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Growth Characters and Proline Levels of Rice Varieties under Various Drought Intensities

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Abstract

Background: Drought has its own impact on agriculture, such as decreased food productivity, including rice. The response of rice plants in the dry season starts with physiological responses in the form of reduced transpiration rate by closing the stomata. This will affect the morphology of the plant, such as reduced crown and leaf size. Various rice varieties have different characteristics in minimizing the impact of drought stress.

Methods: The design used was a completely randomized block design (CRD) with 2 factors and 2 replications. The first factor was the type of variety, namely IR64, Mamberamo, Situbagendit, Way Apo Buru, Inpari32 and IM-70D and the second factor was the irrigation interval, namely 1, 2, 4 and 6 days. This study was conducted in polybags with Entisol soil types in Demangan Village, Sambi District, Boyolali Regency, Central Java, Indonesia, which is located at an altitude of 113 m above sea level.

Result: The type of variety significantly affected the leaf area index and typical leaf weight; the irrigation interval only affected the leaf area index and the interaction of the two treatments affected the typical leaf weight. The highest leaf width index was found in the IR64 variety, while the highest typical leaf weight was found in the Way Apo Buru variety. Proline levels did not differ across all treatment interactions.

Key words: Drought stress, Growth analysis, Physiological response, Proline, Rice varieties.

Introduction

Cereals are essential for feeding billions of people, with rice supplying 50% to 80% of daily caloric intake, especially in Asian countries (Hayat *et al*., 2024; Tefera *et al*., 2025). Rice accounts for nearly 80% of total irrigated freshwater resources and its roots remain submerged during both the vegetative and reproductive stages. The presence of aerenchyma tissue, a specialized structure in rice, enables its growth and development in these submerged conditions (Bhandari *et al*., 2023; Tefera *et al*., 2025).

Direct-seeded rice usually thrives in upland or low-water conditions, where it faces water deficit stress. This stress triggers structural changes in the plant, producing deeper and thicker roots that improve root hydraulic properties, enabling the plant to draw water from deeper soil layers (Molla *et al*., 2023; Zhu *et al*., 2020). These anatomical adaptations under drought stress contribute to higher yields in rainfed rice compared to irrigated rice. Consequently, the physiological traits of rainfed rice enhance its resilience to drought, making upland rice varieties more robust than their irrigated counterparts (Khumar *et al*., 2018).

Under drought stress, rainfed rice varieties generally produce higher levels of proline compared to irrigated varieties. Proline is a neutral amino acid at pH 7, characterized by its rigid structure and high solubility in water. It acts as an effective osmolyte, helping to maintain plant vitality during water scarcity by stabilizing protein structures and preventing protein degradation (Molla *et al*., 2023; Pantuwan *et al*., 2002). Furthermore, proline enhances the activity of metabolic enzymes, particularly ribulose 1,5-bisphosphate carboxylase, which initiates the calvin cycle (Akram *et al*., 2013; Li *et al*., 2015). The accumulation of proline increases cellular osmolarity, which either promotes water influx or reduces water outflow, there by improving pressure potential for cell expansion (Jarin *et al*., 2024; Mote *et al*., 2020; Subedi and Poudel, 2021; Zhu *et al*., 2020).

This study aimed to assess the growth response and proline levels of various rice varieties under different drought intensities.

Materials and Methods

This research was conducted from July to September 2025 in polybags filled with entisol soil in Demangan Village, Sambi District, Boyolali Regency, Central Java, Indonesia. The research area is located between 110°22′ and 110°50′ East Longitude and between 7°7′ and 7°36′ South

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Latitude, at an altitude of 184 meters above sea level, with an average rainfall of 139 mm per month.

The tools used in this study included trays, polybags, pot coasters, shovels, measuring jugs, buckets, sprayers, analytical scales, plastic materials, label paper, stationery, mortars, pestles, volumetric pipettes, beakers, test tubes, cuvettes, test tube stirrers and a UV-Vis spectrophotometer (Genesis 10S). The materials used comprised rice seeds (IR64, Mamberamo, Situbagendit, Way Apo Buru, Inpari 32 and IM-70D), Whatman filter paper, 3% sulfosalicylic acid, glacial acetic acid, ninhydrin acid and toluene.

The research was conducted in two stages: Seed preparation and planting media preparation. Six varieties of rice seeds were sown in separate trays filled with soil. After 14 days, the seedlings were transferred to the planting media, which consisted of a 1:1 mixture of soil and organic fertilizer, totaling 10 kg. This planting media was placed in polybags measuring 40 × 40 cm. The rice plants were irrigated according to four different treatment schedules: daily, every two days, every four days and every six days. NPK fertilization was applied at rates equivalent to 250 kg/ha of urea, 100 kg/ha of SP36 and 50 kg/ha of KCl. Urea was applied at 1.7 g per polybag when the plants were one week old and again at the same rate at four weeks. SP36 was applied at 0.48 g per polybag at one week and KCl was applied at 0.3 g per polybag at the same age. Weed control was performed by manually removing unwanted plants, while pest management involved spraying with systemic pesticides.

Experimental design

This study employed a factorial completely randomized block design with two replications. The first factor considered was the variety type, which included IR64, Mamberamo, Situbagendit, Way Apo Buru, Inpari32 and IM-70D. The second factor was the irrigation interval, categorized as daily (F1), every two days (F2), every four days (F3) and every six days (F4). In total, there were 48 experimental units, each consisting of one polybag with two plants.

Observation parameters

The observed parameters included leaf area index (LAI), specific leaf weight (SLW), net assimilation rate (NAR), crop growth rate (CGR) and proline levels, all recorded 10 weeks after planting. The leaf area index (LAI) is defined as the ratio of the total leaf area of a rice clump to the planting distance or the area shaded by the plant canopy. This value is calculated using the following formula:

$$\text{Leaf area index (LAI)} = \frac{LA}{P}$$

$$\text{Specific leaf weight (SLW)} = \frac{W_l}{LA}$$

Net assimilation rate (NAR) =

$$\frac{W_2 - W_1}{T_2 - T_1} \times \frac{\text{Ln LA2} - \text{Ln LA1}}{\text{LA2} - \text{LA1}} \, gm^{-2}\, d^{-1}$$

$$\text{Crops growth rate (CGR)} = \frac{1}{A} \times \frac{W_2 - W_1}{T_2 - T_1} \, gm^{-2}\, d^{-1}$$

Proline content: (µg proline/ml × ml toluene) $\times \frac{5}{115.13}$ g sample.

Where,

LA= Leaf area.

Leaf weight= W_l.

P= Land area.

LAI= Leaf area index.

ln = Natural logarithm.

W_1 = Dry weight of plant/m^2 recorded at time T_1.

W_2 = Dry weight of plant/m^2 recorded at time T_2.

T_1 and T_2= Each time intervals.

The data was obtained then analyzed using the F test using the Anova table and if there was a significant difference, it was continued with the DMRT test (Duncan multiple range test) at 5% level (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Table 1 indicates that the soil used in this research has the following levels: organic carbon at 1.34% (low), organic matter at 2.28% (low), total nitrogen at 0.22% (low), available P_2O_5 at 9.49 ppm (low), exchangeable potassium at and a neutral pH of 6.52.

According to the analysis of variance presented in Table 2, the rice varieties exhibited highly significant differences in leaf area index and specific leaf weight. However, the net assimilation rate, plant growth rate and proline content showed no significant differences. The watering interval resulted in significant differences only in the leaf area index, while the other parameters remained unaffected. Additionally, the interaction between variety and watering interval resulted in differences solely in specific leaf weight, with no significant differences observed in the other parameters.

Leaf area index

The leaf area index (LAI) is defined as the ratio of leaf surface area to the land area occupied by plants (Hallajian *et al*., 2024; Kumar *et al*., 2020). LAI is crucial for a plant's capacity to absorb light from solar radiation. To effectively capture 95% of the light entering the rice canopy, an LAI value of approximately 4-8 is required for optimal photosynthesis (Khalid *et al*., 2024; Rashid *et al*., 2024). Thus, LAI serves as an indicator of a plant's photosynthetic area (Moturu *et al*., 2024; Usnawiyah *et al*., 2021).

Table 3 illustrates that the IR64 variety achieved the highest LAI, significantly outperforming other varieties. Conversely, the Mamberamo, Way Apo Buru and Inpari32 varieties exhibited the lowest LAI. This variation is influenced by the genetic characteristics of each variety and environmental factors, particularly water availability. The IR64 variety shows a positive response to fertilizer applications, especially nitrogen, which is vital for chlorophyll production-the key molecule in photosynthesis.

Improved photosynthesis contributes to enhanced plant growth and larger leaves (Ouma *et al*., 2024). Dhima *et al*. (2015) and Jarin *et al*. (2024) found that nitrogen fertilizer increases LAI by boosting both the number of tillers and leaf size. Additionally, Badrudin *et al*. (2024) and Li *et al*. (2015) reported that younger seedlings, closer planting distances and urea application result in a higher number of tillers per unit area, further enhancing LAI.

In terms of watering intervals, the LAI for the every-two-day interval did not differ significantly from the daily interval but was distinct from the 4- and 6-day intervals. Notably, the 4-day interval did not show a significant difference from the 6-day interval (Table 3). This indicates that rice plants did not experience water shortages with the two-day watering schedule, as their roots were still able to access water in the growing medium.

Specific leaf weight

Specific leaf weight (SLW) indicates leaf thickness in plants, with higher SLW values corresponding to thicker leaves that contain a greater number of cells than thinner leaves. This increased cell density enhances photosynthetic capability (Ouma *et al*., 2024; Gaballah *et al*., 2022).

As shown in Table 4, the Way Apo Buru variety has the highest specific leaf weight compared to other varieties, while the IM-70D variety has the lowest. The leaf weights of Inpari32 and IM-70D are similar to those of IR64, Mamberamo and Situbagendit. This implies that the Way Apo Buru variety has thicker leaves due to its higher cell count and increased chlorophyll content.

When watered every four days, the Way Apo Buru variety consistently exhibited the highest leaf weight among all treatments, demonstrating its strong tolerance to water

Table 1: Results of chemical analysis of research soil before the experiment.

Types of analysis	Method	Test I	Test II	Test III	Average
Organic C (%)	Walkley and black	1.27	1.41	1.34	1.34
Organic matter (%)	Walkley and black	2.13	2.17	2.44	2.28
N Total (%)	Kjeldhal	0.21	0.24	0.22	0.22
Available P_2O_5 (ppm)	Bray II	8.24	10:48	9.75	9.49
K exchanged (me %)	Extraction of 1 N acetate am. p.h. 7	0.27	0.30	0.28	0.28
pH	Glass electrode	6.40	6.61	6.56	6.52

Table 2: Results of variance analysis of various observed parameters.

SV	DF	Observation				
		LAI	SLW	NAR	CGR	Proline
Treatment	23	5.24^{**}	5.14^{**}	1.14^{ns}	0.93^{ns}	1.59^{ns}
Block	1	1.65^{ns}	1.12^{ns}	26.15^{ns}	23.66^{ns}	22.35^{*}
V	5	12.36^{**}	9.67^{**}	1.63^{ns}	0.99^{ns}	2.82^{ns}
F	3	14.47^{*}	2.42^{ns}	0.05^{ns}	0.20^{ns}	1.67^{ns}
V × F	15	1.02^{ns}	4.18^{**}	1.19^{ns}	1.06^{ns}	1.17^{ns}
Error	23					
Total	47					

SV= Source of diversity, DF= Degrees of freedom, V= Variety, F= Watering frequency, VXF= Interaction between variety and watering frequency.

Table 3: Observations of the leaf area index of various rice varieties at various watering intervals.

Variety	Watering interval				Average
	1 day	2 days	4 days	6 days	
IR64	5.47^{ab}	6.59^{a}	5.27^{ac}	3.47^{be}	5.20^{a}
Mamberamo	3.64^{be}	3.73^{be}	1.73^{e}	1.78^{e}	2.72^{c}
Situbagendit	5.19^{ac}	5.09^{ac}	3.58^{be}	3.26^{ce}	4.29^{b}
Way apo buru	4.17^{bd}	1.98^{e}	1.85^{e}	1.82^{e}	2.72^{c}
Inpari32	3.73^{be}	4.08^{bd}	2.80^{de}	1.89^{e}	3.13^{c}
IM-70D	4.15^{bd}	5.25^{ac}	3.29^{ce}	3.47^{be}	4.04^{b}
Average	4.39^{a}	4.46^{a}	3.09^{b}	2.62^{b}	

Different superscripts in the same column or row indicate a significant difference (p<0.05). Different superscripts in the interaction column indicate a significant difference (p<0.05).

shortages. This moderate-yielding rice variety is well-suited for both irrigated fields and drylands where water scarcity is common.

Net assimilation rate

The Situbagendit variety demonstrated the highest Net Assimilation Rate when watered every four days. The Inpari32 variety, also on a 4-day watering schedule, performed similarly but did not show significant differences compared to the other treatments (Table 5). This indicates that the Situbagendit variety is more efficient in water utilization, even under constraints. Importantly, Situbagendit has been specifically bred for areas that often face water shortages.

Research by Bhandari *et al*. (2023); Mote *et al*. (2020) and Subedi and Poudel. (2021) highlights that drought affects physiological growth and metabolism, providing a vital basis for developing new varieties capable of either escaping or tolerating such conditions.

Plant growth rates

Plant growth rate is the increase in plant weight per unit area occupied by the plant over a specific period (Gardner, 1991). All rice varieties, watering intervals and their interactions showed no differences in plant growth rate (Table 6). This indicates that all tested varieties exhibited similar patterns in responding to drought stress, as evidenced by the similar patterns of leaf area index, Specific leaf weight net assimilation rate. This finding aligns with the findings of several researchers (Aziez, 2022; Dhima *et al*., 2015; Liu *et al*., 2013).

Proline content

The Mamberamo variety exhibited the highest proline levels with a 6-day watering interval, while the Situbagendit variety displayed the lowest proline levels with a 2-day watering interval, similar to those seen with daily watering (Table 7). Proline levels are influenced by the genetic traits of each variety. (Ouma *et al*., 2024; Loho *et al*., 2025) noted that drought stress affects the morphophysiological and biochemical processes of rice plants, including proline levels.

Noelle *et al*. (2018) indicated that genotypic variance in wheat enhances drought tolerance and Usnawiyah *et al*. (2021) suggested that breeding strategies focused on genotypic selection could further improve drought resilience. Morphological traits may significantly impact drought tolerance and in the genomic era, plant phenotypes increasingly determine yield and ecosystem health (Anwar *et al*., 2025).

Water deficit stress delays panicle initiation and can damage respiratory membranes and ATP production in rice (Marimuthu *et al*., 2023). Low water potential during drought is a common occurrence, regulated genetically

Table 4: Observations of spesific leaf weight of various rice varieties at various watering intervals.

Variety	Watering interval				Average
	1 day	2 days	4 days	6 days	
IR64	10.50^{c}	10.50^{c}	17.00^{bc}	26.00^{bc}	16.00^{bc}
Mamberamo	29.00^{bc}	19.50^{bc}	28.50^{bc}	18.50^{bc}	23.88^{bc}
Situbagendit	11.50^{c}	14.00^{bc}	19.50^{bc}	20.00^{bc}	16.25^{bc}
Way apo buru	22.00^{bc}	38.50^{b}	88.00^{a}	26.50^{bc}	43.75^{a}
Inpari32	37.50^{b}	17.00^{bc}	14.50^{bc}	38.50^{b}	26.88^{b}
IM-70D	24.50^{bc}	24.50^{bc}	9.50^{c}	6.00^{c}	12.50^{c}
Average	22.50^{ab}	18.25^{b}	29.50^{a}	22.58^{ab}	

Different superscripts in the same column or row indicate a significant difference (p<0.05).
Different superscripts in the interaction column indicate significant differences (p<0.05).

Table 5: Observations of the net assimilation rate of various rice varieties at various watering intervals ($\times$ 10^{-3} gm^{-2} d^{-1}).

Variety	Watering interval				Average
	1 day	2 days	4 days	6 days	
IR64	330.60^{ab}	343.20^{ab}	492.70^{a}	465.40^{ab}	407.97^{a}
Mamberamo	359.6^{ab}	315.6^{ab}	291.0^{ab}	276.3^{ab}	310.61^{a}
Situbagendit	273.2^{ab}	444.8^{ab}	487.3^{a}	416.7^{ab}	405.51^{a}
Way apo buru	304.9^{ab}	205.4^{ab}	404.2^{ab}	266.9^{ab}	295.34^{a}
Inpari32	421.4^{ab}	265.2^{ab}	167.8^{b}	372.7^{ab}	306.76^{a}
IM-70D	361.5^{ab}	379.8^{ab}	206.7^{ab}	193.1^{ab}	285.28^{a}
Average	341.87^{a}	325.66^{a}	341.63^{a}	331.83^{a}	

Different superscripts in the same column or row indicate a significant difference (p<0.05).
Different superscripts in the interaction column indicate significant differences (p<0.05).

Table 6: Observations of plant growth rates of various rice varieties at various watering intervals ($\times$ 10^{-3} mg cm^{-2} day^{-1}).

Variety	Watering interval				Average
	1 day	2 days	4 days	6 days	
IR64	107.00^{a}	108.50^{a}	159.50^{a}	160.50^{a}	133.88^{a}
Mamberamo	123.50^{a}	108.00^{a}	114.00^{a}	104.50^{a}	112.50^{a}
Situbagendit	89.00^{a}	146.00^{a}	167.00^{a}	145.00^{a}	136.88^{a}
Way Apo Buru	101.50^{a}	79.00^{a}	154.00^{a}	101.00^{a}	108.88^{a}
Inpari32	133.50^{a}	90.50^{a}	59.50^{a}	145.00^{a}	107.25^{a}
IM-70D	122.00^{a}	124.00^{a}	73.00^{a}	66.50^{a}	96.50^{a}
Average	112.75^{a}	109.33^{a}	121.33^{a}	120.50^{a}	

Different superscripts in the same column or row indicate a significant difference ($p<0.05$).

Different superscripts in the interaction column indicate significant differences ($p<0.05$).

Table 7: Observations of proline content of various rice varieties at various watering intervals (µmo g^{-1}).

Variety	Watering interval				Average
	1 day	2 days	4 days	6 days	
IR64	13.04^{ac}	29.48^{a}	12.41^{ac}	16.91^{ac}	17.96^{ab}
Mamberamo	17.78^{ac}	26.19^{ac}	28.08^{ab}	29.39^{a}	25.36^{a}
Situbagendit	19.37^{ac}	7.92^{c}	8.42^{c}	20.63^{ac}	14.09^{b}
Way Apo Buru	9.01^{bc}	14.99^{ac}	18.34^{ac}	14.01^{ac}	14.08^{b}
Inpari32	18.19^{ac}	18.72^{ac}	7.23^{c}	17.45^{ac}	15.40^{b}
IM-70D	11.65^{ac}	22.67^{ac}	7.03^{c}	9.41^{bc}	12.69^{b}
Average	14.84^{a}	19.99^{a}	13.58^{a}	17.97^{a}	

Different superscripts in the same column or row indicate a significant difference ($p<0.05$).

Different superscripts in the interaction column indicate significant differences ($p<0.05$).

based on plant tolerance. This condition affects leaf anatomy and ultrastructure, primarily due to reduced water content, resulting in fewer stomata per micrometer of leaf (Upadhyaya and Panda, 2018; Hou *et al*., 2024; Samiyarsih *et al*., 2022). The consequences include reduced leaf area, decreased chlorophyll content, leaf curling, stomatal closure, a thicker cutin layer on the leaf surface and smaller grain size (Gaballah *et al*., 2022; Zihao *et al*., 2022). Short-term water deficits adversely affect relative water content, leading to osmotic imbalance, lipid peroxidation and membrane damage; in severe cases, necrotic spots have also been reported in rice (Zhu *et al*., 2020; Mishra and Panda, 2017; Panda *et al*., 2021).

CONCLUSION

The variety affected both the leaf area index (LAI) and specific leaf weight (SLW), while the watering interval only influenced the LAI. Furthermore, the interaction between the two treatments impacted the SLW. The IR64 variety showed the highest LAI, while the Way Apo Buru variety had the highest SLW. No differences in proline levels were found among the tested treatments.

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Conflict of interest

The authors declare that they have no conflicts of interest, including financial and non-financial competing interests.

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