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# Drought tolerance and yield stability of M5-G16 red rice mutants: A morpho-physiological and Eberhart–Russell analysis

Anak A.K. Sudharmawan*1), I.G.P. Muliarta Aryana2), Wayan Wangiyana1), Siti Raihanun3)

1) University of Mataram, Graduate Program in Dryland Agriculture, Postgraduate School,
Jalan Majapahit No. 62, Kecamatan Selaparang Mataram 83125 West Nusa Tenggara, Indonesia

2) University of Mataram, Faculty of Agriculture, Study Program of Agroecotechnology,
Jalan Majapahit No. 62, Kecamatan Selaparang Mataram 83125 West Nusa Tenggara, Indonesia

3) University of Mataram, Student of the Graduate Program in Dryland Agriculture,
Jalan Majapahit No. 62, Kecamatan Selaparang Mataram 83125 West Nusa Tenggara, Indonesia

* Corresponding author

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**Highlights**

- Developing drought-tolerant rice with stable yields is crucial in water-limited areas.
- Mutation breeding via gamma irradiation evaluates mutants for drought tolerance.
- Integrated morpho-physiology and yield stability identify superior genotypes.

**Abstract:** Developing drought-tolerant rice varieties is crucial amid climate change, leading to an increased drought frequency and intensity. One strategy to address this issue is mutation breeding. This study was conducted from March to October 2024 to evaluate performance of M5 mutant genotypes of the irradiated G16 red rice line through two experiments. Experiment #1 examined morpho-physiological responses of several mutant genotypes to drought stress under pot culture in greenhouse conditions, investigating four mutant genotypes under three drought stresses (33%, 66%, and 100% field capacity). Experiment #2 evaluated the yield stability of 20 promising mutant genotypes and one comparison variety across three different growing locations (Sumbawa, West Lombok and East Lombok). The combined analysis of yield and stability was performed using Q-Basic and MS Excel. Greenhouse pot experiments evaluating morphological and physiological responses to drought showed genotype G15 (4-20-13) consistently accumulated the highest proline and maintained relatively high chlorophyll content under severe stress, while genotype G27 (16-9-4) preserved flag leaf length during drought and produced the highest grain yield. These complementary traits identify G15 and G27 as superior drought-tolerant candidates based on proline, chlorophyll content, flag leaf length, and yield. Multilocation Eberhart–Russell stability analysis indicated that genotypes G15 (4-20-13), G13 (11-16-11), G15 (2-15-11), and G20 (8-3-5) had regression coefficients ($\beta_i$) near 1 and low regression deviation ($Sd_i^2$), reflecting consistent yields across the three test sites and meeting criteria for broad adaptability and general stability. These results confirm their potential as red rice lines with stable performance across environments.

**Keywords:** drought stress, Eberhart–Russell model, G16 red rice (*Oryza sativa* L.), M5 mutant

## INTRODUCTION

Rice, a staple crop crucial for global food security, faces significant challenges due to drought stress exacerbated by climate change. Research has shown that drought affects various agronomic traits, including plant height, tiller number, and grain yield, with genotype-specific responses observed under limited water conditions (Jarin *et al.*, 2024; Saharia *et al.*, 2024). For example, studies have identified drought-tolerant genotypes such as Basmati 2000 and APO, which demonstrated superior yield performance under stress conditions (Mas-ud *et al.*, 2022; Hallajian, Ebadi and Kordrostami, 2024). Physiologically, drought stress leads to reduced photosynthesis, stomatal conductance, and chlorophyll content, ultimately impairing plant growth and productivity (Ahmad *et al.*, 2022; Hassan *et al.*, 2023). Understanding the molecular mechanisms and genetic variability related to drought tolerance is essential for developing resilient rice varieties, as these traits significantly influence adaptation strategies and breeding programmes aimed at sustaining rice production in increasingly arid environments (Panda, Mishra and Behera, 2021).

Strategies to mitigate drought include developing rice varieties with higher tolerance through mutation breeding. This approach has proven effective in generating genetic diversity that can be utilised to select superior lines (Phunthong *et al.*, 2024). Additionally, the use of modern statistical methods such as stability analysis provides a strong scientific basis for selecting lines with stable adaptive responses across various environmental conditions (Borule *et al.*, 2024). Research on the morpho-physiological characteristics of M5 generation mutant rice lines (G16) under drought stress has shown variation in responses across morphological and physiological parameters, offering insight into plant adaptation mechanisms under water deficit conditions (Hallajian, Ebadi and Kordrostami, 2024). Similar findings were also reported by Bhandari *et al.* (2023), highlighting phenotypic expression differences under drought stress.

An in-depth study of the morphological and physiological responses of mutant rice has revealed structural adjustments, such as root modifications and changes in photosynthetic capacity, in response to drought. Studies by Saharia *et al.* (2024) and Jarin *et al.* (2024) support the finding that these adaptive responses are crucial for sustaining plant growth. This is further corroborated by Ahmad *et al.* (2022), who linked physiological mechanisms with increased drought tolerance. To ensure that mutant rice lines perform well beyond laboratory conditions, yield stability must be evaluated through multi-location trials. The Eberhart and Russell method has been widely used to assess genotype×environment (G×E) interactions and identify lines with stable responses (Aswidinnoor *et al.*, 2023). The study by El-Aty *et al.* (2024) further emphasises the importance of stability analysis in supporting the selection of varieties that are adaptive across various land types, including both dryland and irrigated fields.

Implementing multi-location trials is crucial for accurately evaluating the performance of mutant rice strains under diverse environmental conditions, as highlighted by several studies. Environmental variability significantly influences rice agronomic performance, necessitating multi-location testing to identify genotypes that are stable across conditions (Lee *et al.*, 2023; Prado *et al.*, 2024). Rice genotypes exhibit different responses in dryland versus irrigated environments, underscoring the importance of stability testing in breeding programmes. Furthermore, findings from various studies show that G×E interactions are significant, with certain rice cultivars displaying consistent performance across environments. This is vital for developing resilient varieties that can withstand climate change and abiotic stress (Cooper and Messina, 2023; Ahmed *et al.*, 2024). Thus, the multi-location approach not only enhances understanding of G×E interactions but also aids in selecting superior rice genotypes for sustainable agricultural practices.

The integration of morpho-physiological data with yield stability tests offers a comprehensive approach to selecting mutant rice lines. Findings by Pour-Aboughadareh *et al.* (2019) indicate that integrating morphological and agronomic parameters can reveal the full drought-tolerant potential of genotypes. This approach aligns with the findings of Sircar and Parekh (2019), who emphasise the importance of integrated evaluation in plant breeding.

Integrating morpho-physiological analysis with genotypic stability assessment in mutant rice research enhances the understanding of how adaptation mechanisms work in diverse environments. This multi-location approach enables the identification of stable genotypes with good performance under various conditions, as demonstrated by studies using the additive main effects and multiplicative interaction (AMMI) model, which revealed both specific and general adaptability among rice genotypes across multiple locations (Abdelrahman *et al.*, 2022). Furthermore, evaluations of rice mutants under various abiotic stresses, such as low phosphorus and water limitation, underscore the importance of genotype-environment interactions in determining yield stability (Borule *et al.*, 2024). This combined methodology not only addresses the limitations of laboratory testing but also highlights the importance of physiological and agronomic synergy, ultimately facilitating the development of resilient and superior rice varieties suitable for diverse agricultural settings (Gauch, Piepho and Annicchiarico, 2008; Aswidinnoor *et al.*, 2023).

The use of integrated stability evaluation methods provides a strong methodological foundation for rice breeding programmes. Combining multi-location data with morpho-physiological response analysis allows for the selection of lines that are not only genetically superior but also adaptive to environmental variability (Sircar and Parekh, 2019). Moreover, Oladosu *et al.* (2019) affirms that the application of stability models in field trials is a strategic step toward achieving sustainable yield improvements.

The combined approach of morpho-physiology and yield stability evaluation across different land conditions can significantly contribute to the development of drought-tolerant mutant rice varieties. Therefore, this study aimed to examine morpho-physiological responses of several genotypes of M5 mutant generation to drought stress in controlled pot experiment in a greenhouse, accompanied with multi-location experiments to examine yield stability of all genotypes of M5 mutant generation of red rice across different environments. Based on these objectives, it was hypothesised that there are significant morpho-physiological differences between the M5 mutant genotypes of red rice, with some genotypes showing more adaptive morpho-physiological responses to drought stress. Consequently, these differences may lead to significant variations in yield stability across diverse environmental conditions.

The findings of this research are expected to support more effective breeding strategies in facing climate challenges and to enhance national rice productivity and food security (Selamat and Nadarajah, 2021; Paul *et al.*, 2023). Thus, this integrated approach not only advances innovation in rice research but also provides a scientific foundation for the implementation of sustainability-oriented breeding programmes.

## MATERIALS AND METHODS

This study was conducted from March 13 to October 31, 2024, through two experiments: a greenhouse pot experiment (Experiment #1) and a field experiment in three locations (Experiment #2). Experiment #2 investigated multi-location performance and adaptation of 20 genotypes of M5 mutant genotypes resulted from irradiation of the G16 red rice line (plus one comparison variety), while Experiment #1 used only selected genotypes of the M5 mutants to investigate their morpho-physiological responses to drought. The M5 mutants genotypes were developed by irradiating seeds of the G16 red rice promising line in a Gamma Chamber 4000 facility (PAIR–BATAN) with a Cobalt-60 source at the dose rate of ~91.4 krad·h$^{-1}$ (~914 Gy·h$^{-1}$). The applied doses were 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy to produce M1 population. Subsequent generations (M2, M3, M4, and M5) were selected and characterised based on superior phenotypes, including optimal plant height, high grain yield per panicle, and earlier maturity. Selection was supported by calculations of genetic parameters (phenotypic and genotypic variance) to estimate heritability and potential breeding progress (Sudharmawan *et al.*, 2024).

- **Experiment #1**

The experiment #1 was carried out in the greenhouse of the Faculty of Agriculture, University of Mataram, designed according to factorial completely randomised design (CRD) consisting of two factors. The first factor was drought stress (K), with three treatment levels: K1 = 33% field capacity (*FC*), K2 = 66% *FC*, and K3 = 100% *FC*). The second factor was the M5 mutant genotypes of G16 red rice promising line (P), with four treatment levels: P2 = MD200-G13(11-16-11), P3 = MD300-G20(8-3-5), P4 = MD200-G15(4-20-13), and P5 = MD300-G27(16-9-4). The MD200 genotypes were derived from irradiation at 200 Gy while MD300 genotypes were derived from irradiation at 200 Gy. Each treatment combination was replicated three times.

The growing medium in the greenhouse experiment consisted of field soil. The soil was air-dried, sieved through a 0.5 mm mesh, and mixed with organic fertiliser in a 3:1 ratio. The mixture was then incubated for 14 days. After measurement of its *FC*, this growing medium was used to fill polybag pots (17.5 kg·polybag$^{-1}$), followed by watering with volume depending on the drought stress treatments (33%, 66% or 100% *FC*). The seeds were then sown and the seedlings transplanted 14 days after sowing. Routine maintenance included watering, fertilisation, gap filling, weeding, and pest and disease control.

- **Experiment #2**

In Experiment #2, the rice plants were grown as upland rice on a farmer's land in each location. This multi-location experiment consisted of two factors. The first factor was growing location: L1 = Pernek Village (8°34'07.33" S, 117°26'01.89" E, 46 m a.s.l.) in Sumbawa island, L2 = Nyurlembang Village (8°35'28.06" S, 116°11'05.36" E, 108 m a.s.l.), and L3 = Bagik Payung Village (8°37'23.88" S, 116°33'19.04" E, 138 m a.s.l.) in Lombok island. In each location, the experiment was designed according to the randomised complete block design (RCBD) consisting of 21 treatments: 20 genotypes of M5 mutants and one comparison cultivar, i.e. 'Gamagora' variety. Each treatment was replicated three times, resulting in 63 experimental units per growing location.

Data were analysed using Q-Basic and Microsoft Excel software. The analyses included: grain yield conversion analysis (Mg·ha$^{-1}$), combined analysis of variance, location index analysis, stability analysis, and mean estimation analysis against regression coefficient values. The stability analysis followed the method by Eberhart and Russell (1966).

The formulas used for data analysis are as follows:

- grain yield conversion analysis (Mg·ha$^{-1}$) (Maspary, 2015):

$$\left(\left(\frac{10,000}{Jt}\right)\cdot Ja\cdot Jgb\cdot Bsb\right):10^{9} \tag{1}$$

where: $Jt$ = plant spacing (0.0625 m$^2$), $Ja$ = number of tillers, $Jgb$ = number of filled grains, $Bsb$ = weight of 1,000 grains (g);

- combined analysis of variance for grain yield was done based on Table 1;
- location index ($I_j$) analysis:

$$I_j = \sum(Y_{ij}/i) - \left(\sum\left(\sum Y_{ij}\right)\right)/(i\cdot j) \tag{2}$$

where: $Y_{ij}$ = mean yield of the $i$-th genotype in the $j$-th environment, $i{\cdot}j$ = number of genotype time number of environment;

- yield stability analysis according to Eberhart and Russell (1966):

$$\beta_i = \frac{\sum_j Y_{ij} I_j}{\sum_j I_j^2} \tag{3}$$

where: $\beta_i$ = regression coefficient;

$$Sd_i^2 = \left(\frac{\sum_j \hat{\delta}_{ij}^2}{j-2} - \frac{Se^2}{r}\right) \tag{4}$$

where: $Sd_i^2$ = deviation from regression (variance), $\hat{\delta}_{ij}^2$ = mean combined deviation, $Se^2$ = error in the combined ANOVA, $r$ = replications;

- analysis of the estimation of one mean on the regression coefficient ($\mu$) value

$$\bar{y} - t_{\alpha/2}\frac{S}{\sqrt{n}} < \mu < \bar{y} + t_{\alpha/2}\frac{S}{\sqrt{n}} \tag{5}$$

where: $t_\alpha$ = $t$-table value at significance level $\alpha$, $S$ = standard deviation of the regression coefficient, $n$ = number of treatments.

## RESULTS AND DISCUSSION

### EXPERIMENT #1

Based on the ANOVA results for Experiment #1 (Tab. 2), most variables showed significant interaction effects of the treatment factors, except for the growth variables such as plant height, number of tillers, and number of leaves.

**Table 1.** Model for combined analysis of variance

| Source of variation | *df* | Sum of squares (*SS*) | Mean square (*MS*) | *F* value |
|---|---|---|---|---|
| Location (environment) | $j - 1$ | $SS_j$ | $SS_j/df_j$ | $(SS_j/df_j)/(SS_g/df_g)$ |
| Genotype (line) | $i - 1$ | $SS_i$ | $SS_i/df_i$ | $(SS_i/df_i)/(SS_g/df_g)$ |
| Replication | $j(r - 1)$ | $SS_r$ | $SS_r/df_r$ | $(SS_r/df_r)/(SS_g/df_g)$ |
| Genotype×environment | $(j - 1)(i - 1)$ | $SS_{ij}$ | $SS_{ij}/df_{ij}$ | $(SS_{ij}/df_{ij})/(SS_g/df_g)$ |
| Error | $j(r - 1)(i - 1)$ | $SS_g$ | $SS_g/df_g$ | – |
| Total | $(r \cdot j \cdot i) - 1$ | $\sum SS$ | – | – |

Explanations: *df* = degree of freedom, *j* = location, *i* = strain, *r* = replicate, *g* = error.
Source: own elaboration.

**Table 2.** Summary of ANOVA results for the interaction and main effects of treatment factors on all observed variables

| Treatments | Plant height (cm) | Tiller number ($\text{pot}^{-1}$) | Leaf number ($\text{pot}^{-1}$) | Chlorophyll ($\text{mg·dm}^{-3}$) | Flag leaf length (cm) | Panicle length (cm) | Grain yield ($\text{g·pot}^{-1}$) | Proline content ($\mu\text{mol·g}^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| K1 (33% *FC*) | 44.73c | 2.92c | 10.17c | 19.79b | 15.60c | 14.34c | 0.49c | 128.74a |
| K2 (66% *FC*) | 59.52b | 5.50b | 23.00b | 19.36b | 18.41b | 17.29b | 1.60b | 32.95b |
| K3 (100% *FC*) | 73.96a | 9.92a | 42.00a | 23.00a | 23.10a | 22.50a | 7.36a | 21.76c |
| Tukey's HSD 0.05 | 7.87 | 2.48 | 11.64 | 0.98 | 2.46 | 1.89 | 0.87 | 5.27 |
| P2: MD200-G13 | 57.46b | 5.22a | 20.00a | 20.13b | 16.48b | 17.74b | 2.87b | 42.87c |
| P3: MD300-G20 | 54.81b | 6.67a | 27.78a | 21.34b | 13.30c | 14.38c | 1.58c | 65.99b |
| P4: MD200-G15 | 49.96b | 5.56a | 19.33a | 22.59a | 14.23bc | 17.26b | 3.71ab | 99.31a |
| P5: MD300-G27 | 75.38a | 7.00a | 33.11a | 18.81c | 32.14a | 22.79a | 4.43a | 36.42c |
| Tukey's HSD 0.05 | 10.04 | 3.16 | 14.84 | 1.25 | 3.14 | 2.41 | 1.11 | 6.72 |
| Interaction | ns | ns | ns | *** | ** | * | ** | *** |

Explanations: *FC* = field capacity, HSD = honestly significant difference, for interaction effects: ns = non-significant; *, **, *** = significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, for main effects: mean values in each column followed by the same letters are not significantly different between levels of each factor.
Source: own study.

Evaluation of drought stress tolerance in rice, particularly in the M5 generation of G16 red rice mutants, revealed significant impacts on growth and yield parameters due to reduced water availability. Drought stress leads to morphological changes such as reduced plant height, smaller leaf area, and lower chlorophyll content. These traits are critical for photosynthesis and overall plant health (Jarin *et al.*, 2024). Physiological responses include stomatal closure and reduced transpiration rates, which exacerbate oxidative stress through the production of reactive oxygen species (ROS) (Hassan *et al.*, 2023). Studies have shown that specific genotypes exhibit varying degrees of drought tolerance. Traits such as root volume and chlorophyll stability are positively correlated with yield (Hallajian, Ebadi and Kordrostami, 2024; Saharia *et al.*, 2024).

## Plant height

Table 2 shows that plant height decreased with each reduction in soil moisture contents. When irrigation water is limited during the vegetative phase, one of the symptoms that can be observed in plants is a reduction in height. This occurs due to reduced photosynthetic rates resulted from partial stomata closure under low soil moisture. Effective photosynthesis produces sufficient photosynthates for various cellular activities such as cell division, enlargement, and differentiation processes that contribute to plant growth, including height (Salehi-Lisar and Bakhshayeshan-Agdam, 2020). There was also significant variation in plant height among the mutant genotypes. This may have resulted from gene changes induced by gamma-ray irradiation. Genotypes P2 and P4 were produced using 200 Gy while P3 and P5 were produced using 300 Gy. A 200 Gy dose can significantly reduce plant height as it can alter somatic cells, causing phenotypic changes such as plant height. Tall plant stature is generally not a breeding target because it increases the risk of lodging. Therefore, a suitable breeding strategy is to develop rice varieties with shorter stature. Reduced plant height is also a natural plant response to drought stress, as observed in the genotype P4.

## Number of tillers and leaves

Table 2 also shows lower number of tillers and leaves per clump or per pot under lower soil moisture contents. Since each tiller produces its own leaves, leaf number is a function of tiller number. The inhibition of growth processes related to changes in cell size and cell division under lower water availability is one of the main causes of reduced leaf number. This is associated with increased senescence (premature aging) and leaf abscission (leaf shedding) (Puspitaningrum and Salamah, 2023).The reduction in tiller number as well as leaf number is a plant strategy to reduce evapotranspiration in response to limited water availability in the growing medium (Chaniago and Alim, 2024). In addition, different genotypes also show variation in the number of tillers and leaves. These differences among mutant plants are attributed to the varying doses of gamma radiation. The genetic background of the parental lines can influence a plant's ability to produce tillers and leaves. The number of tillers also affects overall plant productivity, as it is usually positively correlated with the number of productive tillers produced.

### Proline and chlorophyll contents

Both proline and chlorophyll contents were highest in P4 genotype (Tab. 2), and both also showed significant interaction effects between the treatment factors. However, based on the patterns of the interaction effects, proline content was highest in P4 genotype treated with the lowest soil moisture content (33% *FC*), as shown in Figure 1a, but chlorophyll content was highest in P4 genotype having no drought stress (Fig. 1b). Higher proline accumulation under water-deficient conditions reflects the genotype's response to survive in suboptimal environments. Therefore, the differences in proline levels among genotypes indicate genetic variation in their adaptive capacities.

On average, the highest proline accumulation was observed in genotype P4. This genotype differed from P2, P3, and P5. According to Ahmad *et al.* (2022), proline accumulation does not occur exclusively in tolerant varieties; sensitive varieties can also produce proline, although in varying amounts. Selamat and Nadarajah (2021) suggested that variation in proline levels is influenced by plant's genetic factors, as certain genes may express either stronger or weaker stress-response characteristics.

Drought stress significantly affects plant morphology, as evidenced by the reduction in plant height and leaf number under 33% *FC* (Tab. 2), indicating inhibited cell division and expansion. The P5 genotype (MD300-G27(16-9-4)) exhibited superior morphological adaptation by maintaining stable flag leaf and panicle length, suggesting efficient assimilate transport and consistent spikelet formation both of which are crucial for reproductive success (Ahmad *et al.*, 2022). In contrast, genotype P3 showed high vulnerability, with notable reductions in these parameters, reflecting a weaker adaptation to stress (Mas-ud *et al.*, 2022). This differential response is consistent with findings across species, where morphological traits such as stomatal conductance and leaf structure play a key role in drought tolerance (Bhandari *et al.*, 2023; Phunthong *et al.*, 2024). Thus, understanding morphological adaptations is essential for breeding programs aimed at enhancing drought tolerance in rice.

Drought stress affects plant growth and development. According to Ahmad *et al.* (2022), drought can reduce the rate of photosynthesis and leaf area, which are closely related to the plant's chlorophyll content. Chlorophyll levels tend to decrease under drought stress, and Jarin *et al.* (2024) stated that this decline serves as an indicator of the plant's response to the drought conditions. Genotypes P2 and P4 exhibited different chlorophyll content responses under varying *FC* conditions. Both genotypes showed a decrease at 66% *FC* but experienced a slight increase at 33% *FC*. This indicates a level of drought tolerance in P2 and P4, in line with the findings of Saharia *et al.* (2024), who noted that even under stress, tolerant plants can maintain their chlorophyll content. A similar trend was observed in genotype P3. In contrast, genotype P5 showed a consistent decrease in chlorophyll content with each reduction in soil water content.

The chlorophyll content obtained in this study reflects the growth response of the mutant rice plants. Based on previous morphological responses, such as plant height, number of tillers, and number of leaves, all genotypes showed a general decrease as soil moisture contents declined. This is consistent with findings of Bhandari *et al.* (2023), who found that the decline in chlorophyll levels due to drought stress is strongly associated with disrupted cellular metabolism. This disruption leads to morphological changes in the plant, such as reduced leaf number, fewer tillers, smaller leaf area, and lower plant height (Hassan *et al.*, 2023). The differences in chlorophyll content among the observed mutant plants are likely related to differences in the gamma irradiation doses applied earlier (Phunthong *et al.*, 2024), which may have resulted in varying chlorophyll responses under drought stress.

Chlorophyll content showed significant variation among different genotypes under drought stress. Tolerant genotypes such as P2 and P4 maintained higher chlorophyll levels even under reduced water availability. This supports the finding that drought-tolerant plants are able to maintain chlorophyll levels, which is crucial for photosynthesis and growth under stress conditions (Saharia *et al.*, 2024). For example, P4 showed a significant increase in proline accumulation, reaching 237.98 $\mu g \cdot dm^{-3}$, indicating its role as a protective osmolyte that stabilises proteins and membranes during stress. However, the

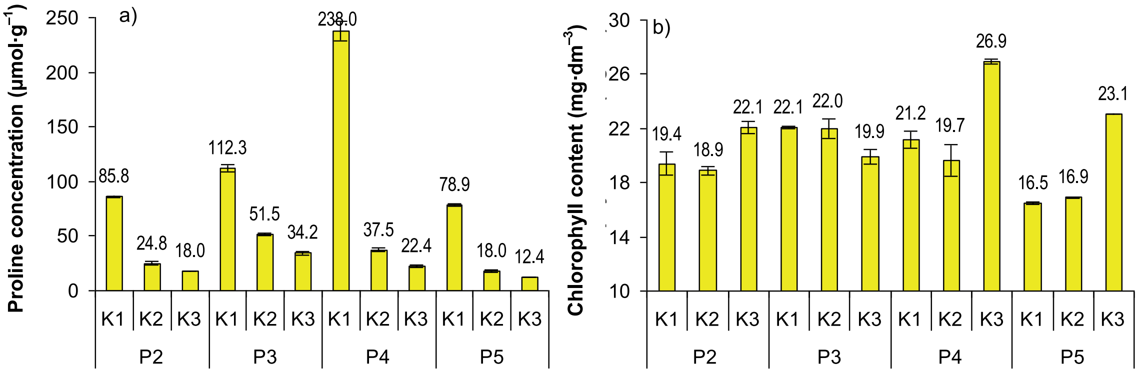

**Fig. 1.** Interaction effect on a) proline concentration and b) chlorophyll content between genotypes (P2: MD200-G13, P3: MD300-G20, P4: MD200-G15, P5: MD300-G27) and drought stress (K1: 33% *FC*, K2: 66% *FC*, K3: 100% *FC*); *FC* = field capacity; source: own study

presence of proline alone does not guarantee drought tolerance, as sensitive genotypes can also produce it under stress (Panda, Mishra and Behera, 2021). Interestingly, P5 maintained agronomic performance despite having lower proline levels. This suggests that its drought tolerance may be more closely associated with structural and metabolic adaptations rather than merely the accumulation of stress markers. This highlights the complexity of drought tolerance mechanisms among different plant genotypes.

### Length of flag leaves and panicles

Both flag leaf length and panicle length showed significant effect of drought stress and genotypes, and both also showed significant interaction effects (Tab. 2). Based on the interaction pattern, flag leaf length in genotype P5 was not affected by drought stress (Fig. 2a). This was due to its ability to maintain consistent flag leaf length across all levels of drought treatment, despite limited water availability. Genotype P3, which received the same gamma radiation dose as P5 (300 Gy), showed similar results without too high differences in flag leaf length. These findings support the statement by Hallajian, Ebadi and Kordrostami (2024) that the same radiation dose does not always produce uniform effects across plant traits, as mutations are random and unpredictable. In contrast, genotypes P2 and P4 responded differently, showing a decrease in flag leaf length as soil moisture contents decreased. This significant reduction reflects the vulnerability of these genotypes to low water availability, whereas P5 demonstrated better adaptive capacity by maintaining its flag leaf length under drought stress.

Plants that adapt to drought stress typically respond by inhibiting their growth, including reducing leaf length and panicle length (Tab. 2). According to Mas-ud *et al.* (2022), a reduction in leaf length under drought stress is a strategy to minimise water loss through transpiration. This occurs because the surface area exposed to sunlight decreases when leaf length is reduced. If plants continue to maintain leaf length as they would under optimal water conditions, it may lead to severe physiological disturbances. This may also increase the risk of death because excessive transpiration without adequate water supply increases water loss.

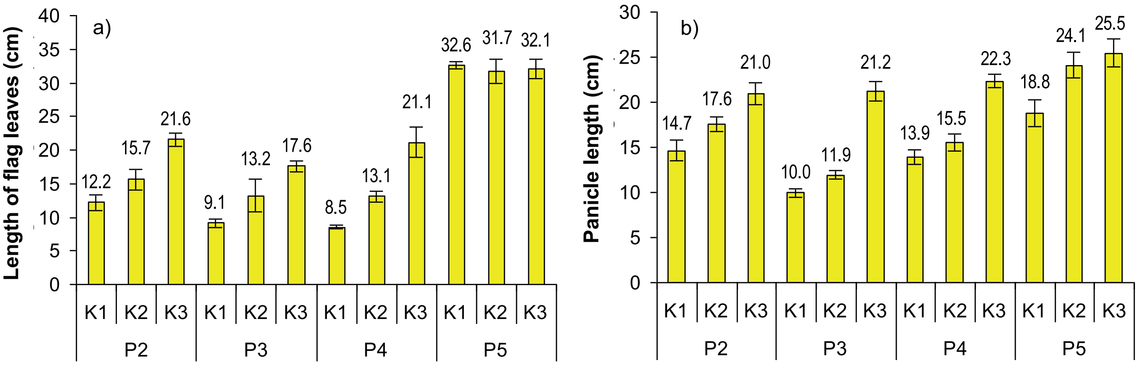

**Fig. 2.** Interaction effect on: a) length of flag leaves, b) panicle length between genotypes and drought stress; P2–P5, K1–K3 as in Fig. 1; source: own study

Similar to flag leaf length, genotype P5 showed the longest average panicle length and P5 consistently produced the longest average panicle length under all drought stress treatments (Fig. 2b). According to Jiao *et al.* (2024), longer panicles contribute to increased production due to a greater potential for grain formation. Furthermore, Jiao *et al.* (2024) emphasised the importance of measuring panicle length, as it directly correlates with the yield potential of rice plants.

Significant differences in panicle length were observed between the 66% and 100% *FC* treatments for all genotypes except P5 (Fig. 2b). These differing responses indicate that some genotypes were susceptible to reductions in panicle length, with the average length beginning to decline under 66% *FC*. In contrast, the genotype P5 maintained stable panicle length under both 66% and 100% *FC*, demonstrating strong adaptive capability. Even though the water availability at 66% *FC* was lower than at 100%, genotype P5 consistently produced the same panicle length. This ability to maintain panicle length under stress conditions indicates that P5 has the potential as a drought-tolerant genotype.

### Grain weight per clump or per pot

Grain weight was affected by both genotype and drought stress treatment. Drought stress significantly reduced grain weight (Tab. 2). However, grain weight also showed significant interaction effect. Based on the interaction pattern (Fig. 3), genotype P5 consistently showed the highest grain weight in each level of drought stress. Water deficiency during the reproductive phase can reduce rice grain weight by up to 60%. This yield reduction is caused by decreased $CO_2$ assimilation, reduced stomatal conductance, impaired starch synthesis and sucrose activity, as well as suboptimal assimilate distribution. These effects ultimately hinder plant growth and productivity (Paul *et al.*, 2023).

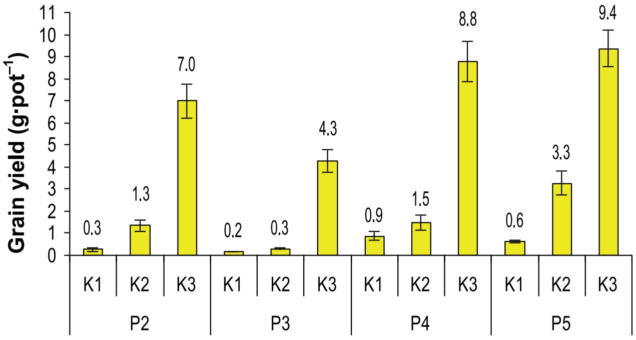

**Fig. 3.** Interaction effect on grain yield per clump between genotypes and drought stress; P2–P5, K1–K3 as in Fig. 1; source: own study

## EXPERIMENT #2

The results of the multi-location test analysis, in general, consist of a combined analysis of the grain yield and an analysis of yield stability of the red rice genotypes at three planting locations.

### Combined yield analysis

The combined yield analysis includes a combined analysis of variance and a combined average grain yield. The results of combined ANOVA on grain yield in the multi-location experiment (Tab. 3) show that the genotypes differ significantly, indicating that there is a difference in grain yield potential among the tested genotypes. Significant differences across locations suggest that yield changes are proportional to environmental productivity. The significant genotype×environment interaction indicates that grain yield of the genotypes varies across growing environments. A genotype with high yield potential in one environment may not necessarily maintain the same high yield in another location due to environmental influences.

**Table 3.** Combined grain yield analysis of variance at three planting locations

| Source of variation | *df* | Sum of squares (*SS*) | Mean square (*MS*) | *F* value |
|---|---|---|---|---|
| Location (environment) | 2 | 1,888.67 | 944.34 | 315.94* |
| Genotype (line) | 20 | 104.03 | 5.2 | 1.74* |
| Replication | 6 | 49.64 | 8.27 | 2.77* |
| Genotype×environment | 40 | 180.95 | 4.52 | 1.51* |
| Error | 120 | 358.77 | 2.99 | – |
| Total | 188 | 2,582.00 | – | – |

Explanations: *df* = degree of freedom; * = significant at $p < 0.05$.

Based on the interaction between locations or environment and the genotypes tested (Tab. 4), grain yield varied among the rice genotypes tested across three different growing locations: Sumbawa, West Lombok, and East Lombok. In general, Sumbawa exhibited the highest productivity of the rice genotypes tested compared to the other two locations. The average grain yield of the genotypes in Sumbawa ranged from 10.20 to 12.38 Mg·ha$^{-1}$, reflecting favourable agroecological conditions that support optimal rice growth and yield in Sumbawa. In addition to high yields, the relatively small error bars for most genotypes in this location indicate good repeatability and performance stability across replications, reinforcing Sumbawa's status as a promising test site.

In contrast, West Lombok showed the lowest productivity (Tab. 4). Some genotypes, such as G12 and G20, even recorded near-zero yields. This suggests that the environment in West Lombok may impose significant constraints on rice cultivation, whether related to climate, soil, or crop management. Nevertheless, some genotypes still performed relatively well in this suboptimal environment, such as G13 (12-2-8) and G63 (17-7-3), which achieved average yield above 6 Mg·ha$^{-1}$, and higher than the comparison variety ('Gamagora'). This highlights the specific adaptability potential of certain genotypes to challenging environments.

**Table 4.** Fluctuation of grain yield (Mg·ha$^{-1}$) of genotypes across three growing locations

| Genotype | Average grain yield in | | |
|---|---|---|---|
| | Sumbawa | West Lombok | East Lombok |
| G12 (7-10-6) | 11.22Aa | 5.20ABCb | 9.75Aa |
| G13 (11-16-11) | 11.54Aa | 4.26ABCb | 9.92Aa |
| G13 (12-2-8) | 12.38Aa | 7.83Ab | 10.45Aab |
| G13 (5-14-7) | 11.06Aa | 4.99ABCb | 10.48Aa |
| G15 (2-15-11) | 12.05Aa | 3.53ABCb | 8.95Aa |
| G15 (4-20-13) | 10.88Aa | 4.46ABCb | 9.61Aa |
| G15 (4-20-20) | 12.15Aa | 3.71ABCc | 7.57Ab |
| G20 (7-8-15) | 10.47Aa | 4.97ABCb | 7.39Aab |
| G20 (8-3-5) | 11.74Aa | 3.38ABCb | 9.30Aa |
| G20 (9-9-3) | 12.20Aa | 2.08BCc | 8.21Ab |
| G27 (1-20-19) | 11.57Aa | 2.43BCc | 9.29Ab |
| G27 (1-20-6) | 11.53Aa | 2.26BCb | 10.06Aa |
| G27 (16-9-4) | 12.03Aa | 1.59Cc | 7.17Ab |
| G27 (17-10-12) | 11.10Aa | 1.70Cb | 7.83Aa |
| G28 (12-20-6) | 12.13Aa | 2.87ABCb | 9.26Aa |
| G52 (15-7-9) | 12.01Aa | 2.24BCc | 7.88Ab |
| G52 (3-9-12) | 11.94Aa | 2.40BCc | 8.17Ab |
| G63 (11-8-6) | 10.60Aa | 5.97ABCb | 8.50Apb |
| G63 (17-7-3) | 10.20Aa | 7.17ABa | 8.67Aa |
| G63 (5-14-10) | 11.85Aa | 3.59ABCb | 10.21Aa |
| 'Gamagora' | 12.19Aa | 6.24ABCb | 8.43Ab |

Remarks: Tukey's HSD: genotypes = 5.12; locations = 3.35.
Explanations: mean values in each column followed by the same uppercase letters are not significantly different between genotypes in each location, while mean values in each row followed by the same lowercase letters indicate non-significant grain yield of a genotype between locations.
Source: own study.

Meanwhile, East Lombok demonstrated moderate to high grain yield, ranging from 7.17 to 10.48 Mg·ha$^{-1}$. Notable performing genotypes in this location included G13 (12-2-8), G13 (5-14-7), G63 (5-14-10), and G27 (1-20-6). Although G20 (9-9-3) had relatively lower yield compared to others, most genotypes in East Lombok showed competitive performance. Overall, there is clear evidence of a significant genotype ×environment (G×E) interaction, where certain genotypes exhibit optimal performance only in specific locations. Genotypes such as G13 (12-2-8) is a strong candidate for elite variety development, as this genotype not only produces high yields in one location but also perform relatively stable in others. Conversely, genotypes such as G27, G28 and G52 require further evaluation due to low or inconsistent yields between locations, particularly in West Lombok (Tab. 4). Therefore, effective varietal selection and dissemination require yield stability-based selection approaches and location-specific genotype mapping. To strengthen these findings, advanced statistical analyses such as combined variance analysis, G×E interaction tests, and GGE biplot analysis are strongly recommended to objectively and quantitatively identify both stable and location-specific superior genotypes.

Figure 4 presents the relationship between the mean yield and the environmental index ($I_j$). The mean yield in Sumbawa was the highest, at 11.56 Mg·ha$^{-1}$, followed by East Lombok at 8.91 Mg·ha$^{-1}$, and West Lombok with the lowest mean yield of 3.95 Mg·ha$^{-1}$. These results correspond with the $I_j$ values of 3.43, −0.77, and −4.20, respectively. The $I_j$ serves as an indicator of environmental productivity. The higher the $I_j$ value, the more optimal the environment. Therefore, Sumbawa is considered the most productive environment.

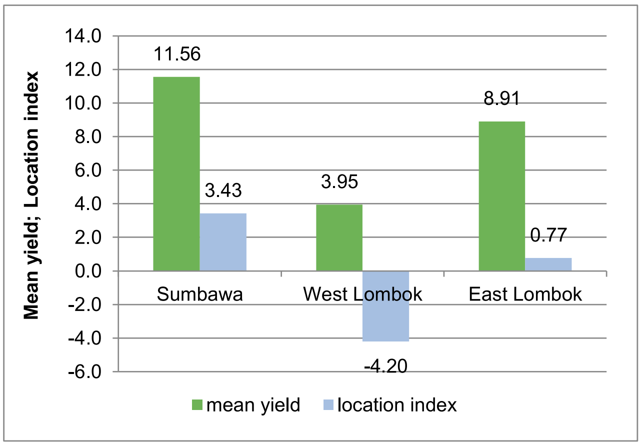

**Fig. 4.** Relationship between mean yield ($\bar{Y}_j$) and location index value ($I_j$); source: own study

The integration of morpho-physiological traits with Eberhart–Russell stability analysis is essential for identifying resilient rice varieties an important step in sustaining rice production in increasingly drought-prone environments (Oladosu *et al.*, 2019; Sircar and Parekh, 2019). The analysis of yield variation across multiple planting sites emphasises the significant influence of agroecological conditions on crop productivity, as evidenced by the highest average yield recorded in Sumbawa (12.38 Mg·ha$^{-1}$), followed by East Lombok (10.48 Mg·ha$^{-1}$), and West Lombok (7.83 Mg·ha$^{-1}$). These findings are consistent with El-Aty *et al.* (2024) and Lee *et al.* (2023), who stressed that environmental factors predominantly affect yield performance and genotype adaptability. Notably, genotypes such as G15, G13, and G20 consistently demonstrated high yields across multiple locations, showing their broad adaptability as elite varieties (Ahmed *et al.*, 2024). In contrast, G27 and G20 (9-9-3) showed low and unstable yields, especially in West Lombok, indicating the need for further evaluation to enhance their performance (Cooper and Messina, 2023). This underscores the importance of location-specific management practices to optimise yield potential based on local environmental conditions.

### Yield stability analysis

The significant genotype×environment interaction effect indicates the need for yield stability assessment for the tested genotypes. According to the Eberhart and Russell (1966) stability analysis, compared with modern methods (Gauch, Piepho and Annicchiarico, 2008), revealed three key stability parameters used for evaluation.

Table 5 shows that stability analysis using the Eberhart–Russell model. It identified several rice genotypes G15 (4-20-13), G13 (11-16-11), G15 (2-15-11), and G20 (8-3-5) that exhibited regression coefficients ($\beta_i$) close to 1 and low deviation from regression ($Sd_i^2$). This indicates their adaptability across diverse environmental conditions (Pour-Aboughadareh *et al.*, 2019; Aswidinnoor *et al.*, 2023). These findings align with the criteria established by Eberhart and Russell (1966) and are further supported by recent studies, including Borule *et al.* (2024) and Abdelrahman *et al.* (2022). In contrast, G63 (11-8-6) and G12 (7-10-6) showed specific adaptation to suboptimal environments, highlighting the need for breeding strategies that combine both general stability and specific adaptability (Prado *et al.*, 2024).

**Table 5.** Yield stability parameters of grain yield across three growing locations

| Genotype | $\bar{Y}_j$ | $\beta_i$ | $Sd_i^2$ |
|---|---|---|---|
| G12 (7-10-6) | 8.72lb | 0.80ako | −0.83sr |
| G13 (11-16-11) | 8.57lb | 0.98s | −0.53sr |
| G13 (12-2-8) | 10.22lb | 0.59ako | −1.00sr |
| G13 (5-14-7) | 8.84lb | 0.83ako | 0.44st |
| G15 (2-15-11) | 8.18lb | 1.11s | −1.07sr |
| G15 (4-20-13) | 8.32lb | 0.87s | −0.48sr |
| G15 (4-20-20) | 7.81lk | 1.24ao | 0.65st |
| G20 (7-8-15) | 7.61lk | 0.69ako | −0.19sr |
| G20 (8-3-5) | 8.14lb | 1.11s | −0.94sr |
| G20 (9-9-3) | 7.50lk | 1.31ao | −0.94sr |
| G27 (1-20-6) | 7.95lk | 1.26ao | 0.92st |
| G27 (1-20-19) | 7.76lk | 1.22ao | −0.55sr |
| G27 (16-9-4) | 6.93lk | 1.33ao | −0.10sr |
| G27 (17-10-12) | 6.87lk | 1.23ao | −1.08sr |
| G28 (12-20-6) | 8.09lk | 1.22ao | −0.99sr |
| G52 (15-7-9) | 7.37lk | 1.26ao | −0.73sr |
| G52 (3-9-12) | 7.50lk | 1.24ao | −0.95sr |
| G63 (11-8-6) | 8.36lb | 0.59ako | −0.93sr |
| G63 (17-7-3) | 8.68lb | 0.38ako | −0.93sr |
| G63 (5-14-10) | 8.55lb | 1.14ao | 0.00sr |
| ‘Gamagora’ | 8.95lb | 0.74ako | 0.76st |
| $\mu$ | 8.14lb | 1.00 | – |

Explanations: $\bar{Y}_j$ = average yield, $\beta_i$ = regression coefficient, $Sd_i^2$ = regression deviation, $\mu$ = general mean, lb = $\bar{Y}_i > \mu$, lk = $\bar{Y}_i < \mu$, ako = $\beta_i < 1$, ao = $\beta_i > 1$, s = average value of $\beta_i$ (1), st = high $Sd_i^2$, sr = low $Sd_i^2$, green colour = high-yielding and stable genotypes under all environmental conditions.
Source: own study.

### Stability according to Eberhart and Russel

This stability analysis uses the regression coefficient ($\beta_i$) and regression deviation ($Sd_i^2$) as parameters. A genotype is considered stable if the $\beta_i$ value is close to 1 and the $Sd_i^2$ value is as small as possible or equal to 0. Genotypes with $\beta_i > 1$ adapt well to the most optimal environment, while genotypes with $\beta_i < 1$ adapt well to less optimal environments (Pour-

Aboughadareh *et al.*, 2019). The $\beta_i$ value indicates the genotype response to environmental conditions, while the $Sd_i^2$ value measures stability (Abdelrahman *et al.*, 2022).

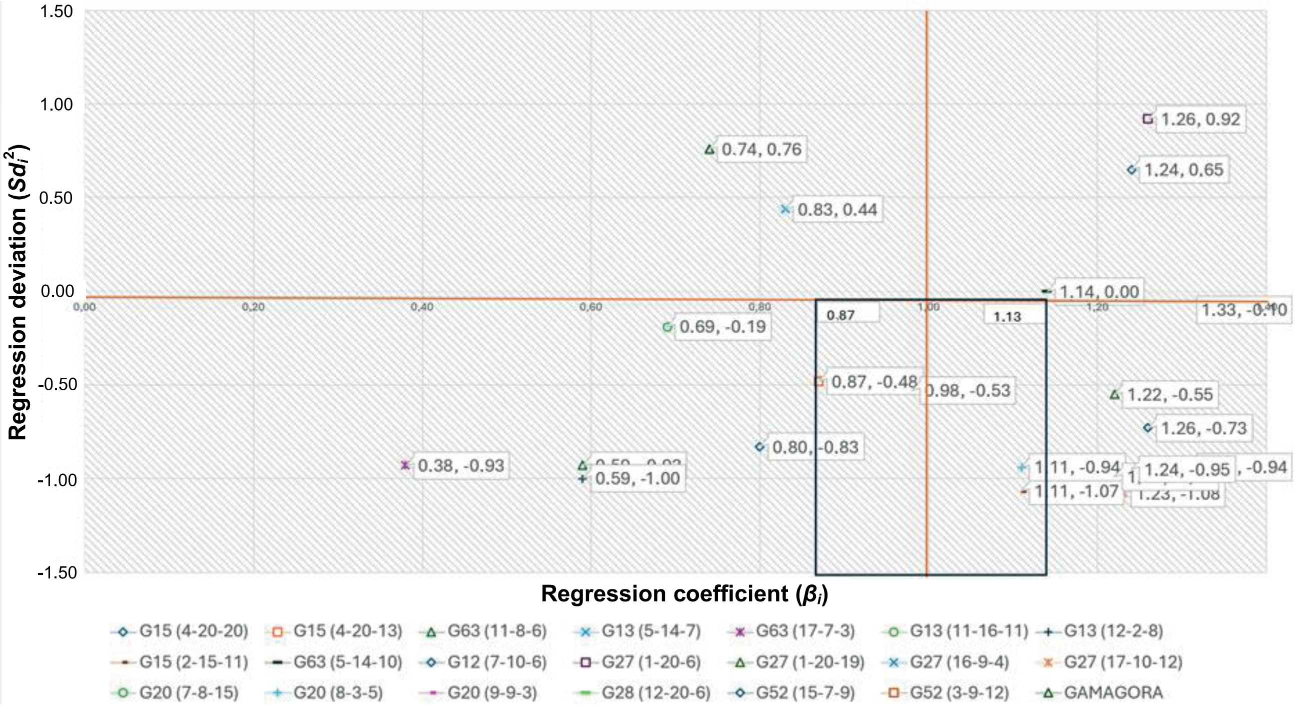

**Fig. 5.** Interpretation of stability analysis based on $\beta_i$ and $Sd_i^2$ according to Eberhart and Russell (1966), four genotypes in the box are high-yielding and stable in all environments, having $\beta_i$ close to 1 and $Sd_i^2 < 0$; source: own study

Figure 5 presents the mean yield of each genotype across the planting locations. Several genotypes demonstrated high yield potential. This aligns with the stability analysis interpretation shown in Figure 5, which identified high-yielding and stable genotypes under all environmental conditions, with $\beta_i = 1$ (s) and $Sd_i^2 < 0$ (sr) (see also Tab. 5), including G15 (4-20-13), G13 (11-16-11), G15 (2-15-11), and G20 (8-3-5). Genotypes such as G63 (11-8-6), G63 (17-7-3), G13 (12-2-8), and G12 (7-10-6) showed high yield potential but were stable only in less optimal environments. Meanwhile, genotype G63 (5-14-10) exhibited high yield but it's only stable in optimal environments. The comparison variety 'Gamagora' showed high yield as well but with unstable performance, although it adapted well to less favourable environments.

This dual approach is essential for optimising yield performance across diverse conditions, as evidenced by the significant G×E interactions observed in various studies (Gauch, Piepho and Annicchiarico, 2008). An integrated approach that combines morpho-physiological analysis and yield stability evaluation has effectively identified genotype MD300-G27(16-9-4) (P5) as a promising drought-tolerant candidate with competitive yield performance. This is an important finding for future red rice breeding programmes in the face of climate change and water scarcity. Previous studies have highlighted substantial genetic variability among rice genotypes, with traits such as relative water content, panicle length, and grain yield showing strong correlations with drought tolerance (Saharia *et al.*, 2024). Genotypes such as Giza 179 and Hybrid 2 have demonstrated resilience under drought conditions, showing stable yields and favourable morpho-physiological traits (Selamat and Nadarajah, 2021; Jiao *et al.*, 2024). These findings emphasise the importance of integrating physiological, agronomic, and yield stability data into breeding strategies to develop adaptive rice varieties that can enhance food security in drought-prone regions (Oladosu *et al.*, 2019; Paul *et al.*, 2023).

## CONCLUSIONS

Based on the results of pot experiments in the greenhouse, testing morphological and physiological responses to drought, genotype G15 (4-20-13) consistently accumulated the highest proline and maintained relatively high chlorophyll levels under the most severe drought conditions. In contrast, genotype G27 (16-9-4) maintained a constant flag leaf length despite stress, and produced the highest grain yield. These two genotypes were identified as superior candidates for drought tolerance based on indicators of proline, chlorophyll, flag leaf length, and grain yield. Multilocation and stability analyses according to the Eberhart–Russell model revealed that genotypes G15 (4-20-13), G13 (11-16-11), G15 (2-15-11), and G20 (8-3-5) had regression coefficients ($\beta_i$) close to 1 and low regression deviations ($Sd_i^2$). This indicated high yield stability in various environments. These four genotypes maintained consistent grain yields across the three test locations, meeting the criteria for broad adaptability ($\beta_i \approx 1$) and general stability. These results confirm the potential of G15 (4-20-13), G13 (11-16-11), G15 (2-15-11), and G20 (8-3-5) as red rice lines with stable yield performance across environments.

## CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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