Cluster heatmap for screening the drought tolerant rice through hydroponic culture

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Abstract : The development of drought rice screening is one of the keys to increase selection effectiveness. This development can be done by developing the analytical method. In general, identification of tolerant rice can be conducted with the cluster analysis. However, the common cluster analysis just was focused on genotype clusters so that the reason for the clustering does not can explain. Therefore, the other analysis approach needs to be done, such as cluster heatmap analysis. The objective of this study is to identify the effectiveness of cluster heatmap used in rice tolerance screening under drought stress. This study was designed with a nested randomized complete group design, where replications were nested in PEG 6000 concentration as a screening environment. The concentration of PEG used in this study was 0% PEG and 20% PEG. The genotype used consisted of 8 genotypes repeated three times. Hydroponic culture used ABmix in culture solution. As for, the number of characters observed was seven morphology characters and three physiological characters. The results of this study showed that cluster heatmap analysis could distinguish between the rice tolerant group drought-tolerant variety control (Salumpikit, Pokkali, and Inpari 29), and sensitive variety control (IR 20). Besides that, the good selection characters in hydroponic drought screening were shoot length, the number of tillers, shoot fresh weight, root fresh weight, and total biomass fresh weight. Based on this study, the cluster heatmap can be recommended as one of the analytical methods in hydroponic drought screening.

1. Introduction
Rice is a staple crop for most people in the world, especially in Asian countries like Indonesia. The potential for rice in Indonesia can be seen from the level of rice consumption and rice production. According to 2019 BPS data, Indonesia's rice consumption reaches 29.6 million tons/year and rice production is 54.6 million tons of GKG. This indicates that increasing rice production is a priority to maintain food stability in Indonesia. However, population growth, changes in land-use experts, and increasing global warming are challenges in increasing rice production in Indonesia [1]. In general, global warming can affect the optimal environment for rice cultivation to become a suboptimal
environment[2]. This suboptimal environment affects rice growth and production in Indonesia. One form of suboptimal environment for rice cultivation is drought stress [3,4].

Drought stress is closely related to the availability of water for plants [5]. The ability of rice plants to regulate the water balance greatly affects the nature of rice tolerance to drought stress. In general, drought stress impacts cell division, turgor stress, and mineral translocation for plants [6, 7, 8]. This will have a direct impact on rice growth and production [9,10]. Therefore, increasing rice production in Indonesia also needs to be supported by the development of varieties that are tolerant of drought stress. However, the development of tolerant varieties must also be supported by the development of drought-tolerant screening. One of them is a statistical approach such as a heatmap cluster analysis.

Heatmap cluster analysis is the development of cluster analysis in general. In general, cluster analysis only focuses on one grouping, but the analysis cannot explain the nature and basis of grouping that occurs [11,12]. This is different from the heatmap cluster analysis which combines two groupings in one dimension, namely the grouping of genotypes and characters so that the basis of the grouping that occurs can be known [13,14]. Besides, the heat map concept in this analysis helps researchers to understand how the interaction pattern between the two dendrograms occurs [15]. The use of this analysis has been carried out in several studies [11,12, 16, 17, 18]. Therefore, this concept can also be applied in the classification of rice tolerance properties to drought stress. The purpose of this study was to identify the effectiveness of using heatmap clusters in screening rice tolerance to drought stress.

2. Materials and methods
The research was conducted at Unhas Lecturer Housing Jl. Algazali Blok BG. 9, Makassar, South Sulawesi in August - September 2020. The study used a nested randomized block design, where nested replicates were in PEG 6000 concentration. The PEG concentrations used consisted of 0% (normal) and 20% (stress). The varieties used consisted of 8 varieties, namely Inpari 34 Salin Agritan (V1), Inpari 29 (V2), IR29 (V3), IR 20 (V4), Salumpikit (V5), Pokkali (V6), Ciherang (V7), dan Jeleteng (V7). This experiment was repeated 3 times, so there were 30 experimental units. Meanwhile, each experimental unit consists of 3 sample units, so there are 90 units of observation.

2.1. Procedure
This experiment uses a modified hydroponic method by Standard Evaluation System (SES) IRRI [19]. The seeds of each variety are sown first on petridish. After seven days after sowing (DAS), the selected seeds were transferred to the treatment container with a solution volume of 8L per container. The concentration of nutrient solution uses AB mix with a dose of 5 mL / L of water in each stock solution A and B. Rice seeds that are transplanted into the treatment tub are inserted into the styrofoam hole by first rolling the seeds with foam. The hole on the styrofoam has a diameter of 2 cm with a distance between the holes that are 4 cm x 5 cm. PEG induction as a selection environment was carried out at age 14 DAS through gradual administration. This is so that the seeds do not experience osmotic shock [20]. The concentration in the first stage is half of the total concentration, namely 10% PEG 6000 or the equivalent of 1000 g PEG per liter. Three days later, the PEG concentration was optimized to be 20% PEG. On the other hand, plants treated with 0% PEG (Control) were grown without adding PEG to ABmix's hydroponic nutrient solution. Meanwhile, the maintenance of seedlings includes providing nutrition, maintaining the pH value, checking the water, and cleaning the nutrient solution container. The pH of the solution was maintained at 5.0-5.1 with the use of 1N NaOH and 1 N HCl.

2.2. Analysis data
Observation of tolerance character and biomass harvest was carried out at 14 days after application. Observation parameters included number of tillers, shoot height (cm), number of leaves, root length (cm), root fresh weight (g), shoot fresh weight (g), total biomass fresh weight (g), chlorophyll a, chlorophyll b, and total chlorophyll. The data obtained were then analyzed using variance and
repeatability using STAR 2.0.1 software. Characters that have a significant interaction effect are continued with an analysis of relative decline. After that, the characters under stress conditions (20% PEG) and relative decline were analyzed together with the heat map cluster analysis. Heatmap cluster analysis using Rstudio software with the gplots package.

3. Results and discussion
The ANOVA results on morphological and physiological characters on drought stress are shown in table 1. Based on this table, genotype diversity significantly affects all characters. Environmental diversity and their interactions with genotypes showed a significant effect on almost all characters, except for the number of leaves for environmental diversity and root length and chlorophyll A for interaction diversity. In general, the diversity of interactions is one of the characteristics in determining indicators of tolerance character [21, 22, 23]. Significant characters in the variety of interactions can show different responses between genotypes to different stress environments [24, 25]. Based on this, all characters, except root length and chlorophyll a were not included in the next analysis.

Table 1. Results from variance character of the morphological and physiological characters of rice in hydroponic drought stress.

| Character | G        | E         | GxE      | e        | Vg       | Vp       | R        | CV      |
|----------|----------|-----------|----------|----------|----------|----------|----------|---------|
| SH       | 578.46** | 4036.63** | 69.05**  | 19.75    | 84.90    | 108.33   | 78.37    | 11.14   |
| NT       | 7.34**   | 76.61*    | 0.27*    | 0.19     | 1.18     | 1.27     | 92.61    | 24.79   |
| NL       | 93.21**  | 966.07    | 28.68**  | 11.36    | 10.76    | 20.55    | 52.34    | 18.33   |
| RL       | 36.98**  | 193.84*   | 4.45     | 2.11     | 5.42     | 6.95     | 78.03    | 9.3     |
| SFW      | 57.58**  | 739.39**  | 43.83**  | 1.98     | 2.29     | 16.94    | 13.52    | 26.15   |
| RFW      | 4.62**   | 44.41*    | 2.61*    | 0.28     | 0.34     | 1.21     | 27.78    | 24.56   |
| BFW      | 92.65**  | 1146.22** | 65.56**  | 3.44     | 4.52     | 26.44    | 17.08    | 24.66   |
| Chl A    | 1354.71* | 208375.81*** | 991.44   | 467.54   | 60.55    | 400.77   | 15.11    | 42.69   |
| Chl B    | 46.84**  | 3440.85** | 38.84**  | 10.44    | 1.33     | 14.50    | 9.20     | 6.12    |
| Chl Tot  | 2157.18* | 299188.92** | 1606.97* | 665.18   | 91.70    | 641.22   | 14.30    | 25.7    |

Note: ** significant effect on 1% level, * significant effect on 5% level, G = Genotype, E = Environment, GxE = Interaction, Vg = genetic variance, Vp = phenotypic variance, R = repeatability, CV = Coefficient of variance, SH = Shoot Height, NT = Number of Tillers, NL = Number of Leaves, RL = Root Length, SFW = Shoot Fresh Weight, RFW = Root Fresh Weight, BBB = Biomass Fresh Weight, Chl A = Chlorophyll A, Chl B = Chlorophyll B, Chl Tot = Total Chlorophyll.

Based on table 1, the root length was not included in further analysis. In general, root length is one of the characteristics of drought tolerance [26, 27, 28]. However, the results of this study show a different response. This difference is due to the type of drought stress given in this study. Stress induction using PEG 6000 can induce physiological drought stress. Physically, the water availability in the environment is in the available status, so that plants can still actively reduce the water potential of the roots and increase the root area to increase water absorption. This concept is different from physical drought, where the availability of water is in a limited status so that the plant will try to minimize root development according to its tolerance ability [12, 27, 29]. Therefore, in hydroponic stress, the susceptible genotype had a root growth rhythm that was relatively the same as the tolerant genotype. This means that there is no significant interaction between genotypes in this study. The chlorophyll A character showed no significant interaction in this study. In general, chlorophyll A is a character that is widely used in assessing tolerance properties [30, 31]. However, in this study, this character has a very high error. This suggests that chlorophyll A should not be included in further analysis.

Based on the repeatability values in table 1, plant height, number of tillers, number of leaves, and root length are characters that have high repeatability values. In general, repeatability is a genetic assessment of its phenotype that can be repeated when the population is tested in the same or similar
environment as the initial test environment [32]. The concept of calculating repeatability has the same concept as the calculation of heritability [25]. Based on this, the four characters are characters that have relatively stable genetic responses to environmental differences. However, the character of the number of leaves and the length of the roots is not influenced by the variety of environment and the variety of interactions, respectively, so that these characters cannot be included as indicators of tolerance character. Meanwhile, other characters show low and moderate repeatability below 50% [32]. A low repeatability value indicates the influence of the environment and high interactions on these characters. Therefore, based on this, plant height, number of tillers, number of leaves, and root length can be used as indicators of a stable tolerance character. However, it is necessary to identify general patterns of tolerance by including potential growth characteristics. This aims to see the general characteristics and classifications between each genotype. One approach that can be done is through a relative reduction analysis and a heat map cluster [12].

Table 2. Relative reduction and phenotype at PEG 20%.

| V        | SH (cm) | NT | NL | SFW (g) | RFW (g) | NFW (g) | Chl B | Chl Tot |
|----------|--------|----|----|---------|---------|---------|-------|---------|
|          | Relative reduction (%) |       |    |         |         |         |       |         |
| Inpari 34 | 37.26  | 43.11 | 35.5 | 82.56  | 47.17   | 74.24   | 27.92 | 93.29   |
| Inpari29  | 29.61  | 48.66 | 36.79 | 84.73  | 69.27   | 80.56   | 29.72 | 90.55   |
| IR29      | 39.02  | 59.29 | 41.74 | 80.02  | 55.9    | 73.08   | 22.56 | 85.02   |
| IR20      | 31.9   | 27.46 | 18.23 | 61.59  | 12.32   | 49.97   | 16.75 | 86.17   |
| Salumpikit | 40.2   | 53.85 | 43   | 90.92  | 76.47   | 86.98   | 34.33 | 87.21   |
| Pokkali   | 42.66  | 60.85 | 41.88 | 88.1   | 67.48   | 83.98   | 22.28 | 84.91   |
| Ciherang  | 35.54  | 29.76 | 33.76 | 76.18  | 50      | 68.89   | 33.58 | 90.16   |
| Jeliteng  | 38.02  | 61.23 | 52.84 | 85.26  | 64.39   | 79.16   | 30.25 | 85.68   |
| Averages  | 36.78  | 48.02 | 37.97 | 81.17  | 55.37   | 74.61   | 27.17 | 87.87   |

| V        | SH (cm) | NT | NL | SFW (g) | RFW (g) | NFW (g) | Chl B | Chl Tot |
|----------|--------|----|----|---------|---------|---------|-------|---------|
|          | Phenotype at PEG 20% |       |    |         |         |         |       |         |
| Inpari 34 | 29.45  | 2.78  | 14.33 | 1.37   | 1.28    | 2.65    | 43.8  | 11.83   |
| Inpari29  | 32.87  | 2.11  | 11.45 | 1.15   | 0.85    | 2       | 44.07 | 18.13   |
| IR29      | 24.8   | 1.45  | 9.78  | 0.8    | 0.71    | 1.51    | 44.4  | 23.33   |
| IR20      | 27.28  | 3.22  | 15.45 | 1.45   | 1.02    | 2.47    | 43.9  | 16.5    |
| Salumpikit | 35.73  | 2     | 13.11 | 1.25   | 1.22    | 2.47    | 44.77 | 28.37   |
| Pokkali   | 44.07  | 2.22  | 13.11 | 2.55   | 1.74    | 4.28    | 44.3  | 23.13   |
| Ciherang  | 27.93  | 3.67  | 17.44 | 1.77   | 1.43    | 3.2     | 44.3  | 21.13   |
| Jeliteng  | 23.72  | 3.45  | 16.56 | 1.27   | 1.27    | 2.54    | 45.2  | 28.83   |
| Averages  | 30.73  | 2.61  | 13.9  | 1.45   | 1.19    | 2.64    | 44.34 | 21.41   |

Note: V = Varieties, SH = Shoot Height, NT = Number of Tillers, NL = Number of Leaves, RL = Root Lenght, SFW = Shoot Fresh Weight, RFW = Root Fresh Weight, BBB = Biomass Fresh Weight, Chl B = Chlorophyll B, Chl Tot = Total Chlorophyll.

The results of relative reduction and phenotype under stress conditions are in table 2. Based on the table, shoot fresh weight and total chlorophyll have the largest relative decrease, while chlorophyll b has the largest relative decrease. The use of relative inheritance is an approach of a character in assessing genotypic traits in particular stress [33]. This has been reported by several researchers [12,21,34,35]. The use of phenotypes in a stressful state can illustrate the adaptability of a genotype to particular stress [25]. Based on the results of the relative decline, there is a variation in the mean yield reduction between characters. Characters with low repeatability have a relatively high decline, while characters with high repeatability have a relative decline which is around 35-50%. Combining all the relative inheritance characters and phenotypes in drought conditions can provide wide variations in the classification process.
The results of the heatmat cluster analysis show that there are two types of dendrogram: a genotype dendrogram with a horizontal position and a character dendrogram with a vertical position (figure 1). Based on the dendrogram genotype, a bit of a tolerant check has a long kinship distance with IR 20 as a drought-sensitive check. This indicates that the grouping process in this screening is going well. In the genotype dendrogram, there are 2 main groups, namely the sensitive group consisting only of IR 20 and the tolerant group consisting of 3 subgroups. The first sub-group in the tolerant group is Salumpikit, Pokkali, and Inpari 29. This sub-group is considered the most tolerant sub-group. This is evidenced by the incorporation of Salumpikit into the subgroup. The second sub-group consists of Jeliteng and IR29. The third sub-group consists of Inpari 34 and Ciherang. Subgroups two and three can be defined as groups that are moderate to drought stress.

Based on dendrogram character grouping, the characters that have a consistently graded grouping pattern are the relative reduction in shoot height, root fresh weight, root fresh weight, biomass fresh weight, and number of tillers. The characters of root fresh weight, root fresh weight, and biomass fresh weight were also assessed as having a stable consistency of color gradations between genotypes. Although the three characters have low repeatability and relatively high decline. However, the different responses in these characters can pattern the nature of tolerance regularly and consistently. This indicates that the response of the fresh weight character is dynamic and highly dependent on the selection environment on static hydroponic drought screening. Also, based on the nature of drought stress, water availability is the main limiting factor for this stress [27,28]. Therefore, the diversity pattern of the fresh weight character is very varied, so that the character of the fresh weight of growth can also be considered as a character of joint selection with shoot height and number of tillers. Based on the grouping of the two dendrograms, the heatmap cluster analysis is considered very informative because the nature of the grouping between genotypes can be seen. This was also stated by [12] on the salinity screening of dihaploid rice lines. Therefore, this analysis is recommended in screening rice tolerance to drought stress, especially in the static hydroponic culture method.
4. Conclusion
Determination of genotype tolerance based on cluster heatmap analysis has a diverse and informative pattern. Pokkali and Inpari 29 have good potential for tolerance to drought stress through hydroponic culture screening, shoot fresh weight character, root fresh weight, total biomass fresh weight, shoot height, and number of tillers are good characters recommended as specific selection characters in screening rice tolerance against drought stress.

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