Morpho-physiological Characterization of Rice Cultivars for Early-season Soil Moisture Stress Response

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Introduction

The majority of rice (*Oryza sativa* L.) grown in Mississippi (MS) is exported overseas. The producers deploy dry direct seeding method followed by one or two flushes to accomplish better seedling establishment. However, the latter not only increases the production cost, but also decreases ground water recharge. Moreover, the limited research on drought tolerance for early season growth has slowed the progress of rice breeding programs in developing drought resistant cultivars. As a consequence, MS farmers every year face a risk of poor stand establishment of rice grown under dry direct seeding practice which adversely affects the economic production of the crop.

Objectives

To identify tolerance to drought stress among rice cultivars based on different levels of soil moisture stresses imposed during the early seedling growth stage.

Hypothesis

It was hypothesized that morpho-physiological traits and appropriate screening tools could be used to identify drought tolerance among the rice cultivars.

Materials and Methodology

Seed Material
The 15 rice genotypes were obtained from the Mississippi State University's Delta Research and Extension Center in Stoneville, MS (33° 42´ N, 90° 92´ W). Seeds were processed in a rice seed laboratory and inspected to ensure they had met the recommended seed quality standards before they were kept in cold storage until use.

Methodology
- The 15 rice genotypes were evaluated at three levels of soil moisture contents (SMC) under greenhouse conditions at Rodney Foil Plant Science Research Center, Mississippi State University, Mississippi State (33° 28´N, 88° 47´W), Mississippi, USA.
- The experiment was arranged in a split-plot RCBD design with soil moisture as main plot factor, cultivar as subplot factor, row as the main plot unit and the plants (pots) as the subplot units.
- Rice seeds were sown in polyvinyl plastic pots, 15.2 cm diameter and 30.5 cm height, filled with sandy loam soil (3:1 by volume of sand and top soil).
- The experiment was organized on four wide benches oriented east to west representing blocks (blocks = replications). In each block (bench) the three levels of soil moisture treatments, 0.160, 0.106, 0.053 $m^3 \cdot m^{-3}$ soil, representing 100, 66, and 33% of field capacity (FC), respectively were randomly assigned to three rows representing main plots. The 15 rice cultivars (sub plots) were randomly placed in each row.
- The three levels of soil moisture contents (SMC) were maintained by inserting moisture sensors (Model EC-5; Decagon Devices, Inc., Pullman, WA, USA) at a depth of 10 cm in four pots per soil moisture treatment until the final harvest, 30 days after sowing (DAS) **(Fig. 1)**. The irrigation amount was determined using following formula.

Irrigation time (min.) = (treatment SMC – measured SMC) / emitter discharge rate

Measurements

Physiological Parameters
Chlorophyll content using SPAD meter (SPAD-502, Minolta Camera Co. Ltd., Japan) and quantum efficiency (Fv'/Fm') that describes the photosynthetic capacity of leaves using Fluor-Pen (FP 100, FluorPen meter, Drasov, Czech Republic) were measured at 25 days after planting (DAP).

Shoot Morphological Parameters
Total number of tillers (TN), plant height (PH), the number of leaves (LN), and total dry weight, and leaf area (LA) using leaf area meter (Li-3100 leaf area meter, Li-COR Inc., Lincoln, NE) were measured at the final harvest, 30 DAP.

Root Parameters
- Roots were cut and separated from the stem after the harvesting. They were then scanned using WinRHIZO Pro software (Regent Instruments, Inc., Quebec, QC, Canada) optical scanner to acquire root images of 800 by 800 dpi resolution.
- Roots images were then analyzed to study 10 root parameters with a computer.
- This includes cumulative root length (RL), root surface area (RSA), average root diameter (RAD), number of forks (RNF), root length per volume (RLPV), number of tips (RNT), root volume (RV), number of crossings (RNC), root number (RN), and longest root length (LRL) **(Table 1)**.

Total Drought Response Index
Cumulative high drought response index (CHDRI) was calculated with sum of all Individual high drought response indices (IHDRI) for each parameter at high drought conditions (33% FC) **(Eq. 1 & 2)**.

$$IHDRI = Ph/Po \quad \text{(Eq. 1)}$$

$$CHDRI = (TLh/TLo)+ (PHh/PHo)+ (LAh/LAo)+LNh/LNo+ (SWh/SWo)+ (LWh/LWo)+ (RWh/RWo)+ (RLh/RLo)+ (PAh/PAo)+ (RSAh/RSAo)+ (RADh/RADo)+ (RLPVh/RLPVo)+ (RVh/RVo)+ (RNh/RNo)+ (LRLh/LRLo)+ (RNTh/RNTo)+ (RNFh/RNFo)+ (RNCh/RNCo)+ (RSh/RSo) \quad \text{(Eq. 2)}$$

Similarly, cumulative moderately drought response index (CMDRI) was calculated.
Total drought response Index (TDRI) = CMDRI + CHDRI
Cultivars were classified based on TDRI and Standard deviation **(Table 2)**.

Data were analyzed using PROC MEANS and PROC MIXED in SAS 9.4 (SAS Institute Inc., 2011) using least significant difference statistic (LSD = 0.05) for comparison.

Figures

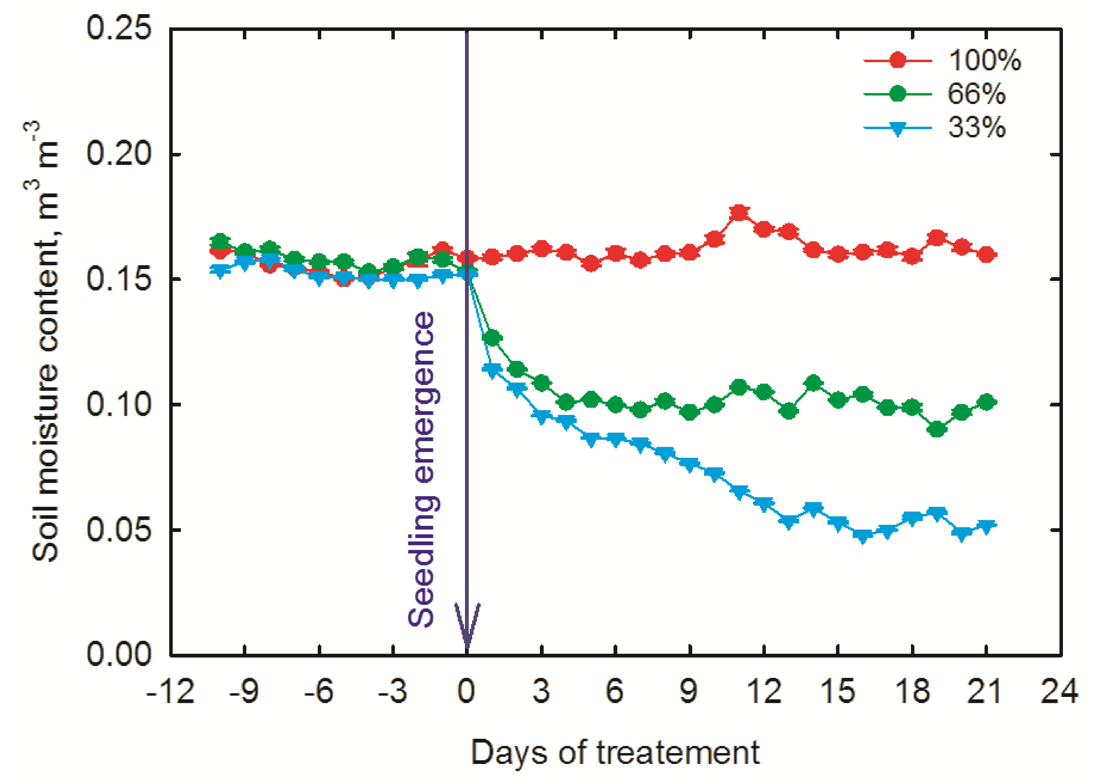

Fig. 1. Volumetric soil moisture content across treatments before and during the experimental period was maintained using sensor-based monitoring and irrigation system. The arrow indicates the day the treatments were imposed. Data are means ± SE (n=4).

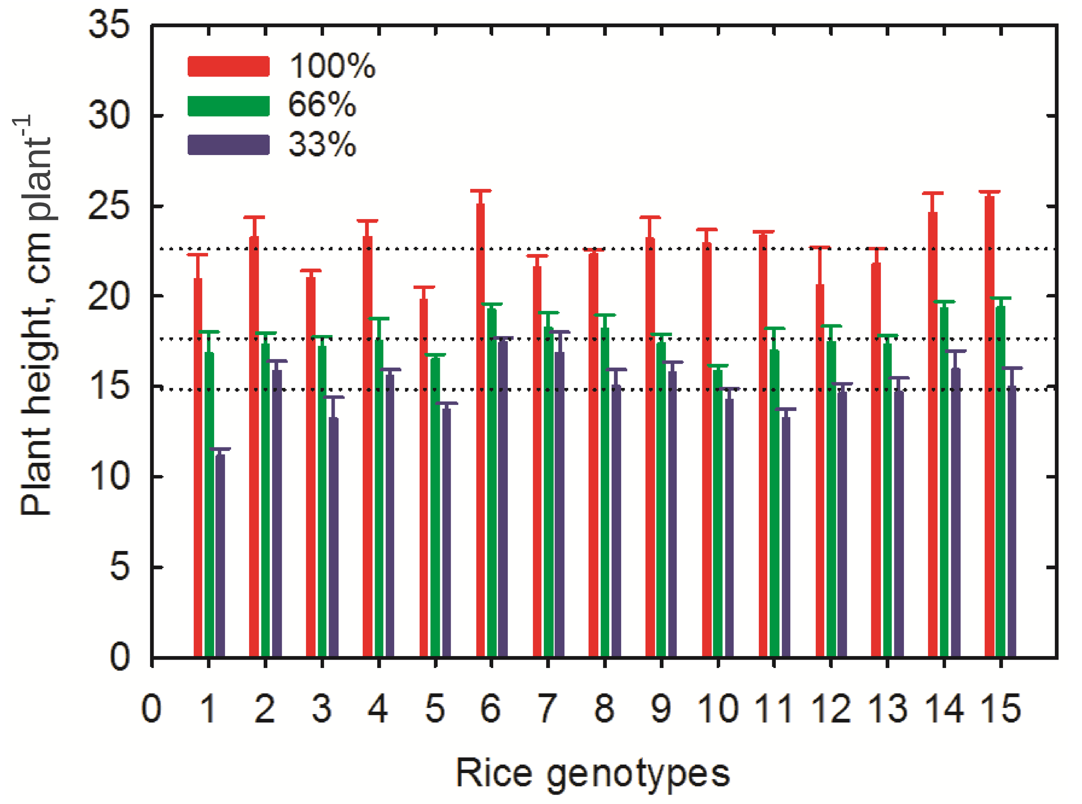

Fig 2. Variability in plant height of 15 rice cultivars at three different soil moisture treatments. Horizontal hatched lines represent 100%, 66%, and 33% field capacity from top to bottom, respectively. Data are means ± SE (n=4).

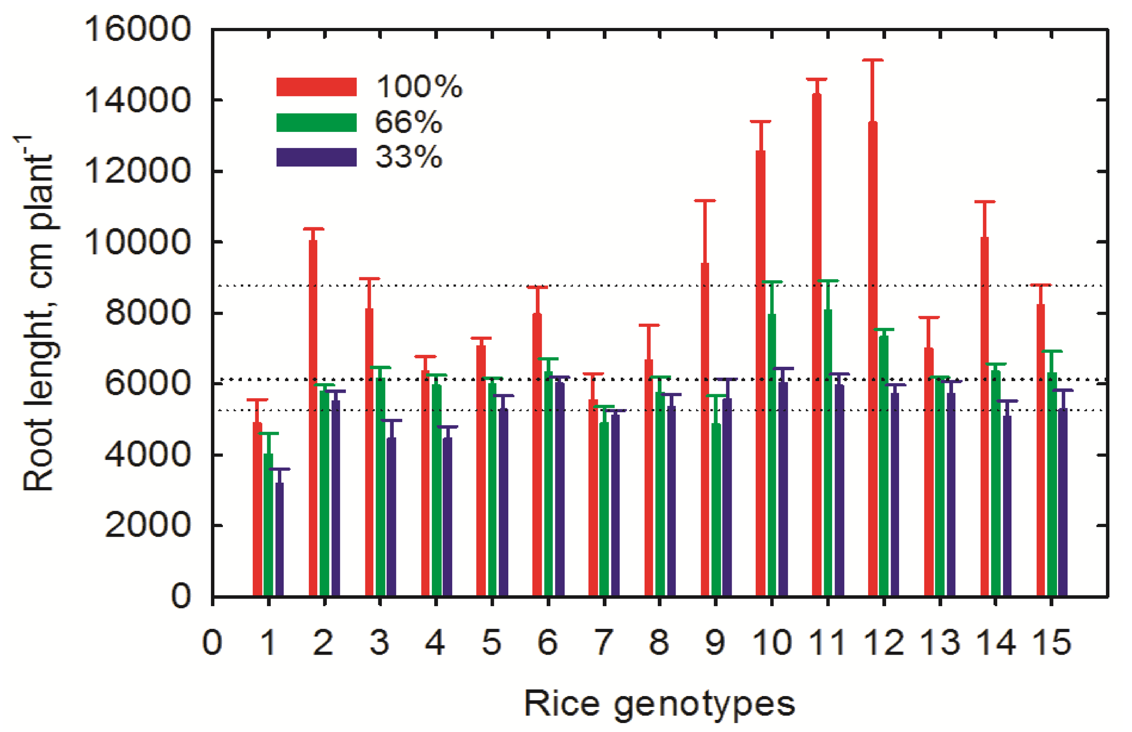

Fig 3. Variability in root length of 15 rice cultivars at three different soil moisture treatments. . Horizontal hatched lines represent 100%, 66%, and 33% field capacity from top to bottom, respectively. Data are means ±SE (n=4).

Tables

Source of variance	RL	PA	SA	Avg. dia.	L/V	RV	Tips	Forks	Cross	RW	LRL	TR	PH	LN	TN	LA	LW	SW	R/S	Fv'/Fm'	SPAD value
Cultivars (100% FC)	***	***	***	***	***	***	***	***	***	***	NS	***	**	***	***	***	***	***	**	NS	*
Cultivars (66% FC)	***	***	***	***	***	***	**	***	***	***	**	***	NS	***	***	***	***	***	NS	NS	***
Cultivars (33% FC)	***	***	***	**	***	***	**	***	**	***	NS	NS	***	***	***	*	***	NS	NS	*	***
Treatment (T)	***	***	***	***	***	***	**	***	***	***	***	***	***	***	***	***	***	***	***	*	NS
Cult X T	***	***	***	***	***	**	***	***	***	***	**	***	NS	***	***	***	***	***	NS	NS	NS

† The significance levels ***, **, *, and NS represent P ≤ 0.001, P ≤ 0.01, P ≤ 0.05, and P > 0.05, respectively.

Field capacity (FC), root length (RL), projected area (PA), surface area (SA), average diameter (Avg. dia.), root length per volume (L/V), root volume (RV), root tips (Tips), root forks (Forks), root crossings (Cross), root weight (RW), longest root length (LRL), total roots (TR), plant height (PH), number of leaves (LN), number of Tillers (TN), leaf area (LA), leaf weight (LW), stem weight (SW), root/shoot ratio (R/S), quantum efficiency (Fv'/Fm').

Table 1. *Analysis of variance across the cultivars (Cult) and treatments (T) and their interaction (Cult X T) based on rice physiological and morphological parameters measured at 25 and 30 days after planting, respectively.*

Low drought-tolerant (TDRI = 22.87-26.11)	Moderately drought-tolerant (TDRI = 26.12-29.35)	High drought-tolerant (TDRI = 29.36-32.59)
CLXL729 (22.87)	CHINERE (27.03)	RU1104122 (30.72)
CL152 (22.91)	RU1304154 (27.04)	CL151 (31.76)
XL753 (23.34)	CL111 (28.62)	CL142-AR (32.21)
CLXL745 (23.44)	MERMENTAU (28.95)	
COCODRIE (24.13)	REX (29.01)	
RU1204122 (24.95)		
LAKAST (25.90)		

Low drought-tolerant (TDRI ≤ minimum TDRI + 1.0 SD)
Moderately drought-tolerant (minimum TDRI + 1.0 SD < TDRI ≤ minimum TDRI + 2.0 SD)
High drought-tolerant (minimum TDRI + 2.0 SD < TDRI ≤minimum TDRI + 3.0 SD)

Table 2. *Classification of 15 rice cultivars based on total drought response index and Standard deviation.*

Results and Discussion

Physiological Parameters
- The study observed significant variability for Fv'/Fm' and SPAD value at 33% FC among the rice cultivars.
- The quantum efficiency (Fv'/Fm') had shown positive and significant correlation with increasing water stress.
- Cultivars Cheniere, Cocodrie, and RU1104122 had higher values for SPAD and Fv'/Fm' at 33% FC than at 66% FC.

Shoot Parameters
- The analysis of shoot parameters in this study indicates significant variation among cultivars, moisture contents, and moisture X cultivar interactions (*P*< 0.001) for LN, TN, LA, and SW **(Table 1)**.
- Shoot growth of the rice cultivars was reduced under the drought stress mainly because of the reduced cell expansion that may result in reduced PH.
- The study observed significantly shorter plants at 66% and 33% FC than at 100% FC. The variability in plant height ranged from 19.8 cm (Rex) to 25.5 cm (RU1304154), with an average PH of 22.61 cm across all the cultivars at 100% field capacity **(Fig. 2)**.

Root parameters
- The analysis of root parameters in this study indicates significant variation among cultivars, moisture contents and, moisture X cultivar interactions (*P*< 0.001) for RL, RSA, RAD, RLPV, RV, RNT, RNF, RNC, RW, LRL, and R/S.
- The study observed higher root to shoot ratio at 33% FC than at 100% FC which may occur due to osmotic adjustment.
- This study observed genetic variability for root traits in response to the moisture stress such that CLXL729 (11) showed greatest reduction in RL i.e. reduced by half on increasing moisture stress from 100% FC to 33% FC **(Fig. 3)**.

Total Drought Response Index
- The cultivars, Cocodrie, Lakast, CL152, XL753, CL XL729, CL XL745, and RU1204122 have been designated as low drought tolerant or drought-sensitive cultivars. Cheniere, Mermentau, Rex, CL111, and RU1304154 were moderately drought tolerant or moderately drought sensitive, and CL142-AR, CL151, and RU1104122 were highly drought tolerant among the 15 cultivars **(Table 2)**.

Conclusion

The cultivars CLXL729 and CL142-AR were identified as the least and the most tolerant to drought, respectively, during the early seedling growth stage. The positively linear coefficient between shoot and root TDRI (R^2 = 0.64) indicate the significance of using any or both of the traits for identifying drought tolerance in rice. Rice genotypes identified as drought tolerant could be selected for dry direct seeded rice cultivation under variable soil moisture conditions in the production environment. However, there is need to test these cultivars under field conditions at different growth stages for moisture stress before formulating any final decision.

Acknowledgement

We are very thankful to Mississippi Rice Promotion Board for funding for this study. A special thanks to Mr. David Brand and other graduate students in Environmental Plant Physiology Laboratory for technical assistance.