Evaluation of growth and physiological characters of local rice under drought conditions

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Abstract. Global warming has increased the frequency of drought events, posing a significant threat to rice production and potentially reducing yields by up to 60%. Drought stress disrupts key physiological processes in rice, particularly carbon fixation, due to stomatal closure. Local rice varieties represent valuable germplasm resources for developing drought-resistant cultivars. This study aimed to evaluate the growth and physiological responses of several local Central Java rice varieties under varying levels of drought stress. This research used a split-plot design with four levels of field capacity (FC) (100%, 75%, 50%, and 25%) as the main plots and seven rice varieties (Ciherang, IR64, Hitam Cempo, Hitam Mutiara, Merah Sengreng, Putih Mentikwangi Karanganyar, and Putih Mutiara) as the sub-plots. Drought stress was applied during the vegetative phase. The observed parameters included agro-morphological traits (plant height, number of tillers, and number of leaves) and physiological traits (relative water content/RWC, photosynthetic rate, stomatal conductance, water use efficiency/WUE, and chlorophyll content). The results indicated that drought stress generally reduced plant height, tiller number, and leaf number in most varieties. Hitam Mutiara exhibited the largest decline in tiller and leaf number, whereas IR64 experienced the smallest reduction. However, the physiological responses varied greatly. Under severe stress conditions (25% FC), local varieties such as Putih Mutiara, Putih Mentik Wangi Karanganyar, and Hitam Cempo demonstrated superior adaptation mechanisms, characterized by increased RWC, photosynthetic rates, and WUE. Conversely, the IR64 variety showed high sensitivity to drought, exhibiting the lowest WUE under stress. Several varieties also increased their total chlorophyll content as a defense mechanism. In conclusion, the local rice varieties Putih Mutiara, Putih Mentik Wangi Karanganyar, and Hitam Cempo demonstrated stronger physiological adaptation mechanisms to drought stress compared to the control varieties. These varieties hold strong potential as genetic resources for the development of drought-tolerant rice cultivars.

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1 Introduction

Global warming has indirectly contributed to drought across many regions and sectors. In agriculture, low average rainfall or high evaporation rates can severely impact crop production. Asia, as the world's largest producer and consumer of rice, is the region most affected. Economic losses caused by water stress depend on the severity of the drought, which is influenced by factors such as low rainfall, high evaporation, and reduced soil moisture [1].

As a semi-aquatic plant, the growth and production of rice are highly sensitive to drought. Its physiological processes become disrupted, particularly during carbon fixation, which is regulated by stomatal closure. Stomatal closure during drought is caused by changes in water content, chlorophyll content, and cell membranes, which affect the rate of photosynthesis. Leaves subjected to water stress often display a rolling appearance, which indicates the plant's effort to reduce transpiration and adapt to excessive water loss [2].

The characteristics of genotipe padi vary depending on whether the genotype is tolerant or sensitive to drought. Although crosses involving drought-tolerant genotypes have been extensively conducted, progress in developing new tolerant varieties has remained slow. This is largely due to the limited availability of donor genotypes with strong tolerance traits, despite the existence of thousands of germplasms worldwide. This is due to the unavailability of truly tolerant genotypes and the lack of suitable screening methods. The International Rice Research Institute (IRRI) has identified 65 accessions as genuinely drought-tolerant genotypes, most of which originate from Bangladesh, India, and Sri Lanka [3].

However, several local rice varieties serve as a source of germplasm to find these traits, thus requiring detailed investigation to develop rice cultivars that are resistant to dry conditions. Physiologically, some drought-tolerant rice varieties exhibit a high recovery index after experiencing water stress. This resilience is reflected in their growth and physiological traits, including efficient water absorption supported by a well-developed root system and the ability to accumulate chlorophyll.

Previous research has shown that certain rice varieties, such as Boawae Seratus Malam (BSM), exhibit growth characteristics that are minimally affected by drought. This cultivar maintains higher shoot and root dry weight, as well as a greater number of leaves, compared to other cultivars under water stress. The BSM cultivar is also able to sustain plant hydration status (RWC) and chlorophyll b levels, displays the lowest degree of cellular damage—as indicated by reduced malondialdehyde (MDA) and electrolyte leakage (EL)—and possesses the highest proline content. Therefore, this study aims to evaluate the growth and physiological characteristics of local Central Java rice under drought conditions at various levels of field capacity.

2 Method

2.1 Place and Time of Research

The research was conducted from September to November. Planting was carried out in polybags inside a screen house located in Jati, Karanganyar, with an average temperature of 30.10°C, air humidity of 77.50%, and light intensity of 9,072.50 lux during the transition to the rainy season. Initial soil water content was measured at the Laboratory of Soil Physics and Conservation, Faculty of Agriculture, Sebelas Maret University. Stomatal density analysis was performed at the Laboratory of Agrotechnology, Faculty of Agriculture, Muhammadiyah University of Yogyakarta. Chlorophyll analysis was conducted at the Laboratory of Plant Physiology, Faculty of Biology, Gadjah Mada University.

2.2 Experimental Design and Stress Treatment

This study employed a Split-plot design within a Randomized Complete Block Design (RCBD), consisting of two factors and three blocks. The main plot factor was the field capacity (FC) treatment at four levels: 100%, 75%, 50%, and 25%. The sub-plot factor was the rice varieties, which included control varieties (Ciherang and IR64) and local varieties (Hitam Cempo, Hitam Mutiara, Merah Sengreng, Putih Mentikwangi Karanganyar, and Putih Mutiara). Each treatment consisted of two polybags: one designated for growth analysis and the other for destructive physiological measurements. Drought stress treatments were initiated during the vegetative phase, approximately 6 Weeks After Planting (WAP), and maintained using the gravimetric method.

2.3 Planting in the Screenhouse

High-quality seeds were sown in trays, with each tray containing only one variety. At 20 days after sowing (DAS), the seedlings were transplanted into 30 × 30 cm polybags filled with a growing medium. The polybags contained a 3:1 mixture of soil and manure. Before transplanting, the growing medium was watered until fully saturated.

The rice plants were watered twice daily, in the morning and evening. The first fertilization was applied during the vegetative stage at 21 days after planting (DAP) using manure. Additional fertilization was carried out at 35 and 42 DAP. The plants were monitored regularly, especially during the generative phase, to protect them from pests and weeds. Organic pesticide applications were performed every two weeks and as needed.

2.4 Application of Drought Treatment

Three polybags, each containing 5 kg of soil, were watered until water drained from the bottom, after which they were weighed to obtain the wet weight. The soil was then left for several days until no further drainage occurred. Next, the soil was oven-dried for 24 hours at 105°C, cooled in a desiccator, and weighed to determine its dry weight. Field capacity was calculated using the following formula:

$$\textit{Field Capacity (FC)} = \textit{wet weight} - \textit{dry weight} / \textit{wet weight} \times 100\% \qquad (1)$$

The treatment was applied by adjusting the soil water content to the desired percentage of field capacity. Soil moisture content was determined using the gravimetric method to calculate the amount of water present in the soil. The treatment was applied using the following formula:

$$\textit{Amount of water added} = [(FC \times n) - \textit{soil moisture content})] \times \textit{soil weight per polybag} \qquad (2)$$

Where: n = the level of FC (100%, 75%, 50%, and 25%).

2.5 Observation of Parameters

2.5.1 Agro-morphological Observation

Observations of agro-morphological variables were conducted from 1 to 7 Weeks After Planting (WAP), focusing on three main parameters: plant height (measured from the stem base to the tip of the highest leaf), number of tillers, and number of leaves.

2.5.2 Physiological Observation

Observations were conducted one week after the stress treatment was applied (7 WAP).

2.5.2.1 Plant Water Status (RWC)

Relative Water Content (RWC) was calculated using the formula:

$$RWC\ (\%) = [(FM - DM) / (TM - DM)]\ X\ 100 \qquad (3)$$

where FM is fresh mass, TM is turgid mass, and DM is dry mass.

2.5.2.2 Photosynthesis Rate, Transpiration Rate, Stomatal Conductance, and WUE

The photosynthetic rate, transpiration rate, and stomatal conductance were measured using a Plant Photosynthesis Meter on the third to fifth leaves from the top. Water Use Efficiency (WUE) was calculated as the ratio of net photosynthetic rate to transpiration rate.

2.5.2.3 Stomatal Density

Leaf sections were examined under a microscope to determine stomatal density (number of stomata per unit area) and stomatal index (the ratio of stomata to epidermal cells). The formulas used to calculate stomatal density and stomatal index are as follows:

$$\textit{Stomatal density} = \textit{number of stomata/unit area of the field of view} \qquad (7)$$

2.5.2.4 Chlorophyll Content

Leaf extracts were analyzed using a spectrophotometer at wavelengths of 645 nm and 663 nm to determine the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll. The formulas used to calculate chlorophyll content are as follows:

$$\textit{Total chlorophyll } (a+b) = (8.02\ x\ E663 + 20.20\ x\ E645) / FW \qquad (4)$$

$$\textit{Chlorophyll } a = (12.7\ x\ E663 - 2.69\ x\ E645)/FW \qquad (5)$$

$$\textit{Chlorophyll } b = (22.9\ x\ E645 - 4.68\ x\ E663)/FW \qquad (6)$$

where a is chlorophyll a, b is chlorophyll b, FW is fresh weight, E_{645} is the absorbance value at a wavelength of 645 nm, and E_{663} is the absorbance value at a wavelength of 663 nm.

2.6 Statistical Analysis

The data were analyzed using ANOVA, and the graphs were generated using R. When significant differences were detected ($p < 0.05$), the analysis was followed by Duncan's test at the 5% significance level. A linearity test was also conducted to evaluate the relationship between water content treatments and plant responses.

3 Results and Discussion

3.1 Agro-Morphological Growth Response

3.1.1 Plant Height

Plant height is an important agronomic trait that characterizes a genotype and is commonly used to assess environmental influences on plant performance. The plant height results for the tested varieties are presented in Table 1.

Table 1. Plant height, umber of tillers and leaves at 5 and 7 WAP for various varieties.

Variety	Plant height (cm)		Reduction proportion (%)	Number of tillers		Reduction proportion (%)	Number of leaves		Reduction proportion (%)
	5 WAP	7 WAP		5 WAP	7 WAP		5 WAP	7 WAP	
1	53.08^{bc}	81.96	21.21	14.75^{c}	23.33^{bc}	16.46	54.25^{c}	87.33^{c}	29.43
2	41.87^{a}	71.01	24.23	7.42^{ab}	17.42^{abc}	44.59	27.58^{ab}	65.50^{abc}	33.19
3	58.86^{bc}	88.92	33.15	5.50^{a}	10.83^{a}	34.15	20.75^{a}	39.92^{a}	37.50
4	52.78^{bc}	84.41	8.34	4.83^{a}	12.75^{a}	66.07	18.58^{a}	44.83^{a}	52.30
5	52.68^{bc}	77.76	20.66	6.75^{ab}	16.00^{ab}	50.00	24.33^{ab}	53.83^{ab}	50.66
6	47.03^{ab}	82.33	23.89	8.75^{ab}	17.67^{abc}	41.67	30.17^{ab}	67.08^{abc}	42.18
7	47.50^{ab}	70.63	13.56	10.58^{bc}	25.33^{c}	23.81	38.67^{b}	84.00^{bc}	30.98

Note: 1: IR64, 2: Ciherang, 3: Hitam Cempo, 4: Hitam Mutiara, 5: Merah Sengreng, 6: Putih Mutiara, and 7: Putih Mentikwangi Karanganyar. Values in the columns for 5 and 7 WAP followed by the same letter are not significantly different according to Duncan's test at $\alpha = 0.05$.

Drought stress led to reduced plant height in most varieties. The linearity test showed that the plant height of IR64, Ciherang, Hitam Cempo, and Putih Mutiara decreased linearly as water availability declined. The extent of height reduction varied depending on drought intensity and genotype. A significant decrease was observed at the 50% FC level for Ciherang, Hitam Cempo, and Putih Mutiara, whereas IR64 experienced its greatest reduction at 25% FC. Hitam Cempo showed the largest proportional reduction (33.15%), while Hitam Mutiara exhibited the smallest (8.34%). Varieties with shorter stature, such as Putih Mentik Wangi, tend to be less sensitive to water stress.

Tillers are produced during the vegetative phase and serve as a key determinant of rice yield. The results for tiller and leaf numbers across the varieties are presented in Table 2. Drought-induced growth reductions also led to decreases in both tiller and leaf production. All varieties exhibited a significant decline in tiller number, ranging from 16.46% to 66.07%, while leaf number decreased by 29.43% to 52.30%. Hitam Mutiara showed the greatest reduction in both parameters, whereas IR64 experienced the smallest decline. The linearity test did not reveal a consistent relationship between tiller or leaf number and water content treatment, indicating that additional factors—such as light availability and nitrogen supply—also play a role in influencing these traits [4].

3.2 Physiological Response

3.2.1 Plant Water Status (RWC) and Photosynthesis

Relative Water Content (RWC) is a key indicator of a plant's hydration status. Under severe stress (25% Field Capacity), the Ciherang, Hitam Cempo, and Putih Mutiara varieties were able to maintain or even increase their RWC, as shown in Figure 1.

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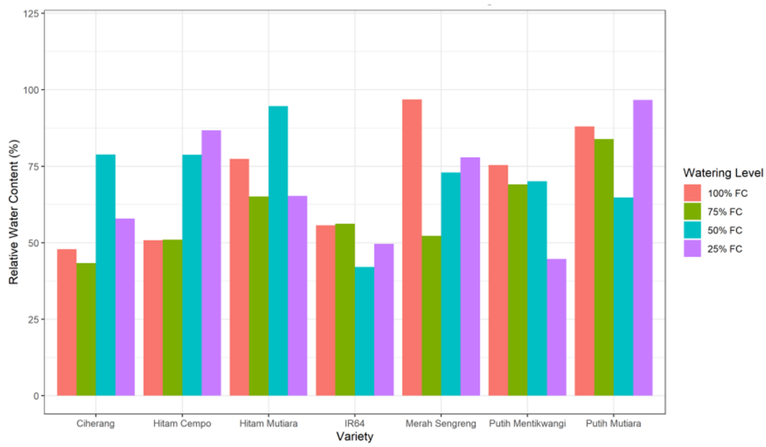

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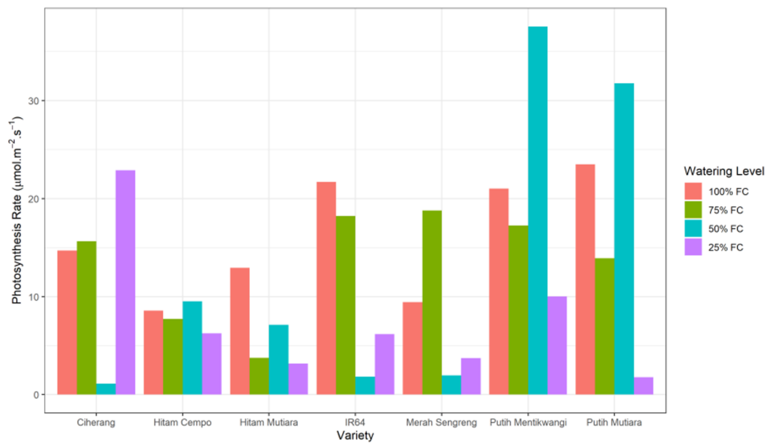

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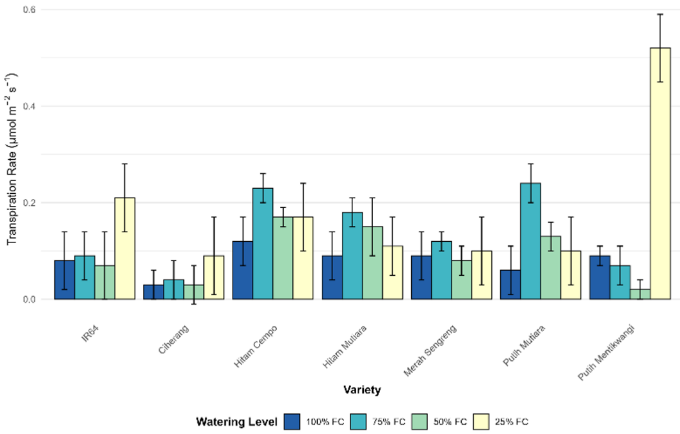
Fig. 1. Physiological response of various varieties to soil water content levels, a) relative water content, b) photosynthesis rate.

Putih Mutiara and Hitam Cempo recorded RWC values above 80%, which supports better overall physiological activity. In Putih Mutiara, the increase in RWC was positively correlated with a substantial rise in photosynthetic rate—from 23.51 $\mu mol\cdot m^{-2}\cdot s^{-1}$ at 100% FC to 31.78 $\mu mol\cdot m^{-2}\cdot s^{-1}$ at 25% FC—indicating an efficient adaptation mechanism. In contrast, varieties such as IR64 showed a decline in photosynthetic rate under the same stress conditions.

3.2.2 Stomatal Response and Water Use Efficiency (WUE)

All varieties exhibited an increase in both transpiration rate (Figure 2a) and stomatal conductance (Figure 2b) at the 25% FC stress level, as depicted in Figure 2.



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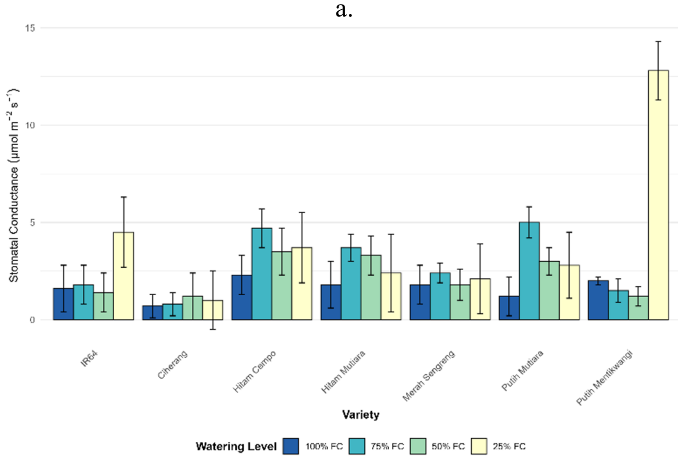

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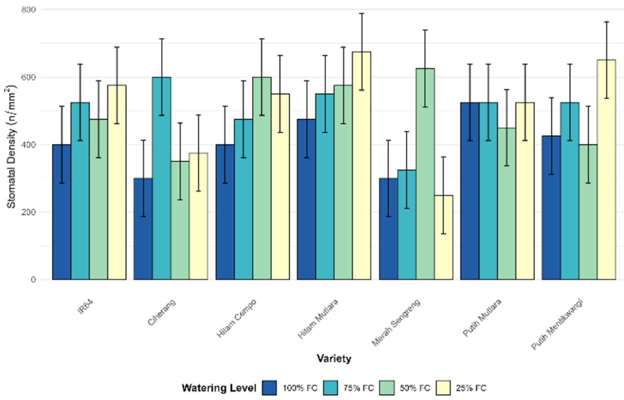
Fig. 2. Physiological response of various varieties to soil water content levels, a) transpiration rate, and b) stomatal conductance.

This was likely influenced by environmental conditions during measurement, particularly increased leaf temperature, which triggers stomatal opening to facilitate cooling [5]. The increase in stomatal conductance was also supported by an increase in stomatal density in most varieties, with the exception of Merah Sengreng (Figure 2c). An increase in stomatal density can be caused by low atmospheric CO_2 concentrations, which in turn boosts stomatal conductance.

Water Use Efficiency (WUE), which reflects the ratio of carbon assimilation to water loss, varied across the tested varieties (Figure 2d). Putih Mutiara, Putih Mentik Wangi Karanganyar, and Hitam Cempo showed increased WUE at 25% FC, indicating their ability to maintain efficient photosynthesis with minimal water loss under drought stress. In contrast, IR64 exhibited the lowest WUE, reinforcing its sensitivity to drought, as illustrated in Figure 3.



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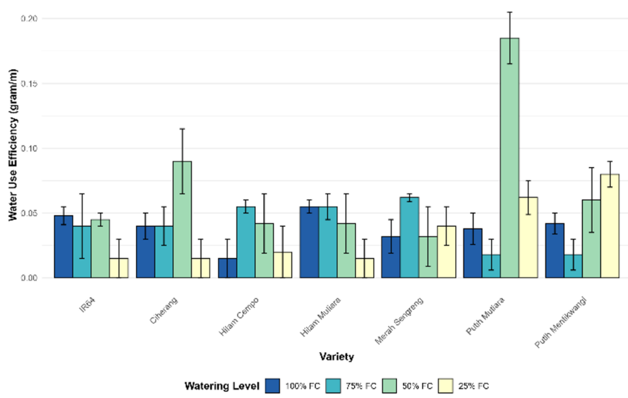
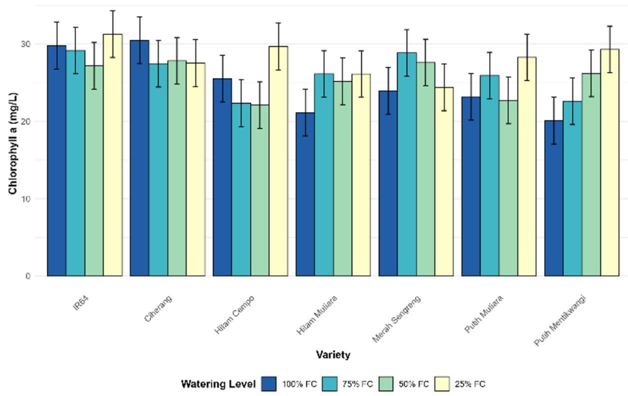


b.

Fig. 3. Physiological response of various varieties to soil water content levels, a) stomatal density, and b) water use efficiency.

3.2.3 Chlorophyll Content

Chlorophyll content can serve as an indicator of the impact of drought stress. At the 25% FC (Field Capacity) stress level, the IR64, Hitam Mutiara, Merah Sengreng, and Putih Mentik Wangi Karanganyar varieties showed an increase in total chlorophyll, as presented in Figure 4. This increase indicates a potential defense mechanism against drought. In contrast, Ciherang, Hitam Cempo, and Putih Mutiara exhibited a reduction in chlorophyll content, which aligns with many reports describing the negative effects of drought on photosynthetic pigments [6].



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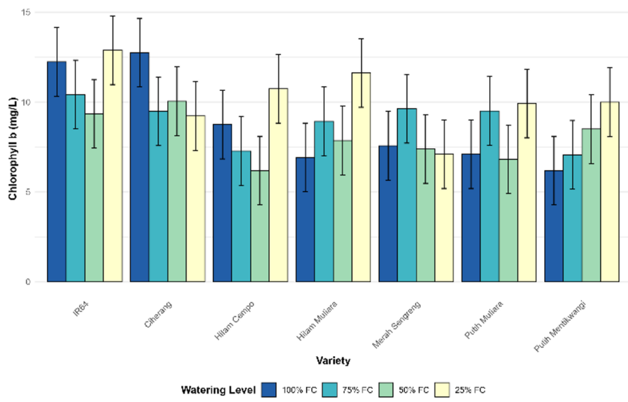

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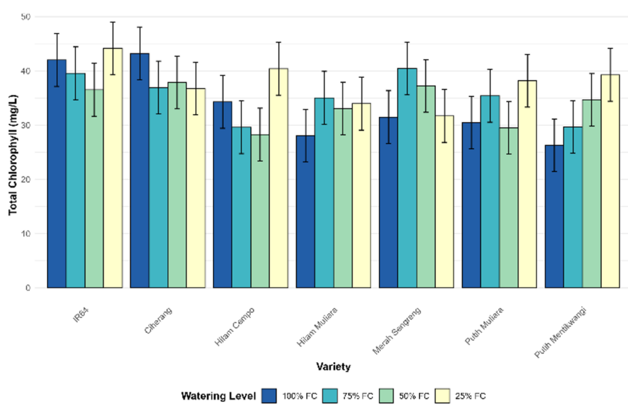

c.

Fig. 4. Physiological response of various varieties to soil water content levels, a) chlorophyll a, b) chlorophyll b, and c) total chlorophyll.

3.3 Discussion

Drought stress affects metabolic activity by reducing turgor pressure, leading to a decrease in plant height. Consistent with this, the linearity test in this study showed that drought stress reduced the plant height of IR64 as water availability decreased to 25% FC. Ciherang, Putih Mutiara, and Hitam Cempo exhibited reduced height at the 50% FC level. In contrast, Merah Sengreng, Hitam Mutiara, and Putih Mentik Wangi Karanganyar showed no consistent relationship between water application level and plant height (Table 1).

The extent of plant height reduction varies depending on both drought intensity and genotype. In this study, water application at 50% FC caused the greatest decrease in plant height. Hitam Cempo experienced the largest reduction while also being the tallest variety, whereas Putih Mentik Wangi showed a smaller reduction and was the shortest plant. Shorter plants generally exhibit lower height reduction because they tend to be less sensitive to water stress [7].

Reduced growth also results in a decline in tiller production. In this study, all varieties experienced a substantial reduction in tiller number, ranging from 16.46% to 66.07% (Table 2). Other factors that can influence tiller growth include planting distance and plant density, the availability of light and nitrogen, and the intensity of photosynthetically active radiation (PAR). A lower ratio of red to far-red light (R:FR) can also reduce the number of tillers [4].

Reduced growth and impaired photosynthesis directly affect leaf development, particularly during the vegetative stage. Under drought stress, plants tend to prioritize survival and adaptive responses rather than continued growth, leading to a decreased number of leaves [8]. In this study, all varieties showed a reduction in leaf number ranging from 29.43% to 52.30% (Table 2).

Impaired leaf growth can also result from reduced water status within the plant tissues. According to Reigosa Roger [9], plants with an RWC of less than 80% exhibit a water potential of approximately -1.5 MPa, which can inhibit various physiological activities, such as the rates of photosynthesis, respiration, and the accumulation of osmotic compounds. In this study, Hitam Cempo and Putih Mutiara maintained RWC values above 80% under the 25% FC treatment. The high RWC observed in Putih Mutiara suggests a higher photosynthetic rate compared to the other varieties. This is supported by the statement from Lawlor and Tezara [10], that water availability affects the rate of photosynthesis, which in turn influences other physiological processes.

A low RWC will affect the transpiration rate, which typically shows a decrease. HoHowever, this study revealed a different pattern, in which all varieties showed an increase in transpiration rate. This was influenced by external factors, as sampling took place during partly cloudy conditions—providing enough sunlight to stimulate transpiration without creating excessively high temperatures. The transpiration rate that affects RWC is also influenced by the silicon content in the soil. The silica layer will act as a protective layer on the leaf epidermis cells to reduce evaporation [11].

The efficiency of photosynthesis and transpiration is reflected in WUE, which indicates a plant's ability to maximize carbon assimilation while minimizing water loss. Plants that are drought-tolerant or adapted to water-limited environments generally exhibit higher WUE. Consequently, WUE varies across plant species. In this study, the varieties with the highest WUE included Putih Mentik Wangi Karanganyar, Putih Mutiara, and Merah Sengreng. In contrast, the variety with the lowest WUE was IR64, which showed high sensitivity, indicating that a genotype that performs well in one environment (non-stressed lowland rice) may be highly susceptible to abiotic stress [12].

A low RWC will also affect stomatal activity, such as the ability of the cells to conduct water vapor into the atmosphere, known as stomatal conductance. Research by Anggaraini et al. [13] states that water application at 30-40% FC causes stomatal conductance to decrease compared to water application at 70-80% FC. However, in this study, water application at 25% FC resulted in higher stomatal conductance compared to the 100% FC treatment. This increase was driven by elevated leaf temperatures, which stimulate wider stomatal opening to facilitate transpiration, thereby directly increasing stomatal conductance [14].

One factor that influences stomatal conductance is stomatal density. The higher the stomatal density, the greater the capacity of the stomata to conduct water vapor. In this study, all varieties except Merah Sengreng exhibited an increase in stomatal density along with higher stomatal conductance. This increase in stomatal density is attributed to low atmospheric CO_2 concentrations, which stimulate the formation of more stomata and wider stomatal openings to meet CO_2 demand, thereby enhancing stomatal conductance [14].

The stomatal index is a more reliable indicator for understanding the relationship between climate change and fluctuations in atmospheric CO_2. However, the effect of water availability on the stomatal index appears to be insignificant. In this study, water application at 100% FC increased the stomatal index in Ciherang, Hitam Cempo, and Merah Sengreng compared to the 25% FC treatment. In contrast, the IR64, Hitam Mutiara, Putih Mutiara, and Putih Mentik Wangi Karanganyar varieties exhibited a decrease in stomatal index. Overall, the stomatal index values tended to remain relatively stable as water availability decreased.

The disruption of various physiological processes affects chlorophyll content, causing it to decrease. Chlorophyll can be used to evaluate the impact of water deficit on plant growth and production [15]. This is consistent with the findings of the present study, in which Ciherang, Hitam Cempo, and Putih Mutiara exhibited reductions in chlorophyll a, chlorophyll b, and total chlorophyll under severe stress (water availability at 25% FC). In contrast, the IR64, Hitam Mutiara, Merah Sengreng, and Putih Mentik Wangi Karanganyar varieties showed increased chlorophyll levels. An increase in chlorophyll content under drought conditions suggests a stronger potential for drought resistance [6].

This phenotypic evidence supports the genetic analysis, which shows that Putih Mutiara, Merah Wangi, and Merah Sengreng share a close phylogenetic relationship with Pokkali. Putih Mutiara exhibited a complex physiological response by increasing RWC, photosynthetic rate, and WUE while maintaining stomatal density, whereas Merah Sengreng responded mainly through an increase in chlorophyll content. Ciherang, despite being more distantly related to Pokkali, also demonstrated strong physiological adaptation to drought by increasing RWC, photosynthetic rate, transpiration rate, conductance, stomatal density, and stomatal index. These findings highlight new opportunities to further investigate the relationship between phylogeny and physiological adaptation to drought stress.

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