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ENHANCING THE TOLERANCE OF FENNEL PLANT TO SALINITY STRESS USING GAMMA RAYS AND LASER AND EVALUATING THE MUTATIONS BY ISSR AND RAPD MARKERS

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ABSTRACT: Salinity causes a decrease in plant growth and productivity, and the problem of salinity increases with the worsening impact of climate change. This work aimed to study the effect of the physical mutants, laser and gamma rays, on the vegetative growth, yield and chemical constituents of *Foeniculum vulgare* plants grown under salinity stress condition, and their ability in inducing salt tolerant mutant. Fennel seeds were irradiated with gamma rays (10 and 20 Kr) and laser (720 and 850 nm). Plants irrigated with saline water at 238 ppm as control plant, 2000 and 4000 ppm. Both radiation types especially gamma rays at 20 and laser at 720 and 850 nm increased plant growth when irrigated with 2000 or 4000 ppm saline water. Two plants were found with better growth under irrigation with 2000 ppm saline water, one was obtained from the treatment of 20 Kr gamma rays and the other from 850 nm laser rays. ISSR and RAPD markers distinguish the two mutants from control. Mutant 2 was more genetically distinct from the control plant.

Keywords: mutants, fennel, antioxidant enzymes, salinity stress, physical mutagen

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) is an annual, aromatic and medicinal herb belonging to the family Apiaceae (Umbelliferae). It is used in the food, cosmetic and medical industries. (Khan and Musharaf, 2014). Fennel has various medicinal uses, including antispasmodic, diuretic, anti-inflammatory, analgesic, galactagogue, secretomotor, eye ointment, and antioxidant remedy (Moura *et al*., 2005). Fennel seeds are chewed, especially after meals for freshening breath and being added to some natural dental pastes. They help soothe intestines and lessen indigestion-related bloating. (Sarla, 2019).

Throughout the world, soil salinity has an impact on agricultural output (Zörb *et al.*, 2004). Salinity issues can decrease yield and quality of the products due to unregulated irrigation, continuous cropping, excessive fertilization, and low-quality water (Cansev and Ozgur, 2010).

Few attempts have been made to improve the fennel crop through genetic manipulation. Because most seed spice crops, including fennel, have modest levels of naturally existing variability, so induced mutation presents a potential alternative for crop improvement. Induced mutation has been widely used for a wide range of genetic variability in a variety of crops and species. Mutation breeding is usually utilized when there is little genetic variability for a specific feature in a gene pool. Physical mutagens that cause mutations were the method that used to create mutant kinds. Types of physical mutagens include gamma rays, laser, and X-rays.

Sixty-four percent of radiation-induced mutant types were created using gamma rays,

while twenty-two percent were created using X-rays. Gamma rays are recognized as a commonly employed mutagen in electromagnetic radiation (Chahal and Gosal, 2002). Because of their easy application, high mutation frequency, strong penetration, reproducibility, and energy. Short-wavelength gamma rays with strong penetration strength interact with atoms or molecules to create free radicals within cells. The most effective physical mutagen for inducing mutations in crop plants is gamma radiation (Jan *et al*., 2011; Verma *et al*., 2012).

Laser beam (non-ionizing radiation has been demonstrated to stimulate the sprouting process, plant growth and production of anise plant (Okla *et al*., 2021). The physical phenomena of laser stimulation depend on the laser's capacity to both absorb and store the light energy that is emitted by plant tissues and cells. The similar phenomenon is seen with seeds; they take in light energy, convert it to chemical energy, and then use it later (Aladjadjiyan, 2012). The energy from a divergent laser beam, physiological and biochemical processes can be altered, improved field performance and crop production (Qiu, *et al*., 2013). laser beam stimulate plant growth and yield, it have an impact on the enzymatic system, which may speed up starch decomposition and seed germination (Podle´sna *et al*., 2015), photosynthesis, transpiration efficiency (Cwintal *et al*., 2010; Wilczek *et al*., 2004; Chen *et al*., 2005), and plant growth and development (Cwintal *et al*., 2013; Wilczek *et al*., 2005). Laser beams also have a mutagenic effect, produce aberration in mitotic divisions. Where Al Aboud (2023) stated that all four phases of mitotic division showed a broad range of chromosomal abnormality on *Vicia faba* when treated with wavelength in the visible region from 660 to 680 nm. One of the most effective methods of biotechnology breeding is the induction of mutations. A novel and simple tool is laser beams mutagenesis. Plant morphology, flowering, chemical composition, and gene mutagenesis can all be impacted by the laser irradiation (Abou-Dahab *et al.*, 2019)

Inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers have been shown effective in studying the genetic diversity and identifying mutants across various crops and medicinal plant (Farajpour *et al.*, 2011). ISSR technique is highly sensitive for identifying the alteration in DNA induced by laser treatments (Osman and Rayan, 2020).

This investigation was done to study the effect of the physical mutagens (laser and gamma rays) on the vegetative growth, yield and chemical constituents of *Foeniculum vulgare* plants grown under salinity stress conditions. Also, to study their ability to produce salt-tolerant mutants and evaluate the genetic differences using ISSR and RAPD markers.

MATERIALS AND METHODS

This experiment was conducted at the farm of the Faculty of Agriculture, Beni Suef University during two consecutive generations of 2020/2021 and 2021/2022.

Plant treatments:

Seeds of *Foeniculum vulgare* (local variety) obtained from Qena governorate, Egypt were irradiated with gamma rays at (0, 10 and 20 Kr) and laser beams at 720 and 850 nm. Dry seeds were treated with gamma rays in the Atomic Energy Authority in Nasr City, Egypt, on October 7th, in the first generation by exposing the treatment of 10 Kr for 30 minutes but the treatment of 20 K exposed to gamma for 60 minutes. This was done using Cs^{137} gamma cells at the dose rate of 633 rad/s. Laser beams were treated in the Institute of Laser Research and Applications at Beni-Suef University, Egypt, on October 18th. The laser pulses were delivered by a mode-locked femtosecond Ti: sapphire MAI TAI HP laser (Spectra-Physics), and a wavelength of 720 and 850 nm were used to push laser pulses using an INSPIRE HF100 laser system for 3 minutes with average power 860 mw.

After the radiation treatments are completed, irradiated and control seeds were sown on 25 cm plastic pots containing clay and sand (1:1). 360 seeds for each treatment were used (9 pots and 40 seeds for each).

Seedlings were transplanted to plastic pots (25 cm) filled with clay soil, after two weeks the salinity stress irrigation treatments (the control with tap water (238 ppm), 2000 and 4000 ppm) were done using 400 ml for each pot and repeated as required during seasons using sodium chloride which obtained from Al-Nasr Chemical Company, Egypt, was used. Chemical soil analysis is shown in Table (1). The soil analysis was performed by the method of Jackson (1973).

Open pollinated seeds were collected from each treatment to obtain the seeds of the second generation (M2) and the control seeds were sown as done in the first generation (except 20 seeds per each treatment per replication were sown in the second generation). Plants were harvested in the second week of May in both generations

Layout of the experiment:

A split plot with three replicates in a randomized complete block design (RCBD) was followed (Gomez and Gomez, 1984). Salt stress was designated as the main plot (A), while both gamma and laser ray treatments were put in the sub-plots (B). Therefore, the interaction treatments (A × B) were 15 treatments (9 plants for each treatment/replicate) in a total of 405 plants

Recorded data:

Growth characteristics:

At the flowering stage, the fresh and dry weights of vegetative growth, length, and fresh weight of roots, plant height (cm), stem diameter (cm), and the number of branches per plant were assessed. Upon maturity, the weight of the fruits per plant and the quantity of umbels per plant were calculated.

Chemical and biochemical estimations:

Using the SPAD meter device, the total chlorophyll content (SPAD unit) was calculated in accordance with Yadava (1986) instructions. Proline content was assessed in mg g^{-1} dry matter using the methodology of Bates *et al*. (1973). We used the Cottenie *et al*. (1982) method to determine the amounts of sodium and potassium in the herb. Fennel fruit essential oil was measured using water distillation methods according to the British Pharmacopeia (1963) method, using 10 g of fruits for three hours (except for mutants where 1.0 g of fruits was used) to extract the essential oil. The essential oil percentage was determined according to Gad *et al*. (1963).

Antioxidant enzymes activity analysis:

Mukherjee and Choudhuri's (1983) method was followed to obtain plant extracts for enzyme activity analysis**.**

Peroxidase activity was assessed following the method of Maehly and Chance (1954).

The method of Aebi (1984) was used to assess the peroxidase activity.

Genetic marker for identification the variability:

Inter-simple sequence repeat (ISSR) technique:

Inter-Simple Sequence Repeat (ISSR) was applied as a molecular fingerprinting technique which designed to evaluate genetic similarity within and among *Foeniculum vulgare* samples. DNA extraction procedure

Table 1. Chemical soil analysis.

Chemical properties			Soluble nutrients		
CaCO3	**E.C (dS/m)**	**pH (1:2.5)**	**P^{++} (ppm)**	**N^{+} (ppm)**	**K^{+} (ppm)**
1.0	0.51	7.60	0.04	10	257
Cations (meq/l)			**Anions (meq/l)**		
Mg^{++}	**Na^{+}**	**K^{+}**	**Ca^{++}**	**HCO_3**	**Cl**	**SO_4**
1.2	2.4	0.04	1.4	2.6	2.5	0.01

for total genomic of *Foeniculum vulgare* samples was done as the protocol of GeneJET Genomic DNA Purification Kit (K0721/ Thermo fisher). DreamTaq PCR Master Mix (2X) (K1071, Thermo fisher. USA) was used as manufacturer protocol to amplify ISSR using four primers (Table, 2). In accordance with Ramadan *et al*. (2019) whole genomic DNA was amplified using a Gene Amp Polymerase Chain Reaction (PCR) system cycler as follows: first cycle 94 °C/2-minute, thirty-five cycle(94 °C/1-minute, 48 °C/2-minute, and 72 °C/2-minute), and last cycle at 72 °C/7-minute. The degrees of primer annealing were adjusted based on each primer's melting point.

Random amplified polymorphic DNA (RAPD) technique:

DNA isolation for total genomic procedure for *Foeniculum vulgare* was done in accordance with Omega Co. (USA. LMt.) manufacturer's.

The whole genomic DNA was amplified using a Gene Amp PCR system cycler. First step is denaturation for five minutes at 94 ºC. The second step involved operating for 44 phases, each consisting of 1 minute of denaturation at 94 ºC, 1 minute of annealing at 42 ºC, and 1 minute of extension at 72 ºC. The third step involved a last extension cycle that lasted seven minutes at 72 ºC. The product was held at 4 ºC (Grover *et al.*, 2011). Four RAPD primers were used (Table, 3).

Then products of PCR were separated on agarose gel electrophoresis using 1.2% agarose solution that equipped by adding 0.75 g agarose for ISSR and 0.60 g agarose for RAPD to 50 ml of 1x TBE electrophoresis buffer. The electrophoresis was adjusted at 80 volts for 100 min. The gel stained with Ethidium bromide for 30 min and photographed using gel documentation system (Geldoc-it UVP, England). Data was analyzed using Totallab analysis software, ww.totallab.com, (Ver.1.0.1).

Statistical analysis:

The CoStat application was used to perform analysis of variance (ANOVA) of the plant data that were obtained during two generations (2020/2021 and 2021/2022). To examine changes between treatments, the least significant difference (LSD) was applied at the 0.05 level of probability (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Growth characteristics:

Tables (4 and 5) show that, without irradiation treatments, the means of vegetative growth characteristics were decreased with increasing salinity stress and this decrease was significant in most cases. Gao *et al*. (2016) concluded that salt stress had a negative impact on the growth and development of seedlings. When compared to control, there was a noticeable increase in ROS levels and genomic DNA damage.

As the main effect, gamma rays at 20 kr and laser at 850 nm significantly increased the plant height compared to control (51.0, 50.2 and 41.3 cm respectively in the first generation and 64.0, 56.0 and 51.2 cm in the

Table 2. Primers code, name, sequence of the used four ISSR primers.

ISSR primer code	ISSR primer name	ISSR primer sequence
Primer 1	49A	CACACACACACAAG
Primer 2	44B	CTCTCTCTCTCTCTCTGC
Primer 3	HB-9	GTGTGTGTGTGTGG
Primer 4	HB-11	GTGTGTGTGTGTCC

Table 3. Primers code, name, sequence of the used four RAPD primers.

RAPD primer code	RAPD primer name	RAPD primer sequence
Primer 1	GCC-176	CAA GGG AGG T
Primer 2	GCC-60	TTG GCC GAG C
Primer 3	GCC-90	GGG GGT TAG G
Primer 4	GCC-176	CAA GGG AGG T

Table 4. Effects of gamma and Laser rays on plant height (cm), number of branches per plant, fresh and dry weights of vegetative growth (g) of *Foeniculum vulgare* L. plants under salinity stress conditions at 2020/2021 and 2021/2022 generations.

Saline water levels (ppm)	Irradiation treatments											
	0.0	Gamma 10 Kr	Gamma 20 Kr	Laser 720 nm	Laser 850 nm	Means (S)	0.0	Gamma 10 Kr	Gamma 20 Kr	Laser 720 nm	Laser 850 nm	Means (S)
	First generation (2020/2021)						Second generation (2021/2022)					
Plant height (cm)												
238	51.8 ab	46.3 a-c	51.3 ab	53.1 ab	44.9 a-c	49.5 a	62.6 a-c	57.3 b-d	64.6 ab	51.1 de	59.3 a-c	59.0 a
2000	40.3 cd	30.6 e	47.6 a-c	44.0 bc	54.0 a	43.3 a	50.0 de	48.5 ef	62.3 a-c	56.3 cd	62.1 a-c	55.8 ab
4000	31.7 de	40.3 cd	54.0 a	43.7 bc	51.6 ab	44.2 a	41.1 f	56.0 cd	65.2 a	51.5 de	46.6 ef	52.1 b
Means	41.3 b	39.0 b	51.0 a	46.9 a	50.2 a		51.2 c	53.9 bc	64.0 a	53.0 bc	56.0 b	
LSD at 5%	S= NS		R= 5.6 ***		S × R = 9.7**		S= 4.29*		R= 4.3***		S × R = 7.5***	
Number of branches per plant												
238	8.0 b-d	8.6 a-c	7.3 de	8.3 a-d	7.3 de	7.9a	8.0 cd	8.3 c	12.3 b	7.0 c-e	15.0 a	10.1 a
2000	7.6 c-e	9.0 ab	9.0 ab	6.0 f	8.3 a-d	8.0 a	8.0 cd	5.8 d-f	6.0 d-f	7.5 c-e	5.3 ef	6.5 b
4000	7.3 de	8.6 a-c	9.3 a	6.6 ef	7.3 de	7.8 a	4.0 fg	2.0 g	4.0 fg	4.0 fg	4.3 f	3.6 c
Means	7.6 bc	8.7 a	8.5 a	7.0 c	7.6 bc		6.6 cd	5.3 e	7.4 bc	6.1 de	8.2 a	
LSD at 5%	S= NS		R= 0.7***		S × R = 1.3**		S= 1.2 ***		R= 1.3 **		S × R = 2.3***	
Fresh weight of vegetative growth (g)												
238	16.2 a-c	8.6 ef	13.3 c-e	15.5 b-d	13.6 c-e	13.4 a	19.6 b-f	21.2 b-e	24.9 b-d	27.1 b	49.6 a	28.5 a
2000	9.6 d-f	20.2 ab	8.2 ef	8.7 ef	13.7 c-e	12.1a	18.7 b-f	11.8 e-g	12.7 e-g	26.5 bc	17.4 c-g	17.4 b
4000	6.2 f	22.3 a	14.2 b-e	9.5 d-f	9.7 d-f	12.4 a	10.5 fg	8.0 g	45.4 a	13.8 e-g	16.4 d-g	18.8 b
Means	10.6 b	17.1 a	11.9 b	11.3 b	12.4 b		16.3 b	13.7 b	27.6 a	22.5 a	27.8 a	
LSD at 5%	S= NS		R= 3.6*		S × R = 6.4***		S= 6.1*		R= 5.5***		S × R = 9.6***	
Dry weight of vegetative growth (g)												
238	4.35 ab	1.39 fg	5.45 a	2.92 cd	2.42 d-f	3.30 a	3.55 d-g	3.57 d-g	4.58 cd	5.15 bc	7.42 a	4.85 a
2000	1.56 e-g	3.62 bc	1.58 e-g	1.22 g	2.70 c-e	2.13 b	2.73 f-i	1.74 i	3.55 d-g	4.51 cd	3.14 e-h	3.69 ab
4000	1.02 g	2.55 c-f	1.78 d-g	1.55 e-g	2.01 d-g	1.78 b	1.86 hi	3.77 d-f	6.15 ab	2.23 g-i	4.48 c-e	3.13 b
Means	2.31 a	2.52 a	2.93 a	1.90 a	2.37 a		2.71 c	3.02 c	4.76 a	3.96 b	5.01 a	
LSD at 5%	S= 1.14*		R= NS		S × R = 1.17***		S= 1.28*		R= 0.79***		S × R = 1.37***	

NS, *, **, *: non-significant, and significant at p= 0.05, 0.01 and 0.001 respectively.**

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

Table 5. Effects of gamma and laser rays on stem diameter (cm), root length (cm), fresh weight of root (g) and number of umbels per plant of *Foeniculum vulgare* L. plants under salinity stress condition at 2020/2021 and 2021/2022 generations.

Saline water levels (ppm)	Irradiation treatments											
	0.0	Gamma 10 Kr	Gamma 20 Kr	Laser 720 nm	Laser 850 nm	Means (S)	0.0	Gamma 10 Kr	Gamma 20 Kr	Laser 720 nm	Laser 850 nm	Means (S)
	First generation (2020/2021)						Second generation (2021/2022)					
Stem diameter (cm)												
238	0.70 a-c	0.56 b-d	0.53 c-e	0.70 a-c	0.83 ab	0.66 a	0.35 c-e	0.33 c-e	0.66 ab	0.53 bc	0.50 bc	0.47 a
2000	0.23 fg	0.63 b-d	0.36 d-g	0.46 c-f	0.66 bc	0.47 b	0.26 de	0.26 de	0.36 c-e	0.80 a	0.26 de	0.39 a
4000	0.16 g	0.98 a	0.96 a	0.26 e-g	0.50 c-f	0.57 ab	0.20 e	0.20 e	0.61 ab	0.46 b-d	0.40 c-e	0.37 a
Means	0.36 c	0.72a	0.62 ab	0.47 bc	0.66 a		0.27 b	0.26 b	0.55 a	0.60 a	0.35 b	
LSD at 5%	S= 0.12 *		R= 0.17**		S × R = 0.29***		S= 0.13 NS		R= 0.11 ***		S × R = 0.20**	
Root length (cm)												
238	24.4 ab	19.3 d-f	22.1 b-d	27.0 a	22.0 b-d	22.9 a	27.1 a	35.8 a	37.5 a	28.1 a	36.1 a	32.9 a
2000	19.8 d-f	20.0 d-f	20.6 c-e	18.5d-f	24.7 ab	21.5 a	21.7 a	22.0 a	26.1 a	27.2 a	34.6 a	26.3 b
4000	16.5 f	24.0 a-c	25.4 ab	18.0 ef	24.0 a-c	20.7 a	23.6 a	18.0 a	22.3 a	17.4 a	24.1 a	21.1 b
Means	20.2c	21.1 bc	22.7 ab	21.1 bc	23.6 a		24.1 b	25.3 b	28.6 ab	24.2 b	31.6 a	
LSD at 5%	S= NS		R= 2.1*		S × R = 3.7 ***		S= 5.7*		R= 4.4**		S × R = NS	
Fresh weight of root (g)												
238	3.0 b-d	1.9 fg	3.3 a bc	4.1 a	3.7 ab	3.2a	5.7 bc	4.0 de	5.9 bc	5.1 cd	7.4 a	5.6 a
2000	2.5 d-f	3.9 ab	2.0 e-g	1.7 g	3.0 b-d	2.6 a	4.7cd	3.0 ef	4.4 d	4.2 de	4.3 d	4.7 a
4000	1.9 fg	4.0 ab	4.1 a	2.7 c-e	2.2 e-g	3.0 a	2.6 f	7.4 a	6.7 ab	3.0 ef	3.9 de	4.1 a
Means	2.5 c	3.3 a	3.2 ab	2.8 bc	2.9 a-c		4.3 cd	4.8 bc	5.7 a	4.1 d	5.2 ab	
LSD at 5%	S= NS		R= 0.5*		S × R = 0.8***		S= NS		R= 0.7**		S × R = 1.3***	
Number of umbels per plant												
238	3.66 cd	4.0 bc	3.66 cd	5.0 a	3.66 cd	4.0 b	7.0 d-f	7.3 c-e	9.3 b	6.0 e-g	13.0 a	8.53 a
2000	3.0 d	3.5 cd	4.0 bc	3.33 cd	3.33 cd	3.43 c	5.66 f-h	5.0 gh	7 d-f	8.66 bc	7.66 cd	6.80 b
4000	3.0 d	5.33 a	4.66 ab	5.0 a	5.0 a	4.6 a	5.33 gh	2.0 i	4.66 gh	4.33 h	7.0 d-f	4.66 c
Means	3.22 b	4.27a	4.11a	4.44a	4.0 a		6.0 c	4.77 d	7.0 b	6.33 c	9.22 a	
LSD at 5%	S= 0.24***		R= 0.51***		S × R = 0.87**		S= 0.86***		R= 0.80***		S × R = 1.38***	

NS, *, **, *: non-significant, and significant at p= 0.05, 0.01 and 0.001 respectively.**

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

second generation. These findings also were found for all other vegetative traits in the M2 generation and stem diameter and root length in the first generation. The treatment of 20 kr gamma rays gave the best results compared to control for number of branches per plant, while gamma rays at 10 kr gave the best result for fresh weight of vegetative growth, both dosages of gamma were the most effect for root fresh weight and stem diameter in the first generation. The highly significant effect of irradiation treatments appeared when plants irrigated with saline water at 4000 ppm for all vegetative growth traits except for root length in the second generation where no significant differences found for the interaction between factors. Also, no significant differences were found between irradiation treatments for dry weight of vegetative growth in the M2 generation.

Number of umbels per plant was increased significantly with all irradiation treatments as the mean effect of irradiation or at 4000 ppm salinity stress in the first generation. Whereas in the second one, both 850 nm laser and 20 kr gamma rays significantly increased this trait compared to control (9.22, 7.0 and 6.0 umbels/plant, respectively) as the mean effect. However, at 4000 ppm salinity stress, the 850 nm laser treatment was the most effective one.

Fruits weight per plant increased significantly when plants were irradiated with laser at 850 nm in both generations as the main effect compared to non-irradiated plants (4.3 and 2.6 for M1, 1.2 and 0.9 for M2). It increased by 65.3 and 33.3% in the first and second generations respectively. As the interaction effect, both laser treatments significantly increased the seeds' weight when plant irrigated with saline water at 2000 ppm (4.4, 6.3 for M1 and 1.8, 1.7 for M2) compared to non-irradiated ones (2.4 and 0.9 for M1 and M2 respectively). In the second generation, gamma rays at 20 kr also significantly increased the fruits weight when plants irrigated by 2000 ppm saline water. While at 4000 ppm saline water, no significant effect between irradiated and non-irradiated plants in both generations was obtained. The stimulating effect of gamma and laser on plants grown under salt stress was also found by Moemen (2012), Gao *et al*. (2015) and Khatiyar *et al*. (2022). He-Ne laser irradiation increased plant growth by reversing physicochemical characteristics, improving cell viability, ameliorate cell wall polysaccharide damage and DNA repair and associated resistance gene expression pattern (Gao *et al*., 2016). Enhanced vegetative growth with laser treatment might be due to the response of phytochrome which modulate the red light spectrum (Thorat, 2024). Low doses of gamma rays stimulated plant growth characteristics in green bean plant as the effect of changes in phytohormones as GA_3 and IAA (Moemen, 2012).

No fruits found in the M2 generation when plants irrigated with 4000 ppm saline water and irradiated with 10 kr gamma rays. This may be due to the mutagenic effect of gamma rays. Differences between 1st and 2nd generations means may be due to seasonal effect as a climate change effect (Epa, 2021)

Chemical and biochemical estimations:

Oil percentage decreased significantly with increasing salinity stress in the absent of irradiation treatments as shown in Table (6). While it increased significantly using laser at 720 nm as the mean effect in the M2 generation (13.3 and 11.33, respectively). The interaction effect between salinity and irradiation for M2 generation, gamma ray at 20 kr increased oil percentage when irrigated with tap water. Plants irrigated with 4000 ppm saline water gave the highest oil percentage when irradiated with laser at 720 nm compared to non-irradiated plant (11.0 and 7.0, respectively). Both laser treatments increased slightly oil percentage when plant irrigated with 2000 ppm saline water (13.0 and 13.5) compared to non-irradiated plants (10.0) while no increment appeared in the M1 generation at 4000 ppm saline water. Plants treated with 850 nm laser and irrigated by 4000 ppm gave atrophic seeds and no oils were extracted.

Table 6. Effects of gamma and laser rays on fruit weight per plant (g), oil percentage and chlorophyll content (SPAD Unit) of vegetative growth (g.) of *Foeniculum vulgare* L. plants under salinity stress condition at 2020/2021 and 2021/2022 generations.

Saline water levels (ppm)	Irradiation treatments											
	0.0	Gamma 10 Kr	Gamma 20 Kr	Laser 720 nm	Laser 850 nm	Means (S)	0.0	Gamma 10 Kr	Gamma 20 Kr	Laser 720 nm	Laser 850 nm	Means (S)
	First generation (2020/2021)						Second generation (2021/2022)					
Fruit weight per plant (g)												
238	3.2 b-d	1.6 e-g	3.7bc	2.5 c-f	4.4 b	3.1 a	1.2 b-d	0.4 f-h	0.3 f-h	1.0 c-e	1.6 ab	0.9 b
2000	2.4 c-f	3.0 b-e	1.9 d-g	4.4 b	6.3 a	3.6 a	0.9 de	0.7 ef	1.4 a-c	1.8 a	1.7 a	1.3 a
4000	2.1 c-g	1.7 d-g	1.2fg	0.7 g	2.1 d-g	1.5 a	0.6 e-g	0.0 h	0.4 f-h	0.4 f-h	0.2 gh	0.3 c
Means	2.6 b	2.1 b	2.3 b	2.5 b	4.3 a		0.9 bc	0.4 d	0.7 c	1.0 ab	1.2 a	
LSD at 5%	S= NS		R= 0.9***		S × R = 1.6**		S= 0.2***		R= 0.3***		S × R = 0.5**	
Oil percentage												
238	1.35 a	0.45 e	0.7 cd	0.95 b	1.0 b	0.89 a	1.6 b-d	1.7 bc	2.0 a	1.5 c-e	1.6 b-d	1.68 a
2000	1.0 b	1.25 a	0.6 de	1.3 a	1.35 a	1.1 a	1.4 de	1.5 c-e	1.3 e	1.4 de	1.0 f	1.32 b
4000	0.85 bc	1.0 b	0.9 b	0.7 cd	0.95 b	0.88 a	1.0 f	0.0 h	0.5 g	1.8 ab	0.0 h	0.66 c
Means	1.06 ab	0.9 c	0.73 d	0.98 bc	1.1 a		1.33 b	1.06 c	1.26 b	1.56 a	0.86 d	
LSD at 5%	S= NS		R= 0.09***		S × R =0.17***		S= 0.25 *		R= 0.12 ***		S × R = 0.21***	
Chlorophyll content (SPAD Unit)												
238	14.43 bc	15.50 b	4.86 i-k	6.66 f-i	1.76 k	8.64 b	29.90 ab	11.23 e-g	8.93 e-g	14.83 de	19.80 cd	16.94 a
2000	9.06 e-g	19.00 a	9.96 d-f	13.16 b-d	11.30 c-e	12.50 a	25.43 bc	8.70 e-g	34.53 a	23.80 bc	12.03 ef	20.90 a
4000	8.26 e-h	5.86 g-j	5.03 h-k	3.16 jk	15.76 ab	7.62 b	5.46 fg	4.40 g	11.30 ef	11.20 e-g	7.40 fg	7.95 b
Means	10.58 b	13.45 a	6.62 c	7.66 c	9.61 b		20.26 a	8.11 c	18.25 a	16.61 ab	13.07 b	
LSD at 5%	S= 2.62*		R= 1.92***		S × R = 3.32***		S= 7.1*		R= 3.95***		S × R = 6.84***	

NS, *, **, *: non-significant, and significant at p= 0.05, 0.01 and 0.001 respectively.**

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

Irradiated plant with 10 kr gamma rays increased significantly chlorophyll content as the mean effect in the M1 generation. Laser at 850 nm combined with 4000 ppm saline water and both laser treatments and 10 kr gamma rays combined with 2000 ppm saline water increased chlorophyll content of plants in the M1 generation. In the M2 generation, 10 kr gamma rays and both laser treatments slightly increased chlorophyll content when plants irrigated with 4000 ppm. While at 2000 ppm salinity, gamma rays at 20 kr increased significantly chlorophyll content.

Sodium content increased with increasing salinity stress in the absence of irradiation treatments as shown in Table (7). At 2000 ppm salinity stress, both dosages of laser in the M1(191.66 and 146.66) and 20 kr gamma rays in the M2 (218.33) decreased sodium content compared to non-irradiated plants (236.66 and 222.33 respectively). The irradiation enables plants to obtain mechanisms to transport sodium outside the cell to maintain it at the down level (Khatiyar *et al*., 2022).

Potassium content slightly increased by 20 kr gamma ray in the M1 and both dosages of laser in the M2 generation at 2000 ppm salinity stress. While 10 kr gamma in the M1 and both laser dosages were more effective in increasing potassium content.

Potassium is one of osmotic regulators and maintains the turgor of the cell, reduce cell acidity and free radicals (Khatiyar *et al*., 2022). Saline stress removes ions of potassium from root of plant so affecting on the synthesis of protein which cause physiological imbalance and decreased growth and yield (Chen *et al*., 2007). Gamma rays enhanced protein synthesis under salinity as reported by (Ling *et al*., 2008).

No significant differences were found for proline content in both generations. But it can be seen that proline content increased significantly when the plant were irrigated with 2000 ppm saline water and decreased when irrigated with 4000 ppm in both generations. All irradiation treatments increased proline content in both generations except for 850 nm laser in the M1 as the mean effect. The same result was found when the plant irrigated with 4000 ppm saline water except for 20 kr gamma rays in the M2 generation.

Verslues and Sharma (2010) reported that the proline increases in the plant under salt stress as a result of increase the expression of P5CS and P5CR genes and reduced in the expression of P5CDH gene. Low level of gamma rays increased the system of plant defense. It decreases lipid peroxidation, and membrane deterioration in plants suffering from salt stress

Salinity reduces growth and yield as it affects many metabolic operations including the inhibition of the synthesis of nucleic acid and protein, decreasing conductivity of stomata, transpiration, photosynthesis and water use efficiency, decreased chlorophyll synthesis, increased uptake toxic ions in the cell and alter gene expression. Numerous researchers observed an increase in amino acids, particularly proline, in plants under salt stress. Proline acts as a free radical scavenger as well as a carbon and nitrogen storage sink (Moemen, 2012). Gamm rays are used to raise abiotic stress tolerance cultivars and change qualitative and quantitative attributes (Khatiyar *et al*., 2022). Gao *et al*. (2016) referred to the effect of laser in the enhancement plant tolerance through plant height, biomass, antioxidant biosynthesis, chlorophyll content and root length

Antioxidant enzymes activity:

Table (7) indicates that there were no discernible variations in the second generation's for catalase activity. Furthermore, no notable differences were discovered in the mean effect of radiation treatments for peroxidase activity. While significant differences were found for interaction effect between salinity concentration. It increased significantly with increasing salinity stress up to 2000 ppm then it decreased peroxidase activity.

Table 7. Effects of gamma and laser rays on sodium (Na^+) content (ppm), potassium (K^+) content (ppm), proline content (mg g^{-1} dry matter) of *Foeniculum vulgare* L. plants under salinity stress condition at 2020/2021 and 2021/2022 generations, peroxidase (POD) and catalase (CAT) activity (unit min^{-1} g^{-1}fresh matter) for second generation.

Saline water levels (ppm)	Irradiation treatments											
	0.0	Gamma 10 Kr	Gamma 20 Kr	Laser 720 nm	Laser 850 nm	Means (S)	0.0	Gamma 10 Kr	Gamma 20 Kr	Laser 720 nm	Laser 850 nm	Means (S)
	First generation (2020/2021)						Second generation (2021/2022)					
Sodium (Na^+) content (ppm)												
238	213.33 cd	246.66bc	250.33 bc	219.66 cd	264.00 ab	238.80 a	178.30 ef	142.00 f	158.00 f	188.33 d-f	243.66 bc	182.06b
2000	236.66 bc	248.33bc	235.00 bc	191.66 d	146.66 e	211.66 a	222.33 b-e	230.66 b-d	218.33 c-e	307.33 a	228.33 b-e	241.4a
4000	242.66 bc	240.00bc	244.33bc	243.00 bc	293.33 a	252.66 a	257.00 bc	251.66 bc	242.00 bc	269.66 ab	260.00 a-c	256.06a
Means	230.88 a	245.00 a	243.22 a	218.11 a	234.66 a		219.22 bc	208.11 c	206.11 c	255.11 a	244.00 ab	
LSD at 5%	S= NS		R= NS		S × R = 37.57***		S= 24.08**		R= 28.95**		S × R = 50.15*	
Potassium (K^+) content (ppm)												
238	55.0 a	44.5 a	59.5 a	39.0 a	56.5 a	50.9 a	33.33 bc	24.66 b-e	37.33 b	26.66 b-e	34.33 bc	31.26 a
2000	50.0 a	32.5 a	59.0 a	24.0 a	46.0 a	42.3 a	19.33 d-f	22.33 c-f	22.66 c-f	52.33 a	27.33 b-e	28.80 a
4000	49.0 a	55.0 a	42.0 a	39.5 a	48.5 a	46.8 a	16.33 ef	10.00 f	10.66 f	31.00 b-d	25.66 b-e	18.73 b
Means	51.33 a	44.0 ab	53.5 a	34.16 b	50.33 a		23.00 bc	19.00 c	23.55 bc	36.66 a	29.11 b	
LSD at 5%	S= NS		R= 11.03*		S × R = NS		S= 6.35*		R= 7.40***		S × R = 12.82**	
Proline content (mg g^{-1} dry matter)												
238	0.22 a	0.80 a	0.80 a	0.43 a	0.25 a	0.50 a	0.63 a	0.85 a	1.28 a	0.80 a	0.66 a	0.84 a
2000	0.87a	0.87 a	0.79 a	0.95 a	0.88 a	0.87 a	0.93 a	1.60 a	0.44 a	0.55 a	0.57 a	0.82 a
4000	0.15 a	0.32 a	0.53 a	0.69 a	0.06 a	0.35 a	0.74 a	0.83 a	0.70 a	2.13 a	1.53 a	1.18 a
Means	0.41 a	0.66 a	0.70 a	0.69 a	0.39 a		0.76 a	1.09 a	0.80 a	1.16 a	0.92 a	
LSD at 5%	S= NS		R= NS		S × R = NS		S= NS		R= NS		S × R = NS	
	Peroxidase (POD) activity [unit min-1 g - 1fresh matter] for M2						**Catalase (CAT) activity [unit min-1 g - 1fresh matter] for M2**					
238	2.10 g	1.00 h	3.60 c-e	3.60 c-e	30 d-g	2.66 b	0.35 a	1.65 a	2.25 a	1.50 a	1.20 a	1.39 a
2000	4.65ab	4.95 a	3.60 c-e	3.90 b-d	3.90 b-d	4.20 a	5.40 a	0.90 a	2.55 a	5.25 a	4.05 a	3.63 a
4000	4.20 a-c	3.60 c-e	2.85 e-g	3.15 d-f	2.55 fg	3.27 ab	11.25 a	1.80 a	0.45 a	0.45 a	2.55 a	3.30 a
Means	3.65a	3.18 a	3.35 a	3.55 a	3.15 a		5.66 a	1.45 a	1.75 a	2.40 a	2.60 a	
LSD at 5%	S= 1.00*		R= NS		S × R =0.92***		S= 5.39 NS		R= NS		S × R = NS	

NS, *, **, *: non-significant, and significant at p= 0.05, 0.01 and 0.001 respectively.**

Values in the same column not followed by the same letter are significantly different at the 5% level of probability.

At 4000 ppm salinity stress, 20 kr gamma, 720 and 850 nm laser decreased significantly peroxidase activity (2.85, 3.15 and 2.55) compared to non-irradiated plants (4.2).

These results are in line with the results in plants at 2000 ppm due to natural acclimation and defense mechanism during stress (Thorat *et al.*, 2024)

Plants are severely impacted by salinity due to the production of reactive oxygen species (ROS) that cause cellular and molecular damage. By closing stomata, it impairs photosynthetic activity. Additionally, it degrades chlorophyll and induces membrane lipid peroxidation, which alters the fluidity and selectivity of the membrane (Munns and Gilliham, 2015).

Thorat *et al*. (2024) reported that laser reduced the harmful effect following salt stress by lowering the antioxidant system's activity.

Induction of variation:

Two salt-tolerant mutants were obtained in the second generation. The mutant 1 (M1) was found from the treatment of 20 Kr gamma rays coupled with 2000 ppm saline water. The mutant 2 (M2) was obtained from the treatments of 850 nm laser coupled with 2000 ppm saline water. The two mutations were superior in most of the traits that were studied (plant height, stem diameter, number of branches, fresh and dry weights of vegetative growth, length and fresh weight of roots, number of umbels per plant, fruits weight, potassium content, oil yield per plant) compared to C2000 that non-irradiated plants and irrigated with the same saline water (2000 ppm) as shown in Table (8). While they have decreased proline content, catalase, and peroxidase activity. Mutant 1 has sodium content similar to that of the control plant (untreated with salinity or radiation).

Salinity-tolerant plants have less content of proline compared to control (Khatiyar *et al*., 2022).

Using mutagenesis, Uddin *et al*. (2007) on rice also produced lines that were resistant to salt. According to Gao *et al.* (2016), pre-illumination with a He-Ne laser increased the salt tolerance of tall fescue seedlings by upregulating the expression levels of many genes related to antioxidant enzymes and the phytochrome B gene.

Laser produced aberration in the mitotic cell division as stickiness, bridge, non-disjunction, laggards, binucleate, polyploidy and nuclear polymorphism, these findings produced changes in germination and plant growth (Al Aboud, 2023).

Previous studies on gamma radiation have already documented its beneficial benefits, which include increased plant germination growth rate, cell division, enzymatic activity, stress tolerance, and production of mutations. (Moemen, 2012). Khatiyar *et al.* (2022) reported that 496 mutant strains of *Oryza sativa* were produced from gamma ray treatments. These mutants were abiotic stress tolerant and improved growth and yield. The two mutants have less proline accumulation compared to non-irradiated plants. These results are in the line with the findings of Munns and Tester (2008). Also have less content of enzyme activity. This need to more studies to obtain the mechanism of their resistance.

Genetic marker:

ISSR finger printing:

Inter simple sequence repeat marker was used to identify the two *Foeniculum* mutants found in the M2 generation as a salt tolerant and control plants.

Mutant 1 is a plant obtained from the treatment of gamma rays at 20 Kr and irrigated with saline water at 2000 ppm.

Mutant 2 is a plant obtained from the treatment of laser at 850 wavelength and also irrigated with saline water at 2000 ppm.

Four primers were used. A total of 5l amplified bands were found with sizes ranging from 950 to 1650 base pairs (bp), bands were polymorphic with 67.25% polymorphism as shown in Table (9).

Table 8. Comparison of the two mutants developed from gamma, laser rays and salt stress with untreated fennel plants.

Traits	C	C2000	M 1	M 2
Plant height (cm)	62.6	50.0	100	96
Stem diameter (cm)	0.35	0.26	0.8	0.5
No. of branches	8.0	8.0	9.0	6.0
Fresh weight of vegetative growth (g)	19.6	18.7	29.1	40.46
Dry weight of vegetative growth (g)	3.55	2.73	9.63	7.7
Root length (cm)	27.1	21.7	22	20.3
Root fresh weight (g)	5.7	4.7	8.04	7.17
No, of umbels	7.0	5.66	9.0	7.0
Fruits weight (g)	1.2	0.9	1.5	2.5
Sodium content (ppm)	178.3	222.33	180	225
Potassium content (ppm)	33.33	19.33	20	27
Proline (mg g^{-1} dry matter)	0.63	0.93	0.5	0.63
Oil percentage %	1.6	1.1	1.3	1.0
Peroxidase activity [unit min^{-1} g^{-1}fresh matter]	2.1	4.65	0.26	0.18
Catalase activity [unit min^{-1} g^{-1}fresh matter]	0.35	5.40	0.09	0.11

C: control (untreated plants), C2000: the treatment of 0.0 radiation coupled with 2000 ppm saline water, M1: mutant 1 (20 Kr gamma rays coupled with 2000 ppm saline water) and M2: mutant 2 (850 nm laser coupled with 2000 ppm saline water).

Table 9. Number of amplified bands, number of polymorphic bands and polymorphism % detected by ISSR marker in the M_2 generation of fennel (*Foeniculum vulgare*) plants treated by gamma, laser rays and/or salt stress.

ISSR primer code	Number of amplified bands	Number of polymorphic bands	Polymorphism %
Primer 1	14	11	78.57
Primer 2	14	13	92.85
Primer 3	9	3	33.33
Primer 4	14	9	64.28
Total	51	36	---
$\bar{x}$	12.75	9.0	67.25

The primer3 (HB-9) gave the lowest number of amplified band (9) and polymorphic band (3) with the 33.33% polymorphism as shown in Table (10).

On the other hand, all the other primers gave the same number of amplified bands (14) but differ from each other in the number of polymorphic bands (11, 13 and 9 for primer1, primer2 and primer4 respectively).

Fig. (1) indicated that the primer 49 A generated three PCR bands (DNA fragment) occurred in all mutants and control plant, these bands are species-specific band, which identifies the species of *Foeniculum*. The primer 44B generated one species-specific band. The primer HB-9 gave five bands, but primer HB-11 gave four species-specific bands.

The bands 1650, 425, 275, 220 and 125 bp were found only in the mutant 2 using primer 49A and only one band 350 and 375 bp using the primer 44B, HB-9 respectively. While primer HB-11 presented five polymorphic bands (1000, 325, 220 and 950 base pair designated for mutant 2 only. So, they can used to identify this mutant.

Mutant 1 can be identified by the unique bands (950,800, bp using primer 49A, 800, 375 and 200 bp bands using primer 44B and 125 bp band using primer HB-11 while the primer HB-9 did not give any extra band designated to mutant 1.

Table 10. Genetic similarity between control and mutants of fennel (*Foeniculum vulgare*) plants obtained by gamma, laser rays and/or salt stress using ISSR marker with four primers.

Mutants	Primer 49A	Primer 44B	Primer HB-9	Primer HB-11	Average genetic distance
Control × Mutant 1	12.0	50.0	6.7	12.5	20.3
Control × Mutant 2	47.0	80.0	20.0	31.9	44.7
Mutant 1× Mutant 2	46.0	50.0	12.5	47.4	39.0

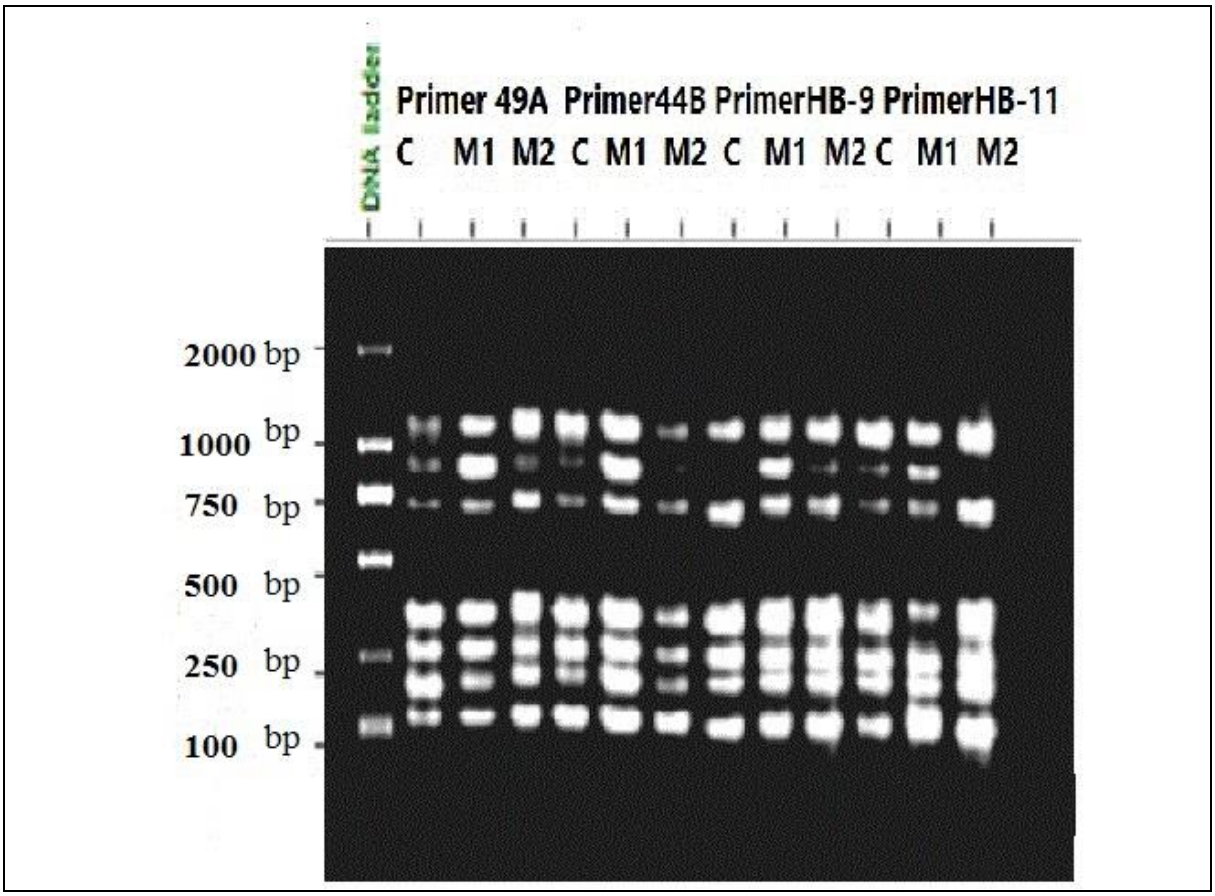

Fig. 1. ISSR genetic marker using four primers (Primer 49A, Primer 44B, Primer HB-9 and Primer HB-11) for M_2-generation of fennel (*Foeniculum vulgare*) treated with gamma and laser rays and grown under salt stress; C: control, M1: mutant 1, M2: mutant 2.

On the contrary, some bands disappeared from mutants compared to control the band 925 bp disappeared from the mutant 1, the bands 250, 200 and 100 bp disappeared from that mutant 2 using the primer 49A .

The bands 1400, 350 and 275 bp disappeared from mutant 2 and the bands 220 and 125 disappeared from the two mutants compared to control using the primer 44B.

One band (325 bp) disappeared from mutant 2 using primer HB-9. The band 800 bp disappeared from the two mutants using the primer HB-11.

These bands can be identified as the mutants. Genetic distance in Table (10) and phylogenetic tree (Fig., 2) indicated that, mutant 2 was more genetically distinct from the control with 44.7 genetic distance. The mutant 1 was less genetically distant from the control with a 20.3 genetic distance and constructed in one group.

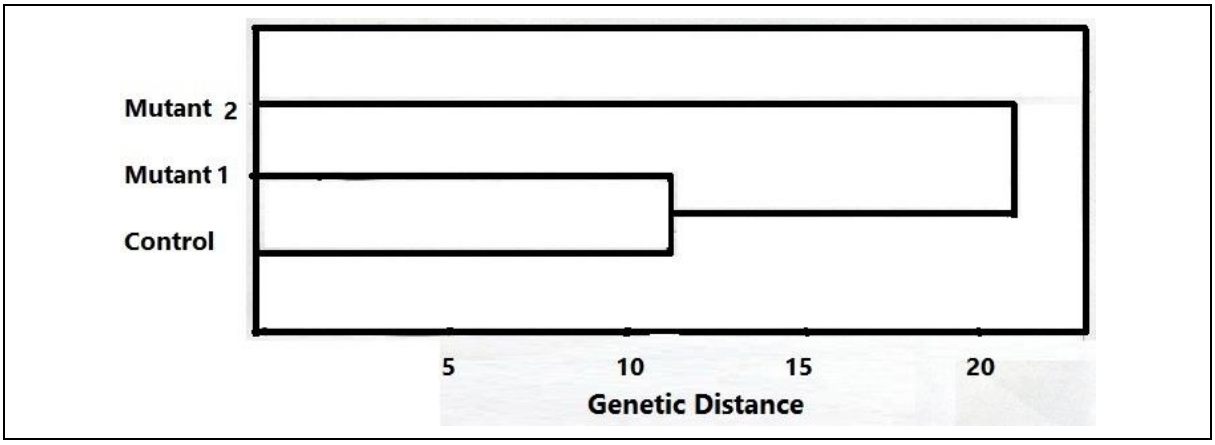

Fig. 2. Tree diagram for M_2 generation of fennel (*Foeniculum vulgare*) plants treated by gamma, laser rays and/or salt stress using ISSR marker with four primer.

These results refer to the mutant effect of laser and gamma rays.

RAPD marker:

Random amplified polymorphic DNA marker was also used to identify the two *Foeniculum* mutants and control plants using four primers. The results revealed that a total of 70 amplified bands were found with sizes ranging from 100 to 1000 base pairs (bp), 65 bands were polymorphic with 93.3% polymorphism as shown in Table (11).

The primer 4 gave the highest polymorphism percentage (100) generated from 16 amplified bands, while primer 2 gave the highest number of amplified and polymorphic bands (22 and 19 respectively) with 86.3% of polymorphism %.

Some bands disappeared from mutants compared to control plants and others generated only on each of the mutants and disappeared from control plants using all primers these bands can used to distinguish the mutant plant (Fig., 3).

Genetic distance in Table (12) and phylogenetic tree Fig. (4) referred to that, the two mutants were differed genetically from control plant and constructed in one group. The mutant 2 was more genetically different from control compared to mutant one with a genetic distance 73.3 and 58.6 and an average similarity value of 26.7 and 44.3 respectively.

These results obtained that RAPD and ISSR markers can used to identify mutants of *foeniculum vulgare* .

Gamma ray is a mutagenic agent. It creates many DNA aberrations, chromosomal breakage, deletion substitution, and rearrangement. So, it can develop plant tolerance to abiotic and biotic stress (Song *et al*., 2012).

The laser is a mutagenic agent that caused aberration in cell division (Al Aboud, 2023). It enhanced salt tolerance probably via interaction between resistance gene expression and cell wall (Gao *et al*., 2016). ISSR markers have investigated the influence of laser on moringa. It showed the appearance and/or absence of some bands. ISSR markers are used as a very sensitive method to distinguish the variability produced after laser treatments (Osman and Rayan, 2020). RAPD and ISSR markers are important in evaluating genetic relationships (El-Sherif *et al*., 2019 and Harb *et al*., 2019).

CONCLUSION

This study looked at how physical mutations, such as laser and gamma rays, affected the yield, vegetative growth, and chemical composition of fennel plants growing under salt stress. Additionally, it aimed to research their capacity to generate salt-tolerant mutants and assess the genetic variations using RAPD and ISSR markers.

Table 11. Number of amplified bands, number of polymorphic bands and polymorphism % detected by RAPD marker in the M_2 generation of fennel (*Foeniculum vulgare*) plants treated by gamma, laser rays and/or salt stress.

RAPD primer code	Number of amplified band	Number of polymorphic band	Polymorphism %
Primer 1	18	17	94.4
Primer 2	22	19	86.3
Primer 3	14	13	92.8
Primer 4	16	16	100
Total	70	65	
$\bar{x}$	17.5	16.25	93.3

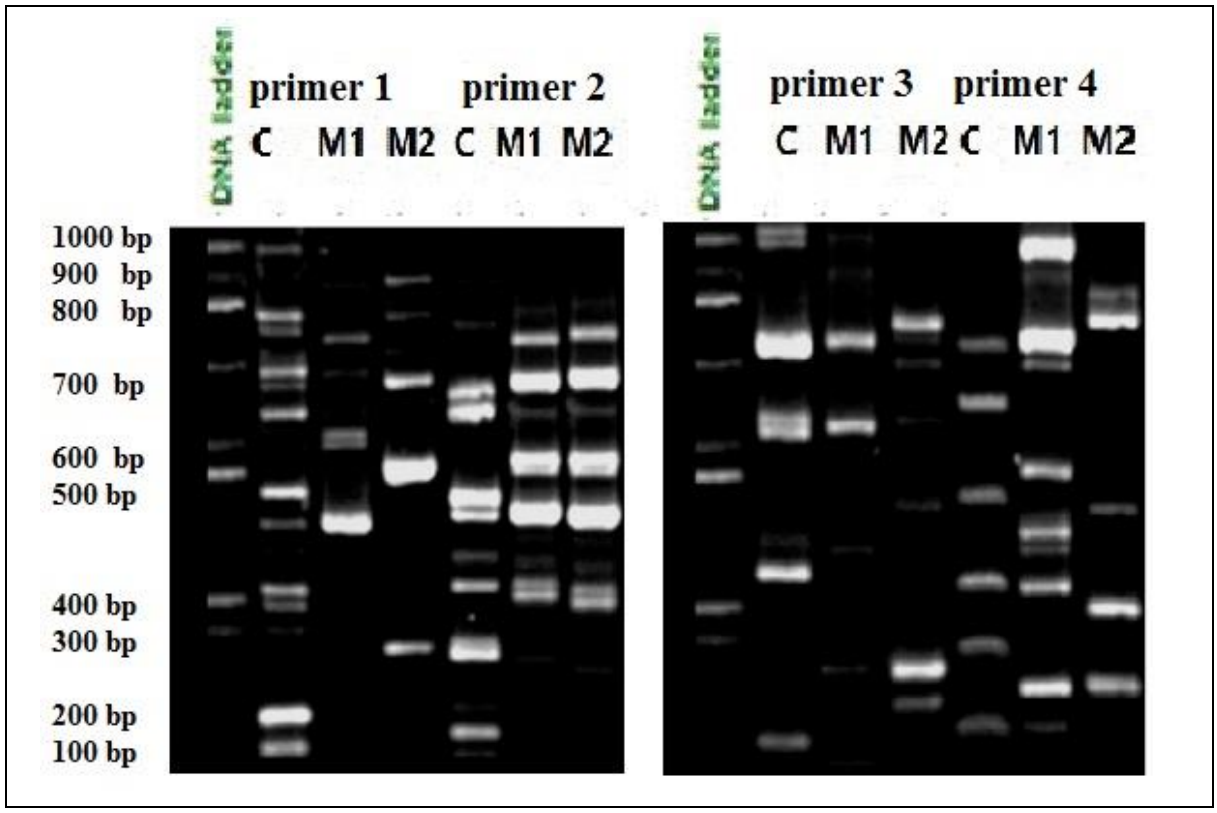

Fig. 3. RAPD genetic marker using four primers for M_2-generation of Fennel (*Foeniculum vulgare*) treated with gamma and laser rays and grown under salt stress; C: control, M1: mutant 1, M2: mutant 2.

Table 12. Genetic distance between control and mutants of fennel (*Foeniculum vulgare*) plants obtained by gamma, laser rays and/or salt stress using RAPD marker with four primers.

Mutants	Primer 1	Primer 2	Primer 3	Primer 4	Average genetic distance
Control × Mutant 1	54.6	60.0	57.2	62.5	58.6
Control × Mutant 2	64.8	75.0	71.5	81.8	73.3
Mutant 1× Mutant 2	84.6	21.8	40.0	76.5	55.7

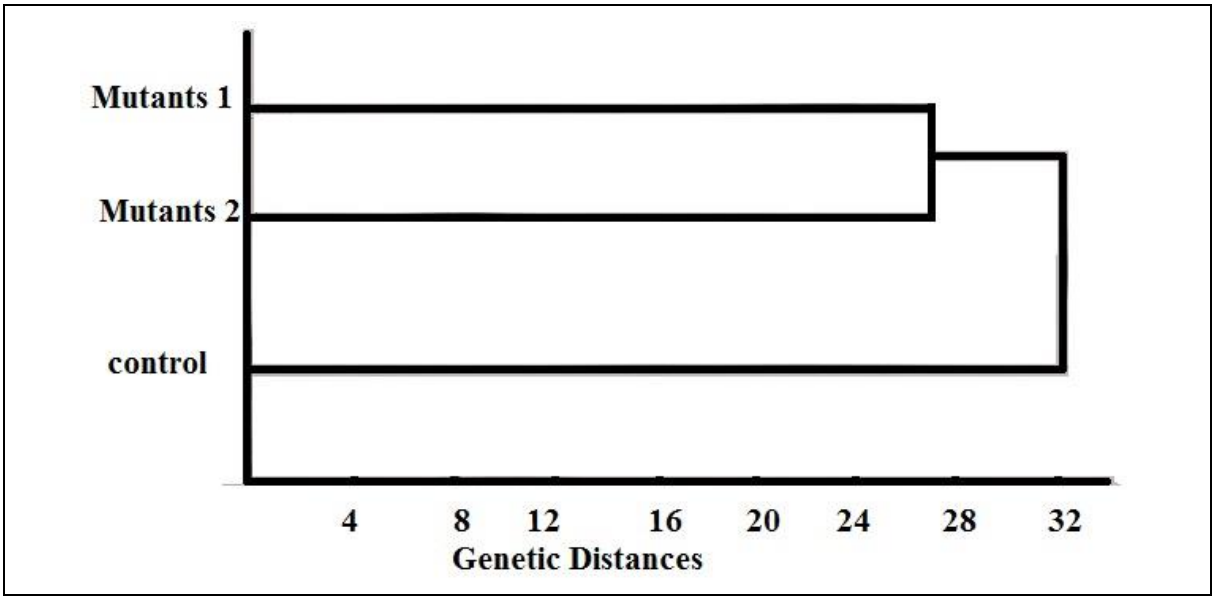

Fig. 4. Based on the ISSR profile obtained from four RAPD primers, a tree diagram was created for the M2-generation of fennel (*Foeniculum vulgare*) plants that were cultivated under salt stress and treated with gamma and laser rays.

Results indicated that both laser treatments at 720 and 850 nm and gamma rays especially at 20 Kr enhanced fennel plant growth and induced two mutants that were more tolerant to salinity. Both RAPD and ISSR markers were used successfully to identify mutants from the control plant. Further investigation is necessary to comprehend the mechanism underlying the effects of laser and gamma rays on improving fennel plant growth and yield under salt stress.

REFERENCES

Abou-Dahab, M.A.D.; Mohammed, T.A.; Heikal, A.A.; Taha, L.S.; Gaber, A.M.M.; Metwally, S.A. and Ali, A.I.R. (2019). In vitro laser radiation induces mutation and growth in *Eustoma grandiflorum* plant. Bull. Natl. Res. Cent., 43(3):1-13.

Aebi, H. (1984). Catalase *in vitro*. Methods in Enzymology, 105:121-126.

Al Aboud, N.M. (2023). Plant genetics a view on the effect of laser irradiation on cell division. J. Plant Sci. Res., 10(1):237-247.

Aladjadjiyan, A. (2012). Physical Factors for Plant Growth Stimulation Improve Food Quality. Food Production - Approaches, Challenges and Tasks. Plovdiv, Bulgaria, 270 p.

Bates, L.S.; Waldren, R.P. and Tear, L.D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39:205-207.

Cansev, A. and Ozgur, M. (2010). Grafting cucumber seedlings on *Cucurbita spp*.: comparison of different grafting methods, scions and their performance. J. Food Agric. Environ., 8:804-809.

Chahal, G.S. and Gosal, S.S. (2002). Principles and Procedures of Plant Breeding: Biotechnology and Conventional Approaches. Alpha Science International, UK, 604 p.

Chen, Y.P.; Yuea, M. and Wang, X.L. (2005). Influence of He-Ne laser irradiation on seeds thermodynamic parameters and seedlings growth of *Isatis indogoitica*. Plant Sci., 168:601-606.

Chen, Z.; Cuin, A.; Zho, M.; Twomey, A.; Naidu, B.P. and Shabala, S. (2007). Compatible solute accumulation and stress mitigating effects in barley genotypes contrasting in their salt tolerance. J. Exp. Bot., 58:4245-4255.

Cottenie, A.; Verloo, M.; Kiekns, L.; Velghe, G. and Comer-lynek, R. (1982). Chemical analysis of plants and soil. Laboratory of Analytical and Agrochemistry, State University, Ghent, Belgium, 63 p.

Cwintal, M. and Dziwulska-Hunek, A. (2013). Effect of electromagnetic stimulation of alfalfa seeds. Int. Agrophys., 27:391-401.

Cwintal, M.; Dziwulska-Hunek, A. and Wilczek, M. (2010). Laser stimulation effect of seeds on quality of alfalfa. Int. Agrophys., 24:15-19.

El-Sherif, F.; Yap, Y.K.; Ibrahim, H.I. (2019). Laser irradiation induces DNA polymorphism and alters phytochemicals compositions as well as growth and yield of *Curcuma longa*. J. Dis. Med. Plants, 5:29-38.

EPA (2021). Seasonality and Climate Change: A Review of Observed Evidence in the United States. U.S. Environmental Protection Agency, USA, 43 p.

Farajpour, M.; Ebrahimi, M.; Amiri, R.; Sadat-Noori, S.H.; Sanjari, S. and Golzari, R., (2011). Study of genetic variation in yarrow using inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. Afr. J. Biotechnology, 54:11137-11141.

Gad, A.M.; El-Dakhakhny, M. and Hassan, M.M. (1963). Studies on the chemical constitution of Egyptian *Nigella sativa*, L. oil. Planta Medica, 11:134-138.

Gao, L.M.; Li, Y.F. and Han, R. (2015). He-Ne laser preillumination improves the resistance of tall fescue (*Festuca arundinacea* Schreb.) seedlings to high saline conditions. Protoplasma, 252:1135-1148

Gao, L.; Li, Y. and Han, R. (2016). Cell wall reconstruction and DNA damage repair play a key role in the improved salt tolerance effects of He-Ne laser irradiation in tall fescue seedlings. Biosci. Biotechnol. Biochem., 80(4):682-693.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research. John Wiley and Sons, New York, USA, 680 p.

Grover, S.; Jakhar, M.L. and Malik, C.P. (2011). Genetic diversity of different varieties of *Foeniculum vulgare* Miller by RAPD markers. Archives of Applied Science Research, 3(5):17-25.

Harb, A.H.; Abu El-Maaty, S.; Drawish, D.S.; Shrief, S.A. and Khater, M.S. (2019). Phenotypic and molecular characterization of M3 lentil lines selected from laser and gamma irradiated Egyptian cultivars. Bioscience Research, 1:337-348.

Jackson, W.T. and Stetler D.A. (1973). Regulation of mitosis, IV. An *in vitro* and ultrastructural study of effects of trifluralin. Canadian Journal of Botany, 51:1513-1518

Jan, S.; Parween, T. and Siddiqi, T.O. (2011). Effect of gamma radiation on morphological, biochemical, and physiological aspects of plants and plant products. Environ. Reviews, 20:17-39.

Khatiyar, P.; Pandey, N. and Keshavkan, S. (2022). Gamma radiation: A potential tool for abiotic stress mitigation and management of agroecosystem. Plant Stress, 5:1-11. https://doi.org/10.1016/j.stress.2022.100089

Khan, M. and Musharaf, S. (2014). *Foeniculum vulgare* Mill. a medicinal herb, A Review, Medicinal Plant Research, 4(6):46-54.

Klapheck, S.; Zimmer, I. and Cosse, H. (1990). Scavenging of hydrogen peroxide in endosperm of *Ricinus communis* by ascorbate peroxidase. Plant Cell Physiol., 31:1005-1013.

Ling, A.P.K.; Chia, J.Y.; Hussein, S. and Harun, A.R. (2008). Physiological responses of *Citrus sinensis* to gamma irradiation. World Appl. Sci. J., 5:12-19

Maehly, A.C. and Chance, B. (1954). The assay of catalase and peroxidase. In: Glick, D. (ed.), Methods of Biochemical Analysis, Vol. 1, Interscience Publishers, Inc., USA, pp. 358-424.

Moemen, A.M.A.H. (2012). Effect of Gamma Rays and Salinity on Growth and Chemical Composition of *Ambrosia maritima* L. Plant. M.Sc. Thesis, Fac. Agric., Cairo University, Egypt, 159 p.

Moura, L.S.; Raul, N.; Carvalho, J.; Mirian, B.; Lin, C. and Angela, A. (2005). Supercritical fluid extraction from fennel (*Foeniculu mvulgare*), global yield, composition and kinetic data. J. Supercritical Fluid, 35:212-219.

Mukherjee, S.P. and Choudhuri, M.A. (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiologia Plantarum, 58:166-170.

Munns, R. and Gilliham, M. (2015). Salinity tolerance of crops, what is the cost? New Phytologist., 208:668-673.

Okla, M.K.; Abdel-Mawgoud, M.; Alamri, S.A.; Abbas; Z.K.; Al-Qahtani, W.H.; Al-Qahtani, S.M. and Abdelgawad, H. (2021). Developmental stages-specific response of anise plants to laser-induced growth, nutrients accumulation, and essential oil metabolism. Plants, 10(12):1-16. https://doi.org/10.3390/plants10122591

Osman, A.S. and Rayan A.W. (2020). The influence of He-Ne laser on agro-morphological criteria, ISSR marker and SDS-PAGE of *Moringa oleifera*. Bulletin of the National Research Centre, 44(3):1-11. https://doi.org/10.1186/s42269-019-0211-x

Podleśna, A.; Gładyszewska, B.; Podleśny, J. and Zgrajka, W. (2015). Changes in the germination process and growth of pea in effect of laser seed irradiation. Int. Agrophys., 29(4):485-92.

British Pharmacopeia (1963). Determination of Volatile Oil in Drugs. The Pharmaceutical Press, London, UK, 1210 p.

Qiu, Z.; Li, J.; Zhang, M.; Bi, Z. and Li, Z. (2013). He–Ne laser pretreatment protects wheat seedlings against cadmium-induced oxidative stress. Ecotoxicol. Environ. Saf., 88:135-141.

Ramadan, W.R.; Shoaib, R.M.; Ali, R.A. and Abdel-Samea, N.S. (2019). Assessment of genetic diversity among some fennel cultivars (*Foeniculum vulgare* Mill.) by ISSR and SCoT Markers. African J. Biol. Sci., 15(1):219-234.

Sarla, G.S. (2019). Saunf: do we really need fennel seeds after a meal?. Journal of Medical Surgical Nursing Practice and Research, 2(1):5-8.

Song, J.Y.; Kim, D.S.; Lee, M.C.; Lee, K.J.; Kim, J.B. and Kim, S.H. (2012). Physiological characterization of gamma-ray induced salt tolerant rice mutants. Aust. J. Crop Sci., 6:421-429

Thorat, S.A.; Srivaishnavi, M.; Kaniyassery, A.; Chandrashekar, K.H.; Mahato, K.K. and Muthusamy, A. (2024). He-Ne laser pre-exposure alleviates salinity stress through physio-biochemical regulation and enhanced withanolide production in *Withania somnifera* (L.) Dunal. Industrial Crops and Products. 216:1-11. https://doi.org/10.1016/j.indcrop.2024.118776

Uddin, M.D.I.; Rashid, M.D.H.; Khan, N.; Perveen, M.S.T.F.; Tai, T.H. and Tanaka, K. (2007). Selection of promising salt tolerant rice mutants derived from cultivar Drew and their antioxidant enzymes activity under salt stress. J. of Breed. and Genet., 39(2):89-98.

Verma, A.K.; Prasad, K.V.; Singh, S.K. and Kumar, S. (2012). *In vitro* isolation of red coloured mutant from chimeric ray florets of chrysanthemum induced by gamma-ray. Indian J. Horticulture, 69:562-567.

Verslues, P.E. and Sharma, S. (2010). Proline Metabolism and Its Implications for Plant-Environment Interaction. Arabidopsis Book, 8:1-23. https://doi.org/10.1199/tab.0140

Wilczek, M.; Koper, R.; Cwintal, M. and Kowalska, K.T. (2004). Germination capacity and the health status of red clover seeds following laser treatment. Int. Agrophys., 18:289-294.

Wilczek, M.; Koper, R.; Cwintal, M.; Kowalska, K.T.（2005). Germination capacity and health status of hybrid alfalfa seeds after laser treatment. Int. Agrophys., 19:257-261.

Yadava, U.L. (1986). A rapid and nondestructive method to determine chlorophyll in intact leaves. HortScience, 21:1148-1150.

Zörb, C.; Schmitt, S.; Neeb, A.; Karl, S.; Linder, M. and Schubert, S. (2004). The biochemical reaction of maize (*Zea mays* L.) to salt stress is characterized by a mitigation of symptoms and not by a specific adaptation. Plant Sci., 167:91-100.

تعزيز تحمل نبات الشمر للاجهاد الملحى باستخدام أشعة جاما والليزر وتقييم الطفرات بواسطة المعلمات الجزيئية ISSR و RAPD

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تتسبب الملوحة فى انخفاض نمو النبات وإنتاجيته، وتزداد مشكلة الملوحة مع تفاقم تأثير التغيرات المناخية. تهدف هذه الدراسة إلى دراسة تأثير الطفرات الفيزيائية، أشعة الليزر وأشعة جاما، على النمو الخضري والمحصول والمكونات الكيميائية لنباتات الشمر المزروعة تحت ظروف الإجهاد الملحي، وقدرتها على إحداث طفرات متحملة للملوحة. تم معاملة بذور الشمر بأشعة جاما (١٠ و ٢٠ كيلو راد) والليزر (٧٢٠ و ٨٥٠ نانومتر). رويت النباتات بمياه مالحة عند ٢٣٨ جزء في المليون كنباتات كنترول، ٢٠٠٠ و ٤٠٠٠ جزء في المليون. كلا نوعي الإشعاع وخاصة أشعة جاما عند ٢٠ والليزر عند ٧٢٠ و ٨٥٠ نانومتر ادى الى زيادة نمو النباتات المروية بمياه مالحة عند ٢٠٠٠ أو ٤٠٠٠ جزء في المليون زيادة معنوية . تم العثور على نباتين متفوقين فى نموهم تحت الري بماء مالح بتركيز ٢٠٠٠ جزء في المليون، أحدهما ناتج من أشعة جاما ٢٠ كيلو راد والآخر من أشعة الليزر ٨٥٠ نانومتر. استخدمت المعلمات الجزيئية ISSR و RAPD بنجاح فى تمييز الطفرتين عن الكنترول. كانت الطفرة ٢ أكثر بعد وراثي عن الكنترول.