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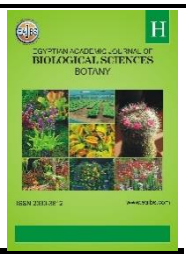

Gamma Radiation as A Mutagenic Agent for Salinity Tolerance Improvement in Some Sweet Sorghum, (*Sorghum bicolor* L.) Genotypes

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ABSTRACT

Salinity is the most common abiotic stress against cultivated crops in the world. Therefore, there are several ways to find out a possible solution to this problem. Mutation breeding approach is one of these ways to elicit salinity tolerant varieties through the induction of beneficial mutations by applying mutagenic agents, either chemically or by radiation. The current study was conducted to apply gamma radiation as a mutagenic agent to induce genetic variation in sorghum plants which can produce different genotypes and give the opportunity to initiate some salinity tolerant genotypes. To proceed with such an experiment, the seeds of four genotypes of sweet sorghum namely., GK Ahron, Brands, Tracy, and Rex were grown under four salt concentrations (control, 3000, 4000, and 5000 ppm) after irradiation by 300 Gy of gamma rays compared with untreated ones at different developmental stages morphologically, biochemically, and by molecular genetic analysis. The results showed that the genotypes GK Ahron, Brands, and Rex had a positive response to salinity tolerance accompanied by improvement in some studied characteristics. Non-Radiated Tracy has died at a salt concentration of 5000 ppm. From the present investigation, it could be recognized that Rex a halophyte plant because of is tolerant to salinity and had the highest quality and yield assessments. On the other hand, Tracy was a glycophytic one that had the lowest ones. All of the examined parameters decreased with increasing salt concentrations, except for proline. Rex genotype was the most responsive to radiation and salinity. The radiated genotypes and non-radiated genotypes have different banding patterns. Therefore, in this study, the possibility of creating beneficial salinity tolerance mutations on sweet sorghum crops occurred by using gamma rays as well as other crops which could be helpful in the salinity breeding program in sweet sorghum.

INTRODUCTION

Sorghum is one of the most important and multipurpose cereal crops and ranked fifth after wheat, rice, maize, and barley. Being a multipurpose crop, it represents various types

of grains, and forage (Berenji, Dahlberg, *et al.,* 2004). Sweet sorghum grains and leaves are very rich in vitamins and minerals, Basic vitamin B consists of thiamin, riboflavin, pyridoxine and phosphorus, potassium, zinc, and iron (Aggarwal, Chand, *et al.,* 2020). So, the sweet sorghum is important and has few fertilizers needs, water, and labor as compared to other crops like sugarcane, rice and maize. Additionally, it has a powerful ability to overcome the effect of adverse climatic conditions during its vegetative stage and can resume its reproductive stage thereafter (Nguyen, *et al.,* 2013, Salama *et al.,* 2020**).** Sweet sorghum as a C4 plant, has high photosynthetic efficiency as well as high carbohydrate production capacity compared to the C3 plant. Subsequently, it is very beneficial in many ways because its leaves and stem can be used such as animal food, juice, and their sugar as a source of bioethanol production as organic fuel and their dregs can be utilized as a raw material for paper making industry (Guigou *et al.,* 2011, Rohowsky *et al.,* 2013). One of the most common reasons for crop failure is soil salinization which may worsen because of incorrect human activity, resulting in severe ecological consequences (Tao *et al.,* 2020). More than 6% of the world's cultivated lands are suffering from salinity and 20% of irrigated lands are salt-affected (Wang, Xu *et al.,* 2015, Yuan, Druzhinina *et al.,* 2016). Salinity as essentially abiotic stress has many effects on crop plants' growth and development from seed germination to harvest. In the last years, augmentation of harmful effects on agricultural productivity has been observed especially in arid and semiarid regions where rainfall is low and evaporation transpiration is high (Jha 2019, Mahpara *et al.,* 2022). Excessive accumulation of salts in the rhizosphere restricts the absorbance of water and nutrients by plant roots. In addition, salinity stress inhibits seed germination, plant growth, and different physiological effects and biochemical reactions in plants (Ahanger *et al.,* 2020).

Plants become acclimatized to salinity by using different mechanisms. Angiosperms plants (Magnoliophyta) are classified into two major groups including glycophytes (intolerant to salinity) and halophytes (salt tolerant) which can survive even under high salt concentrations (Atkinson and Urwin 2012). Most of the field crops belong to the glycophytic group like rice (*Oryza sativa*), wheat (*Triticumaestivum*), maize (*Zea mays*), etc. However, the adaptation strategies to respond to salinity stress are similar regardless of whether these are glycophytes or halophytes (Yokoi *et al.,* 2002). Many plants accumulate high levels of free proline in response to osmotic stress. This amino acid is widely believed to function as a protector or stabilizer of enzymes or membrane structures that are sensitive to dehydration or ionically induced damage. Several investigations have shown that, besides other solutes, the level of free amino acids, especially proline, increases during adaptation to various environmental stresses (Almodares and Hatamipour 2011). Plant breeders want to expand the genetic variation of sorghum to improve grain and crop yields. Natural genetic variation is created *via* spontaneous mutation. However, the frequency of natural mutation is very slow, varying from 5-10 and 8-10 years per loci in higher plants (Jiang, Palma *et al.,* 2010). Inducted genetic variation using physical and chemical mutagens is an alternative method of producing the new genetic variation to tolerant salinity and other factors. Different mutagenic agents have a variable effect on mutations. For example, physical mutagens such as gamma irradiation result in deletion, translocation, and aberrations of chromosomes (Shu *et al.,* 2012). Other than that, if it needs to succeed in inducing genetic variation using mutagenic agents, we must choose the source and know how to apply the dose (Sevanthi *et al.,* 2018**).** Gamma radiation is widely used as a mutagenic agent to create genetic variation in plant breeding programs (Wolabu, Zhang *et al.,* 2016). So, the objective of this investigation was the study of growth, quality, yield parameters, proline free and molecular genetic variations of four gamma-radiated sweet sorghum genotypes under four different levels of salinity stress.

MATERIALS AND METHODS

The experiments of this study were carried out in Giza Agricultural Research Station, Cairo, Egypt, four sweet sorghum genotypes, i.e., Tracy, Brands, Rex, and GK Ahron obtained from Sugar Crops Research Institute, Agricultural Research Center to try to induct the mutation associated to salt tolerance by exposed the seeds to 300 Gy of gamma-ray exposure at the Egyptian Atomic Energy Authority (EAEA).

Planting Experiment:

Irradiated and non-Genotypes were sowing in pots (45 cm diameter) containing the steam-sterilized sandy loam. The soil was mixed with pure sand in a ratio of 2:1 in 5-liter clay pots in the 16th June 2019 and 2020 seasons. For each radiated and non-radiated genotype following parameters were assessed at harvest for the irradiated and control genotypes: were replicated three times. Three factors were studied, i.e., genotypes, radiation, and salt concentrations. The pots were irrigated with three salt concentrations (3000, 4000, and 5000 ppm) and tap water as control. Fertilizers (N.P.K) were applied as recommended.

Growth Parameters and Quality Assessmentsr:

Three growth parameters were used, i.e., plant height, node number per plant, and tillers number per plant. TSS% was recorded using a hand refractometer (Carl Zeiss JENA) after harvest. Sucrose percentage was determined by using the direct polarization method as described by (Chemists and Horwitz 1975**).** Purity % was determined as follows:

$$\text{Purity\%} = \frac{\text{Sucrose \%}}{\text{TSS\%}} \times 100$$

Yield Parameters:

At harvest, stem, leaves, sugar, and ethanol yields per feed were detected. Ethanol yield was calculated by the equal equation of (Lipinsky 1978). Ethanol yield= sucrose % × 6.5× 0.85 × fresh biomass. Whereas: 6.5 = convert index, 0.85 = producing an index, Fresh biomass = tons per fed)

Free Proline as A Biochemical Marker:

Proline was determined according to the method of (Bates, Waldren *et al.,* 1973). Leaf's proline content was determined after 60 DAP (Day after planting) and at harvest. Fresh leaves (0.5g each sample) were homogenized in 5ml of 3% aqueous sulfosalicylic acid. The homogenate was centrifuged at 9000 rpm for 15 minutes then a 2 ml aliquot of the supernatant was mixed with an equal volume of 2ml acetic acid with 2ml acid ninhydrin and then was incubated for 1h at 100° C. The reaction was terminated in an ice bath and extracted with 4 ml of toluene. The extract was vortexed for 20 seconds. The chromatophores containing toluene were then aspirated from the aqueous phase and its absorbance was determined spectrophotometrically at 250nm **(**Beckman 640 D.USA**)** using toluene as a blank.

Molecular Genetic Variation:

Five Inter-specific sequence repeat (ISSR) primers were used. DNA samples were isolated from treated and untreated verities under different levels of salinity. DNA was amplified according to the following protocol. Each PCR reaction mix of 25 µL contained the 30-ng template DNA, 2.5 µL of 10X PCR buffer, 1.5 µL of 25mM MgCl2, 2.5 µL of the dNTPs mix, 30 pmol of ISSR primer, 1.0 U Taq DNA polymerase (Promega, WI, USA). The amplification reaction was performed in a thermal cycler (Applied Bio Systems, USA) programmed for initial denaturation of 5 min at 94°C; 40 cycles of 2 min denaturation at 94°C, 45 Sec. annealing at 50°C, and 2 min extension at 72°C; and final elongation step at 72°C for 7 min. The PCR products were electrophoresed on 1.5% agarose gel containing ethidium bromide 0.5µg/mL in TBE buffer for 2 h at 100 V. After electrophoresis, the gels were observed under a UV-trans illuminator, documented in Gel-Doc XR (Bio-Rad) and

photographed. The size of the amplicons was determined using a 100 bp DNA ladder plus. DNA was amplified according to (Saghai *et al.*, 1994) protocol.

Table 1. Primers sequences information of ISSR markers.

Serial No.	Primers name	Sequence (5′-3′)
1	G-3	GTGTGTGTGTGTGTGTYG
2	G-4	CGCGATAGATAGATAGATA
3	G-6	AGACAGACAGACAGACGC
4	G-7	GATAGATAGATAGATAGC
5	G-10	GACAGACAGACAGACAAT

Data Analysis for DNA:

The generated/ amplified bands were scored visually. The bands were scored as present (1) or absent (0) to create the binary data set. To estimate the genetic similarity, Jaccard's coefficient (Jaccard 1908) was used. A dendrogram was generated by cluster analysis using the non-weighted pair group method of the arithmetic averages (UPGMA) using the SPSS program V1.6.

Statistical Analysis:

The obtained data were subjected to statistical analysis using ANOVA for 3 ways Randomized Blocks. Significant differences among the means of different treatments were carried out by LSD (Least Significant Differences Test, 0.05 probabilities). Bartlett's test (Snedecor and William 1989) is used to examine the null hypothesis and homogeneity of variances for the two season's records. Thus, data from the two seasons were combined for analysis of variance (ANOVA) using MSTAT (Freed *et al.*, 1989).

RESULTS AND DISCUSSION

1. Effect of Salinity and γ-ray on Growth Parameters of The Irradiated and Non-Irradiated Four Sweet Sorghum Genotypes:

Results in Table (2) indicated that there were significant differences at $P \leq 0.05$ among genotypes. Whereas, the highest recorded value was for Tracy (87.52cm) and the lowest one was for Rex (75.04), In addition, there were no significant differences among the other three tested genotypes. These findings revealed that there were no significant differences between radiation (R-) and non-radiation (NR-) treatments. Also, the effect of salt concentrations on plant height appeared that the control treatment possessed the highest value (155.29 cm). Therefore, such character decreased with the increasing concentrations of salt. All the interactions between the two factors were significant, but the triple interaction appeared not significant. The interaction between genotype and radiation (G×T) illustrated that the highest value (98.0 cm) was for NR-Tracy but the lowest one was for NR-Rex (68.17cm). Findings in Table (2) emerged that there were significant differences among genotypes for node number, whereas, the highest recorded value was for Tracy (8.58 node plant^{-1}) and the lowest one was for Rex (6.21 node plant^{-1}). Effects of salt concentrations on node number showed that the control treatment had the highest value (9.96 node plant^{-1}). Such character decreased with the increased salt concentration, up to 5000 ppm (4.33 node plant^{-1}). All interactions owned significant differences except radiation treatments. Results indicated in Table (2) that there were significant differences among tillers' number of genotypes. Where the highest recorded value was for Tracy and Brands (4.93, 4.75 tiller plant^{-1}) respectively, and the lowest one was for GK Ahron (3.54 tiller plant^{-1}). Outcomes of salinity on tillers number plant^{-1} demonstrated that the control treatment had the highest

value (5.04 tiller plant^{-1}) and the last one was 5000 ppm (3.25 tiller plant^{-1}), however, with an increase in salt concentrations, such trait diminished.

Table 2: Effect of radiation, soil salinity treatments, and their interaction on growth parameters of sweet sorghum.

Genotypes (G)	Treatments (T)	Plant height					Nodes number					Tiller number				
		Concentrations of salt					Concentrations of salt					Concentrations of salt				
		Control	3000	4000	5000	**Mean**	Control	3000	4000	5000	**Mean**	Control	3000	4000	5000	**Mean**
Tracy	Non-radiation	163.33	81.00	49.67	-	98.00	14.00	6.33	5.00		8.44	5.67	5.33	4.33	-	5.11
	Radiation	159.67	62.50	57.00	29.00	77.04	14.33	6.33	7.00	4.00	7.92	5.33	4.67	4.33	4.67	4.75
Mean		161.50	71.75	53.33	29.00	87.52	14.17	6.33	6.00	4.00	8.18	5.50	5.00	4.33	4.67	4.93
GK Ahron	Non-radiation	140.67	79.67	43.00	39.33	75.67	9.00	6.33	5.00	5.67	6.50	4.00	2.33	3.33	4.33	3.50
	Radiation	146.00	87.33	41.00	32.00	76.58	9.33	7.00	6.00	4.33	6.67	3.67	3.00	4.33	3.33	3.58
Mean		143.33	83.50	42.00	35.67	76.13	9.17	6.67	5.50	5.00	6.58	3.83	2.67	3.83	3.83	3.54
Brands	Non-radiation	158.67	69.00	31.00	34.33	73.25	10.67	8.00	5.67	5.33	7.42	4.67	3.33	3.67	4.00	3.92
	Radiation	159.33	95.67	27.00	27.00	77.25	10.67	10.0	4.67	4.00	7.33	4.33	3.00	4.00	4.00	3.83
Mean		159.00	82.33	29.00	30.67	75.25	10.67	9.00	5.17	4.67	7.38	4.50	3.17	3.83	4.00	3.88
Rex	Non-radiation	157.00	63.00	30.67	22.00	68.17	6.00	6.33	6.00	6.33	6.17	6.33	5.67	4.33	3.33	4.92
	Radiation	157.67	95.67	50.00	24.33	81.92	5.67	7.33	7.00	5.00	6.25	6.33	6.33	3.33	2.33	4.58
Mean		157.33	79.33	40.33	23.17	75.04	5.83	6.83	6.50	5.67	6.21	6.33	6.00	3.83	2.83	4.75
T × C	Non-radiation	154.92	73.17	38.58	23.92	72.65	9.92	6.75	5.42	4.33	6.60	5.17	4.17	3.92	2.92	4.04
	Radiation	155.67	85.29	43.75	28.08	78.20	10.00	7.67	6.17	4.33	7.04	4.92	4.25	4.00	3.58	4.19
Mean		155.29	79.23	41.17	26.00	75.42	9.96	7.21	5.79	4.33	6.82	5.04	4.21	3.96	3.25	4.11
Genotype (G)		3.40					0.56					0.23				
Treatments (T)		nS					nS					nS				
Concentration (C)		3.33					0.55					0.23				
G × T		4.80					0.79					0.32				
G × C		6.66					1.10					0.45				
C × T		4.71					0.78					0.32				
G × T× C		9.41					1.55					0.64				

The present study was in a line with (Dong, Li *et al.*, 2017) who found that the irradiation dose was negatively correlated with the node number. (Syahruddin, Nur *et al.*, 2021) reported that mutant lines had a better character than the sorghum variety (Numbu) and the stable performance of sweet sorghum characters in various environments has a good performance in a specific environment under salinity. Surya and Soerantoh, (2006) stated that gamma irradiation in sweet sorghum lines gave a different response to Gamma irradiation, and all measured variables were significantly affected and the stability performance of sweet sorghum characters in various environments has a good performance in a specific environment.

2. Effect of Salinity and γ-ray on Quality Parameters Of The Four Sweet Sorghum Genotypes:

The results in Table 3 revealed that there were significant differences in genotype. The highest value was in Rex (9.69%), but the lowest one was for Tracy (8.45%). Outcomes stated that radiation had a lower sucrose % than non-radiation. There were significant differences among concentrations. The control concentration (9.68%) and 5000ppm (7.43%) owned the highest and lowest sucrose%, respectively. Consequently, sucrose% decreased with increasing salt concentrations. For all interactions, there were significant differences except for the interaction between T × C. Findings in Table (3) revealed substantial differences among all treatments and their interactions. Genotypes emphasized that Rex (15%) had genotype the greatest TSS% value, whereas Tracy (13.8%) had the lowest one in the tested seasons. In terms of effect salt concentrations on TSS%, the highest effect was at the control and the lowest was at 5000ppm. Therefore, the salt concentrations had the greatest inversely effect on TSS%, where the concentrations increased, and the TSS% decreased. Regarding radiation treatments, the radiated treatment was significantly greater than non-treatment. In summary, Rex gained the highest quality parameters and Tracy had the least one. Radiation had more significant influences than non-radiation. The present results are in agreement with (Almodares, Taheri et al. 2008) who reported that there were significant differences in Genotype, and sucrose content decreased with the raising of salt

concentrations. In this association of sucrose content with salt concentration, the buildup of soluble sugars inside plants has been commonly stated as a response to salinity (Gill *et al.*, 2001, Juan *et al.*, 2005).

Table 3: Effect of radiation, soil salinity treatments, and their interaction on quality parameters of sweet sorghum

Genotypes (G)	Treatments (T)	Sucrose%					T.S.S%					Purity%				
		Concentrations of salt					Concentrations of salt					Concentrations of salt				
		Control	3000	4000	5000	**Mean**	Control	3000	4000	5000	**Mean**	Control	3000	4000	5000	**Mean**
Tracy	Non-radiation	9.17	8.52	8.66	0.00	6.59	17.0	16.3	15.3	0.0	12.2	54.0	52.2	56.5	0.0	40.7
	Radiation	10.33	9.60	9.33	7.40	9.17	16.3	15.7	15.0	14.7	15.4	63.3	61.1	62.3	50.4	59.3
Mean		9.75	9.06	9.00	3.70	7.88	16.7	16.0	15.2	7.3	13.8	58.6	56.6	59.4	25.2	50.0
GK Ahron	Non-radiation	10.63	9.77	9.61	8.20	9.55	14.3	14.3	14.3	15.0	14.5	74.2	68.2	67.1	54.7	66.0
	Radiation	11.00	9.52	9.63	8.43	9.65	15.3	15.0	15.0	14.3	14.9	71.8	63.4	64.3	58.9	64.6
Mean		10.82	9.65	9.62	8.32	9.60	14.8	14.7	14.7	14.7	14.7	73.0	65.8	65.7	56.8	65.3
Brands	Non-radiation	10.20	9.95	9.00	8.33	9.37	14.7	14.3	15.0	15.7	14.9	69.6	69.5	60.1	53.3	63.1
	Radiation	8.47	8.15	8.59	8.37	8.39	14.7	14.0	15.3	14.0	14.5	57.7	58.2	56.1	59.9	58.0
Mean		9.33	9.05	8.79	8.35	8.88	14.7	14.2	15.2	14.8	14.7	63.6	63.8	58.1	56.6	60.5
Rex	Non-radiation	11.03	8.40	8.07	8.52	9.00	15.3	15.0	14.3	14.0	14.7	71.9	76.7	56.2	60.8	66.4
	Radiation	8.83	11.50	11.01	10.18	10.38	15.3	15.3	15.7	15.0	15.3	57.6	54.8	70.2	68.0	62.7
Mean		9.93	9.95	9.54	9.35	9.69	15.3	15.2	15.0	14.5	15.0	64.8	65.7	63.2	64.4	64.5
T × C	Non-radiation	10.26	9.16	8.83	6.26	8.63	15.3	15.0	14.8	11.2	14.1	67.4	66.6	60.0	42.2	59.1
	Radiation	9.66	9.69	9.64	8.60	9.40	15.4	15.0	15.3	14.5	15.0	62.6	59.4	63.2	59.3	61.1
Mean		9.96	9.43	9.24	7.43	9.01	15.4	15.0	15.0	12.8	14.6	65.0	63.0	61.6	50.8	60.1
Genotype (G)		0.224					0.29					1.55				
Treatments (T)		0.159					0.21					1.09				
Concentration (C)		0.224					0.29					1.55				
G × T		0.317					0.42					2.19				
G × C		0.448					0.59					3.10				
C × T		0.317					0.42					2.19				
G × T× C		0.634					0.83					4.38				

3. Effect of Salinity and γ-ray on Yield Parameters of The Four Sweet Sorghum Genotypes:

The implied results of leaves yield fed^{-1} in Table (4) recorded significant differences in all treatments and their interactions with the exception of triple interaction. The highest value was in Rex followed by GK Ahron. Nevertheless, Tracy was the lowest one. For radiation treatment, radiation was higher than non-radiation. In addition, the greatest concentration was control and the lowest one was 5000 ppm. The data in Table (5) showed significant differences among genotypes. The highest values were Tracy, Rex, GK Ahron and Brands were 678.8, 606.4, 537, and 528 kg/fad, respectively. Data of radiation (627.6 kg/fad) was higher significant than control (547.4 kg/fad). There were significant differences among salt concentrations and the highest and lowest were obtained by control (649.8 kg/fad.) and 5000ppm (479.4 kg/fad.), respectively. All the interactions between two factors and triple interaction were significant. It is demonstrable from the data of ethanol yield presented in Table (5) that all treatments get significant differences in the tested season. Generally, Rex had the highest yield and Brands had the least genotype in tested seasons. The highest salt concentration was documented at 4000 ppm and the last one was at 5000 ppm. Radiated plants gained a higher value than NR-Plant. The present studies are in a line with (Valdineia, Nara *et al.*, 2011) who reported that the variance analysis detected that the high γ-rays dosages, salt concentrations, and interaction between high gamma rays dosages had a significant effect on sorghum genotype survival. These results were in harmony with the findings of (Chen, Chen *et al.*, 2021) whereas similar findings are noticed in the presented study, the control had the highest value for germination parameters, while the lowest one was for the highest salt concentration.

Table 4: Effect of radiation, soil salinity treatments, and their interaction on stems and leaves yields sweet sorghum.

Genotypes (G)	Treatments (T)	Stems yield fed^{-1} (ton/fad)					Leaves yield fed^{-1} (ton/fad)				
		Concentrations of salt					Concentrations of salt				
		Control	3000	4000	5000	**Mean**	Control	3000	4000	5000	**Mean**
Tracy	Non-radiation	6.9	5.5	3.4	0.0	3.9	5.38	4.96	2.45	0.00	3.20
	Radiation	7.2	5.7	5.7	3.4	5.5	5.64	5.73	3.15	1.22	3.94
Mean		7.0	5.6	4.5	1.7	4.7	5.51	5.34	2.80	0.61	3.57
GK Ahron	Non-radiation	5.8	4.9	4.3	2.7	4.4	4.52	4.11	4.08	1.75	3.61
	Radiation	7.3	6.7	4.4	1.9	5.1	4.57	4.52	4.10	2.67	3.96
Mean		6.5	5.8	4.4	2.3	4.7	4.55	4.31	4.09	2.21	3.79
Brands	Non-radiation	6.8	4.0	3.7	1.5	4.0	4.87	4.58	2.86	1.39	3.43
	Radiation	7.0	5.6	4.9	2.1	4.9	5.12	4.78	3.05	2.46	3.85
Mean		6.9	4.8	4.3	1.8	4.4	5.00	4.68	2.95	1.93	3.64
Rex	Non-radiation	8.2	6.8	5.7	4.2	6.2	7.71	6.65	4.41	4.73	5.87
	Radiation	8.7	8.2	7.7	4.7	7.3	8.31	6.83	6.03	6.26	6.86
Mean		8.4	7.5	6.7	4.5	6.8	8.01	6.74	5.22	5.50	6.37
T × C	Non-radiation	6.9	5.3	4.3	2.1	4.6	5.62	5.07	3.45	1.97	4.03
	Radiation	7.6	6.5	5.7	3.0	5.7	5.91	5.46	4.08	3.15	4.65
Mean		7.2	5.9	5.0	2.6	5.2	5.76	5.27	3.77	2.56	4.34
Genotype (G)		0.42					0.230				
Treatments (T)		0.30					0.163				
Concentration (C)		0.42					0.230				
G × T		nS					0.325				
G × C		nS					0.460				
C × T		nS					0.325				
G × T× C		1.20					nS				

Table 5: Effect of radiation, soil salinity treatments, and their interaction on sugar and ethanol yields of sweet sorghum.

Genotypes (G)	Treatments (T)	Sugar yield fed^{-1} (Kg/fad)					Ethanol yield fed^{-1}(Kg/fad)				
		Concentrations of salt					Concentrations of salt				
		Control	3000	4000	5000	**Mean**	**Control**	3000	4000	5000	**Mean**
Tracy	Non-radiation	903.1	829.5	745.8	0.0	619.6	492.8	432.4	401.1	0.0	331.6
	Radiation	787.5	758.7	702.8	702.8	738.0	346.2	348.8	340.7	266.8	325.6
Mean		845.3	794.1	724.3	351.4	678.8	419.5	390.6	370.9	133.4	328.6
GK Ahron	Non-radiation	530.0	493.9	493.9	506.8	506.2	112.5	81.0	122.9	148.6	116.2
	Radiation	707.8	542.0	530.0	491.3	567.8	204.2	120.7	184.0	124.3	158.3
Mean		618.9	518.0	512.0	499.1	537.0	158.3	100.9	153.5	136.5	137.3
Brands	Non-radiation	466.9	531.2	564.9	529.6	523.1	64.8	147.0	184.0	115.6	127.8
	Radiation	554.9	524.8	583.8	467.6	532.8	110.5	110.5	196.6	98.9	129.1
Mean		510.9	528.0	574.3	498.6	528.0	87.6	128.8	190.3	107.2	128.5
Rex	Non-radiation	609.8	428.9	579.8	544.9	540.9	312.5	603.9	759.0	323.4	499.7
	Radiation	638.3	831.4	626.0	592.4	672.0	287.3	513.9	280.7	288.9	342.7
Mean		624.1	630.2	602.9	568.6	606.4	299.9	558.9	519.8	306.2	421.2
T × C	Non-radiation	627.5	570.9	596.1	395.3	547.4	245.6	316.1	366.7	146.9	268.8
	Radiation	672.1	664.2	610.7	563.5	627.6	237.0	273.5	250.5	194.7	238.9
Mean		649.8	617.6	603.4	479.4	587.5	241.3	294.8	308.6	170.8	253.9
Genotype (G)		14.5					25.5				
Treatments (T)		10.3					18.0				
Concentration (C)		14.5					25.5				
G × T		20.5					36.0				
G × C		29.0					50.9				
C × T		20.5					36.0				
G × T× C		41.1					72.0				

4. Effect of Salinity and γ-ray on Free Proline as A Biochemical Marker of Four Sweet Sorghum Genotype:

In Table (6), there were significant differences among genotypes, Rex (44.64) gained the highest value and GK Ahron (38.40) had the lowest one. Findings observed significant between radiation and non-radiation. Moreover, the radiation (39.91) was higher than non-radiation (39.31). Outcomes revealed that there were significant differences among concentrations. Furthermore, the grandest value was at 3000ppm (50.47), while the smallest

amount one was at control was (20.86). A significant difference was found in all interactions. The findings in Table (6) showed that there were significant between genotypes. Whereas, the greatest value was awarded to GK Ahron (50.10) and the lowest to Tracy (38.91). The significant differences between radiation and non-radiation were not recorded. Free proline for stress-tolerant; the proline content in the plant could be an indicator of tolerant salinity stress. That in agree with (Tripathi *et al.*, 2021) who stated that the osmolyte proline accumulates when plants are subjected to abiotic stress. In the present study plant treatments with radiation make plants withstand stress, so radiation plants have less quantity of proline. (Ibrahim 2004) exemplified thus proline is toxicity at high concentrations in plants and the major inhibition effect as a consequence of salinity on plant development and yield has been owing to osmotic effect, ion toxicity that is nutritional imbalance results in a reduction in photosynthetic efficiency and other physiological disorders. Salinity significantly reduced sorghum development and this effect was more noticeable at 250 mM than at 125 mM NaCl.

Table 6: Effect of radiation, soil salinity treatments and their interaction on biochemical parameters of sweet sorghum.

Genotypes (G)	Treatments (T)	Proline 60 DAP					Proline at harvest				
		Concentrations of salt					Concentrations of salt				
		Control	3000	4000	5000	**Mean**	Control	3000	4000	5000	**Mean**
Tracy	Control	23.23	53.93	47.29	-	41.48	22.17	57.07	40.35	-	39.86
	Radiation	22.54	40.91	47.92	36.75	37.03	22.35	43.12	25.84	60.56	37.97
Mean		22.89	47.42	47.60	36.75	39.26	22.26	50.09	33.09	60.56	38.91
GK Ahron	Control	22.70	57.91	50.95	35.71	41.82	22.00	61.78	35.76	65.25	46.20
	Radiation	22.82	27.94	39.84	49.35	34.99	21.75	62.11	64.68	67.48	54.00
Mean		22.76	42.92	45.39	42.53	38.40	21.87	61.95	50.22	66.36	50.10
Brands	Control	22.77	58.26	31.79	39.21	38.01	22.69	59.63	65.31	31.62	44.81
	Radiation	22.73	65.56	40.91	49.31	44.63	22.17	46.85	48.99	26.07	36.02
Mean		22.75	61.91	36.35	44.26	41.32	22.43	53.24	57.15	28.84	40.41
Rex	Control	23.07	56.60	43.04	62.43	46.28	22.20	27.67	57.78	63.27	42.73
	Radiation	23.05	44.76	49.85	54.33	43.00	22.72	42.93	43.19	61.36	42.55
Mean		23.06	50.68	46.45	58.38	44.64	22.46	35.30	50.48	62.31	42.64
T × C	Control	22.94	56.68	43.26	34.34	39.31	22.26	51.53	49.80	40.03	40.91
	Radiation	22.79	44.79	44.63	47.43	39.91	22.25	48.75	45.67	53.86	42.63
Mean		22.86	50.74	43.95	40.89	39.61	22.26	50.14	47.74	46.95	41.77
Genotype (G)		2.631					2.27				
Treatments (T)		2.453					1.58				
Concentration (C)		2.578					2.23				
G × T		3.720					3.22				
G × C		5.155					4.46				
C × T		3.645					3.15				
G × T× C		3.23					6.30				

5. Molecular Analysis:

The interspecific sequence repeat (ISSR) technique was applied by using 5 primers for amplification of the four non-radiated genotypes and their irradiated ones (8 lines) of sorghum, these primers proved their ability in giving polymorphic ISSR products from the studied samples. ISSR primers were able to distinguish between different pear varieties (Monte-Corvo, Goulão *et al.*, 2001). The primers gave PCR products of about 95 bands, most of them were polymorphic, and the percentage of polymorphism was %96.51 (Fig. 1). Traits indicated that ISSR markers could be realistically used to evaluate the genetic diversity and differentiation among sorghum genotypes. The UPGMA clustering (Fig. 2) associated the treatments with two major clusters, separating the tested genotypes. Cluster 1 contains Brands and radiated Brands, cluster 2 contains two sub-cluster for the remaining ones. This results in agreement with (Prakash *et al.*, 2006) who studied sweet sorghum with 21 primers. The molecular genetic analysis appeared differences in banding patterns between radiated genotypes and non-radiated ones, especially in genotype Rex followed by GK Ahron, Brands, and finally Tracy. These results reflected that the radiation treatment

produced genetic variation among genotypes. They can be taken as genetic markers to clarify the genetic variation.

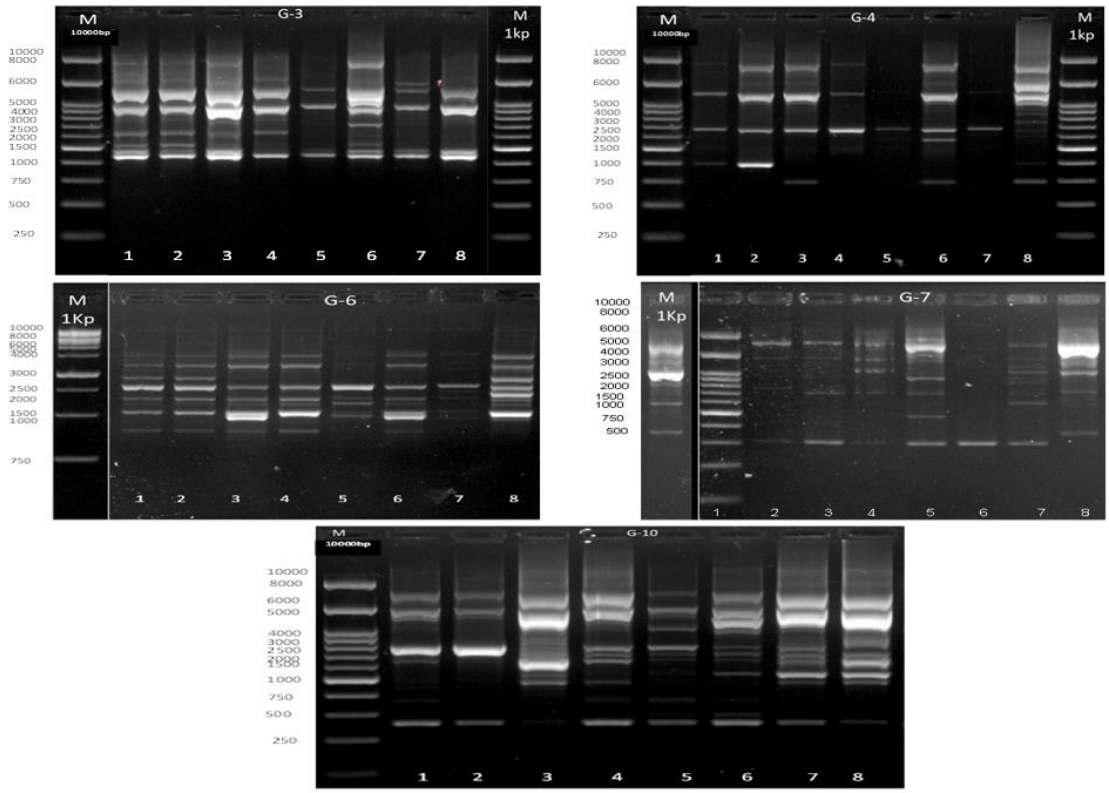

Fig. 1. Five ISSR primer amplification products were obtained from the eight treatments of sweet sorghum.
M = 1 kb ladder; (1) Tracy, (2) R-Tracy, (3) GK Ahron, (4) R-GK Ahron, (5) Brands, (6) R-Brands, (7) Rex and (8) R-Rex

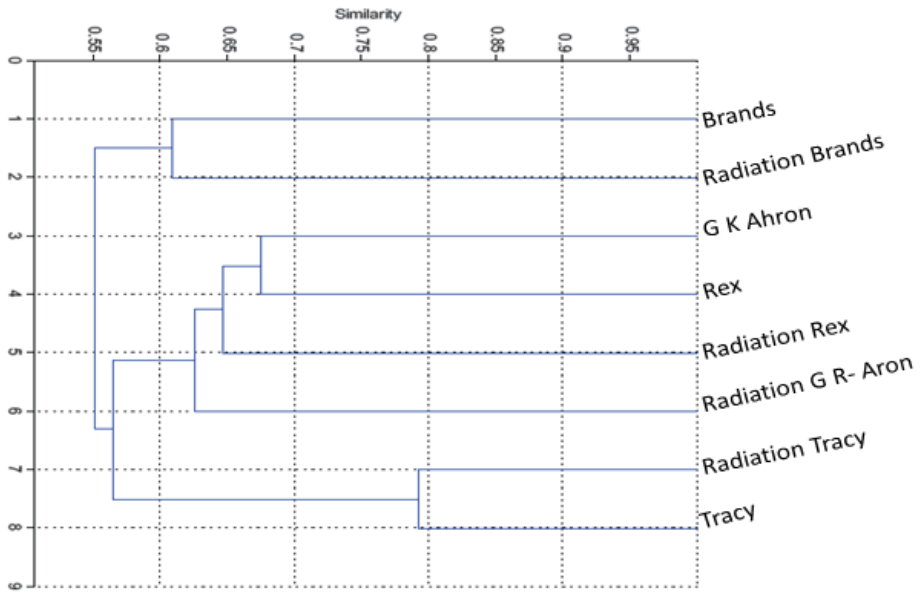

Fig. 2. STATISTICA Dendrogram generated from the genetic similarity coefficients of 8 isolates of sweet sorghum, Tracy, radiation Tracy, GK Ahron, radiation GK Ahron, Brands, radiation Brands, Rex, and radiation Rex.

Conclusion

In general, under radiation and salinity, Rex maintained the steepest values of all growth assessments and all assessments yield except sugar one as well as proline at 60 DAP and minimum ones of plant height and node numbers. On the other hand, Tracy had the highest values of all tested growth parameters as well as sugar yield and lowest of all tested quality parameters and proline at harvest. From our findings, it can be put forward the claim that genotype Rex appeared most responsive to the effect of radiation whereas, it was most tolerant to the salinity, while Tracy was the lowest response. Also, the radiation had a significant effect on reducing sugar content. The ISSR technique proved its efficiency in differentiating the examined genotypes which were grouped in two clusters. This facilitates and enhances their use as promising parents in sorghum breeding and improvement. Finally, in this work, using gamma rays, it may be advised that beneficial salinity tolerance mutations can be produced on sweet sorghum and other crops after repeating these tests for several crops, seasons, and locations .

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