Middle East Journal of Agriculture Research
Volume: 13 | Issue: 03| July – Sept.| 2024

EISSN: 2706-7955 ISSN: 2077-4605
DOI: 10.36632/mejar/2024.13.3.35
Journal homepage: www.curresweb.com
Pages: 643-661

The Importance of Positive Influences for Gamma Rays in Creating of Novel Drought Stress-Tolerant Rice Mutants across ISSR Markers

Rasha Sabry Tawfik [1] and Almoatazbellah Ali El-Mouhamady [2]

[1]Department of Plant Biotechnology, Biotechnology Research Institute, National Research Centre, 33 El Buhouth ST, Postal code 12622, Dokki, Cairo, Egypt.
[2]Genetics and Cytology Department, Biotechnology Research Institute, National Research Centre, 33 El Buhouth ST, Postal code 12622, Dokki, Cairo, Egypt.

Received: 20 June 2024 **Accepted:** 25 August 2024 **Published:** 05 Sept. 2024

ABSTRACT

Given the extreme importance of the rice crop, it is considered a vital source of Egyptian food security, as it provides essential and vital food for the vast majority of Egyptians. Therefore, this study was launched as a serious attempt to genetically improve this strategic crop with the aim of increasing its tolerance to drought stress which has become a major obstacle to rice production under Egyptian conditions. A range of safe doses of gamma rays were used on Egyptian rice line (**GZ1368-S-5-4)** to develop new mutants characterized by tolerance to water stress and high yield. The final results were very promising in deriving new **5 M8 rice** that reached genetic stability in the eighth generation, as they were superior in yield, tolerant to drought stress and stable in various locations and environmental conditions. All data of yield components and physiological traits measurements indicating tolerance were significantly better under the drought experiment compared to the standard treatment. Further, the water stress tolerance indices test for grain yield/ plant trait showed the superiority of the five rice mutants over the Egyptian parent line mentioned above. **Eight ISSR primers** were used for analyzing the molecular genetic differences of the six rice lines including the parent line (GZ1368-S-5-4) which also gave highly polymorphism % reached 100.0% by SR-08, SR-12, SR-13 and SR-21 primers. Further, discovering 35 specific genetic markers for these five promising rice mutants, which will be an important measure for them until they reach and are approved as final rice lines with all the desired attributes mentioned above.

Keywords Rice, ISSR Primers, Gamma Rays, Mutations, Water Stress Tolerance Indices.

Introduction

The rice plant is considered one of the most important strategic food crops at the local and global levels, as it represents the basic and vital food for the vast majority of the world's population. Further, rice crop provides approximately 21% of the total energy of the human body and 15% of the total protein needed by the human body for one individual. Although, rice protein occupies a high rank in terms of nutritional quality compared to the rest of the other crops, the percentage of protein in it is still modest. Also, it is an important source of vitamins, minerals and fiber (Mohidem *et al.,* 2022). In the same context, rice is considered an important food as a very successful fodder for feeding animals and birds, especially from pressing rice straw, as well as converting and recycling it to produce paper. Further, the Arab Republic of Egypt is in the process of cultivating 770,000 feddans of rice, with an average productivity of 4.5 tons per feddan. Also, rice cultivation is spread in most coastal governorates such as Dakahlia, Gharbia, Sharqia, Beheira, and Kafr El-Sheikh (Al-Kordy *et al.,* 2019). Moreover, understanding the process of yield components attributes inheritance provides a greater opportunity for plant breeders to produce higher yielding and quality crops especially in rice regarding the stress tolerance to biotic and abiotic stresses under any conditions and this is what is known as transgenic or

Corresponding Author: Almoatazbellah Ali El-Mouhamady, Genetics and Cytology Department, Biotechnology Research Institute, National Research Centre, 33 El Buhouth ST, Postal code 12622, Dokki, Cairo, Egypt. Email: - elmouhamady@yahoo.com

modified rice crop (Zhao & Zhang 2007). Unfortunately, the Egyptian agricultural belt is among the areas that suffer from great water poverty, especially after the construction of the Grand Ethiopian Renaissance Dam, which has largely forced the Egyptian state to reduce the agricultural area, especially for the most water-hungry crops, such as rice. Accordingly, the need has become urgent for scientists and researchers to devise genetic structures and strains of rice that are more tolerant of water and salt stresses and have high genetic stability, especially under Egyptian conditions (El-Mouhamady *et al.,* 2022 A). One of the most serious damages caused by water stress is the high level of soil salinity due to the low moisture content of the soil due to the lack or absence of washing and cleaning the soil of accumulated salts, especially heavy elements (Heiba *et al.,* 2016 A). Also, water and salt stress cause great damage in all stages of plant growth, physiological, vegetative and biochemical, and also radically affect the expected final output, causing a loss of at least 50 to 60% of the final yield (El-Mouhamady *et al.,* 2019). After this quick review of the most important problems and risks caused by water stress, especially after the limited irrigation water, the most important logical scientific solutions can be summarized by scientists in order to get out of this great crisis. This will only happen by creating rice lines that are highly tolerant to water stress besides, their highly yielding and genetic stability in different environments and conditions during traditional plant breeding methods and the programs of biotechnology and genetic engineering (Melandri *et al.,* 2020). The following is a quick review of the results of the most important research and studies that dealt at some length with traditional plant breeding and modern scientific methods like genetic engineering and biotechnology for improving rice tolerance to water stress. Some rice accessions were various response for drought stress tolerance such as Sakha 103, IET1444 and IET1444 x Giza 178 were recorded highly rank of tolerance for all yield components attributes calculated under normal and stress conditions (El-Mouhamady 2009). Further, (Eldessouky *et al.,* 2016) revealed that some elites rice entries recorded highly level of drought tolerance when tested under the normal treatment compared to the stress experiment besides, determined some yield and its components attributes. The simple selection process in rice genotypes through drought tolerance indices was recorded by Singh *et al.* (2018) where stress susceptibility index, stress tolerance index and stress tolerance level will were identified of water stress-tolerance for rained ecosystem. In the same context, the research of many scientists dealt with the effect of water and salt stresses on changing soil chemistry, which had a bad effect on all morphological, physiological and yield characteristics of some wheat genotypes and the ideal solution to such a crisis was to use the method of breeding by mutations and to create modern high-yielding wheat mutants, through using of different doses of EMS (Heiba *et al.,* 2016 B). Further, (El-Demardash *et al.,* 2017) were able to improve the degree of water stress tolerance in some rice genotypes through using of successful doses of gamma rays. Also, this was clearly reflected in a clear and significant increase in yield and its components attributes under drought treatment compared to the standard experiment. The field of genetic improvement of rice crop through the use of gamma rays was a fertile and vital field, especially in the derivation of 7 rice mutants that are tolerant to water stress from the Egyptian rice variety Giza 178. Moreover, the mutations were high-yielding and were characterized by good physiological characteristics that enabled them to be drought tolerant by producing some organic acids to resist water stress such as proline, glycine betaine and trehalose contents besides, their superior ability to adjust their osmotic pressure so that they could maintain the optimal level of their life through osmotic adjustment, (El-Mouhamady 2023). The comparison was done between two recent rice mutants, the first of which was the ideal flag leaf stomatal mutation, while the second mutation was the mutation grown under water stress conditions. After conducting severe drought tests, three new rice mutants were selected that were filled with grains and high yield. Further, increasing the number of stomata and increasing the stomatal area, (Phunthong *et al.,* 2024). After all that has been presented at some length, it is possible to briefly summarize the aim of this paper, which is to improve the degree of water stress tolerance in rice crop through using different doses of gamma rays. Further, the evaluation of a number of important physiological and yield components traits in this regard. Also, identifying water stress tolerance indices in the promising rice accessions under study.

2. Materials & Methods

2.1. Materials used in this investigation

This work used (GZ1368-S-5-4 Rice Line) with pedigree of (IR1615-31/BG94-2349) which has excellent morphological and physiological traits that qualify it to be highly yielding and distinguished

EISSN: 2706-7955 ISSN: 2077-4605 DOI: 10.36632/mejar/2024.13.3.35

in other agro-morphological characters. As well, it's Biochemical and physiological traits that make it resistance to drought stress. Therefore, this variety is an excellent experimental material that can be used in this investigation.

2.2. Field evaluation

The rice seeds used in the recent investigation were originally performed from Rice research & Training Center, Agriculture Research Centre. Five hundred pure seeds of GZ1368-S-5-4 accessions 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 2010 season (M0). The irradiated materials of all doses were grown and series of selections among the mutant population under normal soil conditions in Sakha location in Kafr El-Sheikh Governorate and this process carried out during 2011-2018 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 conducted at (Sakha city) in Kafr El-Sheikh, Governorate, Egypt during 2021 and 2022 seasons using the original rice cultivar (GZ1368-S-5-4) 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 in 2021 and 2022 growing seasons. 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

Sixty plants were used for determined all attributes under study of each treatments (Normal and drought conditions) where 20 plants for each replicate. Each experiment included three replicated and the six rice entries were grown in a randomized complete block design in both growing seasons (2021 & 2022).

2.4.1. 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):- Osmotic adjustment.

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 & Grattan (1983).

2.5. Water Stress Tolerance Indices

All drought stress tolerance indices were estimated according to Fischer & Maurer (1978); Bouslama & Schapaugh (1984); Lin *et al.* (1986); Hossain *et al.* (1990); Fernandez (1992); Gavuzzi *et al.* (1997) & Golestani & Assad (1998).

2.6. 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).

2.7. Molecular Depiction

Total genomic DNA of all samples was extracted from green six rice leaves using Qiagen DNeasy Plant Minikit following the protocol of the manufacturer (Qiagen Inc, Valencia, CA, USA). The quality of the extracted DNA was assessed on agarose gel electrophoresis. PCR was performed using 8 preselected ISSR primers based on their ability to generate reproducible and informative amplification patterns. Amplification reactions were carried out in Biometra T One Thermal Cycler (Analytik Jena, Jena, Germany). PCR amplification was performed in 25 µl reaction mix which contained 20-30 ng DNA template, 10 pmol of each primer, 2.5 µl of 2 mM Thermo dNTPs, 5 µl of 5X Promega Green GoTaq Flexi Reaction Buffer, 2.5 µl of 25mM Promega MgCl2, and 0.125 µl of 5U/µl Promega GoTaq Flexi DNA polymerase. The reaction was assembled on ice, and amplification was performed at certain conditions as follows: an initial denaturing step at 94 °C for 5 min followed by 35 cycles at 94 °C for 30 s, annealing at 50 °C for 1 min, an extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. The PCR products were assessed on 1.6% agarose gel (Sambrook *et al.,* 1989; Zietkiewicz *et al.,* 1994 & Gezahegn *et al.,* 2010). The banding profile of ISSR were scored using the Labimage program, and the polymorphism percentage was estimated as follows Percent of polymorphism = (number of polymorphic bands/total number of bands) × 100, (Table 1).

Table 1: Band variation and polymorphism percentage in 6 rice lines using 8 ISSR primers.

Primer Code	Sequence(5'→3')	Abbrev.	Mer
SR-01	ACACACACACACACACC	(AC)8C	17
SR-04	ACACACACACACACACYA	(AC)8YA	18
SR-08	AGAGAGAGAGAGGG	(AG)6GG	14
SR-11	AGAGAGAGAGAGAGAGT	(AG)8T	17
SR-12	AGAGAGAGAGAGAGAGYA	(AG)8YA	18
SR-13	AGAGAGAGAGAGAGAGYC	(AG)8YC	18
SR-21	CACACACACACACACAT	(CA)8T	17
SR-40	GTGTGTGTGTGTGTGTYG	(GT)8YG	18

Data was scored for computer analysis on the basis of the presence or absence of the amplified products for each ISSR primer. Pairwise components of the six rice genotypes based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients according to Jaccard (1908). The similarity coefficients were then used to construct dendrograms, using the UN weighted pair group method with arithmetic averages (UPGMA) employing the SAHN (sequential, agglomerative, hierarchical, and nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics Program).
Abbreviations: - - T.B: Total bands, M.B: Monomorphic bands, P.B: Polymorphic bands, U.B or P.S.M: Unique bands or positive specific marker, P%: Polymorphism percentage and R.S (bp): Range size.

3. Results & Discussion

There is no doubt that traditional plant breeding methods succeeded alongside modern methods such as biotechnology, genetic engineering and genetic transfer technology in order to improve the productivity of field crops and raise the degree of their tolerance and resistance to environmental and biological stresses. Also, those scientific methods, such as breeding with mutations, were able to devise new accessions from the original local variety that had an overall superiority in bearing environmental stresses such as water stress besides, its high yielding compared to the original variety. In this context, thousands of papers were launched, which documented the success achieved thanks to breeding with mutations in eliciting of a large number of crops with high productivity and resistance to all stresses, whether biotic or abiotic and with superior quality attributes such as rice, wheat, maize, sorghum, fruit and vegetable crops such as onion (Kishk *et al.,* 2017). However, in this investigation, the most important scientific achievements that were achieved thanks to the use of different doses of gamma rays in the development of rice lines resistant to water stress and highly yielding from the local rice accession

GZ1368-S-5-4 were listed, with a detailed explanation of the physiological and biochemical responses related to tolerance and resistance in this regard.

3.1. Analysis of variance

Data shown in (Tables 2 & 3) associated with (ANOVA) test showed that highly significant differences were observed among all rice accessions (the original line GZ1368-S-5-4 and its five M8-derived mutants) for all attributes studied under normal and water stress conditions during the two growing seasons (2021 and 2022). Also, the percentages of coefficient of variance were ranged from low to medium for all attributes under study in both experiments for the two growing seasons. While, osmotic pressure trait was appeared very high under both conditions for the two seasons where the values were (210.11 & 340.79), respectively. This study succeeded in proving the fact that the analysis of variance or (ANOVA test) to be successful, so first must be significant or even highly significant differences between the varieties or all studied genotypes under normal and drought conditions in both growing seasons. Further, this is the main entrance to the statistical analysis to highlight the most important genetic related to the logical comparison among the promising rice accessions namely; Egyptian rice variety (GZ1368-S-5-4) and its five M8 mutants derived from it. Also, estimating of the coefficient of variation as the only statistical measure that directly expresses the phenotypic differences within the promising rice genotypes was very important and effective at the same time in clarifying and highlighting the genetic behavior of all rice entries used under water stress conditions compared to the standard experiment in the two growing seasons, (Tables 2 & 3). This confirmed beyond any doubt that the use of gamma rays had a strong impact in eliciting new rice mutants genetically different from the local line derived from it and were outperformed it in all yield, physiological and chemical attributes (El-Keredy *et al.,* 2003 a, b, c; Abdel Sattar and El-Mouhamady 2012; Tawfik & El-Mouhamady 2019; El-Mouhamady & Ibrahim 2020 & Mwando 2020).

3.2. Mean Performance

Results viewed in (Tables 4 and 5) succeeded in showing the real and actual comparison between the new six rice mutants and the original local variety or parent (GZ1368-S-5-4) which derived from it in terms of yield, physiological and biochemical traits related to water stress tolerance under the drought experiments compared to the normal treatment during the two growing seasons. Whereas, one of the most important and prominent results obtained in principle was that the six rice mutants formed by exposing the line GZ1368-S-5-4 to different doses of gamma rays had already outperformed the original variety in all the traits under study and showed a great resistance for water stress during the two growing seasons and this result is a major scientific leap in this regard. For example, the highest rice mutants confirmed highly rank of water stress tolerance compared to the original line (GZ1368-S-5-4) were the mutants number (4 & 5) for grain yield/plant where the mean values were (73.62 g & 72.98 g) and (81.05 g & 80.36 g) for the normal and drought conditions for the first growing season and were (58.74 g & 56.14 g) and (66.13 g & 65.83 g) for the two conditions of the two growing season, respectively. Further, the values of proline content were (62.08 & 61.55) and (66.04 & 64.49) under normal conditions and were (109.23 & 110.05) and (96.47 & 94.38) under water stress experiment for the two growing seasons, respectively. This investigation was able to discuss the genetic behavior, environmental and physiological responses related to water stress tolerance for a number of modern promising rice genotypes under stress conditions compared to the standard experiment, (Tables 4 & 5). This study came as a quick response to the use of different doses of gamma rays on the tolerant rice line (GZ1368-S-5-4) to elicit high-yielding rice mutants that are resistance to water stress under Egyptian conditions. Given that the Egyptian agricultural sector suffers greatly from the lack of water resources needed for agriculture, especially after the construction of the Ethiopian Renaissance Dam, which necessitated the development of Egyptian rice varieties and lines with high yields, drought tolerance and low water needs. Accordingly, genetic improvement to abiotic stress tolerance in different crops, especially water stress in rice, was achieved with unparalleled success through the use of different doses of gamma radiation, especially doses that are safe for human health, which proved its superior ability to devise mutations that are tolerant to water deficit conditions and have high yielding. Further, the strategy of this study came, especially that Egypt is located in the belt of scarce countries in terms of the water needs necessary for agriculture and drinking. Therefore, the development of new accessions of rice with high yielding and tolerant to water stress conditions while maintaining an acceptable level

Table 2: Analysis of variance for all studied traits of the six rice lines 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.	Osmotic adjustment.
Genotypes	5	2021	23.12**	17.55**	121.32**	76.03**	25.41**	10.04**
		2022	21.05**	12.04**	117.43**	63.02**	19.87**	8.79**
Replicates	2	2021	17.22**	123.08**	25.78**	156.74**	19.23**	27.84**
		2022	15.12**	110.05**	19.83**	63.22**	16.45**	22.05**
Error	10	2021	1.65	1.41	1.68	2.03	1.85	2.14
		2022	1.42	1.27	1.77	1.95	1.96	2.55
C.V. %		2021	3.89	1.96	2.36	4.25	3.54	189.98
		2022	3.59	1.89	2.45	4.20	3.78	210.11

Table 3: Analysis of variance for all studied traits of the six rice lines 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.	Osmotic adjustment.
Genotypes	5	2021	8.56**	23.05**	11.08**	123.67**	50.11**	18.32**
		2022	12.09**	9.88**	32.02**	117.89**	42.07**	14.05**
Replicates	2	2021	18.55**	14.32**	90.08**	52.09**	35.71**	10.06**
		2022	24.13**	26.19**	71.04**	46.71**	32.07**	17.34**
Error	10	2021	1.73	1.88	1.39	1.20	1.35	1.83
		2022	1.38	1.35	1.62	1.08	1.72	1.59
C.V. %		2021	4.64	2.98	1.43	1.51	1.74	322.08
		2022	4.29	2.61	1.56	1.42	1.99	340.79

Table 4: Mean performance and combined analysis for all studied traits of the six rice lines under normal 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	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**	**2021**	**2022**	**Mean**
GZ1368-S-5-4	28.16	27.05	27.60	38.12	36.97	37.54	39.44	38.94	39.19	22.13	21.78	21.95	17.56	17.03	17.29	0.87	0.85	0.86
Mutant 1	32.31	30.07	31.19	55.80	54.61	55.20	55.13	54.32	54.72	28.44	27.59	28.01	39.76	38.42	39.09	0.73	0.72	0.72
Mutant 2	35.19	34.22	34.70	61.55	60.49	61.02	**62.08**	**61.55**	61.81	31.06	30.05	30.55	42.12	41.08	41.60	0.96	0.94	0.95
Mutant 3	36.02	37.01	36.51	52.83	52.06	52.44	59.56	60.86	60.21	25.43	24.78	25.10	51.84	50.07	50.95	0.71	0.65	0.68
Mutant 4	34.06	33.79	33.92	**73.62**	**72.98**	73.30	46.12	45.55	45.83	41.55	42.08	41.81	33.83	32.11	32.97	0.83	0.81	0.82
Mutant 5	37.04	36.87	36.95	**81.05**	**80.36**	80.70	**66.04**	**64.49**	65.26	52.39	53.01	52.70	44.96	43.26	44.11	0.57	0.59	0.58
Mean	33.79	33.16	33.47	60.49	59.57	60.03	54.72	54.28	54.50	33.50	33.21	33.35	38.34	36.99	37.66	0.77	0.76	0.76
LSD at 5%	1.90	1.71	1.80	1.75	1.66	1.70	1.91	1.96	1.93	2.10	2.06	2.08	2.01	2.07	2.04	2.16	2.36	2.26
LSD at 1%	2.89	2.68	2.78	2.67	2.54	2.60	2.92	3.0	2.96	3.21	3.15	3.18	3.06	3.15	3.10	3.30	3.60	3.45

Table 5: Mean Performance and Combined Analysis for All Studied Traits of the six rice lines 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.			Osmotic adjustment.		
	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean	2021	2022	Mean
GZ1368-S-5-4	22.15	21.03	21.59	29.56	28.32	28.94	57.14	56.55	56.84	54.13	55.07	54.60	28.78	27.94	28.36	0.62	0.59	0.60
Mutant 1	25.11	23.72	24.41	39.44	37.05	38.24	83.39	82.13	82.76	67.32	68.04	67.68	58.43	57.21	57.82	0.55	0.52	0.53
Mutant 2	28.13	27.03	27.58	44.15	42.70	43.42	**109.23**	**110.05**	109.64	74.13	73.88	74.0	67.32	66.45	66.88	0.48	0.44	0.46
Mutant 3	33.01	32.98	32.99	37.87	36.59	37.23	75.58	74.62	75.10	49.52	51.03	50.27	73.42	72.94	73.18	0.28	0.23	0.25
Mutant 4	29.11	27.98	28.54	**58.74**	**56.14**	57.44	69.47	71.03	70.25	86.24	87.94	87.09	80.04	78.45	79.24	0.39	0.32	0.35
Mutant 5	32.56	31.44	32.0	**66.13**	**65.83**	65.98	**96.47**	**94.38**	95.42	103.43	102.79	103.11	92.01	90.87	91.44	0.21	0.17	0.19
Mean	28.34	27.36	27.85	45.98	44.43	45.20	81.88	81.46	81.66	72.46	73.12	72.79	66.66	65.64	66.15	0.42	0.37	0.39
LSD at 5%	1.94	1.73	1.83	2.02	1.71	1.86	1.74	1.88	1.81	1.62	1.53	1.57	1.71	1.94	1.82	2.0	1.86	1.93
LSD at 1%	2.96	2.65	2.80	3.09	2.62	2.85	2.66	2.87	2.76	2.47	2.34	2.40	2.62	2.95	2.78	3.05	2.84	2.94

of quality characteristics has become a major challenge for Egyptian scientists, especially after the near completion from the construction of the Grand Ethiopian Renaissance Dam (El-Mouhamady and Habouh 2019). Further, Among the most prominent and important results obtained is that the new five rice mutants were actually able to achieve a significant superiority in grain yield/plant and 1000-grain weight traits, as their values were higher than the local variety (GZ1368-S-5-4) under water deficit conditions during both growing seasons. Thus, this leads to a significant shift in the field of physiological and genetic changes that caused the state of endurance and resistance to drought stress in the five new rice accessions compared to the original line which derived from it. These promising changes came as a natural reaction to exposure to gamma radiation doses, which proved beyond any doubt the importance of breeding by mutations in creating improved mutations to resist water stress in rice (El-Mouhamady *et al.,* 2016). These results were consistent with those obtained by (El-Mouhamady 2003) when studied and evaluated the physiological and genetic changes that led to an increase in the frequency of salt stress tolerance in some rice genotypes. At the same pace, it is noted that the new promising five rice mutants had also achieved a significant superiority in resistance to water stress by producing large rates of compounds and organic acids responsible for increasing the degree of water stress tolerance, such as proline, glycine betaine, and trehalose contents compared to the original variety descended from it during both growing seasons (Esmail *et al.,* 2016; Ramadan *et al.,* 2016; Darwish *et al.,* 2017; Khatab *et al.* 2017; El-Mouhamady *et al.,* 2019; Khatab *et al.,* 2019; Yassin *et al.,* 2019). These new and promising rice genotypes were also able to achieve a significant physiological change by modifying the osmotic pressure pattern and reducing its rate so that these mutants could maintain the amount of water required for growing and producing besides, reducing its losing rate in what is known as modified osmotic adjustment. These physiological changes are a direct reflection of the genetic improvement of those rice genotypes after exposure to successful doses of gamma radiation (Al-Ashkar *et al.,* 2019; El-Mouhamady and El-Metwally 2020; Ahmed *et al.,* 2022; Duvnjak *et al.,* 2023).

3.3. Drought Stress Tolerance Indices

Results obtained in table (6) revealed that the values of **(YSI & YR)** were lower than the unity for all rice accessions in both years and the same results were observed for **(YI)** parameter except the rice mutant (4 & 5) were showed data higher than the unity in both growing seasons. Also, the five rice mutants were recorded mean values of **(MP & GMP)** higher than the original cultivar (GZ1368-S-5-4) in both growing seasons. Further, the values of **(DTI & DSI)** were showed lower than the unity in the new five rice accessions in both growing seasons except the mutants number (4 & 5) were it recorded mean values higher than one for **(DTI)** parameter through the two years.

Moreover, the results listed in (Table 6) show another evidence of water stress tolerance in the new promising five rice mutants compared to the original variety (GZ1368-S-5-4) when evaluating a number of drought stress tolerance indices during both growing seasons. Where these genotypes, especially mutants number (1, 2 & 3) were able to reduce the rate of yield loss **(YR)** under water stress conditions compared to the standard experiment. Also, these new rice materials were able to reduce the values of **(DTI & DSI)** compared to the original variety during the two growing seasons. This proves its superiority in water stress resistance and that it will be rice varieties classified with highly yielding and highly rank of water stress tolerance in this regard in the future and this is a big goal in this investigation (Khatab *et al.,* 2021 a & b & 2022; El-Mouhamady *et al.,* 2022; Nadeem *et al.,* 2023 & Ndikuryayo *et al.,* 2023).

3.4. Molecular Characterization

Molecular genetic markers has achieved unprecedented success in improving the productivity of field crops, especially rice crop. Also, rice productivity has now exceeded 4.5 tons/ acre and sometimes reaches to 5.0 tons/acre. Further, this success comes as a result of the continuous efforts of plant breeders in breeding and acclimatization to environmental stresses tolerance especially water stress, which has become a major obstacle to crop productivity, (Khatab *et al.,* 2019). Where, molecular markers has been able to bring about a major revolution in the field of genetic transfer, especially for genes related to drought tolerance besides, genes related to disease resistance. This success resulted in the production of highly productive, earlier rice varieties and lines that are tolerant to water and salt stress, especially after using of mutagenic factors that cause positively physiological, biochemical and

Table 6: Estimation of water stress tolerance indices for the 6 rice entries especially for grain yield/plant trait during the two growing season.

Entries	2021 Season									2022 Season								
	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI
GZ1368-S-5-4	38.12	29.56	0.77	0.64	33.84	0.30	33.56	0.23	3.20	36.97	28.32	0.76	0.63	32.64	0.29	32.35	0.24	3.04
Mutant 1	55.80	39.44	0.70	0.85	47.62	0.60	46.91	0.30	**0.91**	54.61	37.05	0.67	0.83	45.83	0.57	44.98	0.33	**0.68**
Mutant 2	61.55	44.15	0.71	0.96	52.85	0.74	52.12	0.29	**0.95**	60.49	42.70	0.70	0.96	51.59	0.72	50.82	0.30	**0.80**
Mutant 3	52.83	37.87	0.71	0.82	45.35	0.54	44.72	0.29	**0.95**	52.06	36.59	0.70	0.82	44.32	0.53	43.64	0.30	**0.80**
Mutant 4	73.62	58.74	0.79	1.27	66.18	1.18	65.76	0.21	**0.29**	72.98	56.14	0.76	1.26	64.56	1.15	64.0	0.24	**0.04**
Mutant 5	81.05	66.13	0.81	1.43	73.59	1.46	73.21	0.19	**0.37**	80.36	65.83	0.81	1.48	73.09	1.49	72.73	0.19	**0.24**

genetic changes that improve the rice crop, such as safe doses of gamma rays, (El-Mouhamady *et al.,* 2014 a & b & 2023). Also, molecular markers were exhibited highly rank for determining genetic diversity in various crops such as in rice, (El-Mouhamady *et al.,* 2012) and in canola, (El-Mouhamady *et al.,* 2013). In the same context, molecular genetic markers has proven to be the most important component, which, if integrated with traditional breeding will lead to significant progress and prosperity for field crops, helping to bring about a major qualitative shift in productivity and resistance to biotic and abiotic stresses alike. Also, molecular markers like ISSR primers were able to identify genetic mechanisms at the molecular level responsible for tolerating environmental stresses such as water stress. These specific markers are considered basic and also classification indicators in determining the tolerant lines in those that are moderate and sensitive to this dangerous environmental factor. Therefore, the actual and vital role of ISSR markers in determining the rice genotypes that are tolerant to water stress will be reviewed in some detail, especially after using safe doses of gamma rays, (Nasser *et al.,* 2024). Results shown in table (7 & Fig. 1) revealed that the eight ISSR primers namely; SR-01, 4, 8, 11, 12, 13, 21 & 40 exhibited 155 amplified fragments. Where, six of them were monomorphic besides, 149 polymorphic bands with 96.12% polymorphism. The polymorphic fragments included 35 unique markers and the range sizes were ranged from 146 bp to 4874 bp, respectively. Also, the highest number of polymorphic bands were observed in SR-01, 04, 12 & 40 primers and the values were (21, 21, 20 & 22), respectively. While, the SR-01 & 40 primers only were recorded highly rank of unique markers (8 & 7). Further, the highest number of polymorphism % were obtained in primers; SR-08, 12, 13 & 21 where the values were (100.0%) for each one of them. Also, the other ISSR primers generated high values of polymorphism % in this regard which indicated the importance role of these primers. These results demonstrated the actual importance of ISSR markers in determining all rice genotypes that are tolerant to water stress compared to those that are moderate or sensitive. Also, these promising primers were able to identify tolerant rice genotypes that were the main cause of the transmission of drought tolerance genes in rice mutants This vital role of molecular genetic markers is not limited to the genetic improvement of rice to water stress tolerance, but also extends to include resistance to various diseases that hinder the path to achieving high productivity in crops. For example, RAPD primers have been used in the genetic improvement of disease resistance in lupine for *(Fusarium oxysporum F. sp Lupini)* according to Zian *et al.* (2013) and Fungal Pathogenic Toxin Filtrate Tolerance in Potato, (Khatab & El-Mouhamady 2022). Further, ISSR primers have produced 35 unique markers, which are considered as guiding tools for identifying promising, high-yielding and water-stress-tolerant rice mutant at the same time, (Nasser *et al.,* 2024).

Table 7: Band variation and polymorphism % in the six rice lines using 8 ISSR markers

No.	ISSR primers	T.B	M.B	P.B	U.B	P %	R. S. (BP)	Sequence
1	**SR-01**	**22**	1	**21**	8	95.45%	4383-175	ACACACACACACACACC
2	**SR-04**	**22**	1	**21**	4	95.45%	4711-280	ACACACACACACACACYA
3	**SR-08**	16	0	16	3	**100.0%**	4580-161	AGAGAGAGAGAGGG
4	**SR-11**	15	1	14	3	93.33%	3985-242	AGAGAGAGAGAGAGAGT
5	**SR-12**	**20**	0	**20**	3	**100.0%**	4856-213	AGAGAGAGAGAGAGAGYA
6	**SR-13**	19	0	19	4	**100.0%**	4597-148	AGAGAGAGAGAGAGAGYC
7	**SR-21**	16	0	16	3	**100.0%**	4668-257	CACACACACACACACAT
8	**SR-40**	**25**	3	**22**	7	88.0%	4874-146	GTGTGTGTGTGTGTGTYG
Total		155	6	149	35	96.12%	4874-146	

Data of unique markers were shown in table (8) recorded 35 specific amplified fragments consisted to 17 positive and 18 negative markers. These special genetic markers have been able to be a distinctive guide for all rice mutants with high tolerance to drought. Further, being molecular tools for classification of these distinctive promising entries to follow up their tracking in the segregation generations to reach high genetic stable and produce new high-yielding rice lines that are tolerant to water stress.

Table 8: Mapping of unique markers in six rice lines using the eight ISSR primers.

ISSR Primers	MS(bp)	L1	L2	L3	L4	L5	L6	(P or N) Marker
SR-01	803	-	+	+	+	+	+	Negative (L1)
	715	-	+	+	+	+	+	Negative (L1)
	650	-	+	+	+	+	+	Negative (L1)
	510	-	-	-	-	+	-	Positive (L5)
	474	+	+	+	+	-	+	Negative (L5)
	232	+	+	+	+	-	+	Negative (L5)
	219	+	+	+	+	-	+	Negative (L5)
	175	-	-	-	-	+	-	Positive (L5)
	599	-	-	-	-	+	-	Positive (L5)
SR-04	473	-	+	+	+	+	+	Negative (L1)
	392	+	-	-	-	-	-	Positive (L1)
	343	+	+	+	+	+	-	Negative (L6)
SR-08	890	-	-	-	-	+	-	Positive (L5)
	796	-	-	-	-	+	-	Positive (L5)
	516	-	-	-	-	+	-	Positive (L5)
SR-11	613	+	+	+	+	-	+	Negative (L5)
	365	+	+	+	+	+	-	Negative (L6)
	299	+	+	+	+	-	+	Negative (L5)
SR-12	297	-	+	+	+	+	+	Negative (L1)
	263	-	+	+	+	+	+	Negative (L1)
	213	-	-	-	-	+	-	Positive (L5)
SR-13	1041	-	-	-	-	+	-	Positive (L5)
	954	-	-	-	-	+	-	Positive (L5)
	394	-	-	-	-	+	-	Positive (L5)
	284	+	+	+	-	+	+	Negative (L4)
SR-21	1002	+	+	+	+	-	+	Negative (L5)
	707	-	+	+	+	+	+	Negative (L1)
	534	-	-	-	+	-	-	Positive (L4)
	1092	-	-	-	-	+	-	Positive (L5)
	937	-	-	-	-	+	-	Positive (L5)
SR-40	646	+	+	+	+	-	+	Negative (L5)
	539	+	+	+	+	+	-	Negative (L6)
	495	-	-	-	-	+	-	Positive (L5)
	376	-	-	-	-	+	-	Positive (L5)
	146	-	-	-	-	+	-	Positive (L5)
Total								17 (Positive) + 18 (Negative)

P: Positive, N: Negative, MS: Molecular Size, L1: Egyptian line (GZ1368-S-5-4), L2:- Mutant 1, L3:- Mutant 2, L4:- Mutant 3, L5:- Mutant 4 & L6:- Mutant 5, respectively.

The following is a detailed presentation of the most important unique markers and their locations in the different rice entries. **SR-01 primer,** generated 6 negative specific markers where three of them were observed in line 1 with molecular sizes of (650bp, 715bp & 803bp) and the other three negative

Middle East J. Agric. Res., 13(3): 643-661, 2024
EISSN: 2706-7955 ISSN: 2077-4605 *DOI: 10.36632/mejar/2024.13.3.35*

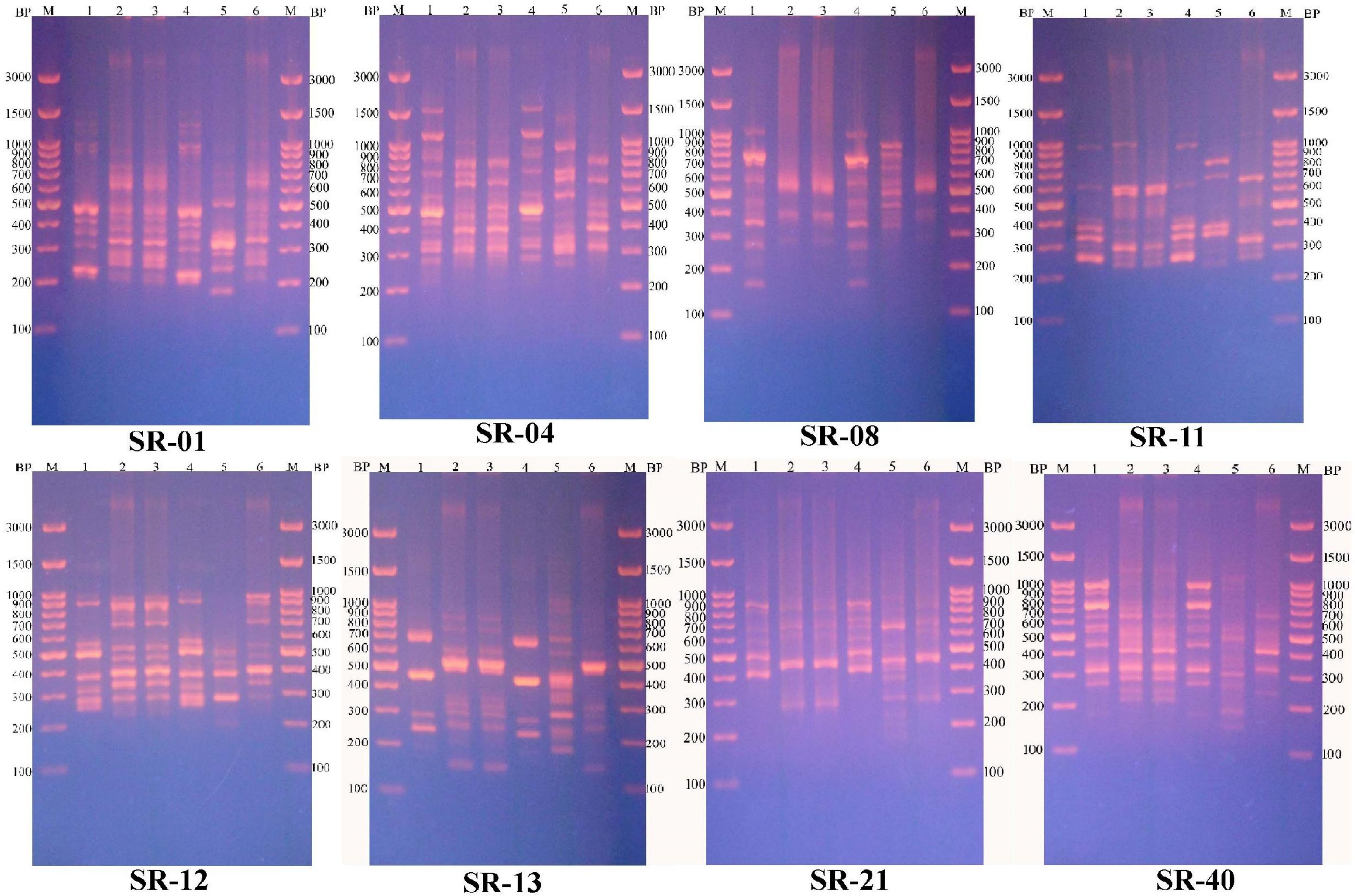

Fig. 1: ISSR primers used in six rice lines namely; SR-1, 4, 8, 11, 12, 13, 21 and 40.

markers were shown in line 5 with sizes of (219bp, 232bp & 474bp), respectively. While, the two positive markers were obtained in line 5 with sizes of (175bp & 510bp).

Also, **SR-04 primer**, recorded four specific markers where two positive markers were showed in lines (1 & 5) with sizes of 392bp and 599bp and two negative unique bands were generated in lines (1 & 6) with sizes of 473bp and 343bp, respectively. Further, **SR-08 primer** was exhibited three positive specific markers in line 5 with sizes of (516bp, 796bp & 890bp), respectively. In the same track, three negative markers were obtained by **SR-11 primer** where two negative markers were in line 5 with sizes 299bp and 613bp and one negative in line 6 with size of 365bp, respectively. Two negative markers at sizes of 263 bp & 297 bp for line 1 besides, one positive marker at size of 213 bp for line 5 were generated by **SR-12 primer**, respectively. While **SR-13 primer**, exhibited three positive specific markers in line 5 at sizes of 394bp, 954 bp & 1041 bp besides, one negative marker at size of 284 bp for line 4 only. Also, **SR-21 primer** was revealed three unique bands two of them were negative for lines (1 & 5) at sizes of 707 bp & 1002 bp and the third marker was positive at size of 534 bp in line 4. Further, SR-40 primer recorded five positive specific markers in line 5 at molecular sizes of (146 bp, 376 bp, 495 bp, 937 bp & 1092 bp) and two negative markers in lines (5 & 6) at sizes of 646 bp & 539 bp, respectively. Thus, it can be said that genetic markers of the ISSR primers type played an effective role in producing genetic evidence at the molecular level that was the real means of identifying new promising rice mutants with high drought tolerance and high yield as well. Further, these five superior mutants reached a high degree of genetic stability under different environments, which qualifies these rice entries to be future rice lines with all attributes desired by plant breeders, (Reddy *et al.,* 2002; Ahmed *et al.,* 2012; El-Badan *et al.,* 2022; Abo-Youssef *et al.,* 2023; Ghazy *et al.,* 2024 & Nasser *et al.,* 2024).

Data obtained in table (9) generated 15 relationships among six rice lines detected about similarity. The values were ranged from 0. 175 to 0.943 with an average of 0.559. Where, the highest rank of similarity was (0.943) within (L2 & L3) and the lowest level was (0.175) among (L1 & L3). Also, the other high similarity values were showed between (L2 & L6) (0.541), (L3 & L6) (0.552) & (L1 & L4) (0.776). Results of cluster analysis in (Fig, 2) revealed the relationships of six rice lines (The original Egyptian line besides, its 5 M8 mutants) included two main cluster where the first one were (L5) and one sub-cluster (L1 & L4). While, the second cluster had (L6) and one sub-cluster (L2 & L3). It is worth mentioning that the results of genetic convergence and the cluster tree shown in this work have proven beyond doubt the high degree of genetic kinship between the five new and promising rice mutants among themselves and between them and the parent line derived from it due to the safe and positive doses of gamma rays. Further, planting these 5 mutants together will be successful due to their very clear degree of convergence and genetic compatibility. Also, this confirms its high genetic stability under different environments, which is close to 100.0%, (Reddy *et al.,* 2002; Ahmed *et al.,* 2012; El-Badan *et al.,* 2022; Abo-Youssef *et al.,* 2023; Ghazy *et al.,* 2024 & Nasser *et al.,* 2024).

Genetic Similarity

Table 9: Similarity % of the six rice lines within using 8 ISSR primers.

Similarity	L1	L2	L3	L4	L5	L6
L1	1.0					
L2	0.176	1.0				
L3	0.175	**0.943**	1.0			
L4	**0.776**	0.216	0.214	1.0		
L5	0.278	0.314	0.322	0.293	1.0	
L6	0.182	**0.541**	**0.552**	0.241	0.216	1.0

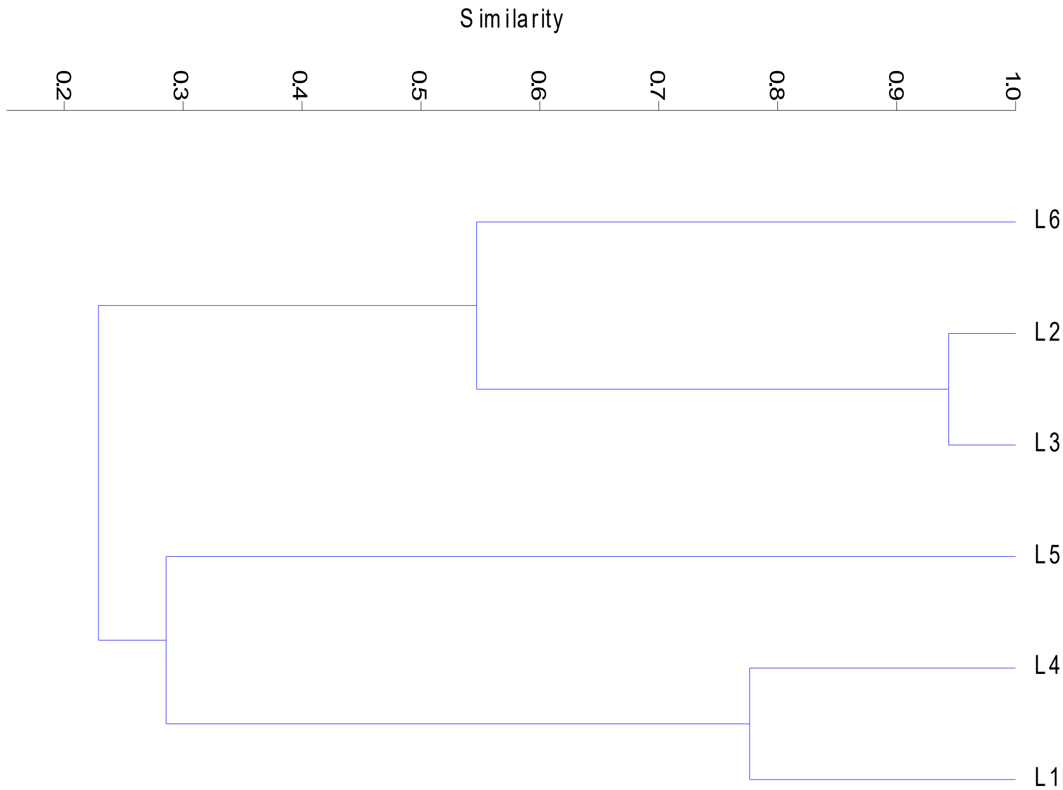

Fig. 2: Dendrogram analysis among the six rice lines across ISSR markers

4. Conclusion

The mutation breeding technique has succeeded in making a significant moral shift in the Egyptian rice line GZ1368-S-5-4 using a group of safe doses of gamma rays. This genetic improvement has resulted in developing of **5 M8 of rice crop** that are highly tolerant to water stress and highly output besides, their genetic stable under various environmental conditions, which qualifies these genotypes to be promising rice lines in the future. Also, the current investigation underwent a set of yield components and physiological tests such as water stress tolerance indices to confirm the degree of acclimatization and genetic improvement that occurred in these five new rice mutants. Further, these physiological tests proved that the safe doses of gamma rays used had indeed succeeded in raising the degree of water stress tolerance in the aforementioned new rice mutants and had no negative impact on the rest of the quantitative traits, but rather brought about a qualitative shift in all of them, most notably the increase in the final yield. ISSR markers demonstrated the presence of genetic differences at the molecular level, which shows the difference between the new five rice mutants and the Egyptian line from which they were derived, through generating of 35 unique bands that are considered a classification and identification tool for those five novel lines, and this fact is a strategic goal in this work.

References

Abdel Sattar, A.A. and A.A. El-Mouhamady, 2012. Genetic analysis and molecular markers for yield and its components traits in faba bean (*Vicia Faba* L.). Aus J of Basic & Applied Sci 6:458–466.

Abo-Youssef, M.I., M. Elbagory, A.B. Elsehely *et al.,* 2023. Biochemical, Anatomical, Genetic, and Yield Assessment of Seven Rice Genotypes (*Oryza sativa* L.) Subjected to Drought Stress. Agronomy*,* 13: 2542. https://doi.org/10.3390/agronomy13102542.

Ahmed, H.G.M.D., Y. Zeng, A.N. Shah, M.M. Yar, A. Ullah and M. Ali, 2022. Conferring of drought tolerance in wheat (*Triticum aestivum* L.) genotypes using seedling indices. Front. Plant Sci., 13: 961049. [CrossRef]

Ahmed, T.A., S.A. Alsamaraee, H.Z. Zaidan and K. Elmeer, 2012. Inter-simple sequence repeat (ISSR) analysis of somaclonal variation in date palm plantlets regenerated from callus. *I.* Proc. of Chem, Biol.l & Envi. Engi., 35: 126-130.

Al-Ashkar, I., A. Alderfasi, S. El-Hendawy, N. Al-Suhaibani, S. El-Kafafi and M.F. Seleiman, (2019. Detecting salt tolerance in doubled haploid wheat lines. Agronomy 9: 1-24.

Al-Kordy, M.A, H.M. Ibrahim, A.A. El-Mouhamady and H.M. Abdel-Rahman, 2019. Genetic stability analysis and molecular depiction in elite entries of rice (*Oryza Sativa* L.). Bull Nat Res Cen., 43:1–15

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant Soil., 39: 205–207.

Bouslama, M. and W.T. Schapaugh, 1984. Stress tolerance in soybean. Part 1: evaluation of three screening techniques for heat and drought tolerance. Crop Sci., 24: 933-937.

Chinard, F.P., 1952. Photometric Estimation of Proline and Ornithine. J. Biol Chem., 1: 91-5.

Darwish, M.A.H., W.M. Fares and H.M.A. Eman, 2017. Evaluation of some bread wheat genotypes under saline soil conditions using tolerance indices and multivariate analysis. J Plant Prod Mansoura Univ., 8:1383–1394.

Duvnjak. J., A. Lonˇcari´c, L. Brkljaˇci´c, D. Šamec, H. Šarˇcevi´c, B. Salopek-Sondi and V. Špani´c, 2023. Morpho-Physiological and Hormonal Response of Winter Wheat Varieties to Drought Stress at Stem Elongation and Anthesis Stages. Plants.,12: 418. https://doi.org/10.3390/ plants12030418

El- Demardash I.S., A.A. El-Mouhamady, H.M. Abdel- Rahman, T.A. Elewa and K. A. Aboud, 2017. Using Gamma Rays for Improving Water Deficit Tolerance in Rice. Curr. Sci. Int., 6: 321-327.

El-Badan, G., A. Amin, F. Ashour and L. El Sadek, 2022. Characterization of the genetic diversity of some species of genus *Vicia* using ISSR and ITS molecular techniques. Egyptian Journal of Botany., 62: 475-492.

El-Demardash, I.S., A.A. El-Mouhamady, H.M. Abdel-Rahman and K.A. Aboud, 2017. Using Gamma Rays for Improving Water Deficit Tolerance in Rice. Current Science International., 6: 321-327.

Eldessouky, S.E.I., S.A.A. Heiba, A.A. El-Mouhamady and Y.M. Abdel-Tawab, 2016. DNA Fingerprinting and Half Diallel sAnalysis of Some Rice Genotypes under Water Deficit Conditions. Research Journal of Pharmaceutical, Biological and Chemical Sciences., 4: 985-997.

El-Keredy, M.S., A.E. Draz, A.Y. Ragab and A.A. El-Mouhamady, 2003a. Combining ability for some quantitative characters in rice (*Oryza sativa* L.) under normal and saline soil conditions. Tenth Conf Agro Octo Suez Canal Univ El-Irish Egypt., 9:7–10

El-Keredy, M.S., A.E. Draz, A.Y. Ragab and A.A. El-Mouhamady, 2003b. Effect of different levels of salinity on some agronomic, yield and its components and grain quality characters in rice. Proc 10th -The tenth conf. of agro. Octo. Suez Canal Univ. Fac. Environ. Agric Sci El-Irish Egypt: 80–89.

El-Keredy, M.S., A.E. Draz, A.Y. Ragab and A.A. El-Mouhamady, 2003c. Studies on rice 9Oryza Sativa L.) II. Genetical analysis and correlation coefficients in some chemical and grain yield and its components characters in rice under saline soil conditions. In: Proceedings of the10th the tenth conf. of agro. Octo. Suez Canal Univ. Fac. Environ. Agric Sci El-Irish Egypt: 121–132.

El-Mouhamady, 2003. Breeding studies for Salt Tolerance in rice. Ph.D Thesis Tan Uni Bra Kafr-She Fac Agric Egypt.

El-Mouhamady, A.A. and M.A.F. Habouh, 2019. Genetic improvement of some rice genotypes for salinity tolerance using generation mean analysis. Cu Sci Int., 8:321–348.

El-Mouhamady, A.A., M.A.F. Habouh and E. Naif, 2022. Elicitation of Novel Sunflower Lines Tolerant to Water Deficit Conditions Through Mutation Breeding. Asian J of Plant Sci., 21: 360-378. DOI: 10.3923/ajps.2022.360.378

El-Mouhamady, A.A.H.M. Abdel-Rahman, A.A. Rizkalla and M.A. El-Metwally, 2019. Assessment of Water Stress Tolerance in Wheat Genotypes Based on Half Diallel Analysis and DNA Fingerprinting. Pak. J. of Biol. Sci., 22:103-116.

El-Mouhamady, A.A., 2009. Breeding for drought tolerance in rice. Ph.D Thesis Fac Agric Kafr-Shei Univ Egypt.

El-Mouhamady, A.A., A.A.M. Gad and G.S.A.A. Karim, 2022. Improvement of drought tolerance in rice using line X tester mating design and biochemical molecular markers. Bull Natl Res Cent., 46: 1.

El-Mouhamady, A.A., and H.F. Ibrahim, 2020. Elicitation of salt stress-tolerant mutants in bread wheat (*Triticum aestivum* L.) by using gamma radiation. Bull of the Nat Res Cen., 44: 1–18.

El-Mouhamady, A.A., Z.M. El-Ashary, F.I. Mohamed, T.A. Elewa and K.A. Aboud, 2016. Study the effect of water stress conditions on some genotypes of bread Wheat (*Triticum aestivum* L.) based on morphological, physiological traits and DNA fingerprinting. RJPBCS., 5:2065–2077

El-Mouhamady, A.A. and M.A. El-Metwally, 2020. Appreciation of Genetic Parameters and Molecular Characterization in Some Promising Accessions of Soybean (*Glycine max* L.). Pak. J. of Biol. Sci., 4: 425-438.

El-Mouhamady, A.A., 2023. Biochemical and Molecular Characterization of Some Rice Accessions Tolerated to Salt Stress. Egypt. J. Chem., 66: 881-891.

El-Mouhamady, A.A., A.A. Abdel-Sattar and E.H. El-seidy, 2013. Assessment of Genetic Diversity and Relationships among canola (Rapeseed) Varieties Using Random Amplified Polymorphic DNA (RAPD) and Specific-PCR Analysis. J. of App. Sci. Res., 9: 1651-1665.

El-Mouhamady, A.A., A.M. El-Ekhtyar and I.S. El-Demardash (2012). Molecular Markers Linked to Some traits in Rice. J. of App. Sci. Res., 8: 2689-2699.

El-Mouhamady, A.A., E.H. El-Seidy and T.A. Elewa, 2014 a. Using Molecular Markers to Study Mechanics Responsible for Drought Tolerance in Some Genotypes of Sorghum. Int. J. Curr. Microbiol. App. Sci., 3: 481-491.

El-Mouhamady, A.A., M.R. Rady and E.H. El-Seidy, 2014 b. Assessment of Genetic Variability for Six Lines of Wheat Using Physiological Traits and Molecular Markers Technique under Normal Irrigation and Water Stress Conditions. W. App. Sci. J., 4: 506-516.

Esmail, R.M., A.A. Abdel Sattar, N. S. Abdel-samea, A.A. El-Mouhamady, E.M. Abdelgany and F.B. Fathallaha, 2016. Assessment of Genetic Parameters and Drought Tolerance Indices in Maize Diallel Crosses. Res. J. of Pharm, Biol & Chem Sci., 7(6): 2409-2428.

Fernandez, G.C.J., 1992. Effective selection criteria for assessing plant stress tolerance. In: Proc of on the Sym Taiw., 25: 257-270.

Fischer, R.A. and R. Maurer, 1978. Drought resistance in spring wheat cultivars. I Grain yield response. Aust J Agric Res., 29: 897–907.

Gavuzzi, P., F. Rizza, M. Palumbo, R.G. Campaline, G.L. Ricciardi and B. Borghi, 1997. Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Can J. Plant Sci., 77: 523-531.

Gezahegn, G., T. Kassahun and B. Endashaw, 2010. Inter Simple Sequence Repeat (ISSR) analysis of wild and cultivated rice species from Ethiopia. Afri J of Biot., 9: 5048-5059.

Ghazy, M.I., S.A. EL-Naem and A.G. Hefeina, *et al.,* 2024. Genome-Wide Association Study of Rice Diversity Panel Reveals New QTLs for Tolerance to Water Deficit Under the Egyptian Conditions. Rice., 17: 29. https://doi.org/10.1186/s12284-024-00703-1.

Golestani, S.A. and M.T. Assad, 1998. Evaluation of four screening techniques for drought resistance and their relationship to yield reduction ratio in wheat. Euphytica., 103: 293-299.

Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for agricultural research, 2th edn. Wiley, New York, p 680

Grieve, C.M. and S.R. Grattan, 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil., 70: 303-307.

Heiba, S.A.A., S.E.I. Eldessouky, A.A. El-Mouhamady, I.S. El-Demardash and A.A. Abdel-Raheem, 2016 b. Use of RAPD and ISSR Assays for the Detection of Mutation Changes in Wheat (Triticumaestivium L.) DNA Induced by Ethyl-Methane Sulphonate (EMS). Int.J. of Chem Tech Research., 9: 42-49.

Heiba, S.A.A., A.A. El-Mouhamady , S.E.I. Eldessouky, H.B.M. Ali and T.A. Elewa, 2016 a. Study the Genetic Variations Related to the Resistance of Heavy metals Toxicity in Some Rice Genotypes Using RAPD Markers. Int. J. Curr. Microbiol. App. Sci., 5: 174-189.

Hossain, A.B.S., A.G. Sears, T.S. Cox and G.M. Paulsen, 1990. Desiccation tolerance and its relationship to assimilate partitioning in winter wheat. Crop Sci., (30): 622-627.

Jaccard. P., 1908. Nouvelles Researchers Sur La IstributionLorale. Bull Soc Vaud Sci Nat., 44: 223–270.

Khatab, I.A., A.A. El-Mouhamady, H.M. Abdel-Rahman, M.A. Farid and I.S. El-Demardash, 2017. Agro-morphological and molecular characterization of sorghum (*Sorghum vulgare* L.) for water stress tolerance. Inter J Cur Res Bio Plant Biol., 4:37–55.

Khatab, I.A., A.A. El-Mouhamady, M.M. El-Hawary, and E. Naif, 2022. Agro-physiological and Genetic Characterization of Three Quinoa (*Chenopodium quinoa* Wild.) Cultivars to Drought Stress. Mid East J of Agri Res .,11:11-34. DOI: 10.36632/mejar/2022.11.1.2

Khatab, I.A., A.A. El-Mouhamady, S.A. Mariey and T.A. Elewa, 2019. Assessment of water deficiency tolerance indices and their relation with ISSR markers in barley (*Hordeum vulgare* L.). Cu Sci Inte 8:83–100.

Khatab, I.A., A.A. El-Mouhamady, S.A. Mariey, M.M. El-Hawary and M.A.F. Habouh, 2021 b. Molecular evaluation and identification of some barley hybrids tolerant to salt stress. Pak J Biol Sci., 24: 997-1014. DOI: 10.3923/pjbs.2021.997.1014

Khatab, I.S., A.A. El-Mouhamady and S.A. Mariey, 2021 a. Comprehensive Selection Criteria for High-Yielding Bread Wheat (*Triticum aestivum* L.) Hybrids under Salinity Stress. Egy. J of Bot., 61: 709-730. DOI: 10.21608/EJBO.2021.65333.1637

Khatab, I.A. and A.A. El-Mouhamady, 2022. In vitro Screening and Molecular Genetic Markers Associated with Fungal Pathogenic Toxin Filtrate Tolerance in Potato. Egypt. J. Chem., 6:-477-487.

Kishk, A.M.S. , H.M. Abdel-Rahman, A.A. El-Mouhamady and K.A. Aboud, 2017. Sophisticated genetic studies on onion through using gamma rays. RJBPCS., 8:202–220

Lin, C.S., M.R. Binns, and L.P. Lefkovitch, 1986. Stability analysis: where do we stand? Crop Sci 26:894–900

Melandri, G., H. AbdElgawad, D. Riewe, J.A. Hageman, H. Asard, G.T. S. Beemster, N. Kadam, K. Jagadish, T. Altamann, C.R. Spira and H. Bouwmeester, 2020. Biomarkers for grain yield stability in rice under drought stress. J. Exp. Bot., 71:669-683

Mohidem, N.A., N. Hashim, R. Shamsudin and M.H. Che., 2022. Rice for Food Security: Revisiting Its Production, Diversity, Rice Milling Process and Nutrient Content. Agric 12: 741. https://doi.org/10.3390/agriculture12060741

Mwando, E., Y. Han, T.T. Angessa, G. Zhou, C.B. Hill, X.G. Zhang and C. Li, 2020. Genome-wide association study of salinity tolerance during germination in barley (*Hordeum vulgare* L). Front Plant Sci., 11:1–15.

Nadeem, T., I.H. Khalil and S.A. Jadoon, 2023. Combining ability analysis for maturity and yield attributes in sweet corn across environments. SABRAO Journal of Breeding and Genetics., 55: 319–28.

Nasser, H., M.S. Youssef, Aziza, A.S. El –Kholy and S.A. Haroun, 2024. EMS-utagenesis, *In vitro* Selection for Drought (PEG) Tolerance and Molecular Characterization of Mutants in Rice (*Oryza sativa* L.) Employing qRT-PCR and ISSR Markers. Egypt. J. Bot., 64: 87-105.

Ndikuryayo, C., A. Ndayiragije, N.L. Kilasi and P. Kusolwa, 2023. "Identification of Drought Tolerant Rice (*Oryza Sativa* L.) Genotypes with Asian and African Backgrounds" Plants *.,4: 922.* https://doi.org/10.3390/plants12040922

Phunthong, C., M.K. Pitaloka, C. Chutteang, S. Ruengphayak, S. Arikit and A. Vanavichit., 2024. Rice mutants, selected under severe drought stress, show reduced stomatal density and improved water use efficiency under restricted water conditions. Front. Plant Sci., 15:1307653.

Ramadan, W.A., H.M. Abdel-Rahman, A.A. El-Mouhamady, M.A.F. Habouh, and K.A. Aboud, 2016. Molecular Genetic Studies on Some Barley Entries for Drought Tolerance. Int. J. of Pharm Tech Res., 12: 265-285.

Reddy, M.P., N. Sarla and E. Siddiq, 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica, 128: 9-17.

Sambrook, J., K.F. Fritsch and T. Maniatis, 1989. Molecular cloning. Second edition (cold spring Harbor New York).

Singh, S.P., A. Kumar, K.M. Satyendra, S. Nahakpam, S. Sinha, S.P. Smirty, S. Kumar and P.K. Singh, 2018. Identification of drought tolerant rice (Oryza sativa L.) Genotypes using drought tolerance indices under normal and water stress condition. Int J Curr Microbiol Appl Sci., 7:4757-4766

Tawfik, R.S. and A.A. El-Mouhamady, 2019. Molecular genetic studies on abiotic stress resistance in sorghum entries through using half diallel analysis and inter-simple sequence repeat (ISSR) markers. Bull Nat Res Cen., 43:1–17. https://doi.org/10.1186/s42269-019-0155-1.

Yassin, M., S.A. Fara, A. Hossian, H. Saneoka, and A. El Sabagh, 2019. Assessment of salinity tolerance bread wheat genotypes: using stress tolerance indices. Fresenius Environ Bull., 28:4199-4217.

Zhao, F.Y. and H. Zhang, 2007. [Transgenic rice breeding for abiotic stress tolerance--present and future]. Sheng Wu Gong Cheng Xue Bao (2007) Chinese 23: 1-6. doi: 10.1016/s1872-2075(07)60001-6. PMID: 17366880

Zian, A.H., I.S. El-Demardash, A.A. El-Mouhamady and E. El-Barougy, 2013. Studies the Resistance of Lupine for Fusarium oxysporum F. sp Lupini) Through Molecular Genetic Technique. W. App. Sci. J., 8: 1064-1069.

Zietkiewicz, E., A. Rafalski and D. Labuda, 1994. Genome Fingerprinting by Simple Sequence Repeats (SSR)-Anchored Polymerase Chain Reaction Amplification. Geno., 20: 176-183.