

Article

Morpho-Physiological Characterization of Diverse Rice Genotypes for Seedling Stage High- and Low-Temperature Tolerance

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Abstract: Extreme temperatures are considered one of the main constraints that limit the growth and development of rice. We elucidated the root and shoot developmental plasticity of 64 rice genotypes during early seedling establishment, using the sunlit plant growth chambers at 22/14 (low), 30/22 (optimum), and 38/30 °C (high) day/night temperatures. Low temperature severely inhibited 23 traits, such as shoot (68%), root (57%), and physiological (35%) attributes. On the contrary, the high temperature positively affected most of the shoot (48%) and root (31%) traits, except root diameter and root/shoot ratio, compared with the optimum. Alternatively, leaf chlorophyll fluorescence-associated parameters declined under low (34%) and high (8%) temperatures. A weak correlation between cumulative high-temperature response index (CHTRI) and cumulative low-temperature response index (CLTRI) indicates the operation of different low- and high-temperature tolerance mechanisms at the early seedling stage. Groups of distinct rice genotypes associated with low or high-temperature tolerance were selected based on CHTRI and CLTRI. The genotypes that commonly performed well under low and high temperatures (IR65600-81-5-2-3, CT18593-1-7-2-2-5, RU1504114, RU1504122, Bowman, and INIA Tacuari) will be valuable genetic resources for breeders in developing early-season high- and low-temperature-tolerant genotypes for a broad range of both tropical and temperate rice-growing environments.

Keywords: cold and heat temperature tolerance; early growth stage; rice; roots; cumulative stress response indices



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1. Introduction

Rice (*Oryza sativa* L.) is one of the major cereal crops grown under different agroecosystems. Globally, the harvested rice area had increased from 120.1 million ha in 1960 to 161.6 million ha in 2018. During this time, the average rice yield doubled from 1.84 to 4.51 tons ha⁻¹ (<http://ricestat.irri.org:8080/wrsv3/entrypoint.htm>). Rice is the staple food for 2.5 billion people globally, particularly in Asia; 1.7 billion people depend solely on rice for their livelihood [1,2]. Although rice is grown in different climatic regions (temperate, tropical, and subtropical), it is challenging to maintain rice productivity at a high level due to its higher sensitivity to unfavorable environmental conditions [3,4]. Crop production faces multiple challenges, with >50% of plant productivity often decreased by various abiotic stresses [5]. Similarly, rice productivity in most rice-growing areas suffers

from one or two extreme production challenges at the same or different periods of any growing season [6]. With the increasing demand for food production, unfavorable environmental conditions such as extreme temperatures (low and high), drought, submergence, and salinity, are becoming threats to sustaining yield [5–7].

In addition to climatic challenges, escalating labor wages and declining labor force for agriculture (0.2% per year) in the rice-growing regions are forcing farmers to change their rice cultivation practices [8]. Thus, in recent years, dry direct-seeded rice cultivation has been increasingly adopted by farmers as a feasible alternative to the traditional puddled-rice method. However, the cultivation transformation has been coupled with an underlying risk of yield loss due to extreme temperatures and soil moisture regimes during early seedling establishment [9]. More than 15 million ha of rice areas suffer from a low-temperature injury at one or more growth stages [10,11]. For example, rice cultivation in the northern part of Vietnam is hampered by cold temperatures at the germination and seedling stages [12,13]. On the other hand, high precipitation, coupled with a colder climate, affects 7% of Africa's total rice area [14]. Similar production challenges have been reported in rice-growing areas of the Mid-South in the USA. Generally, rice is adversely affected by high temperatures in the tropics and lower elevations, and lower temperatures in the temperate regions. The optimum temperature for rice seed germination and emergence is between 25 °C and 30 °C. Temperatures below 15 °C [10,15] or >35 °C [16] severely affect the growth and development of rice during the early growth stages. For instance, extreme temperatures can affect rice growth and development processes, including germination, emergence and seedling establishment, and reproductive and grain-filling stages. The low (cold) temperature in the vegetative stage can cause slow growth; reduced seedling vigor [12,17], number of seedlings, and tillering [18]; and increased plant mortality [11,19]. Cold tolerance at the early stages is essential to obtain the optimum plant population [20] required for successful crop production. Adding to the challenge, temperatures >32 °C negatively affect all growth and development [6,16,21]. Hence, there is a strong need to investigate seedling growth and development influenced by extreme temperature conditions [22].

Death at the seedling stage and uneven seedling establishment cause yield loss under low- and high-temperature stress. Increased productivity under unfavorable environmental conditions can be achieved by improving seedling survival and vigor at the early growth stage. Compared with other crops, rice seems to be more sensitive to extreme (low and high) temperature stress [20,22]. Rice is a cold-sensitive crop; crop growth and development are severely damaged below 15 °C [22]. Temperature <15 °C causes poor germination, late seedling establishment, and non-vigorous plants, which results in loss of seedling and delay in the transplanting period. Breeding genotypes with temperature tolerance could be the best solution for minimizing the influence of low temperature in high-altitude rice-growing areas and temperate zones, or high temperature in tropical, low-altitude rice-growing regions during the early seedling stage in direct-seeding cultivation. Early-season vigor is an essential trait in rice development, playing a crucial role in canopy development and light interception. During seedling establishment under a dynamic environment, low root vigor can potentially affect canopy development at the plant's later stage [16,23,24]. Selection for tolerance based on root traits is often challenging under field conditions and involves tedious work [16,25,26]. Previous rice studies extensively focused on reproductive-stage temperature tolerance due to its high sensitivity and its immediate relevance to grain yield [22,27]. Hence, knowledge on how early-season shoot and root vigor of rice are affected by extreme temperatures is essential for identifying rice genotypes with high levels of low-/high-temperature tolerance to minimize the production risk under direct-seeded cultivation. It is still unclear how different rice subspecies and breeding lines would respond to low and high temperatures at the seedling stage.

Therefore, understanding the temperature tolerance of diverse rice genotypes involving different subspecies (*indica* and *japonica*) and advanced breeding lines would be necessary for formulating any temperature stress mitigation strategy through varietal

improvement. Moreover, few studies that have assessed genetic diversity for temperature tolerance in rice have used selection parameters such as spikelet fertility that can be observed only at later growth stages [22]. Therefore, to address the above knowledge gaps, we utilized a diverse rice panel involving pure lines from both the *indica* and *japonica* major rice subspecies, comprised of both released varieties and breeding lines to evaluate physiological, growth, and development traits using a precise phenotyping approach at the early growth stage. The objectives of this study were to (i) determine variations in low and high temperature tolerance among 64 rice genotypes, (ii) determine which traits among the genotypes are best suited for screening cold and heat stress tolerance, and (iii) classify and rank rice genotypes based on a stress response for cold and heat tolerance. We expect that the identified tolerant genotypes will help breeders develop rice strains or varieties with tolerance to cold and/or heat stress. On the other hand, crop consultants can use stress-response scores as a decision-making tool to select genotypes best suited for rice production in regions where extreme temperatures limit rice growth.

2. Materials and Methods

2.1. Crop Husbandry

We used a rice panel consisting of a diverse set of 64 rice genotypes (31 *indica*, 32 *japonica*, and one *Aus subspecies*, comprised of 27 released varieties and 37 experimental breeding lines under development) that were used in this study (Table S1). The collected genotypes were phenotyped for the low and high temperatures stress-tolerance variability at the early seedling stage using the sunlit soil–plant–atmosphere research (SPAR) [28] chambers at the Mississippi State University, Mississippi State, MS. Each SPAR chamber consists of a steel soil bin (1-m deep × 2-m long × 0.5-m wide) and a 1.27-cm thick Plexiglas chamber (2.5-m tall × 2.0-m long × 1.5-m wide) to accommodate root and aerial plant parts, respectively. The Plexiglas allows 97% of the visible solar radiation to pass without spectral variability in absorption (wavelength 400–700 nm) [29].

Seeds were sown on 17 October 2016 in 576 polyvinyl-chloride pots (10.1-cm diameter and 45.72-cm height) filled with the soil medium consisting of 3:1 sand/topsoil ratio classified as a sandy loam (87% sand, 2% clay, and 11% silt) with a 500 g of gravel at the bottom of each pot. The pots were arranged with 64 in each of the 9 SPAR units. Initially, four seeds were sown in each pot, and 11 d after emergence the seedlings of similar size were replanted to one per pot. Pots were arranged as a randomized complete block in 16 rows, with four pots per row in each SPAR chamber. Plants were irrigated three times a day through an automated, computer-controlled drip system with full-strength Hoagland's nutrient solution [30], delivered at 0700, 1200, and 1700 h, in each SPAR unit. Each SPAR unit's microclimatic environment was continuously monitored using a dedicated computer system [28]. Each chamber's relative humidity was monitored with a humidity and temperature sensor (HMV 70Y, Vaisala, Inc., St. Louis, MO, USA) installed in airline ducts' returning path.

2.2. Temperature Treatments

In this study, 576 plants (64 rice genotypes × three treatments × three replications) were used to explore phenotypic variability among genotypes. The seedlings were grown under the optimum temperature of 30/22 °C (day/night) temperatures with 70% relative humidity and 410 PPM CO₂ for ten days to facilitate uniform emergence. Three temperature treatments (22/14, 30/22, and 38/30 °C, day/night) were randomly arranged in nine SPAR units. All genotypes were moved into three treatments 10 d after planting: control or optimum-temperature (30/22 °C day/night), low-temperature (22/14 °C day/night), and high-temperature (38/30 °C, day/night) treatments. The daytime temperatures were initiated at sunrise and returned to the nighttime temperature 1 h after sunset. Except for the temperature treatments, the other growth conditions were the same during the experiment for all the units. For each treatment, three replications were maintained by using one SPAR unit as one replication.

2.3. Data Collection

2.3.1. Physiology

The chlorophyll index was measured using a SPAD-502 chlorophyll meter (Konica Minolta Inc., Mississauga, ON, Canada) at 30 days after sowing (DAS). Chlorophyll fluorescence (F_v/F_m) was measured using the Fluoropen 1000 (Photo System Instruments, Kolackova, Czech Republic) for OJIP analysis. Application of chlorophyll fluorescence fast-transient analysis (OJIP) is a simple and non-invasive tool for monitoring chloroplast function. The OJIP analysis is a sensitive, reliable, and quick test for the photosynthetic system's functionality and vitality. Minimal fluorescence intensity (F_o), maximal fluorescence intensity (F_m), maximal variable fluorescence (F_v), and F_v/F_m were used to get the maximum potential quantum efficiency of Photosystem II to derive clues about the stress effect on the experimental rice genotypes.

2.3.2. Phenotyping of Agronomic Traits

Tiller number (TN) and the total number of leaves (LN) were counted, and plant height (PH) was measured on all plants at 30 DAS. Leaf area (LA) was measured using the LI-3100 leaf-area meter (LI-COR, Inc., Lincoln, NE, USA). Total dry weights (stem + leaf + root) were measured at 31 DAS (after the harvest) from all plants after oven drying at 75 °C until a constant weight was reached.

2.3.3. Root Phenotypes

Roots were cut and separated from the stems and washed thoroughly, avoiding any disturbance to the root system. The longest root length was determined using a ruler. The cleaned individual root systems were floated in 5 mm of water in a 0.3-by 0.2-m Plexiglas tray. Roots were untangled and separated with a plastic paintbrush to minimize root overlap. The roots were scanned with an Epson Expression 11000XL scanner at a resolution of 800 dots per inch. Acquired images were analyzed for the cumulative root length (RL), root surface area (RSA), average root diameter (RD), root volume (RV), number of roots (RN), number of root tips (RT), number of root forks (RF), number of root crossings (RC), and root:shoot ratio (RS) and images were analyzed using WinRHIZO Pro 2009C software (Regent Instruments, Inc., Québec, QC, Canada).

2.4. Statistical Analysis

The 64 rice genotypes were classified into cold-tolerant and heat-tolerant groups based on the summation of all genotypes' response indices, following the approach used in other crops. Initially, an individual low-temperature stress response index (ILSRI) (22/14 °C) for each of the parameters was calculated as the value of a parameter (P_l) at the low temperature of a given genotype divided by the value for the same parameter (P_o) at the optimum temperature (30/22 °C) (Equation (1)). The individual high-temperature stress response index (IHSRI) (38/30 °C) was determined by dividing the value of a parameter (P_h) at a high temperature of a given genotype by the value for the same parameter (P_o) at the optimum temperature (Equation (2)).

$$\text{ILSRI (low)} = P_l/P_o \quad (1)$$

$$\text{IHSRI (low)} = P_h/P_o \quad (2)$$

Then, the combined low-temperature response index (CLTRI) and combined high-temperature response index (CHTRI) for each genotype (Equations (3) and (4)) were calculated as the sum of 24 ISRIs derived from tiller number (TN), number of leaves (LN), plant height (PH), leaf area (LA), leaf weight (LWT), root dry weight (RWT), above-ground dry weight (SHWT), stem weight (StWT), total seedling dry weight (TdWT), root length (RL), root surface area (RSA), average root diameter (AvgRD), root volume (RV), root number (RN), root tips (RT), root forks (RF), root crossing (RC), root:shoot ratio

(R/S), chlorophyll index (SPAD), minimal fluorescence intensity (F_o), maximal fluorescence intensity (F_m), maximal variable fluorescence (F_v), and F_v/F_m .

$$\begin{aligned} \text{CLTRI} = & (\text{TNI}/\text{TNo}) + (\text{LNI}/\text{LNo}) + (\text{PHI}/\text{PHo}) + (\text{LAI}/\text{LAo}) + (\text{LWTI}/\text{LWTo}) + (\text{RWTI}/\text{RWTo}) + \\ & (\text{SHWTI}/\text{SHWTo}) + (\text{StWTI}/\text{StWTo}) + (\text{TdWTI}/\text{TdWTo}) + (\text{RLI}/\text{RLo}) + (\text{RSAI}/\text{RSAo}) + (\text{AvgRDI}/\text{AvgRDo}) + \\ & (\text{RVl}/\text{RVo}) + (\text{RNI}/\text{RNo}) + (\text{RTI}/\text{RTo}) + (\text{RFI}/\text{RFo}) + (\text{RCI}/\text{RCo}) + (\text{R/SI}/\text{R/So}) + (\text{SPADl}/\text{SPADo}) + (\text{F}_o\text{l}/\text{F}_o\text{o}) + \\ & (\text{F}_m\text{l}/\text{F}_m\text{o}) + (\text{F}_v\text{l}/\text{F}_v\text{o}) + (\text{F}_v/\text{F}_m\text{l}/\text{F}_v/\text{F}_m\text{o}) \end{aligned} \quad (3)$$

$$\begin{aligned} \text{CHTRI} = & (\text{TNh}/\text{TNo}) + (\text{LNh}/\text{LNo}) + (\text{PHh}/\text{PHo}) + (\text{LAh}/\text{LAo}) + (\text{LWTh}/\text{LWTo}) + (\text{RWTh}/\text{RWTo}) + \\ & (\text{SHWTh}/\text{SHWTo}) + (\text{StWTh}/\text{StWTo}) + (\text{TdWTh}/\text{TdWTo}) + (\text{RLh}/\text{RLo}) + (\text{RSAh}/\text{RSAo}) + \\ & (\text{AvgRDh}/\text{AvgRDo}) + (\text{RVh}/\text{RVo}) + (\text{RNh}/\text{RNo}) + (\text{RTh}/\text{RTo}) + (\text{RFh}/\text{RFo}) + (\text{RCh}/\text{RCo}) + (\text{R/Sh}/\text{R/So}) + \\ & (\text{SPADh}/\text{SPADo}) + (\text{F}_o\text{h}/\text{F}_o\text{o}) + (\text{F}_m\text{h}/\text{F}_m\text{o}) + (\text{F}_v\text{h}/\text{F}_v\text{o}) + (\text{F}_v/\text{F}_m\text{h}/\text{F}_v/\text{F}_m\text{o}) \end{aligned} \quad (4)$$

Statistical analyses were carried out using RStudio 3.6.1 (<https://rstudio.com/>). An ANOVA was performed for each of the traits to estimate the significance of genetic variability among rice genotypes under treatments using the library (“agricolae”). Principal component analysis, a multidimensional preference analysis technique that identifies the parameters that best describe tolerance to response variables, was used to separate genotypes into tolerant and sensitive groups. These relative index values were used to perform principal component analysis (PCA) using XLSTAT. A correlation matrix was developed using the library (“corrplot”).

Furthermore, Z scores of the combined low-temperature response index (CLTRI) and combined high-temperature response index (CHTRI) were estimated for each of the genotypes. The computed Z scores were used to group rice genotypes into possible high-temperature-tolerant and low-temperature-tolerant (HTT + LTT), high-temperature-tolerant and low-temperature-sensitive (HTT + LTS), high-temperature-sensitive and low-temperature-tolerant (HTS + LTT), and high-temperature-sensitive and low-temperature-sensitive (HTS + LTS) groups. Sigma Plot 13 was used for plotting graphs and regression analysis.

3. Results and Discussion

Attaining high yield under suboptimal growing conditions is one of the main challenges of this century. It is now clear that the emphasis on stress tolerance in plants has to be addressed from the vegetative to grain-filling stages because of their greater sensitivity to environmental variations [12,16,23]. Current constraints caused by extreme temperatures related to yield losses at the reproductive stage have gained much attention among researchers. However, investigating the multifaceted concerns connecting with growth and development continues to be a primary task. Here, we elucidate the root and shoot developmental plasticity of 64 rice genotypes to low or high temperatures at the early rice seedling stage.

3.1. Shoot Growth and Development

The low temperature had a significant inhibitory effect on all the rice shoot growth and developmental traits, except for leaf number under low temperature compared with the optimum temperature (Figure 1). For example, there was a 73% decrease in plant height due to cold stress compared with its control counterparts (Figure 1A; Table S1). Tiller number, a critical component of grain yield, also decreased by 61.5% under low-temperature conditions (Figure 1B). The direct effect of temperature on the shoot meristem, or its effect on reduced nutrient uptake through the roots, will affect shoot development at suboptimal soil temperatures [31]. Leaf growth is sensitive to various environmental stresses such as low temperature [18,32], water deficits [33], and nitrogen deficiency [34]. Generally, cold temperature slows the leaf initiation rate, reducing the leaf number, and directly lowering the leaf cell division and elongation [35,36], resulting in lower leaf and leaf dry weight. However, there was no effect of cold temperatures on leaf number in the present study (Figure 1C). Still, there was a considerable decline in leaf area (nearly 90%) under the low temperature (Figure 1D). The highest value for leaf area at 22/14 °C was recorded

in FEDEARROZ MOCARE (an *indica* released genotype, 15.68 cm²/plant). In contrast, at 30/22 °C, the highest value was recorded in CT18372-8-1-6-3-1-5 (an *indica* experimental breeding line, 363.55 cm²/plant). Thus, leaf area could be used as a primary selection criterion for the improvement of low-temperature adaptation. As plant growth and development play essential roles in crop production, any factor governing the production of new leaves, leaf expansion duration, and stem extension will affect canopy development and radiation interception and, thus, crop yield [37,38]. Based on the observed results, we conclude that leaf traits (leaf area, leaf weight, and total biomass) have more significant potential to discriminate rice genotypes under low-temperature stress.

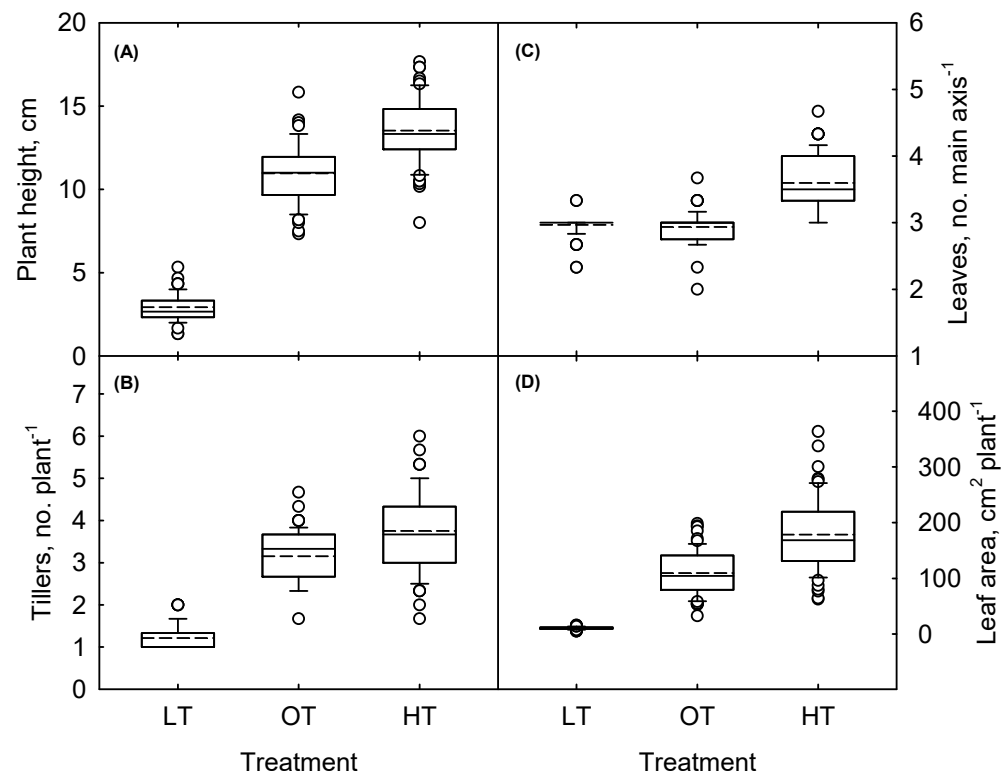


Figure 1. Temperature effect on (A) plant height, (B) tillers number, (C) leaves number, and (D) leaf area of 64 rice genotypes. Measurements were taken at 21 days after sowing. The middle line indicates the median; the box shows the range of the 25th to 75th percentiles of the total data. The whiskers indicate the interquartile range, and the outer dots are outliers: low temperature (LT), optimum temperature (OT), and high temperature (HT).

Unlike the response of shoot and leaf phenotype to low temperature, high temperature positively affected plant height, tiller number, leaf area, and leaf number (Figure 1). However, rice genotypes displayed genetic diversity in response to high temperatures. Plants were generally taller at 38/30 °C with maximum height recorded in N-22 (17.67 cm/plant) in comparison to plants growing at 30/22 °C with the maximum plant height being 15.83 cm/plant (IR86052-32-3-2, an *indica* breeding line). Total leaf number per seedling increased with an increase in temperature across genotypes; however, although very small, differences were recorded among the genotypes. Leaf number ranged between 3/plant to 4.67/plant with an overall average of 3.60/plant at 38/30 °C (Figure 1; Table S1). On average, leaf area increased by 62% under high-temperature conditions compared with optimum conditions among all the genotypes (Figure 1D).

3.2. Dry Weight Traits

Dry matter increased significantly across genotypes at high temperature but declined as temperature treatments decreased relative to optimum temperature conditions (Figure 2;

Table S2). Among the 64 genotypes, significant variation was observed for leaf dry weight (LDW), with a high value of 0.06 g for FEDEARROZ 473 (an *indica* genotype) and CL271 (a released *japonica* genotype), and a low value of 0.02 g for SABINE (a *japonica* released genotype), respectively, with an overall mean of 0.04 g at low temperature (Figure 2C; Table S2). Leaf dry weight increased almost 77% in the genotypes growing at 38/30 °C with the maximum value recorded in RU1204197 (a *japonica* breeding line, 1.13 g/plant). Similarly, the least values for shoot dry weight (Figure 2B), root dry weight (Figure 2C), and total dry weight were observed at 22/14 °C (Figure 2D).

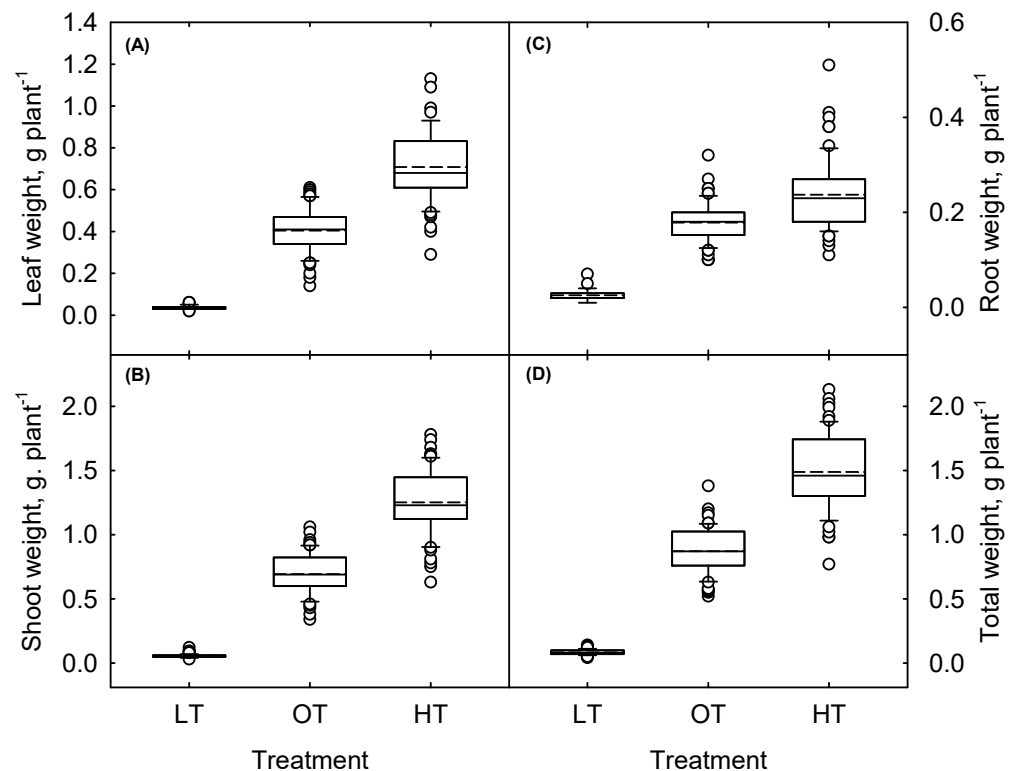


Figure 2. Low temperature (LT), optimum temperature (OT), and high temperature (HT) effects on (A) leaf weight, (B) shoot weight, (C) root weight, and (D) total weight of 64 rice genotypes. Measurements were taken at 31 days after sowing. The middle line indicates the median; the box shows the range of the 25th to 75th percentiles of the total data. The whiskers indicate the interquartile range, and the outer dots are outliers.

There was no significant difference among the genotypes in shoot dry weight at 22/14 °C. An *indica* breeding line, 12DS-GMET-25, showed maximum values for shoot dry weight of 0.9 g/plant and 1.78 g/plant at 30/22 °C and 38/30 °C, respectively. In contrast, IRRI 123 (an *indica* released genotype that is widely grown in Asia) showed the least values for shoot dry weight at optimum and high-temperature conditions. At low and optimum temperatures, root dry weight values ranged from 0.01 (IRRI 123) to 0.07 g (MERMENTAU, a released *japonica* variety) and 0.10 (BOWMAN and CL151—released *japonica* varieties; and RU1304154—a *japonica* breeding line) to 0.32 g (IR86635-2-3-3-3—an *indica* breeding line) with an average of 0.03 and 0.29 g, respectively (Table S2). INIA Tacuari (an *indica* released genotype) showed minimum values, whereas IR78049-25-2-2-2 (an *indica* breeding line) showed maximum values for shoot dry weight and total dry weight at 22/14 °C (Figure 2; Table S2). At high temperature, maximum values for the shoot and total dry weights of 1.78 g/plant and 2.06 g/plant, respectively, were recorded in genotype 12DS-GMET-25, and minimum values (0.63 g/plant for shoot dry weight and 0.77 g/plant for total dry weight) were recorded in IR09L179 (an *indica* breeding line). Low temperature reduces the plants' dry weight content [39–41]. Genotype genetic characteristics might be

responsible for the variability in percentage of shoot dry weight obtained in a day. Rice genotypes having higher total dry weight may have higher temperature stress tolerance than other genotypes.

3.3. Root Growth and Development

Root phenotype co-optimizes root architecture to acquire water and essential nutrients [42] to improve plant productivity under diverse growing conditions [43–46]. There is no single root feature ideal for different environmental stress [42,44]. Total root length, root surface area, root volume, and root diameters indicate the root's function under any given growing conditions [42,44,45]. These are useful characteristics for nutrient uptake efficiency and performance under various stress conditions, including temperature [46]. The roots are the most sensitive part of the plant to temperature [47]. Therefore, we evaluated the root phenotypic responses of the 64 rice genotypes, intending to identify those with synergistic or antagonistic responses to low and high temperatures.

The overall root system under low temperatures was significantly smaller than the roots under high temperatures. All the root growth and developmental parameters decreased (ranged from 2 to 87%) across genotypes under low-temperature conditions, except for root:shoot ratio (Tables S3 and S4). FEDEARROZ MOCARE recorded the maximum value for root length at 22/14 °C (803 cm/plant) and at 30/22 °C (3710 cm/plant). The maximum value was recorded under high temperature in CT18244-9-4-4-2-1-2 (an *indica* breeding line, 5132 cm/plant). Root surface area ranged between 21–83 cm²/plant with an overall average of 48 cm²/plant at low-temperature conditions, whereas, at high-temperature conditions, it ranged from 159–555 cm²/plant with an overall average of 344 cm²/plant (Figure 3C; Table S3). At optimum temperature, the lowest and highest value for root surface area was 114 cm²/plant (RU1504122, a *japonica* breeding line) and 476 cm²/plant (CT18614-4-1-2-3-2, an *indica* breeding line), respectively. There was not much difference in the average root diameter values, neither among the temperature treatments nor among the genotypes. The root volume values (Figure 3D) decreased similarly to the longest root length at low temperatures. However, there was not much difference among the genotypes growing at optimum and high temperatures. Similar to the root observations in this study, reduction or increase in temperature depressed root growth, such as root tips (Figure 3A) [47,48].

Root developmental traits such as the number of root tips, the number of root forks, the number of root crossings, and root numbers increased at 38/30 °C. Still, they decreased at 22/14 °C in comparison with optimum temperature conditions (Table S4). An 81% decrease in the number of root tips and an almost 90% decrease in the number of root forks and crossings were observed at low-temperature conditions compared with high-temperature conditions across rice genotypes (Figure 3A; Table S4). The number of roots ranged from 14.3–49/plant at 30/22 °C with an overall average of 26.7/plant, which decreased to 4.7–16.7/plant at 22/14 °C with an overall average of 26.7 roots/plant (Table S4). There was an increase of 26% in the number of roots in the plants growing at high-temperature conditions. The highest number of roots was recorded in CT186515-1-5-1-2-1 (an *indica* breeding line, 57/plant), whereas SABINE was found to have the least number of roots (18/plant). Genotypes with superior root development traits (number of crossings, RT, and RF densities) suggests developing a more branched root system directly related to the water and nutrient uptake potential of the plant [42]. Higher RCs, RTs, and RFs could lead to a better genotypic ability to compete in the uptake of nutrients and water from the soil profile. Therefore, genotypes with more tips, forks, and crossings should better tolerate extreme-temperature environmental conditions by maximizing nutrient and water uptake than rice genotypes with fewer numbers of those traits [43–46]. On average, rice genotypes under high-temperature conditions showed 26 and 56% lower root:shoot ratios than genotypes under optimum temperature and low-temperature conditions, respectively (Table S4). They were indicating the genetic variability in relative carbon allocation between the shoot and roots under extreme temperature. Compared with other genotypes,

MERMENTAU and RU1304154 (*japonica* breeding line) had the highest root-to-shoot ratio under low temperature (Table S4). As may be expected, the 12DS-GMET-25 and Apo (a drought-tolerant genotype [49]) genotypes had the maximum root:shoot ratio under high temperature relative to control (Table S4). The decrease in root:shoot ratio caused by high soil temperature may be attributed to inhibition of the formation and elongation of the primary root [50,51], decreased distribution of carbohydrates to root [52], and increased respiration [53].

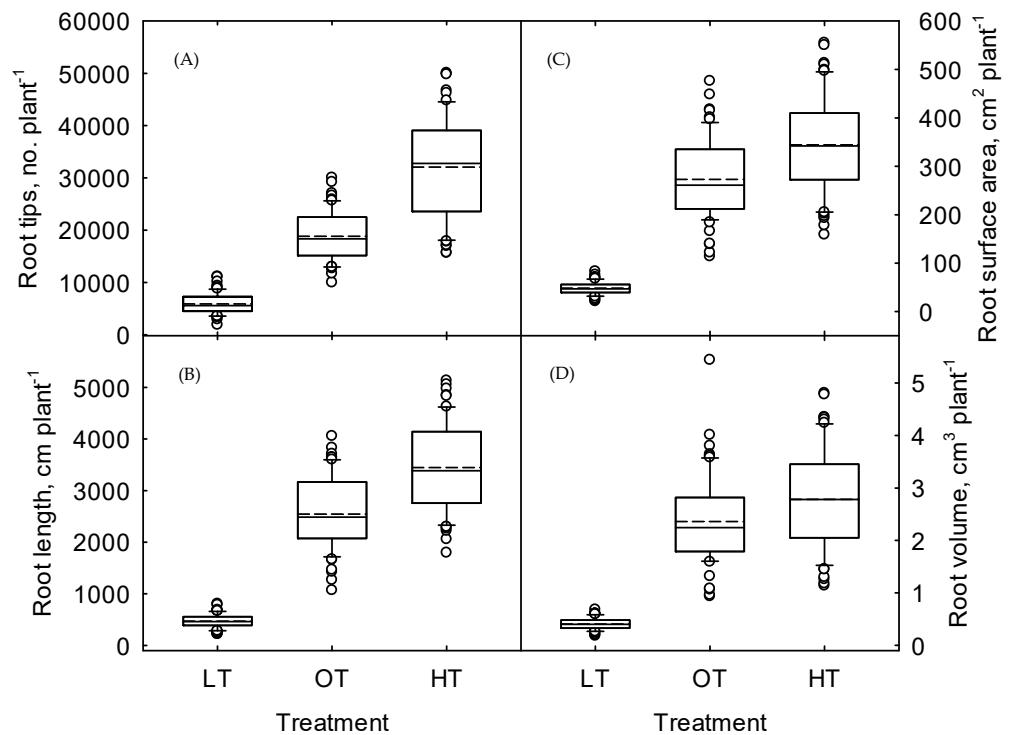


Figure 3. Temperature effects (low temperature—LT, optimum temperature—OT and high temperature—HT) on (A) root tips, (B) root length, (C) root surface area, and (D) root volume of 64 rice genotypes. Measurements were taken at 21 days after sowing. The middle line indicates the median; the box shows the range of the 25th to 75th percentiles of the total data. The whiskers indicate the interquartile range, and the outer dots are outliers.

3.4. Physiological Traits

The chlorophyll index and maximal quantum yield of photosystem II (F_v/F_m) are essential parameters for the photosystem II activity. Cold stress reduces significantly the concentration of chlorophyll in susceptible rice genotypes [36,54]. Chlorophyll index was used as a tool to evaluate the degree of cold tolerance of transgenic plants [55] and ecotype [36], to monitor plant recovery after stress [56], and to compare chilling tolerance between distinct hybrid genotypes during grain filling [52]. On average, the non-destructive leaf chlorophyll index increased by 8% with increased temperature from optimum to high levels (Figure 4A). On the contrary, the chlorophyll index decrement was more substantial (62%) under low temperatures. SPAD values ranged between 5 and 17 with an overall average of 12 at 22/14 °C, whereas it ranged from 25–40 with an overall average of 32 at 30/22 °C (Figure 4A; Table S5). At 38/30 °C the maximum chlorophyll index was recorded in IR65600-81-5-2-3 (an *indica* breeding line, 41), whereas HHZ 1-Y4-Y1 had the lowest value (another *indica* breeding line, 27). Slight differences in the fluorescence among treatments and genotypes (Figure 4B–D) suggest some level of stress-induced photosystem damage or increase in non-photochemical activities under extreme temperature [57]. The reduced chlorophyll index values under low temperature is due to reactive oxygen species (ROS)-

induced impaired pigment biosynthetic pathways, degradation of chloroplast membrane, or increased lipid peroxidation [20,36]. However, the chlorophyll index and fluorescence variability in response to extreme temperature suggest new opportunities for selection and genetic improvement in rice [36] and other C4 crops [57].

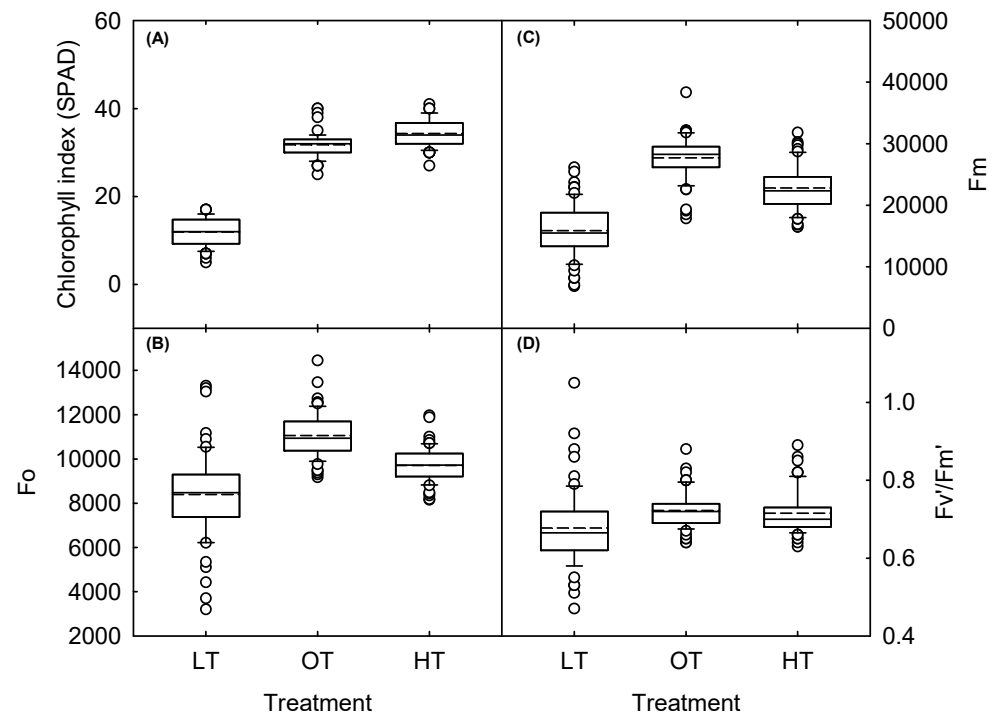


Figure 4. Temperature effects (low temperature—LT, optimum temperature—OT and high temperature—HT) on (A) chlorophyll concentration, (B) minimal fluorescence intensity (F_o), (C) maximal fluorescence intensity (F_m), and (D) chlorophyll fluorescence (F_v/F_m) of 64 rice genotypes. Measurements were taken at 21 days after sowing. The middle line indicates the median, and the box shows the range of the 25th to 75th percentiles of the total data. The whiskers indicate the interquartile range, and the outer dots are outliers.

3.5. Phenotypic Trait Correlations and Contributions of Component Traits to Seedling Vigor

In the present study, principal components (PC) analysis was performed to identify each trait's contributions that best described temperature response. The PCA was performed using relative responses of physiological, root, and shoot traits. Based on the PCA analysis, the first two PC cumulatively explained >43.3% in low temperatures (Figure 5A) and >43.8% under high-temperature (Figure 5B) stress of the total phenotypic variation. The root and shoot traits contributed more than the physiological traits to the separation of the rice genotypes (Figure 5). The rice genotypes variation in the first PC was mostly explained by root parameters (forks, RSA, RL, RV, and RC) in low-temperature stress, and LA (Figure 5A) and StWT under high temperatures (Figure 5B). The second PC variation was explained by seedling vigor traits such as shoot weight (SHWT), LWT, and R/S under low-temperature treatment (Figure 5A). Interestingly, physiological (F_m , F_v , and F_o) and seedling vigor (LWT, SHWT, TdWT, and R/S) were found to be associated with PC2 in high-temperature stress (Figure 5B). Seedling vigor (final seedling biomass) was significantly ($p < 0.05$) correlated with most of its components or related traits in both low and high temperature treatments. However, a significant negative ($p < 0.05$) correlation of shoot weight was observed with root:shoot ratio in low- ($r = -0.37$) and high- ($r = -0.54$) temperature treatments (Figure S1). The response of total seedling dry weight (TdWT) to low-temperature treatment was not significantly ($p > 0.05$) correlated with root:shoot ratio. Unlike in the low-temperature treatment, the correlations of chlorophyll fluorescence parameters (F_o , F_m , and F_v) with R/S and root tips were negative in response to high tem-

perature. However, F_v/F_m 's correlation with leaf area was positive under low temperature ($r = 0.29$), but the same relationship was nonsignificant under high temperature (Figure S1). Similar methodologies have been applied for the successful screening of corn (*Zea mays* L.) hybrids for cold tolerance [32], rice genotypes for drought tolerance [58], and soybean (*Glycine max* [L.] Merr) genotypes for evaluating low- and high-temperature tolerance [41].

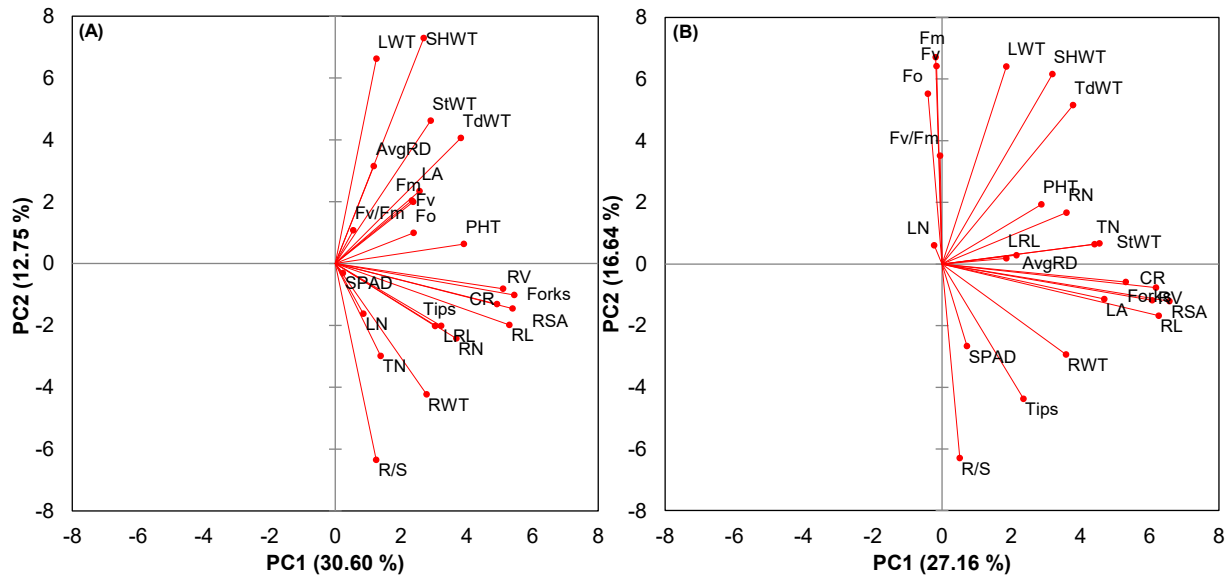


Figure 5. Principle component (PC) analysis for morphological and physiological traits of 64 rice genotypes in response to low temperature (A) and high temperature (B). Plant height (PH), tiller number (TN), number of leaves (LN), leaf area (LA), leaf weight (LWT), stem weight (StWT), shoot weight (SHWT), seedling total dry weight (TdWT), longest root length (LRL), average root diameter (AvgRD), root volume (RV), root tips (RT), root forks (RF), root crossings (RC), root length (RL), root surface area (RSA), root dry weight (RWT), root number (RN), chlorophyll index (SPAD), minimal fluorescence intensity (F_o), maximal fluorescence intensity (F_m), maximal variable fluorescence (F_v), and chlorophyll fluorescence (F_v/F_m).

3.6. Cumulative Low and High-Temperature Response Index

The cumulative temperature response index (CTRI) technique [24,32,40] was used to identify genotypes with low- and high-temperature (low-CLTRI and high-CHTRI) tolerance in the 64 rice genotypes. The most robust correlation coefficient was recorded between CLTRI and root ($r^2 = 0.64$) followed by physiology ($r^2 = 0.64$) low-temperature response index, implying that root and physiological parameters are crucial for low-temperature tolerance (Figure 6A). A weak correlation was observed between CHTRI and physiological traits under high temperature, indicating that root and shoot traits are more useful for selecting vigorous genotypes for heat tolerance at the seedling stage (Figure 6B). High temperature alters cell properties or activities such as enzyme kinetics, protein binding, and membrane fluidity due to excess ROS generation [59]. Heat-stress-induced changes in cell physical properties (degradation of chloroplast membrane or increased lipid peroxidation) partially explain the weaker relationship between CHTRI and physiological traits.

Further, a weak correlation ($r^2 = 0.1$) between CHTRI and CLTRI for shoot, root, and physiological parameters, indicates that heat and cold tolerance mechanisms are distinct. We also tested each trait's relative contribution to CHTRI or CLTRI through multiple linear regression (Table S6). Thirteen traits (PHT, TN, LN, LA, LWT, StWT, RWT, LRL, RT, RF, RC, RN, and SPAD) significantly contributed to CHTRI during seedling development under high-temperature stress treatment. In contrast, only five traits involving root and physiological traits (RWT, RC, RN, SPAD, and F_o) significantly and positively contributed to CLTRI under low temperature for seedling vigor (Table S6). These findings suggest

that developing tolerance for low and high temperatures at the seedling stage may require different selection strategies.

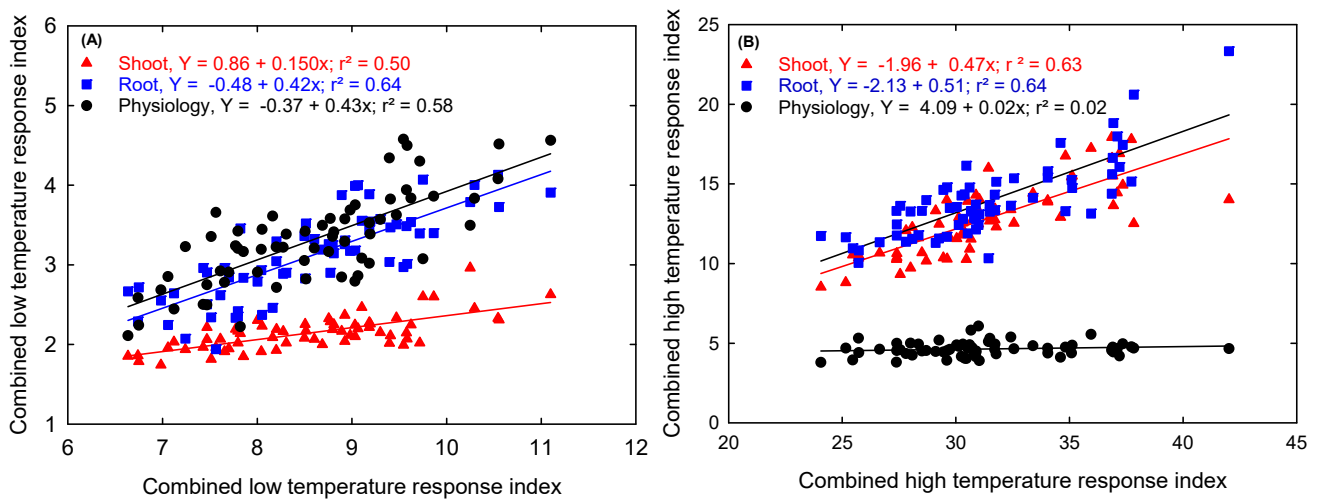


Figure 6. Correlation between combined low-temperature response index (CLTRI, **A**), combined high-temperature response index (CHTRI, **B**), and root, shoot, and physiological of 64 rice genotypes.

Based on the CLTRI values, 13 genotypes were classified as low cold-tolerant (cold-sensitive), 19 were moderately cold tolerant, 24 were highly cold tolerant, and 8 were very highly cold-tolerant genotypes (Table 1). It was observed that among the 19 moderately cold-tolerant genotypes, most of the genotypes were *indica* types, and among the 8 very highly cold-tolerant genotypes, most of them belonged to the *japonica* subspecies (Table 1). This indicated that *japonica* rice genotypes are more tolerant of low temperatures and perform better under such conditions, similar to other studies [12,36,60]. The lowest value of CLTRI was recorded in CT6510-24-1-2 (an *indica* line, 6.63), whereas RU1504114 (a *japonica* line) showed the highest value (11.09). Similarly, based on the CHTRI values, 10 genotypes were classified as low heat-tolerant or heat-sensitive, 31 (around 48%) were moderately heat-tolerant, 12 highly heat tolerant, and 11 were very highly heat-tolerant genotypes. In the highly heat-tolerant and very highly heat-tolerant category, most of the genotypes were *indica* rice genotypes, suggesting the potential of higher tolerance of *indica* compared with *japonica* genotypes to high temperatures. IR65600-81-5-2-3, an *indica* breeding line, had the highest CHTRI value (42.02), whereas IR09L179 (another *indica* line, 24.06) showed the least (Table 2). Based on the CHTRI, eleven genotypes outperformed the well-known reproductive stage tolerant genotype, N-22 [16,27,61]. These observations reveal the existence of different high-temperature stress tolerance mechanisms between vegetative and reproductive in rice. Thus, breeding for heat tolerance combined with vegetative and reproductive or grain filling, would help minimize environmental stresses. The identified heat- or cold-tolerant genotypes in this study could be useful to rice breeders in both tropical and temperate rice-growing countries as genetic donors to develop new rice genotypes that could withstand either low- or high-temperature conditions, or both temperature extremes, during the early season under dry direct seeding cultivation practice [9,14,62]. Such potential new varieties can also widen the timeframe for growing rice in areas affected by low- and/or high-temperature stress early in the growing season, thus avoiding and/or escaping other biotic and abiotic stress that occur later in the season.

Table 1. Classification of rice genotypes into various cold-tolerance groups based on combined low-temperature response index (CLTRI).

Low Cold Tolerance	Moderately Cold Tolerance	High Cold Tolerance	Very High Cold Tolerance
6.6343–7.6464	7.6465–8.6585	8.6585–9.6706	>9.6707
CT6510-24-1-2 (6.6343)	MILYANG 240 (7.6545)	CL163 (8.6878)	RU1504197 (9.7162)
CT18237-13-11-3-3-5-1 (6.7392)	CT6946-9-1-2-M-1P (7.6995)	Rex (8.7552)	NIPONBARE (9.7499)
CT18372-8-1-6-3-1-5 (6.7477)	CT18244-9-4-4-2-1-2 (7.7703)	Sabine (8.7711)	N-22 (9.8631)
12DS-GMET-15 (6.9824)	IR78049-25-2-2-2 (7.7949)	RoyJ (8.7959)	LAKAST (10.2485)
FEDEARROZ 473 (7.0562)	CT18245-4-7-1-1-2-1 (7.7961)	El Paso 144 (8.8085)	CL111 (10.2936)
IRRI 123 (7.1203)	IR65600-81-5-2-3 (7.8207)	Bowman (8.8909)	RU1404156 (10.5430)
CT18615-1-5-1-2-1 (7.2403)	CT19561-3-57-2P-2-1-2-M (7.8536)	RU1504122 (8.9239)	CL271 (10.5537)
FEDEARROZ 2000 (7.4287)	HHZ 1-Y4-Y1 (8.0026)	CL151 (8.9251)	RU1504114 (11.0994)
RU1504083 (7.4676)	IR86635-2-3-3-3 (8.0417)	RU1402174 (8.9802)	
CT18247-12-8-1-4-2-2 (7.4685)	CT18614-4-1-2-3-2 (8.0502)	IR09L179 (9.0320)	
COLOMBIA XXI (7.5123)	CT18233-15-6-6-4-8-1 (8.1615)	JES (9.0341)	
WAB 56-125 (7.5625)	IR86052-32-3-2 (8.1997)	RU1204156 (9.0628)	
RU1504154 (7.6098)	RU1404196 (8.2025)	Taggart (9.1040)	
	12DS-GMET-25 (8.2734)	RU0603075 (9.1826)	
	IR64-EMF NIL (8.3062)	RU1304154 (9.1826)	
	FEDEARROZ 21 (8.4975)	CT18593-1-7-2-2-5 (9.1930)	
	Thad (8.5053)	MERMENTAU (9.2998)	
	Cheniére (8.51322)	IrGA 409 (9.3959)	
	Apo (8.6027)	FEDEARROZ MOCARE (9.4066)	
		RU1204197 (9.4702)	
		RU1303138 (9.5439)	
		IR6 (PAKISTAN) (9.5753)	
		INIA Tacuari (9.5826)	
		RU1504198 (9.6234)	

Table 2. Classification of rice genotypes into various heat-tolerance groups based on combined high-temperature response index (CHTRI).

Low Heat Tolerance	Moderate Heat Tolerance	High Heat Tolerance	Very High Heat Tolerance
24.0647–27.8029	27.8030–31.5410	31.5411–35.2792	35.2793–39.0174
IR09L179 (24.0647)	NIPONBARE (27.8056)	CT18247-12-8-1-4-2-2 (31.7148)	INIA Tacuari (35.9532)
JES (25.1469)	CT19561-3-57-2P-2-1-2-M (28.0089)	IrGA 409 (31.7274)	RU1204197 (36.8627)
IR86052-32-3-2 (25.4620)	CT18233-15-6-6-4-8-1 (28.0844)	CT18237-13-11-3-3-5-1 (31.7754)	CT18593-1-7-2-2-5 (36.8784)
RU1504197 (25.7155)	Thad (28.3560)	Rex (32.4221)	IRRI 123 (36.9060)
12DS-GMET-15 (25.7315)	IR86635-2-3-3-3 (28.5095)	Cheniére (32.5563)	Bowman (36.9310)
CT6946-9-1-2-M-1P (26.6467)	CT6510-24-1-2 (28.6993)	CL111 (33.3937)	RU1504122 (37.1084)
FEDEARROZ 2000 (27.3866)	FEDEARROZ MOCARE (29.1116)	CT18372-8-1-6-3-1-5 (34.0439)	IR78049-25-2-2-2 (37.2010)
CT18615-1-5-1-2-1 (27.4010)	Sabine (29.2603)	IR6 (PAKISTAN) (34.0496)	HHZ 1-Y4-Y1 (37.3457)
RU1303138 (27.4013)	CT18245-4-7-1-1-2-1 (29.4525)	RU0603075 (34.6069)	RU1504114 (37.7292)
Taggart (27.5529)	RU1504154 (29.6024)	LAKAST (34.8213)	MILYANG 240 (37.8333)
	Apo (29.6167)	El Paso 144 (35.0925)	IR65600-81-5-2-3 (42.0246)
	RU1402174 (29.7321)	N-22 (35.1243)	
	COLOMBIA XXI (30.0382)		
	CL271 (30.1294)		
	FEDEARROZ 21 (30.2457)		
	CL163 (30.3132)		
	CL151 (30.3852)		
	RU1204156 (30.4613)		
	WAB 56-125 (30.5225)		
	RU1304154 (30.6095)		
	RoyJ (30.6671)		
	RU1404156 (30.7194)		
	RU1504198 (30.8046)		
	MERMENTAU (30.8833)		
	RU1404196 (30.8856)		
	12DS-GMET-25 (30.8925)		
	IR64-EMF NIL (30.9995)		
	FEDEARROZ 473 (31.0337)		
	CT18614-4-1-2-3-2 (31.4380)		
	RU1504083 (31.4866)		
	CT18244-9-4-4-2-1-2 (31.4997)		

3.7. Distinct Rice Genotypes Associated with Low- and/or High-Temperature Tolerance

Genetic-based approaches for mitigating critical environmental stresses, including extreme temperatures, have been emphasized in rice, starting with identifying genetic donors for breeding [27]. Groups of contrasting rice genotypes were selected based on CHTRI and CLTRI, following a normalized Z distribution method (Figure 7A). Four groups of rice genotypes, representing high- and low-temperature-tolerant (HTT+LTT), high-temperature-tolerant and low-temperature-sensitive (HTT+LTS), high-temperature-sensitive and low-temperature-tolerant (HTS+LTT), and high-temperature-sensitive and low-temperature-sensitive (HTS+LTS) groups were identified and classified as combined cold- and heat-stress resilient, only high-temperature tolerant, only tolerant of low temperature, and heat-stress sensitive, respectively (Figure 7A).

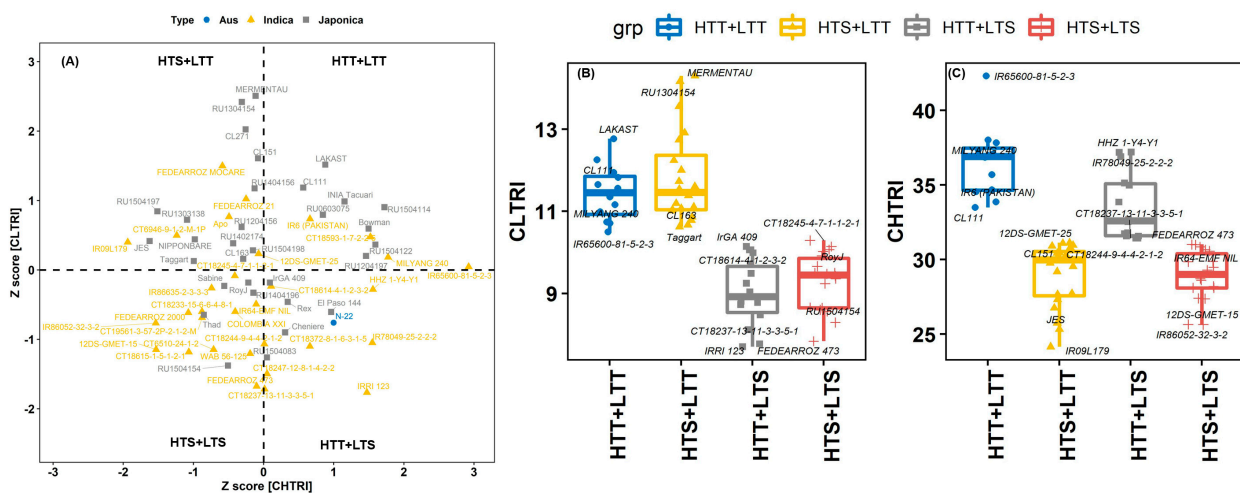


Figure 7. Selection of heat-stress-resilient and -sensitive rice lines based on combined high-temperature response index (CHTRI) and combined low-temperature response index (CLTRI). Normalized Z values were estimated for CHTRI and CLTRI for each line, and the estimated Z score were used to select rice genotypes with a contrasting response under extreme temperature. The figure’s quadrant shows rice genotypes with contrasting responses to low and high-temperature stress (A). The genotypes were grouped into four contrasting groups based on their response to low temperature (B) and high temperature (C) during the seedling stage, and their cumulative phenotypic responses were graphed.

Based on the Z score of CLTRI and CHTRI values, 12 genotypes were classified as low- and high-temperature tolerant (HTT+LTT), 34 were intermediate cold (20, HTS+LTT) or high-temperature tolerant (14, HTT+LTS), 24 were highly cold-tolerant, and 18 were low- and high-temperature-sensitive genotypes (Figure 7A). The selected heat-stress-resilient (HTT+LTT) genotypes had high CHTRI (36.5) and CLTRI (11.5) when exposed to high- and low-temperature stress at the seedling stage, respectively (Figure 7B,C).

Within the heat-stress-resilient group (HTT+LTT), the genotype LAKAST (a released japonica genotype) recorded higher CLTRI (30.4%) than the heat-sensitive group. Likewise, the genotype IR65600-81-5-2-3 had higher CHTRI by 36.5% compared with the sensitive group. On the contrary, the genotypes FEDEARROZ 473 and IR86052-32-3-2 recorded 31.6% and 30% lower CLTRI and CHTRI compared with the HTT+LTT group (Figure 7B,C). Based on the CLTSI performance in this study, five japonicas (breeding line RU1304154, and released varieties CL271, CL151, MERMENAU, and LAKAST) are expected to be more useful for developing varieties for areas with the cold stress that occurs early in the cropping season. On the other hand, three indica genotypes (breeding lines IR65600-81-5-2-3 and HHZ 1-Y4-Y1 and genotype MILYANG 240) and two japonica breeding lines (RU1504114 and RU1504122) performed well under high-temperature stress at the early seedling stage based on CHTSI. Finally, genotypes that performed well under both low- and high-temperature treatments included two indica breeding lines (IR65600-81-5-2-3 and CT18593-1-7-2-2-5), two japonica breeding lines under development (RU1504114 and

RU1504122), and two *japonica* released genotypes (Bowman and INIA Tacuari). Most improved rice varieties grown under current production systems have been developed for optimal growing conditions and were not intentionally bred for unfavorable environmental conditions [27,63]. Therefore, the heat- and/or cold-tolerant genotypes identified in this study, which encompassed a broad range of genetic diversity, involving both the two major rice subspecies of *indica* and *japonica*, could help breeders develop new rice genotypic combinations that would possess low- and or high-temperature tolerance early in the growing season.

4. Conclusions

The 64 rice genotypes examined in this study exhibited substantial variability in their responses for root and shoot traits. By screening a diverse set of rice genotypes, encompassing both the *indica* and *japonica* major rice subspecies, we were able to identify genotypes, including released varieties and experimental breeding lines still under development, that are tolerant of extreme temperatures. Cold stress caused significant reductions in all root, shoot, and physiological traits, whereas a significant increase in all traits was observed under high temperatures among genotypes, compared with optimum temperatures. Our results indicated that rice is more sensitive to low temperatures than high temperatures at the seedling stage. The CTRI-based technique provided a means for quantifying total variability, and thus, may be useful as selection criteria for screening rice genotypes for cold or heat tolerance. This information would help determine which (shoot or root) traits are best suited among rice genotypes for improving cold and heat tolerance. Genotypes identified to be cold-tolerant and heat-tolerant may be used as genetic donors for developing new rice varieties or hybrids that can withstand low-temperature, high-temperature, and/or both extreme conditions under both tropical and temperate climate. With improved plasticity, identified new extreme temperature-resilient genotypes could grow rice under a broader range of geophysical and temporal environments. However, these results should be validated under field conditions to evaluate their performance before recommending them to the producers and breeders to accomplish the maximum benefit.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4395/11/1/112/s1>, Table S1: Plant height (PH), tiller number (TN), leaf number (LN) and leaf area (LA), measured in low-temperature (LT), optimum-temperature (OT) and high-temperature (HT) stress conditions in 64 rice genotypes, Table S2: Dry weight traits measured in low-temperature (LT), optimum-temperature (OT) and high-temperature (HT) stress conditions in 64 rice genotypes, Table S3: Root growth traits: root length (RL), root surface area (RSA), root average diameter (AvgRD), root volume (RV) and longest root length (LRL), measured in low-temperature (LT), optimum-temperature (OT) and high-temperature (HT) stress conditions in 64 rice genotypes, Table S4: Root tips (RT), root forks (RF), root crossings (RC), root number (RN), and root:shoot ratio measured in low-temperature (LT), optimum-temperature (OT) and high-temperature (HT) stress conditions in 64 rice genotypes, Table S5: Chlorophyll index and chlorophyll fluorescence measured in low-temperature (LT), optimum-temperature (OT) and high-temperature (HT) stress conditions in 64 rice genotypes, Table S6: Multiple linear regression of combined high-temperature response index (CHTRI) and combined low-temperature response index (CLTRI) with the individual responses index of twenty-four traits in response to low and high temperature. Figure S1: Correlation matrix among traits in response to low- and high-temperature stress in rice.

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Abbreviations

CHTRI	Cumulative high-temperature response index
CLTRI	Cumulative low-temperature response index
CTRI	Cumulative temperature response index
DAS	Days after sowing
F _m	maximal fluorescence intensity
F _o	minimal Fluorescence Intensity
F _v	maximal variable fluorescence
F _v /F _m	Chlorophyll fluorescence
HTS	High-temperature sensitive
HTT	High-temperature tolerant
IHSRI	Individual heat-stress response index
ILSRI	Individual low-stress response index
LA	Leaf area
LN	Total number of leaves
LTS	Low-temperature sensitive
LTT	Low-temperature tolerant
LWT	Leaf dry weight
PCA	Principal Component Analysis
PH	Plant height
AvgRD	Root average diameter
RC	Root crossings
RF	Root forks
RI	Relative injury
RL	Root length
RN	Root number
R/S	Root:shoot ratio
RSA	Root surface area
RT	Root tips
RV	Root volume
RWT	Root dry weight
SPAD	Chlorophyll index
SPAR	Soil–plant–atmosphere research
StWT	Stem dry weight
SHWT	Above-ground dry weight
TdWT	Total dry weight
TN	Tiller number

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