International Journal of Agriculture and Biosciences 2025 14(1): 84-93.
https://doi.org/10.47278/journal.ijab/2024.200

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Comparative Effect of Drought Stress on Growth and Physiological Performance of Three Different Rice Cultivars

Ploinapat Mahatthanaphatcharakun and Worasitikulya Taratima*

Salt Tolerant Rice Research Group, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002 Thailand
*Corresponding author: worasitikulya@gmail.com

ABSTRACT

Rising global temperatures are causing prolonged droughts that reduce rice growth. This study investigated the impacts of drought stress and the rewatering period on rice's growth and physiological characteristics (*Oryza sativa* L.) cv. KHAO DAWK MALI 105 (KDML105), MALI DAM NONG KHAI 62 (MDNK62) and POKKALI. The 28-day-old rice seedlings of the three cultivars were cultured under water deficit for 19 days (drought stress period) before rewatering for 9 days (rewatering period). Growth and physiological features were investigated at the end of the drought stress and rewatering periods. Drought stress inhibited the growth of all three cultivars, such as tiller number per clump, leaf size, root length, and biomass, while shoot length, leaf rolling score, and drought score of leaf increased compared to the control. All growth features of the three rice cultivars improved after rewatering. The physiological characteristics of KDML105 and MDNK62 rice cultivars differed from POKKALI under drought stress. Chlorophyll a content, total chlorophyll content, Fv'/Fm', Fv/Fm, and relative water content of KDML105 and MDNK62 rice cultivars decreased but increased in POKKALI. Electrolyte leakage percentage and MDA content of the three rice cultivars increased compared to the control group during drought stress and rewatering. Growth and physiological aspects including the drought tolerance index (DTI) for MDNK62 showed moderate adaptation to drought stress, while KDML105 exhibited better adaptability compared to MDNK62 and POKKALI. This research provides important information for rice growth improvement in regions facing drought challenges.

Keywords: Drought stress, Drought tolerance index, Electrolyte leakage, MDA, Rice growth

Article History
Article # 24-871
Received: 06-Oct-24
Revised: 23-Oct-24
Accepted: 15-Nov-24
Online First: 09-Dec-24

INTRODUCTION

The phenomenon of global warming is causing more frequent extreme weather events which are predicted to intensify. Rising temperatures, coupled with droughts, increase water evaporation and disrupt plant reproductive processes and physiology. The process of photosynthesis, which plants use to produce nutrients, becomes less efficient at temperatures above the critical growth threshold (Matsui et al., 2000; Vargas Zeppetello et al., 2022; Yaliang et al., 2020). Water is a major component of plant cells that helps to transport dissolved nutrients and other important substances. Insufficient water causes the plant roots to probe deeper in search of moisture. A lack of water around the roots leads to plant dehydration and death (Hou et al., 2024). Drought affects the growth of rice plants at every stage because they are highly sensitive to water scarcity. Some drought-resistant rice varieties have a good recovery index from drought, which allows them to produce a more complete seed yield compared to varieties with low recovery ability, as observed by shoot length, tiller number per clump, fresh weight and dry weight of plant, and fresh seed weight per panicle (Sandeep & Godi, 2023). After experiencing drought, the ability to maintain good leaf water potential leads to efficient flower development, allowing rice to pollinate and produce full, non-flattened seeds (Farooq et al., 2024). Drought results in stomatal closure due to decreased turgor pressure within the cells. The ABA hormone, a compound in the isoprenoid group, plays a crucial role as a signaling molecule to the guard cells, influencing stomatal closure to reduce water loss (Liu et al., 2022). This can be measured through the relative

Cite this Article as: Mahatthanaphatcharakun P and Taratima W, 2025. Comparative effect of drought stress on growth and physiological performance of three different rice cultivars. International Journal of Agriculture and Biosciences 14(1): 84-93. https://doi.org/10.47278/journal.ijab/2024.200

A Publication of Unique Scientific Publishers

water content (RWC) in leaves which determines the water status of plants. The RWC increases during the early growth stages and decreases when leaves wilt, reflecting tissue metabolic activity and serving as a significant index (Dash et al., 2020; Bharti et al., 2020; Zhang et al., 2024). Stomatal closure also affects the diffusion of carbon dioxide into the cells, impacting the rate of photosynthesis and thus food production)Yang et al., 2020(. Increased temperatures during drought lead to changes in light reaction in the thylakoid membranes within chloroplasts. When plants grow under high-temperature drought stress, the oxygen-evolving complex proteins may be damaged (Eckardt, 2022(, resulting in the dislodgement of the manganese-containing reaction center from the photosystem and the separation of the surrounding light-harvesting complexes from photosystem II. This shift results in slower and less efficient energy activation which delays electron transfer and leads to an accumulation of oxygen from water oxidation reactions that damages cell walls and produces toxic peroxide compounds in plants. These changes can be assessed by measuring the levels of malonaldehyde and the rate of electrolyte leakage in leaves (Taratima et al., 2022). Under high-temperature drought, plant cells activate a mechanism that converts excess light energy into heat through the action of xanthophylls in photosystem II via non-photochemical quenching, with violaxanthin converted to zeaxanthin to absorb excess heat (Sun et al., 2022; Nan et al., 2022). The maximum efficiency of photosystem II is an indicator of the system's ability to use light energy to drive electron transfer which can be determined by measuring chlorophyll fluorescence (Moustakas et al., 2022; Wang et al., 2022a).

KDML105 is a commercially popular rice variety cultivated in Thailand, with good growth, flowering characteristics and high yield. However, the leaves dry out quickly and turn yellow (Rotrujanon et al., 2022). POKKALI rice has a well-developed root system that can efficiently absorb nutrients and water from the soil. The stems are short and strong, making them resistant to high winds and heavy rainfall. Previous research demonstrated that this rice variety is tolerant to salinity and drought (Jacob & Subramannian, 2022). Colored native rice varieties have received certification from the Department of Rice because their characteristics make them resilient to environmental stress (Saleeto et al., 2020.(MDNK62 originated from a selection process within a mixed population of rice varieties, incorporating parental lines of black-husked rice including KDML105, Surin 1, Hom Mali Suko Thai, and black glutinous rice. This rice variety is not sensitive to changes in daylight duration; the leaves are green-purple and stem height reaches 1meter. The grains are slender and dark, producing soft sticky rice with a fragrant aroma and high levels of antioxidants. MDNK62 has excellent resistance to blast disease during the seedling stage and yields over 500kg per hectare. It can be cultivated in both wet and dry seasons. However, information regarding the influence of education on this lineage's prosperity is still lacking (Saleeto et al., 2020). This study evaluated the effects of drought stress on the growth and physiological performance of the three rice seedlings KDML105, MDNK62, and POKKALI. Our findings will advance knowledge to enhance rice genotypes in future breeding initiatives for early drought-resistant cultivars.

MATERIALS & METHODS

Plant Materials and Drought Stress

KDML105 and POKKALI rice seeds were obtained from the Faculty of Agriculture, Khon Kaen University, with MDNK62 obtained from the Rice Research Center, Nong Khai Province, Thailand. Mature seeds were germinated, cultured in soil, and watered daily for 1 week before transferring into 20cm diameter pots with two plants per pot and cultured for 3 weeks. Each pot contained 3.5kg of soil. The 28-day-old seedlings were placed under drought stress for 19 days before rewatering (500mL per pot, once a day) for 9 days. The control and treatment samples were performed in five replicates.

Plant Growth

The control and treatment were conducted with pot-grown plants between July and August 2023 in the greenhouse at the Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand. Shoot length, tiller number per clump, leaf number, leaf size, leaf rolling score, root length, shoot fresh weight, shoot dry weight, roots fresh weight, root dry weight, drought score, and drought recovery score (Beena et al., 2021(were recorded after the drought stress treatment and rewatering treatment. Green intensity was measured using a Konicaminolta SPAD-502 Plus Chlorophyll Meter and expressed in SPAD units. The drought tolerance index (DTI) was calculated according to Taratima et al. (2020) (more than 1 = increase, less than 1 = decrease) as follows:

$$\text{DTI} = \frac{\text{Stress treatment data}}{\text{Nonstress treatment data}}$$

Relative Water Content (RWC)

One cm long leaf at the middle part of leaf was used as initial sample. Fresh weight was recorded and then the sample was transferred into a test tube containing mL10 of deionized water. The tube was sealed tightly and exposed to fluorescent light for 4h. The sample was blotted dry with tissue paper, and the turgid weight was measured. The plant material was then oven-dried at 60°C for 24h. Before dry weight measurement. The RWC was calculated using the following formula according to Basak et al. (2020):

$$\text{Relative water content } = [\%] \left(\frac{\text{FW-DW}}{\text{TW-DW}}\right) \text{x100}$$

where: FW =Leaf fresh weight, DW =Leaf dry weight, TW =Turgid weight

Chlorophyll Content and Chlorophyll Fluorescence

Chlorophyll a, chlorophyll b, and total chlorophyll contents were determined. Mature leaves (30mg) were crushed in a mortar, then dissolved in mL5 of %80acetone and kept in the dark for 48h. The supernatant was measured for absorbance at 645and nm663 wavelengths with 80% acetone operating as the blank. The following formulas were utilized to determine the chlorophyll content based on Vivek et al. (2020):

$$\text{Chlorophyll a (mg g tissue}^{-1}\text{=)} \frac{(12.7A_{663} - 2.69A_{645}) \text{ x V}}{1000 \text{ x W}}$$

Chlorophyll b (mg g tissue^{-1}=) $\frac{(22.9A_{645} - 4.68A_{663}) \times V}{1000 \times W}$

Total chlorophyll (mg g tissue^{-1} =) $\frac{(20.2A_{645} + 8.02A_{663}) \times V}{1000 \times W}$

where V =solution volume)ml(and W =leaf weight.

To analyze chlorophyll fluorescence, mature leaves were determined for both the light-adapted quantum efficiency of PSII (Fv'/Fm') and the maximum or dark-adapted quantum efficiency of PSII (Fv/Fm) using a Chlorophyll Fluorometer (Handy PEA).

Malonaldehyde (MDA)

MDA content was measured according to the technique reported by Heath & Packer (1968). One gram of fresh and expanded leaf was collected, ground with mL10 of %0.1(wv^{-1}) trichloroacetic acid (TCA), and then centrifuged for 5min (14,000rpm). A 2mL aliquot of the supernatant was then transferred to another test tube and heated for 25min at 95°C with 9mL of 0.5% (w/v) thiobarbituric acid (TBA). All reactions were halted by 10min of chilling on ice. The absorbance of the solutions was measured at 532nm (A_{532}) and 600nm (A_{600}) wavelengths with 20% TCA utilized as the blank. MDA was investigated using the following formula:

MDA (µmol gFW^{-1}=) $\frac{(A_{532} - A_{600}) \times Vf \times Ve}{155 \times Va \times FW}$

where V_f = Final volume

V_e = Trichloroacetic acid (TCA) volume

V_a = Solvent volume

FW = Plant sample fresh weight

Electrolyte Leakage (EL)

The EL measurement followed the method of Dionisio-Sese & Tobita (1998) as cited in Altaf et al. (2020) Rice leaves were cut into fragments of approximately 1cm^2. Two pieces were transferred into a test tube containing mL10 of deionized water at room temperature and kept in the dark for 24h. Before electrical conductivity measurement as EC_1. The test tubes were autoclaved at 121°C for 15min before the second conductivity measurement as EC_2 at room temperature. Electrolyte leakage percentages were evaluated using the formula:

Electrolyte leakage = (%) (EC_1 / EC_2) x 100

Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyze the impacts of irrigation and drought on the characteristics of the rice varieties, including at least five replications. Mean value comparisons were analyzed by Duncan's multiple range test at a 95% confidence level. Pearson's correlation, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using Origin 2024 software to examine all the study parameters.

RESULTS

Temperature and Average Relative Humidity in the Greenhouse

During the drought period, the average temperature in the greenhouse was 39.3°C with 53.8% average relative humidity, while during the rewatering period, the average temperature was 36.7°C with 59.3% average relative humidity (Fig. 1).

Growth and Physiological Characteristics

After drought stress treatment, the overall growth performance of the three cultivars dramatically decreased compared to the control group (Fig. 2) including tiller number per clump (TNC), leaf width (LW), leaf length (LL), root length (RL), shoot fresh weight (SFW), shoot dry weight

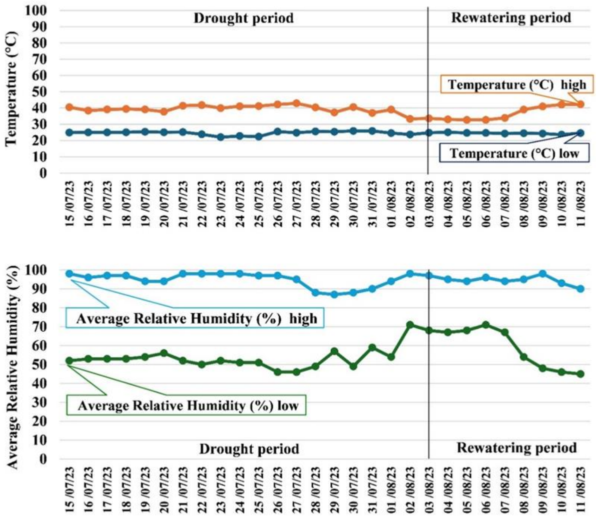

Fig. 1: Average daily temperature and relative humidity inside the greenhouse during the drought and rewatering periods.

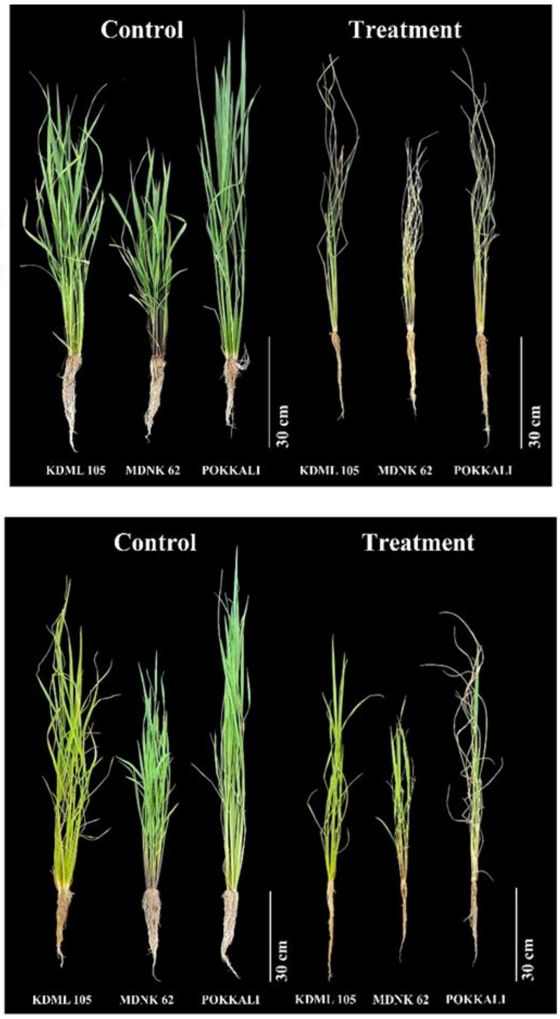

Fig. 2: Adaptation of the three rice cultivars in the vegetative stage after stress treatment in drought period and following rehydration during the rewatering period.

(SDW), root fresh weight (RFW), and root dry weight (RDW) (Table 1). The shoot length (SL), leaf rolling score (LR), and drought score of leaf (DSL) of the three rice cultivars increased compared to the control group during drought stress (Table 1). However, different growth levels were observed in the three rice cultivars after rewatering for 9 days (Fig. 2). The three rice cultivars exhibited delayed growth after rewatering. Some characteristics such as tiller number per clump (TNC), root length (RL), leaf width (LW), leaf length (LL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW) and drought recovery score of leaf (DRSL) decreased, while leaf rolling (LR) and drought score of leaf (DSL) increased. Shoot length (SL) of MDNK62 and POKKALI decreased but increased in KDML105 rice after rewatering for 9 days (Table 1).

Following drought stress treatment, KDML105 and MDNK62 exhibited the same physiological characteristics. Chlorophyll a content, total chlorophyll content, chlorophyll fluorescence measurement in light condition (Fv'/Fm'), chlorophyll fluorescence measurement in dark condition (Fv/Fm) and relative water content (RWC) decreased while chlorophyll b (CH B) content, green intensity (SPAD), electrolyte leakage percentage (EL), and malondialdehyde (MDA) content increased compared to the control group (Table 2). For POKKALI rice, Fv'/Fm', Fv/Fm, and RWC decreased similarly to KDML105 and MDNK62, but the values of other characteristics increased. During the rewatering period, CH A content, CH B content, Total CH content, SPAD and RWC of MDNK62 and POKKALI decreased compared to the control group (Table 2).

Table 1: Growth performance of KDML105, MDNK62 and POKKALI rice seedlings after drought stress and rewatering treatment.

Parameter		Drought stress						Rewatering					
		KDML105	DTI	MDNK62	DTI	POKKALI	DTI	KDML105	DTI	MDNK62	DTI	POKKALI	DTI
TNC	cont.	9.00±0.70b	0.56*	13.00±0.37a	0.56*	7.00±0.81c	0.39*	5.00±0.87bc	0.70*	12.00±0.84a	0.50*	7.00±0.40b	0.61*
	treat.	5.00±0.31d		7.00±0.37c		3.00±0.25e		4.00±0.37c		6.00±0.32b		4.00±0.00c	
LC	cont.	4.00±0.51a	1.25	4.00±0.51a	1.00	4.00±0.51a	1.00	5.00±0.40a	0.80	5.00±0.49a	1.00	5.00±0.20a	1.00
	treat.	5.00±0.51a		4.00±0.00a		4.00±0.00a		4.00±0.25a		5.00±0.25a		5.00±0.25a	
SL	cont.	26.00±2.70ab	1.16	26.20±1.07ab	0.87	21.40±1.50c	1.17	22.40±1.89d	1.57*	29.00±1.89b	0.82*	30.20±0.66bc	0.85*
	treat.	30.20±1.16a		22.80±0.58bc		25.00±0.45bc		35.20±1.39a		23.80±1.24d		25.80±0.66cd	
RL	cont.	92.80±3.06b	0.77	71.60±2.42c	0.76	109.20±2.85a	0.82	109.20±2.65b	0.80*	84.00±1.14c	0.77*	127.40±0.93a	0.53*
	treat.	71.00±3.21c		54.20±2.35d		90.00±1.41b		87.60±2.80c		64.40±1.47d		67.60±7.84d	
LW	cont.	1.11±0.00b	0.61*	1.17±0.01b	0.50*	1.37±0.04a	0.60*	0.97±0.02c	0.88*	1.16±0.01b	0.82*	1.29±0.01a	0.92*
	treat.	0.68±0.04d		0.58±0.01e		0.82±0.06c		0.85±0.02d		0.95±0.02c		1.18±0.03b	
LL	cont.	56.80±2.22a	0.82*	44.60±1.03b	0.66*	60.40±3.46a	0.77*	65.40±1.08b	0.72*	51.00±1.27c	0.79*	79.60±0.68a	0.45*
	treat.	46.80±0.37b		29.40±1.81c		46.80±0.86b		47.40±1.12c		40.40±0.93d		35.60±4.29d	
SFW	cont.	26.89±3.57a	0.19*	30.52±1.12a	0.10*	31.20±3.54a	0.20*	20.19±6.22c	0.35*	41.05±3.96b	0.23*	60.94±6.59a	0.13*
	treat.	5.18±0.28b		3.08±0.33b		6.36±0.32b		6.99±1.02d		9.34±0.64cd		8.20±1.013cd	
SDW	cont.	4.58±0.74a	0.43	5.33±0.21a	0.37	4.85±0.44a	0.39	5.42±2.68b	0.31*	9.41±1.87b	0.20*	21.94±6.66a	0.12*
	treat.	1.99±0.18b		1.96±0.16b		1.89±0.05b		1.68±0.16b		1.93±0.09b		2.66±0.20b	
RFW	cont.	17.78±5.72a	0.05	15.21±2.27ab	0.08	9.16±1.68b	0.10	5.87±2.58c	0.30*	19.80±2.55b	0.10*	29.33±4.14a	0.10*
	treat.	0.93±0.14c		1.28±0.12c		0.88±0.11c		1.78±0.19c		2.05±0.23c		2.97±0.60c	
RDW	cont.	2.52±0.65a	0.23	2.69±0.22a	0.17	3.21±0.55a	0.13	1.76±0.73c	0.33*	4.67±0.57b	0.12*	8.08±1.09a	0.09*
	treat.	0.57±0.06b		0.44±0.04b		0.41±0.04b		0.57±0.07c		0.55±0.06c		0.70±0.02c	
LR	cont.	1.00±0.00b	4.80	1.00±0.00b	5.00	1.00±0.00b	5.00	1.00±0.00c	2.00*	1.00±0.00c	1.40*	1.00±0.00c	2.00*
	treat.	4.80±0.20a		5.00±0.00a		5.00±0.00a		2.00±0.00a		1.40±0.25b		2.00±0.00a	
DSL	cont.	1.00±0.00b	3.80*	0.40±0.25b	8.50*	1.20±0.20b	0.83*	1.00±0.00b	2.20	1.20±0.20b	2.00	1.20±0.20b	3.00
	treat.	3.80±0.37a		3.40±1.12a		1.00±0.00b		2.20±0.20ab		2.40±0.25ab		3.60±1.08a	
DRSL	cont.	-		-		-		8.60±0.40ab	0.86	9.00±0.00a	0.91	8.60±0.40ab	0.86
	treat.	-						7.40±0.40b		8.20±0.49ab		7.40±0.40b	

Values (Means±SE) bearing different letters in a row differ significantly (*P<0.05). TNC, tiller number/clump; LC, leaf number/clump; SL, shoot length (cm); RL, root length (cm); LW, leaf width (cm); LL, leaf length (cm); SFW, shoot fresh weight (g); SDW, shoot dry weight (g); RFW, root fresh weight (g); RDW, root dry weight (g); LR, leaf rolling; DSL, drought score of leaf; and DRSL, drought recovery score of leaf.

Table 2: Physiological aspects of KDML105, MDNK62 and POKKALI rice seedlings after drought stress and rewatering treatment.

Parameter		Drought stress						Rewatering					
		KDML105	DTI	MDNK62	DTI	POKKALI	DTI	KDML105	DTI	MDNK62	DTI	POKKALI	DTI
CH A	cont.	4.88±0.13a	0.76*	4.6±0.08ab	0.88*	4.13±0.1ab	1.19*	2.92±0.24a	1.14	3.82±0.52a	0.81	3.690±0.35a	0.74
	treat.	3.73±0.41b		4.06±0.43ab		4.91±0.28a		3.33±0.32a		3.10±0.36a		2.72±0.40a	
CH B	cont.	1.61±0.07bc	1.05*	1.49±0.05bc	1.23*	1.25±0.04c	1.90*	0.80±0.06a	1.15	1.34±0.35a	0.61	1.10±0.13a	0.69
	treat.	1.69±0.15bc		1.84±0.16b		2.38±0.27a		0.92±0.12a		0.82±0.13a		0.76±0.13a	
Total CH	cont.	6.48±0.20ab	0.84*	6.09±0.13ab	0.97*	5.38±0.13b	1.36*	3.35±0.26a	1.27	4.64±0.78a	0.84	4.31±0.43a	0.81
	treat.	5.42±0.55b		5.90±0.58b		7.29±0.54a		4.26±0.40a		3.91±0.49a		3.48±0.48a	
SPAD	cont.	41.06±0.39a	1.04	42.24±0.82a	1.03	42.70±1.59a	1.02	36.32±1.15abc	0.90	39.60±1.87a	0.83	38.08±0.83ab	0.90
	treat.	42.9±0.57a		43.56±1.46a		43.68±1.97a		32.76±0.23c		32.96±1.25c		34.36±1.70bc	
Fv'/Fm'	cont.	0.77±0.01a	0.68	0.78±0.01a	0.69	0.79±0.00a	0.91	0.77±0.00b	1.01	0.77±0.00b	1.02	0.74±0.00c	1.04
	treat.	0.52±0.08b		0.54±0.11b		0.72±0.02a		0.78±0.00ab		0.79±0.00a		0.77±0.01b	
Fv/Fm	cont.	0.83±0.01a	0.66*	0.84±0.01a	0.70*	0.82±0.01a	0.91*	0.83±0.01a	1.00	0.83±0.00a	1.01	0.83±0.00a	1.00
	treat.	0.55±0.05b		0.59±0.05b		0.75±0.02a		0.83±0.01a		0.84±0.00a		0.83±0.00a	
EL	cont.	31.98±2.54c	2.64*	23.70±1.21d	3.62*	36.75±0.82c	2.50*	22.18±1.06a	1.03	20.24±0.76ab	1.04	17.52±1.30b	1.16
	treat.	84.33±2.45b		85.72±2.21b		91.90±0.50a		22.79±1.53a		20.96±1.34ab		20.37±0.90ab	
MDA	cont.	0.08±0.01c	1.40	0.16±0.06a	1.11	0.10±0.01c	1.55	0.075±0.01bc	1.97*	0.06±0.00b	1.66*	0.07±0.00bc	1.29*
	treat.	0.12±0.02bc		0.18±0.04ab		0.16±0.03ab		0.15±0.02a		0.10±0.01c		0.09±0.01bc	
RWC	cont.	90.77±0.87b	0.39	103.32±3.25a	0.33	99.73±1.12ab	0.31	74.42±4.62ab	0.81	71.98±1.05abc	0.91	77.82±2.38a	0.87
	treat.	35.14±2.08c		33.72±6.37c		30.9±2.09c		60.54±2.87d		65.43±1.42cd		68.05±2.65bcd	

Values (Means±SE) bearing different letters in a row differ significantly (*P<0.05). CH A, chlorophyll a (mg g tissue^{-1}); CH B, chlorophyll b (mg g tissue^{-1}); Total CH, total chlorophyll (mg g tissue^{-1}); SPAD, SPAD unit; Fv'/Fm', light-adapted quantum efficiency of PSII; Fv/Fm, the dark-adapted quantum efficiency of PSII; EL, electrolyte leakage percentage (%); MDA, malonaldehyde (µmole gFW^{-1}); RWC, relative water content (%); DTI, drought tolerance index.

Drought Tolerance Index (DTI)

The drought tolerance index (DTI) values of the three rice cultivars were analyzed (Table 1, 2). Findings indicated that during the drought period, all three rice cultivars exhibited 12 weak characteristics (TNC, RL, LW, LL, SFW, SDW, RFW, RDW, LR, EL, MDA, and RWC) and 2 well-adapted characteristics (CH B and SPAD), while KDML105 had 2 well-adapted DTIs (LC and SL) and 4 weak DTIs (CH A, DSL, Fv′/Fm′, and Fv/Fm). MDNK62 displayed 3 weak DTIs (DSL, Fv′/Fm′, and Fv/Fm), while POKKALI demonstrated 3 well-adapted DTIs (SL, CH A, and Total CH) during the drought period. After rehydration, the three rice cultivars exhibited 9 sensitive growth and physiological DTIs (TNC, LW, LL, RL, SFW, LR, EL, MDA, and RWC) and 3 well-adapted DTI (DRSL, Fv′/Fm′ and Fv/Fm) while MDNK62 showed 3 susceptible DTIs (SL, RFW, RDW) and POKKALI exhibited 6 weak DTIs (SL, RL, SFW, SDW, RFW, and RDW). KDML105 had 4 well-adapted DTIs (SL, CH A, CH B, and Total CH). However, all rice cultivars had well-adapted Fv′/Fm′ and Fv/Fm, approaching a DTI score of 1 (Table 1, 2).

Pearson's Correlation, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA)

All three rice cultivars displayed negative correlations between growth parameters TNC, LW, LL, RL, SFW, SDW, RDW, and drought score of leaf (DSL). All these parameters were associated with RWC and Fv/Fm and tended to be positively correlated with Fv′/Fm′ (Fig. 3A-3F). However, each rice cultivar exhibited unique correlations with specific parameters. KDML105 showed a positive correlation between growth (DSL) and physiological traits (SPAD, EL) in all periods and negative DRSL and LL correlation while DRSL positively correlated with CH A and CH B during the rewatering period (Fig. 3A, 3D). MDNK62 demonstrated a positive correlation between growth (DSL and LR) and the physiological trait (EL) during the drought period and with CH A during the rewatering period. By contrast, DSL was negatively correlated with Fv′/Fm′ and Fv/Fm during the drought period and negatively correlated with SPAD and MDA during the rewatering period (Fig. 3B, 3E). POKKALI exhibited a positive correlation between growth (LR) and physiological traits (CH A, CH B, Total CH, EL, and MDA) during the drought period. During the rewatering period, CH A, CH B, and Total CH were negatively correlated with DSL while Fv′/Fm′ was positively correlated with MDA (Fig. 3C, 3F).

PCA was analyzed using correlation coefficients, with biplots illustrated in Fig. 4A, 4B. The data variability was accounted for by the three principal components (Table 3), explaining 100% of the variance. During the drought period, the first two components, PC1 and PC2, explained 76.20 and 16.70% of the data variance, respectively and were used to construct a PCA biplot, accounting for 92.90% of the variance. During the rewatering period, the first two components, PC1 and PC2, explained 75.0 and 17.2% of the data variance, respectively, and were used to create a PCA biplot, accounting for 92.22% of the variance. During the drought period (Fig. 4A), PC1 was characterized by the positive correlation of rice growth (LR) and physiological traits (SPAD, EL, and MDA) for the three rice cultivars (MDNK62, KDML105, and POKKALI, respectively), with SPAD not correlated during the rewatering period. The parameters TNC, RL, LW, LL, SFW, SDW, RFW, and RDW positively correlated with physiological traits (RWC) across all periods. PC2 showed a closer correlation between LR, DSL, and MDA than KDML105 while EL, Fv′/Fm′, and Fv/Fm were closer to POKKALI and MDNK62 during the rewatering period (Fig. 4B). Results indicated that SL was correlated with CH A, CH B, and Total CH, and most associated with POKKALI during the drought period but closer to KDML105 during the rehydration period.

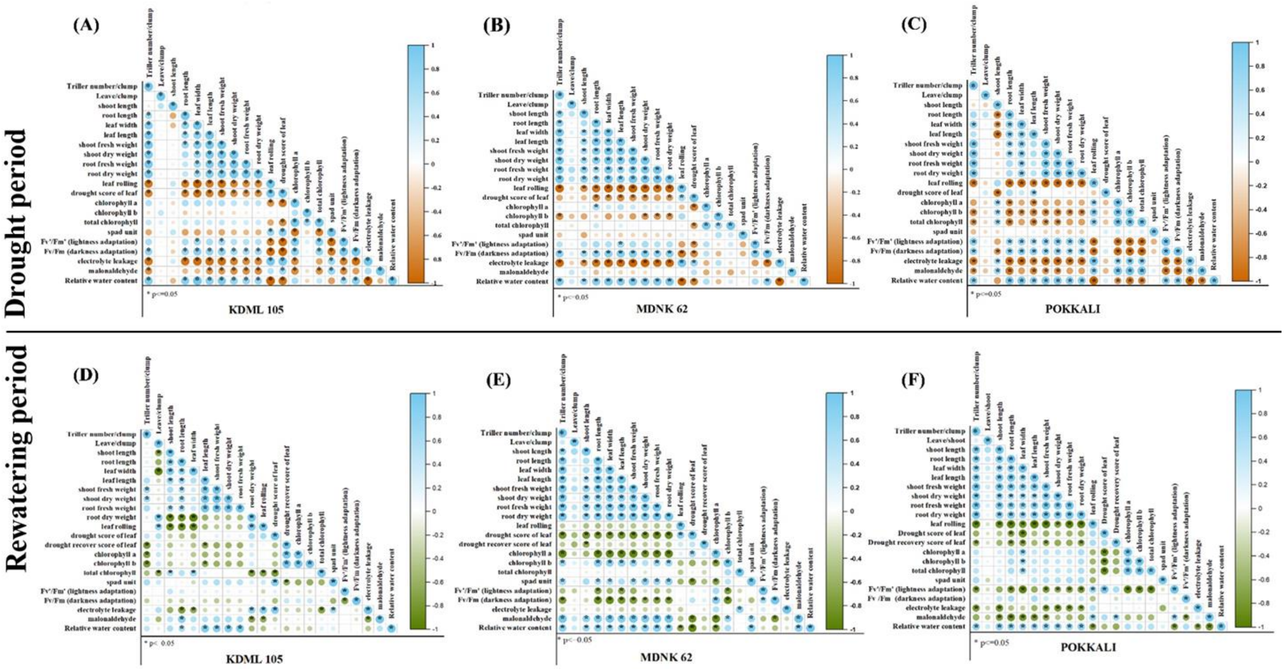

Fig. 3: Pearson's correlation analysis among growth and physiological parameters of the three rice cultivars. **(A)** KDML105, **(B)** MDNK62, and **(C)** POKKALI under drought period and **(D)** KDML105, **(E)** MDNK62, and **(F)** POKKALI under the rewatering period.

Table 3: Loading variables, variance, cumulative and eigenvalues from PCA analysis of growth and physiological parameters of KDML105 seedlings after heat stress.

Parameter	Drought period			Rewatering period		
	PC1	PC2	PC3	PC1	PC2	PC3
1. Tiller number/clump	0.242	-0.127	-0.077	0.243	-0.022	-0.124
2. Leaf number/clump	0.150	-0.299	0.467	0.222	0.012	-0.328
3. Shoot length (cm)	-0.081	0.167	0.732	0.041	0.506	0.029
4. Root length (cm)	0.249	0.057	0.003	0.232	-0.015	0.254
5. Leaf width (cm)	0.249	-0.002	0.088	0.227	-0.099	-0.259
6. Leaf length (cm)	0.241	-0.012	0.215	0.223	-0.059	0.315
7. Shoot fresh weight (g)	0.250	0.001	0.036	0.244	-0.062	0.043
8. Shoot dry weight (g)	0.250	-0.032	0.012	0.244	-0.070	0.034
9. Root fresh weight (g)	0.250	-0.002	-0.053	0.245	-0.046	-0.019
10. Root dry weight (g)	0.248	-0.060	-0.008	0.246	-0.037	0.002
11. Leaf rolling	-0.250	0.022	0.031	-0.220	0.210	-0.138
12. Drought score of leaf	-0.196	-0.305	-0.201	-0.237	0.119	-0.097
13. Drought recovery score of leaf	-	-	-	-0.179	-0.343	0.122
14. Chlorophyll a (mg g tissue^{-1})	0.100	0.485	-0.099	0.187	0.315	0.165
15. Chlorophyll b (mg g tissue^{-1})	-0.188	0.353	-0.007	0.204	0.284	-0.078
16. Total chlorophyll (mg g tissue^{-1})	-0.030	0.528	-0.069	0.119	0.442	0.121
17. SPAD Unit	-0.242	-0.106	0.127	0.236	-0.050	-0.208
18. Fv′/Fm′	0.230	0.209	0.015	-0.239	-0.018	-0.179
19. Fv/Fm	0.228	0.217	-0.017	-0.161	-0.158	0.530
20. Electrolyte leakage (%)	-0.249	-0.010	-0.056	-0.211	-0.035	-0.393
21. Malonaldehyde (µmole gFW^{-1})	-0.219	0.149	0.325	-0.205	0.250	0.199
22. Relative water content (%)	0.249	-0.051	-0.001	0.208	-0.274	0.027
Variance (%)	76.172	16.736	7.092	75.002	17.228	7.770
CV (%)	76.172	92.908	100	75.002	92.230	100.000
Eigenvalue	15.996	3.515	1.489	16.500	3.790	1.709

Abbreviations: Fv′/Fm′, light-adapted quantum efficiency of PSII; Fv/Fm, dark-adapted quantum efficiency of PSII.

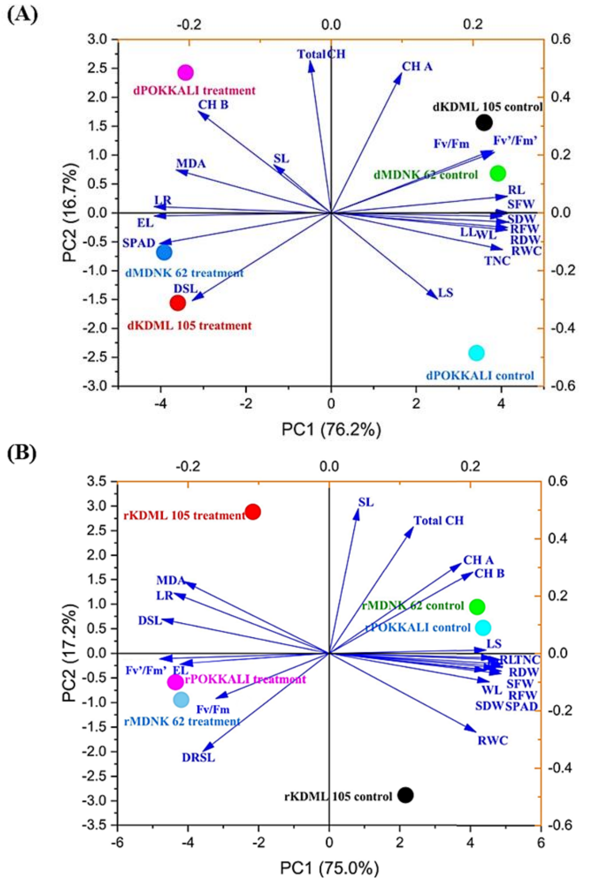

Fig. 4: PCA biplots for PC1, PC2 and PC3 showing the relationships between KDML105, MDNK62 and POKKALI growth and physiological parameters recorded during the drought period **(A)** and rewatering period **(B)**. TNC, tiller number/clump; LC, leaf number/clump; SL, shoot length (cm); RL, root length (cm); LW, leaf width (cm); LL, leaf length (cm); SFW, shoot fresh weight (g); SDW, shoot dry weight (g); RFW, root fresh weight (g); RDW, root dry weight (g); LR, leaf rolling; DSL, drought score of leaf; and DRSL, drought recovery score of leaf.; CH A, chlorophyll a (mg g tissue^{-1}); CH B, chlorophyll b (mg g tissue^{-1}); Total CH, total chlorophyll (mg g tissue^{-1}); SPAD, SPAD unit; Fv′/Fm′, light-adapted quantum efficiency of PSII; Fv/Fm, dark-adapted quantum efficiency of PSII; EL, electrolyte leakage percentage (%); MDA, malonaldehyde (µmole gFW^{-1}); RWC, relative water content (%).

Hierarchical cluster analysis (HCA) and the heatmap were analyzed to explain the overview that the three rice tillering clusters depended on growth and physiological DTI of drought stress and rewatering (Fig. 5). The HCA results were consistent with PCA analysis. Six rice cultivar groups were identified by four clusters of parameters. Cluster III (KDML105 d and MDNK62 d) had similar components, whereas Cluster IV (DTI of POKKALI d) displayed better clustering characteristics during dehydration compared to rehydration. Cluster I (DTI of MDNK62 r and POKKALI r) shared similar components. Conversely, Cluster II (KDML105 r) exhibited growth development and physiological characteristics, with better clustering during rehydration.

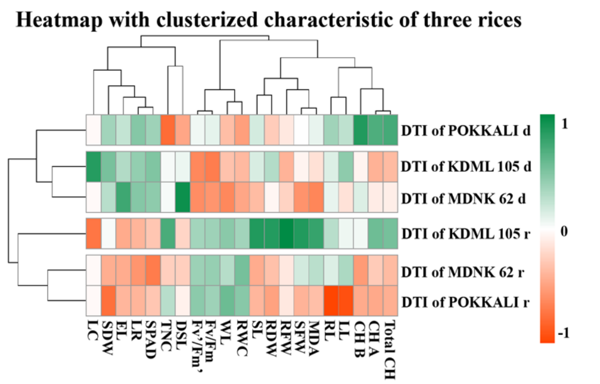

Fig. 5: Hierarchical cluster analysis and a heatmap explaining the DTI of KDML105, MDNK62 and POKKALI tillering during drought (d) and rewatering (r) periods. TNC, tiller number/clump; LC, leaf number/clump; SL, shoot length (cm); RL, root length (cm); LW, leaf width (cm); LL, leaf length (cm); SFW, shoot fresh weight (g); SDW, shoot dry weight (g); RFW, root fresh weight (g); RDW, root dry weight (g); LR, leaf rolling; DSL, drought score of leaf; and DRSL, drought recovery score of leaf.; CH A, chlorophyll a (mg g tissue^{-1}); CH B, chlorophyll b (mg g tissue^{-1}); Total CH, total chlorophyll (mg g tissue^{-1}); SPAD, SPAD unit; Fv′/Fm′, light-adapted quantum efficiency of PSII; Fv/Fm, dark-adapted quantum efficiency of PSII; EL, electrolyte leakage percentage (%); MDA, malonaldehyde (µmole gFW^{-1}); RWC, relative water content (%).

DISCUSSION

This study investigated the responses of KDML105, MDNK62, and POKKALI to drought stress and rewatering under greenhouse conditions. Adverse impacts were seen in growth and physiological characteristics. The drought tolerance index (DTI) as a measure of growth performance decreased by less than 1 point. Results were consistent Xu et al. (2021) who reported that rice seedlings stressed by drought showed decreased germination rate, tiller number per clump, and pale yellow seedlings that died. Leaf size, root length, fresh and dry weight of plants and roots also decreased (Bhandari et al., 2023). The MDNK62 rice variety produced smaller seedlings than the other rice cultivars but had more clumps and was more vulnerable to harm from the sun and extreme heat. The highest DTI of SFW and SDW were recorded throughout the drought treatment. KDML105 rice had higher DTI for SFW and SDW, indicating that leaves with higher fresh and dry weights were more likely to survive during drought. Their robust structure made them more resilient to harm from drought stress and water loss) Bhandari et al., 2023; Sandeep & Godi, 2023(. During the rewatering period, all three rice cultivars showed increased DTI of LW, especially POKKALI rice. This rice variety is known for its ability to withstand drought and salinity due to its broad stem base, narrow dark green and slender leaves with a waxy coating)Jacob & Subramannian, 2022(. POKKALI rice had a higher DTI of LW than the other cultivars, but the DTI of LL decreased significantly during the rewatering period. As a result, the DTI of SFW and SDW during this period were less than MDNK62 and KDML105 rice, which better maintained the DTI of LL. During the rewatering stage, KDML105 rice had a higher DTI of SL than the other cultivars. These traits resulted in greater leaf surface area, enhancing photosynthesis to produce energy and repairing drought-damaged tissues faster than the other rice varieties (Bhandari et al., 2023).

Reduced photosynthesis results in shorter root length)Karim et al., 2024(. In our experiment, the root length index of all three rice cultivars declined during the dry spell. During the rewatering phase, the root length index of POKKALI rice decreased more than MDNK62 and KDML105 rice because the roots of POKKALI rice are extensive and deep)Jacob & Subramannian, 2022(. Compared to the other two rice cultivars, POKKALI rice showed greater root growth when drought stress was experienced. However, when the temperature inside the greenhouse rises, the roots may become heat-stressed and the cells lose more water)Xu et al., 2021; McBrayer et al., 2022; Karim et al., 2024(. Damaged tissue undergoes decomposition during rewatering, causing a reduction in water absorption efficiency and disrupting water transport within cells from the roots to various parts of the rice plant (Panda et al., 2021; Pamuta et al., 2022(. Therefore, the DTI of RFW and RDW in this study decreased. These results concurred with Karim et al. (2024) who studied the comparative characteristics of Moroberekan rice and MR 297 rice. They found that the drought-affected rice group had shorter roots, which led to a decrease in root fresh and dry weight. During the rewatering phase, KDML105 showed increased DTIs of RL, RFW, and RDW. Results suggested that KDML 105 rice had greater root development capacity than the other two cultivars during the rewatering phase after growing through a drought. Furthermore, each of the three rice cultivars showed decreased leaf rolling ability according to the DTI of LR recorded between the droughts and rewatering periods. This indicated adaptation to reduce water loss from the leaves when exposed to drought stress and the leaves expanded when water returned)Cal et al., 2019; Latif et al., 2023(. The leaves of KDML105 and MDNK62 recovered from drought more quickly than POKKALI, as evidenced by the high DTI of DSL during the drought stress period that decreased during the rewatering period.

Zhang et al. (2024) found that the Crop Water Stress Index (CWSI), maximum quantum efficiency of photosystem II (Fv/Fm), and SPAD values were closely related to photosynthetic efficiency and the proficiency of plants to adapt under drought conditions. Cultivars with increased SPAD and Fv/Fm values, along with lower CWSI, were more likely to show better adaptation to drought. This concurred with the high leaf greenness intensity (SPAD unit) of the three rice cultivars under drought stress but was not consistent with the Fv/Fm and Fv′/Fm′ values that decreased during the drought period. Lv et al. (2024) reported that drought priming maintained the SPAD value at the seedling stage to mid-tuber expansion stage of potato, improved the photosynthetic performance, and enhanced the drought tolerance. During drought, water evaporates from rice leaves as they grow to their full potential. The mesophyll cells are consequently closer together. As a result, the distribution of chlorophyll within the chloroplasts is unequal, which impacts the leaf green intensity value. Previous studies reported that the microstructure of plants undergoes various changes when exposed to drought stress such as destruction of the thylakoid membrane, increasing plastoglobulin size and number, swollen grana, disorganized thylakoid membrane system, wider space within the thylakoid and decreased length-to-width ratio and chloroplasts area (Hu et al., 2023). These factors lead to a slowdown in plant growth and cause some plants to have higher levels of chlorophyll in their leaves. Taratima et al. (2022) also reported that the leaf greenness value was related to chlorophyll a, chlorophyll b, and total chlorophyll content. In our experiment, POKKALI rice outperformed the other cultivars in terms of DTIs of CH A and Total CH during the drought stress phase; however, these values dropped during the rewatering phase. Although all three rice varieties had insignificant DTIs for total chlorophyll, chlorophyll a, and chlorophyll b, the increase in the DTI of KDML105 rice was noteworthy. The DTI of CH A, CH B, and Total CH of KDML105 and MDNK62 decreased, resulting in lower DTI of chlorophyll fluorescence. This proved that drought stress impacted the function of photosystem II (PSII). Drought may damage the oxygen-producing Oxygen Evolving Complex (OEC), the reaction center of water splitting, reducing electron transporting efficiency to PSII. Excess oxygen from this reaction then combines with different components within the cell to form free radicals (Reactive Oxygen Species: ROS), which have the potential to cause injury to cells (Eckardt, 2022; Moustakas et al., 2022; Yan et al., 2023) (Fig. 6). KDML105 and MDNK62 returned to similar growth as the control group as well as POKKALI when they were rehydrated after experiencing drought. The pigmentation in the three rice cultivars was a light-harvesting complex consisting of chlorophyll a, chlorophyll b, and carotenoids in the thylakoid membrane. This operates with the water splitting reaction center and provides electrons to the light system even though there are some changes in the structure and size of the antenna)Eckardt, 2022(. MDNK62 rice has anthocyanin in vacuoles that reduces the amount of heat dissipation from light reactions occurring within the mesophyll cells) Dabravolski & Isayenkov, 2023; Khusnutdinov et al., 2021; Li & Ahammed, 2023) (Fig. 6). These characteristics can cause excessive oxygen from water oxidation, leading to the peroxidation of many unsaturated fatty acids (Polyunsaturated fatty acids: PUFA (Mackon et al., 2021) (Fig. 6). For all three rice cultivars, the DTI of MDA was higher than 1 during both the drought and rewatering periods, with an increase in the DTI of EL inside the cells. This also affects the mechanism of the cell membrane recovery during rehydration, through the accumulation of proline, sugar alcohols or other sugar types (Ngcala et al., 2020; Taratima et al., 2022; Zahra et al., 2022) (Fig. 6).

Despite experiencing a scarcity of water throughout the experiment, all three cultivars sustained leaf conditions comparable to the control group throughout the rewatering period. In all three rice cultivars, the DTI of RWC values increased and approached level 1. These results concurred with Wang et al. (2022b) who found that relative leaf water content (RWC) negatively correlated with malondialdehyde (MDA) during drought and rewatering periods in rice. As drought worsened, RWC decreased while MDA, a marker of oxidative stress, increased. Water-saving and drought-resistant rice (HY73) showed better photosynthetic recovery and lower MDA levels compared to drought-sensitive rice (HHZ). HY73 exhibited higher peroxidase (POD) activity and proline levels, while antioxidant enzymes and photosynthetic parameters recovered fully after rewatering, highlighting key physiological traits aiding drought tolerance in HY73.

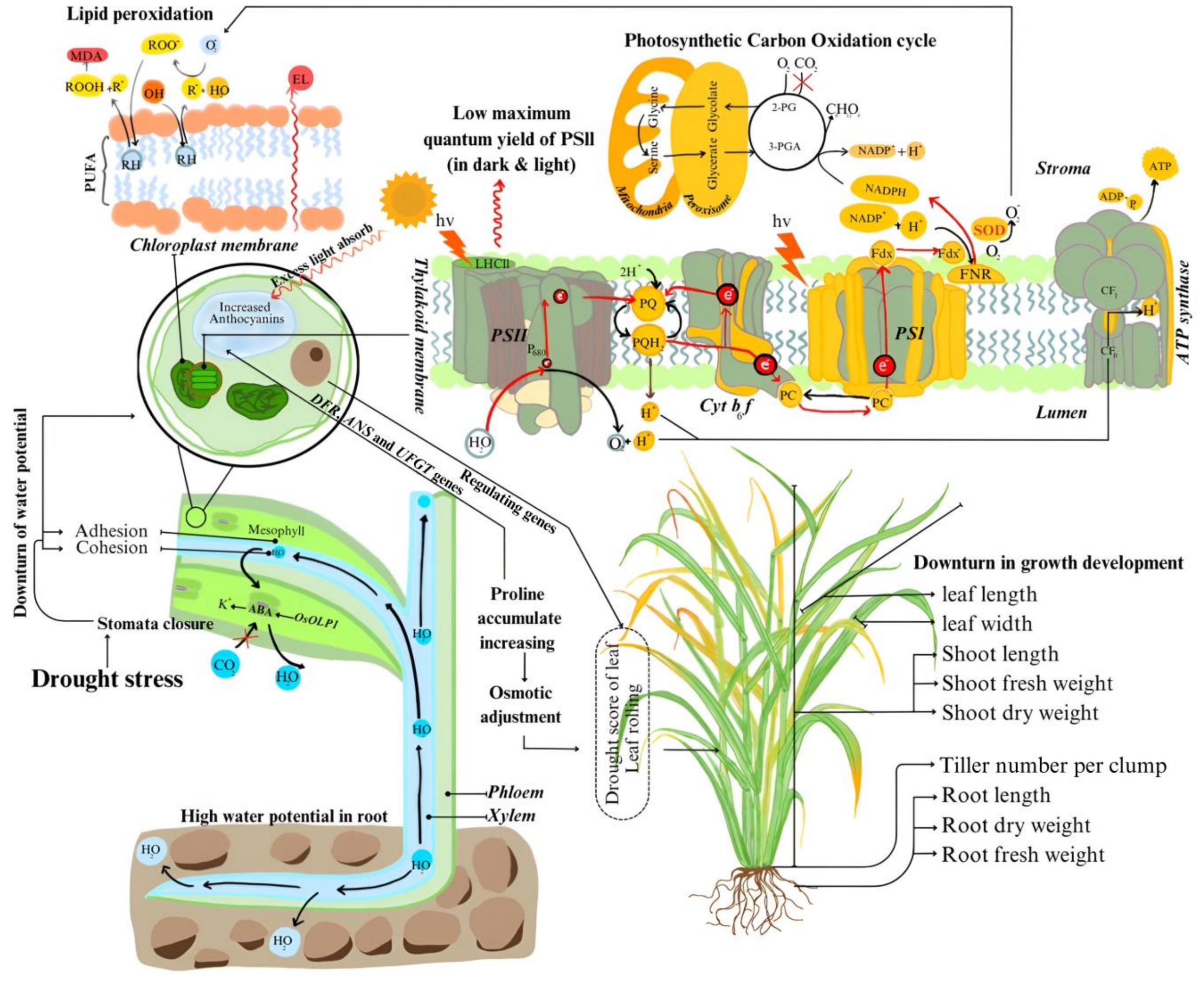

Fig. 6: Growth mechanisms and physiological responses of rice to drought stress. hv, high voltage power; ABA, Abscisic Acid; OsOLP1, *Oryza sativa* oligosaccharide-like protein 1; DFR, Dihydroflavonol 4-reductase; ANS, Anthocyanidin Synthase; UFGT, Uridine flavonoid 3-o-glycosyltransferases; LHC, light-harvesting complex; PQ, Plastoquinone; PC, Plastocyanin; Fdx, ferredoxin; 2-PG, 2-Phosphoglycolate; 3-PGA, 3-Phospoglycerate; EL, electrolyte leakage; MDA, malonaldehyde; SOD, superoxide dismutase; FNR, Ferredoxin-NADP+ reductase; PUFA, Polyunsaturated Fatty Acids.

The Pearson correlation and principal component analysis confirmed that water dehydration in rice caused an increase in leaf dryness factor or drought score of leaf (DSL) resulting in the reduction of growth parameters (TNC, LW, LL, RL, SFW, SDW, and RDW) and physiological traits (RWC, Fv'/Fm', and Fv/Fm) in all three rice cultivars. Drought and high temperatures in greenhouses cause water to evaporate from pots, affecting plant growth. Plant roots then produce more abscisic acid to control the opening and closing of stomata under drought stress conditions) Karim et al., 2024; Santosh Kumar et al., 2021).

Drought-tolerant plants can also retain water by enlarging the size of vessels. This reduces the water potential and pressure in the phloem, resulting in the accumulation of various substances in the form of crystals or vesicles within plant cells (Dabravolski & Isayenkov, 2023). Sarma et al. (2023) found that at the beginning of rice growth, the relative water content in leaves was higher but decreased when the leaves withered. This indicated that various activities occur in cells such as increased electrolyte leakage and malondialdehyde content (Fig. 6). Hierarchical cluster analysis confirmed that KDML105 exhibited lower values of several parameters under drought stress but had higher cell rejuvenation efficiency compared to MDNK62 and POKKALI, which had comparable growth and physiological activities after rehydration.

Conclusion

This study examined the physiological responses of KDML105, MDNK62 and POKKALI to drought stress under greenhouse conditions. The results demonstrated that MDNK62 exhibited moderate potential adaptation to drought stress and also the rewatering period. KDML105 rice showed the highest sensitivity to drought stress compared to the other cultivars but exhibited better growth and physiological characteristics during the rewatering period than MDNK62 and POKKALI by dominant adaptation of shoot length (SL), leaf rolling (LR), leaf per clump (LC), chlorophyll a (CH A), chlorophyll b (CH B), total chlorophyll (Total CH), light (Fv’/Fm’) and dark (Fv/Fm) adapted quantum efficiency of PSII characteristics. Our results can be used as foundational data to develop rice varieties or cultivars that are suitable for regions facing current and future drought challenges.

Author’s Contribution: PM: Investigation, Data curation, Methodology, Data curation, Writing - Original Draft. WT: Conceptualization, Supervision, Validation, Writing - Original Draft, Writing -Review & Editing. All listed authors have approved the final manuscript.

Acknowledgment: This work was financially supported by “Khon Kaen University Research and Graduate Studies Grant No. RP-10-001” and “the research capability enhancement program through graduate student scholarship, Faculty of Science, Khon Kaen University. The authors would like to thank Nong Khai Rice Research Center, Dr.Tidarat Monkham, for the rice seed, and the Department of Biology, Faculty of Science, Khon Kaen University, for facility support.

REFERENCES

Altaf, M.M., Diao, X.P., Ur Rehman, A., Imtiaz, M., Shakoor, A., Altaf, M.A., & Ghani, M.U. (2020). Effect of vanadium on growth, photosynthesis, reactive oxygen species, antioxidant enzymes, and cell death of rice. *International Journal of Agriculture and Biology*, 20(3), 2643–2656. https://doi.org/10.1007/s42729-020-00330-x

Basak, R., Wahid, A.K., Dinh, A., Soolanayakanahally, R., Fotouhi, R., & Mehr, A.S. (2020). Rapid and efficient determination of relative water contents of crop leaves using electrical impedance spectroscopy in vegetative growth stage. *Remote Sensing*, 12(11), 1753.

Beena, R., Kirubakaran, S., Nithya, N., Manickavelu, A., Sah, R.P., Abida, P.S., & Siddique, K.H.M. (2021). Association mapping of drought tolerance and agronomic traits in rice (*Oryza sativa* L.) landraces. *BMC Plant Biology*, 21, 484. https://doi.org/10.1186/s12870-021-03272-3

Bhandari, U., Gajurel, A., Khadka, B., Thapa, I., Chand, I., Bhatta, D., & Shrestha, J. (2023). Morpho-physiological and biochemical response of rice (*Oryza sativa* L.) to drought stress: A review. *Heliyon*, 9, e13744. https://doi.org/10.1016/j.heliyon.2023.e13744

Bharti, P., Prathibha, M.D., Smitharani, J.A., & Sheshshayee, M.S. (2020). Physiological characterization of trait introgressed lines of rice (*Oryza sativa* L.) for drought stress tolerance. *Indian Journal of Pure and Applied Biosciences*, 8(6), 229-234. https://doi.org/10.18782/2582-2845.8457

Cal, A.J., Sanciangco, M., Rebolledo, M.C., Luquet, D., Torres, R.O., McNally, K.L., & Henry, A. (2019). Leaf morphology, rather than plant water status, underlies genetic variation of rice (*Oryza sativa*) leaf rolling under drought. *Plant, Cell & Environment*, 42, 1532–1544. https://doi.org/10.1111/pce.13514

Dabravolski, S.A., & Isayenkov, S.V. (2023). The role of anthocyanins in plant tolerance to drought and salt stresses. *Plants*, 12, 2558. https://doi.org/10.3390/plants12132558

Dash, A.P., De, D.K., Nath, R., Sarkar, A., Mohanty, S., & Bhattacharyya, P.K. (2020). Effects of drought stress on relative water, chlorophyll and proline content in tolerant and susceptible genotypes of lentil (*Lens culinaris* Medik.). *Journal of Crop and Weed*, 16(1), 192-198. https://doi.org/10.22271/09746315.2020.v16.i1.1293

Dionisio-Sese, M.L., & Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Science*, 135, 1–9. https://doi.org/10.1016/S0168-9452(98)00025-9

Eckardt, N.A. (2022). From the archives: Photosynthesis matters; PSII antenna size, photorespiration, and the evolution of C4 photosynthesis. *Plant Cell*, 34, 1145–1146. https://doi.org/10.1093/plcell/koac024

Farooq, M., Wahid, A., Zahra, N., Hafeez, M.B., & Siddique, K.H.M. (2024). Recent advances in plant drought tolerance. *Journal of Plant Growth Regulation*, 43, 3337–3369. https://doi.org/10.1007/s00344-024-11351-6

Heath, R.L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: II. Role of electron transfer. *Archives of Biochemistry and Biophysics*, 125, 850–857. https://doi.org/10.1016/0003-9861(68)90523-7

Hou, D., Liu, K., Liu, S., Li, J., Tan, J., Bi, Q., & Luo, L. (2024). Enhancing root physiology for increased yield in water-saving and drought-resistance rice with optimal irrigation and nitrogen. *Frontiers in Plant Science*, 15, 1370297. https://doi.org/10.3389/fpls.2024.1370297

Hu, F., Zhang, Y., & Guo, J. (2023). Effects of drought stress on photosynthetic physiological characteristics, leaf microstructure, and related gene expression of yellow horn. *Plant Signaling & Behavior, 18*(1), e2215025. https://doi.org/10.1080/15592324.2023.2215025

Jacob, S., & Subramannian, S. (2022). Pokkali: An exquisite climate resilient and organic rice-prawn production system for future. *Kerala Karshakan English Journal*, 10, 4–8.

Karim, M.F., Tajudin, N.H.N., Salmin, S.A., Mohd Rosely, N.F.S., Saimi, N.N., & Che Amri, C.N.A. (2024). Comparative study of root characteristics revealed distinctive responses between *Moroberekan* and MR297 rice varieties subjected to drought stress. *Nusantara Bioscience*, 16, 29–36. https://doi.org/10.13057/nusbiosci/n160104

Khusnutdinov, E., Sukhareva, A., Panfilova, M., & Mikhaylova, E. (2021). Anthocyanin biosynthesis genes as model genes for genome editing in plants. *International Journal of Molecular Sciences*, 22, 8752. https://doi.org/10.3390/ijms22168752

Latif, A., Ying, S., Cuixia, P., & Ali, N. (2023). Rice curled its leaves either adaxially or abaxially to combat drought stress. *Rice Science*, 30, 405–416. https://doi.org/10.1016/j.rsci.2023.04.002

Li, Z., & Ahammed, G.J. (2023). Hormonal regulation of anthocyanin biosynthesis for improved stress tolerance in plants. *Plant Physiology and Biochemistry*, 201, 107835. https://doi.org/10.1016/j.plaphy.2023.

107835
Lv, Z., Zhang, H., Huang, Y., Zhu, L., Yang, X., Wu, L., & Zhu, X. (2024). Drought priming at seedling stage improves photosynthetic performance and yield of potato exposed to a short-term drought stress. *Journal of Plant Physiology*, 292, 154157
Liu, H., Song, S., Zhang, H., Li, Y., Niu, L., Zhang, J., & Wang, W. (2022). Signaling transduction of ABA, ROS, and Ca2+ in plant stomatal closure in response to drought. *International Journal of Molecular Sciences*, 23(23), 14824. https://doi.org/10.3390/ijms232314824
Mackon, E., Jeazet Dongho Epse Mackon, G.C., Ma, Y., Haneef, M., Kashif, M., Ali, N., & Liu, P. (2021). Recent insights into anthocyanin pigmentation, synthesis, trafficking, and regulatory mechanisms in rice (*Oryza sativa* L.) caryopsis. *Biomolecules*, 11(3), 394. https://doi.org/10.3390/biom11030394
Matsui, T., Omasa, K., & Horie, T. (2000). High temperature at flowering inhibits swelling of pollen grains, a driving force for thecae dehiscence in rice (*Oryza sativa* L.). *Plant Production Science*, 3, 430–434. https://doi.org/10.1626/pps.3.430
McBrayer, R.H., Pickens, J.M., Witcher, A.L., Wells, D.E., & Sibley, J.L. (2022). Effects of nursery container color and spacing on root zone temperatures of 'Soft Touch' Holly. *Agriculture*, 12, 2165. https://doi.org/10.3390/agriculture12122165
Moustakas, M., Sperdouli, I., & Moustaka, J. (2022). Early drought stress warning in plants: color pictures of photosystem II photochemistry. *Climate*, 10, 179. https://doi.org/10.3390/cli10110179
Nan, G.-N., Zhou, X.-Q., Zhang, X.-M., Zhang, Q.-S., Hu, Z.-M., Huang, R.-P., & Zhang, D. (2022). Xanthophyll cycle-related non-photochemical quenching protects *Sargassum thunbergii* from high light-induced photoinhibition. *Frontiers in Marine Science*, 9, 1067596. https://doi.org/10.3389/fmars.2022.1067596
Ngcala, M.G., Goche, T., Brown, A.P., Chivasa, S., & Ngara, R. (2020). Heat stress triggers differential protein accumulation in the extracellular matrix of sorghum cell suspension cultures. *Proteomes*, 8, 29. https://doi.org/10.3390/proteomes8040029
Pamuta, D., Siangliw, M., Sanitchon, J., Pengrat, J.L., Siangliw, J., Toojinda, T., & Theerakulpisut, P. (2022). Physio-biochemical traits in improved 'KDML105' jasmine rice lines containing drought and salt tolerance gene under drought and salt stress. *Chilean Journal of Agricultural Research*, 82, 97–110. https://doi.org/10.4067/S0718-58392022000100097
Panda, D., Mishra, S.S., & Behera, P.K. (2021). Drought tolerance in rice: focus on recent mechanisms and approaches. *Rice Science*, 28, 119–132. https://doi.org/10.1016/j.rsci.2021.01.002
Rotrujanon, N., Pamuta, D., Siangliw, M., Sanitchon, J., Siangliw, J.L., & Theerakulpisut, P. (2022). Evaluation of drought and salinity tolerance at seedling stage of introgression lines of 'KDML105' rice carrying drought tolerance QTL and SKC1 salt tolerance gene. *KKU Science Journal*, 50, 146–161.
Saleeto, S., Sinsaithai, N., Jumpaket, R., Sripanom, P., Kaewkhumcheng, C., Thatharak, R., & Grasaetep, J. (2020). RD83 (Mali Dam Nong Khai 62), a non-glutinous rice variety. *Thai Rice Research Journal*, 11, 24–43.
Sarma, B., Kashtoh, H., Lama Tamang, T., Bhattacharyya, P.N., Mohanta, Y.K., & Baek, K.H. (2023). Abiotic stress in rice: Visiting the physiological response and its tolerance mechanisms. *Plants*, 12(23), 3948.
Sun, T., Rao, S., Zhou, X., & Li, L. (2022). Plant carotenoids: recent advances and future perspectives. *Molecular Horticulture*, 2(3). https://doi.org/10.1186/s43897-022-00023-2
Sandeep, T.S.R.S., & Godi, S. (2023). Drought stress tolerance in rice: advances in physiology and genetics research. *Plant Physiology Reports*, 28, 349–361. https://doi.org/10.1007/s40502-023-00743-7
Santosh Kumar, V.V., Yadav, S.K., Verma, R.K., Shrivastava, S., Ghimire, O., Pushkar, S., & Chinnusamy, V. (2021). The abscisic acid receptor OsPYL6 confers drought tolerance to indica rice through dehydration avoidance and tolerance mechanisms. *Journal of Experimental Botany*, 72, 1411–1431. https://doi.org/10.1093/jxb/eraa509
Taratima, W., Ritmaha, T., Jongrungklang, N., Maneerattanarungroj, P., & Kunpratum, N. (2020). Effect of stress on the leaf anatomy of sugarcane cultivars with different drought tolerance (*Saccharum officinarum*, Poaceae). *Revista de Biología Tropical*, 68(4), 1159–1170. https://doi.org/10.15517/rbt.v68i4.41031
Taratima, W., Chuanchumkan, C. Maneerattanarungroj, P., Trunjaruen, A., Theerakulpisut, P., & Dongsansuk, A. (2022). Effect of heat stress on some physiological and anatomical characteristics of rice (*Oryza sativa* L.) cv. KDML105 callus and seedling. *Biology*, 11, 1587. https://doi.org/10.3390/biology11111587
Vargas Zeppetello, L.R., Raftery, A.E., & Battisti, D.S. (2022). Probabilistic projections of increased heat stress driven by climate change. *Communications Earth & Environment*, 3(1), 183. https://doi.org/10.1038/s43247-022-00524-4
Vivek, M., Chandravanshi, P., Nataraju, S.P., Salimath, S., & Kumar Naik, A.H. (2020). Effect of hydrogel on chlorophyll content and chlorophyll stability index of groundnut (*Arachis hypogaea* L.) under rainfed condition. *International Journal of Chemical Studies*, 8(3), 2211–2215
Wang, G., Zeng, F., Song, P., Sun, B., Wang, Q., & Wang, J. (2022a). Effects of reduced chlorophyll content on photosystem functions and photosynthetic electron transport rate in rice leaves. *Journal of Plant Physiology*, 272, 153669. https://doi.org/10.1016/j.jplph.2022.153669
Wang, L., Zhang, X., She, Y., Hu, C., Wang, Q., Wu, L., & He, H. (2022b). Physiological adaptation mechanisms to drought and rewatering in water-saving and drought-resistant rice. *International Journal of Molecular Sciences*, 23(22), 14043. https://doi.org/10.3390/ijms232214043
Xu, Y., Chu, C., & Yao, S. (2021). The impact of high-temperature stress on rice: Challenges and solutions. *Crop Science*, 9(6), 963–976. https://doi.org/10.1016/j.cj.2021.02.011
Yaliang, W., Yikai, Z., Qinghua, S., Huizhe C., Jing X., Guohui H., & Yuping Z. (2020). Decrement of sugar consumption in rice young panicle under high temperature aggravates spikelet number reduction. *Rice Science*, 27(1), 44–55. https://doi.org/10.1016/j.rsci.2019.12.005
Yan, J., Ninkuu, V., Fu, Z., Yang, T., Ren, J., Li, G., & Zeng, H. (2023). OsOLP1 contributes to drought tolerance in rice by regulating ABA biosynthesis and lignin accumulation. *Frontiers in Plant Science*, 14(1), 1163939. https://doi.org/10.3389/fpls.2023.1163939
Yang, J., Li, C., Kong, D., Guo, F., & Wei, H. (2020). Light-mediated signaling and metabolic changes coordinate stomatal opening and closure. *Frontiers in Plant Science*, 11(1), 601478. https://doi.org/10.3389/fpls.2020.601478
Zahra, N., Al Hinai, M.S., Hafeez, M.B., Rehman, A., Wahid, A., Siddique, K.H.M., & Farooq, M. (2022). Regulation of photosynthesis under salt stress and associated tolerance mechanisms. *Plant Physiology and Biochemistry*, 178(1), 55–69. https://doi.org/10.1016/j.plaphy.2022.03.003
Zhang, X., Xu, H., She, Y., Hu, C., Zhu, T., Wang, L., & He, H. (2024). Improving the prediction performance of leaf water content by coupling multi-source data with machine learning in *rice* (*Oryza sativa* L.). *Plant Methods*, 20(1), 48. https://doi.org/10.1186/s13007-024-01168-5