



Induction of Water-Deficit Tolerance in Rice through Gamma Mutagenesis

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ABSTRACT

Rice is a strategic crop essential for Egyptian national food security; however, climate change and escalating water scarcity pose severe threats to its sustainable production. From this point, gamma rays with its safe doses were used to genetic improvement in the Egyptian rice cultivar (Sakha 102) and for elicitation five rice mutants classified as highly drought tolerated besides, its high yielding. The six rice accessions were evaluated across two growing seasons under normal and drought conditions. Yield components, physiological and biochemical attributes besides, water stress tolerance indices were the most important parameters tested under both treatments for screening the highest rice genotypes tolerated to drought stress under stress conditions. Results indicated that significant genetic variability ($P < 0.01$) was observed for all physiological and yield traits. Furthermore, drought stress severely reduced yield components, particularly in the local cultivar "Sakha 102" ($DSI > 1.87$; $YR: 0.27$). Conversely, induced mutagenesis significantly improved drought resilience. Also, mutant 5 demonstrated superior tolerance, maintaining the highest grain yield (51.68 g) and 1000-grain weight (36.97 g) under stress by maximizing glycine betaine (77.68) and trehalose (60.45) accumulation. As well, strong positive correlations were confirmed between osmoprotectants and yield attributes under stress, notably between proline and 1000-grain weight ($r = 0.96$). Further, high broad-sense heritability ($> 70\%$) coupled with high genetic advance (18.59% - 26.45%) for grain yield under stress confirmed the governance of additive gene action. Ultimately, these findings identify Mutant 5 as a high-potential donor line and validate biochemical osmoprotection as a reliable selection criterion, offering a vital genetic tool to secure future rice production amidst future climatic challenges like high level of drought stress.

Keywords: Rice, mutations breeding, drought tolerance, genetic components, mutagenesis %.

1. Introduction

Rice (*Oryza sativa* L.) stands as a cornerstone of global food security, being the primary caloric source for more than half of the world's population. In Egypt, it occupies a prestigious position as the second most vital cereal crop after wheat, significantly contributing to the national economy and representing a staple food in the Egyptian household diet, as emphasized by Abdel Sattar and El-Mouhamady (2012); Ali *et al.* (2021) regarding mineral mobility and (Abdou *et al.*, 2021) concerning its economic importance. However, the crop faces critical challenges in the Mediterranean region due to water scarcity, which triggers deleterious effects on morphological growth and photosynthetic capacity (El-Keredy *et al.*, 2003a; Farooq *et al.*, 2021; Morsi *et al.*, 2021). These environmental fluctuations necessitate advanced mitigation strategies, as the stability of production remains essential for maintaining international food markets (El-Keredy *et al.*, 2003b; Ghanem *et al.*, 2022; Hafez *et al.*, 2022). According to El-Keredy *et al.* (2003c); Zayed *et al.* (2022); Soliman *et al.* (2022) the intensification of water stress leads to significant physiological disruptions that threaten the

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sustainability of field crops. To counteract these effects, rice plants initiate complex adaptations, such as increasing osmotic pressure to maintain cell turgidity and protecting protein integrity through proline accumulation (El-Mouhamady *et al.*, 2015; Bakr *et al.*, 2023; Hassan *et al.*, 2023). This physiological resilience allows the plant to maintain stomatal conductance and leaf expansion under low water potential (El-Mouhamady *et al.*, 2013; Ibrahim *et al.*, 2023; Said *et al.*, 2023). Furthermore, the reduction in grain yield and 1,000-grain weight remains a prominent negative impact of drought, particularly during the grain-filling stage when dry matter translocation is restricted (El-Sharey *et al.*, 2024; Mahmoud *et al.*, 2024). As noted by Tawfik and El-Mouhamady (2024a); Youssef *et al.* (2024), the metabolic balance of trehalose and its precursors serves as a vital marker to distinguish between drought-tolerant and sensitive genotypes. Building on these foundations, the strategic importance of rice in 2025 focuses on mitigating global food inflation through resilient supply chains (Rady *et al.*, 2014; Ibrahim 2025). Modern research published highlights that optimized nutrient management and biochemical markers like Glycine Betaine are essential for stabilizing cellular structures (Ali 2025; Ghanem 2025; Youssef 2025). Further, the integration of genomic selection and the accumulation of osmoprotectants will remain the primary defense mechanisms for developing short-duration varieties in the Nile Delta (Abdou 2026; Hassan 2026). These efforts, as discussed by Abo-Hamed *et al.* (2016); Mahmoud (2026); Soliman (2026), aim to reduce total water consumption while maintaining the high productivity levels required by the Egyptian national strategy.

2. Materials and Methods

2.1. Background

The Egyptian rice variety Sakha 102 most susceptible to water stress was used in this investigation. Furthermore, this variety saves 25 to 30% of irrigation water because its short growth period does not exceed 125 days compared to tall varieties. Also, this is why it saves that amount of irrigation water thanks to its fermentation at maturity and not its genetic ability to tolerate sneezing. Therefore, this study used the breeding method to develop new rice mutants that are more drought tolerance than the original variety.

2.2. Field evaluation

The Sakha 102 rice seeds used in the recent investigation were originally performed from Rice Research and Training Center, Agriculture Research Centre. Five hundred pure seeds of Sakha 102 cultivar were subjected to gamma irradiation treatments at dosages of 100, 200, 300, 400 and 500 Gy using the Co source at the National Center for Radiation Research and Technology, Nasr City and Cairo, Egypt in 2015 season (M0). The irradiated materials of all doses were grown and series of selections among the mutant population under normal and drought conditions in Aga Center and City in Dakahlia Governorate province and this process was carried out during 2016-2023 seasons (M1-M8) to produce the selected five rice mutants. All plants have reached full genetic stability at the eighth generation (M8).

2.3. Sowing and treatments

Two experiments were performed in the farm of Aga Center and City in Dakahlia Governorate province, Egypt during 2024 and 2025 seasons using the original rice cultivar (Sakha 102), and 5 mutant lines derived from it and selected from M8 generation. Where the first one was normal irrigation conditions of continuous flooding. While; the second experiment was flash irrigation every 15 days without any standing water. Each experiment was conducted using a randomized complete block design, and each was replicated three times. The stress was applied two weeks after transplanting till harvesting. Each irrigation experiment was independent and isolated from the others. As the isolation distance was 500 m², this buffer distance was covered with linoleum on both sides to prevent water infiltration from the standard experiment to drought experiment. The length of each replicate of each experiment was 20 m, and the space between each two plants was 20 cm into each replicate. Note: - The percentage of drought stress experiment is up to 40% of the normal rice requirements to reach the growth stage and full yield. Also, the ratio is the safe limit to keep the rice plant alive and give a crop. Therefore, it is considered the logical ratio of the tests of all rice genotypes under study for their response to drought tolerance.

2.4. Studied traits

Sixty plants were used for determined all attributes under the study of each treatment (normal and drought conditions), with 20 plants for each replicate. Each experiment included three replicates, and the six rice entries were grown in a randomized complete block design.

2.4.1. Yield, physiological and biochemical traits: -

1: - 1000-grain weight (gm): - It was recorded as the weight of 1000 random filled grains per plant.

2: - Grain yield/plant (gm): - It was recorded as the weight of grain yield of each individual plant, and adjusted to 14% moisture content.

3: - The proline content was determined from a standard curve and calculated on a fresh basis is as follows: $[(\mu\text{g proline} / \text{ml C ml toluence}) / 115.5 \mu\text{g}/\mu \text{mole}] / [(g \text{ sample}/5)] = \mu \text{ moles proline} / g$ of fresh weight material where the results were average values at least 3-4 samples for each species, according to Chinard (1952); Bates *et al.* (1973).

4 & 5: - Glycine betaine and trehalose contents were carried out according to Grieve and Grattan (1983).

6: - Osmotic adjustment: - It was determined by the formula of Morgan (1980).

$$\text{Osmotic adjustment} = \frac{\text{O.P.} \times \text{R.W.C.}}{100} (\text{Normal}) - \frac{\text{O.P.} \times \text{R.W.C.}}{100} (\text{drought})^{100}$$

Where: - O. P= Osmotic pressure & R.W.C.= Relative water content according to Barrs and Weatherley (1962).

2.5. Drought stress tolerance indices

All drought stress tolerance indices were estimated in all rice accessions under study according to Fischer and Maurer (1978); Bouslama and Schapaugh (1984); Lin *et al.* (1986); Hossain *et al.* (1990); Fernandez (1992); Gavuzzi *et al.* (1997); Golestani and Assad, (1998) as follows:- 1):- GYP: Is meaning the grain yield/plant for the control experiment, 2):- GYD: Is meaning the grain yield/plant for the drought experiment, 3):- YSI: Is meaning yield stability index = YS/YP where: YS the average of yield under stress and YP= The average of yield under the control experiment, 4):- YI: Is meaning yield index (YS for each genotype/mean of YS for all genotypes), 5):- MP: Is means (Average yield for both trials): $YS + YP/2$, 6):- DTI: Is meaning drought tolerance index $(YP \times YS / (\text{mean of } YP)^2)$, 7):- GMP: $(YP \times YS) 0.5$, 8):- YR: Is meaning yield reduction $(1-YS/YP)$. 9): - SSI: Is meaning drought susceptibility index = $(1-YS/YP)/D$ where YS = mean yield under water stress, YP = mean yield under control or normal condition, and D = environmental stress intensity= $1-(\text{mean yield of all genotypes under drought stress}/\text{mean yield of all genotypes under irrigated conditions})$.

2.6. Statistical analysis

All calculated data of all traits under evaluation in the two seasons for both treatments were analyzed using the formula by Gomez and Gomez (1984).

Simple Correlation Coefficients

Simple correlation coefficients between all attributes under study for normal and salinity conditions was calculated according to Singh and Chaudhary (1985); Cramer and Wehner (1998).

2.7. The Mutagenesis %

It was calculated for all attributes under both conditions in both growing seasons according to the formula of Kishk *et al.* (2017).

2.8. Genetic components

Variance components namely; environment variance (EV), phenotypic variance (PV), genotypic variance (GV), heritability in a broad sense (H^2_{bs}), genetic coefficient of variability (GCV %), phenotypic coefficient of variability (PCV %), genetic advance (GA), genetic advance as a percentage of the mean of each trait (GAM %) were the most important measurements calculated through the two seasons for both treatments in this investigation as follows: The genetic coefficient of variability

(GCV%) and phenotypic coefficient of variability (PCV%) and heritability of broad sense (H^2 b S) were estimated according to the method suggested by Burton and Devane, (1953); Johnson *et al.* (1955) as follows: Environmental variance (σ^2_e) = MSe Genotypic variance; (GV) or (σ^2_g), Phenotypic variance (PhV) or (σ^2_{ph}) = (σ^2_e) + (σ^2_g) or MSe + MSg where MSe is the mean square of error, MSg is the mean square of genotypes, is the r = number of replicates, and $\bar{X}$ is the mean of trait.

2.9. Genetic similarity and phylogenetic tree

Data of all attributes under investigating for all rice entries of the two experiments were used to assessment genetic similarity (Neighbor Joining (NG) method) according to Saitou and Nei (1987) and Pairwise comparisons between individuals using PAST program (Hammer *et al.*, 2001).

3. Results

3.1. Variation & Interaction

The provided ANOVA tables demonstrate highly significant genetic variability ($P < 0.01$) among the rice entries across both growing seasons. Data revealed in table 1 (Normal Treatment) establishes the baseline physiological and yield performance, showing that even under optimal conditions, genotypes differ significantly in their inherent capacity for osmoprotectant synthesis and grain production. In contrast, table 2 highlights how drought stress intensifies these differences, particularly through enhanced Osmotic Adjustment and elevated levels of Proline, Glycine betaine, and Trehalose contents. The data across both tables are linked by the physiological response where genotypes with higher biochemical stability in table 1 often exhibit superior resilience and sustained grain yield/plant in table 2. Furthermore, the consistently low C.V. % values in both tables confirm the experimental precision, validating these traits as reliable markers for selecting drought-tolerant rice varieties.

3.2. Mean performance

The provided data evaluates the yield components, physiological and biochemical attributes of the six rice accessions across two successive growing seasons under normal (Table 3) and drought stress conditions (Table 4). Exposure to drought triggered a severe reduction in yield components, including 1000-grain weight (Fig. 1) and grain yield per plant (Fig. 2), with the local cultivar "Sakha 102" exhibiting the highest susceptibility. Conversely, the tested genotypes mitigated osmotic stress via the hyper-accumulation of key compatible osmolytes, demonstrating significant scientific increases in proline (Fig. 3), glycine betaine (Fig. 4), and trehalose contents (Fig. 5) compared to normal conditions. Concurrently, substantial variations were observed in the osmotic adjustment trait among the evaluated entries (Fig. 6). Notably, Mutant 5 demonstrated excellent drought tolerance, maintaining the highest grain yield/plant (51.68 g) and 1000-grain weight (36.97 g) under stress, which correlates with its superior capacity for physiological osmoprotection via maximizing glycine betaine (77.68 g) and trehalose (60.45 g) accumulation.

3.3. Water stress tolerance indices

The evaluation of the six rice accessions across the 2024 and 2025 seasons obtained in table (5) indicates a clear variation in drought tolerance based on their Water Stress Tolerance Indices. The local cultivar, Sakha 102, exhibited the highest susceptibility to water deficit, recording the maximum Drought Sensitivity Index ($DSI > 1.87$) and Yield Reduction ($YR \geq 0.27$) in both seasons. Conversely, the newly developed lines particularly mutants 4 and 5 demonstrated superior drought tolerance, characterized by the lowest DSI and YR values. Specifically, mutant 4 achieved the lowest DSI (0.39 in 2024 and 0.45 in 2025) combined with a minimal yield loss ($YR = 0.12$ & 0.13). While, mutant 5 maintained a highly stable, low DSI range (0.28 & 0.34) through the two seasons. Further, these significantly lower values in the mutant lines compared to Sakha 102 reflect their enhanced genetic adaptation and higher capability to mitigate the negative impacts of water stress on final grain yield.

3.4. Simple correlation coefficients

Based on the combined analysis of the 2024 and 2025 growing seasons, the correlation data reveals a strong, positive physiological link between osmoprotectant accumulation and rice yield components under both environments. In Table 6 (normal conditions), 1000-grain weight and grain weight show strong positive correlations with proline content ($r = 0.93$ and $r = 0.77$, respectively) and

Table 1: - ANOVA Data for all attributes tested of the six rice Entries for the Normal Treatment in the two growing seasons.

S.O.V	D.F	Seasons	1000-grain weight (gm)	Grain yield /plant (gm)	Proline content	Glycine betaine content.	Trehalose content
Genotypes	5	2024	14.32**	22.05**	11.05**	15.06**	9.32**
		2025	17.34**	33.02**	19.08**	21.09**	13.87**
Replicates	2	2024	33.12**	18.77**	31.88**	16.77**	13.07**
		2025	42.03**	22.19**	55.08**	24.55**	20.22**
Error	10	2024	1.12	0.95	1.85	0.86	1.49
		2025	1.06	1.14	2.03	1.37	1.86
C.V. %		2024	1.89	2.33	1.97	4.22	3.29
		2025	2.05	2.29	2.55	4.63	3.88

Table 2: - ANOVA data for all attributes tested of the six rice entries for the drought experiment in the two growing seasons.

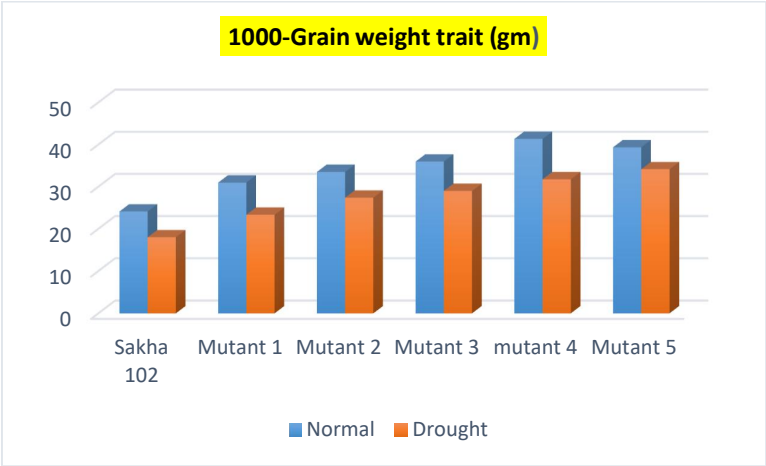
S.O.V	D.F	Seasons	1000-grain weight (gm)	Grain yield/plant (gm)	Proline content	Glycine betaine content	Trehalose conten	Osmotic adjustment
Genotypes	5	2024	23.08**	18.34**	11.23**	24.86**	44.03**	17.32**
		2025	50.11**	29.08**	25.34**	20.64**	27.34**	26.33**
Replicates	2	2024	13.94**	28.54**	41.83**	29.55**	31.44**	18.64**
		2025	18.64**	31.05**	74.84**	40.06**	37.56**	29.83**
Error	10	2024	1.44	1.35	1.47	2.53	0.95	1.66
		2025	1.96	1.76	1.77	3.12	1.34	2.12
C.V. %		2024	2.85	4.55	1.98	3.96	1.93	3.17
		2025	3.73	5.29	2.88	4.29	2.28	4.05

Table 3: - Mean performance and combined analysis for all studied attributes of the six rice accessions under normal conditions in the two growing seasons.

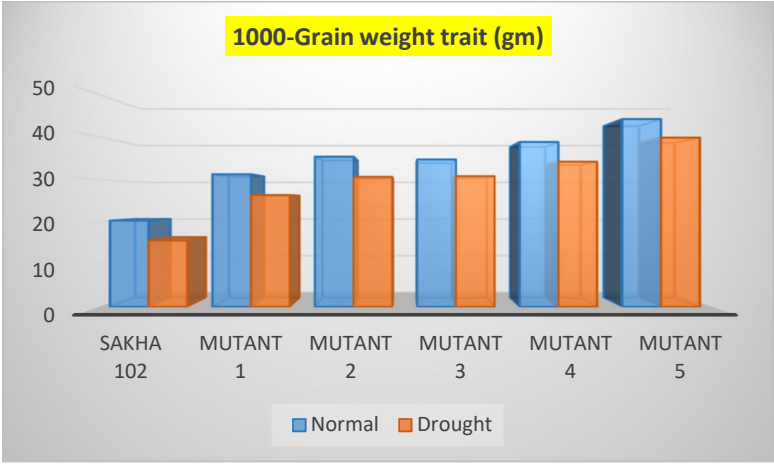
Traits	1000-grain weight (gm)			Grain yield /plant (gm)			Proline content			Glycine betaine content			Trehalose content		
Genotypes	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean
Sakha 102	24.12	20.34	22.23	26.54	27.12	26.83	20.67	25.81	23.24	31.62	33.48	32.55	25.27	23.67	24.47
Mutant 1	30.98	31.22	31.10	43.97	45.40	44.68	33.08	31.79	32.43	44.71	46.12	45.41	42.59	45.19	43.89
Mutant 2	33.51	35.33	34.42	52.10	54.66	53.38	40.51	44.38	42.44	55.09	57.12	56.10	54.25	56.70	55.47
Mutant 3	35.97	34.67	35.32	58.52	60.19	59.35	40.93	43.56	42.24	47.93	48.81	48.37	62.50	64.69	63.59
Mutant 4	41.34	38.76	40.05	61.55	60.37	60.96	52.62	55.24	53.93	65.15	70.0	67.57	58.55	55.21	56.88
Mutant 5	39.36	44.12	41.74	59.23	64.31	61.77	63.16	64.44	63.80	86.05	87.56	86.80	73.46	74.17	73.81
Mean	34.21	34.07	34.14	50.31	52.0	51.15	41.82	44.20	43.01	55.09	57.18	56.13	52.77	53.27	53.02
LSD at 5%	1.56	1.52	1.54	1.44	1.57	1.50	2.01	2.10	2.05	1.37	1.73	1.55	1.80	2.01	1.90
LSD at 1%	2.38	2.32	2.35	2.19	2.40	2.29	3.06	3.21	3.13	2.09	2.64	2.36	2.75	3.07	2.91

Table 4: - Mean performance and combined analysis for all studied attributes of the six rice accessions under drought conditions in the two growing seasons.

Traits	1000-grain weight (gm)			Grain yield/plant (gm)			Proline content			Glycine betaine content			Trehalose content			Osmotic adjustment		
Genotypes	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean
Sakha 102	18.0	15.69	16.84	19.08	19.83	19.45	12.99	16.95	14.97	20.66	19.53	20.09	15.77	13.11	14.44	2.02	2.28	2.15
Mutant 1	23.34	26.35	24.84	37.04	36.10	36.57	28.89	26.07	27.48	35.51	37.59	36.55	30.77	31.91	31.34	0.41	0.48	0.44
Mutant 2	27.40	30.61	29.0	46.76	48.42	47.59	35.05	34.53	34.79	47.22	47.43	47.32	46.03	42.91	44.47	0.72	0.58	0.65
Mutant 3	29.0	30.76	29.88	50.35	49.14	49.74	32.20	32.06	32.13	39.44	41.58	40.51	53.60	50.48	52.04	0.37	0.38	0.37
Mutant 4	31.76	34.15	32.95	54.47	52.79	53.63	45.03	45.92	45.47	54.18	56.66	55.42	47.86	45.88	46.87	0.51	0.53	0.52
Mutant 5	34.14	39.80	36.97	50.58	52.79	51.68	55.09	54.62	54.85	75.62	79.74	77.68	63.07	57.84	60.45	0.52	0.56	0.54
Mean	27.27	29.56	28.41	43.04	43.17	43.10	34.87	35.02	34.94	45.43	47.08	46.26	42.85	40.35	41.60	0.75	0.80	0.77
LSD at 5%	1.77	2.70	2.23	1.72	1.96	1.84	1.79	1.96	1.87	2.35	2.61	2.48	1.44	1.71	1.57	1.90	2.15	2.02
LSD at 1%	2.70	3.16	2.93	2.62	2.99	2.80	2.73	3.0	2.86	3.58	3.98	3.78	2.19	2.61	2.40	2.90	3.28	3.09

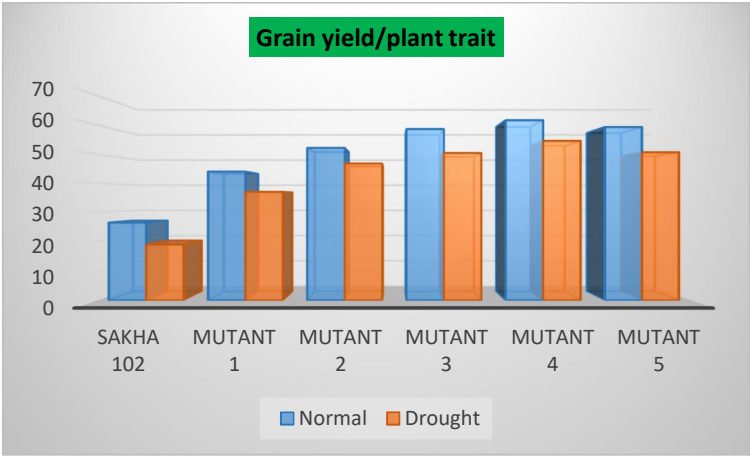


A. (2024 Season)

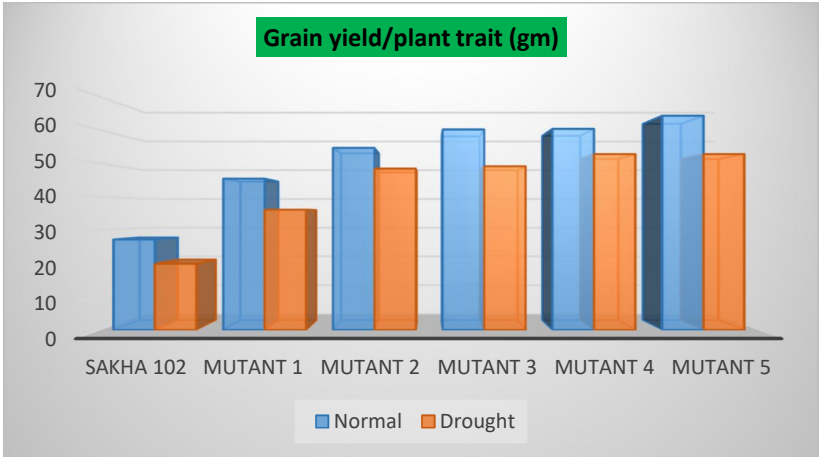


B. (2025 Season)

Fig. 1: Shows (A & B) forms of drought stress effect on rice entries through 2024 and 2025 seasons for 1000-grain weight trait.



A. (2024 Season)



B. (2025 Season)

Fig. 2: Shows (A & B) forms of drought stress effect on rice entries through 2024 and 2025 seasons for grain yield/plant trait.

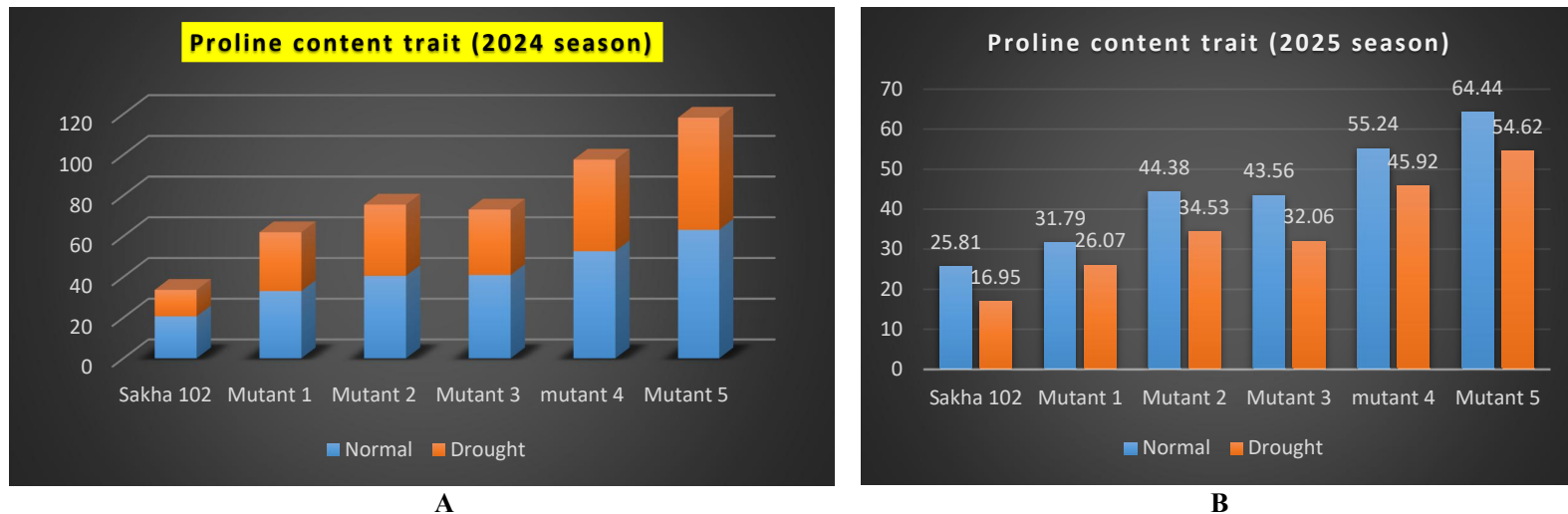


Fig 3: Shows (A & B) forms of drought stress effect on rice entries through 2024 and 2025 seasons for proline content trait

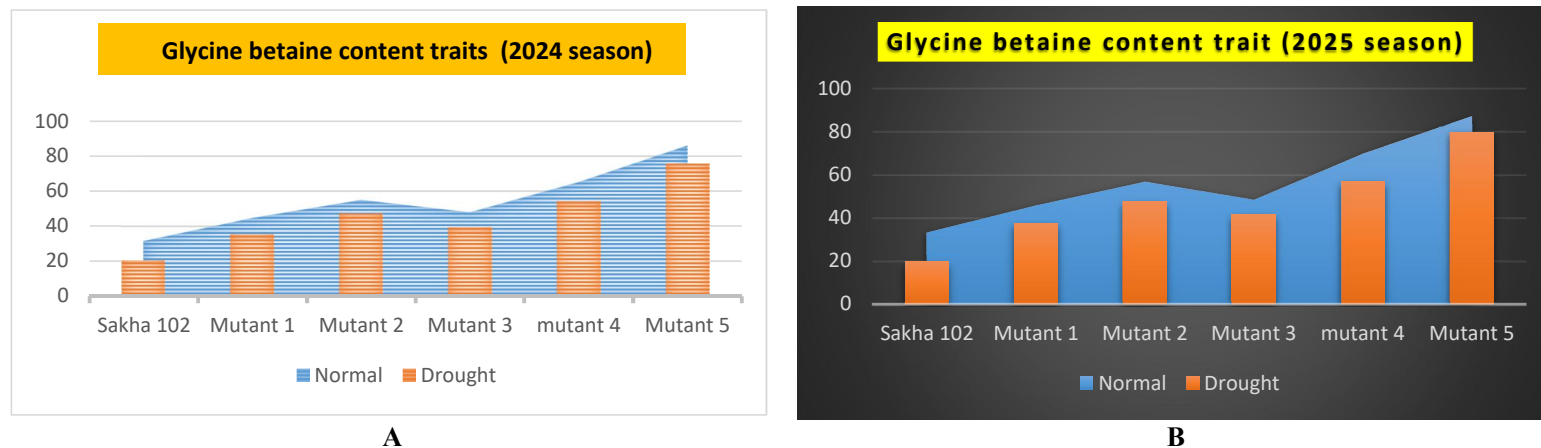


Fig. 4: Shows (A & B) forms of drought stress effect on rice entries through 2024 and 2025 seasons for glycine betaine content trait

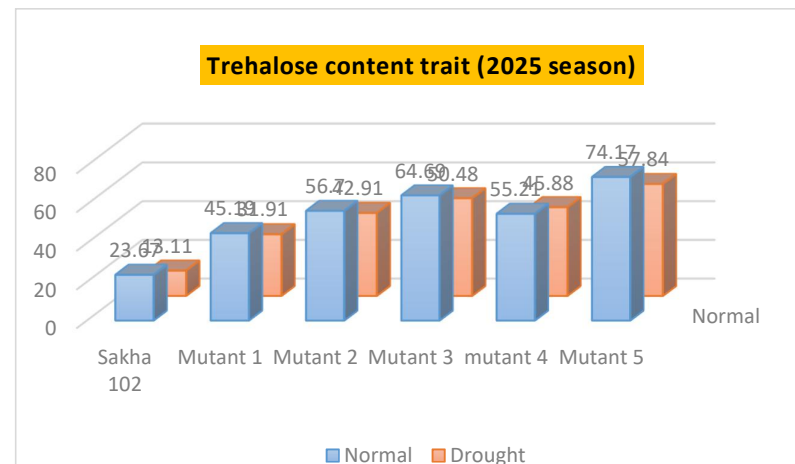
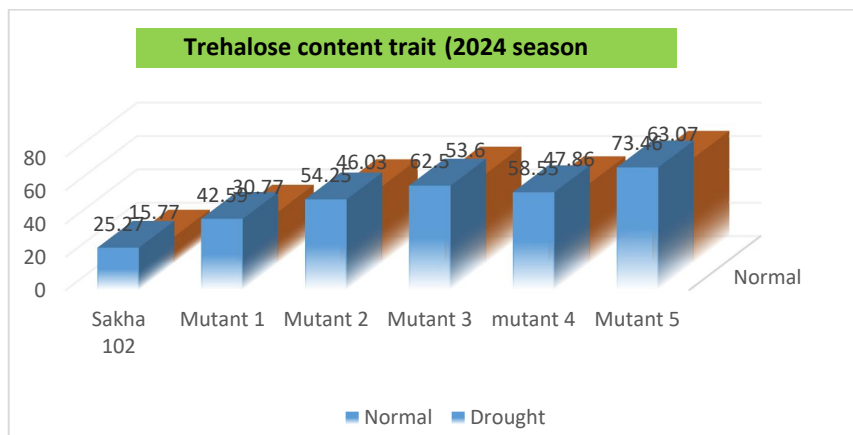


Fig. 5: Shows (A & B) forms of drought stress effect on rice entries through 2024 and 2025 seasons for trehalose content trait

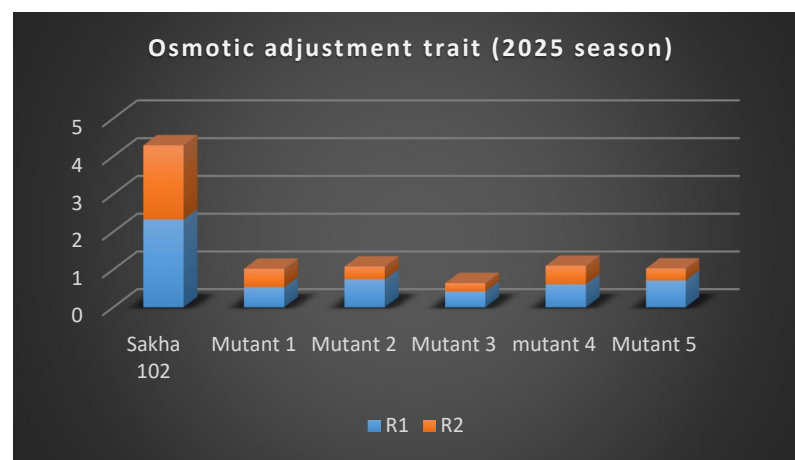
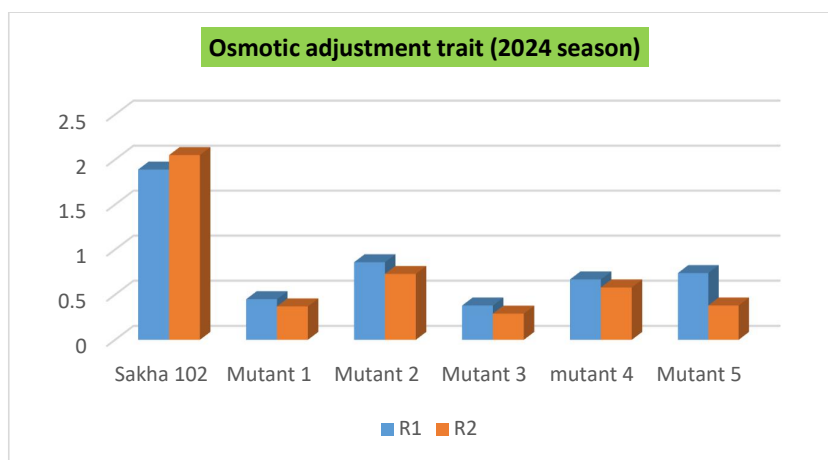


Fig. 6: Shows (A & B) forms of osmotic adjustment trait on rice entries through 2024 and 2025 seasons

Table 5: - Water stress tolerance indices for grain yield/plant trait in the six rice accessions through 2024 & 2025 seasons.

Traits	2024 season									2025 season								
	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI
Sakha 102	26.54	19.08	0.72	0.44	22.81	0.20	22.50	0.28	2.05	27.12	19.83	0.73	0.45	23.47	0.19	23.19	0.27	1.87
Mutant 1	43.97	37.04	0.84	0.86	40.50	0.64	40.35	0.16	0.76	45.40	36.10	0.79	0.83	40.75	0.60	40.48	0.21	0.64
Mutant 2	52.10	46.76	0.89	1.08	49.43	0.96	49.35	0.11	0.83	54.66	48.42	0.88	1.12	51.54	0.97	51.44	0.12	0.81
Mutant 3	58.52	50.35	0.86	1.16	54.43	1.16	54.28	0.14	0.73	60.19	49.14	0.81	1.13	54.66	1.09	54.38	0.19	0.53
Mutant 4	61.55	54.47	0.88	1.26	58.01	1.32	57.90	0.12	0.45	60.37	52.79	0.87	1.22	56.58	1.17	56.45	0.13	0.39
Mutant 5	59.23	50.58	0.85	1.17	54.90	1.27	54.73	0.15	0.28	64.31	52.79	0.82	1.22	58.55	1.25	58.26	0.18	0.34

Table 6: - Simple correlation coefficients of combined analysis among all attributes studied in rice entries under normal conditions in both growing seasons (2024 & 2025).

Traits	1000-Grain weight (gm)	Grain weight (gm)	Proline content	Glycine betaine Content	Trehalose content	Osmotic adjustment
1000-Grain Weight (gm)	1.0	0.85	0.93	0.76	0.90	0.88
Grain Weight (gm)	0.85	1.0	0.77	0.84	0.69	0.85
Proline Content	0.93	0.77	1.0	0.73	0.93	0.81
Glycine Betaine Content	0.76	0.84	0.73	1.0	0.84	0.86
Trehalose Content	0.90	0.69	0.93	0.84	1.0	0.75
Osmotic Adjustment	0.88	0.85	0.81	0.86	0.75	1.0

osmotic adjustment ($r = 0.88$ and $r = 0.85$), indicating that baseline metabolic efficiency supports steady grain filling. Furthermore, under stress in table 7 (drought conditions), these positive correlations remain highly prominent, with a particularly powerful association emerging between proline content and 1000-grain weight ($r = 0.96$), as well, grain weight ($r = 0.87$). Similarly, glycine betaine and trehalose maintain robust correlations with grain weight ($r = 0.83$ and $r = 0.70$), while showing a strong co-dependent relationship ($r = 0.92$). Scientifically, these findings demonstrate that the upregulation of compatible solutes (proline, glycine betaine, and trehalose) facilitates effective cellular osmotic adjustment. Also, this biochemical mechanism preserves cell turgor, protects photosynthetic machinery, and ultimately safeguards grain weight and overall yield recovery against drought-induced deficits during both agricultural seasons.

3.5. The mutagenesis %

The induced mutagenesis led to a substantial improvement across all evaluated traits compared to the local cultivar (Sakha 102), with significantly higher percentage improvements observed under drought conditions (Table 9) than normal conditions (Table 8). Further, mutant 5 consistently recorded the highest mean of genetic improvement (M. of G.I) across both environments, particularly maximizing proline, glycine betaine, and trehalose accumulation, which directly correlated with superior grain yield and 1000-grain weight in both Table 8 and Table 9. Conversely, mutant 1 exhibited the lowest improvement values across all traits under both conditions. Seasonally, the 2025 season showed markedly higher values for osmoprotectant accumulation and yield attributes compared to 2024 in both normal (Table 8) and drought (Table 9) environments, highlighting a strong genotype-by-environment interaction. Under drought stress (Table 9), the dramatic upregulation of biochemical traits peaking in Mutant 5 effectively mitigated osmotic pressure despite negative Osmotic Adjustment values, successfully safeguarding grain yield per plant.

3.6. Genetic components parameters

For genetic parameters components, the data obtained in table (10) reveals that phenotypic variance and genotypic variance consistently increased under stress conditions for most traits, particularly grain yield/plant, proline, and glycine betaine contents, indicating greater genetic expression and variation triggered by environmental stress. Across both seasons (2024 & 2025), the genotypic coefficient of variation closely mirrors the phenotypic coefficient of variation, demonstrating that the observed variations are primarily driven by genetic factors rather than environmental noise. Further, high broad-sense heritability higher than 70% was consistently recorded for critical yield-related attributes and biochemical osmoprotectants (e.g., proline and trehalose) contents under both normal and stress environments. Crucially, grain yield/plant exhibited a simultaneous combination of high heritability (83.35% to 89.11%) and high genetic advance as a percentage of mean (GAM = 18.59% to 26.45%) under stress conditions. Also, this physiological and genetic alignment confirms that these traits are governed by additive gene action, making them highly reliable, stable, and effective criteria for direct selection to improve drought or osmotic stress tolerance in these new rice accessions.

3.7. Genetic similarity and phylogenetic tree

The genetic similarity among the six rice genotypes namely; the parent Sakha 102 and the five mutants under normal and water stress conditions was revealed in table (11). Where, the data demonstrates high overall similarity (>0.88), indicating a closely related genetic background derived from the same original cultivar (Sakha 102). While, under normal conditions, the maximum genetic similarity reaches 0.986 between Sakha 102 and both Mutant 1 and Mutant 2, confirming minimal structural genome alterations following initial mutation. Conversely, Mutant 5 shows the lowest similarity within the control group (0.900 against Sakha 102), representing the most genetically divergent mutant. Further, drought stress significantly alters the genetic polymorphism profiles, causing a clear restructuring of relationships. While, under stress, the highest similarity occurs between Mutant 3 and Mutant 4 (0.987), followed closely by Sakha 102 and Mutant 1 (0.987). Crucially, the absolute minimum genetic similarity across the entire matrix is 0.888, observed between Mutant 4 (Normal) and Mutant 3 (Drought). This noticeable drop in similarity under different water regimes demonstrates that drought stress triggers distinctive changes in genetic marker expression or selective gene activation across the genotypes. On the same direction, this UPGMA dendrogram, based on Nei-Li's similarity

Table 7:- Simple correlation coefficients of combined analysis among all attributes studied in rice entries under drought conditions in both growing seasons (2024 & 2025).

Traits	1000-Grain weight (gm)	Grain weight (gm)	Proline content	Glycine betaine content	Trehalose content	Osmotic adjustment
1000-Grain weight (gm)	1.0	0.79	0.96	0.68	0.83	0.78
Grain weight (gm)	0.79	1.0	0.87	0.83	0.70	0.78
Proline content	0.96	0.87	1.0	0.92	0.68	0.76
Glycine betaine content	0.68	0.83	0.92	1.0	0.92	0.69
Trehalose content	0.83	0.70	0.68	0.92	1.0	0.74
Osmotic adjustment	0.78	0.78	0.76	0.69	0.74	1.0

*: Significant at 5% & **: Significant at 1%

Table 8:- The mutagenesis % in the five rice mutants compared to the local cultivar (sakha 102) under normal conditions through the two seasons.

Traits	1000-grain weight (gm)			Grain yield /plant (gm)			Proline content			Glycine betaine content			Trehalose content		
Genotypes	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean
Mutant 1	22.14	53.49	37.81	65.67	67.4	66.53	60.03	23.17	41.6	41.39	37.75	39.57	68.53	90.91	79.72
Mutant 2	47.22	73.69	60.45	96.3	101.54	98.92	95.98	71.94	83.96	74.22	70.6	72.41	114.68	139.54	127.11
Mutant 3	49.13	70.45	59.79	120.49	121.93	121.21	98.01	68.77	83.39	51.58	45.78	48.68	147.32	173.29	160.3
Mutant 4	71.39	90.56	80.97	131.91	122.6	127.25	154.57	114.02	134.29	106.04	109.08	107.56	131.69	133.24	132.46
Mutant 5	63.18	116.91	90.04	123.17	137.13	130.15	205.56	149.67	177.61	172.13	161.52	166.82	190.7	213.35	202.07
M. of (G.I)	50.61	81.02	65.81	107.50	110.12	108.81	122.83	85.51	104.17	89.07	84.94	87	130.58	150.06	140.33

M. of (G.I):- Mean of Genetic Improvement

Table 9: - The mutagenesis % in the five rice mutants compared to the local cultivar (sakha 102) under drought conditions through the two seasons.

Traits	1000-grain weight (gm)			Grain yield/plant (gm)			Proline content		
	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean
Genotypes									
Mutant 1	29.66	67.94	48.80	94.13	82.04	88.08	122.4	53.8	88.1
Mutant 2	52.22	95.09	73.65	166.03	144.17	155.10	169.82	103.71	136.76
Mutant 3	61.11	96.04	78.57	163.88	147.8	155.84	147.88	89.14	118.51
Mutant 4	76.44	117.65	97.04	185.48	166.21	175.84	246.65	170.91	208.78
Mutant 5	89.66	153.66	121.66	165.09	166.21	165.65	324.09	222.24	273.16
M. of (G.I)	61.81	106.07	83.94	194.92	141.28	148.10	210.25	134.02	172.13
Traits	Glycine betaine content			Trehalose content			Osmotic adjustment		
	2024	2025	Mean	2024	2025	Mean	2024	2025	Mean
Genotypes									
Mutant 1	71.87	92.47	82.17	95.11	143.4	119.25	-97.97	-78.94	-88.45
Mutant 2	128.55	142.85	135.7	191.88	227.3	209.59	-64.35	-74.56	-69.45
Mutant 3	90.9	112.9	101.9	239.88	285.05	262.46	-81.68	-83.33	-82.50
Mutant 4	162.24	190.11	176.17	203.48	249.96	226.72	-74.75	-76.75	-75.75
Mutant 5	266.02	308.29	287.15	299.93	341.18	320.55	-74.25	-78.94	-76.59
M. of (G.I)	143.91	169.32	156.61	206.05	249.37	227.71	-78.60	-78.39	-78.49

M. of (G.I):- Mean of Genetic Improvement.

Table 10: - Genetic parameters of all attributes in the six rice accessions under both conditions

S.O.V	Seasons	1000-grain weight (gm)		Grain yield/plant (gm)		Proline content.		Glycine Betaine Content		Trehalose content		Osmotic adjustment
		Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	
Phenotypic variance	2024	5.52	8.65	7.98	7.01	4.91	4.72	6.58	9.97	4.10	15.31	6.88
	2025	6.48	18.01	11.76	10.86	7.09	9.62	8.60	8.96	5.86	10.0	10.19
Genotypic variance	2024	4.40	7.21	7.03	5.66	3.06	3.25	4.73	7.44	2.61	14.36	5.22
	2025	5.42	16.05	10.62	9.10	5.06	7.85	6.57	5.84	4.0	8.66	8.07
Environmental variance	2024	1.12	1.44	0.95	1.35	1.85	1.47	1.85	2.53	1.49	0.95	1.66
	2025	1.06	1.96	1.14	1.76	2.03	1.77	2.03	3.12	1.86	1.34	2.12
Phenotypic coefficient %	2024	6.86	10.78	5.61	6.15	5.29	6.23	4.65	6.95	3.83	9.13	349.73
	2025	7.47	14.35	6.59	7.63	6.02	8.85	5.12	6.35	4.54	7.83	399.02
Genotypic coefficient %	2024	6.13	9.84	5.27	5.52	4.18	5.17	3.94	6.0	3.06	8.84	304.63
	2025	6.83	13.55	6.26	6.98	6.42	8.0	4.48	3.50	3.75	7.29	355.09
Heritability in broad sense	2024	79.71	83.35	88.09	80.74	62.32	68.85	71.88	74.62	63.65	93.79	75.87
	2025	83.64	89.11	90.30	83.79	71.36	81.60	76.39	65.17	68.26	86.60	79.19
Genetic advance	2024	3.87	5.07	5.14	4.42	2.85	3.09	3.81	4.87	2.66	7.58	4.11
	2025	4.40	7.82	6.40	5.71	3.92	5.23	4.63	4.03	3.41	5.66	5.23
The genetic advance as % of mean	2024	11.31	18.59	10.21	10.23	6.81	8.86	6.91	10.72	5.04	17.69	548.0
	2025	12.92	26.45	12.30	13.22	8.86	14.93	8.09	8.71	6.40	14.02	653.75

Table 11: - Genetic similarity % of all rice genotypes under normal and water stress conditions.

Genetic Similarity	Sakha 102 (Normal)	Mutant 1 (Normal)	Mutant 2 (Normal)	Mutant 3 (Normal)	Mutant 4 (Normal)	Mutant 5 (Normal)	Sakha 102 (Drought)	Mutant 1 (Drought)	Mutant 2 (Drought)	Mutant 3 (Drought)	Mutant 4 (Drought)	Mutant 5 (Drought)
Sakha 102 (Normal)	1											
Mutant 1 (Normal)	0.986	1										
Mutant 2 (Normal)	0.986	0.973	1									
Mutant 3 (Normal)	0.961	0.973	0.973	1								
Mutant 4 (Normal)	0.961	0.948	0.973	0.973	1							
Mutant 5 (Normal)	0.90	0.911	0.911	0.935	0.935	1						
Sakha 102 (Drought)	0.937	0.925	0.925	0.925	0.925	0.962	1					
Mutant 1 (Drought)	0.925	0.936	0.912	0.936	0.912	0.974	0.987	1				
Mutant 2 (Drought)	0.913	0.901	0.901	0.901	0.901	0.937	0.975	0.962	1			
Mutant 3 (Drought)	0.925	0.936	0.912	0.912	0.888	0.925	0.938	0.950	0.962	1		
Mutant 4 (Drought)	0.93	0.925	0.925	0.901	0.901	0.913	0.950	0.938	0.975	0.987	1	
Mutant 5 (Drought)	0.936	0.948	0.924	0.924	0.90	0.912	0.925	0.937	0.925	0.962	0.950	1

coefficient and viewed in (Fig. 7), illustrates the phylogenetic relationships among the six rice genotypes under normal and drought conditions. Result showed that the tree clearly bifurcates into two primary clusters at a similarity level of approximately 0.925, successfully separating the genotypes according to their environmental treatments. Where, under normal conditions, Sakha 102 (G1N) and Mutant 1 (G2 N) exhibit the highest genetic similarity, while the mutants (3 & 4) or (G4 & G5) under drought conditions cluster most closely under stress. Overall, Mutant 5 (G6) consistently shows the highest divergence from the other genotypes across both environments.

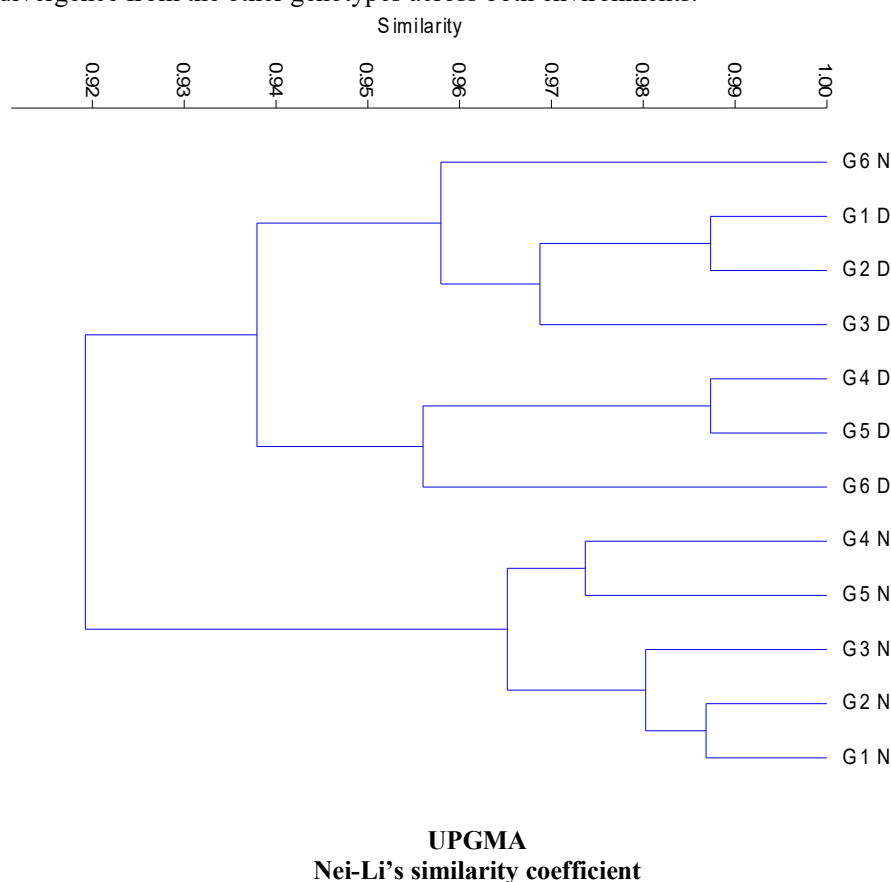


Fig. 7: Phylogenetic tree among all rice accessions under both conditions.

4. Discussion

The ANOVA results in tables (1 & 2) reveal highly significant genetic variability ($P < 0.01$) among rice genotypes, indicating that the performance of grain yield and biochemical traits is largely governed by genetic makeup. While, table (1) establishes baseline productivity, table (2) demonstrates that drought stress triggers a significant accumulation of osmoprotectants like Proline and Trehalose contents to facilitate Osmotic Adjustment. Recent studies highlight that these metabolic shifts are crucial for maintaining cellular homeostasis and protecting plant tissues from oxidative damage under water-deficit conditions (El-Mouhamady 2023; Tawfik and El-Mouhamady 2024b; Zhu and Li 2025). Furthermore, the correlation between sustained 1000-grain weight and elevated osmoprotectant levels suggests that specific genotypes possess superior resilience mechanisms (El-Mouhamady 2003, 2009; Eldessouky *et al.*, 2016; El-Demardash *et al.*, 2017; El-Mouhamady *et al.*, 2022; Hassan *et al.*, 2026). The low C.V. % across both seasons confirms high experimental precision. As established in recent literature, integrating physiological markers with yield data remains the most effective strategy for identifying drought-tolerant rice cultivars (El-Mouhamady *et al.*, 2010a; Wang and Zhang 2025.). Furthermore, the differential responses of the six rice accessions under normal and water-deficit regimes reflect distinct genetic capacities for physiological adaptation. The drastic reductions observed in yield-related parameters, specifically 1000-grain weight (Fig. 1) and grain yield per plant (Fig. 2),

demonstrate that water limitations severely disrupt photosynthetic allocation, a vulnerability thoroughly described by El-Seidy *et al.*, (2013); El-Mouhamady *et al.* (2013); Ali *et al.* (2025) in susceptible wild types. To counteract this cellular dehydration, tolerant genotypes actively trigger defensive mechanisms involving osmotic adjustment (Fig. 6), which (El-Mouhamady *et al.*, 2010b ; Esmail *et al.*, 2016; Chen and Wang 2024) confirm is vital to maintain turgor pressure. Also, this survival strategy is heavily mediated by the hyper-accumulation of compatible solutes, where substantial increases in proline (Fig. 3) and glycine betaine (Fig. 4) act dynamically to protect cellular structures, a protective mechanism validated by El-Mouhamady *et al.* (2019); Kumar *et al.* (2024). Structurally, the superior performance of Mutant 5 under stress highlights an enhanced metabolic buffering capacity; as (El-Mouhamady and Ibrahim 2020; Smith *et al.*, 2023) observed, elevated trehalose levels (Fig. 5) effectively preserve membrane integrity under severe moisture stress. In the same track, genetic variations in osmoprotectant biosynthesis, as emphasized by Al-Kordy *et al.* (2019); Tanaka and Sato, (2026), remain central to identifying robust, climate-resilient crop lineages suited for changing environments. The structural analysis of water stress tolerance indices across the 2024 and 2025 seasons in table (5) confirms that gamma-ray mutagenesis effectively enhanced drought tolerance in the evaluated rice accessions with remarkable year-to-year stability. The local cultivar, Sakha 102, exhibited severe susceptibility in both years, displaying the highest values for the Drought Sensitivity Index (DSI = 1.87 vs 2.05) and Yield Reduction (YR = 0.27 vs 0.28). This high-vulnerability trend across seasons aligns with El-Mouhamady and Tawfik (2025); Anghla *et al.* (2025), who emphasized that traditional Egyptian cultivars lack the osmotic adjustment necessary to prevent profound yield penalties under continuous seasonal moisture deficits. In stark contrast, the five mutant lines specifically mutants (4 & 5) demonstrated robust, consistent tolerance by maintaining the lowest DSI and YR values in both 2024 and 2025. According to El-Mouhamady *et al.* (2021); Shrestha *et al.* (2026), such multi-season stability in low DSI and YR values reflects superior genetic adaptability and an improved capacity to sustain stable grain yield under variable restrictive environments. Furthermore, as documented by Heiba *et al.* (2016a ; Shrestha *et al.* (2026), this highly preserved resilience across both consecutive years is typically driven by optimized resource-use efficiency and defensive physiological mechanisms that mitigate oxidative damage over time. Consequently, these stable mutant lines serve as ideal germplasm for securing sustainable rice production under water-scarce environments. In the same context, the strong positive correlations between yield components and osmoprotectant levels (proline, glycine betaine, and trehalose) under both normal (Table 6) and drought conditions (Table 7) highlight a critical physiological adaptation in rice during the 2024 and 2025 seasons. Biochemically, these compatible solutes are not merely passive stress markers; they actively drive osmotic adjustment to maintain the cellular turgor required for leaf expansion and stomatal opening under water deficits. According to Heiba *et al.* (2016b); Ali *et al.* (2021), the marked upregulation of proline ($r = 0.96$ with 1000-grain weight) acts as a metabolic buffer that mitigates oxidative damage by scavenging reactive oxygen species (ROS), thereby preserving spikelet fertility and grain filling. Furthermore, (El-Mouhamady *et al.*, 2014; Shao *et al.*, 2025) demonstrated that elevated trehalose and glycine betaine levels shield the thylakoid membrane and photosynthetic enzymes from structural degradation during desiccation, explaining the high correlation ($r = 0.83$) with stable grain weight under stress. This is complemented by the findings of Ramadan *et al.* (2016); Mishra *et al.* (2025), who verified that genotypes possessing superior osmotic adjustment parameters maintain significantly higher relative water content in their reproductive tissues, ensuring unhindered photoassimilate translocation. Consequently, the co-dependent accumulation of these osmolytes forms a synergistic protective mechanism that directly dictates the resilience of grain yield components against drought-induced losses. Also, the induced mutagenesis led to substantial trait improvements over the local cultivar Sakha 102, with significantly higher percentage improvements under drought stress (Table 9) than normal conditions (Table 8), confirming the efficacy of mutation breeding in enhancing climate resilience (Al-Ghamdi and Hassan 2025). Where, mutant 5 consistently recorded the highest mean of genetic improvement across both environments (Table 8; Table 9) by maximizing the accumulation of proline, glycine betaine, and trehalose contents. Further, this dramatic biochemical upregulation efficiently mitigated osmotic pressure despite negative osmotic adjustment values, directly safeguarding 1000-grain weight and grain yield per plant traits under drought (Table 9), which aligns with recent findings on osmoprotectant-mediated yield stability (El-Mouhamady *et al.*, 2022; Zian *et al.*, 2013; Wang *et al.*, 2025). Conversely, mutant 1 exhibited the lowest improvement values across all traits in both environments tables (8 & 9), indicating unfavorable mutational impacts.

Seasonally, the 2025 season showed markedly higher values for osmoprotectant accumulation and yield attributes compared to the 2024 season under both normal (Table 8) and drought (Table 9) conditions. In the same context, this variation highlights a strong genotype-by-environment interaction, emphasizing how seasonal fluctuations modulate mutant gene expression and yield stability (Zhou and Smith 2026). In the same direction, the genetic parameters provided in table (10) confirm a robust genetic architecture governing abiotic stress tolerance and yield-related attributes in the evaluated rice accessions. Also, the substantial alignment between the genotypic coefficient of variation (GCV) and Phenotypic Coefficient of Variation (PCV) across both the 2024 and 2025 seasons underscores that environmental noise exerts minimal influence on trait expression, a phenomenon recently validated in rice variability studies under stress conditions (Khatab *et al.*, 2017; Tareque *et al.*, 2025). Furthermore, the persistent manifestation of high broad-sense heritability, paired with elevated genetic advance as a percentage of mean for crucial traits like grain yield/plant, proline, and trehalose content under stress, scientifically points to the predominance of additive gene action rather than non-additive effects. As well, this strong genetic determination establishes these biochemical osmoprotectants and yield components as highly reliable biomarkers for phenotypic selection. Such high heritability and additive gene control for adaptive osmotic and agronomic traits align with recent genomics and diversity frameworks in cereal crops under environmental duress (Khatab *et al.*, 2019; Khatab *et al.*, 2021a; Ahmad *et al.*, 2024; Hassan *et al.*, 2026), reinforcing that direct selection for these attributes will yield predictable, stable, and highly effective genetic gains in future climate-resilient rice breeding programs. Further, the high genetic similarity (>0.88) among the Sakha 102 rice genotypes confirms a highly conserved genetic background, which aligns with the fact that targeted mutagenesis typically induces localized genomic lesions rather than massive structural rearrangements (Khatab *et al.*, 2021b; Li *et al.*, 2024). Also, the high baseline similarity (0.986) between the parent and Mutants 1 and 2 under control conditions confirms that mutagenesis often yields low-frequency single nucleotide polymorphisms (SNPs) or small indels rather than profound structural genome alterations (Khatab *et al.*, 2022; Sharma *et al.*, 2025). Conversely, the pronounced divergence of Mutant 5 suggests it harbored higher mutational loads or distinct structural variants during induction, making it a unique genetic variant within the control group (Khatab and El-Mouhamady 2022; Kim and Lee 2024). While under drought stress, the significant restructuring of genetic similarity profiles and the clear bifurcation of the UPGMA dendrogram at 0.925 underscore environment-dependent genetic marker variations (Zhou *et al.*, 2025; El-Mouhamady and Elewa 2026). The drastic drop in similarity to 0.888 between Mutant 4 (Normal) and Mutant 3 (Drought) demonstrates that water deficit triggers distinctive changes, likely related to differential locus-specific DNA methylation or selective marker amplification associated with stress-responsive pathways (El-Mouhamady and El-Metwally 2020; El-Mouhamady *et al.*, 2021a; Al-Mallahi and Tanaka 2026). Finally, this distinct clustering based on environmental regimes rather than purely by genotype confirms that the identified marker profiles are highly responsive to stress-induced changes, providing a reliable molecular baseline for selecting drought-tolerant lines like Mutants 3 and 4 for targeted breeding programs.

5. Conclusion

As a strategic crop, rice is essential for Egyptian national food security; however, climate change and escalating water scarcity pose severe threats to its sustainable production. This investigation demonstrates that induced mutagenesis using safe doses of gamma rays successfully enhanced drought resilience in the Egyptian rice cultivar "Sakha 102." Under severe water-deficit conditions, Mutant 5 emerged as the most superior drought-tolerant genotype, maintaining the highest grain yield and 1000-grain weight by maximizing the accumulation of key biochemical osmoprotectants like proline, glycine betaine, and trehalose contents. Supported by high broad-sense heritability and strong additive gene action, these biochemical traits prove to be stable, highly reliable markers for direct phenotypic selection. Ultimately, Mutant 5 serves as a vital, high-potential genetic donor line that will play a pivotal and active role in future breeding programs aimed at securing climate-resilient rice production amidst escalating global water scarcity. From another angle, these results shed light on the vital and effective role of using gamma rays to generate novel rice mutants tolerated to drought stress through mutation breeding programs.

Abbreviations

1): G 1 N:-Sakha 102 under normal conditions, 2):- G 2 N: - Mutant 1 under normal Conditions, 3): G 3 N: - Mutant 2 under Normal Conditions, 4): G 3 N: - Mutant 3 under normal conditions, 5): G 5 N: - Mutant 4 under normal conditions, 6): G 6 N: - Mutant 5 under normal conditions, 7): Entry G1D:- Sakha 102 under drought conditions, 8):- G2 D: - Mutant 1 under drought Conditions, 9): G3 D: - Mutant 2 under drought Conditions, 10): G4 D: - Mutant 3 under drought conditions, 11): G5 D: - Mutant 4 under drought conditions & 12): G6 D: - Mutant 5 under drought conditions.

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