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RESEARCH ARTICLE

Image Based-Phenotyping and Selection Index Based on Multivariate Analysis for Rice Hydroponic Screening under Drought Stress

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ABSTRACT The development of rice varieties that are tolerant of drought stress needs to be detected with Image-based phenotyping. This Image-based phenotyping method in combination with selection index and multivariate analysis can characterize the morphological response easily within a short time, which makes it is suitable for rice screening under drought stress. Therefore, this study aims to determine the selection index based on multivariate analysis and assess the effectiveness of using image-based phenotyping in drought rice screening. This study was conducted in two stages, the first was in static hydroponic and the second was in dynamic hydroponic. In static hydroponic, a split-plot design was used, where the levels of drought were the main plots and varieties were the subplots. However, in dynamic hydroponic, a nested design was used, where the replicates were nested in the drought level treatments. Also, The drought level factors used were PEG 0%, PEG 10%, and PEG 20%, and the variety factor consisted of 5 varieties which were repeated three times. The results showed that the selection index for static hydroponic consisted of shoot area (0.421), green shoot area (0.4177), and the area growth rate (0.4192). Meanwhile, the selection index in dynamic hydroponics consisted of object extent Y from the side (0.4516) and convex hull from the side (0.4177). The regression of the two-selection index has a good determination of 0.84. Hence, these results showed that rice screening based on image-based phenotyping can be recommended for rapid screening under drought stress.

Keywords Drought stress, Image-based phenotyping, Multivariate analysis, RGB, Rice

INTRODUCTION

Rice is a commodity that plays an important role in Indonesia's economy and is generally consumed by approximately 90% of the population as a staple food (Donggulo *et al.* 2017). In Indonesia, rice production in 2020 has decreased from 59.2 million tons in the previous year to 54.6 million tons (Iswara *et al.* 2021). This decline in production was influenced by several factors, which includes drought stress. According to Bouman and Tuong (2001), approximately 80% of the cultivation area of rice and other cereals is affected by drought as a limiting factor for production. In addition, several studies have shown that drought reduces the rate of growth and rice yield (Supriyanto 2013; Sujinah and Jamil 2016; Rusmawan *et al.* 2018). Therefore, the development of drought-tolerant varieties becomes a solution for increase rice production.

The development of drought-tolerant varieties requires an effective and efficient screening process, which is carried out artificially or directly in the targeted environment. Moreover, the environment for artificial selection is considered easier to restrain, especially the

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control of stress concentration levels, which makes this method effective and efficient in the increase of the screening process (Anshori *et al.* 2019). In drought stress, artificial screening is carried out using a hydroponic concept with a polyethylene glycol (PEG) selection agent (Swapna and Shylaraj 2017; Mangansige *et al.* 2018; Osmolovskaya *et al.* 2018). A previous study on the use of this method under drought stress conducted by Ai *et al.* (2010), Wening and Susanto (2017), and Farid *et al.* (2021) stated that the use of PEG 6000 with certain concentrations in the early (vegetative) phase can function as an alternative for quick or early selection. This concept is very important in increasing the effectiveness of selection, however, the vegetative screening under salinity stress has to validate or correlate with the reproductive phase, such as the dynamic hydroponic. Meanwhile, this hydroponic makes it possible to measure the tolerance screening until the reproductive phase with a controlled environment is achieved (Farid *et al.* 2021). Therefore, the use of PEG in static and dynamic hydroponic systems can be a good alternative in the screening process of drought tolerance of rice, however, it requires an accurate and precise assessment approach.

Assessment of drought stress tolerance through screening is a common method that is often considered inaccurate. Based on the genetic constitution, drought tolerance nature is polygenic and is indicated by the QTL report that encodes these characteristics (Lanceras *et al.* 2004), therefore, qualitative screening is not involved in the assessment of drought. Observations based on morphology are generally considered to be highly biased (Brereton *et al.* 2015), while physiological and molecular are considered effective and have a relatively low bias, but these approaches are expensive (Nuraida 2012). Therefore, an accurate and precise approach with a relatively low cost is needed in identifying the nature of rice tolerance to drought stress. One method that is considered effective is a digital technology approach through image-based phenotyping.

Image-based phenotyping technology is currently being developed to characterize morphological and physiological responses of a plant using digital images. This technology makes it easy to calculate phenotypes by analyzing plants through images accurately in large numbers within a short time (Das Choudhury *et al.* 2018). Several studies have reported the use of an effective and efficient image-based phenotyping selection method in rice (Hairmansis *et al.* 2014; Siddiqui *et al.* 2014; Duan *et al.* 2018; Guimaraes *et al.* 2020; Kim *et al.* 2020; Vishal *et al.* 2020; Anshori *et al.* 2021a) and maize (Asaari *et al.* 2019; Li *et al.* 2021). However, there are still few studies that examined the development of image-based phenotyping based on selection index and multivariate analysis on the static hydroponic systems.

A selection index is a breeding approach that combines several criteria in one formula (Anshori *et al.* 2021b). This approach is commonly used in plant breeding because it considers all selection criteria with current economic value on each criterion in genotype selection (Rajamani *et al.* 2016; Islam *et al.* 2017). The selection index often combines with multivariate analysis to increase its effectiveness (Anshori *et al.* 2019; Fadhli *et al.* 2020; Akbar *et al.* 2021; Anshori *et al.* 2021b; Farid *et al.* 2021). This multivariate analysis is used to analyze or integrate the variance of large variable data into a simpler and more comprehensive manner for the results to be easily understood (Mattjik and Sumertajaya 2011). Furthermore, several studies have reported the effectiveness of selection with the use of multivariate analysis (Anshori *et al.* 2018; Kose *et al.* 2018; Anshori *et al.* 2019; Fadhli *et al.* 2020; Farid *et al.* 2020; Akbar *et al.* 2021; Anshori *et al.* 2021; Farid *et al.* 2021). Based on these analyzes, the use of image-based phenotyping with the selection index based on multivariate analysis in static and dynamic hydroponic screening is necessary. Therefore, this study aims to determine the selection index based on multivariate analysis and assess the effectiveness of using image-based phenotyping in drought rice screening.

MATERIALS AND METHODS

The two experiments conducted in this study include the destructive test, which was carried out on static hydroponic (floating raft system), and the second was the non-destructive test carried out on dynamic hydroponic

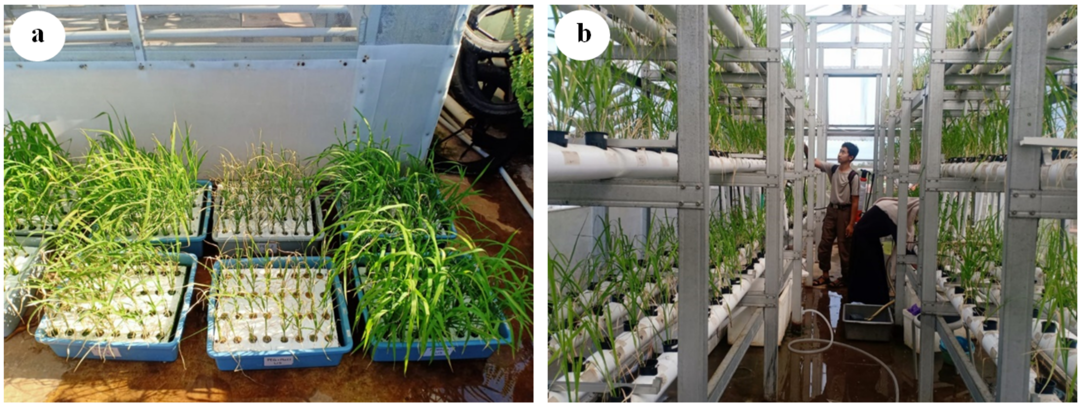

Fig. 1. Images of static hydroponic system at 20 DAS (a) and dinamic hydroponic system at 33 DAS (b).

(deep flow technique system). These studies were carried out simultaneously in the greenhouse, Unhas Lecturer Residence, Makassar City, South Sulawesi Province, Indonesia (22.4 m above sea level [asl]) from August to November 2020.

Experimental design and procedures

The static hydroponic study used a split-plot design with the levels of drought as the main plot and varieties as the subplots (Fig. 1a). However, the dynamic hydroponic used a nested design, where the replicates were nested in the drought level treatments (Fig. 1b) and the drought level factors were PEG 0%, PEG 10%, and PEG 20%. Furthermore, the varieties used were Inpari 34, IR 20 (drought-sensitive check), Salumpikit (drought-resistant check), Ciherang, and Jeliteng.

In this study, the medium used was ABmix with a concentration of 8 mL/L, and the PEG concentration treatments were gradually given to the hydroponic nutrient solution. The first stage was given 1/2 concentration of PEG treatment at the age of 13 DAS, while the second stage was given at the age of 16 DAS to prevent osmotic shock. The pH control was applied to keep it constant at the desired value, which from range 5.8 to 6.2. Meanwhile, the pH control was carried out by adding HCl or NaOH to lower or raise the pH. Data collection on the static hydroponic method was carried out 2 weeks after the treatment application or 30 DAS, while dynamic hydroponics data were collected at 68 DAS.

The observations for static hydroponic included shoot and root fresh weight (actual measurements), 2nd, 3rd, and 4th leaf length, shoot and green shoot areas from the side view, ratio of green shoot to shoot area from the side view, red, green, blue, ratio of red to green, and area growth rate. The traits measured in dynamic hydroponic included shoot and root fresh weight (actual measurements), convex hull from the top view, shoot area from the top view, green shoot area from the top view, the ratio of green shoot area to shoot area from the top view, number of leaves from the top view, red, green, blue, the ratio of red to green, the ratio of shoot area to the convex hull from the top view, object extent X from a side view, object extent Y from a side view, shoot area from a side view, green shoot area from a side view, a ratio of green shoot area to the shooting area from a side view, convex hull from a side view, and the ratio of shoot area to convex hull area from a side view (Table 1).

Image analysis

The images were taken with a Canon EOS 1200D RGB camera in a portable photo studio with a 75 cm × 75 cm × 75 cm. A white background with two 8 watt white LED lights in the studio with camera settings (5.6 F-stops, 1/160 seconds exposure time, ISO 800, and without flash) was used. The images were taken from a top view (0^o) and the side view (90^o) of the plant. For static hydroponic, the images were taken twice at 13 DAS (early treatment) and 30 DAS (after treatment) as shown in Fig. 2. For dynamic hydroponic, images were taken at 13 DAS (early treatment) and 68 DAS (after treatment) (Fig. 3 and the images results were analyzed using the Fiji application.

Table 1. Phenotyping characters used to analyze growth and drought-related traits.

Characters	Description
Shoot fresh weight (SFW)	The weight of the fresh shoot at the end of observation.
Root fresh weight (RFW)	The weight of the fresh root at the end of observation.
2nd leaf length (LL2)	The length of the 2nd leaf from the tip of the plant.
3rd leaf length (LL3)	The length of the 3rd leaf from the tip of the plant.
4th leaf length (LL4)	The length of the 4thleaf from the tip of the plant.
Shoot area (SA)	The number of pixels scaled in cm on the shoot of the plant.
Green shoot area (GSA)	The number of pixels that have been scaled in cm in the shoot section has a hue value of 50.
Convex hull area (CT for top and CS for side)	The smallest area is covered by theouter contour of an object.
Object extend X (XT for top and XS for side)	The length of the X-axis of the rectangle covering the object is used to measure the width of the plant.
Object extend Y (YT for top and YS for side)	The length of the Y-axis of the rectangle covering the object is used to measure the height of the plant.
Number of leaves (NL)	The numberof leaf tips visible from above the plant.
Area growth rate (AGR)	Dividing the difference in shoot area between the drought stress treatment intervals by drought stress treatment time (days).
Red (R)	Color is measured by averaging the Red color value of all pixels in the shoot. RGB values are between 0 (no color) and 255 (maximum color).
Green (G)	Color is measured by averaging the Green color value of all pixels in the shoot. RGB values are between 0 (no color) and 255 (maximum color).
Blue (B)	Color is measured by averaging the Blue color value of all pixels in the shoot. RGB values are between 0 (no color) and 255 (maximum color).

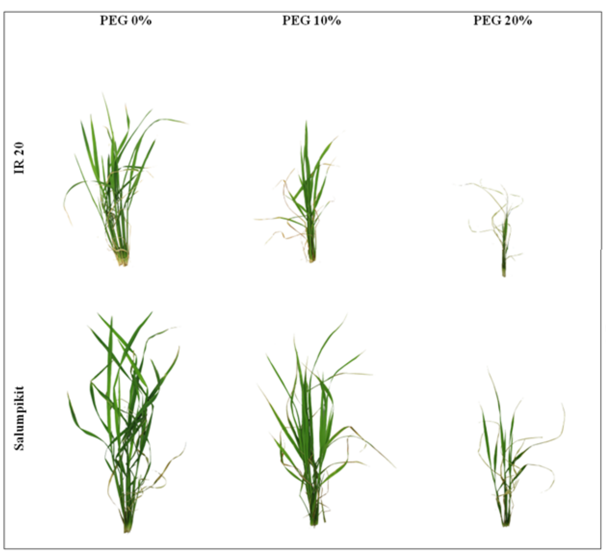

Fig. 2. RGB images of IR 20 and Salumpikit under drought stress at 30 DAS in static hydroponic.

Furthermore, the plant color was analyzed using hue channels with a hue 50 (green area) and the area growth rate was calculated using the projection of shoot area over two time periods.

Data analysis

The data were analyzed independently for variance and the characters that have significant interactions with each other were further analyzed using the Pearson correlation test and Principal Component Analysis (PCA) with STAR 2.01 software. After static hydroponic has been tested by correlation analysis, it was followed by path analysis. All further analysis based on the stress tolerance index (STI) value of each character on individual PEG concentration (0% PEG (normal) −10% PEG (drought stress) = STI 1 and 0% PEG (normal)−20% PEG (drought stress) = STI 2). This concept was reported by Anshori *et al.* 2019, as follows:

Table 2. Means square value of analysis of variance on the static hydroponic method.

Characters	D	V	D × V
SFW	178.73 **	28.92 **	10.37 **
RFW	24.77 **	7.30 **	1.96 **
LL2	1,486.95 **	326.19 **	14.68 ns
LL3	1,471.64 **	466.95 **	49.37 *
LL4	669.49 **	188.37 **	28.07 ns
SA	65,556.88 **	13,117.85 **	3,438.49 **
GSA	61,237.72 **	9,849.18 **	3,261.87 **
R	3,460.07 **	86.95 ns	748.95 ns
G	879.98 Ns	87.35 ns	1,498.13 ns
B	1,680.60 *	96.75 ns	272.54 ns
RRG	0.60 Ns	0.80 ns	0.96 ns
RGSS	0.57 Ns	0.02 ns	0.03 ns
AGR	202.25 **	32.11 **	10.60 **

D: Drought level, V: Variety, *: Significant effect at $P \leq 0.05$, **: Significant effect at $P \leq 0.01$, ns: Not significant, SFW: Shoot freshweight, RFW: Root fresh weight, LL2: 2nd leaf length, LL3: 3rd leaf length, LL4: 4th leaf length, SA: Shoot area, GSA: Green shoot area, R: Red, G: Green, B: Blue, RRG: Ratio of red to green, RGSS: Ratio of green shoot area to shoot area, AGR: Area growth rate.

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

$$STI = \frac{Yp \times Ys}{\bar{Y}p^2}$$

Note: Yp = The character value of each variety in normal / non-stressed conditions.

Ys = The character value of each variety in a stressed condition.

Ȳp = Average character values of all varieties in normal / non-stressed conditions.

The selected characters from the PCA analysis results were used to obtain the index value. The validation of static hydroponic was conducted by the regression test toward the dynamic hydroponic.

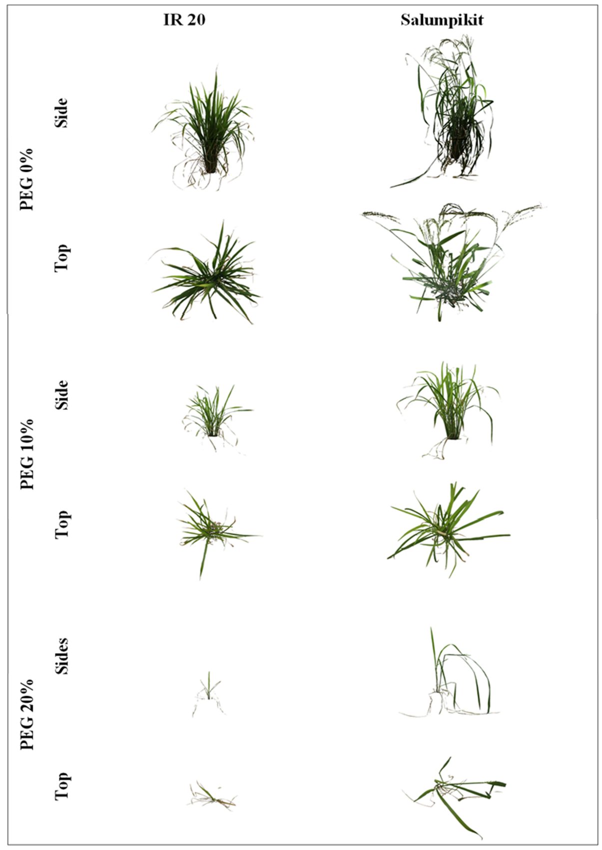

Fig. 3. RGB images of IR 20 and Salumpikit under drought stress at 68 DAS in dynamic hydroponic.

RESULTS

Static hydroponic system

In the static hydroponic method, the analysis of variance showed that the diversity of PEG concentrations had a significant effect on almost all the characters, except for the green, ratio of red to green, and the ratio of green shoot area to shoot area (Table 2). The genotype variance analysis also showed a significant effect on the shoot and root fresh weight, 2nd, 3rd, and 4th leaf length, shoot area, shoot green area, and area growth rate. Meanwhile, the characters that were affected significantly by drought-genotype interactions included shoot and root fresh weight, 3rd leaf length, shoot area, green shoot area, and area growth rate. All characters that significantly affected the interaction effect were continued by deep analysis on the static hydroponic system. Meanwhile, the phenotype varieties on these characters are shown in Supplementary 1.

Table 3. Correlation analysis of STI values on the static hydroponic method.

	RFW	LL3	SA	GSA	AGR	SFW
RFW	1.000	1.000	1.000	1.000	1.000	1.000
LL3	0.745**	0.789**	0.991**	0.977**	0.981**	
SA	0.983**	0.748**	0.984**	0.978**		
GSA	0.984**	0.802**	0.981**			
AGR	0.963**	0.769**				
SFW	0.982**					

The numeric in table indicate the correlation value. *: Significant correlated at $P \leq 0.05$, **: Significant correlated at $P \leq 0.01$, SFW: Shoot freshweight, RFW: Root fresh weight, LL3: 3rd leaf length, SA: Shoot area, GSA: Green shoot area, AGR: Area growth rate.

Table 4. Path analysis of the STI value on shoot fresh weight on the static hydroponic method.

Characters	Direct influence	Indirect influence					Residual
		RFW	LL3	SA	GSA	AGR	
RFW	0.600**		−0.006	−0.108	−0.059	0.555	0.589
LL3	−0.009	0.447		−0.086	−0.045	0.461	−0.007
SA	−0.109	0.590	−0.007		−0.059	0.567	−0.107
GSA	−0.060	0.590	−0.006	−0.108		0.562	−0.059
AGR	0.576**	0.578	−0.007	−0.108	−0.059		0.565

R^2: 0.86, **: Significant direct effect at $P \leq 0.01$, RFW: Root fresh weight, LL3: 3rd leaf length, SA: Shoot area, GSA: Green shoot area, AGR: Area growth rate.

The correlation analysis on static hydroponic results was based on the static hydroponic Stress Tolerance Index (STI) value (Table 3). The results showed that the 3rd leaf length (0.769 and 0.745), shoot area (0.981 and 0.983), green shoot area (0.978 and 0.984), and area growth rate (0.981 and 0.961) had a significant correlation on the shoot and root fresh weights, respectively. These correlation analyzes were followed by independent path analysis on the shoot and root fresh weights, which were the main characters of actual morphology under artificial vegetative screening with different roles. Therefore, this independent concept analysis can determine the specific relationship among image-based phenotyping characters to the conventional morphology characters.

Path analysis on the STI value of shoot fresh weight in static hydroponic showed representative results with a determinant coefficient value of 0.86 (Table 4). Based on this analysis, root fresh weight is the character that has the highest direct effect (0.600) on shoot fresh weight. Similarly, based on the image-based phenotyping character, the area growth rate is the character that has the highest direct effect (0.576) on shoot fresh weight. Therefore, the area growth rate directly affects the variance of shoot fresh weight as one of characters selection.

Moreover, the path analysis on the STI value of root fresh weight in static hydroponic showed representative results with a determinant coefficient value of 0.87 (Table 5). Based on this analysis, shoot fresh weight was the character that had the highest direct effect (0.548) on root fresh weight. Based on image-based phenotyping characters, the shooting area (0.493) and green shoot area (0.377) were the characters that had a significant direct effect on root fresh weight. Meanwhile, the area growth rate has a negative significant direct effect (−0.417) on the root fresh weight. This showed that the high correlation of area growth rate to root fresh weight was due to the indirect effect of the shoot and green shoot areas. Therefore, shoot area and green shoot area as image-based phenotyping characters can represent the root fresh weight variance, while the area growth rate is unable to be a selection

Table 5. Path analysis of the STI value on root fresh weight on the static hydroponic method.

Characters	Direct influence	Indirect influence					Residual
		LL3	SA	GSA	AGR	SFW	
LL3	−0.013		0.389	0.282	−0.335	0.421	−0.010
SA	0.493**	−0.010		0.374	−0.411	0.538	0.485
GSA	0.377**	−0.010	0.488		−0.408	0.536	0.371
AGR	−0.417**	−0.010	0.485	0.369		0.537	−0.402
SFW	0.548**	−0.010	0.484	0.369	−0.409		0.538

R^2: 0.87, **: Significant direct effect at $P \leq 0.01$, SFW: Shoot fresh weight, LL3: 3rd leaf length, SA: Shoot area, GSA: Green shoot area, AGR: Area growth rate.

Table 6. Principle component analysis of the STI value on the static hydroponic method.

Variables	PC1	PC2	PC3	PC4	PC5	PC6
RFW	0.4162	−0.2267	0.6382	−0.195	0.4902	0.2996
SFW	0.4183	−0.1602	−0.1349	−0.7478	−0.4166	−0.2201
LL3	0.3527	0.9258	0.1169	−0.0058	−0.053	0.0447
SA	**0.4210**	−0.1139	0.0357	0.3858	0.1809	−0.7918
GSA	**0.4177**	−0.2224	0.1076	0.5005	−0.6192	0.3613
AGR	**0.4192**	−0.0579	−0.7403	0.0585	0.4089	0.3198
CP	0.9284	0.9881	0.9935	0.9975	0.9989	1
EV	5.5707	0.3578	0.0324	0.0243	0.0083	0.0064

The numeric in table indicate the eigenvector value, CP: Cumulative proportion, EV: Eigenvalues, PC: Principal component, SFW: Shoot fresh weight, RFW: Root fresh weight, LL3: 3rd leaf length, SA: Shoot area, GSA: Green shoot area, AGR: Area growth rate.

character to represent root fresh weight variance.

The principal components analysis showed that there was one main component that can be used as the basis for the selection index (Table 6), namely the first principal component (PC1) with a cumulative proportion of 0.928 which is approximately 1. Based on PC1, shoot fresh weight (0.4183) and root fresh weight (0.4183) in drought stress were in the same direction with phenotyping characters of shoot area (0.4210), green shoot area (0.4177), and area growth rate (0.4192). These three image-based phenotyping characters were combined in a static hydroponic selection index using the formula as follows:

Static hydroponic index = 0.421 shoot area + 0.4177 shoot green area + 0.4192 growth rate area

(Equation 1)

Dynamic hydroponic system

Analysis of variance in the dynamic hydroponic method in Table 7 showed that the PEG treatment as drought stress has an impact on all image-based-phenotyping characters, except on the ratio of red to green from the top view and the ratio of green shoot area to shoot area from the top view. Variety variance has a significant effect on the number of leaves, red, and blue from the top view, the ratio of shoot area to the convex hull from the top view, object extends X, and Y from a side view, and convex hull from a side view. Meanwhile, the characters that were significantly affected by the interaction include red and blue from the top view, the ratio of shoot area to the convex hull from the top view, object extend Y from a side view, and convex hull from a side view. All characters that were significantly affected by the interaction effect in the dynamic hydroponic system were correlated with the selection index on static hydroponic. This occurred to detect the selected characters that have a relationship with the static hydroponic. The varieties phenotype on these characters are shown in Supplementary 2.

Table 7. Means square value of analysis of variance on the dynamic hydroponic method.

Characters	D	V	D × V
SFW	8,042.24 **	319.95 ns	376.25 ns
RFW	467.01 **	53.23 ns	14.64 ns
CT	81,129,688.30 **	4,224,903.16 ns	1,562,942.10 ns
SAT	5,571,152.36 **	474,034.51 ns	97,115.97 ns
GSAT	3,072,273.82 **	333,330.03 ns	80,272.07 ns
NLT	2,478.87 **	617.19 *	156.84 ns
RT	3,946.91 **	462.79 **	189.34 *
GT	4,734.46 **	241.02 ns	59.00 ns
BT	505.53 **	193.20 **	74.72 *
RRGT	0.0051 ns	0.0082 ns	0.0099 ns
RGSST	0.0083 ns	0.0674 ns	0.0480 ns
RSCT	0.0226 **	0.0085 **	0.0010 *
XS	300.57 **	174.52 *	66.56 ns
YS	1,636.09 **	147.66 **	110.49 **
SAS	3,696,517.43 **	280,606.15 ns	91,206.26 ns
GSAS	1,651,359.59 **	212,567.33 ns	68,283.65 ns
CS	48,116,645.30 **	6,716,755.96 **	947,650.84 *
RGSSS	0.0853 *	0.0141 ns	0.0082 ns
RSCS	0.0463 **	0.0063 ns	0.0024 ns

D: Drought level, V: Varieties, *: Significant effect at $P \leq 0.05$, **: Significant effect at $P \leq 0.01$, ns: Not significant, SFW: Shoot fresh weight, RFW: Root fresh weight, CT: Convex hull from top view, SAT: Shoot area from top view, GSAT: Green shoot area from top view, NLT: Number of leaves from top view, RT: Red from top view, GT: Green from top view, BT: Blue from top view, RRGT: Ratio of red to green from top view, RGSST: Ratio of shoot green area to shoot area from top view, RSCT: Ratio of shoot area to convex hull from top view, XS: Object extend X from side view, YS: Object extend Y from side view, SAS: Shoot area from side view, GSAS: Green shoot area from side view, CS: Convex hull from side view, RGSSS: Ratio of green shoot area to shoot area from side view, RSCS: Ratio of shoot area to convex hull from side view.

Significant characters on dynamic hydroponic were correlated with the selection index on static hydroponic. Based on this analysis (Table 8), the object extends Y from the side view (0.91) and convex hull from the side view (0.91) have a significant correlation with the selection index of static hydroponic. Therefore, these characters can be continued in PCA analysis to develop a selection index of a dynamic hydroponic system.

The principal components analysis on dynamic hydroponic results was shown in Table 9, which showed that PC1 has the highest cumulative proportion with a value of 58.83% of the total diversity of the initial data. Therefore, PC1 can be the basis in the weighting of selection characters such as the static hydroponic index. However, red from the top view and blue from the top view have negative eigenvectors. In contrast, the characters of the ratio of shoot area to the convex hull from the top view, object extend Y and convex hull from the side view have positive eigenvectors. This showed that the YS and CS characters were combined into a selection index in dynamic hydroponic screening using the formula stated below:

Dynamic hydroponic index = 0.4516 object extend Y from the side view + 0.4177 Convex hull from the side view

(Equation 2)

Selection index

The index value of each variety on static and dynamic hydroponics was shown in Table 10. Salumpikit variety at a PEG concentration of 10% (2.03) showed the highest value, while IR 20 at a PEG concentration of 20% (0.11)

Table 8. Correlation analysis of STI values on the dynamic hydroponic method.

	RT	BT	RSCT	YS	CS	SH index
RT	1.00					
BT	0.88**	1.00				
RSCT	−0.74*	−0.84**	1.00			
YS	−0.48ns	−0.24ns	0.30ns	1.00		
CS	−0.30ns	−0.03ns	0.14ns	0.97**	1.00	
SH index	−0.16ns	−0.04ns	0.19ns	0.90**	0.91**	1.00

The numeric in table indicate the correlation value, *: Significant correlated at $P \leq 0.05$, **: Significant correlated at $P \leq 0.01$, ns: Not significant, RT: Red from top view, BT: Blue from top view, RSCT: Ratio of shoot area to convex hull from top view, YS: Object extend Y from side view, CS: Convex hull from side view, SH index: Static hydroponic selection index.

Table 9. Principle component analysis of the STI value on the dynamic hydroponic method.

	PC1	PC2	PC3	PC4	PC5
RT	−0.5098	0.2535	−0.4404	0.4184	−0.5539
BT	−0.4794	0.4091	−0.3104	−0.3116	0.6399
RSCT	0.4142	−0.3275	−0.8412	−0.0971	0.0643
YS	0.4516	0.4988	−0.0229	0.6615	0.3305
CS	0.3671	0.6422	−0.0396	−0.53	−0.4128
CP	0.5883	0.8544	0.9536	0.9801	1
EV	2.9414	1.3306	0.496	0.1322	0.0997

The numeric in table indicate the eigenvector value, CP: Cumulative proportion, EV: Eigenvalues, PC: Principal component, RT: Red from top view, BT: Blue from top view, RSCT: Ratio of shoot area to convex hull from top view, YS: Object extend Y from side view, CS: Convex hull from side view.

Table 10. Selection index on static and dynamic hydroponics.

Varieties	Treatments	SH index	DH index
Inpari 34	PEG 10%	0.61	0.31
IR 20	PEG 10%	0.27	0.23
Salumpikit	PEG 10%	2.03	0.53
Ciherang	PEG 10%	0.59	0.35
Jeliteng	PEG 10%	0.78	0.34
Inpari 34	PEG 20%	0.12	0.22
IR 20	PEG 20%	0.11	0.22
Salumpikit	PEG 20%	0.39	0.36
Ciherang	PEG 20%	0.25	0.27
Jeliteng	PEG 20%	0.16	0.30

SH index: Static hydroponic selection index, DH index, Dynamic hydroponic selection index.

showed the lowest value in static hydroponics. Based on dynamic hydroponic, salumpikit variety at a PEG concentration of 10% (0.53) remained the best, while Inpari 34 (0.22) and IR 20 (0.22) were the varieties with the lowest index value. Furthermore, the regression analysis results showed that the static hydroponic index has a significant linear regression to the dynamic hydroponic index as shown in Fig. 4. Similarly, this figure also showed that the Salumpikit as the tolerant check variety has a good value index than IR 20 as a sensitive check variety on both 10% and 20% PEG.

DISCUSSION

The significant interaction in ANOVA is one of the early indicators in stress screening. Meanwhile, a previous study by Akçura and Çeri (2011); Anshori *et al.* (2019); Farid *et al.* (2020) showed that characters that are influenced by interactions had different patterns of decreased response between genotypes on normal and stressful environments.

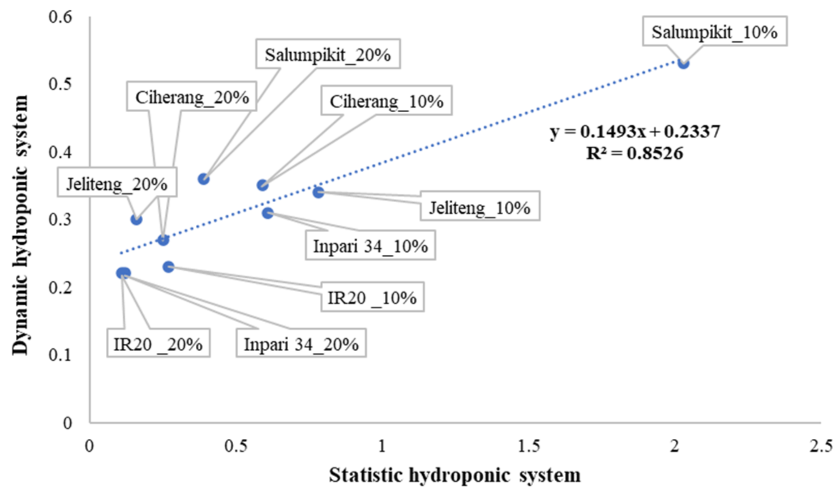

Fig. 4. Analysis of static hydroponic selection index regression on dynamic hydroponic.

This difference can be used as a parameter to identify tolerance traits between genotypes in stress (Ali *et al.* 2014; Safitri *et al.* 2016; Fadhli *et al.* 2020). Based on this analysis, the image-based phenotyping of 3rd leaf length, shoot area, shoot green area, and area growth rate on static hydroponic screening as well as red, blue, and the ratio of shoot area to the convex hull from the top view, object extend Y, and convex hull from the side view in dynamic hydroponic screening are used as a candidate to selection criteria in stress tolerance. These characters were also significantly influenced by the variance of PEG concentrations and varieties, which indicated that the variations in interactions between genotypes on environmental differences are shown directly (Farid *et al.* 2020; Farid *et al.* 2021). Therefore, in-depth analysis with several series of multivariate analyzes was required to obtain the best selection criteria for each drought screening process.

The assessment of varieties' response to stress needs to be carried out using a tolerance index. Meanwhile, a study by Mau *et al.* (2014), Aboughadareh *et al.* (2019), Anshori *et al.* (2019); Aboughadareh *et al.* (2020), and Farid *et al.* (2021) stated that the Stress Tolerance Index (STI) is appropriate in characterizing the most tolerant of drought stress. This index is classified as dynamic because it considers the general response of the population means, even though the general response on this index focused on normal conditions (Anshori *et al.* 2019). Therefore, the STI value becomes the basis for deeper analysis as shown in previous studies conducted by Anshori *et al.* (2019) on rice against salinity stress, Fadhli *et al.* (2020) on maize against drought stress, and Farid *et al.* (2021) on wheat against drought stress. Hence, the use of STI as the basis for a more in-depth analysis was also carried out for static hydroponic and dynamic screenings.

The correlation and path analysis on the static hydroponic were focused on the shoot and root fresh weight characters. A previous study showed that these fresh weights have a close relationship with drought tolerance (Saha *et al.* 2019). Moreover, the relationship between image-based phenotyping characters on these two characters can be the basis to determine the best selection criteria for drought stress. Similarly, the concepts of correlation and path analysis are often combined to determine the selection criteria (Safitri *et al.* 2011). This correlation still cannot describe the magnitude of the importance of a character to the diversity of the main characters (Singh and Chaudhary 2007; Rohaeni and Permadi 2012; Anshori *et al.* 2018). The use of this analysis has been widely reported by several studies (Aman *et al.* 2020; Ashraf *et al.* 2020; Saleh *et al.* 2020), therefore, it needs to be carried out on the character of the image-based phenotyping on the two fresh weights.

The correlation and path analyses results on static hydroponic results showed that shoot and green shoot areas, as well as area growth rate can be used as selection criteria. This is in line with a study by Hairmansis *et al.* (2014) on the potential of these image-based phenotyping characters on rice under salinity stress and barley under drought stress, while Kim *et al.* (2020) were on rice under drought stress. Based on the path analysis results, the area growth rate has an inverse orientation to the shoot and the green shoot areas. This difference in orientation is shown in the direct and indirect effect on the character of the shoot and root fresh weights (Table 4 and 5). This showed that the shoot and root fresh weights become the middle of the three image-based phenotyping characters. However, the combination of the three characters can make the selection easier because each character has specific potential. This is in line with a study by Acquaah (2007) and Anshori *et al.* (2021) which showed that the selection index formed by combining characters with opposite orientations has the potential to increase the selectivity of a selection. Therefore, these three characters are still used as selection criteria. However, the crucial thing in the formation of the selection index of the three characters is the weighting value of the selection, which can be determined using the principal component analysis.

The principal component is a multivariate analysis that aims to extract important information from large data into a new simpler set of orthogonal variables (Ilmaniati and Putro 2019). This analysis has been reported as a weighting indicator for the selection index by Anshori *et al.* (2019), Alsabah *et al.* (2019), Fadhli *et al.* (2020), Akbar *et al.* (2021) and Farid *et al.* (2021). Based on the PCA results in Table 6, the characteristics of shoot area, green shoot area, and area growth rate have the same eigenvector direction and variance with the shoot and root fresh weights. This showed that the three characters generally have the same variance direction, although, in the broad cross-section, the growth of the shoot has a different orientation from the other two characters. However, due to the inclusion of the two fresh weight characters, the three characters generally have the same range direction that can be combined into the selection index in Equation 1.

Validation of image-based phenotyping characters in static hydroponics was carried out by identifying the relationship between these characters and dynamic hydroponic characters. Relatively, dynamic hydroponic has a complex growth rate than static hydroponic (Sagita *et al.* 2020). This was because dynamic hydroponic observations were carried out in the flowering phase, while static hydroponic were in the vegetative phase. Since the application of the selection agent in the two methods was carried out at the same time in the 21 days after sowing, therefore, the static hydroponic selection index assessment can be related to the growth character of dynamic hydroponic as part of its validation.

The dynamic hydroponic ANOVA showed that red, blue, and ratio of shoot area to the convex hull from the top view, object extend Y and convex hull from a side view were the characters that were significantly affected by the interaction of drought level–genotype treatments. Meanwhile, shoot and root fresh weights showed an insignificant effect on the genotype and interaction of drought level–genotype. This was due to the high error of these characters and the variance domination from drought level treatment, hence, the genotype and its interaction are difficult to be determined on both fresh characters. Therefore, the shoot and root fresh weights are inappropriate as the main characters in detecting the selection characters from image-based phenotyping in this dynamic hydroponic system.

Moreover, the correlation analysis of image-based phenotyping characters on dynamic hydroponic that have significant interactions is a method of validating static hydroponic selection indexes. Based on the dynamic hydroponic screening correlation results, object extend Y and convex hull from the side view can be used as selection characters. This is in line with a study by Duan *et al.* (2018) and Kim *et al.* (2020) which stated that the leaves on plants that are stricken with drought turn yellow and bend, which caused a reduction in the calculated convex hull. However, the results of this study also need to be supported by other analyzes such as PCA and regression between selection indices (Fig. 4) as stated by Anshori *et al.* (2018) in determining the selection criteria.

Based on PCA results, the object extends Y and the convex hull from the side view has the same eigenvector direction. Meanwhile, the eigenvectors that are relatively similar in both dimensions showed the closeness of the variance between the two variables (Anshori *et al.* 2018).

This showed that both characters can be used as validation characters through the selection index. Furthermore, the dynamic hydroponic selection index can be used as the Y factor in a regression. Based on the regression results, the static hydroponic index has good adherence to the dynamic hydroponic index. The differences in tolerance groups between the Salumpikit as the tolerant check variety and IR 20 varieties as the sensitive check variety had a significant distance, which is in line with a study by Hairmansis *et al.* (2020) and Kartina *et al.* (2019). Meanwhile, the use of control varieties can be one of the proofs to detect effective selection. This concept had reported in previous studies by Ali *et al.* (2014), Safitri *et al.* (2016) and Anshori *et al.* (2020) on salinity stress, Anshori *et al.* (2019) and Wening *et al.* (2019) on submergence, Akbar *et al.* (2018) and Akbar *et al.* (2021) on drought stress. Furthermore, the use of Salumpikit and IR 20 have been reported as a check or control of rice drought tolerance by Akbar *et al.* (2018) and Akbar *et al.* (2021). Based on these results, the selection index on static hydroponic screening has the potential to be used to identify the tolerance properties of rice to drought stress.

Therefore, the use of multivariate analysis on image-based phenotyping characters has the potential to increase the effectiveness of selection in drought stress tolerance screening. The selection index for static hydroponic is 0.421 shoot area + 0.4177 green shoot area + 0.4192 area growth rate. Also, the character selection index for dynamic hydroponic is 0.4516 objects extend Y from the side view + 0.4177 convex hull from the side view. These results showed that rice screening based on image-based phenotyping can be recommended as a more effective and efficient method for the rapid screening of drought stress.

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