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Effect of drought stress on morphological, anatomical, and physiological characteristics of Cempo Ireng Cultivar Mutant Rice (*Oryza sativa* l.) strain 51 irradiated by gamma-ray

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Abstract. Drought stress is a factor that affects plant growth and development, both in terms of morphology, anatomy, and physiology. Mutant *Oryza sativa* L. strain 51 of Cempo Ireng cultivar as the result of gamma-ray irradiation is superior mutant black rice strain which has a faster planting period of 10-20 days than its control and shorter plant height. This study aims to determine the morphological, anatomical, and physiological responses, especially the proline content inside the leaves of mutant black rice strain 51. The study used a completely randomized design (CRD) with the treatment of drought stress using PEG 6000 in Yoshida liquid media. The seedlings were planted for 21 days in untreated media, then treated for 14 days. Observation of morphological characters was carried out by measuring plant height, root length, leaf area, and plant biomass. Observations of anatomical characters were carried out by observing the cross-section of the root. Observation of physiological character was carried out by measuring leaf proline levels. The results showed that drought stress with PEG 6000 inhibited the growth and development of mutant rice strain 51. Drought stress reduces plant height, root length, leaf area, plant biomass and the area of root aerenchyma. Proline leaf content increased significantly at a PEG concentration of 30%. Mutant rice strain 51 showed a tolerant response to drought stress with the significant increased of proline, the increased of root stele diameter and the constant number of metaxylem.

1. Introduction

Indonesia experiences many agricultural problems including drought and high soil salinity so that agricultural productivity decreased. According to the 2017 National Disaster Management Agency (BNPB) data, drought has hit 105 districts/ cities in Java and Nusa Tenggara. About 56,334 hectares of agricultural land experienced drought so that 18,516 hectares of it experienced crop failure [1]. In the research of rice plants (*Oryza sativa* L.), drought conditions will lower the photosynthetic activity of plants so it will reduce plants' productivity [2]. Drought stress also causes a decrease in plant height, plant dry weight, root canopy ratio, the relative water content of leaves also increases leaf rolling and drying scores [3].

Oryza sativa L. or black rice has the advantage of being safe for diabetics because it contains anthocyanin pigments and high antioxidant activity [4]. Black rice has a weakness that is the harvest time requires a long time, approximately 129 days. Another weakness is the height of black rice plants which is ± 145cm makes it easily falls when exposed to the wind [5]. Efforts to improve black rice varieties have been carried out among others by the induction of mutations through radiation.

Induction of mutations by gamma-ray irradiation at the right dose can increase agricultural crop production, including higher productivity and more tolerant of environmental stress [6]. Cempo Ireng black rice resulting from gamma-ray irradiation is useful as an initial crop in efforts to improve black rice varieties in the future [7]. Cempo Ireng mutant rice produced by irradiation of 200 gray gamma-ray produces several superior strains, one of which is strain 51 with superior characteristics, namely plant height which is shorter and uniform than other strains.

Plant tolerance to environmental stress is by making adjustments of cell osmotic so that turgidity remains high. Osmotic adjustment of these cells with the accumulation of organic osmolyte materials such as proline, glycine betaine, sugar, polyamines and other proteins [8]. Proline can accumulate in leaf organs because water deficits can modulate chemical signals from the roots of one of them abscisic acid (ABA) which is transferred to the canopy so that it can induce the accumulation of proline in the leaves [9,10]. The other rice plants research also reported that rice plants (*O. Sativa* L.) of genotype MR269 experienced a significant increase of proline accumulation under drought stress conditions. The irradiated MR269 rice plant had a higher increase of proline than the non-irradiated treatment under drought stress conditions [11].

Proline has a key role in stabilizing cellular proteins and cell membranes [12]. Proline accumulation also has a role in reducing the levels of ROS (Reactive Oxygen Species) such as H_2O_2. During drought stress, increased ROS causes cell death. In addition, the accumulation of proline compounds can be used in cell walls hardening to maintain cell resistance [13]. Based on the explanation above, observation of morphological, anatomical and physiological responses to the drought stress of Cempo Ireng black rice strain 51 resulting from gamma-ray irradiation is needed to open the potential of dry land use in Indonesia.

2. Materials and Methods

2.1 Materials
Materials used in the study include seeds of Cempo Irengblack rice strains 51 resulting from gamma rays irradiation. Other ingredients are PEG 6000, distilled water , 3% sulfosalicylic acid, ninhydrin acid, glacial acetic acid, toluene, *L-proline* , FAA, alcohol, xylol, *sodium hypochlorite* (NaOCl) 5%, safranin 1%, *fast green* 1%, paraffin, glycerin, wax , filter paper and Yoshida nutritional media.

2.2 Research design
This study used a Completely Randomized Design (CRD) with a treatment in the form of variations in the concentration of PEG 6000 (0% , 10 %, 20%, and 30%) which was applied to the rice strain 51 growing media.

2.3 Methods

2.3.1 Sterilization and germination of rice seeds. Rice seed strains 51 were sterilized by means soaked in a solution of *sodium hypochlorite* 5% for 10 minutes and then rinsed with aqua dest. Rice seed germination was carried out on 2 filters paper in a petri dish for 7 days.

2.3.2 Rice Planting and Drought Stress Treatment. Rice planting stage in Yoshida nutritional media[14] with the treatment of PEG (*Polyethylene Glycol)* 6000 as a drought stress treatment. The PEG 6000 solution is best used for osmotic control of an experiment [15]. Paddy plants aged 7 days were transferred to Yoshida nutrient medium without treatment for 21 days. Drought stress treatment with the addition of PEG 6000 concentration of 0%, 10%, 20% and 30% was added for 14 days after planting without treatment.

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2.3.3 Morphological Character Observation. Morphological characters observed included plant height, root length, leaf area and plant biomass.

2.3.4 Observation of Anatomical Character. Anatomical characters observed were a cross-section of the root using the paraffin embedding method with safranin and fast green dyes. The preparations were observed under a digital microscope and analyzed with the Image-J application.

2.3.5 Observation of Physiological Characters
The physiological characters measured were leaf proline levels. Leaf proline levels were measured by the method of Bates which has been in the modification that the sample ends 0,1g leaves crushed and extracted with 5 ml of acid sulfosalisilat 3%. Extract as much as 2 ml plus 2 ml of ninhydrin acid and 2 ml of glacial acetic acid. The extract was incubated at 100 ^{o}C for 1 hour. After that, the tube containing the sample is inserted into an ice cube until it reaches a room temperature of ± 29 ^{o}C measured with a thermometer. The sample was extracted again with 4 ml of toluene and the absorbance was calculated with a UV-visible spectrophotometer at a wavelength of 520 nm. The standard solution used is L-proline. The proline concentration (M/g fresh weight) is calculated based on a standard curve [16].

3. Results And Discussion

3.1 Morphological Character
Morphological characters were observed as a response to drought stress with the application of PEG 6000 in plants strain 51, namely plant height, root length, leaf area and plant biomass. Based on the results of this study, drought stress significantly affected the morphological characters measured except root length and plant height (Table 1). Drought stress inhibits the growth of strains of rice plants 51. The higher the concentration of drought stress, plant growth is increasingly inhibited. Plant growth is inhibited in drought conditions due to decreased cell turgor resulting in low cell metabolism so that cell division and elongation are inhibited [17].

Table 1. Morphological Characteristics of Strains 51 in Drought Stress conditions

Treatment	Plant Height (cm)	Root Length (cm)	Leaf area (cm^2)	Plant Dry Weight (g)
CONTROL	64.7 ± 7.14 a	13.36 ± 1.91 a	154.68 ± 65.6361 a	0.8463 ± 0.2878 a
PEG10%	52.18 ± 9.49 a	13.08 ± 2.35 a	77.3519 ± 40.9073 b	0.4399 ± 0.2385 b
PEG20%	57.38 ± 4.49 a	13.06 ± 0.79 a	75.9637 ± 16.1869 b	0.3854 ± 0.0553 b
PEG30%	55.44 ± 8.48 a	12.94 ± 0.73 a	77.0349 ± 27.0905 b	0.4006 ± 0.1274 b

Note: value with different letter annotation in the same column show significant differences ($P < 0.05$)

The treatment of drought stress had no significant effect on the root length and plant height of the Rice strain 51. This is the same as research by the reference, drought stress had no significant effect on the root length of rice plants [18]. In contrast to the research of reference, drought stress was significantly able to inhibit the root lengthening of non-irradiated and irradiated rice plants [19]. The difference in response is thought to be due to differences in the genotype of each variety that gives rise to different responses.

3.2 Anatomical Character

Drought stress has a significant effect on the area ofstrain 51 root aerenchyma (Table 2). This is in accordance with the research of reference, wider aerenchyma roots of a plant are experienced on optimum water availability rather than the condition of lacking water [20]. Aerenchyma serves to keep the plant in order to survive in enough water environment by arranging the sustainability of the oxygen diffusion in aerobic respiration.

Table 2. The Width of black rice strain 51 root aerenchyma in drought stress conditions

Treatment	Area of aerenchyma (x10 ^{3}μm ^{2})
Control	192,6500 ± 1.89 a
PEG 10%	62.8411 ± 2.06 b
PEG 20%	21.6588 ± 0.34 d
PEG 30%	50,7011 ± 2.21 c

Note: The value with different letters in the same column shows significant differences (P <0.05).

The diameter of the stele looks wider in the drought stress treatment (Figure 1). Root cross-section of plant 51 shows there is no change in the number of metaxylem, it means that the root stele of plant strain 51 is more complex in drought stress condition which shows an indication of resistance to drought stress. This is consistent with the study of reference who reported that rice variety Nagina22 in drought conditions reduced the area of aerenchyma, expanded the diameter of the root stele and increased the xylem [21]. The rice is rice that is tolerant to drought.

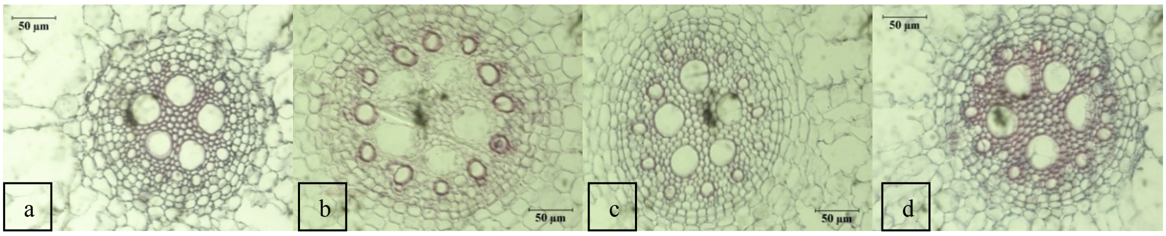

Figure 1. Root stele of plant 51 control treatments (a), PEG 10% (b), PEG 20% (c), PEG 30% (d)

3.3 Physiological character

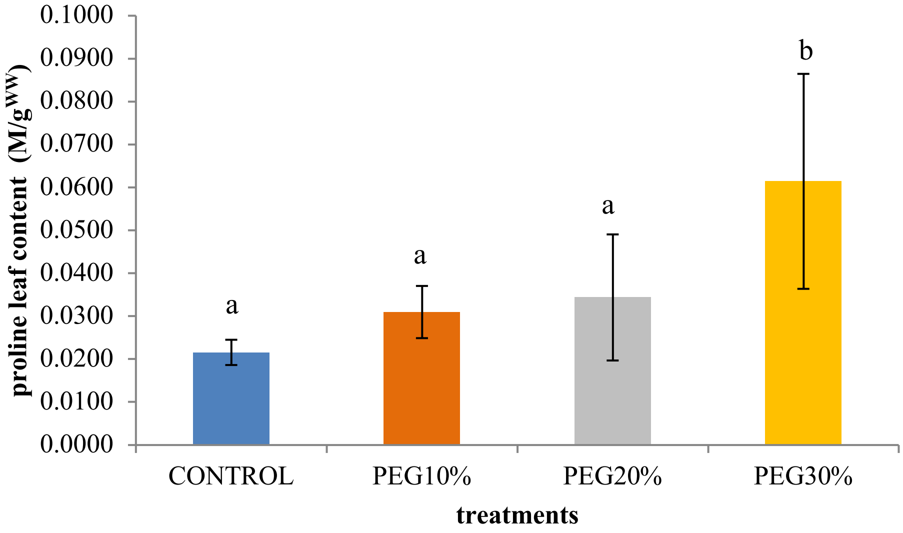

Figure 2. Proline leaf content of Rice Strain 51 in drought stress conditions. Note: Different letters show significant differences (P <0.05).

The average response of proline levels of rice plants strain 51 to drought stress has a higher score than normal conditions (Figure 2). The higher the concentration of drought stress given will further increase proline levels. In accordance with the research of reference, proline levels of black rice increase under drought stress conditions as the stress concentration increases [22]. Based on the results of this study plant strain 51 was suspected of having an indication of the resistance character to drought stress because leaf proline levels increased significantly. It fits with researches from reference, the rice of rood Zayande cultivars have an increase in the levels of proline significant at drought stress conditions [23].

Drought stress is a trigger for plants to increase proline accumulation [24]. The existence of proline accumulation is one of the *Osmotic Adjustment* (OA) as the process of protecting cell turgor when the plant is in drought stress . Proline can be useful for maintaining the integrity of cell membranes and also maintaining the stability of enzymes or proteins [10].

Black rice strain 51 showed a response to drought conditions by reducing root length, number of leaves and plant biomass more numerous so that the growth of plant height did not decrease significantly. The tolerant ability of strain 51 is indicated by the significant increase in proline, larger and complex root stele, and smaller aerenchyma area under drought stress conditions. The stomata density that does not change in drought stress conditions causes strain 51 to respond by reducing the amount and area of leaves and plant biomass to deal with drought stress.

4. Conclusion

Drought stress caused Cempo Ireng cultivar mutant rice (O. *Sativa*) strain 51 irradiated by gamma-ray decreased the growth response character of plant height, root length, leaf area, plant biomass and aerenchyma area except for leaf proline content. Strain 51 showed an indicative response of the existence of drought stress resistance with the increase of proline content significantly, aerenchyma with a small area and complex root stele in drought stress conditions.

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