

# Evaluation of Some Drought Tolerant M5 Rice Genotypes on Proline Content and Yield Components at Different Levels of Field Capacity

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Received: August 30, 2024  
Revised: September 27, 2024  
Accepted: October 25, 2024  
Published: October 31, 2024

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DOI: [10.29303/jossed.v5i2.9008](https://doi.org/10.29303/jossed.v5i2.9008)

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**Abstract:** Massive conversion of agricultural land, especially on wetlands, has resulted in declining rice productivity, so extending cultivation to drylands is considered appropriate. However, limited water availability is a major constraint that cannot be ruled out, so the procurement of drought-adaptive high-yielding varieties is considered to be the most effective solution. The purpose of this study was to evaluate drought-tolerant mutant genotypes based on proline content and yield component characters at various percentages of water availability. The experiment was arranged using a factorial complete randomized design, the first factor is drought stress consisting of 100% field capacity (K3), 66% (K2), 33% (K1) and the second factor is genotype consisting of inpagu unram (P1), MD200-G13-3-11-5 (P2), MD300-G20-8-3-5 (P3), MD200-G24-17-10-8 (P4), MD300-G27-16-9-5 (P5). The observed characters consisted of proline content, flag leaf length, panicle length, filled grain weight and hollow grain weight. The results showed that MD300-G27-16-9-5 (P5) is a mutant plant that has the greatest potential to obtain drought-tolerant traits, although the level of proline produced is not as high as Inpagu Unram (P1) and MD200-G24-17-10-8 (P4), but the consistency of adaptation shown by MD300-G27-16-9-5 (P5) when experiencing drought stress tends not to cause a significant decrease in all yield component characters, namely flag leaf length, panicle length, filled grain weight and unfilled grain weight.

**Keywords:** Drought stress; Mutant genotype; Proline; Tolerant; Yield componente

## Introduction

Population growth that continues to increase from year to year is a challenge, especially for the agricultural sector. Indirectly, this will increase the demand for food, so the availability of food must be further increased to support the needs of the community. On the other hand, rice production has recently decreased. Based on the Central Bureau of Statistics (2024), rice production in Indonesia in 2022 reached 54.75 million tons, while production in 2023 reached 53.98 million tons. The decline in production is due to the narrowing of the harvest area, as the wet harvest area decreased by around 24 hundred ha in 2023. The decline in the size of the cropping area is partly triggered by the massive conversion of agricultural land, especially wetlands (paddy fields), because wetlands are considered to have

a higher fertility level than other lands, especially drylands (Wunangkolu et al., 2019).

Drylands are generally characterized by agroecosystem conditions with limited availability of water sources. Water availability is a very crucial aspect in supporting the life cycle of plants, because none of the plant metabolism can run without the availability of water (Chaniago et al., 2022). Limited water availability in drylands is often the main trigger for low crop production, but the high area of drylands available in Indonesia has the potential to increase national food production, especially rice. Rice production in drylands in 2018 touched 4.17 million tons with a utilized harvest area of around 1.27 million ha (Directorate General of Food Crops, 2022b). This utilized land area is still very far from the potential land area for agricultural development with a land area of 63.4 million ha (Faizinia

## How to Cite:

Raihanun, S., Sudharmawan, A., Kisman, K., Wangiyana, W., & Yakop, U. M. (2024). Evaluation of Some Drought Tolerant M5 Rice Genotypes on Proline Content and Yield Components at Different Levels of Field Capacity. *Journal of Science and Science Education*, 5(2), 113-120. <https://doi.org/10.29303/jossed.v5i2.9008>

et al., 2023). If this condition continues, it will be difficult to optimize drylands.

The use of high-yielding seed varieties is part of the solution offered by the Directorate of Cereals, especially the Subdirector of Rainfed and Dryland Rice, as an effort to increase the potential for dryland utilization while increasing rice production (Directorate General of Food Crops, 2022a). This is a challenge for plant breeders, because the varieties created must be adapted to drought conditions. Drought conditions stated by Bhandari et al. (2023) adversely affect the vegetative phase, but drought stress at the reproductive stage is considered more severe because it can substantially reduce yields by up to 60%. Therefore, before selection, mutation induction is carried out on the genotypes evaluated to increase diversity, so that it can facilitate breeders to create drought-adaptive varieties.

Gamma-ray mutation induction reported by Suliartini et al. (2022) successfully produced most of the superior varieties of plants, where radiation doses of 200-300 Gy successfully changed the quantitative characters of rice plants (Tumanggor et al., 2022). In previous studies, several mutant genotypes have been obtained that have been irradiated with 200 Gy and 300 Gy gamma rays, but these genotypes have not been evaluated for tolerance to drought stress. According to Nurmalasari (2018); Larasani and Violita (2021), indicators of drought tolerance in plants can be evaluated through proline levels produced by plants. Proline plays a role in maintaining cell turgidity, as well as functions in storing N elements, enzyme protectors, preventing damage and denaturation of protein structures and as an osmoregulator (Mudhor et al., 2022). Basically, every plant, both sensitive and tolerant, is able to produce proline when it is stressed, but the difference lies in the accumulation of proline produced. Tolerant plants tend to produce higher proline levels than sensitive plants (Susetio et al., 2019). The high level of proline produced is thought to be a form of plant defense, because stressed plants are believed to have better water holding capacity.

This study aims to evaluate drought-tolerant mutant genotypes based on proline levels and yield-supporting component characters at various percentages of water availability.

## Method

This research was conducted from November to May 2024 and took place in the greenhouse of the Faculty of Agriculture, Mataram University. Physiological analysis for plant proline levels was carried out at the Immunobiology Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University. The tools used consisted of eppendorf, erlenmeyer, scissors, label

paper, glass cuvette, meter, oven, ruler, UV plastic, centrifuges, soil scoop, soil moisture sensor, spectrophotometer, marker, falcon tubes, test tubes, tarpaulin, analog scales, digital scales, vortex and water bath. Materials such as glacial acetic acid, ninhydrin acid, sulfosalicylic acid, rice genotype seeds, buffer solution and toluene were used in this experiment.

The study was organized using a completely randomized factorial design (CRFD) consisting of two factors. The first factor was drought stress (K) which consisted of three treatment levels: 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 (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.

Determination of field capacity begins with drying the planting media, sieving, mixing organic fertilizer with planting media in a ratio of (3:1) and incubating the planting media. Determination of field capacity begins with determining the volume of soil per experimental unit, then the soil sample is sieved with a 0.5 mm diameter sieve. Previously, the cup and soil sample were weighed three times, then the sample was 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):

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

b = initial weight of soil before oven (g)

c = final weight of soil after oven (g)

The percentage of field capacity that has been obtained is used as a basis for determining the amount of water volume to be given to each treatment. The volume of water is determined by equation 2.

$$\text{Water volume (l)} = \text{field capacity (\%)} \times \text{volume of planting media/polybag (kg)} \quad (2)$$

The volume of water that has been obtained for each field capacity is scaled using a soil moisture sensor measuring instrument. The scale value obtained from the soil moisture sensor tool indicates the moisture level in the planting media after watering. The volume of water previously obtained is given to the planting media only once, then if a decrease in the scale value is detected, watering is carried out by adding water to the planting

media slowly until it shows the initial scale that has been obtained previously.

The observation characters consisted of proline, flag leaf length, panicle length, filled grain weight and unfilled grain weight. These characters were further tested using Duncan Multiple Range Test 5%. The level of tolerance of genotypes to proline levels produced was determined through the drought sensitivity index (DSI) with Formulation 3:

$$DSI = (1 - \frac{Y_c}{Y_o}) / (1 - \frac{X_c}{X_o}) \quad (3)$$

- Yc = Average genotypes under drought stress conditions
- Yo = Average genotypes at optimum condition
- Xc = Average of all genotypes under drought stress conditions
- Xo = Average of all genotypes at optimum condition

The closeness between characters is determined through phenotypic correlation using the equation (Ujianto et al., 2021):

$$r_{x_1x_2} = \frac{\sum(X_{1i} - \bar{X}_1)(X_{2i} - \bar{X}_2)}{\sqrt{\sum(X_{1i} - \bar{X}_1) \cdot \sum(X_{2i} - \bar{X}_2)}} \quad (4)$$

## Result and Discussion

Phenotypic correlation is used to express the degree of relationship between certain characters and other characters. The degree of relationship in phenotypic correlation is shown through positive and negative values. Correlation with a positive value means that an increase in a particular character is proportional to an increase in the intended character, on the other hand, a negative number means that an increase in a character will have implications for reducing the intended character (Akbar et al., 2019). According to Sarwono (2006) in (Tanjung & Muliyani, 2021) the correlation coefficient criteria are classified into no correlation if it is 0, very weak correlation if the value is 0-0.25, moderate correlation if the value is 0.25-0.5, strong correlation if the value is 0.5-0.75, very strong correlation if the value is 0.75-0.99 and perfect correlation if the value is 1.

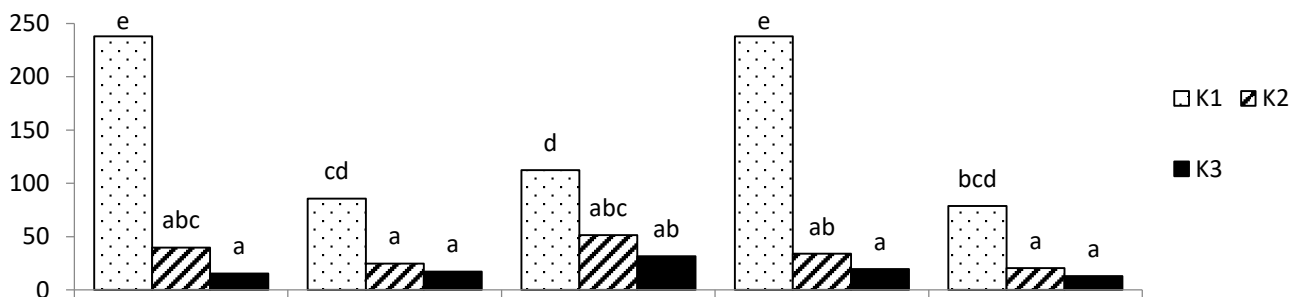
**Table 1.** Phenotypic Correlation Between Observed Characters

Observation Character	Flag Leaf Length	Panicle Length	Filled Grain Weight	Unfilled Grain Weight	Proline
Flag Leaf Length	1				
Panicle Length	0.827*	1			
Filled Grain Weight	0.555*	0.724*	1		
Unfilled Grain Weight	0.480	0.782*	0.787*	1	
Proline	-0.460	-0.525*	-0.428	-0.504	1

Notes: \* = significant base on r-tabel 5% = 0,514

Based on Table 1, all observation characters showed a negative correlation with proline content. Moderate correlation was shown by the characters of flag leaf length and filled grain weight, while the characters of flag leaf length and unfilled grain weight were strongly correlated with proline levels. These results indicate that an increase in proline levels indirectly causes a decrease in flag leaf length, filled grain weight, panicle length and

unfilled grain weight, while an increase in these characters causes proline levels to decrease. The response shown by all observation characters is influenced by drought stress treatment, because the more severe the drought stress experienced by plants, the higher the proline levels produced by plants (Figure 1).



**Figure 1.** Average Proline Content (µg/g). K1 = 33% field capacity, K2 = 66% field capacity, K3 = 100% field capacity, P1 = Inpago unram, P2 = MD200-G13-3-11-5, P3 = MD300-G20-8-3-5, P4 = MD200-G24-17-10-8, P5 = MD300-G27-16-9-5. The same letter indicates the treatment is not significantly different according to the DMRT further test at the 5% level

Bates et al. (1973) stated that the evaluation of the level of tolerance of a variety to stress can be determined

through the accumulation of proline produced from plant tissue. The presence of proline compounds when

the stress is so important, because proline plays a role in regulating the osmotic pressure of plants with the aim of defending themselves from drought stress conditions (Rahayu et al., 2016). Low water content will result in a greater amount of energy expended by plants to absorb water, so plants need to stabilize osmotic potential by accumulating non-toxic solutes in the cell (Sudrajat et al., 2021). A variety can be declared tolerant if the level of proline produced increases with the severity of drought

stress experienced by plants. Because, according to Mudhor et al. (2022) proline accumulation can not only be produced in tolerant varieties, but sensitive varieties are also able to produce proline so that the tolerant level of a variety is determined by the level of proline produced. The tolerance levels of the genotypes evaluated referring to the levels of proline produced are available in Table 2 as follows:

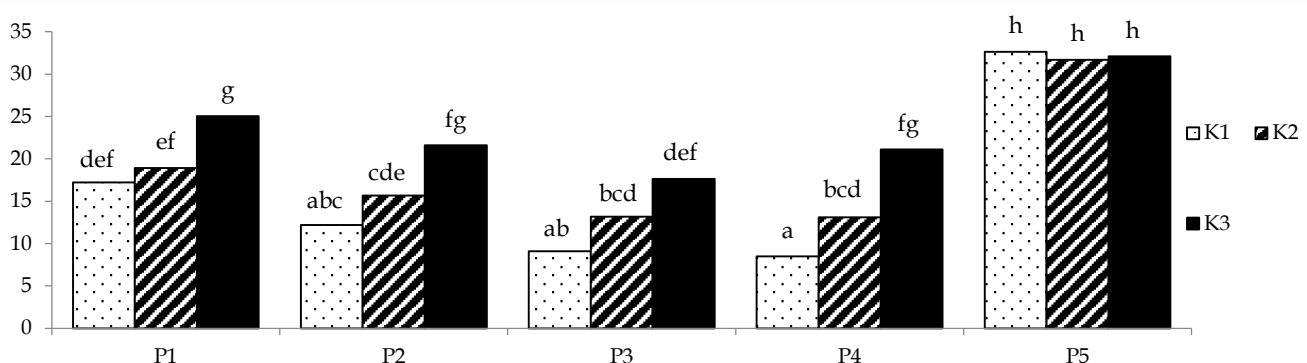
**Table 2.** Drought Sensitivity Index of Aall Genotypes to Prolin

Genotype	66% field capacity (K2)		33% field capacity (K1)	
	Value	Criteria	Value	Criteria
Inpago Unram (P1)	2.12	Sensitive	2.07	Sensitive
MD <sub>200</sub> -G13-3-11-5 (P2)	0.59	Moderate	0.58	Moderate
MD <sub>300</sub> -G20-8-3-5 (P3)	0.38	Tolerant	0.83	Moderate
MD <sub>200</sub> -G24-17-10-8 (P4)	1.64	Sensitive	0.96	Moderate
MD <sub>300</sub> -G27-16-9-5 (P5)	0.75	Moderate	0.75	Moderate

The criteria shown from the drought sensitivity index value can be used as a measure of genotype resistance to drought stress (Adeputri, 2024). The level of plant tolerance can be classified into tolerant if the value of DSI  $\leq 0.5$ , moderate if the value of  $0.5 < \text{DSI} \leq 1.0$  and sensitive if the value of DSI  $> 1.0$  (Rohaeni & Susanto, 2020). The lower the drought sensitivity value, the higher the genotype's resistance to stress. Genotypes that are able to adapt well even though drought stress has been increased indicate the nature of a genotype's resistance to stress is getting better. Based on Table 2, it can be determined that the P3 genotype is the only tolerant genotype at 66% field capacity, but after drought stress was increased to 33% field capacity, the criteria decreased to somewhat sensitive. Unlike the P2 genotype and P3 genotype which showed a rather sensitive adaptation pattern to mild drought stress and severe drought stress. The response shown by these

mutant genotypes needs to be evaluated further, because the genotypes are still in the form of strains so that segregation can still occur although the possibility is small. This provides an opportunity for mutant genotypes, especially the P3 genotype, to obtain better drought adaptive traits.

The high and low levels of proline produced by genotypes are strongly influenced by drought stress treatment, because the more severe the drought stress experienced by the genotype, the higher the increase in proline levels of the genotype (Figure 1). During drought stress, solute accumulation will cause cell water potential to decrease so that the concentration of proline compounds will increase, which can maintain plant cell turgidity (Sinay et al., 2015). However, this increase in proline levels has the implication of reducing flag leaf length, panicle length, filled grain weight and unfilled grain weight of the genotypes evaluated.



**Figure 2.** Average Flag Leaf Length (cm). K1 = 33% field capacity, K2 = 66% field capacity, K3 = 100% field capacity, P1 = Inpago unram, P2 = MD<sub>200</sub>-G13-3-11-5, P3 = MD<sub>300</sub>-G20-8-3-5, P4 = MD<sub>200</sub>-G24-17-10-8, P5 = MD<sub>300</sub>-G27-16-9-5. The same letter indicates the treatment is not significantly different according to the DMRT further test at the 5% level

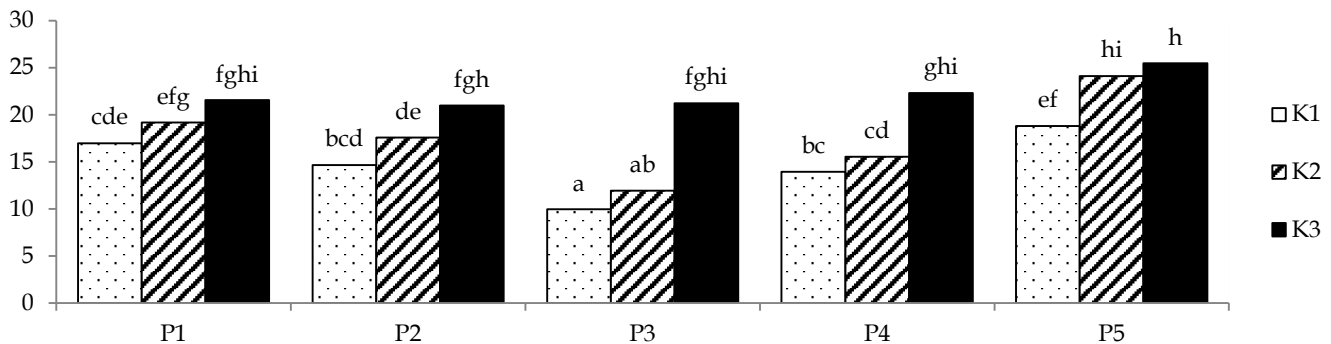
Figure 1 shows that the highest proline content was obtained at 33% field capacity for genotypes P1 and P4

with a mean value of 237.98  $\mu\text{g/g}$ , while the lowest mean proline content of 13.06  $\mu\text{g/g}$  was produced by genotype

P5 at 100% field capacity. These results were inversely proportional to the character of flag leaf length (Figure 2), because the longest flag leaf (32.63 cm) was obtained by genotype P5 at 100% field capacity and the shortest flag leaf (8.50 cm) was obtained by genotype P4 at 33% field capacity. The longest panicle character (25.47 cm) was also produced by genotype P5 at 100% field capacity, but the shortest panicle (9.97cm) was obtained by genotype P3 at 33% field capacity (Figure 3).

The treatment without drought stress at 100% field capacity consistently showed the best flag leaf length and panicle length, while the shortest flag leaf length and panicle length were always found when the genotypes

were under severe drought stress. This response is very common, because a decrease or inhibition in the growth process is an adaptation of plants to overcome water limitations. This is in accordance with the statement of Sinay (2015) that the decrease in leaf length is an effort to prevent water loss through transpiration, because the reduction in leaf length will indirectly reduce the leaf surface area exposed to sunlight. Reduced surface area, stomatal closure and decreased protoplasmic activity are mechanisms carried out by plants when drought stress occurs (Subantoro, 2014). These activities directly inhibit the process of photosynthesis which ultimately results in production not being maximized.



**Figure 3.** Average Panicle Length (cm). K1 = 33% field capacity, K2 = 66% field capacity, K3 = 100% field capacity, P1 = Inpago unram, P2 = MD200-G13-3-11-5, P3 = MD300-G20-8-3-5, P4 = MD<sub>200</sub>-G24-17-10-8, P5 = MD300-G27-16-9-5. The same letter indicates the treatment is not significantly different according to the DMRT further test at the 5% level

Mardiyah et al. (2022) stated that the length of the flag leaf contributes to determining yield, because the flag leaf is the closest source that acts to supply photosynthate to the panicle. Activities related to the character of flag leaf length indirectly affect the character of panicle length, because the length of the flag leaf is very strongly positively correlated with panicle length (Table 1). The longer the flag leaf, the longer the panicle and vice versa. Hasan et al. (2021) stated that the short length of panicles is related to the productivity that will be produced by plants. Long panicles have the potential to increase production, because the longer the panicle, the higher the chance of grain formation (Umam et al., 2018). This is thought to be because long panicles

indicate whether or not photosynthate is optimally distributed to plants.

Photosynthesis greatly affects the high and low distribution of photosynthates to plants, if photosynthate translocation is disrupted, it can have a negative impact on the formation and development of rice grains (Tse et al., 2022). The grain itself is the formation of grain, where grain weight is stated by Sarwendah et al. (2021) to be a criterion in evaluating genotype tolerance to drought. The characters of filled grain weight and unfilled grain weight are supporting components of yield related to rice production measured through. The characters of filled grain weight and unfilled grain weight show a very strong correlation with the character of panicle length (Table 1).

**Table 3.** Average of Filled Grain Weight Characters

Drought stress	Genotype					Average
	Inpago Unram (P1)	MD <sub>200</sub> -G13-3-11-5 (P2)	MD <sub>300</sub> -G20-8-3-5 (P3)	MD <sub>200</sub> -G24-17-10-8 (P4)	MD <sub>300</sub> -G27-16-9-5 (P5)	
33% field capacity (K1)	0.70	0.06	0.01	0.23	0.29	0.26a
66% field capacity (K2)	1.36	0.21	0.02	0.46	1.06	0.62a
100% field capacity (K3)	4.35	1.71	0.88	4.50	5.46	3.38b
Average	2.14bc	0.66ab	0.30a	1.73abc	2.27c	

Notes: The same letter indicates the treatment is not significantly different according to the DMRT further test at the 5% level

Filled grain weight character showed a similar pattern with panicle length character, where the highest

grain weight (5.46 g) was obtained in genotype P5 with 100% field capacity and the lowest weight (0.01 g) was

obtained in genotype P3 with 33% field capacity (Table 3). The results showed that among the genotypes evaluated under all drought stresses, the average weight of filled grain produced was much lower than the average weight of unfilled grain (Table 3 & Table 4). All genotypes except P5 produced higher unfilled grain weight than the filled grain weight of each genotype.

**Table 4.** Average of Unfilled Grain Weight Characters

Drought stress	Genotype					Average
	Inpago Unram (P1)	MD <sub>200</sub> -G13-3-11-5 (P2)	MD <sub>300</sub> -G20-8-3-5 (P3)	MD <sub>200</sub> -G24-17-10-8 (P4)	MD <sub>300</sub> -G27-16-9-5 (P5)	
33% field capacity (K1)	1.11	0.26	0.16	0.65	0.35	0.50a
66% field capacity (K2)	1.86	1.13	0.28	1.01	2.21	1.30b
100% field capacity (K3)	4.49	5.29	3.40	4.30	3.91	4.28c
Average	2.49	2.23	1.28	1.98	2.16	

Notes: The same letter indicates the treatment is not significantly different according to the DMRT further test at the 5% level

Optimal water availability at 100% field capacity treatment tended to produce higher unfilled grain weight compared to mild stress (K2) and severe stress (K1) conditions in the genotypes evaluated (Table 4). The highest unfilled grain weight (5.29 g) was obtained by genotype P2 at 100% field capacity and the lowest grain weight (0.16 g) was obtained by genotype P3 at 33% field capacity. Kasim et al. (2018) stated that the high and low grain weight is influenced by the number of productive tillers, because productive tillers play a role in panicle formation and will directly affect yield characters. In fact, long panicles do not always guarantee high grain weight, because other factors such as temperature and non-optimal photosynthate distribution can trigger high unfilled grain weight.

Based on the results of the study, it can be said that the proline levels of the mutant genotypes evaluated cannot be used as the main reference indicator of drought tolerance, because high proline levels cause flag leaf length, panicle length, filled grain weight and unfilled grain weight to increase along with high proline levels. Genotype P4 which produces the highest proline content has not shown a stable response to the yield component characters, on the other hand, genotype P5 with the lowest average proline content tends to show the best adaptation pattern among other mutant genotypes. Genotype P5 is the only mutant that shows the consistency of adaptation to the characters of flag leaf length, panicle length, filled grain weight and unfilled grain weight, and can even offset the adaptation of the Inpago Unram variety which has been proven to be drought adaptive.

## Conclusion

Based on the results of the study, it can be concluded that MD300-G27-16-9-5 (P5) is a mutant plant

This may be caused by one of the temperature factors, because temperatures that are too high without being balanced by adequate water availability in the generative phase can interfere with the photosynthesis process which will have implications for increasing unfilled grain.

that has the greatest potential to obtain drought-tolerant traits, although the level of proline produced is not as high as Inpago Unram (P1) and MD200-G24-17-10-8 (P4), but the consistency of adaptation shown by MD300-G27-16-9-5 (P5) when experiencing drought stress tends not to result in a significant decrease in all yield component characters, namely flag leaf length, panicle length, filled grain weight and unfilled grain weight.

## Author Contributions

All authors have real contributions in completing this manuscript.

## Funding

This research received no external funding

## Conflicts of Interest

The authors declare no conflict of interest.

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