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The morpho-physiological and gene expression of East Timor's local rice plant (*Oryza sativa*) response to drought and salinity stress

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Abstract. *Quintao G, Ubaidillah M, Hartatik S, Khofifa RAN, Siswoyo TA. 2023. The morpho-physiological and gene expression of East Timor's local rice plant (*Oryza sativa*) response to drought and salinity stress. Biodiversitas 24: 4548-4556.* East Timor is rich in local rice varieties, such as *Silaun*, *Ime Buci*, *Me Klan*, *Me Likan*, *Me Meak*, *Me Taek*, and *Umak Klete*. These varieties are tolerant to drought and salinity stress. These superior characteristics emerge as the coping strategy of the rice plants toward environmental conditions such as mountainous, upland planting areas without irrigation networks and coastal regions with high salinity levels. This study determined the tolerance response of East Timor's local rice to drought and salinity stresses. This study was designed in a Completely Randomized Design (CRD) with two factors. The first factor, i.e., rice varieties, consisted of 8 varieties, i.e., *Silaun* (V1), *Ime Buci* (V2), *Me Klan* (V3), *Me Likan* (V4), *Me Meak* (V5), *Me Taek* (V6), *Umak Klete* (V7), and *IR-64* (V8) and the second factor was the abiotic stress treatments, namely no treatment or control (P0), 10% PEG treatment (P1), and 150 mM NaCl treatment (P2). The parameters observed were morpho-physiology and biochemical characteristics of the plants. The results showed that the *Silaun* local rice variety from East Timor was tolerant to drought and salinity, supported by the gene expressions for tolerance to drought and salinity stress.

Keywords: Abiotic, drought, East Timor, local rice, salinity, stress

INTRODUCTION

Rice is a staple food crop for 3.5 billion people worldwide, 90% of which are Asian people (Stallworth et al. 2021). The East Timor region, a part of Asia, has unique rice germplasm diversity in each region. This diversity emerges from the different geographical conditions and microclimate of each district in East Timor, namely mountainous areas that result in drought-tolerant rice plant variety and the coastal regions that produce salinity-tolerant rice variety. East Timor rice germplasm with superior properties of environmental stress tolerance includes *Silaun*, *Ime Buci*, *Me Klan*, *Me Likan*, *Me Meak*, *Me Taek*, and *Umak Klete*. So far, identifying the environmental stress tolerance is limited to phenotypic changes, which indicates that the variety has not been well characterized, and its tolerance to drought or salinity is still being determined. Moreover, the environmental condition has been more challenging recently due to global warming, which can harm rice production due to increased rainfall in a short time and drought in a relatively long time (Arham and Adiwibowo 2022).

Drought and saline soils are environmental stresses caused by global warming. Drought stress has deleterious effects on plant morphology and physiology, including changes in external morphology and internal structure of roots, stems and leaves, osmotic regulation, drought-induced proteins, and plant ROS metabolism (Yang et al. 2021). Meanwhile, salinity causes stress from poor osmotic regulation and ion poisoning due to saline soil conditions (Ullah et al. 2021), significantly impacting farmers. This loss can be reduced if farmers apply the superior varieties. Superior varieties are obtained from breeding activities. Breeding activities can increase the variety's potential and support production (Aristya and Taryono 2019). Therefore, rice variety breeding can become an option to respond to the global warming challenge. Breeding can be performed by providing an advanced genetic resource. Selected genetic sources generally come from the superior characteristics of the local variety.

The superior characteristics of a local variety are obtained from the region where the variety grows, a dry and hot area that can grow plants tolerant to dry and hot conditions. It is possible to obtain genetic resources for drought- and saline-tolerant plants from several areas confirmed to have dry and saline soils. Sahoo et al. (2019) showed that 2 of 21 rice varieties in the arid Northeastern region of India were tolerant (*Tampa*) and sensitive (*Chandan*) to drought stress, based on the PEG exposure and further biochemical analysis. The study also found the presence of a solid antioxidative defense mechanism in the *Tampa* variety, which led to reduced ROS accumulation and increased photosynthetic activity, indicating that it is more tolerant under PEG-induced drought stress than *Chandan*. During the germination phase in saline soil (Rasel et al. 2021), the growth and physiological rate of the rice plant decreases due to stress. Still, several varieties from Bangladesh have the lowest reduction, such as

Ghunsi, *Nonabokra*, *Hogla*, *Holdegotal*, *Vusieri*, and *Kanchon*, due to increased proline content, catalase, and ascorbic peroxidase activity. Meanwhile, the accumulation of H_2O_2 and MDA was reported to be relatively lower.

Considering the description above, it is necessary to screen and identify the superior characteristics of each rice variety from East Timor through stress treatment during the germination phase to obtain tolerant varieties. The tolerance level in the following varieties can later be used as a donor combined with other superior germplasm properties to support the growth process and produce a high rice yield. The success of these efforts will produce new superior rice genotypes tolerant to climate change and have high production levels to meet future food needs. Thus, morpho-physiological changes and genetic expression of East Timor's local rice plants under drought and salinity stress were analyzed further to obtain potential drought and saline-tolerant rice varieties.

MATERIALS AND METHODS

Study area

This study was conducted from August - December 2022 at the Nutraceutical and Pharmaceutical Laboratory, Center for Development of Advanced Sciences and Technology (CDAST), and the Agrotechnology Laboratory, Faculty of Agriculture, Universitas Jember, East Java, Indonesia.

Procedures

The rice morphological responses under stress conditions were analyzed in the Agrotechnology Laboratory. Seven local rice of East Timor varieties and one high-yielding rice variety (*IR-64*) were soaked for 24 hours with stress treatment. Each rice variety was divided into three abiotic stress treatment groups: no treatment (control), 10% PEG 6000 treatment, and 150 mM NaCl treatment. No treatment group varieties were prepared by soaking each rice variety in aquadest solution as a treatment under normal conditions. The 10% PEG 6000 treatment group was prepared by soaking each rice variety in 10% PEG 6000 solution as a drought treatment. At the same time, the 150 mM NaCl treatment group was prepared by soaking each rice variety in 150 mM NaCl solution as a salinity treatment. After the rice seeds had been soaked for 24 hours and germinated, they were transferred to a pot and installed with the following treatment. The growth of rice plant seedlings was observed after seven days.

Observations

The physiological and biochemical responses of rice plants after salinity and drought stress exposure were analyzed at the Nutraceutical and Pharmaceutical Laboratory. The physiological responses of rice plants after drought and salinity stresses were observed in height, shoot fresh weight, root wet weight, shoot dry weight, and root dry weight. Leaves from each rice variety with and without 10% PEG 6000 treatment were taken and analyzed to obtain the following parameters: total chlorophyll content, phenolic compounds, flavonoid compounds, DPPH test, and gene expression.

Total chlorophyll content

100 mg samples of the third leaf were homogenized using 5 mL of 95% ethanol. The samples were then centrifuged at 12,000 rpm for 10 minutes. The absorbance of the supernatant absorbance was measured using a spectrophotometer with 664 nm and 649 nm wavelengths.

chlorophyll A = (13.36 * Abs 664) − (5.19 * Abs 649)
chlorophyll B = (27.43 * Abs 649) − (8.12 * Abs 664).

Phenolic compounds

Samples were extracted with 1 mL of 80% ethanol, 5 mL of distilled water, and 0.5 mL of 50% Folin-Ciocalteu before homogenizing and allowed to stand for 5 minutes. Then, 1 mL of 2% Na_2CO_3 was added and left in the dark for ± 60 minutes with gallic acid as a standard. The absorbance value at 750 nm wavelength was then converted into total phenol in mg GAE/g sample weight unit.

Flavonoid compounds

Samples were extracted with 450 mL of distilled water, 40 mL of methanol, and 30 mL of 5% $NaNO_2$ and incubated for 5 minutes. Samples were added with 30 mL of 10% alcohol and incubated for 6 minutes, then added with 240 mL of distilled water and 200 mL of 1 M NaOH. The absorbance of flavonoid compounds was measured using a spectrophotometer at λ = 415 nm.

DPPH test

This test determines the maximum α by preparing 3 mL of DPPH 0.2 solution and allowing it to stand for ± 10 minutes. Time stability was determined by making 25 mL of 200 ppm extract solution. The extract solution was taken at 4.5 mL and added with 0.2 mm of DPPH solution at 1.5 mL before finding the time stability after and before incubation among 5-60 minutes with 5-minute intervals. The sample was measured at λ max, and the stability time was obtained. In this test, each extract was dissolved at 100 ppm concentration, and 4.5 mL of the extract was taken, and 1.5 mL of DPPH solution was added (the ratio of soluble extract and DPPH solution was 3:1) at 0.2 mm concentration in 99% ethanol (v/v). The extract was incubated at 37ºC with the same time stability obtained in the previous step. Then, the extract was put into a cuvette to measure its absorbance at λ max = 515 nm with three-time measurements (triplicates). The absorbance data obtained from each concentration of each extract was calculated by the % value of its antioxidant activity. This value was obtained by the formula:

$$\text{Antioxidant activity (\%)} = \frac{(ao\text{-}ac)}{ac} \times 100\%$$

ao = Absorbance control
ac = Absorbance samples

Gene expression analysis

Expressed genes observed in this study were *OsAPX1*, *OsCATA*, *OsCAT*, *Cytosolic APX*, *Mn-SOD*, *GPOD*, and *Cu/ZnSOD* as antioxidative responsible gene, *OsLEA* as drought and saline stress responsible gene, and *OsAbI3* as drought stress-responsive gene. Meanwhile, *OsActin1* serves as a housekeeping gene. Callus sampling was conducted in the second and fourth weeks after planting in regeneration media. The stages in gene expression analysis are RNA isolation, cDNA synthesis, and PCR. Total RNA at 1000 ng from callus was extracted following the *Ribospin™ Plant kit* (*GeneAll*) procedure, and then cDNA synthesis was carried out following the *ReverTra Ace® qPCR RT Master Mix* (Toyobo) procedure. The Polymerase Chain Reaction (PCR) (Table 1) was performed with a total volume of 15 µL, following the *GoTaq® Green Master Mix* (Promega) procedure. The amplified PCR product was then electrophoresed in 2% agarose gel stained with EtBr and visualized with a UV transilluminator. The electrophoretic gel, placed on the UV-transluminator, had a glowing-orange color from the formed DNA fragments. The DNA fragments were documented and observed for the band size.

Data analysis

Data were analyzed using a one-way Analysis of Variance (ANOVA). If there was a significant difference among the treatments, a further test was carried out using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Meanwhile, the data from the gel electrophoresis were analyzed using a qualitative descriptive analysis with a visual presentation.

RESULTS AND DISCUSSION

Morphological analysis of East Timor local rice under drought and salinity stress

The growth characteristics of rice plants under drought stress conditions with 10% PEG 6000 exhibited different morphological traits for each rice type. These different growth characteristics of rice plants were due to the morphological characteristics of each rice type. Under drought stress conditions, the tested plants exhibited different morphological conditions (Table 2).

Drought treatment on all rice varieties (Table 2) caused plant height and shoot fresh weight reductions. Regarding plant height, the lowest reduction value was found in *Silaun*, *Uma Klete*, and *Me Klan* (17.98%;8.85%;7.6%). For shoot fresh weight observation, the lowest reduction value was obtained in *Me Taek*, *Silaun*, and *Me Klan* (18.26%; 16.68%;14.54%).

The root fresh weight did not show any significant differences. In contrast to the fresh root weight, the *Me Taek* variety showed an increased shoot dry weight, which differed from other varieties that obtained a reduction value. The lowest shoot dry weight reduction was found in *Ime Buci*, *Silaun*, and *Me Klan* (8.33%; 5.19%; 2.05%), and the weight gain in *the Me Taek* variety was 2.28%. In root dry weight, varieties with the lowest reduction value were *Me Klan*, *Uma Klete*, and *Me Taek* (10.52%;10.31%;0%). Based on this condition, with *IR-64* as a control sample, the most drought-tolerant varieties are *Me Klan*, *Silaun*, and *Uma Klete*.

In addition to drought stress treatment, the salinity stress treatment with 150 mM NaCl produced different morphological conditions in each rice type (Table 3).

Table 1. Primer sequence for antioxidant gene expression analysis

Gene	Primer	References
OsAPX1	F: 5’ CCA AGG GTT CTG ACC ACC TA 3’ R: 5’ CAA GGT CCC TCA AAA CCA GA 3’	Kim et al. 2018
OsCATA	F: 5’ CGG ATA GAC AGG AGA GGT TCA 3’ R: 5’ AAT CTT CAC CCC CAA CGA CT 3’	Kim et al. 2018
CAT	F: 5’ CAT CTG GCT CTC CTA CTG GTC T 3’ R: 5’ CAG GAG AAA CGT GTC TTC AGG T 3’	Kim et al. 2007
Cytosolic APX	F: 5’ GCG AAA TCC ATG TGA TAC AAG A 3’ R: 5’ CAA TGC TGA AGG TGT AGC TGA G 3’	Kim et al. 2007
Mn-SOD	F: 5’ GGA AAC AAC TGC TAA CCA GGA C 3’ R: 5’ GCA ATG TAC ACA AGG TCC AGA A 3’	Kim et al. 2018
GPOD	F: 5’ ACC GTG AGC GAG GAC TAC CT 3’ R: 5’ AGC GTC AAG TGA GCC TTA GC 3’	Kim et al. 2007
Cu/ZnSOD	F: 5’ CAA TGC TGA AGG TGT AGC TGA G 3’ R: 5’ GCG AAA TCC ATG TGA TAC AAG A 3’	Kim et al. 2018
OsAbI3	F: 5’ CCC AAC AAC AAA AGC AGG AT 3’ R: 5’ CCT TTG TAT TGG ACG AGA CG 3’	Zhou et al. 2020
OsLEA	F:5’ CCCAAGCTTAAAATGGCGTCGAGGCA G GACA 3’ R:5’ TGCTCTAGATCATGGCAAGACTGCTG A TGTATGG 3’	Zhou et al. 2020
OsACTIN1	F: 5’ TCC ATC TTG GCA TCT CTC AG 3’ R: 5’ GTA CCC GCA TCA GGC ATC TG 3’	Kim et al. 2018

Salinity treatment in all varieties of rice plants (Table 3) caused a plant height reduction. Plants with the lowest height reduction were *Silaun*, *Uma Klete*, and *Me Taek* varieties (21.64%; 19.65%; 18.66%). In shoot wet weight, varieties that showed the lowest reduction value were *Me Clan*, *Ime Buci*, and *Me Taek* (19.99%; 18.26%; 8.69%), whereas the *Silaun* variety had an increased shoot wet weight at 5.01%. There was no significant difference in the root fresh weight. Meanwhile, the lowest shoot dry weight was obtained from *Silaun*, *Uma Klete*, and *Me Klan* (18.70%; 14.02%; 3.16%). In the root dry weight, the lowest reduction was found in *Me Meak*, *Melikan*, and *Meclan* (21.09%;16.55%;13.16%). Compared to *IR64* as a control, *Silaun*, *Me Taek*, and *Uma Klete* are the most saline-tolerant varieties.

Morphologically, the *Me Klan* variety is the most tolerable local rice variety from East Timor to drought stress conditions (Figure 1), compared to *IR-64* rice as a moderate positive control and *Silaun* as a quite tolerable rice variety to drought stress. However, the *Silaun* and *Me Taek* varieties are superior to *Me Klan* in saline exposure. This can be shown in the morphological reduction in Figure 1.

Table 2. Rice morphological characteristics under drought stress condition

Variety x Treatment	Plant Height (cm)	Shoot Wet Weight (mg)	Root Wet Weight (mg)	Shoot Dry Weight (mg)	Root Dry Weight (mg)
IR_C	10.08 ± 0.58 ef	66.00 ± 1.73 efg	28.33 ± 7.50	9.43 ± 0.50 efgh	4.10 ± 0.10 defg
IR_D	8.73 ± 0.29i	56.66 ± 4.04 gh	29.00 ± 9.16	8.86 ± 1.62 efgh	5.32 ± 0.65 fghi
MK_C	13.93 ± 0.57 a	36.66 ± 2.51 a	17.00 ± 1.00	6.33 ± 0.73 a	3.80 ± 0.10 de
MK_D	12.87± 0.63b	31.33 ± 2.08 b	14.33 ± 4.50	6.20 ± 0.45 ab	3.40 ± 0.20 ab
IB_C	12.25 ± 0.37 c	38.33 ± 2.08 defg	13.33 ± 10.78	6.36 ± 0.66 efgh	2.57 ± 0.25J
IB_D	9.63± 1.15fg	33.33 ± 5.85 gh	16.33 ± 1.52	5.83 ± 0.90 gh	3.63 ± 0.25 efgh
SI_C	12.29 ± 1.17 c	50.00 ± 6.55 bc	23.66 ±11.06	7.70 ± 1.32 abcdef	4.03 ± 1.88 cd
SI_D	10.08 ± 1.34 ef	41.66 ± 2.88 def	21.33 ± 7.23	7.30 ± 0.50 bcdefgh	4.60 ± 1.80 cd
UK_C	11.30 ± 0.50 d	52.00 ± 6.00 bc	29.33 ± 11.23	8.56 ± 0.90 abc	4.13 ± 0.15 de
UK_D	10.30 ± 0.66 e	36.66 ± 5.03 efgh	23.66 ± 4.16	6.70 ± 0.98 defgh	3.70 ± 0.44 efgh
ML_C	13.54 ± 1.04 a	56.33 ± 6.35 b	27.66 ± 6.65	8.33 ± 1.05 abcd	4.47 ± 0.93 bc
ML_D	9.52 ± 0.97 fg	41.66 ± 4.93 def	24.00 ± 8.88	7.50 ± 0.26 bcdefg	5.57 ± 0.25 a
MM_C	13.50 ± 0.43 a	45.33 ± 3.51 cd	15.00 ± 1.73	7.26 ± 3.07 abcd	3.13 ± 0.25 hi
MM_D	9.18 ± 0.58 ghi	32.66 ± 2.88 gh	16.33 ± 0.57	5.46 ± 0.64 h	3.43 ± 0.15 fghi
MT_C	11.47 ± 0.57 d	38.33 ± 3.21 defg	20.33 ± 8.08	5.70 ± 0.79 gh	2.97 ± 1.93 fghi
MT_D	8.17 ± 0.63 j	31.33 ± 5.50 gh	15.00 ± 1.73	5.83 ± 1.12 gh	2.97 ± 0.06 ij

Note: *IR-64* (*IR*), *Me Klan* (*MK*), *Ime Buci* (*IB*), *Silaun* (*SI*), *Umak Klete* (*UK*), *Me Likan* (*ML*), *Me Meak* (*MM*), and *Me Taek* (*MT*) after drought stress exposure (D). A further test used the DMRT test with a significance level of 0.05

Table 3. Rice morphological characteristics under salinity stress condition

Variety x Treatment	Plant Height (cm)	Shoot Wet Weight (mg)	Root Wet Weight (mg)	Shoot Dry Weight (mg)	Root Dry Weight (mg)
IR_C	10.08 ± 0.58 ef	66.00 ± 1.73 efgh	28.33 ± 7.50	9.43 ± 0.50 efgh	4.10 ± 0.10 defg
IR_S	6.53 ± 0.94 k	42.3 ± 4.61 h	18.33 ± 2.51	7.26 ± 0.92 fgh	4.13 ± 0.64 ghi
MK_C	13.93 ± 0.57 a	36.66 ± 2.51 a	21.33 ± 5.03	6.33 ± 0.73 a	3.80 ± 0.10 de
MK_S	9.03 ± 0.29 ghi	29.33 ± 2.88 de	15.33 ± 1.15	6.13 ± 0.20 bcdefgh	3.30 ± 0.10 cd
IB_C	12.25 ± 0.37 c	38.33 ± 2.08 defg	14.33 ± 4.50	6.36 ± 0.66 efgh	2.57 ± 0.25j
IB_S	8.87 ± 0.14 hi	31.33 ± 0.00 gh	16.33 ± 1.52	6.36 ± 0.89 efgh	3.57 ± 0.47 efghi
SI_C	12.29 ± 1.17 c	33.33 ± 5.85 bc	23.66 ± 11.06	7.70 ± 1.32 abcdef	4.03 ± 1.88 cd
SI_S	9.63 ± 0.63 fg	35.00 ± 3.60 efgh	15.66 ± 4.72	6.26 ± 1.02 efgh	3.00 ± 2.77 k
UK_C	11.30 ± 0.50 d	52.00 ± 6.00 bc	29.33 ± 11.23	8.56 ± 0.90 abc	4.13 ± 0.15 de
UK_S	9.08 ± 0.91 ghi	36.66 ± 5.03 efgh	16.66 ± 6.50	7.36 ± 1.16 bcdefg	4.87 ± 0.35 bc
ML_C	13.54 ± 1.04 a	56.33 ± 6.35 b	27.66 ± 6.65	8.33 ± 1.05 abcd	4.47 ± 0.93 bc
ML_S	7.87 ± 1.46 j	34.33 ± 3.51 fgh	18.00 ± 1.00	6.76 ± 1.36 cdefgh	3.73 ± 0.72 def
MM_C	13.50 ± 0.43 a	45.33 ± 3.51 cd	15.00 ± 1.73	7.26 ± 3.07 abcd	3.13 ± 0.25 hi
MM_S	9.47 ± 1.00 fgh	31.66 ± 3.05 gh	11.33 ± 0.57	5.80 ± 0.60 gh	2.47 ± 0.50 j
MT_C	11.47 ± 0.57 d	38.33 ± 3.21 defg	20.33 ± 8.08	5.70 ± 0.79 gh	2.97 ± 1.93 fghi
MT_S	9.33 ± 1.50 ghi	35.00 ± 5.50 efgh	18.00 ± 2.00	8.00 ± 0.72 abcde	3.77 ± 0.06 efg

Note: *IR-64* (*IR*), *Me Klan* (*MK*), *Ime Buci* (*IB*), *Silaun* (*SI*), *Umak Klete* (*UK*), *Me Likan* (*ML*), *Me Meak* (*MM*), and *Me Taek* (MT) after salinity stress exposure (S). A further test used the DMRT test with a significance level of 0.05

Physiological analysis of rice in East Timor under drought and salinity stress

The physiological response to drought stress with PEG treatment in each variety (Table 4) showed decreased chlorophyll and increased DPPH values. The lowest chlorophyll reduction was found in *Ime Buci* (24.53%), *Silaun* (24.76%), and *Me Klan* (36.83%), and the highest decrease was found in *IR-64* (72.04%), *Me Meak* (61. 67%), and *Me Likan* (60.56%). In the DPPH parameter, the varieties with the highest value were *Me Likan*, *Silaun*, and *Me Meak*. In addition, a decreased DPPH value occurred in the *Ime Buci* and *Me Taek* varieties. Each variety's phenolic and flavonoid contents showed different results, contradictory to *the Me Klan* variety, which had an increased phenolic content. Still, they showed a reduced flavonoid content, while *IR-64* showed a decreased phenolic content but an increased flavonoid content. Furthermore, *Silaun* and *Me Taek* varieties experienced a decrease in both phenolic and flavonoid contents. Varieties with the highest phenolic content were *Me Meak*, *Me Klan*, *Ime Buci*, *Uma Klete*, and *Silaun*. Varieties with the lowest phenolic content were *IR-64* and *Me Taek*. Varieties with the most significant flavonoid reduction were *Me Likan*, *Ime Buci*, *Me Klan*, *Me Taek*, and *Me Meak*. Meanwhile, the varieties with the most significant flavonoid increase were *Silaun*, *Uma Klete*, and *IR-64*.

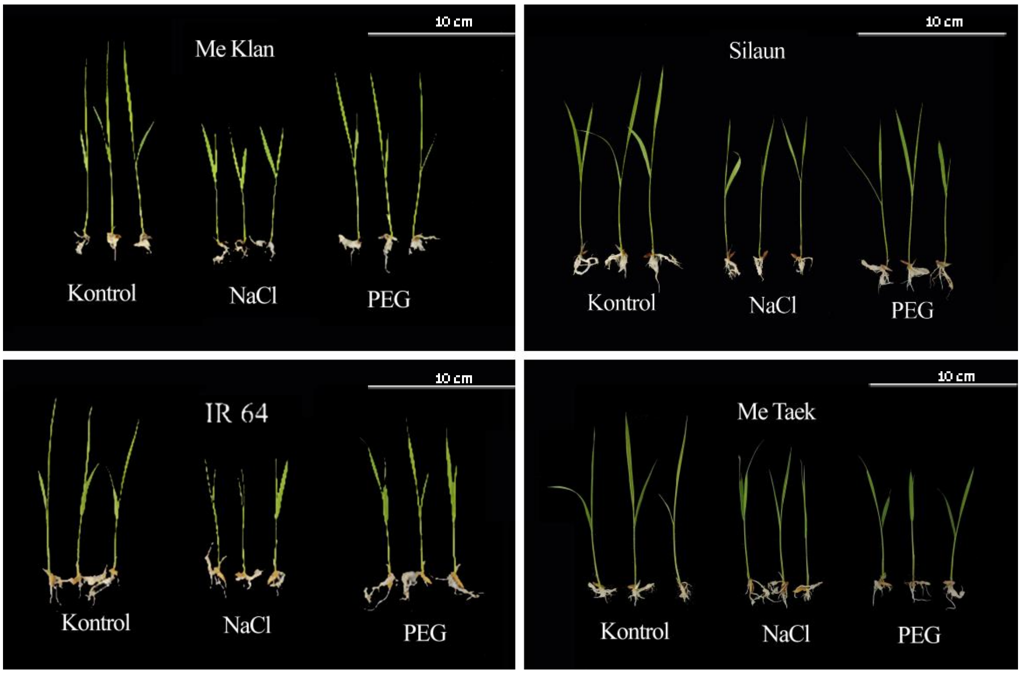

Figure 1. Morphology of rice plants under abiotic stress conditions (salinity and drought)

Table 4. Physiological characteristics of rice under drought stress condition

Variety x Treatment	PEG Treatment			
	Chlorophyll	**Phenolic**	**Flavonoid**	**DppH**
IR_C	8.62 ± 0.67 d	1.51 ± 0.02 j	1.92 ± 0.14 p	0.44 ± 0.07 r
IR_D	2.41 ± 0.25 jk	1.31 ± 0.03 n	3.26 ± 0.14 h	1.16 ± 0.03 p
MK_C	3.34 ± 0.13 i	0.98 ± 0.03 s	6.90 ± 0.07 e	1.25 ± 0.07 p
MK_D	2.11 ± 0.25 kl	1.77 ± 0.02 f	3.12 ± 0.14 i	1.65 ± 0.25 n
IB_C	3.22 ± 0.36 i	1.15 ± 0.02 r	6.72 ± 0.14 f	4.43 ± 0.27 h
IB_D	2.43 ± 0.12 jk	1.85 ± 0.02 d	2.93 ± 0.14 k	1.95 ± 0.13 m
SI_C	4.28 ± 0.16 h	1.82 ± 0.02 e	3.30 ± 0.07 h	1.41 ± 0.04 o
SI_D	3.22 ± 0.81 i	1,83 ± 0.02 de	6.87 ± 0.07 e	4.42 ± 0.03 h
UK_C	4.58 ± 1.05 gh	0.91 ± 0.02 t	1.95 ± 0.07 op	2.22 ± 0.01 l
UK_D	2.12 ± 0.43 kl	2.08 ± 0.02 b	5.51 ± 0.07 g	4.41 ± 0.04 h
ML_C	6.11 ± 0.48 f	2.76 ± 0.03 a	9.78 ± 0.07 a	0.85 ± 0.08 q
ML_D	2.41 ± 1.22 jk	1.31 ± 0.03 n	3.02 ± 0.07 j	4.10 ± 0.05 i
MM_C	7.25 ± 1.08 e	0.97 ± 0.02 s	8.05 ± 0.07 c	2.81 ± 0.02 k
MM_D	2.79 ± 0.51 ij	1.90 ± 0,02 c	7.72 ± 0.07 d	5.02 ± 0.02 g
MT_C	11.16 ± 1.42 b	1.72 ± 0.03 gh	8.20 ± 0.07 b	3.49 ± 0.03 j
MT_D	10.19 ± 1.66 c	1.72 ± 0.02 g	3.28 ± 0.12 h	2.26 ± 0.16 l

Note: *IR-64* (*IR*), *Me Klan* (*MK*), *Ime Buci* (*IB*), *Silaun* (*SI*), *Umak Klete* (*UK*), *Me Likan* (*ML*), *Me Meak* (MM), and *Me Taek* (MT) after PEG (drought) treatment (D). A further test used the DMRT test with a significance level of 0.05

The physiological response to salinity stress in each variety (Table 5) decreased the chlorophyll content and increased the DPPH value. The lowest reduction in the chlorophyll content was in *Me Klan* (28.44%), but the *Silaun* and *Uma Klete* varieties experienced an increase in the amount of chlorophyll as much as 188.551%. And 158.297%. At the same time, the highest reduction of chlorophyll was observed in *Me Taek* (51.62%) and *IR64* (72.51%). The highest increase in DPPH values was observed in Me Klan, Silaun, and IR64 varieties, while the varieties that experienced the lowest increases *were Ma Taek*, *Ime Buci*, and *Me Meak*. The results of phenolic and flavonoid content of each variety showed different results. As in the *Me Klan* variety, which experienced an increase in phenolic content but experienced a reduction in flavonoid content after being treated, it was inversely proportional to the control variety *IR64*, which experienced a decrease in phenolic content but an increase in flavonoid content. At the same time, the *Silaun* and *Me Taek* variety experienced a reduction in phenolic and flavonoid content values. The varieties that experienced the highest reduction in phenolic content were *Me Likan, Silaun, Me Taek* and *IR64.* At the same time, the varieties that showed the highest increase in phenolic content were *Me Klan, Ime Buci, Me Meak*, and *Uma Klete*. The varieties that experienced a reduction of flavonoids were only *IR64*.

Tolerant gene expression in rice

Stress conditions showed different morphological and physiological responses to each rice variety. Based on morphological and physiological analysis, several abiotic stress tolerance genes were expressed in the selected East Timor local rice variety (Figure 2), e.g., the *Silaun* variety. This variety had relatively high tolerance in both types of treatment compared to other varieties. *Silaun*, as a Timor Leset's local rice variety, expressed more tolerance genes than the *IR-64* rice variety. *Osapx* is detected more clearly in the *Silaun*, while some tolerance genes like *OsLEA* and *OsABI3* were only expressed in the *Silaun*. However, other genes, such as *OsCATA*, *OsCAT*, *CuZnSOD*, *GPOD*, *Cytosolic APX*, *MNSOD*, and *Osactin1*, were expressed in both *IR-64* and *Silaun* varieties.

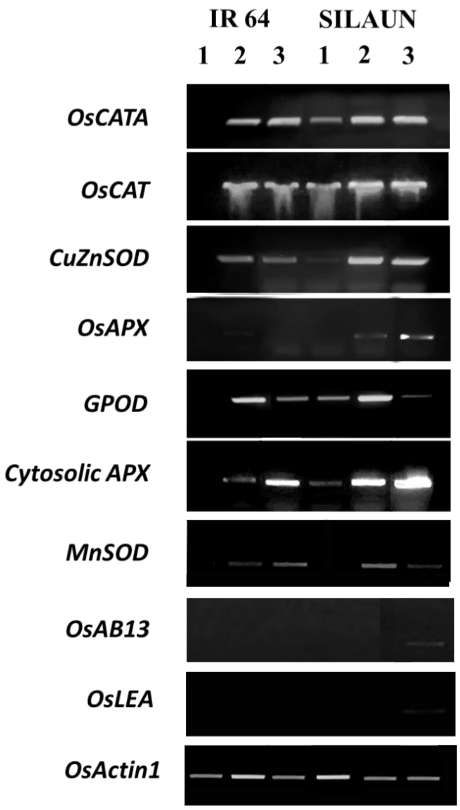

Figure 2. Expression of tolerance genes to abiotic stress in rice plants. Note: 1: Control; 2: PEG 10%; 3: NaCl 150mM

Table 5. Physiological data of rice under saline stress conditions

Variety x Treatment	NaCl Treatment			
	Chlorophyll	Phenolic	Flavonoid	DppH
IR64_C	8.62 ± 0.67 d	1.51 ± 0.02 j	1.92 ± 0.14 p	0.44 ± 0.07 r
IR64_S	2.37 ± 0.49 jk	1.24 ± 0.02 o	2.09 ± 0.07 n	7.31 ± 0.24 d
MK_C	3.34 ± 0.13 i	0.98 ± 0.03 s	6.90 ± 0.07 e	1.25 ± 0.07 p
MK_S	2.39 ± 0.13 jk	1.70 ± 0.02 h	2.53 ± 0.07 l	12.32 ± 0.69 a
IB_C	4.28 ± 0.26 h	1.15 ± 0.02 r	6.72 ± 0.14 f	4.43 ± 0.27 h
IB_S	3.22 ± 0.36 i	1.66 ± 0.02 i	3.05 ± 0.07 ij	7.19 ± 0.04 e
SI_C	4.28 ± 0.16 h	1.82 ± 0.02 e	3.30 ± 0.07 h	1.41 ± 0.04 o
SI_S	12.35 ± 1.09 a	1.20 ± 0.03 p	2.00 ± 0.07 o	8.44 ± 0.08 b
UK_C	4.58 ± 1.05 gh	0.91 ± 0.02 t	1.95 ± 0.07 op	2.22 ± 0.01 l
UK_S	11.83 ± 0.77 a	1.18 ± 0.05 q	1.92 ± 0.14 p	8.14 ± 0.15 c
ML_C	6.11 ± 0.48 f	2.76 ± 0.03 a	9.78 ± 0.07 a	0.85 ± 0.08 q
ML_S	1.76 ± 0.54 l	1.44 ± 0.02 k	2.24 ± 0.07 m	6.92 ± 0.10 f
MM_C	7.25 ± 1.08 e	0.97 ± 0.02 s	8.05 ± 0.07 c	2.81 ± 0.02 k
MM_S	2.02 ± 0.42 kl	1.39 ± 0.02 l	2.59 ± 0.14 l	8.41 ± 0.02 b
MT_C	10.19 ± 1.66 c	1.72 ± 0.03 gh	8.20 ± 0.07 b	3.49 ± 0.03 j
MT_S	4.93 ± 1.47 g	1.35 ± 0.02 m	2.54 ± 0.14 l	6.88 ± 0.15 f

Note: *IR-64* (*IR*), *Me Klan* (*MK*), *Ime Buci* (*IB*), *Silaun* (*SI*), *Umak Klete* (*UK*), *Me Likan* (*ML*), *Me Meak* (*MM*), and *Me Taek* (MT) after salinity exposure treatment with NaCl (S). A further test used the DMRT test with a significance level of 0.05

Discussion

Rice (*Oryza sativa* L.) is a widely planted crop in East Timor, but its characterization against the environmental challenge due to global warming still needs to be improved. Global warming can cause many effects on rice, such as drought or saline soil, due to excessive evaporation. Thus, varieties found around East Timor regions, planted in Coastal or dry mountain areas, may be used to find the best varieties that are tolerant to drought and saline conditions. Based on the morphological observation after drought stress exposure, *Me Klan*, *Silaun*, and *Uma Klete* are the most susceptible varieties against drought stress. At the same time, *Silaun* and *Taek* are the most tolerant rice varieties against salinity stress (Figure 1).

The morphological observation results (Table 2) showed that each variety experienced a reduction value of the observed variables due to drought stress, which aligns with the results of Maemunah et al. (2021). The concentration of PEG caused these reductions applied to the seeds, which could bind water, so roots could not absorb water for the metabolic process. This process is similar to the mechanism of drought that generally occurs in seeds and plants. Upadhyaya and Panda (2019) highlighted that morphological responses indicated the response of rice plants to drought stress, e.g., decreased leaf area, less number of stomata, leaf necrosis, and reduced plant biomass due to low roots and crowns formed, leaf surface area, and percentage of flowering. Drought stress also affects the cell life cycle, which causes growth retardation due to a lack of water content in the soil. The fresh and dry weight of the rice plant biomass significantly decreased due to excessive dehydration. However, not all plants produce a reduction mechanism due to drought; some varieties showed an increased root dry weight, such as *Me Klan* and *Uma Klete*. However, the wet root weight had no significant difference, which caused the root variable in drought stress to be unable to be used as an observational variable, as mentioned in Mangansige et al. (2018).

The response of rice plant morphological changes to salinity stress on plant morphology (Table 3) is closely related to growth inhibition and yield loss (Haque et al. 2021). Salinity stress can increase the osmotic pressure, thus decreasing the water absorption by roots and water potential in plant cells and tissues, which causes a poor growth rate. High salinity levels in plant tissues can inhibit growth and productivity, affecting germination, photosynthesis, and plant nutrient balance. The morphological response produced by plants when being exposed to salinity stress in rice plants is stomata closure, which can impact the assimilation of CO_2 levels and cause the disruption of the photosynthesis process in rice, so the rice biomass will also decrease (Giordano et al. 2021). Decreased plant biomass includes decreased roots and crowns, leaf surface area, and flowering percentage, which also causes a productivity decline.

Similarly, roots in salinity stress exposure treatment also cannot be used as a benchmark to assess the salinity stress tolerance because fresh root weight had no significant values. However, root dry weight due to salinity stress significantly differed in several varieties, even an occurrence of dry weight increase in *IR-64*, *Ime Buci*, *Uma Klete*, and *Me Taek*. However, different responses of the rice variety to drought and salinity stresses in the forms of an increased root dry weight were attributed to the following varieties' tolerance. For example, *IR-64,* as a control variety, is highly tolerant to drought (Salsinha et al. 2020) but sensitive to saline conditions (Saini et al. 2018); the results were associated with the increased root dry weight in the salinity stress treatment.

Several varieties have different responses based on the physiological response of rice plants to drought (Table 4) and salinity stress (Table 5). Stress affects the biochemical photosynthesis properties, i.e., chlorophyll. Chlorophyll is an essential component of photosynthetic activity, which can be used as a variable in the initial screening process of plants to determine whether plants are affected by stress (Swapna and Shylaraj 2017). The total chlorophyll content can decrease due to low water availability, which elevates the transpiration process and chlorophyll disintegration (Hendriyani and Setiari 2009). However, the *Uma Klete* and *Silaun* varieties under salinity stress experienced an increased chlorophyll content, similar to the results of Kanawapee et al. (2012) on drought-sensitive rice varieties found to be tolerant to salinity stress.

In contrast, the chlorophyll content increased, although the susceptible varieties experienced a decreased chlorophyll content. This condition may occur due to the defense mechanism of tolerant plant varieties to reduce free radicals as a stress product, producing more chlorophyll to protect plant cells from damage. Generally, plants sensitive to drought stress are prone to have the greatest and sharpest reductions than drought-tolerant plants (Mishra et al. 2019).

In addition to chlorophyll, phenolic is a secondary metabolite compound in plants that acts as a natural defense against unfavorable environmental conditions. Hopkins (1999) stated that secondary metabolites could accumulate in tissues when plants were subjected to environmental stress. Bidwell (1979) also stated that stress caused complex changes in cytoplasm and plant biochemical process imbalance. This imbalance causes the accumulation of various secondary metabolites, such as phenolics and flavonoids. Flavonoids are secondary metabolites widely found in plant tissues and are one of the mechanisms for surviving stress. This has an impact on the value of phenolic and flavonoid contents, which depends on the mechanism of the variety to face stress, as mentioned in this study with *Me Klan* that had high phenolic content after being exposed to drought and salinity stress but showed a decreased flavonoid content. According to Abdalla et al. (2022), The DPPH test is used to determine the antioxidant activity in plants. A higher antioxidant activity indicates more active genes responsible for counteracting the free radicals due to stress. However, this makes it less correlated with phenolics because various other antioxidant compounds may have an effect.

In stressed conditions, rice plants activate or express their tolerance genes (Figure 2). The *Silaun* variety was chosen because it was the most resistant in all treatments, and for the observed variables, we looked at its gene expression and compared it to IR64 as a control. The *OsABI3* and *OsLEA* genes were used as parameters to observe the mechanism of plant tolerance to drought stress. Fatima et al. (2020) and Duan and Cai (2012) used the Late Embryogenesis Abundant (*LEA*) gene to demonstrate drought or cold and high salinity stress that occurred in water-associated plants. The Abscisic Acid Insensitive 3 (*ABI3)* gene mediates plant stress, especially in ABA signaling response, which can be highly expressed when exposed to drought stress (Koramutla et al. 2021). *OsABI3* and *OsLEA* genes were expressed in *Silaun* var. gene expression, which means that Silaun is resistant to drought stress compared to *IR64*.

Meanwhile, several enzymes, such as Catalase (CAT), Superoxide Dismutase (SOD), Guaiacol Peroxidase (GPX/GPOD), and Ascorbic Peroxidase (APX), were analyzed to determine the effect of NaCl concentrations, that could upregulate these enzymes during salinity stress (Siddiqui et al. 2022). The genes that control these enzymes were expressed in *IR64* and *Silaun* var., so they were considered resistant/tolerant to salinity stress. The *OsActin1* was used as a control gene for normalization (Lin et al. 2020). Therefore, based on morphological conditions, Me Klan, Silaun, and Uma Klete are the most tolerant varieties to drought stress. Based on the physiological response, the most tolerant varieties to drought stress are *Ime buci*, *Silaun*, and *Me Klan*.

Meanwhile, the most tolerant varieties of salinity stress on the morphological response are *Silaun*, *Uma Klete*, and *Me Taek*. The most tolerant varieties based on the physiological response are *Silaun*, *Me Taek*, and *Me Klan*. These varieties have unique tolerance mechanisms in phenolic content or the DPPH test. However, the most tolerant rice variety to drought and salinity stress is the *Silaun* variety, as evidenced by the various expressions of defensive genes and enzymes to tolerate stress exposure.

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REFERENCES

Abdalla H, Adarosy MH, Hegazy HS, Abdelhameed RE. 2022. Potential of green synthesized titanium dioxide nanoparticles for enhancing seedling emergence, vigor and tolerance indices and DPPH free radical scavenging in two varieties of soybean under salinity stress. BMC Plant Biol 22: 560. DOI: 10.1186/s12870-022-03945-7.

Arham IL, Adiwibowo S. 2022. Pengaruh kemarau panjang 2019 sebagai indikasi perubahan iklim terhadap kesejahteraan rumah tangga petani padi Desa Tenajar Kidul, Indramayu. Jurnal Sains Komunikasi dan Pengembangan Masyarakat. 6 (1): 86-100. DOI: 10.29244/jskpm.v6i1.960. [Indonesian]

Aristya VE, Taryono. 2019. Pemuliaan tanaman partisipatif untuk meningkatkan peran varietas padi unggul dalam mendukung swasembada pangan nasional. Agrinova 2 (1): 26-35. DOI: 10.22146/agrinova.51985. [Indonesian]

Bidwell RCS. 1979. Plant Physiology. Macmillan Publishing co. Inc., New York.

Duan J, Cai W. 2012. OsLEA3-2, an abiotic stress induced gene of rice plays a key role in salt and drought tolerance. PLoS One 7 (9): 1-11. DOI: 10.1371/journal.pone.0045117.

Fatima P, Chand P, Singh SK, Purushottam, Mishra A, Kumar P. 2020. Effects of water stress on biochemical character and transcription factors expression in rice (*Oryza sativa* L.). J Pharmacogn Phytochem 9 (5): 2773-2776.

Giordano M, Petropoulos S, Rouphael Y. 2021. Response and defence mechanisms of vegetable crops against drought, heat and salinity stress. Agriculture 11 (5): 463. DOI: 10.3390/agriculture11050463.

Haque MA, Rafii MY, Yusoff MM, Ali NS, Yusuff O, Datta DR, Anisuzzaman M, Ikbal MF. 2021. Advanced breeding strategies and future perspectives of salinity tolerance in rice. Agronomy 11: 1631. DOI: 10.3390/agronomy11081631.

Hendriyani IS, Setiari N. 2009. Kandungan klorofil dan pertumbuhan kacang panjang (*Vignas sinensis*) pada tingkat penyediaan air yang berbeda. J Sains Mat 17 (3): 145-150. [Indonesian]

Hopkins WG. 1999. Introduction to Plant Physiology. Jhon Wiley and Sons Inc., Toronto.

Kanawapee N, Sanitchon J, Lontom W, Threerakulpisut P. 2012. Evaluation of salt tolerance at the seedling stage in rice genotypes by growth performance, ion accumulation, proline and chlorophyll content. Plant Soil 358: 235-249. DOI: 10.1007/s11104-012-1179-6.

Kim DW, Shibato J, Agrawal GK, Fujihara S, Iwahashi H, Kim DH, Shim IS, Rakwal R. 2007. Gene transcription in the leaves of rice undergoing salt-induced morphological changes (*Oryza sativa* L.). Mol Cell 24 (1): 45-59.

Kim Y, Mun BG, Khan AL, Waqas M, Kim HH, Shahzad R, Imran M, Yun BW, Lee IJ. 2018. Regulation of reactive oxygen and nitrogen species by salicylic acid in rice plants under salinity stress conditions. PLoS One 13 (3): 1-20. DOI: 10.1371/journal.pone.0192650.

Koramutla MK, Negi M, Ayele BT. 2021. Roles of glutathione in mediating abscisic acid signaling and its regulation of seed dormancy and drought tolerance. Genes 12 (10): 1620. DOI: 10.3390/genes12101620.

Lin F, Li S, Wang K, Tian H, Gao J, Zhao Q, Du C. 2020. A leucine-rich repeat receptor-like kinase, OsSTLK, modulates salt tolerance in rice. Plant Sci 296: 110465. DOI: 10.1016/j.plantsci.2020.110465.

Maemunah M, Samudin S, Alfiana A, Mustakim M, Yusran Y. 2021. Toleransi kekeringan beberapa kultivar padi gogo (*Oryza sativa* L.) pada konsentrasi PEG 6000 fase perkecambahan. Agroland 28 (1): 72-80. DOI: 10.22487/agrolandnasional.v28i1.704. [Indonesian]

Mangansige C, Ai NS, Siahaan PS. 2018. Panjang dan volume akar tanaman padi lokal Sulawesi Utara saat kekeringan yang diinduksi dengan polietilen glikol 8000. Mipa Unsrat Online 7 (2): 12 -15. DOI: 10.35799/jm.7.2.2018.20618.

Mishra SS, Behera PK, Panda D. 2019. Genotypic variability for drought tolerance-related morpho-physiological traits among indigenous rice landraces of Jeypore tract of Odisha, India. J Crop Improv 33: 254-278. DOI: 10.1080/15427528.2019.1579138.

Rasel M, Tahjib-Ul-Arif M, Hossain MA, Hassan L, Farzana S, Brestic M. 2021. Screening of salt-tolerant rice landraces by seedling stage phenotyping and dissecting biochemical determinants of tolerance mechanism. J Plant Growth Regul 40: 1853-1868. DOI: 10.1007/s00344-020-10235-9.

Sahoo S, Saha B, Awasthi JP, Omisun T, Borgohain P, Hussain S, Panigrahi J, Panda SK. 2019. Physiological introspection into differential drought tolerance in rice cultivars of North East India. Acta Physiol Plant 41: 53. DOI: 10.1007/s11738-019-2841-x.

Saini S, Kaur N, Pati PK. 2018. Reactive oxygen species dynamics in roots of salt sensitive and salt tolerant cultivars of rice. Anal Biochem 550: 99-108. DOI: 10.1016/j.ab.2018.04.019.

Salsinha YCF, Indradewa DI, Purwestri YA, Rachmawati D. 2020. Selection of drought-tolerant local rice cultivars from East Nusa Tenggara, Indonesia during vegetative stage. Biodiversitas 21 (1): 170-178. DOI: 10.13057/biodiv/d210122.

Siddiqui ZS, Wei X, Umar M, Abideen Z, Zulfiqar F, Chen J, Hanif A, Dawar S, Dias DA, Yasmeen R. 2022. Scrutinizing the application of saline endophyte to enhance salt tolerance in rice and maize plants. Front Plant Sci 12: 770084.DOI: 10.3389/fpls.2021.770084.

Stallworth S, Shrestha S, Schumaker B, Roma-Burgos N, Tseng TM. 2021. Screening diverse weedy rice (*Oryza sativa* ssp.) mini germplasm for tolerance to heat and complete submergence stress during seedling stage. Front Agron 3: 642335. DOI: 10.3389/fagro.2021.642335.

Swapna S, Shylaraj KS. 2017. Screening for osmotic stress responses in rice varieties under drought condition. Rice Sci 24 (5): 253-263. DOI: 10.1016/j.rsci.2017.04.004.

Ullah A, Bano A, Khan N. 2021. Climate change and salinity effects on crops and chemical communication between plants and plant growth-promoting microorganisms under stress. Front Sustain Food Syst 5: 618092. DOI: 10.3389/fsufs.2021.618092.

Upadhyaya H, Panda SK. 2019. Drought stress responses and its management in rice In: Hasanuzzaman M, Fujita M, Nahar K, Biswas JK (eds). Advances in Rice Research for Abiotic Stress Tolerance: 177-200. Woodhead Publishing, Cambridge. DOI: 10.1016/B978-0-12-814332-2.00009-5.

Yang X, Lu M, Wang Y, Wang Y, Liu Z, Chen S. 2021. Response mechanism of plants to drought stress. Horticulturae 7: 50. DOI: 10.3390/horticulturae7030050.

Zhou C, Lin Q, Lan J, Zhang T, Liu X, Miao R, Mou C, Nguyen T, Wang J, Zhang X, Zhou L, Zhu X, Wang Q, Zhang X, Guo X, Liu S, Jiang L, Wan J. 2020. WRKY transcription factor OsWRKY29 represses seed dormancy in rice by weakening abscisic acid response. Front Plant Sci 11: 691. DOI: 10.3389/fpls.2020.00691.