Response growth and yield of local black rice variety mutiara to drought stress

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Abstract. Plant tolerance under drought stress has mechanisms to adapt to adverse impacts such as synthesizing proline compounds, increasing root length, changing physiological processes, inhibiting growth, and reducing yield of paddy. The purpose of this study was to determine the level of tolerance of local black rice variety mutiara in response to growth and yield to drought stress. The research used was a factorial Randomized Complete Block Design (RCBD) with two factors and 4 block. The first factor was watering interval (every 3 and 6 day). The second factor was soil moisture (100, 80, 60, and 40% Field Capacity/FC). The variables observed were longest roots, total grain weight, grain yield, chlorophyll content, transpiration rate and proline content. The results showed that 6 day watering interval can reduce total grain weight, grain yield, and increase longest roots and proline content compared to the 3-day watering interval. Soil moisture at FC up to 40% can reduce total grain weight, grain yield, chlorophyll content, and transpiration rate from 100% FC.

1. Introduction

There are several regions in Indonesia that produce black rice such as Boyolali, Surakarta, Bantul, Gunungkidul, and Kulon Progo. Black rice produces rice that can be consumed as a functional food. Meanwhile, based on [1] , the area of black rice cultivation in Boyolali Regency has increased drastically by 25,35 ha from 2017-2020, but black rice production has decreased in 2020 by 2,56 quintal.ha^{-1} compared to 2019. One of the causes of decreased production is drought stress.

Drought is one of the most damaging environmental stresses for plants in different soil types. Water deficit can inhibit the absorption of nutrients in plants because water is a transportation medium in transporting nutrients from soil to the plant. [2]. Although the soil contains a lot of plant nutrients, if the availability of water is low, it can result in a decrease in nutrient transportation to the plant [3]. Water availability supports plant uptake of nutrients through the mechanism of mass flow by roots [4]. This is because water can dissolve ions from the soil so that can be absorbed by plant roots [5].

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Plants that experience drought stress will affect plant growth and development and inhibit plant production. Drought stress causes a decrease in water content which reduces stomatal activity and cell growth [6]. In addition, drought also reduces leaf area, cell size, intercellular volume, meristematic activity, thus inhibiting root elongation. Photosynthetic rate under drought stress decreases because it can damage pigments, photosystems, gas exchange, and key photosynthetic enzymes, affecting the photosynthetic pathway [7]. This can result in a decrease in plant biomass and yield.

Plants under drought stress mitigate the adverse effects by signaling osmoprotectants, osmoprotectant utilizes various group of low molecular weight molecular compound [8]. Metabolites that regulate plant osmoprotectants under drought stress are proline [9]. Proline is one of the most common compatible osmolytes in plants against drought stress. Proline functions in regulating osmotic pressure, water transport, inhibiting stomatal opening, and regulating transpiration rate [10]. Based on [11], Rice under drought stress showed a 16,19% increase in proline levels, which was higher than that of the control. Proline accumulation that occurs is a mechanism in plants to be able to maintain the growth and development of drought stress.

Local black rice varieties are varieties that have been cultivated for generations by local farmers and are sensitive to drought. Local rice varieties are able to adapt to climate change compared to introduced varieties [12]. However, black rice under drought stress experiences reduced growth and yield. According to research [13], the plant height of black rice under drought stress at 50% field capacity was 315,31 cm, compared to 384,07 cm in the control. This indicates that drought stress negatively affects rice growth, which in turn impacts yield.

Drought tolerance in plants is dynamic and is influenced by factors such as the stage of plant growth. Drought stress occurring at any growth phase can reduce yield and lead to crop failure. In this study, drought stress was induced by manipulating soil moisture based on field capacity and adjusting the watering time intervals. Drought stress up to 40% FC can reduce chlorophyll content, stomatal density, and percentage of full grain per panicle in black rice variety Jeliteng [14]. Based on research [15], a 6-day watering interval can reduce plant height, number of leaves, chlorophyll content, number of tillers, and number of seeds in upland rice. The purpose of this study was to determine the level of tolerance of local black rice pearl varieties in response to growth and yield to drought stress.

2. Material and methods

The black rice of Mutiara variety were the main plant material used in this study. The cultivation of black rice was conducted at the screen house of the Faculty of Agriculture, UNS, located in Jumantono District, Karanganyar Regency. Moreover, soil analysis was carried out at the Soil Physics and Conservation Laboratory, Faculty of Agriculture, UNS, while proline content analysis was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Brawijaya University, Malang. The research was conducted for 5 months in November 2023-March 2024.

The black rice was planted in polybags (35 x 40 cm), filled with a mixture of Andosol soil and cow manure. NPK fertilizer (16-16-16) and urea fertilizer were also applied to maintain nutrient levels. The research employed a factorial Randomized Complete Block Design (RCBD) with two factors and four blocks.The first factor was watering interval (3 and 6 days). The second factor is soil moisture (100%, 80%, 60%, 40% FC) Based on these factors, 8 combinations were obtained, each treatment included 4 groups, so there were 32 experimental units. Drought stress treatment was carried out when the plants were 3 weeks old using the gravimetric method by maintaining soil weight based on soil moisture [16], namely 100% FC of 10,1 kg, 80% FC of 9,5 kg, 60% FC of 8,9 kg, 40% FC of 8,3 kg which was given water every 3 days or 6 days.

The variables observed were 1) the longest root (cm) measured at harvest by measuring the base of the root to the tip of the longest root using a tape meter, 2) total grain weight (g) measured at harvest by weighing the total grain using digital scales, 3) grain yield (ton.ha^{-1}) refers to the number of clumps and total grain weight and also correction factor (0,8), 4) chlorophyll content (mg.g^{-1}) refers to the calculation method of Arnon (1949), 5) transpiration rate (µmol.m^{-2}.s^{-1}) measured period the maximum vegetative phase of the leaf using a plant photosynthetic meter NY-1020, 6) proline content (µmol proline.g^{-1}) measured using modified method [17]. Observational data obtained were analyzed using analysis of variance (ANOVA) with a 5% error rate, if there was a significant difference, it would be followed by Duncan's Multiple Range Test (DMRT) with a 5% error rate. The program used in data analysis is IBM SPSS version 25

3. Result and discussion

3.1 Longest root

Roots are one of the important indicators to obtain drought stress tolerant plants. Table 1 shows an increase in root length in the 6-day watering interval treatment, with an improvement of up to 10.41% compared to the 3-day watering interval condition. This is in line with research [18] that there was an increase in the longest roots with increasing watering intervals in corn up to 13,54% of the control. Soil moisture on plant height experienced a relatively similar increase in the longest root. At 40% FC there was an increase of 8,63% from 100% FC. In line with research [19] that there was an increase in the longest roots up to 10.11% of 80% FC in the Gaung lake rice variety.

The increase longest root in black rice is an adaptation mechanism to drought stress [20]. Plants that lack water have the ability to take water by lengthening and increasing the depth of the root system as a form of tolerance to drought stress [21]. Drought stress can change root cells, among others, by increasing or reducing the number or size [22]. This mechanism is a form of plant adaptation to drought stress to obtain nutrients and water absorption optimally [23].

Tabel 1. Effect of watering interval and soil moisture on longest root

Watering Inteval (once a day)	Soil Moisture (FC)				Average
	100%	80%	60%	40%	
3	44,45	44,69	45,38	48,68	45,80b
6	49,35	49,43	51,73	53,98	51,12a
Average	46,90	47,06	48,55	51,33	(-)

Note: Numbers followed by the same notation in the same column group show no significant difference in DMRT at the 5% level. (-) indicates there is no interaction

3.2 Total grain weight

One of the most important yield components in black rice is total grain weight. Total grain weight is related to the weight of empty grain and the weight of full grain [24]. Table 2 shows that the 6-day watering interval reduced the weight by up to 44.60%, compared to the 3-day watering interval, which amounted to 49.11 g. According to [25], an increase in the watering/irrigation interval of 6 days once can reduce 40-60% grain weight from the interval of 3 days once. Soil moisture at 100% FC produced the highest treatment of 50,92 g and there was a decrease that was not significantly different at FC to 60%. However, the greatest decrease occurred in the 40% FC treatment, with a reduction of up to 54.32% compared to the 100% FC treatment. This finding is consistent with research by [26], which indicates that 20%-40% FC can reduce grain weight by 50-80%. The decrease was attributed to insufficient water availability, which reduced the percentage of filled grains and increased the percentage of hollow grains [27].

Table 2. Effect of watering interval and soil moisture on total grain weight (g)

Watering Interval (once a day)	Soil Moisture (FC)				Average
	100%	80%	60%	40%	
3	63,91	51,31	44,97	36,25	49,11a
6	37,92	30,57	30,07	10,27	27,21b
Average	50,92a	40,94a	37,52a	23,26b	(-)

Note: Numbers followed by the same notation in the same column group show no significant difference in DMRT at the 5% level. (-) indicates there is no interaction

3.3 Grain yield

Grain yield is a value that shows the average yield of plants per unit hectare. Grain yield is influenced by various related factors, one of which is water availability [28]. Table 3 shows that the 6-day watering interval can reduce up to 44,67% compared to 3-day watering interval which amounted to 6,29 tons.ha^{-1}. Soil moisture at 100% FC produced the highest treatment of 6,52 tons.ha^{-1} and there was a decrease that was not significantly different at FC up to 60%. However, the highest decrease occurred in the 40% FC treatment up to 54.30% of 100% FC. The decrease in grain yield against drought stress is due to decreased primary metabolism, shrinkage of leaf area, and photosynthetic activity [29].

Table 3. Effect of watering interval and soil moisture on grain yield (ton.ha^{-1})

Watering Interval (once a day)	Soil Moisture (FC)				Average
	100%	80%	60%	40%	
3	8,18	6,57	5,76	4,64	6,29a
6	4,85	3,91	3,85	1,31	3,48b
Average	6,52a	5,24a	4,80a	2,98b	(-)

Note: Numbers followed by the same notation in the same column group show no significant difference in DMRT at the 5% level. (-) indicates there is no interaction.

3.4 Chlorophyll content

Chlorophyll is a green pigment found in plants. Chlorophyll functions as a photosynthetic pigment that absorbs light [30]. Table 4 shows that 100% FC gives the highest average value of chlorophyll a of 0,89 mg.g^{-1} and a decrease that is not significantly different from 80% FC. However, the largest decrease in chlorophyll a occurred at 40% FC, with a reduction of up to 39.32% compared to 100% FC, and this was not significantly different from the 60% FC treatment. In chlorophyll b, 100% FC gave the highest average value of 0,74 mg.g^{-1} and there was a decrease that was not significantly different from 60% FC. There was largest decrease in chlorophyll b at 40% FC up to 66,22% of 100% FC. This is in line with research [31] that 40% FC can reduce chlorophyll a and chlorophyll b. Total chlorophyll showed the highest average value at 100% FC of 1,24 mg.g^{-1} but there was a decrease that was not significantly different from 80% FC. There was a significant decrease at 40% FC up to 51.53% and not significantly different from 60% FC from the condition of 100% FC. The decrease in chlorophyll content to drought stress as a plant response in adapting under drought stress conditions. Lack of water content in the leaves will affect chlorophyll synthesis, accelerate chlorophyll decomposition, reduce light absorption capacity, and inhibit photosynthesis [32]. The decrease in chlorophyll is also due to the inhibition of carbon and nitrogen metabolic activities resulting in nitrogen and carbon deficiency under drought stress conditions [33].

Table 4. Effect of watering interval and soil moisture on chlorophyll content ($mg.g^{-1}$)

Treatment	Chlorophyll content ($mg.g^{-1}$)		
	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Watering Interval (once a day)			
3	0,77	0,49	1,25
6	0,66	0,53	1,22
Soil Moisture (FC)			
100%	0,89a	0,74a	1,63a
80%	0,76ab	0,59ab	1,39ab
60%	0,66bc	0,46ab	1,13bc
40%	0,54c	0,25b	0,79c
Interaction	-	-	-

Notes: Numbers followed by the same notation in the same column group show no significant difference in DMRT at the 5% level. (-) indicates there is no interaction

3.5 Transpiration rate

Transpiration is the process of losing water in the form of vapor from plant tissues. Transpiration is one of the mechanisms regulating plant physiology related to conditions in adaptation to the environment [34]. Table 5 shows that soil moisture with 100% FC gives the highest average value of 0,54 $\mu mol.m^{-2}.s^{-1}$ and a decrease that is not significantly different up to 60% FC. There was the largest decrease in transpiration rate at 40% FC up to 75,93% of 100% FC. Based on research [35] that at 40% FC there was a decrease in transpiration rate up to 61,9% compared to the control in cotton plants. The lower the transpiration, the lower the water absorbed by the plant, but this has a negative impact on plant growth and yield [36].

Decreased transpiration rate in black rice is a physiological response of plants in adapting to drought stress. The decrease in transpiration rate is characterized by the closure of stomata to reduce conductance thereby reducing water loss through transpiration [37]. The opened and closed of stomata is determined by the turgor pressure of the guard cells which is influenced by the amount of water contained [38]. The decrease in transpiration rate can also be caused by abscisic acid because it can regulate stomatal closure by blocking K^+ ions that flow to guard cells in the opening of stomata so that stomatal closure occurs and reduces the transpiration rate [39].

Table 5. Effect of watering interval and soil moisture on transpiration rate ($\mu mol.m^{-2}.s^{-1}$)

Watering Interval (once a day)	Soil Moisture (FC)				Average
	100%	80%	60%	40%	
3	0,61	0,45	0,33	0,17	0,39
6	0,47	0,40	0,19	0,09	0,29
Average	0,54a	0,43a	0,26ab	0,13b	(-)

Notes: Numbers followed by the same notation in the same column group show no significant difference in DMRT at the 5% level. (-) indicates there is no interaction.

3.6 Proline content

Proline is a compound produced by plants against drought stress. Proline as a compatible compound that detoxifies Reactive Oxygen Species (ROS) and as carbon and nitrogen storage in plants experiencing drought stress [40]. Table 6 shows that there was a significant increase in proline levels at the 6-day watering interval up to 58% of the 3-day watering interval by 0,21 µmol proline.g^{-1}. In soil moisture, there was a relatively similar increase in proline levels. The increase in proline levels at 40% FC up to 57,14% of the 100% FC condition amounted to 0.24 µmol proline.g^{-1}. This is in line with research [41] that there was an increase in proline levels at 40% field capacity by 38,89% of 100% field capacity in *Phaseolus vulgaris* plants.

Increase proline in black rice is a plant adaptation mechanism to drought stress. Under drought or osmotic stress conditions, plants usually produce and accumulate cellular osmolytes as a stress tolerance mechanism to stabilize subcellular structures and balance homeostatis such as proline [42]. Proline is also a stress tolerance mechanism in maintaining cell turgor and stabilizing the membrane [43].

Table 6. Effect of watering interval and soil moisture on proline content (µmol proline.g^{-1})

Watering Interval (once a day)	Soil Moisture (FC)				Average
	100%	80%	60%	40%	
3	0,18	0,10	0,25	0,32	0,21b
6	0,30	0,36	0,53	0,81	0,50a
Average	0,24	0,23	0,39	0,56	(-)

Note: Numbers followed by the same notation in the same column group show no significant difference in DMRT at the 5% level. (-) indicates there is no interaction

4. Conclusion

In conclusion, the results of this study indicate that a 6-day watering interval reduced total grain weight (44.60%), grain yield (44.67%), while increasing the longest roots (10.41%) and proline content (58%) compared to a 3-day watering interval. Additionally, soil moisture at 40% field capacity (FC) resulted in significant reductions in total grain weight (54.32%), grain yield (54.30%), chlorophyll a (39.32%), chlorophyll b (66.22%), total chlorophyll (51.53%), and transpiration rate (75.93%) compared to the 100% FC condition.

Suggestions that can be given from the results of research that has been carried out should be in the cultivation of black rice variety mutiara in drought conditions can use watering intervals every 3 days with FC up to 60% so that plants can adapt under drought stress. It is also recommended to conduct research on the use of local black rice in soil moisture with different watering intervals to obtain drought-tolerant local black rice varieties.

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