4.8	2.1	5.6	2.9
67	RU1402134	2.1	1.0	1.9	0.7	4.0	1.7	4.7	2.4
68	RU1402149	1.3	0.8	2.0	0.8	3.3	1.6	3.9	2.3
69	RU1402174	1.4	0.9	1.3	0.7	2.7	1.6	3.1	2.2
70	RU1402189	1.6	1.0	1.8	1.1	3.4	2.1	4.0	2.8
71	RU1402195	2.3	1.2	1.6	1.0	3.9	2.2	4.4	2.9
72	RU1403107	1.3	0.9	1.9	0.5	3.1	1.4	3.8	1.9
73	RU1403126	1.9	0.8	2.3	1.1	4.2	1.9	5.1	2.5
74	RU1404122	1.3	0.8	1.6	0.5	2.9	1.3	3.6	1.9
75	RU1404154	2.3	0.8	2.0	0.8	4.3	1.7	5.0	2.1
76	RU1404156	1.5	0.7	1.6	1.0	3.1	1.7	3.7	2.3
77	RU1404157	1.1	0.7	1.1	1.0	2.1	1.6	2.6	2.3
78	RU1404191	2.3	0.9	1.7	0.7	4.1	1.6	4.8	2.2
79	RU1404193	1.7	1.1	1.6	0.9	3.3	1.9	4.0	2.7
80	RU1404194	1.4	0.7	1.5	0.9	2.9	1.6	3.4	2.1
81	RU1404196	0.6	0.6	1.5	0.4	2.1	1.0	2.6	1.5
82	RU1404198	1.7	1.0	2.0	1.0	3.7	1.9	4.6	2.7
83	RU1504083	1.7	0.7	1.9	1.1	3.6	1.8	4.4	2.4
84	RU1504100	1.9	0.8	2.0	0.9	3.9	1.7	4.8	2.6
85	RU1504114	2.0	0.9	2.0	0.7	4.0	1.6	4.6	2.0
86	RU1504122	2.3	1.0	2.5	1.5	4.8	2.4	5.5	3.1
87	RU1504154	1.6	1.0	2.1	1.1	3.8	2.0	4.7	2.8
88	RU1504156	1.7	0.9	1.9	1.0	3.6	2.0	4.2	2.6
89	RU1504157	1.9	1.0	2.2	0.9	4.2	1.9	4.9	2.4
90	RU1504186	1.6	1.0	2.2	1.1	3.8	2.1	4.4	2.9
91	RU1504191	1.8	0.9	1.8	0.9	3.6	1.7	4.3	2.4
92	RU1504193	1.6	1.0	1.9	0.6	3.5	1.6	4.2	2.3
93	RU1504194	1.3	0.4	1.8	0.9	3.1	1.3	3.7	1.6
94	RU1504196	2.1	0.9	1.7	0.9	3.8	1.8	4.5	2.5
95	RU1504197	2.0	0.9	1.8	0.9	3.8	1.9	4.6	2.6
96	RU1504198	1.6	0.7	1.7	0.8	3.3	1.5	3.9	2.1
97	Sabine	1.2	0.9	1.9	1.2	3.1	2.1	3.8	2.7
98	Taggart	1.7	0.9	2.2	0.9	3.9	1.7	4.6	2.4
99	Thad	2.0	0.8	2.2	0.9	4.1	1.7	4.9	2.3
100	N-22	1.4	1.3	2.5	1.1	4.0	2.4	4.7	3.4
	Mean	1.8	0.9	2.0	0.9	3.8	1.8	4.6	2.5
	Genotypes	***	*	NS	***	**	***	***	***
	Gen. X Treatment		**		NS		*		*

These findings are in agreement with Islam (1999) that found moisture stress in early vegetative stages to hamper plant height due to the inhibition of the increase in

cell length as well as to reduction in cell division under water deficit. LA was also drastically reduced in all genotypes under drought conditions. The drastic reduction of leaf area can be attributed to limited photosynthesis due to a decline in Rubisco activity (Bota et al., 2004). Leaf area being an important benchmark for virtual plant performance under drought as leaf area declines according to the onset and rate of senescence, thus determining the amount of green leaf area maintained throughout plant life (Borrell et al., 2000). Water stress can also affect leaf area by speeding the rate of leaf senescence (Murty and Murty, 1982). Farooq et al, (2010) suggested that total dry biomass can be exploited as a stress parameter to estimate drought tolerance. Under drought stress, the reduction in dry matter can be attributed to the reduction of leaf area leading to slow photosynthesis rate resulting in limited assimilates under drought (Mostajeran and Rahimi-Eichi, 2009). Under drought condition, RSR changed significantly with an average increment of 86 per cent.

Root Traits

Roots play a crucial role for nutrient and water acquisition and are targeted to enhance plant productivity under a broad range of growing conditions including drought (Paez et al., 2015). Under different types of drought stress, plasticity in root length density or total root length (Tran et al, 2014) and lateral root length and/or branching (Kano et al, 2011) has been observed to improve shoot biomass, water uptake, and photosynthesis under drought in rice. Major root growth parameters like RL, RSA, and RN were severely affected under drought stress conditions in most of the rice genotypes. Average RL in controlled conditions was 6009 cm as compared to 5498 cm under drought conditions. Under drought conditions, maximum RL of 7455 cm was

expressed by genotype N-22 and minimum CRL of 3136 cm by Cheniere. The ARD of 0.50 mm was found to be similar under both control and drought stress treatments, with the genotype RU1401067 having the maximum ARD of 0.52 mm (Table 5.4).

Table 5.4 Drought effects on root parameters viz root length (RL, cm plant^{-1}), root surface area (RSA, cm^2 plant^{-1}), average root diameter (ARD, mm plant^{-1}), root volume (RV, cm^3 plant^{-1}), and number of root tips (NRT, no. plant^{-1}). Measurements were taken at harvesting time, 28 days after sowing.

Genotype	RL		RSA		ARD		RV		NRT	
name	C	D	C	D	C	D	C	D	C	D
14CLPYT033	6041	3397	694	559	0.4	0.4	7.8	7.2	31758	20908
14CLPYT108	5270	5394	756	804	0.5	0.5	8.6	9.6	36543	32795
14CVPYT094	6242	4302	964	641	0.5	0.4	11.9	8.0	29504	27114
14CVPYT144	6260	4571	862	513	0.4	0.4	9.5	7.5	38393	26787
COLORADO	5901	4087	597	588	0.4	0.4	7.0	6.6	34943	45111
Bowman	7184	5729	995	805	0.5	0.4	11.2	9.0	30939	33218
CAFFEY	7377	6167	1052	867	0.5	0.5	12.1	9.8	32550	30728
CHENIERE	6480	3136	911	456	0.4	0.4	10.3	5.9	38025	18491
CL Jazzman	7719	6199	1572	946	0.6	0.5	25.9	11.8	42080	35988
CL111	6172	5558	868	815	0.5	0.5	10.2	9.8	32586	33571
CL142-AR	5879	5628	887	860	0.5	0.5	10.8	10.5	34831	29363
CL151	6027	5109	834	602	0.4	0.4	9.2	8.4	30265	21258
CL152	7248	6370	986	705	0.4	0.4	10.7	7.7	40278	40359
CL163	5625	6200	551	908	0.4	0.5	6.2	10.6	36794	34950
CL172	5825	5977	815	730	0.5	0.4	9.5	8.0	34546	27188
CL271	6993	5055	1088	791	0.5	0.5	13.6	10.1	31395	25237
Cocodrie	5630	6399	787	938	0.4	0.5	9.0	10.9	33545	33564
NIPONBARE	6139	5131	853	675	0.4	0.4	9.6	7.1	33959	44868
ANTONIO	6909	4947	957	679	0.4	0.4	10.8	7.6	39350	41717
El Paso 144	7290	6259	804	976	0.4	0.5	9.3	12.2	31847	26864
GSOR100390	7023	6137	797	765	0.5	0.4	9.9	9.0	27327	32512
GSOR100417	5685	5339	903	813	0.5	0.5	11.8	10.0	35984	47327
GSOR101758	4777	4925	713	808	0.5	0.5	8.5	10.7	22618	35597
RU1104122	6285	6284	884	932	0.5	0.5	10.1	11.1	35498	31532
CLJZMN	6105	5725	864	897	0.5	0.5	10.1	11.2	44233	31747
INIA Tacuari	6311	6429	524	919	0.4	0.5	5.1	10.5	29196	26821
IRGA409	5803	5716	965	883	0.5	0.5	13.5	11.0	28347	36644
JES	5218	5885	813	868	0.5	0.5	10.5	10.3	26679	30797
JUPITER	6565	6056	1151	829	0.5	0.5	16.4	9.6	37192	27008
LA 2008	5779	3349	659	443	0.4	0.4	8.0	4.7	34126	21804
LA 2134	6026	5683	874	748	0.5	0.4	10.2	6.6	42621	28870
LAKAST	5947	6152	877	902	0.5	0.5	10.3	10.6	26579	27815
MERMENTAU	5727	6331	872	882	0.5	0.4	10.7	10.0	32272	33286
Presidio	5331	5936	483	722	0.3	0.4	4.4	8.6	41565	34805
Rex	6777	6031	969	929	0.5	0.5	11.1	11.5	30130	41999
RoyJ	7352	5408	1138	718	0.5	0.4	14.2	8.1	29153	35800
RU0603075	6705	5345	1338	902	0.6	0.5	21.6	12.6	43374	43138
RU1201024	5778	5946	840	878	0.5	0.5	9.8	10.4	27668	39538
RU1201047	5612	5254	798	780	0.5	0.5	9.0	9.2	26485	26557
RU1201136	5300	4911	702	657	0.4	0.4	7.7	7.1	20912	24851
RU1204156	4371	6099	659	772	0.5	0.4	8.0	10.0	28734	28895
RU1204197	6835	7182	912	1288	0.4	0.4	10.0	11.1	35127	28760
RU1301084	6859	6027	763	873	0.4	0.5	8.5	10.1	31602	41481
RU1301093	4938	5636	729	898	0.5	0.5	9.0	11.5	24344	33795
RU1301102	6664	4683	874	609	0.4	0.4	9.2	6.4	33505	35040
RU1302192	6138	5956	988	884	0.5	0.5	12.7	10.6	28019	32047
RU1303138	7891	6035	1487	955	0.6	0.5	22.7	12.4	37536	45647

Table 5.4 (Continued)

RU1303181	5544	5149	748	839	0.4	0.5	8.1	11.0	31829	21817
RU1304114	6105	5286	849	787	0.4	0.5	9.5	9.5	30465	23699
RU1304122	7569	5963	1120	983	0.5	0.5	13.5	13.5	34389	29399
RU1304154	6304	5861	985	936	0.5	0.5	12.5	11.9	36738	28035
RU1304156	6647	5841	799	906	0.4	0.4	8.7	11.4	35151	36203
RU1305001	7482	6568	1149	845	0.5	0.4	14.1	9.6	29797	43435
RU1401067	5126	5153	675	835	0.4	0.5	7.6	10.9	28948	27572
RU1401070	6013	5156	808	669	0.4	0.4	8.7	7.0	32415	37780
RU1401090	5659	4381	794	623	0.4	0.4	8.9	7.1	30363	32690
RU1401099	5979	5559	880	856	0.5	0.5	10.4	10.7	25809	31867
RU1401102	6311	6228	868	800	0.4	0.4	9.5	11.0	32882	33409
RU1401145	4694	5562	586	870	0.4	0.5	7.0	10.9	22772	40820
RU1401161	5757	5835	772	804	0.4	0.4	8.2	8.8	30581	34938
RU1401164	6742	4414	1127	622	0.5	0.4	15.4	8.4	31391	28624
RU1402005	5571	6507	801	766	0.5	0.4	9.4	10.5	31656	29392
RU1402031	6530	5708	915	625	0.5	0.4	10.3	8.4	34059	40013
RU1402065	6006	4766	953	733	0.5	0.5	12.0	9.0	38037	23424
RU1402115	6039	5955	967	610	0.5	0.4	12.7	9.4	37939	28999
RU1402131	7605	6811	1085	1045	0.5	0.5	12.4	12.8	35181	35032
RU1402134	6447	5976	899	866	0.4	0.5	10.0	10.1	32752	42093
RU1402149	5188	6016	669	847	0.4	0.4	6.9	9.5	27483	37050
RU1402174	2985	5392	403	673	0.4	0.4	4.5	9.7	16928	28757
RU1402189	5468	5628	728	861	0.4	0.5	7.9	10.5	31059	26915
RU1402195	4854	6330	700	924	0.5	0.5	8.1	10.8	28259	34494
RU1403107	6753	4178	702	585	0.4	0.4	7.3	8.3	38290	26595
RU1403126	6532	4892	1094	721	0.5	0.5	14.6	8.5	22839	28394
RU1404122	4261	5068	632	761	0.5	0.5	7.5	9.4	25497	39265
RU1404154	6218	4803	809	615	0.4	0.4	8.8	6.3	25607	34838
RU1404156	5688	5475	733	753	0.4	0.4	7.6	8.3	27317	35010
RU1404157	4736	4870	659	655	0.4	0.4	7.4	7.5	36871	35874
RU1404191	6098	4079	893	569	0.5	0.4	10.6	8.2	37397	38782
RU1404193	5985	5755	845	919	0.4	0.5	9.5	11.7	42759	29338
RU1404194	6166	4971	613	663	0.3	0.4	5.5	7.1	35612	21290
RU1404196	3308	4707	399	649	0.4	0.4	3.9	7.1	29477	24809
RU1404198	5509	4925	564	755	0.3	0.5	5.3	9.2	34761	31803
RU1504083	5902	3396	764	482	0.4	0.4	7.9	5.3	30714	24462
RU1504100	6262	6211	989	892	0.5	0.5	12.4	10.3	34431	25518
RU1504114	6082	4553	873	620	0.5	0.4	10.0	5.6	34966	32121
RU1504122	6243	5896	879	823	0.4	0.4	10.1	9.3	34979	31523
RU1504154	6579	5875	948	869	0.5	0.5	11.1	10.3	33640	31169
RU1504156	4803	5533	697	801	0.4	0.5	8.3	9.2	30333	31892
RU1504157	6459	5285	924	646	0.5	0.4	10.6	7.3	28373	28039
RU1504186	5652	6971	762	977	0.4	0.4	8.2	11.0	28881	33596
RU1504191	5868	5866	864	819	0.5	0.4	10.1	9.1	34134	36980
RU1504193	5459	6335	794	986	0.5	0.5	9.7	12.3	24541	25225
RU1504194	4917	3718	622	446	0.4	0.4	6.3	4.3	28345	34680
RU1504196	6045	6297	881	857	0.5	0.4	10.3	9.3	29576	28129
RU1504197	6304	5575	888	877	0.5	0.5	10.2	11.1	28418	27006
RU1504198	5509	4871	782	744	0.5	0.4	8.9	7.6	29937	22923
Sabine	5556	5214	736	778	0.4	0.5	8.0	9.3	30795	27502
Taggart	5669	5644	816	875	0.5	0.5	9.5	10.9	30078	30770
Thad	6342	5243	864	757	0.4	0.5	9.5	8.8	29780	28841
N-22	5355	7455	830	1172	0.5	0.5	10.5	14.7	25729	31417
MEAN	6009	5498	845	788	0.5	0.5	10.0	9.4	32059	31884
Genotypes	*	***	**	*	*	NS	***	**	*	NS
Gen. X Treatment	*		NS		NS		*		NS	

Drastic reduction in RN was observed under drought conditions where it ranged between 20.00 and 50, with an average RN of 32. Maximum RN under drought was found in the genotype N-22 and minimum RN was in RU1401090. Plant roots optimize their architecture to acquire water and essential nutrients. Under drought conditions, the number of forks and crossings differed significantly among the rice genotypes, with genotype RU1204197 having maximum NRF of 89898 and the genotype RU1402005 with minimum NRF of 13078 and average NRF of 63434 under drought conditions. Significant reduction was observed in NRC between the control (7076) and drought treatment (4603). There was no significant effect of drought on NRT, with the genotype GSOR100417 having maximum NRT of 47327 and genotype Cheniere with minimum NRT of 18491 under drought conditions, as compared to the average NRT of 32059 under control conditions (Table 5.5). Lateral roots are responsible for the larger quantities of water and nutrient absorption (Yoshida and Hasegawa, 1982) because they account for approximately 77% of the surface area of the root system in any crop (Parker et al, 2000). The ARD of 0.50 mm was found to be similar under both control and drought stress treatments, with the genotype RU1401067 having the maximum ARD of 0.52 mm. This finding suggests that the comparable values of ARD under stress is probably because of enhanced cell elongation that provides drought resistance because of enhanced penetration ability (Clark et al., 2008).

Table 5.5 Drought effects on root parameters viz number of root forks (NRF, no. plant^{-1}), number of root crossings (NRC, no. plant^{-1}), longest root length (LRL, cm plant^{-1}), number of roots (RN, no. plant^{-1}), root dry weight (RDW, g plant^{-1}) root shoot ratio (RSR). Measurements were taken at harvesting time, 28 days after sowing.

Genotype name	NRF		NRC		LRL		RN		RDW		R/S	
	C	D	C	D	C	D	C	D	C	D	C	D
14CLPYT033	68558	45785	4712	2494	40	46	54	22	0.7	0.3	0.2	0.1
14CLPYT108	59218	62470	4343	4027	46	47	46	33	0.6	0.6	0.2	0.4
14CVPYT094	72833	73910	6262	5039	47	47	47	34	0.9	0.6	0.2	0.4
14CVPYT144	75391	37629	7105	2703	44	46	49	30	0.7	0.5	0.2	0.3
COLORADO	30764	84610	10209	5917	39	48	73	29	0.6	0.5	0.2	0.3
Bowman	79284	67015	10186	4874	52	46	43	42	0.9	0.6	0.2	0.3
CAFFEY	82116	72517	9745	5307	49	44	52	32	0.7	0.6	0.2	0.4
CHENIERE	90928	28846	8995	2342	46	43	52	27	0.7	0.3	0.2	0.2
CL Jazzman	98635	77855	8460	5338	51	50	69	35	1.7	0.8	0.2	0.4
CL111	73663	63238	8263	4118	48	50	53	27	0.7	0.7	0.2	0.4
CL142-AR	70911	61635	5763	4028	53	50	43	29	0.8	0.7	0.3	0.4
CL151	69514	69820	8127	4850	49	43	47	27	0.7	0.7	0.2	0.5
CL152	94284	70310	9055	5349	47	43	53	42	0.8	0.8	0.2	0.5
CL163	43964	68106	5408	4704	47	55	46	27	0.6	0.8	0.2	0.4
CL172	81129	54776	8348	4838	44	52	51	28	0.6	0.6	0.2	0.4
CL271	93078	58762	9236	4209	50	49	73	35	0.8	0.7	0.2	0.4
Cocodrie	66235	79882	5735	5395	51	49	47	29	0.5	0.8	0.1	0.5
NIPONBARE	69209	58252	5655	3895	42	43	65	34	0.8	0.4	0.2	0.3
ANTONIO	86597	49798	8472	3453	46	47	54	35	0.7	0.6	0.2	0.4
El Paso 144	101535	85439	8972	6223	51	50	55	32	1.0	0.8	0.2	0.3
GSOR100390	78000	76030	6088	5665	49	50	51	39	0.6	0.8	0.2	0.4
GSOR100417	81110	60166	6344	3841	40	50	55	43	0.7	0.7	0.2	0.5
GSOR101758	68255	65031	4176	3782	43	43	49	34	0.6	0.7	0.2	0.5
RU1104122	69487	72876	8219	5154	47	46	61	32	0.7	0.8	0.2	0.4
CLJZMN	75392	72771	8243	4844	48	47	55	39	0.7	0.8	0.2	0.4
INIA Tacuari	52647	79214	8097	5822	48	53	45	26	0.6	0.7	0.2	0.3
IRGA409	86001	73409	6551	4779	54	54	48	33	0.9	0.7	0.3	0.3
JES	62148	71870	4973	4824	51	52	34	23	0.7	0.9	0.2	0.4
JUPITER	114342	67663	7295	5071	56	52	71	30	1.0	0.6	0.2	0.3
LA 2008	50404	31498	5362	2316	48	39	40	26	0.7	0.3	0.2	0.3
LA 2134	85662	74463	7125	5172	43	44	62	26	0.6	0.7	0.2	0.3
LAKAST	62119	70781	5407	5155	48	49	49	32	0.8	0.7	0.2	0.4
MERMENTAU	65393	79066	4950	6088	41	52	63	30	0.8	0.7	0.2	0.4
Presidio	69114	54080	5015	4186	46	46	43	23	0.5	0.8	0.2	0.4
Rex	64889	68233	9429	4819	51	51	59	44	0.8	0.8	0.2	0.4
RoyJ	74966	58404	8932	3963	47	46	72	28	1.0	0.6	0.2	0.3
RU0603075	95602	80475	8449	4884	47	44	66	37	1.0	0.6	0.2	0.3
RU1201024	69135	66852	5743	4393	46	47	48	36	0.8	0.7	0.2	0.4
RU1201047	59965	57973	5052	4223	49	54	38	30	0.7	0.6	0.2	0.4
RU1201136	46063	49444	4512	3623	49	50	46	25	0.7	0.6	0.2	0.4
RU1204156	54823	56391	6796	5173	42	43	56	31	0.6	0.8	0.2	0.4
RU1204197	88884	89898	9329	8555	50	51	51	43	0.8	0.8	0.2	0.4
RU1301084	71340	63121	8167	4217	53	50	62	38	1.0	0.7	0.2	0.5
RU1301093	45382	61397	3888	4281	48	47	52	34	0.8	0.7	0.2	0.3
RU1301102	100896	48855	10176	3410	50	43	53	42	0.8	0.5	0.3	0.3
RU1302192	91038	70093	9028	4940	52	50	57	37	0.8	0.7	0.2	0.3
RU1303138	111293	76638	9703	5372	52	43	77	41	1.1	0.7	0.2	0.3
RU1303181	63272	66739	5275	4088	50	47	44	33	0.6	0.7	0.2	0.4
RU1304114	99701	59316	8058	4429	42	48	61	27	0.7	0.7	0.2	0.3
RU1304122	140774	78169	14381	6032	49	49	49	42	1.0	0.8	0.2	0.4
RU1304154	76104	77051	5381	5081	47	49	61	40	0.8	0.8	0.2	0.4
RU1304156	95739	69377	8224	4146	48	47	45	23	0.8	0.7	0.2	0.4
RU1305001	107388	68004	8274	5215	51	44	55	27	0.9	0.8	0.2	0.4
RU1401067	79793	56595	8847	3672	42	46	49	34	0.6	0.8	0.2	0.5
RU1401070	66326	50458	6224	4992	53	51	42	25	0.6	0.6	0.2	0.4
RU1401090	60077	47756	5164	2999	51	50	42	20	0.6	0.5	0.2	0.3

Table 5.5 (Continued)

RU1401099	70521	61887	6358	4059	49	51	42	31	0.8	0.7	0.2	0.3
RU1401102	66861	76018	4955	4621	46	48	59	34	0.7	0.6	0.2	0.2
RU1401145	51459	62671	3113	5076	42	49	48	31	0.7	0.8	0.2	0.4
RU1401161	59192	63311	5431	4631	50	51	44	33	0.7	0.7	0.2	0.4
RU1401164	98540	53231	7567	3760	48	41	59	31	0.8	0.6	0.2	0.3
RU1402005	63647	13078	6420	7374	43	43	54	29	0.8	0.7	0.2	0.3
RU1402031	72977	54008	8420	5312	51	40	43	32	0.7	0.6	0.2	0.3
RU1402065	92428	53332	7916	3621	51	43	63	32	0.8	0.6	0.2	0.3
RU1402115	81259	73678	6956	5675	48	39	64	31	0.8	0.7	0.2	0.4
RU1402131	113164	87864	10315	5920	50	49	57	25	0.8	0.9	0.2	0.4
RU1402134	78096	68525	7086	4608	43	44	55	29	0.7	0.7	0.2	0.4
RU1402149	58986	70881	5233	4795	47	50	43	24	0.6	0.7	0.2	0.4
RU1402174	36099	73206	2805	3720	47	49	49	27	0.4	0.6	0.2	0.4
RU1402189	65699	67998	6983	4357	47	47	43	33	0.6	0.7	0.2	0.3
RU1402195	34795	71442	8239	5585	45	49	51	43	0.5	0.7	0.1	0.3
RU1403107	76407	41575	10347	2954	43	47	55	36	0.7	0.5	0.2	0.3
RU1403126	83546	59875	6285	4088	55	46	77	36	0.9	0.6	0.2	0.3
RU1404122	58721	53259	5383	3409	42	48	38	28	0.7	0.6	0.3	0.5
RU1404154	70793	48565	6922	3877	45	50	38	26	0.8	0.4	0.2	0.3
RU1404156	56081	65313	5132	4618	45	43	54	26	0.6	0.6	0.2	0.3
RU1404157	50252	77871	4146	4530	47	38	49	28	0.5	0.7	0.2	0.4
RU1404191	85908	60946	6782	4480	46	41	54	23	0.8	0.6	0.2	0.4
RU1404193	68448	67882	5467	4301	48	51	49	38	0.7	0.7	0.2	0.4
RU1404194	83833	52347	10919	4422	53	54	37	32	0.6	0.5	0.2	0.3
RU1404196	60368	45763	6856	5190	41	47	37	30	0.5	0.5	0.3	0.6
RU1404198	29442	56006	9739	3614	52	47	63	35	0.9	0.7	0.3	0.4
RU1504083	68840	48212	6909	3759	49	37	43	28	0.8	0.5	0.2	0.3
RU1504100	76401	70149	6043	5547	49	54	59	29	0.9	0.8	0.2	0.5
RU1504114	74629	50749	6243	3879	46	48	56	21	0.7	0.5	0.2	0.3
RU1504122	98946	70813	11697	5134	45	50	60	31	0.8	0.6	0.2	0.3
RU1504154	88534	66421	8464	4632	47	49	52	30	1.0	0.7	0.3	0.4
RU1504156	50237	59414	4225	3982	43	47	62	35	0.6	0.7	0.2	0.3
RU1504157	77871	47382	7160	3411	49	44	47	30	0.8	0.5	0.2	0.3
RU1504186	68582	82925	6054	6081	51	57	58	49	0.6	0.8	0.2	0.4
RU1504191	73074	67301	8770	4844	45	47	39	34	0.7	0.7	0.2	0.4
RU1504193	54019	81137	4646	6057	52	50	50	32	0.7	0.8	0.2	0.5
RU1504194	58100	34251	6044	2685	46	42	57	25	0.6	0.3	0.2	0.2
RU1504196	70747	65800	6327	5418	49	48	49	28	0.7	0.7	0.2	0.4
RU1504197	76783	66529	7789	4434	52	52	48	29	0.7	0.8	0.2	0.4
RU1504198	57681	47584	5226	3844	52	51	40	28	0.7	0.6	0.2	0.4
Sabine	63439	59691	6158	4136	47	49	52	35	0.6	0.7	0.2	0.3
Taggart	56582	64907	4987	4294	50	49	60	34	0.7	0.7	0.2	0.4
Thad	51585	58503	7119	4302	48	50	53	33	0.7	0.6	0.2	0.4
N-22	53175	80254	8077	6923	46	51	46	50	0.7	1.0	0.2	0.4
MEAN	72781	63434	7076	4603	48	48	52	32	0.7	0.7	0.2	0.4
Genotypes	***	***	***	**	***	NS	*	*	***	***	NS	**
Genotypes X Treatment	***		***		NS		NS		*		*	

Generally, if root length exceeds a certain size, the branching process starts by initiation, emergence, and growth of lateral roots from the root pericycle and epidermis (Abe and Morita 1994). In soils which are water stressed, there is reduced oxygen supply, with a physical barrier like hardpans, and poor adaptation of roots to aerobic condition.

These in turn limit exploitation of deeper soil layers hence reducing root length and biomass production (Samson and Wade, 1998). Drastic reduction in RN was observed under drought conditions. Plant roots optimize their architecture to acquire water and essential nutrients. The number of root tips, forks, and crossings play an important role in root architecture because they have potential to enhance penetration through soil layers, resulting in a positive effect on plant nutrient uptake. Under drought conditions, unlike NRT the number of forks and crossings differed significantly among the rice genotypes.

Physiological Traits

Many physiological factors may be involved in drought stress injury as drought stress both damages the photosynthetic apparatus and diminishes chlorophyll content (Fu and Huang 2001). The multiplicity of factors involved in drought stress injury suggests that screening studies of many kinds may be useful for characterizing drought resistance (Hura et al, 2007). In the present study, significant differences were observed among all genotypes under control and drought condition though with narrow range (Table 5.6). Under drought conditions, the maximum SPAD was observed for genotype RU1301084 (45.73) and the minimum in genotype Niponbare (32.83), with an average value of 41.00, which is higher than the control average of 38.78. F_v/F_m ratio under drought conditions averaged at 0.692 with maximum value in genotype CL271 and minimum in RU1504100, with 73 per cent of the genotypes falling below the average values. Individually, F_v and F_m results under both control and drought conditions were significant for cultivars as well as for their genotype X drought interactions.

Table 5.6 Drought effects on physiological parameters measured viz chlorophyll content (SPAD), minimal fluorescence intensity ($F_{o)}$, maximal fluorescence intensity ($F_{m)}$, maximal variable fluorescence ($F_{v)}$ and fluorescence (Fv/Fm). Measurements were taken at 28 days after sowing.

Genotype name	SPAD		Fo		Fm		Fv		Fv/Fm	
	C	D	C	D	C	D	C	D	C	D
14CLPYT033	36.07	39.90	9444.67	8506.67	30891.67	26296.67	22113.67	17790.00	0.72	0.67
14CLPYT108	38.07	41.30	11037.67	7252.33	29563.33	22730.33	19192.33	15478.00	0.64	0.68
14CVPYT094	38.13	41.57	11183.33	10371.33	34999.00	28783.00	20482.33	18411.67	0.58	0.64
14CVPYT144	39.60	39.77	11411.33	9124.33	34602.67	26246.67	23191.33	17122.33	0.67	0.65
COLORADO	36.77	39.10	10695.67	8940.33	31297.00	24459.00	20601.33	15518.67	0.66	0.63
Bowman	42.67	40.93	8897.33	8604.33	28263.00	26799.67	19365.67	18195.33	0.68	0.68
CAFFEY	38.27	41.77	9710.00	7537.33	30447.67	19032.67	17404.33	14828.67	0.57	0.86
CHENIERE	35.10	38.50	9753.33	6853.00	30733.67	16651.67	20980.33	13132.00	0.68	0.89
CL Jazzman	43.73	37.87	11931.33	6204.33	33193.67	15533.67	24595.67	9329.33	0.76	0.60
CL111	37.03	41.97	10772.00	7122.00	33030.67	24070.33	22258.67	16948.33	0.67	0.70
CL142-AR	39.47	43.40	8788.67	8311.67	29845.00	26279.33	21056.33	17967.67	0.71	0.68
CL151	37.33	33.90	11368.00	9384.67	36282.00	25933.00	24914.00	16548.33	0.69	0.64
CL152	33.90	37.87	10435.67	7987.00	33969.33	24686.67	20200.33	16699.67	0.58	0.67
CL163	37.17	43.47	11107.67	10100.00	36119.67	30278.33	25012.00	20178.33	0.69	0.66
CL172	38.63	42.13	9374.00	8431.00	34257.67	26702.67	24883.67	16938.33	0.73	0.64
CL271	40.00	39.80	11866.33	7477.33	37387.33	21593.33	25521.00	20782.67	0.68	1.03
Cocodrie	42.37	40.43	11525.00	8496.00	34125.67	21587.00	23267.33	13091.00	0.68	0.60
NIPONBARE	38.37	32.83	10197.67	9276.33	33768.00	24871.00	23570.33	15594.67	0.70	0.63
ANTONIO	36.97	40.83	9926.67	8851.67	30389.00	24403.00	17129.00	17218.00	0.57	0.71
El Paso 144	35.73	38.40	11292.33	8550.67	33183.33	27992.33	21891.00	19441.67	0.66	0.69
GSOR100390	34.60	40.80	9341.67	9135.33	31275.67	25601.67	21934.00	21133.00	0.70	0.84
GSOR100417	33.97	42.20	9753.33	8409.67	28410.33	25413.33	21990.33	17003.67	0.77	0.67
GSOR101758	36.33	40.10	9156.67	5232.67	22605.00	20544.33	15781.67	16311.67	0.71	0.88
RU1104122	36.07	41.23	11757.67	7738.00	35155.33	20666.67	23397.67	16262.00	0.66	0.84
CLJZMN	35.87	41.07	9493.67	8875.33	30408.67	30545.00	20915.00	18336.33	0.69	0.59
INIA Tacuari	38.33	42.60	10425.00	8358.00	34949.33	22031.67	24524.33	16673.67	0.70	0.82
IRGA409	42.57	42.43	11845.00	7429.33	36293.00	22813.33	24448.00	16717.33	0.67	0.75
JES	38.60	40.67	10999.33	10522.67	32727.33	29910.00	25061.33	19387.33	0.80	0.65
JUPITER	42.87	43.40	10241.00	7005.00	31893.33	19813.67	21652.33	16142.00	0.68	0.87
LA 2008	42.60	40.10	10013.33	9406.67	31329.67	25109.33	21316.33	15702.67	0.68	0.62
LA 2134	37.37	39.23	11297.00	9352.00	37463.00	28458.33	25499.33	19106.33	0.68	0.67
LAKAST	39.70	43.80	9265.33	8257.67	20345.33	23939.00	8746.67	15681.33	0.44	0.65
MERMENTAU	35.77	37.43	10966.33	7801.00	31784.67	25626.67	20818.33	12533.00	0.64	0.49
Presidio	35.60	38.60	12224.67	7363.67	34088.67	20955.67	26530.67	16270.12	0.81	0.87
Rex	39.27	41.30	10208.33	9991.67	35350.33	27005.67	28475.33	17014.00	0.83	0.63
RoyJ	40.47	40.53	9839.67	9623.33	31286.33	27667.00	18113.33	18043.67	0.60	0.65
RU0603075	36.07	41.37	10284.00	8322.67	39138.33	26698.00	28854.33	15042.00	0.74	0.55
RU1201024	41.30	45.10	10252.00	9200.33	29076.00	26626.33	20824.00	17426.00	0.73	0.65
RU1201047	35.80	43.63	9720.33	8127.67	33215.00	23895.33	23494.67	15767.67	0.71	0.65
RU1201136	37.67	42.83	10685.00	8358.00	34440.00	26224.00	23755.00	19199.33	0.69	0.73
RU1204156	35.43	34.27	11769.00	9211.33	40671.33	25694.33	28902.33	16483.00	0.70	0.64
RU1204197	38.37	39.73	9915.67	8452.67	32424.33	25326.00	22508.67	16873.33	0.69	0.67
RU1301084	40.30	45.73	10273.00	9547.00	33069.67	28431.33	21796.67	18884.33	0.66	0.66
RU1301093	43.10	45.40	9937.33	8853.33	34370.33	28652.67	22433.00	19799.33	0.65	0.69
RU1301102	39.00	43.57	9775.00	8258.00	31849.67	23798.00	22074.67	15540.00	0.69	0.65
RU1302192	40.43	43.33	11097.00	9563.00	30516.33	28989.67	22419.33	17093.33	0.77	0.59
RU1303138	33.43	39.60	9518.67	9075.33	28328.67	26247.67	20797.00	16172.33	0.75	0.61
RU1303181	39.47	42.07	9262.67	7380.00	30278.33	19202.67	20015.67	9822.67	0.66	0.53
RU1304114	38.50	38.33	10089.33	9482.33	33998.00	26648.33	23908.67	17166.00	0.70	0.64
RU1304122	39.70	42.10	11075.33	9015.67	33937.00	31404.33	22861.67	20722.00	0.68	0.66
RU1304154	41.03	39.27	9634.00	7362.33	28653.00	21923.33	22352.33	17894.33	0.81	0.89
RU1304156	38.13	43.83	9233.00	8670.00	31611.67	25272.33	22378.67	19935.67	0.71	0.86
RU1305001	41.07	42.97	9493.00	10013.00	32033.67	29075.33	22540.67	19062.33	0.70	0.65
RU1401067	41.60	41.70	9850.67	7741.00	33237.00	24697.67	23386.33	16956.67	0.70	0.68
RU1401070	41.50	44.23	10067.33	7900.00	35794.00	19712.33	25726.67	15145.67	0.72	0.80
RU1401090	39.03	39.23	11801.67	8175.00	37127.33	15093.67	25325.67	14884.00	0.68	0.98

Table 5.6 (Continued)

RU1401099	40.30	41.97	10490.33	9233.00	38309.00	28793.67	27818.67	19560.67	0.72	0.68
RU1401102	36.67	42.13	11650.00	8647.67	36303.67	18618.00	24653.67	9970.33	0.68	0.53
RU1401145	40.17	45.23	10100.00	9417.33	33264.67	22530.00	23164.67	16446.00	0.70	0.78
RU1401161	38.47	36.43	9645.00	8212.67	28035.33	19834.67	21723.67	15036.00	0.79	0.84
RU1401164	37.60	42.63	11043.00	8528.67	33508.33	23625.00	22465.33	18429.67	0.67	0.83
RU1402005	42.13	40.87	9365.33	6803.33	29043.33	25465.00	18011.33	15585.67	0.62	0.61
RU1402031	38.87	41.10	11758.00	9504.00	36975.67	27775.33	25217.67	18271.33	0.68	0.66
RU1402065	40.00	41.83	11501.00	8051.67	33919.67	24870.67	23418.67	16819.00	0.69	0.66
RU1402115	41.43	39.07	9514.67	8821.00	31654.67	24469.67	22140.00	15648.67	0.70	0.62
RU1402131	38.97	40.37	10804.33	8409.33	36488.00	21728.00	25683.67	13318.67	0.70	0.61
RU1402134	34.93	41.53	11671.67	8398.33	33898.33	20275.67	22226.67	15210.67	0.65	0.78
RU1402149	37.13	42.07	11086.00	8236.00	33561.67	26030.33	22475.67	17794.33	0.67	0.68
RU1402174	33.67	40.30	8575.33	9178.67	21279.00	23603.00	20370.33	17540.33	1.04	0.78
RU1402189	36.93	41.93	11107.67	7102.67	34927.33	18520.33	23819.67	11417.67	0.68	0.62
RU1402195	40.33	41.03	9774.67	8214.33	32803.33	23169.33	23028.67	14955.00	0.70	0.64
RU1403107	39.03	40.10	11844.67	9005.33	36542.00	25304.33	24697.33	16299.00	0.68	0.64
RU1403126	38.47	41.73	10912.67	7806.33	36997.33	15802.00	26084.67	9995.67	0.70	0.66
RU1404122	43.23	43.73	11227.00	9255.00	37669.33	26529.33	26442.33	17274.33	0.70	0.65
RU1404154	39.53	38.87	11390.33	7705.00	36978.33	20222.00	24254.67	15850.33	0.66	0.84
RU1404156	39.27	37.97	11498.00	9081.33	37691.00	27591.00	26193.00	18509.67	0.69	0.67
RU1404157	35.60	35.10	12570.67	5837.00	35978.67	25118.00	23408.00	12947.67	0.64	0.51
RU1404191	40.17	44.13	10663.33	7197.33	31975.33	27999.00	24645.33	17468.33	0.79	0.62
RU1404193	36.60	40.97	8471.00	8485.33	28287.00	22400.00	20149.33	13914.67	0.71	0.62
RU1404194	45.90	43.03	9498.67	9395.33	24946.67	26778.00	16114.67	17382.67	0.64	0.64
RU1404196	33.73	37.57	9937.00	8626.00	27655.67	25770.33	21052.00	17144.33	0.82	0.66
RU1404198	37.07	38.93	10056.67	9038.00	31260.00	22758.00	21203.33	17053.33	0.68	0.84
RU1504083	39.23	38.97	9905.00	8127.67	33893.67	23483.33	23988.67	15355.67	0.70	0.66
RU1504100	43.00	41.20	9449.67	7575.00	31975.67	19528.33	15859.33	7953.33	0.48	0.45
RU1504114	40.03	38.03	12581.33	8843.33	37268.00	27580.33	24686.67	18737.00	0.66	0.68
RU1504122	38.10	42.03	11482.00	8864.67	29606.67	26041.33	18791.33	17176.67	0.63	0.65
RU1504154	38.13	41.27	10295.00	9244.00	31076.33	26561.33	20781.33	17317.33	0.67	0.65
RU1504156	36.53	43.90	10284.00	7197.67	35047.00	21252.67	24763.00	17388.33	0.71	0.92
RU1504157	36.10	40.87	10633.00	6818.33	37961.67	18322.67	26594.00	9837.67	0.70	0.56
RU1504186	39.60	41.80	10230.00	7477.33	32955.00	21121.00	22725.00	13643.67	0.69	0.64
RU1504191	38.30	42.20	10977.67	7726.67	34039.00	21023.67	26394.67	16630.33	0.79	0.85
RU1504193	40.50	39.80	9883.33	6851.33	28967.33	17723.67	22417.33	10872.33	0.79	0.62
RU1504194	39.80	41.57	9102.67	6949.67	27385.00	22213.33	18282.33	18597.00	0.66	0.91
RU1504196	38.63	44.13	10858.67	7868.00	37908.00	23705.00	27049.33	19170.33	0.71	0.88
RU1504197	42.93	43.10	11703.67	7781.00	39511.33	19441.67	27807.67	11660.67	0.70	0.60
RU1504198	42.23	41.67	10501.33	7367.00	32034.33	18059.00	21533.00	10692.00	0.66	0.59
Sabine	43.63	41.20	10869.33	8897.00	30690.33	28609.67	19821.00	19712.67	0.64	0.69
Taggart	41.30	42.77	10121.67	8138.67	31687.33	24827.67	21565.67	16689.00	0.68	0.67
Thad	39.80	43.47	11454.67	9644.67	38156.67	31308.00	26702.00	21663.33	0.70	0.69
N-22	37.67	42.30	10057.00	7748.33	29190.33	23125.67	17133.33	15377.33	0.58	0.66
Mean	38.78	41.00	10478.28	8359.04	32847.92	24141.54	22605.50	16317.21	0.69	0.69
Genotypes	***	**	***	NS	***	*	***	***	*	NS
Gen. X Treatment	NS		*		***		***		NS	

Decline in SPAD values is a progressive phenomenon under drought conditions as chlorophyll degradation is one of the consequences of drought stress that may result from sustained photo inhibition and photo-bleaching (Long et al., 1994).

Even though other plant processes, such as cell division and cell expansion are the earliest to respond to water deficit stress, a decline in SPAD index is a sensitive and readily measurable trait that could be used to screen for stress tolerance (O'Neill et al, 2006). Chlorophyll fluorescence measurements of Fv/Fm represent the maximum photochemical efficiency of PSII and indicate that the effect of drought stress in the fluorescence parameter Fv/Fm, which is a measure of accumulated photooxidative damage to PSII (Ambavaram et al, 2014). For F_v/F_m 73 percent of the genotypes fell below the average value under drought conditions. Drought stress generally results in decreased F_v/F_m, which will be mainly expressed by drought-susceptible genotypes. Stress-induced reduction in F_v/F_m is indicative of photoinhibition associated with an over-reduction of PSII (Maxwell and Johanson, 2000). The ability to maintain high F_v/F_m under drought stress thus indicates a high efficiency of radiation use possibly for photochemistry and carbon assimilation. Colom and Vazzana (2003) reported similar correlations between F_v/F_m and drought tolerance in *Erasgrostis curvula* cultivars, with high F_v/F_m values being associated with drought tolerance and low F_v/F_m values being associated with susceptibility to drought stress. Individually, F_v and F_m results under both control and drought conditions were significant for cultivars as well as for their genotype X drought interactions.

Root traits using image analysis

Roots play a vital role in withstanding drought stress, as the primary defense mechanism involved in strengthening acquisition of more water from soil, which purely depends on root architectural plasticity. In the present study, a scanner-based image

analysis was used to unravel root architecture of rice genotypes. The visual appearance of root scan results revealed correspondence of early vigor parameters with the root scan images among the respective response group genotypes where in CDRI values were effective in classifying the genotypes based on the overall responses (Figure 5.3).

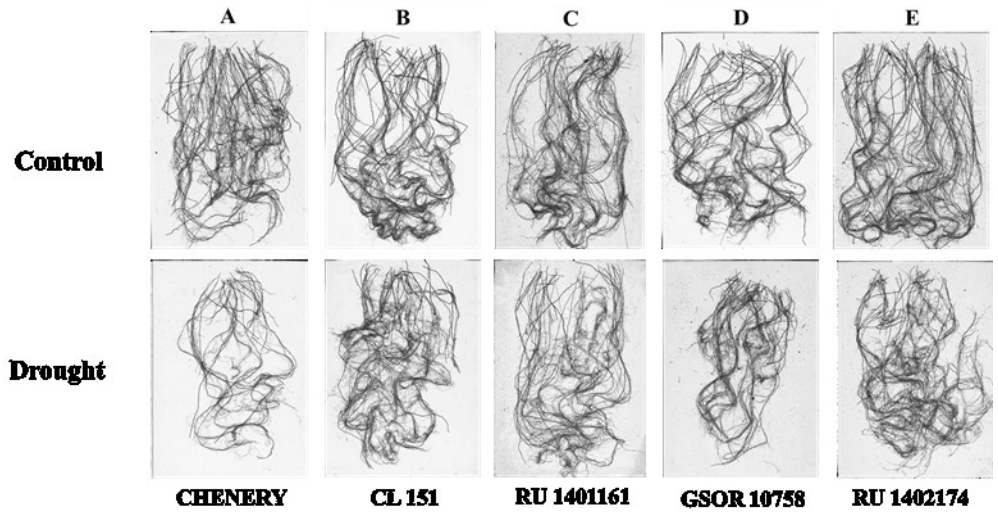

Figure 5.3 Representative scanned root images from control and drought sets in each drought stress response class: A) Highly sensitive; B) Sensitive; C) Moderately sensitive; D) Tolerant; and E) Highly tolerant.

The genotype (GSOR 10758) and rice breeding line (RU 1402174) contributed a strong, well-structured root system, and higher abundant root hairs while genotypes (CHENERY, CL 151) and rice breeding line RU 1401161 exhibited a less-structured root system with reduced RN and RNL at the drought treatment. Comparatively, all rice breeding lines and genotypes designated as drought tolerant in this study had larger, more robust and branched root systems with higher values for root parameters, whereas

drought sensitive rice breeding line and genotypes showed less organized root structures with low values for root parameters.

The makeup of plant root morphological parameters and above ground shoot development will reflect on the overall performance of a plant. Improving the understanding of the interaction between root function and drought in rice could have a significant impact on global food security (Gowda et al., 2011). Therefore, improving the root system with deep root and high-water uptake ability would be the key to developing elite rice varieties suitable for water-limited and/or water use-efficient farming systems.

Classification of rice genotypes based on drought response

Present systems for classifying genotypes for drought tolerance include filed based rating scales such as those in the Standard Evaluation System (IRRI, 2014). The identified tolerant genotypes in this study could withstand early season drought stress under dry direct seeding practice. Moreover, farmers could use the identified tolerant genotypes to manipulate flushing practice following planting. Using mini-hoop structures in combination with developmental, root, and physiological traits, this study effectively developed a scoring system for early season drought tolerance in rice. Using mini-hoop structures in combination with developmental, root, and physiological traits, this study effectively developed a scoring system for early season drought tolerance in rice. Results indicated that by using two indices (CDSRI and IDSRI), all rice genotypes could be classified into different groups viz highly sensitive, sensitive, moderately sensitive, tolerant, and highly tolerant to drought based on their cumulative

response for all shoot and root parameters. The CDSRI values ranged from 14.70 (highly sensitive) for the genotype Cheniere to 27.96 (highly tolerant) for the genotype RU1402174. Based on the resilience to drought, six genotypes were classified as highly drought tolerant and five genotypes were grouped in the highly sensitive class, with the majority of genotypes (45) falling under moderate response class (Table 5.7). The rice breeding lines (RU1402195, RU1401145, and RU1402174) and genotypes (INIA Tacuari, CL163, and N-22) were identified to be the most drought tolerant genotypes (Table 5.7). Thus, they may be used along with other water saving strategies to improve crop yields in commercial rice production. Singh et al (2017); Massey et al (2014) have also determined similar or higher yields for (RU1104122, CL151, CL142-AR, and CL111) when grown under intermittent flooding as compared to continuous flooding. The high and very high drought tolerant rice breeding lines and genotypes in this study may have inherent tolerance under variable drought levels which could let them fit well with different water saving strategies.

Table 5.7 Classification of rice genotypes into five drought response groups based on the cumulative drought stress response index (CDSRI), along with individual scores in parenthesis.

Highly sensitive (14.701-16.989)	**Sensitive (16.990- 19.278)**	**Moderate (19.279- 21.567)**	**Tolerant (21.568-23.855)**	**Highly tolerant (23.856 <)**
CHENIERE (14.701), CL Jazzman (15.397), LA 2008 (16.012), RU1504157 (16.662), RU1403126 (16.731)	JUPITER(17.001), RU0603075 (17.178), RU1504114 (17.181), 14CVPYT144 (17.251), RU1401164 (17.273), RU1402065 (17.573), RU1303138 (17.679), RU1404191 (17.690), RU1504194 (17.730), 14CLPYT033 (17.836), RoyJ (17.854), NIPONBARE (17.980), CL271 (18.054), RU1403107 (18.103), RU1504083 (18.106), RU1404154 (18.157), RU1401090 (18.210), RU1504198 (18.228), RU1402115 (18.390), RU1301102 (18.427), RU1402031 (18.771), ANTONIO (18.865), RU1304114 (19.046), Bowman (19.062), IRGA409 (19.079), 14CVPYT094 (19.100), RU1504100 (19.195), CL151 (19.269),	RU1305001 (19.293), CL152 (19.314), LA 2134 (19.319) RU1302192 (19.425), RU1304122 (19.431), RU1504122 (19.434), RU1504154 (19.549), CL172 (19.573), RU1504197 (19.671), CL111 (19.742), RU1504191 (19.748), RU1401070 (19.829), RU1402005 (19.884), CAFFEY (19.909), RU1402131 (19.930), El Paso 144 (20.026), RU1504196 (20.181), Thad (20.205), CL142-AR(20.211), RU1401099 (20.293), RU1201136 (20.310), RU1304156 (20.319), RU1401102 (20.347), RU1404156 (20.365), RU1402134 (20.399), RU1301084 (20.427), RU1404194 (20.495), RU1304154 (20.685), GSOR100417 (20.714), Rex (20.791), RU1201047 (20.863), MERMENTAU (20.868), Taggart (21.050), COLORADO (21.147), RU1303181 (21.153), RU1104122 (21.216), Sabine (21.266), RU1204156 (21.314), RU1404193 (21.395), RU1402189 (21.422), 14CLPYT108 (21.427), CLJZMN (21.457), RU1404122 (21.518), RU1201024 (21.526), RU1401161 (21.557)	RU1504193 (21.831), RU1401067 (21.884), RU1504156 (21.910), RU1404157 (21.978), RU1404198 (21.990), JES (22.160), GSOR100390 (22.307), RU1204197 (22.381), RU1301093 (22.639), RU1504186 (22.864), Cocodrie (22.998), RU1402149 (23.074), LAKAST (23.076), Presidio (23.225), GSOR101758 (23.472), RU1404196 (23.763),	INIA Tacuari (23.989), RU1402195 (24.527), CL163 (24.709), RU1401145 (25.376), N-22 (25.638), RU1402174 (27.955),

The correlation coefficient (R^2) between the combined root drought response index and cumulative drought stress response index using CDSRI for drought tolerance is positively correlated ($R^2 = 0.91$ for root and $R^2 = 0.48$ for shoot at P = 0.0001, n = 100) This implies that greater importance of root parameters than shoot parameters in identifying drought tolerant rice lines using these indices. The plot of shoot drought response index relative to the root drought stress response index also revealed positive correlation coefficient ($R^2 = 0.45$, p = 0.0001, n = 100) (Figure 5.4 and 5.5).

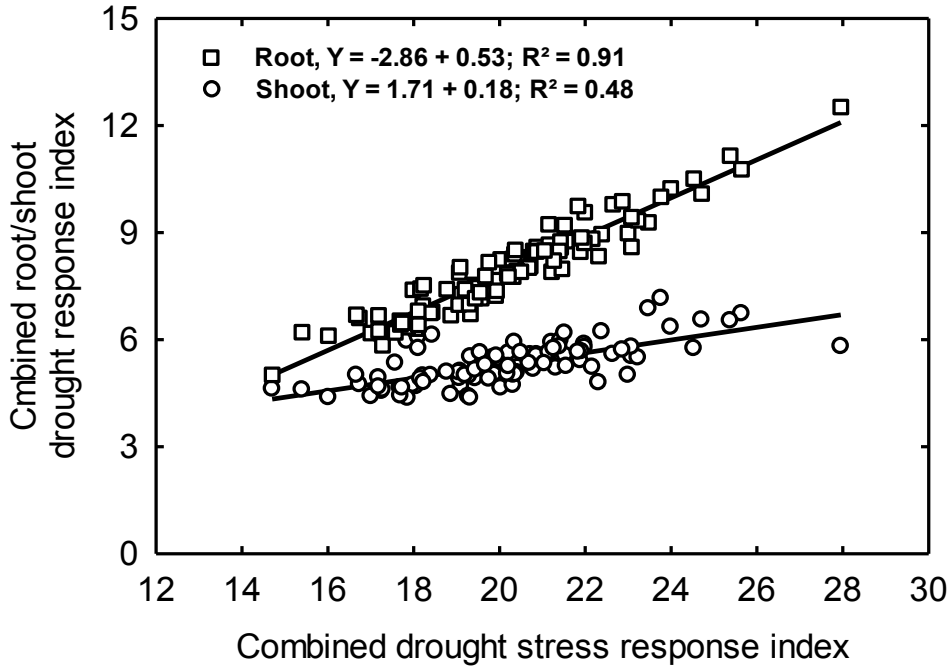

Figure 5.4 The relationship between cumulative drought response index and cumulative drought stress response index of root and shoot indices of 100 elite rice genotypes.

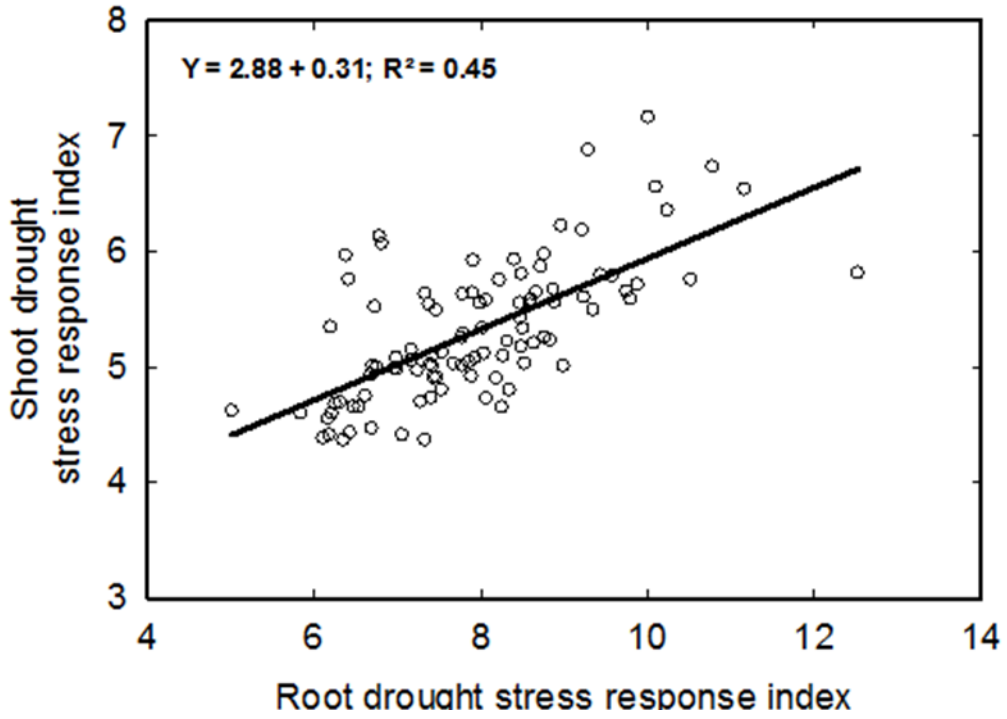

Figure 5.5 The relationship between root and shoot drought response index of 100 elite rice genotypes.

CHAPTER VI

GENOME-WIDE ASSOCIATION STUDY FOR DROUGHT STRESS INVOLVING 100 RICE GENOTYPES

Abstract

Drought is one of the key abiotic stress that affects rice growth and development during the seedling stage. The objectives of this study were to estimate genetic diversity among drought-tolerance rice genotypes using Single Nucleotide Polymorphisms (SNP) markers and apply Genome Wide Association Study (GWAS). A total of 100 rice genotypes were screened for drought tolerance based on 21 morpho-physiological traits. A wide spectrum of phenotypes based on growth as well as drought in morpho-physiological traits. A total of 7098 polymorphic SNPs was initially identified among the 100 rice genotypes. After removing the markers and the accessions with the missing values larger than 20% and the markers with Minor Allele Frequency (MAF) less than 0.05, 4,947 SNP markers were used for association analysis. They distributed on 12 chromosomes ranging from 305 SNPs on chromosome 5 to 603 SNPs on chromosome 1. A phylogenetic tree based on SNPs revealed evolutionary relationship among the genotypes. Two clusters each formed two sub-populations and a third sub-population was more homogenous. Genome-wide association mapping found 33 SNPs distributed on chromosomes 1, 3, 4, 5, 6, 8, 9, 10, 11, and 12 that were significantly associated (p-value 9.7 x 10-5 for GLM and 9.7 x 10-4 for MLM) with most of the morpho-physiological

traits under drought conditions. This study provided preliminary evidence for discovering genes in cultivated rice genotypes. The genotypes with allelic combinations of the 33 significant SNP loci with major effects are potentially useful resources for breeding and genetic studies of drought tolerance.

Introduction

Rice has been cultivated for 10 thousands years and is a staple food in over a hundred countries. The genus Oryza belongs to the tribe Oryzeae of the family Poaceae. There are 12 genera within the Oryzeae tribe. The genus Oryza has 20 wild species and 2 cultivated species including 22 Asian *Oryza sativa* and African *Oryza glaberrima* (Vaughan et al., 2003). O. glaberrima is primarily grown in West African countries while *O. sativa* is the most widely grown throughout the world. The *O. sativa* species is further divided into Indica and Japonica populations, a distinction formed over one thousand years ago (Callaway, 2014). Many researchers have taken the task of identifying the genetic diversity of these varieties and their possible lineages using pedigree, origin, morphological traits and genetic markers.

Among all the abiotic stresses, drought is one of the key factors limiting plant production. It is estimated that drought affects approximately 23 million ha of rice growing areas worldwide (Serraj et al 2011), resulting in losses of up to 40% of total production. Drought resistance is a complex phenomenon, involving a number of morpho-physiological processes playing at different levels within the organism, and at different developmental stages (Tripathy et al., 2000). In the past 30 years, many bi-parental mapping populations have been developed in rice and used to evaluate shoot and root morphological parameters, with the aim of identifying quantitative trait loci (QTLs)

related to drought tolerance. Different types of populations were used, including backcross inbred lines (Kato et al., 2008), recombinant inbred lines (Courtois et al., 2003), doubled haploid lines (Babu et al., 2003), and F_2 families (Price and Tomos, 1997). Genome-wide association study (GWAS) mapping, which is based on linkage disequilibrium (LD) in natural populations to identify associations between markers and quantitative traits (Gaut and Long 2003), has recently emerged as a promising approach for revealing the genetic basis of phenotypic variation.

For the assessment of gene diversity, molecular markers have generally been superior to morphological, pedigree, heterosis and biochemical data (Melchinger et al., 1991). Genetic diversity is commonly measured by the genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weir, 1990) and has the potential for evaluating variations in genetic diversity over time and space (Duwick, 1984). According to previous research, the Japonica population is genetically less diverse than the Indica population when studied using different genetic markers, regardless of sample size of rice accessions (Zhang et al., 1992; Ni et al., 2002). However, this is not always true of genetic diversity for specific traits. Bai et al. (2016) identified common and different alleles between indica and japonica populations for panicle architecture. Ntanos and Koutroubas (2002) showed a significant difference between indica and japonica populations for biomass and grain yield. For biotic and abiotic stresses, there was no significant difference between indica and japonica a tolerance to rice blast pathogen (Zhu et al., 2016). Within populations, there were also differences among varieties regarding drought tolerance along rice accessions (Munasinghe and Price, 2016).

Molecular markers allow selection for the traits to be made by simple laboratory tests on a small amount of plant tissue, rather than direct measurement of the character itself on the entire plant (Christopher et al., 2004). There are several types of molecular markers that may be suitable for this type of marker assisted selection (MAS). These include older markers that are no longer used because they are too much work, which include Restriction Fragment Length Polymorphisms (RFLP) and Amplified Fragment Length Polymorphisms (AFLP), both of which rely on DNA hybridization. Some are not used because they are not repeatable between laboratories, such as Random Amplified Polymorphism DNA (RAPD). Others which rely on Polymerase Chain Reaction (PCR) techniques and are fast and accurate include Sequence Characterized Amplified Regions (SCAR), Cleaved Amplified Polymorphic Sequences (CAPS), and Simple Sequence Repeats (SSR); however, these require knowledge of sequence information in the genome (Botstein et al., 1980; Williams et al., 1990; Paran and Michelmore, 1993; Konieczny and Ausubel, 1993; Litt and Luty, 1989; Vos et al., 1995). The variation within a restricted range of *Oryza sativa* germplasm including Japonica and Indica types to identify suitable parents for linkage map construction and QTL identification for the traits can be done. Moreover, variable patterns of amplified products may be used as potential genetic markers in genome mapping studies (Monna et al., 1994). Association mapping exploits linkage disequilibrium to identify relationships between phenotypic variation and genetic polymorphisms (Breseghello and Sorrells, 2006; Yu and Buckler, 2006) provided an accurate estimation of the population and linkage disequilibrium structures is also made with the markers. Association mapping explores all historical recombination events and mutations in a given population, and thus considered more advantageous over linkage

mapping. The type and the number of genetic markers may have a direct effect on the resolution of the association between genotype and phenotype (Mei et al., 2005). The qualities and imperfections of each type of marker depends on the specific objectives of the study. Dendrogram constructed based on molecular polymorphism reveals genotypic specific DNA bands for selected genotypes. These distinct markers have the potential to be used as genetic fingerprints for future varietal classification and identification (Shivapriya and Shailaja, 2006).

Genome Wide Association Study (GWAS) identifies Single Nucleotide Polymorphisms (SNP) markers that are significantly associated with a trait of interest across a diverse range of natural accessions. For species such as rice, Arabidopsis, maize, wheat, barley and other crops, GWAS has contributed to revealing rich genetic architectures underlying complex traits (Atwell et al., 2010; Xue et al., 2013; Chen et al., 2016). Genome-wide association study (GWAS) was developed as an innovative method for studying associations between genotypes and phenotypes. In a broad sense, GWAS identifies single nucleotide polymorphism markers that are significantly associated with a trait of interest across a diverse population. GWAS method has proved to be very useful for dissecting complex quantitative traits based on a linkage disequilibrium mapping approach (Huang et al. 2010). The main difference to traditional genomic mapping is the ability of GWAS to use SNPs as molecular markers which precisely position genetic markers in the genome (Si et al., 2016).

As genomic and expressed sequence tag sequence information began to accumulate (Adams et al., 1991), the main molecular markers tools changed from DNA fragment lengths to the SNP level, since SNPs consist of the most basic level of variation

and together, the largest numbers of polymorphisms in different genomes (Buso et al., 1998; Zhu et al., 2016). Using single nucleotide polymorphisms (SNPs) as molecular markers for genotypic data is becoming a conventional technique for estimating genetic diversity and relatedness in plant populations, cultivars and genotypes (Martin et al., 1997, Buso et al., 1998). Single nucleotide polymorphisms are sites along the DNA that differ by a single base when two or more individuals are compared, and currently represent the most popular genetic markers. SNPs are now widely used in many breeding and research programs.

Genotyping by sequencing, or next-generation genotyping, is a genetic screening method for discovering novel plant and animal SNPs for genotyping and mapping studies (Poland and Rife, 2012). Sequence-based genotyping provides a lower-cost alternative to arrays for studying genetic variation. SNPs are not only the most abundant form of genetic variation in eukaryotic organisms, being present in both coding and non-coding regions of nuclear and plastid DNA (Edwards and Batley 2010), but they are also stable, efficient, amenable to automation and increasingly cost-effective (Duran et al., 2009). Unlike multi-allelic SSR loci, SNP markers are bi-allelic, and the specific base change detected as a SNP is expected to have occurred only once in evolutionary time. This makes them suitable for studies of widely divergent materials, but they are also widely used in many breeding and research programs. They are exploited for marker-assisted selection programs, for QTL and association mapping studies as well as for QTL positional cloning approaches. Many successful studies have demonstrated that SNPs are powerful markers in terms of assessment of the range of alleles available for a specific

gene in a germplasm collection and their combined use in plant improvement for a target environment (Jannink et al. 2010; Moose and Mumm 2008).

Linkage disequilibrium (LD) is defined as the non-independence of alleles at different loci, and different methods have been proposed to calculate it. The most popular measures are: D, which incorporates information about allelic association and allele frequencies; D', in which the allelic association is normalized with the allele frequencies and thus not dependent on marginal allele frequencies; and r^2, which takes into account the recombination rate between two markers and the effective population size. To date, association mapping applied to drought resistance gene discovery in rice has been performed only in target regions of candidate genes (Serraj et al., 2011). In this study, 100 elite rice genotypes were selected, phenotyped in drought conditions and screened for Single-Nucleotide Polymorphisms (SNP) in several drought-related candidate genes. However, the big drawback of targeted genotyping is the need to identify candidate genes before the screening, whereas drought stress affects thousands of genes. Genome-Wide Association (GWA) mapping, in contrast, can detect new regions associated with the trait of interest by testing the statistical associations between the variation of the trait and SNP variation at the whole genome level. Moreover, GWAS can handle millions of SNPs and 10,000 natural accessions as a mapping population (Lipka et al., 2012; and Chen et al., 2014).

The success of GWAS relies on thorough and accurate phenotyping for the traits of interest coupled with a cost-effective high-throughput genotyping technology, enabling rapid scan the largest number of markers across the largest set of genotypes to yield high-density/quality haplotype maps. Rice, as a self pollinating species with a large extent of

LD, has been shown to be a good candidate for GWAS and does not need more than several thousand SNPs to cover all LD blocks. In two separate studies, Zhao et al. (2011) genotyped 413 and 383 globally distributed rice landraces using an Affimetrix 44K SNP custom array and identified several genes with large effects in determining yield, morphology, stress tolerance and nutritional quality traits. Huang et al. (2010) performed a GWAS for 14 agronomic traits, using a direct low-depth resequencing approach coupled with a novel data imputation method of 317 indica landraces, and identified several loci explaining on average about 36% of the phenotypic variance. Furthermore, GWAS has revealed association signals and identified genes related to rice domestication, physiological, and yield-related traits as well as genes involved in biotic and abiotic conditions (Zhao et al., 2011; Kang et al., 2015; Ueda et al., 2015).

In this study, 100 japonica genotypes widely grown throughout the Midsouth U.S. were collected to evaluate drought tolerance at early vigor stage using two different criteria and were genotyped with a 7K SNP array. Then, a genome-wide association analysis (GWAS) was performed on the generated genotypic and phenotypic data to identify new SNPs for drought tolerance in japonica rice. These results will be useful for improving drought tolerance in rice breeding, and for discovering new QTLs or genes associated with drought tolerance.

Materials and methods

Plant material and experiment

The phenotypic portion of the experiment was conducted at the North Farm Environmental Plant Physiology Laboratory, Mississippi State University, Mississippi State, MS (lat. 33° 28′ N, long. 88° 47′ W). A total of 100 rice genotypes (Table 6.1) were sown in PVC pots (6" diameter by 24" high) arranged in a randomized complete block design with four replications (4 pots per genotypes).

Table 6.1 100 rice genotypes were used in this study

No.	Genotype name	No.	Genotype name	No.	Genotype name	No.	Genotype name
1	14CLPYT033	26	INIA Tacuari	51	RU1304154	76	RU1404156
2	14CLPYT108	27	IRGA409	52	RU1304156	77	RU1404157
3	14CVPYT094	28	JES	53	RU1305001	78	RU1404191
4	14CVPYT144	29	JUPITER	54	RU1401067	79	RU1404193
5	COLORADO	30	LA 2008	55	RU1401070	80	RU1404194
6	Bowman	31	LA 2134	56	RU1401090	81	RU1404196
7	CAFFEY	32	LAKAST	57	RU1401099	82	RU1404198
8	CHENIERE	33	MERMENTAU	58	RU1401102	83	RU1504083
9	CL Jazzman	34	Presidio	59	RU1401145	84	RU1504100
10	CL111	35	Rex	60	RU1401161	85	RU1504114
11	CL142-AR	36	RoyJ	61	RU1401164	86	RU1504122
12	CL151	37	RU0603075	62	RU1402005	87	RU1504154
13	CL152	38	RU1201024	63	RU1402031	88	RU1504156
14	CL163	39	RU1201047	64	RU1402065	89	RU1504157
15	CL172	40	RU1201136	65	RU1402115	90	RU1504186
16	CL271	41	RU1204156	66	RU1402131	91	RU1504191
17	Cocodrie	42	RU1204197	67	RU1402134	92	RU1504193
18	NIPONBARE	43	RU1301084	68	RU1402149	93	RU1504194
19	ANTONIO	44	RU1301093	69	RU1402174	94	RU1504196
20	El Paso 144	45	RU1301102	70	RU1402189	95	RU1504197
21	GSOR100390	46	RU1302192	71	RU1402195	96	RU1504198
22	GSOR100417	47	RU1303138	72	RU1403107	97	Sabine
23	GSOR101758	48	RU1303181	73	RU1403126	98	Taggart
24	RU1104122	49	RU1304114	74	RU1404122	99	Thad
25	CLJZMN	50	RU1304122	75	RU1404154	100	N-22

Initially, six seeds were sown per pot, which were thinned to one after 11 days of emergence. The experiment was irrigated three times daily in the morning (8.00am) afternoon (1.00pm) and evening (6.00pm) with water until emergence of all seeds, then irrigated with standard Hoagland Nutrition Solution until the final harvest. Drought treatment using 50% filed capacity (0.08 volumetric water content (m3/m3) was initiated after an emergence. Plant height (PH), tiller number (TN), and leaf number (LN) were measured on the 28th day of sowing. Leaf area was measured using the LI-3100 leaf-area meter (LI-COR, Inc. Lincoln Nebraska, USA) at harvest followed by measurement of plant components. Leaf dry weight (LDW), stem dry weight (SDW), shoot dry weight (SHDW) and total dry weights (TDW) were measured from all plants after oven drying at 75°C until constant weight was reached. From the shoot and root dry weight, root/shoot (RSR) was estimated in all rice plants and treatments. To account for genotypic differences, all comparisons were done with respect to the control. Instant chlorophyll measurements were recorded in all genotypes using a SPAD meter (SPAD 502 Minnolota Inc. Ontario, Canada) on the 25th day after sowing. Chlorophyll fluorescence was measured using the Fluropen 1000 (Photo System Instruments, Kolackova, Czech Republic) for OJIP Analysis. Roots were cut and separated from the stems and washed thoroughly, avoiding any disturbance to the root system. Longest root length (LRL) was determined using a metric ruler. The cleaned individual root systems were floated in 5 mm of water in a 0.3- by 0.2-m Plexiglas tray. Roots were untangled and separated with a plastic paintbrush to minimize root overlap. The tray was placed on top of a specialized dual-scan optical WinRHIZO scanner (Regent Instruments, Inc. Quebec, Canada, 2009), linked to a computer software system. Gray-

scale root images were acquired according to the same procedure described previously (Brand et al, 2016: Reddy et al, 2017; Wijewardana et al, 2015) by setting the parameters to high accuracy (resolution 800 x 800 dpi). Acquired images were analyzed for the cumulative root length (CRL), root surface area (RSA), average root diameter (ARD), root volume (RV), number of roots (RN), number of root tips (NRT), number of root forks (NRF), and number of root crossings (NRC) using the WinRHIZO optical scanner and associated software. Thorough phenotypic screening for root morphological features was performed using enabling to address deep root development, coupled with WinRHIZO analyses, an image analysis software specifically designed for root characterization. GWAS performed on the first set of phenotypic traits allowed the identification of SNP markers located within QTLs previously shown to be associated with drought avoidance root traits.

Genomic DNA extraction

Fresh green leaves were collected from all rice lines in the early morning to avoid wilting and wrapped in tissue paper. The samples were then placed in small paper bags inside ziplock bags and stored at -80 C^o temporarily. Samples were freeze-dried for one week before grinding. Gridding was done by using SPEX CertiPrep 2000 Geno/Grinder.

Good quality DNA was extracted from the collected leaf samples that were stored at -80 until use. The step-wise protocol of DNA extraction used involved the following,

1. 140 mg lyophilized, ground tissue was added to 5 mL labelled tubes from each.
2. Warm CTAB buffer to 65°C in incubator then add 1 mL of BME (per 100 mL CTAB) to heated CTAB buffer.

3. Add 3 mL heated CTAB-BME mixture to each labeled 5 mL tube w/sample in a capped polypropylene tube.
4. Incubate at 65°C, rock for 60 minutes, and then remove samples from incubation, let them cool for 5 minutes at room temperature.
5. Add 1.4 mL chloroform/octanol (24:1) and rock again for 10 min at room temperature with cap tubes.
6. Centrifuge at room temperature at 2000 rpm for 10 minutes and pipette off aqueous layer (~ 2.5 mL) into new 5 mL tubes.
7. Again add 1.4 mL chloroform/octanol (24:1), followed by gentle mixing inversion androck for 10 min at room temperature.
8. Centrifuge at room temperature at 2000 rpm for 10 minutes.
9. Add 17 µL of 10 mg/mL or 8.5. Add 17 µL of 10 mg/mL or 8.5 µL of 20 mg/mL RNAase A to new 5 mL tubes.
10. Transfer aqueous layer (~2.5mL) into new 5 mL tubes. Incubate again at 37°C for about 30 minutes or at room temperature for 60 minutes.
11. Add 2 mL of ice-cold isopropanol. Invert 1-2 times to precipitate DNA.
12. Hook out DNA with glass hooks, wash DNA with 70% ethanol, and blot dry with Kim wipes.
13. Dissolve DNA in labeled 1.5 mL tube containing 200 µL TE for ~5 min followed by gentle mixing and rocking overnight. Clean up including autoclaving glass hooks.
14. Analysis, each sample with the Nano-drop Spectrophotometer to quantify DNA concentration, and quality can be observed by running quality gel (1 % (w/v)

agarose gel). Further, working dilutions were made from the stock as required Appendix 1, 2, and 3.

Quantification of DNA:

Thermo Fisher Scientific Nano-drop spectrophotometer (ND 1000) was used to quantify the concentration of extracted genomic DNA of all the genotypes. The optical density of each sample was calculated by measuring the absorption of ultraviolet light of wavelength 260 nm. The absorbance of light is depended upon the concentration of DNA, and 260/280 ratio is the measure of purity and should range from 1.8 to 2.0 for good quality DNA.

The procedure of measuring the concentration of extracted genomic DNA is as follows,

1. Swicth on Nano spectro-photometer instrument carefully.
2. First, take a water sample reading by taking a drop of water (d_3H_2O) the help of micropipette and pour it onto the cuvette (lower lens) of Nano-drop instrument (wipe out the lens after each reading).
3. Then, take a blank reading by putting a drop of TE buffer and pressing blank reading only once.
4. The calibration is needed to be set at 260 nm.
5. Take 1 ul of diluted DNA, gently drop it on the lower lens, press the measurement and wait for double click of the pedestal and the graph to appear on the screen.
6. Repeat the same procedure to measure the optical density of the extracted DNA of all samples for their concentration.

DNA Quality measurement

The quality of extracted genomic DNA was determined on 1 % (w/v) agarose gel by comparing bands to standard bands of known concentrations.

Protocol of 1% agarose gel

The protocol for making 1% agarose gel is given bellow,

1. Measure 150 mL of 1X TAE buffer in a measuring cylinder and pour into the bottle.
2. Measure 1 g of agarose on electronic weigh balance, add into the bottle containing TAE buffer and swirl gently. After homogenization the lid of the bottle was loosely tight.
3. Boil the mixture by putting the bottle into the microwave oven until the gel particles disappear.
4. After boiling the total volume of the mixture should make equal to as was prior to boiling.
5. Let the gel cool down at room temperature. After cooling add 3 uL of ethidium bromide.
6. Prepared the gel casting tray by wrapping its edges by blockers and putting combs in the tray in such a way that the combs do not touch the bottom of the tray to avoid holes in the gel.
7. Gently pour the gel into the gel casting tray (Sunrise 96 gel system) ensuring that no bubbles get into the gel and leave it to solidify for 30-40 minutes and then pull off the combs carefully.

Gel Electrophoresis

The solidified gel was transferred to a Bio-Rad electrophoresis unit containing 1% TAE buffer. The DNA samples were mixed with a 1/3rd volume of gel loading-cum-tracking dye (0.25 % Sucrose: 0.25 % Bromophenol blue) and loaded on the gel. 1.5 µl of

1 kb ladder was loaded on one end (first well) and about 3-5 µl of the mixture of loading dye and DNA samples were loaded in to individual wells carefully.

The gel was allowed to run for about 45-60 min at 80-90 V and later it was documented. After checking the concentration of the DNA based on the band intensity, the DNA samples were diluted to 20 ng µl-1 for further polymerase chain reaction (PCR) analysis (Vilber Lourmat BLX312 UV crosslinker) (appendix 4 and 5).

Data analysis:

Genotyping and population structure analysis [SNP]

All 100 rice genotypes were genotyped from Illumina company (https://www.illumina.com/) which uses SNP arrays (7K SNP-Chips) to determine allelic variation. The 7000 Illumina SNPs derive from over two million common SNPs (minor allele frequency MAF ≥0.05) in the Rice HapMap data (Bradbury et al. 2007). DNA amplification, fragmentation, and chip hybridization, washing, and staining were performed according to the Infinium assay standard protocol (Infinium HD Assay Ultra Protocol Guide,http://www.illumina.com/). HiScan scanner (Illumina Inc., San Diego, CA) was used for chip scanning, and GenomeStudio software was used for raw data analysis. In the present genome wide association study, we used a subset of 5776 SNP markers selected from the whole SNP array by applying the following thresholds: missing data ratio > 95% and markers with the frequency of a minor allele (MAF) > 0.05. A neighbor-joining (NJ) tree was constructed using TASSEL 2.3.3 software and Structure 2.3.4 software.

Genome wide Association analysis:

Associations between marker alleles and rice phenotypic traits data was performed with the TASSEL (trait analysis by association, evolution, and linkage) software package, Version 2.3.3 (Bradbury et al. 2007). TASSEL is a software package that evaluates a trait's associations with sequence polymorphisms (generally, SNPs and insertion/deletion or InDels), evolutionary patterns, and linkage disequilibrium. It provides powerful statistical approaches to association mapping including the General Linear Model (GLM) and the Mixed Linear Model (MLM). MLM is an implementation of the technique which reduces Type I error in association mapping with complex pedigrees, families, founding effects and population structure. In the current study, the association between markers and phenotypic traits was done using the Mixed Linear Model, where markers tested, and subpopulation data (Q matrix) were considered as fixed-effect factors, and a kinship matrix was considered as a random-effect factor. The P value determining significance of each marker/trait association and the r^2 value, indicating the fraction of the total variation explained by the marker, were reported. The SNPs with $p < 7.9$ x 10-5 in GLM and the SNPs with $p < 7.9$ x 10-4 in MLM, were considered to be significant. For GLM and MLM, optimum compression level was used in TASSEL 2.3.3 (Bradbury et al., 2007). QQ plots and Manhattan plots of – Log10 (P) values for each SNP vs. the chromosomal position were generated in the TASSEL results. The population structure underlying the genotyped collection of accessions was analyzed using STRUCTURE v 2.3.4 (Pritchard et al. 2000). STRUCTURE's quantitative clustering method uses a Bayesian approach to identify subpopulations and to assign individuals to these populations. Given a sample of individuals, K populations

are assumed, and individuals are assigned to these populations. In case of the SNP data, the bases were numerically coded as follows: A=1, C=2, G=3, T=4, and missing data were coded as 999 as suggested in the user manual (Peakall and Somouse 2012; Yan et al., 2009). Software STRUCTURE V2.3.4 was applied to infer historical lineages that show clusters of similar genotypes (Pritchard et al., 2000). The membership of each genotype was run for a range of genetic clusters from the value of K= 1 to 10 with the admixture model and correlated allele frequency. Each run was implemented with a burn-in period of 10,000 followed by 100,000 Monte Carlo Markov Chain replicates (Prithard et al., 2000). Ln (PD) was derived for each K and then plotted to find the plateau of the ΔK values (Evanno et al., 2005). Online available program "Structure Harvester" was used (http://taylor0. biology.ucla.edu) to calculate and found the correct final population structure. The proportion of the genome of an individual that belongs to each inferred population (admixture) was estimated).

Results and discussions

Marker analysis

A total of 7098 polymorphic SNPs was initially identified among the 100 rice genotypes and are shown in Figure 6.1. After removing the markers and the accessions with missing values larger than 20% and the markers with MAF less than 0.05, 4,947 SNP markers were used for association analysis. They are distributed on the 12 rice chromosomes ranging from 305 SNPs on chromosome 5 to 603 SNPs on chromosome 1 (Figure 6.1). The fact that Chromosome 1 contributed the most polymorphic markers (12.19% of the total) and Chromosome 5 the least (6.16%; Figure 6.2) is in congruence with the physical sizes of the chromosomes.

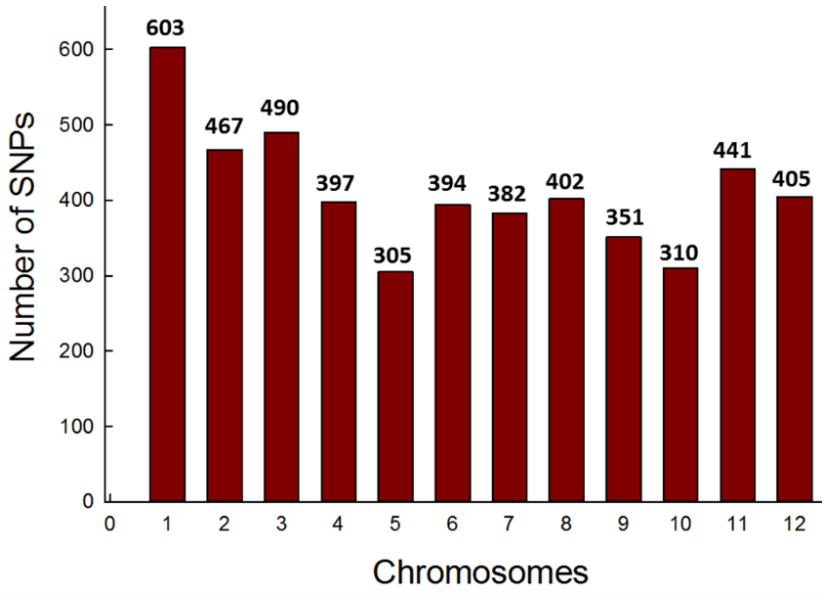

Figure 6.1 Distributions of the mapped polymorphic markers with a MAF (minor allele frequency) greater than 0.05. Number of SNPs on each. The number at the top of each column represents the number of polymorphic markers on each chromosome.

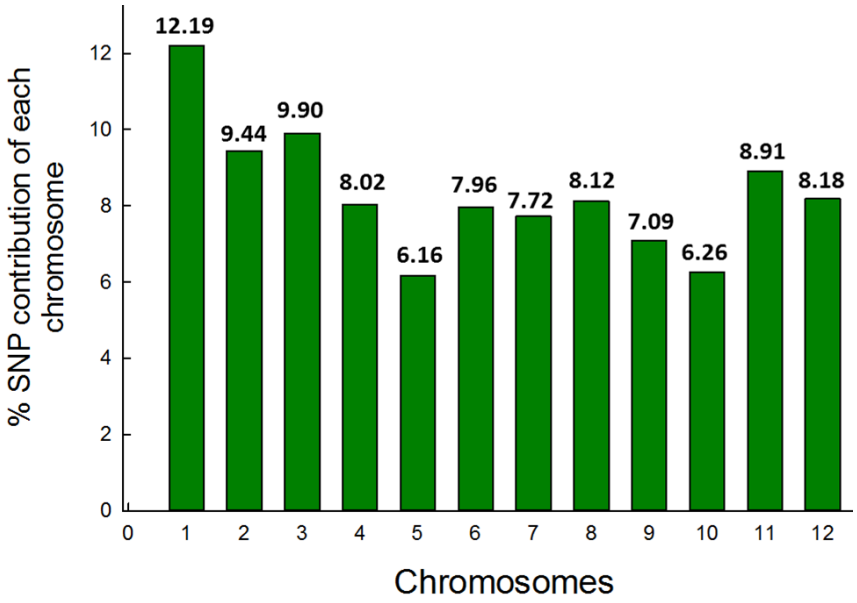

Figure 6.2 Percent SNP contribution of each chromosome to the total number of SNPs. The number at the top of each column represents the number of % SNP contribution.

Selection of a representative rice germplasm diversity panel

The GWAS analysis of shoot, root, and physiological traits was carried out on a rice diversity panel including 100 genotypes. This pilot subset was selected with the aim to explore the broadest range of genotypic/phenotypic diversity of rice cultivated in the Mid-south of the U.S. A a set of drought-tolerant genotypes, which was selected by Cumulative Drought Stress Response Index (CDSRI) and principal component analysis (PCA) well adapted to our agro-climatic conditions was included to enrich allelic variation associated with drought avoidance. A genetic diversity analysis of the rice genotypes used in the present study was estimated using TASSEL software v. 2.3.3 (Bradbury et al., 2007) by creating a Cladogram with arthmetic Neighbor-Joining. This analysis separated the 100 rice entries into three clusters (Figure 6.3a) . The first cluster

of 52 genotypes is composed of two subgroups including 32 rice breeding lines in one and and 20 released cultivars in the other (Figures 6.3b). Similarly, the second group of 36 genotypes is clustered into two subgroups including 24 rice breeding lines in one and 12 released cultivars in the other, and three of Mississippi rice varieties (Bowman, Thad, and CL163) belonged to this subgroup. (Figures 6.3c). The third cluster contained only 12 rice genotypes including four rice breeding lines and 8 of the released cultivars in one homogenous group and Rex that is one of Mississippi rice variaty belong to this subgroup (Figure 6.3d). SNP markers have been used successfully to identify genetic diversity in several rice collections which represented many of the world's rice-growing regions (Xu et al., 2004; Eizenga et al., 2006).

In other studies, the accessions under study were limited to a specific geographical area such as the USA (Lu et al., 2005), Southeast Asia (Garris et al., 2003), Argentina (Giarrocco et al., 2007), Indonesia (Thomson et al., 2007). Almost all diversity analyses identified clusters for the two rice subspecies, indica, and japonica, and often subsequently delineate both temperate and tropical japonica sub-clusters. Agrama et al. (2007) observed most accessions were classified into one of the three groups, which corresponded to the traditional rice sub-species indica (29 accessions), temperate japonica (32), and tropical japonica (17) separated by relatively large genetic distance. By the above reports mentioned indica and japonica genotypes formed separate groups and subclusters indicating the diversity among them. Thus, it was shown that the genotypes used for SNP mapping study are diverse. The genetic diversity analysis with SNP markers will contribute to maximize the selection of diverse parents and broaden the germplasm base in the future rice breeding program or development of drought tolerant

cultivars. In addition, it will help in identifying efficient strategies for the sustainable management of genetic resources of rice crops to cope with the climate change.

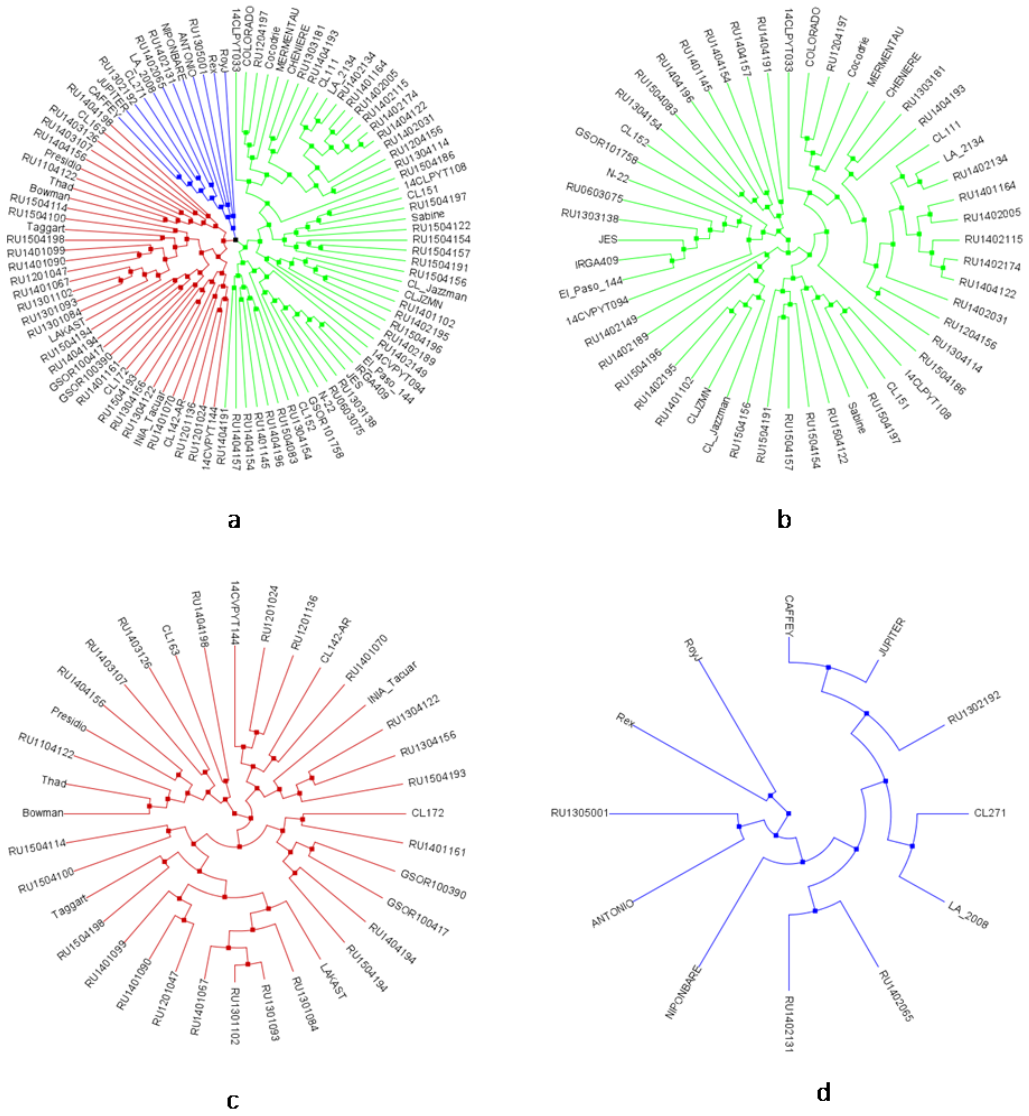

Figure 6.3 Distance tree constructed using the Neighbor-Joining method based on 100 rice genotypes (a). The three subgroups identified from tree are color-coded in b, c, and d, respectively.

Population Structure and Relative Kinship

We ran STUCTURE software for K (number of fixed subgroups or clusters) ranging from 1 to 10 on the entire set of rice genotypes using all SNPs scored. The mean likelihood value of this analysis is shown in Figure 6.4. Likelihood increases regularly, and no obvious inflection point was observed for SNP. Likelihood increases linear from K1 to K5 and no obvious inflection point was observed for SNP.

Though, the most significant change was observed increased when K was increased from one to two then to three, maybe due to the rice genotypes, which most them are rice breeding line and another set of released varieties.

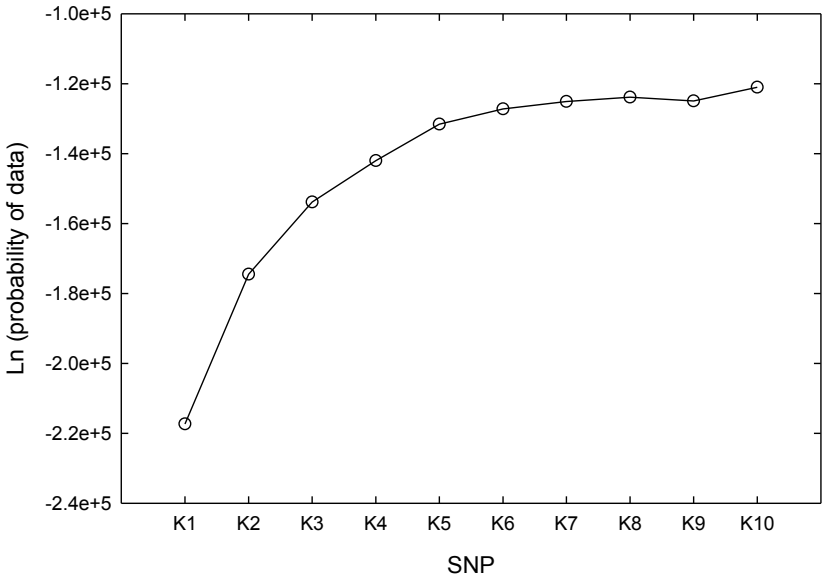

Figure 6.4 Estimated ln (probability of data). Ln (probability of data) was calculated for K ranging from 1 to 10 for SNPs.

A further study of the partitioning of rice genotypes can be seen in Figure 6.5, which is the Structure using a graphical representation of the placement of each genotype

in the study into its corresponding cluster, for K ranging from 2–10. This graph shows the percent mixing of each genotype within each cluster, the number of genotypes in each cluster, and useful visualization of admixture. The most significant change was observed when K = 3 (Figure 6.5 and Appendix 7 and 8).

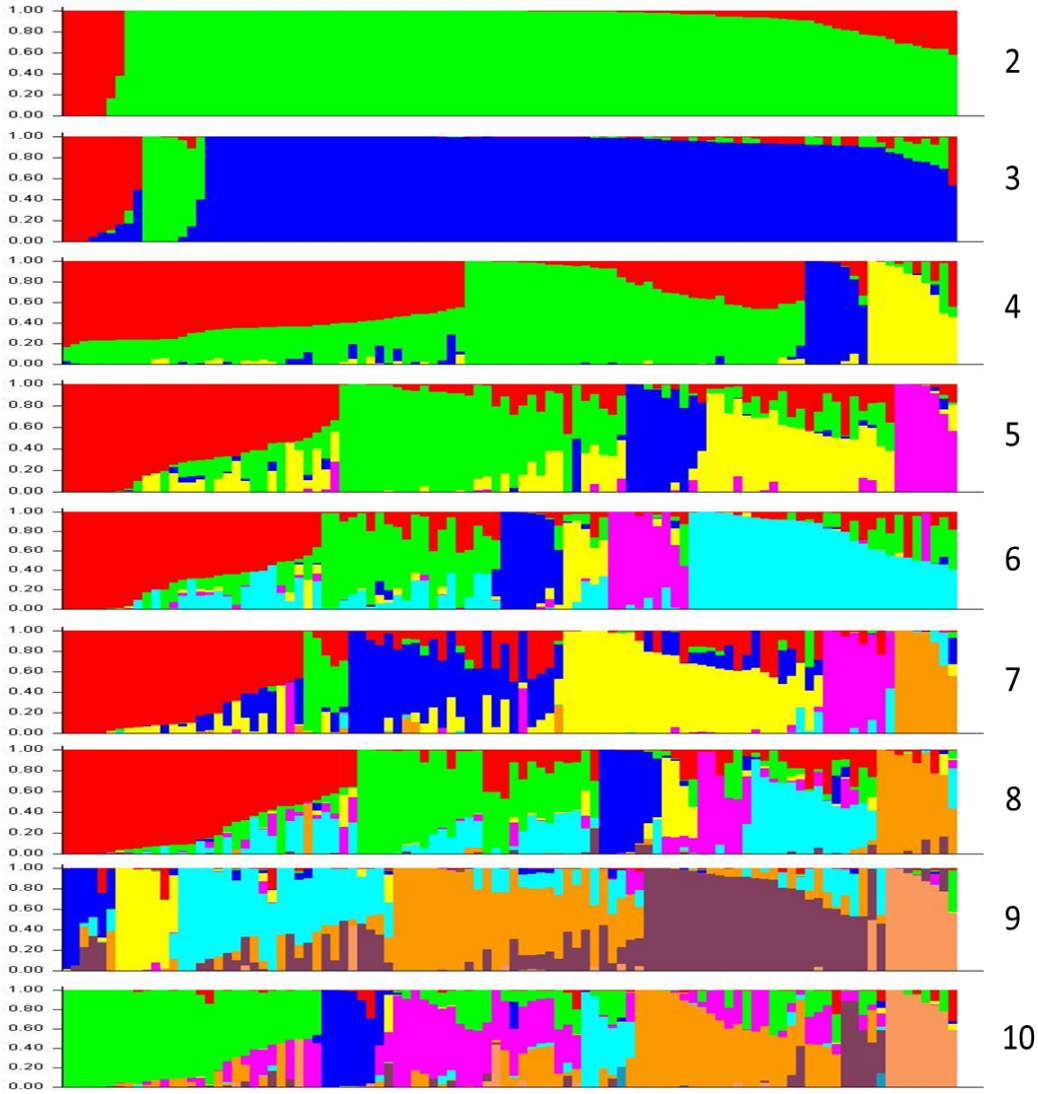

Figure 6.5 Estimated population structure of the diverse rice genotypes in the study. Each of the 100 individuals is represented by a thin vertical line, which is partitioned into k colored segments that represent the individual estimated membership to the k clusters.

SNPs/traits association significant

Unlike Indica genotypes, Japonica genotypes are characterized by lower genetic diversity, which is difficult to study in GWAS, so we chose to conduct GWAS for only the Japonica genotypes population to refute or confirm this claim. The list of the significant (*P*-value < 0.00005) associations detected in this preliminary study is reported in Table 6.2. GWAS for shoot growth and development traits identified 12 highly significant associated regions on chromosomes 3, 5, 6, 8, and 12 for leaf area trait while 7 significantly associated regions on chromosome 5, 6, and 12 for plant height trait. Nine genes were identified for leaf area including Os03g0693600, Os06g0495500, Os06g0315900, Os03g0670200, Os03g0423800, Os03g0739700, Os06g0581151, Os05g0408200, and Os08g0154950, which accounted for 33, 33, 28, 25, 25, 23, 21, 19, and 19% of the phenotypic variance, respectively. 6 genes (Os05g0419100, Os12g0525300, Os06g0484600, Os05g420500, Os05g0410200, and Os05g0418000) were detected only in PH explaining 25, 21, 19, 19, 18, and 18% of the phenotypic variance, respectively. In the case of component dry weight traits, five significantly SNP clusters on chromosome 1, 3, 4, 6, and 12 were revealed for leaf dry weight, stem dry weight, and shoot dry weight. 4 genes were identified only in LDW, SDW, and SHDW including Os03g0566600, Os01g0329075, Os12g0525300, and Os01g0328900, which accounted for 14, 15, 16, and 15% of the phenotypic variance, respectively. Ten chromosomal regions were significantly associated with root growth and development traits, on chromosome 1, 4, 8, and 11 for root dry weight, longest root length, number of root forks, number of root, and root surface area Table 6.2.

Table 6.2 SNP/Trait associations significant at the $p < 9.99E-5$ level, and all genes within a window of ± 10Kbp of the SNP.

Trait	Chromosome	[a]Position	Range (position ± 10 kbp)		Genes	*P*-value	[b]R^2
Leaf area (LA)	3	27761403	27751403	27771403	Os03g0693600	7.81E-11	0.33
	6	11830140	11820140	11840140	-	8.48E-11	0.33
	6	17273260	17263260	17283260	Os06g0495500	1.04E-10	0.33
	6	12183428	12173428	12193428	Os06g0315900	4.64E-09	0.28
	12	1626010	1616010	1636010	-	7.98E-09	0.27
	3	26456796	26446796	26466796	Os03g0670200	5.28E-08	0.25
	3	17674269	17664269	17684269	Os03g0423800	7.85E-08	0.25
	3	30342031	30332031	30352031	Os03g0739700	1.45E-07	0.23
	6	22669171	22659171	22679171	Os06g0581151	7.92E-07	0.21
	8	15216843	15206843	15226843	-	1.71E-06	0.2
	5	19928571	19918571	19938571	Os05g0408200	2.21E-06	0.19
	8	3164982	3154982	3174982	Os08g0154950	3.51E-06	0.19
Plant height (PH)	5	20551103	20541103	20561103	Os05g0419100	1.50E-07	0.25
	12	20644305	20634305	20654305	Os12g0525300	2.36E-06	0.21
	6	16508748	16498748	16518748	Os06g0484600	3.89E-06	0.19
	5	20621852	20611852	20631852	Os05g0420500	7.39E-06	0.19
	5	20046475	20036475	20056475	Os05g0410200	1.03E-05	0.18
	5	20488479	20478479	20498479	Os05g0418000	1.03E-05	0.18
leaf dry weight (LDW)	3	27761403	27751403	27771403	Os03g0566600	9.96E-05	0.14
Stem dry weight (SDW)	1	12679816	12669816	12689816	Os01g0329075	8.58E-05	0.15
Shoot dry weight (SHDW)	12	20644305	20634305	20654305	Os12g0525300	3.47E-05	0.16
	1	12679816	12669816	12689816	Os01g0328900	6.38E-05	0.15
	6	14485044	14475044	14495044	-	7.72E-05	0.15
Root dry weight (RDW)	4	34477098	34467098	34487098	Os04g0675101	7.85E-05	0.14
Longest root length (LRL)	1	39282883	39272883	39292883	Os01g0901900	3.58E-05	0.16
	1	39342234	39332234	39352234	Os01g0903800	3.58E-40	0.16
Number of root forks (NRF)	8	26922186	26912186	26932186	Os08g0538300	1.05E-40	0.17
Number of root (RN)	6	3598843	3588843	3608843	Os06g0171700	9.05E-07	0.22
	6	3547983	3537983	3557983	Os06g0170500	3.28E-05	0.16
	6	3632725	3622725	3642725	Os06g0171900	3.28E-05	0.16
	11	25969407	25959407	25979407	Os11g0650700	9.06E-05	0.16
Root surface area (RSR)	4	34477098	34467098	34487098	Os04g0675101	3.89E-05	0.16
SPAD	12	25127540	25117540	25137540	Os12g0597800	1.68E-05	0.17

a- For each trait, SNP with the highest P-value is reported.

b- phenotypic variance explained

Nine genes (Os04g067510, Os01g0901900, Os01g0903800, Os08g0538300, Os06g0171700, Os06g0170500, Os06g0171900, Os11g0650700, and Os04g0675101) were detected only in RDW, LRL, NRF, RN, and RSA explaining 14, 16, 16, 17, 22, 16, 16, 16 and 16% of the phenotypic variance, respectively. However, there was only one significant associated region on chromosome 12 for SPAD trait. Os12g0597800 was detected as associated with SPAD explaining 17% of the phenotypic variance. The different regions on all chromosomes above indicate partially overlap with that identified for the shoot, root, and physiological traits. All the detected associations with regions previously reported being involved in drought tolerance or growth and development traits based on SNPs mapping studies (Courtois et al., 2003). A preliminary exploration of a region spanning 150 kb surrounding the identified peaks revealed several candidate genes shown to be related to shoot and root growth characteristics**.** The peak signal of the GWAS loci sometime appeared in the region near but not within the known genes (Huang et al., 2010). In this study, we found similar cases of four known genes involved in plant height: (Spielmeyer et al., 2002), (Jiang et al., 2005), (Du et al., 2012), and (Giri et al., 2011).

Genome-wide association analyses

In a broad sense, GWAS identifies single nucleotide polymorphisms (SNPs), Insertion/Deletion and any other sequence changes along the chromosome that are significantly associated with a trait of interest across a diverse population. To get insight into the genetic variants associated with drought tolerance at the seedling growth stage in rice, a GWAS was conducted for a stress tolerance index using 21 morpho-physiological traits. The 21 traits were classified into four categories, namely growth and development

traits (plant height (PH), tillers number (TN), leaf number (LN), and leaf area (LA)); component dry weight (leaf dry weight (LDW), stem dry weight (SDW), shoot dry weight (SHDW), and total dry weight (TDW)); root growth and development (cumulative root length (CLR), root surface area (RSA), average root diameter (ARD), longest root length (LRL), root volume (RV), number of root (RN), number of tips (NRT), number of root forks (NRF), number of root crossing (NRC), root dry weight (RDW), and root-shoot ratio (RSR); physiological characteristics (fluorescence (Fv/Fm) and SPAD). All these traits were considered for GWAS with SNPs assayed from a chip.

To assess the effectiveness of GWAS on our SNP dataset and diversity panel, we first conducted an association analysis for the each of the shoot, root, and physiological traits individually. Phenotypic data from these traits were obtained from a parallel pots experiment, in which the 100 genotypes used in this study were evaluated for agronomic performances (data show in Chapter 5). Although GWAS conducted in rice were performed on large highly-structured diversity panels (Huang et al. 2010; Zhao et al. 2011); we ran only the 100 rice genotypes. Because of the detection of sub-population-specific alleles, the analysis had to be restricted to the particular sub-population (Zhao et al 2011). Two statistical models were used to perform GWAS analyses: GLM and MLM (see Methods). GWAS was then carried out on the selected drought avoidance shoot traits. For each character, we decided to perform the analysis using both statistical models and to choose the most appropriate based on the Quantile-Quantile (Q-Q) plots generated. The Manhattan plot of $-\log_{10}$(p-values) and the Q-Q (quantile-quantile) plots of expected (under a Gaussian distribution) vs observed p-values for SNP-based genotype-phenotype associations for the shoot and root morphological traits under examination are reported in

Figs 6.6, 6.7, 6.8, 6.9, 6.10, 6.11, 6.12, and 6.13. Additionally, the Manhattan and Q-Q plots for the physiological characteristics are shown in Fig 6.14.

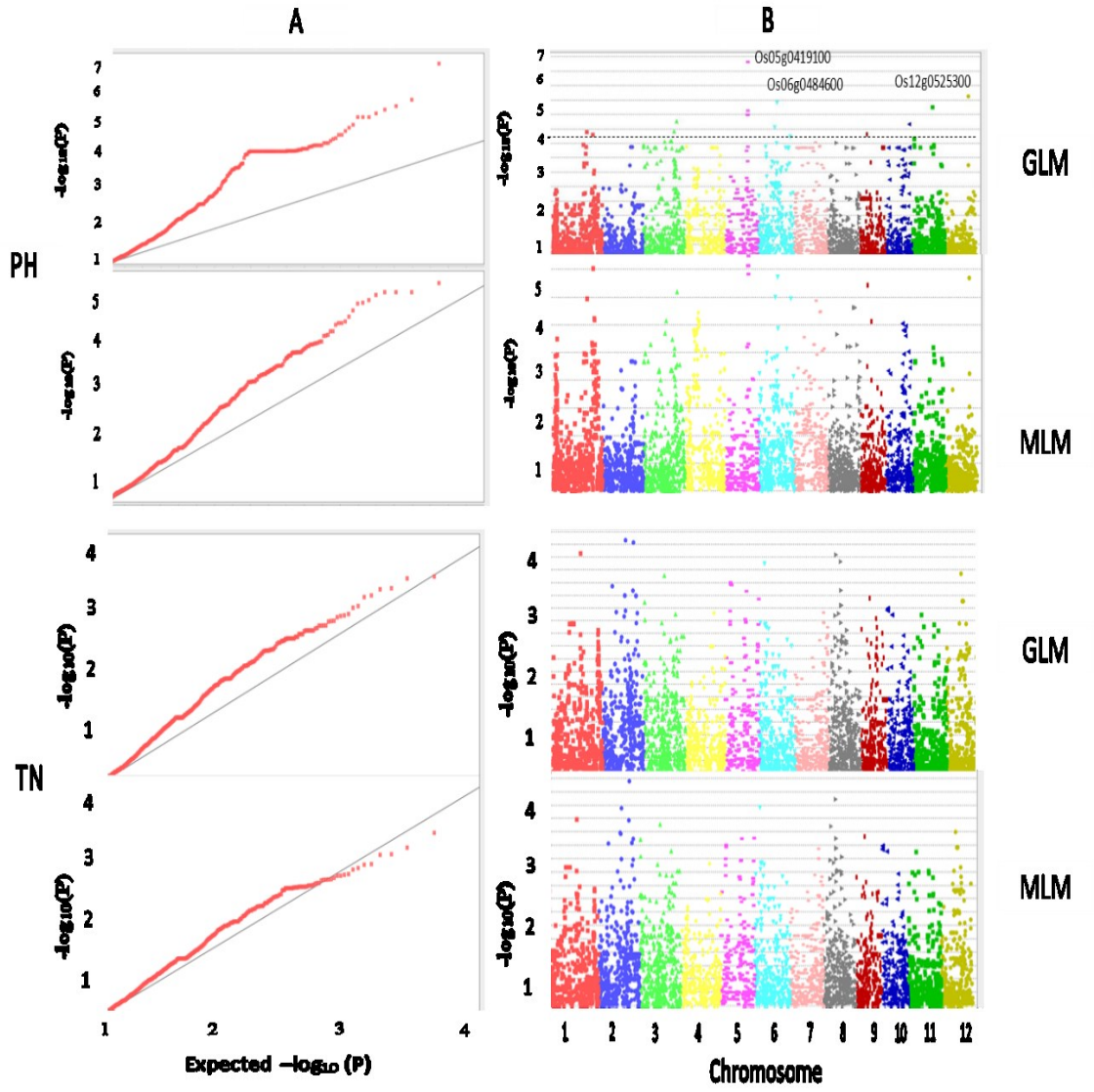

Figure 6.6 GWAS analysis for plant height (PH) and tillers number (TN). For each of the two-statistical model employed, the Quantile-Quantile plot (A) and the Manhattan plot (B) is shown. A threshold of p<1.0E-05 for GLM-P was applied.

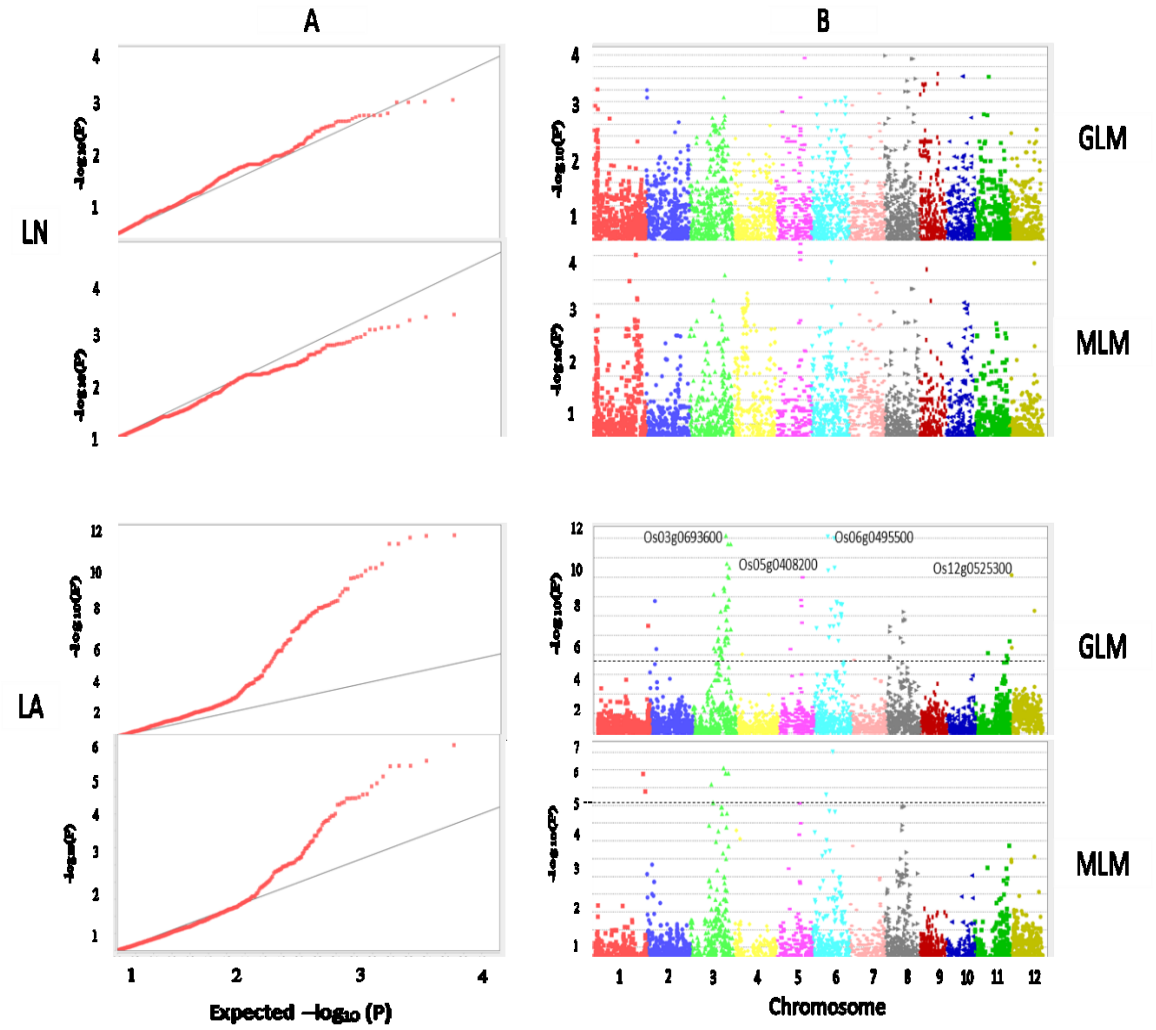

Figure 6.7 GWAS analysis for leaf number (LN) and leaf area (LA). For each of the two-statistical model employed, the Quantile-Quantile plot (A) and the Manhattan plot (B) is shown. The black circles indicate the SNP significantly associated with qsw5. A threshold of p<1.0E-04 for MLM and of p<1.0E-05 for GLM-P was applied.

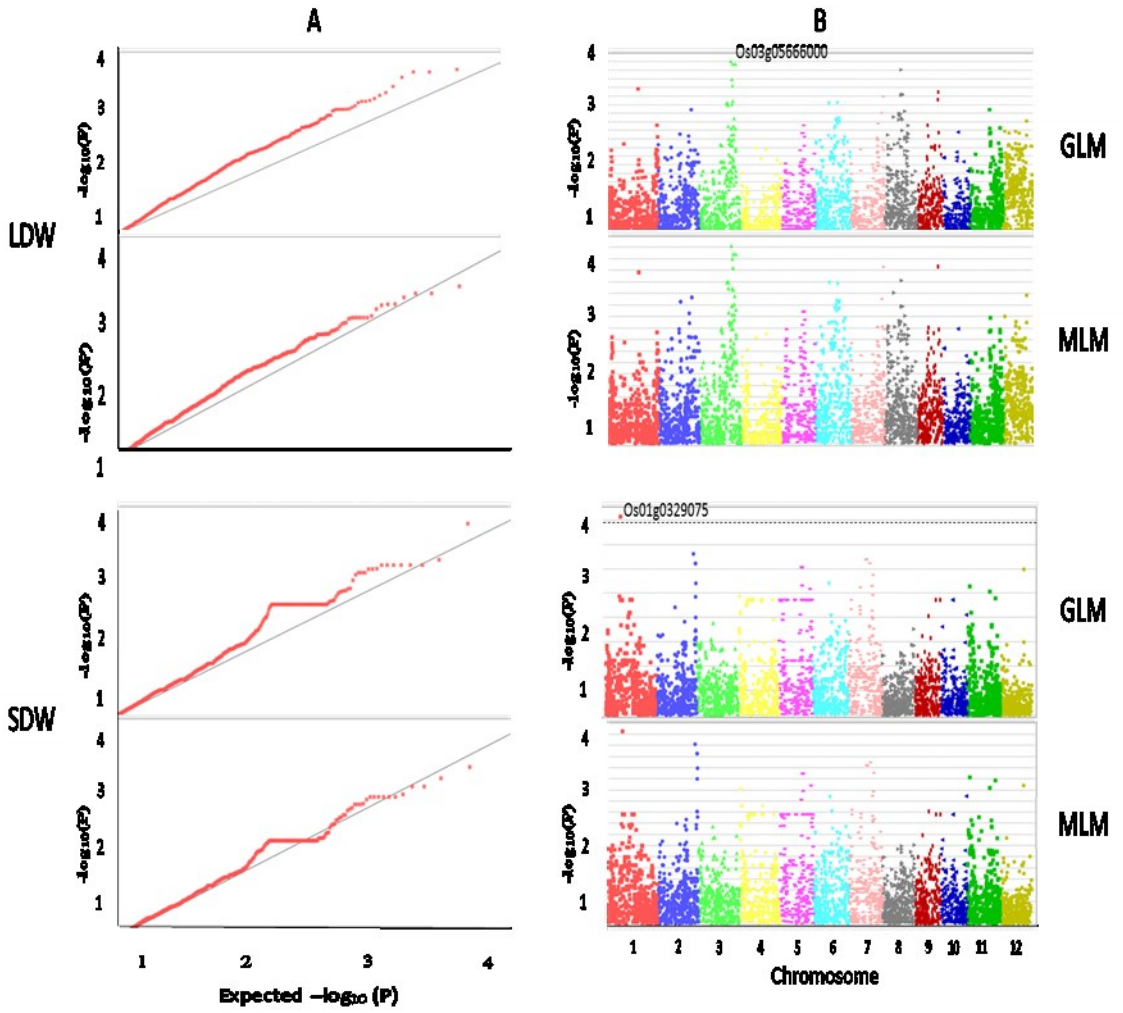

Figure 6.8 GWAS analysis for leaf dry weight (LDW) and stem dry weight (SDW). For each of the two-statistical model employed, the Quantile-Quantile plot (A) and the Manhattan plot (B) is shown. A threshold of p<1.0E-05 for GLM-P was applied.

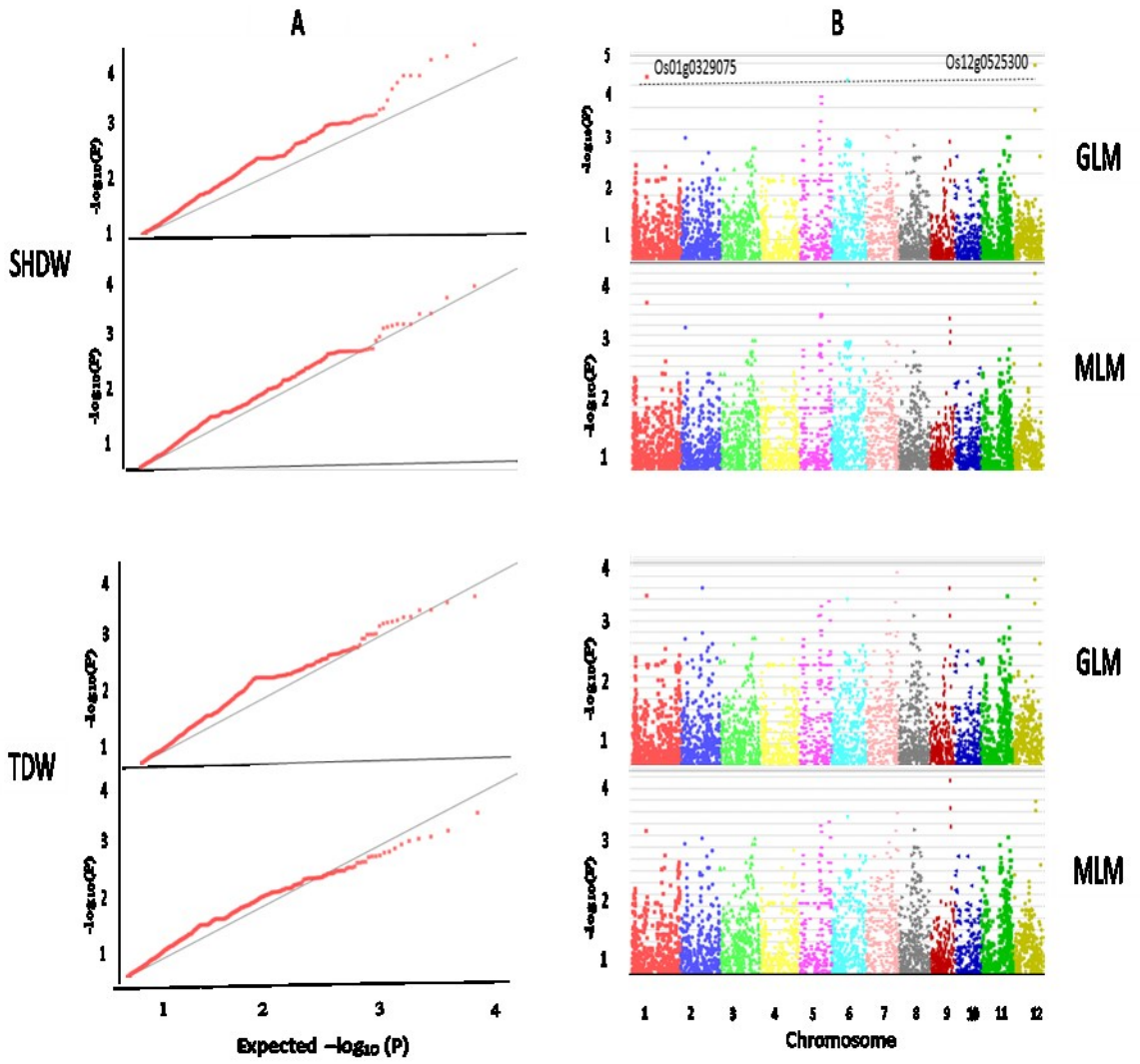

Figure 6.9 GWAS analysis for shoot dry weight (SHDW) and total dry weight (SDW). For each of the two-statistical model employed, the Quantile-Quantile plot (A) and the Manhattan plot (B) is shown.

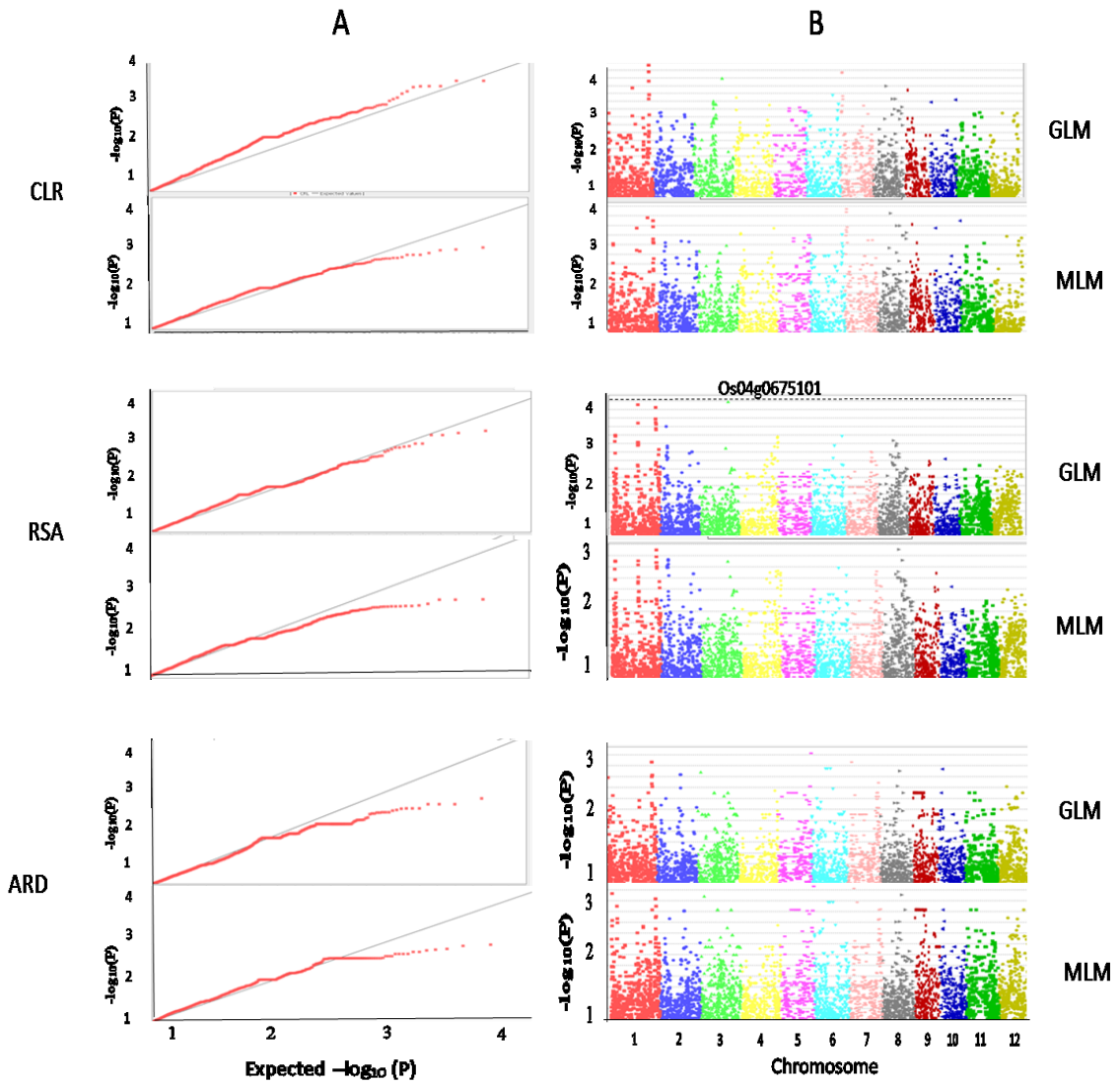

Figure 6.10 GWAS analysis for cumulative root length (CRL), root surface area (RSA), and average root dimeter (ARD). For each of the two-statistical model employed, the Quantile-Quantile plot (A) and the Manhattan plot (B) is shown.

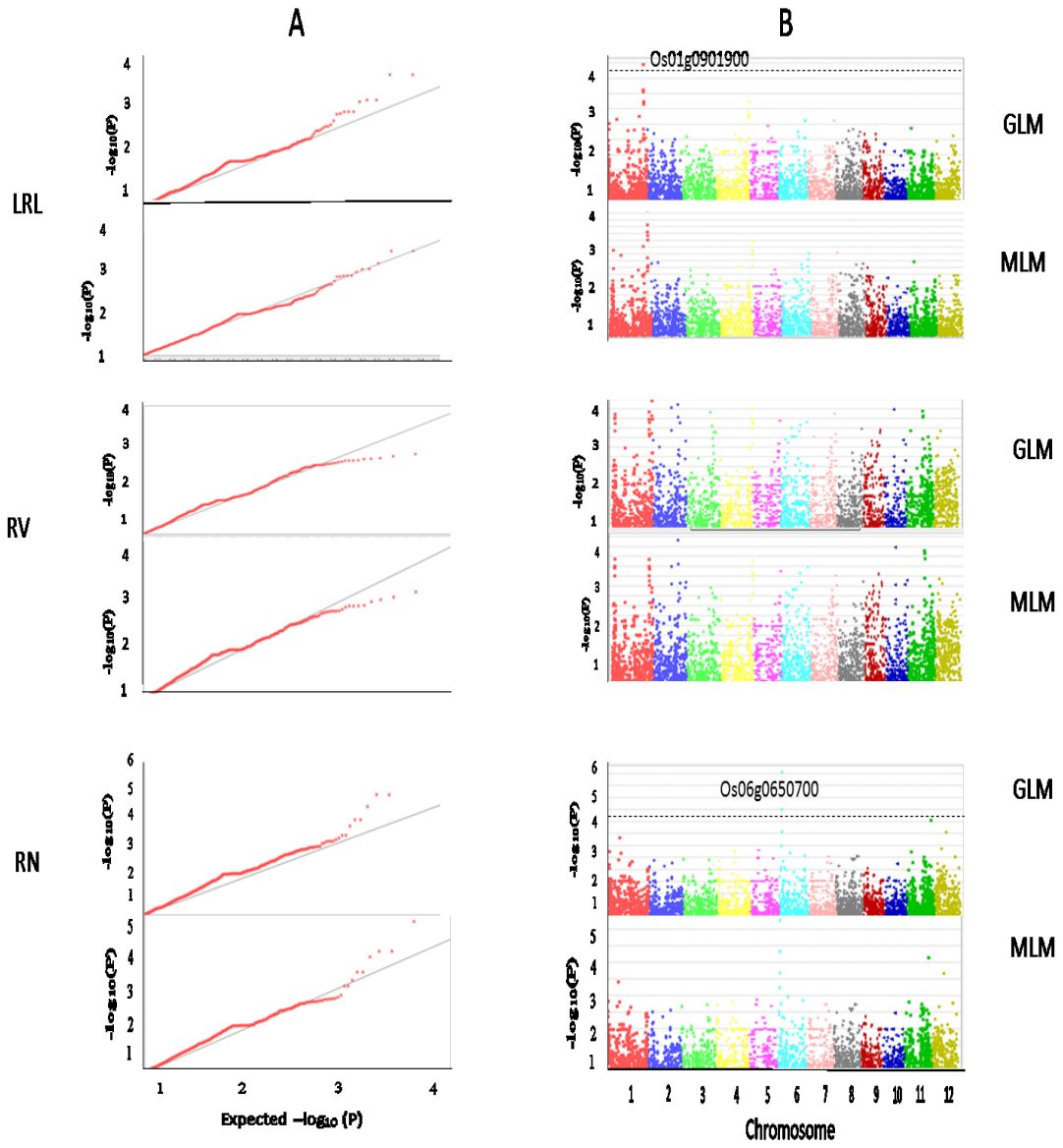

Figure 6.11 GWAS analysis for longest root length (LRL), root volume (RV), and root number (RN). For each of the two-statistical model employed, the Quantile-Quantile plot (A) and the Manhattan plot (B) is shown. A threshold of p<1.0E-05 for GLM-P was applied.

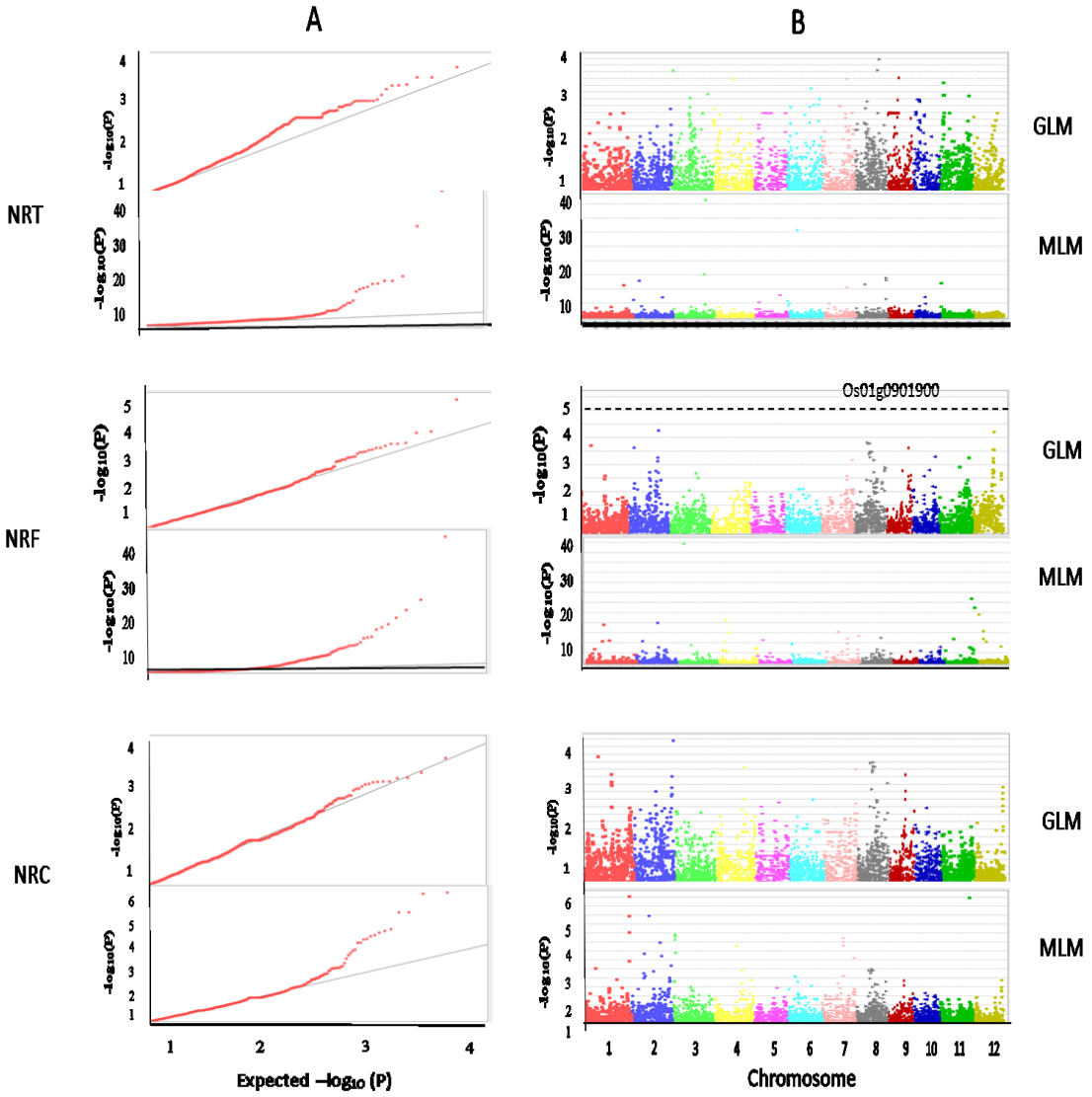

Figure 6.12 GWAS analysis for number of root tips (NRT), number of root forks (NRF), and number of root crossing (NRC). For each of the two-statistical model employed, the Quantile-Quantile plot (A) and the Manhattan plot (B) is shown. A threshold of p<1.0E-04 for MLM and of p<1.0E-05 for GLM-P was applied.

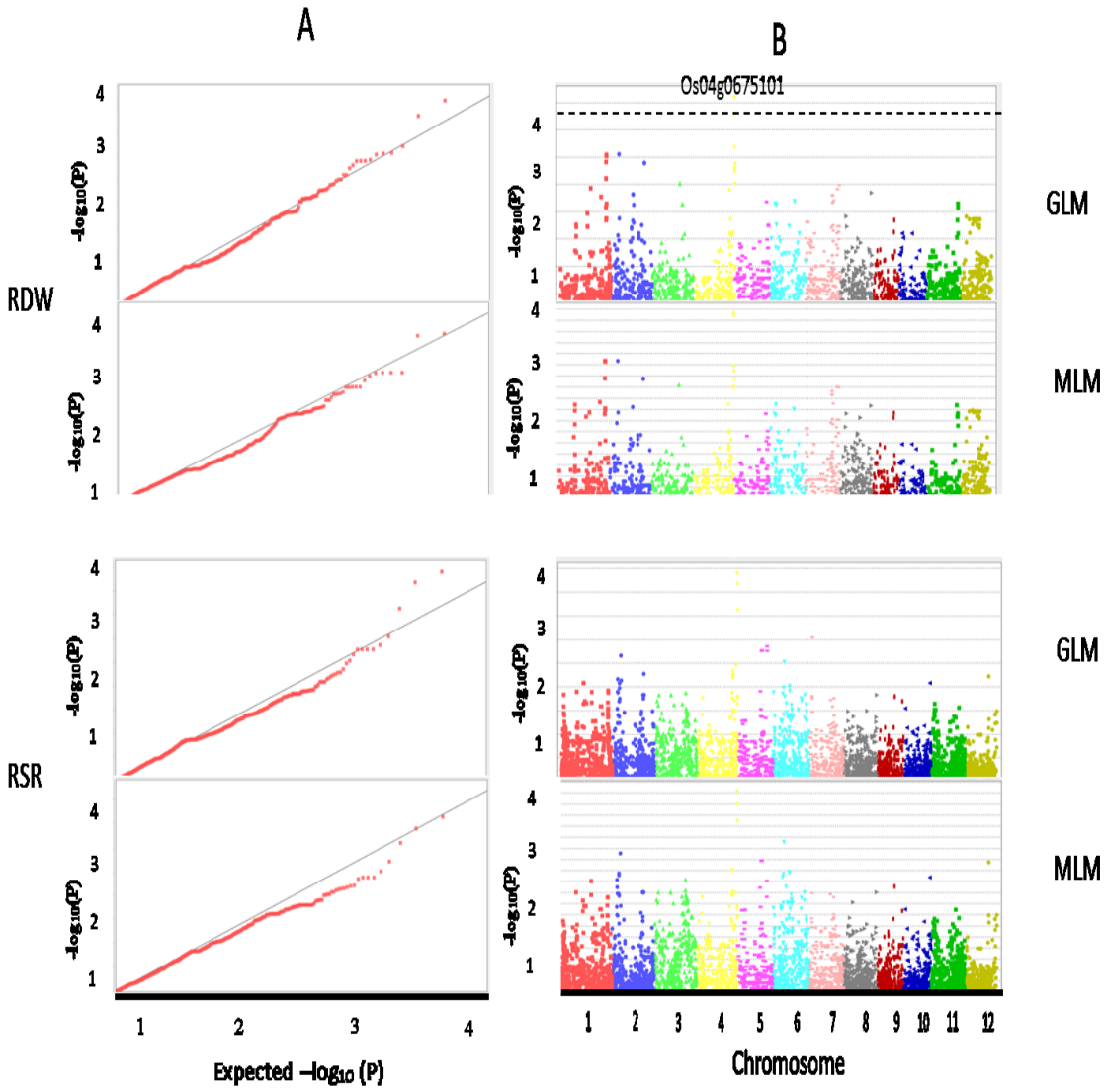

Figure 6.13 GWAS analysis for root dry weight (RDW) and root shoot ratio (RSR). For each of the two-statistical model employed, the Quantile-Quantile plot (A) and the Manhattan plot (B) is shown. A threshold of for GLM-P was applied.

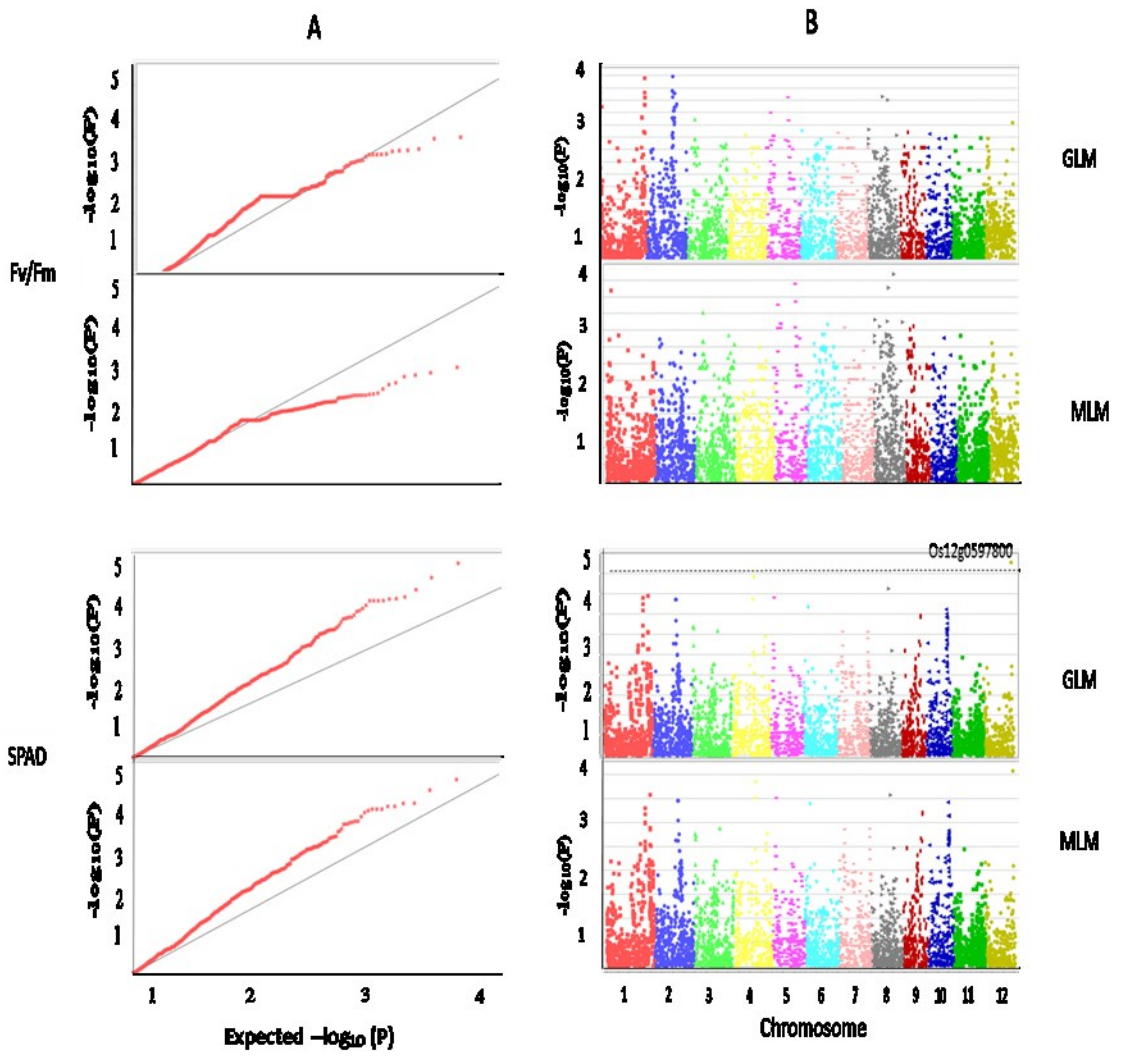

Figure 6.14 GWAS analysis for Fluorescence (Fv/Fm) and SPAD. For each of the two-statistical model employed, the Quantile-Quantile plot (A) and the Manhattan plot (B) is shown.

In the Manhattan plots, all SNPs are plotted on the x-axis according to their position on each chromosome, against their association with cotinine level, as shown on the y-axis as –log10 p-value (the significance of the association). The observed distribution of p-values (y-axis) against the expected distribution of p-values under the null hypothesis (x-

axis) are presented in the QQ plot for GLM (above) and MLM (below). As shown in Figures 6.6, 6.7, 6.8, 6.9, 6.10, 6.13, and 6.14, strong association signals located were detected in the PH, LA, SDW, SHDW, LRL, RN, RSR, and SPAD, respectively when using the GLM model. Accordingly, the GLM model was chosen for GWAS analysis in case of all these traits. Even though MLM allowing to correct for population structure and relatedness respectively should eliminate false positive signals increasing the power in detecting true associations, the simple MLM model performed worse than the GLM model for all these traits considered. However, as shown in figure 1.10 strong association signals located were detected in the NRF and NRC when using the MLM model. Accordingly, the MLM model was chosen for GWAS analysis in case of NRF and NRC traits. The simple GLM model (which does not consider population structure) performed worse than the MLM model for NRF and NRC traits. When the GLM model was used, the distribution of observed $-\log_{10}$ (P-values) in the Q-Q plot analysis strongly deviated from the expected distribution in case of no association (P-values lying on the diagonal line), which lead to a high level of false positive signals.

In the GWAS analysis, population structure and close relationship between kins that is unaccounted for in the model may cause a spurious association between traits and markers (Yu et al., 2006). General linear model corrects for population structure, while MLM takes both population structure and familial relatedness into account. Both GLM and MLM control the genomic inflation effectively and have been widely used in GWAS of soybean traits (Zhang et al., 2016; Wen et al., 2015). Another hand, MLM, allowing to correct for population structure and relatedness respectively should eliminate false positive signals due to close kinship, thus increasing the power in detecting true

associations. The number of significant associated SNPs and their phenotypic contributions are similar to reports of drought tolerances in Brassica napus, alfalfa and soybean (Buckler et al., 2009: Huang et al., 2008; Barrett et al., 2005). These results confirmed that genotypes set, despite its small size, are still finding some SNPs associated with traits of interest. When these two models were used for TN, LN, LDW, TDW, CLR, RSA, ARD, RV, NRT, RDW and Fv/Fm the distribution of observed $-\log_{10}$ (P-values) in the Q-Q plot analysis has strongly deviated from the expected distribution in case of no association (P-values lying on the diagonal line), which may indicate a high level of false positive signals. However, this seemed to be less of a problem with the MLM, in most traits.

In conclusion, this preliminary GWAS on drought-shoot, root, and physiological traits indicated that a substantial phenotypic and genotypic diversity exists in the rice genotypes, despite the predicted narrow genetic basis. This study demonstrates that GWAS of rice genotypes can be used for genetic mapping of several traits with each other at a soft resolution. Moreover, direct resequencing of rice genotypes provides a wealth of sequence polymorphisms and high association resolution in GWAS, despite modest rates of significant of SNPs in this study.

CHAPTER VII

GENERAL SUMMARY AND CONCLUSIONS

There is an urgent need to understand genotypic variability among crop genotypes and to improve crop tolerance to abiotic stresses to meet the food security challenges for growing global population. Since it takes 10-30 years to introduce a novel trait of importance through breeding, developing screening tools to characterize morpho-physiological and genetic traits across a wide range of crop genotypes is urgently warranted to augment breeding efforts. In this study, the objectives of this study were to characterize the genetic variability of 100 elite rice lines for early-season vigor, growth and physiological plasticity, and drought and temperature tolerance. To accomplish these objectives, five studies were conducted. In study 1, 100 rice elite genotypes consisting of several cultivars and experimental breeding lines were characterized for early-season vigor using several shoot and root morphological and physiological traits. In study 2, variability in growth and yield and physiological plasticity were evaluated during vegetative and grain-filling stages. In study 3, genotypic variability in response to drought stress tolerance using morpho-physiological traits including roots was assessed under pot-culture conditions in a mini-greenhouse environment. In study 4, low- and high-temperature tolerance was assessed on select rice cultivars/hybrids during early-season. In study 5, the 100 rice genotypes were used to identify and validate SNP

markers, and genome-wide association analysis to generate genotypic and phenotypic data with the objective of identifying new genetic loci controlling drought stress traits.

Significant genotypic variability was observed for morpho-physiological traits for early season vigor. The limited physiological parameters such as chlorophyll content using SPAD and fluorescence (Fv/Fm) measured in this study were not different among the genotypes and may not be useful as screening tools in rice during early season. The cumulative vigor response index (CVRI) derived from the root, shoot, and physiological parameters, however, showed not only variability among the rice genotypes, but also showed a significant positive correlation with the shoot and root parameters. This infers that shoot and root morphological traits are important in identifying variability and to classify rice genotypes into various vigor groups. Also, using principal component analysis, we were able to identify total plant weight, root length, leaf weight, total tiller numbers, root volume, surface area, and numbers, and shoot weight as the best parameters to define the vigor response stability of rice genotypes. The principal component component analysis (PCA) and CVRI methods used collectively in the present study identified low, moderate and high stable rice genotypes. Based on these screening methods, the rice genotypes, N-22, REX, IRGA 409, RU1303138, and RU0603075, were identified as high vigor genotypes and may be useful to develop new rice lines with high vigor indices.

The physiological parameters such as photosynthesis, stomatal conductance and water-use efficiencies along with several growth and developmental traits during vegetative and grain-filling stages showed significant variability among the 100 rice

genotypes. Total tillers, specific leaf area, carotenoids and stomatal conductance at prior to flowering and photosynthesis during grain failing stages contributed significant variability among the rice lines. In addition, shoot weight, individual grain weight, and grain production efficiency at maturity also added variability among the rice lines. These traits could be helpful for breeders to be used in large populations as screening tools for the respective during vegetative andgrain filling stages, and yield attributes. Based on CVRI and standard deviation values, five vigor groups were identified; (4, 18, 36, 34, and 8%) of genotypes were classified as very low, low, moderate, high, and very high vigor index , respectively at vegetative stage, while (6, 28, 36, 20, and 10%) of genotypes were classified as very low, low, moderate, high, and very high vigor index, respectively at grain filling stage, and (11, 25, 44, 15, and 5%) of genotypes were classified as very low, low, moderate, high, and very high vigor index, respectively at harvest stage. Rice genotypes N-22, 14CLPYT033, and REX were identified as very high and high vigor response index groups, respectively. On the otherhand, genotypes RU1401145, RU14031126, RU1504198, RU1201024, LAKAST, RU1303138, 14CVPYT094, and CAFFEY showed moderate and low vigor response index groups, respectively. Significantly high correlation between CVRI and physiological vigor response indices ($R^2 = 0.82$) indicate that gas exchnage traits could be used as selection criterion in indentifying high vigor groups during vegetative and grain-filling stages.

In another study, selected rice cultivars (four) and hybrids (two) were evaluted for temperature responses. The very low temperature treated plants showed significantly lower shoot and root growth and developmental parameters. Leaf area and chlorophyll

content (SPAD) were greatly affected by low temperature treatments compared to other traits measured. On the otherhand, very high temperature caused significant decrease in some parameters like leaf area, root dry weight, root length, and number of root tips. However, high temperature showed a significant increase in the root, shoot, and physiological traits, compared to optimum temperature. The highest significant increase was noted in leaf dry weight (201%) and the lowest significant increase was observed in chlorophyll content (101%). These results indicate that very low-temperature is more harmful than a very high temperature to rice plant growth and development. The moderate coefficient of determination between total low and total high temperature response indices ($r^2 = 0.69$; n=6; $p > 0.01$) indicates that cold and heat tolerance mechanisms are different and selection must be made independently in developing tolerance to low and high temperatures. However, a strong, positive, and linear coefficient of determination between total low or high temperature response indices and total shoot and root low- or high-temperature response indices, respectively, for the studied six rice cultivars. This implies that shoot and root traits are vital for selecting cold and heat tolerance during early establishment of rice cultivars. Breeder could potentially use low and high-temperature tolerant cultivars identified in this study to improve high yielding genotypes for future environments that could benefit for rice producers aiming to increase rice yield.

In final study, the 100 rice genotypes revealed ample genetic diversity with respect to their response for all traits measured under early season drought stress. The low-cost prefabricated module used as screening platform enabled efficient dissection

of plant traits. Drought exposure significantly affected all of the genotypes for the growth parameters that influenced processes essential for healthy canopy establishment, which subsequently is pivotal for reproductive growth and final yield recovery. Root scan imaging using WinRHIZO revealed extensive insight into the functional architecture of roots under stress and results revealed coherence of the scan capture with the overall performance of the genotype that can potentially be exploited for screening purposes. Based on the CDSRI-based classification, the genotypes N-22 and RU1402174 were the most tolerant with respect to most of the traits measured under drought stress and Cheniere was the most sensitive among the genotypes to drought. The cultivar N-22 is a rice genetic donor that has been used extensively in previous drought response studies and has shown tolerance to heat and other stresses as well. Retrospective insight on the phenotypic potential of a genotype is essential for its effective use to meet research objectives. Thorough early season drought stress tolerance screening using the platform in this study resulted in the identification of promising genotypes can be harnessed by breeders for increasing the level and improving the sustainability of rice production to meet future demands. Further, there is need to test these rice genotypes under different growth stage including reproductive stage for their response to soil moisture stress condition.

A genome-wide association analysis conducted using high-density SNP markers and 21 morpho-physiological traits indicators of the drought stress in a mapping population consisting of 100 rice genotypes were conducted. For the general linear model (GLM) and Mixed linear model (MLM), a total of 33 SNPs distributed on chromosome.

1, 3, 4, 5, 6, 8, 9, 10, 11, and 12 were significantly associated with most of the morpho-physiological traits. With the implementation of GLM and MLM models, the major significant drought stress SNP for morpho-physiological traits was confirmed for leaf area on chromosomes 6 with p-value 6.5×10^{-11}, a minor significant drought stress for shoot growth traits was confirmed for number of root on chromosomes 11 with 9.1×10^{-5}. Diversity analysis using the neighbor-joining method defined the three subgroups of 100 rice genotypes were poorly discriminated. We also found some of the rice genotypes with a long branch length. The number of SNPs per chromosome of all morpho-physiological traits was ranged from 305 SNPs on chromosome 5 to 603 SNPs on chromosome 1. The increased availability of low-cost genome-scale sequencing technologies has led to a dramatic decrease in sequencing costs. Single nucleotide polymorphism (SNP) is currently the most preferred approach for sequencing DNA genomes. The GWAS and forward genetic screening approaches have identified association signals and novel potential candidate genes that can be useful for genetic transformation. The newly identified significant SNP markers for drought stress at the seedling stage will benefit breeders in developing drought tolerant cultivars by assisting in parent lines selection, trait introgression, and evaluation of germplasm.

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APPENDIX A

MATERIALS AND APPARATUS FOR DNA EXTRACTION

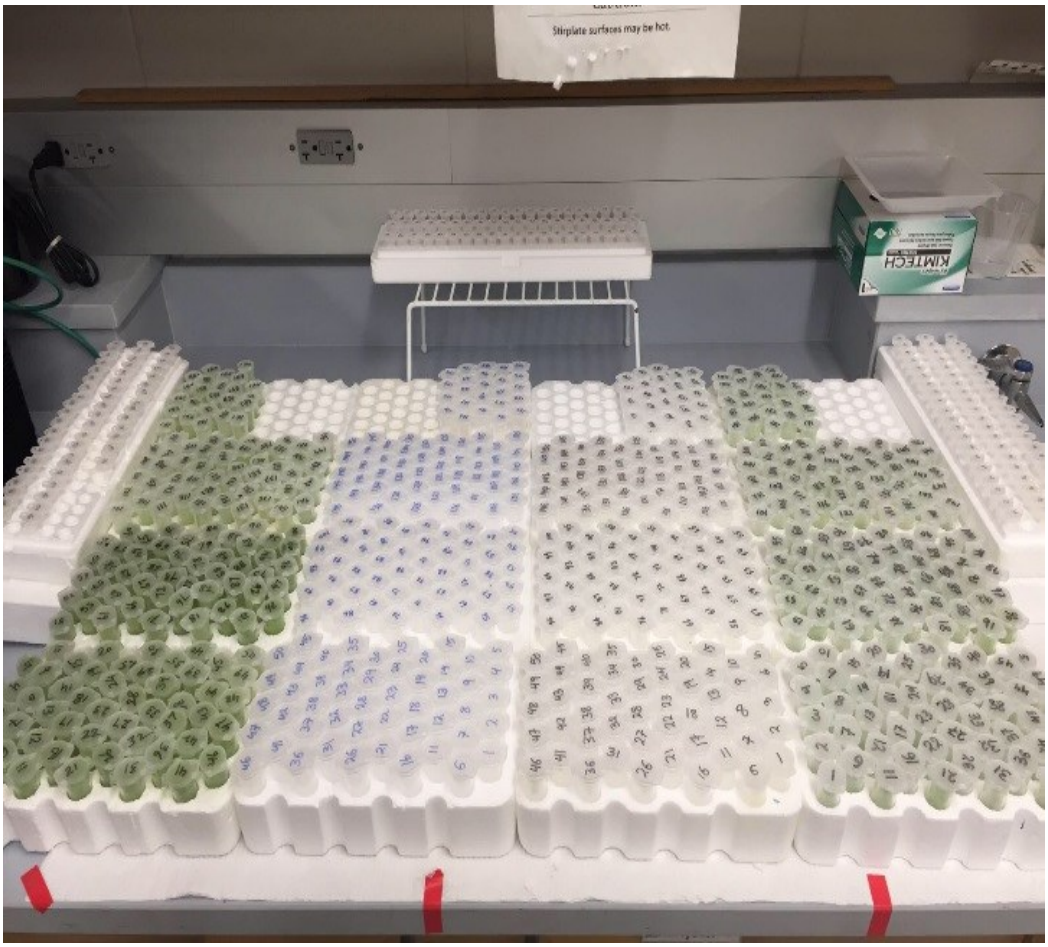

Figure A.1 Collected and ground samples of 100 rice genotypes for DNA extraction

Figure A.2 Transferring aqueous layer into new tubes under fume hood

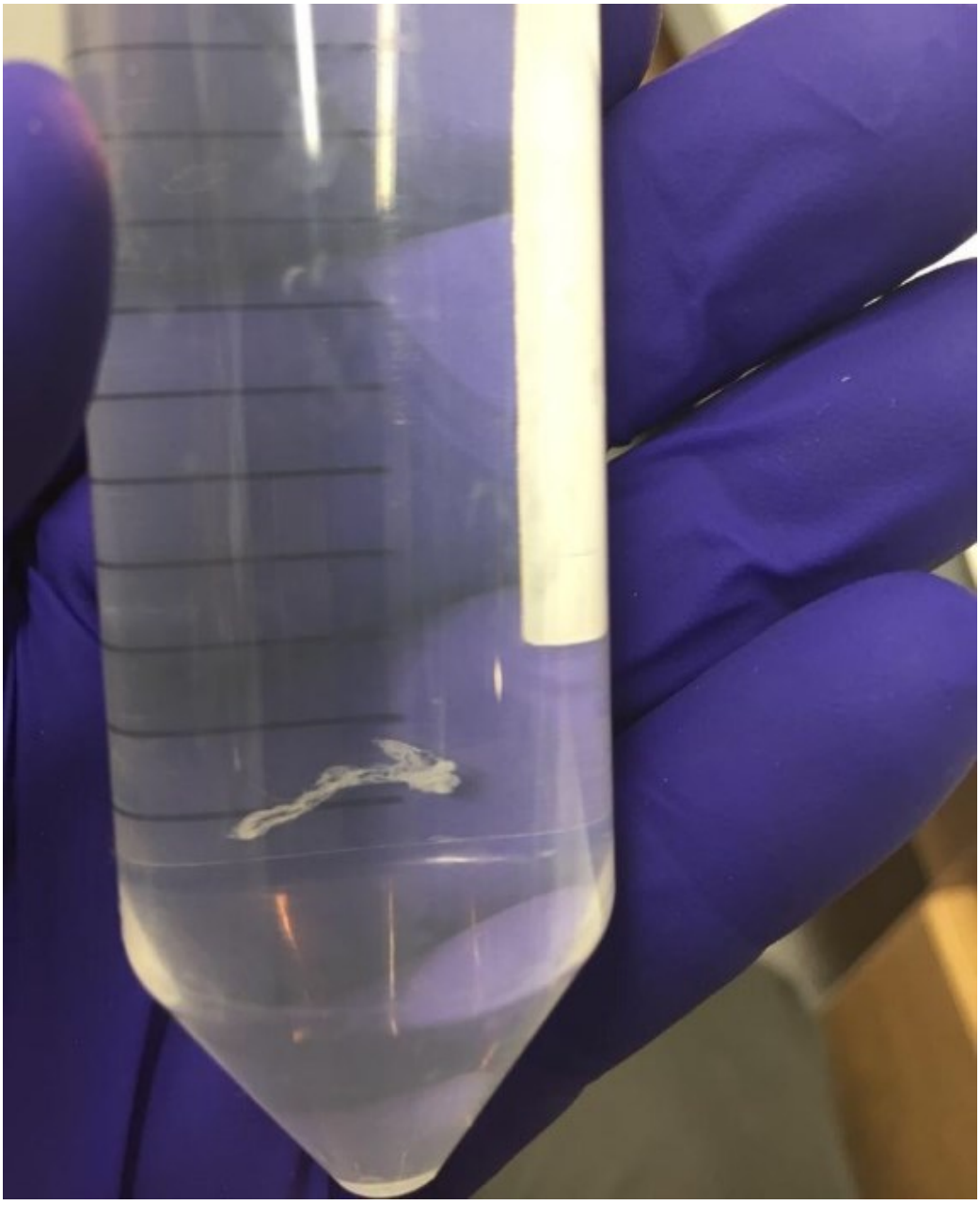

Figure A.3 Condensed DNA after precipitation

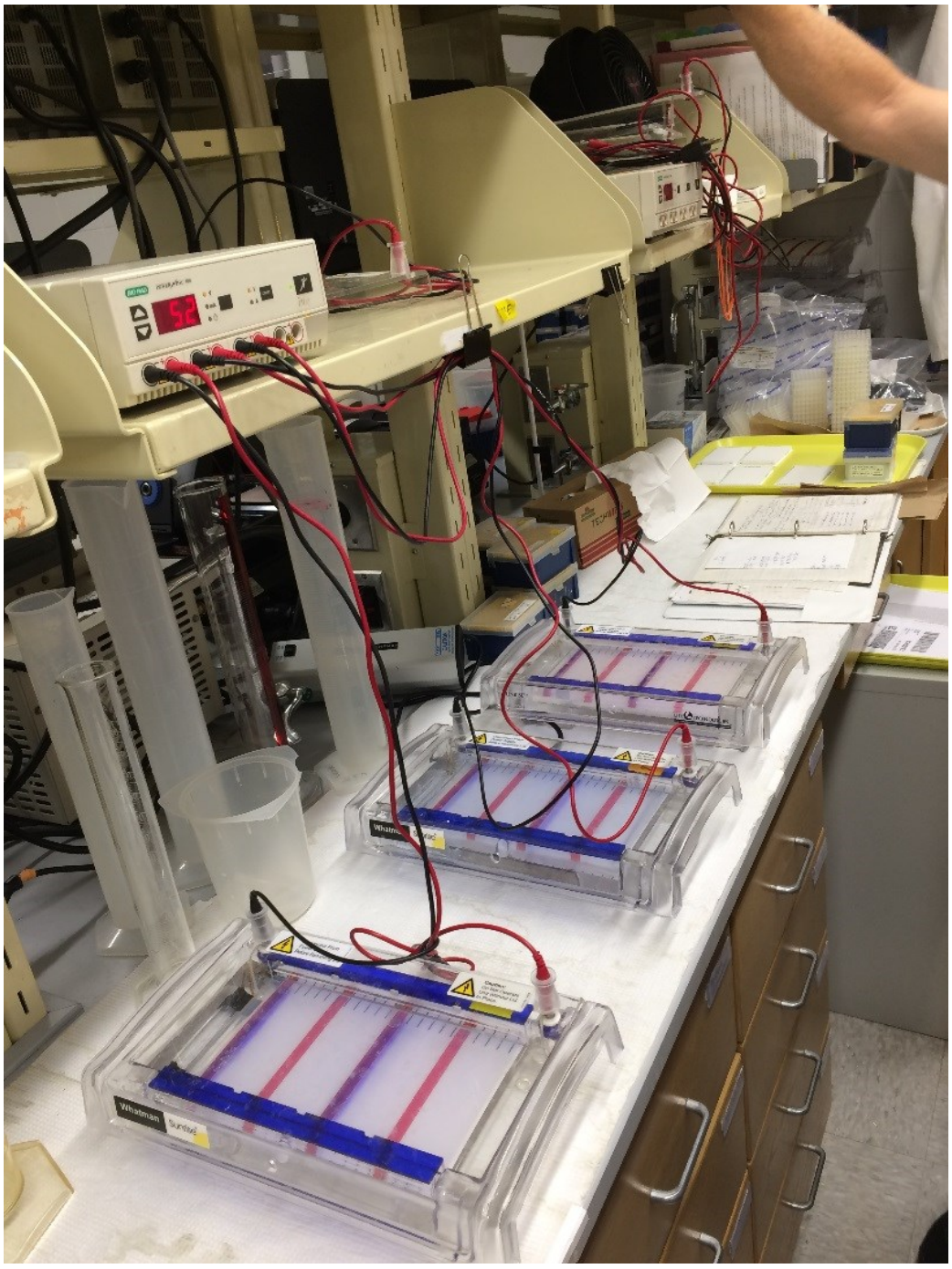

Figure A.4 Gel electrophoresis apparatus running for DNA quality determination (quality gel)

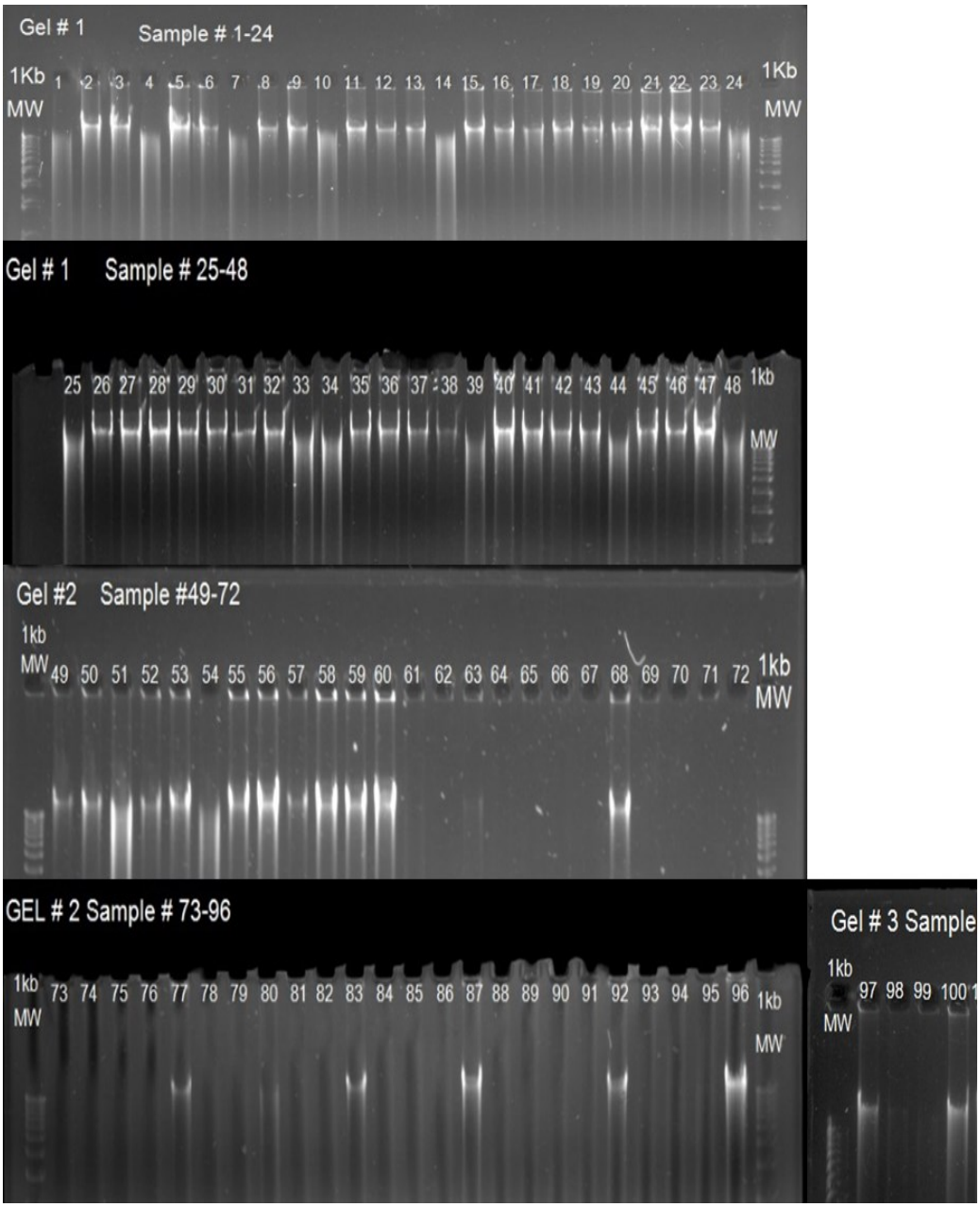

Figure A.5 Annotated quality gel pics, the intensity of bands show the quality of extracted DNA for each sample.

Figure A.6 Nano-drop Spectrophotometer (ND-1000) for the determination of DNA concentration

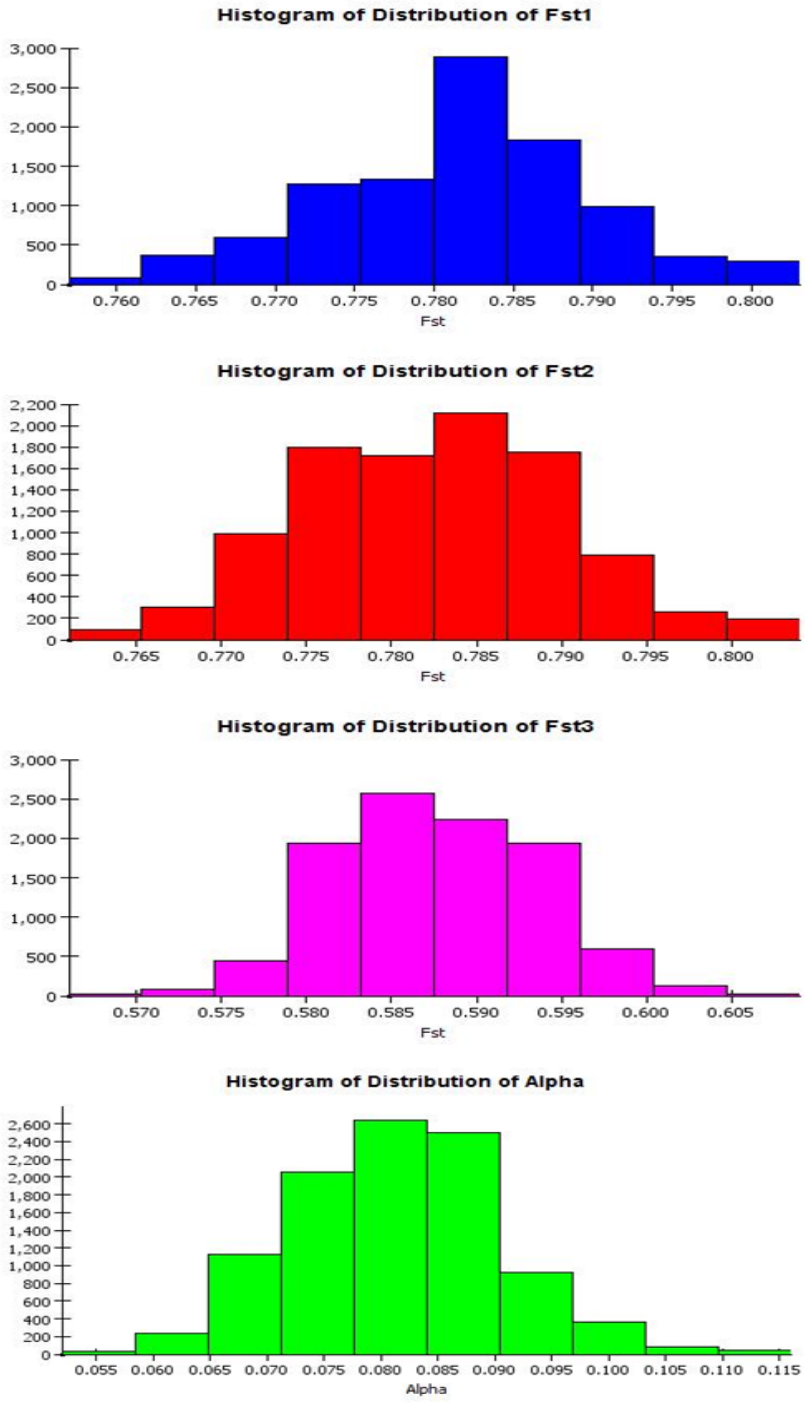

Figure A.7 Histogram of distribution of Fst1-3 and Alpha

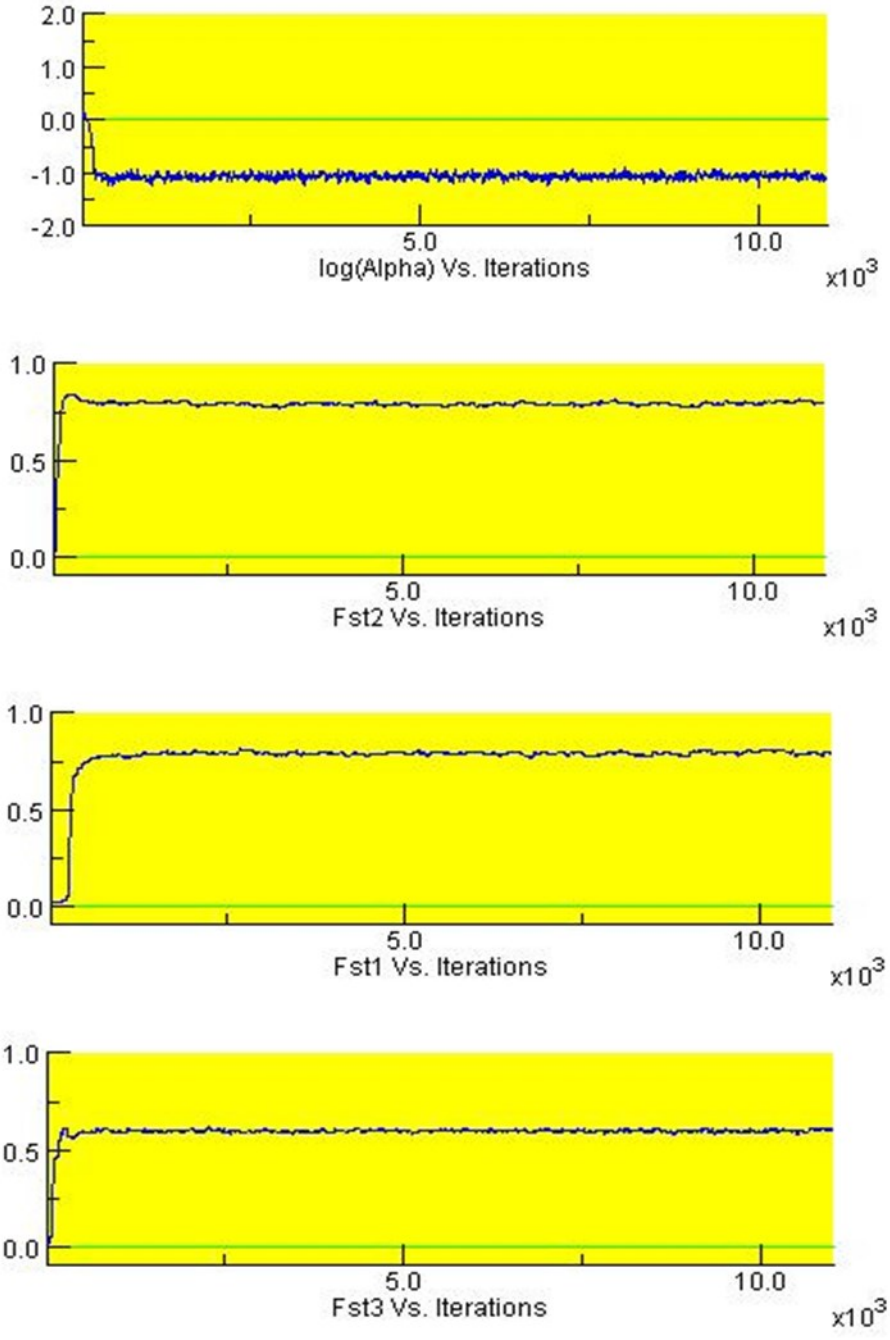

Figure A.8 Fst1-3 for k-cluster 3.