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

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SUMMARY

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, ten 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 sensitive to heat, CL152 was moderately sensitive to cold and heat, and XL 753 was highly cold and heat tolerant 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.
**Key words:** Rice (*Oryza sativa* L.), temperature, morpho-physiological parameters, root growth and developmental traits, SPAR, Total Drought Response index (TDRI), TLTRI, Total low temperature response index; THTRI, total high temperature response index; SD, standard deviation; $r^2$, coefficient determination; PH, plant height; TN, tillers number; LN, leaves number, LA, leaf area; LW, leaf weight; SW, stem weight; RW, root weight; AGW, above ground weight; TW, total weight; RS, root/shoot; RL, root length; RSA, root surface area; RAD, root average diameter; RV, root volume; RNT, number of tips; RNF, number of forks; RNC, number of crossing; SPAD, chlorophyll content; Fv'/Fm', fluorescence

**Key findings:** Temperature affects all shoot, root and physiological parameters. Cultivar differences were observed for low and high-temperature responses during the early-season.

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for about 50% of the world’s population and plays a 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 affects crop production of many staple crops such as rice, wheat, maize, rice, and cotton (Hoogenboom, 2000; Fahad *et al*., 2016, 2017; Gbetibouo and Hassan, 2005; Reddy *et al*., 2017). Plants have developed diverse defense mechanisms to cope with stresses and thus minimizing 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 a 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.

Farm managers 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, 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, researchers at germination, seedling, and reproductive stages (Singh et al., 2017a, b) have estimated genetic variability for heat and cold tolerance. 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 during all phases of growth from germination until grain filling (Ye et al., 2009). Cold tolerance at seedling emergence and during early vegetative stages is 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) and
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 would 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 has 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. Also, little information is available about the response of japonica cultivars to high temperature during the early seedling stage. Moreover, there is no or at best minimal information on the competitive response of rice grown in the US midSouth, where rice is initially dry drill-seeded in rows of 6 to 10 inches, but the entire field is flooded.
permanently at the beginning of the tillering 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 CO₂ concentration was monitored and maintained at 400 µmol mol⁻¹ using a dedicated LI-6250 CO₂ 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 (TACAURI/3/CYPRESS/L-202/TEBONNET/4/CL161) released by Louisiana State University (LSU) and Horizon Ag in 2013, Bowman (CI9881/PI331581/L201)/863270 (Mars/Newrex//Tebonnet/Bellmont) released by Mississippi State University in 2008, Antonio (Cypress/Cocodrie) released by Texas A& M University in 2012, and Mermentau (AR1188/COCODRIE/9502088/LAGRU E) released by LSU in 2012, along with two proprietary hybrids bred by RiceTec, namely, XL 753 and CLXL 745, were obtained from the Mississippi State University’s Delta Research and Extension Center, Mississippi State (33° 42´ N, 90° 92´ W), Mississippi, USA. 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 to 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 were 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, ten 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, cm² 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, cm² plant⁻¹), average root diameter (RAD, mm plant⁻¹), root volume (RV, cm³ 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, Inc., Cary, NC, USA). 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, USA). The correlation of the morphophysiological 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].

IVLTRI = Pvl/Po

ILTRI = Pl/Po

IHTRI = Ph/Po

IVHTRI = Pvh/Po

\[ \text{CVLRI} = \left( \frac{\text{PH}}{\text{PHo}} \right) + \left( \frac{\text{TN}}{\text{TNo}} \right) + \left( \frac{\text{LN}}{\text{LNo}} \right) + \left( \frac{\text{LA}}{\text{LAo}} \right) + \left( \frac{\text{LW}}{\text{LWo}} \right) + \left( \frac{\text{SW}}{\text{SWo}} \right) + \left( \frac{\text{RW}}{\text{RWo}} \right) + \left( \frac{\text{AGW}}{\text{AGWo}} \right) + \left( \frac{\text{Tw}}{\text{TwO}} \right) + \left( \frac{\text{SAPAD}}{\text{SAPADo}} \right) \]  

\[ \text{CLTRI} = \left( \frac{\text{PH}}{\text{PHo}} \right) + \left( \frac{\text{TN}}{\text{TNo}} \right) + \left( \frac{\text{LN}}{\text{LNo}} \right) + \left( \frac{\text{LA}}{\text{LAo}} \right) + \left( \frac{\text{LW}}{\text{LWo}} \right) + \left( \frac{\text{SW}}{\text{SWo}} \right) + \left( \frac{\text{RW}}{\text{RWo}} \right) + \left( \frac{\text{AGW}}{\text{AGWo}} \right) + \left( \frac{\text{Tw}}{\text{TwO}} \right) + \left( \frac{\text{SAPAD}}{\text{SAPADo}} \right) \]  

\[ \text{IHTRI} = \left( \frac{\text{Ph}}{\text{Pho}} \right) + \left( \frac{\text{RTR}}{\text{RTo}} \right) + \left( \frac{\text{RNF}}{\text{RNo}} \right) + \left( \frac{\text{RNC}}{\text{RNo}} \right) + \left( \frac{\text{SPAD}}{\text{SPADO}} \right) \]  

\[ \text{IVHTRI} = \left( \frac{\text{Pvh}}{\text{Pvho}} \right) + \left( \frac{\text{RTR}}{\text{RTo}} \right) + \left( \frac{\text{RNF}}{\text{RNo}} \right) + \left( \frac{\text{RNC}}{\text{RNo}} \right) + \left( \frac{\text{SPAD}}{\text{SPADO}} \right) \]
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 hybrid were determined according to the results from the classification vigor indices 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 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
Table 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’).

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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 \( P \leq 0.05 \).
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 a 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 the 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.

**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 (Figure 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 in plant height varies among cultivars/hybrids, and the quadratic functions showed an increase and decline of the plant height for genotypes. CLXL 745 and Bowman increased by 4.58 cm and decreased -0.07 cm 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 -0.06 cm 1 °C$^{-1}$ (Figure 1A). Kondo and Okamura (1931) and Osada et al. (1973) revealed that the plant height increased with the increase in 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 (Figure 1B). 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 1; Figures 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.
Figure 1. Temperature effect on (A) plant height, (B) tillers number, (C) number of leaves, and (D) leaf area of rice cultivars. Measurements were taken at 39 days after sowing. Standard errors of the mean ± five observations are presented if the values are larger than the symbols.
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), aiding nutrient uptake efficiency and performance under various stress conditions including temperature (Hammer et al., 2009; Rosolem et al., 1994) (Figure 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. The quadratic response was observed in all cultivars/hybrids studied. CLXL 745 and XL 753 exhibited greater root length at the five temperatures tested (Figure 2A).

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 a quadratic response to temperature. The responses, however, were different among the cultivars and hybrids; hybrids XL 753 and CLXL 745 showed greater root length at the five temperatures tested compared to the other four cultivars, Mermentau, CL 152, Antonio, and Bowman (Figures 2B and 5), with maximum values observed at 35/27 °C. However, there were no differences in the root volume and average root diameter among the cultivars and hybrids tested, and they increased 2.18 cm³ and 0.09 mm, respectively per 1 °C⁻¹ (Figures 2C and D). Root forks, root crossing, and root tips increased quadratically across temperatures in all cultivars and hybrids (Figures 3A, B, and C). 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).

Physiological parameters

Chlorophyll content and maximal quantum yield of PSII photochemistry (Fv/Fm) are essential parameters for PSII activity. Any decrease 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 (Figure 4A). Variability of
Figure 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 ± five observations are presented if the values are larger than the symbols.

Figure 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 ± five observations are presented if the values are larger than the symbols.
Figure 4. Temperature effect on (A) Chlorophyll content (SPAD) and Fluorescence (Fv′/Fm′) of rice cultivars. Measurements were taken at 36 days after sowing. Standard errors of the mean ± five observations are presented if the values are larger than the symbols.

Figure 5. Root images for selected rice cultivars/hybrids grown at various temperatures, harvested 39 days after sowing. These root images for this picture were taken before they were split into two images for analysis purpose as they were large root systems.
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 (Figure 4A). However, there were no differences in the fluorescence among the cultivars and hybrids tested, and fluorescence increased by 0.08 per 1 °C\(^{-1}\) (Figure 4B). Both negative response relationships between temperature and total chlorophyll content were reported by Nagai 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.

**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 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).

**Total low or high-temperature response index**

The use of indices derived from combining several primary parameters in plant competition experiments is well documented (Weigelt and Jolliffe, 2003). The combination of several measurements into a single index help researchers analyze and present
Table 2. Temperature effect on leaf dry weight, stem dry weight, root dry weight, above ground dry weight, total dry weight, and the root-shoot ratio of rice cultivars. Measurements were taken 39 DAS.

<table>
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<tr>
<th>Parameters</th>
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<th>25/17</th>
<th>30/22</th>
<th>35/27</th>
<th>40/32</th>
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<td></td>
<td></td>
<td>g plant⁻¹</td>
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<td>0.25</td>
<td>0.21</td>
<td>0.17</td>
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Table. 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.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Cultivar</th>
<th>TLTRI</th>
<th>Classification</th>
<th>Cultivar</th>
<th>THTRI</th>
</tr>
</thead>
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<td>Cold sensitive</td>
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<td>(18.48)</td>
<td>Heat sensitive</td>
<td>Antonio</td>
<td>(42.01)</td>
</tr>
<tr>
<td>TLTRI ≤ 20.06</td>
<td>Antonio</td>
<td>(19.54)</td>
<td>CL XL 745</td>
<td>(43.59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CL XL 745</td>
<td>(19.80)</td>
<td>Mermentau</td>
<td>(44.09)</td>
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</tr>
<tr>
<td></td>
<td>Mermentau</td>
<td>(19.82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>CL152</td>
<td>(20.28)</td>
<td>Moderate</td>
<td>CL 152</td>
<td>(44.54)</td>
</tr>
<tr>
<td>20.07 &lt; TLTRI ≤ 21.63</td>
<td></td>
<td></td>
<td>Heat tolerant</td>
<td>Bowman</td>
<td>(46.33)</td>
</tr>
<tr>
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<td>XL 753</td>
<td>(23.15)</td>
<td></td>
<td>XL 753</td>
<td>(48.82)</td>
</tr>
<tr>
<td>Cold tolerant</td>
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<td></td>
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<tr>
<td>21.64 &lt; TLTRI ≤ 23.20</td>
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</table>

†SD = 1.57. Cold sensitive: TLTRI ≤ TLTRI \(\text{min} + 1.0\) SD; Moderate: TLTRI \(\text{min} + 1.0\) SD < TLTRI ≤ TLTRI \(\text{min} + 2.0\) SD; Cold tolerant: TLTRI \(\text{min} + 2.0\) SD < TLTRI ≤ TLTRI \(\text{min} + 3.0\) SD.

‡SD = 2.37. Heat sensitive: THTRI ≤ THTRI \(\text{min} + 1.0\) SD; Moderate: THTRI \(\text{min} + 1.0\) SD < THTRI ≤ THTRI \(\text{min} + 2.0\) SD; Heat tolerant: THTRI \(\text{min} + 2.0\) SD < THTRI ≤ THTRI \(\text{min} + 3.0\) SD.

Results in a way that better describes plant performance and competitive ability than the use of just a single measure (Hunt, 1982). 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 sub-optimal 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 3). 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 six rice cultivars and hybrids. 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 six 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 (Figure 6). 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 (Figure 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
Figure 6. Correlation between total low-temperature response index (TLTRI) and shoot or root traits of six rice cultivars, measured at 39 days after sowing.

Figure 7. Correlation between total high-temperature responses index (THTRI) and shoot or root traits of six rice cultivars, measured at 39 days after sowing.

Figure 8. Correlation between total low-temperature response index (TLTRI) and total high-temperature response index (TLTRI) of six rice cultivars, measured at 39 days after sowing.
and heat tolerance during the early establishment of rice cultivars. The regression coefficient between total low-temperature response index and the total high-temperature response index was 0.69 (Figure 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., 2017a).

The identified heat or cold tolerant cultivars in this study could be 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. Using the response indices may also identify new genetic donors in addition to those found already using primary measurements (Tenorio et al. 2013). The information generated could also be useful in the identification of new genes or quantitative trait loci (QTL) underlying cold and heat tolerance, based not just on primary measurements, but also on the derived response indices. It would be interesting, for example, to determine whether the chromosomal locations of QTLs already mapped for cold (Ye et al. 2010) and heat (Ye et al. 2012) stress tolerance would correspond those of QTLs identified based on these derived indices.

Moreover, 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 (Figure 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 (Figure 9).

**Figure. 9.** Principal component analysis for the first three principal components (PC) scores, PC1 and PC2 related to the classification of six rice cultivars and hybrids for low and high-temperature tolerance.
CONCLUSION

Selected rice cultivars (four) and hybrids (two) were evaluated 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 other hand, very high temperature caused a significant decrease in some parameters like leaf area, root dry weight, root length, and a 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 may imply that shoot and root traits are vital for selecting cold and heat tolerance during early establishment of rice cultivars. The breeder could potentially use low and high-temperature tolerant cultivars identified in this study to improve high yielding genotypes and identify novel genes and QTLs for future environments that could benefit rice producers aiming to increase rice yield.

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