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Amylose Accumulation Under Water Deficit in Glutinous Rice (*Oryza sativa* L. Var. glutinosa)

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ABSTRACT

The research aims to obtain a genotype of local glutinous rice tolerant to drought stress by investigating yield and physiological responses. The experiment is conducted in May-September 2020 in West Lombok, West Nusa Tenggara. The factorial experiment uses a Randomized Block Design, including the first factor: glutinous rice varieties, namely Me'e, Kala, Samada, and Paketih. The second factor is water supply, consisting of 2,250 ml, 3,375 ml, 4,500 ml, and 5,625 ml. The variables are observed stomata density, the content of proline and chlorophyll, panicle length, number and weight of grains, starch, and amylose content. The result shows that the stomata density has about 39 to 54.74 mm^2 caused by water application on all glutinous rice strains. The chlorophyll content decreases to 50% following the declining amount of water application, while proline content on all varieties reaches 40% by water supply at 2,250 ml. In addition, the amylose content reaches 40% with 4,500 ml of water and 5,625 ml of water in all varieties. By contrast, increasing the amount in water supply affect several variables observed, an increase in panicle length of around 10% and the number and weight of the grains at 20% and 40%, respectively.

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food consumed by more than 60% of the world's population and is the dominant staple food of Indonesians. Glutinous rice (*Oryza sativa glutinous*) is among the most popular rice varieties (Lei et al., 2021). Glutinous rice has opaque grains with low amylose content and high amylopectin; therefore, it is sticky. West Nusa Tenggara is one of Indonesia's provinces with several glutinous rice varieties. Some of these varieties are Me'e, Kala, and Samada. Based on data from the West Nusa Tenggara Province Food Crops Agriculture Office (2019), in the last few years, there has been a decline in upland rice production; in the year 2014 (212,527 tons), 2015 (207,186 tons), 2016 (179,252 tons), 2017 (173,113 tons), 2018 (129,153 tons). Several factors that cause the decrease in upland rice production are the reduction in planting area and changes in rainfall intensity hence the plants cultivated under drought stress. The declining rainfall intensity in 5 years, regarding the data from the Meteorology and Geophysics Agency (2019), rainfall in West Nusa Tenggara from 2014 to 2018 was 1231.5 mm, 1324 mm, 2185 mm, 1844.75 mm, and 1278 mm. The West Nusa Tenggara Food Crops Protection Center (2019) stated that during the last five years, there were drought conditions due to climate change in a row from 2015 - 2019 about 366.60 ha, 899 ha, 6.30 ha, 6,722 ha, and 367 ha. Water is an essential factor for plant growth and development. Some of the functions of water are (a) as a solvent, in which there are gases, salts, and other solutes, which move in and out of cells, (b) as a reagent in photosynthesis and various hydrolysis processes; and (c) water is essential to maintain turgidity including enlargement cells, stomata opening (Bhatla, 2018). Plants experiencing drought stress have a variety of mechanisms in terms of morphology, physiology, biochemistry, cellular, and molecular (Fang & Xiong, 2015), including reduction of leaf area (Darmadi et al., 2021) as well as a repressed number of stomata, transpiration rate, panicle initiation, flowering, inhibition of growth

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and yield reduction (Gaballah et al., 2021), and genetic differentiation, by determining specific DNA markers related to drought tolerance using Simple Sequence Repeats (SSR, and a marked decrease in photosynthetic growth rate at all growth stages (Zhu et al., 2020).

Furthermore, the result of the experimental field by Das & Biswas (2022) shows that drought stress can reduce the concentration of fat, non-structural carbohydrates such as starch and sugar, and minerals. By contrast, inconsistencies are found in sugar concentration. Following this result, the amylose content in seeds decreased due to drought stress (Pandey et al., 2014), and the secondary metabolites which synthesized and produced by plants as plant protection metabolism (Iwuala & Alam, 2017) and part of the defense as against biotic and abiotic stresses. The amino acid proline is the protein that responds to drought, acting as compatible osmolytes, possessing antioxidant properties, and contributing to cell recovery after stress (Boyidi et al., 2021). In the case of rice cultivation, increasing drought levels cause a decline in plant growth and yield (Mawardi et al., 2016). The results of research by Mottaleb et al. (2015) showed that different levels of drought caused the decline of rice yields in respectively by 10% (less drought), 50% (moderate drought), and 100% (heavy drought). Therefore, one of the efforts to maintain productivity in fewer water conditions, the research might be investigated using several drought-tolerant strains. The study aims to obtain a genotype of local glutinous rice tolerant to drought stress by investigating physiological responses and yields.

MATERIALS AND METHODS

Plant Materials

The materials used in this study were four (4) glutinous rice consisting of 3 (three) varieties of local West Nusa Tenggara's glutinous rice, namely Me'e varieties, Kala varieties, Samada varieties, and 1 (one) National glutinous rice, Paketih.

Experimental Designs

The research was conducted from May - September 2020 in Labuapi, West Lombok, West Nusa Tenggara. The study used a Randomized Block Design with two factors: the first factor was the glutinous rice strains, and the second factor was the amount of water supply. The first factor is Vm (Me'e variety), Vk (Kala variety), Vs (Samada variety), and Vp (Paketih variety). The second factor is the water supply W1 (2,250 ml), W2 (3,375 ml), W3 (4,500 ml), and W4 (5,625 ml). They obtained 16 treatment combinations. Each treatment combination was repeated 3 times, so it was obtained 48 experimental units. Each experiment consisted of 4 plant polybags and obtained 192 plant populations. The amount of water supply is calculated based on the field capacity of the soil.

Procedures

The planting medium is sifted and dried. Next, 10 kg of soil is mixed with 160 g of organic fertilizer and put in a polybag. Planted three grains of rice in each polybag and, after two weeks, thinned them. The treatment was applied when the plant began to tiller and completed at the beginning of the flowering phase.

Measurement of Variables Associated with Drought Tolerance

The observation variables consisted of stomata density, proline content, chlorophyll content, panicle length, number of grains per panicle, the weight of whole grains, starch, and amylose content.

The stomata density using fresh leaves was observed when the plant entered the flowering phase. Leaf samples were taken from the third leaf of each plant. The lower surface of the leaves is smeared with clear nail polish and covered with clear tape. Then remove the tape and place it on the slide. Stomata observation using an Olympus microscope connected to a photomicroscope (400x magnification). Calculation of stomata density using the formula (1):

$$\text{The stomata density } (mm^2) = \frac{\text{Number of stomata}}{\text{Wide field of view}} \quad \ldots\ldots\ldots (1)$$

The chlorophyll content was measured by the Spectrophotometer method by Winterman de Most. A total of 0.1 g of leaves was crushed in a mortar and mixed with 10 ml of 80% acetone in a 15 ml tube. The mixture was centrifuged at 402xg for 10 minutes. The absorbance of the supernatant was read at wavelengths 663 and 646 nm.

The proline content was observed using the Spectrophotometer method when the plant entered the flowering phase (Bates et al., 1973). A sample of 0.25 g of leaves was weighed and crushed in a mortar with 10 ml of 3% sulfosalicylic acid and then centrifuged at 402xg for 10 minutes. Two mililiters (2 ml) sample was put into a new tube and added 2 ml of ninhydrin and 2 ml of glacial acetic acid. The mixture was heated in a water heater at 100°C for 1 hour, incubated on ice for 5 minutes, then added 4 ml of toluene and vortexed for 15 seconds. The absorbance was read in a 520 nm wavelength spectrometer.

Panicle length was observed by measuring the length of all panicles in one clump and dividing by the number of panicles. The number of grains per panicle was observed by counting the number of seeds in all panicles in one clump and calculating the average. While, the weight of total grains was observed by weighing complete grains in one clump and carried out after harvesting.

The starch content was analyzed using the Nelson Somogy method. First, 0.2 g of rice flour was put in a centrifuge tube and washed with 80% hot ethanol (v/v). Next, the residue was added with 52% (v/v) pecrolic acid and then centrifuged to obtain a residue. The top filtrate is discarded, the remainder is filtered, and anthrone is added. The filtrate was heated at 100°C for 12 minutes. Then the absorbance was measured at a wavelength of 607nm.

The amylose content was analyzed using the Spectrophotometer method. One hundred miligrams (100 mg) of rice flour was put in an Erlenmeyer, and added 1 ml of 96% alcohol and 9 ml of 1 N NaOH. The solution was heated at 100°C for 10 minutes and then diluted with distilled water. Next, 5 ml was pipetted into a 100 ml volumetric flask, added 2 ml of 2% I2 and 1 ml of 0.5 N acetic acids. The solution was diluted with distilled water, shaken, and allowed to stand for 20 minutes (amylose operating time) until the solution turned blue. Next, the absorbance at the maximum wavelength and operating time of amylose were measured. The blank used was Aquades with 2% I2 added.

Data Analysis

Observational data were analyzed using analysis of variance (5% F test). If the treatment significantly affects the observed variables, it is further tested with the DMRT (Duncan Multiple Range Test).

RESULTS AND DISCUSSION

Effect of the Amount of Water Supply on Plant Physiology

Stomata Density

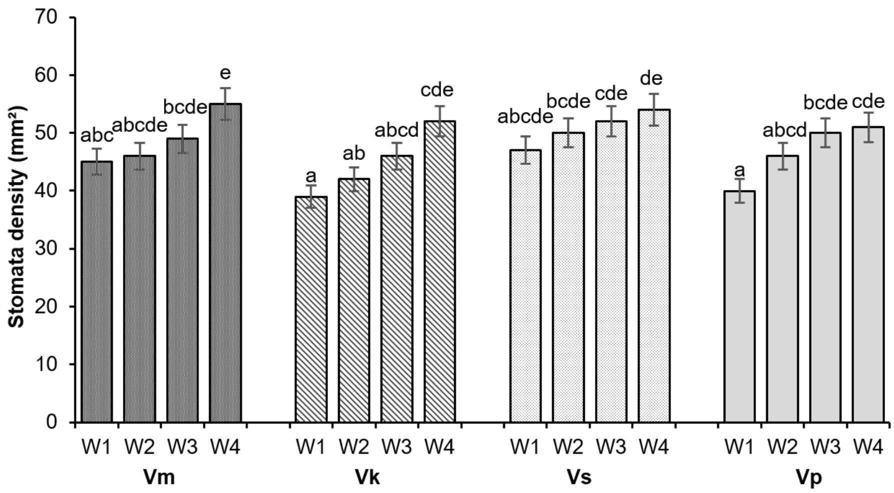
The graph of stomata density in glutinous rice varieties under different amounts of water supply is presented in Fig. 1. All glutinous rice varieties generally show increased stomata density that tends to be similar to water treatment, with rates observed at about 39 to 54.74 mm^2. In detail, the Me'e variety (Vm) in the water supply at about 5,625 ml (W4) had a higher stomata density than other treatments. However, it was not significantly different from the treatments VmW2, VmW3, VkW4, VsW1, VsW2, VsW3, VsW4, VpW3, and VpW4. In contrast, the lowest stomata density was Kala variety (Vk) and Paketih (Vp), both the same affected by water supply at about 2,250 ml (W1). However, the two treatments were not significantly different from the VpW2, VsW1, VkW2, VkW3, VmW1, and VmW2 treatments.

The stomata density has a role in photosynthesis and respiration activities which absorb CO_2 from the air. Thus, the stomata density on plant leaves will directly affect plant photosynthesis. The high stomata density in glutinous rice plants shows that these plants have advantages over other glutinous rice, hence increasing the rate of photosynthate, which in turn increases yields. The low stomata density decreases by deficit water at about 2,250 ml on Kala and Paketih. Basu et al. (2016) stated that the experiment results showed a lack of stomata as the response of leaves to drought conditions. Physiological processes in the plant inhibit plant growth and development. Following the results of research by Wu et al. (2014), Sofy et al. (2020) and Fatima et al. (2020), that the disruption of plant metabolism, both in the absorption of water and nutrients in photosynthesis rate, causes plant stunted or dwarf growth. Due to the lack of stomata, leaves reduce the diffuse amount of CO_2. Therefore, leaves will respond due to decreased leaf cell turgor (Yodhia et al., 2020). Closure of stomata reduces water loss through transpiration and CO_2 and nitrogen uptake from the atmosphere and will change the rate of biomass accumulation (Sham et al., 2015; Ma et al., 2020; Lei et al., 2021). Thus it might be able to decrease total yield cultivation (Salsinha et al., 2021).

Chlorophyll Content

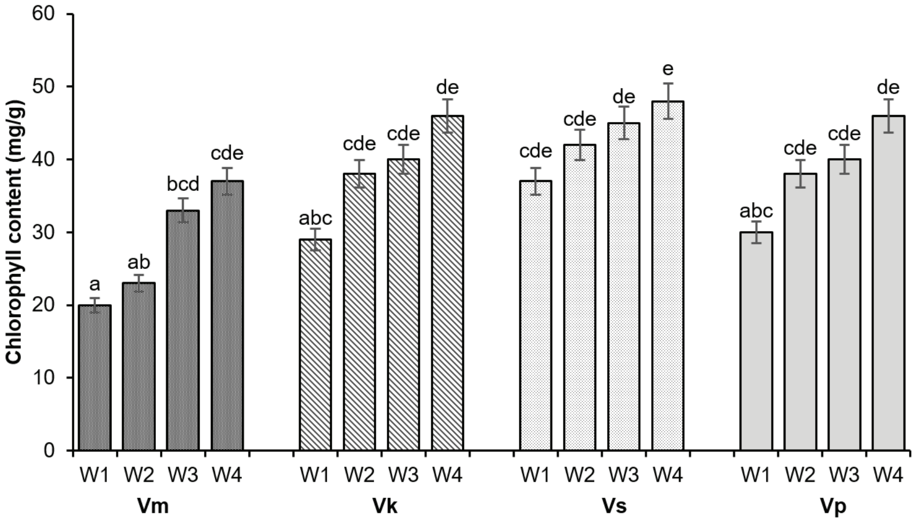
Measurement of physiological parameters such as chlorophyll content is one approach to studying the effect of water shortage on growth and production yields because these parameters are closely related to chloroplast activities (Batool et al., 2020; Xu et al., 2021). The graph of at about chlorophyll content in the glutinous rice grains in different amounts of water supply is presented in Fig. 2. In general, the glutinous rice varieties have the chlorophyll content increase by 33.3% to 50% with increasing water supply. Samada variety (Vs) provision of water supply at about 5,625 ml (W4) had a higher chlorophyll content than the other treatments. However, it is no different compared to VmW4, VkW2, VkW3, VkW4, VsW1, VsW2, VsW3, VpW2, VpW3 and VpW4. In comparison, the lowest chlorophyll content was Me'e variety (Vm) at 2,250 ml (W1), and by contrast, the treatment was not significantly different from VmW2, VkW1, and VpW1.

The low chlorophyll content decrease under deficit water at about 2,250 ml because it is not sufficient and inhibit on plant growth and development. According to the research results by Hussin et al. (2017) and Nugraha (2018), decreased chlorophyll content in water shortage conditions can block chloroplast synthesis, hence reduced chloroplast activity. Ali et al. (2014), Tian et al. (2014) and Wang et al. (2021a), convinced that the decrease of chlorophyll content reduced the photosynthetic apparatus photosynthesis in term of limitation of irrigation volume from 100% water supply to 60% and 20%. Following reducing chlorophyll by 18.90% and 60.36%, respectively (Ridwan et al., 2016).



Remarks: Vm = Me'e variety, Vk = Kala variety, Vs = Samada variety, and Vp = Paketih variety; W1 = water supply 2,250 ml, W2 = water supply 3,375 ml, W3 water supply = 4,500 ml, and W4 = water supply 5,625 ml

Fig. 1. Graph of stomata density varieties of glutinous-rice due to different amounts water supply



Remarks: Vm = Me'e variety, Vk = Kala variety, Vs = Samada variety, and Vp = Paketih variety; W1 = water supply 2,250 ml, W2 = water supply 3,375 ml, W3 water supply = 4,500 ml, and W4 = water supply 5,625 ml

Fig. 2. Graph of chlorophyll content varieties of glutinous-rice due to different amounts water supply

By contrast, increasing water increase the amount of the chlorophyll content in the leaves. The chlorophyll content of rice leaves increased by 6.99% and 6.67%, respectively, in T2 cm and T3 (Pascual & Wang, 2017) and reached 100% soil moisture level (Suete et al., 2017). According to research result by Wang et al. (2021b), under water sufficient conditions, the chlorophyll content in plants will increase significantly, hence able to elevate the activity of photosynthesis. This study shows that the water supply of about 3,375 ml, 4,500 ml, and 5,625 ml can increase glutinous rice's chlorophyll content by 35%.

The chlorophyll content of Kala, Samada, and Paketih tends to be higher than that of the Me'e variety. This is due to differences in plant genetic responses to droughts defending themselves from stress. Regarding the results of research by Iqbal et al. (2019), that drought stress can cause changes in the decrease in chlorophyll content in varies between varieties. It is because plants have different defense mechanisms against drought stress. Hence, plants with higher chlorophyll content will efficiently use solar radiation energy for photosynthesis (Gu et al., 2017). This means that the plants' growth under water supply conditions can be presented by looking at the yield indicators of the number of seeds per panicle and the weight of whole grains.

Proline Content

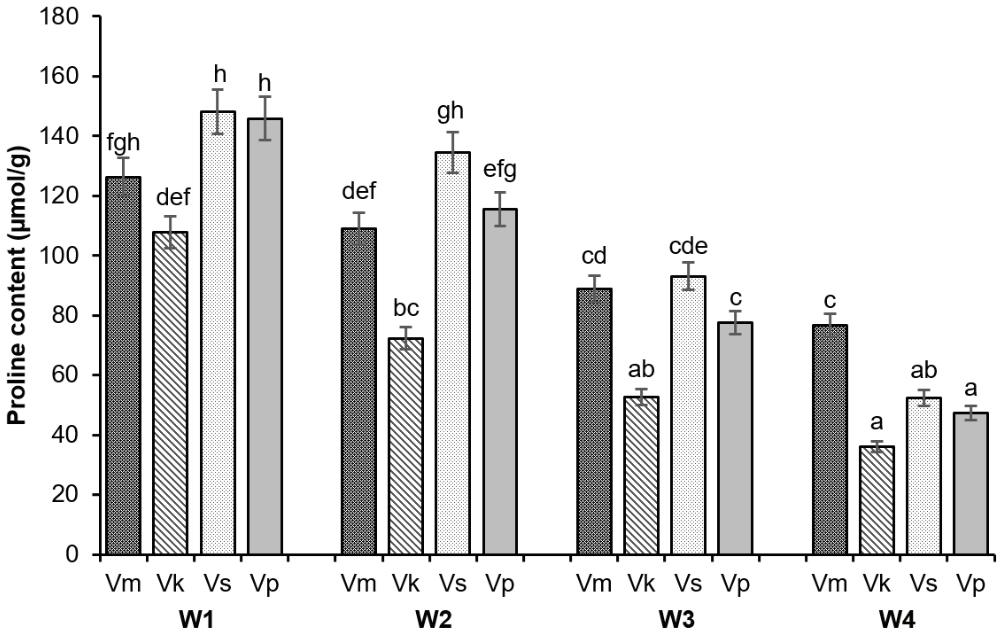
Plant metabolism regards their defense against water deficit which depicts amino produced by plants. Proline is one of the amino compounds which, as the amount in rice varieties, caused different water supply levels, as presented in Fig. 3. All varieties generally show a decrease in proline content followed by sufficient water supply. Barunawati et al. (2016) stated that there is a correlation between the repressed water capacity on proline accumulation and root length development. The yield production of wheat obtains the grains, and proline storage might be affected by plant water status. The highest proline content was obtained in the Samada variety, and the lowest was in the Kala variety. Me'e (Vm), Samada (Vs), and Paketih (Vp) had a significantly higher proline content of 40% in deficit water at about 2,250 ml (W1). However, the three treatments were not significantly different from the Samada variety (Vs) in the water supply at about 3,375 ml (W2).

Meanwhile, the Me'e variety (Vm) and Kala variety (Vk) in the water supply at about 2,250 ml (W1) were not significantly different from the Me'e variety (Vm) and Paketih (Vp) in the water supply at about 3,375 ml (W2). However, it was significantly different from other treatments. Kala variety (Vk) and Paketih (Vp) in the water supply at about 5,625 ml (W4) were significantly lower than the other treatments. However, it was not significantly different from the Samada variety (Vs) in the water supply at about 5,625 ml (W4) and Kala (Vk) in the water supply at about 4,500 ml (W3).

Physiologically, to maintain their metabolic processes under water stress, plants regulate the osmotic potential of their cells to remain negative by regulating Potassium (K). The regulation of osmotic metabolite as proline widely synthesized and accumulated in various plant tissues, especially in leaves under drought stress. This situation follows the statement of Zadehbagheri et al. (2014), Michaletti et al. (2018) and Khan et al. (2019), which stated that as a result of underwater sufficient, osmotic accumulation, in the form of free proline, increases in the leaves which function to maintain water potential. Comparing the varieties, the Samada and Paketih had higher proline content than the Me'e and Kala variety. This is tended to be due to the genetic ability to accumulate higher proline content as a response to lack of water during their development, as the research results by Dewi et al. (2019) showed that 25% of field capacity showed the highest proline content compared to 50% field capacity and 75% field capacity. Based on the ability of Samada gains to drought, yields remain stable. Hence, the variety shows higher yields, particularly in the number of grains per panicle and weight of grains compared to Paketih. This means that Samada varieties are addressed to the study by Vishwakarma et al. (2020). Furthermore, according to the research results by Rahayu et al. (2016), upland rice plants grown at 50% field capacity produced proline with a higher content than those grown at 100% field capacity.

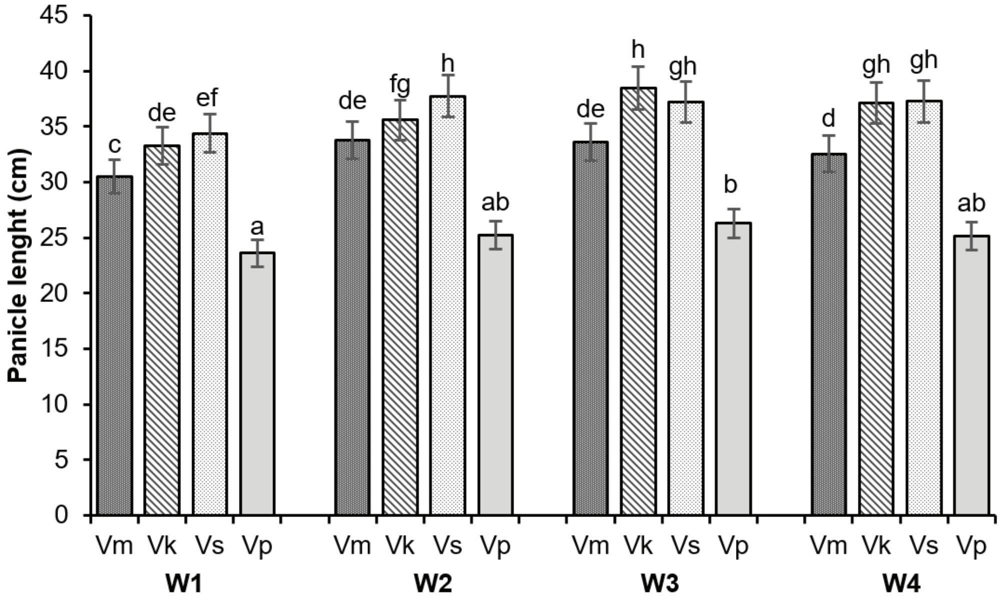
Effect of the Amount of Water Supply on Yield

The yield observation includes the graph of the panicle length of varieties at different water supply amounts presented in Fig. 4. In general, all glutinous rice shows with panicle lengths tended to be the same in all amounts of water treatment. However, local Me'e variety (Vm), Kala (Vk), and Samada (Vs) had lengths of panicle at about 30-39 cm, higher 30% compared to Paketih (National of varieties) with lower panicle lengths (24-27 cm). In detail, the Samada varieties (V3) had similar lengths of panicle under water supply of 3,375 ml (W2), 4,500 ml (W3), and 5,625 ml (W4). By contrast, no significant difference with Kala varieties (Vk) in water supply 4,500 ml (W3) and 5,625 ml (W4). The Me'e and Paketih show length of panicles which tend to be similar in all water supplies (30 cm and 24 cm).



Remarks: Vm = Me'e variety, Vk = Kala variety, Vs = Samada variety, and Vp = Paketih variety; W1 = water supply 2,250 ml, W2 = water supply 3,375 ml, W3 water supply = 4,500 ml, and W4 = water supply 5,625 ml

Fig. 3. Graph of proline content varieties of glutinous-rice due to different amounts water supply



Remarks: Vm = Me'e variety, Vk = Kala variety, Vs = Samada variety, and Vp = Paketih variety; W1 = water supply 2,250 ml, W2 = water supply 3,375 ml, W3 water supply = 4,500 ml, and W4 = water supply 5,625 ml

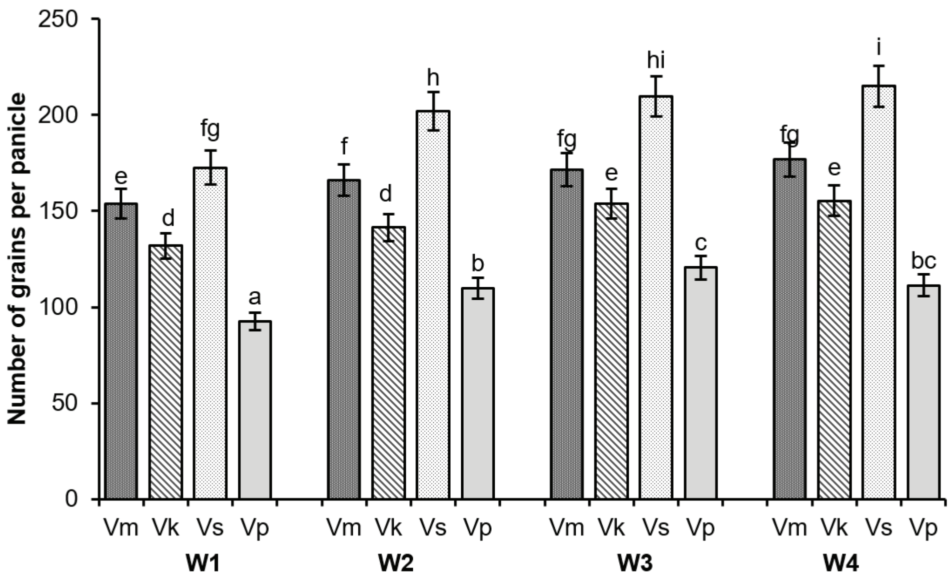
Fig. 4. Graph of panicle length varieties of glutinous rice due to different amounts water supply

The graph number of grains per panicle of several varieties caused the level of water supply is presented in Fig. 5. In general, the Samada variety (Vs) has a similar number of grains per panicle (200-220 seeds) under 5,625 ml (W4), 4,500 ml (W3), and 3,375 ml (W2). However, it differs from 2,250 ml (W1) (140 seeds). Samada variety is dominant in the number of grains compared to other varieties. In detail, following Samada, the Me'e variety had remained stable on grains number at all water supply treatments, reached at 160 seeds each panicle. At the same time, the Kala variety had fewer grains than Me'e, which had 140 seeds under all water treatments. In brief comparison, the Paketih variety had about 100 as the lowest number of grains per panicle. Interestingly, the Paketih variety had a grain number per panicle at about 90, which seems to be the lowest result under a 2,250 ml water supply. In brief, a water supply of about 2,250 ml (W1) on all rice strains can reduce the number of grains per panicle to 20%.

The graph of the weight of full grains glutinous rice varieties at different amounts of water supply is presented in Fig. 6. In general, all varieties increased the weight of full grains by 40% with water supply. The Samada variety (Vs) in all water supply weigh full grains higher than the other variety. Following Kala variety show the weight of full grains remains stable on the water supply at about 3,375 ml (W2), 4,500 ml (W3), and 5,625 ml (W4). At the same time, it reached lower on the deficit water 2,250 ml (W1), likewise with the Me'e variety and Paketih.

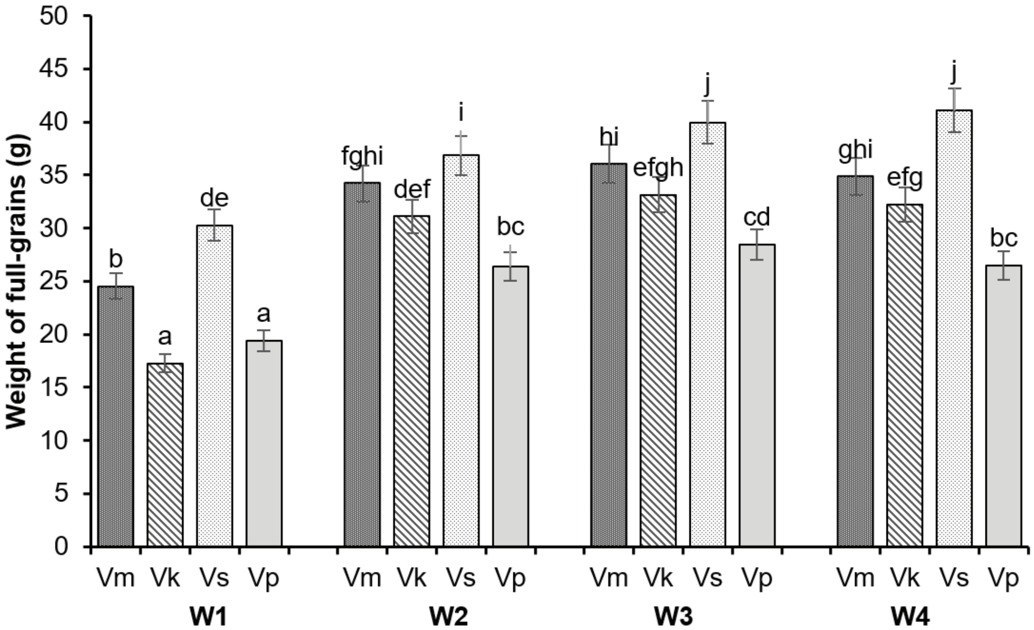
This study result shows that the panicle length and the number of grains per panicle in all varieties decreased by low deficit water at about 2,250 ml. This result is due to decreased plant metabolism due to the lack of water availability in the soil. This causes the process of transpiration and photosynthesis to decrease, reducing plant yields. The research by Mawardi et al. (2016) found that water shortage conditions affect the growth and yield of rice plants. The drought level condition, tend to lower the growth and yield of rice plants. Upland rice experiencing drought stress a significant decrease in panicle length (Munawaroh et al., 2016). While in soybeans, the difference in water content treatment of soybean plants significantly affected plant height, number of leaves, panicle length, and productivity (Hussain et al., 2021).

The glutinous rice varieties had a lower weight of grains, causing deficit water at about 2,250 ml, thought to be caused by the plant's inability to maintain a water balance on all organs. The disruption of the growth process causes low crop yields. Following the results of the study by Pascual & Wang (2017), the grains yield was significantly reduced at drainage by water height at 2, 3, and 4 cm compared to the grains watered at a height of 5 cm.



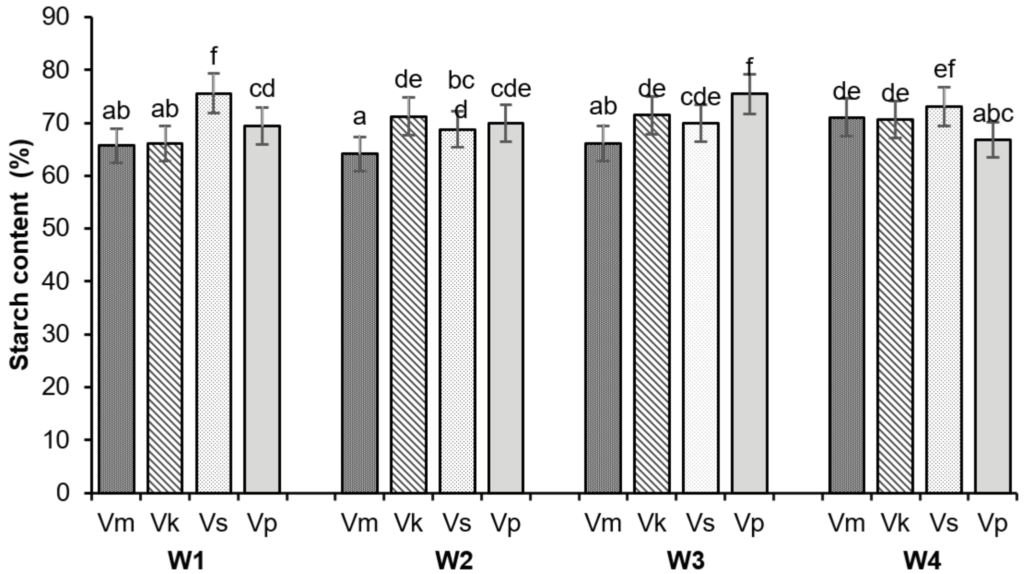
Remarks: Vm = Me'e variety, Vk = Kala variety, Vs = Samada variety, and Vp = Paketih variety; W1 = water supply 2,250 ml, W2 = water supply 3,375 ml, W3 water supply = 4,500 ml, and W4 = water supply 5,625 ml

Fig. 5. Graph number of grains per panicle varieties of glutinous-rice due to different amounts water supply



Remarks: Vm = Me'e variety, Vk = Kala variety, Vs = Samada variety, and Vp = Paketih variety; W1 = water supply 2,250 ml, W2 = water supply 3,375 ml, W3 water supply = 4,500 ml, and W4 = water supply 5,625 ml

Fig. 6. Graph weight of full-grains varieties glutinous-rice due to different amounts water supply



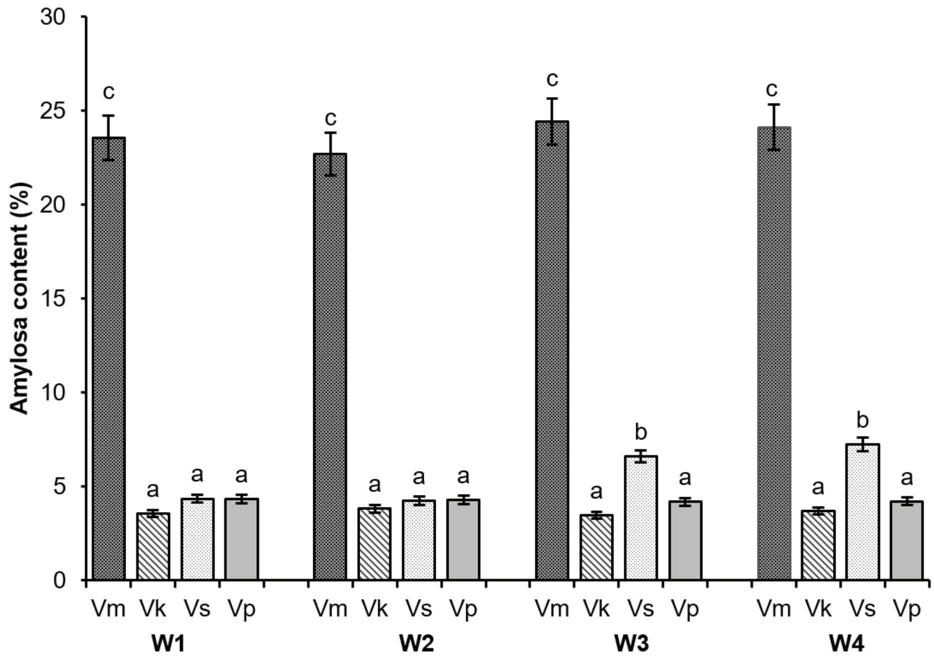
Remarks: Vm = Me'e variety, Vk = Kala variety, Vs = Samada variety, and Vp = Paketih variety; W1 = water supply 2,250 ml, W2 = water supply 3,375 ml, W3 water supply = 4,500 ml, and W4 = water supply 5,625 ml

Fig. 7. Graph starch content varieties of glutinous-rice due to different amounts water supply

In other cases, the water supply of barley was planted at 25% field capacity, resulting in the lowest weight of grains per clump compared to water at 50% field capacity and 75% field capacity (Dewi et al., 2019). This is due to the lack of water experiencing a decrease in metabolic processes in terms of protein and carbohydrate since the amount of assimilation is reduced. The decrease in assimilation causes limited ability of plants to produce full grains. Thus, the expected-glutinous rice, which had adapted well to insufficient water supply can still maintain producing grain's weight per panicle. Therefore, addressing to the weight of full grains, the Samada strain is considered local glutinous rice, which is genetically capable of producing well even under insufficient drainage. That indicates that glutinous rice strains can adapt well, particularly in drought conditions, by specific characters, leading to stable yields (Basu et al., 2016; Jalil & Ansari, 2021; Ainsworth & Long, 2021).

Starch Content and Amylose

The graph of the starch content of the glutinous rice varieties at different water supplies is shown in Fig. 7. In general, the starch content of the varieties tends to be similar under different amounts of water content, which ranged at around 65-77 ppm. The Samada variety (Vs) obtained the highest starch content, and the lowest obtained Me'e variety (Vm). Inconsistent starch content is thought to be affected by differences in glutinous rice varieties' response, which is assumed by differences in gene expression. Based on the research results by Rabara et al. (2021), each variety has different genetic, morphological, and physiological characteristics. Different types of rice can affect diversity; thus, it affects starch metabolism and accumulation. Following the results of research by Nisah (2018), the functional properties of starch influenced variety, natural conditions, and the place of the plant origin. By contrast, drought stress in wheat significantly reduced starch accumulation but did not affect the ratio of amylose and amylopectin (Lv et al., 2021; Dai et al., 2021; Wang et al., 2021a). The percentage of type B-starch granules is significantly enhanced in wheat plants under conventional irrigated conditions. On the other hand, type A-starch granules decreased under a rainfed irrigated system (Dai et al., 2021). It is assumed that the composition of granule starch types is strongly affected by water shortage or excess water conditions.



Remarks: Vm = Me'e variety, Vk = Kala variety, Vs = Samada variety, and Vp = Paketih variety; W1 = water supply 2,250 ml, W2 = water supply 3,375 ml, W3 water supply = 4,500 ml, and W4 = water supply 5,625 ml

Fig. 8. Graph amylose content varieties of glutinous rice due to different amounts water supply

The graph of amylose content of glutinous rice varieties due to different amounts of water is presented in Fig. 8. Me’e variety (Vm) in all water supply shows that amylose content is significantly higher by 80% compared to other varieties. Meanwhile, the Kala variety (Vk) and Paketih (Vp) had amylose content which tended to be the same in all water supplies. However, the Samada variety showed a different response. It increased amylose content at an increase of water supply at about 4,500 ml (W3) and 5,625 ml (W4). According to opinion Fahmy et al. (2022), water availability in soil media is one factor that affects the quality of the chemical properties of rice. Meanwhile, genetic factors obviously regulate and contribute to the difference in amylose content. Following Jakšić et al. (2020) research, the Wx gene genetically controls amylose content by encoding a waxy protein (starch synthase bound to granules). The amylose content which is controled by amylose synthesis and regulated by Waxy (*Wx*) (LOC_*Os06g04200*) encoding Granule-Bound Starch Synthase I (GBSSI). In that case the amylose which has the contrast of amylopectin content in grains. (Xu et al., 2021).

Me’e variety shows amylose content is about 23.69%, higher fifth fold than other varieties. The high amylose content in Me’e grains is thought to have higher anthocyanin storage in the vacuole; hence its varieties have a darker epidermis coat color. Meanwhile, Kala, Samada, and Paketih varieties tend to be lighter in the color of seeds and cause the presence of genes that regulate aleurone (Aminah et al., 2019). This convinced Saputra et al. (2022) that each variety had color and amylose content characteristics. Amylose content determines the physical appearance of rice in the form of color and texture, so rice is classified into high amylose content (>25%), medium (20-24%), and low (<20%) (Luna et al., 2015). Rice with a high amylose content is not sticky (Anugrahati et al., 2017). In addition, based on the research by Nandariyah et al. (2018), one of the strains, namely Matesih and Klaten, had dark-color, high amylose content with black glutinous rice at around 23.61% and 23.44%.

CONCLUSION

The results of the research present that all varieties of glutinous rice under water supply rates were observed had the stomata density at about 39 to 54.74 mm^2, the chlorophyll content decreased (33.3% to 50%), by contrast, the proline content increased by 40% under deficit water at about 2,250 ml. Meanwhile, the water supply at about 3,375 ml shows an increase in the number of grains per panicle and the weight of full grains on all varieties. Interestingly, the highest amylose and weight of full grains reached 40% by the Samada variety, which tends to adapt well compared to other varieties under insufficient water (2,250 ml).

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