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RESEARCH ARTICLE

Evaluation of the drought tolerance level of Malaysian *indica* rice genotypes using biochemical markers at reproductive stage

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ABSTRACT

Drought is a serious limiting factor to rice (*Oryza sativa* L.) production in Malaysia, and becoming a more significant issue with respect to the global climate change. This study selected 15 widely cultivated Malaysian *indica* rice genotypes whose drought tolerance level is still unknown. The study aimed to determine the changes in some biochemical responses of selected rice genotypes under drought condition was designed in completely randomized design in rain-out shelter. The results showed that all genotypes had significantly increased total soluble sugar (TSS), proline, catalase (CAT), ascorbate peroxidase (APX) and membrane electrolyte leakage (EL) upon drought stress. The study revealed maximal 154.88% and 166% increase in CAT and APX respectively during the stress. However, MR167 showed maximal 46.79% decrease in total chlorophyll and MARDI WARNA98 revealed maximal 41.95% decrease in total protein as a result of water stress. The correlation analysis among the biochemical traits under drought condition showed positive and significant relationship except EL. Multivariate principal component analysis (PCA) showed that total protein and TSS were important criteria for identifying drought-tolerant genotypes. MR185, MR211, MR253, MR269, MR284, MR303, and MR307 had better performances in all of the biochemical analyses and contributed better to most of the variations in the study.

Key words: Ascorbate peroxidase, catalase, drought, electrolyte leakage, *indica* rice, *Oryza sativa*.

INTRODUCTION

Rice (*Oryza sativa* L.) feeds over half the world's population, is a staple food for a large number of people (Yadav et al., 2023). The higher nutritional quality and easy digestibility enabled rice to be considered the queen among cereals (Verma et al., 2018). Rice is subjected to numerous abiotic and biotic stresses, including drought, flood, salinity, alkalinity, insect pests, and diseases and these variables have significant impact on rice output and productivity, resulting in substantial losses (Yadav et al., 2023).

Drought stress negatively impacts the physiological, morphological and biochemical characteristics of rice, limiting plant productivity and hence the economy of rice production. The turgidity and osmotic balance of crop plants are altered as a result of drought (Saud et al., 2017). The plant responds to drought stress by adjusting its osmotic balance to mitigate the stress induced loss. The defence mechanisms of plants trigger build-up of osmolytes and osmoprotectants during stress. Organic compounds such as sugars (trehalose, fructan, etc.), prolines, polyols, alkaloids, mannitols, sorbitols, betaines, polyamines, and D-inositol are directly related to drought induced osmotic adjustment (Zafar et al., 2020). Again, water stress typically causes plant damage via oxidative stress, which generates reactive oxygen species (ROS) (Sarker

and Oba, 2018). Plants increase antioxidant accumulation and induce ROS detoxification during the stress. Catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione *S*-transferase (GST), guaiacol peroxidase (POX), and peroxiredoxin (PRX) are the major enzymes involved in this system (The PLOS ONE Editors, 2022).

Malaysia is among the Asia-Pacific countries most vulnerable to natural disasters such as floods, landslides, droughts, and climate change (Alam et al., 2020). Around 30 million people in Malaysia feed on rice. A previous study by Herman et al. (2015) stated that the El Niño phenomenon has disrupted agricultural activities, which has led to a 20% decrease in overall rice production. Precipitation fell off throughout this time, resulting in longer and intense dry seasons (Bong and Richard, 2020). The prolonged effect of water stress resulted in a significant loss in rice production in Malaysia amounting to approximately RM21.6 million between the years 2017-2021 as the drought destroyed around 9000 ha paddy fields nationwide (Azmi, 2021). The Malaysian government has set a goal of 100% self-sufficiency level (SSL) in rice production line in order to meet demand, while currently achieves a considerably lower level of 75% SSL (Rahim et al., 2017).

Malaysian Agricultural Research and Development Institute (MARDI) has so far released 52 rice genotypes (Sunian et al., 2022) since 1964. The MARDI released genotypes have their own characteristics, such as plant height, maturation days, yield, and disease resistance. However, there is lack of information about their drought tolerance. According to prior studies, reproductive stage of rice is the most sensitive to water deficiency (Kumar et al., 2021). Hence, 15 rice genotypes were selected for the study to evaluate drought tolerance level through biochemical markers at the reproductive growth stage.

MATERIALS AND METHODS

Study materials

Fifteen rice (*Oryza sativa* L.) genotypes collected from Malaysian Agricultural Research and Development Institute (MARDI), were used for the study: MARDI WANGI88 (accession number-MRGB13020), MARDI WARNA98 (MRGB13141), MR157 (MRGB08636), MR167 (MRGB08646), MR185 (MRGB08455), MR211 (MRGB11629), MR219 (MRGB11633), MR220 (MRGB11634), MR253 (MRGB12095), MR263 (MRGB12133), MR269 (MRGB12120), MR284 (MRGB12140), MR297 (MRGB13019), MR303 (MRGB13001) and MR307 (MRGB13005).

Seed sterilization and germination

The seeds were sterilized and germinated according to the method by Evamoni et al. (2023). Firstly, the seeds of all genotypes were surface sterilized through immersion in 70% ethanol for 30 s. The seeds were then rinsed once with distilled water to remove the ethanol. Again, seeds were stirred in 40% sodium hypochlorite solution for 20 min. Finally, seeds were rinsed with sterile distilled water four to five times.

Seeds were germinated by evenly distributing 10 seeds in a glass jar for each replicate lined with a layer of Whatman filter paper (90 mm size) for 10 d in a growth room at 25 ± 2 °C, relative humidity 50%-70%, and 12:12 h photoperiod.

Experimental design and treatment

A pot experiment was conducted in a rainout shelter in a completely randomized design (CRD). Ten days-old seedlings were transferred to pots. Each of the 15 genotypes was grown in three replicates under two treatments: Control (well-watered) and drought, where water stress was applied at early reproductive stage and continued until maturity. For control, consistent water supply was maintained during whole experiment. A basal application of N:P:K (150:60:50, kg ha^{-1}) was applied during the experiment (Kamarudin et al., 2020). Drought was applied at the early reproductive stage and continued until maturity. During this period, the moisture content in the soil surface ranged between 15% and 25%, causing a mild to moderate drought (Huang et al., 2019), measured with LCD digital moisture meter (E11910, OEM, Guangdong, China).

Biochemical analyses
Third or fourth fully expanded leaves from the tip of the shoots in each replicate per genotype were collected for biochemical analyses after 15 d of drought imposition (Saha et al., 2020). Total chlorophyll, total soluble sugar (TSS), proline, membrane stability, total protein, catalase (CAT), and ascorbate peroxidase (APX) were estimated to determine their variable availability in control and drought stressed plants.

Determination of total chlorophyll
A dry water bath was adjusted at 70 °C to extract the leaf sample (300 mg) in 1 mL 80% ethanol for 20 min. The extract was then transferred to another test tube and the extraction was repeated for 20 min until they went entirely white. The pooled extract was then analysed with a spectrophotometer (U-1900, Hitachi, Tokyo, Japan) at three different wavelengths: 470, 649, and 665 nm for chlorophyll *a*, chlorophyll *b*, and carotenoid, respectively (Lichtenthaler and Wellburn, 1983).

Determination of total soluble sugar (TSS)
Fresh leaves (150 mg) were ground in 2 mL 80% ice-chilled ethanol. The homogenate was centrifuged at 13000 g for 7 min at 4 °C. The reaction mixture (100 µL supernatant and 3 mL freshly prepared anthrone reagent) was then heated for 10 min in a water bath at 95 °C. The reaction was stopped by placing the tubes in an ice box to cool down. Then, the absorbance of the mixture was measured at 620 nm with the spectrophotometer (Watanabe et al., 2000). The TSS content was calculated from a linear equation based on a standard curve produced with D-glucose.

Determination of proline content
The procedure of Bates et al. (1973) was followed for proline content estimation with minor modifications; 250 mg leaf sample was frozen in liquid nitrogen, then ground in an ice-chilled mortar with 3 mL aqueous sulfosalicylic acid (pH 7.8). The reaction mixture was incubated in a water bath at 100 °C for 1 h; 4 mL toluene was added with mixture after cooling down. The reaction mixture was then vortexed for 4 min at 40 rpm. Finally, the upper layer was collected to measure proline content at 520 nm spectrophotometrically. Proline content was evaluated using a linear equation derived from a pure L-proline standard curve.

Determination of cell membrane stability (electrolyte leakage)
A mixture of 250 mg leaf samples and 10 mL deionized water were heated in a water bath (40 °C) for 30 min. Initial electrical conductivity (EC1) was determined with an electric conductivity meter (Thermo Scientific, Eutech Instruments, Singapore) from the mixture after cooling down. The mixture was again heated at 100 °C for 30 min to completely destroy the cell tissues and release all electrolytes. Then the final electrical conductivity (EC2) was measured. Lastly, the electrolyte leakage (EL) was calculated following the formula: $EL = EC1/EC2 \times 100$.

Analysis of total soluble protein
To obtain protein extract, rice leaves (250 mg) were frozen using liquid nitrogen. The samples were ground separately in an ice-chilled mortar with 2 mL 62.5 mM Tris-HCL (pH 6.7) and centrifuged at 13 000 g 10 min at 4 °C (Muchate et al., 2019). The supernatant was collected and analysed for total soluble protein, catalase, and ascorbate peroxidase activity. Total soluble protein was assayed using a slightly modified of Bradford (1976), 1 mL reaction mixture was prepared consisting of 100 µL protein extract and 900 µL Bradford reagent. The reaction mixture was measured at 595 nm with a spectrophotometer (Multiskan SkyHigh, Thermo Scientific).

Assay of enzyme activities
For catalase (CAT) activity determination, reaction mixture (1 mL) was prepared containing 100 µL enzyme extract, 100 µL 15 mM hydrogen peroxide, as well as 800 µL 50 mM potassium phosphate buffer (pH 7.0). To determine CAT activity, absorbance was measured at 240 nm every 15 s. Absorbance changes resulted because of the decomposition of H_2O_2 (Zhang et al., 2015). For

determination of ascorbate peroxidase (APX) activity, 1 mL reaction mixture was prepared consisting of 200 µL enzyme extract, 200 µL 0.5 mM ascorbic acid, 200 µL 0.1 mM H_2O_2 and 400 µL 50 mM potassium phosphate buffer (pH 7.0). The reaction was commenced by applying H_2O_2, and APX activity was detected at a wavelength of 290 nm every 15 s for 1 min using the slightly modified procedure of Nakano and Asada (1981).

Statistical analyses

ANOVA was used to infer the significance of the data with a DSAASTAT version of 1.101, and the standard error (SE) of the means was calculated. Duncan's multiple range test (DMRT) ($P \leq 0.05$) was used to compare the means where ANOVA indicated a significant difference. Principal component analysis (PCA) for the 15 genotypes for 7 traits was done using Minitab version 19.0 (Minitab Ltd., Coventry, UK). Pearson's correlation was studied to find out the inter-correlations among the traits using SPSS window version 26 (IBM Corp., Armonk, New York, USA).

RESULTS

Effect of drought on biochemical traits

Drought stress at the reproductive stage significantly influenced the performance of biochemical traits, i.e., total chlorophyll content, total soluble sugar (TSS), proline content, and electrolyte leakage (EL). A significant reduction in total chlorophyll content was observed in water stressed plants (Table 1). The genotype MR303 had the maximum value of total chlorophyll (21.20 mg mL^{-1}), whereas the minimal chlorophyll content was recorded in MR219 (12.91 mg mL^{-1}) during the stress. MR167 revealed maximal loss in total chlorophyll (46.79%) during the stress while compared with control. On the other hand, MR263 showed minimal decrease in chlorophyll content (12.42%). TSS content ranged from 3.89-6.75 mg mL^{-1} in MARDI WARNA98 and MR211, respectively, representing a significant increase under stress. Proline content in the leaves of rice increased significantly for all the tested genotypes (Table 1). Proline content under irrigated condition revealed a value between 32.04-38.88 mg L^{-1}, whereas during drought it increased between 40.43 and 52.22 mg L^{-1}. MR219 and MR253 represented minimal and maximal proline content under stress condition respectively. MR 307 and MR211 resulted in 60.39% and 51.41% increase in proline content respectively compared to the control condition. On the contrary, MR219 had 21.20% representing minimal increase in proline content during the stress. In terms of EL, the results suggested that MR303 (107.36 dS m^{-1}) had a minimum EL, thereby implying higher membrane stability under stress condition, whereas MARDI WARNA98 (133.40 dS m^{-1}) revealed a maximum EL.

Water stress caused a decrease in the protein content of leaves in all genotypes (Figure 1). Among them, MR211 showed the minimal decrease (9.48%), on the contrary MARDI WARNA98 exhibited the maximal decrease (41.95%) in protein content. On the other hand, drought increased CAT and APX activities in all genotypes. The change in protein, CAT, and APX has been shown (Figure 1). The current finding revealed MR211 showed maximal value in CAT content, followed by 307, representing 4.71 and 4.47 units mg^{-1} protein respectively. However, the genotype MR303 revealed maximal increase (154.88%) in CAT, on the contrary, MR220 had minimal increase (58.20%) as a result of drought stress. Additionally, MR307 revealed a maximum value of 4.69 units mg^{-1} protein for APX, which was very close to the 4.68 units mg^{-1} protein presented by MR 211 as a result of water stress, thereby conferring stronger drought tolerance. Again, maximal increase in APX was showed by MR307 (166.00%), on the other hand, minimal increase of 16.71% was revealed by MR220 during the stress.

Table 1. Biochemical traits of 15 rice genotypes under control and drought conditions. Means ± SE in the same column with the same letter do not differ significantly according to Duncan's Multiple Range test at $P \leq 0.05$. *, **Significant at the 0.05 and 0.01 level of probability, respectively; ^{ns}: nonsignificant; SE: standard error of the mean.

Genotypes	Total chlorophyll			Total soluble sugar			Proline			Electrolyte leakage		
	Control	Drought	Decrease	Control	Drought	Increase	Control	Drought	Increase	Control	Drought	Increase
	mg mL^{-1}		%	mg mL^{-1}		%	mg L^{-1}		%	dS m^{-1}		%
MARDI WANGI88	24.78 ± 1.37^{ab}	16.35 ± 1.21^{e-i}	34.04	1.75 ± 0.29^{d}	5.40 ± 0.98^{abc}	209.61	33.64 ± 1.33^{gh}	45.51 ± 1.02^{a-f}	35.26	82.78 ± 5.32^{d}	123.71 ± 3.58^{ab}	49.44
MARDI WARNA98	19.93 ± 1.01^{c-g}	16.07 ± 2.16^{f-i}	19.36	1.72 ± 0.23^{d}	3.89 ± 0.34^{c}	126.72	36.63 ± 2.93^{d-h}	47.32 ± 4.13^{a-d}	29.19	86.74 ± 4.12^{d}	133.40 ± 4.69^{a}	53.80
MR157	20.76 ± 1.36^{a-f}	13.57 ± 0.75^{hi}	34.61	1.79 ± 0.41^{d}	4.69 ± 0.29^{abc}	162.32	38.12 ± 1.43^{c-h}	47.24 ± 1.60^{a-d}	23.91	84.49 ± 2.74^{d}	125.84 ± 6.30^{ab}	48.94
MR167	25.51 ± 0.47^{a}	13.57 ± 1.02^{hi}	46.79	1.16 ± 0.09^{d}	4.32 ± 0.44^{bc}	271.18	33.58 ± 2.31^{gh}	46.67 ± 5.42^{a-e}	38.96	81.76 ± 3.27^{d}	119.81 ± 3.51^{bc}	46.55
MR185	21.53 ± 1.82^{abc}	17.41 ± 0.68^{d-i}	19.12	1.51 ± 0.33^{d}	6.34 ± 0.07^{ab}	318.87	36.32 ± 2.19^{e-h}	50.70 ± 2.42^{ab}	39.59	81.48 ± 6.40^{d}	116.80 ± 4.31^{bc}	43.36
MR211	21.16 ± 1.13^{a-e}	18.06 ± 0.75^{d-h}	14.66	1.62 ± 0.29^{d}	6.75 ± 0.72^{a}	317.83	34.34 ± 2.98^{gh}	52.00 ± 1.35^{a}	51.41	86.25 ± 3.39^{d}	115.91 ± 3.27^{bc}	34.39
MR219	20.19 ± 0.94^{b-g}	12.91 ± 0.44^{i}	36.07	1.67 ± 0.55^{d}	4.63 ± 0.22^{abc}	177.63	33.36 ± 1.94^{gh}	40.43 ± 4.47^{b-h}	21.20	83.03 ± 4.10^{d}	126.69 ± 6.81^{ab}	52.58
MR220	18.93 ± 1.85^{d-g}	15.43 ± 0.87^{ghi}	18.52	1.63 ± 0.25^{d}	4.44 ± 0.64^{bc}	172.20	32.19 ± 2.86^{h}	43.67 ± 3.02^{a-g}	35.68	80.39 ± 3.47^{d}	127.12 ± 2.90^{ab}	58.12
MR253	19.36 ± 1.14^{d-g}	16.72 ± 1.40^{d-i}	13.64	1.59 ± 0.22^{d}	6.28 ± 0.06^{ab}	294.65	38.88 ± 2.48^{c-h}	52.22 ± 4.73^{a}	34.31	81.95 ± 4.56^{d}	114.64 ± 3.46^{bc}	39.90
MR263	19.06 ± 1.40^{d-g}	16.69 ± 1.69^{d-i}	12.42	1.74 ± 0.07^{d}	6.28 ± 0.35^{ab}	260.49	34.08 ± 3.27^{gh}	51.22 ± 3.72^{a}	50.31	86.26 ± 2.83^{d}	120.90 ± 3.04^{ab}	40.16
MR269	20.75 ± 3.33^{a-f}	16.54 ± 1.08^{a-i}	20.29	1.70 ± 0.42^{d}	6.65 ± 0.28^{a}	290.59	32.80 ± 2.86^{gh}	46.89 ± 4.77^{a-e}	42.95	83.10 ± 3.64^{d}	116.85 ± 3.64^{bc}	40.60
MR284	20.31 ± 2.69^{b-g}	17.10 ± 0.73^{d-i}	15.79	1.77 ± 0.39^{d}	6.19 ± 0.87^{ab}	248.87	36.61 ± 3.88^{d-h}	48.45 ± 4.17^{abc}	32.33	84.96 ± 3.54^{d}	119.11 ± 3.84^{bc}	40.19
MR297	24.39 ± 2.11^{abc}	17.78 ± 1.36^{d-i}	27.09	1.56 ± 0.04^{d}	4.78 ± 0.36^{abc}	206.09	33.58 ± 3.42^{gh}	46.72 ± 4.51^{a-e}	39.13	80.31 ± 1.39^{d}	119.44 ± 2.19^{bc}	48.73
MR303	25.19 ± 0.93^{a}	21.20 ± 1.13^{a-e}	15.85	1.50 ± 0.56^{d}	6.12 ± 0.22^{ab}	309.55	35.38 ± 3.46^{fgh}	50.70 ± 2.00^{ab}	43.32	76.25 ± 2.76^{d}	107.36 ± 3.10^{c}	40.80
MR307	24.70 ± 0.93^{abc}	20.36 ± 1.02^{b-g}	17.56	1.64 ± 0.74^{d}	6.63 ± 0.46^{a}	304.89	32.04 ± 3.10^{h}	51.39 ± 0.57^{a}	60.39	83.08 ± 4.51^{d}	116.09 ± 2.14^{bc}	39.73
Genotype (G)	**			ns			ns			*		
Treatment (T)	**			**			**			**		
G × T	ns			ns			ns			ns		

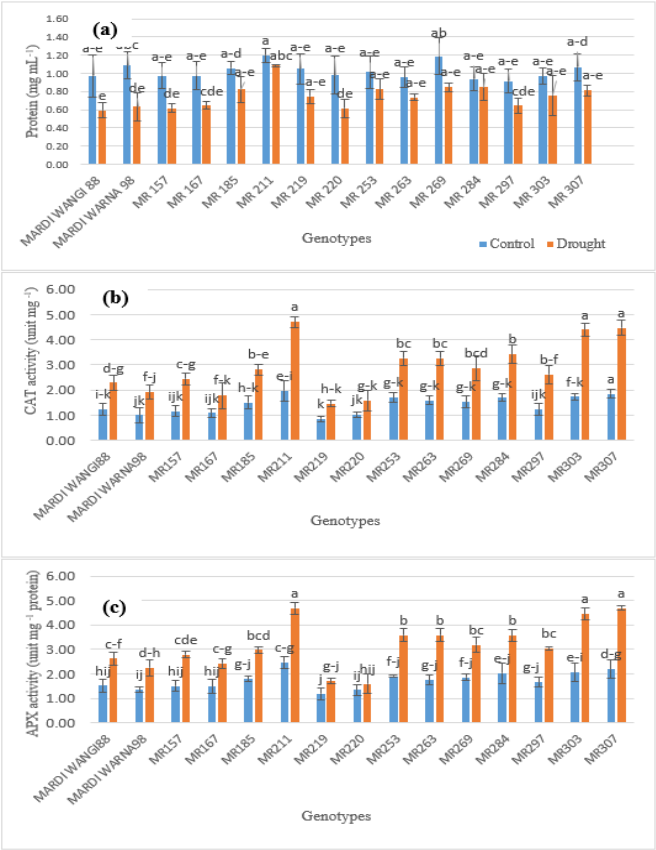


Figure 1. The effect of drought stress on protein concentration (a), catalase (CAT) activity, and (b) ascorbate peroxidase (APX) activity (c) of rice leaf. Each value is the mean of three replicates; the bars represent ± standard error.

Correlations among the studied traits under control and drought stress

Due to significant interaction between genotypes and environment, Pearson correlation was evaluated separately for control and drought conditions (Tables 2 and 3, respectively). The TSS represented a significant negative correlation with total chlorophyll (-0.538*), whereas APX had a significant positive correlation with CAT (0.973**) during control condition. All other traits had nonsignificant intercorrelation. Under drought stress, all biochemical traits were significantly correlated to each other except total protein and total chlorophyll (0.418). Among them, most significant relationship was observed between APX and CAT (0.987**). Proline had a strong positive correlation with CAT (0.821**) and APX (0.847**), respectively. However, EL revealed significant negative correlation with all the traits.

Table 2. The correlation coefficient for biochemical traits in rice genotypes in the control condition. *, **Significant at the 0.05 and 0.01 levels of probability respectively. TSS: Total soluble sugar; CAT: catalase; APX: ascorbate peroxidase; EL: electrolyte leakage.

	Total chlorophyll	TSS	Total protein	CAT	APX	Proline	EL
Total chlorophyll	1						
TSS	-0.538*	1					
Total protein	-0.218	0.088	1				
CAT	0.090	0.063	0.282	1			
APX	0.175	-0.010	0.350	0.973**	1		
Proline	-0.321	0.160	-0.145	0.124	0.062	1	
EL	-0.507	0.503	0.364	0.037	0.030	0.152	1

Table 3. The correlation coefficient for biochemical traits in rice genotypes in the drought condition. *, **Significant at the 0.05 and 0.01 levels of probability respectively. TSS: Total soluble sugar; CAT: catalase; APX: ascorbate peroxidase; EL: electrolyte leakage.

	Total chlorophyll	TSS	Total protein	CAT	APX	Proline	EL
Total chlorophyll	1						
TSS	0.642**	1					
Total protein	0.418	0.788**	1				
CAT	0.829**	0.822**	0.724**	1			
APX	0.806**	0.809**	0.685**	0.987**	1		
Proline	0.678**	0.706**	0.559*	0.821**	0.847**	1	
EL	-0.694**	-0.760**	-0.568*	-0.765**	-0.778**	-0.644**	1

Principal component analysis (PCA) of the biochemical traits

An analysis of principal components (PCs) is presented in Table 4 for both control and drought stress, where different biochemical traits contributing to the total variation calculated for each component is mentioned. A cumulative count of 88.8% of total variability by PC1, PC2, PC3, and PC4 justifies the major contribution of the first four principal components towards tracing out the important biochemical traits of the rice genotypes under control condition (Table 4). In PC1, EL (0.473) contributed mostly to the variation, followed by TSS (0.420), whereas total chlorophyll contributed negatively to all four PCs. PC2 was mostly influenced by TSS (0.303). Proline (0.761) and total protein (0.507) had major contributions to PC3 and PC4, respectively. On the contrary, cumulative 86.7% variability was exhibited by PC1 and PC2 under drought stress, where PC1 and PC2 contributed 77.3% and 9.4% of total variance, respectively. In PC1, all traits had a positive influence on variability except EL (-0.364), while CAT (0.418) and APX (0.415) represented major contributions for PC1. Total protein (0.737), TSS (0.322), EL (0.087) positively contributed to PC2 whereas PC2 was negatively influenced by total chlorophyll (-0.545), CAT (-0.071), APX (-0.112), and proline (-0.177) under stress condition.

Table 4. Eigenvalues, variability and factor loadings of principal components (PCs) generated by principal component analysis (PCA) executed on all parameters of rice genotypes in control and drought conditions. TSS: Total soluble sugar; CAT: catalase; APX: ascorbate peroxidase; EL: electrolyte leakage.

	Control condition				Drought condition	
Variable	PC1	PC2	PC3	PC4	PC1	PC2
Total chlorophyll	-0.407	-0.413	-0.053	-0.157	0.356	-0.545
TSS	0.420	0.303	0.051	-0.674	0.386	0.322
Total protein	0.376	-0.147	-0.571	0.507	0.329	0.737
CAT	0.363	-0.542	0.189	-0.161	0.418	-0.071
APX	0.338	-0.580	0.105	-0.098	0.415	-0.112
Proline	0.217	0.127	0.761	0.477	0.369	-0.177
EL	0.473	0.264	-0.207	0.035	-0.364	0.087
Eigenvalue	2.2941	2.1023	1.1398	0.6826	5.4122	0.6595
Proportion	0.328	0.300	0.163	0.098	0.773	0.094
Cumulative	0.328	0.628	0.791	0.888	0.773	0.867

The scree plot for both conditions is presented in Figure 2, which makes it clearly apparent that four principal components (PCs) showed most of the variability under control while, two PCs influenced most of the variation under water stress. Hence, selection of genotypes with better performances in the first two PCs will be significant under drought condition (Mounika et al., 2021). Figure 3 represents a genotype × trait biplot from the PCA of seven traits and 15 genotypes of rice for both control and drought stress conditions. The PCA biplot summarized the relationships between the variables and the genotypes (Turin et al., 2021). From the biplot, PC1 was positively influenced by eight genotypes under drought stress. These are MR185, MR211, MR253, MR263, MR269, MR284, MR303, and MR307. However, PC2 was positively influenced by MR 185, MR211, MR219, MR253, MR269, as well as MR284 under drought stress.

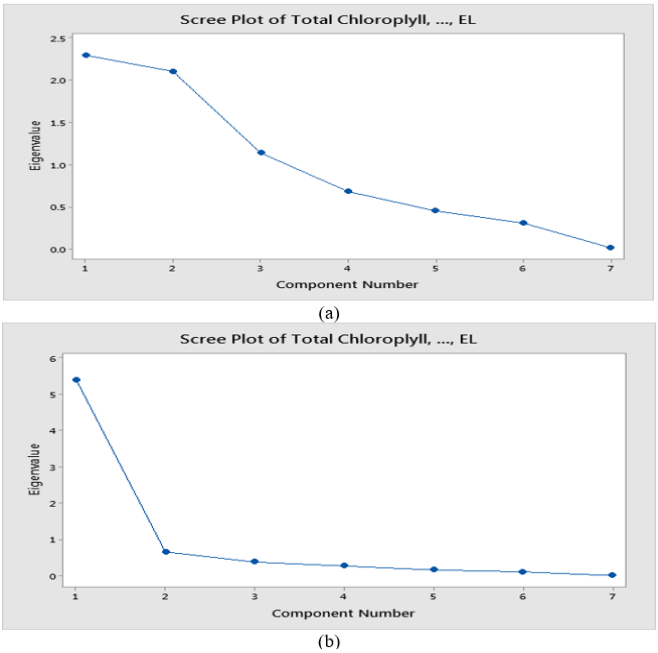
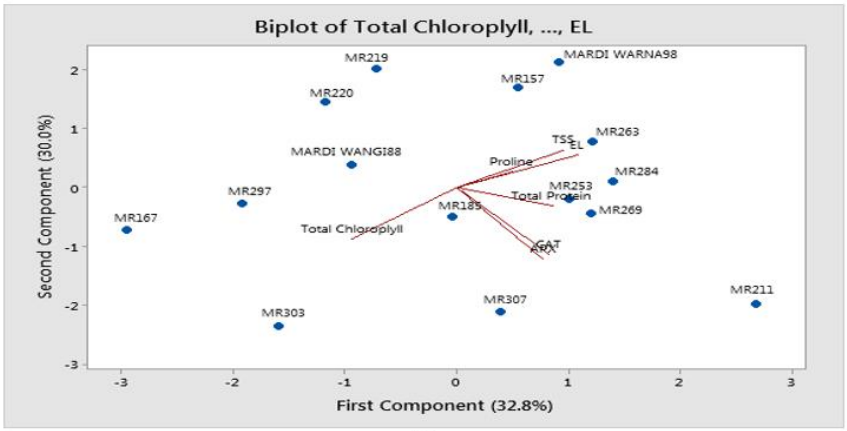
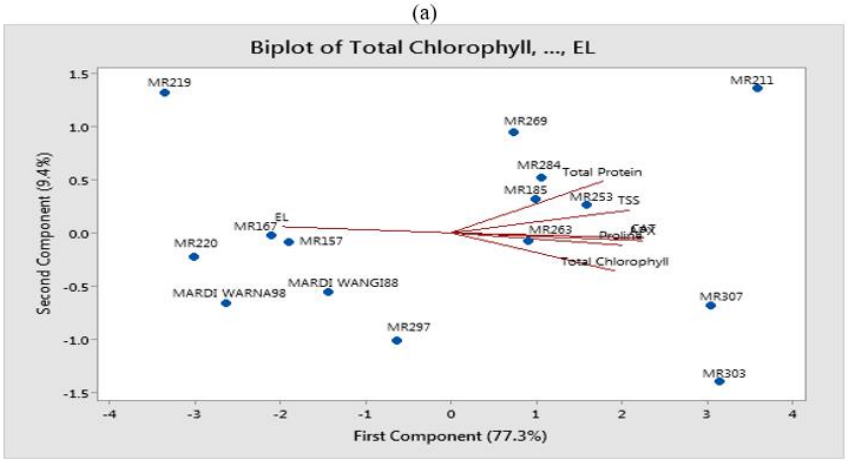


Figure 2. Scree plot of principal component analysis (PCA) between eigenvalue and component number under control (a) and drought conditions (b). The number of principal components (PCs) is on the x-axis and the associated eigenvalues–which indicate the amount of variance are on the y-axis.



(a)



(b)

Figure 3. Biplot of the first two principal components of principal component analysis (PCA) executed on biochemical traits under control (a) and drought conditions (b). TSS: Total soluble sugar; CAT: catalase; APX: ascorbate peroxidase; EL: electrolyte leakage.

DISCUSSION

The results obtained from the present work clearly demonstrated that the rice genotypes displayed distinct variation in biochemical analyses during the reproductive growth stage. Saha et al. (2020) reported a vast depletion in chlorophyll *a* and *b*, total chlorophyll, and carotenoid content in drought-stressed rice. There was a substantial reduction in total chlorophyll in the current study ranged from 12.42% to 46.79% during the stress while compared with control. Chlorophyll content is a significant marker to assess stress tolerance capacity in plants, while high value of total chlorophyll suggests little impact on drought-induced loss (Kumar et al., 2014).

Plants accumulate soluble sugars, including sucrose, trehalose, glucose, and fructose and proline in water-stressed condition, which help to maintain cellular water balance and regulate important ion absorption (e.g., K^+, Ca^{2+}, Mg^{2+}) (Zafar et al., 2020). The increase in proline and total soluble sugars is considered an important defensive strategy displayed in response to water stress (Nahar et al., 2016). The current finding reports noteworthy changes in sugar as well as proline content. It is well documented that drought-stressed plants with higher proline concentrations have stronger osmotic adjustment capabilities, which leads to greater tolerance. Proline assists in osmotic regulation, stabilizes the structure of proteins and cell membranes, protects enzymes, and works as a free radical scavenger and antioxidant. Hence, changes in proline and sugar content are good indicators for screening drought tolerant varieties under water

stress condition (Dien et al., 2019). In comparison with control condition, the study revealed maximal 60.39% increase in proline content among the genotypes. On contrary, MR219 had 21.20% increase representing minimal proline content during the stress. In a study by Kamarudin et al. (2020) also showed MR219 to represent 29.9% increase in proline content during water stress.

Increased EL induced by membrane damage resulting from oxidative stress would be one of the principal reasons for growth depression. A considerable decrease in membrane injury was correlated with increase in proline, protein, as well as total phenols and flavonoids (Tourky et al., 2023). Electrolyte leakage represents the level of cell membrane damage produced by K^+ efflux in plants. The current study resulted in remarkable enhancements in EL for all genotypes as a result of water stress. Thus, genotypes with stronger tolerance have lower EL, while MR303 represented minimal EL.

Additionally, drought changes the regular metabolic process by altering gene expression, which leads to variation in total protein contents. Consequently, the change in total protein content is a sign of a stress response against drought (Nahar et al., 2016). Moreover, some proteins get expressed only under certain stress conditions. Therefore, when plants are exposed to abiotic stresses, both qualitative and quantitative changes are expected. The current study revealed that all the genotypes had reduced protein content after the stress. Among them, MR211 showed the least reduction in protein, which may be related to its stronger tolerance to drought. This was in agreement with the previous results that tolerant rice had a comparatively higher protein content than susceptible genotypes under water stress condition (Kumar et al., 2014).

Reactive oxygen species (ROS) act as a signalling molecule that maintains various physiological processes in plant cells. However, extreme drought causes the synthesis of superoxide ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydroxyl ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) (Rajput et al., 2021) that result in oxidative damage through interfering with the physiological and biochemical processes. The ROS has crucial functions in plant reproduction, particularly in pollen formation and fertilization. Enhancing natural antioxidants (enzymatic and non-enzymatic) could be an approach to minimize or halt oxidative damage and strengthen drought resistance (Hasanuzzaman et al., 2013). In a study, Kamarudin et al. (2020) stated that enzymatic defence is commonly thought to be the most effective. These enzymes either scavenge ROS directly or protect plants indirectly by controlling non-enzymatic defences (Tourky et al., 2023). The CAT is critical for ROS detoxification in peroxisomes under stress, which have the fastest turnover rates and immediately dismutate H_2O_2 into H_2O and O_2. The current study revealed maximal 154.88% and 166.00% increase in CAT and APX content during the stress. It may be attributed that antioxidant enzyme (CAT and APX) activity and proline accumulation were related to dry mass production and, consequently, with the drought tolerance of rice (Lum et al., 2014). A previous study by Saha et al. (2020) suggested that less reduction in protein content and higher activity of antioxidant enzymes might be related to the drought tolerance mechanisms in rice. These findings resemble those of Wang et al. (2019), in which different antioxidant enzymes and osmolyte build up conferred drought tolerance in rice.

Principal component analysis (PCA) revealed the relative contribution of each characteristic for total variation. According to Mounika et al. (2021), the genotypes detected on the extremely positive side of both X and Y axes had superior performances for PC1 and PC2 contributing features. Therefore, the results of PCA showed the high level of genetic variation among the genotypes and explained the traits that were significant for this diversity. This indicates the potentiality of PCA to identify important traits, distinguish selected genotypes based on similarities in one or more traits, and classify the genotypes into separate groups. The PCA for the study clarified that the two representative variables from each of the principal components (TSS and total protein) were sufficient to capture most of the variation in the data (Table 4 and Figure 3b) during the stress. Hence, these two traits could be used to screen the rice genotypes for drought tolerance (Bhattarai and Subudhi, 2019), as both of the traits contributed positively to both PC1 and PC2. On the other hand, total chlorophyll, CAT, APX, and proline influenced PC1 positively and significantly and can also be suggested as important variables because PC1 presented 77.3% of total variability. The EL had no contribution to the variance shown for PC1 and PC2 under drought condition, as also revealed by the correlation study.

CONCLUSIONS

The response of rice plants to drought is complex, as the stress involves changing their molecular, biochemical, and physiological mechanisms and their morphology. The rice genotypes in this study showed differential responses for the biochemical traits studied. Hence, the drought tolerance of these genotypes seems to be linked to the activities of total protein, antioxidant enzymes, proline, total soluble sugar (TSS), and total chlorophyll. Finally, this study provides an insight into drought tolerance for biochemical traits. According to the results in this study, the genotypes MR185, MR211, MR253, MR263, MR269, and MR284, MR303, and MR307 had better performances in all of the biochemical analyses. Again, total protein and TSS can be used as selection criteria for identifying drought-tolerant genotypes. Therefore, the study identified the presence of diversity among genotypes for the majority of the analysed variables, providing an opportunity to select genotypes with superior performances under drought stress.

Author contribution

Conceptualization: F.Z.E., R.N. Methodology: F.Z.E., R.N. Software: F.Z.E. Validation: R.N. Formal analysis: F.Z.E. Investigation: R.N. Resources: R.N. Data curation: F.Z.E., R.N. Writing-original draft: F.Z.E. Writing-review & editing: R.N. Visualization: F.Z.E. Supervision: R.N. Project administration: R.N. Funding acquisition: R.N. All co-authors reviewed the final version and approved the manuscript before submission.

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