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Morphological and Physiological Responses of Local Rice Cultivars of Central Java under Drought Stress

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ABSTRACT

Exploration of the potential resilience of local rice cultivars could be one strategy to overcome drought. This study was aimed at selecting local drought-resistant rice cultivars. The result showed that drought stress significantly reduced the morphological characters. The morphological analysis of plants (plant height, the number of productive tillers, panicles and grain weight per clump) showed that Jelitheng Karanganyar had the best characteristics for tolerating drought. The highest proline content during drought conditions was in Wonogiri black rice. Based on screening, Salatiga black rice and Sragen black rice showed a high-stress tolerance index (STI) values and were identified as drought-tolerant cultivars at 70% FC.

Key words: Black rice, drought, proline, red rice, stress tolerance index

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary food for most Indonesians. Rice has essential cultural, social and economic impacts on the life of people. The enhancement of rice production is a priority to address the rice supply shortage. Indonesia has various kinds of rice, but black and red rice get less attention than white rice. Both (black and red rice) contain nutrients that white rice lacks. Black rice (*Oryza sativa* L.) contains high protein, fat and crude fiber, while red rice (*Oryza nivara*) contains high iron and zinc (Rathna *et al.,* 2019). Anthocyanin is an antioxidant that contributes a major role in health benefits and obviates non-communicable diseases (Sangma and Parameshwari, 2021). The nutritional value contained in both the types of rice should have the potential to be developed. Black and red rice can be a source of carbohydrates and functional foods that are beneficial to human health. It can attempt to meet the Indonesian people's food and nutritional needs.

Indonesia has 8.1 million ha of rice area consisting of 2.25 million ha for rainfed lowland, 4.76 million ha of irrigated lowland and 1.09 million ha of upland land (Rumanti *et al.,* 2018). Significant climate change can cause drought in rice fields and negatively impact rice production. Rice requires a large amount of water to produce. Drought stress has become the main limiting factor to crop productivity. It causes a risk of crop failure and could endanger food security. The response of plants to drought stress depends upon the duration of stress, severity of the stress and the crop growth stage (Khan *et al.,* 2017).

Drought stress in any or all of the growth stages can reduce yield. Hossain *et al.* (2015) argued that drought stress in the vegetative phase influenced the formation of the grains per panicle. Water deficit conditions induce several plant responses, such as physiological, morphological, biochemical and molecular changes (Murtaza *et al.,* 2016). Aslam *et al.* (2015) suggested that the root length, root density, root volume and the number of roots were the morphological changes that were disturbed under drought. Drought-tolerant varieties tend to have deep and thick roots (Ramamoorthy *et al.,* 2018). Water deficit causes water potential decrease and stomata closure so that it could inhibit CO_2 absorption and photosynthetic activity. Accumulating compatible solutes such as proline is an adaptive mechanism developed by plants to deal with drought conditions (Joseph *et al.,* 2015).

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Proline contributes to drought tolerance in plants by regulating the osmotic potential of cells. It could avoid cell damage due to ROS reduction (Sahebi *et al.,* 2018).

The strategy to deal with drought stress is to develop drought-tolerant local rice varieties by exploring the potential for local rice resistance. The local rice used in this study is black rice and aromatic red rice from various regions in Central Java. Several studies on local black and red rice of Central Java under drought have not led to research on the essential aspects, such as morphology and physiology. These aspects are used to select parents in the plant breeding program. In addition, the identification of changes in morphological and physiological characteristics of plants under drought stress is essential for increasing rice yields. This study investigated the morphological and physiological responses to the application of drought stress based on field capacity. The study aimed at selecting potential local black and red rice of Central Java that were tolerant to drought stress.

MATERIALS AND METHODS

The experiment was carried out in the screen house from June to December 2021. The research was located at Depok Village, Kandeman District, Batang, Central Java. Field capacity test was done in laboratory of soil chemical, Universitas Sebelas Maret. The proline content was estimated in The Integrated Research and Testing Laboratory, Universitas Gadjah Mada. The experiment was conducted in factorial completely randomized design (CRD). The materials were six Central Java local rice samples collected from four locations in Central Java province, Indonesia. The local rice consisted of Wonogiri black rice (V1), Jelitheng Karanganyar (V2), Salatiga black rice (V3), Sragen black rice (V4), Wonogiri aromatic red rice (V5) and Karanganyar aromatic red rice (V6). The level of drought treatments used variations in field capacity, namely, 100% FC (K1) for control, 70% FC (K2) and 40% FC (K3). There were 18 treatment combinations with three repetitions. This study used three sample plants per treatment per replication. In total, there were 162 plant units.

Drought treatment was carried out when the plants were 35 to 95 days after planting (DAP). The observation of morphological characteristics was plant height, the number of productive tillers and panicle and grain weight per clump. Stress tolerance index (STI) was estimated to identify cultivars that can perform well under drought-stressed or non-stressed conditions. Plant height was measured 60 DAP. The number of productive tillers, number of panicles and grain weight per clump were measured after harvest. The proline content as physiological character was measured at 60 DAP. The stress tolerance index (STI) was calculated based on the total grain weight using the formula STI = (Ypi x Ysi)/Yp^2. Ypi was the total grain weight of the cultivar in non-stress conditions (100% FC), Ysi was the total grain weight cultivar in stress conditions (70% FC and 40% FC), and Yp was the mean total grain weight in overall cultivars at 100% FC condition. The categories for determining the level of drought tolerance were if STI <0.5 as a sensitive cultivar, the moderate tolerant cultivar if $0.5<\text{STI}\leq 1.0$ and the tolerant-drought cultivar if STI >1.0.

Drought treatment was carried out by determining the field capacity of the soil planting medium: manure (2 : 1). The variations in the field capacity level were 100, 70 and 40% by measuring the field capacity (FC) using the gravimetric method. Two polybags were filled with 1.000 g of soil medium. The poly bags were watered until saturated and left for 3 x 24 h until the water did not drip as wet weight (WW). The growing media was oven-dried for 24 h at 100°C and cooled in a desiccator, then it weighed as the dry weight of the soil (Dw). Field capacity was calculated as:

$$\text{The field capacity (FC)} = \frac{\text{WW} - \text{Dw}}{\text{Dw}} \times 100\%$$

Research data were analyzed using two-way ANOVA with P=0.05 and 95% confidence level using R-studio software. If it had a significant effect, further analysis was determined by the HSD test at α = 0.05. The proline content and STI were analyzed descriptively.

RESULTS AND DISCUSSION

Plant height indicated growth and could predict grain yield and biomass. Statistical tests showed no interaction effect between rice cultivars and drought stress treatment in affecting plant height. A single factor of cultivar and drought stress significantly affected plant height. Drought stress at 40% FC decreased to 8.72% and was significantly lower than watering at 100% FC (Table 1). Jelitheng Karanganyar had the lowest plant height (103.29 cm). The most significant reduction in plant height was observed in Sragen black rice treated with 40% FC (13.63%). Plant height also decreased in weedy rice accessions by 29% under drought (Piveta *et al.,* 2020). According to Singh *et al.* (2018), this was due to reduced turgor activity which affected cell division and elongation activity.

The number of productive tillers and panicles could also measure the response of rice plants under drought stress. Tiller number determined panicle number and grain yield in rice plants. Drought stress significantly reduced the number of productive tillers and panicle (Table 2). The statistical tests showed that a 70% FC drought treatment decreased the number of productive tillers by up to 27.2% compared to a 100% FC condition. Increased stress conditions also reduced the number of productive tillers of wheat (Kumar *et al.,* 2018). Environmental conditions affected the number of productive tillers during the initiation of tiller shoots and advanced developmental stages. Drought affected stomata closure and leaf curling. Plants experienced osmotic adjustment and increased cell wall elasticity. These could disturb gas exchange activity and translocation of water and nutrients for photosynthesis (Abid *et al.,* 2018; Keipp *et al.,* 2020). A decrease in photosynthesis would significantly impact a decrease in biomass, plant height, number of tillers and yield.

Table 2. Effect of drought stress on the number of productive tillers and panicle on local black rice and red rice of Central Java

Drought stress (% FC)	Productive tillers	No. of panicles
100	17.56a	17.33a
70	12.78b	12.44b
40	13.56ab	12.06b

Numbers followed by the same letter in the same column were not significantly different in the HSD test at a 95% confidence level.

According to Tang *et al.* (2019), plant height and number of productive tillers affected seed yields because the above-ground part of the plant was associated with the interception of solar radiation, the site of photosynthesis, and supported yield vessels.

In this study, the number of panicles was not affected by the interaction of cultivar and drought stress nor cultivar. However, drought stress significantly reduced the number of panicles. The number of panicles were significantly decreased from the 70% FC drought treatment up to 28.21%. The decrease in panicles was due to the drought stress period during the vegetative to reproductive phases. This opinion is also in line with Kang *et al.* (2017), that drought stress during the tillering phase reduced the number of tillers so that effective panicles decreased during the booting stage.

Almost all cultivars experienced reduced grain weight per clump as the field capacity decreased (Table 3). Drought stress reduced the grain weight per clump and reached 25.01% at 40% FC. The average grain weight per clump in all cultivars decreased to 41.34% and significantly differed from the 100% FC condition. Sragen black rice and Jelitheng

Table 1. Plant height of local black and red rice cultivars of Central Java at 60 DAP under drought stress

Rice cultivars	Drought treatments			
	100% FC	70% FC	40% FC	Mean
Wonogiri black rice	161.33	149.63	146.10	152.35a
Jelitheng Karanganyar	107.30	99.50	103.07	103.29b
Salatiga black rice	153.27	148.67	132.37	144.77a
Sragen black rice	155.43	149.73	145.47	150.21a
Wonogiri red rice	114.57	113.17	104.60	110.78b
Karanganyar red rice	103.60	112.27	94.50	103.46b
Mean	132.58a	128.83ab	121.02b	

Numbers followed by the same letter in the same column were not significantly different in the HSD test at a 95% confidence level.

Table 3. Grain weight per clump (g) of local black and red rice cultivars in central Java under drought stress

Rice cultivars	Drought treatments		
	100% FC	70% FC	40% FC
Wonogiri black rice	27.53	15.10	12.02
Jelitheng Karanganyar	23.83	18.68	17.33
Salatiga black rice	34.10	26.75	12.25
Sragen black rice	29.56	25.12	17.43
Wonogiri red rice	28.71	11.88	17.37
Karanganyar red rice	17.18	23.03	17.99
Mean	26.82a	20.09ab	15.73b

Number followed by the same letter in the column and the same treatment showed that there was no significant difference between treatments in the HSD test at a 95% confidence level.

Karanganyar showed the lowest loss percentage of grain weight per clump under drought stress. However, Wonogiri red rice under 70% FC condition experienced the highest reduction in grain weight per clump (58.62%). It was assumed that drought stress during the vegetative to the generative phase reduced the number of fertile spikelets. The water shortage during the generative phase could increase the spikelet's abortion and caused a decrease in spikelet number per panicle (Mayumi *et al.,* 2017). According to Pandey *et al.* (2014), water stress during the flowering stage would promote the remobilization of stored carbon stocks, thereby increasing plant aging and accelerating seed filling. It caused a decrease in seed weight. The decrease in all morphological characters indicated that drought was one of the major factors that affected the growth of rice plants. Different drought tolerance indexes were measured to determine whether genotypes were drought-tolerant or drought-sensitive (Table 4). According to Fadhli *et al.* (2020), the stress tolerance index (STI) was one of the tolerance indexes widely used to identify the tolerant level of varieties under stress conditions. Drought tolerance index for total grain weight of six local rice cultivars of Central Java ranged from 0.4 to 1.44. The results showed that the Karanganyar red rice cultivar (0.4) indicated the lowest index value in the 40% FC drought treatment. Salatiga black rice showed the highest tolerance index (1.44) at 70% FC drought. Meanwhile, this cultivar had a moderate tolerance index (0.69) at 40% FC. The highest tolerance index at 40% FC drought stress was Jelitheng Karanganyar (0.71), which indicated that Jelitheng Karanganyar belonged to a moderate tolerant cultivar.

Based on STI analysis, Salatiga and Sragen black rice cultivars were drought-resistant in the STI_1 group. However, in the STI_2 group, there was no cultivar belonging to the tolerance cultivar. Moderately drought-tolerant cultivars in the group of STI_1 were Wonogiri black rice, Jelitheng Karanganyar and Karanganyar red rice. Karanganyar red rice was classified as a drought-sensitive cultivar on STI_2, and the other cultivars were classified as moderate tolerant rice cultivars. Only Wonogiri red rice under 70% FC condition (STI_1) was classified as a sensitive cultivar to drought. According to Jaleel *et al.* (2009), the reduction in absorption and nutrient metabolism, photosynthetic rate, translocation, respiration and seed filling were all affected by drought stress. It caused a decline in grain yield.

Under drought stress, the proline content increased in Jelitheng Karanganyar cultivar (Fig. 1). Accumulation of proline content under

Table 4. Drought tolerance index for the total grain weight of six local black and red rice cultivars in Central Java at the level of drought stress

Rice cultivars	Mean of STI		Tolerance characters	
	STI_1	STI_2	STI_1	STI_2
Wonogiri black rice	0.64	0.51	Moderate	Moderate
Jelitheng Karanganyar	0.57	0.71	Moderate	Moderate
Salatiga black rice	1.44	0.69	Tolerance	Moderate
Sragen black rice	1.13	0.69	Tolerance	Moderate
Wonogiri red rice	0.46	0.69	Sensitive	Moderate
Karanganyar red rice	0.65	0.4	Moderate	Sensitive

The tolerance index for each cultivar is the average STI value (STI_1=70% FC; STI_2=40% FC) against the control (100% FC condition).

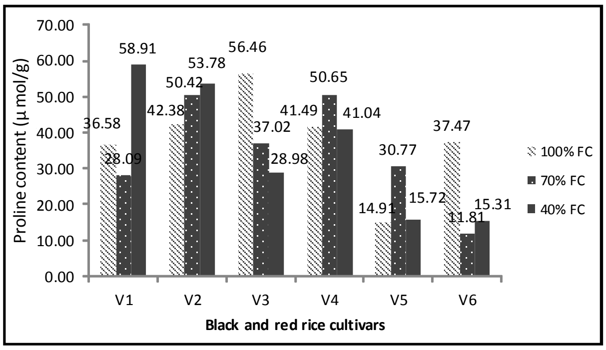

Fig. 1. Proline content of local black and red rice cultivars under drought stress: (V1= Wonogiri black rice, V2=Jelitheng Karanganyar, V3=Salatiga black rice, V4=Sragen black rice, V5=Wonogiri red rice and V6=Karanganyar red rice).

water deficit might keep the leaf cells' osmotic potential low. An increase in the proline content was shown in Wonogiri black rice and Jelitheng Karanganyar at 40% FC. It could mean that the cultivars were more responsive to drought than the other. Research by Dien *et al.* (2019) also showed an increase in drought stress from moderate to severe drought, causing an increase in proline content of all rice plant cultivars. The ability of proline accumulation in plants under drought indicated the drought tolerance of plants. According to Khan and Bano (2019), proline as stress tolerance will maintain cell turgor and stabilize membranes. It might prevent electrolyte leakage and keep the ROS (reactive oxygen species) range normal.

CONCLUSION

Each cultivar of black rice and red rice of Central Java gave different morphological and physiological responses to drought stress. Drought stress significantly reduced plant height, the number of productive tillers, panicles and grain weight per clump. In this study, Jelitheng Karanganyar showed the lowest decline of these characters. Wonogiri black rice showed the highest proline content under drought stress. Salatiga black rice and Sragen black rice were classified as drought-tolerant cultivars based on STI analysis at 70% FC.

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