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Early Identification of Potentially Drought-Tolerant Doubled Haploid Rice Lines During the Seedling Stage

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Abstract

Climate change and reduced crop yields caused by drought stress have increased the demand for drought-tolerant varieties. The anther culture technique allows the production of improved varieties with high homozygosity in a short time. The study aimed to select the drought-tolerant double haploid lines at the seedling stage. This research was conducted in the greenhouse of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Instrument Standard Testing (BBPSI Biogen), Bogor, from August to November 2023. The drought-tolerant selection of 12 doubled-haploid rice lines (AE1-AE12) and four check varieties, i.e., two commercial checks ("Inpari 18" Tadah Hujan Agritan or AE13, "Bioni63" Ciherang Agritan or AE14), one drought-tolerant check ("Salumpikit" or AE15), and one drought-sensitive check ("IR20" or AE16). The research used a randomized complete block design with three replications. The characters observed were leaf rolling, leaf drying, recovery ability, and plant fresh and dry weight. The Friedman test results showed that the lines with the lowest rankings, three lines (AE2, AE5, and AE12) with mild tolerant criteria for leaf rolling, five lines (AE1, AE2, AE5, AE8, and AE12) with mild tolerant criteria for leaf drying, and three lines (AE1, AE5, and AE12) with tolerant criteria for recovery ability. The selection index for drought tolerance at the seedling stage identified seven lines with positive values. Based on the Friedman test, selection index, and heatmap visualization, AE12, AE1, AE5, and AE8 exhibited a tolerance similar to "Salumpikit" and were deemed suitable based on drought tolerance characters.

Keywords: abiotic stress, El Niño, genetic improvement, vegetative

Introduction

Rice is cultivated in over 100 countries, and 90% of the total production comes from Asia (Fukagawa and Ziska, 2019). Rice is a major staple food in the Asian region, including Indonesia. Based on data from the Central Bureau of Indonesian Statistics (BPS, 2024), there have been fluctuations in Indonesia's rice production from 2020 to 2024. In 2024, there was a decrease of 1.64% and 2.45% in dry milled rice production and harvested area, respectively, compared to the previous year (BPS 2024). Therefore, it is crucial for Indonesia to keep sustainable rice productivity to avoid a further potential deficit.

Various factors affect rice production, including climate change (Ndikuryayo et al., 2023; Rajan, 2023). Drought is an impact of climate change where the dry season period is longer (Kumar et al., 2019; Botahala et al., 2021), thus affecting plant growth and yield (Baniya et al., 2020; Oo et al., 2020; Panda et al., 2021). The El Niño can also exacerbate drought conditions by delaying rainfall and decreasing rice planting in Indonesia's rice-growing regions (Naylor et al., 2007). Furthermore, the long dry season in Indonesia has resulted in limited rice planting, drought, and crop failure (Sukarman et al., 2018). Based on data from the Indonesian Ministry of Agriculture and the Indonesian Agency for Meteorological, Climatological and Geophysics from 2016 to 2023, rice fields have experienced drought, with an average area of 255,974 ha, and 29.39% of them failed to harvest (Kementan, 2021; BMKG, 2024). Climate change and drought stress that cause reduced yields (Bhandari et al., 2023; Malau et al., 2023) have increased the need for drought-tolerant rice varieties (Sabouri, 2022). Hence, it is important to develop rice varieties that adapt to climate change to maintain yields while minimizing the harmful effects of abiotic stress (Hassan et al., 2023).

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Plant breeding can be done to obtain new superior varieties of rice based on desired characteristics. This effort can be achieved quickly through anther culture technique (Dewi and Purwoko, 2012). The anther culture technique can reduce the breeding process duration by up to 8 generations compared to conventional methods (Purwoko et al., 2010). Based on the research findings of Hadianto et al. (2023), the selected doubled haploid (DH) irrigated rice lines demonstrate favorable agronomic traits and high yield potential. Therefore, further testing, such as drought stress evaluation, is required for these lines. The selected DH lines were derived from KP4 × "BioNL 6-1" (AE1-AE6) and "Inpari 45" × KP4 (AE7-AE12).

Drought tolerance evaluation can be conducted at the vegetative and generative phases. Rice has three growth stages most sensitive to drought stress: early seedling, vegetative, and anthesis (Singh et al., 2012). Rapid testing in selecting drought-tolerance lines at the seedling stage based on the Standard Evaluation System (SES) of rice from IRRI has been carried out by other researchers and can provide an overview of how the tolerance of the tested lines (Opalofia et al., 2018; Kartina et al., 2019; Susanto et al., 2019; Herawati et al., 2021; Tirtana et al., 2021). According to Ambikabathy et al. (2019), some lines identified as tolerant to drought stress in the seedling stage are also tolerant to drought stress in the generative phase. This study aims to determine the tolerance of DH rice lines to drought stress and select tolerant lines at the seedling stage.

Materials and Methods

Study Sites and Design

This research was conducted in the greenhouse of the Indonesian Center for Biotechnology and Agricultural Genetic Resources Instrument Standard Testing (BBPSI Biogen), Bogor, from August to November 2023. The climate data during the experiment is presented in Table 1. The drought-tolerant test was conducted using a completely randomized block design with three replications. Fourteen seeds per line were planted in rows on a planting trough (3 m × 0.6 m × 0.8 m) at a spacing of 5 cm × 5 cm. Nitrogen, phosphorus, and potassium fertilizers were applied at 200 kg.ha^{-1} in a 16:16:16 ratio, dissolved in water, and given seven days after planting (DAP).

Genetics Materials

In the present study, 12 DH rice lines (AE1-AE12) and four check varieties consisting of two commercial checks ("Inpari 18" or AE13 and "Bioni63" or AE14), one drought-tolerant check ("Salumpikit" or AE15), and one drought-sensitive check ("IR20" or AE16) were used for drought screening. Lines AE1-AE6 were derived from KP4 × "BioNL 6-1", whereas lines AE7-AE12 were derived from "Inpari 45" × KP4.

Observation and Measurement

The crop was irrigated until 14 DAP and left without irrigation until the drought-sensitive check plants showed leaf drying at a score of 9. The doubled haploid (DH) rice lines were visually screened for drought-tolerant variables: leaf rolling, leaf drying, and recovery ability. Assessment of the plant responses to drought stress was conducted based on the Standard Evaluation System (SES) of rice from "IRRI" (2014) (Table 2-4). The assessment of leaf rolling in the tested DH lines was conducted when the leaves of the "IR-20" had completely rolled (score 9). The evaluation of leaf drying in the tested DH lines was conducted when all the leaves of the "IR-20" had dried entirely (score 9), which occurred at 48 DAP. The tested DH lines were then rewatered for 10 days, and the recovery ability assessment was conducted.

Other growth responses measured in this study were the fresh and dry weights. Plant fresh weight data were collected by weighing all plant parts (leaf, stem, and root). The root part had been cleaned from the soil. Plant samples that had been weighed fresh were put into separate envelopes for each experimental unit and dried using an oven at 60^{o}C for 3x 24 h. The dried plant samples were then weighed with an analytical balance to obtain the dry weight of the plants.

Table 1. Climate data of the study area

Months	Precipitation (mm per month)	Minimum temperature (°C)	Maximum temperature (°C)	Humidity (%)
August	144.7	20.3	34.4	75.5
September	62.2	19.6	35.8	72.0
October	102.1	21.6	36.0	74.9
November	1068.0	22.2	34.8	85.1
Mean	344.3	20.9	35.3	76.9

Source: BPS (2024)

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Data Analysis

Data from the scoring of the drought-tolerant test of doubled haploid (DH) rice lines were calculated based on the Friedman test (Conover 1999; Pereira et al. 2015; Liu and Xu, 2022):

$$R_j = \sum_{i=1}^{b} R(X_{ij}) \;; A = \sum_{i=1}^{b} \sum_{j=1}^{k} [R(X_{ij})]^2 \;; C = \frac{bk(k+1)^2}{4}$$

$$T_1 = \frac{(k-1)}{A-C}\, x \left[\sum_{j=1}^{k} R_j^2 - bxC\right] ; T_2 = \frac{(b-1)T_1}{b(k-1)T_1}$$

R_j = Sum of rank values of each line in the whole block, A = Sum of squares of each observation value, C = Correction factor, T_1 = Friedman test statistic based on chi-square distribution, T_2 = Friedman test statistic based on F distribution, b = Blocks, k = Number of lines, i = 1, 2, 3 (block), j = 1, 2, 3, ..., n (lines).

If the Friedman test showed differences among the tested lines, then the LSD test was conducted on the drought-sensitive and drought-tolerant checks (Conover, 1999; Pereira et al., 2015):

$$|\overline{R_b} - \overline{R_a}| > t_{((\alpha/_2)x(k-1)x(b-1))} x \sqrt{\frac{2(bxA - \sum R_j^2)}{(b-1)(k-1)}}$$

$\overline{R_a}$ = Average number of ranks of line a, $\overline{R_b}$ = Average number of ranks of line b, R_j = Sum of ranks of values of each line in the whole block, b = Blocks, k = Number of lines, t = t table value, A = Sum of squares of each observation value.

Each drought test character and plant weight were subjected to correlation analysis. The correlation between characters was calculated based on Spearman's rank correlation (Spearman, 1904; Zar, 2005):

$$r_s = 1 - \frac{6 \sum d_i^2}{n(n^2-1)}$$

r_s = Spearman's rank correlation coefficient, d_i^2 = The square of the difference in the rank of the observation of characters x and y, n= Number of genotypes.

The degree of relatedness between the characters analyzed can be determined based on the correlation coefficient (r) criteria. Specifically: 0.7 < r ≤ 1.0 indicates a high correlation, 0.4 < r ≤ 0.7 indicates a medium correlation, 0.2 < r ≤ 0.4 indicates a low correlation, and r ≤ 0.2 indicates no correlation (Djarwanto and Subagyo, 1993).

The weighted selection index ranks the DH lines based on the character of rice plants against leaf rolling, leaf drying, and recovery ability after drought. The determination of the weighted selection index was based on the following Falconer and Mackay

Table 2. Drought tolerance categories based on leaf rolling

Scale	Categories	Description
0	Highly tolerant	The leaves are healthy
1	Tolerant	The leaves start to fold (shallow)
3	Mild tolerant	The leaves are folding (deep V-shape)
5	Moderate	The leaves are fully cupped (U-shape)
7	Mild sensitive	The leaf margins are touching (0-shape)
9	Sensitive	The leaves are tightly rolled

Source: IRRI (2014)

Table 3. Drought tolerance categories based on leaf drying

Scale	Categories	Description
0	Highly tolerant	No symptoms
1	Tolerant	Slight tip drying
3	Mild tolerant	Tip drying extended up to 1/4 length in most leaves
5	Moderate	One-fourth to 1/2 of all leaves dried
7	Mild sensitive	More than 2/3 of all leaves are fully dried
9	Sensitive	All plants are dead

Source: IRRI (2014)

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(1996) and Zhang and Amer (2021):

$$I = \sum b_i z_i = b_1 z_1 + b_2 z_2 + \cdots + b_n z_n$$

I = Selection index, b_i = Weight of the character, z_i = Standardized phenotypic value (leaf rolling, leaf drying, and recovery ability), i = 1, 2, 3, …, n where n = number of traits.

$$z_n = \frac{(x_i - \bar{x})}{\sqrt{\sigma^2_{error}}}$$

z_n = Standardized phenotypic value, x_i = Phenotypic value in original scale, $\bar{x}$ = Mean of character, σ^2_{error} = Variance of character

In this study, there are differences in the weighting of characters observed in the drought test experiment based on the importance of a character in selection activities. Weighing on the recovery ability character is given a value of 2x greater than the character of leaf rolling and leaf drying, which aims to maximize the selection index model with the aim of plant breeding, because recovery ability is an important character in plant adaptation to drought stress. The direction of selection on these three drought test characters is left, so the index model is given a negative sign.

In addition to the selection index, the results were visualized with heatmaps using R software with the package "pheatmap" to illustrate the tolerance of each line of the three characters (leaf rolling, leaf drying, and recovery ability) and the grouping of lines and characters. Data analysis in the experiment was conducted using Microsoft Excel, SAS OnDemand for Academics, and R Studio.

Results and Discussion

Fourteen doubled haploid (DH) rice lines and four check varieties were screened for drought stress at the seedling stage based on the Friedman test (Table 5). Each of the observed drought-related characters had significant effects. Based on the value of the chi-square test ($\mathcal{X}^2$) and the F test, the characters of leaf rolling, leaf drying, and recovery ability were significant at 5% and 1% levels. This condition indicates a difference in ranking among the tested DH rice lines in response to the drought test. The ranking values obtained from the Friedman test indicated the tolerance level of the tested DH lines to drought stress. The smaller the Friedman test ranking value of a line, the higher its tolerance to drought stress. Based on the Friedman test, differences in rankings for the identified characters can be further analyzed to determine the ranking differences of DH lines compared to the check varieties.

Leaf Rolling Response

Leaf rolling is an early plant response mechanism to drought stress (Ali et al., 2022) and is a consequence of soil drying (Cal et al., 2019). This response is a physiological adaptation to drought that helps reduce water loss (Wang et al., 2023), similar to stomatal closure and increased oxidative stress (Yang et al., 2022). Leaf rolling is the plant's mechanism to maintain water status. Under water deficit, leaf rolling may enable partial stomatal conductance (Kartika et al., 2020). The lines with the smallest Friedman test rank, equal to Salumpikit on leaf rolling characters, are AE2, AE5, and AE12, classified as mild tolerant. No DH lines are classified as tolerant for leaf rolling characters (Table 6).

Table 4. Drought tolerance categories based on recovery ability

Scale	Categories	Recovery ability (%)
1	Tolerant	90-100
3	Mild tolerant	70-89
5	Moderate	40-69
7	Mild sensitive	20-39
9	Sensitive	0-19

Source: IRRI (2014)

Table 5. Friedman test results on drought tolerance characters in doubled haploid rice lines at the seedling stage with model adjustment for data with the same scoring value (adjusted for ties)

Characters	$\mathcal{X}^2$-value	P-value $\mathcal{X}^2$	F-value	P-value
Leaf rolling	25.68 *	0.04	2.66 *	0.01
Leaf drying	39.19 **	<0.01	13.49 **	<0.01
Recovery ability	29.62 *	0.01	3.85 **	<0.01

Notes: df= degree of freedom, $\mathcal{X}^2$ = chi-square, *= significant at 5% alpha level, **= significantly at 1% alpha level.

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Leaf Drying Response

Observations of leaf drying characters (Table 7) showed diverse scoring results for all tested lines. Leaf drying is an advanced response to leaf rolling (Mustikarini et al., 2022). Leaf drying is caused by an oxidative reaction that leads to the leaves drying out and aging (Krieger-Liszkay et al., 2019). The lines with the smallest Friedman test rank, equal to Salumpikit on leaf drying characters, are AE1, AE2, AE5, AE8, and AE12 with mild tolerant criteria. However, no DH lines are classified as tolerant for leaf drying characters. In this case, AE2, AE5, and AE12 lines consistently responded relatively well to drought tolerance characteristics. Drought-tolerant lines are assumed to retain water more effectively and dry out more slowly. This mechanism includes stomatal closure (Shen et al., 2021) to reduce transpiration, increased water retention capacity in tissues (Yang et al., 2020), and osmotic adjustment to maintain cellular turgor (Yu et al., 2024).

Recovery Ability Response

Observations of recovery ability characters (Table 8) showed diverse scoring results for all tested lines. Recovery ability is a post-drought resistance response characterized by the growth of new young leaves (Hassan et al., 2023). Good recovery ability allows plants to survive after drought stress. The lines with the smallest Friedman test rank, equal to "Salumpikit" for recovery ability characters, are AE1, AE3, AE5, AE8, AE11, and AE12, a distribution ranging from mild tolerant to tolerant. AE1, AE5, and AE12 are classified as tolerant, the same as "Salumpikit". AE12 exhibits recovery ability with a total Friedman test rank value and tolerant classification identical to "Salumpikit". Plants with good recovery ability from drought stress were indicated by osmotic adjustment through increased solute activity to increase water diffusion into the plant and maintain high turgor potential (Taryono et al., 2023).

Correlation Values

The correlation values between drought-tolerant characters (Figure 1) showed significant results for all characters at the 1% level. It explains that each character has a relationship. Two characters are correlated if a change in one character is consistently followed by a change in the other character, either in the same or opposite direction (Bewick et al., 2003). The direction of correlation was expressed as a positive (+) or negative (-) relationship between characters (Kiernan, 2014), while the strength of the relationship is indicated by the magnitude of the correlation coefficient (Gomez and Gomez, 1995). Recovery ability was an important characteristic in the drought test (Chen et al., 2015; Hasanuzzaman et al., 2022; Taleb et al., 2023). In our study, they have

Table 6. Leaf rolling scores and total Friedman ranking of doubled haploid rice lines, and check varieties for the drought test

Genotypes	Leaf rolling	Criteria	Sum of rank Friedman value
AE1	5.24	Moderate	27.00 ab
AE2	4.08	Mild tolerant	14.00 b
AE3	5.27	Moderate	27.00 ab
AE4	5.35	Moderate	29.00 ab
AE5	4.24	Mild tolerant	15.00 b
AE6	6.53	Mild Sensitive	38.00 a
AE7	5.29	Moderate	24.00 ab
AE8	4.71	Moderate	19.00 ab
AE9	5.64	Moderate	32.00 ab
AE10	5.46	Moderate	29.00 ab
AE11	5.38	Moderate	30.00 ab
AE12	3.21	Mild tolerant	13.00 b
"Inpari-18"	6.52	Mild Sensitive	31.00 ab
"Bioni 63"	5.76	Moderate	29.00 ab
"Salumpikit" (CT)	0.57	Tolerant	3.00 b
"IR-20" (CS)	8.51	Sensitive	48.00 a

Notes: CT= check tolerant, CS= check sensitive, a= significantly from the "Salumpikit" with LSD test at 5% alpha level, b= significantly from the IR20 with LSD test at 5% level, LSD critical value = 15.11.

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Table 7. Leaf drying scores and total Friedman ranking of doubled haploid rice lines, and check varieties for the drought test

Genotypes	Leaf drying	Criteria	Sum of rank Friedman value
AE1	3.37	Mild tolerant	10.00 b
AE2	3.33	Mild tolerant	9.00 b
AE3	5.20	Moderate	31.00 ab
AE4	4.37	Mild tolerant	21.00 ab
AE5	3.80	Mild tolerant	13.00 b
AE6	5.82	Moderate	36.00 a
AE7	4.85	Moderate	26.00 ab
AE8	3.92	Mild tolerant	17.00 b
AE9	6.26	Moderate	39.50 a
AE10	5.68	Moderate	34.50 a
AE11	5.19	Moderate	27.00 ab
AE12	3.73	Mild tolerant	16.00 b
“Inpari-18”	5.59	Moderate	34.00 a
“Bioni 63”	6.72	Mild sensitive	43.00 a
“Salumpikit” (CT)	1.15	Tolerant	3.00 b
“IR-20” (CS)	9.00	Sensitive	48.00 a

Notes: CT = check tolerant, CS = check sensitive, a = significantly different from "Salumpikit" with LSD test at α5% alpha level, b significantly from the IR20 with LSD test at 5% level, LSD critical value = 14.63.

Table 8. Recovery ability scores and total Friedman ranking of doubled haploid rice lines and check varieties for the drought test

Genotypes	Recovery ability	Criteria	Sum of rank Friedman value
AE1	1.67	Tolerant	14.00 b
AE2	3.67	Mild tolerant	27.50 ab
AE3	3.00	Mild tolerant	21.50 b
AE4	4.33	Moderate	33.00 a
AE5	1.67	Tolerant	14.00 b
AE6	4.33	Moderate	31.50 a
AE7	3.67	Mild tolerant	28.00 ab
AE8	2.33	Mild tolerant	16.00 b
AE9	5.00	Moderate	39.50 a
AE10	4.33	Moderate	33.00 a
AE11	3.00	Mild tolerant	21.50 b
AE12	1.67	Tolerant	10.00 b
“Inpari-18”	4.33	Moderate	31.50 a
“Bioni 63”	4.33	Moderate	31.50 a
“Salumpikit” (CT)	1.67	Tolerant	10.00 b
“IR-20” (CS)	6.33	Mild sensitive	45.50 a

Notes: CT= check tolerant, CS= check sensitive, a= significantly from the "Salumpikit" with LSD test at 5% alpha level, b= significantly from the IR20 with LSD test at 5% level, LSD critical value = 14.59.

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a positive and high correlation with leaf rolling (0.86) and leaf drying (0.83), as well as a negative and high correlation with plant fresh weight (-0.82) and plant dry weight (-0.79).

The positive and high correlation indicates that when a line has a smaller Friedman test ranking value for leaf rolling and leaf drying characters, its tolerance to drought stress increases, and its recovery ability will also improve accordingly. For the characteristics of recovery ability, the fresh weight and dry weight of the plant have negative and high correlation values. This correlation indicates that as the plant's fresh and dry weight increases, the Friedman test ranking for recovery ability decreases, indicating better tolerance. Based on this, recovery ability can be prioritized in selection during the seedling stage drought test.

Selection Index and Heatmap

The weighted selection index was based on the selection objectives and the economic value of a character (Oliveira et al., 2014; Cui et al., 2014; Sarwendah et al., 2022). The selection index values (Table 9) for measuring the drought test characters showed that seven lines had positive selection index values, namely, AE1, AE2, AE3, AE5, AE8, AE11, and AE12. A positive selection index value indicates that these lines have good tolerance to drought stress at the seedling stage. These lines can be utilized as genetic resources in rice breeding programs to develop drought-tolerant lines (Sabouri et al., 2022).

Visualization of selection index results with heatmaps can provide an overview of the tolerance of each line based on the three drought characters (leaf rolling, leaf drying, and recovery ability), as well as the grouping of lines and test characters. Based on Figure 2, there were two groupings of lines based on drought tolerance. There are lines that are grouped with IR-20, which is a drought-sensitive check, and there are lines with "Salumpikit", which is a drought-tolerant check. AE12, AE1, AE5, and AE8 were clustered with "Salumpikit" as a tolerant check. It explains that the four lines have similar and good seedling phase tolerance based on drought testing characters.

Conclusions

The results of the drought test on doubled haploid rice lines at the seedling stage and the Friedman test revealed significant differences in the rankings of the tested lines for leaf rolling, leaf drying, and recovery ability characters. Recovery ability showed a positive and high correlation with leaf rolling and leaf drying

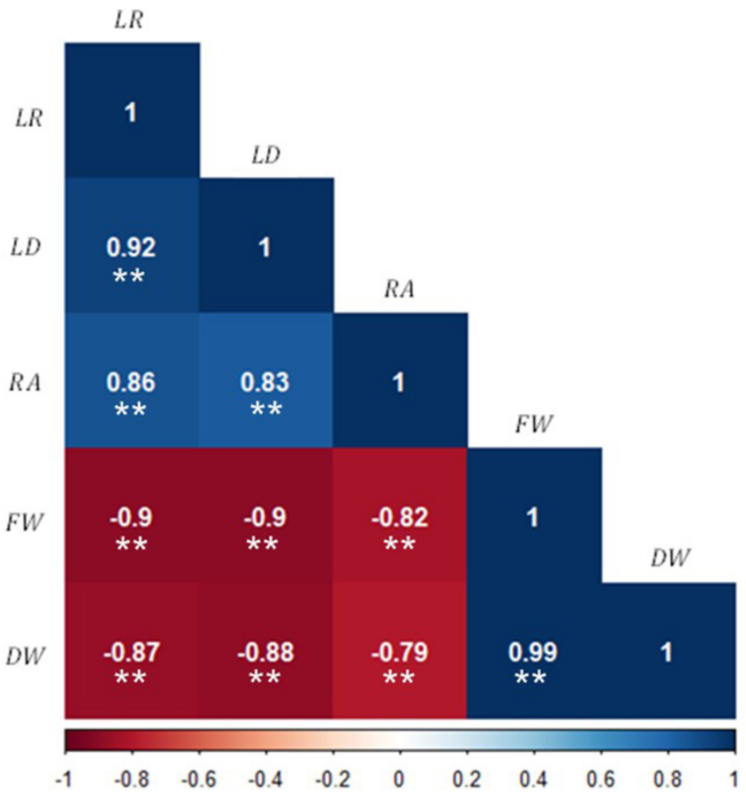

Figure 1. Spearman rank correlation value for drought tolerance characters of doubled haploid rice lines at the seedling stage. Notes: **= significantly different at α= 0.01. LR= leaf rolling, LD= leaf drying, RA= recovery ability, FW= plant fresh weight, DW= plant dry weight

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Table 9. The selection index values based on leaf rolling (LR), leaf drying (LD), and recovery ability (RA) characteristics

Genotypes	LR	LRC	LD	LDC	RA	RAC	Index
AE1	5.24	M	3.37	MT	1.67	T	3.35
AE2	4.08	MT	3.33	MT	3.67	MT	1.19
AE3	5.27	M	5.20	M	3.00	MT	0.38
AE4	5.35	M	4.37	MT	4.33	M	-1.11
AE5	4.24	MT	3.80	MT	1.67	T	3.70
AE6	6.53	MS	5.82	M	4.33	M	-2.63
AE7	5.29	M	4.85	M	3.67	MT	-0.39
AE8	4.71	M	3.92	MT	2.33	MT	2.39
AE9	5.64	M	6.26	M	5.00	M	-3.32
AE10	5.46	M	5.68	M	4.33	M	-1.92
AE11	5.38	M	5.19	M	3.00	MT	0.32
AE12	3.21	MT	3.73	MT	1.67	T	4.34
“Inpari-18”	6.52	MS	5.59	M	4.33	M	-2.50
“Bioni 63”	5.76	M	6.72	MS	4.33	M	-2.68
“Salumpikit” (CT)	0.57	T	1.15	T	1.67	T	7.37
“IR-20” (CS)	8.51	S	9.00	S	6.33	MS	-8.48

Notes: Index , LR= leaf rolling, LRC= leaf rolling criteria, LD= leaf drying, LDC= leaf drying criteria, RA= recovery ability, RAC= recovery ability criteria, S= sensitive, MS= mild sensitive, M= moderate, MT= mild tolerant, T= tolerant, CT= check tolerant, CS= check sensitive.

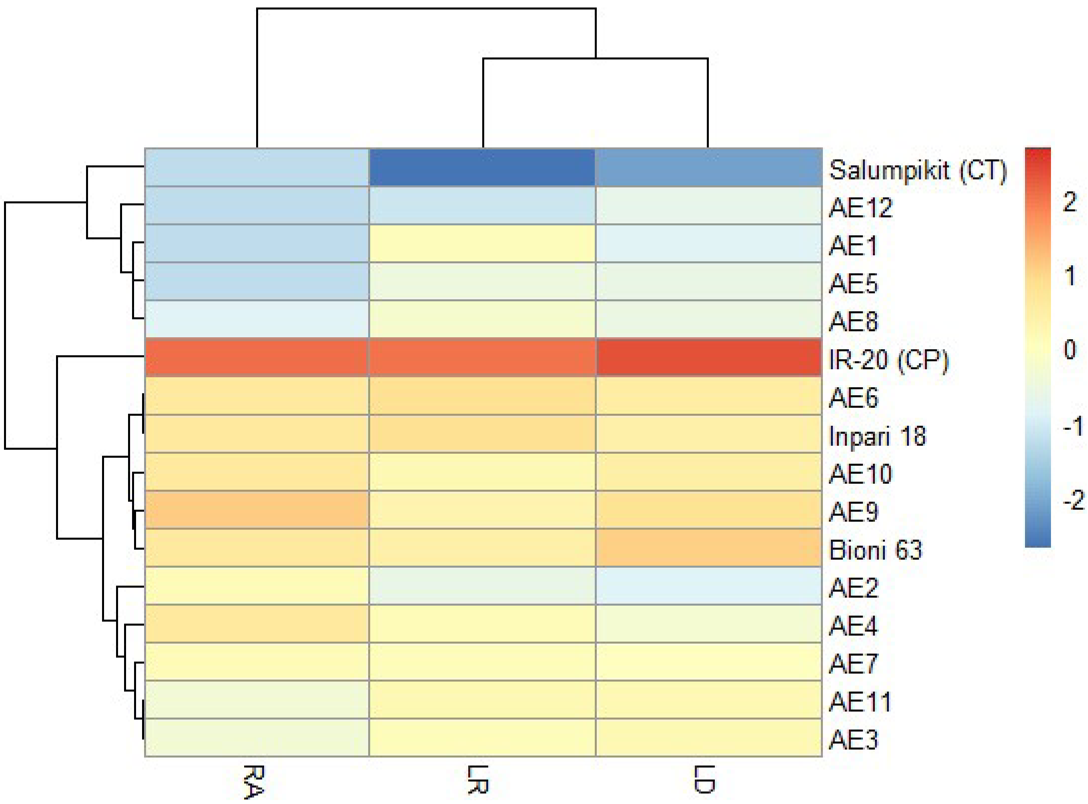

Figure 2. Heatmap of doubled haploid rice lines based on drought test characters. LR= leaf rolling, LD= leaf drying, RA= recovery ability

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characters, and a negative and high correlation with fresh and dry plant weight characters. The selection index values for drought tolerance identified seven lines with positive selection index values, namely AE1, AE2, AE3, AE5, AE8, AE11, and AE12. Based on the heatmap visualization, four lines, AE12, AE1, AE5, and AE8, exhibited a seedling-stage tolerance level similar to the "Salumpikit" drought-tolerant check and were deemed suitable based on drought tolerance characters.

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References

Ali, Z., Merrium, S., Habib-ur-Rahman, M., Hakeem, S., Saddique, M.A.B., and Sher, M.A. (2022). Wetting mechanism and morphological adaptation; leaf rolling enhancing atmospheric water acquisition in wheat crop—a review. *Environmental Science and Pollution Research* **29**, 30967–30985. DOI: https://doi.org/10.1007/s11356-022-18846-3.

Ambikabathy, A., Banumathy, S., Gnanamalar, R.P., Arunchalam, P., Jeyaprakash, P., Amutha, R., and Venkatraman, N.S. (2019). Evaluation of rice genotypes for seedling and reproductive stage drought tolerance. *Electronic Journal of Plant Breeding* **10**, 1122–1132. DOI: https://doi.org/10.5958/0975-928X.2019.00143.1.

Baniya, S., Thapa. L.B., and Pokhrei, C.P. (2020). Effect of water-deficit stress on the selected landraces and improved varieties of rice (*Oryza sativa* l.) in Nepal. *Agrivita Journal of Agricultural Science* **42**, 381-392. DOI: https://doi.org/10.17503/agrivita.v42i2.2554.

Bewick, V., Cheeck, L., and Ball, J. (2003). Statistics review 7: Correlation and regression. *Critical Care* **7**, 451-459. DOI: https://doi.org/10.1186/cc2401.

Bhandari, U., Gajurel, A., Khadka, B., Thapa, I., Chand, I., Bhatta, D., Pundel, A., Pandey, M., Sherstha, S., and Sherstha, J. (2023). Morpho-physiological and biochemical response of rice (*Oryza sativa* L.) to drought stress: A review. *Heliyon* **9**, e13744. DOI: https://doi.org/10.1016/j.heliyon.2023.e13744.

[BMKG] Indonesian Agency for Meteorological, Climatological and Geophysics. (2024). "Pemantauan Musiman Juli-September (Q3) 2023". https://cdn.bmkg.go.id/Web/Q3_2023.pdf. [August 26, 2024].

Botahala, L., Djasibani, H.R., Oualeng, A., Makanmoy, Y.R., and Botahala, D.E. (2021). Mencegah laju kekeringan sungai akibat pemanasan global. *Jurnal Pemberdayaan Masyarakat Berkarakter* **4**.

[BPS] Central Bureau of Indonesian Statistics. (2022). "Rice Harvest Area, Production and Productivity by Province 2020-2022". https://www.bps.go.id/indicator/53/1498/1/luas-panen-produksi-dan-produktivitas-padi-menurut-provinsi.html [May 15, 2024].

[BPS] Central Bureau of Indonesian Statistics. (2023). "Harvest Area and Rice Production in Indonesia 2023 (Provisional Figures)". https://www.bps.go.id/id/pressrelease/2023/10/16/2037/luas-panen-dan-produksi-padi-di-indonesia-2023--angka-sementara-html [May 15, 2024].

[BPS] Central Bureau of Indonesian Statistics. (2024). "Jumlah Curah Hujan dan Jumlah Hari Hujan (mm), 2022-2023". https://bogorkota.bps.go.id/id/statistics-table/2/MTUzIzI=/jumlah-curah-hujan.html [March 18, 2025].

Cal, A.J., Sanciangco, M., Rebolledo, M.C., Luquet, D., Torres, R.O., McNally, K.L., and Henry, A. (2019). Leaf morphology, rather than plant water status, underlies genetic variation of rice leaf rolling under drought. *Plant Cell, and Environment* **42**, 1532-1544. DOI: https://doi.org/10.1111/pce.13514.

Chen, D., Wang, S., Cao, B., Leng, G., Li, H., Yin, L., Shan, L., and Deng., X. (2016). Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Frontiers in Plant Science* **6**, 1241. DOI: https://doi.org/10.3389/fpls.2015.01241.

Conover, W.J. (1999). "Practical Nonparametric Statistics". 3rd Edition. John Wiley and Sons.

Cui, Y., Li, R., Li, G., Zhang, F., Zhu, T., Zhang, Q., Ali, J., Li, Z., and Xu., S. (2020). Hybrid breeding of rice via genomic selection. *Plant Biotechnology Journal* **18**, 57-67. DOI: https://doi.org/10.1111/pbi.13170.

Received 30/10/2024; Revised 30/03/2025; Accepted 23/04/2025
https://doi.org/10.29244/jtcs.12.02.346-357

Dewi, I.S., and Purwoko, B.S. (2012). Kultur antera untuk percepatan perakitan varietas padi di Indonesia. *Jurnal AgroBiogen* **8**, 78-88.

Djarwanto, S., P. (2005). “Statistik Induktif”. 5th ed. BPFE.

Falconer, D.S., and Mackay T.F.C. (1996). “Introduction to Quantitative Genetics”. 4th ed. Longman Essex.

Fukagawa, N.K., and Ziska, L.H. (2019). Rice: Importance for global nutrition. *Journal of Nutritional Science and Vitaminology* **65**, S2-S3. DOI: https://doi.org/10.3177/jnsv.65.s2.

Gomez, K.A., and Gomez, A.A. (1995). “Prosedur Statistik untuk Penelitian Pertanian”. UI Press.

Hadianto, W., Purwoko, B.S., Dewi, I.S., Suwarno, W.B., and Hidayat, P. (2023). Selection index and agronomic characters of doubled haploid rice lines from anther culture. *Biodiversitas* **24**, 1511-1517. DOI: https://doi.org/10.13057/biodiv/d240321.

Hasanuzzaman, M., Shabala, L., Brodribb, T.J., Zhou, M., and Shabala, S. (2022). Understanding the role of physiological and agronomical traits during drought recovery as a determinant of differential drought stress tolerance in barley. *Agronomy* **12**, 2136. DOI: https://doi.org/10.3390/agronomy12092136.

Hassan, M.A., Dahu, N., Hongning, T., Qian, Z., Yueming, Y., Yiru, L., Shimei, W. 2023. Drought stress in rice: morpho-physiological and molecular responses and marker-assisted breeding. *Frontiers in Plant Science* **14**, 1215371. DOI: https://doi.org/10.3389/fpls.2023.1215371.

Herawati, R., Alnopri, Masdar, Simarmata, M., Sipriyadi, and Sutrawati, M. (2021). Identification of drought tolerant markers, DREB2Aand BADH2genes, and yield potential from single-crossing varieties of rice in Bengkulu, Indonesia. *Biodiversitas* **22**, 785-793. DOI: https://doi.org/10.13057/biodiv/d220232.

[IRRI] International Rice Research Institute. (2014). “Standard Evaluation System (SES) for Rice”. http://www.knowledgebank.irri.org/images/docs/rice-standard-evaluation-system.pdf [July 7, 2024].

Kartika, K., Sakagami, Jun-Ichi, Lakitan, B., Yabuta, S., Wijaya, A., Kadir, S., Widuri, L.I., Siaga, E., and Nakao, Y. (2020). Morpho-physiological response of *Oryza glaberrima* to gradual soil drying. *Rice Science* **27**, 67-74. DOI: https://doi.org/10.1016/j.rsci.2019.12.007.

Kartina, N., Purwoko, B.S., Dewi, I.S., Wirnas, D., and Nindita, A. (2019). Skrining awal toleransi galur-galur dihaploid padi gogo terhadap cekaman kekeringan pada stadia bibit. *Indonesian Journal of Agronomy* **47**, 1-8. DOI: https://doi.org/10.24831/jai.v47i1.22766.

[Kementan] Indonesian Ministry of Agriculture and the Indonesian Agency. (2021). “Rencana Strategis Kementerian Pertanian Tahun 2020-2024”. https://ppid.pertanian.go.id/doc/1/Draft%20Renstra%202020-2024%20edited%20BAPPENAS%20(Final).pdf [July 31, 2023].

Kiernan, D. (2014). “Natural Resources Biometrics”. Open SUNY Textbooks.

Krieger-Liszkay, A., Krupinska, K., and Shimakawa, G. (2019). The impact of photosynthesis on initiation of leaf senescence. *Physiologia Plantarum* **166**, 148-164. DOI: https://doi.org/10.1111/ppl.12921.

Kumar, K.P., Binodh, A.K., Saravanan, S., Senthil, A., and Kumar, N.S. (2019). Rapid screening for drought tolerance in traditional landraces of rice (*Oryza sativa* L.) at seedling stage under hydroponics. *Electronic Journal of Plant Breeding* **10**, 636-644. DOI: http://dx.doi.org/10.5958/0975-928X.2019.00080.2.

Liu, J., and Xu, Y. (2022). T-Friedman test: a new statistical test for multiple comparison with an adjustable conservativeness measure. *International Journal of Computational Intelligence Systems* **15**, 1-19. DOI: https://doi.org/10.1007/s44196-022-00083-8.

Malau, L.R.E., Rambe, K.R., Ulya, N.A., and Purba, A.G. (2023). Dampak perubahan iklim terhadap produksi tanaman pangan di Indonesia. *Jurnal Penelitian Pertanian Terapan* **23**, 34-46. DOI: https://doi.org/10.25181/jppt.v23i1.2418.

Mustikarini, E.D., Lestari, T., Santi, R., Prayoga, G.I., Cahya, Z. (2022). Short communication: Evaluation of F6 generation of upland rice promising lines for drought stress tolerance. *Biodiversitas* **23**, 3401-3406. DOI: https://doi.org/10.13057/biodiv/d230712.

Received 30/10/2024; Revised 30/03/2025; Accepted 23/04/2025
https://doi.org/10.29244/jtcs.12.02.346-357

Naylor, R.L., Battisti, D.S., Vimoont, D.J., Falcon, W.P., and Burke, M.B. (2007). Assessing risks of climate variability and climate change for Indonesian rice agriculture. *PNAS* **104**, 7752-7757. DOI: https://doi.org/10.1073/pnas.0701825104.

Ndikuryayo, C., Ndayiragije, A., Kilasi, N.L., and Kusolwa, P. (2023). Identification of drought-tolerant rice (*Oryza sativa* L.) genotypes with Asian and African backgrounds. *Plants* **12**, 1-17. DOI: https://doi.org/10.3390/plants12040922.

Oliveira, R.L., Pinho, R.G.V., Ferreira, D.F., Pires, L.P.M., and Melo, W.M.C. (2014). Selection index in the study of adaptability and stability in maize. *The Scientific World Journal* **2014**,1-6. DOI: https://doi.org/10.1155/2014/360570.

Oo, N.A., Ngwe, K., and Myint, N.O. (2020). Evaluation of drought tolerance for improved rice lines in terms of yield, chlorophyll content, and water use efficiency. International *Journal of Environmental and Rural Development* **11**, 25-31. DOI: https://doi.org/10.1155/2014/360570.

Opalofia, L., Yusniwati, and Swasti, E. (2018). Drought tolerance in some of red rice line based on morphology at vegetative stage. International *Journal of Environment, Agriculture and Biotechnology* **3**, 1995-2000. DOI: https://doi.org/10.22161/ijeab/3.6.6.

Panda, D., Mishra, S.S., and Behere, P.K. (2021). Drought tolerance in rice: focus on recent mechanisms and approaches. *Rice Science* **28**, 119-132. DOI: https://doi.org/10.1016/j.rsci.2021.01.002.

Pereira, D.G., Afonso, A., and Medeiros, F.M. (2015). Overview of Friedman's test and post-hoc analysis. *Communications in Statistics-Simulation and Computation* **44**. DOI: https://doi.org/10.1080/03610918.2014.931971.

Purwoko, B.S., Dewi I.S., and Khumaida, N. (2010). Rice anther culture to obtain double-haploids with multiple tolerances. *Asia Pacific Journal of Molecular Biology and Biotechnology* **18**.

Rajan, J. (2023). "5 Emerging Challenges in Rice Cultivation Faced by Farmers: Organica's Solutions to High Yield and Crop Management". https://organicabiotech.com/5-emerging-challenges-in-rice-cultivation-faced-by-farmers-organicas-solutions-to-high-yield-and-crop-management/ [May 15, 2023].

Sabouri, A., Dadras, A.R., Azari, M., Kouchesfahani, A.S., Taslimi, M., and Jalalifar, R. (2022). Screening of rice drought tolerant lines by introducing a new composite selection index and competitive with multivariate methods. *Scientific Reports* **12**, 2163. DOI: https://doi.org/10.1038/s41598-022-06123-9.

Sarwendah, M., Lubis, I., Junaedi, A., Purwoko, B.S., Sopandie, D., and Dewi, A.K. (2022). Application of selection index for rice mutant screening under a drought stress condition imposed at reproductive growth phase. *Biodiversitas* **23**, 5446-5452. DOI: https://doi.org/10.13057/biodiv/d231056.

Shen, C., Zhang, Y., Li, Q., Liu, S., He, F., An, Y., Zhou, Y., Liu, C., Yin, W., and Xia, X. (2021). PdGNC confers drought tolerance by mediating stomatal closure resulting from NO and H2O2 production via the direct regulation of PdHXK1 expression in Populus. *New Phytologist* **230**, 1868-1882. DOI: https://doi.org/10.1111/nph.17301.

Singh, C.M., Kumar, B., Mehandi, S., and Chandra, K. (2012). Effect of drought stress in rice: a review on morphological and physiological characteristics. *Trends in Biosciences* **5**.

Spearman, C. (1904). The proof and measurement of association between two things. *The American Journal of Psychology* **15**, 72-101 DOI: https://doi.org/10.2307/1412159.

Sukarman, Mulyani, A., and Purwanto, S. (2018). Modifikasi metode evaluasi kesesuaian lahan berorientasi perubahan iklim. *Jurnal Sumberdaya Lahan* **12**.

Susanto, U., Rohaeni, W.R., and Sasmita, P. (2019). Selecting traits for drought tolerance screening in rice In "The 1st International Conference on Agriculture and Rural Development", Banten, Indonesia. IOP Conference Series: Earth and Environmental Science 383. DOI: https://doi.org/10.1088/1755-1315/383/1/012049.

Taleb, M.H., Majidi, M.M., Pirnajmedin, F., and Maibody, S.A.M.M. (2023). Plant functional trait responses to cope with drought in seven cool-season grasses. *Scientific Reports* **13**, 5285. DOI: https://doi.org/10.1038/s41598-023-31923-y.

Taryono, Supriyanta, Basunanda, P., Wulandari, R.A., Nurmansyah, Ambarwati, E., Arsana, I.G.K.D.,

Received 30/10/2024; Revised 30/03/2025; Accepted 23/04/2025
https://doi.org/10.29244/jtcs.12.02.346-357

Aristya, V.E., Purba, A.E., Aisya, A.W., and Alam, T. (2023). Selection of drought-tolerant rice genotypes under cajuput (*Melaleuca cajuputi subsp. cajuputi*) agroforestry system. *Biodiversitas* **24**, 4791-4802. DOI: https://doi.org/10.13057/biodiv/d240920.

Tirtana, A., Purwoko, B.S., Dewi, I.S., and Trikoesoemaningtiyas. (2021). Selection of upland rice lines in advanced yield trials and response to abiotic stress. *Biodiversitas* **22**, 4694-4703. DOI: https://doi.org/10.13057/biodiv/d221063.

Wang, X., Huang, J., Peng, S., and Xiong, D. (2023). Leaf rolling precedes stomatal closure in rice (*Oryza sativa*) under drought conditions. *Journal of Experimental Botany* **74**, 6650–6661. DOI: https://doi.org/10.1093/jxb/erad316.

Yang, Y., Guo, Y., Zhong, J., Zhang, T., Li, D., Ba T., Xu T., Chang, L., Zhang, Q., and Sun, M. (2020). Root physiological traits and transcriptome analyses reveal that root zone water retention confers drought tolerance to *Opisthopappus taihangensis*. *Scientific Reports* **10**, 2627. DOI: https://doi.org/10.1038/s41598-020-59399-0.

Yang, Y., Yu, J., Qian, Q., and Shang, L. (2022). Enhancement of heat and drought stress tolerance in rice by genetic manipulation: a systematic review. *Rice* **15**, 2-18. DOI: https://doi.org/10.1186/s12284-022-00614-z.

Yu, B., Chao, D., and Zhao, Y. (2024). How plants sense and respond to osmotic stress. *Journal of Integrative Plant Biology* **66**, 394-423. DOI: https://doi.org/10.1111/jipb.13622.

Zhang, X., and Amer, P. (2021). A new selection index percent emphasis method using subindex weights and genetic evaluation accuracy. *Journal of Dairy Science* **104**, 5827-5842. DOI: https://doi.org/10.3168/jds.2020-19547.

Zar, J.H. (2005). “Spearman Rank Correlation: Overview in Encyclopedia of Biostatistics”. 2nd ed. John Wiley and Sons. DOI: https://doi.org/10.1002/0470011815.b2a15150.