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RICE SELECTION CRITERIA BASED ON MORPHOLOGICAL AND IMAGE-BASED PHENOTYPING UNDER DROUGHT- AND SALINITY-STRESS CONDITIONS

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SUMMARY

Image-based phenotyping in selecting drought- and salinity-tolerant rice lines is a potential approach to complement other selection criteria. This study aimed to determine tolerance response and selection criteria on drought and salinity stresses based on a morphological and image-based phenotyping character. The experiment, set up in a screen house of the Department of Agronomy, Faculty of Agriculture, Hasanuddin University, Indonesia, consisted of a nested randomized complete group design. The nested replication included stressed environments with two factors and three repetitions. The level of environmental stresses comprised the first factor, i.e., normal (without NaCl and PEG), salinity (60 and 120 mM NaCl), drought (10% and 20% PEG), and combination of drought-salinity (10% PEG + 60 mM NaCl). The second factor entailed the rice genotypes. Observations of the morphological and image-based phenotyping characters ensued. The results indicated that salinity stress had a wider diversity than drought stress, while the multiple stresses had a relatively stable variety compared with single stress. Morphological and image-based phenotyping character increased precision in assessing the tolerance or adaptability of rice to drought stress, salinity, and its combination. The morphological characters that can serve as rice selection criteria in a combination of drought-salinity stress included the shoot and root fresh weights and the root length. As for the image-based phenotyping character, the shoot phenotype width can serve as the selection criterion. Image-based phenotyping characters, especially the shoot phenotype area, were recommended as criteria for precise selection in assessing rice genotypes' potential tolerance and adaptability to drought stress, salinity, and its combinations.

Keywords: Rice, adaptability, drought, salinity, image processing, multiple stresses

Key findings: The results showed that the most promising criteria for efficient rice selection under salinity-drought stress consist of the morphological characteristics, i.e., fresh shoot weight, root length, and fresh root weight. Meanwhile, the image-based phenotypic trait criterion consists of the shooting area phenotypes. The study also recommended that combining image-based morphological and phenotypic characters could improve rice tolerance or adaptation to drought, salinity, and combined stress.

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INTRODUCTION

Rice is a strategic commodity that plays a vital role in the global economy and food security, especially in Indonesia. In 2025, national rice demand estimates will reach 65.8 million t. However, rice production is still at 54.4 million t (Statistics Indonesia, 2021). This condition indicates the need for more efforts to increase rice production and make it a top priority. Yet, population growth and vast land conversion caused ineffective agricultural extensification (Rumanti *et al*., 2018). Thus, effective production per area or intensification becomes the prime solution to increase rice production.

Indonesia's potential as an archipelago has its challenges in food stability, policy, and natural challenges. The ever-dynamic climate changes impact the environment and result in environmental stresses. It causes the inability of plants to grow and produce (Förster, 2011). Drought is an environmental stress that causes a huge impact globally (Akbar *et al*., 2018; Fadhli *et al*., 2020). However, its effects on archipelagos will be more severe with additional stress, such as, salinity. Sea level rise caused by global warming indirectly increases the soil salinity in coastal areas planted with rice (Rumanti *et al*., 2018; Anshori *et al*., 2019). According to Rad *et al*. (2012), the increase of salinity up to 6 dS m^{-1} could decrease up to 50% of rice productivity and 100% at 12 dS m^{-1}. Therefore, the drought and salinity stresses, especially on rice plants, become the main issue to be solved.

Selection of rice-tolerant cultivars against drought and salinity can be done artificially or directly on the target environment (Safitri *et al*., 2016; Akbar *et al*., 2018; Anshori *et al*., 2019). The most common approach conducted for this research was artificial selection. It easily controls the artificial environment that provides focus on the targeted tolerant characteristics (Ali *et al*., 2014; Anshori *et al*., 2018), especially in the early vegetative phase. The vegetative phase has a shorter duration compared with the reproductive phase (Ali *et al*., 2014; Mondal and Borromeo, 2016), with a more complex metabolism (Mondal and Borromeo, 2016; Anshori *et al*., 2020). It becomes more efficient and effective in the selection process, specifically in the hydroponic culture (Kashenge-Killenga *et al*., 2013). However, artificial screening requires selection criteria to determine tolerant genotypes (Anshori *et al*., 2018, 2019).

Selection criteria development can be done through morphology, biophysical, and OMICS approaches (Horgan and Kenny, 2011; Sopandie, 2014). Generally, morphological characters are the common criteria used to determine tolerant genotypes. However, the environment heavily influences these criteria, needing detailed observation and experience (Anshori *et al*., 2019) to avoid increased bias in the selection. Hence, the need to combine these selection criteria with other approaches, one of which is image-based phenotyping.

Image-based phenotyping allows the selection process to be more precise. This approach minimizes the time required in determining tolerant plants toward drought and salinity stress (Siddiqui *et al*., 2014). This imaging technique has increased observation precision and objectivity (Asaari *et al*., 2019). Various researches have reported the effectiveness of image-based phenotyping (Hairmansis *et al*., 2014; Siddiqui *et al*., 2014; Asaari *et al*., 2019). Despite wide reports of the application, none of these studies described its use for selection criteria on drought and salinity stress in rice. Also, there are still very few of its development in Indonesia. Therefore, selection criteria based on conventional morphological characters and image-based phenotyping is crucial. This research aimed to determine the tolerance response of the traits to identify promising selection criteria on drought and salinity stress, as well as, their combination.

MATERIALS AND METHODS

The research was conducted from July to August 2020 at the screenhouse facility of Plant Breeding and Tissue Culture Laboratory, Department of Agronomy, Hasanuddin University, Indonesia. The average maximum temperature and humidity were 40°C and 66.28%, respectively, and the minimum temperature and humidity were 24.34°C and 42.96%, respectively. The experiment, set up in a nested randomized complete group design, consisted of nested replication of stressed environments with two factors and three replications. The first factor comprised the level of environmental stresses, i.e., normal

Table 1. Rice genotypes used in the study.

Genotypes	Origin and Crossing
Inpari 34 Salin Agritan	BR41XIR61920-3B-22-2
Inpari 29	IR69502/KAL9418 // Pokkali/ Angke
IR 29	Salinity susceptible check
IR 20	Drought susceptible check
Salumpikit	Drought tolerant check
Pokkali	Salinity tolerant check
Ciherang	IR18349-53-1-3-1-3/IR19661-131-3-1//IR19661-131-3-1-///IR64/////IR64
Jeliteng	Ketan Hitam /Pandan Wangi Cianjur

Figure 1. Rice plants in static hydroponic culture.

(without NaCl and PEG), medium and high salinity (60 and 120 mM NaCl), medium and high drought (10% and 20% PEG), and combination of medium drought and salinity (10% PEG + 60 mM NaCl). The second factor comprised the rice genotypes. Eight rice cultivars were used in this research, including popular rice cultivars and check cultivars for drought and salinity tolerance (Table 1).

Rice screening

The hydroponic system evaluated rice on its early vegetative phase following the previous study (Sakleh *et al*., 2020; Farid *et al*., 2021) with a modification. Rice seeds were sown in the seeding trays for seven days, with the seedling being transplanted to the nutrient culture media by placing the seedlings on a styrofoam tray floated in a plastic container filled with 8 L nutrient solution. Each styrofoam had 18 mm diameter holes and a 5 cm × 4 cm plant spacing. The seedlings planted in styrofoams were rolled with a thin foam layer, enabling the seedlings to float well on the culture media (Figure 1). The culture media used consisted of AB mix (EC ±3 ds m^{-1}), changed weekly. The solution pH was maintained at around 5.5–6.5 by applying NaOH or HCl 1 N. Stress was imposed on the 10th day after transplanting (DAT). Gradual stress was applied to avoid osmotic shock.

Salinity

Sodium chloride (NaCl) application in the first stage was 50% of the stress concentration, and three days after increasing the application to full concentration based on the treatment,
NaCl 0 mM (Normal)
NaCl 60 mM (Medium stress): 30 mM NaCl + 30 mM NaCl
NaCl 120 mM (High stress): 60 mM NaCl + 60 mM NaCl

Drought

PEG (polyethylene glycol) application on the first stage was 50% of the stress concentration, and after three days increasing the application to full concentration based on the treatment,
PEG 0% (Normal)
PEG 10% (Medium stress): 5% PEG + 5% PEG
PEG 20% (High stress): 10% PEG + 10% PEG

Drought-Salinity

In the combination treatment, the use of a concentration of 50% (5% PEG + 30 mM NaCl)

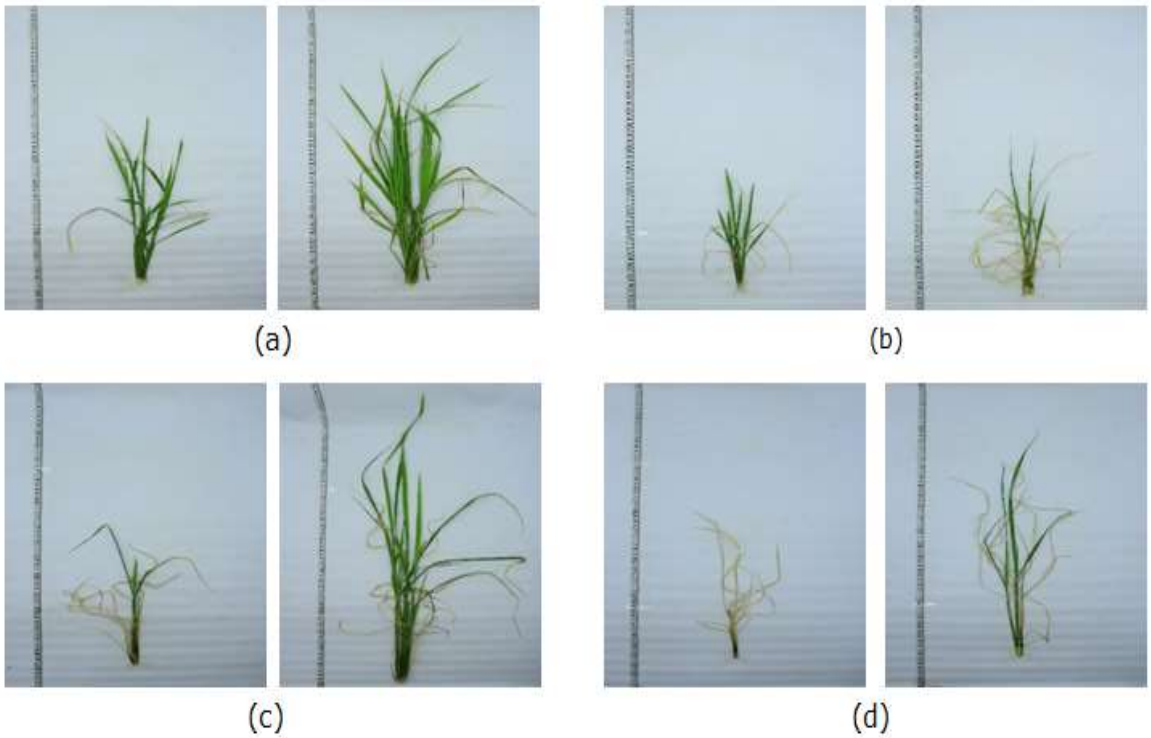

Figure 2. Examples of the phenotyping result on sensitive and tolerant rice varieties (a) IR 20 and Salumpikit on 10% PEG, (b) IR 20 and Salumpikit on 20% PEG, (c) IR 29 and Pokkali on 60 mM NaCl, (d) IR 29 and Pokkali on 120 mM NaCl.

in the first stage of treatment has induced high symptoms in rice plants, therefore, discontinuing additional stress application.
0% PEG + 0 mM NaCl (Normal condition)
5% PEG + 30 mM NaCl (Combination drought-salinity)

Image analysis

Figure 2 shows the captured images 14 days after the application using a Canon EOS 1200D camera). Image acquisition used a portable photo studio sized 75 cm × 75 cm × 75 cm and white background with two 8-watt LEDs in the studio. The photos got captured with 5, 6 F-stop, 1/160 seconds exposure time, and ISO 800 without flash (Laraswati *et al*., 2021). Capturing the images used a hole positioned above the studio. The photos were then analyzed using Image-J software.

Data analysis

Observation on the number of characters ensued 14 days after the applications for the following morphological characters: plant height (cm), length of longest root (cm), number of tillers, number of leaves, shoot fresh weight (g), root fresh weight (g), and biomass fresh weight (g); and image-based phenotyping characters: second leaf length (cm), third leaf length (cm), fourth leaf length (cm), shoot area (cm^2), index red, index green, index blue, red/green ratio, and green area percentage (%).

Independent analysis for all of the observations used the analysis of variance between salinity, drought, and a combination of drought and salinity. Characters significant to the minimal interaction on two stress lines received further analysis. The adaptability index determined the best concentration for selection. The adaptability index calculation combined the responses to stressed condition and its relative decrease, which was normalized. The most significant deviation between the adaptability of tolerant and sensitive cultivars became the basis for determining the best selection environment for each stress. The Principal Component Analysis (PCA) evaluated the relative decrease in chosen concentration using RStudio extra package. Using the Software Minitab17, the adaptability index data of the selected concentration served in the factor analyses to obtain the selection criteria. The chosen selection criteria evaluated the general tolerance characteristics of cultivars from the average adaptability index on the three selection environments using Heatmap Cluster Analysis (HCA) and RStudio gplots package.

RESULTS

Analysis of variance

Drought stress

The analysis of variance on drought stress indicated that the genotype, growing environment, and interaction variance significantly influenced the plant height, root length, shoot fresh weight, root fresh weight, biomass fresh weight, second leaf length, third leaf length, fourth leaf length, and shoot area (Table 2). Based on the variance analysis, the interaction variance on drought stress affected three image-based phenotyping characters. It indicates that image-based phenotyping can serve to differentiate genotype characteristics on drought stress.

Salinity stress

The analysis of variance for rice characters under salinity stress suggested that genotype, growing environment, and interaction variance considerably affected root length, shoot fresh weight, root fresh weight, biomass fresh weight, third leaf length, shoot area, and green per area (Table 3). For image-based phenotyping characters, the third leaf length and width acquired an effect by the interaction variance. Yet, the number of characters significantly affected by salinity was fewer compared with the ones affected by drought stress.

Drought-salinity stress

The variance analysis on traits treated with the combination of drought and salinity stress showed that the genotype, environment, and the interactions of genotype and environment greatly affected the plant height, shoot fresh weight, root fresh weight, biomass fresh weight, third leaf length, and shoot area (Table 4). The analysis of variance also showed two characteristics of image-based phenotyping influenced by the interaction (third leaf length and shoot area).

Considering the intersection of interaction variance between environments in the analysis of variance (Tables 2, 3, and 4), the plant height, root length, shoot fresh weight, root fresh weight, biomass fresh weight, third leaf length, and shoot area serve as promising candidates for the selection criteria. An explanation of this comprised the consistent characteristics that have at least two types of selection environments.

Table 2. Analysis of variance on drought stress.

Characters	Mean squares		
	Genotypes	Environments	Interactions
Morphological traits			
Plant height	960.03**	2070.44**	38.53*
Number of tillers	9.78**	38.81*	1.94*
Number of leaves	128.49**	559.07**	18.92ns
Root length	70.544**	238.07*	8.45**
Shoot fresh weight	79.95**	371.00**	23.02**
Root fresh weight	8.08**	49.31**	1.73**
Biomass fresh weight	124.11**	613.43**	34.53**
Image-based phenotype			
2nd Leaf length	739.81*	3009.81**	51.87*
3rd Leaf length	682.05**	2723.02**	69.74**
4th Leaf length	613.59**	2206.02**	80.58*
Shoot area	19417.14**	115195.23**	5174.44**
Red	409.00ns	8390.93**	556.98ns
Green	469.97ns	3735.14*	1037.61ns
Blue	269.63ns	3482.54**	242.62ns
Red/green ratio	0.48ns	0.29ns	0.60ns
Green area (%)	0.03ns	0.15**	0.02ns

**, *: Significant at P≤0.01 and P≤0.05, respectively, ns: Nonsignificant

Table 3. Analysis of variance on salinity stress.

Characters	Mean squares		
	Genotypes	Environments	Interactions
Morphological traits			
Plant height	1010.01**	1445.22**	22.98ns
Number of tillers	10.14**	34.99*	1.92ns
Number of leaves	147.14**	274.42**	19.13ns
Root length	69.02**	46.57**	11.71*
Shoot fresh weight	107.52**	291.42**	18.34**
Root fresh weight	7.34**	17.87**	1.65**
Biomass fresh weight	168.37**	451.37**	29.49**
Image-based phenotype			
2nd Leaf length	869.21**	1414.41**	48.41ns
3rd Leaf length	930.12**	1446.20**	55.16*
4th Leaf length	840.38**	840.22**	27.03ns
Shoot area	34834.99**	94151.30**	4190.65**
Red	1126.51ns	7550.66**	246.06ns
Green	735.24ns	2094.03*	391.17ns
Blue	337.64*	3697.94**	103.36ns
Red/green ratio	0.66ns	0.11ns	0.02ns
Green area (%)	0.13**	2.02**	0.45*

**, *: Significant at P≤0.01 and P≤0.05, respectively, ns: Nonsignificant

Table 4. Analysis of variance on drought-salinity stress.

Characters	Mean squares		
	Genotypes	Environments	Interactions
Morphological traits			
Plant height	641.37**	3857.63**	48.62**
Number of tillers	5.33**	76.66*	4.12ns
Number of leaves	66.80ns	370.24*	88.50ns
Root length	20.69**	223.26**	7.74ns
Shoot fresh weight	61.53**	586.74**	40.76**
Root fresh weight	4.93**	26.12**	2.61*
Biomass fresh weight	98.16**	860.48**	62.17**
Image-based phenotype			
2nd Leaf length	534.16**	5032.14**	14.09ns
3rd Leaf length	604.69**	4412.36**	87.94*
4th Leaf length	532.38**	1932.81**	68.10ns
Shoot area	15365.39**	184395.46**	9102.09**
Red	613.08ns	7764.03**	269.45ns
Green	1338.17ns	6526.10**	442.76ns
Blue	114.20ns	1400.22**	64.65ns
Red/green ratio	0.06ns	0.03ns	0.03ns
Green area (%)	0.01ns	0.34**	0.00ns

**, *: Significant at P≤0.01 and P≤0.05, respectively, ns: Nonsignificant

Adaptability index

Drought stress

The adaptability index from drought stress of 10% PEG showed that Inpari 29 and Pokkali cultivars have a positive inclination to eight candidates of selection criteria (Table 5). On the contrary, Inpari 34 and Jeliteng cultivars consistently tend to have negative adaptability index to these eight characters. Meanwhile, the Salumpikit cultivar relatively had a better adaptability index than the IR 20 cultivar at 10% PEG treatment.

Based on the 20% PEG stress, Pokkali and IR 20 cultivars had a positive character adaptability index. Inversely, IR 29, Salumpikit, and Jeliteng cultivars had an adaptability index that of a negative inclination. Comparing the IR 20 and Salumpikit cultivars showed that the IR 20 cultivar had a relatively better character adaptability index value than the Salumpikit cultivar, except for the character of shoot fresh weight and third leaf length.

Table 5. Adaptability index of selected characters to drought stress.

Cultivars	PH	RL	SFW	RFW	BFW	3LL	SA
Drought 10% PEG							
Inpari 34	-4.4	-1.84	-1.2	0.51	-1.03	-2.32	-2.04
Inpari 29	1.56	-0.16	7.87	3.22	1.36	0.18	0.69
IR 29	3.17	2.34	-4.24	0.89	1.99	-4.62	0.87
IR 20	-3.43	0.27	-7.78	0.6	0.2	0.19	1.12
Salumpikit	-0.79	1.94	-0.79	2	2.02	-0.03	2.81
Pokkali	7.48	1.18	6.5	0.97	0.95	2.88	0.55
Ciherang	0.66	-1.09	-2.07	-4.05	-2.29	4.86	-0.81
Jeliteng	-4.24	-2.63	1.71	-4.13	-3.18	-1.13	-3.2
Drought 20% PEG							
Inpari 34	-0.86	-0.87	2.28	1.92	0.11	-6.53	-3.49
Inpari 29	5.61	-2.73	-1.63	-4.87	-3.63	3	-6.02
IR 29	-3.98	-3.2	-7.19	-4.23	-3.51	0.08	5.11
IR 20	1.74	6.04	-1.96	4.6	5.42	2.05	1.33
Salumpikit	-0.13	-4.09	4.9	-2.74	-3.6	4.08	-0.94
Pokkali	1.77	3.81	8.56	3.04	3.34	0.23	2.02
Ciherang	-0.37	3.26	-1.85	2.85	3.3	2.75	5.58
Jeliteng	-3.78	-2.23	-3.1	-0.57	-1.44	-5.67	-3.59

PH: Plant height, RL: Root length, SFW: Shoot fresh weight, RFW: Root fresh weight, BFW: Biomass fresh weight, 2LL: 2nd Leaf length, 3LL: 3rd Leaf length, SA: Shoot area.

Salinity stress

The adaptability index on the 60 mM NaCl salinity stress showed that Inpari 34, Inpari 29, and Pokkali cultivars have a positive character adaptability index to the eight selected criteria (Table 6). Conversely, IR 29, IR 20, Salumpikit, and Jeliteng cultivars tend to have a negative adaptability index to these eight criteria. Meanwhile, Pokkali and Inpari 34 had a relatively better adaptability index than IR 29 at 60 mM NaCl salinity.

Based on the 120 mM NaCl stress, the Salumpikit cultivar had a relatively consistent positive adaptability index to eight selection criteria. On the other hand, Inpari 34, IR 29, and IR 20 cultivars tended to have a negative adaptability index. Meanwhile, comparing IR 29 and Pokkali cultivars showed that Pokkali had better character adaptability index values compared with the IR 29 cultivar. However, the score index differences between the 120 mM NaCl concentration and the 60 mM NaCl concentration are near equivalent.

Table 6. Adaptability index of the selected characters against two salinity stress concentrations.

Cultivars	PH	RL	SFW	RFW	BFW	3LL	SA
60 mM NaCl							
Inpari 34	2.38	2.83	2.14	1.87	2.20	1.02	3.23
Inpari 29	4.58	2.47	5.62	3.39	5.16	2.62	4.52
IR 29	-6.93	-7.00	-5.28	-6.91	-5.93	-3.94	-7.59
IR 20	-2.11	-5.25	-3.88	2.62	-1.41	-5.29	-4.64
Salumpikit	-0.48	-3.23	-4.69	-4.68	-5.14	-2.74	-2.81
Pokkali	3.12	1.92	5.73	4.53	5.25	4.86	7.13
Ciherang	0.58	6.43	1.59	0.33	1.24	5.65	2.07
Jeliteng	-1.14	1.82	-1.23	-1.15	-1.37	-2.16	-1.90
120 mM NaCl							
Inpari 34	-0.17	0.6	-5.92	-2.46	-5.26	-3.74	-2.64
Inpari 29	0.01	-0.1	5.03	1.33	3.98	6.21	6.08
IR 29	0.59	-9.37	-5.27	4.68	-0.04	1.32	-5.3
IR 20	-6.77	-2.74	-0.01	-1.63	-0.38	0.35	-3.77
Salumpikit	4.81	5.19	4.15	4.5	4.12	2.32	5.29
Pokkali	1.26	6.13	2.72	-0.4	0.92	2.47	6.03
Ciherang	-0.36	-4.01	1.59	-0.93	0.66	-2.51	-1.57
Jeliteng	0.63	4.33	-2.3	-5.1	-3.99	-6.43	-4.09

PH: Plant height, RL: Root length, SFW: Shoot fresh weight, RFW: Root fresh weight, BFW: Biomass fresh weight, 3LL: 3rd Leaf length, SA: Shoot area.

Table 7. Adaptability index of selected characters to drought stress and salinity stress combinations.

Cultivars	PH	RL	SFW	RFW	BFW	3LL	SA
5% PEG + 30 mM NaCl							
Inpari 34	-2.67	4.79	1.90	2.24	1.84	-5.40	0.87
Inpari 29	4.96	3.92	2.33	5.10	3.51	-0.23	5.64
IR 29	2.65	-1.45	3.42	4.19	3.80	4.68	0.88
IR 20	-4.46	-1.60	-4.39	-4.79	-4.85	-6.13	-3.94
Salumpikit	-1.23	-4.73	2.06	0.41	1.56	-0.09	-0.08
Pokkali	4.51	5.67	-0.51	-2.15	-1.15	5.70	0.28
Ciherang	-1.95	-0.82	0.53	0.98	0.79	1.70	2.89
Jeliteng	-1.81	-5.77	-5.34	-5.99	-5.51	-0.23	-6.54

PH: Plant height, RL: Root length, SFW: Shoot fresh weight, RFW: Root fresh weight, BFW: Biomass fresh weight, 3LL: 3rd Leaf length, SA: Shoot area.

Drought-salinity stress

The results of the adaptability index on the drought stress and salinity combination showed that Inpari 29, Inpari 34, IR 29, and Ciherang cultivars tended to have a positive adaptability index to the selection criteria (Table 7). On the contrary, Inpari 20 and Jeliteng cultivars consistently tended to have a negative adaptability index to these eight criteria. Meanwhile, Inpari 29 showed a relatively better adaptability index than all cultivars in the combination of drought and salinity stress. The principal component analysis illustrates the overall results of the best adaptability index.

The study results revealed that the concentrations of PEG 10% and NaCl 60 mM seemed the optimal selection conditions for drought and salinity stress, respectively, based on the differences and consistency of the adaptability index.

Principal component analysis

The principal component analysis (PCA) of eight cultivars in three types of selection environment through static hydroponic screening showed the total variance of the PCA biplot at 72.1% (dimension (Dim) 1 = 59.6% and Dim 2 = 12.5%) (Figure 3). The salinity stress showed wide variation in Dim 1, which was the dimension with the widest variation. The combination of drought and salinity stress is a selection environment with a fairly enormous and stable cultivar composition in both dimensions. The drought stress consists of a relatively low variance compared with other stress environments and is concentrated around the center of the PCA Biplot.

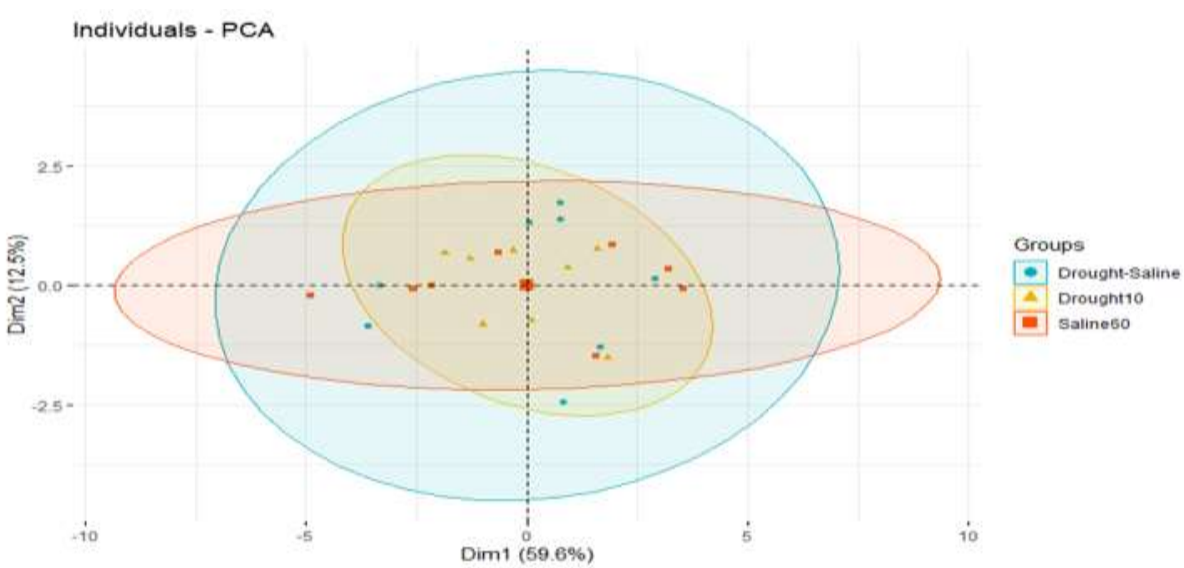

Figure 3. Principal component analysis (PCA) of eight rice cultivars in three types of selection environments through static hydroponic screening.

Table 8. Factor analysis of rice based on morphological and image-based phenotyping on screening of drought, salinity, and combination of drought-salinity through hydroponic culture.

Variables	Factor-1	Factor-2	Factor-3	Factor-4	Communality
PH	0.032	-0.395	0.021	0.233	0.785
RL	-0.28	0.126	0.941	0.012	0.926
SFW	0.306	-0.304	-0.539	-0.16	0.836
RFW	0.567	0.266	-0.135	0.166	0.957
BFW	0.385	0.121	-0.013	0.061	0.951
3LL	-0.297	-0.749	-0.056	-0.016	0.885
SA	0.079	0.068	0.327	-0.114	0.924

PH: Plant height, RL: Root length, SFW: Shoot fresh weight, RFW: Root fresh weight, BFW: Biomass fresh weight, 3LL: 3rd Leaf length, SA: Shoot area.

Factor analysis

The results of the factor analysis showed there are four main factors with total diversity of 90.5% from the initial data (Table 8). The first factor comprised the largest data diversity compared with other factor variables. Factor 1 contains root fresh weight and biomass fresh weight as the main characteristics that determine the diversity of tolerance between cultivars, based on the three stress environments. Factor 2 indicates that plant height and third leaf length have a large diversity. However, both characters had a negative response to factor 2. Factor 3 showed that root length and area were the main positive characters in the said factor. Factor 4 showed the same pattern as factor 2, where the number of tillers was the main factor 4, but with a negative value.

Heatmap cluster analysis

The heatmap cluster analysis demonstrates the grouping between genotypes in the chosen selection criteria. The heatmap analysis in Figure 4 illustrates that the selection criteria dendrogram (column in figure) consists of two groups of characteristics, namely, the shoot area and root length, and the biomass fresh weight and root fresh weight. The cultivar dendrogram (row in figure) also consists of two major groups, which were divided into the adaptive cultivars and sensitive cultivars. The two groups each had four cultivars. The difference between the two groups resulted from the character root length based on the degradation of yellow and orange color in the figure. Meanwhile, the characteristics of shoot area, biomass fresh weight, and root fresh weight divided these cultivars proportionally.

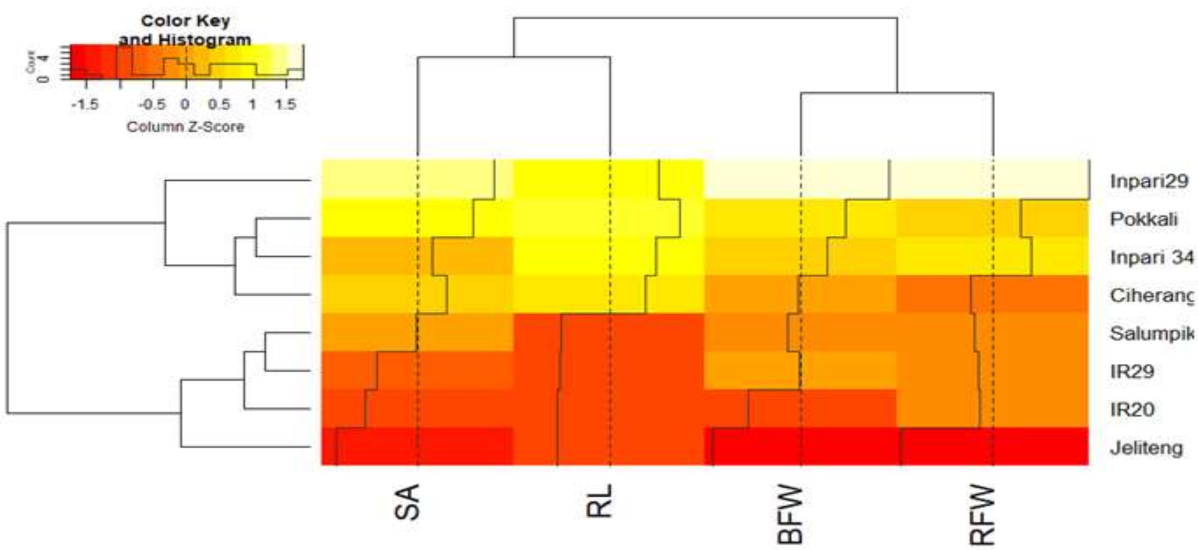

Figure 4. Cluster heatmap analysis of eight rice cultivars on four selection criteria. SA: Shoot area, RL: Root length, BFW: Biomass fresh weight, RFW: Root fresh weight.

The first group consists of the adaptive cultivars containing Inpari 29, Pokkali, Inpari 34, and Ciherang. Inpari 29 displays as an adaptive cultivar to all selection criteria. The second group makes up a sensitive cultivar group consisting of the Salumpikit, IR 29, IR 20, and Jeliteng. Jeliteng had a lower shoot area, biomass fresh weight, and root fresh weight compared with the three other cultivars in its group.

DISCUSSION

The ANOVA results showed that several characters had significant consistency toward the three sources of variance, especially the variance of interactions. Interaction variance is an essential factor in determining the criteria of selection in a screening tolerance (Sitaresmi *et al*., 2016; Anshori *et al*., 2019; Farid *et al*., 2021). The notable interaction indicated the difference in responses between the genotypes to the normal and stressed environment for characters that have pronounced consistency to the three variance sources, especially interaction variance (Kan *et al*., 2010; Fadhli *et al*., 2020; Farid *et al*., 2020). Relatively tolerant genotypes can minimize damage due to stress, resulting in a low relative decrease or sloped declination graph. On the other hand, susceptible genotypes suffer severe damage, so the decline becomes relatively large or a steep declination graph (Anshori *et al*., 2018b). Therefore, an interaction variance can be an early indicator of selection criteria. Based on the intersection of interaction variance between environments (Tables 2, 3, and 4), the plant height, root length, shoot fresh weight, root fresh weight, biomass fresh weight, third leaf length, and shoot area can serve as candidates for the selection criteria. Hence, an in-depth analysis needs attention to determine the best selection criteria.

One of the approaches to assess the selection criteria is to combine the potential from relative decrease to its adaptive nature in a stressed environment. According to Anshori *et al*. (2020), the combination of relative decrease and phenotype in the saline state will form a heat map cluster that is directed toward tolerance properties. The combination of the two concepts can be done using the selection index approach (Rajamani *et al*., 2016), thus, the formed index can be expressed as a stress adaptability index. According to the stress adaptability index (Tables 5, 6, and 7), this index can distinguish between tolerant and sensitive check cultivars for several characters in each type and stress concentration. This difference indicates this index serves well in distinguishing tolerance characters. However, determining the best selection environment can increase the chances of choosing the best selection criteria. Anshori *et al*. (2018b) and Bakhtiar (2010) also reported a similar finding, in which the assessment of the selection criteria and the tolerance trait resulted from the tolerance screening environment. Therefore independently determining the selection environment has previous research indicating the best selection criteria, particularly in drought stress and salinity environments separately.

Determining the selection environment for each stress type can be done by comparing the difference in the value of the adaptability index. A good selection environment can differentiate between tolerant and sensitive genotype responses (Anshori *et al*., 2018b). Based on this concept, the 10% PEG concentration stood out as the best selection environment, with consistent differences between the Salumpikit and IR 20 cultivars. In contrast, the 20% PEG concentration proved less receptive due to the instability of the differences in characters between the two cultivars. It is in line with the research of Farid and Ridwan (2018), which states that the tolerance limit for rice resistance to drought was at a PEG concentration of 10% (-0.67 MPa). In a salinity stress environment, the 60 mM NaCl concentration resulted as the best concentration, with the difference in index between Pokkali and IR 29 more incredible than the 120 mM concentration. Although several reports also stated that the 120 mM NaCl concentration came out as the best selection environment (Safitri *et al*., 2018; Anshori *et al*., 2020). This may be due to the difference in nutrient solutions used. The recent study used ABmix as a nutrient solution, which differed from the research of Anshori *et al*. (2020) and Safitri *et al.* (2018), which used the Yoshida solution as a nutrient solution. Based on the differences and consistency of the adaptability index, it can be concluded that the concentrations of PEG 10% and NaCl 60 mM stand out as the most promising selection environments for drought and salinity stress, respectively, in this study.

The Principal Component Analysis (PCA) illustrated the overall results of the best adaptability index. PCA is a statistical technique that linearly changes the form of a set of original variables into a smaller set of uncorrelated ones that can represent information from the original set of variables

(Dunteman, 1989). This analysis can map the variance of the initial data in the form of a new dimension or variable (Fadhli *et al.*, 2020; Farid *et al*., 2020; Farid *et al*., 2021; Widyawan *et al*., 2020) so that the determination of the variance pattern of each group can be identified. Based on this research, all selection environments overlapped or interacted with each other. Salinity stress appeared to have the most significant variance, especially in dimension 1. Dimension 1 was the largest dimension of the variance of the initial data, so this dimension became the initial basis for assessing the general variance of the existing data (Farid *et al*., 2020; Jolliffe, 2002).

Salinity stress is relatively dynamic stress. It was also stated by Ismail *et al*. (2013) and Kranto *et al*. (2016), who reported about the dynamics of salinity tolerance based on the growth phase. Salinity stress harms the entire crop cycle, especially at the pollination and fertilization stages (Reddy *et al*., 2017). Wide variation of salinity is due to the potential for this stress which can induce several stresses, such as, osmotic, oxidative, homeostasis, and toxic stress. The resulting variance is relatively diverse compared with other stresses (Ghosh and Ali, 2016). The combined stress includes all the variance in each independent stress environment so that the mapping of this variance becomes balanced in each dimension. Therefore, determining the selection criteria based on the variance of the three selection environments can serve as basis for assessing the stable genotype tolerance trait to multiple drought-salinity stresses. Determination of the selection criteria can be done by using factor analysis.

Factor analysis plays a role to identify the key or significant dimensions in the multivariate domain (Acquaah, 2007). Factor analysis aims to select specific factors that have the same direction as the main factor and eliminate those that do not have a relevant effect on the main character (Mattjik and Sumertajaya, 2011; Farid *et al*., 2020). Based on Table 8, root fresh weight (0.567 in factor 1), biomass fresh weight (0.385 in factor 1), root length (0.941 in factor 3), and shoot area (0.327 in factor 3) proved the recommended characters as selection criteria. The explanation shows the four characters have a loading factor exceeding 0.32 (Yong and Pearce, 2013). Based on this, the four characters can determine the nature of tolerance to drought-salinity double stress. One analysis that can look into the tolerance character is the cluster heat map analysis (Anshori *et al*., 2020).

Cluster analysis is a method often used in plant breeding. Two main functions of the cluster analysis application include 1) the measurement to identify outliers and 2) the classification of sample subtypes (Zhao *et al*., 2014). However, in current developments, cluster analysis is often combined with heat map analysis (Anshori *et al*., 2020; Virga *et al*., 2020). The concept of heatmaps can facilitate a graphical representation of the data of an individual value in the form of normalized-color gradations (Tiessen *et al*., 2017). Based on the heatmap cluster analysis, the four characteristics could specifically identify the double-stress tolerance trait among the cultivars. The first group has good adaptive characteristics against multiple stresses. Pokkali, Inpari 29 and Inpari 34 Salin consist of the cultivars reported having good tolerance to salinity stress (Safitri *et al*., 2016; Sembiring *et al*., 2019; Anshori *et al*., 2020; Nasaruddin *et al*. 2020).

Meanwhile, reports said the Ciherang cultivar has good adaptive properties in various environments, therefore, it was known as a megacultivar. In contrast, other reports stated that the IR 20 and IR 29 are control cultivars sensitive to drought stress and salinity, respectively (Akbar *et al*., 2018; Anshori *et al*., 2018). The Jeliteng cultivars are known as black cultivars with high antioxidants (Suarni *et al*., 2020). The antioxidant is active during the reproductive phase and causes a purplish to black color on the grains (Dwiatmini and Afza, 2018), so their tolerance to double stress is less optimal in the vegetative phase. The Salumpikit cultivar is a genotype only tolerant of drought stress. However, its potential is still better than the IR 20, IR 29, and Jeliteng. Therefore, based on the results of the heat map cluster, root fresh weight, biomass fresh weight, root length, and image area have the potential for the selection criteria in drought and salinity multiple stress screening.

The characteristics for selection criteria in this study proved that drought-salinity multiple stresses affected all parts of rice morphology during the early vegetative phase. Several studies have shown that the response to root growth can be a selection criterion identical to drought and salinity stress (Susanto *et al*., 2019; Salleh *et al*., 2020). Although in several studies, the root character did not have a significant interaction in differentiating the tolerant and sensitive cultivars to salinity stress (Yamamoto *et al*., 2011; De Leon *et al*., 2015; Anshori *et al*., 2020). However, in this study, the root character exhibited more dominance in distinguishing large groups from the drought-salinity multiple stress adaptive traits. In contrast, the shoot characters tended to differentiate the specific adaptability traits between the cultivars in each group, specifically the broad character of the shoot image area. The shoot image area becomes a good selection indicator in determining rice genotypes' adaptability to salinity stress, drought, and multiple drought-salinity stress. Studies by Berger *et al*. (2012) and Wu *et al*. (2020) also supported this finding and showed that a shoot image (crop canopy) area has a good linear correlation in assessing plant growth. Therefore, the broad character of the shoot image can be recommended as a selection criterion in identifying the adaptability trait of the rice genotype to multiple drought-salinity stress.

Crop canopy structure is determined by genetic characteristics, physiological and biochemical processes, planting patterns, and growth status. So far, little emphasis has been placed on understanding how canopy traits contribute to yield differences between rice ecotypes grown in water-stressed conditions. This idea has long been floated as a potential avenue for developing new rice plant cultivars. Given that crop canopies belong to an integrated photosynthetic and matter production system, crop canopy structure is critical to its function (Guo *et al*., 2015; Ouyang *et al*., 2021). According to Ouyang (2021), rice genotypes with different canopy architectures may respond to water deficit differently. Guo *et al*. (2015) demonstrated that the distribution of the leaf area index is the most critical factor in determining leaf physiological characteristics in crop canopies. Also, Wang *et al*. (2016) recently determined abiotic stress (critical N concentrations) using rice canopy cover information extracted from digital images rather than aboveground dry weight.

As an alternative to measuring the canopy cover, the multispectral remote sensing technology can quickly and easily measure growth parameters in various plants. The method, however, is sensitive to measurement saturation caused by a dense canopy and thus underestimates canopy growth parameters (Yu *et al*., 2020). Therefore, the near-surface hyperspectral technique is an excellent way to estimate canopy structure parameters and reduce measurement saturation through an optimized vegetation index. It became the first step to developing image-based phenotyping, especially in its use in screening rice in drought-salinity stress.

CONCLUSIONS

Salinity stress had a wider variance than drought stress, while the combination of drought-salinity stress had a relatively stable variance compared with independent stress. The combination of morphological characters and image-based phenotyping could increase precision in assessing the tolerance or adaptability of rice to drought, salinity, and combined stress. Morphological characters that could be used as rice selection criteria in multiple drought-salinity stress comprised the shoot fresh weight, root length, and root fresh weight. The image-based phenotyping character suggested as a selection criterion was the width of the shoot phenotype. Image-based phenotyping characters, specifically the shoot phenotype area, are recommended as a character for precise selection in assessing the potential tolerance or adaptability of rice genotypes either to drought stress, salinity, or the combination of both.

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