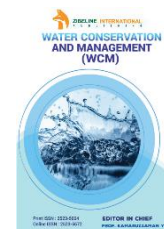


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RESEARCH ARTICLE

SCREENING RICE (*Oryza sativa* L.) GENOTYPES FOR RESISTANCE AGAINST DROUGHT

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ABSTRACT

Drought is regarded as one of the limiting factors in rice production nationally and globally. The present study was conducted to study morpho-physiological and biochemical responses of rice genotypes to drought stress, to identify potential traits for use as a selection criterion in breeding drought-tolerant rice at seedling stage and finally to identify rice genotype resistant to drought stress for use as parents in future breeding. The experimental design used was a split-plot design with three replications, with drought stress as the main plot and rice genotypes as the sub-plot. The main plots consisted of control (normal irrigation) and drought stress. The sub-plots consisted of twelve rice genotypes namely Apami (V1), Boewani (V2), Basmati 370 (V3), Cica-4 (V4), Dular (V5), Jarom mas (V6), Kalarata (V7), Biris (V8), Haiboq (V9), Moroberekan (V10), MR 297 (V11) and Aerob 1 (V12). Results showed that drought stress led to a decrease in plant height, leaves size, root length, total dry weight, and number of leaves but an increment in proline content. The genotype Apami and Kalarata were found to accumulate higher proline content indicating potential resistant ability towards drought stress. Dular and Aerob1, along with tolerant control genotype, Moroberekan, on the other hand, recorded a lower SES score. Leaf size, root length, and plant height could also be used as a selection criterion in breeding drought-tolerant rice due to high values of broad-sense heritability and genetic advance by percentage of mean (GAM). Nevertheless, further study on the genetics and physiological basis of tolerant ability at reproductive growth stages are necessary in order to assess grain yield potential of the potentially tolerant genotype reported in this study.

KEYWORDS

Drought resistance, rice, breeding for abiotic stress, genetic analysis, heritability.

1. INTRODUCTION

Most rice cultivars are susceptible to drought due to its small root system, thin cuticular wax, and swift stomata closure. Seed germination and early seedling growth are among the most critical stages for water stress (Ahmad et al., 2009). During the seedling growth stage, the effects of water deficit lead to inhibition of cell growth, expansion, and division (Jaleel et al., 2008). Drought is also a major problem that limits the adoption of high-yielding rice varieties in drought-prone rainfed rice environments where high sensitivity to even short periods of water deficit constitutes a risk that farmers cannot afford to take (Lafitte et al., 2007). To improve crop productivity, breeding is one of the suggested approaches, and it is, therefore, necessary to understand the mechanism of plant responses to drought conditions before any breeding programme for drought resistance is initiated. Therefore, this study was conducted to assess the sensitivity and tolerant ability of selected rice genotypes to drought stress at seedling stage with the ultimate aim to develop selection criteria for drought resistance and identify resistant genotypes for use as parents in future the breeding program.

2. MATERIALS AND METHODS

Twelve accessions of rice from the IIUM rice collection (Table 1) were used

in the experiment. The accessions were originally obtained from the Rice Gene Bank, MARDI Seberang Perai, Pulau Pinang, Malaysia. Drought-tolerant rice genotype Moroberekan (V10) was used as a resistant check and MR297 (V11) as susceptible control following (Salleh et al., 2018).

Table 1: List of rice genotypes

Rice genotype number	Genotype Name	Country of origin
V1	APAMI	Malaysia
V2	BOEWANI	Suriname
V3	BASMATI 370	India
V4	CICA 4	Colombia
V5	DULAR	India
V6	JAROM MAS	Malaysia
V7	KALARATA 1-24	India
V8	BIRIS	Malaysia
V9	HAIBOQ	China
V10	MOROBEREKAN	Guinea
V11	MR219	Malaysia
V12	PADI AEROB	Malaysia

The experiment was conducted using a split plot design with two drought stress treatments, namely well-watered condition (T1) and drought stress at -60 kPa of soil water potential (T2). Seeds were soaked for 24 hours

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with distilled water and followed by incubation for another 48 hours. Pre-germinated seeds were then sown in a polybag of size 5x5 inch containing about 500 g of topsoil with three seeds per polybag. Drought stress treatment was imposed for seven consecutive days starting from 21 day after sowing (DAS) by withholding water application and removing any ponded water from the soil surface. The polybags under well-watered conditioned were placed in a tray full of water. Water application for the well-watered treatment was made by providing water (into the tray) once in every three days.

The phenotyping scoring index assessment was conducted to evaluate the sensitivity and tolerant ability of rice genotypes to drought stress based on the Modified Standard Evaluation System (SES) for rice following the International Rice Research Institute (IRRI) guidelines (IRRI, 2013) as shown in Table 3.2. The assessment was made at 28 DAS.

Table 2: Modified Standard Evaluation System (SES) scoring for drought in rice

Score	Observation	Rating
1	Leaves start to fold (shallow)	Highly tolerant
3	Leaves folding (deep V-shape)	Tolerant
5	Leaves fully cupped (U-shape)	Moderately tolerant
7	Leaf margins touching (O-shape)	Sensitive
9	Leaves tightly rolled	Highly sensitive

Scoring index assessment (SES) was calculated to evaluate the sensitivity and tolerant ability of rice genotypes to drought stress using the Modified Standard Evaluation System (SES) method for rice developed by IRRI (2013). Morphological parameters recorded were plant height, size of leaves, number of leaves, length of roots, and total dry weight collected and recorded at the end of stress treatment (at 28 DAS).

The proline content in the leaves was estimated based on the method described by Bates et al. (1973). Genotypic variance, phenotypic variance, phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), broad sense heritability and expected genetic advance (GA) were computed based on methods described by Burton (1952). Statistical analysis system (SAS) software was used in data analysis.

3. RESULTS AND DISCUSSION

The SES drought scoring is shown in Table 2. The average value of SES scoring ranged from '5' in V10 (Moroberekkan) to '9' in V8 (Haiboq). It was observed that rice genotypes V8 recorded the highest average value for SES scoring, which was '9'. According to IRRI (2013), the scale '9' indicated that the leaves were tightly rolled. Therefore, the higher the value of SES scoring would indicate that the genotype was profoundly affected by the drought stress (very susceptible). Meanwhile, V10, V5, and V12 recorded SES scoring of '5', '6', and '6', respectively (delayed leaf rolling and drying), indicating that those genotypes were a little bit tolerant to drought stress (i.e., having some degree of resistance). According to Hsiao (1982), leaf rolling was induced by the loss of turgor, and poor osmotic adjustment in rice and delayed leaf rolling is an indication of turgor maintenance and dehydration avoidance (Blum,1989).

Table 3: Average value of SES drought scoring in leaf rolling for 12 genotypes.

Rice genotype	Average for SES score
V1	8
V2	8
V3	8
V4	7
V5	6
V6	8
V7	8
V8	9
V9	8
V10	5
V11	8
V12	6

The analysis of variance indicated that all traits were significant at $p \leq 0.05$ except for the total dry weight (Table 3). This indicated prevalence of

sufficient genetic variability in the studied materials for further selection and improvement of genotypes. Selection for these characters might have resulted in a positive impact on genetic improvement. The mean comparison of the phenotypic performance of 12 rice genotypes at the seedling stage in all morpho-physiological traits and leaf's biochemical traits were summarized in table 4.

Table 4: Mean squares of traits under drought stress condition.

Traits	Genotype (df=11)	Block (df=2)	Error
PH	39.9378*	53.5219*	10.4683
LS	80.5296*	45.8611 ^{ns}	15.7075
NOL	0.3430*	1.9820*	0.1556
RL	57.9400*	13.3633 ^{ns}	18.5642
TDW	9.8485 ^{ns}	4.7500 ^{ns}	6.9621
PRO	1.4926*	8.5708*	0.5874

*Significant at $p \leq 0.05$, ^{ns}: non-significant PH= Plant height, LS= Leaves size, NOL= Number of leaves, RL= Root length, TDW= Total dry weight, PRO= Proline.

The effects of drought on morphological traits of rice are presented in Figure 1, Table 4, and Table 5. As shown in Figure 1, under drought conditions, plant height ranged from 5.6 cm in V11 (MR 297) to 18.27 cm in V6 (Jarom mas) with an overall mean of 14.78 cm while the average of plant height under the control treatment was 18.13 cm. V11 was observed to be the most affected. According to Singh et al. (2018), drought stress would reduce metabolic activity due to lack of water. Consequently, reduction in turgor pressure would affect cell division and cell elongation activities of the plant. As a result, the plant height would also be reduced. Under drought conditions, about 67% of the rice genotypes exceeded the average value of overall plant height at 14.78 cm.

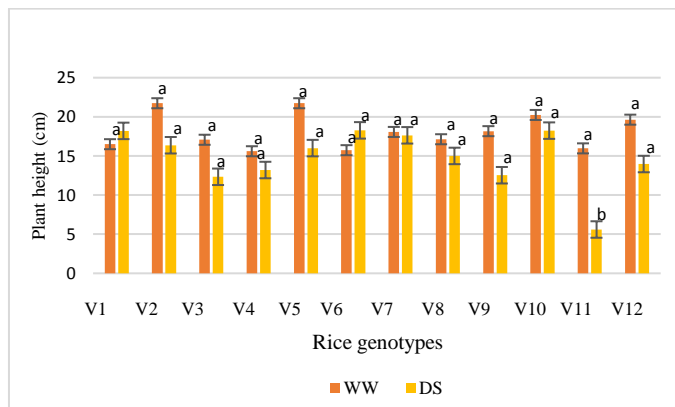


Figure 1: Effect Of Drought On Plant Height

As shown in Table 4, the leaves size was decreased in genotypes under drought conditions ranging from 10.59 cm² in V11 (MR 297) to 29.31 cm² in V10 (Moroberekkan), with an average of 20.17 cm² when compared to leaf size of 33.08 cm² under well-watered treatment. The maximum reduction in leaves size of about 64.5% was observed in V11. In detail, the leaf size of V11 under drought conditions recorded 10.59 cm² as compared to 29.84 cm² under well-watered conditions. Drought stress is commonly known to reduce the overall rate of photosynthesis due to decreasing in both leaf area and photosynthetic rate per unit leaf area (Schuppler et al., 1998). Bigger leaf size under drought stress was recorded in V10 at 29.31 cm² followed by V12 at 26.61 cm² and V5 at 23.67 cm², respectively.

The average value for the number of leaves under drought stress ranged from 7.33 (V11) to 13.67 (V9), as shown in Table 4, with an average of 11.67 when compared with the average number of leaves at 17.81 under a well-watered treatment. The V4 and V9 showed a significant reduction in the number of leaves between well-watered and drought stress conditions. This reduction indicated that drought stress would reduce the number of leaves in rice genotypes. Similar results were also reported by Singh et al. (2018), who observed that water deficit significantly reduced the number of leaves in rice. In addition, the number of tillers, as well as leaves, was reduced due to limited growth and photosynthesis processes in the plant (Quampah et al., 2011). Total dry weight was also found to be affected by the drought stress, with an average decline of about 0.61 g (Table 4). The average mean under well-watered condition was recorded at 1.68 g while the overall mean under drought was recorded at 1.07g. The maximum reduction of about 63% in total dry weight was observed in the

genotype V9 (Biris) under drought conditions.

Results presented in Table 5 showed that there was no significant effect of drought stress on the root length. The maximum root length under drought stress at 29.7 cm was recorded in V6 while the minimum in V8 at 13.7 cm. The average root length under well-watered, however, was recorded at 24.94 cm. According to Wijewardana et al. (2015), root systems for any crop are difficult to study because of their highly structured underground distribution pattern, the complexity of vigorous interactions with the immediate environment, and their functional diversity. To date, very little information has been available describing the effects of drought on rice root morphology and root-related traits at the early growth stage (Lone et al. 2019). Therefore, a deep understanding of

how shoot and root parameters respond to early drought is important as these parameters provide a greater foundation for canopy development and are useful in improving selection criteria in breeding drought-tolerant cultivars.

The highest plant height was observed in V6, followed by V10 and V7. For leaves size, a genotype that has a higher mean value was V10, followed by V12 and V5. In the number of leaves, V9, V1, and V2 had significantly higher mean value compared to other genotypes. The most extended root length was observed in V6, followed by V10 and V5. In the case of total dry weight, the higher mean value was observed in V7, followed by V5 and V2. Therefore, these results suggested that V5 and V6 were more tolerant to drought based on their plant growth traits performance.

Table 5: Mean genotypic performance on leaves size, number of leaves, and total dry weight of 12 genotypes under well-watered and drought stress.

Genotype	Leaf size		Number Of leaves		Total dry weight	
	Well-watered	Drought Stress	Well-watered	Drought stress	Well-watered	Drought stress
V1	24.25 ^a (±2.18)	22.46 ^a (±2.97)	13.33 ^a (± 0.31)	13.33 ^a (±0.32)	1.31 ^a (±2.40)	1.12 ^a (±0.33)
V2	25.42 ^a (±6.74)	17.89 ^a (± 2.11)	16.67 ^a (±0.31)	12.67 ^a (±0.41)	1.46 ^a (±1.45)	1.16 ^a (±1.33)
V3	30.53 ^a (± 8.52)	18.3 ^a (± 1.40)	17.33 ^a (±0.06)	12.33 ^a (±0.55)	1.30 ^a (±1.86)	1.10 ^a (±0.67)
V4	30.90 ^a (±10.86)	17.53 ^a (± 0.85)	22 ^a (±0.36)	10.67 ^b (±0.24)	1.95 ^a (±3)	1.03 ^b (± 2.96)
V5	38.07 ^a (±8.24)	23.67 ^a (± 2.72)	12.33 ^a (±0.49)	9.33 ^a (± 0.26)	1.48 ^a (±4.00)	1.60 ^b (±0.88)
V6	35.76 ^a (± 0.78)	23.27 ^a (±3.37)	22 ^a (± 0.16)	9.67 ^a (± 0.24)	1.87 ^a (± 4.16)	0.74 ^b (± 2.67)
V7	40.95 ^a (±10.30)	20.34 ^a (± 3.81)	20.67 ^a (±0.53)	11.33 ^a (±0.31)	2.37 ^a (±1.67)	1.81 ^b (±1.66)
V8	28.87 ^a (± 8.72)	16.95 ^a (±0.05)	19 ^a (±0.51)	12 ^a (±0.49)	1.78 ^a (±1.15)	1.0 ^a (±1.52)
V9	20.93 ^a (±1.91)	15.13 ^a (±0.44)	23.67 ^a (±0.17)	13.67 ^b (±0.09)	2.07 ^a (±1.45)	0.77 ^b (±1.66)
V10	52.09 ^a (±12.36)	29.31 ^a (±2.61)	12.67 ^a (± 0.46)	10.67 ^a (±0.25)	1.85 ^a (±1.76)	1.02 ^a (±0.67)
V11	29.84 ^a (±7.42)	10.59 ^a (± 0.59)	20 ^a (± 0.25)	7.33 ^a (±0.20)	1.6 ^a (±4.58)	0.66 ^a (±0.33)
V12	39.35 ^a (±13.81)	26.61 ^a (± 4.03)	14 ^a (± 0.24)	11 ^a (± 0.18)	1.10 ^a (±3.21)	0.83 ^a (±1)

Means followed by the same small letter within a row is not significantly different between well-watered conditions and drought stress treatment from each other at $P \leq 0.05$.

Table 6: Mean genotypic performance on root length of 12 genotypes under well-watered and drought stress.

Genotype	Root length	
	well-watered	Drought stress
V1	25.37 ^a (±2.34)	22.77 ^a (±1.49)
V2	23.53 ^a (± 1.25)	22.73 ^a (± 0.27)
V3	19.17 ^a (±2.03)	18.63 ^a (± 2.32)
V4	26.87 ^a (±1.13)	24.33 ^a (± 0.33)
V5	27.23 ^a (±1.89)	25.6 ^a (± 2.46)
V6	29.83 ^a (±0.44)	29.7 ^a (± 2.40)
V7	24.6 ^a (± 2.26)	23.77 ^a (± 2.15)
V8	25.77 ^a (± 2.71)	18.93 ^a (± 4.53)
V9	22.83 ^a (± 0.44)	13.7 ^a (± 3.69)
V10	26.43 ^a (± 1.72)	26.13 ^a (± 1.44)
V11	25.2 ^a (±1.33)	18.03 ^a (± 3.03)
V12	22.43 ^a (± 0.58)	25.27 ^a (± 1.71)

Means followed by the same small letter within a row is not significantly different between well-watered conditions and drought stress treatment from each other at $P \leq 0.05$.

The proline analysis presented in Table 6 showed that there was no significant difference in the leaf's proline content among rice genotypes under well-watered conditions. However, there was a large variation in proline accumulation between genotypes under drought stress conditions. Proline accumulation could be regarded as an early response of plants under water deficit conditions (Anjum et al., 2011). Therefore, proline accumulation does not only happen in a tolerant genotype but also in the susceptible genotype. Nonetheless, drought-tolerant genotype may

accumulate proline for a more extended period of time than the susceptible genotype (Saruhan et al., 2006). This trend could be seen in the V7 (Kalarata) with an average mean of 2.58 $\mu\text{mol g}^{-1}$ followed by the V1 (Apami) at 2.09 $\mu\text{mol g}^{-1}$. The leaf proline content of genotype V7 increased significantly from 1.15 $\mu\text{mol g}^{-1}$ under well-watered to 2.58 $\mu\text{mol g}^{-1}$ under drought conditions. Similarly, an increase in the leaf proline content of V10 was also observed from 0.44 $\mu\text{mol g}^{-1}$ under well-watered to 0.61 $\mu\text{mol g}^{-1}$ under drought conditions. In brief, V7 and V1 accumulated higher proline content than other genotypes under drought stress conditions.

Table 7: Mean genotypic performance on proline content in leaves of 12 genotypes under well-watered and drought stress.

Genotype	Proline content	
	well-watered	Drought stress
V1	1.59 ^a (±0.84)	2.10 ^a (±0.72)
V2	1.39 ^a (±0.5)	0.90 ^a (±0.69)
V3	1.52 ^a (±0.6)	0.77 ^a (±0.33)
V4	1.33 ^a (±0.73)	1.96 ^a (±1.13)
V5	1.17 ^a (±0.4)	1.45 ^a (±0.9)
V6	1.40 ^a (±0.66)	0.47 ^a (±0.28)
V7	1.15 ^a (±0.39)	2.58 ^b (±0.56)
V8	2.82 ^a (±1.59)	1.36 ^a (±0.65)
V9	0.51 ^a (±0.23)	0.88 ^a (±0.55)
V10	0.44 ^a (±0.07)	0.61 ^a (±0.3)
V11	0.55 ^a (±0.3)	1.60 ^a (±0.73)
V12	1.03 ^a (±0.73)	0.36 ^a (±0.27)

Means followed by the same small letter within a column are not significantly different between well-watered conditions and drought stress treatments from each other at $P \leq 0.05$.

V7 and V1 accumulated higher proline content than other genotypes. This result may suggest that they might also possess the tolerant ability to drought as they accumulated higher proline content than other genotypes. According to Maisura et al. (2014), drought-tolerant genotype may accumulate higher proline content and total sugar as compared to the susceptible one. Yue et al. (2006) also reported that the mechanism of drought tolerance through osmotic adjustment as the increased accumulation of solutes, such as proline and total sugar.

The results of the genetic analysis are presented in Table 7. Estimates of the phenotypic (VP), genotypic (VG), and environmental (VE) variances and the phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were displayed in Table 7. The lower genotypic coefficients of variation were 8.78 for total dry weight, and higher GCV is 43.74 for proline. Similarly, the lower phenotypic coefficients of variation were 25.06 for root length and higher PCV 75.34 for proline. In this study, the GCV values were lower than that of PCV, indicating that the environment had an important role in the expression of these characters. Generally, quantitative characters are highly influenced by the environment. A quantitative trait is a measurable phenotype that depends on the cumulative actions of many genes and the environment. These traits can vary among individuals, over a range, to produce a continuous distribution of phenotypes. According to Deshmukh et al. (1986), PCV and GCV values greater than 20% are regarded as high, whereas values less than 10% are considered to be low and values between 10 and 20% to be medium. Based on this argument, proline, leaf size, plant height, and number of leaves recorded high GCV and PCV while the root length had medium GCV but high PCV values. A similar finding was reported by Singh et al. (2018) that plant height and proline content in leaves had high PCV and GCV values.

A heritability estimate for characters under study was as in table 4.7. The estimates of heritability act as a predictive instrument in expressing the potential inheritance value of the phenotypic traits. Therefore, a high heritability value would indicate an effective selection for a particular trait. Dabholkar (1992) classified broad-sense heritability estimates as low (5-10%), medium (10-30%), and high (>30%), respectively. In the present study, heritability was ranged from 12.14% for total dry weight to 57.9% for leaf size. In detail, plant height (48.4%), leaves size (57.9%),

root length (41.3%), and proline (33.7%) were observed to be highly heritable traits. In contrast, number of leaves (27.27%) and total dry weight (12.14%) recorded lower heritability values. Similar findings were also reported by Patel et al. (2012) that plant height recorded high heritability value in rice. In addition, Islam et al. (2016) also reported that high heritability value for leaf area in rice. High heritability values indicate that the traits under study are less influenced by the environment in their expression. The plant breeder, therefore, may make his selection safely based on phenotypic expression of these traits in the individual plant by adopting simple selection methods.

However, the broad-sense heritability value itself may not be a precision estimate of potential genetic advance due to the presence of both additive and non-additive gene action. Therefore, the broad-sense heritability estimate should be coupled by the genetic advance in the percentage of mean (GAM). Genetic advance in the percentage of mean gives more precise results in comparison to only genetic advance (Adhikari et al. 2018). According to Jonhson et al. (1955), high heritability estimates along with the high genetic advance as per mean (GAM) are usually more helpful in predicting gain under selection than heritability alone.

In addition, Johnson et al. (1955) also classified genetic advance as a percentage of mean (GAM) values from 0-10% as low, 10-20% as moderate, and more than 20% as high. High GAM was recorded for the plant height (43.68%), leaves size (47.48%), root length (33.22%), number of leaves (47.18%), and proline content in leaves (90.10%) (Table 4.7). Hence, it indicated the predominance of additive gene action in controlling these characters. Therefore, these characters could be potentially improved through selection. Hence, the results of the present study showed that plant height, leaves size, and root length. Therefore, these traits could be potentially used as a selection criterion in breeding drought-tolerant rice at the seedling stage. However, even though the number of leaves has a high value of PCV and GCV, it has a lower value of heritability. The same goes for proline even though it has a high value of GAM, PCV, and GCV, and proline is considered to have a low value of heritability, which is only (33.7%). Thus, this morphological trait and biochemical trait are less preferable traits as a selection in breeding for drought tolerance rice.

Table 8: Genetic variance of 6 morpho-physiological and biochemical traits of rice genotype under drought stress treatment.

TRAITS	MEAN	VG	VE	VP	PCV (%)	GCV (%)	H ² (%)	GA	GAM
PH	14.77778	9.82	10.47	20.29	30.48118	21.2054	48.4	6.455397	43.68313
LS	20.17056	21.61	15.71	37.32	30.28676	23.04674	57.9	9.576231	47.47628
RL	22.46667	13.13	18.56	31.69	25.05662	16.12849	41.43	7.46448	33.22469
TDW	11.16667	0.962	6.96	7.922	25.20541	8.783424	12.14	2.020481	18.09385
NOL	1.069472	0.06	0.16	0.22	43.8573	22.90373	27.27	0.504595	47.18168
PRO	1.252203	0.3	0.59	0.89	75.33907	43.74072	33.7	1.128308	90.10587

4. CONCLUSION

The screening of 12 rice genotypes in this study revealed ample genetic diversity in concerning their response for all traits measured at the early stages of growth under drought stress. Physiological characters of rice genotypes differed in their response to drought stress. In general, drought stress reduced plant height, number of leaves, leaves size, root length, total dry weight, but increased proline content. In the present study, tolerant genotypes under drought stress at seedling stages could be identified which are consist of highly tolerant, V10, tolerant genotypes are V5 and V12 and moderately tolerant are V1, V6, and V7. Meanwhile, V11 is highly susceptible genotypes under drought stress and other genotypes considered as susceptible genotypes under drought stress. For heritability and GAM result, plant height, root length, and leaves size showed high heritability and GAM. Thus, these traits would be a favourable phenotypic trait for selection criterion in breeding for drought-tolerant rice. In conclusion, early-season drought stress tolerance screening using the platform in this study resulted in the identification of promising genotypes that can be harnessed by breeders for increasing the level and improving the sustainability of rice production to meet future demands.

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