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GAMMA-RAYS AND MICROWAVE IRRADIATION INFLUENCE ON GUAR (*CYAMOPSIS TETRAGONOLOBA*): I - MARKERS ASSISTED SELECTION FOR RESPONDING TO MUTAGENIC AGENTS

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SUMMARY

The recent investigation was carried out to determine the effect of different gamma-ray doses and 900 W (2450 MHz) microwave radiation with various exposure times, separately or in combinations, on the yield, yield components, and chemical properties of guar (*Cyamopsis tetragonoloba*), as well as, to detect variation induction. The cDNA-SCoT technique was used to obtain molecular markers related to some traits. SSR technique was used to sequence the target fragment related to plant height. Gamma-ray doses of 150 and 250 Gy alone, and in combination with 900 W microwaves irradiation applied with different duration or time span (1, 2, 3, and 4 min) influenced the plant height significantly, as well as, number of tillers plant^{-1} and fresh and dry forage yield, and fresh and dry leaf stem^{-1} ratio. In the second sample, seed yield at harvest time, e.g., pods plant^{-1}, weight of pods plant^{-1}, whole plant dry weight, number of seeds pod^{-1}, length of pod, 100-seed weight, and seeds yield were affected by irradiation with different and varied responses. In the M_1 generation, the 18 SCoT primers produced 327 bands ranging between 151–2895 bp in size, out of which 282 were polymorphic (86.24%). In the M_2, the 18 SCoT primers produced 328 bands ranging between 212–2661 bp in size, out of which 299 were polymorphic (91.16%). The M_1 and M_2 generations exhibited 89 positive and 39 negative bands, which could be used as marker assisted-selection in response to treated guar plants with different gamma ray doses, separately or in combinations with microwave treatments.

Keywords: Gamma irradiation, microwave heating, guar grain, yield and yield components, quality analysis, SCoT

Key findings: Some positive and negative bands were exhibited in M_1 and M_2 generations, which could be used as marker-assisted selection for responding to treated guar plants with different gamma ray doses, separately or in combinations, with microwave treatments.

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INTRODUCTION

Insufficiency of feed supply is the main handcuffs for any additional increase in animal production in Egypt. Moreover, farm animals sustain malnutrition, especially in the summer seasons, when fresh green forages with sensible protein content are insufficient. Some trials were carried out to insert new green forages or silages, including elevated protein content, viz., guar (Gabra *et al*., 1990). Guar (*Cyamopsis tetragonoloba*) is a high economic and social significance forage crop and multi-purpose plant. It has been used recently as a source of galactomannan gum (guar gum) that is used as a stabilization source in foods, i.e*.,* ice cream, salad dressings, and yogurt, as well as, in other industries, such as*,* paper manufacturing, cosmetics, pharma, and textile (Mudgil *et al*., 2014).

Guar is generally cultivated in subtropical and semi-arid areas (Ecocrop, 2010). Guar is drought-tolerant and sturdy. It is well adapted to semi-arid and arid climates with high temperatures, but can also be cultivated in sub-humid conditions. This crop is of high adaptation toward erratic rainfall, too. Guar, like other legumes, promotes nitrogen availability in soils, and the plowed crop remains have been shown to enhance the successive crop yields significantly with low input requirements (Lubbe and Verpoorte, 2011). It is applied to reclamation of low fertility, high alkalinity, and high saline soils (Ecocrop, 2010). It is unsuitable for grazing due to its unpalatability and hairy leaves (Göhl, 1982). It is occasionally grazed to decrease the hazard of bloat in ruminants (Wong and Parmar, 1997). Palatability improves after mowing and wilting (Göhl, 1982). The preferable time for mowing guar for fodder is throughout the flowering and early pod formation stages (Wong and Parmar,1997).

Despite the world speeding up agricultural renovations, we are still concerned about food security and animal feeding. To meet the considerable increase in population's requirements, there is a demand for 70% more food by 2050. To cope with this situation, we have to improve our existing crop varieties and make them varied genetically, climate change adapted, input use efficient, highly productive, enhanced in nutritional traits, and adapted to a wide range of agro-ecosystems, as well as, environment-friendly. Among the numerous applications of breeding to improve crop varieties, mutation breeding plays a critical function in improving genetic variation among themselves. Over the past 50 years, mutation breeding has become more common, and 3,362 mutant plant varieties from 240 different plant species in more than 75 countries have been released. The various breeders have used different physical, chemical, and combined mutagens to induce genetic variability in multiple crops. Mutation breeding improves several qualitative and quantitative characteristics of crop plants and is successfully applied in cereal grains, legumes, oilseed, vegetables, fruits, medicinal and ornamental plants, and fodder crops. With the progression of different plant breeding, genetics, and biotechnological tools, mutation breeding contributes to the increase in international food and agriculture production, which overcomes overall hunger and improves the nutritional status worldwide (Pandit *et al*., 2021)

Ionizing radiation (gamma rays, electrons, and x-rays) can modify the irradiated materials' physical, chemical, and biological properties. Gamma radiation could react with atoms and molecules to produce free radicals in cells, which are fit to alter significant motifs of plant cells (Amer *et al*., 2001, 2008; Azzam, 2004; Azzam and Abbas, 2005). These radicals have been demonstrated to manipulate plants' morphological, physiological, biochemical, and anatomical characteristics based on irradiation doses (Azzam and Zein, 2012; Azzam and Khalifa, 2016; Ashmawy *et al*., 2016). The effects of gamma ray include changes in the plant cell structure and metabolism, such as, adjustment of the antioxidative system, alter in malondialdehyde levels as a sign of free radicals, expansion of thylakoid membranes, and increase of phenolic compounds (Kovacs and Keresztes, 2002; Kim *et al*., 2004; Wi *et al*., 2005; Azzam *et al*., 2007a b, 2008, 2014).

Gamma irradiations are commonly used to generate genetic variability in field crops and for the development of new genotypes through plant breeding (Shabana *et al*., 1994 a, b, c; Khalifa *et al*., 2006; Ashmawy and Azzam, 2011; Abdalla *et al*., 2017, 2018). As conventional breeding programs take a long time, it is eligible for mutation practices in field crops. Furthermore, when all other techniques of generating variation fail, it is simple for breeders to use mutation breeding. Moreover, gamma-ray irradiation does not pose an impediment to human beings and the environment. It is a supportive procedure for conventional breeding programs.

Unlike GMOs (genetically modified organisms), there is no insertion of new foreign

genes, so no biosafety control is required. Gamma irradiations could be practiced for various field crops. Induced mutagenesis via gamma irradiations can contribute to the release of plant genetic resources' potentials, and provide plant breeders the required raw materials to generate new promising field crop varieties (Azzam, and El-Sawy, 2005; Al-Taweel *et al*., 2021). Field crop varieties developed via gamma irradiation contribute to universal food and nutritional security and improve livelihoods (Singh, 2017). Correspondingly, the generality of non-invasive techniques contains treatment with microwave, electromagnetic waves, ultrasound, optical emissions, magnetic fields, and blue light-UV irradiation. They have been used to improve seeds germination of field crops, increase productivity, and yield biologically active components (Wang *et al*., 2018). Microwaves have different effects on the biological systems of the entire organism, cell, tissue, and molecular level (Roux *et al*., 2006; Hamada, 2007).

Microwaves are a form of electromagnetic irradiation, with a frequency between 30 and 300 GHz, significantly stimulating germination and enhancing growth (Gaurilcikiene *et al*., 2013). Microwaves at a specific power can effectively activate several enzymes involved in seed germination (Radzevičius *et al*., 2013), significantly improving the germination rate (GR) and increasing the synthesis of specific biological components in the seeds (Stan *et al*., 2014). Microwave pre-treatment promotes the gene expression of the isozymes, e.g., peroxidase (POD) and superoxide dismutase (SOD) (Aladjadjiyan, 2012). It significantly increases germination potential, GR, stem length, root length, and total seed mass (Radzevičius *et al*., 2013). A positive correlation between these increases and microwave power was found (Łupinska *et al*., 2009; Han, 2010). In addition, these increases were microwave radiation dose-dependent (Hamada, 2007). The dose-dependency could be linked to the changes in the protein structure of the enzyme due to the microwave treatment (Damm *et al*., 2012). Most of these studies focused on very weak (> 0.5 mW cm-2) and low-frequency magnetic fields to determine their toxic or side effects. Nevertheless, microwave enhances germination, plant height, and fresh mass (Aladjadjiyan, 2002; Belayavskaya, 2004; Racuciu *et al*., 2006). Microwaves generate rotation in dielectric molecules under an electromagnetic field, producing system heating. This rotation may destabilize bio-molecules, including DNA.

Molecular markers are used to identify genotypes and determine gene expression of targeted genes related to abiotic stress (Khaled *et al*., 2015). Markers, such as, amplified fragment length (AFLP), simple sequence repeats (SSR), and start codon targeted marker (SCoT), are used to identify the gene expression and QTLs in plants (Khaled *et al*., 2018; AL-Taweel *et al*., 2019; Salah *et al*., 2021; and Khaled *et al*., 2022). A cDNA SCoT (cDNA starts codon-targeted) molecular technique has been proposed to be an appropriate and powerful tool for identifying gene expression variations, stress tolerance, and genetic stability in plants (Al-Taweel *et al*., 2019; Abou-Sreea *et al*., 2021). This method is advantageous compared with all other existing ones because it is cheaper, relatively more efficient, simpler to operate, faster, and readily reproduces the results (Luo *et al*., 2014). The cDNA-SCoT markers were used to determine the gene expression in *Saccharum officinarum*, *Phoenix dactylifera*, *Mangifera indica, Olea europaea* tree, and *Dendrobium officinales* (Munns and Tester, 2008; Chen *et al*., 2013; AL-Janabi and Al-Rawi, 2018).

The recent investigation was carried out to determine the effect of different gamma-ray irradiated doses and different power and time treatment of microwaves heating, separately or in combinations, on forage yield, productivity, and chemical properties of guar (*C. tetragonoloba*) to detect variation induction, as well as, detect some marker-assisted selection for important traits in guar.

MATERIALS AND METHODS

Irradiation with gamma ray doses

Dry seeds of the local variety of guar (Shandaweel 9) were exposed to 0, 50, 150, and 250 Gy of gamma ray doses of Cobalt-60 source at a dose rate of 7.03 Gy min^{-1}. The source of irradiation was that installed at the National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority , Cairo, Egypt.

Irradiation with microwave heating treatments

The microwave source was a Japanese Panasonic NN – K597WS microwave oven with variable power, from 200 to 900 W, and a

Table 1. The different gamma-ray doses and different power and time of microwave and the code used across the manuscript.

Microwave treatments		Gamma-ray doses	Code	Treatments
Time	Power			
0	0	0	GM Control	1
0	0	50Gy	G50	2
1 min	900 W = 2450MHz	50Gy	G501T	3
2 min	900 W = 2450MHz	50Gy	G502T	4
3 min	900 W = 2450MHz	50Gy	G503T	5
4 min	900 W = 2450MHz	50Gy	G504T	6
0	0	150Gy	G150	7
1 min	900 W = 2450MHz	150Gy	G1501T	8
2 min	900 W = 2450MHz	150Gy	G1502T	9
3 min	900 W = 2450MHz	150Gy	G1503T	10
4 min	900 W = 2450MHz	150Gy	G1504T	11
0	0	250Gy	G250	12
1 min	900 W = 2450MHz	250Gy	G2501T	13
2 min	900 W = 2450MHz	250Gy	G2502T	14
3 min	900 W = 2450MHz	250Gy	G2503T	15
4 min	900 W = 2450MHz	250Gy	G2504T	16
1 min	900 W = 2450MHz	0	M Control 1T	17
2 min	900 W = 2450MHz	0	M Control 2T	18
3 min	900 W = 2450MHz	0	M Control 3T	19
4 min	900 W = 2450MHz	0	M Control 4T	20

frequency of 2450 MHz. Seeds were exposed to 900 W (2450 MHz) microwave radiation in varying exposure times, separately or in combination, with the different gamma ray doses (Table 1).

Field experiments of M_1 and M_2 generations

A field trial was conducted during the two successive summer seasons of 2019 and 2020 at Agricultural Research Station, ARC, Giza Governorate, Egypt. The preceding crop in the two summer seasons was barley. The experiment was laid out in a split-plot design with three replications. Each plot size was 42 m^2 (3 m × 14 m) and consisted of 20 rows. The seed was hand-drilled in rows 20 cm apart at the seeding rate of 47.6 kg ha^{-1}. Before sowing, chemical fertilizer was applied in the forms of 557 kg ha^{-1} calcium superphosphate (15.5% P_2O_5) and 238 kg ha^{-1} potassium sulfate (48% K_2O). Nitrogen fertilizer was applied as urea (46% N) and added in three equal doses 10 days after planting, and the other two doses were applied prior to the following two successive irrigation times. Guar's seed was sown on May 15, 2019 and May 20, 2020. The other agronomic practices were done as recommended up to harvest time.

The experimental plots were divided into two equal parts: the first was for estimating growth, yield component, fresh and dry yields (ton ha^{-1}), while the second was left to the stage of flowering and seed formation to estimate seed yield (kg ha^{-1}). According to the methods adopted for growing guar crops, other cultural practices were followed.

Data recorded

The data of vegetative growth (forage yield) were recorded as follows: plant height (cm), number of tillers $plant^{-1}$ and fresh and dry forage yield (ton ha^{-1}), as well as, fresh and dry leaf $stem^{-1}$ ratio. Data included in the second sample were seed yield at harvest time, e.g., number of pods $plant^{-1}$, weight of pods $plant^{-1}$ (g), whole plant dry weights (g), number of seeds pod^{-1}, length of pods (cm), 100-seed weight (g), and seeds yield (kg ha^{-1}).

Fresh forage yield (ton ha^{-1}) was estimated according to Krishnasamy and Seshu (1990) as follows: plants were hand clipped and weighed in kg $plot^{-1}$, then converted to ton ha^{-1}. Then an average of 10 normal seedlings were calculated from each replication. As for the dry forage yield (ton ha^{-1}), it was estimated as follows: 100g plant samples from each plot were dried at 105 °C until reaching constant weight and dry matter percentage (DM%). The dry forage yield (ton ha^{-1}) was

Table 2. Details of 18 SCoT primers sequences used in PCR reactions.

Temp. (˚C)	GC%	Primers sequence (5`-3`)	Oligo	Primer
53.9	50.00	AAGCAATGGCTACCACCA	SCOT 11	1
58.4	61.00	ACGACATGGCGACCAACG	SCOT 12	2
58.4	61.00	ACGACATGGCGACCATCG	SCOT 13	3
60.7	67.00	ACGACATGGCGACCGCGA	SCOT 15	4
58.4	61.00	ACCATGGCTACCACCGAC	SCOT 16	5
58.4	61.00	ACCATGGCTACCACCGAG	SCOT 17	6
60.7	67.00	ACCATGGCTACCACCGCC	SCOT 18	7
60.7	67.00	ACCATGGCTACCACCGGC	SCOT 19	8
60.7	67.00	ACCATGGCTACCACCGCG	SCOT 20	9
58.4	61.00	ACGACATGGCGACCCACA	SCOT 21	10
56.1	56.00	AACCATGGCTACCACCAC	SCOT 22	11
58.4	61.00	CACCATGGCTACCACCAG	SCOT 23	12
56.1	56.00	CACCATGGCTACCACCAT	SCOT 24	13
60.7	67.00	ACCATGGCTACCACCGGG	SCOT 25	14
58.4	61.00	ACCATGGCTACCACCGTC	SCOT 26	15
58.4	61.00	ACCATGGCTACCACCGTG	SCOT 27	16
60.7	67.00	CCATGGCTACCACCGCCA	SCOT 28	17
63.0	72.00	CCATGGCTACCACCGGCC	SCOT 29	18

calculated by multiplying the fresh forage yield (ton ha^{-1}) with the DM%. Chemical analysis followed the conventional method recommended by the Association of the Official Agricultural Chemists **(**A.O.A.C., 1980**)** on the dried forage sample at 70 °C for the two seasons to determine crude protein (CP %), crude fiber (CF %), and ash (%).

Statistical analysis

The data were statistically analyzed according to the procedures outlined by Snedecor and Cochran (1980)**.** A combined analysis of the two experimental seasons was carried out based on the results of Bartlett's test. Means were compared using the least significant difference (LSD) at 5% probability levels.

cDNA SCoT PCR reaction and amplification conditions

The cDNA-SCoT technique was used as described by Al-Taweel *et al*. (2019). The RNA was extracted from 40 guar populations according to the Trizol method (Luo *et al*., 2014). The RNA synthesized cDNA by adding 1 µl of oligo dT to RNA and incubating at 66 ºC for 5 min. After thawing on ice for 2 min, reverse transcriptase 1 µl 5 × buffers, 2 µl of dNTPase, and 1 µl of reverse transcriptase enzyme were added. Every guar sample was incubated for one cycle in PCR at 42 °C for 1 h, followed by another termination cycle at 70 °C for 5 min. The cDNA concentration was measured using a Fluorometer, and 100 ng of cDNA was used to conduct the reaction for all guar samples. The cDNA-SCoT technique was applied to compare the 40 guar populations and find molecular markers linked to some traits due to different gene expressions. Eighteen primers (cDNA-SCoT Oligo primer, macro gene Company) were used (Table 2). The reaction mixture components (25 µL) were gathered to amplify and evolve the SCoT markers. PCR reaction was implemented on ABI 2700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Initial denaturation was set out at 95 °C for 4 min, followed by 40 cycles at 95 °C with 51 °C for 1 min, 72 °C for 1 min, and a final extension at 75 °C for 5 min. The amplification products were separated on an agarose gel (1.3%), which contained ethidium bromide against a 100 bp DNA ladder. Fragments were detected on a UV transilluminator, and photographed with a gel documentation system (Alpha Ease FC, Alphimager^{Tm} 2200, USA).

Molecular data analysis

The banding patterns generated by SCoT primers were scored as present (1) or absent (0) for each primer using 1D software (Total Lab software v2009, Nonlinear Dynamics, UK). A total number of bands, polymorphic, monomorphic, and polymorphism percentages were recorded by observing the banding patterns produced by different SCoT primers. The genetic similarities were computed following Dice, and the dendrogram was created using SPSS Windows Version 25.

RESULTS AND DISCUSSION

Vegetative growth and forage yield

The overall measurement results of the vegetative growth and forage yield are summarized in Figure 1. Plant height of guar plants was not affected by irradiation with 50 Gy gamma-ray dose alone, as well as, the irradiation at 900 W microwaves applied for different times (1, 2, 3, and 4 min) alone in both M_1 and M_2. In contrast, gamma-ray doses of 150 and 250 Gy alone or in combination with the irradiation of 900 W microwaves applied for different times (1, 2, 3, and 4 min) influenced the plant height significantly (P<0.05), as well as, irradiation with 50 Gy gamma-ray dose in combination with the irradiation of 900 W microwaves, applied for different times (1, 2, 3, and 4 min) in both generations. The highest guar plants appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation of 900 W microwaves applied for 3 and 4 min in both M_1 (153.32 and 142.99 cm, respectively) and M_2 (155.99 and 146.40 cm, respectively) generations, which is an approximate increase of 53.2% for M_1 and 42.9% for M_2, compared with the GM control. In M_1 generation, it was also higher at 53.6 and 44.2 cm for 3 and 4 min, respectively, as well as, in M_2, at 34.3 and 25.3 cm for 3 and 4 min, respectively, as compared with G2503T and G2504T in M_1 at 32.8 and 24.6, respectively, and with G2503D and G2504D in M_2 (Figure 1). In contrast, Mahla *et al*. (2018) reported that the genotypic response concerning plant height was less conspicuous and the reduction was progressive, starting from 100 to 800 Gy. Also, the plant height was increased by irradiation in only one of the two investigated varieties. An increase in induced variation in M_2 progenies of guar cv. RGC 197 obtained by gamma irradiation (10, 30, 50, 60, 70, and 80 kR) was noticed in plant height (Amrita and Jain, 2003; Mahla *et al*., 2018). Arora and Pahuja (2008) have observed an increase in peduncle length and plant height. They reported that the doses of 100 to 200 kR have been quoted to be lethal.

The number of tillers per guar plant was significantly (P<0.05) affected by irradiation with all used gamma-ray doses alone and all microwave irradiation treatments alone and their combinations, except the irradiation at 900 W microwaves applied for 3 min in M_1 generation. The highest number of tillers per guar plants appeared in irradiated plants with 250 Gy gamma-ray dose, in irradiation combined with the irradiation at 900 W microwaves applied for 4 min in both M_1 (13.55 tiller plant^{-1}) and M_2 (13.88 tiller plant^{-1}), with an estimated increase of 99.9% and 93.0%, respectively, compared with the GM control in M_1 and M_2 generations (Figure 1). In contrast, the guar plants in M_1 and M_2 generations were characterized by non-branching habit, reduced plant height, increased cluster size, synchronous and early maturity and above all, the main shoot either terminated into a leaf or an inflorescence. The populations developed by artificial hybridization of determinate mutants with the normal (indeterminate) plants indicated that two genes controlled determinate habit and that at least two dominant alleles were required for the expression of the character (Singh *et al*., 1981).

The fresh forage yield of guar plants was significantly affected by irradiation at 900 W microwaves applied for 2 min alone in M_1 generation, at 24.5 tons ha^{-1}. Likewise, irradiation with all gamma-ray doses used alone or in combination with the irradiation at 900 W microwaves applied for different times (1, 2, 3, and 4 min) significantly (P<0.05) increased the guar fresh forage yield in both M_1 and M_2 generations, except the treatment of irradiation with 150 Gy gamma-ray dose alone (G150) in M_2 generation, as well as, the irradiation with 250 Gy gamma-ray dose in combination with 900 W microwaves irradiation applied for 4 min (G2504T). The guar plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 3 min produced the highest fresh forage yield of 29.5 tons ha^{-1} in M_1 and 29.9 tons ha^{-1} in M_2 generations. The said enhanced produce is estimated to be higher by 23.6% and 21.8%, respectively, as compared with the GM control in M_1 and M_2 generations (Figure 1).

All gamma-ray doses and all microwave treatments and their combinations significantly (P<0.05) increased the guar dry forage yield in M_1 and M_2 generations. The guar plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min (G2504T) produced the highest dry forage yield of 7.3 tons ha^{-1} in M_1 and 7.8 tons ha^{-1} in M_2 generations. The estimated increase of 188.7% and 146.6%, respectively, was observed compared with the GM control in M_1 and M_2 generations (Figure 1).

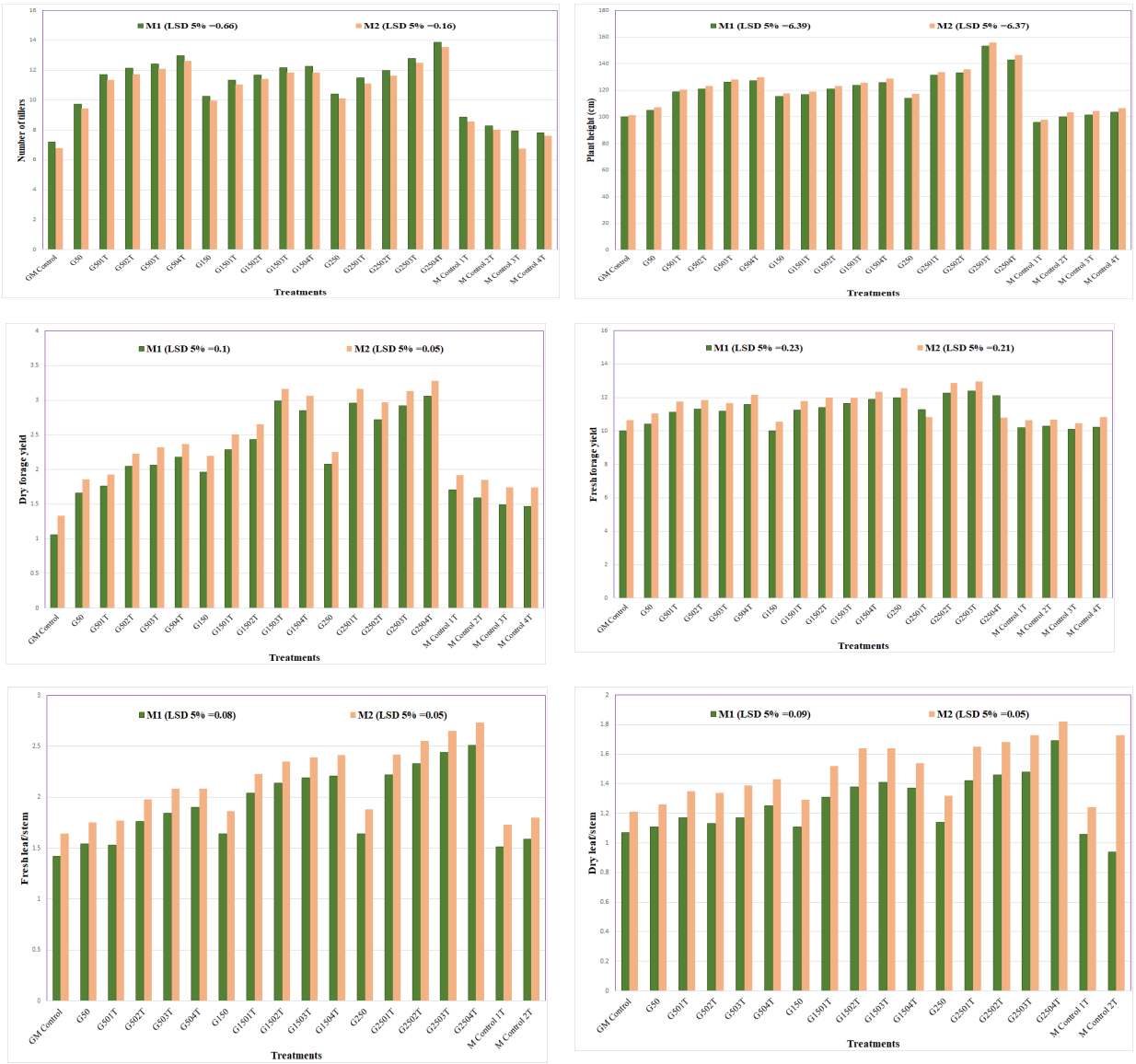

Figure 1. Effect of different gamma-ray doses and 900 W microwave irradiation applied for different time treatments separately or in combinations on the vegetative growth of guar (*Cyamopsis tetragonoloba*) in M_1 and M_2 generations**.**

The fresh leaf stem^{-1} ratio of guar plants was significantly (P<0.05) affected by irradiation with all the gamma-ray doses used alone or in combination with the irradiation at 900 W microwaves applied for 1, 2, 3, and 4 min separately, as shown in Figure (1). The highest fresh leaf stem^{-1} ratio of guar plants appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min in both M_1 (2.51%) and M_2 **(**2.73%), with an estimated increase of 76.8% and 66.5%, respectively, compared with the GM control in M_1 and M_2 generations (Figure 1).

In M_1 generation, all gamma-ray doses alone did not affect the dry leaf stem^{-1} ratio of guar plants, as well as, all microwave treatments, except M Control 2T (the irradiation at 900 W microwaves applied for 2 min, in both M_1 and M_2, as it significantly (P<0.05) decreased the dry leaf stem^{-1} ratio. In contrast, the dry leaf stem^{-1} ratio of guar plants was significantly (P<0.05) increased due to the irradiation with all used gamma-ray

doses alone or in combination with the irradiation at 900 W microwaves applied for 3 and 4 min in M_2 generation (Figure 1).

The highest dry leaf stem^{-1} ratio of guar plants appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min in both M_1 (1.69%) and M_2 (1.82%), with an estimated increase of 57.9% and 50.4%, respectively, as compared with the GM control in M_1 and M_2 generations (Figure 1). The gradual increase in sterile plants indicated their inducibility in response to radiation treatment. The mutations with respect to M_1 damage first increased, with the increasing dose of gamma ray up to 300 Gy, thereafter, gradually reduced, indicating a decline in the rate of recovery of mutations with respect to increasing M_1 damage (Mahla *et al*., 2018).

The frequency of mutants dose dependent mutation frequency was recorded while working with physical (gamma rays) and chemical (EMS) mutagens in guar by Bhosale and Kothekar (2010), Velu *et al*. (2012), and Mahla *et al*. (2018). Mutagenesis is a powerful tool for creating variation in a crop like a guar, where exploitable and favorable genetic variability is very meager (Arora and Pahuja, 2008). Some morphological mutants were also reported earlier in guar (Bhosale and Kothekar, 2010; Patil and Rane, 2015; Mahla *et al*., 2018).

Seed yield and yield components

The overall measurement results of the seed yield and yield components are summarized in Figure (2). The number of pods per plant of guar plants was significantly (P<0.05) affected by irradiation with all used gamma-ray doses alone or in combination with the irradiation at 900 W microwaves applied for different times (1, 2, 3, and 4 min) in both M_1 and M_2 generations, as well as, the irradiation treatment at 900 W microwaves applied for 3 and 4 min in M_2 generation. The highest number of pods per guar plants appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min in both M_1 (203.20 pods plant^{-1}) and M_2 (209.87 pods plant^{-1}), which is estimated to be higher by 42.1% and 41.8%, respectively, as compared with the GM control in $M_1$and M_2 generations (Figure 2).

Pods weight per guar plants and whole plant dry weight were significantly (P<0.05) increased by irradiation with all used gamma-ray doses alone and all microwave irradiation treatments alone and their combinations, except the irradiation treatment at 900 W microwaves applied for 4 min in M_1 generation. The highest pods weight per guar plants and whole plant dry weight appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min in both M_1 and M_2 (190.87 and 196.87 g pods weight plant^{-1}, respectively and 480.58 and 493.25 g whole plant dry weight, respectively), which is higher by 36.4% and 36.8%, respectively for pods weight per guar plants, and 152.8% and 146.1%, for whole plant dry weight, respectively, as compared with the GM control in M_1 and M_2 generations (Figure 2).

The number of seeds per pod of guar plants was significantly (P<0.05) increased by irradiation with all used gamma-ray doses alone or in combination with the irradiation at 900 W microwaves applied for different times (1,2,3, and 4 min) in both M_1 and M_2 generations, as well as, the irradiation treatment at 900 W microwaves applied for 2 min in both M_1 and M_2 generations. In contrast, the irradiation treatment at 900 W microwaves applied for 4 min in both M_1 and M_2 generations significantly (P<0.05) decreased the number of pods per guar plant. The highest number of seeds per pod appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min in both M_1 (12.11 seeds) and M_2 (12.59 seeds), which is higher by 94.1% and 90.5%, respectively, as compared with the GM control in M_1 and M_2 generations (Figure 2).

Length of guar pods was decreased significantly or non-significantly by irradiation with all used gamma-ray doses alone or the irradiation at 900 W microwaves applied for different times (1, 2, 3, and 4 min) alone, except the irradiation treatment at 900 W microwaves applied for 3 min in M_2 generation. In contrast, it was significantly (P<0.05) increased by the combination of all gamma-ray doses and the irradiation at 900 W microwaves applied for different times (1, 2, 3, and 4 min) in both M_1 and M_2 generations, except G501T in M_1, G502T in both $M_1$and M_2 generations, as shown in Figure (2). The highest length of guar pods appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min in both M_1 (8.57 cm) and M_2 (8.84 cm), with an estimated increase of 33.5% in $M_1$and M_2 generations, as compared with the GM control (Figure 2).

Figure 2. Effect of different gamma-ray doses and 900 W microwave irradiation applied for different time treatments separately or in combinations on the yield and yield components of guar (*Cyamopsis tetragonoloba*) in M_1 and M_2 generations**.**

The 100-seed weight of guar plants was significantly or non-significantly (P<0.05) decreased by irradiation at 900 W microwaves applied for different times (1, 2, 3, and 4 min) alone. In contrast, it was significantly (P<0.05) increased by irradiation with all used gamma-ray doses alone or in combination with the irradiation at 900 W microwaves applied for different times (1, 2, 3, and 4 min) in both M_1 and M_2 generations, except G50, G501T, and G502T in M_1 generation (Figure 2). The highest 100-seed weight of guar plants appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min in both M_1 (4.82 g) and M_2 (4.99g), with an estimated increase of 118.1% and 117.9%, respectively, in M_1 and M_2 generations, as compared with the GM control, shown in Figure 2.

Seed yield per hectare of guar plants was significantly (P<0.05) enhanced by irradiation with all used gamma-ray doses alone or in combination with the irradiation at 900 W microwaves applied for different times (1, 2, 3, and 4 min) in both M_1 and M_2 generations, as well as, the irradiation treatment with 900 W microwaves applied for 1and 4 min in M_2 generation. The guar plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min produced the highest seed yield of 1.29 tons ha^{-1} in M_1 and 1.35 tons ha^{-1} in M_2 generations. The estimated increase of 107.0% and 102.8%, respectively, was recorded, as compared with the GM control in M_1 and M_2 generations (Figure 2).

Amrita and Jain (2003) mentioned an increase in induced variation in M_2 progenies of guar cv. RGC 197 that was obtained by gamma irradiation (10, 30, 50, 60, 70, and 80 kR) in the number of clusters per plant, number of pods per cluster, number of pods per plant, and seed yield per plant, but induced variation generally reduced the number of seeds per pod and pod length. Based on progeny means, some progenies recorded higher values for all the traits except 100-seed weight and number of clusters per plant. Various types of manifestations, such as, seed yield, number of seeds per pod, pod length, number of clusters, number of pods per cluster, number of pods, seed yield, protein content, gum content, and early maturity in the mutated material, have been reported in the materials treated with mutagens and their progenies (Arora and Pahuja, 2008). Also, Choudhary *et al*. (1973) observed an increase in yield in an M_2 population generated through irradiation with low doses (e.g., 2, 5, 10, 15, and 20 kR) of gamma rays. High doses of gamma rays (e.g., 10 to 80 kR) have been shown to be unsuitable for pods per plant and seed yield (Yadav *et al*., 2004).

Chemical properties of guar

The overall measurement results of the chemical properties of guar are summarized in Figure (3). Crude protein% and ash% of guar plants were significantly (P<0.05) increased by irradiation with all used gamma-ray doses alone and all microwave irradiation treatments alone and their combinations, except the irradiation at 900 W microwaves applied for 4 min alone in M_2 generation for protein%, and G50 in M_1 for ash%. In contrast, G150 significantly (P<0.05) decreased ash% in M_1 generation. The highest crude protein% in guar plants appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 4 min in both M_1 (22.44%) and M_2 (22.74%), which is estimated to have an increase of 27.6% and 25.6%, respectively, as compared with the GM control in M_1 and M_2 generations (Figure 3).

As noted, the highest ash% in guar plants appeared in plants irradiated with 250 Gy gamma-ray dose in combination with the irradiation at 900 W microwaves applied for 1 and 2 min in both M_1 (15.88% and 15.86%, respectively) and M_2 (16.13% and 16.06%, respectively), with an estimated increase of 24.7% and 24.6%, respectively, as compared with the GM control in the M_1 generation, 29.8% and 29.2%, respectively in M_2, as compared with the GM control in $M_1$and M_2 generations (Figure 3).

On the other hand, crude fiber percentage of guar plants was significantly (P<0.05) decreased by irradiation with all used gamma-ray doses alone and all microwave irradiation treatments alone and their combinations, except the irradiation at 900 W microwaves applied for 2 and 3 min in M_1 and the irradiation at 900 W microwaves applied for 3 min in M_2, as shown in Figure (3). The effect of M Control 3T was non-significant on this trait. Chaudhary *et al*. (1973) observed an increase in protein and gum contents in an M_2 population generated through irradiation with low doses gamma rays (2, 5, 10, 15, and 20 kR).

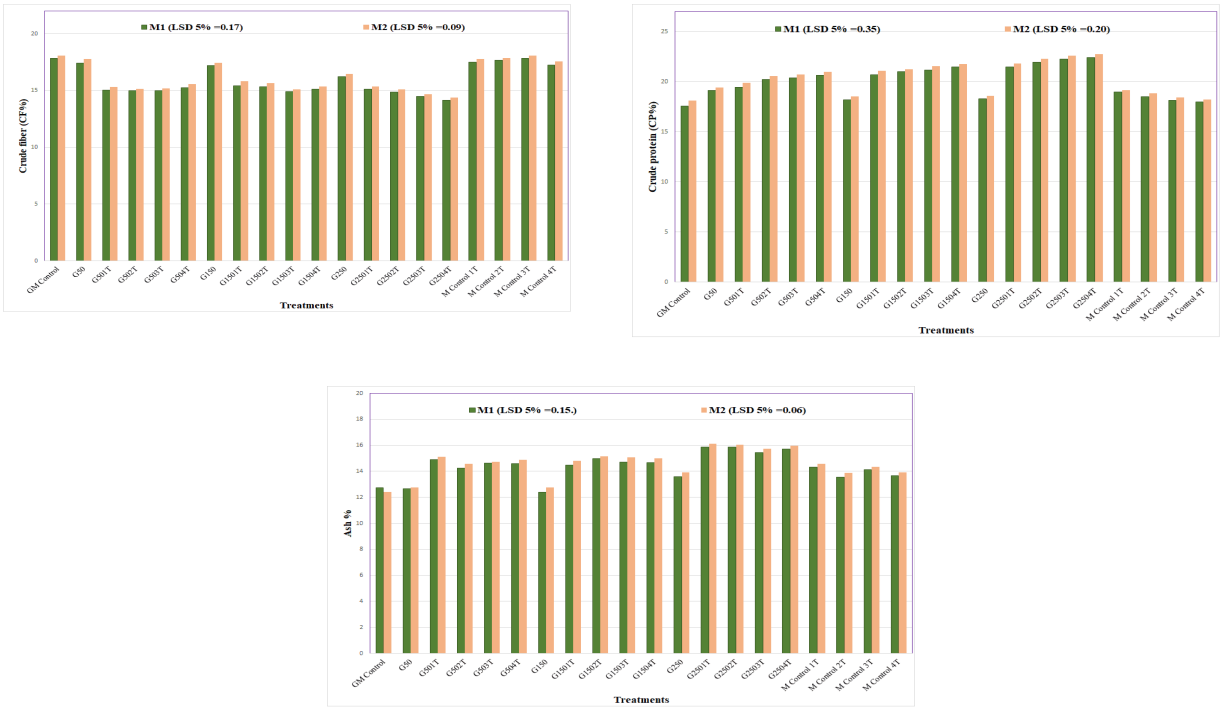

Figure 3. Effect of different gamma-ray doses and 900 W microwave irradiation applied for different time treatments separately or in combinations on the chemical properties of guar (*Cyamopsis tetragonoloba*) in M_1 and M_2 generations.

Molecular analysis

Agarose gels containing PCR products of 18 SCoT primers were carefully visualized for analyzing variations induced in guar variety treated with 20 treatments, including different gamma-ray doses separately or in combinations with microwave treatments beside different microwave treatments (Figures 4 to 6). The 18 SCoT primers exhibited various bands among the treated plants (Table 3) and as compared with the control (guar variety shandaweel 9) (Figures 4 to 6). In M_1 generation, the 18 SCoT primers produced a total of 327 bands, ranging between 151–2895 bp in size (Table 3), out of which 282 bands were found to be polymorphic (86.24% polymorphism). These polymorphic bands could be used as marker-assisted selection for the response of guar plants to different gamma-ray doses separately or in combinations with different microwave treatments.

In M_2 generation, the 18 SCoT primers produced a total of 328 bands ranging between 212–2661 bp in size, out of which 299 bands were found to be polymorphic (91.16% polymorphism) (Table 3). The availability of genetic diversity conserved in the germplasm determines the success of any crop improvement program. Therefore, this study was conducted using gamma rays and microwave irradiation treatments to create valuable genetic diversity in guar. Based on DNA markers, Kumar *et al*. (2017) reported the existence of poor genetic diversity in guar. Further, exploitation of available diversity through hybridization is cumbersome due to the small flower size and very poor seed setting in manually hybridized buds. Therefore, mutation breeding may be the preferred choice in such situations for creating variability and isolating desirable mutants for specific purposes. Though the mutability of guar has amply been demonstrated through various studies, systematic work is still lacking (Arora and Pahuja, 2008; Velu *et al*., 2012; Mahla *et al*., 2018). The successful use of induced mutation depends on the efficiency to create desirable changes with the least undesirable effects (Pathak, 2015). Thus, basic information is needed for the proper application of mutagenesis in guar improvement.

In this study, the SCOT molecular data provided a lot of information concerning clustering results and the genetic variation

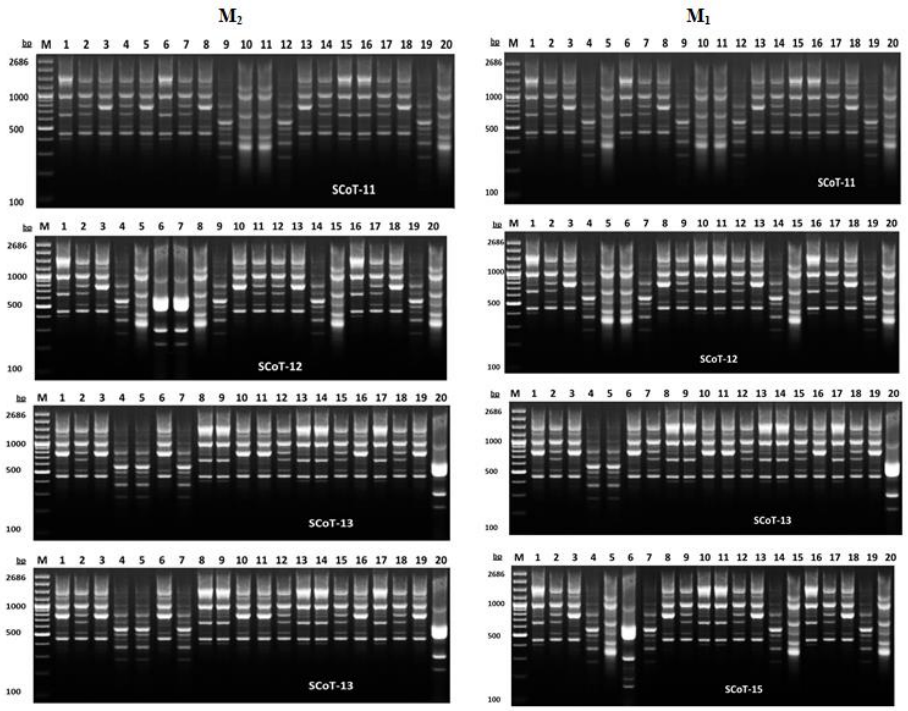

Figure 4. SCOT patterns of *Cyamopsis tetragonoloba* generated by primers SCoT 11, 12, 13, and 15. Lane M is double-digested k DNA (EcoRI and HindIII) DNA ladder and lanes 1–20 represent different treatments as listed in Table 1 in M_1 (left) and M_2 (right) generations.

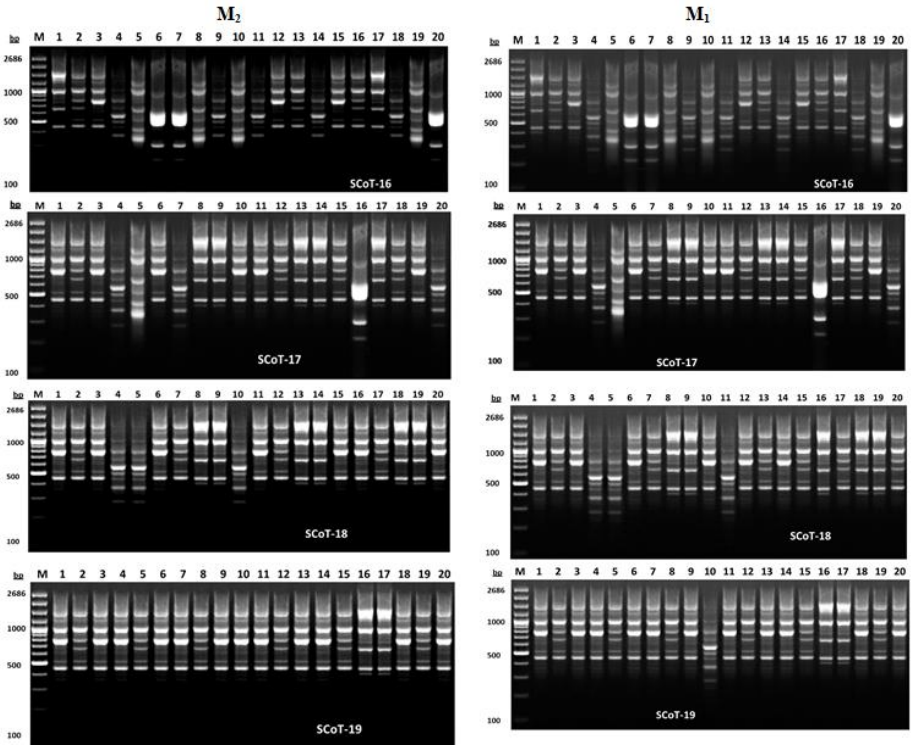

Figure 5. SCOT patterns of *Cyamopsis tetragonoloba* generated by primers SCoT 16, 17, 18, and 19. Lane M is double-digested k DNA (EcoRI and HindIII) DNA ladder and lanes 1–20 represent different treatments as listed in Table 1 in M_1 (left) and M_2 (right) generations.

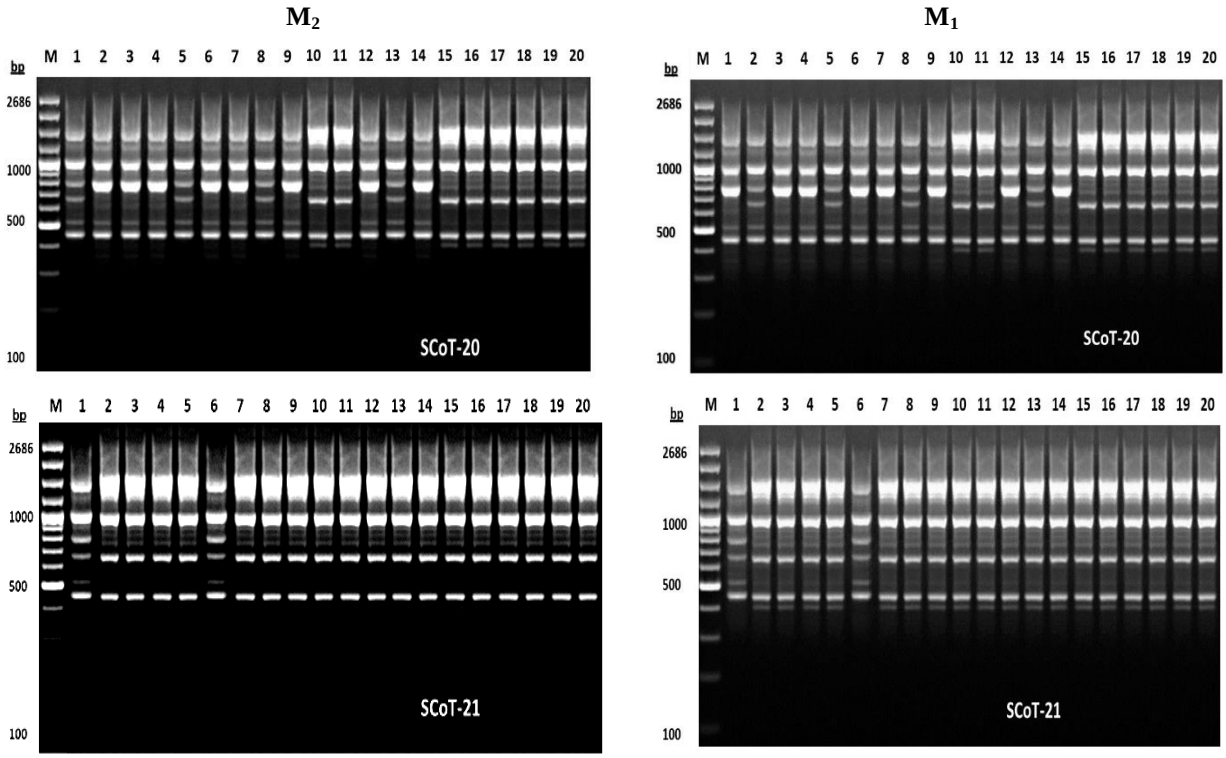

Figure 6. SCOT patterns of *Cyamopsis tetragonoloba* generated by primers SCoT 20 and 21. Lane M is double-digested k DNA (EcoRI and HindIII) DNA ladder and lanes 1–20 represent different treatments as listed in Table 1 in M_1 (left) and M_2 (right) generations.

Table 3. SCOT marker ranged bands produced by M_1 and M_2 generations across 20 treatments applied to *Cyamopsis tetragonoloba.*

M_2			M_1			SCOT primer
Bp	PPB (%)	TB	Bp	PPB (%)	TB	
282-2345	100.0	23	363-2506	95.65	23	SCOT 11
215-2282	100.0	24	279-2342	100.0	22	SCOT 12
216-2294	100.0	18	212-2895	100.0	24	SCOT 13
156-2295	100.0	25	151-2521	100.0	27	SCOT 15
219-2380	100.0	23	220-2306	100.0	23	SCOT 16
218-2520	100.0	24	217-2595	100.0	25	SCOT 17
278-2652	83.33	18	278-2686	85.71	21	SCOT 18
406-2553	76.92	13	280-2520	85.00	20	SCOT 19
406-2520	78.57	14	406-2520	75.00	12	SCOT 20
402-2658	69.23	13	405-2686	64.71	17	SCOT 21
264-2049	84.62	13	220-956	22.22	9	SCOT 22
405-2512	80.00	15	452-2540	30.00	10	SCOT 23
279-2630	90.00	20	355-2627	81.25	16	SCOT 24
279-2661	88.24	17	279-2513	94.12	17	SCOT 25
402-2536	78.57	14	217-2529	90.48	21	SCOT 26
121-2522	100.0	19	215-2188	16.67	6	SCOT 27
276-2497	88.89	18	280-2477	88.24	17	SCOT 28
280-2522	88.24	17	278-2554	88.24	17	SCOT 29
212-2661	91.16	18.22	151-2895	86.24	18.17	Mean
		328			327	Total

TB total band, PPB polymorphic band %

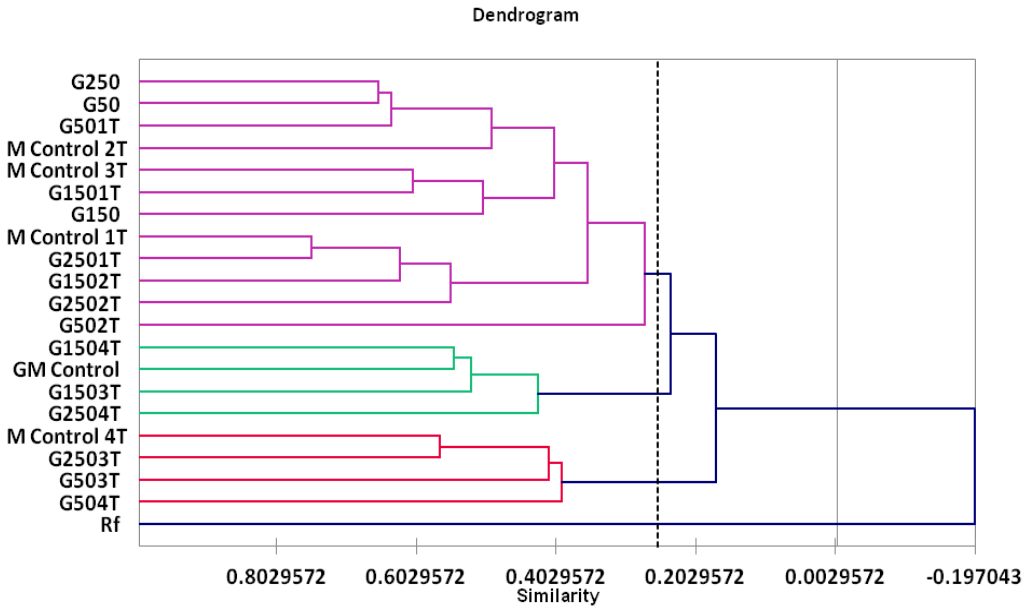

Figure 7. Phylogenetic tree of guar plants as a response to gamma-ray doses and/or in combination with microwave irradiation.

within and between the treatments in M_1 and M_2 of the guar check variety (Shandaweel 9) and the degree of gene differentiation suggested a relatively high genetic diversity, which occurred due to the combination of the irradiation with gamma-ray doses and microwave heating treatments, that can be used in guar breeding programs. These results are in agreement with past findings published by Azzam and El-Sawy (2005), Azzam *et al*. (2007), Azzam and Zein (2012), Sharma *et al*. (2014), Azzam and Khalifa (2016), Abdalla *et al*. (2017, 2018), and Kumar and Agrawa (2019).

Genetic similarity indices varied among the genotypes treated with 20 gamma-ray doses and microwave irradiation treatments (Table 4). The highest similarity (75.3%) was recorded between guar plants with treatment of G250-1T and M control-1T, whereas a low similarity (17.3%) was recorded between guar plants with treatment of G250-2T and G50-3T. These results agreed with those obtained by Ashmawy *et al*. (2016), Azzam and Khalifa (2016), Abdalla *et al*. (2017), Abdalla *et al*. (2018), Al-Taweel *et al*. (2021), and Ashry *et al*. (2021), who demonstrated that gamma irradiations are commonly used to generate genetic variability in field crops and for the development of new genotypes in plant breeding.

Genetic variability is essential for any crop improvement program. The creation and management of genetic variability is the basic need of crop breeding. Mutation breeding is an important alternative employed in crop breeding nowadays. The induction of mutations is an important source of genetic variability (Singh *et al*., 2007). In legumes, genetic variability has been exhausted due to natural selection, hence conventional plant breeding methods are not useful (Wani *et al*., 2001). Lack of sufficient genetic variability is one of the reasons for the failure of active breakthroughs in the self-pollinating crops like legumes as compared with the cereals, hence mutation breeding is the best method to induce genetic variability in the crops within a short time and played a significant role in the development of many crop varieties (Azzam and El- Sawy, 2005; Azzam *et al*., 2007; Azzam and Zein, 2012; Azzam and Khalifa, 2016; Abdalla *et al*., 2017, 2018).

A dendrogram representing the relationships among the genotypes treated with 20 gamma-ray doses and microwave irradiation treatments is presented in Figure 7. The dendrogram separated the treatments into three main clusters. The first main cluster is divided into three subclusters. The second is divided into two subclusters and the third one is also divided into two subclusters. The divided subclusters revealed the similarity indices of genotype treatments.

Table 4. Similarity indices of guar plants as response to gamma-ray doses and /or in combination with microwaves irradiation.

Treatments	GM Control	G50	G50 1T	G50 2T	G50 3T	G50 4T	G150	G150 1T	G150 2T	G150 3T	G150 4T	G250	G250 1T	G250 2T	G250 3T	G250 4T	M Control 1T	M Control 2T	M Control 3T
GM Control																			
G50	0.46																		
G501T	0.46	0.64																	
G502T	0.24	0.39	0.43																
G503T	0.24	0.40	0.34	0.31															
G504T	0.51	0.41	0.46	0.25	0.40														
G150	0.33	0.61	0.54	0.41	0.21	0.38													
G1501T	0.24	0.51	0.59	0.28	0.36	0.26	0.51												
G1502T	0.35	0.61	0.50	0.50	0.31	0.32	0.42	0.52											
G1503T	0.53	0.42	0.56	0.27	0.46	0.45	0.35	0.41	0.32										
G1504T	0.55	0.44	0.47	0.37	0.29	0.28	0.40	0.31	0.39	0.54									
G250	0.45	0.66	0.64	0.42	0.26	0.33	0.50	0.49	0.49	0.32	0.44								
G2501T	0.37	0.60	0.58	0.37	0.44	0.46	0.42	0.60	0.65	0.44	0.28	0.47							
G2502T	0.31	0.48	0.50	0.49	0.17	0.34	0.53	0.47	0.62	0.38	0.36	0.36	0.56						
G2503T	0.34	0.54	0.58	0.26	0.41	0.40	0.55	0.54	0.32	0.45	0.45	0.49	0.41	0.31					
G2504T	0.43	0.47	0.46	0.33	0.25	0.37	0.32	0.42	0.44	0.52	0.44	0.31	0.48	0.32	0.49				
M Control 1T	0.35	0.56	0.48	0.32	0.40	0.36	0.40	0.50	0.63	0.40	0.32	0.41	0.75	0.55	0.41	0.52			
M Control 2T	0.34	0.61	0.60	0.34	0.33	0.40	0.48	0.51	0.60	0.40	0.53	0.50	0.57	0.47	0.51	0.43	0.51		
M Control 3T	0.27	0.47	0.50	0.47	0.28	0.33	0.58	0.61	0.47	0.49	0.36	0.52	0.38	0.42	0.48	0.51	0.44	0.41	
M Control 4T	0.21	0.33	0.32	0.27	0.48	0.40	0.42	0.35	0.28	0.36	0.39	0.19	0.37	0.24	0.57	0.38	0.32	0.33	0.32

CONCLUSIONS

Gamma-ray doses of 150 and 250 Gy alone or in combination with the irradiation at 900 W microwaves applied for different times (1, 2, 3, and 4 min) significantly influenced the plant height of guar (*Cyamopsis tetragonoloba*), as well as, the number of tillers plant^{-1},fresh and dry forage yield, and fresh and dry leaf stem^{-1} ratio. Data included in the second sample were about seed yield at harvest time, i.e., pods plants^{-1}, weight of pods plant^{-1}, whole plant dry weight, seeds pod-^{1}, length of pods, 100-seeds weight, and seed yield; these were affected by irradiation with different and varied responses. In M_1, the 18 SCoT primers produced a total of 327 bands ranging between 151–2895 bp in size, with 86.24% polymorphism. Meanwhile in M_2 generation, it produced a total of 328 bands ranging between 212–2661 bp in size, with 91.16% polymorphism. The M_1 and M_2 generations exhibited positive and negative bands, which could be used as marker-assisted selection for responding to treated guar plants with different gamma-ray doses separately or in combinations with microwave treatments.

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