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1 **Image Based-Phenotyping and Multivariate Analysis for Rice Rapid Screening under**
2 **Drought Stress**

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10 **Abstract**

11 The development of rice varieties that are tolerant of drought stress should be detected early with
12 Image-based phenotyping. The Image-based phenotyping combined with multivariate analysis
13 can characterize the morphological response easily and in a short time, so this method is suitable
14 for rice rapid screening under drought stress. Therefore, the aim of this study is to determine the
15 selection criteria and assess the effectiveness of using image-based phenotyping based on
16 multivariate analysis in drought stress rapid screening. This study was conducted in two stages,
17 the first stage was conducted in static hydroponic and the second stage in dynamic hydroponic. In
18 static hydroponic, the study used a split-plot design where the levels of drought were the main
19 plots and varieties were the subplots. However, in dynamic hydroponic, the study used a nested
20 design, where the replicates were nested in the drought level treatments. The drought level factors
21 used in this study were PEG 0%, PEG 10% and PEG 20%. The variety factor consisted of 5
22 varieties which were repeated three times. The results of this study showed that the selection
23 index for static hydroponic consisted of shoot area (0.421), green shoot area (0.4177), and the
24 area growth rate (0.4192). Meanwhile, the selection index in dynamic hydroponics consisted of
25 object extent Y from side (0.4516) and convex hull from side (0.4177). The regression of two

26 selection index has a good determination of 0.84. Based on this study, rice screening based on
27 image-based phenotyping can be recommended for rapid screening under drought stress.

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

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57 **Introduction**

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59 Paddy is a commodity that plays an important role in Indonesia's economy. In general, rice
60 is consumed by approximately 90% of the Indonesia population as a staple food (Donggulo *et al.*
61 2017). Rice production in Indonesia at 2020 has decreased from the previous year, from 59.2
62 million tons to 54.6 million tons (BPS 2021). The decline in production can be influenced by
63 various factors, one of which is drought stress. According to Mawardi *et al.* (2016), about 80% of
64 the cultivation area of paddy and other cereals are affected by drought as a limiting factor for
65 production. In addition, several studies have shown that drought can reduce the rate of growth
66 and rice yield (Supriyanto 2013; Sujinah and Jamil 2016; Rusmawan *et al.* 2018). Therefore, the
67 development of drought tolerant varieties is a solution to increasing rice production.

68 The development of drought tolerant variety requires an effective and efficient screening
69 method. In general, drought tolerant screening can be carried out artificially or directly in the
70 target environment. However, the artificial selection environment is considered easier to control,
71 especially the control of stress concentration levels, hence the use of this method can increase the
72 effectiveness and efficiency in screening (Anshori *et al.* 2019). In drought stress, artificial
73 screening can be carried out with a hydroponic concept with a polyethylene glycol (PEG)
74 selection agent (Swapna and Shylaraj 2017; Mangansige *et al.* 2018; Osmolovskaya *et al.* 2018).
75 The use of this method under drought stress has been reported by Farid *et al.* (2021) in wheat
76 crop. In addition, according to Ai *et al.* (2010) and Wening and Susanto (2017), the use of PEG
77 6000 with certain concentrations in the early (vegetative) phase can be used as an alternative for
78 quick or early selection. This concept is very important in increasing the effectiveness of
79 selection. Therefore, the use of PEG in a static hydroponic system in the vegetative phase can be

80 a good alternative in screening process of drought tolerance of paddy. However, this screening
81 requires an accurate and precise assessment approach.

82 Assessment of drought stress tolerance through scoring is a common assessment. However,
83 this assessment is considered inaccurate. Based on the genetic constitution, drought tolerance
84 nature is polygenic. It is indicated by the QTL report that encode these characteristics (Lanceras
85 *et al.* 2004), so that in the assessment of drought, a qualitative scoring should not be used.
86 However, observations based on morphology are generally considered to be a highly bias
87 (Brereton *et al.* 2015). In addition, physiological and molecular approaches are considered
88 effective and have a relatively low bias, but these approaches are expensive (Nuraida 2012).
89 Therefore, an accurate and precise approach with a relatively low cost is needed in identifying the
90 nature of rice tolerance to drought stress. One method that is considered effective is a digital
91 technology approach through image-based phenotyping.

92 Image-based phenotyping technology is currently being developed to characterize plant
93 morphological and physiological responses using digital images. This technology makes it easy
94 to calculate phenotypes by analyzing plants through images accurately in large numbers and in a
95 short time, hence technique does not require a long time (Das Choudhury *et al.* 2018). Several
96 studies have reported the use of an effective and efficient imaged-based phenotyping selection
97 method in rice (Hairmansis *et al.* 2014; Siddiqui *et al.* 2014; Duan *et al.* 2018; Guimaraes *et al.*
98 2020; Kim *et al.* 2020; Vishal *et al.* 2020) and maize (Asaari *et al.* 2019; Li *et al.* 2021).
99 However, there are still few studies that examine the development of imaged-based phenotyping
100 based on multivariate analysis on static hydroponic system. In general, multivariate analysis is
101 able to analyze or integrate the diversity of large variable data into a simpler and more
102 comprehensive manner, so that the results are easier to understand (Mattjik and Sumertajaya
103 2011). Several studies have reported the effectiveness of selection with the use of multivariate

104 analysis (Anshori *et al.* 2018; Kose *et al.* 2018; Akbar *et al.* 2019; Anshori *et al.* 2019; Fadhli *et*
105 *al.* 2020; Farid *et al.* 2020; Farid *et al.* 2021). Therefore, the use of image-based phenotyping
106 with a multivariate analysis approach as a rapid screening for static hydroponic is necessary.
107 Meanwhile, the aim of this study was to determine the selection criteria and assess the
108 effectiveness of using image-based phenotyping based on multivariate analysis in drought stress
109 rapid screening.

110

111 **Materials and Methods**

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113 This study was conducted out in two experiments. The first experiment was the destructive
114 test carried out on static hydroponic (floating raft system), while the second test was the non-
115 destructive experiment carried out on dynamic hydroponic (deep flow technique system). These
116 studies was carried out parallelly in the greenhouse, Unhas Lecturer Residence, Makassar City,
117 South Sulawesi Province, Indonesia (22.4 m above sea level (asl) in August to November 2020.

118

119 **Experimental Design and Procedures**

120 The study in static hydroponic used a split-plot design where the levels of drought were the
121 main plot and varieties were the subplots. However, in dynamic hydroponic used a nested design,
122 where the replicates were nested in the drought level treatments. The drought level factors used in
123 this study were PEG 0%, PEG 10% and PEG 20%. The varieties used in this study were Inpari 34,
124 IR 20 (drought-sensitive check), Salumpikit (drought-resistant check), Ciherang, and Jeliteng.
125 The drought stress environment used in this study was PEG 0% (normal), PEG 10% and PEG
126 20%.

127 The medium used was ABmix with a concentration of 8 mL/L. The PEG concentration
128 treatments were given to the hydroponic nutrient solution gradually. The first stage was given ½
129 concentration of PEG treatment at the age of 13 DAS. The second stage was given at the age of
130 16 DAS, this was done to prevent osmotic shock. pH control was applied to keep it constant. The
131 desired pH was in the range 5.8-6.2. pH control was done by adding HCl or NaOH to lower or
132 raise the pH. Data collection on the static hydroponic method was carried out 2 weeks after the
133 treatment application or 30 DAS. As for dynamic hydroponics, data collection was carried out at
134 68 DAS.

135 Observations for static hydroponic carried out on shoot fresh weight, root fresh weight, 2nd,
136 3rd and 4th leaf length, shoot area from the side view, green shoot area from the side view, ratio
137 of green shoot area to shoot area from the side view, red, green, blue, ratio of red to green, and
138 area growth rate. Observations for dynamic hydroponic carried out on root fresh weight, shoot
139 fresh weight, convex hull from top view, shoot area from top view, green shoot area from top
140 view, ratio of green shoot area to shoot area from top view, number of leaves from top view, red,
141 green, blue, ratio red to green, ratio of shoot area to convex hull from top view, object extent X
142 from side view, object extent Y from side view, shoot area from side view, green shoot area from
143 side view, ratio of green shoot area to the shoot area from side view, convex hull from side view,
144 and ratio of shoot area to convex hull area from side view (Table 1).

145

146 **Image Analysis**

147 Images were taken using Canon EOS 1200D RGB camera in a portable photo studio with a
148 studio 75 cm x 75 cm x 75 cm. The background used was a white background with two 8 watt
149 white LED lights in the studio with camera settings (5.6 F-stops, 1/160 second exposure time,
150 ISO 800, and without flash). Image were acquired from a top view (0°) and from the side view

151 (90°) of the plant. Images were taken twice, at 13 DAS (early treatment) and 30 DAS (after
152 treatment) for static hydroponic (Figure 1). For dynamic hydroponic, images were taken at 13
153 DAS (early treatment) and 68 DAS (after treatment) (Figure 2). The results of the images were
154 analyzed using the Fiji application. The plant color was analyzed using hue channels with a hue
155 50 (green area). The area growth rate was calculated using the projection of shoot area over two
156 time periods.

157

158 **Data analysis**

159 Data were analyzed independently for variance, then the characters that have significantly
160 interactions at each character were further analyzed with the pearson correlation test and
161 Principal Component Analysis (PCA) with STAR 2.01 software. For static hydroponic, after
162 being tested by correlation analysis, it was followed by path analysis. All futher analysis based on
163 the stress tolerance index (STI) value of each character (Anshori *et al.* 2019), as follow:

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

165
$$STI = \frac{Y_p \times Y_s}{\bar{Y}_p^2}$$

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

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

168 \bar{Y}_s = Average character values of all varieties in normal / non-stressed conditions.

169 The selected characters from the PCA analysis results are used to obtain the index value.

170 The validation of static hydroponic was conducted by the regression test toward the dynamic
171 hydroponic.

172

173

174 **Results**

175

176 **Static Hydroponic System**

177 Analysis of variance in the static hydroponic method showed that the diversity of PEG
178 concentrations had a significant effect on almost all characters, except for the green, ratio of red
179 to green, and ratio of green shoot area to shoot area (Table 2). Genotype diversity also showed a
180 significant effect on shoot fresh weight, root fresh weight, 2nd, 3rd, and 4th leaf length, shoot
181 area, shoot green area, and area growth rate. Meanwhile, the diversity of drought-genotype
182 interactions only had a significant effect on shoot fresh weight, root fresh weight, 3rd leaf length,
183 shoot area, green shoot area and area growth rate.

184 The results of correlation analysis on growth characters of static hydroponic are based on
185 the static hydroponic Stress Tolerance Index (STI) value (Table 3). The results of the analysis
186 showed that the 3rd leaf length (0.7891), shoot area (0.981), green shoot area (0.9781), and area
187 growth rate (0.9805) had a significant correlation on shoot fresh weight and root fresh weight.
188 Based on the result of the correlation analysis, then followed by path analysis on shoot fresh
189 weight and root fresh weight.

190 Path analysis on the STI value of shoot fresh weight in static hydroponic shows
191 representative results with a determinant coefficient value of 0.86 (Table 4). Based on this
192 analysis, root fresh weight is the character that has the highest direct effect (0.600) on shoot fresh
193 weight. Meanwhile, based on the image-based phenotyping character, area growth rate is the
194 character that has the highest direct effect (0.576) on shoot fresh weight.

195 Path analysis on the STI value of root fresh weight in static hydroponic shows
196 representative results with a determinant coefficient value of 0.87 (Table 5). Based on this
197 analysis, shoot fresh weight was the character that had the highest direct effect (0.548) on root

198 fresh weight. Meanwhile, based on image-based phenotyping characters, the shoot area (0.493)
199 and green shoot area (0.377) were characters that had a significant direct effect on root fresh
200 weight. On the other hand, the area growth rate has a negative significant direct effect (-0.417) on
201 the root fresh weight.

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

209 Static hydroponic index = 0.421 shoot area + 0.4177 shoot green area + 0.4192 growth rate area
210 (Equation 1)

212 **Dynamic Hydroponic System**

213 Analysis of variance in the dynamic hydroponic method in Table 7 show that PEG
214 treatment as drought stress has an impact on all image-based-phenotyping characters, except for
215 ratio of red to green from top view and ratio of green shoot area to shoot area from top view.
216 Variety diversity has a significant effect on the number of leaves from top view, red from top
217 view, blue from top view, ratio of shoot area to convex hull from top view, object extend X from
218 side view, object extend Y from side view, and convex hull from side view. Meanwhile, the
219 characters that are significantly affected by the interaction are red from top view, blue from top
220 view, ratio of shoot area to convex hull from top view, object extend Y from side view, and
221 convex hull from side view.

222 Significant characters were correlated with the selection index on static hidroponic. The
223 results of the correlation analysis of dynamic hydroponic phenotyping characters were also based
224 on the dynamic hydroponic stress tolerance index (STI) values (Table 8). Based on this, the
225 object extend Y from side view (0.91) and convex hull from side view (0.91) have a significant
226 correlation with the selection index of static hydroponic.

227 The result of principal components analysis on dynamic hydroponic are shown in Table 9.
228 Based on this table, PC1 has the largest cumulative proportion with a value of 58.83% of the total
229 diversity of the initial data. Red from top view and blue flom top view have negative eigenvectors.
230 In contrast, the characters of rasio of shoot area to convex hull from top view, object extend Y
231 and convex hull from side view have positive eigenvectors. Based on this, the YS and CS
232 characters were combined into a selection index in dynamic hydroponic screening using the
233 formula:

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

237 **Selection Index**

238 The index value of each variety on static and dynamic hydroponics are shown in Table 10.
239 Salumpikit variety at a PEG concentration of 10% (2.03) shows the highest value, while IR 20 at
240 a PEG concentration of 20% (0.11) shows the lowest value in static hydroponics. In addition,
241 based on dynamic hydroponic, salumpikit variety at a PEG concentration of 10% (0.53) remained
242 the best, while Inpari 34 (0.22) and IR 20 (0.22) were the varieties with the lowest index value.
243 Meanwhile, the results of the regression analysis show that the image-based phenotyping
244 character in static hydroponic have a significant linear regression to the image-based phenotyping
245 character in dynamic hydroponic (Figure 3).

246 **Discussion**

247
248 The significant interaction on anova is one of the early indicators in screening for a stress.
249 This has been reported by Akçura and Çeri (2011); Anshori *et al.* (2019); Farid *et al.* (2020),
250 where characters influenced by interactions show different patterns of decreased response
251 between genotypes on normal and stressful environments. This difference can be a parameter in
252 identifying tolerance traits between genotypes in a stress (Ali *et al.* 2015; Safitri *et al.* 2016;
253 Fadhli *et al.* 2020). Based on this analysis, the image-based phenotyping of 3rd leaf length, shoot
254 area, shoot green area and area growth rate on static hydroponic screening as well as red from top
255 view, blue from top view, ratio of shoot area to convex hull from top view, object extend Y from
256 the side view, and convex hull from the side view in dynamic hydroponic screening can be used
257 as candidate to selection criteria in stress tolerance. In addition, these characters were also
258 significantly influenced by the diversity of PEG concentrations and varieties, therefore the
259 differences in interactions between genotypes on environmental differences can be seen clearly
260 and direct (Farid *et al.* 2020; Farid *et al.* 2021). However, more in-depth analysis with several
261 series of multivariate analyzes were required in order to obtain the best selection criteria for each
262 drought screening method.

263 The assessment of varieties response to stress should be done using a tolerance index. It has
264 been reported by Mau *et al.* (2014), Aboughadareh *et al.* (2019), Anshori *et al.* (2019);
265 Aboughadareh *et al.* (2020), and Farid *et al.* (2021) that the Stress Tolerance Index (STI) is an
266 appropriate index to characterize the most tolerant of drought stress. This index is classified as
267 dynamic because it considers the general response of the population mean. Even though on this
268 index the general response focuses on normal conditions (Anshori *et al.* 2019). This STI value
269 becomes the basis for a deeper analysis. This was reported by Anshori *et al.* (2019) on rice

270 against salinity stress, Fadhli *et al.* (2020) on maize against drought stress, and Farid *et al.* (2021)
271 on wheat against drought stress. Therefore, the use of STI as the basis for a more in-depth
272 analysis was also carried out, both for static hydroponic screening and for dynamic screening.

273 Correlation analysis and path analysis on static hydroponic were focused on the shoot fresh
274 weight and root fresh weight characters. Both of these fresh weights have been reported to have a
275 close relationship with drought tolerance (Saha *et al.* 2019). The relationship between image-
276 based phenotyping characters on these two characters can be the basis for determining the best
277 selection criteria for drought stress. Meanwhile, the concepts of correlation and path analysis are
278 often combined in determining the selection criteria (Safitri *et al.* 2011). This is because the
279 correlation still cannot describe how big the pure role of a character is to the diversity of the main
280 characters (Singh and Chaudhary 2007; Rohaeni and Permadi 2012; Anshori *et al.* 2018). The use
281 of this analysis has been widely reported by several studies (Aman *et al.* 2020; Saleh *et al.* 2020;
282 Dutt *et al.* 2020; Ashraf *et al.* 2020). Therefore, the use of this analysis also needs to be done on
283 the character of the image-based phenotyping on the two fresh weights.

284 The results of correlation analysis and path analysis on static hydroponic show that shoot
285 area, green shoot area, and area growth rate can be used as selection criteria. The potential of this
286 image-based phenotyping characters were also reported by Hairmansis *et al.* (2014) on rice under
287 salinity stress, Honsdorf *et al.* (2014) on barley under drought stress and Kim *et al.* (2020) on rice
288 under drought stress. Based on the results of the path analysis, the area growth rate has an inverse
289 orientation to the shoot area and the green shoot area. This difference in orientation can be seen
290 from the direct effect and indirect effect on the character of shoot fresh weight and root fresh
291 weight (Table 4 and 5). This indicates that shoot fresh weight and root fresh weight become the
292 middle of the three image-based phenotyping characters. However, the combination of the three
293 characters can make the selection more selective because each character has specific potential.

294 This has been reported by Acquaah (2007) and Anshori *et al.* (2021, In press), a selection index
295 formed from combining characters with opposite orientations can increase the selectivity of a
296 selection. Therefore, these three characters are still used as selection criteria. However, crucial
297 thing in the formation of the selection index of the three characters is the weighting value of the
298 selection. One method that can be used in determining the weighted value of the selection index
299 is through principle component analysis.

300 Principle component analysis is a multivariate analysis that aims to extract important
301 information from big data into a new simpler set of orthogonal variables (Ilmaniati and Putro
302 2019). This analysis has been reported as a weighting indicator for selection index by Akbar *et al.*
303 (2019), Anshori *et al.* (2019), Alsabah *et al.* (2019), Fadhli *et al.* (2020) and Farid *et al.* (2021).
304 Based on the PCA results in Table 6, the characteristics of shoot area, green shoot area, and area
305 growth rate have the same eigenvector direction and variance with shoot fresh weight and root
306 fresh weight. This support that the three characters generally have the same variance direction,
307 even though in the broad cross-section, the growth of the shoot has a different orientation from
308 the other two characters. However, because of the inclusion of the two fresh weight characters,
309 the three characters generally have the same range direction and can be combined into the
310 selection index in Equation 1.

311 Validation of image-based phenotyping characters in static hydroponics was done by
312 identifying the relation of these characters to the image-based phenotyping characters in dynamic
313 hydroponic. Relatively, dynamic hydroponic has a growth rate that is more complex than the
314 growth rate in static hydroponic (Sagita *et al.* 2020). This was because dynamic hydroponic
315 observations were carried out in the flowering phase, while static hydroponic were carried out in
316 the vegetative phase. However, the application of selection agent in the two methods were carried

317 out at the same time in the 21 days after sowing. Therefore, the static hydroponic selection index
318 assessment can be related to the growth character of dynamic hydroponic as part of its validation.

319 Correlation analysis of image-based phenotyping characters on dynamic hydroponic that
320 have significant interactions is one method of validating static hydroponic selection indexes.
321 Based on the results of the dynamic hydroponic screening correlation, object extend Y from the
322 side view, and convex hull from the side view can be used as selection characters. This result has
323 also been reported by Duan *et al.* (2018) and Kim *et al.* (2020) that stated the leaves on plants
324 that are stricken with drought will turn yellow and bend, thereby reducing the calculated convex
325 hull. However, the results of this study also need to be supported by other analyzes such as PCA
326 and regression between selection indices (Figure 3). The use of these two analyzes was also
327 reported by Anshori *et al.* (2018) in determining the selection criteria.

328 Based on the results of PCA, the object extend Y from the side view and the convex hull
329 from the side view have the same eigenvector direction. The eigenvectors that are relatively the
330 same in both dimensions show the closeness of the variance between the two variables (Anshori
331 *et al.* 2018). This indicates that both characters can be used as validation characters through the
332 selection index. The dynamic hydroponic selection index can be used as the Y factor in a
333 regression. Based on the regression results, the static hydroponic index has good adherence to the
334 dynamic hydroponic index. In addition, the differences in tolerance groups, between the
335 Salumpikit and IR 20 varieties, also show quite a distance. This result is also consistent with the
336 research of Hairmansis *et al.* (2019) and Kartina *et al.* (2019). Based on the overall results, the
337 selection index on static hydroponic screening can be used to identify the tolerance properties of
338 rice to drought stress.

339 In conclusions, the use of multivariate analysis on image-based phenotyping characters
340 can increase the effectiveness of selection in drought stress tolerance screening. The selection

341 index for static hydroponic is 0.421 shoot area + 0.4177 green shoot area + 0.4192 area growth
342 rate. Meanwhile, the character selection index for dynamic hydroponic is 0.4516 object extend Y
343 from the side view + 0.4177 convex hull from the side view. Based on this study, rice screening
344 based on image-based phenotyping can be recommended as a more effective and efficient method
345 for rapid screening of drought stress.

346

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350

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

Characters	Description
2nd leaf length	The length of the 2nd leaf from the tip of the plant.
3rd leaf length	The length of the 3rd leaf from the tip of the plant.
4th leaf length	The length of the 4th leaf from the tip of the plant.
Shoot area	The number of pixels scaled in cm on the shoot of the plant.
Green shoot area	The number of pixels that have been scaled in cm in the shoot section that have hue value of 50.
Convex hull area	The smallest area covered by the outer contour of an object.
Object extend X	The length of the X axis of the rectangle covering the object, used to measure the width of the plant.
Object extend Y	The length of the Y axis of the rectangle covering the object, used to measure the height of the plant.
Number of leaves	Number of leaf tips visible from above of the plant.
Area growth rate	Dividing the difference in shoot area between the drought stress treatment intervals by drought stress treatment time (days).
Red	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	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	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).

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510 Table 2. *Analysis of variance on the static hydroponic method*

Characters	D	V	D x V
SFW	178.73 **	28.92 **	10.37 **
RFW	24.77 **	7.30 **	1.96 **
LL2	1486.95 **	326.19 **	14.68 tn
LL3	1471.64 **	466.95 **	49.37 *
LL4	669.49 **	188.37 **	28.07 tn
SA	65556.88 **	13117.85 **	3438.49 **
GSA	61237.72 **	9849.18 **	3261.87 **
R	3460.07 **	86.95 tn	748.95 tn
G	879.98 tn	87.35 tn	1498.13 tn
B	1680.60 *	96.75 tn	272.54 tn
RRG	0.60 tn	0.80 tn	0.96 tn
RGSS	0.57 tn	0.02 tn	0.03 tn
AGR	202.25 **	32.11 **	10.60 **

511 Note: D= Drought level; V= Variety; *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$;
512 SFW= Shoot fresh weight; RFW= Root fresh weight; LL2= 2nd leaf length; LL3= 3rd leaf length;
513 LL4= 4th leaf length; SA= Shoot area; GSA= Green shoot area; R= Red; G= Green; B= Blue;
514 RRG= Ratio of red to green; RGSS= Ratio of green shoot area to shoot area; AGR= Area growth
515 rate.

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

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

524 Note: *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; SFW= Shoot fresh weight; RFW=
 525 Root fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green shoot area; AGR= Area
 526 growth rate.

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

Characters	Direct	Indirect Influence					Residual
	Influence	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

542 Note: $R^2 = 0.86$; RFW= Root fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green
 543 shoot area; AGR= Area growth rate.

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559 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	SGA	GRA	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
SGA	0.377**	-0.010	0.488		-0.408	0.536	0.371
GRA	-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

560 Note: $R^2 = 0.87$; SFW= Shoot fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green
 561 shoot area; AGR= Area growth rate.

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577 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

578 Note: CP= Cumulative proportion; EV= Eigenvalues; PC= Principal component; SFW= Shoot
 579 fresh weight; RFW= Root fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green
 580 shoot area; AGR= Area growth rate.

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594 Table 7. Analysis of variance on the dynamic hydroponic method

Characters	D	V	D x V
SFW	8042.24 **	319.95 tn	376.25 tn
RFW	467.01 **	53.23 tn	14.64 tn
CT	81129688.30 **	4224903.16 tn	1562942.10 tn
SAT	5571152.36 **	474034.51 tn	97115.97 tn
GSAT	3072273.82 **	333330.03 tn	80272.07 tn
NLT	2478.87 **	617.19 *	156.84 tn
RT	3946.91 **	462.79 **	189.34 *
GT	4734.46 **	241.02 tn	59.00 tn
BT	505.53 **	193.20 **	74.72 *
RRGT	0.0051 tn	0.0082 tn	0.0099 tn
RGSST	0.0083 tn	0.0674 tn	0.0480 tn
RSCT	0.0226 **	0.0085 **	0.0010 *
XS	300.57 **	174.52 *	66.56 tn
YS	1636.09 **	147.66 **	110.49 **
SAS	3696517.43 **	280606.15 tn	91206.26 tn
GSAS	1651359.59 **	212567.33 tn	68283.65 tn
CS	48116645.30 **	6716755.96 **	947650.84 *
RGSSS	0.0853 *	0.0141 tn	0.0082 tn
RSCS	0.0463 **	0.0063 tn	0.0024 tn

595 Note: D= Drought level; V= Varieties; *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; SFW= Shoot fresh
596 weight; RFW= Root fresh weight; CT= Convex hull from top view; SAT= Shoot area from top view; GSAT= Green
597 shoot area from top view; NLT= Number of leaves from top view; RT= Red from top view; GT= Green from top
598 view; BT= Blue from top view; RRG: Rasio of red to green from top view; RGSST= Ratio of shoot green area to
599 shoot area from top view; RSCT= Ratio of shoot area to convex hull from top view; XS= Object extend X from side
600 view; YS= Object extend Y from side view; SAS= Shoot area from side view; GSAS= Green shoot area from side
601 view, CS= Convex hull from side view; RGSSS= Ratio of green shoot area to shoot area from side view; RSCS=
602 Ratio of shoot area to convex hull from side view.

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604 Table 8. *Correlation analysis of STI values on the dinamyc 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	-0.48	tn	-0.24	tn	0.30	tn	1.00				
CS	-0.30	tn	-0.03	tn	0.14	tn	0.97	**	1.00		
SH Index	-0.16	tn	-0.04	tn	0.19	tn	0.90	**	0.91	**	1.00

605 Note: *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; RT= Red from top view; BT= Blue
 606 from top view; RSCT= Ratio of shoot area to convex hull from top view; YS= Object extend Y
 607 from side view; CS= Convex hull from side view; SH Index= Static hydroponic selection index.

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622 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
Cumulative Proportion	0.5883	0.8544	0.9536	0.9801	1
EigenValues	2.9414	1.3306	0.496	0.1322	0.0997

623 Note: CP= Cumulative proportion; EV= Eigenvalues; PC= Principal component; RT= Red from
 624 top view; BT= Blue from top view; RSCT= Ratio of shoot area to convex hull from top view;
 625 YS= Object extend Y from side view; CS= Convex hull from side view.

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639 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

640 Note: SH Index= Static hydroponic selection index; DH Index= Dynamic hydroponic selection
 641 index.

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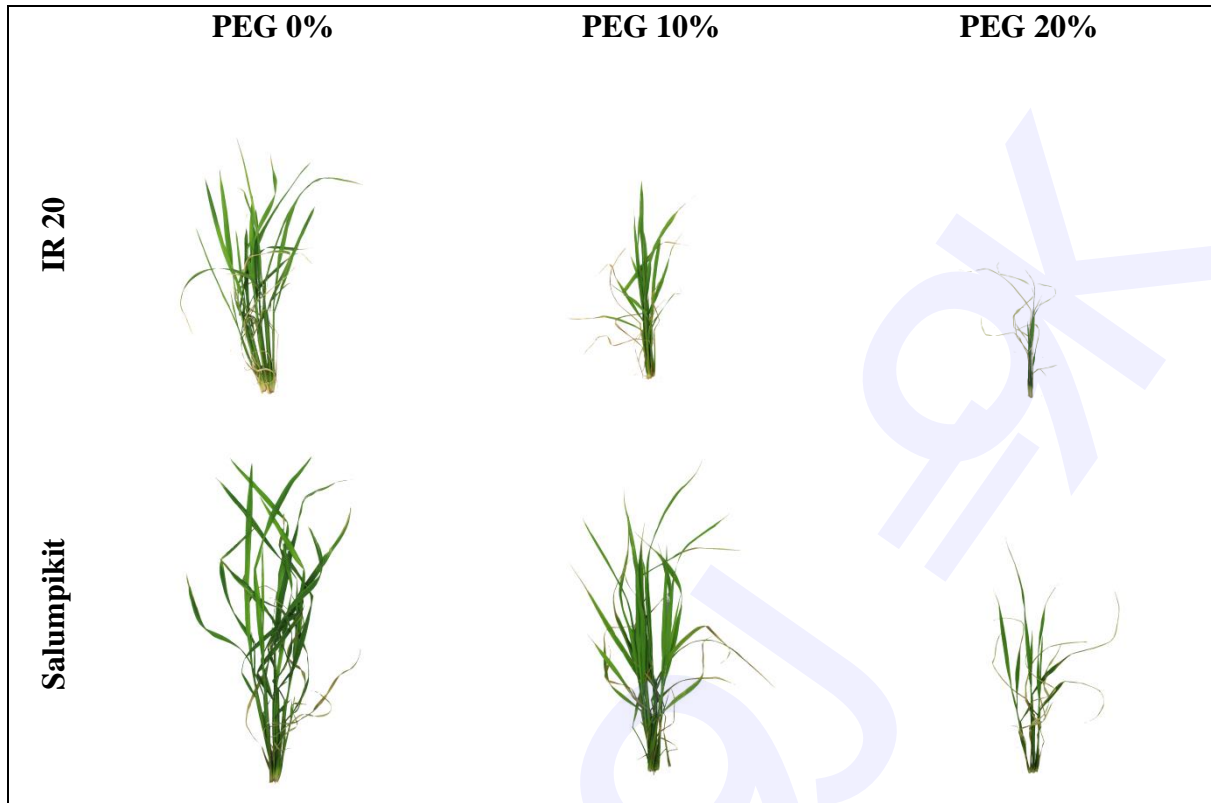
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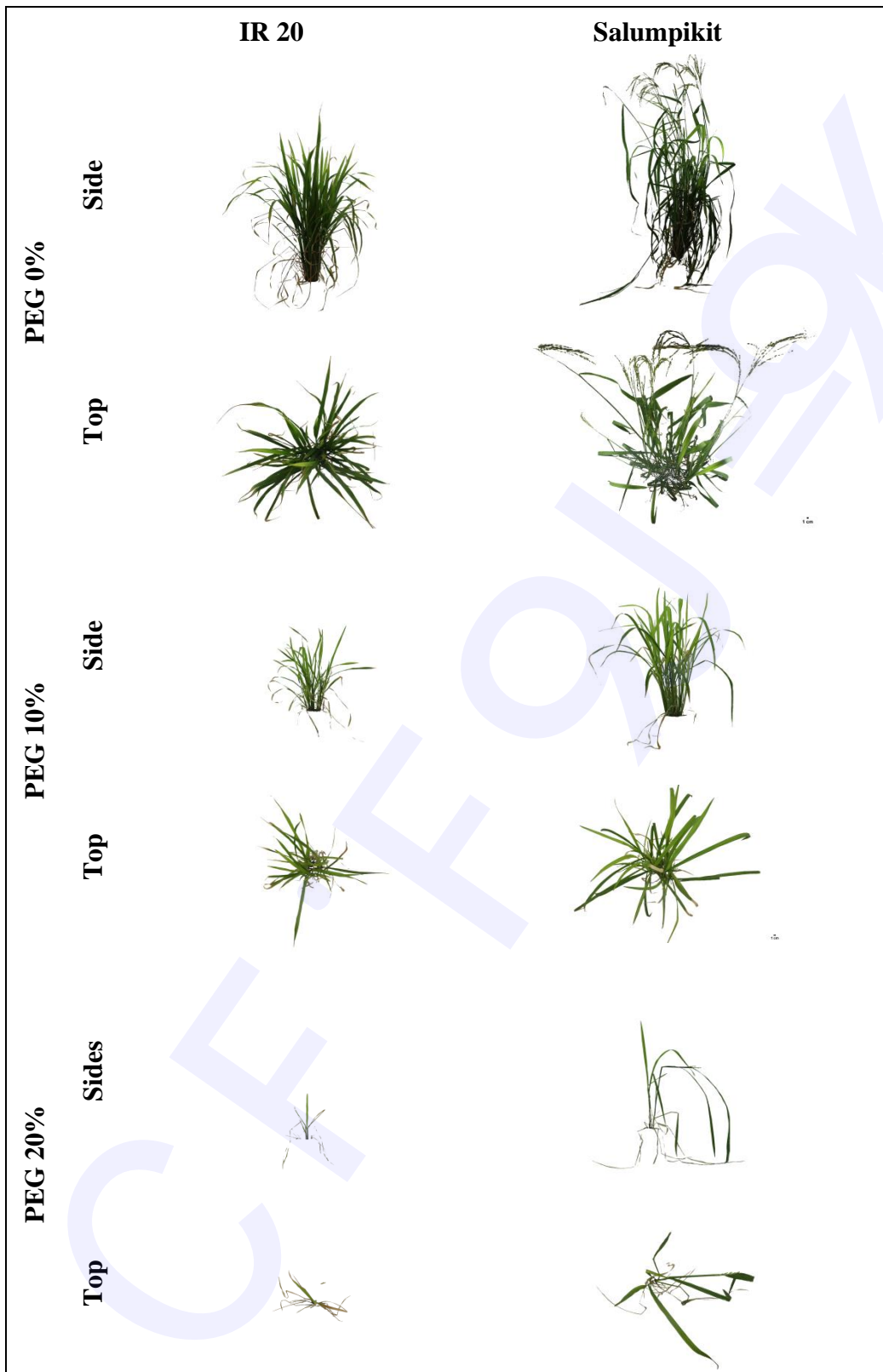
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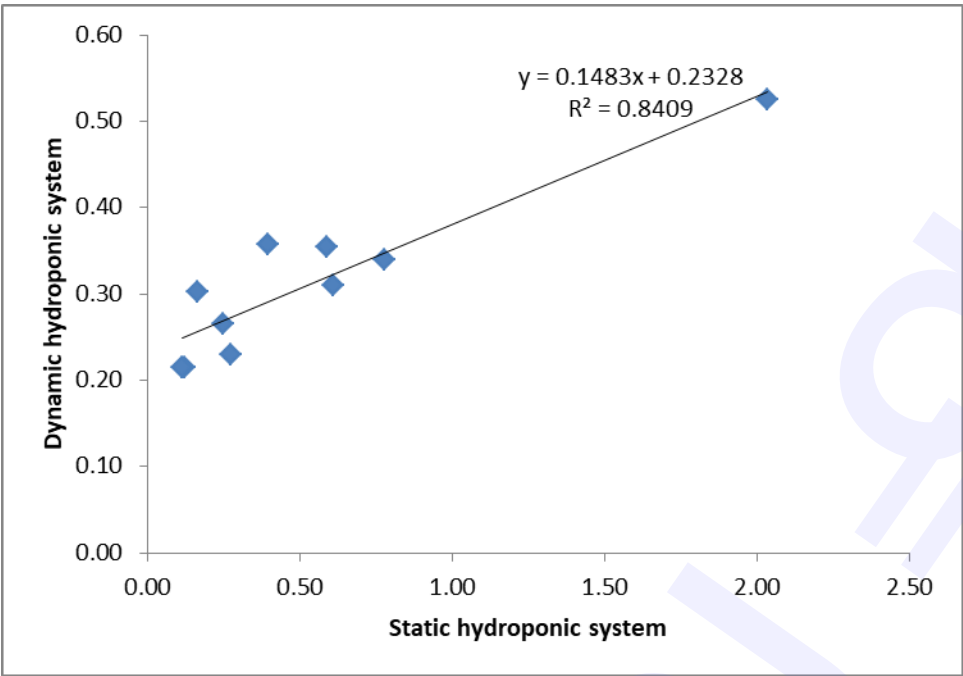


654 Figure 1. RGB images of IR 20 and Salumpikit under drought stress at 30 DAS in static
655 hydroponic.

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668 Figure 2. RGB images of IR 20 and Salumpikit under drought stress at 68 DAS in dynamic
 669 hydroponic.



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Figure 3. Analysis of static hydroponic selection index regression on dynamic hydroponic.

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1 **Image Based-Phenotyping and Selection Index Based on Multivariate Analysis for Rice**
2 **Hydroponic Screening Under Drought Stress**

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10 **Abstract**

11 The development of rice varieties that are tolerant of drought stress should be detected with
12 Image-based phenotyping. The Image-based phenotyping combined with selection index and
13 multivariate analysis can characterize the morphological response easily and in a short time, so
14 this method is suitable for rice screening under drought stress. Therefore, this study aims to
15 determine the selection index based on multivariate analysis and assess the effectiveness of using
16 image-based phenotyping in drought rice screening. This study was conducted in two stages, the
17 first stage was conducted in static hydroponic and the second stage in dynamic hydroponic. In
18 static hydroponic, the study used a split-plot design where the levels of drought were the main
19 plots and varieties were the subplots. However, the study used a nested design in dynamic
20 hydroponic, where the replicates were nested in the drought level treatments. The drought level
21 factors used in this study were PEG 0%, PEG 10% and PEG 20%. The variety factor consisted of
22 5 varieties which were repeated three times. The results of this study showed that the selection
23 index for static hydroponic consisted of shoot area (0.421), green shoot area (0.4177), and the
24 area growth rate (0.4192). Meanwhile, the selection index in dynamic hydroponics consisted of
25 object extent Y from the side (0.4516) and convex hull from the side (0.4177). The regression of
26 the two selection index has a good determination of 0.84. Based on this study, rice screening
27 based on image-based phenotyping can be recommended for rapid screening under drought stress.

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

29
30 **Introduction**

31
32 Paddy is a commodity that plays an important role in Indonesia's economy. In general, rice
33 is consumed by approximately 90% of the Indonesia population as a staple food (Donggulo *et al.*
34 2017). Rice production in Indonesia at 2020 has decreased from the previous year, from 59.2
35 million tons to 54.6 million tons (BPS 2021). The decline in production can be influenced by
36 various factors, one of which is drought stress. According to Mawardi *et al.* (2016), about 80% of
37 the cultivation area of paddy and other cereals are affected by drought as a limiting factor for
38 production. In addition, several studies have shown that drought can reduce the rate of growth
39 and rice yield (Supriyanto 2013; Sujinah and Jamil 2016; Rusmawan *et al.* 2018). Therefore, the
40 development of drought tolerant varieties is a solution to increasing rice production.

41 The development of drought tolerant variety requires an effective and efficient screening
42 method. In general, drought tolerant screening can be carried out artificially or directly in the
43 target environment. However, the artificial selection environment is considered easier to control,
44 especially the control of stress concentration levels, hence the use of this method can increase the
45 effectiveness and efficiency in screening (Anshori *et al.* 2019). In drought stress, artificial
46 screening can be carried out with a hydroponic concept with a polyethylene glycol (PEG)
47 selection agent (Swapna and Shylaraj 2017; Mangansige *et al.* 2018; Osmolovskaya *et al.* 2018).
48 The use of this method under drought stress has been reported by Ai *et al.* (2010), Wening and
49 Susanto (2017), and Farid *et al.* (2021). In addition, according to Ai *et al.* (2010) and Wening and
50 Susanto (2017), the use of PEG 6000 with certain concentrations in the early (vegetative) phase
51 can be used as an alternative for quick or early selection. This concept is very important in
52 increasing the effectiveness of selection. Nevertheless, the vegetative screening under salinity stress
53 has to validate or correlate with generative phase, one of them is the dynamic hydroponic. This
54 hydroponic can measure the tolerance screening until generative phase with controlled

55 environment (Farid *et al.* 2021). Therefore, the use of PEG in static and dynamic hydroponic
56 systems can be a good alternative in the screening process of drought tolerance of paddy.
57 However, this screening requires an accurate and precise assessment approach.

58 Assessment of drought stress tolerance through scoring is a common assessment. However,
59 this assessment is considered inaccurate. Based on the genetic constitution, drought tolerance
60 nature is polygenic. It is indicated by the QTL report that encode these characteristics (Lanceras
61 *et al.* 2004), so that in the assessment of drought, a qualitative scoring should not be used.
62 However, observations based on morphology are generally considered to be a highly bias
63 (Brereton *et al.* 2015). In addition, physiological and molecular approaches are considered
64 effective and have a relatively low bias, but these approaches are expensive (Nuraida 2012).
65 Therefore, an accurate and precise approach with a relatively low cost is needed in identifying the
66 nature of rice tolerance to drought stress. One method that is considered effective is a digital
67 technology approach through image-based phenotyping.

68 Image-based phenotyping technology is currently being developed to characterize plant
69 morphological and physiological responses using digital images. This technology makes it easy
70 to calculate phenotypes by analyzing plants through images accurately in large numbers and in a
71 short time, hence technique does not require a long time (Das Choudhury *et al.* 2018). Several
72 studies have reported the use of an effective and efficient imaged-based phenotyping selection
73 method in rice (Hairmansis *et al.* 2014; Siddiqui *et al.* 2014; Duan *et al.* 2018; Guimaraes *et al.*
74 2020; Kim *et al.* 2020; Vishal *et al.* 2020) and maize (Asaari *et al.* 2019; Li *et al.* 2021).
75 However, there are still few studies that examine the development of imaged-based phenotyping
76 based on selection index and multivariate analysis on static hydroponic system.

77 The selection index is a breeding selection approach with combining some selection criteria
78 in one formula (Anshori *et al.* 2021). This approach is famous to use in plant breeding since it

79 considers all selection criteria with current economic value on each criterion in genotype
80 selection (Rajamani *et al.* 2016; Islam *et al.* 2017). The selection index often to combine with
81 multivariate analysis to increase the effectiveness selection (Akbar *et al.* 2019; Anshori *et al.*
82 2019; Fadhli *et al.* 2020; Farid *et al.* 2021; Anshori *et al.* 2021). Multivariate analysis is able to
83 analyze or integrate the variance of large variable data into a simpler and more comprehensive
84 manner, so that the results are easier to understand (Mattjik and Sumertajaya 2011). Several
85 studies have reported the effectiveness of selection with the use of multivariate analysis (Anshori
86 *et al.* 2018; Kose *et al.* 2018; Akbar *et al.* 2019; Anshori *et al.* 2019; Fadhli *et al.* 2020; Farid *et*
87 *al.* 2020; Farid *et al.* 2021). Therefore based on these, the use of image-based phenotyping with
88 the selection index based on multivariate analysis in static and dynamic hydroponic screening is
89 necessary. The objective of this study was to determine the selection index based on multivariate
90 analysis and assess the effectiveness of using image-based phenotyping in drought rice screening.

91

92 **Materials and Methods**

93

94 This study was conducted out in two experiments. The first experiment was the destructive
95 test carried out on static hydroponic (floating raft system), while the second test was the non-
96 destructive experiment carried out on dynamic hydroponic (deep flow technique system). These
97 studies was carried out parallely in the greenhouse, Unhas Lecturer Residence, Makassar City,
98 South Sulawesi Province, Indonesia (22.4 m above sea level (asl) in August to November 2020.

99

100 **Experimental Design and Procedures**

101 The study in static hydroponic used a split-plot design where the levels of drought were the
102 main plot and varieties were the subplots (Figure 1a). However, in dynamic hydroponic used a

103 nested design, where the replicates were nested in the drought level treatments (Figure 1b). The
104 drought level factors used in this study were PEG 0%, PEG 10% and PEG 20%. The varieties
105 used in this study were Inpari 34, IR 20 (drought-sensitive check), Salumpikit (drought-resistant
106 check), Ciherang, and Jeliteng. The drought stress environment used in this study was PEG 0%
107 (normal), PEG 10% and PEG 20%.

108 The medium used was ABmix with a concentration of 8 mL/L. The PEG concentration
109 treatments were given to the hydroponic nutrient solution gradually. The first stage was given $\frac{1}{2}$
110 concentration of PEG treatment at the age of 13 DAS. The second stage was given at the age of
111 16 DAS, this was done to prevent osmotic shock. pH control was applied to keep it constant. The
112 desired pH was in the range 5.8-6.2. pH control was done by adding HCl or NaOH to lower or
113 raise the pH. Data collection on the static hydroponic method was carried out 2 weeks after the
114 treatment application or 30 DAS. As for dynamic hydroponics, data collection was carried out at
115 68 DAS.

116 Observations for static hydroponic carried out on shoot fresh weight, root fresh weight, 2nd,
117 3rd and 4th leaf length, shoot area from the side view, green shoot area from the side view, ratio
118 of green shoot area to shoot area from the side view, red, green, blue, ratio of red to green, and
119 area growth rate. Observations for dynamic hydroponic carried out on root fresh weight, shoot
120 fresh weight, convex hull from top view, shoot area from top view, green shoot area from top
121 view, ratio of green shoot area to shoot area from top view, number of leaves from top view, red,
122 green, blue, ratio red to green, ratio of shoot area to convex hull from top view, object extent X
123 from side view, object extent Y from side view, shoot area from side view, green shoot area from
124 side view, ratio of green shoot area to the shoot area from side view, convex hull from side view,
125 and ratio of shoot area to convex hull area from side view (Table 1).

126

127 **Image Analysis**

128 Images were taken using Canon EOS 1200D RGB camera in a portable photo studio with a
129 studio 75 cm x 75 cm x 75 cm. The background used was a white background with two 8 watt
130 white LED lights in the studio with camera settings (5.6 F-stops, 1/160 second exposure time,
131 ISO 800, and without flash). Image were acquired from a top view (0°) and from the side view
132 (90°) of the plant. Images were taken twice, at 13 DAS (early treatment) and 30 DAS (after
133 treatment) for static hydroponic (Figure 2). For dynamic hydroponic, images were taken at 13
134 DAS (early treatment) and 68 DAS (after treatment) (Figure 3). The results of the images were
135 analyzed using the Fiji application. The plant color was analyzed using hue channels with a hue
136 50 (green area). The area growth rate was calculated using the projection of shoot area over two
137 time periods.

138

139 **Data analysis**

140 Data were analyzed independently for variance, then the characters that have significantly
141 interactions at each character were further analyzed with the pearson correlation test and
142 Principal Component Analysis (PCA) with STAR 2.01 software. For static hydroponic, after
143 being tested by correlation analysis, it was followed by path analysis. All futher analysis based on
144 the stress tolerance index (STI) value of each character (Anshori *et al.* 2019), as follow:

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

146
$$STI = \frac{Y_p \times Y_s}{\bar{Y}_p^2}$$

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

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

149 \bar{Y}_s = Average character values of all varieties in normal / non-stressed conditions.

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

153

154 **Results**

155

156 **Static Hydroponic System**

157 Analysis of variance in the static hydroponic method showed that the diversity of PEG
158 concentrations had a significant effect on almost all characters, except for the green, ratio of red
159 to green, and ratio of green shoot area to shoot area (Table 2). Genotype diversity also showed a
160 significant effect on shoot fresh weight, root fresh weight, 2nd, 3rd, and 4th leaf length, shoot
161 area, shoot green area, and area growth rate. Meanwhile, the diversity of drought-genotype
162 interactions only had a significant effect on shoot fresh weight, root fresh weight, 3rd leaf length,
163 shoot area, green shoot area and area growth rate.

164 The results of correlation analysis on growth characters of static hydroponic are based on
165 the static hydroponic Stress Tolerance Index (STI) value (Table 3). The results of the analysis
166 showed that the 3rd leaf length (0.7891), shoot area (0.981), green shoot area (0.9781), and area
167 growth rate (0.9805) had a significant correlation on shoot fresh weight and root fresh weight.
168 Based on the result of the correlation analysis, then followed by path analysis on shoot fresh
169 weight and root fresh weight.

170 Path analysis on the STI value of shoot fresh weight in static hydroponic shows
171 representative results with a determinant coefficient value of 0.86 (Table 4). Based on this
172 analysis, root fresh weight is the character that has the highest direct effect (0.600) on shoot fresh

173 weight. Meanwhile, based on the image-based phenotyping character, area growth rate is the
174 character that has the highest direct effect (0.576) on shoot fresh weight.

175 Path analysis on the STI value of root fresh weight in static hydroponic shows
176 representative results with a determinant coefficient value of 0.87 (Table 5). Based on this
177 analysis, shoot fresh weight was the character that had the highest direct effect (0.548) on root
178 fresh weight. Meanwhile, based on image-based phenotyping characters, the shoot area (0.493)
179 and green shoot area (0.377) were characters that had a significant direct effect on root fresh
180 weight. On the other hand, the area growth rate has a negative significant direct effect (-0.417) on
181 the root fresh weight.

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

189 Static hydroponic index = 0.421 shoot area + 0.4177 shoot green area + 0.4192 growth rate area
190 (Equation 1)

191

192

193

194 **Dynamic Hydroponic System**

195 Analysis of variance in the dynamic hydroponic method in Table 7 show that PEG
196 treatment as drought stress has an impact on all image-based-phenotyping characters, except for

197 ratio of red to green from top view and ratio of green shoot area to shoot area from top view.
198 Variety diversity has a significant effect on the number of leaves from top view, red from top
199 view, blue from top view, ratio of shoot area to convex hull from top view, object extend X from
200 side view, object extend Y from side view, and convex hull from side view. Meanwhile, the
201 characters that are significantly affected by the interaction are red from top view, blue from top
202 view, ratio of shoot area to convex hull from top view, object extend Y from side view, and
203 convex hull from side view.

204 Significant characters were correlated with the selection index on static hidroponic. The
205 results of the correlation analysis of dynamic hydroponic phenotyping characters were also based
206 on the dynamic hydroponic stress tolerance index (STI) values (Table 8). Based on this, the
207 object extend Y from side view (0.91) and convex hull from side view (0.91) have a significant
208 correlation with the selection index of static hydroponic.

209 The result of principal components analysis on dynamic hydroponic are shown in Table 9.
210 Based on this table, PC1 has the largest cumulative proportion with a value of 58.83% of the total
211 diversity of the initial data. Red from top view and blue flom top view have negative eigenvectors.
212 In contrast, the characters of ratio of shoot area to convex hull from top view, object extend Y
213 and convex hull from side view have positive eigenvectors. Based on this, the YS and CS
214 characters were combined into a selection index in dynamic hydroponic screening using the
215 formula:

216 $\text{Dynamic hydroponic index} = 0.4516 \text{ object extend Y from the side view} + 0.4177 \text{ Convex hull}$
217 $\text{from the side view}$

218 **Selection Index**

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

221 a PEG concentration of 20% (0.11) shows the lowest value in static hydroponics. In addition,
222 based on dynamic hydroponic, salumpikit variety at a PEG concentration of 10% (0.53) remained
223 the best, while Inpari 34 (0.22) and IR 20 (0.22) were the varieties with the lowest index value.
224 Meanwhile, the results of the regression analysis show that the image-based phenotyping
225 character in static hydroponic have a significant linear regression to the image-based phenotyping
226 character in dynamic hydroponic (Figure 4). The figure also showed the Salumpikit as the
227 tolerant check variety has good value index than IR 20 as sensitive check variety on both 10%
228 and 20% PEG.

229

230 **Discussion**

231

232 The significant interaction on ANOVA is one of the early indicators in screening for a
233 stress. This has been reported by Akçura and Çeri (2011); Anshori *et al.* (2019); Farid *et al.*
234 (2020), where characters influenced by interactions show different patterns of decreased response
235 between genotypes on normal and stressful environments. This difference can be a parameter in
236 identifying tolerance traits between genotypes in a stress (Ali *et al.* 2014; Safitri *et al.* 2016;
237 Fadhli *et al.* 2020). Based on this analysis, the image-based phenotyping of 3rd leaf length, shoot
238 area, shoot green area and area growth rate on static hydroponic screening as well as red from top
239 view, blue from top view, ratio of shoot area to convex hull from top view, object extend Y from
240 the side view, and convex hull from the side view in dynamic hydroponic screening can be used
241 as candidate to selection criteria in stress tolerance. In addition, these characters were also
242 significantly influenced by the diversity of PEG concentrations and varieties, therefore the
243 differences in interactions between genotypes on environmental differences can be seen clearly
244 and direct (Farid *et al.* 2020; Farid *et al.* 2021). However, more in-depth analysis with several

245 series of multivariate analyzes were required in order to obtain the best selection criteria for each
246 drought screening method.

247 The assessment of varieties response to stress should be done using a tolerance index. It has
248 been reported by Mau *et al.* (2014), Aboughadareh *et al.* (2019), Anshori *et al.* (2019);
249 Aboughadareh *et al.* (2020), and Farid *et al.* (2021) that the Stress Tolerance Index (STI) is an
250 appropriate index to characterize the most tolerant of drought stress. This index is classified as
251 dynamic because it considers the general response of the population mean. Even though on this
252 index the general response focuses on normal conditions (Anshori *et al.* 2019). This STI value
253 becomes the basis for a deeper analysis. This was reported by Anshori *et al.* (2019) on rice
254 against salinity stress, Fadhli *et al.* (2020) on maize against drought stress, and Farid *et al.* (2021)
255 on wheat against drought stress. Therefore, the use of STI as the basis for a more in-depth
256 analysis was also carried out, both for static hydroponic screening and for dynamic screening.

257 Correlation analysis and path analysis on static hydroponic were focused on the shoot fresh
258 weight and root fresh weight characters. Both of these fresh weights have been reported to have a
259 close relationship with drought tolerance (Saha *et al.* 2019). The relationship between image-
260 based phenotyping characters on these two characters can be the basis for determining the best
261 selection criteria for drought stress. Meanwhile, the concepts of correlation and path analysis are
262 often combined in determining the selection criteria (Safitri *et al.* 2011). This is because the
263 correlation still cannot describe how big the pure role of a character is to the diversity of the main
264 characters (Singh and Chaudhary 2007; Rohaeni and Permadi 2012; Anshori *et al.* 2018). The use
265 of this analysis has been widely reported by several studies (Aman *et al.* 2020; Saleh *et al.* 2020;
266 Dutt *et al.* 2020; Ashraf *et al.* 2020). Therefore, the use of this analysis also needs to be done on
267 the character of the image-based phenotyping on the two fresh weights.

268 The results of correlation analysis and path analysis on static hydroponic show that shoot
269 area, green shoot area, and area growth rate can be used as selection criteria. The potential of this
270 image-based phenotyping characters were also reported by Hairmansis *et al.* (2014) on rice under
271 salinity stress, Honsdorf *et al.* (2014) on barley under drought stress and Kim *et al.* (2020) on rice
272 under drought stress. Based on the results of the path analysis, the area growth rate has an inverse
273 orientation to the shoot area and the green shoot area. This difference in orientation can be seen
274 from the direct effect and indirect effect on the character of shoot fresh weight and root fresh
275 weight (Table 4 and 5). This indicates that shoot fresh weight and root fresh weight become the
276 middle of the three image-based phenotyping characters. However, the combination of the three
277 characters can make the selection more selective because each character has specific potential.
278 This has been reported by Acquah (2007) and Anshori *et al.* (2021), a selection index formed
279 from combining characters with opposite orientations can increase the selectivity of a selection.
280 Therefore, these three characters are still used as selection criteria. However, crucial thing in the
281 formation of the selection index of the three characters is the weighting value of the selection.
282 One method that can be used in determining the weighted value of the selection index is through
283 principle component analysis.

284 Principle component analysis is a multivariate analysis that aims to extract important
285 information from big data into a new simpler set of orthogonal variables (Ilmaniati and Putro
286 2019). This analysis has been reported as a weighting indicator for selection index by Akbar *et al.*
287 (2019), Anshori *et al.* (2019), Alsbah *et al.* (2019), Fadhli *et al.* (2020) and Farid *et al.* (2021).
288 Based on the PCA results in Table 6, the characteristics of shoot area, green shoot area, and area
289 growth rate have the same eigenvector direction and variance with shoot fresh weight and root
290 fresh weight. This supports that the three characters generally have the same variance direction,
291 even though in the broad cross-section, the growth of the shoot has a different orientation from

292 the other two characters. However, because of the inclusion of the two fresh weight characters,
293 the three characters generally have the same range direction and can be combined into the
294 selection index in Equation 1.

295 Validation of image-based phenotyping characters in static hydroponics was done by
296 identifying the relation of these characters to the image-based phenotyping characters in dynamic
297 hydroponic. Relatively, dynamic hydroponic has a growth rate that is more complex than the
298 growth rate in static hydroponic (Sagita *et al.* 2020). This was because dynamic hydroponic
299 observations were carried out in the flowering phase, while static hydroponic were carried out in
300 the vegetative phase. However, the application of selection agent in the two methods were carried
301 out at the same time in the 21 days after sowing. Therefore, the static hydroponic selection index
302 assessment can be related to the growth character of dynamic hydroponic as part of its validation.

303 Correlation analysis of image-based phenotyping characters on dynamic hydroponic that
304 have significant interactions is one method of validating static hydroponic selection indexes.
305 Based on the results of the dynamic hydroponic screening correlation, object extend Y from the
306 side view, and convex hull from the side view can be used as selection characters. This result has
307 also been reported by Duan *et al.* (2018) and Kim *et al.* (2020) that stated the leaves on plants
308 that are stricken with drought will turn yellow and bend, thereby reducing the calculated convex
309 hull. However, the results of this study also need to be supported by other analyzes such as PCA
310 and regression between selection indices (Figure 4). The use of these two analyzes was also
311 reported by Anshori *et al.* (2018) in determining the selection criteria.

312 Based on the results of PCA, the object extend Y from the side view and the convex hull
313 from the side view have the same eigenvector direction. The eigenvectors that are relatively the
314 same in both dimensions show the closeness of the variance between the two variables (Anshori
315 *et al.* 2018). This indicates that both characters can be used as validation characters through the

316 selection index. The dynamic hydroponic selection index can be used as the Y factor in a
317 regression. Based on the regression results, the static hydroponic index has good adherence to the
318 dynamic hydroponic index. In addition, the differences in tolerance groups, between the
319 Salumpikit as the tolerant check variety and IR 20 varieties as the sensitive check variety, also
320 show quite a distance. This result is also consistent with the research of Hairmansis *et al.* (2020)
321 and Kartina *et al.* (2019). The use control varieties could be one of proofs to detect effectiveness
322 selection. This concept has been reported by Ali *et al.* (2014), Safitri *et al.* (2016) and Anshori *et*
323 *al.* (2020) on salinity stress, Wening *et al.* (2019) and Anshori *et al.* (2019) on submergence,
324 Akbar *et al.* (2018) and Akbar *et al.* (2019) on drought stress. Besides that, the use of Salumpikit
325 and IR 20 have been reported as check or control of rice drought tolerance by Akbar *et al.* (2018)
326 and Akbar *et al.* (2019). Based on the overall results, the selection index on static hydroponic
327 screening can be used to identify the tolerance properties of rice to drought stress.

328 In conclusions, the use of multivariate analysis on image-based phenotyping characters
329 can increase the effectiveness of selection in drought stress tolerance screening. The selection
330 index for static hydroponic is $0.421 \text{ shoot area} + 0.4177 \text{ green shoot area} + 0.4192 \text{ area growth}$
331 rate . Meanwhile, the character selection index for dynamic hydroponic is $0.4516 \text{ object extend Y}$
332 $\text{from the side view} + 0.4177 \text{ convex hull from the side view}$. Based on this study, rice screening
333 based on image-based phenotyping can be recommended as a more effective and efficient method
334 for rapid screening of drought stress.

335

336

337

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341

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

Characters	Description
2nd leaf length	The length of the 2nd leaf from the tip of the plant.
3rd leaf length	The length of the 3rd leaf from the tip of the plant.
4th leaf length	The length of the 4th leaf from the tip of the plant.
Shoot area	The number of pixels scaled in cm on the shoot of the plant.
Green shoot area	The number of pixels that have been scaled in cm in the shoot section that have hue value of 50.
Convex hull area	The smallest area covered by the outer contour of an object.
Object extend X	The length of the X axis of the rectangle covering the object, used to measure the width of the plant.
Object extend Y	The length of the Y axis of the rectangle covering the object, used to measure the height of the plant.
Number of leaves	Number of leaf tips visible from above of the plant.
Area growth rate	Dividing the difference in shoot area between the drought stress treatment intervals by drought stress treatment time (days).
Red	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	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	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).

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535 Table 2. *Analysis of variance on the static hydroponic method*

Characters	D	V	D x V
SFW	178.73 **	28.92 **	10.37 **
RFW	24.77 **	7.30 **	1.96 **
LL2	1486.95 **	326.19 **	14.68 tn
LL3	1471.64 **	466.95 **	49.37 *
LL4	669.49 **	188.37 **	28.07 tn
SA	65556.88 **	13117.85 **	3438.49 **
GSA	61237.72 **	9849.18 **	3261.87 **
R	3460.07 **	86.95 tn	748.95 tn
G	879.98 tn	87.35 tn	1498.13 tn
B	1680.60 *	96.75 tn	272.54 tn
RRG	0.60 tn	0.80 tn	0.96 tn
RGSS	0.57 tn	0.02 tn	0.03 tn
AGR	202.25 **	32.11 **	10.60 **

536 Note: D= Drought level; V= Variety; *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$;

537 SFW= Shoot fresh weight; RFW= Root fresh weight; LL2= 2nd leaf length; LL3= 3rd leaf length;

538 LL4= 4th leaf length; SA= Shoot area; GSA= Green shoot area; R= Red; G= Green; B= Blue;

539 RRG= Ratio of red to green; RGSS= Ratio of green shoot area to shoot area; AGR= Area growth

540 rate.

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

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

549 Note: *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; SFW= Shoot fresh weight; RFW=
 550 Root fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green shoot area; AGR= Area
 551 growth rate.

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

Characters	Direct	Indirect Influence					Residual
	Influence	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

567 Note: $R^2 = 0.86$; RFW= Root fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green
 568 shoot area; AGR= Area growth rate.

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584 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

585 Note: $R^2 = 0.87$; SFW= Shoot fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green
 586 shoot area; AGR= Area growth rate.

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602 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

603 Note: CP= Cumulative proportion; EV= Eigenvalues; PC= Principal component; SFW= Shoot
 604 fresh weight; RFW= Root fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green
 605 shoot area; AGR= Area growth rate.

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619 Table 7. Analysis of variance on the dynamic hydroponic method

Characters	D	V	D x V
SFW	8042.24 **	319.95 tn	376.25 tn
RFW	467.01 **	53.23 tn	14.64 tn
CT	81129688.30 **	4224903.16 tn	1562942.10 tn
SAT	5571152.36 **	474034.51 tn	97115.97 tn
GSAT	3072273.82 **	333330.03 tn	80272.07 tn
NLT	2478.87 **	617.19 *	156.84 tn
RT	3946.91 **	462.79 **	189.34 *
GT	4734.46 **	241.02 tn	59.00 tn
BT	505.53 **	193.20 **	74.72 *
RRGT	0.0051 tn	0.0082 tn	0.0099 tn
RGSST	0.0083 tn	0.0674 tn	0.0480 tn
RSCT	0.0226 **	0.0085 **	0.0010 *
XS	300.57 **	174.52 *	66.56 tn
YS	1636.09 **	147.66 **	110.49 **
SAS	3696517.43 **	280606.15 tn	91206.26 tn
GSAS	1651359.59 **	212567.33 tn	68283.65 tn
CS	48116645.30 **	6716755.96 **	947650.84 *
RGSSS	0.0853 *	0.0141 tn	0.0082 tn
RSCS	0.0463 **	0.0063 tn	0.0024 tn

620 Note: D= Drought level; V= Varieties; *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; SFW= Shoot fresh
621 weight; RFW= Root fresh weight; CT= Convex hull from top view; SAT= Shoot area from top view; GSAT= Green
622 shoot area from top view; NLT= Number of leaves from top view; RT= Red from top view; GT= Green from top
623 view; BT= Blue from top view; RRG: Ratio of red to green from top view; RGSST= Ratio of shoot green area to
624 shoot area from top view; RSCT= Ratio of shoot area to convex hull from top view; XS= Object extend X from side
625 view; YS= Object extend Y from side view; SAS= Shoot area from side view; GSAS= Green shoot area from side
626 view, CS= Convex hull from side view; RGSSS= Ratio of green shoot area to shoot area from side view; RSCS=
627 Ratio of shoot area to convex hull from side view.

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629 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	-0.48	tn	-0.24	tn	0.30	tn	1.00				
CS	-0.30	tn	-0.03	tn	0.14	tn	0.97	**	1.00		
SH Index	-0.16	tn	-0.04	tn	0.19	tn	0.90	**	0.91	**	1.00

630 Note: *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; RT= Red from top view; BT= Blue
 631 from top view; RSCT= Ratio of shoot area to convex hull from top view; YS= Object extend Y
 632 from side view; CS= Convex hull from side view; SH Index= Static hydroponic selection index.

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647 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
Cumulative Proportion	0.5883	0.8544	0.9536	0.9801	1
EigenValues	2.9414	1.3306	0.496	0.1322	0.0997

648 Note: CP= Cumulative proportion; EV= Eigenvalues; PC= Principal component; RT= Red from
 649 top view; BT= Blue from top view; RSCT= Ratio of shoot area to convex hull from top view;
 650 YS= Object extend Y from side view; CS= Convex hull from side view.

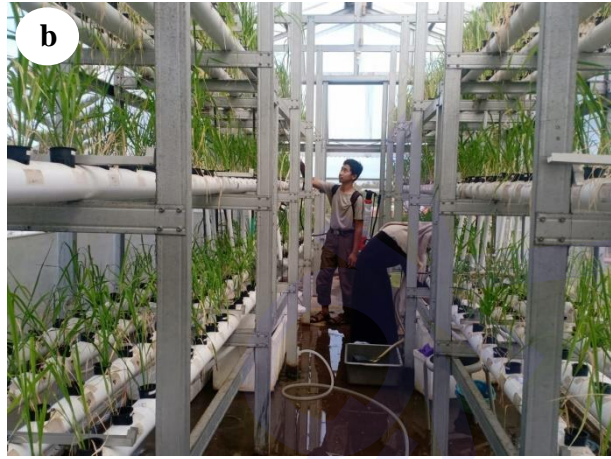
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664 Table 10. *Selection index on static and dynamic hydroponics*

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

665 Note: SH Index= Static hydroponic selection index; DH Index= Dynamic hydroponic selection
 666 index.

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

681 DAS (b).

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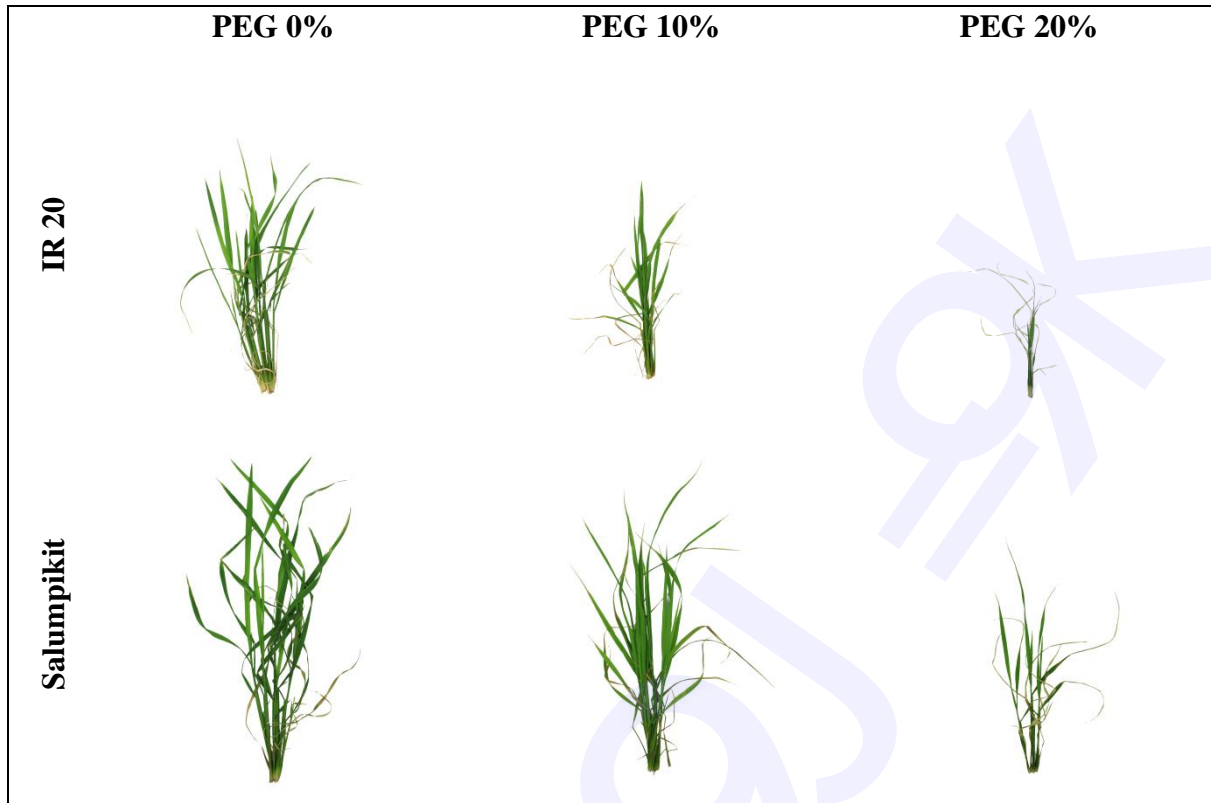
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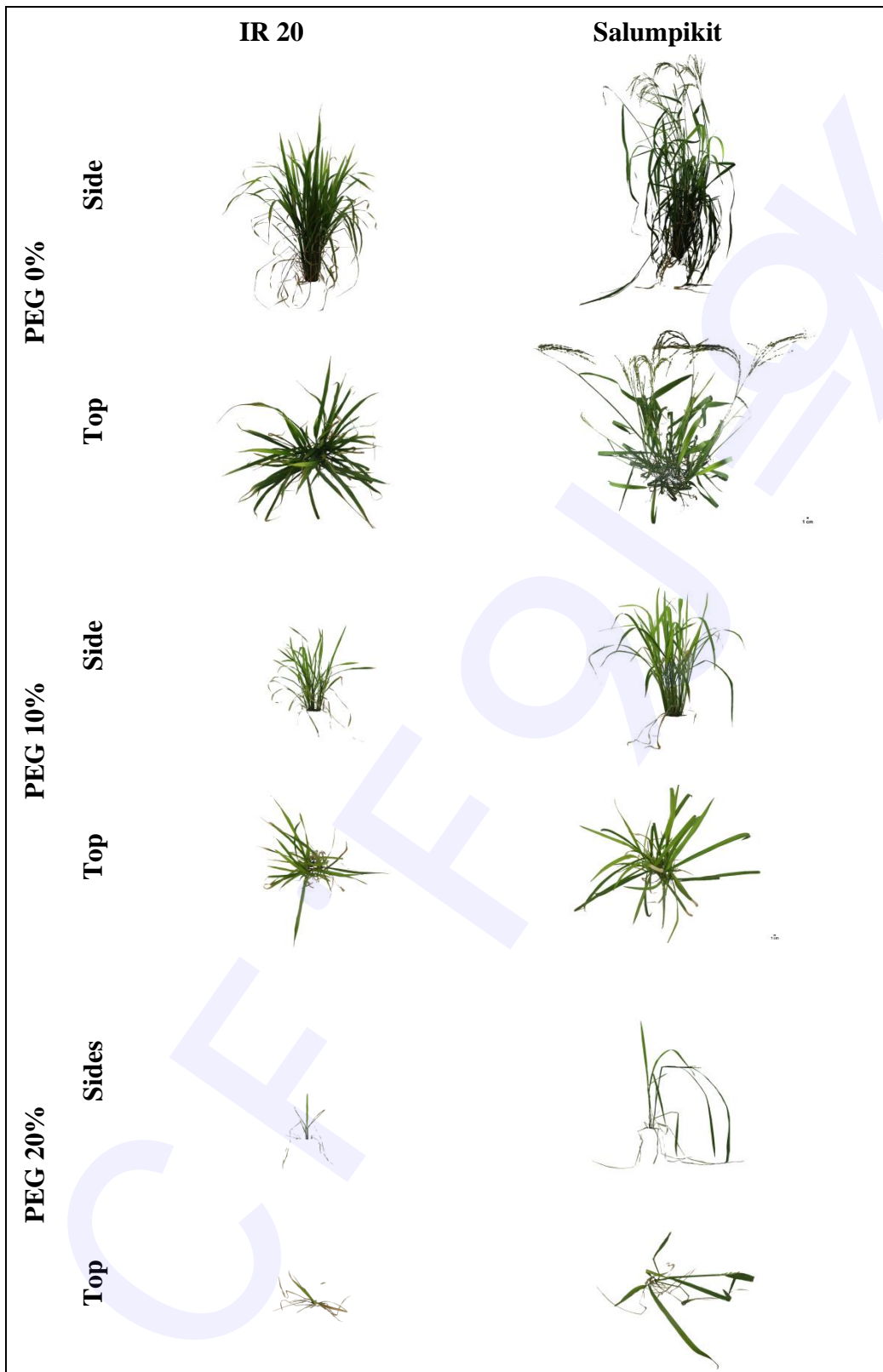
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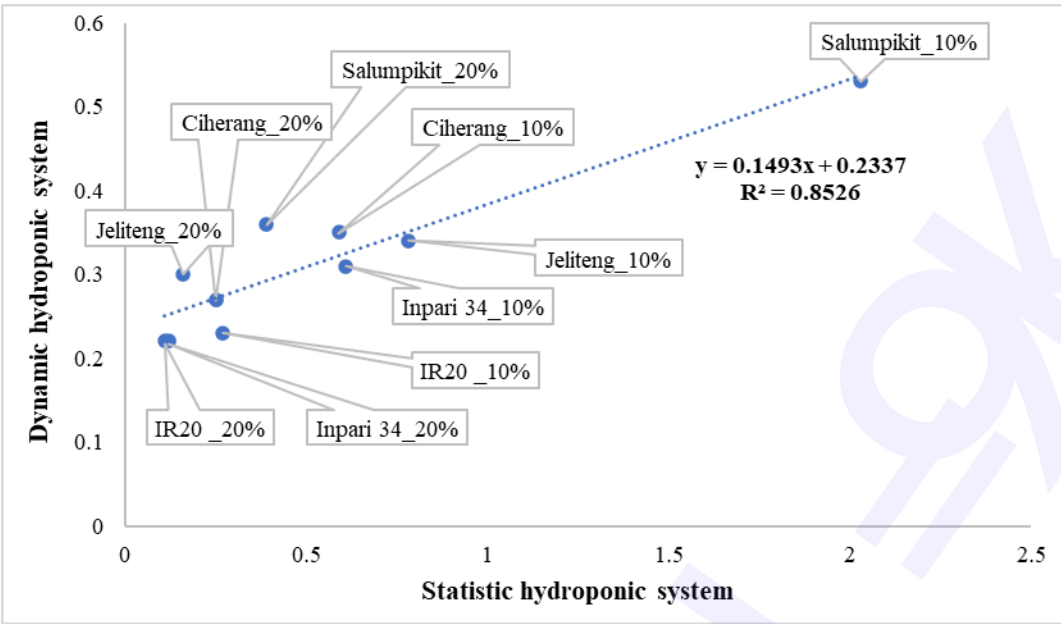


697 Figure 2. RGB images of IR 20 and Salumpikit under drought stress at 30 DAS in static
698 hydroponic.

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



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714 Figure 4. Analysis of static hydroponic selection index regression on dynamic hydroponic.

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

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11 **Abstract**

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

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

30 **Introduction**

31
32 Paddy is a commodity that plays an important role in Indonesia's economy. In general, rice
33 is consumed by approximately 90% of the Indonesian population as a staple food (Donggulo *et al.*
34 2017). Rice production in Indonesia in 2020 has decreased from the previous year, from 59.2
35 million tons to 54.6 million tons (BPS 2021). The decline in production can be influenced by
36 various factors, one of which is drought stress. According to Mawardi *et al.* (2016), about 80% of
37 the cultivation area of paddy and other cereals is affected by drought as a limiting factor for
38 production. In addition, several studies have shown that drought can reduce the rate of growth
39 and rice yield (Supriyanto 2013; Sujinah and Jamil 2016; Rusmawan *et al.* 2018). Therefore, the
40 development of drought-tolerant varieties is a solution to increasing rice production.

41 The development of drought-tolerant varieties requires an effective and efficient screening
42 method. In general, drought-tolerant screening can be carried out artificially or directly in the
43 target environment. However, the artificial selection environment is considered easier to control,
44 especially the control of stress concentration levels, hence the use of this method can increase the
45 effectiveness and efficiency of screening (Anshori *et al.* 2019). In drought stress, artificial
46 screening can be carried out with a hydroponic concept with a polyethylene glycol (PEG)
47 selection agent (Swapna and Shylaraj 2017; Mangansige *et al.* 2018; Osmolovskaya *et al.* 2018).
48 The use of this method under drought stress has been reported by Ai *et al.* (2010), Wening and
49 Susanto (2017), and Farid *et al.* (2021). In addition, according to Ai *et al.* (2010) and Wening and
50 Susanto (2017), the use of PEG 6000 with certain concentrations in the early (vegetative) phase
51 can be used as an alternative for quick or early selection. This concept is very important in
52 increasing the effectiveness of selection. Nevertheless, the vegetative screening under salinity
53 stress has to validate or correlate with the generative phase, one of them is the dynamic
54 hydroponic. This hydroponic can measure the tolerance screening until the generative phase with

55 a controlled environment (Farid *et al.* 2021). Therefore, the use of PEG in static and dynamic
56 hydroponic systems can be a good alternative in the screening process of drought tolerance of
57 paddy. However, this screening requires an accurate and precise assessment approach.

58 Assessment of drought stress tolerance through scoring is a common assessment. However,
59 this assessment is considered inaccurate. Based on the genetic constitution, drought tolerance
60 nature is polygenic. It is indicated by the QTL report that encodes these characteristics (Lanceras
61 *et al.* 2004) so that in the assessment of drought, qualitative scoring should not be used. However,
62 observations based on morphology are generally considered to be highly biased (Brereton *et al.*
63 2015). In addition, physiological and molecular approaches are considered effective and have a
64 relatively low bias, but these approaches are expensive (Nuraida 2012). Therefore, an accurate
65 and precise approach with a relatively low cost is needed in identifying the nature of rice
66 tolerance to drought stress. One method that is considered effective is a digital technology
67 approach through image-based phenotyping.

68 Image-based phenotyping technology is currently being developed to characterize plant
69 morphological and physiological responses using digital images. This technology makes it easy
70 to calculate phenotypes by analyzing plants through images accurately in large numbers and in a
71 short time, hence the technique does not require a long time (Das Choudhury *et al.* 2018). Several
72 studies have reported the use of an effective and efficient image-based phenotyping selection
73 method in rice (Hairmansis *et al.* 2014; Siddiqui *et al.* 2014; Duan *et al.* 2018; Guimaraes *et al.*
74 2020; Kim *et al.* 2020; Vishal *et al.* 2020) and maize (Asaari *et al.* 2019; Li *et al.* 2021).
75 However, there are still few studies that examine the development of image-based phenotyping
76 based on selection index and multivariate analysis on the static hydroponic systems.

77 The selection index is a breeding selection approach with combining some selection criteria
78 in one formula (Anshori *et al.* 2021). This approach is famous to use in plant breeding since it

79 considers all selection criteria with current economic value on each criterion in genotype
80 selection (Rajamani *et al.* 2016; Islam *et al.* 2017). The selection index often combines with
81 multivariate analysis to increase the effectiveness of selection (Akbar *et al.* 2019; Anshori *et al.*
82 2019; Fadhli *et al.* 2020; Farid *et al.* 2021; Anshori *et al.* 2021). Multivariate analysis is able to
83 analyze or integrate the variance of large variable data into a simpler and more comprehensive
84 manner so that the results are easier to understand (Mattjik and Sumertajaya 2011). Several
85 studies have reported the effectiveness of selection with the use of multivariate analysis (Anshori
86 *et al.* 2018; Kose *et al.* 2018; Akbar *et al.* 2019; Anshori *et al.* 2019; Fadhli *et al.* 2020; Farid *et*
87 *al.* 2020; Farid *et al.* 2021). Therefore based on these, the use of image-based phenotyping with
88 the selection index based on multivariate analysis in static and dynamic hydroponic screening is
89 necessary. The objective of this study was to determine the selection index based on multivariate
90 analysis and assess the effectiveness of using image-based phenotyping in drought rice screening.

91

92 **Materials and Methods**

93

94 This study conducted two experiments. The first experiment was the destructive test carried
95 out on static hydroponic (floating raft system), while the second test was the non-destructive
96 experiment carried out on dynamic hydroponic (deep flow technique system). These studies was
97 carried out parallely in the greenhouse, Unhas Lecturer Residence, Makassar City, South
98 Sulawesi Province, Indonesia (22.4 m above sea level (asl) from August to November 2020.

99

100

101 **Experimental Design and Procedures**

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

108 The medium used was ABmix with a concentration of 8 mL/L. The PEG concentration
109 treatments were given to the hydroponic nutrient solution gradually. The first stage was given $\frac{1}{2}$
110 concentration of PEG treatment at the age of 13 DAS. The second stage was given at the age of
111 16 DAS, this was done to prevent osmotic shock. pH control was applied to keep it constant. The
112 desired pH was in the range of 5.8-6.2. pH control was done by adding HCl or NaOH to lower or
113 raise the pH. Data collection on the static hydroponic method was carried out 2 weeks after the
114 treatment application or 30 DAS. As for dynamic hydroponics, data collection was carried out at
115 68 DAS.

116 Observations carried out for static hydroponic were shoot fresh weight (actual
117 measurements), root fresh weight (actual measurements), 2nd, 3rd, and 4th leaf length, shoot area
118 from the side view, green shoot area from the side view, ratio of green shoot area to shoot area
119 from the side view, red, green, blue, ratio of red to green, and area growth rate. Traits measured
120 in dynamic hydroponic included shoot fresh weight (actual measurements), root fresh weight
121 (actual measurements), convex hull from the top view, shoot area from the top view, green shoot
122 area from the top view, the ratio of green shoot area to shoot area from the top view, number of
123 leaves from the top view, red, green, blue, ratio red to green, the ratio of shoot area to the convex
124 hull from the top view, object extent X from a side view, object extent Y from a side view, shoot
125 area from a side view, green shoot area from a side view, a ratio of green shoot area to the

126 shooting area from a side view, convex hull from a side view, and the ratio of shoot area to
127 convex hull area from side view (Table 1).

128

129 **Image Analysis**

130 Images were taken using Canon EOS 1200D RGB camera in a portable photo studio with a
131 studio 75 cm x 75 cm x 75 cm. The background used was a white background with two 8 watt
132 white LED lights in the studio with camera settings (5.6 F-stops, 1/160 second exposure time,
133 ISO 800, and without flash). Image were acquired from a top view (0°) and from the side view
134 (90°) of the plant. Images were taken twice, at 13 DAS (early treatment) and 30 DAS (after
135 treatment) for static hydroponic (Figure 2). For dynamic hydroponic, images were taken at 13
136 DAS (early treatment) and 68 DAS (after treatment) (Figure 3). The results of the images were
137 analyzed using the Fiji application. The plant color was analyzed using hue channels with a hue
138 50 (green area). The area growth rate was calculated using the projection of shoot area over two
139 time periods.

140

141 **Data analysis**

142 Data were analyzed independently for variance, then the characters that have significant
143 interactions at each character were further analyzed with the Pearson correlation test and
144 Principal Component Analysis (PCA) with STAR 2.01 software. For static hydroponic, after
145 being tested by correlation analysis, it was followed by path analysis. All further analysis based
146 on the stress tolerance index (STI) value of each character on each PEG concentration (0% PEG
147 (normal) –10% PEG (drought stress) = STI 1 and 0% PEG (normal)–20% PEG (drought stress) =
148 STI 2). This concept has been reported by Anshori *et al.* 2019, as follow:

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

150

$$STI = \frac{Y_p \times Y_s}{\bar{Y}_p^2}$$

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

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

153 \bar{Y}_p = Average character values of all varieties in normal / non-stressed conditions.

154 The selected characters from the PCA analysis results are used to obtain the index value.

155 The validation of static hydroponic was conducted by the regression test toward the dynamic
156 hydroponic.

157

158 **Results**

159

160 **Static Hydroponic System**

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

170 The results of correlation analysis on static hydroponic were based on the static hydroponic
171 Stress Tolerance Index (STI) value (Table 3). The results of this analysis showed that the 3rd leaf
172 length (0.769 and 0.745), shoot area (0.981 and 0.983), green shoot area (0.978 and 0.984), and

173 area growth rate (0.981 and 0.961) had a significant correlation on shoot fresh weight and root
174 fresh weight, respectively. Based on these correlation analyses, then followed by independent
175 path analysis on shoot fresh weight and root fresh weight. Both characters had known as the main
176 characters of actual morphology under artificial vegetative screening with different roles. So, this
177 independent concept analysis could determine the specific relationship among image-based
178 phenotyping characters to the conventional morphology characters.

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

185 Path analysis on the STI value of root fresh weight in static hydroponic showed
186 representative results with a determinant coefficient value of 0.87 (Table 5). Based on this
187 analysis, shoot fresh weight was the character that had the highest direct effect (0.548) on root
188 fresh weight. Meanwhile, based on image-based phenotyping characters, the shooting area (0.493)
189 and green shoot area (0.377) were characters that had a significant direct effect on root fresh
190 weight. On the other hand, the area growth rate has a negative significant direct effect (-0.417) on
191 the root fresh weight. It meant that the high correlation of area growth rate to root fresh weight
192 was due to the indirect effect of the shooting area and green shoot area. Therefore, shoot area and
193 green shoot area as image-based phenotyping characters could represent the root fresh weight
194 variance, while area growth rate could not be a selection character to represent root fresh weight
195 variance.

196 The principal components analysis showed there was one main component that can be used
197 as the basis for the selection index (Table 6), namely the first principal component (PC1) with a
198 cumulative proportion of 0.928 which is almost close to 1. Based on PC1, shoot fresh weight
199 (0.4183) and root fresh weight (0.4183) in drought stress were in the same direction with
200 phenotyping characters of shoot area (0.4210), green shoot area (0.4177), and area growth rate
201 (0.4192). These three image-based phenotyping characters are then combined in a static
202 hydroponic selection index using the formula:

$$203 \text{ Static hydroponic index} = 0.421 \text{ shoot area} + 0.4177 \text{ shoot green area} + 0.4192 \text{ growth rate area} \\ 204 \dots\dots\dots \text{(Equation 1)}$$

205

206 **Dynamic Hydroponic System**

207 Analysis of variance in the dynamic hydroponic method in Table 7 showed that PEG
208 treatment as drought stress has an impact on all image-based-phenotyping characters, except for
209 the ratio of red to green from the top view and the ratio of green shoot area to shoot area from the
210 top view. Variety variance has a significant effect on the number of leaves from the top view, red
211 from the top view, blue from the top view, the ratio of shoot area to the convex hull from the top
212 view, object extend X from a side view, object extend Y from a side view, and convex hull from
213 a side view. Meanwhile, the characters significantly affected by the interaction are red from the
214 top view, blue from the top view, the ratio of shoot area to the convex hull from the top view,
215 object extend Y from a side view, and convex hull from a side view. All characters significantly
216 affected by the interaction effect in the dynamic hydroponic system were correlated with the
217 selection index on static hydroponic. It aimed to detect the selected selection characters that have
218 a relationship with the static hydroponic. As for, the varieties phenotype on these characters
219 showed in Supplementary 2.

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

225 The result of principal components analysis on dynamic hydroponic was showed in Table 9.
226 Based on this table, PC1 has the highest cumulative proportion with a value of 58.83% of the
227 total diversity of the initial data, so that this PC could be the basis in the weighting of selection
228 characters like the static hydroponic index. Red from the top view and blue from the top view
229 have negative eigenvectors. In contrast, the characters of the ratio of shoot area to the convex hull
230 from the top view, object extend Y and convex hull from the side view have positive eigenvectors.
231 Based on this, the YS and CS characters were combined into a selection index in dynamic
232 hydroponic screening using the formula:

$$233 \text{ Dynamic hydroponic index} = 0.4516 \text{ object extend Y from the side view} + 0.4177 \text{ Convex hull} \\ 234 \text{ from the side view} \dots\dots\dots \text{(Equation 2)}$$

236 **Selection Index**

237 The index value of each variety on static and dynamic hydroponics was showed in Table 10.
238 Salumpikit variety at a PEG concentration of 10% (2.03) shows the highest value, while IR 20 at
239 a PEG concentration of 20% (0.11) shows the lowest value in static hydroponics. In addition,
240 based on dynamic hydroponic, salumpikit variety at a PEG concentration of 10% (0.53) remained
241 the best, while Inpari 34 (0.22) and IR 20 (0.22) were the varieties with the lowest index value.
242 Meanwhile, the results of the regression analysis showed static hydroponic index has a significant
243 linear regression to the dynamic hydroponic index (Figure 4). The figure also showed the

244 Salumpikit as the tolerant check variety has a good value index than IR 20 as sensitive check
245 variety on both of 10% and 20% PEG.

246

247 **Discussion**

248

249 The significant interaction on ANOVA is one of the early indicators in screening for stress.
250 It had reported by Akçura and Çeri (2011); Anshori *et al.* (2019); Farid *et al.* (2020), where
251 characters influenced by interactions show different patterns of decreased response between
252 genotypes on normal and stressful environments. This difference can be a parameter in
253 identifying tolerance traits between genotypes in stress (Ali *et al.* 2014; Safitri *et al.* 2016; Fadhli
254 *et al.* 2020). Based on this analysis, the image-based phenotyping of 3rd leaf length, shoot area,
255 shoot green area, and area growth rate on static hydroponic screening as well as red from the top
256 view, blue from the top view, the ratio of shoot area to the convex hull from the top view, object
257 extend Y from the side view, and convex hull from the side view in dynamic hydroponic
258 screening can be used as a candidate to selection criteria in stress tolerance. In addition, these
259 characters were also significantly influenced by the variance of PEG concentrations and varieties.
260 It indicated the differences in interactions between genotypes on environmental differences could
261 be seen clearly and direct (Farid *et al.* 2020; Farid *et al.* 2021). However, more in-depth analysis
262 with several series of multivariate analyzes was required to obtain the best selection criteria for
263 each drought screening method.

264 The assessment of varieties' response to stress should be done using a tolerance index. It
265 had reported by Mau *et al.* (2014), Aboughadareh *et al.* (2019), Anshori *et al.* (2019);
266 Aboughadareh *et al.* (2020), and Farid *et al.* (2021) that the Stress Tolerance Index (STI) is an
267 appropriate index to characterize the most tolerant of drought stress. This index is classified as

268 dynamic because it considers the general response of the population means. Even though the
269 general response on this index focuses on normal conditions (Anshori *et al.* 2019). The STI value
270 becomes the basis for a deeper analysis. It had reported by Anshori *et al.* (2019) on rice against
271 salinity stress, Fadhli *et al.* (2020) on maize against drought stress, and Farid *et al.* (2021) on
272 wheat against drought stress. Therefore, the use of STI as the basis for a more in-depth analysis
273 was also carried out, both for static hydroponic screening and dynamic screening.

274 Correlation and path analysis on the static hydroponic were focused on the shoot fresh
275 weight and root fresh weight characters. Both of these fresh weights had been reported to have a
276 close relationship with drought tolerance (Saha *et al.* 2019). The relationship between image-
277 based phenotyping characters on these two characters can be the basis for determining the best
278 selection criteria for drought stress. Meanwhile, the concepts of correlation and path analysis are
279 often combined in determining the selection criteria (Safitri *et al.* 2011). The correlation still
280 cannot describe how large the pure role of a character is to the diversity of the main characters
281 (Singh and Chaudhary 2007; Rohaeni and Permadi 2012; Anshori *et al.* 2018). The use of this
282 analysis has been widely reported by several studies (Aman *et al.* 2020; Saleh *et al.* 2020; Dutt *et*
283 *al.* 2020; Ashraf *et al.* 2020). Therefore, the use of this analysis also needs to be done on the
284 character of the image-based phenotyping on the two fresh weights.

285 The results of correlation analysis and path analysis on static hydroponic show that shoot
286 area, green shoot area, and area growth rate can be used as selection criteria. The potential of
287 these image-based phenotyping characters was also reported by Hairmansis *et al.* (2014) on rice
288 under salinity stress, Honsdorf *et al.* (2014) on barley under drought stress, and Kim *et al.* (2020)
289 on rice under drought stress. Based on the results of the path analysis, the area growth rate has an
290 inverse orientation to the shooting area and the green shoot area. This difference in orientation
291 can be seen from the direct effect and indirect effect on the character of shoot fresh weight and

292 root fresh weight (Table 4 and 5). This indicates that shoot fresh weight and root fresh weight
293 become the middle of the three image-based phenotyping characters. However, the combination
294 of the three characters can make the selection more selective because each character has specific
295 potential. It has been reported by Acquah (2007) and Anshori *et al.* (2021), a selection index
296 formed from combining characters with opposite orientations can increase the selectivity of a
297 selection. Therefore, these three characters are still used as selection criteria. However, the
298 crucial thing in the formation of the selection index of the three characters is the weighting value
299 of the selection. One method that can be used in determining the weighted value of the selection
300 index is principal component analysis.

301 Principle component analysis is a multivariate analysis that aims to extract important
302 information from big data into a new simpler set of orthogonal variables (Ilmaniati and Putro
303 2019). This analysis has been reported as a weighting indicator for the selection index by Akbar
304 *et al.* (2019), Anshori *et al.* (2019), Alsabah *et al.* (2019), Fadhli *et al.* (2020), and Farid *et al.*
305 (2021). Based on the PCA results in Table 6, the characteristics of shoot area, green shoot area,
306 and area growth rate have the same eigenvector direction and variance with shoot fresh weight
307 and root fresh weight. This supports that the three characters generally have the same variance
308 direction, even though in the broad cross-section, the growth of the shoot has a different
309 orientation from the other two characters. However, because of the inclusion of the two fresh
310 weight characters, the three characters generally have the same range direction and can be
311 combined into the selection index in Equation 1.

312 Validation of image-based phenotyping characters in static hydroponics was done by
313 identifying the relation of these characters to the image-based phenotyping characters in dynamic
314 hydroponic. Relatively, dynamic hydroponic has a growth rate that is more complex than the
315 growth rate in static hydroponic (Sagita *et al.* 2020). This was because dynamic hydroponic

316 observations were carried out in the flowering phase, while static hydroponic were carried out in
317 the vegetative phase. However, the application of the selection agent in the two methods was
318 carried out at the same time in the 21 days after sowing. Therefore, the static hydroponic
319 selection index assessment can be related to the growth character of dynamic hydroponic as part
320 of its validation.

321 The dynamic hydroponic ANOVA showed that red from top view, blue from top view,
322 ratio of shoot area to the convex hull from the top view, object extend Y from a side view, and
323 convex hull from a side view was the characters significantly affected by the interaction of
324 drought level – genotype treatments. Meanwhile, shoot fresh weight and root fresh weight
325 showed not significantly affected on the genotype and interaction of drought level – genotype
326 effect. The not significantly affect on shoot fresh weight and root fresh weight due to the high
327 error of these characters and the variance domination from drought level treatment, so that the
328 genotype and its interaction are difficult to sign on both fresh characters. Therefore, the shoot
329 fresh weight and root fresh weight does not appropriate as the main characters in detecting the
330 selection characters from image-based phenotyping in this dynamic hydroponic system.

331 Correlation analysis of image-based phenotyping characters on dynamic hydroponic that
332 have significant interactions is one method of validating static hydroponic selection indexes.
333 Based on the results of the dynamic hydroponic screening correlation, object extend Y from the
334 side view, and convex hull from the side view can be used as selection characters. This result has
335 also been reported by Duan *et al.* (2018) and Kim *et al.* (2020) that stated the leaves on plants
336 that are stricken with drought will turn yellow and bend, thereby reducing the calculated convex
337 hull. However, the results of this study also need to be supported by other analyzes such as PCA
338 and regression between selection indices (Figure 4). The use of these two analyzes was also
339 reported by Anshori *et al.* (2018) in determining the selection criteria.

340 Based on the results of PCA, the object extends Y from the side view and the convex hull
341 from the side view has the same eigenvector direction. The eigenvectors that are relatively the
342 same in both dimensions show the closeness of the variance between the two variables (Anshori
343 *et al.* 2018). It indicates that both characters can be used as validation characters through the
344 selection index. The dynamic hydroponic selection index can be used as the Y factor in a
345 regression. Based on the regression results, the static hydroponic index has good adherence to the
346 dynamic hydroponic index. In addition, the differences in tolerance groups, between the
347 Salumpikit as the tolerant check variety and IR 20 varieties as the sensitive check variety, also
348 show quite a distance. This result is also consistent with the research of Hairmansis *et al.* (2020)
349 and Kartina *et al.* (2019). The use of control varieties could be one of the proofs to detect
350 effectiveness selection. This concept had reported by Ali *et al.* (2014), Safitri *et al.* (2016) and
351 Anshori *et al.* (2020) on salinity stress, Wening *et al.* (2019) and Anshori *et al.* (2019) on
352 submergence, Akbar *et al.* (2018) and Akbar *et al.* (2019) on drought stress. Besides that, the use
353 of Salumpikit and IR 20 have been reported as a check or control of rice drought tolerance by
354 Akbar *et al.* (2018) and Akbar *et al.* (2019). Based on the overall results, the selection index on
355 static hydroponic screening can be used to identify the tolerance properties of rice to drought
356 stress.

357 In conclusion, the use of multivariate analysis on image-based phenotyping characters can
358 increase the effectiveness of selection in drought stress tolerance screening. The selection index
359 for static hydroponic is $0.421 \text{ shoot area} + 0.4177 \text{ green shoot area} + 0.4192 \text{ area growth rate}$.
360 Meanwhile, the character selection index for dynamic hydroponic is $0.4516 \text{ objects extend Y}$
361 $\text{from the side view} + 0.4177 \text{ convex hull from the side view}$. Based on this study, rice screening
362 based on image-based phenotyping can be recommended as a more effective and efficient method
363 for the rapid screening of drought stress.

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367

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

Characters	Description
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 4th leaf 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 that have hue value of 50.
Convex hull area (CT for top and CS for side)	The smallest area covered by the outer 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, 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, used to measure the height of the plant.
Number of leaves (NL)	Number of leaf tips visible from above of 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).

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560 Table 2. Analysis of variance on the static hydroponic method

Characters	D	V	D x V
SFW	178.73 **	28.92 **	10.37 **
RFW	24.77 **	7.30 **	1.96 **
LL2	1486.95 **	326.19 **	14.68 ns
LL3	1471.64 **	466.95 **	49.37 *
LL4	669.49 **	188.37 **	28.07 ns
SA	65556.88 **	13117.85 **	3438.49 **
GSA	61237.72 **	9849.18 **	3261.87 **
R	3460.07 **	86.95 ns	748.95 ns
G	879.98 ns	87.35 ns	1498.13 ns
B	1680.60 *	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 **

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

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

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

574 Note: *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; SFW= Shoot fresh weight; RFW=
 575 Root fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green shoot area; AGR= Area
 576 growth rate.

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

Characters	Direct	Indirect Influence					Residual
	Influence	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

592 Note: $R^2 = 0.86$; RFW= Root fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green
 593 shoot area; AGR= Area growth rate.

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609 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

610 Note: $R^2 = 0.87$; SFW= Shoot fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green
 611 shoot area; AGR= Area growth rate.

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627 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

628 Note: CP= Cumulative proportion; EV= Eigenvalues; PC= Principal component; SFW= Shoot
 629 fresh weight; RFW= Root fresh weight; LL3= 3rd leaf length; SA= Shoot area; GSA= Green
 630 shoot area; AGR= Area growth rate.

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644 Table 7. Analysis of variance on the dynamic hydroponic method

Characters	D	V	D x V
SFW	8042.24 **	319.95 ns	376.25 ns
RFW	467.01 **	53.23 ns	14.64 ns
CT	81129688.30 **	4224903.16 ns	1562942.10 ns
SAT	5571152.36 **	474034.51 ns	97115.97 ns
GSAT	3072273.82 **	333330.03 ns	80272.07 ns
NLT	2478.87 **	617.19 *	156.84 ns
RT	3946.91 **	462.79 **	189.34 *
GT	4734.46 **	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	1636.09 **	147.66 **	110.49 **
SAS	3696517.43 **	280606.15 ns	91206.26 ns
GSAS	1651359.59 **	212567.33 ns	68283.65 ns
CS	48116645.30 **	6716755.96 **	947650.84 *
RGSSS	0.0853 *	0.0141 ns	0.0082 ns
RSCS	0.0463 **	0.0063 ns	0.0024 ns

645 Note: D= Drought level; V= Varieties; *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; ns= Not significant;

646 SFW= Shoot fresh weight; RFW= Root fresh weight; CT= Convex hull from top view; SAT= Shoot area from top

647 view; GSAT= Green shoot area from top view; NLT= Number of leaves from top view; RT= Red from top view;

648 GT= Green from top view; BT= Blue from top view; RRG: Ratio of red to green from top view; RGSST= Ratio of

649 shoot green area to shoot area from top view; RSCT= Ratio of shoot area to convex hull from top view; XS= Object

650 extend X from side view; YS= Object extend Y from side view; SAS= Shoot area from side view; GSAS= Green

651 shoot area from side view, CS= Convex hull from side view; RGSSS= Ratio of green shoot area to shoot area from

652 side view; RSCS= Ratio of shoot area to convex hull from side view.

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654 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	-0.48	ns	-0.24	ns	0.30	ns	1.00				
CS	-0.30	ns	-0.03	ns	0.14	ns	0.97	**	1.00		
SH Index	-0.16	ns	-0.04	ns	0.19	ns	0.90	**	0.91	**	1.00

655 Note: *= Significant at $P \leq 0.05$; **= Significant at $P \leq 0.01$; ns= Not significant; RT= Red from
 656 top view; BT= Blue from top view; RSCT= Ratio of shoot area to convex hull from top view;
 657 YS= Object extend Y from side view; CS= Convex hull from side view; SH Index= Static
 658 hydroponic selection index.

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672 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
Cumulative Proportion	0.5883	0.8544	0.9536	0.9801	1
EigenValues	2.9414	1.3306	0.496	0.1322	0.0997

673 Note: CP= Cumulative proportion; EV= Eigenvalues; PC= Principal component; RT= Red from
 674 top view; BT= Blue from top view; RSCT= Ratio of shoot area to convex hull from top view;
 675 YS= Object extend Y from side view; CS= Convex hull from side view.

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689 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

690 Note: SH Index= Static hydroponic selection index; DH Index= Dynamic hydroponic selection
 691 index.

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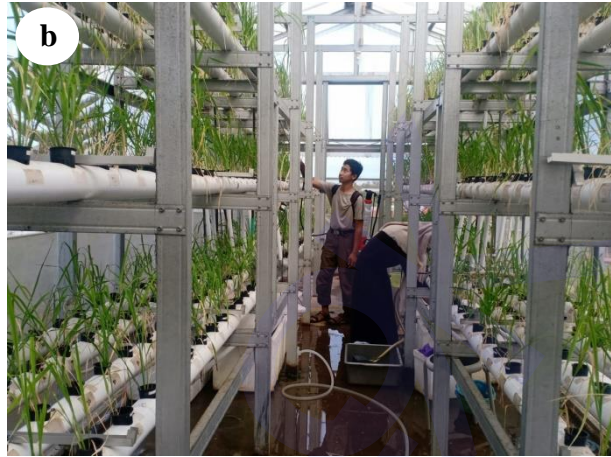
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705 Figure 1. Images of static hydroponic system at 20 DAS (a) and dynamic hydroponic system at 33
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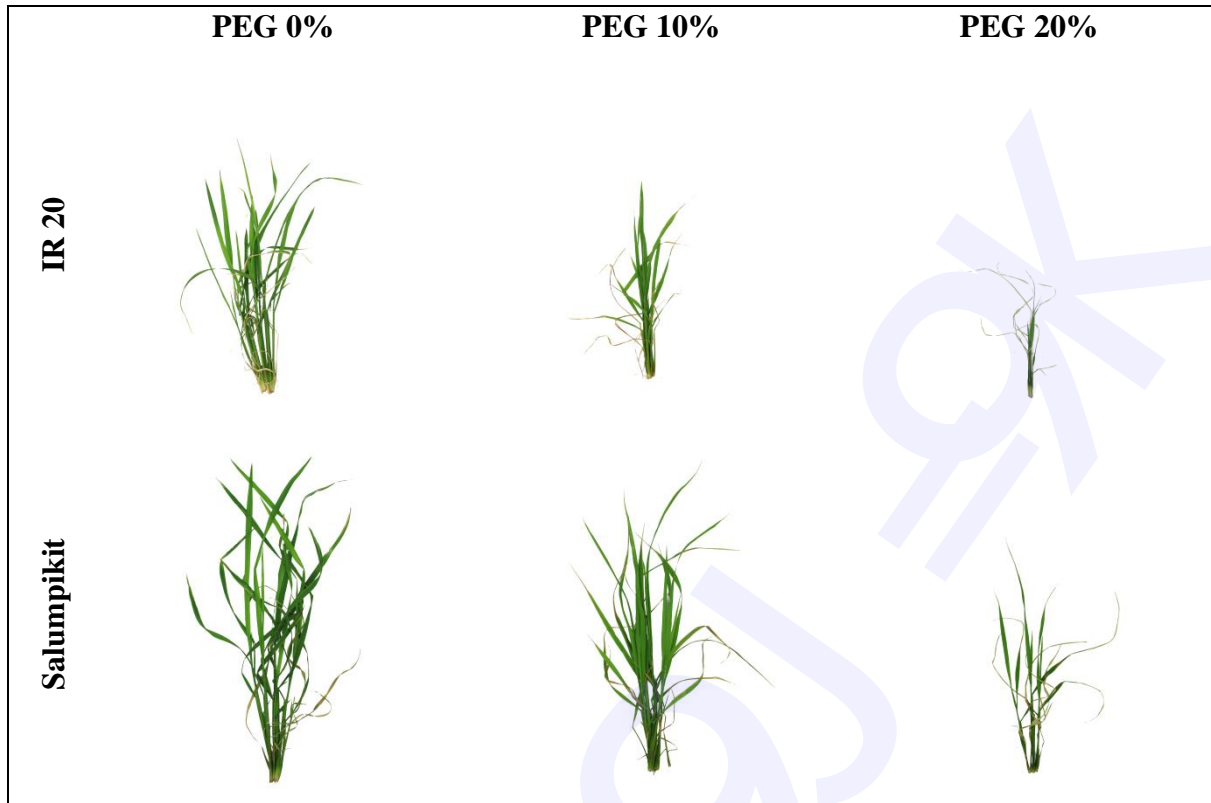
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722 Figure 2. RGB images of IR 20 and Salumpikit under drought stress at 30 DAS in static
723 hydroponic.

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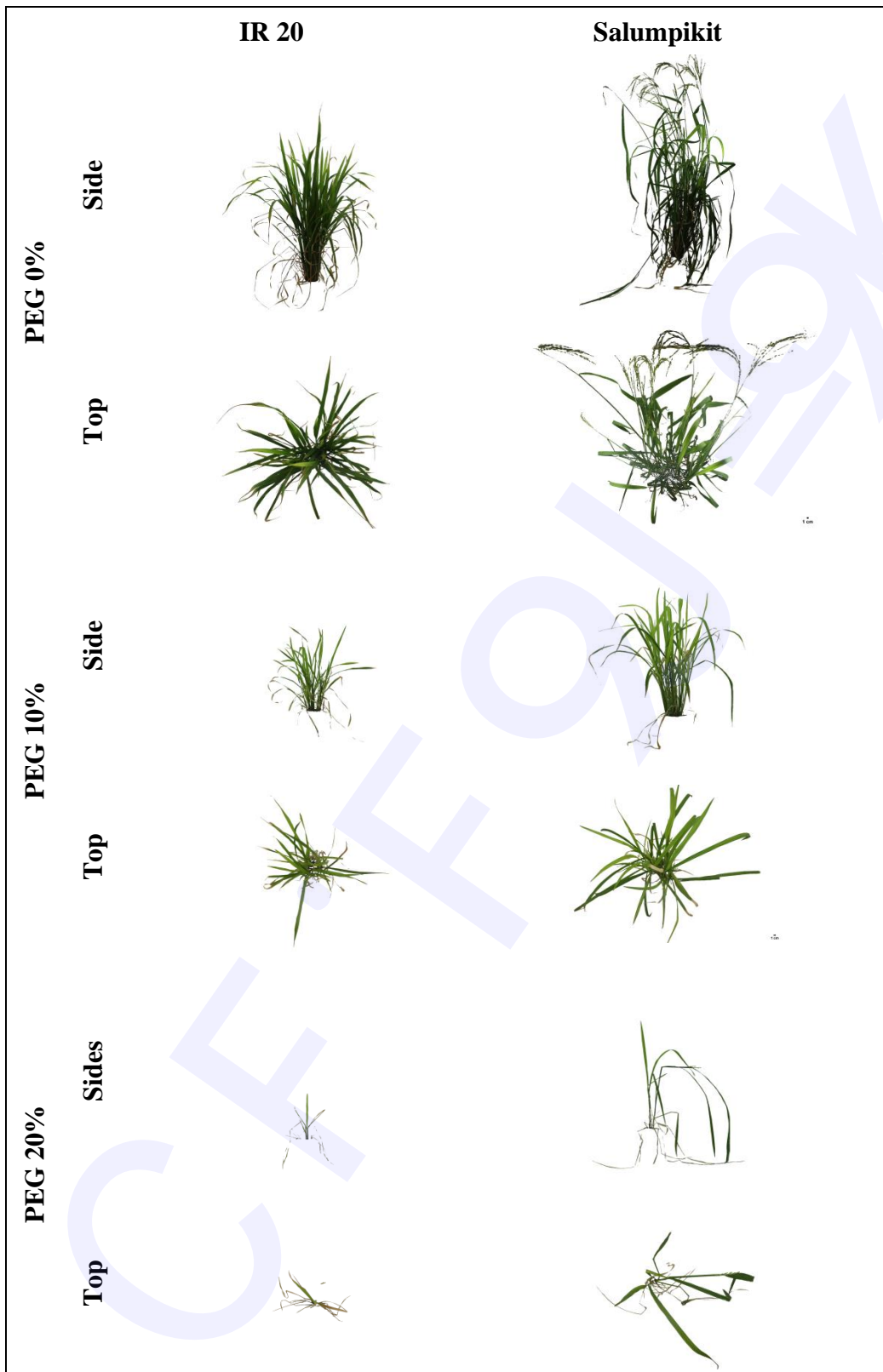
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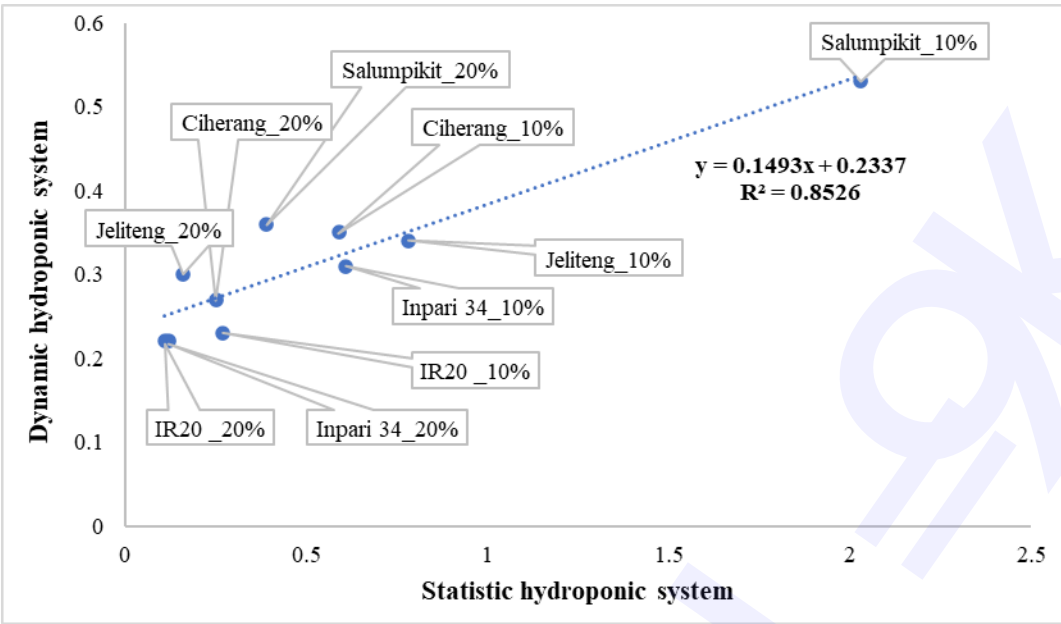
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736 Figure 3. RGB images of IR 20 and Salumpikit under drought stress at 68 DAS in dynamic
 737 hydroponic.



738
739 Figure 4. Analysis of static hydroponic selection index regression on dynamic hydroponic.

740

741 Supplementary 1. Average of root fresh weight, shoot fresh weight, 3rd leaf length, shoot area,
 742 green shoot area, and area growth rate on the static hydroponic method

V	D	RFW (g)	SFW (g)	LL3 (cm)	SA (cm ²)	GSA (cm ²)	AGR (cm ² /day)
Inpari 34	0 %	2.42	7.86	48.78	156.34	138.52	7.24
IR 20	0 %	1.16	3.77	39.37	90.07	75.46	4.38
Salumpikit	0 %	5.17	13.80	62.59	282.75	254.95	14.14
Ciherang	0 %	2.86	7.42	36.65	154.76	132.87	6.74
Jeliteng	0 %	3.57	8.64	37.47	186.66	167.74	8.80
Inpari 34	10 %	3.82	5.05	38.39	106.65	88.87	4.48
IR 20	10 %	2.43	3.33	35.48	79.58	69.76	3.80
Salumpikit	10 %	5.75	8.48	47.44	187.75	154.93	8.87
Ciherang	10 %	3.18	5.07	37.65	111.36	90.86	4.33
Jeliteng	10 %	3.50	5.12	33.16	114.55	94.57	4.79
Inpari 34	20 %	1.28	1.37	21.84	33.83	15.70	0.44
IR 20	20 %	1.02	1.44	25.75	36.08	23.94	1.38
Salumpikit	20 %	1.22	1.25	35.57	49.40	29.96	1.18
Ciherang	20 %	1.43	1.77	25.12	57.72	42.58	1.35
Jeliteng	20 %	1.27	1.27	19.25	35.87	20.80	0.42
Inpari 34	STI 10 %	1.00	0.58	0.93	0.55	0.52	0.48
IR 20	STI 10 %	0.30	0.18	0.69	0.23	0.22	0.24
Salumpikit	STI 10 %	3.26	1.70	1.47	1.74	1.65	1.83
Ciherang	STI 10 %	0.97	0.54	0.68	0.57	0.51	0.43
Jeliteng	STI 10 %	1.36	0.64	0.62	0.71	0.67	0.62
Inpari 34	STI 20 %	0.33	0.16	0.52	0.17	0.09	0.04
IR 20	STI 20 %	0.13	0.08	0.51	0.11	0.08	0.09
Salumpikit	STI 20 %	0.72	0.25	1.10	0.46	0.32	0.23
Ciherang	STI 20 %	0.43	0.19	0.46	0.28	0.23	0.12
Jeliteng	STI 20 %	0.49	0.16	0.37	0.22	0.15	0.05

743 Note: V= Variety; D= Drought level; SFW= Shoot fresh weight; RFW= Root fresh weight; LL3=
744 3rd leaf length; SA= Shoot area; GSA= Green shoot area; AGR= Area growth rate.



745 Supplementary 2. Average of red from top view, blue from top view, ratio of shoot area to convex
 746 hull from top view, object extend Y from side view, and convex hull from side view on the
 747 dynamic hydroponic method

V	D	RT	BT	RSCT	YS	CS (cm ²)
Inpari 34	0 %	57.69	30.49	0.22	66.43	4421.01
IR 20	0 %	57.75	31.95	0.23	64.94	4835.01
Salumpikit	0 %	62.73	40.00	0.19	86.33	7697.93
Ciherang	0 %	59.21	31.69	0.25	67.12	5575.29
Jeliteng	0 %	51.03	26.41	0.29	68.34	5696.44
Inpari 34	10 %	79.77	34.97	0.21	59.77	3272.07
IR 20	10 %	95.45	45.78	0.18	48.04	1898.39
Salumpikit	10 %	80.93	37.13	0.21	59.96	4610.10
Ciherang	10 %	81.80	40.80	0.20	59.38	3788.83
Jeliteng	10 %	77.81	33.88	0.28	60.18	3113.71
Inpari 34	20 %	99.73	48.79	0.16	47.25	1607.00
IR 20	20 %	106.95	54.42	0.14	48.03	1522.07
Salumpikit	20 %	79.26	42.45	0.14	46.90	2695.94
Ciherang	20 %	79.57	39.31	0.18	51.30	2203.88
Jeliteng	20 %	73.59	33.52	0.20	56.57	2565.48
Inpari 34	STI 10 %	1.37	1.04	0.79	0.79	0.46
IR 20	STI 10 %	1.65	1.42	0.73	0.63	0.29
Salumpikit	STI 10 %	1.53	1.44	0.76	1.03	1.16
Ciherang	STI 10 %	1.49	1.27	0.91	0.80	0.66
Jeliteng	STI 10 %	1.23	0.90	1.45	0.83	0.55
Inpari 34	STI 20 %	1.73	1.47	0.61	0.63	0.22
IR 20	STI 20 %	1.85	1.70	0.57	0.62	0.23
Salumpikit	STI 20 %	1.49	1.64	0.50	0.81	0.66
Ciherang	STI 20 %	1.46	1.25	0.80	0.69	0.38
Jeliteng	STI 20 %	1.16	0.88	1.02	0.77	0.45

748 Note: V= Variety; D= Drought level; RT= Red from top view; BT= Blue from top view; RSCT=
749 Ratio of shoot area to convex hull from top view; YS= Object extend Y from side view; CS=
750 Convex hull from side view; SH Index= Static hydroponic selection index.

751

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

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11 **Abstract**

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

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

31 **Introduction**

32

33 Rice is a commodity that plays an important role in Indonesia's economy and is generally
34 consumed by approximately 90% of the population as a staple food (Donggulo *et al.* 2017). In
35 Indonesia, rice production in 2020 has decreased from 59.2 million tons in the previous year to
36 54.6 million tons (BPS 2021). This decline in production was influenced by several factors,
37 which includes drought stress. According to Mawardi *et al.* (2016), approximately 80% of the
38 cultivation area of rice and other cereals is affected by drought as a limiting factor for production.
39 In addition, several studies have shown that drought reduces the rate of growth and rice yield
40 (Supriyanto 2013; Sujinah and Jamil 2016; Rusmawan *et al.* 2018). Therefore, the development
41 of drought-tolerant varieties becomes a solution for increase rice production.

42 The development of drought-tolerant varieties requires an effective and efficient screening
43 process, which is carried out artificially or directly in the targeted environment. Moreover, the
44 environment for artificial selection is considered easier to restrain, especially the control of stress
45 concentration levels, which makes this method effective and efficient in the increase of the
46 screening process (Anshori *et al.* 2019). In drought stress, artificial screening is carried out using
47 a hydroponic concept with a polyethylene glycol (PEG) selection agent (Swapna and Shylaraj
48 2017; Mangansige *et al.* 2018; Osmolovskaya *et al.* 2018). A previous study on the use of this
49 method under drought stress conducted by Ai *et al.* (2010), Wening and Susanto (2017), and
50 Farid *et al.* (2021) stated that the use of PEG 6000 with certain concentrations in the early
51 (vegetative) phase can function as an alternative for quick or early selection. This concept is very
52 important in increasing the effectiveness of selection, however, the vegetative screening under
53 salinity stress has to validate or correlate with the reproductive phase, such as the dynamic
54 hydroponic. Meanwhile, this hydroponic makes it possible to measure the tolerance screening

55 until the reproductive phase with a controlled environment is achieved (Farid *et al.* 2021).
56 Therefore, the use of PEG in static and dynamic hydroponic systems can be a good alternative in
57 the screening process of drought tolerance of rice, however, it requires an accurate and precise
58 assessment approach.

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

69 Image-based phenotyping technology is currently being developed to characterize
70 morphological and physiological responses of a plant using digital images. This technology
71 makes it easy to calculate phenotypes by analyzing plants through images accurately in large
72 numbers within a short time (Das Choudhury *et al.* 2018). Several studies have reported the use
73 of an effective and efficient image-based phenotyping selection method in rice (Hairmansis *et al.*
74 2014; Siddiqui *et al.* 2014; Duan *et al.* 2018; Guimaraes *et al.* 2020; Kim *et al.* 2020; Vishal *et al.*
75 2020) and maize (Asaari *et al.* 2019; Li *et al.* 2021). However, there are still few studies that
76 examined the development of image-based phenotyping based on selection index and
77 multivariate analysis on the static hydroponic systems.

78 A selection index is a breeding approach that combines several criteria in one formula
79 (Anshori *et al.* 2021). This approach is commonly used in plant breeding because it considers all
80 selection criteria with current economic value on each criterion in genotype selection (Rajamani
81 *et al.* 2016; Islam *et al.* 2017). The selection index often combines with multivariate analysis to
82 increase its effectiveness (Akbar *et al.* 2019; Anshori *et al.* 2019; Fadhli *et al.* 2020; Farid *et al.*
83 2021; Anshori *et al.* 2021). This multivariate analysis is used to analyze or integrate the variance
84 of large variable data into a simpler and more comprehensive manner for the results to be easily
85 understood (Mattjik and Sumertajaya 2011). Furthermore, several studies have reported the
86 effectiveness of selection with the use of multivariate analysis (Anshori *et al.* 2018; Kose *et al.*
87 2018; Akbar *et al.* 2019; Anshori *et al.* 2019; Fadhli *et al.* 2020; Farid *et al.* 2020; Farid *et al.*
88 2021). Based on these analyzes, the use of image-based phenotyping with the selection index
89 based on multivariate analysis in static and dynamic hydroponic screening is necessary.
90 Therefore, this study aims to determine the selection index based on multivariate analysis and
91 assess the effectiveness of using image-based phenotyping in drought rice screening.

92

93 **Materials and Methods**

94

95 The two experiments conducted in this study include the destructive test, which was carried
96 out on static hydroponic (floating raft system), and the second was the non-destructive test
97 carried out on dynamic hydroponic (deep flow technique system). These studies were carried out
98 simultaneously in the greenhouse, Unhas Lecturer Residence, Makassar City, South Sulawesi
99 Province, Indonesia (22.4 m above sea level (asl) from August to November 2020.

100

101

102 **Experimental Design and Procedures**

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

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

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

126 area to the shooting area from a side view, convex hull from a side view, and the ratio of shoot
127 area to convex hull area from a side view (Table 1).

128

129 **Image Analysis**

130 The images were taken with a Canon EOS 1200D RGB camera in a portable photo studio
131 with a 75 cm x 75 cm x 75 cm. A white background with two 8 watt white LED lights in the
132 studio with camera settings (5.6 F-stops, 1/160 second exposure time, ISO 800, and without flash)
133 was used. The images were taken from a top view (0°) and the side view (90°) of the plant. For
134 static hydroponic, the images were taken twice at 13 DAS (early treatment) and 30 DAS (after
135 treatment) as shown in Figure 2. For dynamic hydroponic, images were taken at 13 DAS (early
136 treatment) and 68 DAS (after treatment) (Figure 3 and the images results were analyzed using the
137 Fiji application. Furthermore, the plant color was analyzed using hue channels with a hue 50
138 (green area) and the area growth rate was calculated using the projection of shoot area over two
139 time periods.

140

141 **Data analysis**

142 The data were analyzed independently for variance and the characters that have significant
143 interactions with each other were further analyzed using the Pearson correlation test and Principal
144 Component Analysis (PCA) with STAR 2.01 software. After static hydroponic has been tested by
145 correlation analysis, it was followed by path analysis. All further analysis based on the stress
146 tolerance index (STI) value of each character on individual PEG concentration (0% PEG (normal)
147 -10% PEG (drought stress) = STI 1 and 0% PEG (normal)-20% PEG (drought stress) = STI 2).
148 This concept was reported by Anshori et al. 2019, as follows:

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

150
$$STI = \frac{Y_p \times Y_s}{\bar{Y}_p^2}$$

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

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

153 \bar{Y}_p = Average character values of all varieties in normal / non-stressed conditions.

154 The selected characters from the PCA analysis results were used to obtain the index value.

155 The validation of static hydroponic was conducted by the regression test toward the dynamic
156 hydroponic.

157

158 **Results**

159

160 **Static Hydroponic System**

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

170 The correlation analysis on static hydroponic results was based on the static hydroponic
171 Stress Tolerance Index (STI) value (Table 3). The results showed that the 3rd leaf length (0.769
172 and 0.745), shoot area (0.981 and 0.983), green shoot area (0.978 and 0.984), and area growth

173 rate (0.981 and 0.961) had a significant correlation on the shoot and root fresh weights,
174 respectively. These correlation analyzes were followed by independent path analysis on the shoot
175 and root fresh weights, which were the main characters of actual morphology under artificial
176 vegetative screening with different roles. Therefore, this independent concept analysis can
177 determine the specific relationship among image-based phenotyping characters to the
178 conventional morphology characters.

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

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

196 The principal components analysis showed that there was one main component that can be
197 used as the basis for the selection index (Table 6), namely the first principal component (PC1)
198 with a cumulative proportion of 0.928 which is approximately 1. Based on PC1, shoot fresh
199 weight (0.4183) and root fresh weight (0.4183) in drought stress were in the same direction with
200 phenotyping characters of shoot area (0.4210), green shoot area (0.4177), and area growth rate
201 (0.4192). These three image-based phenotyping characters were combined in a static hydroponic
202 selection index using the formula as follows:

$$203 \text{ Static hydroponic index} = 0.421 \text{ shoot area} + 0.4177 \text{ shoot green area} + 0.4192 \text{ growth rate area} \\ 204 \dots\dots\dots \text{(Equation 1).}$$

206 **Dynamic Hydroponic System**

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

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

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

232 Dynamic hydroponic index = 0.4516 object extend Y from the side view + 0.4177 Convex
233 hull from the side view (Equation 2)

234

235 **Selection Index**

236 The index value of each variety on static and dynamic hydroponics was shown in Table 10.
237 Salumpikit variety at a PEG concentration of 10% (2.03) showed the highest value, while IR 20
238 at a PEG concentration of 20% (0.11) showed the lowest value in static hydroponics. Based on
239 dynamic hydroponic, salumpikit variety at a PEG concentration of 10% (0.53) remained the best,
240 while Inpari 34 (0.22) and IR 20 (0.22) were the varieties with the lowest index value.
241 Furthermore, the regression analysis results showed that the static hydroponic index has a
242 significant linear regression to the dynamic hydroponic index as shown in Figure 4. Similarly,

243 this figure also showed that the Salumpikit as the tolerant check variety has a good value index
244 than IR 20 as a sensitive check variety on both 10% and 20% PEG.

245

246 **Discussion**

247

248 The significant interaction in ANOVA is one of the early indicators in stress screening.
249 Meanwhile, a previous study by Akçura and Çeri (2011); Anshori *et al.* (2019); Farid *et al.* (2020)
250 showed that characters that are influenced by interactions had different patterns of decreased
251 response between genotypes on normal and stressful environments. This difference can be used
252 as a parameter to identify tolerance traits between genotypes in stress (Ali *et al.* 2014; Safitri *et al.*
253 2016; Fadhli *et al.* 2020). Based on this analysis, the image-based phenotyping of 3rd leaf length,
254 shoot area, shoot green area, and area growth rate on static hydroponic screening as well as red,
255 blue, and the ratio of shoot area to the convex hull from the top view, object extend Y, and
256 convex hull from the side view in dynamic hydroponic screening are used as a candidate to
257 selection criteria in stress tolerance. These characters were also significantly influenced by the
258 variance of PEG concentrations and varieties, which indicated that the variations in interactions
259 between genotypes on environmental differences are shown directly (Farid *et al.* 2020; Farid *et al.*
260 2021). Therefore, in-depth analysis with several series of multivariate analyzes was required to
261 obtain the best selection criteria for each drought screening process.

262 The assessment of varieties' response to stress needs to be carried out using a tolerance
263 index. Meanwhile, a study by Mau *et al.* (2014), Aboughadareh *et al.* (2019), Anshori *et al.*
264 (2019); Aboughadareh *et al.* (2020), and Farid *et al.* (2021) stated that the Stress Tolerance Index
265 (STI) is appropriate in characterizing the most tolerant of drought stress. This index is classified
266 as dynamic because it considers the general response of the population means, even though the

267 general response on this index focused on normal conditions (Anshori *et al.* 2019). Therefore, the
268 STI value becomes the basis for deeper analysis as shown in previous studies conducted by
269 Anshori *et al.* (2019) on rice against salinity stress, Fadhli *et al.* (2020) on maize against drought
270 stress, and Farid *et al.* (2021) on wheat against drought stress. Hence, the use of STI as the basis
271 for a more in-depth analysis was also carried out for static hydroponic and dynamic screenings.

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

283 The correlation and path analyses results on static hydroponic results showed that shoot and
284 green shoot areas, as well as area growth rate can be used as selection criteria. This is in line with
285 a study by Hairmansis *et al.* (2014) on the potential of these image-based phenotyping characters
286 on rice under salinity stress and barley under drought stress, while Kim *et al.* (2020) were on rice
287 under drought stress. Based on the path analysis results, the area growth rate has an inverse
288 orientation to the shoot and the green shoot areas. This difference in orientation is shown in the
289 direct and indirect effect on the character of the shoot and root fresh weights (Table 4 and 5).

290 This showed that the shoot and root fresh weights become the middle of the three image-based
291 phenotyping characters. However, the combination of the three characters can make the selection
292 easier because each character has specific potential. This is in line with a study by Acquah (2007)
293 and Anshori *et al.* (2021) which showed that the selection index formed by combining characters
294 with opposite orientations has the potential to increase the selectivity of a selection. Therefore,
295 these three characters are still used as selection criteria. However, the crucial thing in the
296 formation of the selection index of the three characters is the weighting value of the selection,
297 which can be determined using the principal component analysis.

298 The principal component is a multivariate analysis that aims to extract important
299 information from large data into a new simpler set of orthogonal variables (Ilmaniati and Putro
300 2019). This analysis has been reported as a weighting indicator for the selection index by Akbar
301 *et al.* (2019), Anshori *et al.* (2019), Alsbah *et al.* (2019), Fadhli *et al.* (2020), and Farid *et al.*
302 (2021). Based on the PCA results in Table 6, the characteristics of shoot area, green shoot area,
303 and area growth rate have the same eigenvector direction and variance with the shoot and root
304 fresh weights. This showed that the three characters generally have the same variance direction,
305 although, in the broad cross-section, the growth of the shoot has a different orientation from the
306 other two characters. However, due to the inclusion of the two fresh weight characters, the three
307 characters generally have the same range direction that can be combined into the selection index
308 in Equation 1.

309 Validation of image-based phenotyping characters in static hydroponics was carried out by
310 identifying the relationship between these characters and dynamic hydroponic characters.
311 Relatively, dynamic hydroponic has a complex growth rate than static hydroponic (Sagita *et al.*
312 2020). This was because dynamic hydroponic observations were carried out in the flowering
313 phase, while static hydroponic were in the vegetative phase. Since the application of the selection

314 agent in the two methods was carried out at the same time in the 21 days after sowing, therefore,
315 the static hydroponic selection index assessment can be related to the growth character of
316 dynamic hydroponic as part of its validation.

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

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

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

338 This showed that both characters can be used as validation characters through the selection index.
339 Furthermore, the dynamic hydroponic selection index can be used as the Y factor in a regression.
340 Based on the regression results, the static hydroponic index has good adherence to the dynamic
341 hydroponic index. The differences in tolerance groups between the Salumpikit as the tolerant
342 check variety and IR 20 varieties as the sensitive check variety had a significant distance, which
343 is in line with a study by Hairmansis *et al.* (2020) and Kartina *et al.* (2019). Meanwhile, the use
344 of control varieties can be one of the proofs to detect effective selection. This concept had
345 reported in previous studies by Ali *et al.* (2014), Safitri *et al.* (2016) and Anshori *et al.* (2020) on
346 salinity stress, Wening *et al.* (2019) and Anshori *et al.* (2019) on submergence, Akbar *et al.*
347 (2018) and Akbar *et al.* (2019) on drought stress. Furthermore, the use of Salumpikit and IR 20
348 have been reported as a check or control of rice drought tolerance by Akbar *et al.* (2018) and
349 Akbar *et al.* (2019). Based on these results, the selection index on static hydroponic screening has
350 the potential to be used to identify the tolerance properties of rice to drought stress.

351 Therefore, the use of multivariate analysis on image-based phenotyping characters has the
352 potential to increase the effectiveness of selection in drought stress tolerance screening. The
353 selection index for static hydroponic is $0.421 \text{ shoot area} + 0.4177 \text{ green shoot area} + 0.4192 \text{ area}$
354 growth rate . Also, the character selection index for dynamic hydroponic is $0.4516 \text{ objects extend}$
355 $\text{Y from the side view} + 0.4177 \text{ convex hull from the side view}$. These results showed that rice
356 screening based on image-based phenotyping can be recommended as a more effective and
357 efficient method for the rapid screening of drought stress.

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548 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 4th leaf 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 the outer 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 number of 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).

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

Characters	D	V	D x V
SFW	178.73 **	28.92 **	10.37 **
RFW	24.77 **	7.30 **	1.96 **
LL2	1486.95 **	326.19 **	14.68 ns
LL3	1471.64 **	466.95 **	49.37 *
LL4	669.49 **	188.37 **	28.07 ns
SA	65556.88 **	13117.85 **	3438.49 **
GSA	61237.72 **	9849.18 **	3261.87 **
R	3460.07 **	86.95 ns	748.95 ns
G	879.98 Ns	87.35 ns	1498.13 ns
B	1680.60 *	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 **

552 Note: D= Drought level; V= Variety; *= Significant effect at $P \leq 0.05$; **= Significant effect at P
553 ≤ 0.01 ; ns= Not significant; SFW= Shoot fresh weight; RFW= Root fresh weight; LL2= 2nd leaf
554 length; LL3= 3rd leaf length; LL4= 4th leaf length; SA= Shoot area; GSA= Green shoot area; R=
555 Red; G= Green; B= Blue; RRG= Ratio of red to green; RGSS= Ratio of green shoot area to shoot
556 area; AGR= Area growth rate.

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

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

565 Note: the numeric in table indicate the correlation value, *= Significant correlated at $P \leq 0.05$;

566 **= Significant correlated at $P \leq 0.01$; SFW= Shoot fresh weight; RFW= Root fresh weight;

567 LL3= 3rd leaf length; SA= Shoot area; GSA= Green shoot area; AGR= Area growth rate.

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

Characters	Direct	Indirect Influence					Residual
	Influence	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

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

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

Characters	Direct	Indirect Influence					Residual
	Influence	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

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

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618 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

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

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

Characters	D	V	D x V
SFW	8042.24 **	319.95 Ns	376.25 ns
RFW	467.01 **	53.23 Ns	14.64 ns
CT	81129688.30 **	4224903.16 Ns	1562942.10 ns
SAT	5571152.36 **	474034.51 Ns	97115.97 ns
GSAT	3072273.82 **	333330.03 Ns	80272.07 ns
NLT	2478.87 **	617.19 *	156.84 ns
RT	3946.91 **	462.79 **	189.34 *
GT	4734.46 **	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	1636.09 **	147.66 **	110.49 **
SAS	3696517.43 **	280606.15 Ns	91206.26 ns
GSAS	1651359.59 **	212567.33 Ns	68283.65 ns
CS	48116645.30 **	6716755.96 **	947650.84 *
RGSSS	0.0853 *	0.0141 Ns	0.0082 ns
RSCS	0.0463 **	0.0063 Ns	0.0024 ns

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

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645 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	-0.48	ns	-0.24	ns	0.30	ns	1.00				
CS	-0.30	ns	-0.03	ns	0.14	ns	0.97	**	1.00		
SH Index	-0.16	ns	-0.04	ns	0.19	ns	0.90	**	0.91	**	1.00

646 Note: the numeric in table indicate the correlation value, *= Significant correlated at $P \leq 0.05$;

647 **= Significant correlated at $P \leq 0.01$; ns= Not significant; RT= Red from top view; BT= Blue

648 from top view; RSCT= Ratio of shoot area to convex hull from top view; YS= Object extend Y

649 from side view; CS= Convex hull from side view; SH Index= Static hydroponic selection index.

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663 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
Cumulative Proportion	0.5883	0.8544	0.9536	0.9801	1
EigenValues	2.9414	1.3306	0.496	0.1322	0.0997

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

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680 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

681 Note: SH Index= Static hydroponic selection index; DH Index= Dynamic hydroponic selection
682 index.

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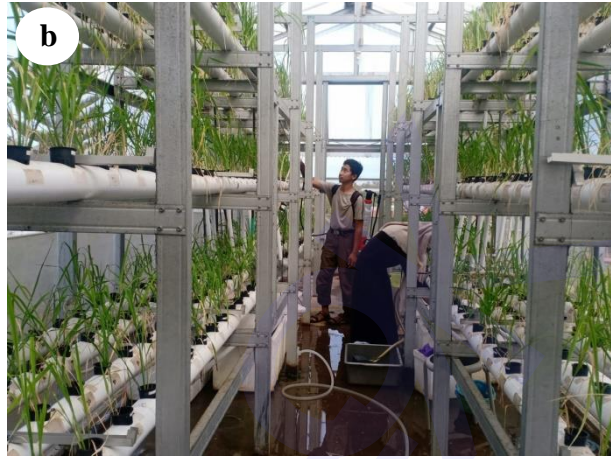
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696 Figure 1. Images of static hydroponic system at 20 DAS (a) and dinamic hydroponic system at 33

697 DAS (b).

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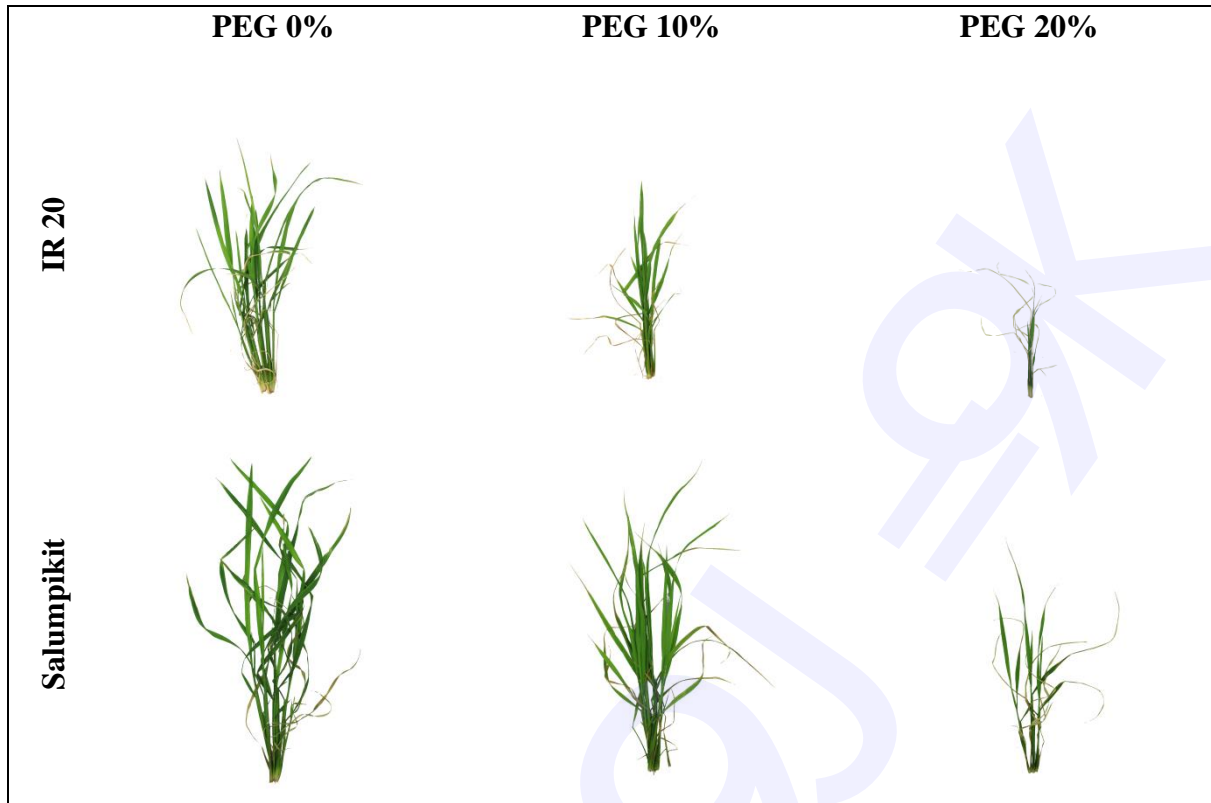
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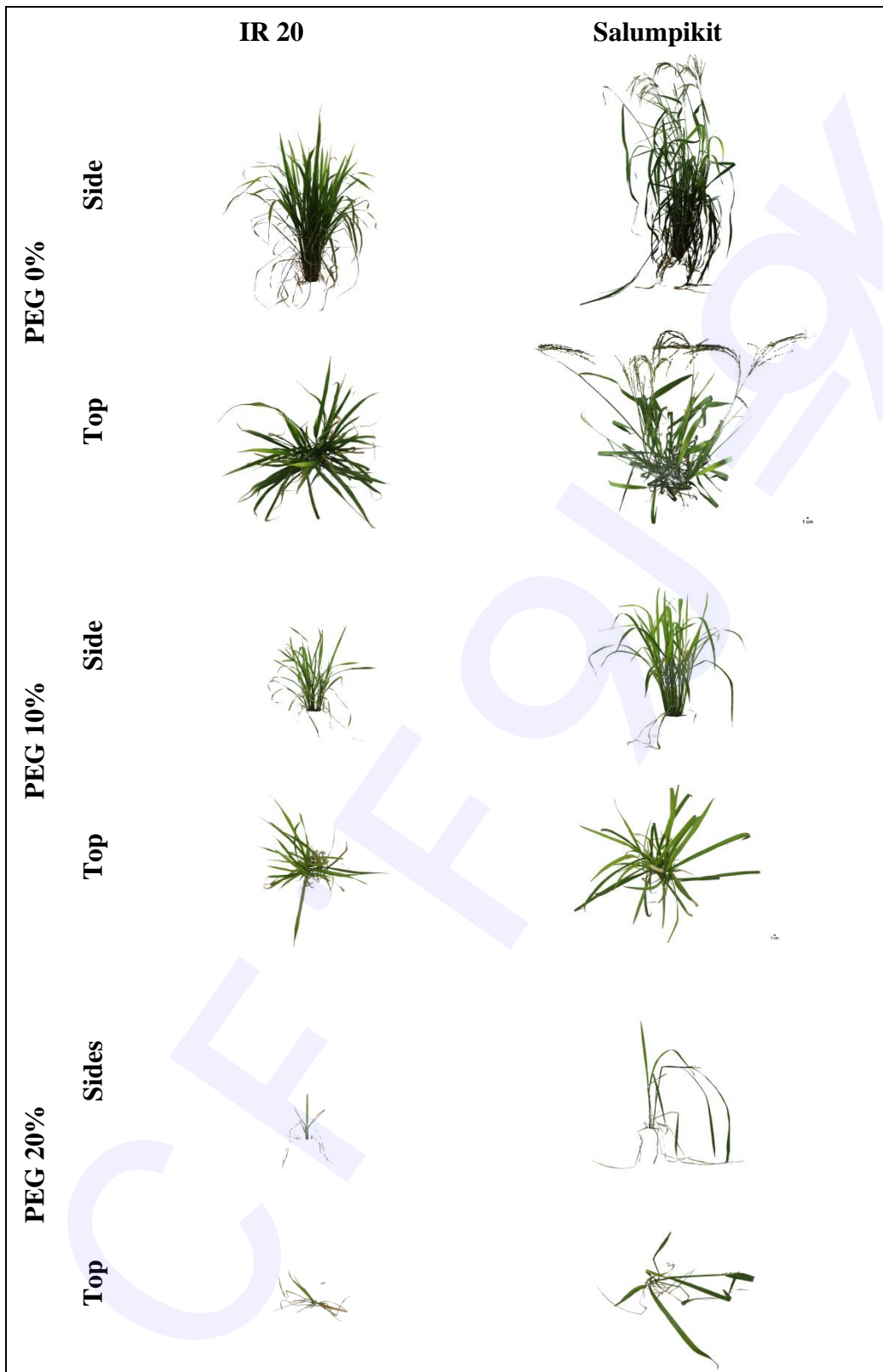
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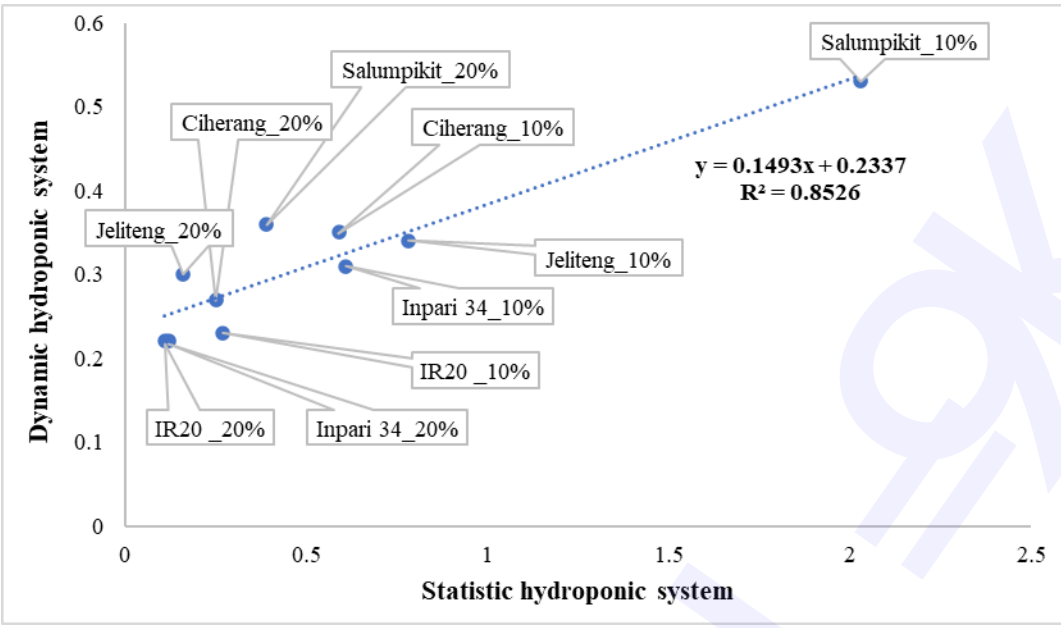


713 Figure 2. RGB images of IR 20 and Salumpikit under drought stress at 30 DAS in static
714 hydroponic.

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



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