Vol. 14, No. 4 (2025): 1148 - 1155 https://doi.org/10.23960/jtepl.v14i4.1148-1155

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Morpho-Physiological Responses of Purbalingga and Purwokerto Local Black Rices to Drought Stress

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Article History:

Received : 15 August 2024
Revised : 08 October 2024
Accepted : 10 December 2024

Keywords:

Black Rice,
Drought Stress,
Morphological Response,
Physiological Response.

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ABSTRACT

Black rice (Oryza sativa L. indica) is a pigmented rice plant that has high antioxidant content. Drought is an abiotic stress that can inhibit the growth and productivity of rice plants. Planting of local black rice with several drought treatments using PEG 6000 was observed in this study. The aim of this research is to determine the morphological and physiological response of local black rice plants in Purbalingga and Purwokerto at various levels of drought stress. This research used a Completely Randomized Design (CRD) with 2 factors. The first factor is 2 local black rice plants, namely Purbalingga and Purwokerto. The second factor was drought stress treatment via PEG 6000 with 4 treatments, namely 0% PEG (control), 5%, 10%, and 15%. The results showed that when applying 15% PEG, local black rice in Purbalingga and Purwokerto experienced a decrease in plant height and number of leaves but increased root length. Based on the physiological response, when addition 15% PEG there was a decrease in the amount of chlorophyll and an increase in the content of H_2O_2 *and anthocyanins in both Purbalingga and Purwokerto local black rice.*

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of crop commodities that serves as the main food source for the Indonesian population to meet their daily needs. The increasing population in Indonesia has led to a corresponding rise in the demand for rice consumption. Black rice is one kind of rice that is getting more popular recently and consumed as functional food due to the usefulness to health. Black rice is one of pigmented rice varieties highly beneficial for health, primarily due to antioxidants properties. It contains several bioactive components including phytochemicals and anthocyanins (Zahroh & Agustini, 2021). Black rice has a higher fiber content than white rice. Black rice containing fiber 20.1g/100g (Nurhidajat *et al*., 2018), meanwhile white rice contains fiber 2.4g/100g (Kristamtini *et al*., 2014). Additionally, black rice has carbohydrate content of 76.9g/100g (Kristamtini *et al*., 2014), while white rice contains carbohydrate of 77 to 78 g/100g (Salah *et al*., 2019).

Unpredictable season changes and extreme conditions like prolonged drought causes plants has a stress. Drought is an abiotic stress that affects plants when they receive inadequate water, leading to reduced rice productivity. Drought stress can trigger a range of morphological and physiological changes in rice plants at various growth stages (Kim *et al*., 2020). Plant responds, adapt, and survive under drought stress through the regulation of various morphological, physiological, and biochemical responses. The plant's response to drought stress begins with physiological adjustments such as reducing of transpiration to conserve water by closing stomata and reducing leaf surface area (Sujinah & Jamil, 2016).

Drought stress can lead to the production of reactive oxygen species (ROS) such as hydrogen peroxide, which can damage cell membrane phospholipids. This cellular damage caused by ROS can be mitigated by regulating cell osmotic potential through the accumulation of antioxidant compounds. The high content of phenolic compounds and antioxidants in black rice helps protect the plant from drought stress, enabling it to adapt to its environment. One of the antioxidant compounds found in black rice is anthocyanin. Anthocyanins are water-soluble pigments naturally present in various plant species, particularly those with pigmentation (Sholikhah *et al*., 2019). The accumulation of anthocyanins, as one of the antioxidant compounds in plants, serves as a protective mechanism against drought stress (Kaur *et al*., 2022).

One method to assess the impact of drought stress on black rice involves the use of PEG 6000. Polyethylene glycol (PEG) can decrease the water potential in a nutrient solution, thereby inducing drought conditions in plants. PEG 6000 has a high capacity to absorb water, which can lead to plant cell dehydration, making it suitable for mimicking drought stress in plants (Murillo-Amador *et al*., 2002). Therefore, it is important to conduct research to determine the effects of drought stress on the morphological and physiological responses of local black rice varieties from Purbalingga and Purwokerto.

2. MATERIALS AND METHOD

2.1. Plant Materials

The research conducted in UPA Taman Agroteknologi and UPA Pengelolaan Limbah dan Laboratorium Terpadu, Jember University, spanned from October 2023 to March 2024. Two black rice local accessions from Central Java namely Purbalingga and Purwokerto were used in this experiment.

2.2. Experimental Design

Plant materials, growth conditions and drought treatments: Seeds of Purbalingga and Purwokerto local black rices. The seeds were planted in pot with 28 cm diameter. Each pot contained 10 kg of soil. A factorial randomized complete design with three replications was used to facilitate the combination of two factors. The first factor was two accessions of black rices (Purbalingga and Purwokerto) and second factor was PEG application with four levels (Control, 5%, 10%, and 15%). The different drought stress levels were obtained by dissolving PEG 6000 in distilled water. The control was maintained using distilled water only. The drought stress treatment on rice plants was conducted starting at 38 days after planting (DAP). The drought stress treatment lasted for 7 days (International Network for Genetic Evaluation of Rice, 1996).

2.3. Observation of Morphological Characters

Plant morphology characters, i.e. plant height, number of leaves, and root length were observed on 3 days after treatment. Plant height and root length was measured by ruler.

2.4. Analysis of chlorophyll content

As much as 0.03 g of fresh rice leaves cut into pieces and leaving the leaf veins. The samples were extracted using 96% ethanol and allowed to stand for 24 h and was measured by spectrophotometer under a wavelength 649 nm and 665 nm. Chlorophyll calculation was conducts using the formula by Wintermans & De Mots (1965):

$$C = (20.2 \times \text{Abs } 649) - (6.1 \times \text{Abs } 665) \quad (1)$$

2.5. Analysis of Hydrogen Peroxide

As much as 0.1 g fresh rice leaves homogenized with 1 mL of 0.1% Trichloroacetic acid (TCA), and then centrifuge at 12.000 rpm for 10 minutes at 4°C and collect 0.5 mL of the supernatant solution and transferring it to a 1.5 mL microtube. Add 0.5 mL of 10mM phosphate buffe pH 7 and 1 mL of 1M potassium iodide and incubate at room temperature for 30 minutes. Measured the sample by spectrophotometer at a wavelength of 390 nm. Determine the hydrogen peroxide concentration based on the standard curve values obtained from various dilutions.

2.6. Analysis of Anthocyanin Content

As much as 0,1 g of fresh rice leaves were ground in a mortar, homogenized with HCl and Ethanol in a 15:85 (v/v) and then centrifuge at 12,000 rpm for 10 minutes at 4°C. Divide the supernatant into two samples. The first sample add KCl for pH 1.0 and second sample add Sodium Acetate Monohydrate for pH 4.5. Then, the solution of different pH conditions was measured by using spectrophotometer at a wavelength of 520 nm and 700 nm. The total anthocyanin content is calculated using the following formula:

$$\text{Total anthocyanin} = (A \times MW \times DF \times 1000)/(E \times 1) \quad (2)$$

where: A = (A520nm – A700nm) pH 1 – (A520nm – A700nm) pH 4.5; E = molar extinction coefficient (26,900 L/mol/cm); MW = molecular weight (449.2 g/mol); and DF = dilution factor

2.7. Data Analysis

The collected data were analyzed and the differences between treatments were tested using Analysis of Variance (ANOVA). If significant differences were found, data were analyzed by Duncan Multiple Range Test (DMRT) at a 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Plant Height

Plants in drought conditions will exhibit responses, one of which is by changing their morphological structure to increase water use efficiency through the regulation of transpiration rates (Sujinah & Jamil., 2016). This indicates that when plants cell has sufficient water intake it allows for cell elongation compared to plants with limited water intake. Plants receiving limited water supply has shorter height more than plants that receiving adequate water supply. This aligns with results in Figure 1 showing that increasing concentration of PEG in the growth medium resulted in higher

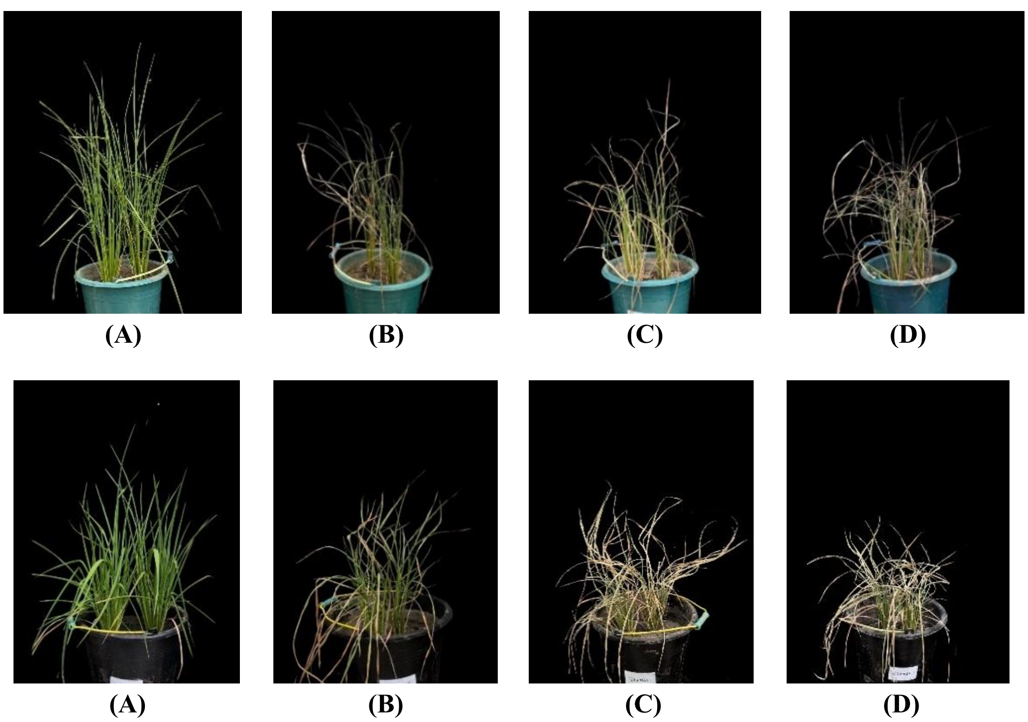

Figure 1. Morphological responses of Purbalingga (top) and Purwokerto (bottom) local black rices during drought stress treatment: (A) Control, (B) PEG 5%, (C) PEG 10%, and PEG 15% (D).

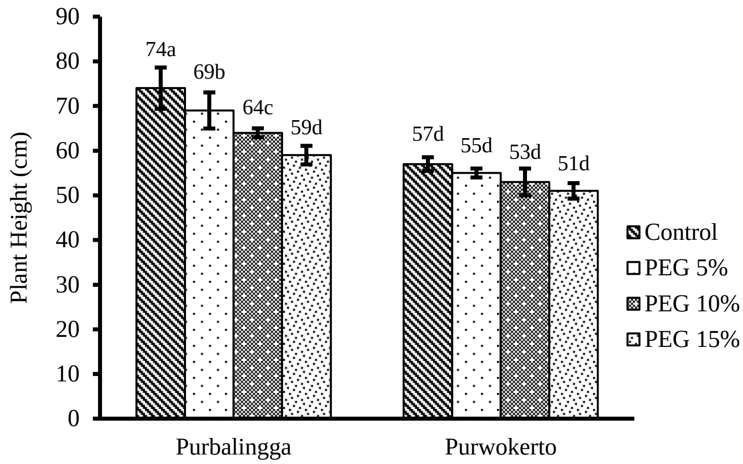

Figure 2. Plant height for Purbalingga and Purwokerto local black rice at various levels of drought stress, followed by notation indicating significance in DMRT at 5%.

osmotic pressure, so it can inhibit the growth of rice plants. Based on the analyzed research results, the interaction of application PEG 6000 and variety have a significant effect on plant height. From Figure 2 it is seen that the control treatment in the both of local black rice varieties shows the highest plant height, which is 74 cm for the Purbalingga variety and 57 cm for the Purwokerto variety. Purbalingga variety has decrease 0.79% the plant height to 59cm on the treatment of PEG 15%. Purwokerto variety has decrease 0.89% the plant height to 51cm on the treatment of PEG 15%. It relevant with research from Pandey & Shukla (2015), that water deficiency in plants can alter membrane permeability, reduce cell turgidity, inhibit cell division, and cell enlargement so it consequently affecting in plant height growth.

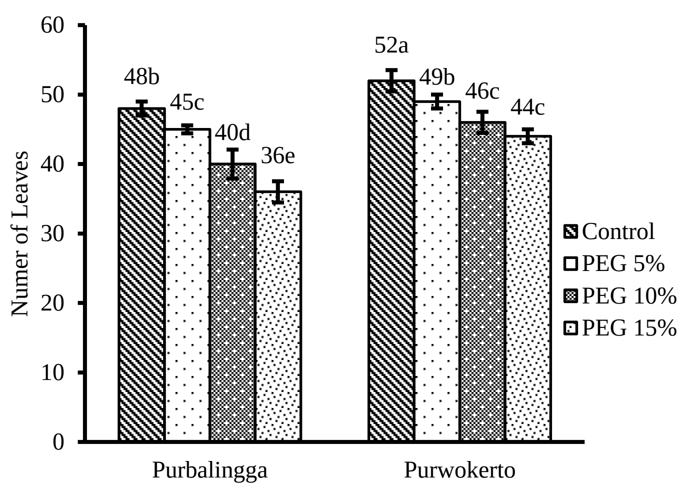

Figure 4. Number of leaves for Purbalingga and Purwokerto local black rice at various levels of drought stress, followed by notation indicating significance in DMRT at 5%.

3.2. Number of Leaves

It was observed that drought stress decreased number of leaves in both local black rice Purbalingga and Purwokerto from 0% PEG (Control) to 15% PEG. Results in Figure 3 indicates that the overall trend of number of leaves for Purbalingga and Purwokerto local black rice was decreased. Based on the analyzed research results, the interaction of Purbalingga and Purwokerto local black rice at various levels of drought stress on the parameter of leaf number shows significantly different results. Figure 3 shows that the control treatment produced the highest number of leaves, with the Purbalingga variety having 48 leaves and Purwokerto variety having 52 leaves. Purbalingga variety has decrease 0.75% number of leaves to 36 leaves on the treatment of PEG 15%. Purwokerto variety has decrease 0.84% to 44 leaves on the treatment of PEG 15%. Reason for decrease in number of leaves is when plants are under drought condition, they will response by adjusting the distribution of assimilates to limiting leaf expansion to reduce transpiration (Ilyani *et al.*, 2017). In this condition, cell division activities may decrease so there is no increase in leaf numbers. This aligns with research finding, results in Figure 3 shows that higher concentration of PEG stress on plants resulting in a decrease the number of leaves for both local black rice varieties.

3.3. Root Length

Plants absorb water and minerals through their roots to sustain life and compete for nutrients. Under drought stress, the development of roots determines the plants' tolerance to these conditions (Riaz *et al.*, 2013). The elongation of roots into deeper soil layers during drought conditions demonstrates the plant's resilience and highlights potential root morphological characteristics (Torey *et al.*, 2013). The results in Figure 4 indicate that the root length of both local black rice varieties increased across all treatments. The research results show a significant difference in root length between the Purbalingga and Purwokerto local black rice varieties under various levels of drought stress. Figure 4 reveals that the control treatment resulted in a root length of 43 cm for the Purbalingga variety and 50 cm for the Purwokerto variety. In contrast, the longest root lengths were observed under the PEG 15% treatment. Purbalingga variety increase 1.25% the root length to 54cm and Purwokerto variety has increase 1.14% the root lenght to 57cm. Root elongation in rice is a strategy for finding water and nutrients to support plant growth and development (Nasrudin *et al.*, 2020). Under drought conditions, rice plants have the ability to elongate their roots as a resilience and adaptation mechanism, providing them with a greater opportunity to absorb water by reaching deeper soil layers.

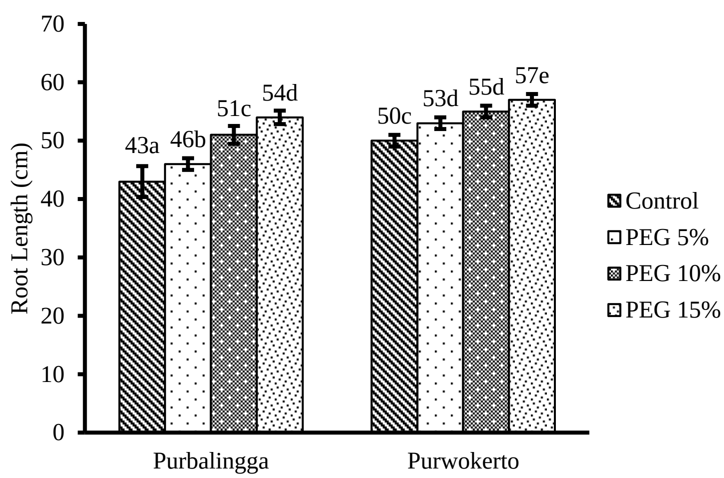

Figure 4. Root length for Purbalingga and Purwokerto local black rices at various levels of drought stress, followed by notation indicating significance in DMRT at 5%.

3.4. Chlorophyll Content

Chlorophyll is a pigment in plants plays a crucial role in plant metabolism under normal and stress conditions. Measuring chlorophyll levels can serve as a physiological indicator to assess a plant's resistance to drought stress. The content of chlorophyll indicates a plant's tolerance to drought because chlorophyll biosynthesis is closely linked to photosynthesis, which is sensitive to water deficiency (Nio *et al.*, 2019). Plants that are tolerant to stress exhibit a smaller decrease in chlorophyll content compared to non-resistant plants. The reduction in chlorophyll content during drought stress in rice plants is believed to be due to water scarcity, which results in suboptimal nutrient uptake. The result in Figure 5 indicate that drought stress can decrease chlorophyll content. Based on the analyzed research results, the interaction of Purbalingga and Purwokerto local black rice varieties at various levels of drought stress shows significantly different results in terms of chlorophyll content. Figure 5 shows that the highest chlorophyll content was produced by the control treatment, which is the Purbalingga variety having 15.69 mg/L and Puwokerto variety having 19.1 mg/L. In contrast, the lowest of chlorophyll content were observed under the treatment of PEG 15%. Purbalingga variety decrease 0.73% the chlorophyll content to 54 cm and Purwokerto variety has decrease 0.68% the chlorophyll content to 57cm. The reduction in chlorophyll content may occur due to inhibited chlorophyll formation and the plant inabilty to absorb nutrients such as nitrogen and magnesium which are crucial for chlorophyll synthesis (Song & Banyo, 2011). Chlorophyll damage can also results from the overproduction of Reactive Oxygen Species (ROS) like O_2 and H_2O_2 which can lead lipid peroxidation that impact on lipid degradation and disrupting cell membranes, thereby damaging chlorophyll molecules (Ahmadikhah & Marufinia, 2016).

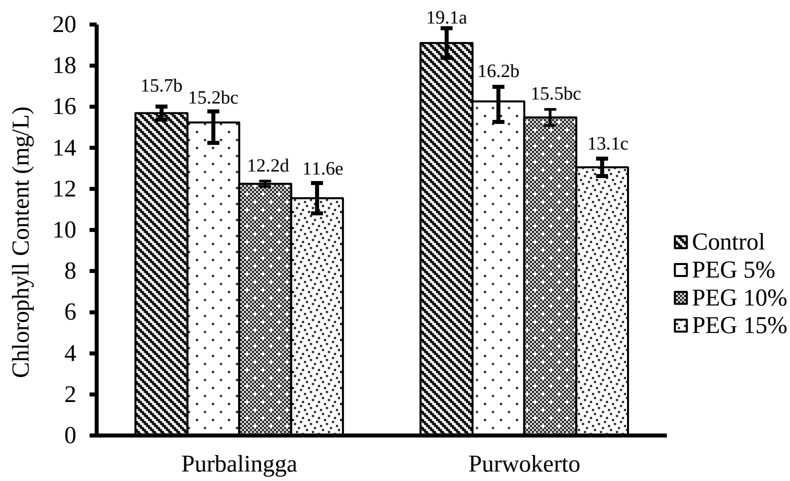

Figure 5. Chlorophyll content for Purbalingga and Purwokerto local black rices at various levels of drought stress, followed by notation indicating significance in DMRT at 5%.

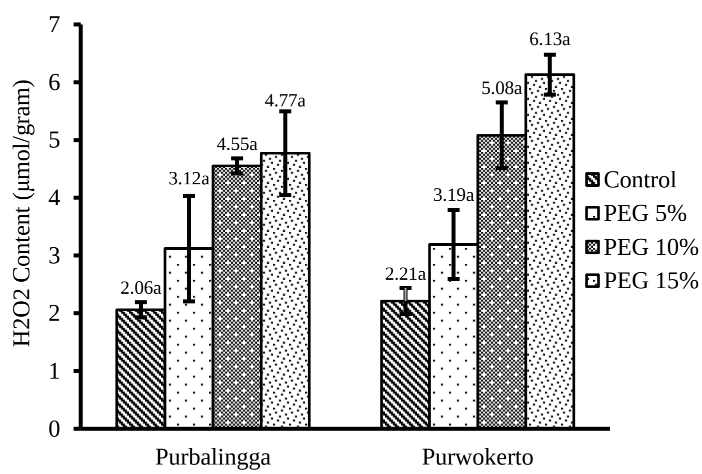

Figure 6. Hydrogen peroxide content for Purbalingga and Purwokerto local black rices at various levels of drought stress.

3.5. Hydrogen Peroxide Content (H_2O_2)

Figure 6 shows that the interaction of Purbalingga and Purwokerto local black rice at various levels of drought stress on the Hydrogen Peroxide (H_2O_2) content parameter showed no significant difference. Drought stress can trigger oxidative stress in plants, leading to increase in Reactive Oxygen Species (ROS) levels. ROS are reactive and toxic to plants. Among various of ROS, hydrogen peroxide (H_2O_2) has a relatively longer half- life compared to other ROS so it often used as a parameter to determine the level of stress in plants. Plants that tolerant of drought stress will accumulate fewer ROS compared to stress sensitive plants. Based the analyzed research results, However, based on Figure 6 shows that each treatment increased H_2O_2 content in both of local black rice. Figure 6 indicates that the control treatment produced the lowest H_2O_2 content with Purbalingga variety has 2.06 µmol/gram and Purwokerto has 2.21 µmol/gram. Meanwhile, the highest H_2O_2 content was produced by 15% treatment of PEG. Purbalingga variety has increase 2.31% the H_2O_2 content to 4.77 µmol/gram and Purwokerto variety has increase 2.85% the H_2O_2 content to 6.13 µmol/g. Hydrogen peroxide (H_2O_2) can play dual roles when plants are under stress. Increased H_2O_2 content can activate antioxidant defense system to prevent cell death due to stress so plant can growth (Simbolon *et al.*, 2020).

3.6. Anthocyanin Content

Anthocyanins are natural antioxidant compounds that act as natural scavengers of free radicals. The role of anthocyanin is often correlated with a plant's tolerance to abiotic stress which is the higher anthocyanin content in plants indicates greater tolerance to drought stress (Naing & Kim., 2021). Based on the analyzed research results, the interaction of Purbalingga and Purwokerto local black rice at various levels of drought stress on the anthocyanin total parameter showed no significant difference. However, based on Figure 7 shows that each treatment increased the anthocyanin total in both local black rice. Figure 7 indicates that the control treatment produced the lowest anthocyanin content with Purbalingga variety has 0.04mg/L and Purwokerto has 0.04mg/L. Meanwhile, the highest anthocyanin content was produced by 15% treatment of PEG. Purbalingga variety has increase 3.5% the anthocyanin content to 0.14mg/L and Purwokerto variety has increase 4.25% the anthocyanin content to 0.17mg/L. Figure 8 indicates that the 0% PEG treatment (control) has lower anthocyanin content than local black rice under drought condition. Increased of anthocyanin content is one of strategies of plant to protect for ROS. Anthocyanins can act as osmotic regulators during periods of drought and low temperatures so it suggests that increased anthocyanin content can enhance the plant's antioxidant responses in survival under biotic and abiotic stress (Priska *et al.*, 2018).

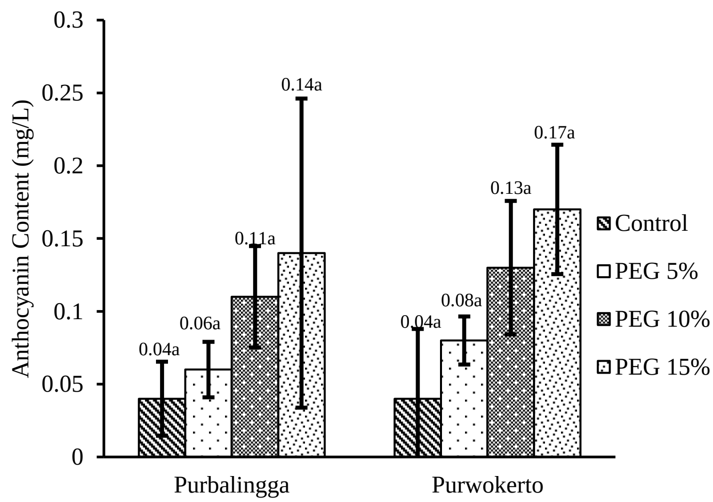

Figure 7. Anthocyanin content for Purbalingga and Purwokerto local black rices at various levels of drought stress.

4. CONCLUSION

Based on the research conducted, it can be concluded that drought stress using PEG 6000 at concentrartion of 0% (Control), 5%, 10%, and 15% can affect the morphology and physiology of Purbalingga and Purwokerto locak black rice. Both of Purbalingga and Purwokerto local black rices can survived in PEG 15% treatment, so it can be concluded that both of them resistance in drought stress with 15% PEG. The treatment of PEG 6000 can decrease plant height as 0.86%, number of leaves 0.83%, and chlorophyll content as 0.82% but increase the root length as 1.16%, hiydrogen peroxide content as 2%, and anthocyanin content as 2.58% in Purbalingga local black rice. The treatment of PEG 6000 also decrease plant height by 0.92%, number of leaves 0.88%, chlorophyll content as 0.77% and increase the root length as 1.1%, hydrogen peroxide content as 2.19%, and anthocyanin content as 3.16% in Purwokerto black rice.

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