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Elicitation of Novel Rice Mutants Tolerated to Drought stress through Using of Gamma Rays

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ABSTRACT

Rice is a strategic food crop at both global, local levels and represents vital importance for Egyptian National Food Security. However, the environmental challenges that reduce the productivity and spread of rice cultivation are many, especially water stress due to the lack of water needed for its cultivation. Accordingly, this investigation set out with great efficiency in order to infer new rice mutants characterized by their high water deficit tolerance as well, its high yielding across using several safe doses of gamma rays. Further, the present study succeeded in genetic improvement for rice variety Sakha 101 after exposure it to several doses of gamma rays, through the events of large physiologically, genetically and biochemical changes that had the greatest impact on raising its drought tolerance degree besides, eliciting five improved novel rice mutants from it. Also, the six rice accessions were evaluated under normal and drought through estimating some yield, physiological and biochemical attributes for screening the most mutants tolerated for water stress in 2022 and 2023 seasons. The final results confirmed that the rice mutants number (3, 4 & 5) were recorded the biggest trend of drought tolerance then followed by the mutants number (1 & 2) compared to the local cultivar under water stress treatment compared to the standard experiment. Therefore, these novel rice mutants will be future high-yielding and drought-tolerant rice varieties and this is the biggest goal in this work.

Keywords: plant breeding, mutations, rice lines, gamma rays, phylogenetic tree

1. Introduction

Rice is one of the most important strategic food crops at both local and global levels where it is the true measure of food security for the largest population of the world. Further, drought stress is considered one of the most important serious environmental obstacles that threaten global food security for all peoples of the earth as it has many devastating negative effects on all aspects of agriculture and agricultural development areas alike especially in countries suffering from a severe shortage of irrigation water resources, (El-Mouhamady 2003 & 2009; FAO, 2015). Also, water stress also has devastating effects on agricultural soil properties as the number of washing cycles of salts decreases, leading to higher in summer crops such as in rice and maize (Esmail *et al.,* 2016). As, the high levels of salt stress especially in agricultural land prepared in advance for the cultivation of summer crops such as rice, maize and sugarcane is a real challenge in destroying the final yield of those crops and taking those lands out of agricultural service, (Fahad *et al.,* 2017). One of the most important destructive negative effects of water stress on crops in general and on rice crop in particular is a sharp decrease in the final output which ranged from 40 to 50%, (El-Mouhamady *et al.,* 2019). Due to the great importance of this crop, its cultivation has spread too many countries of the world, especially the United States, Australia, Southeast Asian countries such as India, China, Japan, the Philippines, and also in North Africa, such as the Arab Republic of Egypt. However, the determining factor for the spread of

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rice cultivation in Egypt is the availability of water resources necessary for cultivation and growth, which is the core of the scientific problem under discussion in this paper which must discuss something of the insights, (Melandri *et al.,* 2020). In the same path, the exacerbation of the pace of water stress leads to a deficiency and acute destruction of all morphological and physiological factors, including the formation of the final output and its components, which ultimately gives a low yield that does not meet the required nutritional needs, (Panda *et al.,* 2021). This deficiency occurs after a series of destructive physiological and biochemical changes such as a high percentage of empty grains, a decreasing in the total chlorophyll content of the leaves and a general collapse in the osmotic system of the cells, which is due to the exit of water from the cells during high temperatures, (Khatab *et al.,* 2021a). Further, this crop was able to save millions of Chinese people from starvation after World War II, representing our storehouse of a number of vitamins, carbohydrates and proteins important for providing calories for the human body, (Mohidem *et al.,* 2022). What has led to the exacerbation of this problem is the limited irrigation water, especially needed for agriculture, irrigation and drinking for the Arab Republic of Egypt after the construction of the Ethiopian Renaissance Dam. Where this Ethiopian project had devastating effects on agriculture and Egyptian desire alike and this includes reducing the annual quota of Blue Nile water and wasting a large proportion of the water stored behind the dam, which called on decision-makers in Egypt to develop quick and alternative solutions to contain this serious crisis. (El-Mouhamady, 2023). Further, the lack of rainfall rates as well, the lack of water resources necessary for agriculture, growth and crop production in the tropical and subtropical regions has led to the exacerbation and increase of areas affected by salt stress, especially in many areas of northern Egypt, such as the New Valley, (El-Mouhamady *et al.,* 2021; Mohidem *et al.,* 2022; El-Mouhamady, 2023). In the same context, it is noted that water stress negatively affects most metabolic processes, including raising the respiration rate, which negatively affects the process of photosynthesis and dry matter formation, especially in the two stages of germination, starter formation and flowering alike, (Duvnjak *et al.,* 2023). This called for the Egyptian state to develop short and long-term plans as a serious attempt to deal with the seriousness of drought stress, which actually seemed to reduce the size of agricultural land, especially in the valley and delta region. In particular, the rice crop is a crop that is sensitive to water, which is essential for the completion of growth processes and all stages of physiological, biochemical and morphological metabolism, especially in the summer. Also, one of the most important efforts of the Egyptian state was the inference of new accessions of some strategic crops like rice and barley with high tolerance to water stress and high yielding as well, their genetic stable under different environments, (Al-Kordy *et al.,* 2019; El-Mouhamady and Tawfik, 2025). From another angle, work in the fields sciences of plant breeding, biotechnologies and genetic engineering in order to elicitation of new rice maturity entries where their water needs are low compared to long-lasting genotypes. For achieving this strategy, safe doses of gamma rays were used to derive drought-tolerant and high-yielding rice lines through a number of isolation generations in order to obtain promising rice genotypes through which to exit the water stress crisis with minimum losses to Egyptian food security and this is the purpose of this investigation.

2. Materials and Methods

2.1. History of plant materials

The present investigation used 5 mutant rice lines derived from the Egyptian rice variety **Sakha 101** which classified as tolerate for drought stress after treated this Egyptian variety for safe dosages of gamma irradiation (100, 200, 300, 400 and 500 Gy) using the Co source at the National Center for Radiation Research and Technology, Nasr City and Cairo in 2012 season (M0). The pedigree of the original Egyptian variety Sakha 101 was (Giza 176/Milyang 9).

2.2. Field evaluation

The rice seeds of the Egyptian cultivar Sakha 101 used in the recent investigation were originally performed from Rice Research & Training Center, Agriculture Research Centre. Also, one thousand pure seeds of Sakha 101 variety were subjected for gamma irradiation treatments 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 2012 season (M0). Further, the irradiated materials of all doses were grown and series of selections among the mutant population under normal soil conditions in the farm of Aga Center and City in Dakahlia Governorate province and this process carried out during 2013-

2020 seasons (M1-M8) to produce the selected five mutant rice lines and 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 2022 and 2023 seasons using the original rice cultivar (Sakha 101) besides, 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 cultivated as a randomized complete block design where each one was replicated three times. The stress was applied two weeks after transplanting till harvesting. Each irrigation experiment was a completely independent experiment and completely isolated from the other experiment. 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 among each two plants was 20 cm into each replicate.

2.4. Studied traits

Ninety plants were used for determined all attributes under study of each treatments (Normal and water stress conditions) where 30 plants for each replicate. Each experiment included three replicated and the six rice accessions were grown in a randomized complete block design in both growing seasons (2022 & 2023).

2.5. Yield, physiological and biochemical traits

1):- 1000-grain weight (gm), 2):- Grain yield/plant (gm), 3):-Proline Content, 4):- Glycine betaine Content, 5):- Trehalose Content, 6):- Maximum root Length

The proline content was determined from a standard curve and calculated on a fresh basis is as follows: [(µg proline / ml C m1 toluence) / 115.5 µg / µ mole] / [(g sample/5)] = µ 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) while glycine betaine and trehalose contents were carried out according to Grieve and Grattan, (1983).

2.6. Water Stress Tolerance Indices

All drought stress tolerance indices were estimated for grain yield/plant trait 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).

2.7. Statistical analysis

All calculated data of all traits under evaluation in two seasons for both treatments were analyzed using the formula by Gomez and Gomez, (1984). All graphs were done using (Graph Pad Prism model 8).

2.8. Genetic similarity and phylogenetic tree

Data of all attributes under investigating for all rice accessions 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

There is no doubt that environmental stresses, especially water stress, are considered one of the most serious challenges in the field of genetic improvement of strategic field crops such as rice. Therefore, the efforts of scientists and researchers have gone to try to make a big and moral leap in the field of genetic improvement of rice crop using safe doses of gamma rays. Further, this crop represents an important source of national food security in the Egyptian state. In the following, the results of this investigation are presented in detail under water stress conditions compared to the standard experiment of two agricultural seasons.

3.1. Variation & interaction

Data viewed in (Tables 1 & 2) and related to the (ANOVA) test revealed that highly significant differences were obtained between all rice genotypes (the original line Sakha 101 and its five M8-derived mutants) for all studied traits tested under both normal and drought conditions during the two growing seasons (2022 and 2023). Also, data of C.V. % were generated tracking ranged from low to medium form for all traits under investigation for the two experiments of the two growing seasons.

3.2. Mean performance

Results shown in tables (3 & 4) revealed the mean values of all studied traits for the new five M8 rice mutants derived from the Egyptian rice variety Sakha 101 evaluated under both normal and drought condition through the two growing seasons (2022 & 2023). It is noteworthy that the five M8 rice mutants have already been able to outperform the original variety Sakha 101 which derived from it in both the studied traits grain yield /plant, 1000-grain weight and maximum root length. Also, these new materials have been excelled in the biochemical traits related to water stress tolerance like proline, glycine betaine and trehalose contents where they gave morally high values under stress conditions compared to the experimental experiment in both growing seasons. These results prove beyond doubt that the use of safe doses of gamma rays of the Egyptian rice variety Sakha 101 has already secreted new rice accessions that reached the highly level of genetic stable and were already superior in all traits under study besides, its water stress tolerant to a greater extent than the original variety. Therefore, the combined analysis of all mean performances obtained in tables (3 & 4) during the two growing seasons for all attributes under testing proves beyond doubt the natural and obvious effect of water stress on the six rice accessions which highlights and confirms the extent to which the new rice variants can tolerate water stress, figures (1 to 6).

3.3. Drought stress tolerance indices

Water stress tolerance indices parameters were calculated for grain yield/plant trait in both growing seasons in table (5). The values of (YSI & YR) were recorded results lower than one for the six rice genotypes in the two growing seasons. Further, the same track were obtained for (YI) parameter except the rice mutant (3 & 4) for 2022 season and (3 & 4 & 5) for 2023 season where these materials exhibited values higher than the unity. In the same track, the novel five rice materials were generated values of **(MP & GMP)** parameters higher than the original rice cultivar (Sakha 101) in both growing seasons. Also, the data of **(DTI)** parameter were viewed lower than one in all rice entries in both growing seasons except the rice mutants (4 & 5) in 2022 season only were these two rice materials recorded results higher than one. From these results, it is noteworthy that the previous statistics have clearly revealed the genetic and physiological behavior associated with water stress tolerance of rice crop in the novel five rice mutants compared to the original variety (Sakha 101).

3.4. Similarity indices and cluster analysis

Data observed in table (6) showed 55 relationships among 12 rice accessions detected about similarity. Results were ranged from 0.376 to 0.625 with an average of 0.500. Where, the biggest level of similarity was (0.625) between (G6 & G12). While, the lowest rank was (0.376) within (G2 & G10). Further, other high similarity values were obtained in this investigation for example not limited between (G4 & G7) (0.604), (G4 & G8) 0.617), (G4 & G10) (0.602), (G5 & G7) (0.620), (G5 & G12) (0.606) and (G8 & G12) where it recorded (0.609), respectively. Data of phylogenetic tree (Fig, 7) revealed that the cluster analysis of 12 rice genotypes (the six rice accessions under normal and water stress conditions) were consisted to two main cluster. Where, the first one was contained (Sakha 101 and Mutant 2 under normal conditions besides, Mutant 4 only under drought conditions). While, the second cluster had four sub-cluster. Where, these groups were (Mutant 5 under both treatments, Sakha 101 for drought conditions and Mutant 4 for normal treatment, Mutant 1 for drought conditions and Mutant 3 for normal conditions and Mutants 2 & 3 for drought treatment), respectively.

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Table 1: Analysis of variance for all studied traits of the six rice accessions under normal conditions 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	Maximum root length
Genotypes	5	2022	35.12**	11.67**	14.98**	29.05**	30.21**	10.05**
		2023	28.43**	13.22**	22.03**	41.05**	28.54**	14.21**
Replicates	2	2022	15.32**	16.28**	107.03**	94.06**	46.83**	18.55**
		2023	19.04**	15.89**	88.14**	54.23**	58.23**	24.05**
Error	10	2022	1.88	1.32	1.17	1.41	1.28	1.62
		2023	1.54	1.22	1.28	1.84	1.59	1.39
C.V. %		2022	4.25	2.25	3.36	2.37	2.20	2.97
		2023	3.90	2.26	2.82	2.62	2.40	2.62

Table 2: Analysis of variance for all studied traits of the six rice genotypes under water stress conditions 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	Maximum root length
Genotypes	5	2022	19.67**	55.12**	103.11**	38.01**	103.44**	7.23**
		2023	12.06**	75.03**	86.14**	42.012**	93.05**	10.15**
Replicates	2	2022	38.01**	51.33**	65.02**	133.22**	117.56**	93.16**
		2023	78.05**	44.06**	72.11**	127.04**	123.49**	78.31**
Error	10	2022	1.63	2.04	1.74	1.14	1.25	1.93
		2023	1.54	1.84	1.83	1.19	1.37	1.77
C.V. %		2022	4.59	3.20	2.46	1.70	1.67	2.70
		2023	4.67	3.08	2.47	1.79	1.74	2.56

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

Traits / Genotypes	1000-grain weight (gm)			Grain yield /plant (gm)			Proline content			Glycine betaine content			Trehalose content			Maximum root length (cm)		
	2022	**2023**	**Mean**	**2022**	**2023**	**Mean**	**2022**	**2023**	**Mean**	**2022**	**2023**	**Mean**	**2022**	**2023**	**Mean**	**2022**	**2023**	**Mean**
Sakha 101	25.34	24.89	25.11	29.34	28.75	29.04	29.66	32.14	30.90	34.25	32.98	33.61	34.15	31.97	33.06	28.54	30.07	29.30
Mutant 1	29.81	30.14	29.97	44.12	46.03	45.07	33.86	35.02	34.44	39.53	42.01	40.77	49.54	51.03	50.28	35.78	41.45	38.61
Mutant 2	33.96	31.78	32.87	48.32	46.54	47.43	40.03	42.35	41.19	44.85	46.05	45.45	41.84	43.09	42.46	42.13	45.08	43.60
Mutant 3	31.18	33.02	32.10	52.03	54.18	53.10	37.22	35.89	36.55	53.73	56.04	54.88	52.11	54.75	53.43	55.13	57.36	56.42
Mutant 4	35.62	34.77	35.19	63.14	62.54	62.84	51.45	49.64	50.54	61.09	63.56	62.32	61.08	63.31	62.19	39.72	42.05	40.88
Mutant 5	37.41	35.86	36.63	68.34	66.44	67.39	43.18	45.02	44.10	66.84	69.02	67.93	68.55	70.04	69.29	55.02	53.12	54.07
Mean	32.22	31.74	31.97	50.88	50.74	50.81	39.23	40.01	39.62	50.04	51.61	50.82	51.21	52.36	51.78	42.72	44.85	43.81
LSD at 5%	2.02	1.83	1.92	1.69	1.63	1.66	1.60	1.67	1.63	1.75	2.00	1.87	1.67	1.86	1.76	1.88	1.74	1.81
LSD at 1%	3.09	2.79	2.94	2.59	2.49	2.54	2.44	2.55	2.49	2.67	3.06	2.86	2.55	2.84	2.69	2.87	2.65	2.76

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Table 4: Mean performance and combined analysis for all studied traits of the six rice genotypes under water stress conditions in the two growing seasons

Traits / Genotypes	1000-grain weight (gm)			Grain yield /plant (gm)			Proline content			Glycine betaine content			Trehalose content			Maximum root length		
	2022	**2023**	**Mean**	**2022**	**2023**	**Mean**	**2022**	**2023**	**Mean**	**2022**	**2023**	**Mean**	**2022**	**2023**	**Mean**	**2022**	**2023**	**Mean**
Sakha 101	21.09	18.33	19.71	26.12	25.45	25.78	41.05	42.85	41.95	45.12	47.03	46.07	46.03	48.02	47.02	37.11	30.05	33.58
Mutant 1	26.32	24.11	25.21	39.22	38.54	38.88	46.96	49.56	48.26	55.23	57.03	56.13	62.13	60.08	61.10	44.15	46.03	45.09
Mutant 2	28.05	29.03	28.54	41.19	39.18	40.18	52.12	55.08	53.60	52.12	55.29	53.70	58.72	60.14	59.43	52.18	53.86	53.02
Mutant 3	29.02	30.15	29.58	45.16	47.32	46.24	48.13	51.03	49.58	62.44	64.11	63.27	72.13	75.04	73.58	61.04	63.41	62.22
Mutant 4	31.07	28.11	29.59	56.01	54.42	55.21	69.42	67.83	68.62	78.14	80.03	79.08	79.22	77.15	78.18	48.91	52.36	50.63
Mutant 5	31.07	29.49	30.28	60.08	58.57	59.32	63.07	61.69	62.38	83.34	86.19	84.76	81.19	83.04	82.11	64.42	65.03	64.72
Mean	27.77	26.53	27.15	44.63	43.91	44.26	53.45	54.67	54.06	62.73	60.69	63.83	66.57	67.24	66.90	51.30	51.79	51.54
LSD at 5%	1.88	1.83	1.85	2.11	2.00	2.05	1.95	2.00	1.31	1.57	1.61	1.59	1.65	1.73	1.69	2.05	1.96	2.00
LSD at 1%	2.88	2.79	2.83	3.22	3.06	3.14	2.97	3.05	3.01	2.40	2.46	2.43	2.52	2.64	2.58	3.13	3.00	3.06

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Table 5: Estimation of drought tolerance indices for the 6 rice accessions especially for grain yield/plant trait during the two growing season

Entries	2022 Season									2023 Season								
	GYP	**GYD**	**YSI**	**YI**	**MP**	**DTI**	**GMP**	**YR**	**DSI**	**GYP**	**GYD**	**YSI**	**YI**	**MP**	**DTI**	**GMP**	**YR**	**DSI**
Sakha 101	**29.34**	**26.12**	**0.89**	**0.58**	**27.73**	**0.29**	**27.68**	0.11	0.91	28.75	25.45	0.88	0.57	27.10	0.28	27.04	0.12	0.78
Mutant 1	**44.12**	**39.22**	**0.88**	**0.87**	**41.67**	**0.66**	**41.95**	0.12	0.56	46.03	38.54	0.83	0.87	42.28	0.68	42.11	0.17	0.29
Mutant 2	**48.32**	**41.19**	**0.85**	**0.92**	**44.75**	**0.76**	**44.61**	0.15	0.48	46.54	39.18	0.84	0.89	42.86	0.71	42.70	0.16	0.62
Mutant 3	**52.03**	**45.16**	**0.86**	**1.01**	**48.59**	**0.90**	**48.47**	0.14	0.51	54.18	47.32	0.87	1.07	50.75	0.99	50.63	0.13	0.48
Mutant 4	**63.14**	**56.01**	**0.88**	**1.25**	**59.57**	**1.36**	**59.46**	0.12	0.34	62.54	54.42	0.87	1.23	58.48	1.32	58.33	0.13	0.36
Mutant 5	**68.34**	**60.08**	**0.87**	**0.87**	**64.21**	**1.58**	**64.07**	0.13	0.69	66.44	58.57	0.88	1.33	62.50	1.51	62.38	0.12	0.43

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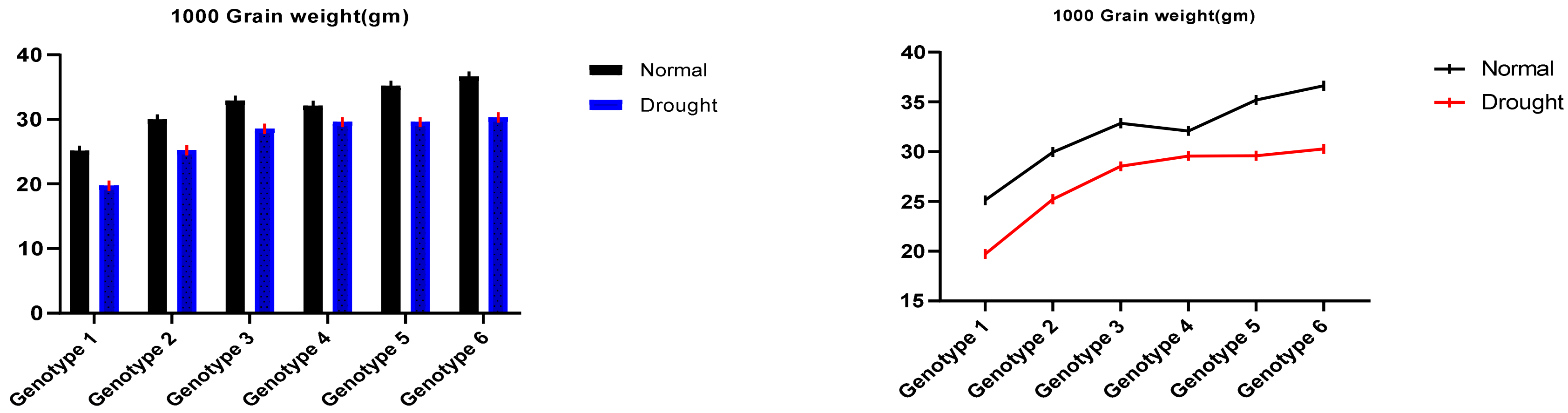

Fig. 1: Effect of drought stress on the six rice accessions for 1000-grain weight trait

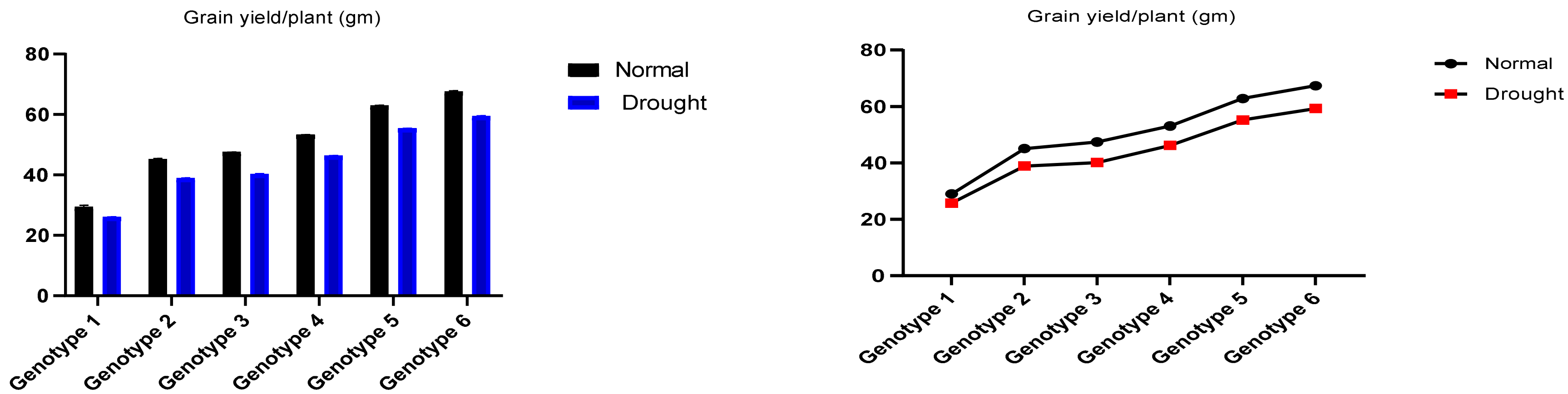

Fig. 2: Effect of drought stress on the six rice accessions for grain yield/plant trait

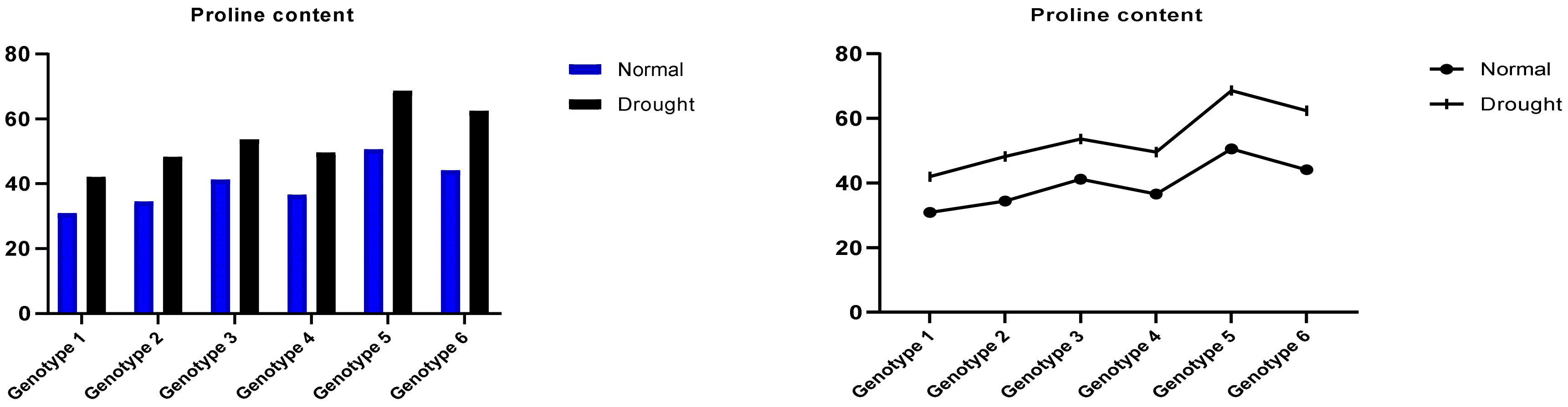

Fig. 3: Effect of drought stress on the six rice accessions for proline content trait

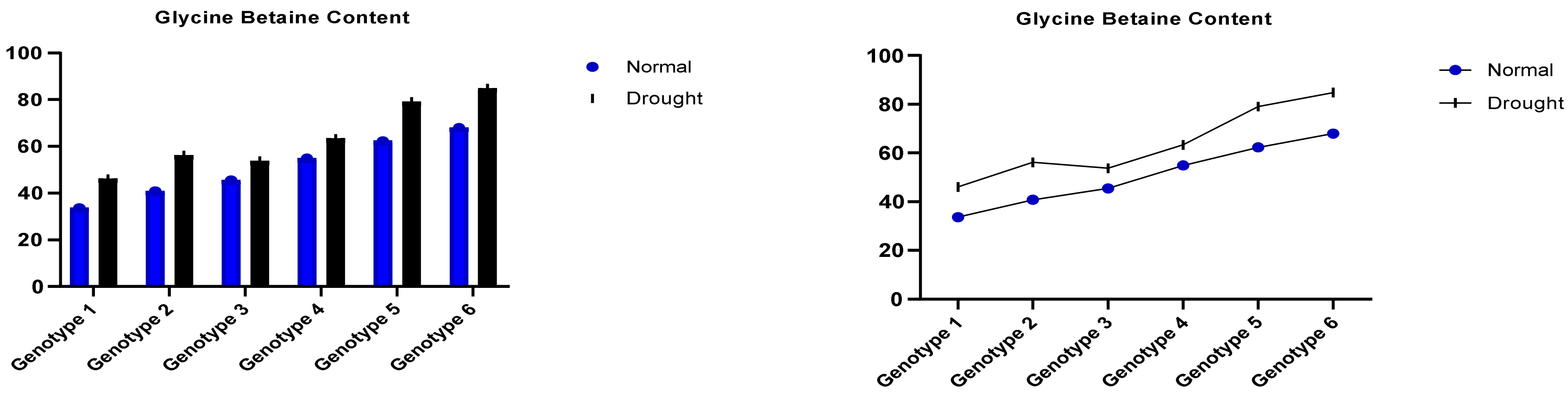

Fig. 4: Effect of drought stress on the six rice accessions for glycine betaine content trait

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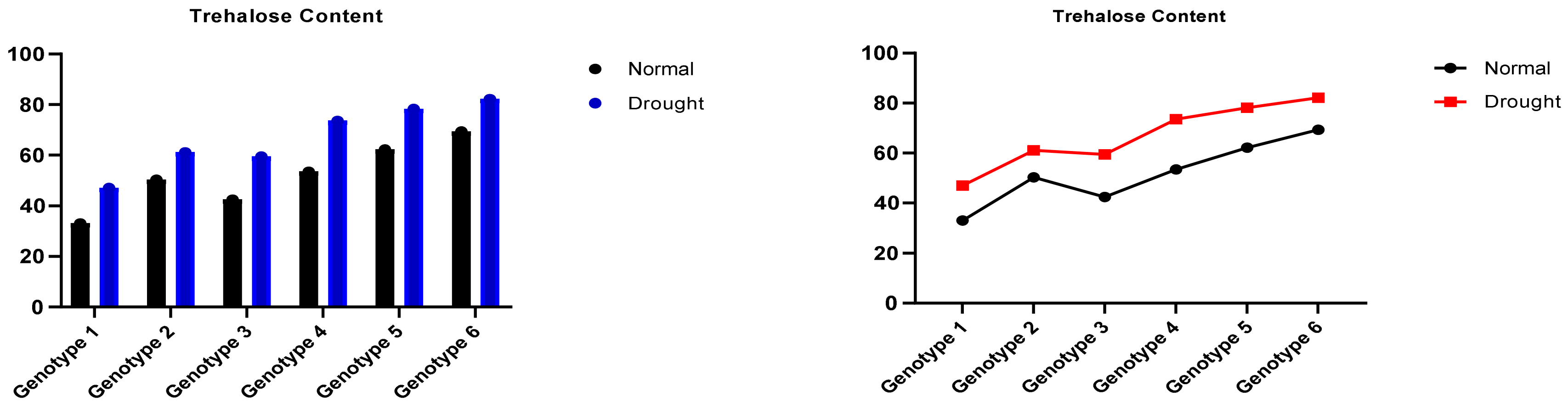


Fig. 5: Effect of drought stress on the six rice accessions for trehalose content trait

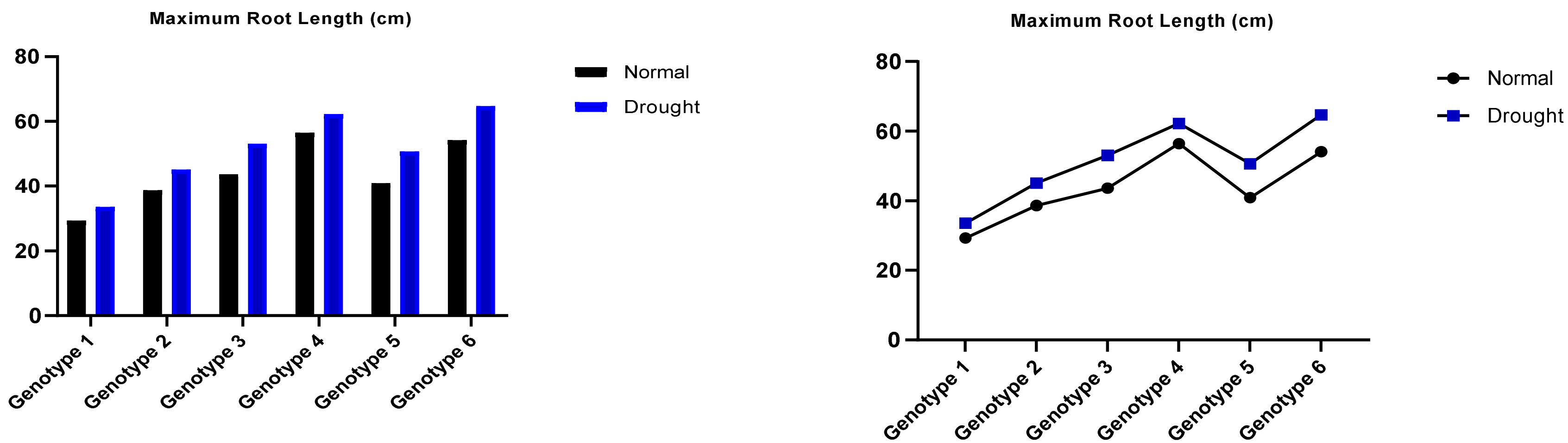
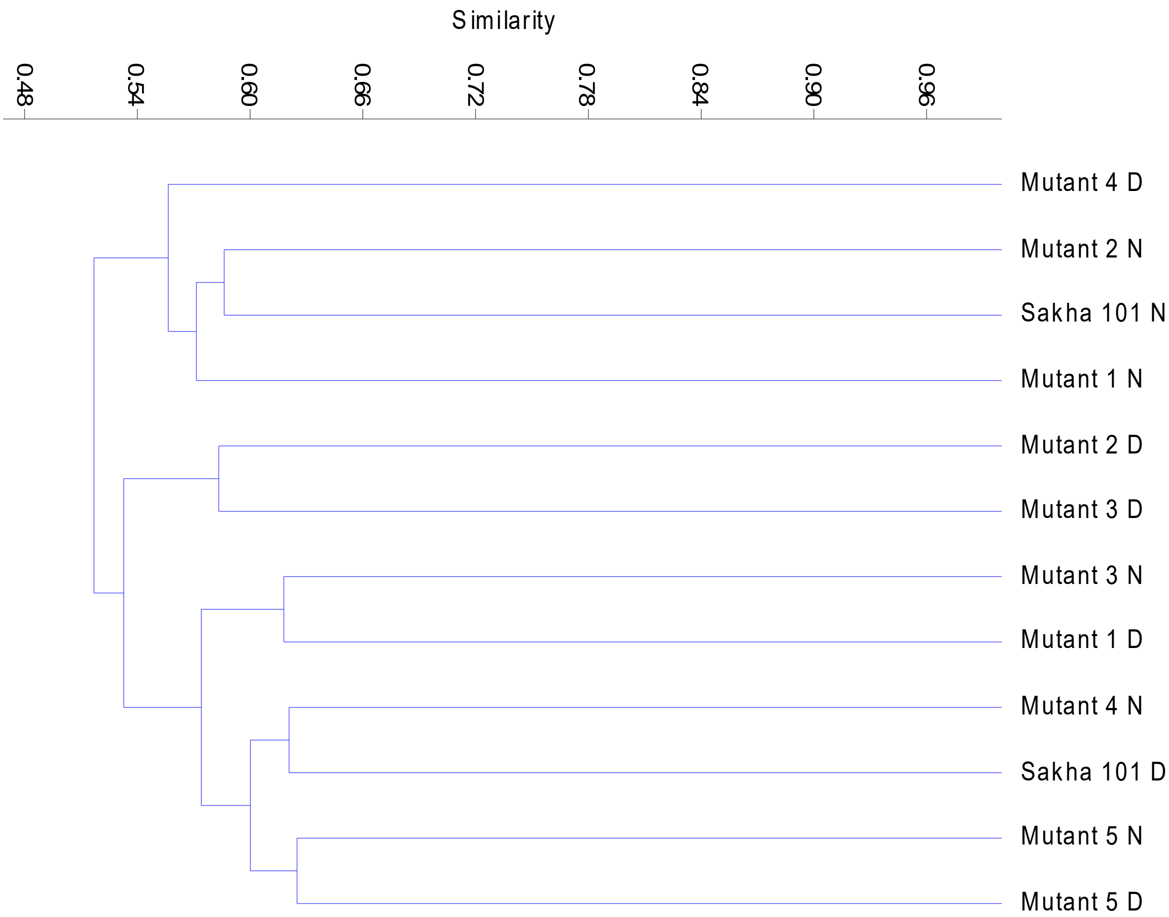


Fig. 6: Effect of drought stress on the six rice accessions for maximum root length content trait

Table 6: Similarity % of the six rice genotypes under normal and drought conditions

Similarity %	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
G1	1.0											
G2	0.571	1.0										
G3	0.586	0.571	1.0									
G4	0.559	0.478	0.576	1.0								
G5	0.555	0.505	0.505	0.597	1.0							
G6	0.573	0.540	0.521	0.563	0.577	1.0						
G7	0.527	0.477	0.494	**0.604**	**0.620**	**0.620**	1.0					
G8	0.556	0.439	0.522	**0.617**	0.494	0.561	0.586	1.0				
G9	0.574	0.408	0.489	0.582	0.527	0.432	0.468	0.545	1.0			
G10	0.472	0.376	0.440	**0.602**	0.528	0.511	0.569	0.477	0.583	1.0		
G11	0.555	0.558	0.555	0.547	0.526	0.494	0.549	0.527	0.544	0.563	1.0	
G12	0.620	0.483	0.549	0.574	**0.606**	**0.625**	0.595	**0.609**	0.590	0.556	0.537	1.0



UPGMA
Nei-Li's similarity coefficient

Fig. 7: Phylogenetic tree among six rice accessions under normal and drought conditions

4. Discussion

Traditional genetic improvement methods of strategic crops have been able to go a long way in the field of inferring novel entries resistant to environmental stresses let alone water stress. These mechanisms are centered on the capture and screening of plant lines carrying genes for resistance to environmental stresses during simple hybridization between native varieties sensitive to those stresses with wild accessions carrying resistance genes, (Eldessouky *et al.,* 2016; El-Demardash El-Mouhamady *et al.,* 2022). Then, the process of following the new hybrids through segregation generations which carried out to reach genetically stable, high-yielding and resistant to these stresses, especially water stress. However, modern scientific trends have used biotechnological programs, genetic engineering, and gene transfer as a novel scientific ways for genetic improvement of crops to counter environmental stresses. As those methods have shortened the time in extrapolating plant lines resistant to environmental stresses such as drought. Further, it has contributed with traditional methods in making successful accessions to produce of drought-resistant rice varieties while maintaining a largely satisfactory final output, (Zian *et al.,* 2013; He *et al.,* 2018; Wai *et al.,* 2020, El-Mouhamady & Ibrahim 2020; Naik *et al.,* 2023). One of the most prominent achievements of biotechnology in the field of genetic improvement of field crops, especially rice crop under Egyptian conditions for water stress tolerance, is the inference of short-lived, high-yielding and drought-tolerant varieties, especially what has been achieved in the last two decades. This technique succeeded in reducing the period of total maturity at the expense of floral growth, which pushed rice plants to a radical physiological change that consists of escaping from the period of drought extreme, (Ma *et al.,* 2024). Furthermore, the use of several safe doses of gamma rays played a significant role in the events of a series of physiological and biochemical changes in the five new rice mutants, which gained a higher water stress tolerance compared to the original variety (Sakha 101). This will discuss in some detail by explaining the side effects of gamma rays in the physiological and genetic behavior of the new drought-tolerant rice mutants across all studied traits under water stress conditions compared to the standard experiment. Results shown in tables (1 & 2) were able to clarify and confirm the extent of significant differences between the six rice accessions under investigation under the two treatments within the two growing seasons, which is a matter of great importance in designing and conducting experiments. Also, the five rice mutants were able to significantly and noticeably outperform the original rice cultivar (Sakha 101) from which they descended. These results confirm beyond any doubt that the basic approach to genetic statistical analysis has indeed been achieved based on significant differences between the six rice genotypes, (Banoth *et al.,* 2023; Ma *et al.,* 2024). Also, all results of statistical analysis within several replicates are considered evidence and a real test of the normal rate of success of significance tests for various plant accessions under different conditions, including water stress experiments, (Luo *et al.,* 2024). All of the above proves that breeding and improvement programs for field crops' tolerance to environmental stresses, especially water stress, are important and necessary at the same time, along with the typical statistical analysis to ensure their genetic and moral divergence, which confirms the success of the genetic improvement process. Before, starting to explain all results of morphological, physiological and biochemical measurements attributes responsible for water stress tolerance in rice, it is first necessary to clarify the fruitful role of traditional plant breeding and improvement programs for enhancing abiotic stress tolerance in crops. Further, this effective and fruitful role came in parallel with the use of gamma rays with the aim of inducing physiological and molecular genetic changes including the secretion of water stress tolerant genotypes, (Moustakas, 2025). Where, the biochemical and physiological evidence responsible for water stress tolerance in crops was able to create a clear path that served as a strong light that illuminated the way for molecular genetics to stand on those essential genes that carry those tolerance traits, (Gilad *et al.,* 2025). In the same track, the applications of biotechnology and molecular genetics have succeeded in capturing the genetic factors and alleles responsible for environmental stress tolerance from wild plant lineages and transferring them to susceptible local varieties and accessions, with the aim of shorting the time needed to elicitation lines which highly output, tolerance to abiotic stress besides, its high stable under various conditions, (Zhang *et al.,* 2025). Results shown in tables (3 & 4) proved the superiority of the five M8 rice mutants over the original variety (Sakha 101) derived from it in all estimated traits under water stress treatment compared to the standard experiment during the two growing seasons. Furthermore, the levels of superiority divided into two levels such that mutants number (3, 4 & 5) exhibited the first track while mutants' number (1 & 2) came in the second direction of superiority for all attributes under study of the two treatments within 2022 and 2023 seasons, Figures (1 to 6). Further, the five M8 rice mutants exhibited highly rank of the three physiological and biochemical attributes namely; proline, glycine betaine and trehalose contents compare to the original rice cultivar (Sakha 101) under drought conditions compared to the control treatment during the two growing seasons. These promising results of the five high-yielding, water-stress-tolerant rice mutants came as a logical consequence of the physiological, biochemical, and genetic changes induced by exposure to safe doses of gamma rays, (Zangani *et al.,* 2023; Hadipanah *et al.,* 2025). Also, safe doses of gamma rays were able to achieve a positive leap in the path of physiological improvement of water stress tolerance in the M8 five rice mutants by increasing the maximum depth of the root system, (Hadipanah *et al.,* 2025). Where, the process of genetic improvement of drought tolerance began through physiological changes and increasing the fertility rate represented by producing highly yielding and 1000-grain weight attributes under water stress conditions compared to the standard experiment of the novel five rice mutants compared to the original variety (Sakha 101) has confirmed this scientific fact during the two growing seasons, (Fatima *et al.,* 2024). In this context, the process of genetic improvement in rice for water deficit stress tolerance under Egyptian soil conditions has been successfully achieved through the events of molecular genetic changes in DNA of the local rice variety Sakha 101, which resulted in the production of five promising rice mutants. This succeeded was done especially after exposure to significant doses of gamma rays, which have also been labeled as safe for human health in the future, (Fatima *et al.,* 2024; Hadipanah *et al.,* 2025). Those positive results are in agreement with many findings of a large number of researchers in the field of genetic improvement in various crops for environmental stress tolerance using gamma rays. The same strategy used by (Esmail *et al.,* 2016) for breeding of drought tolerance in maize. Further, many studies for salt and drought stress tolerance in wheat and barley genotypes were performed using gamma rays like results observed by (Khatab *et al.,* 2021a and B). Also, for example, not limited (El-Mouhamady *et al.,* 2019; El-Mouhamady, 2023) have succeeded for enhancing drought and salinity tolerance in wheat and rice lines through using different doses of gamma rays. In the same track, safe doses of gamma rays besides, molecular markers techniques were the best ways for increasing water deficit stress tolerance in rice, (Banoth *et al.,* 2023). Therefore, biochemical attributes and molecular markers were used for identification the alleles responsible for salinity tolerance in barley accessions through using gamma rays, (El-Demardash *et al.,* 2017; El-Mouhamady and Tawfik, 2025) and so on. Data observed in table (5) and related to drought stress tolerance indices for grain yield/plant trait confirm beyond doubt the recent physiological changes enjoyed by the five novel rice mutants, which intermittently proved their water stress tolerance during the two growing seasons (2022 & 2023), (El-Seidy *et al.,* 2013; El-Mouhamady *et al.,* 2010 & 2013; Khatab *et al.,* 2017). Where, these new rice entries were able to reduce the final loss rate in yield grain to reasonable limits and did not affect the final output under the influence of water stress treatment compared to the standard experiment during the two growing seasons. Further, the values of (DSI) parameter were less than the unity in the five rice mutants compared with the original cultivar Sakha 101 which indicated that the highly rank of drought tolerance for these new rice materials. Thus, these novel rice genotypes are considering the largest evidence of the successful use for gamma rays in genetic improvement of water stress tolerance in strategic crops under Egyptian conditions, (Fatima *et al.,* 2024, Tawfik & El-Mouhamady 2024 A & B; and Hadipanah *et al.,* 2025). Results of the cluster tree analysis showed that there is very high concordance and similarity at the genetic level for a number of rice accessions in the current investigation under normal and water deficit conditions. Further, multiple and safe doses of gamma rays succeeded in adding new genes that were responsible for the acquisition of novel rice mutants with the ability to drought stress tolerance, as clearly shown through the phylogenetic tree in table (7) and Fig. 7, (Ramadan *et al.,* 2016; El-Mouhamady and Tawfik, 2025). Also, among the most important results obtained from genetic affinity degrees analysis for the local rice variety (Sakha 101) and the five novel rice mutants derived from it was the presence of great genetic homology of these rice mutants number (3, 4 & 5). In the same track, the previous three new rice mutants were confirmed highly genetic stable and drought tolerance under water stress treatment compared to the standard experiment. While, rice mutants number (1 & 2) ranked second in terms of genetic similarity and drought tolerance, (Heiba *et al., 2016* a&b; Khatab *et al.,* 2022; Fatima *et al.,* 2024 and Hadipanah *et al.,* 2025).

5. Conclusion

The current study focused on discussing the most important genetic, physiological and biochemical factors and mechanisms associated and causing water stress tolerance in some promising rice accessions. Also, this work demonstrated the vital and effective role of gamma rays in the genetic improvement of rice genotypes with the aim of increasing its drought tolerance besides, ultimately giving a high output. One of the most important results obtained is the inference of five novel rice mutants improved, water-stress tolerant and high-yielding from the local rice variety Sakha 101. It is worth mentioning that the ascertainment of water stress tolerance for these new rice mutants was not a mere coincidence but came after a number of yield, physiological and biochemical measurements under normal irrigation conditions compared to the drought experiment. Further, water stress tolerance indices of grain yield/plant trait was done for the five six rice accessions. From this point of view, it follows that the strategy of this investigation is to elicitation novel drought-tolerant, high-yielding and genetically stable rice mutants through using of safe doses of gamma rays to be used as local rice varieties containing all these advantages in the future.

Abbreviations

G1: Sakha 101 under normal Conditions, **G2:** Mutant 1 under normal conditions, **G3:** Mutant 2 under normal conditions, **G4:** Mutant 3 under normal conditions, **G5:** Mutant 4 under normal conditions, **G6:** Mutant 5 under normal conditions, **G7;** Sakha 101 under drought conditions, **G8;** Mutant 1 under drought conditions, **G9;** Mutant 2 under drought conditions, **G10;** Mutant 3 under drought conditions, **G11;** Mutant 4 under drought conditions & **G12:** Mutant 5 under drought conditions.

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