Genetic Variation in Drought Resistance of Rice (Oryza sativa L.) at the Germination Stage

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Article

Genetic Variation in Drought Resistance of Rice (*Oryza sativa* L.) at the Germination Stage

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Statements and Declarations

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Abstract

Drought stress is a major abiotic factor limiting rice growth and productivity. Establishing scientifically screening methods at the germination stage is critical for identifying superior drought-adapted genotypes. This study aimed to develop a novel discriminant equation for evaluating drought resistance in rice at the germination stage, with field yield data used as a benchmark to calibrate laboratory-based assessments. Seventy-six rice accessions from southern China were used as experimental materials. Drought resistance during germination was assessed using nine key physiological indicators, including vigor index and root length. Three statistical methods—Membership Function Comprehensive Evaluation Value (MFSV), Principal Component Analysis Comprehensive Evaluation Value (PCASV), and Grading Coefficient (GC)—were applied to classify drought resistance levels from different analytical perspectives. Field drought treatment was conducted throughout the growth period, and final grain yield was used to represent the comprehensive drought resistance (CDR) of each accession. Results showed that the nine indicators responded variably to drought stress. Drought resistance from the three statistical methods were inconsistent, with only 45% of accessions showing consistent classification across all methods, indicating that the choice of analytical approach influences the outcome. Correlation analysis revealed that MFSV, PCASV, and GC were all positively correlated with CDR, supporting the use of CDR as a reliable reference factor for integrating laboratory-based evaluations. Finally, a Fisher discriminant function was established using MFSV, PCASV, and GC as independent variables (X) and CDR as the dependent variable (Y), providing a more comprehensive and scientifically grounded method for assessing drought resistance during rice germination.

Keywords: rice; drought; germination; Fisher discriminant

1. Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population, and rice yield losses pose a major threat to food security [1]. Rice cultivation involves substantial water consumption, and irrigated rice represents 53% of the global cultivated rice area [2]. As global agricultural water resources dwindle, enhancing drought adaptability in rice has become pivotal for ensuring stable production [3].

Rice is highly sensitive to drought stress and displays distinct morphological responses at different growth stages [4]. It is susceptible to drought stress during the germination and maturity stages [5–8], which limits rice production and yield stability. The germination stage is more critical to drought because it determines of later seedling growth and maturation of rice. Therefore, screening for rice cultivars that can tolerate drought stress at the germination stage will provide new insights for rice breeding [9].

Large-scale screening of drought-resistant rice germplasm is fundamental for elucidating the molecular mechanisms underlying drought tolerance. The Department of Agronomy at Bangabandhu Sheikh Mujibur Rahman Agricultural University evaluated 100 rice (*Oryza sativa* L.) genotypes for drought tolerance at the germination and early seedling stages using the Standard Evaluation System, with germination index, seedling height, and dry weight as key assessment criteria [10]. Similarly, Han et al. evaluated drought resistance in 667 rice germplasm accessions using seedling root length under drought stress as the key indicator. They employed a genome-wide association study (GWAS) analysis and identified a root-related gene designated as ROOT LENGTH 1 (RoLe1) [11]. Further investigation revealed that the OsNAC41-RoLe1-OsAGAP module promotes root development and drought resistance in upland rice[11]. Additionally, the drought resistance of 99 rice accessions was evaluated under five distinct natural drought conditions, using mean trait values for statistical analysis. Results identified chlorophyll stability index, leaf rolling degree, days to 50% heading, chlorophyll content, root volume, and root biomass as optimal predictors of grain yield under stress. A subsequent GWAS based on these drought-resistant phenotypes revealed haplotype variations in key genomic regions on chromosomes 1, 4, 6, and 11 [12]. In recent decades, a substantial number of quantitative trait loci have been identified using bi-parental or multi-parental populations [13–15]. GWAS technology, based on population phenotyping results, has increasingly become a vital method for deciphering the genetic mechanisms underlying complex traits [16–21]. These studies highlight the importance of accurate drought resistance phenotyping of large rice populations for mechanistic studies. However, the degree of drought susceptibility in rice varies significantly depending on growth phase, drought duration, stress intensity, cultivar characteristics, and environmental conditions [22]. The variability in evaluation criteria and statistical methodologies across studies has resulted in inconsistent results and the absence of standardized protocols for assessment criteria, growth stages, and metrics [23]. This lack of standardization hinders progress in deciphering the mechanisms underlying drought resistance in rice. Consequently, there is an urgent need to integrate multi-trait screening approaches and develop scientifically robust predictive models to advance research in this field.

To address this research gap, this study aims to systematically evaluate the drought resistance of rice during the germination stage using multiple indicators and analytical methods, and to propose a novel assessment framework for evaluating drought tolerance in germinating rice. This study selected 76 rice germplasm resources from southern China for evaluation under drought stress conditions during the germination stage. Nine key parameters were measured: germination potential (GP), germination rate (GR), bud index (BI), vitality index (VI), relative seedling water content (SRWC), germination drought resistance index (GDRI), root length (RL), plant height (PH), and root-to-shoot ratio (RSR).

This study employed three statistical approaches, membership function synthetic value (MFSV), principal component analysis synthetic value (PCASV), and grading coefficient (GC), to assess drought resistance during the germination stage of 76 rice accessions from multiple dimensions. In this study, Fisher's discriminant function was established by designating the germination-stage drought resistance results derived from the three statistical methods as the independent variable (X) and the comprehensive drought resistance (CDR) of rice accessions as the dependent variable (Y), thereby proposing a novel and more scientific methodology for drought resistance evaluation. This study optimized the existing drought-resistance evaluation system for the rice germination stage and provided a theoretical foundation for drought-resistant rice breeding.

2. Results

Evaluation of Indicators during the Rice Germination Period

The key indicators for assessing rice germination capacity under drought stress were GP, GR, BI, VI, GDRI, PH, RL, RSR, and SRWC. The results for all indicators following drought stress are shown in Figure 1. The GP was measured as the relative germination percentage on the fourth day of drought stress. A few highly drought-resistant rice materials exhibited GP values close to 100%, whereas drought-sensitive materials exhibited GP values clustered within the 0–2% range. GR, defined as the relative germination percentage on the eighth day of drought treatment, approached 100% in most rice samples, indicating that drought stress delayed germination but had a limited impact on the final GR. GI describes the germination status from days 1 to 8, whereas GDRI applies a decreasing weight over the same period to represent germination dynamics. As shown in Figure 1, the GI and GDRI exhibited consistent trends. Nearly all materials reached their maximum germination rate by the sixth day, suggesting that an extended duration had minimal effects on germination. VI reflects both the number of germinated seeds and the growth status, as indicated by plant height and root length, under drought conditions. Higher VI values indicated stronger drought tolerance during germination. For example, rice strain AS65 showed a GR of 1.0 and a relative VI of 1.61. RSR values ranged from 0.54 to 1.79, with approximately 50% of individuals exceeding 1.0. Comparisons between PH and RL revealed that drought stress generally reduced plant height, whereas root length increased in some cases, with a maximum RL value of 1.67. These results suggest that certain rice genotypes develop longer roots and higher root-to-shoot ratios under drought stress during the seedling stage. Coefficient of variation analysis indicated that GP, VI, RL, and RSR were considerably influenced by drought stress, whereas GR and SRWC were less affected. Correlation analysis among the indicators (Figure 2) showed that RSR was not significantly correlated with VI, GP, or GI, and that SRWC showed no significant correlation with any of the other indicators.

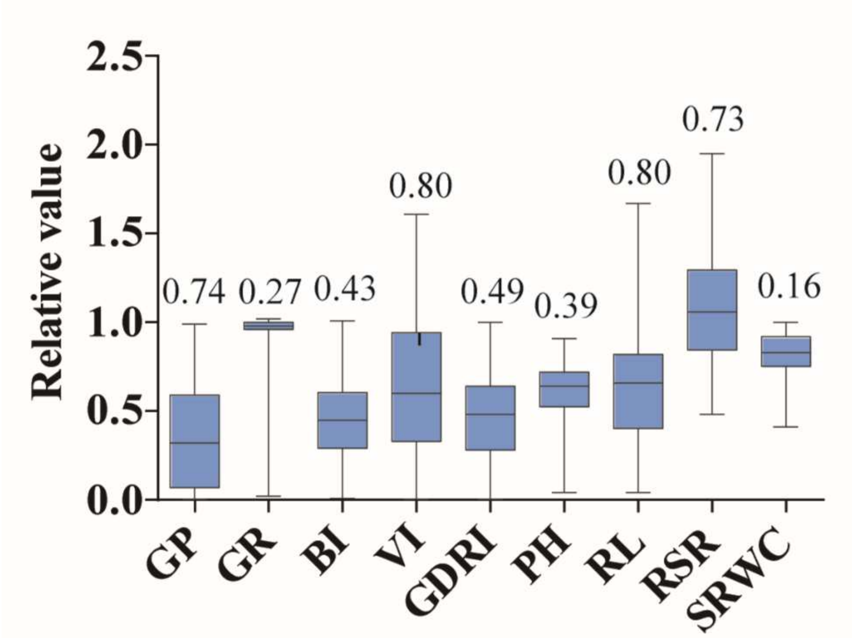

Figure 1 Box and whisker plots for the relative values of the 9 indicators across 76 rice samples. The whisker below the box represents the first quartile or the fifth percentile showing the first 25% data distribution in this range. In contrast, the whisker above the box represents the third quartile or 95th percentile showing the last 25% of the data distribution. The length of the box is called the interquartile range or (25th to 75th percentile),

shows 25% to 75% of the data distribution for that particular trait, and the horizontal line in the box indicates the median value. Coefficient of Variation values shown on the bar.

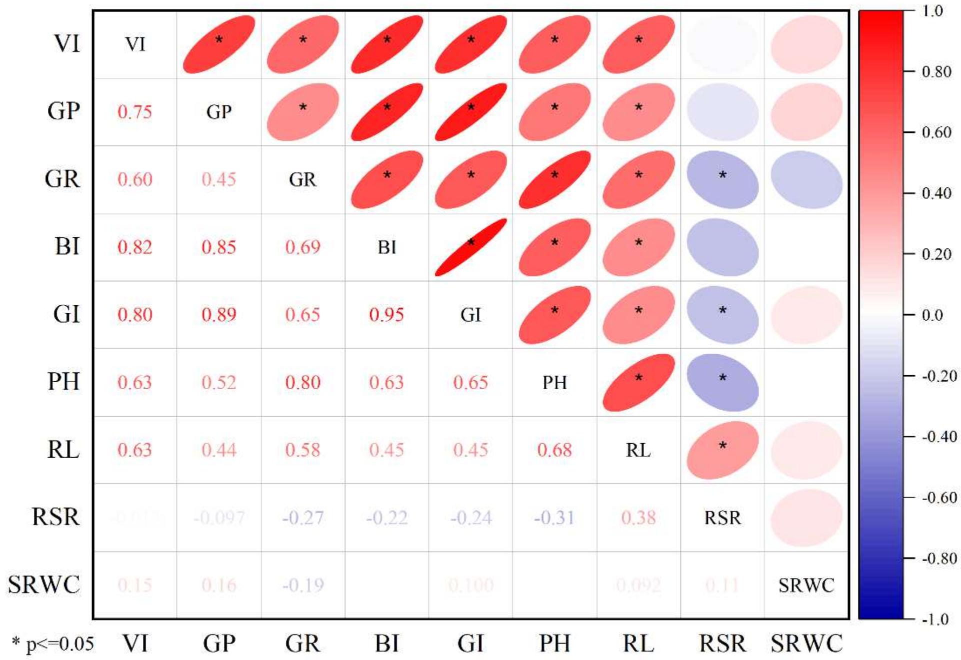

Figure2 Correlation analysis among the nine indicators

The numerical values in the figure represent correlation coefficients between the indicators. Larger absolute values indicate stronger correlations, while negative values denote negative correlations. An asterisk (*) denotes P < 0.05 (statistically significant). The eccentricity of the ellipses reflects the strength of the correlation—more elongated ellipses indicate weaker associations.

MFSV

The MFSV calculation incorporated the differential weighting of the nine indicators by computing the membership function value for each indicator, ultimately deriving the MFSV for each rice accession (Supplementary Table 2). As shown in Figure 3a, higher MFSV values indicate stronger drought resistance during the germination stage. The MFSV values ranged from 0.32 to 0.61, with the majority falling between 0.40 and 0.50, suggesting that most rice accessions exhibited moderate drought resistance. Notably, AS9, AS10, AS45, and AS61 demonstrated relatively strong germination-stage drought tolerance in this population.

PCASV

The PCASV was calculated by performing principal component analysis (PCA) on nine indicators, followed by the determination of the membership values of the principal components using a membership function, ultimately obtaining the PCASV value for each rice variety (Supplementary Table 3). The PCA results (Figure 3b) showed that the cumulative contribution rate of the first three principal components reached 85.2%, indicating that PC1, PC2, and PC3 effectively captured the variation characteristics of the original data. The PCASV results revealed that a higher PCASV value corresponded to stronger drought resistance during the germination stage. The PCASV values ranged from 0.21 to 0.62, with only a few rice materials exceeding 0.5, whereas the remaining materials exhibited a relatively uniform distribution (Figure 3c). Among the tested varieties, AS11, AS65, AS3, AS12, and AS63 exhibited the strongest drought resistance during germination.

Notably, the PCASV analysis results showed some discrepancies compared with those of the MFSV analysis.

GC

GC analysis was performed by assigning weighted scores to each measured parameter based on the standard deviation and mean values, ultimately generating a comprehensive GC score for each rice cultivar (Supplementary Table 4). Higher GC scores indicated greater drought tolerance. Analysis of the GC scores identified AS18, AS20, AS3, AS63, and AS65 as exhibiting superior drought resistance during the germination phase (Figure 3d). Notably, the GC evaluation results demonstrated partial inconsistencies with the MFSV and PCASV analytical approaches.

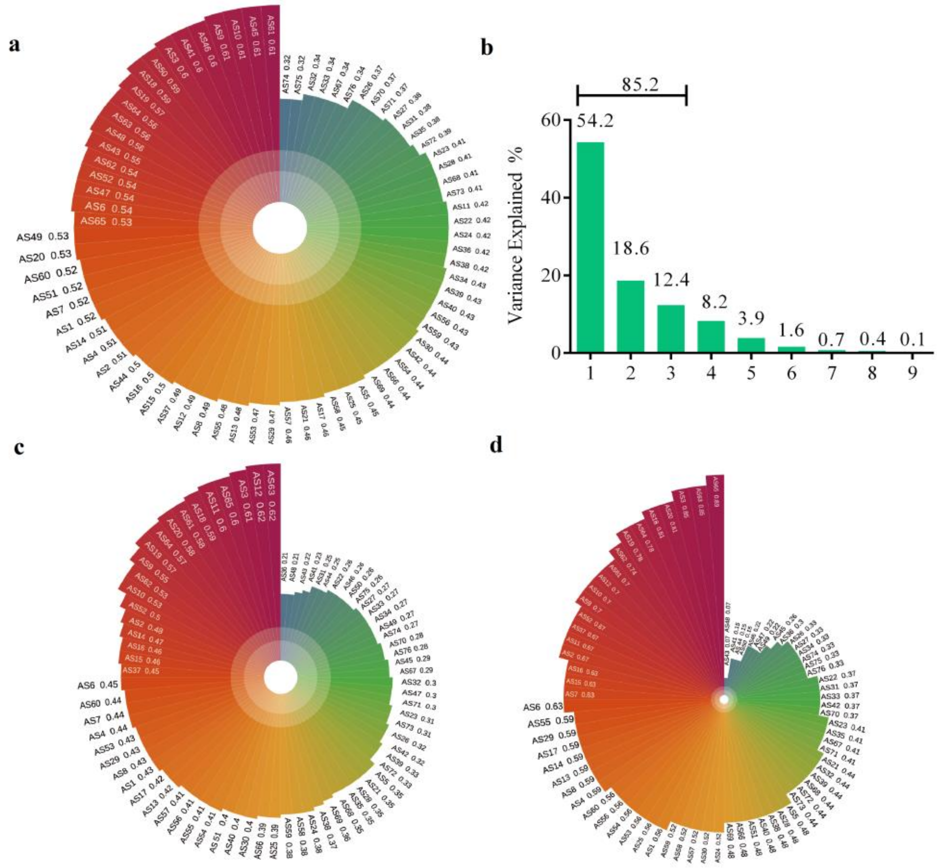

Figure 3. Evaluation of Drought Resistance in 77 Rice Accessions. (a) Drought resistance assessment based on MFSV; (b) Principal component analysis of nine indicators; (c) Drought resistance evaluation based on PCASV; and (d) Drought resistance assessment based on GC.

The results obtained from the three statistical methods (MFSV, PCASV, and GC) were separately ranked from weakest to strongest drought resistance, and subsequently classified into four drought resistance levels (Levels 1 to 4). Each rice accession was assigned three separate "drought resistance level" identifications (Figure 4). These results were utilized for the subsequent Venn diagram analysis.

variety	A	B	C	variety	A	B	C	variety	A	B	C	variety	A	B	C
AS1	2	2	2	AS20	2	1	1	AS39	3	3	3	AS58	3	3	3
AS2	2	2	2	AS21	3	3	3	AS40	3	2	3	AS59	2	2	2
AS3	1	1	1	AS22	3	4	3	AS41	1	4	4	AS60	3	1	1
AS4	2	2	2	AS23	3	3	3	AS42	3	3	3	AS61	1	1	2
AS5	3	3	3	AS24	3	3	2	AS43	2	4	4	AS62	2	2	1
AS6	2	2	2	AS25	3	2	2	AS44	2	4	4	AS63	2	1	1
AS7	2	2	2	AS26	4	3	3	AS45	1	3	4	AS64	1	1	1
AS8	2	2	2	AS27	4	3	3	AS46	1	4	4	AS65	2	1	1
AS9	1	2	1	AS28	3	3	3	AS47	2	3	4	AS66	3	3	3
AS10	1	2	1	AS29	2	2	2	AS48	2	4	4	AS67	4	3	3
AS11	3	1	2	AS30	3	2	2	AS49	2	3	4	AS68	3	3	3
AS12	2	1	2	AS31	4	4	3	AS50	1	4	4	AS69	3	3	3
AS13	2	2	2	AS32	4	3	3	AS51	2	2	3	AS70	4	3	3
AS14	2	2	2	AS33	4	3	3	AS52	2	2	2	AS71	4	3	3
AS15	2	2	2	AS34	3	4	3	AS53	2	2	2	AS72	3	3	3
AS16	2	2	2	AS35	4	3	3	AS54	3	2	2	AS73	3	3	3
AS17	3	2	2	AS36	3	4	4	AS55	2	2	2	AS74	4	4	3
AS18	1	1	1	AS37	2	2	2	AS56	3	2	2	AS75	4	4	4
AS19	1	1	1	AS38	3	3	3	AS57	3	2	2	AS76	4	3	4

Figure 4. Predicted drought resistance classifications of 76 rice varieties.

Numerical labels correspond to individual rice accessions. Panels A, B, and C represent drought resistance levels value based on MFSV, PCASV, and GC, respectively. Numerical values 1 through 4 denote specific resistance grades, with blue representing Grade 1, orange Grade 2, green Grade 3, and yellow Grade 4. Circles indicate outliers excluded due to inconsistent classifications across A, B, and C.

Through Venn analysis of the drought resistance levels derived from the three statistical methods (MFSV, PCASV, and GC) (Figure 5), it was observed that 45% of the accessions showed consistent classification across all three methods. Consistency between the MFSV and PCASV was observed in 55% of the population, between the MFSV and GC in 58%, and between the GC and PCASV in 79%. Overall, 97% of the accessions exhibited consistent drought resistance levels when using at least two of the three methods.

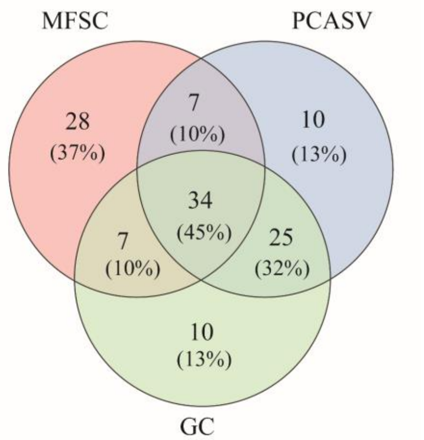

Figure 5. Venn diagram of drought resistance results analyzed by MFSV, PCASV, and GC statistical methods

Assessment of Comprehensive Drought Resistance in Rice

Under drought conditions, yield serves as the most direct indicator of rice drought resistance. All rice samples were direct-seeded and cultivated in both well-watered paddy fields and moisture-deficient dryland plots. After harvest, grain yield was measured, and the relative yield of each sample was calculated (Table 1). Under drought stress, rice varieties with differing drought resistance exhibited distinct relative yields. Based on these relative yield values, the rice population was classified into four drought resistance grades (Table 1).

Further correlation analysis between yield and values derived from the three statistical methods revealed positive relationships in all cases (Figure 6). Specifically, yield demonstrated a Pearson's correlation coefficient of 0.77 with the GC, indicating a statistically significant positive correlation. These results confirm the reliability of using yield as an indicator for CDR in rice, providing a solid foundation for subsequent development of discriminant functions.

Table 1. Drought resistance classification and relative yield performance of rice.

No.	Relative yield	level	No.	Relative yield	level	No.	Relative yield	level	No.	Relative yield	level
AS1	0.74	2	AS20	0.80	1	AS39	0.54	3	AS58	0.54	3
AS2	0.64	2	AS21	0.55	3	AS40	0.42	3	AS59	0.72	2
AS3	0.75	1	AS22	0.54	3	AS41	0.23	4	AS60	0.76	1
AS4	0.72	2	AS23	0.51	3	AS42	0.55	3	AS61	0.76	1
AS5	0.54	3	AS24	0.45	3	AS43	0.17	4	AS62	0.75	2
AS6	0.71	2	AS25	0.74	2	AS44	0.27	4	AS63	0.77	1
AS7	0.74	2	AS26	0.58	3	AS45	0.45	3	AS64	0.77	1
AS8	0.72	2	AS27	0.52	3	AS46	0.16	4	AS65	0.78	1
AS9	0.77	1	AS28	0.56	3	AS47	0.71	2	AS66	0.54	3
AS10	0.75	1	AS29	0.64	2	AS48	0.33	4	AS67	0.44	3
AS11	0.67	2	AS30	0.61	2	AS49	0.68	2	AS68	0.53	3
AS12	0.72	2	AS31	0.27	4	AS50	0.33	4	AS69	0.48	3
AS13	0.73	2	AS32	0.51	3	AS51	0.67	2	AS70	0.53	3
AS14	0.70	2	AS33	0.49	3	AS52	0.65	2	AS71	0.41	3
AS15	0.72	2	AS34	0.47	3	AS53	0.72	2	AS72	0.52	3
AS16	0.64	2	AS35	0.53	3	AS54	0.73	2	AS73	0.36	3
AS17	0.65	2	AS36	0.33	4	AS55	0.70	2	AS74	0.32	4
AS18	0.79	1	AS37	0.62	2	AS56	0.64	2	AS75	0.20	4
AS19	0.77	1	AS38	0.55	3	AS57	0.61	2	AS76	0.26	4

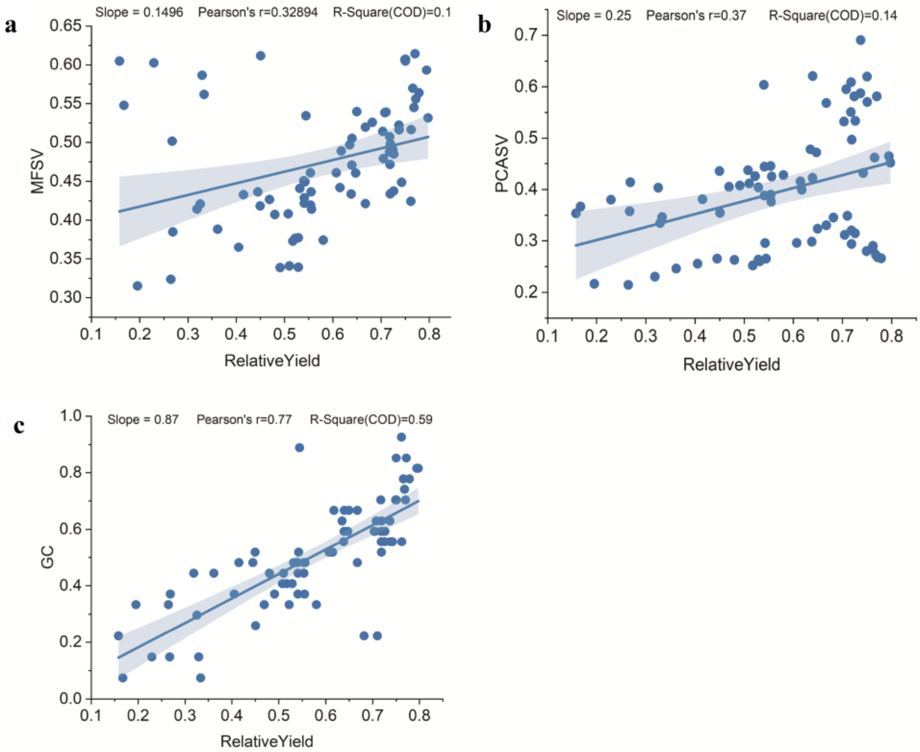

Figure 6. Correlation analysis between yield and MFSV, PCASV, and GC values.

Establishment of Fisher Discriminant Function

Using the drought resistance values obtained from the MFSV, PCASV, and GC methods as independent variables (X) (figure 4) and the CDR of rice accessions as the dependent variable (Y) (table 1), Fisher discriminant function equations were constructed. The Fisher discriminant function established four distinct categories: drought-sensitive, moderately drought-resistant, drought-resistant, and highly drought-resistant. The validation results indicate that the discriminant function achieved a reliability of 94.6%.

The Fisher discriminant functions were established as follows:

$y = 257.64X_1 + 6.4X_2 + 232.96X_3 + 168.71$, for drought-sensitive

$y = 209.74X_1 + 13.21X_2 + 181.1X_3 + 110.09$, for moderately drought-resistant

$y = 169.44X_1 + 9.64X_2 + 135.5X_3 + 67.04$, for drought-resistant

$y = 169.19X_1 + 4.86X_2 + 98.21X_3 + 53.15$, for highly drought-resistant

In these equations, X_1, X_2, and X_3 represent MFSV, PCASV, and GC, respectively, and the constant term accounts for random error. The drought resistance category was determined by selecting the equation that yielded the highest y-value. The estimated accuracy of the discriminant function exceeded 94.6%, demonstrating that this model provides highly accurate predictions for assessing drought resistance in rice germplasm during the germination stage.

3. Discussion

Screening for rice varieties with strong drought resistance is a prerequisite for research on both drought resistance mechanisms and breeding programs. Traditional field-based drought resistance screening is highly susceptible to environmental interference, resulting in poor reproducibility, high labor intensity, and low efficiency. In contrast, laboratory-based screening methods focusing on germination and seedling stages offer several advantages, such as simplicity, short experimental cycles, and high-throughput capacity, enabling the rapid identification of drought-tolerant rice germplasm [28–29]. However, existing evaluation approaches often rely on limited indicators and simplistic computational methods, resulting in suboptimal assessments of drought resistance and reduced practical applicability. Accordingly, we have developed a relatively comprehensive and scientific discriminant method. Admittedly, this approach has certain limitations, such as an insufficient number and diversity of samples, a narrow range of evaluation metrics, and a lack of physiological evidence. However, to our knowledge, this study represents the most extensive methodology currently available for assessing drought resistance in rice during the germination stage, encompassing the broadest set of influencing factors. In subsequent research, we will expand the sample size and diversity, incorporate physiological indicators such as ABA and GA, and strive to maximize the refinement of this evaluation system.

Indicators such as GP, GR, and BI reflect the germination performance of seeds under drought conditions, whereas SRWC, GDRI, and RSR help elucidate the impact of drought on seedling establishment following germination. The VI integrates both early germination capacity and subsequent seedling growth traits, collectively providing a multidimensional assessment of drought resistance throughout the germination phase in rice. GP and GR are the most commonly used indicators for evaluating germination capacity plants because they directly reflect the extent of damage incurred by seeds under stress [30–32]. Changes in plant height and root length occur when rice is subjected to drought during germination. Drought-resistant plants typically exhibit minimal changes in these traits; in some cases, root length may even increase under drought stress. This response is attributed to the activation of inherent drought resistance mechanisms, in which longer roots facilitate enhanced water uptake, thereby improving drought tolerance [33]. Among the materials tested, AS12 and AS20 showed reduced plant height but a significant increase in root length after drought treatment, resulting in a root-to-shoot ratio greater than 1. Therefore, these accessions are of considerable value for research on drought resistance in rice. SRWC and GDRI are specific indicators designed to assess drought resistance. However, our study revealed minimal variation in SRWC and no significant correlation with other metrics, suggesting that SRWC during early seedling establishment may not fully capture the degree of drought-induced damage. In summary, different indicators contribute to the evaluation of drought resistance during germination. Therefore, approaches that rely solely on individual indicators, or assume equal weighting across metrics, may introduce bias and inaccuracy. Many studies have evaluated drought resistance in rice using limited indicators, often employing membership function analysis for assessment [34,35]. Although methodologically feasible, this approach may overlook the CDR profiles of certain materials.

MFSV, PCASV, and GC are the three most commonly used analytical methods for phenotypic statistics. Both MFSV and PCASV are based on the principle of membership function, yet they differ in their computational approach: MFSV incorporates the contributions of all nine indicators, whereas PCASV first applies principal component analysis (PCA) to these indicators and subsequently calculates the values using the top three principal components. GC is a grading method that averages the contributions of nine indicators, ranks all individuals in the population based on the resulting values, and evaluates the drought resistance of each accession. Our results revealed inconsistencies between the three methods. Only 45% of the rice accessions showed consistent drought resistance across all three methods, whereas 97% showed agreement in at least two of the three evaluations. This suggests that employing too many statistical methods may introduce interference in the final assessment, which is why many studies rely on a single analytical approach[36]. Furthermore, classifying all individuals in a population into drought resistance levels allows for the stratification of their drought tolerance into distinct intervals, providing a scientific framework for evaluation and conclusions supported by our study. For instance, accession AS59 was ranked 24th, 23rd, and 34th by MFSV, PCASV, and GC, respectively; however, all three methods assigned it a drought-resistance level of 2.

Based on these findings, we propose a new method for determining drought resistance in rice at the germination stage that holistically integrates the influencing factors captured by each statistical method while avoiding inconsistencies resulting from multi-method discrepancies. Fisher discriminant analysis, also known as canonical discriminant analysis, identifies and establishes discriminant functions based on existing classification criteria and is widely applied in fields such as medicine, civil engineering, and agriculture. The principle of discriminant function analysis involves projecting high-dimensional independent variables (MFSV, PCASV, and GC) into a low-dimensional discriminant space through linear transformation, with the objective of maximizing inter-class separation among samples with different drought resistance levels while minimizing intra-class variability among samples of the same category.

Consequently, prior knowledge of the drought resistance attributes of the samples is required before constructing the discriminant function to ensure accurate positioning within the projected space. In this study, yield performance under field drought stress conditions was utilized as the criterion for assessing drought resistance in rice, primarily because yield serves as a direct manifestation of drought tolerance and represents the most compelling evaluation metric. However, the methodological approach of calibrating germination-stage drought resistance using whole-growth-period drought resistance data in the discriminant function may be subject to criticism. However, this study revealed significant positive correlations between CDR and values derived from MFSV, PCASV, and GC methods, demonstrating the scientific validity of utilizing yield as an evaluation criterion for drought resistance in rice. To our knowledge, the methodology established in this study represents one of the most comprehensive and scientifically rigorous evaluation systems developed to date for assessing drought resistance at the rice germination stage. Future research will validate the discriminant equation using rice germplasm resources with broader geographical origins and genetic backgrounds.

4. Materials and Methods

Plant Materials and Growth Conditions

The experiment was conducted at the Institute of Crops and Nuclear Technology Utilisation, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang, China. Seventy-six rice germplasm lines collected from southern China were used as experimental materials (Supplementary Table S1). Plump and uniformly sized seeds were selected from each accession and soaked in distilled water at 28 °C for 12 h. Subsequently, 50 seeds were evenly placed in sterile 90-mm-diameter Petri dishes lined with double-layer sterilized filter paper. For the drought stress treatment group, 15 mL of 20% polyethylene glycol (PEG) solution was added to each dish, whereas the control group received an equal volume of distilled water [24]. Three replicates were performed for each treatment group. All dishes were maintained in a growth chamber under controlled conditions of constant temperature (28 °C), relative humidity (70%), and a 10/14-h (light/dark) photoperiod for 10 d. The liquid level in each dish was monitored daily, and distilled water was replenished based on day one measurements to maintain a consistent volume throughout the experimental period.

Trait Measurement

All rice seeds were observed starting from the 2nd day of treatment. The germination standard was defined as the radicle breaking through the seed coat by 1 mm and the plumule reaching half the seed length. The number of germinated seeds was recorded at the same time on the 2nd, 4th, 6th, and 8th days. Germination potential was calculated on the 4th day, and the germination rate was calculated on the 8th day. On the 8th day, 15 plants were randomly selected from each petri dish to measure root length and plant height. All samples in each petri dish were individually weighed for fresh mass, then placed in an oven at 105 °C for 0.5 h, followed by drying at 80 °C until a constant mass was reached, after which the dry mass was weighed.

The formulas for calculating germination potential, germination rate, germination index, vitality index, seedling water content, germination drought resistance index, plant height, root length, and root-to-shoot ratio are as follows:

GP = G4 / 50 × 100, where G4 is the number of germinated seeds on the 4th day, and 50 is the number of seeds per dish.

GR = G8 / 50 × 100, where G8 is the number of germinated seeds on the 8th day, and 50 is the number of seeds per dish.

BI = ∑(DG/DT), where DG is the number of daily germinated seeds, and DT is the corresponding germination time for DG.

VI = BI × (plant height + longest root length), where BI is the seed germination index.

SRWC = (seedling fresh mass - seedling dry mass) / seedling fresh mass × 100.

GI = (1.00) nd2 + (0.75) nd4 + (0.50) nd6 + (0.25) nd8, where nd2, nd4, nd6, and nd8 are the seed germination rates on the 2nd, 4th, 6th, and 8th days respectively, and 1.00, 0.75, 0.50, and 0.25 are the weight coefficients assigned to the corresponding growth days.

GDRI = germination index of seeds under drought stress / germination index of seeds under control conditions.

RSR = root length / plant height.

RL = longest root length

PH = the height from the base of the plant root to the longest leaf

The data for all the above indicators are expressed as relative values:

Relative value of the indicator = measured value of the indicator under stress / measured value of the indicator under control conditions.

Calculation of MFSV, PCASV, and GC

The MFSV and PCASV were both calculated using the membership function method [25], while the GC value was obtained through the graded evaluation method [26]. The membership function value was calculated using the following formula:

$$u(X_j) = \frac{X_j - X_{min}}{X_{max} - X_{min}} \quad (1)$$

where j=1,2,3,…,n, Xj denotes the value of the j-th indicator, Xmin and Xmax represent the minimum and maximum values of the j-th indicator, respectively. The membership value μ for each indicator of each variety is computed accordingly.

The weight coefficient W is calculated as follows:

$$W_j = r_j / \sum_{j=1}^{n} r_j \quad (2)$$

where rj denotes the coefficient of variation of the j-th indicator.

The comprehensive evaluation value D is then determined using:

$$D = \sum_{j=1}^{n} [(X_j) \times W_j] \quad (3)$$

where j = 1, 2, 3,…, n, Xj represents the membership value of the j-th indicator for each variety and Wj is the weight coefficient of the j-th indicator. A higher value of D indicates a better performance of the trait.

The MFSV was obtained based on the aforementioned membership function calculation formula, where Wj represents the weight coefficient and j = 1, 2, 3, 4, 5, 6, 7, 8, and 9, corresponding to the following nine indicators: germination potential, germination rate, seedling water content, germination index, vigor index, germination drought resistance coefficient, plant height, root length, and root-to-shoot ratio. The MFSV is the sum of Xj × Wj for the nine indicators of each rice variety. Higher MFSV values indicate stronger drought resistance, whereas lower values indicate weaker drought resistance.

The PCASV was obtained by performing principal component analysis on the nine indicators and then applying the aforementioned membership function calculation formula. Three principal components were extracted each indicator. The weight coefficient Wj was calculated based on the contribution rate of the principal components, where j = 1, 2, and 3, corresponding to three comprehensive indicators: principal component 1, principal component 2, and principal component 3. PCASV is the sum of Xj × Wj for the three principal components for each variety. Higher PCASV values indicate stronger drought resistance, whereas lower values indicate weaker drought resistance.

GC Calculation Method: The mean value ($\bar{X}j$) of each of the nine indicators was used as the benchmark, and the standard deviation (S) was used as the criterion for grade classification. Each indicator value Xj was assigned a grade as follows:

Grade 1: $Xj \geq \bar{X}j + S$, Grade 2: $\bar{X}j + S > Xj \geq \bar{X}j$, Grade 3: $\bar{X}j > Xj \geq \bar{X}j - S$, Grade 4: $\bar{X}j - S > Xj$.

GC was calculated as follows:

$$GC = (nm - \sum Xj) / (nm - n) \quad (4)$$

where n is the number of indicators, m is the number of grades, and Xj denotes the graded evaluation value of the j-th indicator. The sum of Xj values represents the drought resistance grade value of each sample. A larger GC value indicates stronger drought resistance in the variety.

Evaluation of Rice Drought Resistance at the Germination Stage

The Fisher discriminant function takes MFSV, PCASV, and GC as independent variables and "drought resistance level" as the dependent variable. In this study, the method for obtaining the "drought resistance level" of rice was as follows: the results of the three groups (MFSV, PCASV, and GC) were sorted from weak to strong. The top 15% of the total number after sorting were classified as level 1, 16%–50% as level 2, 51%–85% as level 3, and 86%–100% as level 4 for the “drought resistance level” division [27].

Assessment of CDR in Rice

For each variety, 400 plump and uniform seeds were selected. After 1 day of soaking, the seeds were direct-seeded. The control group was established in conventional paddy fields, while the drought treatment group was planted in relatively moist upland soil. The plot size for both conditions was 1 × 10 square meters, and an equal amount of base fertilizer was applied to all plots before sowing. The water management protocol for the drought treatment group was as follows: after sowing, a small amount of water was sprayed every 5 days for a total of 2-3 times until seedling emergence, after which water supply was stopped. During the heading stage, plots were irrigated every 4 days for a total of 3-4 times. No additional manual irrigation was provided at other times.

At maturity, all plants from both the control and treatment groups were harvested and weighed. The moisture content was standardized to 14.5% for japonica rice and 13.5% for indica rice for yield calculation. The relative yield for each sample was calculated (Relative Yield = Drought Treatment Group / Control Group) and ranked from highest to lowest. The top 15% of the total number after sorting were classified as level 1, 16%–50% as level 2, 51%–85% as level 3, and 86%–100% as level 4 for the "CDR"。

Fisher Discriminant

Based on the drought resistance ratings derived from MFSV, PCASV, and GC, Fisher discriminant functions were constructed as follows [6-7]:

$$y = u_1X_1 + u_2X_2 + u_3X_3 + P$$

where X_1, X_2, and X_3 represent the drought resistance ratings based on MFSV, PCASV, and GC, respectively; u_1, u_2, and u_3 denote the coefficients corresponding to X_1, X_2, and X_3; P is the constant term; and y indicates the level of drought resistance. In this study, four discriminant functions were established: y_1 for highly drought-resistant, y_2 for drought-resistant, y_3 for moderately drought-resistant, and y_4 for drought-sensitive.

Statistical Analysis

Data were processed using Microsoft Office 2010 software(Microsoft Corp., Redmond, WA, USA). All experimental treatments were conducted in triplicate. Statistical significance and error analysis were performed using SPSS 26 (IBM Corp., Armonk, NY, USA), with significant differences defined at $^*p \leq 0.05$ according to Tukey's test. The membership function values were calculated using SPSS 26. Significant difference diagrams were generated using Origin 2022 (OriginLab Corp., Northampton, MA, USA), and box plots were created using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA).

5. Conclusions

This Drought stress is a highly destructive abiotic factor that limits rice growth and development. Establishing an efficient and rapid screening technique during the germination stage is crucial for identifying genotypes that are best suited to limited water conditions. Traits such as GP, GR, BI, VI, GDRI, PH, RL, RSR, and SRWC reflect various aspects of rice growth from germination to early seedling establishment under drought stress. Drought resistance evaluations derived from the MFSV, PCASV, and GC statistical methods showed discrepancies, with only 45% of the accessions exhibiting consistent results across all three methods. Therefore, screening for drought-resistant germplasm should incorporate multiple statistical approaches. Based on the results of these three methods, and using CDR as a calibration reference factor, we propose a new discriminant equation approach for assessing drought resistance levels during rice germination. The drought-resistant genotypes identified in this study can be used to explore the mechanisms underlying drought tolerance, which are essential for achieving and maintaining global food security.

Supplementary Materials:

Supplementary Table 1. Information on 76 rice accessions;

Supplementary Table 2. Statistical analysis of drought resistance during germination stage using MFSV;

Supplementary Table 3. Statistical analysis of drought resistance during germination stage using PCASV;

Supplementary Table 4. Statistical analysis of drought resistance during germination stage using GC;

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