



## Utilizing Stress Tolerance Index and Principal Component Analysis for Rice Selection in Hydroponic Drought Screening Based on Physiological Traits

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### ABSTRACT

The environment and selection criteria are critical during the seedling phase of artificially hydroponic drought screening. Notably, the selection environment is intricately linked to the chosen selection criteria. Physiological parameters, which offer precise insights into genotype performance, must be complemented by rigorous statistical analyses. In this study, the stress tolerance index (STI) and principal component analysis (PCA) were instrumental in defining the optimal selection environment for rice under hydroponic drought conditions. This research was conducted at the screen house using a nested and randomized block design, whereby replications were nested in the level of drought stress. Three levels of drought stress were applied across eight rice varieties, each with three replications. Physiological observations especially photosynthetic characteristics were observed. The results demonstrated that STI and PCA are effective tools for screening rice genotypes for drought tolerance under hydroponic conditions. The character of solar radiation, including absorption and transmission, is used as a correction for the chlorophyll character. The STI under 10% PEG stress predicted tolerance under 20% PEG stress with a high determination coefficient ( $R^2 = 0.76$ ). Thus, a 10% PEG concentration is recommended as the selection environment for hydroponic drought screening at the seedling stage. These findings have significant implications for developing drought-tolerant rice varieties, which are crucial for ensuring global food security in the face of climate change.

**Keywords:** Drought stress, Hydroponic screening, *Oryza sativa*, PCA, STI

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### INTRODUCTION

The rising global population is driving an unprecedented increase in food demand, particularly for rice (Walker, 2016; Adam, 2021). This surge in rice production poses a formidable challenge, especially in the context of global warming (Rumanti et al., 2018; Rondhi et al., 2019), which is exacerbating the frequency and severity of drought stress in rice cultivation (Fahad et al., 2017; Muhammad et al., 2018; Godoy et al., 2021). These stresses have a detrimental effect on rice growth and productivity, as evidenced by the findings of various studies (Fahad et al., 2017; Upadhyaya and Panda, 2019; Yang et al., 2019; Panda et al., 2021; Akbar et al., 2021). Therefore, it is crucial to develop tolerant rice varieties as part of the efforts to ensure global food security.

Tolerant variety development is an effective and

efficient method of resolving the problem of drought stress. However, such assembly requires an effective line screening method (Anshori et al., 2018). Artificial line screening is more precise since it helps manage stress levels, lowering the variance of randomization bias (Anshori et al., 2018; Akbar et al., 2018; Farid et al., 2021a). One type of artificial screening that can be performed is on the seedling phase using Polyethylene Glycol (PEG) hydroponics to determine abiotic tolerance (Purbajanti et al., 2019; Al Azzawi et al., 2020; Altaf et al., 2021; Farid et al., 2021b; Hussain et al., 2021). Therefore, the selection on rice drought tolerance screening through PEG 6000 static hydroponics can increase the effectiveness screening. Nevertheless, PEG 6000 screening also requires development in determining the critical environment and character selection (Haroon et al., 2022).

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Determination of an optimal selection environment is highly dependent on the selected character. The physiological characteristics are associated with planting metabolic processes (Bhanu et al., 2016; Swapna and Shylaraj, 2017; Farid et al., 2021b; Sheldon et al., 2021), which influence the morphological characteristics of the plant both directly and indirectly (Bhanu et al., 2016; Khadka et al., 2020), resulting in physiological characters having a higher level of precision than morphological characters. Photosynthetic efficiency is a key physiological trait frequently utilized in assessing plant performance. As the primary metabolic process responsible for sugar synthesis, photosynthesis is crucial for plant growth and development (Bhanu et al., 2016; Kapoor et al., 2020; Al Azzawi et al., 2020; Sheldon et al., 2021). Stress conditions, such as drought, have a direct impact on this metabolic process (Brito et al., 2019; Kapoor et al., 2020; Khadka et al., 2020). Consequently, identifying the optimal selection environment based on photosynthetic characteristics is vital for effectively screening rice genotypes for drought tolerance using PEG 6000 in static hydroponic systems.

Selection traits are commonly utilized in conjunction with the stress tolerance index (STI) to evaluate a plant's potential to withstand stress (Anshori et al., 2019; Farid et al., 2021a; Singh et al., 2015). However, this index has the shortcoming of being limited to a single character selection. According to Anshori et al. (2019, 2020), assessing tolerance potential based on many correlated characters can improve precision using principal component analysis (PCA) (Zafar et al., 2023). PCA can partition and compress big data more straightforwardly while retaining most of the initial data's total diversity (Jolliffe & Cadima, 2016; Widyawan et al., 2020; Zafar et al., 2022). Anshori et al. (2019), Farid et al. (2021a, b), and Singh et al. (2015) demonstrated the efficacy of the stress tolerance index (STI) in conjunction with multivariate analysis. However, the research concentrated on the primary characteristic of productivity. Therefore, STI and multivariate analysis also apply to determining the character and environment of rice drought screening selection in the vegetative phase. Therefore, the research aims are to evaluate the effectiveness of the stress tolerance index and principal component analysis in determining the criteria and selection environment under rice drought screening using PEG 6000 static hydroponics.

## MATERIALS & METHODS

### Experimental Design

The experiment was conducted at the greenhouse of Hasanuddin University, Makassar, South Sulawesi Province, Indonesia, from August to November 2020, with average and maximum drinking temperatures of 24 and 32 °C, respectively. The study employed a nested and randomized block design, with replications nested within different levels of drought stress induced by polyethylene glycol (PEG) 6000: 0% (control), 10%, and 20%. PEG 6000 is an inert substance that is not absorbed by plants but affects the osmotic conditions of the growing environment, which makes it difficult for plants to absorb water or is physiologically drought stress (Datir and

Inamdar 2019; Purbajanti et al., 2019; Sallam et al., 2019). This makes PEG 6000 an effective agent for simulating drought stress in screening studies. The other factor in this study was varieties effect. The varieties used include Salumpikit (check drought-tolerant), Inpari 29, Ciherang, Inpari 34, IR 20 (check drought-sensitive), Jeliteng, IR 29 (check salinity sensitive), and Pokkali (check salinity tolerant). The treatment combination was repeated thrice, producing 72 experimental units of 4 samples.

### Research Procedure

The experimental procedures followed in this research were based on the methodologies described by Laraswati et al. (2021) and Fatimah et al. (2023). The treatment was started by sowing the seeds in a Petri dish for seven days. Subsequently, the seeds were transferred to ABmix hydroponic media at a concentration of 5mL L<sup>-1</sup>, which was given to prevent osmotic shock stress. In the first stage, the concentration of PEG was applied to hydroponic media 13 days after planting (DAP). After three days, it was increased to the maximum level according to the drought stress. The pH nutrients were maintained between 5.8 and 6.2 using HCl and NaOH to lower or raise the pH. Physiological observations were made ten days after completing the PEG application or 24 DAP. Chlorophyll a, b, and total chlorophyll were determined using CCM 200, as well as radiation parameters such as absorption, transmission, reflection, and scope using Miniature Leaf Spectrometer CI-710, and stomatal characters such as intensity and stomata opening.

### Observation and Data Analysis

The observation data on photosynthetic physiology were analyzed using variance analysis first. Subsequently, the significant characters affected by the level of drought stress were analyzed by principal component analysis, which became the basis for forming physiological indices at each level of PEG drought stress. Before forming the physiological index, each eigenvector on PC1 is adjusted to the value of the variance proportion (Anshori et al., 2021). After that, each genotype value was changed to a stress tolerance index (STI) under 10% and 20% PEG concentrations. Finally, the STI was validated using simple regression analysis between 10% and 20% PEG. The following shows the formulation of the stress tolerance index (STI).

The stress Tolerance Index (STI) is calculated by the equation (Fernandez 1992):

$$STI = (Y_p \times Y_s) / (\bar{Y}_p)^2 \quad (1)$$

$Y_p$  = Character values of each variety under normal/unstressed conditions.

$Y_s$  = Character values of each variety in a stress condition.

$\bar{Y}_p$  = The average character value of all varieties under normal/unstressed conditions.

## RESULTS

The analysis of variance showed that the different levels of stress treatments, varieties, and their interactions significantly affected the chlorophyll A, chlorophyll B, and total chlorophyll characters (Table 1). Based on the

spectrophotometer character, absorption and transmission are affected considerably by PEG stress treatment. However, the stomatal physiological traits were not significantly affected by the different stress treatments or variety types. The average of all physiological characters is shown in Supplementary 1.

The principal component analysis results shown in Table 2 are focused on PC1. For each stress treatment, the proportion of variance explained by PC1 was greater than 0.5. Furthermore, PC1 at 10% PEG had the highest proportion of variance (PV) (0.719), while PEG at 20% had the lowest PV (0.5952). The chlorophyll character eigenvectors point in the same direction at all stress levels. On the other hand, except for 10% PEG, the spectrophotometer characters, absorption and

transmission, were inversely proportional to the chlorophyll. Additionally, the eigenvector values for chlorophyll were higher than those for the spectrophotometric traits. Consequently, to normalize the variance in PC1 under normal conditions (0% PEG), the eigenvector values for PC1 at 10% PEG and 20% PEG were multiplied by -1.

The 3D PC plots showed several groupings of genotypes based on three PC PEG interactions (0, 10, and 20%) (Fig. 1). The Jeliteng and Salumpikit varieties were relatively categorized in the same group. The varieties Ciherang, Inpari 29, and Inpari 34 also showed clustering potential. Inpari 34 also showed the potential for grouping. Meanwhile, the Pokkali and IR 29 varieties also showed similar grouping potential. In contrast, IR 20 was the lowest base of the potential interaction of the three PCs.

**Supplementary 1:** Average of all Physiological Characters

Varieties	PEG (%)	Chlorophyll a ( $\mu\text{mol m}^{-2}$ )	Chlorophyll b ( $\mu\text{mol m}^{-2}$ )	Chlorophyll total ( $\mu\text{mol m}^{-2}$ )	Absorption (%)	Transmission (%)	Reflection (%)	Scope	Stomatal (n $\text{mm}^{-2}$ )	Intensity	Width of Stomata Opening (mm)
Salumpikit	0	449.12	204.55	665.66	14.72	16.97	5.66	3699.42	160.79		0.0832
Inpari 29	0	380.30	188.18	575.63	15.38	17.22	5.74	3765.37	200.42		0.0680
Ciherang	0	432.95	200.08	644.03	15.49	17.05	5.69	3716.63	229.30		0.0581
Inpari 34	0	342.75	182.33	528.92	15.09	16.84	5.70	3732.04	198.73		0.0468
IR20	0	206.51	158.24	357.87	15.72	16.87	5.66	3705.39	212.88		0.0874
Jeliteng	0	401.29	194.34	604.07	15.11	16.97	5.65	3694.56	242.89		0.0748
IR 29	0	295.00	172.03	467.35	16.13	17.36	5.78	3793.69	190.80		0.0406
Pokkali	0	289.08	170.96	459.88	14.76	17.27	5.71	3746.73	258.74		0.0390
Average		349.63	183.84	537.93	15.30	17.07	5.70	3731.73	211.82		0.0622
Salumpikit	10	224.71	162.37	381.92	15.49	16.29	5.46	3581.47	164.19		0.0638
Inpari 29	10	263.90	166.46	428.12	15.67	17.03	5.67	3717.97	161.36		0.0529
Ciherang	10	181.36	154.57	326.95	16.21	17.03	5.67	3715.65	214.01		0.0594
Inpari 34	10	274.43	168.49	441.51	14.84	14.80	5.63	3688.03	333.47		0.0552
IR20	10	136.43	148.77	272.65	15.11	17.06	5.67	3704.09	247.42		0.0542
Jeliteng	10	203.09	157.47	353.34	15.24	17.15	5.71	3739.84	199.86		0.0526
IR 29	10	197.57	156.53	346.40	17.38	17.10	5.71	3745.80	156.83		0.0714
Pokkali	10	193.30	155.85	341.09	16.33	16.59	5.53	3629.90	214.01		0.0597
Average		209.35	158.81	361.50	15.78	16.63	5.63	3690.34	211.39		0.0586
Salumpikit	20	19.36	134.28	98.07	17.37	20.61	6.65	4426.96	169.29		0.0343
Inpari 29	20	10.94	132.22	65.75	18.07	20.29	6.73	4385.49	133.05		0.0523
Ciherang	20	15.43	132.84	74.07	17.93	17.15	5.72	3761.32	151.17		0.0589
Inpari 34	20	17.01	131.37	57.47	18.09	18.56	6.08	3987.57	156.26		0.0481
IR20	20	2.68	131.76	41.05	16.45	18.20	6.10	3993.37	272.89		0.0458
Jeliteng	20	53.64	135.65	100.62	18.20	19.09	6.36	4181.49	120.59		0.0395
IR 29	20	10.94	133.22	82.98	19.17	19.57	6.49	4367.61	154.00		0.0597
Pokkali	20	1.31	132.94	71.44	16.45	17.70	5.94	3894.33	270.63		0.0377
Average		16.41	133.04	73.93	17.72	18.90	6.26	4124.77	178.49		0.0470

**Table 1:** Analysis of variance (ANOVA) of photosynthetic physiological characters in rice screening through PEG. Hydroponics.

Characters	Stress (S)	Varieties (V)	SxV	CV
Chlorophyll a	0.0008**	0.0002**	0.0134**	26.92
Chlorophyll b	0.0026**	0.0005**	0.0049**	5.86
Chlorophyll total	0.0010**	0.0009**	0.0493**	22.21
Absorption	0.0223*	0.1340ns	0.8561ns	8.33
Transmission	0.0386*	0.4839ns	0.6524ns	9.08
Reflection	0.0770ns	0.7716ns	0.8745ns	8.35
Scope	0.0675ns	0.6109ns	0.7578ns	7.92
Stomatal Intensity	0.3226ns	0.3480ns	0.8422ns	28.8
Width of stomata opening	0.1640ns	0.9504ns	0.8136ns	31.27

Notes: CV = coefficient of variance, \*\*: Significantly influential at 1% level ( $P \leq 0.01$ ); \*: Significantly influential at 5% level ( $0.01 \leq P \leq 0.05$ ); ns: No significant.

**Table 2:** Principal component (PC) 1 of various stress levels.

Variables	Principal component analysis			Index PC1 corrected at each environment.		
	PC1 0%	PC1 10%	PC1 20%	PC1 0%	PC1 10%	PC1 20%
Chlorophyll a	0.5401	-0.5094	-0.4876	0.355	-0.367	-0.290
Chlorophyll b	0.5431	-0.5155	-0.5201	0.357	-0.371	-0.310
Chlorophyll total	0.5407	-0.5105	-0.5398	0.355	-0.367	-0.321
Absorption	-0.3262	0.2188	-0.326	-0.214	0.157	-0.194
Transmission	-0.1211	0.4078	-0.3067	-0.080	0.293	-0.183
Proportion of Variance	0.6571	0.7196	0.5952			
Cumulative Proportion	0.6571	0.7196	0.5952			
EigenValues	3.2855	3.5979	2.9761			

Notes: PC = Principal component

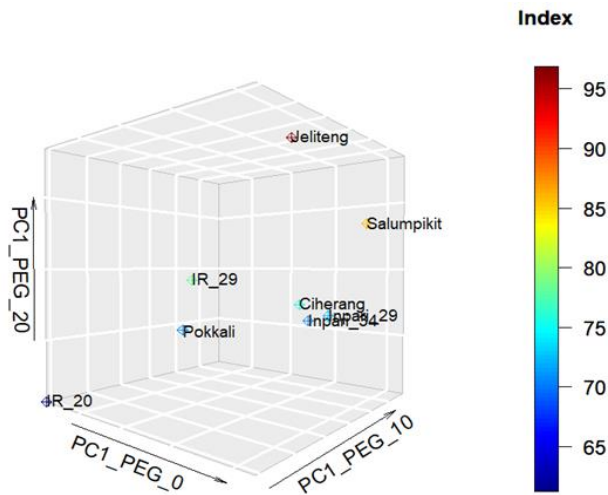


Fig. 1: 3Dplot in interaction analysis among three plant environments

The physiological index analysis results at each level of PEG stress showed that under normal conditions (0 PEG), the highest average index was 376.18. In contrast, 20 PEGs had the most minor average index at 76.59, as shown in Table 3. Under normal conditions, Salumpikit is the best variety in the physiological index with 464.39; at 10% PEG condition, the Inpari 34 variety had the best physiological index with 318.61; at 20% PEG condition, the Jeliteng variety had the best physiological index with 96.90. Based on the results of the stress tolerance index (STI) analysis in Table 3, the 10% PEG has an average STI (0.70), which is better than the 20% PEG (0.30). At STI 10% PEG, almost all varieties were classified as moderate, except for the IR 20, which was classified as sensitive. The Salumpikit variety has the best STI value at 10% PEG. At 20% PEG, all varieties were classified as sensitive varieties. Meanwhile, the Jeliteng variety has the highest STI value, while the IR 20 variety has the lowest STI value.

Table 3: Physiological index and stress tolerance index (STI) at each level of PEG concentration.

Varieties	Physiology Index			STI 10% PEG	Classification	STI 20% PEG	Classification
	0% PEG	10% PEG	20% PEG				
Salumpikit	464.39	275.68	85.83	0.90	Moderate	0.35	Sensitive
Inpari 29	401.98	308.29	72.44	0.88	Moderate	0.33	Sensitive
Ciherang	449.20	236.38	76.01	0.75	Moderate	0.26	Sensitive
Inpari 34	370.06	318.61	70.97	0.83	Moderate	0.33	Sensitive
IR 20	252.20	197.97	61.27	0.35	Sensitive	0.18	Sensitive
Jeliteng	421.81	255.23	96.90	0.76	Moderate	0.36	Sensitive
IR 29	327.30	249.99	78.37	0.58	Moderate	0.29	Sensitive
Pokkali	322.46	246.53	70.91	0.56	Moderate	0.26	Sensitive
Average	376.18	261.09	76.59	0.70		0.30	

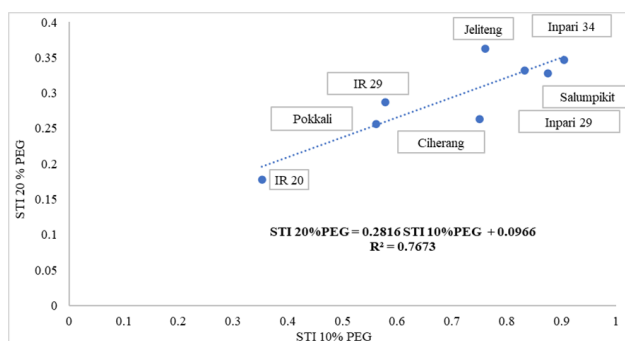


Fig. 2: Regression analysis between STI PEG 10% and PEG 20%.

The regression analysis results between STI PEG 10% and 20% (Fig. 2) showed good linear regression with a determination value of 0.767. Based on this Fig., the eight varieties are grouped into 3 clusters. The first cluster consisted of Salumpikit, Inpari 29, Inpari 34, and Jeliteng varieties. The second consists of IR 29, Pokkali, and Ciherang, while the third consists of only IR 20.

## DISCUSSION

The results of the physiological analysis showed that the chlorophyll and spectrophotometer characters, in general, had a direct impact on drought stress, as previously reported by Al Azzawi et al. (2020) and Kapoor et al. (2020). However, the chlorophyll character differentiates genotypes' responses to drought stress levels from the solar radiation character. The chlorophyll character is directly related to photosynthesis (Abid et al., 2017; Kapoor et al., 2020; Singh et al., 2020a; Shan et al., 2023). Chlorophyll functions as an antenna for plants, capturing solar radiation, particularly in the blue and red light spectra (Ritchie, 2010; Chowdhury et al., 2017; Yavari et al., 2021). Damage to this photosynthetic antenna impairs the process of water photolysis, which is essential for photosynthesis, ultimately leading to plant starvation (Osakabe et al., 2014). As plants experience drought, ROS experiences a drastic increase and causes damage to the photosynthetic system and mitochondria (Gharibi et al., 2016; Karimpour, 2019; Kapoor et al., 2020; Singh et al., 2020a; Shin et al., 2021; Kumar et al., 2023). Additionally, because water is the primary ingredient of photosystem 1, the net photosynthetic net is significantly reduced, degrading some photosynthetic apparatus, including chlorophyll, during the development of some energy balance in plants (Abid et al., 2017; Brito et al., 2019; Kapoor et al., 2020; Singh et al., 2020b; Kumar et al., 2023; Shan et al., 2023). Therefore, drought stress from PEG will significantly affect the amount of chlorophyll present in plants. However, this impact cannot be separated from the genetic composition of the plant.

Plants have a genetic tolerance mechanism for stress management, including optimizing their chlorophyll content. The degree of tolerance in a genotype is typically inversely correlated with the impact of drought stress it experiences (Khadka et al., 2020; Panda et al., 2021; Sakinah et al., 2024). The varieties used in this study exhibit varying levels of tolerance to salinity and drought stress, which results in differences in chlorophyll content under drought conditions. According to Anshori et al. (2018), the effectiveness of the selection environment assessment is based on the diversity of the tested genotypes; hence, the influence of variety and the variety-stress interaction was significantly related to the chlorophyll character. Additionally, these results showed that chlorophyll is used as a physiological selection character in screening for drought stress.

The spectrophotometer's characteristic relates to how plants absorb solar radiation, which has two opposite properties: photon energy and wavelength. However, both of these properties are used by plants in photosynthesis (Ritchie, 2010; Amthor, 2010; Ullah et al., 2019). In general,

solar radiation is not absorbed by plant leaves for photosynthesis. Instead, they reflect and transmit radiation (Kume, 2017; Mubarak and June, 2019) because leaves have special antennae to capture these energy rays, one of which is chlorophyll (Kume, 2017; Ullah et al., 2019). Therefore, the characteristics of chlorophyll and spectrophotometers are related. In this research, the spectrophotometer characters, including absorption and transmission, did not show different responses between varieties when exposed to drought stress. This indicates that both tolerant and sensitive genotypes have the same apparatus and response when receiving solar radiation, implying that this character does not accurately describe the potential for tolerance between genotypes. However, this character is still considered in the selection process to correct the more dominant tolerance trait of the chlorophyll character.

Multiple characters can be combined in PCA analysis and incorporated into the selection index formula, as reported by Alsbah et al. (2019), Anshori et al. (2019, 2020), Akbar et al. (2021), Karima et al. (2021), Tirtana et al., (2021), and Farid et al., (2021a; 2024), PC1 is the PC with the most significant variance in the initial data diversity partitioning and compaction process (Jolliffe and Cadima, 2016; Dueñas et al., 2024) which indicates it is representative of the initial data set. Therefore, the establishment of a selection index through PC1 becomes effective. Based on this research, the PC1 was considered adequate in describing the diversity of the initial data with PV exceeding 50%, indicating that these characters have variations centered in a particular direction. Hence, the diversity in PC1 has a high PV value. According to the eigenvector values, both standard and 10% PEG conditions exhibit a high degree of diversity, supported by PV, because both conditions have variables with distinct directions. On the other hand, 20% of PEG has relatively low diversity. All characters have the same direction with slightly different eigenvector values, indicating that PEG 10% is more effective as a selection environment than PEG 20%. According to Anshori et al. (2018) and Sakinah et al. (2024), a good selection environment shows excellent diversity. However, this needs to be strengthened by other estimates, one of which is through analysis of relative decline (Ali et al., 2014; De Leon et al., 2015; Anshori et al., 2020).

The results of the PEG physiology index showed that PEG 20% had a very drastic decrease compared to PEG 10%. The relative decline in PEG 20% reached 70.67% (1-76.59/376.18), while the relative decrease in PEG 10% only reached 30.59% (1-261.09/376.18). This decrease implies that 20% PEG induces plastic lines in rice plants compared to 10% PEG. The finding aligns with Sopandie (2014) and Anshori et al. (2018), who report that increasing stress levels results in a drastic or exponential decrease. Additionally, these results support the previous analysis, where 20% stress is not recommended as a selection environment. According to Anshori et al. (2018, 2020) and Farid et al. (2021b), a selection environment is favorable when the average relative decline reaches 50%. Therefore, based on the relative decreasing value of the PEG

physiological index, 10% PEG was considered an artificial drought screening environment compared to 20% PEG, which is still retained in the stress tolerance index-analysis process.

The adaptability and tolerance of genotypes are determined using a value of one in the stress tolerance index analysis. This index represents the intersection of dynamic plant adaptability and stress tolerance (Singh et al., 2015; Anshori et al., 2019; Farid et al., 2021b; Kumar et al., 2024; Sakinah et al., 2024), as indicated using the average genotype under normal conditions as a reference (Anshori et al., 2019). Numerous research, including (Ferreira et al., 2020, Hussain et al., 2021) on drought stress, Anshori et al. (2018, 2019, 2021) on salinity conditions, and Shandilya and Tanti (2019) on aluminum stress, have used this index in the rice tolerance screening process. Based on the STI analysis, the genotypes tested at 10% PEG dominantly had moderate tolerance, except for IR 20. On the other hand, at 20% PEG STI, all genotypes were classified as sensitive. Therefore, Salumpikit and IR 20 have different tolerance potentials and are further used as tolerant (Salumpikit) and sensitive (IR 20) controls in rice drought stress screening. These differences in properties were also reported by Widyastuti et al. (2017), Borah et al. (2017), Akbar et al. (2018), and Farid et al. (2021a), indicating that 10% PEG stress was effective in differentiating tolerance traits in control varieties.

Subsequently, regression analysis between 10% and 20% PEG STI can be used to determine the effectiveness of 10% PEG as a drought screening environment. Anshori et al. (2018), Farid et al. (2021b), Laraswati et al. (2021), and Okasa et al. (2021) reported on the concept of regression analysis in assessing the effectiveness of a selection environment. The results showed that 10% STI PEG could predict 20% STI with a high determination value of 0.767. Also, it describes the grouping of tolerance traits, where the Salumpikit, Inpari 29, Inpari 34, and Jeliteng varieties are grouped as more tolerant than other varieties, especially IR 20. Inpari 34 is also a salinity-tolerant rice variety (Anshori et al., 2018; Subekti et al., 2020). According to Reddy and Jabeen (2016), drought and salinity tolerance have similarities, which indicates they can be used as a supporting control in screening for drought stress. Based on this analysis, 10% PEG stress was effective as a selection environment. According to Widyastuti et al. (2017) and Akbar et al. (2018), 20% PEG is effective in rice drought screening conducted in the germination phase. According to Islam et al. (2018), the germination phase was relatively linear compared to the quadratic seedling phase, implying that the critical point of PEG concentration in seedling conditions is lower than in germination. As a result of these physiological properties, using 10% PEG as a suitable selection environment for rice screening under drought stress is recommended. It also has been used and reported by Quintao et al. (2023), Sakinah et al. (2024), and Fatimah et al. (2023). Additionally, a selection index based on various physiological characteristics is adequate for assessing the selection environment. This concept can also be recommended in screening for stress tolerance, especially drought stress.

## Conclusion

The Stress tolerance index and principal component analysis combination were considered adequate in screening rice drought tolerance through the PEG static hydroponic system. The physiological trait of chlorophyll content is a robust indicator for selecting drought-tolerant rice varieties. To accurately assess this trait, the parameters of solar radiation, including absorption and transmission, are employed to correct for variations in chlorophyll measurement. Based on the findings from the static hydroponic system, a PEG concentration of 10% is recommended as the optimal selection environment for screening rice drought tolerance during the vegetative phase. It is also suggested that the efficacy of this selection environment be further evaluated using a population of crossed lines to validate and potentially enhance the robustness of the selection criteria.

**Conflict of Interest:** The authors declare there is no conflict of interest.

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