Research article

Water-efficient rice performances under drought stress conditions

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Abstract: The use of varieties that are able to adapt well to extreme environments is one strategy to overcome the challenges of decreasing production in sub-optimal land. Indonesian tropical rice varieties (Jatiluhur, IPB 9G, IPB 3S, Hipa 19, Mentik Wangi, Ciherang, Inpari 17, and Mekongga) have been tested and established as water-used-efficient varieties in an optimal environment. However, to date, these varieties have not been examined in the suboptimal area, in particular, drought stress conditions. Therefore, this study aimed to evaluate the adaptation response of production, morphological, and physiological character of several water-efficient rice varieties under drought stress in the field. The study was designed in a split-plot with two factors and 4 replications, where the first factor (main plot) was drought stress stages i.e. vegetative (Dv), reproductive (Dr), generative (Dg), and control (Dc). The second factor was rice varieties, consisting of eight varieties, i.e., Jatiluhur, IPB 3S, IPB 9G, Hipa 19, Mentik Wangi, Ciherang, Inpari 17, and Mekongga. The experiment was conducted from May to December 2018 in Muneng Kidul Village, Probolinggo Regency, East Java Province. The experimental variables were morphology, production, leaf scrolling score during drought stress, drought sensitivity index, water use efficiency, physiology and root anatomy. The result showed that upland rice varieties were more tolerant to drought stress and had a higher water use efficiency than lowland rice varieties. This shows that Jatiluhur and IPB 9G which are indicated to be adaptive to drought stress, and have the ability to regulate water use more efficiently when drought stress occurs. Therefore, water use efficiency could be used as selection characters under drought conditions in rice particularly tropical upland rice. Moreover,
morphological characters, i.e., grain yield per plot, weight of pithy grain, weight of shoot biomass and weight of roots could be the selection characters to predict drought tolerant tropical rice. According to physiological characters, photosynthesis rate, transpiration rate, proline content, malondialdehyde content, leaf water potential and leaf greenness could be used as a selection tool to predict water use efficient genotypes in rice. However, further studies are needed to understand the complex mechanisms of water use efficiency by combining various approaches.

**Keywords:** correlation analysis; malondialdehyde; leaf water potential; proline; upland rice

1. Introduction

Rice is the main food source for most of the world’s population. The area of rice harvested in Indonesia in 2020 has decreased compared to 2019 by 0.19 percent and rice production in 2020 has increased compared to 2019 by 0.08 percent [1]. The rice planting period in Indonesia follows the climatic conditions generally divided into two planting seasons, namely the rainy season and the dry season. Extreme climate changes almost every 3 to 5 years occur namely El-Nino and LaNina which have an impact on rice production [2]. Climate change effects are increasingly evident: flooding, droughts and environmental degradation are more severe; high temperatures are disturbing cropping patterns whilst rising sea levels are having detrimental consequences in coastal cities and communities [3]. One of the abiotic stresses that is a limiting factor in rice production in Indonesia is drought stress. Drought stress is a condition of water shortages in plants caused by water limitations in the growing environment. Drought stress in plants can also be caused by excessive water demand from leaves so that the rate of evapotranspiration exceeds the rate of water absorption even though the available groundwater condition is sufficient [4].

The use of varieties that are able to produce optimally in a limited amount of water is an opportunity to overcome drought disasters in various regions during the dry season. Bramley et al. [5] reported that one theoretical way of increasing yield with less water is through manipulating the relationship between carbon gain (photosynthesis) and water loss (transpiration). The comparison between these two parameters is called water use efficiency (WUE). The results of research by Supijatno et al. [6] under optimal environmental conditions in greenhouses showed that the Jatiluhur variety is very efficient in its water use compared to other varieties. The value of water use efficiency (WUE) is based on the weight of grain produced compared to water consumption in one season [6,7]. Hu and Xiaoang [8] stated that new plant varieties with drought tolerance and efficient use of water have been enhanced through conventional breeding approaches using molecular assisted selection.

The impact of drought stress on plants can be seen visually through plant morphology including leaf scrolling, leaf damage, decreased plant height, decreased number of productive tillers, late flowering and decreased yield. Drought that occurs in the vegetative phase inhibits plant height growth, the development of the number of tillers and leaves [9], the reproductive phase reduces of productive tillers, decreases 1000 grain weight and decreases the harvest index [10]. Visual performance which is also affected by drought is leaf senescence [11]. This is closely related to the increase in reactive oxide species that induces senescence in leaves [12], so that tolerance can be observed in the degree of leaf damage known as the leaf dryness score [13]. Apart from visual
appearance, the impact of drought also affects morphological, agronomic, and physiological growth characters. This was also reported by Kamarudin et al. [14], Lawas et al. [15], and Yang et al. [16]. The difference in the impact of drought on these characters can explain the tolerance traits of a genotype [13]. However, this impact was largely determined by the critical phase in plants when a stress occurs [17].

Rice response to drought stress was determined by three critical phases, namely the vegetative, reproductive and generative phases [10]. According to Tubur et al. [10] the difference in critical phase show differences in morphological diversity in drought stress. In addition, according to Fischer et al. [18] drought stress in the generative phase was drought that occurs at the heading could be severe impact on yield components and plant yield. Therefore, identification of the response pattern to the effect of drought in several phases of rice is an important study to determine the relationship between patterns of water use efficiency and growth characteristics. The purpose of this study was to determine the response of several types of rice which are efficient in using water in drought conditions, climate type E (Oldeman) in the field.

2. Materials and methods

2.1. Time and location of research

The experiment was conducted in Muneng, Probolinggo Regency, East Java, from May to November 2018. Chlorophyll analysis was performed at the post-harvest laboratory of the Department of Agronomy and Horticulture, Faculty of Agriculture, IPB. Root anatomy was performed at the microtechnical laboratory of the Department of Agronomy and Horticulture, Faculty of Agriculture, IPB. Leaf water potential was analyzed at the Physiology Stress Laboratory of the Indonesian Institute of Sciences, Cibinong. Proline and malondialdehyde analyses were conducted at the Soil Biology Laboratory, BB Biogen Research Center, Bogor.

2.2. Research design and agro-morphological analysis

The experiment used a split-plot design consisting of two treatment factors. First factor (main plot) was four levels of drought stress treatment during the growing period consisting of control (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). The second factor (subplot) was 8 rice varieties, i.e., Jatiluhur (upland rice), IPB 9G (upland rice), IPB 3S (lowland rice), Hipa 19 (lowland rice), Cihang (lowland rice), Inpari 17 (lowland rice), and Mekongga (local lowland rice), Mentik Wangi (local lowland rice). These varieties have been tested for water consumption in two seasons (rainy season and dry season) in 2018 with water use efficiency values of 2.3, 2.4, 1.5, 1.8, 1.7, 1.7, 1.5 and 1.8 [19]. In total, there were thirty-two treatment combinations with four replications, so that there were 128 units of the experiment. Experiment was conducted on a plot of 5 m x 6 m size. The planting space used was 20 cm x 20 cm with one seedling per hole. Planting was conducted in stages (staggered planting) to facilitate the application of drought stress at the same time. The first nursery was for the control treatment (Dc) and the generative phase of drought (Dg). Then the second was for the reproductive phase drought (Dr) and the vegetative phase drought. Plant maintenance was carried out by administering fertilizer with an equivalent dose of 250 kg ha^{-1} Urea, 200 kg ha^{-1} SP-18, and 100 kg ha^{-1} KCl.
Urea was given three times, while SP-18 and KCl were given simultaneously in two weeks After Planting (WAP). Pest and disease control was adjusted according to the level of attack in the field.

Drought regulation was performed by arranging to plant the generative phase drought treatment and control that were planted together, planting of the reproductive phase, and finally planting of the vegetative phase. Furthermore, stoppage of water administration at the same time according to treatments was performed, i.e., at the age of thirty-nine Days After Planting (DAP) for the vegetative phase drought (Dv), at the age of fifty-four DAP for the reproductive phase drought (Dr) and the age of seventy-five DAP for the generative phase drought (Dg). In the control (Dc), water administration was continued until before harvest. The water surface level during inundation was around 3.5 cm from the ground. Observation of the drought sensitivity was carried out based on the level of leaf rolling until it reached a score of 9. Moreover, the experimental plot was re-irrigated until before harvest. Water supply into the plot is measured using a flowmeter to calculate daily water consumption. Table 1 showed the data of water consumption of eight rice varieties in the field.

| Varieties   | Water consumption per clump (Liter) | Dc  | Dv  | Dr  | Dg  |
|-------------|------------------------------------|-----|-----|-----|-----|
| Jatiluhur   |                                    | 32.3| 26.6| 28.8| 31.6|
| IPB 9 G     |                                    | 29.1| 26.6| 28.8| 32.3|
| IPB 3 S     |                                    | 29.1| 26.6| 28.8| 30.9|
| Ciherang    |                                    | 30.7| 27.6| 29.8| 33.6|
| Inpari 17   |                                    | 29.1| 27.1| 29.3| 30.2|
| Mekongga    |                                    | 30.7| 27.1| 29.3| 31.8|
| Mentik Wangi|                                    | 29.1| 27.1| 29.3| 30.9|
| Hipa 19     |                                    | 27.5| 26.6| 28.8| 31.6|
| Average     |                                    | 29.7| 26.9| 29.1| 31.6|

Variables observed in this study were plant height, flowering age, yield productivity, leaf rolling score, dryness sensitivity index, water use efficiency, leaf greening level, leaf water potential, stomatal density, total chlorophyll, proline and malondialdehyde contents. Analysis of total chlorophyll was according to the method of [20]. Proline content analysis followed Bates et al. [21] with slight modification. Malondialdehyde (MDA) content was analyzed following [22]. Leaf water potential analysis was performed using a dewpoint water potential instrument. The level of leaf rolling was determined visually based on the 2013 IRRI standard, with a score range of 1–9. Plant productivity was calculated by the formula = 10,000 m²/area of tile x tiled pithy grain weight. The drought stress sensitivity index (DSI) was determined based on [23] and became the basis for grouping the characteristic of drought tolerance. Drought stress sensitivity index (DSI) was calculated using the formula: DSI = (1 – Yc/Yo)/(1 – Xc/Xo), where Yc = certain average of drought stress condition genotypes, Yo = the average of an optimum condition genotype, Xc = the average of all drought stress condition genotypes, and Xo = the average of all optimum condition genotypes. The criterion for determining the level of tolerance to drought stress was if the DSI value = 0.5 then the genotype is tolerant, if 0.5 < DSI = 1.0 then the genotype is quite tolerant, and if DSI > 1.0 then the genotype is sensitive. The water use efficiency was calculated using the formula = total water consumption in the treatment plot/the pithy grain weight in the plot.
Irrigation uses artificial irrigation which was pumped from wells with a depth of 80 m. Addition of water in the area was done once every day, every addition of water was recorded. Water surface was maintained at a height of 3.5 cm from the ground. Water supply was stopped 14 days before harvest. Harvesting was done by looking directly at panicles and grains of rice when they were 90 to 95% yellow.

2.3. Proline analysis

Proline content was analyzed following the method of Bates et al. [21] with slightly modified. Ninhydrin (Merck) was prepared as a reactant by dissolving 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid (Sigma). The solution was cooled and stored at 4 °C. Leaf samples (0.5 g) were extracted in 10 mL of 3% sulfosalicylic acid (Merck) and centrifuged for 25 minutes at 4 °C at 3600 rpm. A total of 2 mL of the supernatant was taken and quickly added with 2 mL of ninhydrin acid and 2 mL of glacial acetic acid in the 15 mL tube, followed by heating in a waterbath (80 °C) for 1 h. The solution was then immediately cooled at room temperature and stored in a refrigerator (4 °C). The mixture was directly extracted using 4 mL of Toluene (Merck) and vortex for 10 seconds. The solution was pipetted and measured at a wavelength (λ) of 520 nm using a spectrophotometer. As a blank, toluene was used. The proline concentration (µmol/g) was determined from the standard proline curve and calculated against the fresh weight. The equation used according to Akbar, (2018) was \( y = 0.013x - 0.0002 \), where (y) was the absorbance value and (x) was referred to the content of proline.

2.4. Analysis of Malondialdehyde (MDA)

Malondialdehyde (MDA) content was obtained based on Wang et al. [22]. An amount of 0.3 g leaves were ground with a mortar, added 5 mL 0.1% (w/v) Trichloroacetic acid (TCA), and then centrifuged at 10.000 g for 5 minutes. After centrifugation, 1 mL of supernatant was moved to a new tube, added by 4 mL of 0.1% (w/v) Thiobarbituric acid (TBA) in 20% (w/v) TCA. The solution was then incubated at 80 °C in a water bath for 30 minutes and then cooled to room temperature. The absorbance of the TBA-MDA complex was measured using a spectrophotometer at a wavelength (λ) of 532 nm and 450 nm, whereas non-specific absorbance measured at a wavelength (λ) of 600 nm [24]. Plant roots without Al treatment were used as a control. The MDA content was determined based on the following calculation:

\[
C_{MDA} \left( \frac{\mu mol}{g \cdot FW} \right) = [6.45 \times (A532 - A600)] - [0.56 \times A450]
\]

2.5. Total Chlorophyll Content

Leaf chlorophyll content was determined following the methods of Sim and Gamons [20]. An amount of 0.1 g of rice leaves was ground using the mortar and homogenized by adding 10 ml of 100% acetone. The mixture was then centrifuged at 1000 rpm for one minute. The supernatant was removed and then re-centrifuged at 2500 rpm for ten minutes. The absorbance of the supernatant was measured using a spectrophotometer at wavelengths 537, 647 and 663 nm. The experiment was conducted with three biological replications and three technical replications. Calculation of total
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chlorophyll content including chlorophyll a, chlorophyll b, and total chlorophyll was carried out following the formula:

\[
Chlorophyll \ a = \frac{(0.01373 \times A_{663}) - (0.000897 \times A_{537}) - (0.003046 \times A_{467})}{\text{Fresh weight}} \times 8 \quad (2)
\]

\[
Chlorophyll \ b = \frac{(0.02405 \times A_{647}) - (0.004305 \times A_{537}) - (0.005507 \times A_{663})}{\text{Fresh weight}} \times 8 \quad (3)
\]

Total Chlorophyll = Chlorophyll a + Chlorophyll b \quad (4)

2.6. Data Analysis

Data analysis included the F-test, Pearson correlation, and path analysis. F-test was conducted to determine the significance of the treatment difference. If these results showed a significant effect, then Duncan's further test at a level of 5% was performed. Pearson correlation was used to determine the relationship of each characteristic. Path analysis was used to determine the main components that affected the productivity of each tested characteristic. F-test, Duncan Multiple Range Test (DMRT), and Pearson correlation were performed using the STAR IRRI program. Path analysis was carried out using the R studio program with the agricolae package.

3. Results and discussion

3.1. Ecological Condition of Muneng Kidul Village, Probolinggo-East Java

This experimental field was located at 7°48′7.2″ (latitude) 113°9′32.4″ (longitude), This study was conducted in drought extreme growing environmental conditions. dos Santos et al. [25] stated that the extreme environment is an environment that gives rise to stress on plants. The extreme rainfall conditions at the study site from May to November 2018 in the village of Muneng Kidul, Probolinggo Regency, East Java was quite dry (Figure 1). Probolinggo Regency has an altitude of 10 m above sea level (above sea level), climate type E1 (Oldeman). Probolinggo Regency has two types of climate change every year, i.e., the dry season and the rainy season. The dry season ranges from April to October with an average rainfall of ± 29.5 mm per rainy day, while the rainy season ranges from November to April with an average rainfall of ± 229 mm per rainy day. High rainfall from December to March, the average rainfall is ± 360 mm per rainy day. Rainfall in these months (2018) was very low at 0 mm per day. The minimum air temperature was 23.5 °C and the maximum air temperature was 34.0 °C. The average humidity was around 78.36%. Altitude 10 m above sea level (above sea level). The analysis showed that the pH of the media (H2O) was 6.5 in the normal category; organic matter content C was 1.2%, N content was 0.09%, C/N was 13.33, sand, dust and clay texture were 42%, 38% and 22%, respectively.
Figure 1. Ecological conditions of Muneng Kidul, rainfall (mm³), average temperature (°C) and average humidity (%) from May–November 2018. The data source of the micro climatology station in the Muneng station of Balitkabi-Probolinggo, East Java.

3.2. Performance of morphological characters on optimum conditions and drought stress

Morphological and production characteristics form the initial basis for assessing the effect of drought stress [26,27]. The results of the recapitulation of variance in Table 2 showed that the interaction of drought stress affects all morphological characters (plant height, number of productive tillers, flowering age, root length, shoot weight, root weight, weight of pithy grain per plant, productivity, weight of tuber, sensitivity index, dryness and water use efficiency), physiological characters (leaf greenness, leaf water potential, photosynthesis, transpiration, total chlorophyll, proline and malonaldehyde). Plant height, leaf width, weight of pithy grain and weight of plant crown are growth variables that can be observed from the increase in plant size. Increasing plant height after drought stress is a form of positive growth for the development of varieties in rainfed land [28,29]. Drought affects plant height, flowering age, gene expression, and rice yields in various plants [29,30]. In this study, drought stress in the generative phase lasted for 15 days, drought stress in the reproductive phase and the vegetative phase lasted for 21 days.

Impact of drought was reduced roots and changes in leaf properties (shape, epiculate layer, color) [31]. In this study, it was confirmed that there were differences in response between the vegetative, reproductive, and generative phases at certain plant heights under drought stress conditions in tropical rice (Table 3). Based on Table 3, the response to the number of productive tillers shows that all the included varieties have a drought tolerant response, each variety is able to regrow (recovery) has increased the number of productive tillers an average of 6.42% after drought stress occurred in the vegetative phase and on average 1.83% on average in the reproductive phase. This explains that the time of stress treatment/phase plant growth determines the response of the character.

Morphological and production characters were the initial basis for seeing the effect of drought stress [32]. Based on Table 3, the effect of drought on the generative phase compared to control did
not affect the difference in plant height. This is because drought stress in the generative phase has no effect on the character of plant height, because plant height growth occurs in the vegetative phase. Plant height increased by 5.57% due to drought in the vegetative phase and 0.40% due to drought in the reproductive phase. Afrianingsih [33] reported that lengthening of the canopy or plant height after drought stress is a positive growth for the development of these varieties in rainfed land. This shows that the eight varieties experienced a drought recovery mechanism during drought stress in the reproductive phase and the vegetative phase. Plant height increased by 5.57% due to drought in the vegetative phase and 0.40% due to drought in the reproductive phase (Table 1). All of these varieties underwent a drought recovery mechanism on plant height characteristics when they were stressed by drought in vegetative and reproductive phases.

Table 2. Recapitulation of variance and correlation of morphological, production, EPA and physiological characters of eight rice varieties on productivity varieties were in optimum condition (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018. Muneng Kidul, Probolinggo-East Java.

| Character                              | D   | V   | D x V | CD (%) |
|----------------------------------------|-----|-----|-------|--------|
| Morphology, production and WUE         |     |     |       |        |
| Plant height (cm)                      | **  | **  | **    | 2.12   |
| Number of productive tillers (stems)  | **  | **  | **    | 6.62   |
| Flowering age (HSS)                   | **  | **  | **    | 0.57   |
| Root length (cm)                      | **  | **  | **    | 6.20   |
| Dry canopy weight (g)                 | **  | **  | **    | 4.49   |
| Dry root weight (g)                   | **  | **  | **    | 5.02   |
| Weight of pithy grain (g)             | **  | **  | **    | 4.45   |
| Productivity (t/ha)                   | **  | **  | **    | 7.39   |
| Grain yield per plot (kg)             | **  | **  | **    | 7.20   |
| Drought sensitivity index (unit)      | **  | **  | **    | 26.62  |
| Water use efficiency (g/L)            | **  | **  | **    | 8.13   |
| Physiology                            |     |     |       |        |
| leaf greenness (unit)                 | **  | **  | **    | 2.31   |
| Leaf water potential (MPa)            | **  | **  | *     | 10.48  |
| Photosynthesis (μmol CO₂ m⁻² s⁻¹)     | **  | **  | tn    | 3.51   |
| Transpiration (μmol H₂O m⁻² s⁻¹)      | **  | *   | **    | 15.05  |
| Total chlorophyll (mg/g)              | **  | **  | **    | 56.47  |
| Proline (μmol/g)                      | **  | **  | **    | 16.40  |
| Malonaldehyde (μmol/g)                | **  | **  | **    | 8.65   |

Note: CD = coefficient of diversity, D = drought treatment at various growth phases, V = variety. db D = 3, db V = 7. ** = significantly different at α 0.01, * = significantly different at α 0.05, tn = not significantly different at α 0.05.
Table 3. Response of plant height and number of productive tillers of eight rice varieties at optimum conditions (Dc), drought in the vegetative phase (Dv), drought in the reproductive phase (Dr), and drought in the generative phase (Dg). Dry season May–November 2018. Muneng Kidul, Probolinggo-East Java.

| Varietas   | Plant height (cm) | Number of tillering (clump) |
|------------|-------------------|----------------------------|
|            | Dc    | Dv    | Dr    | Dg    | Dc    | Dv    | Dr    | Dg    |
| Jatiluhur  | 82.1 a | 86.3 a | 87.2 a | 82.0 a | 8.1 c | 7.9 cd | 7.5 c | 7.3 a |
| IPB 9 G    | 78.9 ab | 83.9 ab | 79.9 b | 80.0 a | 8.3 c | 8.0 d | 7.6 c | 7.1 a |
| IPB 3 S    | 78.0 b | 82.2 b | 78.4 b | 79.4 a | 6.6 d | 6.4 e | 5.7 d | 5.2 a |
| Cihetang   | 60.7 d | 69.8 c | 62.5 c | 60.4 c | 11.0 a | 9.1 bc | 9.5 a | 7.9 a |
| Inpari 17  | 61.4 d | 70.0 c | 62.5 c | 61.1 c | 11.1 a | 13.8 b | 8.5 abc | 7.5 a |
| Mekongga   | 59.8 d | 63.9 d | 63.3 d | 59.5 c | 9.8 b | 9.1 abc | 8.2 bc | 7.6 a |
| Mentik     | 66.9 c | 70.3 c | 71.0 c | 67.1 b | 11.5 a | 8.2 d | 8.7 abc | 7.6 a |
| Hipa 19    | 62.2 d | 70.5 c | 62.9 d | 61.0 c | 11.2 a | 10.1 a | 9.4 c | 8.2 a |
| Average    | 68.8  | 73.7  | 70.9  | 68.7  | 9.7   | 9.1   | 8.1   | 7.3   |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of α = 5%. Plant height and number of productive tillers were measured 7 days before harvest.

Drought stress was very significant in the characters of 50% age and root length (Table 4). This shows that drought in the vegetative and reproductive phases significantly affected plant age 15.26% and 15.02%, respectively, compared to the control treatment. Saxena et al. [31] stated that the impact of drought stress affects a longer plant life. The drought stress that occurs in the middle of the growing season causes delays in the flowering phase of up to 2 to 3 weeks [23].

Table 4. Responses to flowering age and root length of eight rice varieties at optimum conditions (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018. Muneng Kidul, East Java.

| Varieties   | Age flowering (DAP) | Root length (cm) |
|-------------|---------------------|------------------|
|            | Dc    | Dv    | Dr    | Dg    | Dc    | Dv    | Dr    | Dg    |
| Jatiluhur   | 83.3 d | 97.5 cd | 97.8 b | 83.5 d | 20.5 a | 17.0 a | 16.3 a | 16.8 a |
| IPB 9 G     | 83.3 d | 97.5 cd | 97.5 bc | 83.5 d | 19.2 ab | 16.3 a | 16.7 a | 16.3 a |
| IPB 3 S     | 83.3 d | 96.5 d | 96.5 c | 83.5 d | 19.0 ab | 15.8 ab | 15.7 a | 15.8 ab |
| Cihetang    | 92.3 a | 99.8 a | 99.5 a | 92.5 a | 14.5 c | 11.8 de | 11.3 b | 12.2 cd |
| Inpari 17   | 85.0 c | 98.8 ab | 98.5 ab | 85.3 c | 13.9 cd | 12.8 cd | 11.5 b | 11.8 d |
| Mekongga    | 87.0 b | 99.0 ab | 98.5 ab | 87.3 b | 12.0 d | 10.6 e | 11.3 b | 11.2 d |
| Mentik Wangi| 83.8 d | 98.8 ab | 98.3 b | 84.0 d | 18.7 ab | 14.0 bc | 12.6 b | 14.0 bc |
| Hipa 19     | 83.8 d | 98.0 bc | 97.8 b | 83.5 d | 17.3 b | 13.8 bc | 12.7 b | 12.7 cd |
| Rata-rata   | 85.2  | 98.2  | 98.1  | 85.4  | 16.9  | 14.0  | 13.5  | 13.9  |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of α = 5%. Flowering age of each variety was calculated after 50% in the flowering plot. The root length was calculated after harvest, the roots of the plant were dismantled to a depth of 30 cm. The roots were cleaned from the soil and then measured the longest roots from the base to the longest end of the roots.
Late flowering is a positive response for plants as a plant adaptation to drought-stressed environments. When drought is gripped, plants use various mechanisms to survive. Some of the mechanisms that can be seen visually are leaf rolling, maintaining leaf greenness, the ability to maintain tillers when drought stress and forming productive tillers after drought stress. Leaf rolling was an initial response when plants were stressed by drought [10] at this time the plant is implementing a drought avoidance mechanism which was related to the ability to adjust the transpiration rate to maintain the potential for leaf water to remain high in drought conditions [10]. The ability to form tillers after the plants are stressed is a mechanism of recovery. Drought recovery is a healing mechanism after a plant is gripped by drought. This mechanism was usually when drought stress occurs early in growth [34].

The response to root length shows a very significant effect on root length between varieties and a significant effect on recovery after drought stress, namely the reproductive phase and the vegetative phase. Nio and Torey [35] reported that root elongation during drought stress shows that the plant is resistant to drought stress. Root length is one of the morphological characters related to drought resistance in rice [35,36].

Table 5. Response of canopy dry weight and root weight of eight rice varieties was in optimum condition (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018. Muneng Kidul, Probolinggo-East Java.

| Varieties    | Dry canopy weight (g) | Dry root weight (g) |
|--------------|-----------------------|---------------------|
|              | Dc  | Dv  | Dr  | Dg  | Dc  | Dv  | Dr  | Dg  |
| Jatiluhur    | 13.5 a | 12.5 a | 12.0 a | 11.5 a | 3.5 a | 3.3 a | 3.3 a | 3.4 a |
| IPB 9 G      | 13.7 a | 12.7 a | 12.1 a | 11.7 a | 3.5 a | 3.1 ab | 3.1 a | 3.3 a |
| IPB 3 S      | 12.7 b | 11.0 b | 10.1 c | 9.7 b | 3.3 a | 2.6 c | 2.6 b | 2.7 b |
| Ciherang     | 12.4 b | 10.2 cd | 10.1 c | 9.8 b | 2.5 b | 2.1 d | 2.1 c | 2.2 c |
| Inpari 17    | 11.0 c | 9.9 c  | 11.0 b | 10.0 b | 2.6 b | 2.3 d | 2.4 bc | 2.1 c |
| Mekongga     | 12.3 b | 10.3bcd | 9.8 c  | 9.3 b | 2.6 b | 2.4 d | 2.3 bc | 2.2 c |
| Mentik Wangi | 13.0 ab | 10.1 b | 10.0 c | 9.6 b | 3.6 a | 3.0 b | 2.9 ab | 3.1 a |
| Hipa 19      | 11.5 c | 10.8 bc | 10.4 bc | 10.0 b | 2.5 b | 2.3 cd | 2.4 bc | 2.1 c |
| Rata-rata    | 12.53 | 10.94 | 10.70 | 10.20 | 2.89 | 2.65 | 2.64 | 2.66 |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of $\alpha = 5\%$.

Based on Table 5, upland rice, especially IPB 9 G, has a relatively low decrease in canopy dry weight compared to lowland rice. In addition, the two upland rice also have a relatively high response to root and canopy weight in dry conditions. However, IPB 9 G has a lower relative decline compared to Jatiluhur. The effect of drought was also very significant on the canopy weight and root weight characters (Table 5). Canopy weight has a greater average relative decline than root weight. This shows that when drought is gripped, all varieties maintain root growth relatively in order to supply more water for the crown. This was also stated by Yue et al. [38] and Deng et al. [27] stated that in drought, the reproductive phase of rice plants was more dominant in implementing avoidance adaptation mechanisms by increasing absorption and maintaining water loss.

Table 5 also showed that canopy dry weight and root weight of drought tolerant varieties (IPB 9...
G and Jatiluhur) have a higher weight than sensitive varieties (six other varieties). Afrianingsih [33] reported that the Salumpikit variety, which is a drought tolerant variety, has high dry canopy weight and dry root weight compared to other genotypes tested for drought stress. Genotypes with greater root weights during the dry season were more resistant to water shortages [38,39].

Drought stress has a significant effect on plant productivity in all varieties and growth phases of drought stress. The large relative weight reduction of pithy grain (Table 6) indicated that the weight of pithy grain could be an effective selection character in the drought stress of the generative phase, although the relative decrease does not reach an average of 50%.

The decreased weight of pithy grains in the generative phase showed a fairly high decrease, namely 34.24%, then the reproductive phase reached an average of 25.11% and an average of 18.26% in the vegetative phase. The reduction in weight of paddy rice in lowland rice was higher than that of lowland rice in all phases. This was also reported by Tubur et al. [10] and Yang et al. [16], drought stress treatment in the generative phase has a major impact on decreasing productivity. Drought stress has a very significant effect on plant productivity in all varieties and growth phases of drought stress. Upland rice varieties (IPB 9 G and Jatiluhur) show higher productivity than lowland rice varieties (six other varieties).

Table 6. Response to weight of pithy grain and productivity potential of eight rice varieties was in optimum condition (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018. Muneng Kidul, Probolinggo-East Java.

| Varieties   | Weight of pithy grain (g) | Productivity potential (t/ha) |
|-------------|---------------------------|-------------------------------|
|             | Dc | Dv | Dr | Dg | Dc | Dv | Dr | Dg |
| Jatiluhur   | 26.3 a | 20.5 a | 18.2 a | 16.9 a | 4.76 a | 4.24 a | 3.95 a | 3.23 a |
| IPB 9 G     | 22.9 b | 19.5 a | 17.9 ab | 16.7 a | 4.71 a | 4.26 a | 4.11 a | 3.36 a |
| IPB 3 S     | 22.0 bc | 17.7 b | 16.9 abc | 14.2 b | 4.20 bc | 3.17 bc | 3.13 b | 2.52 b |
| Ciharing    | 21.0 bcd | 17.6 b | 15.5 cd | 13.9 b | 4.58 a | 3.53 b | 3.09 b | 2.39 b |
| Inpari 17   | 19.5 d | 18.1 bcd | 13.1 d | 13.8 b | 3.40 e | 2.54 e | 2.45 c | 1.84 c |
| Mekongga    | 20.5 cd | 14.8 d | 14.5 d | 11.9 c | 4.54 ab | 3.31 bc | 2.84 bc | 2.20 bc |
| Mentik Wangi | 22.4 bc | 15.8 bc | 14.3 cd | 12.1 c | 3.73 d | 2.99 cd | 2.77 bc | 2.13 bc |
| Hipa 19     | 20.9 bcd | 17.2 cd | 16.2 bcd | 15.3 c | 4.12 c | 2.89 cd | 2.85 b | 2.33 bc |
| Average     | 21.9 | 17.9 | 16.4 | 14.4 | 4.39 | 3.46 | 3.28 | 2.89 |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of α = 5%. Potential productivity is calculated from the grain yield per plot weight * (10000: plot area), the grain yield per plot * 1600, the calculation results are converted to t/ha.

Drought stress research was conducted in the field and during the dry season showed that the productivity decline in the generative phase is greater than the reproductive phase and the vegetative phase. The reduction were 40% in the generative phase, 30% in the reproductive phase and 20% in the vegetative phase. Drought stress has resulted in decreased yields and components of rice yields [34,40]. The following is the performance of plants in the Muneng experimental garden (Figure 2). Research on drought stress in the field showed that plant morphophysiological responses are not only caused by water stress but also associated with high temperatures [34]. Rice plants are very sensitive to high temperature stress in the flowering phase so that it can cause pollen...
sterility to decrease production [42], while in the vegetative phase rice plants tend to be more tolerant. High temperatures can affect the growth and development of rice plants [43]. High temperature >33.7 °C in rice plants with a duration of 1 hour at the time of anthesis can reduce fertility in rice spikelets [44]. Yield decreases by 10% for every 1 °C increase in temperature [45]. The conditions of rainfall, temperature and humidity in the location of this study can be seen in Figure 1. Rainfall at the research location was very low, reaching 0 mm/day during the research.

Figure 2. Plant performance before drought stress treatment in the generative phase (a), plant performance after drought stress in the generative phase (b), in the Probolinggo-East Java. 2018.

3.3. Characteristics of water use efficiency in optimum conditions and drought stress

Table 7 showed that the Jatiluhur and IPB 9 G varieties have higher WUE values compared to other varieties in drought stress conditions in the field. The decrease in WUE due to dry stress occurred in the generative phase with an average of 41.4%, then the reproductive phase was 25.7% and the lowest decrease in WUE occurred in the vegetative phase by 13.8%. The lowest reduction in WUE occurred in the vegetative phase because plants had a longer recovery time after drought stress treatment. Table 7 also showed that it was consistent with the grain yield per plot of the vegetative phase which was higher than the generative and reproductive phases. Jatiluhur and IPB 9 G varieties had the highest WUE values in the three growth phases in the drought stress treatment.
**Table 7.** Character response of grain yield per plot and water use efficiency of eight rice.  
Dry season May–November 2018. Muneng Kidul, Probolinggo-East Java.

| Varietas       | Grain yield per plot (kg) | Water use efficiency (g/L) |
|----------------|--------------------------|----------------------------|
|                | Dc  | Dv  | Dr  | Dg  | Dc  | Dv  | Dr  | Dg  |
| Jatiluhur      | 2.95 a | 2.65 a | 2.47 a | 2.35 a | 0.66 a | 0.63 a | 0.55 a | 0.48 a |
| IPB 9 G        | 2.98 a | 2.66 a | 2.57 a | 2.42 a | 0.64 a | 0.63 a | 0.56 a | 0.51 a |
| IPB 3 S        | 2.62 bc | 1.98 bcd | 1.95 b | 1.58 b | 0.59 ab | 0.47 bc | 0.43 b | 0.32 b |
| Cihering       | 2.86 a | 2.21 b | 1.93 b | 1.50 bc | 0.61 a | 0.52 b | 0.42 b | 0.31 b |
| Inpari 17      | 2.12 e | 1.59 e | 1.53 c | 1.15 d | 0.45 c | 0.38 d | 0.33 c | 0.24 b |
| Mekongga       | 2.84 ab | 2.07 bc | 1.77 b | 1.37bcd | 0.61 a | 0.49 bc | 0.39 bc | 0.28 b |
| Mentik Wangi   | 2.33 d | 1.87 cd | 1.73 bc | 1.33 cdc | 0.52 bc | 0.45 bc | 0.38 bc | 0.27 b |
| Hipa 19        | 2.58 c | 1.81 cd | 1.78 b | 1.46 bc | 0.58 ab | 0.43 cd | 0.39 bc | 0.29 b |
| Rata-rata      | 2.66  | 2.10  | 1.97  | 1.78  | 0.58  | 0.50  | 0.43  | 0.34  |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of α = 5%. WUE = grain weight: water consumption.

Recent research related to WUE at optimal conditions in the eight varieties tested by Darmadi et al. 2019, namely IPB 9 G and Jatiluhur have the highest WUE compared to other varieties. Furthermore, the adaptation test of the eight varieties in drought-stressed conditions in the field showed that IPB 9 G and Jatiluhur also had the highest WUE compared to other varieties tested. Tubur et al. [10] reported varieties Jatiluhur shows a moderate drought response in several studies of drought stress periods based on the drought sensitivity index parameter.

**3.4. Physiological character on optimum conditions and drought stress**

Physiological characters are characters that have a better level of accuracy than morphological characters [34,46]. Based on Table 8, drought stress can also reduce the leaf greenness and leaf water potential. The decrease in the generative phase was 13.68% higher than the control. Different leaf color levels indicate differences in leaf pigment content including chlorophyll pigment, namely dark green leaves contain higher chlorophyll than leaves with light green or yellowish green color [27]. The chlorophyll content is related to the photosynthetic process which is then related to the seed filling process.

Table 8 showed the average leaf water potential of the control treatment (without drought stress) higher than the average of all drought stress treatments. The potential for leaf water when rice is experiencing drought stress has decreased [25,47]. Upland rice has a higher leaf water potential than lowland rice in the generative phase (Table 8). This shows that upland rice maintains air circulation in the leaves to improve its photosynthesis compared to lowland rice, so that the water potential has a lower decrease compared to lowland rice.

The photosynthetic rate response (Table 9) showed that drought stress affects the rate of photosynthesis in each growth phase. The control treatment had a higher photosynthetic rate than the treatment of all growth phases that were given drought stress. The reduction in the rate of photosynthesis compared to the control in the reproductive phase was higher, namely 15.13% compared to the generative phase 8.94% and 12.64% in the vegetative phase. Akram et al. [48] reported that drought stress caused a significant reduction on the rate of photosynthesis in all phases of plant growth.
Table 8. Response of leaf greenness and leaf water potential of eight rice varieties was in optimum condition (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg).

| Varieties     | Leaf greenness (unit) | Leaf water potential (MPa) |
|---------------|-----------------------|---------------------------|
|               | Dc        | Dv        | Dr        | Dg        | Dc        | Dv        | Dr        | Dg        |
| Jatiluhur     | 36.2 bc   | 33.6 d    | 33.8 bc   | 33.1 bc   | −2.1 a    | −3.0 a    | −2.6 a    | −2.7 a    |
| IPB 9 G       | 39.6 a    | 37.2 a    | 36.2 a    | 35.2 a    | −2.4 a    | −2.9 a    | −2.8 a    | −2.6 a    |
| IPB 3 S       | 39.0 a    | 36.8 ab   | 35.8 a    | 35.4 a    | −2.6 a    | −3.2 a    | −3.2 ab   | −3.5 b    |
| Ciherang      | 35.3 c    | 32.6 d    | 28.9 d    | 32.6 a    | −2.1 a    | −2.8 a    | −3.1 ab   | −3.1 ab   |
| Inpari 17     | 36.6 bc   | 35.4 bc   | 33.6 bc   | 31.6 c    | −2.9 a    | −3.1 a    | −3.2 ab   | −3.0 ab   |
| Mekongga      | 35.7 bc   | 33.7 bc   | 32.2 c    | 31.9 c    | −2.3 a    | −3.1 a    | −3.9 c    | −3.5 b    |
| Mentik Wangi  | 31.8 d    | 29.2 e    | 28.1 d    | 27.1 d    | −2.4 a    | −3.3 a    | −3.7 bc   | −3.3 ab   |
| Hipa 19       | 37.2 b    | 35.6 ab   | 34.9 ab   | 34.8 ab   | −2.3 a    | −2.8 a    | −3.0 a    | −3.3 ab   |
| Average       | 36.4      | 34.2      | 32.9      | 31.9      | −2.6      | −3.2      | −3.3      | −3.1      |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of α = 5%. Measurement of leaf greenness and leaf water potential of Dc and Dg at the same age 90 DAP. Phase Dr at the age of 54 DAP, phase Dv at the age of 39 DAP.

Table 9. The response rate of photosynthesis and transpiration rate of eight rice varieties was in optimum condition (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018. Muneng Kidul, Probolinggo-East Java.

| Varieties     | Photosynthesis rate (μmol CO₂ m⁻² s⁻¹) | Transpiration rate (μmol H₂O m⁻² s⁻¹) |
|---------------|----------------------------------------|--------------------------------------|
|               | Dc        | Dv        | Dr        | Dg        | Dc        | Dv        | Dr        | Dg        |
| Jatiluhur     | 33.2 a    | 30.8 a    | 27.8 c    | 34.0 a    | 11.7 a    | 12.7 a    | 12.2 ab   | 10.0 a    |
| IPB 9 G       | 34.0 ab   | 30.6 a    | 32.5 a    | 30.8 c    | 10.8 ab   | 12.5 a    | 12.2 ab   | 10.8 a    |
| IPB 3 S       | 35.8 a    | 30.0 a    | 30.5 ab   | 31.2 bc   | 9.4 ab    | 12.3 a    | 11.6 b    | 12.6 a    |
| Ciherang      | 35.7 a    | 31.3 a    | 30.9 ab   | 31.9 bc   | 11.0 a    | 11.6 a    | 15.9 a    | 11.3 a    |
| Inpari 17     | 33.7 ab   | 31.0 a    | 32.5 a    | 30.9 bc   | 10.9 ab   | 11.5 a    | 11.6 b    | 9.8 a     |
| Mekongga      | 35.6 a    | 30.8 a    | 29.4 bc   | 33.1 ab   | 7.2 b     | 12.2 a    | 11.6 b    | 10.8 a    |
| Mentik Wangi  | 35.7 a    | 29.3 a    | 30.7 ab   | 31.4 bc   | 8.6 ab    | 11.0 a    | 11.2 b    | 11.5 a    |
| Hipa 19       | 35.1 ab   | 29.3 a    | 27.1 c    | 33.3 ab   | 11.8 a    | 12.1 a    | 11.4 b    | 9.7 a     |
| Average       | 34.8      | 30.4      | 30.2      | 32.1      | 10.2      | 12.0      | 12.2      | 10.8      |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of α = 5%. Measurement of photosynthetic rate and transpiration rate of Dc and Dg at the same age 90 DAP. Phase Dr at the age of 54 DAP, phase Dv at the age of 39 DAP.

Transpiration rate response during drought stress (Table 9) showed that different transpiration rates occur according to the plant growth phase. The highest increase in transpiration rate was in the reproductive phase of the pregnant plant condition (bothing phase), namely 16.4%, then the vegetative phase was 15.0% and the lowest was the generative phase, 5.5%. Table 9, showed that the transpiration rate of the generative phase during drought stress has decreased compared to the optimum conditions (Dc). The decrease in transpiration rate as a form of adaptation of plants to
drought stress is by closed and reducing the number of stomata so that the transpiration rate can be reduced [49].

Another physiological character that has been identified is total chlorophyll. Based on Table 10, the effect of drought significantly reduced the total chlorophyll of all varieties. Total chlorophyll is a general physiology that is carried out against stresses, including drought.

**Table 10.** The mean and standard deviation of total chlorophyll content of eight rice varieties at optimum conditions (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018.

| Varieties | Dc   | Dv       | Dr       | Dg       |
|-----------|------|----------|----------|----------|
| Jatiluhur | 0.14 ± 0.0 b | 0.97 ± 0.3 a | 0.99 ± 0.4 c | 0.09 ± 0.1 a |
| IPB 9 G   | 0.15 ± 0.1 b | 0.80 ± 1.0 ab | 1.10 ± 0.4 c | 0.10 ± 0.1 a |
| IPB 3 S   | 0.18 ± 0.2 b | 0.75 ± 0.5 ab | 2.06 ± 0.1 a | 0.12 ± 0.0 a |
| Cihering  | 0.36 ± 0.1 b | 0.10 ± 0.0 b  | 1.19 ± 1.1 bc | 0.10 ± 0.1 a |
| Inpari 17 | 0.40 ± 0.1 b | 0.36 ± 0.3 ab | 1.15 ± 1.1 bc | 0.04 ± 0.0 a |
| Mekongga  | 0.58 ± 0.7 ab | 0.32 ± 0.2 ab | 0.85 ± 0.6 c  | 0.48 ± 0.6 a |
| Mentik Wangi | 1.31 ± 0.7 a | 1.00 ± 0.7 a  | 1.88 ± 0.3 ab | 0.24 ± 0.1 a |
| Hipa 19   | 0.25 ± 0.2 b | 0.62 ± 0.5 ab | 1.28 ± 0.9 bc | 0.02 ± 0.0 a |
| Average   | 0.42 | 0.61     | 1.31      | 0.15      |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of α = 5%. Chlorophyll analysis for total Dc and Dg was carried out at the age of 90 DAP, while the Dr treatment was at the age of 75 DAP and Dv at the age of 60 DAP.

The generative phase shows a higher level of drought than the other phases. The decrease response for each variety was different depending on the growth phase. The data table 10 showed that varieties that have low total chlorophyll produce high productivity in Jatiluhur and IPB 9 G varieties. There are other factors that are more influential, such as genetic factors [50] or the environment [51].

Variety with the lowest decrease in chlorophyll content was Jatiluhur, but this reduction pattern was not followed by IPB 9 G as an upland rice variety with Jatiluhur. The decrease in total chlorophyll content can be compared between drought stress at the generative phase and control because they have the same age at the time of the drought stress treatment. Table 10 showed the decrease in total chlorophyll content in all varieties in the generative phase. Lowest decrease was in the Hipa 19 variety of 92.0%. The decrease in total chlorophyll content of all varieties was an average of 64.3%. The decline in upland rice varieties was 34.4% on average, then in the lowland type, the decline was 67.5%. Response of varieties to total chlorophyll during stress shows that this parameter is very responsive to unfavorable environmental changes. This shows that chlorophyll content can be a characteristic character of drought tolerance in this study.

Physiological character of proline is also a character that is often identified when plants experience drought [14,15,47]. Based on Table 11, giving drought stress show a very real response to each rice variety. Provision of drought stress at all phases growth was able to increase proline drastically. Table 11 showed that the proline content of the control treatment is relatively low compared to stress treatment at various phases of drought stress. Guo et al. [52] stated that plant
physiological responses to drought stress occur together with a decrease in osmotic potential, then the accumulation of proline and betaine increases in roots and shoots. This shows that the accumulation of proline is getting higher due to drought stress. Lum et al. [53] reported a drastic increase in proline during drought stress up to 10 times normal. Therefore, this character can characterize the tolerance between upland rice and paddy fields. Leaf proline content has increased due to drought stress [54,55].

Table 11. Response to the proline content and MDA content of eight rice varieties at optimum conditions (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018.

| Varieties | Proline content (μmol/g) | MDA content (μmol/g) |
|-----------|--------------------------|----------------------|
|           | Dc | Dv | Dr | Dg | Dc | Dv | Dr | Dg |
| Jatiluhur | 17.59 a | 41.84 a | 47.50 a | 64.62 a | 1.34 d | 1.04 d | 1.73 d | 1.96 c |
| IPB 9 G   | 14.86 ab | 39.78 a | 38.10 b | 51.43 b | 1.62 d | 1.13 d | 1.92 d | 2.18 c |
| IPB 3 S   | 7.95 c | 8.78 d | 6.30 d | 5.34 c | 2.84 a | 3.10 c | 2.94 c | 3.20 b |
| Ciherang  | 7.60 c | 14.10 c | 12.57 d | 5.23 c | 2.70 ab | 3.03 b | 3.91 ab | 3.43 ab |
| Inpari 17 | 8.22 c | 11.13 cd | 17.64 c | 11.22 c | 2.39 bc | 3.61 b | 3.74 b | 3.72 a |
| Mekongga  | 13.71 ab | 14.94 c | 8.62 d | 9.98 c | 2.20 c | 3.94 ab | 3.58 b | 3.49 ab |
| Mentik Wangi | 14.84 ab | 19.00 b | 6.60 d | 7.57 c | 2.82 a | 2.94 c | 2.83 c | 3.23 b |
| Hipa 19   | 11.11 bc | 8.14 d | 6.80 d | 8.64 c | 2.62 ab | 4.21 a | 4.13 a | 3.16 b |
| Average   | 11.98 | 19.71 | 18.01 | 20.50 | 2.32 | 2.88 | 3.07 | 3.05 |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of α = 5%.

The last physiological character observed was the content of malondialdehyde (MDA). This character is closely related to the level of reactive oxide species (ROS) in plant tissue [27]. Based on Table 10, drought stress gave significantly different levels of MDA. In general, all varieties experienced an increase in ROS, except for IPB 3 S and Ciherang. Meanwhile, Jatiluhur and IPB 9 G had a low increase in MDA content, but Hipa 19 as a lowland rice variety also experienced a relatively high increase. This makes the MDA content specific as a characteristic of drought stress. MDA content is the main response to abiotic stress, namely aluminum stress [56], NaCl stress [17], and drought stress [34,57].

The oxidative damage of lipids was evaluated by measuring the changes in malondialdehyde (MDA) content [58]. MDA character have complex arrangements in rice and are controlled by many genes [29,56]. This study confirms that the character of MDA affects rice yields under drought stress, especially negative correlation to productivity and efficiency of water use (Table 13). Malondialdehyde (MDA) is the end product of lipid peroxide and its presence can indicate the level of oxidative stress that occurs in plants. The degree of root cell damage due to lipid peroxide is different for each species, even for each variety within one species. Varieties that were sensitive to drought stress experienced higher lipid peroxidation than tolerant varieties [59]. Based on all physiological character, including a relative decrease in leaf water potential and an increase in the relative content of proline can be a characteristic between the two types of rice. In addition, chlorophyll can also be used as a tolerance character for varieties.
3.5. Performance of root anatomical characters in optimum conditions and drought stress

Roots are the part that changes during drought stress. Following figure shows the anatomical appearance of plant roots in optimum conditions and drought (Figure 3). The role of roots during drought is very important to absorb water and nutrients from the soil [60].

![Figure 3](image)

Note: Diameter of stele (i), thickness of cortex (ii) and xylem (iii).

**Figure 3.** Root anatomy of control plants/without stress (a), roots are stressed by drought (b).

The root response when there is drought is anatomical and physiological changes [61]. The cross-section of the roots of rice plants in optimum conditions (control) is circular, while the roots of plants that are affected by drought are flat. Research conducted by Rosawanti et al. [60], soybean plants that were stricken with drought showed a change in the cross-sectional shape of the roots in varieties that were gripped by drought. Changes that occur in these roots are a decrease in stele diameter, cortex thickness and diameter during drought stress.

3.6. Identification of drought tolerance of eight rice varieties

Drought tolerance identification is a standard response that is carried out to identify the nature of plant tolerance to drought stress [11,13]. Based on Table 1, almost all lowland rice showed a sensitive response to drought stress, on the other hand upland rice has a better tolerance level than lowland rice. This shows that the difference in tolerance response between upland rice and lowland rice is highly determined by the ecology of growth [32].

Table 12 showed the different DSI values according to the plant growth phase. The response of Jatiluhur and IPB 9 G varieties in the reproductive and generative phases gave a moderate response, then the vegetative phase gave a tolerant response. This shows the variety response according to the phase when stress occurs, IPB 9 G and Jatiluruh are varieties that have the best tolerance levels compared to other varieties. The stress that occurs in the vegetative phase gives time for plants to recover after drought. The Mentik Wangi variety gave a moderate response to the stress of the vegetative phase. Mentik Wangi is a local variety that has several characters that support recovery, namely total chlorophyll content, photosynthesis rate, high proline content, shoot weight and root weight. Furthermore, the Mentik Wangi variety has a low transpiration rate and flowering age compared to varieties belonging to the sensitive group. DSI correlation with morphophysiological characters is presented in Table 13. The correlation value between DSI and WUE is -0.7 and has a
very significant effect. This shows that the more tolerant the variety is, the smaller the DSI value, the higher the WUE value, meaning that the variety is more efficient in using water. Furthermore, DSI measurement is used to assess the reduction in yield in the suboptimal environment compared to the optimum environment [18]. A low DSI value indicates that the varieties tested at sub-optimum conditions did not show a large decrease, so it can be said that the variety was tolerant [13]. Water stress will increase the efficiency of water use although slightly reduce yields [62].

**Table 12.** The average drought sensitivity index (DSI) of eight rice varieties at optimum conditions (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018

| Varieties       | Drought sensitivity index (unit) | Dv Response | Dr Response | Dg Response |
|-----------------|--------------------------------|-------------|-------------|-------------|
|                 |                                | Dv          | Dr          | Dg          |
| Jatiluhur       | 0.48 b                         | Tolerant    | 0.78 bc     | 0.95 b      | Moderate    |
| IPB 9 G         | 0.50 b                         | Tolerant    | 0.65 c      | 0.87 b      | Moderate    |
| IPB 3 S         | 1.20 a                         | Sensitive   | 1.22 ab     | 1.90 a      | Sensitive   |
| Ciherang        | 1.07 ab                        | Sensitive   | 1.57 a      | 2.28 a      | Sensitive   |
| Inpari 17       | 1.18 a                         | Sensitive   | 1.32 ab     | 2.18 a      | Sensitive   |
| Mekongga        | 1.31 a                         | Sensitive   | 1.77 a      | 2.46 a      | Sensitive   |
| Mentik Wangi    | 0.90 ab                        | Moderate    | 1.20 ab     | 2.02 a      | Sensitive   |
| Hipa 19         | 1.43 a                         | Sensitive   | 1.50 a      | 2.01 a      | Sensitive   |
| Average         | 1.04                           | 1.11        | 1.18        |             |

Note: The numbers followed by the same letter in the same column show no significant difference based on the DMRT test at the level of α = 5%. The size of the plot is 2.5 m x 2.5 m. DSI based on grain yield per plot.

The characters were chosen for the DSI value test were plot weight characters (Table 12). The highest correlation between various characters and production was the plot weight character and water use efficiency of 1.0 and of WPG 0.8 had a very significant effect at the 0.01 level. The results of the DSI calculation on the tested characters can be used as a reference for determining drought tolerance.

### 3.7. Correlation and path analysis between morphophysiological characters on rice production

The correlation value that affects the character of production is divided into two, i.e., negative and positive correlation. The character that have a negative correlation with rice production are MDA, DSI, FA50, TR, CT and WC with values of −0.7, −0.7, −0.2, −0.1, −0.1 and −0.1 respectively. The character of MDA has a negative effect on rice production because in drought conditions rice experiences a reactive oxygen species (ROS) reaction in the tissue. This situation is then indicated by the lipid peroxidation value on the membrane and further makes the MDA value relatively high. High MDA content will decrease in rice production. The DSI character has a negative effect on rice production during drought because in drought conditions rice has increased leaf temperature. The increase in leaf temperature can then disrupt the metabolic processes in the processes of photosynthesis and respiration and ultimately make rice more sensitive to drought. A high DSI value will further result in a low production of rice. FA50 character has a negative effect on production, namely late flowering due to drought stress has an effect on production. Rice plants try to be...
adaptive to drought stress by extending the vegetative phase of the plant so that flowering will be late. Sujinah et al. [63] reported that the longer the flowering period, the less grain yields. The growing age of flowering causes plants to run out of energy to produce optimally due to drought. TR, CT and WC have a negative effect on production due to drought-stressed plants. When drought is gripped, there is a decrease in the transpiration rate and chlorophyll content as a plant physiological mechanism to reduce water loss and survive with limited chlorophyll content. The decrease in transpiration rate and chlorophyll content causes the rate of photosynthesis to decrease, then the assimilate produced decreases so that production will also decrease. WC has a negative effect on production because plants need more water to survive drought-stressed conditions. The rate of growth is stunted and all metabolic processes of the plant are interrupted, which ultimately results in plant productivity which drops dramatically compared to optimal conditions.

On the other hand, character that have a positive correlation with rice production factors are WUE, GYP, DCW, RL, DRW, PH and NPT (Table 13). The agronomic character that greatly influence the production value are PW and WUE. The weight of the ubinan directly affects the value of rice production in drought conditions. The results of the correlation analysis showed that the morphological characters had a positive and very significant effect on the weight of plot with water use efficiency (r = 1.0 **, P <0.01), grain yield per plot (r = 0.80 **, P <0.01 ), dry canopy weight (r = 0.70 **, P <0.01), root length (r = 0.6 **, P <0.01), dry root weight (r = 0.6 **, P <0.01), plant height (r = 0.3 **, P <0.01)), the number of productive tillers (r = 0.2 **, P <0.01). The higher the plant, the higher production. However, plant height is not included in the selection of plants to increase yield because it is related to lodging plant [64]. Physiological characters had a positive and very significant effect on the potential characters of leaf water (r = 0.6 **, P <0.01), leaf greenness (r = 0.5 **, P <0.01), proline (r = 0.5 **, P <0.01), photosynthesis rate (r = 0.4 **, P <0.01). An important physiological response is the ability of plants to maintain turgor pressure by reducing the osmotic potential as a tolerance mechanism to drought stress [54]. Factors that can help maintain turgor in times of drought stress are a decrease in osmotic potential and the ability to accumulate dissolved compounds, namely sugars and amino acids, especially proline [65].

Drought stress in rice affects plant morpho-physiology and has further implications for production values [13,29]. The physiological characters of plants can be analyzed using two approaches, namely path analysis and correlation between various characters [66]. Path analysis is an analysis that can determine the main character that directly affect productivity. Path analysis is used to identify components that have a direct or indirect impact on the character of rice yields [67,68]. Physiological character can be divided into two influences on path analysis, namely direct and indirect effects. Path analysis to partition the results of the correlation into direct and indirect effects [69]. The high direct effect indicates the magnitude of the proportion of the influence of these characters on the diversity of the main characters. Path analysis was conducted on character that were correlated with productivity both on morphological, physiological, production and other characters that were thought to have an effect on productivity. If a variable has a low coefficient value of direct influence on results, it is necessary to pay attention to the value of the influence of these variables on results indirectly through other variables. Drought stress in this study affected the morphological characters of rice and ultimately affects productivity. Based on the path analysis, it showed that the morphological characters of AF50, WUE, PH, DRW and WPG have a significant direct effect on productivity (Table 14). The productivity of eight varieties were directly influenced by the characters of WUE (7.21), FA50 (7.80), PH (4.79), DRW (2.18) and WPG (0.85).
Table 13. Pearson correlation of morphological characters, production, water use efficiency and physiology of eight rice varieties to drought stress at optimum conditions (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018. Muneng Kidul, Probolinggo-East Java.

| Character | LG  | LWP  | PR    | CT    | TR   | Prolin | MDA   | PH   | FA50 | NPT | WPG   | RL | DRW   | DCW | WC   | WUE | DSI | YGP | Prod |
|-----------|-----|------|-------|-------|------|--------|-------|------|------|-----|-------|----|-------|-----|------|-----|-----|-----|------|
| LG        |     |      |       |       |      |        |       |      |      |     |       |    |       |     |      |     |     |     |      |
| LWP       | 0.4** |      |       |       |      |        |       |      |      |     |       |    |       |     |      |     |     |     |      |
| PR        | 0.3** | 0.5** |     |       |      |        |       |      |      |     |       |    |       |     |      |     |     |     |      |
| CT        | −0.1 | −0.2 | −0.3** |       |      |        |       |      |      |     |       |    |       |     |      |     |     |     |      |
| TR        | −0.1 | −0.2** | −0.4** | 0.2** |     |        |       |      |      |     |       |    |       |     |      |     |     |     |      |
| Prolin    | 0.1  | 0.2** | −0.1 | 0.0   | 0.0  |        |       |      |      |     |       |    |       |     |      |     |     |     |      |
| MDA       | −0.4** | −0.4** | −0.2** | 0.0  | 0.0  | −0.6** |      |      |      |     |       |    |       |     |      |     |     |     |      |
| PH        | 0.2* | 0.4** | 0.6** | −0.4** | −0.3** | 0.1 | −0.3** |     |      |      |     |       |    |       |     |      |     |     |     |      |
| AF50      | −0.2** | −0.4** | −0.6** | 0.5** | 0.4** | 0.0 | 0.2** | −0.9** |     |      |      |     |       |    |       |     |      |     |     |     |      |
| NPT       | −0.1 | 0.3** | 0.3** | 0.0  | 0.0  | −0.2* | 0.1  | −0.2* | 0.1 |     |       |    |       |     |      |     |     |     |      |
| WPG       | 0.6** | 0.7** | 0.4** | 0.0  | −0.1 | 0.1  | −0.6** | 0.3** | −0.3** | 0.3** |     |       |    |       |     |      |     |     |     |      |
| RL        | 0.5** | 0.5** | 0.3** | −0.1 | 0.0  | 0.3** | −0.7** | 0.5** | −0.4** | −0.1 | 0.7** |     |    |       |     |      |     |     |     |      |
| DRW       | 0.2** | 0.4** | 0.2** | 0.1  | −0.1 | 0.5** | −0.7** | 0.4** | −0.3** | −0.2 | 0.6** | 0.8** |    |       |     |      |     |     |     |      |
| DCW       | 0.4** | 0.7** | 0.6** | −0.2 | −0.2* | 0.2* | −0.6** | 0.5** | −0.5** | 0.2 | 0.7** | 0.6** | 0.5** |     |       |     |     |     |      |
| WC        | −0.2 | 0.0  | 0.3** | −0.3** | −0.2* | 0.0 | 0.0  | 0.6** | −0.6** | −0.2* | −0.2* | −0.1 | 0.0 | 0.1 |     |     |     |     |      |
| WUE       | 0.5** | 0.6** | 0.3** | 0.0  | −0.1 | 0.5** | −0.7** | 0.1 | −0.1 | 0.2* | 0.8** | 0.6** | 0.6** | 0.6* | −0.3** |     |     |     |      |
| DSI       | −0.4** | −0.7** | −0.5** | 0.1  | 0.1  | −0.3** | 0.6** | −0.4** | 0.4** | −0.2* | −0.7** | −0.6** | −0.6** | −0.7** | 0.0 | −0.7** |     |     |     |      |
| YGP       | 0.5** | 0.6** | 0.4** | −0.1 | −0.1 | 0.5** | −0.7** | 0.3** | −0.2* | 0.2 | 0.8** | 0.6** | 0.6** | 0.7** | −0.1 | 1.0** | −0.7** |     |     |      |
| Prod      | 0.5** | 0.6** | 0.4** | −0.1 | −0.1 | 0.5** | −0.7** | 0.3** | −0.2* | 0.2 | 0.8** | 0.6** | 0.6** | 0.7** | −0.1 | 1.0** | −0.7** | 1.0** |     |      |

Note: LG: leaf greenness, LWP: leaf water potential, PR: photosynthesis, CT: total chlorophyll, TR: transpiration rate, MDA: malondialdehyde, PH: plant height, FA50: 50% flowering age, NPT: number of productive tillers, WPG: weight of pithy grain, RL: root length, DRW: dry root weight, DCW: dry canopy weight, WC: water consumption per seasonal plant, WUE: water use efficiency per plant, DSI: drought sensitivity index, YGP: yield grain per plot, Prod: potential productivity based on the weight of the plots.
Table 14. Path analysis of morphological characters and water use efficiency on the productivity of eight rice varieties to drought stress at optimum conditions (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018. Muneng Kidul, Probolinggo-East Java.

| Character | DE | PH | FA50 | NPT | WPG | RL | DRW | DCW | WC | WUE |
|-----------|----|----|------|-----|-----|----|-----|-----|----|-----|
| PH        | 4.79 | −4.68 | 0.79 | 0.70 | −9.93 | 1.85 | −1.97 | 5.20 | 3.68 |
| FA50      | 7.80 | −2.87 | −0.39 | −0.34 | 7.11 | −1.33 | −3.60 | −7.36 | 0.58 |
| NPT       | −0.96 | −3.97 | 3.20 | −0.44 | 5.72 | −1.22 | 3.60 | −2.54 | −3.89 |
| WPG       | 0.85 | 3.97 | −3.12 | 0.50 | −9.00 | 1.68 | −5.11 | 6.22 | 4.54 |
| RL        | −11.68 | 4.07 | −5.15 | 0.47 | 0.65 | 1.79 | −1.28 | 8.88 | 2.45 |
| DRW       | 2.18 | 4.07 | −4.76 | 0.54 | 0.65 | −9.58 | −0.70 | 5.71 | 2.09 |
| DCW       | −11.61 | 0.81 | 2.42 | 0.30 | 0.37 | −1.29 | 0.13 | 4.57 | 4.90 |
| WC        | −12.69 | −1.96 | 4.53 | −0.19 | −0.41 | 8.18 | −0.98 | 4.18 | −0.50 |
| WUE       | 7.21 | 2.44 | 0.62 | 0.52 | 0.53 | −3.97 | 0.63 | −7.89 | 0.89 |

Residual effect: 0.15. Note: DE: direct effect, PH: plant height, AF50: flowering age 50%, NPT: number of productive tillers, WPG: weight of pithy grain, RL: root length, DRW: dry root weight, DCW: dry canopy weight, WC: water consumption, WUE: efficient use of water.

In this study, there is new information that rice productivity in drought-stressed conditions can be directly affected by the flowering age of 50%, the value of water use efficiency, plant height, root weight and weight of pithy grains.

Table 15. Path analysis of physiological characters on the productivity of eight rice varieties to drought stress at optimum conditions (Dc), vegetative phase drought (Dv), reproductive phase drought (Dr), and generative phase drought (Dg). Dry season May–November 2018. Muneng Kidul, Probolinggo-East Java.

| Character | DE | LG | PR | CT | TR | Proline | MDA |
|-----------|----|----|----|----|----|---------|-----|
| LG        | −0.06 | −0.50 | 0.15 | 0.16 | 0.02 | 0.58 |
| PR        | 1.65 | 0.02 | −0.08 | −0.26 | 0.04 | −1.43 |
| CT        | −0.18 | 0.05 | 0.73 | −0.27 | −0.02 | −0.80 |
| TR        | 0.44 | −0.02 | −0.99 | 0.11 | 0.01 | 0.55 |
| Proline   | −0.08 | 0.01 | −0.69 | −0.03 | −0.04 | 1.24 |
| MDA       | −1.77 | 0.02 | 1.34 | −0.08 | −0.14 | 0.06 |

Residual effect: 0.04. Note: DE: direct effect, LG: leaf greenness, PR: photosynthesis rate, CT: total chlorophyll, TR: transpiration rate, MDA: malondialdehyde.

Bezerra et al. [51] reported similarities between path analysis and genetic correlations for flowering age and 100 grain weight related to productivity. In addition, the high WUE character indicates that in drought conditions the plants are more efficient in using water. High water use efficiency has a direct effect on the productivity value. This shows that the drought tolerant plants use water efficiently during drought stress. Based on Table 15, path analysis showed that the
physiological characteristics of PR (1.65) and TR (0.44) have a significant direct effect on productivity. Physiologically, plant productivity can still be maintained during drought stress with a reduced transpiration rate with more optimal stomatal closure than under normal conditions. Water stress directly affects the rate of photosynthesis due to decreased CO₂ availability due to stomatal closure [70].

4. Conclusion

Upland rice has a better level of drought tolerance and water use efficiency compared to lowland rice. The more tolerance of rice to drought stress shows a correlation with the level of efficiency of water use in three growing phases. The drastic decrease in the value of water use efficiency was due to a very drastic yield reduction during drought-stressed conditions while water consumption remained high. Furthermore, physiological characters, such as proline content, malondialdehyde content, photosynthetic rate, transpiration rate, leaf greenness and total chlorophyll could describe the physiological mechanism on variety tolerant response to drought stress.

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Conflict of interest

All authors declare that have no conflict of interest in this paper.

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