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Anatomical and Physiological Responses of M5 Mutant Red Rice G16 from Gamma Irradiation to Drought Stress Condition in Vegetative Phase

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Abstract: Food security and sustainability are facing challenges due to the increase in population and the large amount of dry land that has not been optimized for utilization. Rice cultivated in paddy fields is a problem that must be considered. The use of local varieties should be further studied because it is one of the efforts to increase plant productivity against drought stress. This study aims to assess the anatomical and physiological responses of M5 mutant G16 red rice from gamma irradiation to drought stress in the vegetative phase. The method used was experimental method with field experiments. The experiment was conducted from November 2023 to July 2024 at the Greenhouse of the Faculty of Agriculture, University of Mataram. The experimental design used was Faktorial Completely Randomized Design (CRD) with two factors, the first factor is drought stress consisting of 33% Field Capacity (K1), 66% Field Capacity (K2), 100% Field Capacity (K3) and the second factor is genotype consisting of Inpago Unram (P1), MD200-G13-18-16 (P2), MD300-G20-16-9-5 (P3), MD200-G24-17-10 (P4), MD300-G27-8-3-5 (P5). The result of this study show that MD200-G24-17-10 (P4) was the most responsive genotype to drought stress conditions in the vegetative phase. This was indicated by a decrease in stomatal density and chlorophyll content, as well as an increase in xylem diameter and stele diameter.

Keywords: Drought stress; Mutant; Anatomy; Physiology; Vegetative

Introduction

Rice is one of the most widely cultivated food crops because the majority of the world's population consumes rice as a staple food, one of which is in Indonesia. Rice plants generally live in rice fields with a lot of water needs, so it will be a problem if rice plants live in drought conditions. Awanis et al. (2022) stated that the decline in productivity in rice plants is caused by drought factors influenced by climate change. Land conditions in Indonesia are also an influential factor. Most of the land in Indonesia is dry land. This is indicated by Hikmat et al. (2022) that of the total land area as a whole about 75.6% is dry land. Regions in Indonesia are also divided into two types of climate, namely areas with wet climates and areas with dry climates. One of the areas with dry climate distribution is West Nusa Tenggara Province, so optimal utilization of dry land must be done.

The use of rice genotypes that are adaptive to drought stress conditions is one of the technologies that can anticipate the impact of climate change and to optimize the use of dry land. One of the plant genotypes that have drought stress tolerance according to Mustikarini et al. (2018) is a stable red rice genotype, because it can provide relatively the same yield when planted in dry or wet environments. Awanis et al. (2022) stated that local brown rice has the potential as a gene source because it has a high tolerance to drought stress. The use of local varieties as an effort to increase plant productivity against drought stress has been widely studied, but little is found in local brown rice.

West Nusa Tenggara has quite a lot of local cultivar brown rice germplasm that can be used as a gene source to obtain new varieties for breeding activities (Umam et al., 2018). One of them is the red rice genotype G16, the result of a cross between the Cere cultivar and the Bulu

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cultivar that was conducted by Sudharmawan et al. (2008). The parents of the genotype have the advantage of a large number of tillers and are drought tolerant but still have a long harvest period. Genetic improvement through mutant selection with gamma-ray induction has been carried out on the G16 genotype. Superior varieties resulting from mutation induction according to Suliartini et al. (2020) were produced through mutation induction with gamma rays. Therefore, further studies related to the response of mutant plants resulting from irradiation with gamma rays to drought stress must be carried out.

A way to determine the effects of drought stress on plants can be done by giving water below field capacity. Drought stress on plants will affect their growth and development. In the vegetative phase, plants will focus on the growth and development of organs which will affect the resulting crop production. Drought stress according to Setiawan et al. (2015) in Salsadilla & Hariyono (2022) is an environmental condition where plants do not receive sufficient water intake, so that plant growth and development cannot be carried out optimally which results in decreased production.

Water deficit conditions can trigger biological stress in plants, so that the physiological processes of plants become disrupted. Mudhor et al. (2022) informed that the closure and narrowing of stomata is the first response of rice plants to water deficit conditions that can affect plant physiology. One of the physiological responses of plants to water shortage reported by Nio et al. (2019) is a decrease in chlorophyll levels, which can interfere with the photosynthesis process. Water is the most important ingredient in the photosynthesis process, so the physiological process in plants is closely related to the root system. Rice genotypes that have deep roots and many branching are reported as characteristics of plant mechanisms in avoiding drought stress (Sandar et al., 2022).

This study aims to assess the anatomical and physiological responses of the M5 mutant of red rice G16 from gamma irradiation to drought stress in the vegetative phase.

Method

This research was conducted from November 2023 to July 2024 and took place in the greenhouse of the Faculty of Agriculture, Mataram University. Observations of leaf anotomy were carried out at the Plant Breeding Laboratory of the Faculty of Agriculture, Root Anatomy at the Center for Agarwood Studies Laboratory and Plant Embryology Laboratory and physiological analysis for Chlorophyll Levels at the Analytical Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences (MIPA), Mataram University. The tools used consisted of soil sieve, sprayer, stationery, 50 ml plastic bottle, bamboo, hoe, cup, bucket, plastic cup, measuring cup, scissors, sack, filter paper, label paper, planting media, mortar, binocular microscope, optical microscope, object glass, object cover glass, oven, ruler, anatomical tweezers, drip pipette, plastic clip, UV plastic, 40 x 40 cm polybag, erlenmeyer tube, rapia rope, tarpaulin, marker, meter, ruler, analytical balance, clear tape, shovel, soil moisture sensor, razor blade, UV Vis spectrophotometer, and standing pouch. The materials used consisted of water, athonic 6.0 L, distilled water, G16 rice mutant seeds of genotypes MD200-G13-18-16, MD¬200-G24-17-10, MD¬300-G20-16-9-5, MD300-G27-8-3-5, Inpago Unram 1 rice seeds, nail polish, entellan, fungicide, insecticide, cruiser 350 EC solution, NPK fertilizer, chicken compost, soil, 70% ethanol, and 1% safranin.

The design used in this study was aFaktorial Completely Randomized Design (CRD) consisting of two factors. The first factor is drought stress (K) which consists of three treatment levels, namely 33% field capacity (K1), 66% field capacity (K2) and 100% field capacity (K3). The second factor is mutant (P) which consists of five treatment levels namely Inpago Unram 1 (P1), MD200-G13-3-11-5 (2), MD300-G20-8-3-5 (3), MD200-G24-17-10-8 (4) and MD300-G27-16-9-5 (5). A total of 15 combinations were obtained from both factors and each factor was repeated three times, resulting in 45 experimental units.

The implementation of this research experiment begins with the preparation of planting media, namely taking soil in the experimental field of Mataram University, then drying, sifting, mixing with organic fertilizer in a ratio of (3:1) and finally incubating for 14 days. The next stage is the determination of field capacity, starting with sieving with a diameter of 0.5 mm, weighing the cup and soil sample three times, then the sample is transferred into the soil ring with the bottom of the ring covered with filter paper. The soil sample is watered with water until saturated and will be stopped when the first water droplet comes out. The soil samples were allowed to stand for 2x24 hours and baked for 48 hours at 107°C, and weighed again after the soil samples cooled. The field capacity value is determined using the equation (Kusumawati, 2021):

$$\text{Field capacity (\%)} = ((b-c))/((b)) \times 100\% \quad (1)$$

b = initial weight of soil sample before oven (g)
c = final weight of soil sample before oven (g)

Furthermore, the determination of water volume, the percentage of field capacity that has been obtained is used as a basis for determining the amount of water volume to be given for each treatment. The volume of water is determined by the following equation:

Water volume (l) = field capacity (%) × volume of planting media/polybag (kg) (2)

The volume of water that has been obtained is then applied to the planting media in accordance with the predetermined field capacity treatment, the planting media that has been watered, then determined the humidity using a soil moisture sensor with the acquisition of 2 for K1, 3 for K2, and 4 for K3. The moisture scale obtained is a reference for further watering, which when the planting media shows a decrease in the moisture scale, it will be watered slowly until the predetermined initial scale value.

After that is the experimental stage in the greenhouse which begins with putting the planting media that has been known to AKL into polybags as much as 17.5 kg per experimental unit. The next stage of seed preparation, the seeds used were seeds that were nutritious, after which they were soaked for 12 hours with water, then soaked again with 1 ml/l cruiser and 2 ml/l athonic solution for 10 minutes each, then matured for 48 hours. Next, it is sown and transplanted when the rice plants are 14 days old. Then maintenance was carried out such as watering, fertilizing, replanting, weeding, and controlling pests and diseases.

The observation characters consisted of stomatal density, cortex thickness, xylem diameter, stele diameter, and chlorophyll content. Stomatal density was observed at the age of 6 weeks after planting, the leaves used were the second leaves after the top of the plant, before being observed the leaves were cleaned of sand or soil and then smeared with nail polish covering 1 cm in three parts of the leaf; tip, middle, and base. Observations were made using an ocular lens configured WF10x/22mm. Furthermore, it was calculated using the equation (Mudhor et al., 2022):

Stomatal Density = (Number of Stomata)/(Area of Field of View) (3)

The thickness of the cortex, xylem diameter, and stele diameter were observed at the age of 6 weeks after planting by first taking samples and then cleaning them with water. after that, they were fixed using 70% ethanol for 1x24 hours, then cross-section preparations were made and colored using 1% safranin. Next, the glass preparation was closed with the edge glued with entellan. Observations were made with a 40x magnification light microscope, then measured using an optical microscope that was connected to an opticlab. Chlorophyll content analysis was conducted when the plants were 8 weeks old. After sampling, 0.25 leaf per sample was taken and crushed until smooth using a mortar. Next, it was dissolved using 70% ethanol as much as 20 ml, then filtered using a glass funnel in a test tube. The extract that has been obtained is then analyzed using a spectrophotometer with wavelengths of 645 nm and 663 nm. Furthermore, it was calculated using the following equation (Prasetyo & Laily, 2015):

Chlorophyll a = 12.7 A_663mµ - 2.69 A_645mµ mg/l
Chlorophyll b = 22.9 A_645mµ - 4.68 A_663mµ mg/l
Total chlorophyll = 20.2 A_645mµ + 8.02 A_663mµ mg/l

The data obtained were analyzed using Analysis of Variance (Anova) at the 5% level, then significantly different data were further tested using Duncan Multiple Range Test (DMRT) 5%. The closeness between characters was determined through phenotypic correlation using the equation (Ujianto et al., 2021):

$$r_{X1X2} = \frac{\sum(X_{1i}-\overline{X}_1)(X_{2i}-\overline{X}_2)}{\sqrt{\sum(X_{1i}-\overline{X}_1).\sum(X_{2i}-\overline{X}_2)}} \quad (4)$$

Result and Discussion

Stomatal Density

Stomata are important plant parts in the photosynthesis process. Lack of water causes plants to close their stomata as a mechanism to avoid drought stress conditions. Sumadji & Purbasari (2018) informed that the inhibition of photosynthesis process is due to the narrowing of stomata. This is related to the distribution of water in the plant body and a decrease in CO_2 flow in the leaves. Drought stress conditions result in a decrease in the number of stomata in plants with the aim of reducing water loss during transpiration (Hidayati et al. 2017).

The results shown in Figure 1, genotypes P4 and P5 have properties that are responsive to drought stress, because there is a decrease in stomatal density along with a decrease in field capacity. Changes in morphological, anatomical, physiological, and biochemical responses are prone to occur when water availability is reduced, such as leaf curling, loss of cell turgor, and stomatal closure (Zampieri et al., 2023). In contrast to other mutant genotypes, there was an increase in stomatal density in P2 and P3 during severe stress. The increase in stomatal density in Sumadji & Purbasari (2018) is thought to be influenced by wide leaves, so that the number of stomata obtained is more. As informed by Widianti et al. (2017) in Salsabila et al. (2021) that leaf size has an influence on the number of stomata, broad leaves usually have a greater number of stomata while narrower leaves have fewer stomata.

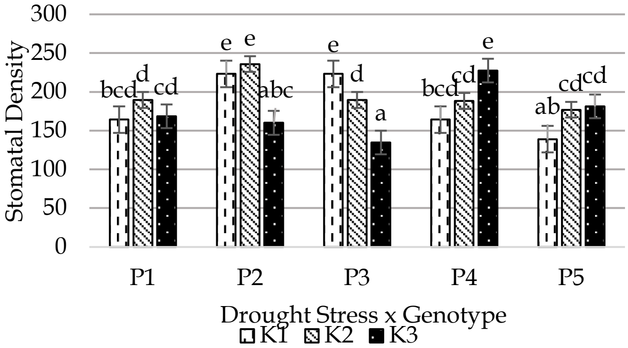

Figure 1. Effect of drought stress (K) x genotype (P) interaction on stomatal density

Cortex Thickness

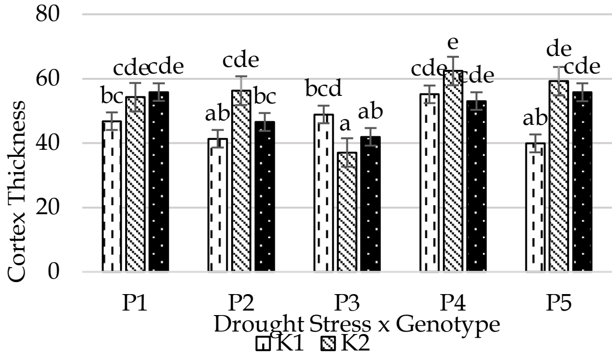

Figure 2. Effect of drought stress (K) x genotype (P) interaction on cortex thickness

The most important part of the plant that must be considered during drought stress conditions is the root system. Roots are the main plant organ that detects changes in soil conditions and play an important role in responding to water stress (Kim et al., 2020). Water and nutrients needed for plant growth and development are obtained from the roots, therefore the anatomical characteristics of the roots play a major role in shoot growth and overall production. One of the root anatomy is by observing the thickness of the cortex. Phoura et al. (2020) mentioned that drought stress causes thinner cortex in rice plants.

Figure 2 shows that control plants (P1), mutant genotypes P2 and P5 have a thinner cortex under severe stress conditions (K1). This indicates that there is plant responsiveness to drought conditions. One of the adaptation mechanisms to increase drought tolerance reported by Saputri et al. (2022) is the thinning of the cortex on plant roots. The results obtained in this study as a whole found diversity, meaning that each decrease in field capacity did not show a response to a decrease in cortex thickness as well. This is thought to be due to the influence of gamma irradiation on mutant plants in responding to field capacity conditions. As found in genotypes P3 and P4, there is an increase in cortex thickness, in accordance with the results of research by Youssef et al. (2023) that the crossed genotypes used increased the thickness of the cortex when experiencing water shortages.

Diameter of xylem

The characteristic of drought-tolerant rice is to have a larger xylem diameter with thicker roots (Gowda et al., 2011; Sandar et al., 2022). Genotype P3 showed an increase in xylem diameter during severe stress conditions (K1) which can be seen in Figure 3, while other genotypes showed a decrease in xylem diameter along with a decrease in field capacity conditions. This indicates that the P3 genotype is responsive to drought stress. The results of research by Patmi et al. (2020) showed the same thing, namely the increase in xylem diameter when drought stress occurs. The increase in xylem diameter affects root hydraulic conductivity which can determine plant productivity during water deficit conditions (Gokce & Chaudhry, 2020). large hydraulic conductivity according to Richards & Passiora (1989) in Sandar et al. (2022) results in less conservative water use so that the risk of cavitation is greater, this will affect the flow of water in the xylem so that the plant has difficulty in its physiological processes. Therefore, small xylem diameter also needs to be considered in the process of plant selection against drought stress.

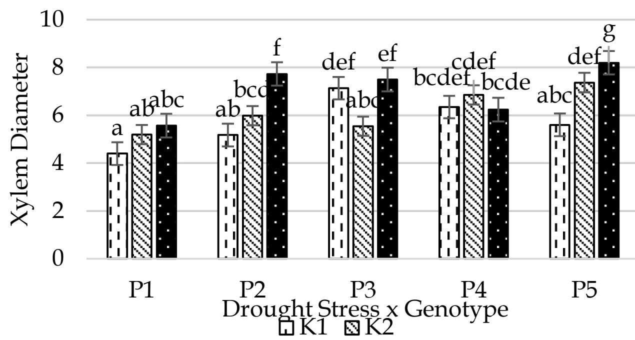

Figure 3. Effect of drought stress (K) x genotype (P) interaction on xylem diameter

Stele Diameter

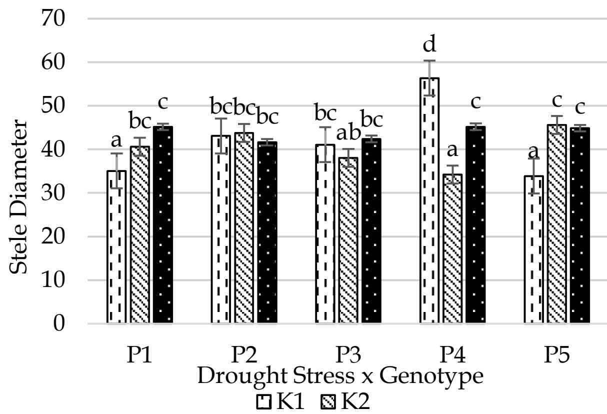

Figure 4. Effect of drought stress (K) x genotype (P) interaction on stele diameter

A wider stele size under severe stress conditions is one form of plant adaptation to drought. The increase in stele diameter under drought stress conditions can maintain the ability of plants to retain and store water in vascular tissues (Kim et al., 2020). Stele diameter in genotype P4 shown in Figure 4 increased when the field capacity was 33%, while P1 and P5 decreased and P2 and P3 produced constant stele diameter in each drought stress treatment. The increased stele diameter in genotype P4 is expected to result in increased production, in accordance with the research of Jeong et al. (2013) in Phoura et al. (2020) that there are two rice mutants that have a larger stele size with a greater number of steles resulting in higher production under drought conditions.

Chlorophylle Content

Drought stress can reduce leaf chlorophyll levels. Chlorophyll levels are the plant's response to water deficit, causing changes in plant physiology (Meihana et al., 2022). There are two types of chlorophyll, namely chlorophyll a with a bluish green colour, while chlorophyll b is yellowish green. Figure 5 shows that there was no interaction between the two factors on chlorophyll a, but chlorophyll a responded to drought stress and the genotypes observed. Drought stress condition with the highest chlorophyll a in K3 condition and the lowest in K2 condition, while the highest chlorophyll a level was found in genotype P4. In chlorophyll b, there was a decrease in genotypes P2, P4, and P5 in severe stress conditions.

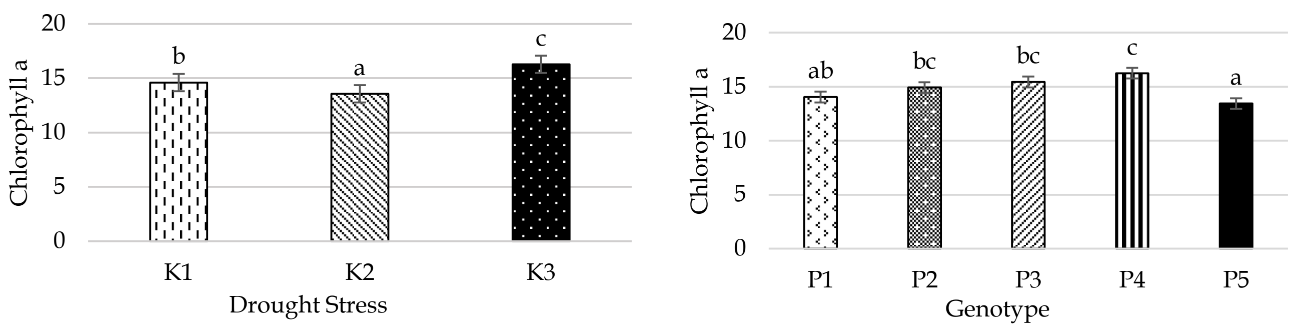

Figure 5. Effect of drought stress factor (K) and genotype factor (P) on chlorophyll a content

Respon tanaman terhadap cekaman kekeringan akan menunjukkan penurunan kandungan klorofil a dan rasio klorofil a/b namun terjadi peningkatan kandungan klorofil b (Maisura et al., 2014; Maisura et al., 2015). Berdasarkan hasil penelitian yang telah diperoleh, genotipe P1, P2 dan P4 menunjukkan peningkatan ratio klorofil a/b seiring dengan penurunan klorofil b pada kondisi cekaman kekeringan. Hal ini menunjukkan terjadinya peningkatan klorofil a sehingga kemampuan tanaman dalam reaksi merubah energi radiasi cahaya semakin meningkat yang mana proses fotosintesis dapat berjalan dengan optimal.

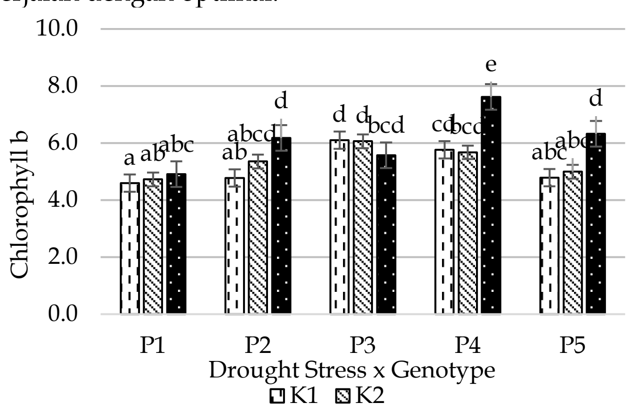

Figure 6. Effect of drought stress factor (K) and genotype factor (P) on chlorophyll b content

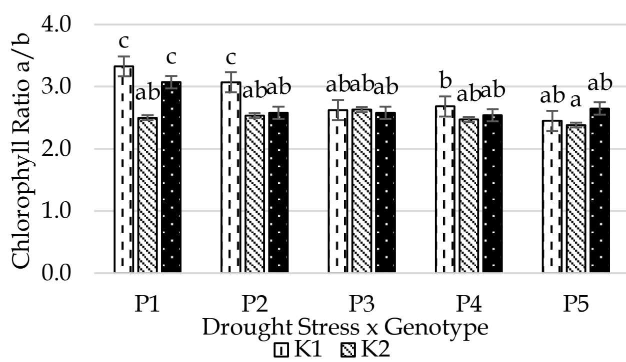

Figure 7. Effect of drought stress factor (K) and genotype factor (P) on chlorophyll ratio a/b

Drought stress affects the chlorophyll content of plants, which will result in a decrease in chlorophyll content. According to Syamsia et al. (2018) in Mudhor et al. (2022) that one form of plant response to drought stress is a decrease in chlorophyll content. In this study, the lowest overall chlorophyll content in each genotype used was in the 66% field capacity condition, while the condition with the most severe stress, namely 33% field capacity, produced higher chlorophyll levels. This is thought to be due to the effect of mutation with gamma rays given to mutant plants, so that mutant genotypes give different responses to chlorophyll levels in drought stress environments.

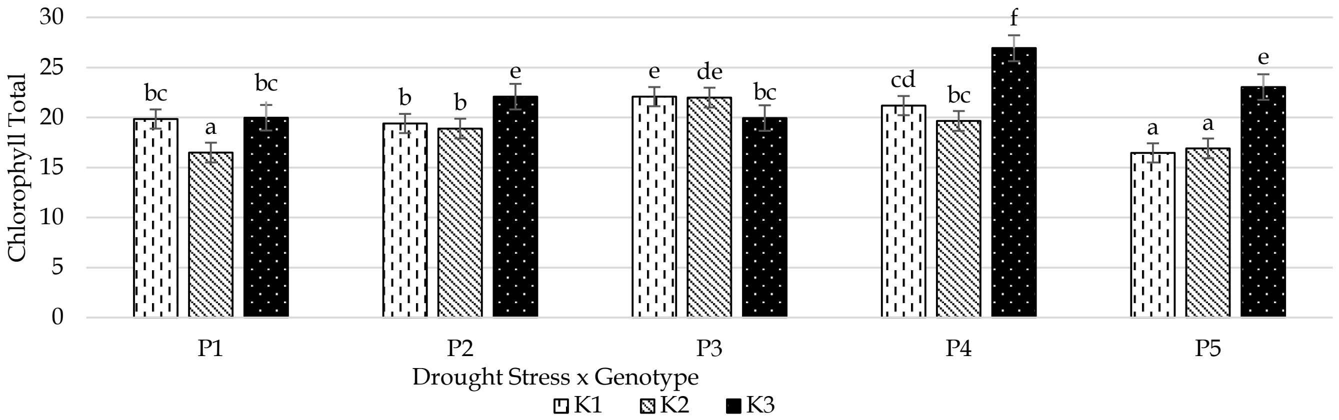

Figure 8. Effect of drought stress factor (K) and genotype factor (P) on chlorophyll total

Relationship between Anatomical and Physiological Characters

Table 1. Correlation between characters and chlorophyll content

	Stomata Density	Cortex Thickness	Xylem Diameter	Stele Diameter	Chlorophylle
Stomata Density	1,00				
Cortex Thickness	0,07	1,00			
Xylem Diameter	0,09	0,39	1,00		
Stele Diameter	0,22	0,45	0,27	1,00	
Chlorophylle	0,09	-0,06	0,26	0,27	1,00

The closeness of the relationship between observed characters can be determined by phenotypic correlation. In this study, the correlation between anatomical characters and plant physiological characters, namely stomatal density, cortex thickness, xylem diameter, and stele diameter with chlorophyll content was observed. The relationship between anatomical and physiological characters can be seen in Table 1. The table shows that the overall character shows a positive relationship to chlorophyll levels except for the cortex thickness character which shows a negative relationship. This shows that if there is an increase in stomatal density, xylem diameter, and stele diameter, there will also be an increase in chlorophyll levels. Conversely, if there is an increase in cortex thickness, chlorophyll levels will decrease. In accordance with the opinion of Hariyadi et al. (2023) that a positive relationship shows that any increase in the value of a character will be followed by an increase in the value of other characters, while if it decreases it will be followed by a decrease in the value of these characters. The negative relationship shows the reverse response, namely if the value of a character increases, it will be followed by a decrease in the value of another character, and vice versa.

The increase in stomatal density will result in an increase in the number of stomata as well. Stomata play a role in the exchange of CO2 and O2 gases, so that the large number of stomata can increase the absorption of CO2 (Putri et al., 2017), which will be utilised by chlorophyll in the photosynthesis process (Purwanto et al., 2021). The diameter of xylem and stele is related to the transport of water and nutrients in plants. The increase can have a positive effect on plants, when the diameter of xylem and stele is larger, the leaves will receive sufficient water supply so that it can support higher chlorophyll levels. High chlorophyll levels will affect the absorption of light and the process of photosynthesis, where the higher the chlorophyll level of the plant, the higher the rate of photosynthesis that occurs, so that the assimilate produced can be sufficient for growth and development (Wang et al., 2022).

Different things are shown by the thickness of the cortex, an increase in the thickness of the cortex can inhibit the process of water absorption in the soil, where the thicker the cortex, the longer it takes for water to reach the transport network (Karlova et al., 2021). Obstructed water distribution will result in decreased water supply to the leaves so that the level of chlorophyll produced will also decrease.

However, the results of this study show a very low and low level. According to the correlation coefficient criteria classified by Sugiono (2002) in Hariyadi et al. (2023), 0 - 0.199 (very low), 0.200 - 0.399 (low), 0.400 - 0.599 (medium), 0.600 - 0.799 (strong), and 0.800 - 1.000 (very strong). This shows that there is no strong relationship between characters in the vegetative phase. In accordance with the opinion of Rofidah et al. (2018) that the closeness of a relationship between observation responses is indicated by the magnitude of the correlation coefficient obtained.

Conclusion

MD200-G24-17-10 (P4) was the most responsive genotype to drought stress conditions in the vegetative phase. This was indicated by a decrease in stomatal density and chlorophyll content, as well as an increase in xylem diameter and stele diameter.

Author Contributions
All authors have real contributions in completing this manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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