

GROWTH PERFORMANCE OF SELECTED ADVANCED MUTANT RICE LINES UNDER DROUGHT STRESS ENVIRONMENT

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Abstract

Water scarcity caused millions of ringgit losses in a vast Malaysia from 2017 to 2021 in vast area of rice fields. The development of drought tolerant varieties is vital as most Malaysian rice varieties are drought-susceptible. Hence the aim of this project was to study the performance of advanced mutant rice lines (*Oryza sativa* L.) on phenotypic and genotypic coefficients of variation, broad sense heritability, genetic gain and correlations. The experiment was laid out in a randomized complete block design (RCBD) with three replications in the rice plot area of Sekinchan, Selangor during the 2020/2021 cropping season. All traits were drastically affected by the drought stress and significant reduction in their performance was also observed. Analysis of variance showed significant differences for all phenotypic and agronomic traits observed in a stressful environment. Genotypes differed significantly at $p < 0.05$ for all the traits studied, which implies that the genotypes constitute a pool of germplasm with adequate genetic variability. Genotypic coefficients of variation were lower than the corresponding phenotypic coefficients in all the traits studied, indicating a considerable influence of the environment on the expression of the traits. Heritability showed two phenotypic and agronomic traits, namely, days to UFG and SF. These traits showed high heritability both under non-stress (control) (56.60-57.87) and stress (47.94-50.36%) environments. FLA had medium heritability under non-stress environment (15.98%) but low heritability under stress environment (7.37%). Several significant positive as well as negative correlations in a stressful environment were observed among the traits. Similar trends of correlation were also observed in non-stress environments. Grain yield exhibited a significantly positive correlation with the number of tillers per plant ($r = 0.48^*$), filled grains per panicle ($r = 0.55^*$) and spikelet fertility ($r = 0.69^*$). Therefore, the results suggest that these traits can be used for grain yield selection.

Keywords: Advanced breeding lines, Broad sense heritability, drought stress, genotypic coefficient of variation, phenotypic coefficient of variation, yield components

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Introduction

Rice (*Oryza sativa* L.) is the main staple food in the world and provides more than 60% of the total calories needed by the human population on a daily basis. The Food and Agriculture Organization, (FAO, 2022) stated that about 90% of the global rice is produced and consumed in Asia with Malaysia alone produced an average of 2.32 million tons from 1994 to 2020. However, rice production worldwide has declined and has become a major concern for food security to meet the demand and sustain an increasing population. In Malaysia, rice production decreased to around 2.57 million tons of rice in 2017 compared to 2.74 million tons in 2016 and continues to drop to 2.32 million tons in 2020 (FAO,

2022). This large yield gap is due to different environmental strains. Environmental strains are defined as natural occurrences that impact harvest production and sustainability in agriculture. Biotic factors such as pests, insects and diseases; and abiotic factors including floods, salinity, drought stress, extreme temperature, air contamination, mineral deficits, adversative pH and the toxicity of heavy metals may cause stress on plant growth thus reducing rice yield. Drought is one of the most devastating abiotic factors, and more than 50% of the arable land worldwide is expected to be threatened in 2050 by drought (Tang, 2019).

Drought is also one of the major problems to rice farmers in Malaysia. It significantly inhibits the growth and production of plants and agricultural characteristics, leading to a decrease in crop yields. For many decades, Malaysia's climate has been predictable. However, due to the effects of climate change, El-Niño phenomenon is becoming more common in Malaysia causing widespread drought in rice cultivating areas of the country. Pauzi (2016) reported that paddy farmers in Kerian, Perak lost about RM 56 million due to the El-Nino series which occurred in the beginning of 2016.

An appropriate number of cultivars related to drought tolerance would control the balance of supply and demand for food (Suprasanna et al., 2014; Kamarudin et al., 2019). Until now, production of drought-resistance rice is very low, which could not contribute for improving rice yields to achieve designated self-sufficiency level (SSL). One of the alternative solutions to improve rice production during El-Nino is the introduction of new cultivars with improved drought resistance. One strategy is the use of mutational approach ion beam irradiation for breeding program in order to obtain superior alleles in rice genome. Mutation induction by ion beams has led to the improvement of yields (Oladosu et al., 2016; Hasan et al., 2021), which plays a major part in enhancing global food security. New varieties of food crops, developed through induced mutations, have significantly increased crop production. As a result, people should have direct access to these crops, which exhibit observable characteristics that are easier to produce. Through mutation induction, more than 20 drought rice varieties had managed to be developed worldwide, in which had resulted in increasing food security (Johnson et al., 2017). However, there are only a few studies on growth performance and morphological changes under water stress environments at different stages of crop growth particularly at the reproductive stage. Therefore, this study is an attempt to evaluate four advanced rice lines to identify tolerant rice lines based on selected morphology and agronomy traits. The study would give a better understanding on relationships and responses of the particular traits of the lines to drought stress environment. This information would be useful to rice breeders in selecting the potential lines for developing drought tolerant rice varieties based on morphological characteristics associated with drought tolerance. Besides, the data collected from this study will be compiled in the information system in Malaysian Nuclear Agency's (ANM) Genebank for future use.

Methods

Plant materials

A total of four advanced breeding lines were selected from the Genebank of the Malaysian Nuclear Agency, Selangor. Two check varieties with a broad range of drought tolerance namely NMR151 and NMR152 and one check variety susceptible to drought, MR219, were included in the experiment.

Experimental design

The field evaluation was conducted in an experimental plot at Sekinchan, Selangor (3°32'47.5" N, 101°05'45.6" E) with an average ambient temperature of 29°C during the off-season of 2021 (March 2021 - June 2021) and main season of 2021 (September 2021 - December 2021). The experiment used a randomized complete block design (RCBD) with four replications for each treatment. The entire experimental area was 18 x 30 meters in size and divided into two smaller plots. Each replication includes seven different rice types, each of which was planted in a 2 x 2-meter plot with a 0.5-meter space between them. Each hill was planted with a single seedling, and the hills were spaced apart by 0.25 x 0.25 meters.

Drought stress imposition

From the reproductive stage to plant maturity, well-water and drought stress treatments were administered on the 30th day after transplanting (DAT) while maintaining 5 cm of standing water in the well-watered plot (Kamarudin et al., 2020). Two tensiometers were set at a depth of 30 cm on each treatment plot, and they were examined often. As soon as the tensiometer value falls below -50kPa and the rolling of leaves occurs, reirrigation on the drought stress plot will be carried out to prevent severe damage to the plant. Weeding and spraying were referred from Nwilene et al. (2008) aside from a recommendation from Malaysia's Department of Agriculture for fertilizer (Berahim et al., 2014).

Phenotypic and morphological data collection

Observations were recorded at different stages of crop growth until maturity for both treatments. The phenotypic data recorded were as follows: days to 50% flowering (DTF) = Number of days from sowing until 50% of the plants in a plot had flowering tillers; plant height (PH) = Distance from the ground to the tip of the panicle on the main tiller at maturity from three random plants in each plot; flag leaf length (FLL) = Length of the flag leaf from the ligule to the tip of the blade; flag leaf width (FLW) = Width at the widest portion of the flag leaf; tiller number (TN) = Total number of grain-bearing and non-bearing tillers of five plants; panicle number (PN) = Number of panicles per plant at early ripening stage; panicle length (PL) = Length of main axis of panicle measured from the panicle base to the tip; and grain yield (GY) = Paddy was harvested at physiological stage of maturity and grain moisture content adjusted to 14%. Visual scores of leaf rolling (LR) were recorded after two weeks of exposure to stress. The evaluation for drought stress was done at the reproductive stage following the Rice Evaluation Standard developed by IRRI (Anon, 1996) using scores of 1 to 9. Score 1 was given to accessions showing green and normal leaves indicating tolerance to drought and score 9 to accessions with completely rolled leaves indicating high susceptibility to drought.

Statistical analysis

The phenotypic observations were analyzed using the Statistical Tools for Agricultural Research (STAR) to determine the mean, range, LSD values, broad sense heritability (H²) and F values. The significance levels were verified by the F values at $p < 0.05$. Correlations among the traits under stress and non-stress (control) were estimated using Pearson's.

Result and Discussion

Performance of Malaysian rice germplasm under stress environment

The mean, range, standard deviation and test of significance for the different traits under stress and non-stress environments are presented in Table 1. All nine phenotypic and agronomic traits differed significantly among the genotypes in non-stress and stress environments. However, only unfilled grains per panicle showed a non-significant difference in this study. Drought stress decreased the number of unfilled grains per panicle of BRRRI dhan66 rice varieties (Urmi et al., 2023). It is not strongly correlated with performance under stress (Sarwendah et al. 2022), thus unfilled grains per panicle is not an effective trait in selecting drought tolerance varieties variety. All other traits were drastically affected by the drought stress. Seed fertility was reduced by 11.4% under stress compared to non-stress plants (Table 1). However, for all the other traits, there were significant reductions in their performance under stress. PH was reduced by 5.29%, PL by 1.19%, TN by 8.66%, FLA by 7.72%, FGP by 0.83%, CC by 1.39% and GWP by 7.4% (Table 1). The standard deviation and test of significance clearly showed that there was wider variation for agronomic traits under a non-stress condition and greater differential response of genotypes under drought stress.

The decrease in spikelet fertility observed under stress conditions in the present study agrees with earlier reports by several workers (Liu et al., 2006; Rang et al., 2011; Salleh et al., 2022). Drought stress caused an enhanced accumulation of reactive oxygen species (ROS) in the spikelets of rice genotypes thereby, impeding spikelet fertility. According to Pantuwan et al. (2002), decreased spikelet fertility under drought stress could be a good measurement of plant responses and adaptability to drought tolerance, as well as an efficient selection criterion for distinguishing drought susceptible and resistant genotypes.

Findings in this study also reported the negative effect of drought on performance of many traits including yield. Study was aligned with Pantuwan et al. (2002), Zhang et al., (2018) and Takahashi et al., (2020). Hence, selections for overly sensitive traits like productive tiller number, spikelet fertility, and grain yield can be considered as an important breeding objective for rice varieties in water stress conditions. Drought has an extremely adverse effect on meiosis and anthesis, which directly affects grain number.

Drought decreased rice yield and caused a severe reduction in plant height, biomass, spikelet fertility during flowering under mild, moderate, and severe stress by 17.0%, 27.8%, and 32.0%, respectively (Zhang et al., 2018). This causes a substantial reduction in grain yield. Hu et al. (2021) stated that exposure to stressful environments during the panicle development stage can result in delayed flowering time, reduced number of spikelets and poor grain filling. The reduction is mainly because of water shortage in the plant causing more respiration and reduced photosynthesis, leading to less biomass accumulation and less grain yield (Zhou et al., 2020). One of the major causes of yield loss under drought is poor panicle exertion especially in the late flowering genotypes. Panicles unable to exert fully resulted in parts or whole panicles to still remaining in the flag leaf sheath causing a reduction in grain yield (Sikuku et al., 2012; Wei et al., 2017).

Table 1. Mean, range and standard deviation for morphological characteristics, agronomic traits and physiological change of 7 Malaysian rice during the main season.

Traits Environment	Mean		Range		Std Dev	
	C	D	C	D	C	D
PH	119.72	113.38	96.40 - 136.20	101.60 - 129.00	13.01	6.87
PL	29.30	28.95	24.90 - 32.97	26.74 - 32.09	2.13	1.48
TN	23.12	20.58	18.40 - 29.20	15.20 - 29.00	2.60	3.17
FLA	107.00	98.73	67.30 - 158.02	60.16 - 159.13	23.67	23.95
FGP	137.63	136.48	105.31 - 161.78	72.78 - 199.89	11.76	27.76
UFGP	23.71	41.28	5.67 - 48.56	19.22 - 80.11	11.64	16.76
CC	38.81	38.27	35.82 - 43.64	31.96 - 41.72	2.03	1.86
SF	85.89	76.09	74.16 - 96.00	47.73 - 88.45	6.28	11.15
GYP	67.10	62.13	47.68 - 89.16	36.44 - 76.77	8.51	9.22

PH = Plant height, PL = Panicle length, TN = Number of tillers, FLA = Flag leaf area, FGP = Filled grain per panicle, UFGP = Unfilled grain per panicle, CC = Chlorophyll content, SF = Spikelet fertility, GYP = Grain yield per plant, C = Well-watered condition, D = Drought stress condition

Heritability of agronomic traits

The broad-sense heritability (H₂) of the phenotypic and agronomic traits under different environments was estimated and presented in Table 2. Broad-sense heritability is defined as $H_2 = VG/VP$, estimates the proportion of phenotypic variation due to genetic values that may include effects due to dominance and epistasis (Wray and Visscher 2008). Heritability values of agronomic traits were classified based on Hanson et al. (1956) as follows: 0 – 30% (low), 30 – 60% (moderate), and >60% (high). All the traits showed low to moderate heritability under non-stress (control) and stress environments. Traits such as UFG and SF were moderately heritable under both environments. Heritability values under non-stress (control) and stress environments were 57.87% and 50.36% for UFG and 56.60% and 47.94% SF, respectively.

For PL and CC, moderate heritability (38.84% and 37.77%) was obtained under non-stress (control) condition and low heritability (29.97% and 26.34%) under stress environment. This indicated that the phenotypic variation observed in this study was contributed by the genotypes with minimum environmental effects. Moderate heritability in that trait under drought stress environment was also observed in many studies (Akinwale et al., 2011; Gyawali et al., 2018; Nath & Kole, 2021; Awad-Allah et al., 2022). Low heritability in plant height, tiller number, flag leaf area and filled grains per panicles under drought stress environment were observed in this study (Lestari et al., 2015; Govintharaj et al.,

2017; Somchit et al., 2017; Tezera, 2021). Low heritability indicates that characters are mainly influenced by environmental factors. Low heritability of these traits indicates the ineffectiveness of direct selection for these traits. It was concluded that the high to moderate heritability traits were due to preponderance of additive gene action and suitable for direct selection in improving drought resistance in rice (Gomez et al. 2006). In this study, the moderate heritability values for yield and yield related traits indicated that they were genetically controlled by additive gene action and can be used as a selection parameter under non-stress as well as stress environments.

Table 2. Broad-sense heritability (H^2) (%) of phenotypic and agronomic traits of 4 advanced breeding lines under stress and non-stress environments.

Trait	Stress	Non-stress
PH	29.96	28.59
TN	14.93	1.01
PL	38.84	29.79
FLA	7.37	15.98
FGP	19.41	30.27
UFG	57.87	50.36
SF	56.60	47.94
CC	37.77	26.34

PH = plant height, PL = panicle length, TN = number of tillers, FLA = flag leaf area, FGP = filled grain per panicle, UFGP = unfilled grain per panicle, CC = chlorophyll content

Correlation of agronomic traits

Knowledge of the correlation among different traits is crucial to construct an implicit breeding strategy for any crop. The yield and characters are quantitatively inherited and influenced by genetic effects and the interaction between genotype and environment. Therefore, direct selection to develop yield could be intricate and laborious due to some selected characters being expressed late in plant development. Thus, indirect selection is much easier and preferable. Accordingly, it is proper to determine and practice the selection of highly correlated characters (Sohrabi et al., 2012; Laslita-Zapico et al., 2010). Similar correlation trends were also observed in non-stress environments. Grain yield exhibited a significantly positive correlation with the number of tillers per plant ($r = 0.58^{**}$), panicle weight ($r = 0.60^*$) and number of grains per panicle ($r = 0.52^*$). Therefore, the results suggest that these traits can be used for grain yield selection.

The correlation coefficient was estimated for all eight phenotypic and agronomic traits in non-stress (control) (Table 3) and stress environments (Table 4). Under a stressful environment, GY showed a significant negative correlation with PH, UFGP and CC. Plant height has a negative correlation with yield indicating that taller plants have low yield. This negative correlation was also observed in earlier studies by Lafitte et al. (2004), Zahid et al. (2006), Kamarudin et al. (2018) and Oladosu et al. (2018). The genotypes with lower plant height normally have higher yields than the genotypes that high plant high (Liu et al., 2018). This indicated that plants that have shorter plant height will produce higher grain yield compared to taller plants that are prone to lodging, thus reducing the grain yield (Zhou et al. 2010). Temporary or permanent water shortages can inhibit plant growth and development more than other environmental factors. Plants cannot absorb ground water in drought, so essential elements become less available to plants.

A significant positive correlation was observed between GY with TN, FGP and SF under stress environment (Table 4). This showed that increasing the TN, FGP and SF will consequently increase grain yield. A significant positive correlation between GY with TN under stress was also observed by Tiwari et al. (2019). A significant positive correlation was also observed between FGP and SF. These traits appeared to be promising secondary traits for selection to improve the yield under stress and consequently develop high-yielding drought tolerant rice varieties.

Table 3. Correlation analysis for morphological characteristics, agronomic traits and physiological change of 7 Malaysian rice during stress.

Traits	PH	PL	TN	FLA	FGP	UFGP	CC	SF	GY
PH	1.00								
PL	-0.09	1.00							
TN	0.36*	-0.02	1.00						
FLA	0.24	0.29*	0.18	1.00					
FGP	0.17	0.17	0.30*	0.00	1.00				
UFGP	0.01	-0.27*	-0.14	-0.22	-0.32*	1.00			
CC	0.37*	-0.19	0.47*	0.16	0.24	0.00	1.00		
SF	0.10	0.24	0.20	0.22	0.54*	-0.92*	0.09	1.00	
GY	-0.25	0.42*	0.44*	0.09	0.37*	-0.59	-0.21	0.47*	1.00

*Correlation is significant at 0.05 level ($p < 0.05$)

Table 4. Correlation analysis for morphological characteristics, agronomic traits and physiological change of 7 Malaysian rice during non-stress.

Traits	PH	PL	TN	FLA	FGP	UFGP	CC	SF	GY
PH	1.00								
PL	-0.21	1.00							
TN	0.02	0.21	1.00						
FLA	-0.09	0.13	0.11	1.00					
FGP	0.09	0.25	0.23	0.25	1.00				
UFGP	-0.06	-0.28*	-0.42*	-0.21	-0.49*	1.00			
CC	0.24	-0.10	0.00	-0.10	0.04	0.09	1.00		
SF	0.07	0.29*	0.42*	0.25	0.62*	-0.94*	-0.09	1.00	
GY	-0.01	0.21	0.48*	0.07	0.55*	-0.64*	-0.11	0.69*	1.00

*Correlation is significant at 0.05 level ($p < 0.05$)

Some traits under a non-stress environment showed similar trends of correlation with traits under a stress environment. The PH showed a negative correlation with GY; and strong positive correlation with SF; while, FLL had a negative correlation with CC and PN; a positive correlation was observed in TN and PH, FGP, CC and SF. The grain yield under a well-watered environment is important in determining the grain yield under stress environment. Although there is no genetic relationship between yield potential and drought tolerance, the trait contributed to higher grain production under a stress environment (Lanceras et al. 2004). Similar trends in correlation among the traits observed under stress and non-stress environments indicated that grain yield under drought can be improved without affecting its yield potential under normal situations. It is also possible to simultaneously increase the yield under non-stress and stress environments.

Conclusion

This study provides a better understanding on the responses of Malaysian rice germplasm to drought stress environment. Many yield component traits such as spikelet fertility showed potential traits for direct selection in improving drought resistance in rice. These traits have medium heritability with strong correlations among them. Selection based on these yield component traits is important for selecting donor parents for developing drought tolerance varieties.

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Author Contribution

MAN Ja'afar -Conduct research and writing manuscript, NA Hasan – supervision, A Mohamed Azzeme- review & editing, F Ahmad- review & editing, S Hussein- review & editing, AR Harun- review & editing, A Abu Hasan- review & editing, N Abd Aziz Shamsudin- review & editing

Conflict of Interest

Authors declare no conflict of interest.

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