RESEARCH ARTICLE

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Identification and characterization of drought-tolerant rice (*Oryza sativa* L.) genotypes using morpho-physiological and biochemical traits

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Abstract

Drought stress is a major constraint and the primary cause of low rice production in rainfed ecosystems. The results obtained from the study revealed the existence of significant variation among all the genotypes studied for different physiological and biochemical parameters. Genotypic and phenotypic coefficients of variation were wide-ranging, with traits like PC and grain yield showing higher values. Moderate to high heritability and genetic advance percentage of mean suggested the feasibility of selection for improved traits. The mean performance of genotypes revealed specific genotypes, such as Gin, excelling in root-related traits and filled grains per panicle. Leaf rolling and drying responses varied among genotypes, providing insights into drought tolerance. Positive correlations between grain yield and traits like flag leaf length, panicle length, and spikelet fertility were observed. Path coefficient analysis identified direct relationships between yield and traits such as flag leaf breadth, chlorophyll content, and root volume. So, this study provides a comprehensive understanding of genetic variability and trait relationships among rice genotypes, offering valuable insights for the selection of resilient varieties with improved root, shoot, and yield traits, particularly crucial for addressing drought stress.

Keywords: Drought indices, leaf rolling, morphology, rice, yield

Introduction

Rice (*Oryza sativa* L.) holds a crucial position as the primary cereal crop in South and Southeast Asian nations, where over 90% of the population relies on it as a staple food. On a global scale, rice ranks as the third most vital cereal after wheat and maize. Rice, botanically categorized as a semiaquatic annual grass within the Poaceae family, is cultivated across diverse environments. It demonstrates remarkable adaptability across a wide range of altitudes, from below sea level to as high as 3000 meters above mean sea level. India, with its extensive latitude span from 8°N to 34°N, is the foremost rice-producing nation, thriving in diverse climatic conditions. In India, rice is cultivated across approximately 46.38 million hectares, yielding a productivity of 2809 kg per hectare and a production volume of 130.29 mt (Anonymous 2022). North-East region of India is considered the secondary center of origin and is enriched with landraces and primitive cultivars of special importance (Durai et al. 2015). About 72% of the total cultivated area is under agricultural cultivation practices in upland, lowland, and water-fed areas (Ranjan et al. 2015). The indigenous farmers of the hilly areas are still practicing their landrace or cultivar, which suits the local microclimate and adaptation. These local cultivars are reservoirs for novel genes that can be used against biotic and abiotic stresses.

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Rice consumes a relatively huge quantity of water during its growth period compared to other crops (Pandey and Shukla 2015). About 5000 liters of water is required to produce 1-kg of rice grain. Crop yield is affected by agronomic factors and various environmental variables such as water availability and temperature (Hatfield et al*.* 2015). Reduced water supply to the crop or a condition of water stress not only affects the grain yield but also adversely affects the grain quality. Drought is one of the biggest constraints on rice production in many parts of the world (Herdt 1991). Rice under drought stress conditions displays poor vegetative and reproductive growth and development of different morpho-physiological characters (Kadam et al., 2017; Quinones et al., 2017). The high intensity of stress during the growth period affects yield by up to 90% (Venuprasad et al. 2007; Daryanto et al. 2017). The recent trends in climate change leading to unpredictable, harsh weather have also escalated the necessity for climateresilient crops (Upadhyaya and Panda 2019). Therefore, to improve the productivity potential of rice to withstand drought situations, the development of drought-tolerant varieties is only the best option to sustain it.

Drought stress indices are vital tools for early warning systems, assessing drought severity, and allocating resources efficiently during drought events. They play a crucial role in climate change adaptation by providing essential information for assessing climate-related risks and building resilience strategies. Overall, they are indispensable for understanding, monitoring, and mitigating the impacts of drought, enhancing resilience to water scarcity and climate variability. Although, several studies have been carried out earlier on this aspect, still the indigenous varieties of the north-east region lack information on drought tolerance. The local cultivars are reservoirs for novel genes that can be used against biotic and abiotic stress. Therefore, the present study was conducted to address the above issue of utilizing the new breeding tools and strategies to phenotyping the rice genotypes of Manipur and Assam for traits associated with drought tolerance.

Materials and methods

Experimental material and layout

Sixty-six upland rice genotypes of Assam and Manipur along with four checks, two drought tolerant viz., Sahbaghi Dhan and an aerobic rice variety MAS-26 and two susceptible (IR-64 and Swarna) were used in the study. The present study was conducted for two years at the Genetics and Plant Breeding farm, College of Agriculture, Central Agricultural University, Imphal, during *kharif* 2021-22 and in the experimental field of Biotech Hub, BNCA, Assam Agricultural University during *kharif* 2022-23. The seeds of the genotypes were obtained from Rice Research Station, Wangbal, Manipur and Advanced Level Biotech Hub, BNCA, Assam Agricultural University. The names of the rice genotypes are given in Table 1. The material was planted in PVC pipes under normal and moisture stress conditions in a completely randomized design (CRD) to study the root and shoot traits. Moisture stress condition was created in one set of the PVC pipes by removing the water for 20 days in the panicle initiation stage. Life-saving irrigation was given at that time.

Observations recorded

The observations were recorded on three randomly sampled competitive plants in all three replications for 18 morphophysiological and biochemical traits. *viz*., relative water content (RLW), flag leaf length (FLL), flag leaf breadth (FLB), days to panicle emergence (DE), total chlorophyll content (TCC), proline content (PC), plant height (PH), panicle length (PL), days to 50% flowering (DF), days to maturity (DM), effective tillers per plant (ET), total grains per panicle (TG), filled grains per panicle (FG), spikelet fertility (SF), 100-grain weight (GW), yield per plant in gram (YP), root length (RL) and root volume (RV) after the end of the stress period.

Statistical analysis

The data obtained were subjected to analysis of variance following the standard protocol given by Panse and Sukhatme (1967). Estimation of genetic parameters of variation was estimated by Singh and Choudhury (1988). The standard methods of Burton and Devane (1953), Lush (1945) and Johnson et al. (1955) were used to estimate the variability parameters, heritability and genetic advance. Both genotypic and phenotypic coefficients of correlation between all pairs of characters were determined by using variance and covariance components, as suggested by Al-Jibouri et al. (1958). Path coefficients were calculated as suggested by Wright (1921) and Dewey and Lu (1959). The drought scores, leaf rolling and leaf drying observations were taken as per SES method on a 1–9 scale (IRRI, 1996).

Stress indices

The following stress indices were calculated based on yield under normal and moisture stress conditions (Table 2). Where Ypi and Ysi, are the yield of ith variety under normal and moisture stress conditions, respectively. Ys and Yp are the average yields of all varieties under normal and moisture-stress conditions.

Results and discussion

The highly significant differences in the mean sum of squares among the genotypes for all the 18 morphophysiological and biochemical traits under both normal and moisture stress conditions revealed the presence of high genetic variability for all the characters studied. This indicates that there is ample scope for the selection of promising genotypes from the present diverse genotypes for root, shoot and yield traits. Significant variability in root-

Varieties	Code	Varieties	Code	Varieties	Code	Varieties	Code
Pari	V ₁	Leima	V19	Basudev	V37	Ranjit	V ₅₅
Ashima-a	V ₂	Machang	V ₂₀	Betu	V38	Laslua Sali	V ₅₆
Ayuang Leima	V3	Meinei Phou	V ₂₁	Boga Bhepa	V39	Luit	V ₅₇
Bumanmur	V ₄	Moirang Phou	V22	Bogi Lahi	V40	Malbhog	V58
Chakia-58	V ₅	Moliro	V ₂₃	Dharmeshwar	V ₄₁	Moni Sali	V59
Changta Rice	V ₆	Mozinlu	V ₂₄	Dhepa	V42	Moran Beji	V60
Damudar	V7	Napadai	V ₂₅	Dhusuri Bao	V43	Numoli	V61
Durai	V8	Ngodainap	V26	Dikhow	V44	Panchanan	V62
Ereima	V9	Noi Noi Phou	V ₂₇	Disang	V45	Panindra	V63
Gin	V10	Tevahmah	V28	Disang Lahi	V46	Podumoni	V64
Heitup	V11	Thangjing	V29	Dungum Bao	V47	Ranga Dhepa	V65
Hemang Phou	V12	Thoibi phou	V30	Gitesh	V48	Ranga Sali	V66
Kaosan	V13	Tulshi	V31	Gomi	V49	IR-64	V67
Keibi Phou	V14	Tungou	V32	Kajoli Sakua	V50	MAS-26	V68
Khamah	V15	Yaiphabi	V33	Kanaklata	V ₅₁	Sahabhagi	V69
Khok Machang	V16	Ad Bao	V34	Kedo	V ₅₂	Swarna	V70
Khula	V17	Amona Bao	V ₃₅	Khoju Lahi	V ₅₃		
Kyiya Tungla	V18	Badal Bao	V36	Kholihoi Bao	V ₅₄		

Table 1. List of different varieties and their codes used in this study

Table 2. Stress tolerance indices applied for data anlaysis

Stress tolerance indices	Formula	Reference	
Mean relative performance (MRP)	$(Ysi/Ys) + (Ypi/YP)$	Benjamin et al. 2003	
Stress susceptibility indices (SSI)	$Ypi-Ysi/2(yp) \times 100$	Darkwa et al. 2016	
Stress tolerance (TOL)	Ypi – Ysi	Rosielle and Hamblin13	
Relative efficiency index (REI)	(Ysi/Ys) x (Ypi/Yp)	Manjeru et al. 1995	
Stress Tolerance Index (STI)	$(Ysi x Ypi) / (Yp)^{2}$	Lambers et al. 2008	
Drought tolerance efficiency (DTE)	(Ysi/Ypi) x 100	Fischer and Wood 1981	

related component traits in rice has also been observed earlier in different set of material (Verma et al*.* 2019). The study further revealed a wide range of phenotypic and genotypic coefficients of variation with respect to all the traits studied (Table 3). A higher magnitude of the genotypic and phenotypic coefficient of variation was recorded for proline content and grain yield under both normal and moisture-stressed conditions. While studying physiological characterization and allelic diversity in rice, Mishra et al. (2016) also reported high genetic and phenotypic variation for different traits. The PCV values were slightly higher than the GCV values for all the characters in both normal and moisture-stressed conditions which may be due to a higher degree of interaction of genotypes with the environment. Davatgar et al. (2009) studied the morpho-physiological response to drought stress conditions and reported wide genetic variation among the rice genotypes studied. The high heritability of any trait indicates that environmental factors least influence the trait. The heritability (h^2) and genetic advance as a percent of the mean (GAM) were either moderate (30<H<60; 10<GA<20) or high (High above 60; Genetic Advance above 20) in the traits. High heritability coupled with high genetic advance was observed under normal and moisture stress conditions in all the characters except relative water content, proline content, days to flowering and days to maturity.

The mean performance of all the 66 genotypes used in the present study indicated considerable variation in root distribution both in normal and moisture-stress conditions. The root length was recorded as the highest under stress conditions because genotypes will penetrate their root system into deeper soil layers in search of moisture. The root is a very vital plant organ to uptake water and nutrients from the soil and therefore, root system efficiency in combating drought stress conditions is of utmost importance to be considered in breeding for drought tolerance (Comas et al. 2013; Panda et al. 2021). The entire root system, including the primary root, secondary roots, thickness, root dry mass

Score	Numbers	Varieties	Scale
$\mathbf{0}$	12	Khok Machang, Tulshi, Ad bao, Amona Bao, Badal Bao, Dharmeshwar, Dhusuri Bao, Ranga Sali, MAS-26, Sahabhagi, Pari, Gin	Highly tolerant
$1 - 2$	15	Ereima, Ranjit, Chakia-58, Changta Rice, Hemang Phou, Kaosan, Keibi Phou, Leima, Machang, Moirang Phou, Thulshi, Yaiphabi, Bogi Lahi, Dikhow, Disang Lahi, Dungum Bao, Kholihoi Bao, Ranga Dhepa	Tolerant
$3 - 4$	7	Ayaung Leima, Bumanmur, Khamah, Khula, Thoibi Phou, Basudey, Panchanan, Machang.	Moderately toelrant
$5-6$	13	Heitup, Kaiya Tungla, Leima, Meinei Phou, Mozinlu, Napadai, Ngodainap, Dhepa, Disang, Gomi, Dungum Bao, Panchanan, Podumoni, Panindra	Moderately susceptible
$7 - 8$	9	Ashima-A, Kaosan, Kyiya Tungla, Moliro, Gitesh, Kajoli Sakua, Kanaklata, Laslua Sali, Moni Sali, Numoli, Swarna	Susceptible
9	10	Damudar, Durai, Tungou, WR 1911, Thangjing, Betu, Boga Dhepa, Moran Beji, Luit, IR-64	Highly susceptible

Table 4. Classification of genotypes based on leaf rolling score (SES, IRRI)

Table 5. Classification of genotypes based on leaf drying score (SES, IRRI)

Score	Numbers	Varieties	Scale
$\mathbf{0}$	5	Thoibi Phou, Tulshi, Bogi Lahi, Kholihoi Bao, Sahabhagi, Pari, Gin	Highly tolerant
$1 - 2$	15	Leima, Machang, Yaiphabi, Ad Bao, Amona Bao, Badal Bao, Basudev, Boga Dhepa, Dharmeshwar, Dhusuri Bao, Kurmi Sali, MAS-26	tolerant
$3 - 4$	19	Chakia-58, Changta Rice, Damudar, Ereima, Keibi Phou, Khamah, Khok Machang, Khula, Meinei Phou, Moirang Phou, Napadai, Noi Noi Phou, Disang, Disang Lahi, Gitesh, Khoju Lahi, Laslua Sali, Moran Beji, Ranga Dhepa, Ranga Sali	Moderately tolerant
$5 - 6$	17	Bumanmur, Heitup, Kaosan, Kyiya Tungla, Machang, Mozinlu, Numoli, Panchanan, Panindra, Podumoni, Gitesh, KajoliSakua, Kedo, Malbhog, Moni Sali, Moran Beji, Ranga Sali, IR-64	Moderately susceptible
$7 - 8$	4	Ashima-A, WR 1911, Thangjing, Swarna	Susceptible
9		Luit	Highly susceptible

and length and depth, play a significant role in water and nutrient uptake (Uga et al. 2013; Hussain et al. 2018; Panda et al. 2021; Kim et al. 2020). The results on root length have been reported earlier, which proved that root length is higher under stress conditions (Ganapathy et al. 2010). The maximum root length was recorded in the variety Gin under stress conditions in both seasons. Other characteristics like the highest RLW, Total chlorophyll content, proline content and filled grain per panicle were shown by Badal bao, Pari, Ad bao and Gin, respectively.

It was observed in the present study also that genotypes with higher grain yield per plant showed lower leaf rolling and drying with higher RWC of the leaf. Beena et al. (2021) reported that Spearman's rank correlation coefficients indicated a significant negative association between leaf drying and grain yield. Reduced RWC resulted in increased spikelet sterility. The genotype with higher leaf rolling also recorded higher leaf drying in rice. They advocated that traits like chlorophyll stability index, leaf rolling, chlorophyll content, and root biomass were the most important predictors of grain yield under drought. The present findings were also supported by the results of Roy et al. (2023) in rice germplasm evaluation.

Leaf rolling and drying in the rice leaf was scored as per the Standard Evaluation System (SES) in rice developed by IRRI with a 0-9 score rating. Genotypes Damudar, Durai, Tungou, WR 1911, Thangjing, Betu, Boga Dhepa, Moran Beji, Luit, and IR-64 showed clear water stress symptoms and recorded tightly rolled score of 9 after 20 days of the imposition of water stress and hence, these genotypes are characterized as very sensitive to drought condition. However, genotypes Khok Machang, Tulshi, Ad bao, Amona bao, Badal bao, Dharmeshwar, Dhusuri bao, Kurmi Sali, Ranga Sali, MAS-26, and Sahabhagi showed no morphological symptoms of stress with a score of 0 (Table 4). Similarly, genotypes Pari, Gin, Thoibi Phou, Tulshi, Bogi Lahi, Kholihoi bao, and Sahabhagi showed no water stress symptoms with no drying and as they recorded a score of 0 and hence, they are not sensitive to drought conditions. However, genotype Luit showed morphological symptoms of complete drying of leaves with a score of 9 (Table 5). Different morphological parameters are being used to assess the plant response to drought stress (Upadhyaya and Panda, 2019). Drought-induced low-water potential limits leaf growth, reduces leaf area, leaf rolling, wilting, thickened leaf size, early senescence, stomatal closure, and cutinized

Table 8. Estimates of Stress Tolerance indices for different rice genotypes

Varieties	MRP	SSI	TOL	REI	STI	DTE
Pari	2.658	2.620	1.181	1.657	0.944	94.751
Ashima-A	1.286	9.100	4.102	0.410	0.233	68.763
Ayuang Leima	1.319	5.972	2.692	0.424	0.242	78.479
Bumanmur	0.977	12.481	5.626	0.238	0.136	51.412
Chakia-58	1.576	5.637	2.541	0.600	0.342	82.489
Changta Rice	2.097	32.597	14.694	1.083	0.617	44.580
Damudar	1.650	27.617	12.449	0.665	0.379	41.906
Durai	1.490	33.981	15.318	0.502	0.286	30.198
Ereima	2.449	11.939	5.382	1.466	0.835	77.061
Gin	2.986	1.329	0.599	2.075	1.182	97.586
Heitup	1.105	21.720	9.791	0.289	0.165	35.898
Hemang Phou	1.008	24.551	11.067	0.224	0.127	27.667
Kaosan	1.567	32.677	14.730	0.573	0.326	33.649
Keibi Phou	3.278	33.005	14.878	2.686	1.531	59.012
Khamah	1.604	32.830	14.799	0.603	0.344	34.361
Khok Machang	2.122	33.808	15.240	1.106	0.630	43.702
Khula	1.841	44.789	20.190	0.746	0.425	27.694
Kyiya Tungla	1.217	32.287	14.554	0.310	0.177	24.293
Leima	2.634	39.874	17.974	1.713	0.976	45.535
Machang	2.763	19.790	8.921	1.892	1.078	68.459
Meinei phou	1.362	4.849	2.186	0.448	0.255	82.558
Moirang Phou	2.715	6.717	3.028	1.760	1.003	87.455
Moliro	2.248	22.313	10.058	2.612	1.488	69.504
Mozinlu	1.839	17.548	7.910	0.845	0.481	60.613
Napadai	2.094	21.687	9.776	2.371	1.351	69.001
Ngodainap	2.684	36.513	16.459	1.791	1.020	49.262
Noi Noi Phou	2.035	29.083	13.110	1.027	0.585	47.554
WR 1911	0.712	4.836	2.180	0.125	0.071	69.777
Thangjing	1.684	35.317	15.920	0.661	0.377	33.436
Thoibi Phou	2.802	3.019	1.361	1.844	1.050	94.279
Tulshi	2.388	10.504	4.735	1.388	0.791	79.004
Tungou	1.190	13.994	6.308	0.354	0.201	54.127
Yaiphabi	2.830	44.253	19.948	1.972	1.123	44.374
Ad Bao	1.597	5.342	2.408	0.615	0.350	83.505
Amona Bao	2.653	21.172	9.544	1.751	0.998	65.653
Badal Bao	1.178	12.112	5.460	0.347	0.198	58.362
Basudev	1.380	14.604	6.583	0.476	0.271	57.474
Betu	1.160	19.742	8.899	0.328	0.187	41.284
Boga Dhepa	2.101	37.376	16.848	1.068	0.609	39.658
Bogi Lahi	2.271	75.827	34.181	2.406	1.371	29.569
Dharmeshwar	1.662	33.702	15.192	0.650	0.370	34.720

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layer on the leaf surface are some of the morphological traits associated with drought stress (Mishra and Panda,

2017; Hussain et al. 2018; Panda et al. 2021). Recently, Veerala et al. (2024) genotypes identified a few suitable drought tolerant genotypes considering leaf rolling and senescence as selection criteria based on molecular analysis of drought tolerance genes in basmati rice.

In this study, the inter-relationship between grain yield per plant and its contributing traits was determined by correlation (Tables 6 and 7). Grain yield per plant was significantly and positively correlated with flag leaf length, panicle length, effective tillers per plant, filed grains per panicle, total grains per panicle and spikelet fertility under both moisture stress and non-stress conditions. Moisture stress during the vegetative period causes delayed panicle initiation, followed by late maturity (Singh et al. 2012), which is directly correlated with yield reduction. However, most of the damage of drought stress on grain yield occurs during the reproductive growth stage. A short time stress during this phase severely curbs the rice grain yield by diminishing panicle length, poor seed setting, reduced number of kernels per panicle, and poor spikelet development and pollination, resulting in poor seed setting and reduced grain size and grain number (Davatgar et al., 2009; Wei et al., 2017). Therefore, it is suggested that the direct selection of these characters may improve the grain yield.

Different stress tolerance indices were calculated and presented in Table 8. Genotype Keibi Phou recorded the highest value for MRP, so this genotype can be used in breeding programs as the higher the MRP higher the

Fig. 1. Scree plot of Principal Component Analysis between Eigen value and PC under normal and stress conditions

tolerance capacity. Similarly, the highest value of SSI, TOL was recorded by Bogi Lahi and the lowest value was recorded by Dhusuri Bao. Smaller values of TOL are preferred to select tolerant genotypes since larger values indicate a higher susceptibility to stress. STI and REI were recorded highest by Keibi Phou and lowest by WR1911 and DTE was highest for Dhusuri bao and lowest for Luit. So, the genotypes Keibi Phou and Dhusuru bao can be selected as drought-tolerant genotypes and WR1911 and Luit as susceptible as higher the STI, RET and DTE higher the tolerance ability of the genotypes. Drought Selection indices are well proven and important parameters for selecting the suitable tolerant genotypes for yield under drought stress and normal conditions in maize (Kumar et al. 2016) and may be applicable to other cereal crops. Based on the indices, a few genotypes were found to be relatively less droughtsensitive. Selection is based on the high value of mean yield performance under drought and irrigated conditions (Mehraban et al. 2018).

Path coefficient analysis was conducted to partition out the simple correlations into direct and indirect effects on yield. In the present investigation, path coefficient analysis revealed that yield had a positive direct relationship with flag leaf breadth, total chlorophyll content, panicle length, filled grains per panicle, 100-grain weight, spikelet fertility and root volume. These findings corroborate the observations of Reddy et al. (2008) for panicle length and spikelets per panicle. So, we can go for a direct selection of these traits. On the other hand, grain yield was observed to have a negative direct correlation with relative leaf water, flag leaf length, proline content, total grains per panicle and root length at both normal and drought conditions. It might be due to the presence of undesirable linkage between these characters and to break this linkage, recombination breeding will be helpful.

PCA was performed using yield and yield-contributing components on the rice genotypes. Out of eighteen, six principal components (PCs) in both controlled and stressed conditions exhibited more than 1 Eigenvalue and showed about 72.88 and 75.63% total variability. (Fig. 1). In the present study, the first component is positively influenced by DF, DM, DE, PL, PC and ET in controlled whereas by DF, DM and DE in stressed conditions (Table 9). The genotypes located in the first and second quarters had the most influential characters. The positive and negative loadings enunciate the presence of positive and negative correlation trends between the variables and the components. Hence, the characters that load high values positively or negatively contributed more to the diversity and they were the ones that differentiated the clusters. Drought is a very complex phenomenon and therefore, we have to have more understanding about the modern breeding techniques and marker-assisted selections, which are considered suitable tools for introgression of the known drought tolerance genes into lines to develop drought-tolerant rice varieties (Hassan et al. 2023).

Authors' contribution

Conceptualization of research (PK, AB, DDS); Designing of the experiments (PK, AB, MKS, DDS); Contribution of experimental materials (PK, MKS, DDS); Execution of field/ lab experiments and data collection (DDS, MK, DB); Analysis of data and interpretation (DDS, TNS); Preparation of the manuscript (DDS, PK).

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