agronomy

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Response of U.S. Rice Cultivars Grown under Non-Flooded Irrigation Management

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Received: 25 November 2019; Accepted: 20 December 2019; Published: 31 December 2019

Abstract: Achieving food security along with environmental sustainability requires high yields with reduced demands on irrigation resources for rice production systems. The goal of the present investigation was to identify traits and germplasms for rice breeding programs that target effective grain production (EGP) under non-flooded field systems where the crop can be subjected to intermittent water stress throughout the growing season. A panel of 15 cultivars was evaluated over three years regarding phenological and agronomic traits under four soil moisture levels ranging from field capacity (29% volumetric water content; VWC) to just above the wilting point (16% VWC) using subsurface drip irrigation. An average of 690 ha-mm ha^{-1} water was applied for the 30% VWC treatment compared to 360 ha-mm ha^{-1} for the 14% VWC treatment. The average soil moisture content influenced several traits, including grain quality. Regression analysis identified six traits that explained 35% of the phenotypic variability of EGP. Four varieties (PI 312777, Francis, Zhe 733, and Mars) were found possessing significant slopes for 10 or more traits that respond to a range in soil moisture levels, indicating that they may offer promise for future rice breeding programs. Furthermore, based on the contrasting responses of four parent cultivars, two mapping populations were identified as potential genetic resources for identifying new quantitative trait loci/genes for improving EGP of *tropical japonica* rice varieties.

Keywords: drought; water stress; yield; rice; water productivity; food security

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops on the planet [1]. Although rice yield improvements attributed to breeding and cultural advances were achieved in the 20th century, trends over the last 20 years indicate a deceleration in the rate of yield gains for many rice-producing regions [2,3]. In addition, by 2050, the global population is expected to reach nearly 10 billion, which will further strain agricultural systems and natural resources to produce enough to feed the world. There are worldwide concerns for the long-term sustainability of irrigation-intensive crops; projections indicate 20–60 Mha of cropland may change from irrigated to rainfed management systems in the next 30 years, highlighting the need for reducing the water required for food production [4]. Rice production utilizes the most irrigation resources, followed by wheat and corn [5,6]. The United States of America (U.S.A.), although not a major producer of rice, exports half of its production, accounting for 7% of the rice in world trade, and is among the top five rice exporting countries [7]. The south-central region of the United States (U.S.) is the primary rice growing area, with the state of Arkansas producing half of the country's crop. Despite moderate rainfall in this area, approximately 80% of the irrigation demands for rice are met by pumping from underground aquifers, especially the Mississippi River Valley alluvial aquifer [8,9]. Over the past four decades, this alluvial aquifer has been depleted at an alarming rate of

30 to 45 cm a year [10–12]. Moreover, with increasing population growth, there is increased competition between agricultural and urban sectors for freshwater resources, which is expected to grow further in the next 30 years, increasing the need for sustainable food production [13–15]. Davies, et al. [16] suggest that irrigation management must shift from maximizing production per unit area to per unit of water applied, which is often termed as water productivity, but Blum [17] argued that effective use of water for grain yield (GY) should be the breeding target compared to the water use efficiency under limited moisture conditions. Rice research and on-farm field trials have demonstrated that several irrigation management systems, such as alternate wetting and drying (AWD), multiple-inlet, furrow, pivot irrigation, and zero-grade fields have the potential to increase water savings and the long-term sustainability of rice production [18–23]. Furthermore, rice produced under AWD, aerobic, or low soil moisture conditions has additional benefits, such as reduced greenhouse gas emissions and lower arsenic accumulation in the grain [24,25]. Despite these benefits, a major concern for producing commercial rice under reduced irrigation is GY penalties ranging from 13% to 32% when compared to conventional flood irrigation, but genetic variation in rice germplasm may serve to correct this concern [24,26,27] by deploying alleles through breeding that result in effective use of water and crop resiliency to abiotic stress [17,28]. There has been worldwide interest in evaluating rice germplasm for production under reduced irrigation systems, particularly in areas where access to irrigation resources is limited and/or incidence of drought is expected to increase [29–32]. However, such efforts have been more limited in the U.S. where rice research has historically been focused on achieving higher yield potential without production constraints [26,30].

With the availability of whole genome sequencing there is a greater understanding of the genetic population structure found in cultivated rice [33,34]. Most U.S. cultivars trace to the *tropical japonica* (TRJ) subpopulation [35], whereas most global rice acreage is planted with cultivars derived from the *indica* sub-population [36]. Genomic analysis offers the opportunity to identify gene-trait linkages that will facilitate breeding efforts to develop new cultivars that efficiently use water for grain production. Recently, several comprehensive efforts for screening rice germplasm for field performance under water deficit conditions have been conducted [29,37–39]. These studies showed that reoccurring water stress commonly seen while using water-saving irrigation practices may reduce rice GY by 10%–59%. In addition, germplasm identified to be tolerant to drought at a particular stage may be different from that which is tolerant to a range of drought timings and severities [38]. In general, biochemical, physiological, and biomass production traits were found to be related to higher grain yield under drought stress, and a few genebank accessions were identified as being suitable for rice breeding for drought tolerance [26,29,40–42]. However, no consistent relationship was observed between component traits (such as root dry weight, drought response index, or stomatal conductance) and potential GY. This may be due to the complexity of traits that respond to drought, such as plant height, flowering time, flowering duration, loading and unloading rates of xylem and phloem, that may have positive or negative effects on GY depending on the timing, severity, and duration of the drought [43]. Recently it has been demonstrated that crops which accumulate carbohydrates in leaves and maintain stomatal conductance under water stress conditions sustain GY production [26,44,45]. In other reports, robust quantitative trait loci (QTL) and traits with high heritability under water stress conditions have been identified, indicating that improvement of such a complex trait is possible through breeding [46,47]. However, the traits of importance may vary depending on the spatial and temporal levels (i.e., seedling, pre- heading, heading, or grainfill) of the water stress and may be dictated by whether the stress tolerance is based on escape, avoidance, or resistance mechanisms [14,48,49]. In addition, field evaluations can be compromised by variability in soil moisture and the amount and frequency of uncontrolled precipitation during the cropping season.

The present study was undertaken to evaluate TRJ and *indica* rice cultivars adapted to the southern U.S. with the goal to identify key traits and genetic resources related to the resiliency of GY production when grown under aerobic field conditions supplemented using four irrigation levels.

2. Materials and Methods

2.1. Plant Material, Experimental Design, and Cultural Management

The 15 varieties chosen for this study (Table 1) represent cultivars that were grown on major commercial acreage and span approximately 30 years of breeding in the southern U.S.A. Among these, 11 cultivars were from the TRJ subpopulation and four were international introductions from the *indica* subpopulation—all of which have been used in southern U.S. rice breeding programs. The cultivars include long and medium grain market classes as well as varieties like Saber and Rondo that have been grown on minor acreage because they are used for specialty purposes such as parboiling and rice flour markets, respectively. From these 15 cultivars, 10 have also been used as parents in developing five gene mapping populations (designated from A to E, Table 1) at the Dale Bumpers National Rice Research Center (DBNRRC), United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Stuttgart, AR, U.S.A. These bi-parental crosses included A: PI 312777/Katy; B: Cybonnet/Saber; C: Francis/Rondo; D: KBNT-1-1 (a low phytic acid mutant of Kaybonnet)/Zhe 733; and E: Lemont/Teqing. The selection of this genetic panel thus focuses on germplasm that is commercially relevant to on-going southern U.S. breeding efforts.

Table 1. Genetic subpopulation structure, southern U.S.A. commercialization, grain shape and bi-parental mapping populations developed among 15 varieties used in this study.

Variety	Sub-Population [1]	Year of Release for Production in U.S.A.	Commercialized Acreage in Southern U.S.A.	Grain Shape	Parents of Mapping Population	Bi-parental Mapping Population
Katy	TRJ	1990	Major	Long	A1	A
PI 312777	*Indica*	.	None	Short	A2	
Cybonnet	TRJ	2006	Minor	Long	B1	B
Saber	TRJ	2004	Minor	Long	B2	
Francis	TRJ	2007	Major	Long	C1	C
Rondo	*Indica*	2010	Minor	Long	C2	
Kaybonnet	TRJ	1994	Major	Long	D1	D
Zhe 733	*Indica*	.	None	Long	D2	
Lemont	TRJ	1985	Major	Long	E1	E
Teqing	*Indica*	.	None	Medium	E2	
Roy J	TRJ	2013	Major	Long	.	.
Lagrue	TRJ	1995	Major	Long	.	.
Mars	TRJ	1979	Major	Medium	.	.
CL 151	TRJ	2011	Major	Long	.	.
Jupiter	TRJ	2006	Major	Long	.	.

[1] TRJ = *Tropical japonica*.

Field experiments were conducted over three years (2014–2016) at the DBNRRC and the University of Arkansas Rice Research and Extension Center, in Stuttgart, AR, U.S.A. (34.46286° N, 91.39944° W). Soil at the location is characterized as a Dewitt silt loam (fine, smectitic, thermic, Typic Albaqualfs; https://soilseries.sc.egov.usda.gov/OSD_Docs/D/DEWITT.html) with a slope generally < 1% and the A and E horizons ranging from 18 to 60 cm in depth. Prior to planting, a subsurface drip irrigation (SDI) system was installed with 15 mil drip irrigation tapes (Jain Irrigation, India) placed in the rhizosphere at 20 cm below the soil surface with one drip tape between the two rows of each plot and 81.2 cm between irrigation tapes in adjacent plots (Supplemental Figure S1). Time domain transmissometry (TDT) moisture sensors (Digital TDT, Acclima, Meridian, ID, USA.) were installed in each treatment zone between the two experimental rows at a depth of 18 cm, just above the placement of the irrigation tapes. The targeted irrigation levels were based on plant available soil moisture calculated as the difference between field capacity and the wilting point for the Dewitt silt loam using soil-water characteristics derived from Saxton and Rawls [50]. The percent plant available water was calculated to determine depletions of 0%, 15%, 30% and 60%. These depletions correspond to the volumetric water content (VWC): 30% VWC [irrigation treatment 1, equivalent to field capacity (FC)]; 24% VWC (treatment 2); 20% VWC (treatment 3); and 14% VWC (treatment 4) as measured by the TDT moisture sensors and portable dielectric sensors (Theta probe, Dynamax Corporation, Fresno, CA, USA.) calibrated to the soil. The total amount of rainfall was determined from an on-site weather station. Irrigation water was applied with an emitter discharge rate of 3.20 L h^{-1}.

Approximately one week prior to planting, the field was sprayed with a non-selective herbicide to kill winter weed growth. The field was then tilled at a depth of 3 cm just prior to sowing. Plots were drill seeded at a depth of 2.5 cm using a seed planter (Almaco Nevada, IA, USA) with 40.6 cm row spacing on 26 May 2014, 12 June 2015, and 4 May 2016. Two-row plots, 2.1 m in length, were sown using a seeding rate of 108 kg ha^{-1}. All plots were flood irrigated for 48 h after sowing to fully saturate the soil to assure uniform seedling stands after germination. Three days after seedling emergence, 135 kg urea N ha^{-1} was applied as a single dose, and the field was fully irrigated two more times before initiating the irrigation treatments. Pre-and post-emergence herbicides were applied as per the recommendations for the region. At V5, the five-leaf stage [51], the plots were thinned to achieve a plant population of 19 plants m^{-2}, and plants in the middle of the plot were used for subsequent measurements. Fungicide was applied before booting and at the heading stage as a preventative measure.

The experiment was conducted using a split-plot design with three replications, four irrigation treatments as the main plots (laid out in un-replicated strips), and 15 varieties as the subplots (Figure S1). Because there was a 0.83% slope in the field perpendicular to the irrigation emitter tapes, four buffer rows (cv. RoyJ) were planted between each irrigation zone to prevent water seepage between irrigation treatments, and the treatments were arranged in the same manner each year with the field capacity treatment at the low end of the field. In addition to the TDT sensor in each irrigation treatment, soil volumetric water content (% VWC) was monitored at the 5 cm soil depth for each experimental unit using a portable SM-150 probe (in year 2014) (Dynamax Corporation, Houston, TX, USA) or a POGO Pro (Stevens Water Monitoring Systems Inc., Portland, OR, USA) (in years 2015 and 2016) during the growing season every 1 to 2 weeks depending on rainfall and prior to irrigation being applied. The seasonal soil moisture was determined for each experimental unit as the average of these approximate bi-weekly soil moisture readings. The irrigation treatments were maintained until physiological maturity and harvest of all varieties. In 2015, irrigation treatment 3 was dropped from the study due to heavy bird predation in most of the plots.

2.2. Trait Measurements

Days to heading was determined in 2015 and 2016 for each plot based on the number of days from plant emergence to 50% heading in the plot. Determination of days to maturity was based upon the number of days from plant emergence to physiological maturity. Days of grainfill was calculated as the difference between days to maturity and days to heading. Final plant height (cm) was measured from the soil surface to the tip of the panicle just prior to harvest. Growing degree days (GDD) were determined for each experimental unit according to the method of Counce et al. [52] with GDD1 being based upon the period between the date of emergence and the date of maturity, whereas GDD2 was determined between the date of heading and the date of maturity (i.e., grainfill). In addition, during 2016, plant growth rates were determined for the periods prior to heading and during grainfill. For the vegetative phase, the plant height was measured at 71 DAE, which was prior to heading in any of the plots, to calculate the growth rate (cm day^{-1}). For the reproductive phase, the growth rate was determined from the height gained between 71 DAE and the maximum plant height (at heading) divided by the number of days during this period and is presented as cm day^{-1}.

At panicle maturity, the R9 stage [51], two to four of the spaced plants were hand harvested, threshed, and the grains were dried to 12% moisture for determining the GY per plant (g $plant^{-1}$). For each experimental unit we calculated the effective grain production (EGP) as a ratio of GY (g $plant^{-1}$):average seasonal volumetric water content (% VWC). A subsample of approximately 20 g of rough rice was hulled using a rice sheller (model TH035A, Satake Engineering Co. Ltd., Tokyo, Japan) to produce whole grain brown rice. Grain length (mm), grain width (mm), and grain chalk (% opaque area) were determined on approximately 100 grains of brown rice with WinSEEDLE ProV.2007E (Regents Instruments Inc., Sainte-Foy Quebec, Canada) software and an Epson Perfection photo scanner (model V700, Epson America Inc., Long Beach, CA, USA) [53]. The grain length:width ratio was calculated, and thousand kernel weight (TKW, g) was also determined. Grain thickness (mm) was determined on approximately 20 brown rice kernels using hand calipers.

The plots were scored for leaf and panicle stress at the R7 stage in 2014 and 2016. Leaf stress was recorded using a scale of 1 (little or no leaf rolling) to 5 (severe leaf rolling). Panicle stress was determined on a scale of 1 (panicles fully emerged from the boot and little sterility) to 8 (panicles not emerged from the boot or highly sterile). Canopy temperature (°C) was recorded in 2014 as an average of three plants using the upper most leaf or flag leaf during the V13 (prior to booting) and R4 (at heading) stages of plant development using a digital laser infrared thermometer (Etekcity Corporation, Anaheim, CA, USA) between 10:00 and 14:00; all measurements were collected on the same day for consistency [54]. In 2016, canopy temperature was measured in the same method as in 2014 but at 85 DAE. On this same date in 2016, a SPAD 502 Pulse (Spectrum Technologies, Aurora, IL, USA) was used to measure the chlorophyll content of the most recent fully emerged leaf as explained in Barnaby et al. [26].

Ascorbic acid (AsA) content was measured in 2016 using the uppermost leaf or the leaf just below the flag leaf of each plot (84 DAE). Total AsA content was determined by the ascorbate oxidase assay adapted to a 96-well plate format as previously described [55]. Briefly, all the leaf samples were collected from the field on the same day, immediately frozen in liquid nitrogen, and stored at −80 °C until analysis. Frozen tissue was ground in a freshly prepared 6% *meta*-phosphoric acid solution in deionized water using a mortar and pestle. The resulting slurry was transferred to a plastic tube and centrifuged for 5 min in a benchtop centrifuge at 15,000 g speed. The supernatant was transferred to a fresh Eppendorf tube, and a 300 µL reaction mixture was prepared by mixing the tissue extract with 100 mM potassium phosphate pH 6.9. When all samples were ready for assay, the reaction mixture was transferred to a 96-well plate, and each sample was spiked with 1 unit of ascorbate oxidase (Sigma, St. Louis, MO, USA). The reduced form of AsA was determined by measuring the decline in absorbance A265 relative to the control sample (no enzyme added). The oxidized form of AsA was determined in a similar 300 µL reaction mixture including 40 mM dithiothreitol (DTT), incubating at room temperature in the dark for 30 min, and measuring the absorbance A265. Total AsA was calculated as the sum of reduced and oxidized AsA contents. For calibration purposes, a standard curve with pure L-ascorbic acid (Sigma, St. Louis, MO, USA) was run in parallel to the experiment.

2.3. Statistical Analysis

Analysis of variance (ANOVA) was performed using the generalized linear model (GLM) procedure using SAS v9.3 (SAS Institute, Cary, NC, USA) with year, irrigation, and variety as fixed effects. All factors were tested by the experimental error except for the irrigation effect, which was tested using the irrigation × replication term. The main effects in the model were tested using least square means at a probability level of p <0.05. To determine whether a variety was responsive to differences in soil moisture for each of the plant traits measured, regression analysis was performed by variety, with the dependent variable being the plant trait, the independent variable being average soil moisture, and the Year being included as a fixed effect. A variety was considered sensitive (i.e., responsive) to seasonal soil moisture levels if the slope was significant ($p < 0.10$) and stable if the slope was nonsignificant. Positive slopes indicated that the trait increased in response to increasing soil moisture. The SAS v9.3 Proc Reg procedure was used to develop a regression model with EGP being the dependent variable and using the "stepwise" method. The defaults for entry or removal of a variable into the model were at a significance level of $p = 0.15$. Pearson correlation coefficients (r) among the traits were determined to summarize the strength of the linear relationships between each pair of response variables using the multivariate platform in JMP13.2 (SAS Institute, Cary, NC, USA).

3. Results

During the three cropping seasons of the study, a wide range in annual rainfall occurred, with the highest (396.49 mm) in 2014 and the least (261.62 mm) in 2015 (Figure S2). Although the 2015 study was planted the latest (Julian date 163), it was a relatively cool season with an overall accumulated GDD of 1870 °C, whereas the 2016 planting was the earliest (Julian date 125) and was, at the time, the hottest season on record at the location (an overall accumulated GDD of 2322 °C) (http://www.climatecentral.org/news/2016-was-second-hottest-year-for-us-21034). The average soil moisture, %VWC, over the three years for each irrigation treatment was close to the target values as described in Materials and Methods section and were significantly different, with Irrigation-1 having 29.14 ± 0.50% VWC, Irrigation-2 having 26.14 ± 0.53% VWC, Irrigation-3 having 22.75 ± 0.56% VWC, and Irrigation-4 having 16.47 ± 0.40% VWC ($p < 0.0001$) (Figure 1).

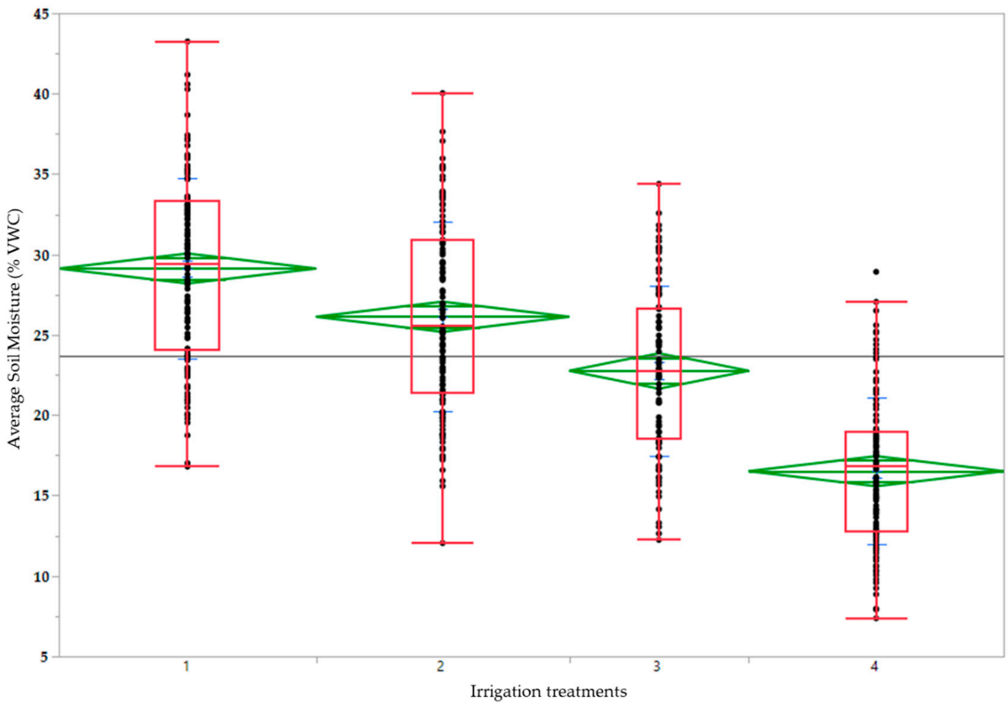

Figure 1. Volumetric water content (% VWC) for 15 varieties evaluated over three years for each of the four irrigation treatments applied via SDI in the field study. The X axis shows four irrigation treatments. The Y axis shows soil moisture (% VWC). Overlapping black dots represent values for each experimental unit within each irrigation treatment. The green diamond shows the mean value of each irrigation treatment. The top and bottom of each green diamond represent the 95% confidence interval. The red rectangles are box plots defined by the quartiles with the median shown as a red horizontal line in each box. The horizontal gray line at 23.71% VWC shows the grand mean across the four irrigation treatments.

3.1. Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) demonstrated that there were significant differences for essentially all traits measured over the three years due to Year and Variety effects ($p < 0.05$) (Table 2). Although the irrigation treatments were significantly different based upon the average seasonal soil moisture (Table 3), differences in days to maturity, EGP, leaf stress score, grain length:width ratio, grain chalk, grain thickness, grainfill days, GDD1, and GDD2 were not detected due to irrigation at $p < 0.05$ (Table 2). Irrigation treatment effects were significantly different for eight of the plant traits measured during the study (Tables 2 and 3). Varieties accounted for most of the model variation except for the traits days to heading and days to maturity, which were explained more by the Year effect, whereas average seasonal soil moisture and canopy temperature were explained more by the Irrigation effect. Interestingly, there were only five traits that had a significant Variety × Irrigation interaction ($p < 0.05$), but the proportion of the total sum of squares in the model accounted for by each of these traits was less than 5%. This demonstrates that even with this small panel of southern U.S. germplasm, the variation due to rice cultivars is far greater than other sources of variation in the model.

3.2. Variety and Trait Response to Soil Moisture Levels

To further explore these genetic differences, regression analysis was performed for each variety to determine which traits were most responsive to a range of seasonal soil moisture levels (Table 4a,b). This regression analysis was performed for 13 traits listed in Table 2, excluding EGP, seasonal soil moisture, GDD1 and GDD2. Depending on the variety, significant slopes were determined for six to twelve traits indicating that these were sensitive to increasing seasonal soil moisture levels ($p < 0.01$). The most responsive varieties (PI 312777, Francis, Zhe 733, and Mars) had significant slopes for ten or more traits. The most stable varieties (Cybonnet, Lemont, RoyJ, and Lagrue) had significant slopes for six or seven traits. However, maturity, grain length:width ratio, and leaf stress were relatively insensitive to increasing soil moisture with four or fewer varieties having significant slopes for these traits. All varieties had significant slopes for days to heading, plant height, GY, and panicle stress, with positives slopes for plant height and GY indicating these traits increased with soil moisture. For canopy temperature, all but one variety (RoyJ) had a significant negative slope, indicating higher temperatures at the lower soil moisture levels. This demonstrates that varieties differ in response to soil moisture for growth and development traits (heading, height), grain dimension traits (length, width, thickness), yield and TKW, and indicators of stress (canopy temperature, panicle stress). However, in this genetic panel, other traits that were not sensitive to soil moisture were more affected by Year (maturity), Variety (chalk, length:width ratio), or unexplained variation (leaf stress).

Table 5a,b summarize the results from the parental pairs that we have used to develop five mapping populations (A–E, Table 1). Here, the populations are compared (Table 5a) for the number of responsive and stable traits having significant or non-significant slopes, respectively, in Table 4. The comparison was made on the number of responsive traits (Table 5b) that were common between the parental pairs and the number of responsive traits that were complementary (e.g., one mapping parent of a population is responsive, and another is stable). Based on the results of the mapping parents, the PI 312777/Katy and the Francis/Rondo populations (A and C, Tables 1 and 5) appear to offer the greatest potential for breeding, as they have the highest combined number of responsive and complementary traits (12) for optimizing production under varying irrigation inputs. Although both populations offer wide genetic diversity (TRJ × *Indica*), the Francis and Rondo parents differ significantly for more traits (chalk, grain weight, grain thickness, grain length:width ratio and leaf stress), thus offering greater selection potential, and both parents have more desirable grain quality traits by the U.S. long grain market as compared to population A parents (Tables 1 and 5b). The Cybonnet/Saber population (B, Tables 1 and 5) offers a somewhat different approach to breeding as compared to Francis/Rondo. Similar to population C, the B parents have five complementary traits and, although both are conventional U.S. long grains, the parents differ significantly for grain quality traits: chalk, grain length, grain width, TKW, and length:width ratio (Table 5b).

Table 2. Analysis of variance from study conducted over three years with four irrigation treatments and 15 rice varieties.

Trait	Total Sums of Squares	Year Effect		Variety Effect		Irrigation Effect		Variety × Irrigation Effect		% of Total Sums of Squares Explained by Significant Interaction
		Year Sums of Squares	*p* Value > *F*	Variety Sums of Squares	*p* Value > *F*	Irrigation Sums of Squares	*p* Value > *F*	Variety × Irrigation Sums of Squares	*p* Value > *F*	
Days to heading	56,372	27,257	<0.0001	13,472	<0.0001	2161	<0.022	1056	ns	-
Days to maturity	47,553	17,453	<0.0001	12,790	<0.0001	295	ns	166	ns	-
Plant height	87,473	22,272	<0.0001	24,948	<0.0001	11,583	<0.006	3908	<0.0003	4.5
GY	115,592	573	ns	31,832	<0.0001	12,046	<0.05	220	<0.005	0.2
EGP	295	21	<0.0001	86	<0.0001	3	ns	15	ns	-
Avg soil moisture	25,072	4869	<0.0001	933	<0.0001	11,716	<0.0001	783	ns	-
Panicle stress	1372	69	<0.0001	135	<0.0001	378	<0.002	63	ns	-
Leaf stress	215	0.05	ns	24	<0.0001	11	ns	20	ns	-
Grain length	132	0.12	<0.05	116	<0.0001	1	<0.001	1.16	ns	-
Grain width	23	0.05	<0.0013	19	<0.0001	0.39	<0.012	0.25	<0.03	1.1
Length:Width	99	0.06	<0.006	90	<0.0001	0.09	ns	0.23	ns	-
Chalk	15,401	553	<0.0001	10,422	<0.0001	49	ns	619	<0.02	4.0
TKW	1661	142	<0.0001	761	<0.0001	161	<0.0001	56	ns	-
Grain thickness	3.55	0.04	<0.0001	2.57	<0.0001	0.015	ns	0.13	<0.02	3.6
Grainfill days	14,892	3055	<0.0001	3146	<0.0001	166	ns	1580	ns	-
Canopy temp ^	4251	111	<0.0003	222	<0.02	1322	<0.012	310	ns	-
GDD1 ^	16,679,867	12,509,660	<0.0001	157,448	<0.0001	40,353	ns	195,696	ns	-
GDD2 ^	3,280,359	420,046	<0.0001	1,043,113	<0.0001	104,726	ns	308,436	ns	-

^ Determined in two years only; TKW denotes one thousand kernel weight; [ns] denotes the values that are not significant at $p < 0.05$; GDD denotes growing degree days.

Table 3. Comparison of means for average soil moisture and eight plant traits having a significant F test for Irrigation treatment.

Irrigation Treatment	Avg Soil Moisture (%VWC)	Days to Heading	Plant Height (cm)	GY (g plant^{-1})	Panicle Stress Score	Grain Length (mm)	Grain Width (mm)	TKW (g)	Canopy Temperature (°C) ^
1	29.1 ^{a}	86.6 ^{c}	91.3 ^{a}	26.2 ^{a}	2.4 ^{d}	6.27 ^{a}	2.27 ^{a}	17.9 ^{a}	31.8 ^{c}
2	26.1 ^{b}	89.5 ^{b}	89.6 ^{a}	26.3 ^{a}	3.2 ^{c}	6.25 ^{a}	2.23 ^{b}	17.8 ^{a}	32.2 ^{c}
3	22.8 ^{c}	90.4 ^{b}	84.5 ^{b}	23.2 ^{a}	3.9 ^{b}	6.18 ^{b}	2.21 ^{b}	17.1 ^{b}	33.6 ^{b}
4	16.5 ^{d}	93.8 ^{a}	79.4 ^{c}	14.4 ^{b}	5.3 ^{a}	6.13 ^{b}	2.18 ^{c}	16.3 ^{c}	35.1 ^{a}

Means values within a column followed by a different letter are significantly different at $p < 0.05$; ^ Determined in 2014 and 2016 only. TKW denotes one thousand kernel weight.

Table 4. Direction and significance of the slope of the regression line for agronomic, grain quality and stress-related traits for each of 15 varieties in response to average growing season soil moisture after controlling for the Year effect. Positive slope indicates response to increasing soil moisture. (**a**) Plant traits; (**b**) Seed traits.

(**a**) Plant traits.

Variety	Canopy Temperature (°C)			Days to Heading			Plant Height (cm)			Grain Yield (g)			Leaf Stress Score			Panicle Stress Score		
	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept
Katy	−0.261	0.012	39.710	−0.610	0.012	108.934	1.322	<0.0001	59.779	0.758	0.001	−8.622	−0.037	0.154	4.086	−0.262	0.000	11.599
PI 312777	−0.180	0.046	37.106	−0.708	<0.0001	112.015	1.201	<0.0001	51.639	1.461	<0.0001	−2.746	−0.004	0.790	3.444	−0.130	0.031	6.840
Cybonnet	−0.139	0.004	36.851	−0.389	0.039	95.517	0.520	0.003	70.348	0.620	0.017	1.323	−0.019	0.352	3.554	−0.166	<0.0001	7.747
Saber	−0.144	0.026	35.740	−0.616	0.001	100.968	0.717	0.002	70.587	0.478	0.089	13.648	0.030	0.375	2.229	−0.145	0.001	6.463
Francis	−0.143	0.010	36.223	−0.539	0.001	95.810	0.909	<0.0001	72.617	1.205	0.001	5.592	0.053	0.041	1.775	−0.156	0.000	6.734
Rondo	−0.172	0.034	37.438	−0.394	0.003	102.252	0.816	<0.0001	59.837	0.494	0.163	20.171	−0.010	0.468	3.775	−0.127	0.005	5.768
Kaybonnet	−0.166	0.039	37.183	−0.290	0.057	96.007	0.641	0.000	81.809	0.575	0.019	−1.320	0.011	0.680	2.777	−0.232	<0.0001	9.290
Zhe733	−0.243	0.000	39.525	−0.322	0.012	78.234	0.800	0.000	57.996	0.985	0.000	1.686	−0.032	0.057	4.574	−0.201	<0.0001	8.518
Lemont	−0.199	0.009	38.341	−0.506	0.001	105.133	0.677	0.004	58.616	0.668	0.002	−3.289	−0.031	0.321	3.860	−0.135	0.017	8.354
Teqing	−0.186	0.052	36.513	−0.663	0.002	111.764	1.634	<0.0001	49.407	1.964	0.002	−1.897	−0.028	0.190	3.546	−0.272	0.000	9.406
CL151	−0.217	0.004	37.987	−0.634	0.002	97.723	0.491	0.006	76.299	0.848	0.043	3.685	0.018	0.497	2.758	−0.105	0.037	6.531
Jupiter	−0.217	0.001	38.411	−0.262	0.051	91.575	0.563	0.002	68.532	0.576	0.098	8.544	−0.025	0.232	4.051	−0.114	0.004	6.456
Lagrue	−0.115	0.091	35.421	−0.841	0.000	110.081	1.012	0.001	67.501	0.578	0.021	3.717	−0.042	0.036	4.185	−0.118	0.052	7.627
Mars	−0.134	0.093	37.810	−0.604	0.008	100.445	0.714	0.003	74.084	0.784	0.002	−6.670	−0.003	0.895	3.377	−0.151	0.012	8.136
RoyJ	−0.064	0.158	33.686	−0.483	0.001	107.706	0.534	0.006	85.231	0.898	0.000	4.065	−0.049	0.008	3.996	−0.193	<0.0001	8.877

Determined in 2014 and 2016 only.

(**b**) Seed traits.

Variety	Chalk (%)			Grain Length (mm)			Grain Width (mm)			Grain Thickness (mm)			1000 Kernel Weight (g)			Length:Width Ratio			No. of Traits with Significant Slope *
	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept	Slope	*p* Value	Intercept	
Katy	−0.017	0.534	2.528	0.018	0.051	6.179	0.005	0.043	1.852	0.003	0.073	1.475	0.137	0.027	12.057	0.000	0.916	3.352	9
PI 312777	−0.405	<0.0001	13.234	0.009	0.000	4.926	0.007	0.003	2.387	0.001	0.080	1.692	0.133	0.000	13.744	−0.002	0.083	2.077	12
Cybonnet	−0.059	0.012	3.906	0.001	0.909	6.593	−0.001	0.755	2.123	0.001	0.311	1.584	0.040	0.325	15.903	0.002	0.653	3.082	6
Saber	−0.026	0.163	3.022	0.016	<0.0001	5.759	0.007	<.0001	1.803	0.001	0.407	1.559	0.112	0.000	11.996	−0.003	0.094	3.197	9
Francis	−0.079	0.035	4.481	0.020	<0.0001	5.978	0.002	0.114	2.080	−0.001	0.199	1.662	0.075	0.012	15.680	0.008	0.001	2.852	10
Rondo	−0.001	0.959	1.727	0.016	0.000	6.072	0.004	0.001	2.087	0.001	0.094	1.579	0.126	<0.0001	14.598	0.001	0.352	2.919	8
Kaybonnet	0.001	0.951	1.539	0.008	0.050	6.384	0.003	0.074	1.893	0.002	0.078	1.502	0.082	0.020	13.397	−0.002	0.571	3.383	9
Zhe733	0.502	0.021	9.007	0.011	0.000	5.811	0.003	0.020	2.377	−0.003	0.005	1.849	0.069	0.056	18.512	0.001	0.722	2.460	11
Lemont	−0.014	0.669	2.941	0.006	0.149	6.553	0.003	0.049	2.122	−0.001	0.439	1.682	0.143	0.039	14.744	−0.001	0.618	3.076	7
Teqing	−0.162	0.139	11.884	0.021	<0.0001	4.876	0.012	<0.0001	2.347	0.006	<0.0001	1.654	0.237	<0.0001	12.748	−0.002	0.178	2.082	9
CL151	−0.116	0.054	6.422	0.005	0.055	6.312	0.003	0.053	2.112	0.000	0.724	1.642	0.069	0.007	15.918	−0.003	0.258	2.998	9
Jupiter	−0.006	0.770	1.911	0.010	<0.0001	5.202	0.007	0.000	2.421	0.002	0.006	1.736	0.122	<0.0001	15.473	−0.001	0.357	2.123	9
Lagrue	−0.007	0.775	2.649	0.009	0.105	6.478	0.001	0.269	2.030	0.000	0.639	1.620	0.038	0.212	17.181	0.003	0.163	3.167	6
Mars	0.022	0.266	1.990	0.007	0.047	5.544	0.008	0.049	2.187	0.003	0.052	1.609	0.107	0.037	14.455	−0.006	0.183	2.556	10
RoyJ	−0.032	0.121	2.463	0.005	0.067	6.761	0.001	0.486	2.013	0.000	0.894	1.667	0.052	0.095	17.150	0.001	0.339	3.354	7

Determined in 2014 and 2016 only; * combined values from Table 4a,b at $p < 0.01$.

Table 5. Comparison of mapping population parents for number of responsive and stable traits as a function of seasonal average soil moisture. (**a**) Summary table; (**b**) Traits table.

(**a**) Summary table.

Parent of Mapping Populations	Variety	Sub-Population *	No. of Responsive Traits for Each Mapping Parent [1]	No. of Responsive Traits in Common between Mapping Parents [2]	No. of Stable Traits in Common between Mapping Parents [3]	No. of Complimentary Traits between Mapping Parents [4]
A1	Katy	TRJ	9	9	1	3
A2	PI 312777	*Indica*	12			
B1	Cybonnet	TRJ	6	5	3	5
B2	Saber	TRJ	9			
C1	Francis	TRJ	10	6	1	6
C2	Rondo	*Indica*	8			
D1	Kaybonnet	TRJ	9	8	2	3
D2	Zhe 733	*Indica*	11			
E1	Lemont	TRJ	7	7	4	2
E2	Teqing	*Indica*	9			

* TRJ = *Tropical japonica*; [1] Total number of traits for each mapping parent having a significant slope; [2] Number of traits in common between mapping parent pairs having a significant slope; [3] Number of traits in common between mapping parent pairs not having a significant slope; [4] Number of traits differing in significance of slope between mapping parents.

(**b**) Traits table.

Parent of Mapping Populations	Chalk (%)	Canopy Temp (°C) ^	Grain Length (mm)	Grain Width (mm)	Length: Width Ratio	Grain Thickness (mm)	TKW (g)	Days to Heading	Days to Maturity	Plant Height (cm)	GY (g plant^{-1})	Leaf Stress Score	Panicle Stress Score
A1	1.94 ^{b}	33.2 ^{a}	6.6 ^{a}	1.98 ^{a}	3.34 ^{a}	1.53 ^{a}	15.4 ^{a}	97 ^{a}	126 ^{b}	93 ^{a}	10 ^{a}	3.1 ^{a}	4.6 ^{a}
A2	5.18 ^{a}	33.3 ^{a}	5.13 ^{a}	2.53 ^{a}	2.03 ^{b}	1.74 ^{a}	16.69 ^{a}	97 ^{a}	134 ^{a}	76 ^{a}	27 ^{a}	3.4 ^{a}	3.9 ^{a}
Mean of A	3.56	33.3	5.87	2.26	2.69	1.64	16.05	97	130	85	19	3.3	4.3
B1	2.09 ^{b}	33.3 ^{a}	6.57 ^{a}	2.09 ^{a}	3.14 ^{a}	1.61 ^{a}	16.73 ^{a}	87 ^{a}	120 ^{a}	83 ^{a}	18 ^{a}	3.0 ^{a}	3.3 ^{a}
B2	2.34 ^{a}	32.6 ^{a}	6.12 ^{b}	1.97 ^{b}	3.13 ^{b}	1.59 ^{a}	14.46 ^{b}	90 ^{a}	121 ^{a}	86 ^{a}	25 ^{a}	2.9 ^{a}	3.0 ^{a}
Mean of B	2.22	32.9	6.35	2.03	3.14	1.60	15.60	89	121	85	22	3.0	3.2
C1	2.67 ^{a}	33.2 ^{a}	6.42 ^{a}	2.12 ^{b}	3.02 ^{a}	1.63 ^{a}	17.23 ^{a}	85 ^{a}	122 ^{a}	92 ^{a}	32 ^{a}	3.0 ^{b}	3.0 ^{a}
C2	1.61 ^{b}	33.4 ^{a}	6.47 ^{a}	2.19 ^{a}	2.95 ^{b}	1.61 ^{b}	17.66 ^{a}	93 ^{a}	127 ^{a}	79 ^{a}	33 ^{a}	3.6 ^{a}	2.7 ^{a}
Mean of C	2.14	33.3	6.45	2.16	2.99	1.62	17.45	89	125	86	33	3.3	2.9
D1	1.26 ^{b}	33.2 ^{a}	6.58 ^{a}	1.97 ^{a}	3.35 ^{a}	1.55 ^{a}	15.29 ^{a}	90 ^{a}	120 ^{a}	97 ^{a}	13 ^{a}	3.1 ^{b}	3.3 ^{a}
D2	21.9 ^{a}	33.6 ^{a}	6.08 ^{a}	2.45 ^{a}	2.48 ^{b}	1.78 ^{a}	20.15 ^{a}	71 ^{a}	116 ^{a}	75 ^{a}	10 ^{a}	3.8 ^{a}	3.5 ^{a}
Mean of D	11.58	33.4	6.33	2.21	2.92	1.67	17.72	81	118	86	12	3.5	3.4
E1	2.57 ^{a}	33.1 ^{a}	6.71 ^{a}	2.2 ^{a}	3.05 ^{a}	1.68 ^{b}	18.22 ^{a}	94 ^{a}	126 ^{a}	75 ^{a}	13 ^{a}	3.0 ^{a}	4.6 ^{a}
E2	9.86 ^{a}	32.6 ^{a}	5.33 ^{b}	2.6 ^{a}	2.05 ^{a}	1.79 ^{a}	17.81 ^{a}	98 ^{a}	135 ^{a}	84 ^{a}	41 ^{a}	2.9 ^{a}	3.2 ^{a}
Mean of E	6.22	32.8	6.02	2.40	2.55	1.74	18.02	96	131	80	27	3.0	3.9

^ Determined in 2014 and 2016 only; Letters within a column compare means for each pair of parental lines in all five mapping populations. The shaded rows are means of mapping populations A–E. TKW denotes one thousand kernel weight.

Cybonnet and Saber also have five responsive traits and three stable traits (days to maturity, leaf stress, and grain thickness) (Table 5a). Unlike Francis/Rondo, both B parents are from the TRJ subpopulation, but due to new high-density genotyping platforms, marker assisted breeding approaches are viable in genetic materials that are from the same subpopulation [56].

During 2016, four additional traits were determined to better assess physiological responses of rice genotypes to the irrigation treatments. These traits included growth rate (rate of height increase) during the vegetative and reproductive (post-heading) phases, chlorophyll content (SPAD readings), and leaf AsA content at the V8, eight leaf, stage [51]. Similar to the combined three-year analysis, varieties were significantly different ($p < 0.05$) for most traits with the exception of leaf ascorbic acid (Table 6).

Table 6. Analysis of variance of 2016 study with 15 varieties and four irrigation treatments.

Trait	* p Value > F			% of Total Sum of Squares Explained by Significant Interaction
	Variety	Irrigation	Variety × Irrigation	
Days to heading	0.0001	ns	0.04	6
Days to maturity	0.0001	ns	0.02	16
Grainfill days	0.0001	ns	ns	.
GDD2	0.0001	ns	ns	.
GDD1	0.0001	ns	0.02	16
Plant height	0.0001	ns	0.02	14
GY	0.0001	ns	0.02	13
EGP	0.0001	ns	ns	.
Avg soil moisture	0.03	0.0001	ns	.
Panicle stress score	0.0001	0.0002	ns	.
Leaf stress score	0.0001	0.04	0.02	11
Grain length	0.0001	0.01	ns	.
Grain width	0.0001	0.005	ns	.
Length:Width ratio	0.0001	ns	ns	.
Chalk	0.0001	ns	0.0001	11
TKW	0.0001	0.0001	ns	.
Grain thickness	0.0001	0.02	ns	.
Canopy temp	0.0005	0.04	ns	.
Vegetative height rate	0.001	ns	ns	.
Reproductive height rate	0.0001	ns	ns	.
Leaf ascorbic acid at V8	Ns	0.02	ns	.
SPAD	0.0001	0.002	ns	.

* ns denotes the values that are not significant at $p < 0.05$. TKW denotes one thousand kernel weight, GDD1 and GDD2 denote growing degree days from emergence to maturity and from heading to maturity, respectively.

Irrigation treatments were significantly different for 10 of the 22 traits, including stress indicators (chlorophyll content, leaf and panicle stress, and leaf AsA), grain dimensions, and TKW (Table 6). In 2016, the Variety × Irrigation interaction was significant ($p < 0.05$) for seven traits: days to heading, days to maturity, GDD1, plant height, GY, leaf stress score, and chalk with all but days to heading explaining 11–16% of the total model variation. Although plant height was generally the greatest in Irrigation-1, for five of the varieties, the tallest plants were observed in Irrigation-2 or Irrigation-3. Likewise, GY was generally highest in Irrigation-1, but for seven of the varieties, the highest GY were observed in the irrigation treatments 2 and 3. While the lowest level of leaf stress was generally observed at Irrigation-1, the Variety × Irrigation interaction could be explained by the lowest scores being observed at Irrigation-2 for three of the varieties, although these had less than one-unit difference from the next lowest scores. Chalk percentage was generally highest at the lowest soil moisture level; however, Zhe 733 had significantly higher chalk at Irrigation-1, and three other varieties had higher levels under the intermediate irrigation levels.

3.3. Correlations Between Plant Traits

To better understand the relationship between agronomic, phenological and stress-related traits measured in 2016, correlations were determined (Table 7; $p < 0.0001$). Many of the large correlations were found in expected relationships, such as correlations among grain dimension traits (e.g., grain length with grain width $r = -0.79$) and with chalk percentage (e.g., with grain thickness $r = 0.56$), and are not discussed.

Table 7. Correlation coefficients among plant traits and soil moisture determined across four irrigation treatments in 2016 study (N = 159–175).

Trait 1	Trait 2	* Correlation Coefficient
Reproductive height rate	Vegetative height rate	−0.72
GDD1	Days to heading	0.70
GDD1	Days to maturity	0.99
GDD1	Reproductive height rate	−0.44
Chalk	Reproductive height rate	0.42
GDD2	Grainfill days	0.98
Grain thickness	GDD2	0.44
TKW	Avg soil moisture	0.39
Plant height	Avg soil moisture	0.39
Leaf ascorbic acid at V8	Avg soil moisture	0.39
Canopy temp	Avg soil moisture	−0.57
Canopy temp	Leaf stress score	0.49
Canopy temp	SPAD	0.41
Avg soil moisture	SPAD	−0.57
Days to maturity	SPAD	0.39
Panicle stress score	SPAD	0.43
Panicle stress score	Leaf stress score	0.42

* All correlation coefficient values are at $p < 0.0001$.

In 2016, plant growth rate was determined during the vegetative phase (emergence to heading) and the reproductive phase (during grainfill period). Averaged across the varieties and irrigation treatments, the growth rates during the two phases were very similar (vegetative phase, 0.93 cm day^{-1}; and grainfill, 1.01 cm day^{-1}), but the two were inversely correlated ($r = -0.72$) with each other.

This finding suggests that the genotypic differences in phenology are balanced, with some growing faster earlier, but others compensating with rapid later growth. Varieties having a large season-long GDD (i.e., GDD1), were later to head ($r = 0.70$) and to mature ($r = 0.99$). However, the plant growth rate during the grainfill phase was generally slower ($r = -0.44$) in varieties having a larger GDD1, and there was no significant correlation between GDD1 except with plant growth rate during the vegetative phase. Interestingly, there was a positive correlation between grain chalk and plant growth rate during the grainfill phase ($r = 0.42$). This suggests a possible source-sink imbalance that leads to inadequate starch deposition in the grain, resulting in chalky areas. Plants having a large GDD during grainfill (i.e., GDD2) had a longer grainfill phase ($r = 0.98$), but there was no significant relationship with plant growth rates during the vegetative or grainfill phases. GDD2 was also positively correlated with grain thickness ($r = 0.44$).

Increasing soil moisture was associated with increased TKW, final plant height, and leaf AsA content ($r = 0.39$). A negative correlation was observed between soil moisture and canopy temperature ($r = -0.57$). Canopy temperature was also positively correlated with leaf stress symptoms ($r = 0.49$) and chlorophyll content ($r = 0.41$). Similarly, soil moisture was negatively correlated with chlorophyll content ($r = -0.57$). Thus, plants with low soil moisture had higher canopy temperatures, were darker green, and had lower leaf AsA contents. Plants with higher SPAD scores had delayed maturity ($r = 0.39$) and greater panicle stress scores ($r = 0.43$). This result indicated that under reduced soil moisture conditions or for genotypes that scavenge a large amount of soil moisture, AsA does not serve as a protectant and the higher chlorophyll contents might be due to reduced remobilization of assimilates from the leaves to the grains. In fact, panicle stress was positively correlated with chlorophyll content ($r = 0.43$) and leaf stress ($r = 0.42$).

3.4. Identification of Traits Related to Crop Resiliency to Non-Flooded Irrigation Conditions

Stepwise regression was performed to determine which variables were most important in explaining EGP. Analyses were performed separately for the three-year data and for 2016 alone, as the latter contained additional physiological variables not previously measured (vegetative height rate, reproductive height rate, SPAD, and leaf AsA content). Regression models were developed for the three-year data and 2016 data, with R^2 = 0.36, C(p) = 6.35 and R^2 = 0.38, C(p) = 5.51, respectively (Table 8a,b). Both models resulted in the same six variables with the 2016 analysis also including vegetative height rate. The parameter estimates used in both models were all positive except for maturity and panicle stress. Decreasing panicle stress would be evidenced by full emergence of the panicle from the boot and low floret sterility; the latter is a factor involving both pollen viability and grain filling. Surprisingly, in both models, increasing canopy temperature was related to greater EGP. To explore this result, regression analysis using the three-year data was performed by each irrigation treatment. The analysis revealed that only under the driest treatment (14% VWC) canopy temperature was significant (and positive) factor in the model (data not shown). A scatter plot of the data showed that although there was a wide range in canopy temperatures observed with irrigation treatment 4, none of the varieties with the highest EGP had low canopy temperatures (Figure S3). In addition, all but one of these plots, having both higher EGP and canopy temperature, were from the 2016 environment which was noted as having record high air temperatures. In addition, over half of these varieties were *indica* introductions (Teqing, Rondo, and PI 312777) which originated from sub-tropical areas in PR China and Taiwan. This suggests that these varieties demonstrated tolerance to a combination of water stress and high air temperature stress by being able to produce high EGP even when canopy temperatures were elevated.

Table 8. Stepwise regression towards effective grain production for (**a**) all three years combined and (**b**) for 2016 alone.

(a) All three years combined						
Step	**Variable Entered**	**Partial R^2**	**Model R^2**	**C(p)**	***F* Value**	***p* Value > *F***
1	Panicle stress score	0.11	0.11	52.90	18.81	<0.0001
2	Canopy temp	0.11	0.22	29.84	21.32	<0.0001
3	GDD1	0.07	0.28	16.58	14.10	0.0002
4	Plant height	0.01	0.30	15.75	2.64	0.1063
5	Chalk	0.02	0.32	12.10	5.43	0.0211
6	Days to maturity	0.03	0.35	6.35	7.79	0.0059
(b) 2016 alone						
Step	**Variable Entered**	**Partial R^2**	**Model R^2**	**C(p)**	***F* Value**	***p* Value > *F***
1	Panicle stress score	0.11	0.11	57.55	18.81	<0.0001
2	Canopy temp	0.11	0.22	33.92	21.32	<0.0001
3	GDD1	0.07	0.28	20.31	14.10	0.0002
4	Vegetative height rate	0.03	0.31	16.14	5.75	0.0178
5	Chalk	0.01	0.33	14.65	3.30	0.0711
6	Days to maturity	0.04	0.36	7.82	8.78	0.0035
7	Plant height	0.02	0.38	5.51	4.38	0.0381

4. Discussion

There are many countries which subsist largely on rice production but lack access to irrigation resources and, thus, drought threatens their food security. In these parts of the world where upland rice is grown, germplasm screening trials have used drought stress levels ranging from −40 kPa to −60 kPa [38]. However, U.S. rice production will be likely not be economically viable under upland conditions because there are alternative crops that have lower water consumption that can be grown in these areas. Moreover, U.S. rice growers have focused on efficiently utilizing rainwater, aquifers, and recycled water (tail-water recovery) rather than attempting to grow rice under upland conditions [21]. Thus, this research targets water-saving production systems like intermittent flood and alternate wetting and drying where the rice crop may be subjected to a range of soil moisture levels throughout the growing season. For such non-flooded systems, the goal is to develop varieties that have effective grain production (EGP) per unit of water applied. In this study, we used the SDI system to deliver season-long stress, versus stress at a particular growth stage, that ranged from 29.14% VWC (equivalent to field capacity) to 16.47% VWC (equivalent to the wilting point) in DeWitt Silt loam soils; levels which are considered relevant to the water-saving rice production systems in the USA We compared a set of rice varieties for EGP as defined by GY per unit of available soil moisture (%VWC) and determined which plant traits are most important for production under a water limited system. Decreased soil moisture resulted in delayed heading, reduced plant height, lower GY, panicle emergence delay and blanking, smaller grains, and higher canopy temperatures. Other traits such as days to maturity, EGP, leaf stress score, grain length:width ratio, grain chalk, grain thickness, and GDD (GDD1: season-long, and GDD2: grainfill period) did not show significant effect due to irrigation level, which may be because our irrigation stress treatments were not extreme.

We observed that differences among varieties accounted for, by far, most of the variation and that there was relatively little interaction with irrigation treatments. Other researchers made similar observations, finding that the heritability of most traits is similar under flood and drought conditions, that QTLs for traits overlap regardless of water stress level, and that selection under well-watered systems is also effective for drought [47,49]. Palanog et al. [46] demonstrated that even though drought stress is considered a complex trait, bulk segregate analysis (BSA), which is usually reserved for major genes, is effective for selecting across water stress levels.

Ultimately, we aim to develop genetic markers that are linked to resilient crop performance under non-flooded irrigation production practices that are being used in the U.S. Identifying contrasting parent pairs for use in developing mapping populations is a first step towards gene discovery [57,58]. We compared 10 parents used in five mapping populations that we have developed and found that the Francis/Rondo population (C, Tables 1 and 5a,b) holds the most promise for breeding and gene discovery under a reduced irrigation production system. This population is segregating with genes from different rice subpopulations (TRJ and *indica*) that trace to different global regions of adaptation and types of production systems. In addition, the parents possess a number of traits that are responsive and complimentary for both stress tolerance (panicle stress) traits [14], as well as important agronomic and grain quality traits, critical for varieties utilized by the U.S. rice industry. Similarly, the Cybonnet/Saber mapping population (B, Tables 1 and 5a,b) is a potential candidate for marker discovery and breeding as it has five responsive traits, five complementary traits and three stable traits between the parents. Both parents of the Cybonnet/Saber population are from the TRJ subpopulation making them a unique germplasm resource without concern for adaptability to the U.S. Additionally, the current investigation identified varieties with contrasting sensitivities for other important traits. For example, four varieties (PI 312777, Francis, Zhe 733, and Mars) had 10 or more traits with significant slopes, indicating that they were responsive to soil moisture availability. The most stable varieties were Cybonnet, Lemont, RoyJ and Lagrue, with only six or seven traits with significant slopes. New genetic populations using such parents could provide excellent breeding material for deficit irrigation production systems.

An important goal of modern rice breeding efforts is to optimize EGP, the amount of grain produced per unit of water used. Stepwise regression analyses identified six traits that were the most related to EGP: panicle stress, GDD1 days to maturity, grain chalk, plant height, and canopy temperature. The positive slope of canopy temperature was primarily associated with suboptimum soil moisture levels (irrigation treatment-4) where greater water use efficiency is observed (versus field capacity) and in combination with an environment where high field temperatures occurred (2016). Increasing canopy temperature is indicative of disturbed homeostasis of stomatal biology that involves complex relationships between environment (e.g., air temperature, vapor pressure deficit, available soil moisture) and the plant (e.g., biomass, architecture, biochemical adjustments). A negative correlation that was observed between soil moisture and canopy temperature (r = -0.57) has also been reported in rice and other crop plants [14,59–62]. Barnaby et al. [26] reported that cultivars that sustained photosynthesis and stomatal conductance under water stress resulted in low canopy temperature and protection from yield loss. Similarly, simulation analysis also showed that exploitation of photosynthesis-related traits may contribute towards increasing rice productivity [63]. However, there is likely a threshold for canopy temperature above which it may affect the biochemistry of plant cells and cell growth, and ultimately lead to the disruption of cell membranes and carbohydrate metabolism [48,64]. Fukuda et al. [65] reported a *CTd11* allele from a high yielding indica rice performed better in regulation of canopy temperature, stomatal conductance and photosynthetic rates compared to the *japonica* allele associated with moderate yield. We also observed an increase in chlorophyll content under extreme water stress, which is in contrast to other reports [66]. Under water stress, panicle elongation and seed set were reduced, resulting in a loss of a sink for carbohydrate remobilization and an increase in stay green canopy [14,47,48]. Although ascorbic acid has been commonly associated with response to abiotic stress, in this study, we observed a positive relationship of ascorbic acid content with soil moisture availability. This has also been observed in other studies using rice, wheat and soybeans [67–69].

5. Conclusions

New water-saving rice production practices are rapidly being adopted in the USA However, because U.S. rice varieties have been developed for production in continuously flooded paddies, it is not known whether current varieties possess the genetic potential for use in breeding programs that desire to combine high yield potential and resilience to soil moisture fluctuations as seen in intermittent flood and AWD systems. The objective of the study was to identify traits and germplasm for rice breeding programs targeted at increasing EGP in the southern USA. In this investigation, the varieties were chosen to explore the genetic diversity among those that are well adapted to the southern USA, have proven commercial success due to demonstrated yield potential, and are thus relevant to on-going breeding efforts among southern U.S. rice breeders. The majority of the variation for GY and EGP was due to the genotype effect. The stepwise regression approach identified six variables (viz., panicle stress score, canopy temperature, GDD1, plant height, grain chalk, days to maturity) that explained 35% of the phenotypic variability for EGP. The Variety × Irrigation interaction was significant only for very few traits, and these explained less than 5% of the total variation in the model. Four rice genotypes (viz., PI 312777, Francis, Zhe 733, and Mars) were identified with 10 or more traits having significant slopes in response to a range in soil moisture levels. Three bi-parental mapping populations (viz., A: PI 312777/Katy; B: Cybonnet/Saber; C: Francis/Rondo) were also identified in this study as having the most promise in identifying QTLs/genes associated with crop resiliency to water stress. The results indicate the potential to develop improved rice varieties that have high yield potential and are optimized for production under non-flooded irrigation systems that use comparatively less water. However, other factors such as disease resistance and weed competition may be equally important to include as breeding targets in these systems.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/1/55/s1. Figure S1: Graphic representation of field layout using sub-surface drip irrigation system to evaluate 15 rice cultivars under four irrigation treatments. Row-to-row distance of the planted rice was 40.6 cm and the drip tape-to-drip tape distance was at 81.2 cm, so the rice row-to-drip tape distance was 20.3 cm. The blue lines indicate the position of drip tape. The green lines show the position of the planted rice genotypes. The red dots indicate the position of TDT sensors (for further details see Materials and Methods); Figure S2: Overlay plot showing rainfall (mm) values (in red) using left Y axis, growing degree days (GDD, °C day^{-1}) values (in blue) using right Y axis, from planting until harvest during 2014 (a), 2015 (b), and 2016 (c) at rice research station, Stuttgart, Arkansas; Figure S3: Plot of canopy temperatures and effective grain production in irrigation treatment-4 across the whole study. The X axis shows effective grain production (EGP) as the ratio of GY (g plant^{-1}):average seasonal volumetric water content (% VWC). The Y axis shows canopy temperature (°C). The circle identifies plots that had highest EGP. None of which had relatively low Canopy Temperatures.

Author Contributions: Conceptualization, A.M.M., J.S.R. and C.G.H.; Data curation, A.M.M., J.S.R., C.G.H. and A.L.; Formal analysis, A.M.M. and J.S.R.; Funding acquisition, A.M.M. and C.G.H.; Investigation, A.M.M. and J.S.R.; Methodology, A.M.M., J.S.R., C.G.H. and A.L.; Project administration, A.M.M.; Resources, A.M.M. and C.G.H.; Writing—original draft, J.S.R.; Writing—review and editing, A.M.M., J.S.R., C.G.H. and A.L. All authors have read and agreed to the published version of the manuscript.

Funding: The research was partly supported by Arkansas Rice Research and Promotion Board.

Acknowledgments: This research utilized facilities and assistance provided by USDA Agricultural Research Service, Dale Bumpers National Rice Research Center. The authors acknowledge the help from Jason Gaspar, Phil Sims, Laduska Sells, Jonathan Moser, Tim Scott, Jamison Murray, Tiffany Sookaserm, and Courtland Hemphill for performing all aspects of the field study. Appreciation is extended to Jarrod Creameans and Kendl Fisher for AsA analysis. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or the University of Arkansas and does not imply its approval to the exclusion of other products that also can be suitable. The authors also thank Jeremy Edwards, Debbie Boykin, and Sara Duke for input on statistical analyses, and David Gealy and Courtland Hemphill for their critical peer review. USDA is an equal opportunity provider and employer. All experiments complied with the current laws of the United States, the country in which they were performed.

Conflicts of Interest: The authors declare no conflict of interest.

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