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GAMMA-RAYS AND MICROWAVES IRRADIATION INFLUENCE ON GUAR (*CYAMOPSIS TETRAGONOLOBA*): II – PROTEOMIC ANALYSIS LINKED TO PLANT HEIGHT AND CRUDE PROTEINS

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SUMMARY

Guar is an economically significant forage crop and a multi-purpose plant. In Egypt, with a limited gene pool of guar, gamma ray (γ-rays) and microwave pre-treatment, individually or in association, were used to generate genetic variability and develop new high-yielding genotypes. Guar seeds of the variety Shandaweel-9 were irradiated with different γ-ray doses (*i.e.*, 0, 150, 250, and 350 Gray "Gy"), either individually or along with the irradiation with 900 W microwave treatments applied for different exposure times (1, 2, 3, and 4 min), then grown at Agricultural Research Center, Giza, Egypt in 2019, and 2020 summer seasons. The results indicated that gamma-ray doses of 150 and 250 Gy, stand-alone or with microwave treatments, significantly influenced guar plant height and crude protein in M_1 and M_2. Fragments with 925, 1427, and 2145 bp linked to plant height and crude protein were eluted from the gel, sequenced, and then registered on GenBank. The fragment linked to plant height received accession No. LC681484.1 and fragments linked to crude protein received accession No. of OK617330.1 and OK617331.1. The sequenced fragments underwent translation to protein, then proceeded to proteome analyses. Depending on the sequence analysis, the 925 bp fragment consisted of a 302 bp ORF (open reading frame) encoded with 302 amino acids. However, the fragment of 1427 bp has 433 ORF, and the fragment of 2145 bp has 705 ORF. The LC681484.1 might have a role in plant elongation. On the other hand, OK617330.1 and OK617331.1 might be responsible for cell wall protein organization.

Keywords: γ-rays irradiation, microwave heating, plant height, quality analysis, SCoT, plant height- and crude protein-linked genes

Key findings: Proteome analysis revealed three predicted proteins: the first is responsible for plant elongation (plant height), and the others are responsible for cell wall protein characterization (crude protein contents). Registration was done for a gene linked to plant height in *Cyamopsis tetragonoloba* (Accession No. LC681484.1), as well as, genes linked to crude protein (OK617330.1 and OK617331.1) on GenBank`s database.

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INTRODUCTION

Guar (*Cyamopsis tetragonoloba*) is a self-pollinating crop (2n=14), an economically significant forage crop, and a multi-purpose plant. It has been used recently as a source of galactomannan gum (Guar gum) used as a stabilizer in foods, viz., ice cream, salad dressings, and yogurt, and in other industries, viz., paper manufacturing, cosmetics, pharma, and textiles (Mudgil *et al*., 2014). Gamma irradiations are commonly used in field crops to generate genetic variability and develop new genotypes (Shabana *et al*., 1994; Azzam and Abbas, 2005; Azzam and El- Sawy, 2005; Azzam *et al*., 2008; Ashmawy and Azzam, 2011; Azzam and Zein, 2012; Azzam and Khalifa, 2016; Abdalla *et al*., 2017; Al-Taweel *et al*., 2021). Microwave pre-treatment promotes gene expression of the isozymes, *i.e*., superoxide dismutase (SOD) and peroxidase (POD) (Aladjadjiyan, 2012). It significantly exceeds the potential of germination, root length, stem length, GR, and total seed block (Radzevičius *et al*., 2013).

Molecular techniques are applied to detect gene expression and identify genotypes influenced by abiotic stress (Khaled *et al*., 2015). The identification of gene expression and QTLs in plants used SCoT (the start codon targeted marker), AFLP (Amplified fragment length), and SSR (Simple Sequence Repeats) (Khaled *et al*., 2018; Al-Taweel *et al*., 2019; Salah *et al*., 2021). The cDNA SCoT (cDNA starts codon-targeted) molecular technique came highly proposed as a great tool suitable for identifying variations in gene expression, stress tolerance, and plants` genetic stability (Al-Taweel *et al*., 2019; Abou-Sreea *et al*., 2021). cDNA-SCoT markers were applied to detect the gene expression in *Saccharumofficinarum*, *Phoenix dactylifera*, *Mangiferaindica, Oleaeuropaea* tree, and *Dendrobiumofficinales* (Chen *et al*., 2013; Al-Janabi and Al-Rawi, 2018). Plants developed many molecular pathways to recognize and confront the environment quickly. Proteins play an essential role because a) They regulate the physiological processes to adapt to environmental changes; they form new phenotypes, and b) Proteins are the critical expression of cellular machinery and have a vital role in maintaining homeostasis within the cell. The individual protein behavior did not mirror this complicated signals network and biological regulation that influence plant response to environmental changes. Therefore, several proteins respond to environmental stress (Ingolia, 2014; Feussner and Polle, 2015; Wang *et al*., 2019). Consequently, it is essential to know proteins and their role during stress exposure.

The current research progressed to determine the influence of γ-ray doses and various time treatments of microwaves, either individually or in association, on guar`s plant height and crude protein to detect variations induced via mutagenic agents, to generate genetic variability and develop new high-yielding genotypes. Also, it sought to identify and sequence genes linked to plant height and crude protein and perform proteomic analysis.

MATERIALS AND METHODS

Irradiation with γ-rays doses

Exposure of guar seeds of the local cultivar Shandaweel-9 to 0, 50, 150, and 250 Gy of γ-rays of Cobalt-60 source at a dose rate of 7.03 Gy min^{-1} proceeded at the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt.

Irradiation with microwave heating treatments

A Japanese Panasonic NN-K597WS microwave oven, with a variable power range of 200 to 900 W and a frequency of 2450 MHz, served as the source of the microwaves. A 900 W (2450 MHz) microwave radiation, applied to seeds alone or with different γ-ray doses for varying exposure times, transpired (Table 1). In a previous study, a field trial was conducted on the guar cultivar Shandaweel-9 to study the influence of various γ-rays doses and 900 W (2450 MHz) microwave radiation at varying times of exposure, individually or in combination (Table 1) on forage yield, productivity and chemical composition (Khaled *et al*., 2022). This investigation focused on plant height (cm) and crude protein (CP %). Crude protein determination followed the conventional method recommended by the Association of the Official Agricultural Chemists (AOAC, 1980) on the dried forage sample at 70°C.

Statistical analysis

Statistical analysis of data proceeded outlined by Snedecor and Cochran (1980). A combined analysis of the two experimental generations took place based on the outcomes of Bartlett's test. The application of the least significant

Table 1. The various γ-rays doses and various power and exposure time of microwaves and the code used across the manuscript.

Treatment	Code	γ-rays dose	Microwave heating treatment	
			Power	Time
1	GM Control	0	0	0
2	G-50	50Gy	0	0
3	G-50-1T	50Gy	900 W = 2450MHz	1 min
4	G-50-2T	50Gy	900 W = 2450MHz	2 min
5	G-50-3T	50Gy	900 W = 2450MHz	3 min
6	G-50-4T	50Gy	900 W = 2450MHz	4 min
7	G-150	150Gy	0	0
8	G-150-1T	150Gy	900 W = 2450MHz	1 min
9	G-150-2T	150Gy	900 W = 2450MHz	2 min
10	G-150-3T	150Gy	900 W = 2450MHz	3 min
11	G-150-4T	150Gy	900 W = 2450MHz	4 min
12	G-250	250Gy	0	0
13	G-250-1T	250Gy	900 W = 2450MHz	1 min
14	G-250-2T	250Gy	900 W = 2450MHz	2 min
15	G-250-3T	250Gy	900 W = 2450MHz	3 min
16	G-250-4T	250Gy	900 W = 2450MHz	4 min
17	M Control-1T	0	900 W = 2450MHz	1 min
18	M Control-2T	0	900 W = 2450MHz	2 min
19	M Control-3T	0	900 W = 2450MHz	3 min
20	M Control-4T	0	900 W = 2450MHz	4 min

difference (LSD) at a 5% probability level compared means.

PCR -cDNA SCoT reaction and conditions of amplification

Al-Taweel *et al*. (2019) described the technique used for PCR-cDNA-SCoT amplification. RNA extraction of 40 guar populations used the Trizol method (Luo *et al*., 2014). Then cDNA was synthesized using QuantiTect Reverse Transcription Kit (Qiagen company) and the company instructions. Employing the technique of cDNA-SCoT helped compare guar samples (40 populations) to find markers related to several characteristics depending on various gene expressions. Eighteen primers (Oligo primer of cDNA- SCoT, provided by Macrogene Company) were used (Table 2). The performed reaction followed the method described by Al-Taweel *et al*. (2019). Running the products on agarose (1.3%) went against a 100 bp DNA ladder. Then gel was visualized and photographed using a gel documentation system after being detected on a UV transilluminator.

Analysis of molecular data

The generated banding patterns were scored as 1 or 0 for the presence or absence of each band, using 1D software, v2009 of Total Lab software provided by Nonlinear Dynamics.

Data scoring and statistical analysis

Banding patterns produced by 18 cDNA-SCoT primers got scored for absence and presence as 0 and 1 of bands. The recording of polymorphic and monomorphic bands, the total number of bands, and polymorphism% depended on banding patterns observed and produced by SCoT primers. In addition, it assessed the potential of markers for genetic variability estimation by measuring resolving power (RP), effective multiplex ratio (EMR), marker index (MI), and polymorphism information content (PIC). Calculating the Polymorphic Information Content (PIC) for dominant markers used the algorithm:

$$PIC = 1- (f^2 + [1 - f]^2)$$

Where 'f' is the frequency of the marker in the data set (the frequency of the i^{th} allele), PIC for dominant markers has a maximum of 0.5 for 'f'=0.5 (De-Riek *et al*., 2001).

Calculating the effective multiplex ratio (EMR) used the formula: EMR = n × β, where n is the mean amplified bands by accession to a particular marker (multiplex ratio), and β is calculated from polymorphic (PB) and nonpolymorphic (MB) loci; β = PB/(PB + MB). Multiplying the PIC by the EMR determined the primer`s marker index (MI) (Varshney *et al*., 2007).

Table 2. Sequences, GC%, and annealing temperature (Tm) of SCoT primers used in the PCR reactions.

Primer ID	Primer Sequence 5` to 3`	GC%	Tm (°C)
SCOT-11	AAGCAATGGCTACCACCA	50.00	53.9
SCOT-12	ACGACATGGCGACCAACG	61.00	58.4
SCOT-13	ACGACATGGCGACCATCG	61.00	58.4
SCOT-15	ACGACATGGCGACCGCGA	67.00	60.7
SCOT-16	ACCATGGCTACCACCGAC	61.00	58.4
SCOT-17	ACCATGGCTACCACCGAG	61.00	58.4
SCOT-18	ACCATGGCTACCACCGCC	67.00	60.7
SCOT-19	ACCATGGCTACCACCGGC	67.00	60.7
SCOT-20	ACCATGGCTACCACCGCG	67.00	60.7
SCOT-21	ACGACATGGCGACCCACA	61.00	58.4
SCOT-22	AACCATGGCTACCACCAC	56.00	56.1
SCOT-23	CACCATGGCTACCACCAG	61.00	58.4
SCOT-24	CACCATGGCTACCACCAT	56.00	56.1
SCOT-25	ACCATGGCTACCACCGGG	67.00	60.7
SCOT-26	ACCATGGCTACCACCGTC	61.00	58.4
SCOT-27	ACCATGGCTACCACCGTG	61.00	58.4
SCOT-28	CCATGGCTACCACCGCCA	67.00	60.7
SCOT-29	CCATGGCTACCACCGGCC	72.00	63.0

EMR*PIC = MI. The primer`s resolving power (RP) was estimated using the formula of Prevost and Wilkinson (1999). Rp = Σ Ib (band informativeness)

Where Ib revealed the informative bands, the Ib ranged between 0 and 1 by the following formula:

$$Ib = 1 - (2\,[0.5 - pi])$$

Where *pi* is the proportion of accessions containing the i^{th} band.

Sequence analysis of gene linked to plant height and crude protein

The fragments linked to plant height and crude protein were sequenced, then translated online using Expasy tools (https://web.expasy.org/translate/). DNA and protein sequences were aligned for the homology study with BLASTN and BLASTP (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The protein structure was analyzed using PyMOL Molecular Graphics System version 2.5.2 (Schrodinger, LLC, https://pymol.org) based on the three-dimensional (3D) X-ray crystallography structure. The sequenced fragments were submitted to GenBank and afterward obtained accession numbers.

RESULTS AND DISCUSSION

Guar plant characteristics

The 50 Gy γ-rays did not influence the guar's plant height, as well as, heating irradiation with 900 W microwave heating treatments, applied at different times (1, 2, 3, and 4 min) individually in M_1 and M_2. However, γ-rays: 150 and 250 Gy, either individually or along with the heating irradiation with microwaves (1, 2, 3, and 4 min), significantly influenced plant height ($P < 0.05$) (Table 3). Irradiation with 50 Gy γ-rays with microwave treatments (1, 2, 3, and 4 min) followed the same trend in both generations. Plants irradiated with 250 Gy γ-rays and heating with microwave heating treatments for three and four minutes occurred the tallest in M_1 (153.3 and 143.0 cm, respectively) and M_2 (156.0 and 146.4 cm, respectively). Percent increases scored at 53.2% and 42.9%, respectively, compared with M_1's GM control, 53.6% and 44.2%, respectively in M_2, 34.3% and 25.3%, respectively, compared with G-250-3T and G-250-4T in M_1, 32.8% and 24.6%, respectively compared with G-250-3D and G-250-4D in M_2.

The individual irradiation of 50 Gy γ-rays or 900 W microwaves applied various times (1, 2, 3, and 4 min) did not influence guar`s plant height in the two mutational

Table 3. The influence of various γ-rays doses and 900 W microwave heating treatments applied for different time treatments, either individually or in combination, on guar plant height and crude protein in M_1 and M_2 generations.

Treatment	Character	Plant height (cm)		Crude protein (CP%)	
		Generation			
		M_1	M_2	M_1	M_2
GM-Control		100.07	101.54	17.58	18.11
G-50		105.15	107.33	19.14	19.44
G-50-1T		118.85	120.60	19.47	19.87
G-50-2T		121.06	123.22	20.22	20.55
G-50-3T		126.10	128.10	20.38	20.74
G-50-4T		127.40	129.78	20.64	20.98
G-150		115.29	117.62	18.22	18.55
G-150-1T		116.76	119.09	20.71	21.10
G-150-2T		120.85	123.25	21.02	21.26
G-150-3T		123.66	125.55	21.17	21.54
G-150-4T		125.82	128.88	21.50	21.77
G-250		114.14	117.50	18.28	18.58
G-250-1T		131.40	133.61	21.48	21.81
G-250-2T		133.04	135.71	21.97	22.27
G-250-3T		153.32	155.99	22.25	22.58
G-250-4T		142.99	146.40	22.44	22.74
M Control-1T		95.93	97.97	18.97	19.15
M Control-2T		100.03	103.36	18.52	18.85
M Control-3T		101.39	104.36	18.13	18.43
M Control-4T		103.63	106.47	17.98	18.23
L.S.D 5%		6.39	6.37	0.35	0.20

generations. However, γ-rays: 150 and 250 Gy alone or with microwave heating treatments for 1, 2, 3, and 4 min significantly increased plant height ($P < 0.05$). The same trend appeared at irradiation with 50 Gy γ-rays combined with irradiation with microwave heating treatments for 1, 2, 3, and 4 min. in the two mutational generations. Furthermore, Mahla *et al*. (2018) discovered that the reduction in genotypic response for plant height was progressive, starting at 100 Gy to 800 Gy. Also, irradiation enhanced plant height in M_2 generations of cv. RGC 197 when subjected to gamma radiation doses of 10, 30, 50, 60, 70, and 80 kR (Mahla *et al*., 2018; Amrita and Jain, 2003). Arora and Pahuja (2008) have noticed increasing peduncle length and plant height, but 100–200 kR γ-rays have proven as lethal. According to Damm *et al*. (2012), the dose dependency may relate to modifications made to the enzyme's protein structure by microwave heating treatment. To determine their toxicity or side effects, most of these studies concentrated on weak (> 0.5 mW cm^{-2}) and low-frequency magnetic fields. However, the use of a microwave boosted plant height, fresh mass, and germination done by Aladjadjiyan (2002), Belayavskaya (2004), Łupinska *et al*. (2009), Han (2010), and improved crop yield and quality (Pandit *et al*., 2021). System heating arises from the rotating dielectric molecules caused by microwaves in an electromagnetic field. This rotation may destabilize bio-molecules, including DNA.

Results show that guar crude protein% was significantly ($P < 0.05$) increased with the individual use of γ-rays doses and all microwave treatments, either individually or in combination, in the two mutational generations, except for the individual irradiation with microwaves for four minutes in M_2. The greatest crude protein% in guar plants appeared in plants irradiated with 250 Gy γ-rays with microwave irradiation for four minutes in both M_1 (22.44%) and M_2 (22.74%) (Table 3).

The present results agreed with Chaudhary *et al*. (1973). They noticed that low doses of gamma (i.e., 2, 5, 10, 15, and 20 kR) increased the protein and gum contents of the M_2 population. The function of a protein depends on its subcellular localization because various cell contents are a consequence of several biochemical and physiological processes. Most biological processes and pathways in the cell alter the localization of subcellular proteins, such as transcription factor nucleocytosolic shuttling and relocating the mitochondrial protein during apoptosis (Lundberg and Borner, 2019).

Molecular analysis

PCR products of 18 SCoT primers were visualized on agarose gels and analyzed for variants induced in guar cultivar treated with 20 treatments, including various γ-rays doses, individually or with microwaves. The 18 SCoT primers exhibited various bands among the treated plants (Table 4, Figures 1 and 2). In M_1, 327 bands emerged from SCoT primers, ranging between 151–2895 bp, with 282 bands as polymorphic (p% = 86.24). The average PIC was 0.43, EMR (671), MI (3.11), and RP (1679). The maximum value of PIC (0.50) came from SCoT-24, SCoT-25, and SCoT-29, whereas SCoT-23 and SCoT-27 revealed minimum PIC values of 0.13 and 0.12, respectively. The lowest EMR and MI values originated with SCoT-27 (0.93 and 0.12, respectively). The SCoT- 21 recorded the greatest value of RP (20.3), whereas the lowest one by SCoT-27 (Table 4, Figure 1).

Table 4. Scot marker parameters calculated in M_1 and M_2 generations across 20 treatments applied to the guar.

Generation	Scot primer	TB	PB	MB	PPB %	Uniq band		PIC	EMR	MI	Rp
						+	-				
	SCOT-11	23	22	1	95.65	-	-	0.47	8.37	3.93	17.5
	SCOT-12	22	22	0	100.0	2	-	0.48	8.77	4.21	18.4
	SCOT-13	24	24	0	100.0	6	3	0.46	8.70	4.00	17.4
	SCOT-15	27	27	0	100.0	5	2	0.44	8.85	3.89	17.7
	SCOT-16	23	23	0	100.0	2	-	0.45	7.80	3.51	15.6
	SCOT-17	25	25	0	100.0	4	3	0.46	9.15	4.21	18.3
	SCOT-18	21	18	3	85.71	1	1	0.50	8.36	4.18	19.5
	SCOT-19	20	17	3	85.00	3	1	0.50	8.16	4.07	19.2
	SCOT-20	12	9	3	75.00	-	-	0.48	5.33	2.56	14.2
M_1	SCOT-21	17	11	6	64.71	3	1	0.48	6.57	3.16	20.3
	SCOT-22	9	2	7	22.22	1	-	0.31	1.62	0.50	14.6
	SCOT-23	10	3	7	30.00	-	1	0.13	2.97	0.39	18.6
	SCOT-24	16	13	3	81.25	1	-	0.50	6.87	3.42	16.9
	SCOT-25	17	16	1	94.12	2	-	0.50	7.77	3.88	16.5
	SCOT-26	21	19	2	90.48	5	1	0.47	7.01	3.27	15.5
	SCOT-27	6	1	5	16.67	-	-	0.12	0.93	0.12	11.2
	SCOT-28	17	15	2	88.24	4	1	0.49	6.57	3.23	14.9
	SCOT-29	17	15	2	88.24	-	1	0.50	7.02	3.49	15.9
	Average/primer	18.17	15.67	2.50	86.24	3.00	1.50	0.43	6.71	3.11	16.79
	SCOT-11	23	23	0	100.0	3	-	0.42	6.75	2.80	13.5
	SCOT-12	24	24	0	100.0	1	-	0.44	7.90	3.49	15.8
	SCOT-13	18	18	0	100.0	2	2	0.48	7.30	3.52	14.6
	SCOT-15	25	25	0	100.0	5	-	0.42	7.40	3.09	14.8
	SCOT-16	23	23	0	100.0	1	-	0.42	7.00	2.96	14.0
	SCOT-17	24	24	0	100.0	8	1	0.43	7.35	3.12	14.7
	SCOT-18	18	15	3	83.33	1	-	0.49	6.42	3.14	15.4
	SCOT-19	13	10	3	76.92	1	-	0.49	5.77	2.82	15.0
	SCOT-20	14	11	3	78.57	1	-	0.50	5.58	2.79	14.2
M_2	SCOT-21	13	9	4	69.23	1	-	0.49	4.99	2.46	14.4
	SCOT-22	13	11	2	84.62	1	-	0.49	6.26	3.07	14.8
	SCOT-23	15	12	3	80.00	5	3	0.47	7.36	3.49	18.4
	SCOT-24	20	18	2	90.00	5	1	0.48	7.16	3.43	15.9
	SCOT-25	17	15	2	88.24	5	4	0.50	7.54	3.77	17.1
	SCOT-26	14	11	3	78.57	1	-	0.49	6.25	3.07	15.9
	SCOT-27	19	19	0	100.0	3	1	0.43	5.95	2.56	11.9
	SCOT-28	18	16	2	88.89	6	1	0.49	6.67	3.24	15.0
	SCOT-29	17	15	2	88.24	-	1	0.50	6.75	3.34	15.3
	Average/primer	18.22	16.61	1.61	91.16	2.94	1.75	0.47	6.69	3.12	15.04

TB = Total number of bands, PB = Polymorphic bands, MB= Monomorphic bands, PPB %= Polymorphism%, PIC = Polymorphism information content, MI= Marker index, EMR = Effective multiplex ratio, Rp= Resolving power.

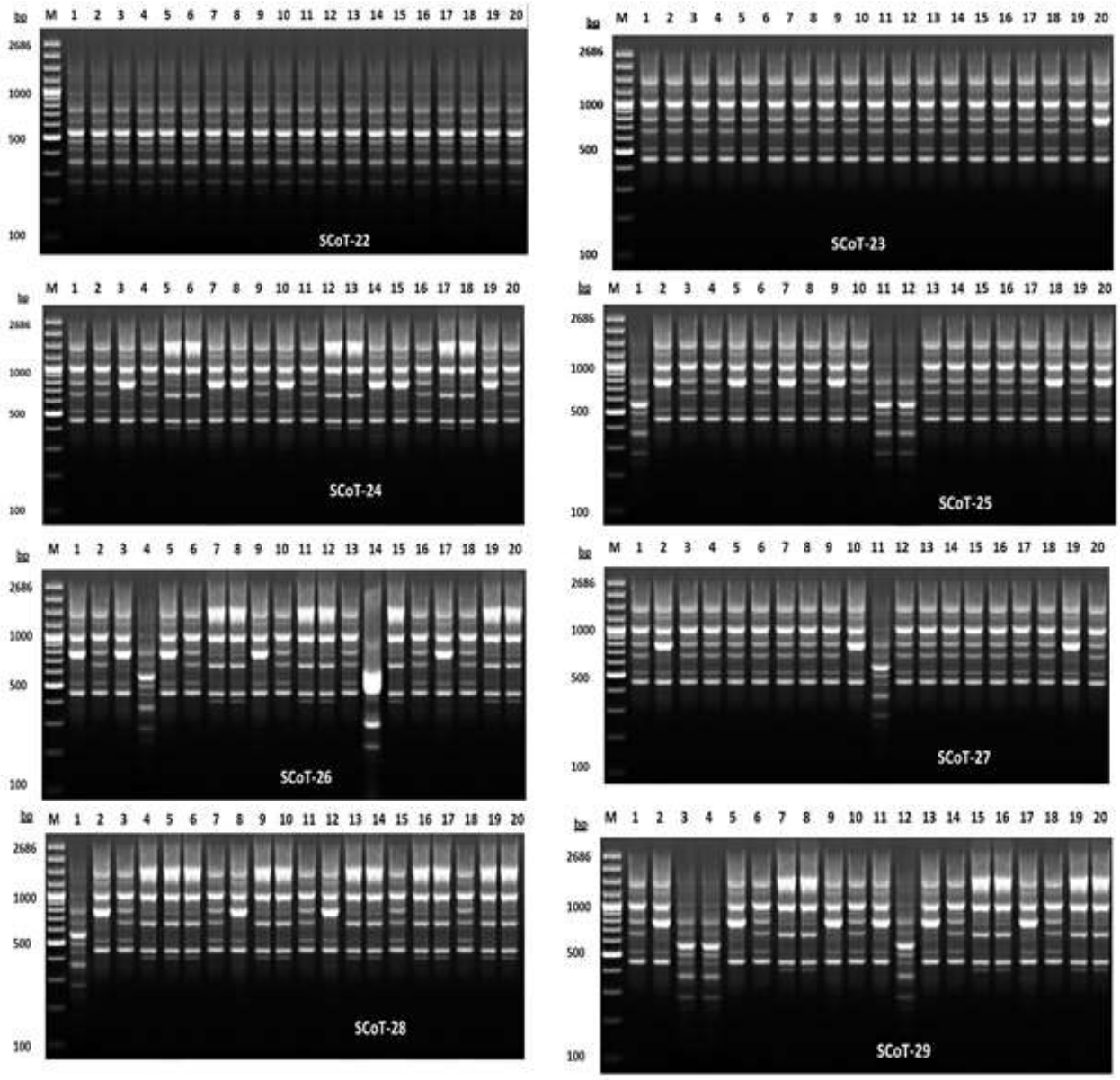

Figure 1. SCOT patterns of *Cyamopsis tetragonoloba* generated by primers SCoT-22:29. Lane M is double-digested k DNA (EcoRI and HindIII) DNA ladder and lanes 1–20 represent the different treatments of M_1 generation.

In the M_2 generation, the SCoT primers provided 328 bands, ranging between 212–2661 bp. Two hundred ninety-nine bands showed polymorphic (p% = 91.16) (Table 4). The average of PIC (0.47), EMR (6.69), MI (3.12), and RP (15.04) appear in Table 4. Among various SCoT primers used, the maximum value of PIC (0.5) recorded came from SCoT-25 and SCoT-29. The greatest MI (3.77) was created by SCoT-25. SCoT-23 revealed the highest RP value of 18.4, with the lowest produced by SCoT-27 (11.9). The most number of unique negative bands (four bands) was revealed by SCoT-25 at the molecular size of 1419.4, 1267.4, 1000.0, and 414.5 bp in the genotype developed from treatments number 12 (G-250) that irradiated with 250 γ-rays (Table 4, Figure 2).

One of the reasons for the failure of active developments in self-pollinating crops like legumes compared with cereals is a lack of sufficient genetic variability. As a result, mutation breeding is the best method for inducing genetic variability in crops in a short

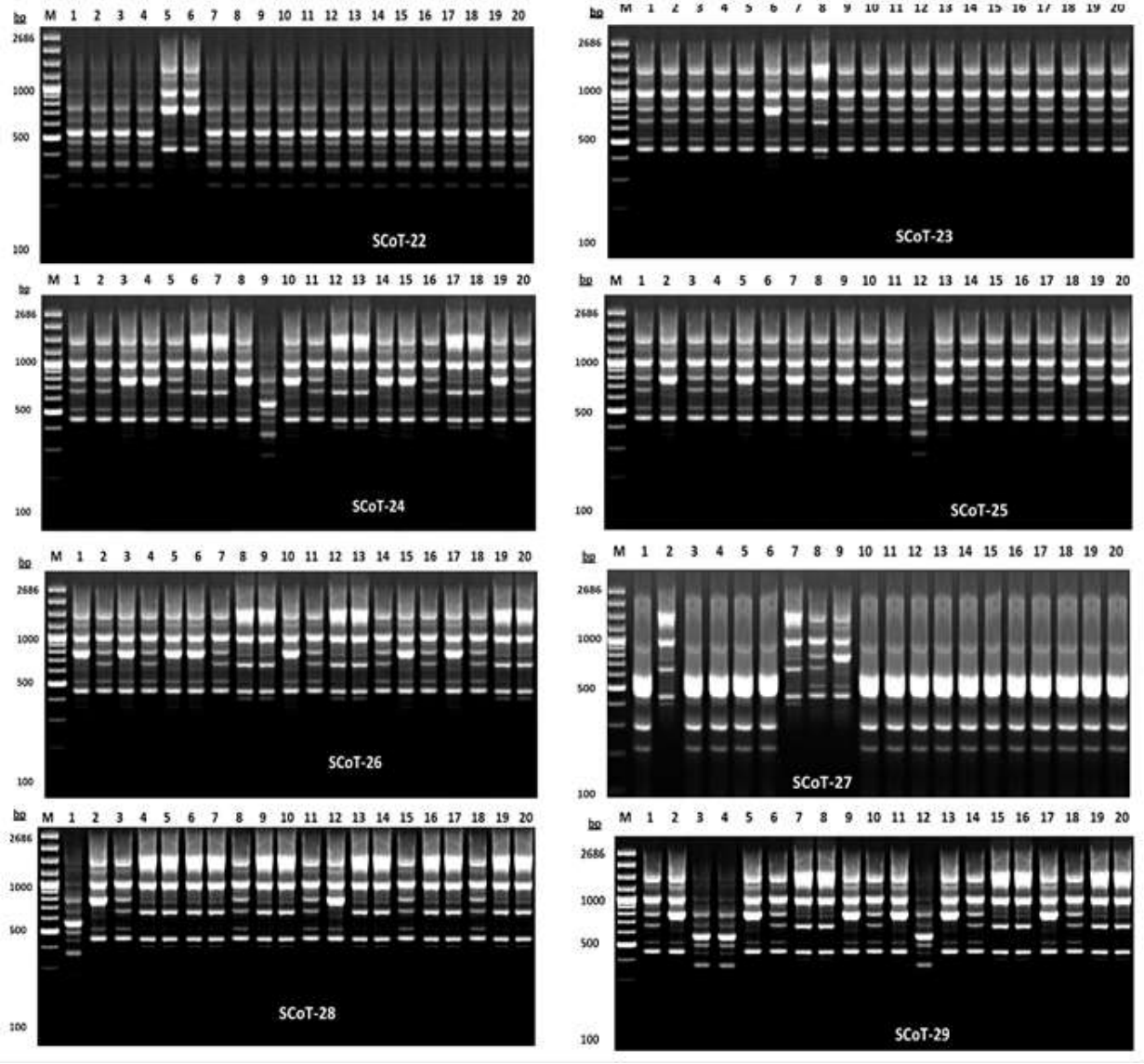

Figure 2. SCOT patterns of *Cyamopsis tetragonoloba* generated by primers SCoT-22:29. Lane M is double-digested k DNA (EcoRI and HindIII) DNA ladder and lanes 1–20 represent the different treatments of M_2 generation.

period and plays a vital role in developing diverse crop varieties (Azzam and El- Sawy, 2005; Azzam and Zein, 2012; Azzam and Khalifa, 2016; Abdalla *et al*., 2017). The SCOT molecular data in this study provided much information about clustering results and genetic variation within and between treatments in M_1 and M_2 of guar. The degree of gene differentiation suggested a relatively high genetic diversity. These results are in agreement with past findings published by Azzam and El-Sawy (2005), Azzam and Zein (2012), Sharma *et al*. (2014), Azzam and Khalifa (2016), Abdalla *et al*. (2017), and Kumar and Agrawa (2019).

Sequence analysis of gene linked to plant height and crude protein

Exploring the fragment of 900 bp on the gel revealed 921 bp when sequenced, and fragments with 1178 and 2496 bp occurred to be 1329 and 2210 bp, respectively. The fragment linked to plant height received accession No. LC681484.1 and fragments linked to crude protein received accession No. OK617330.1 and OK617331.1.

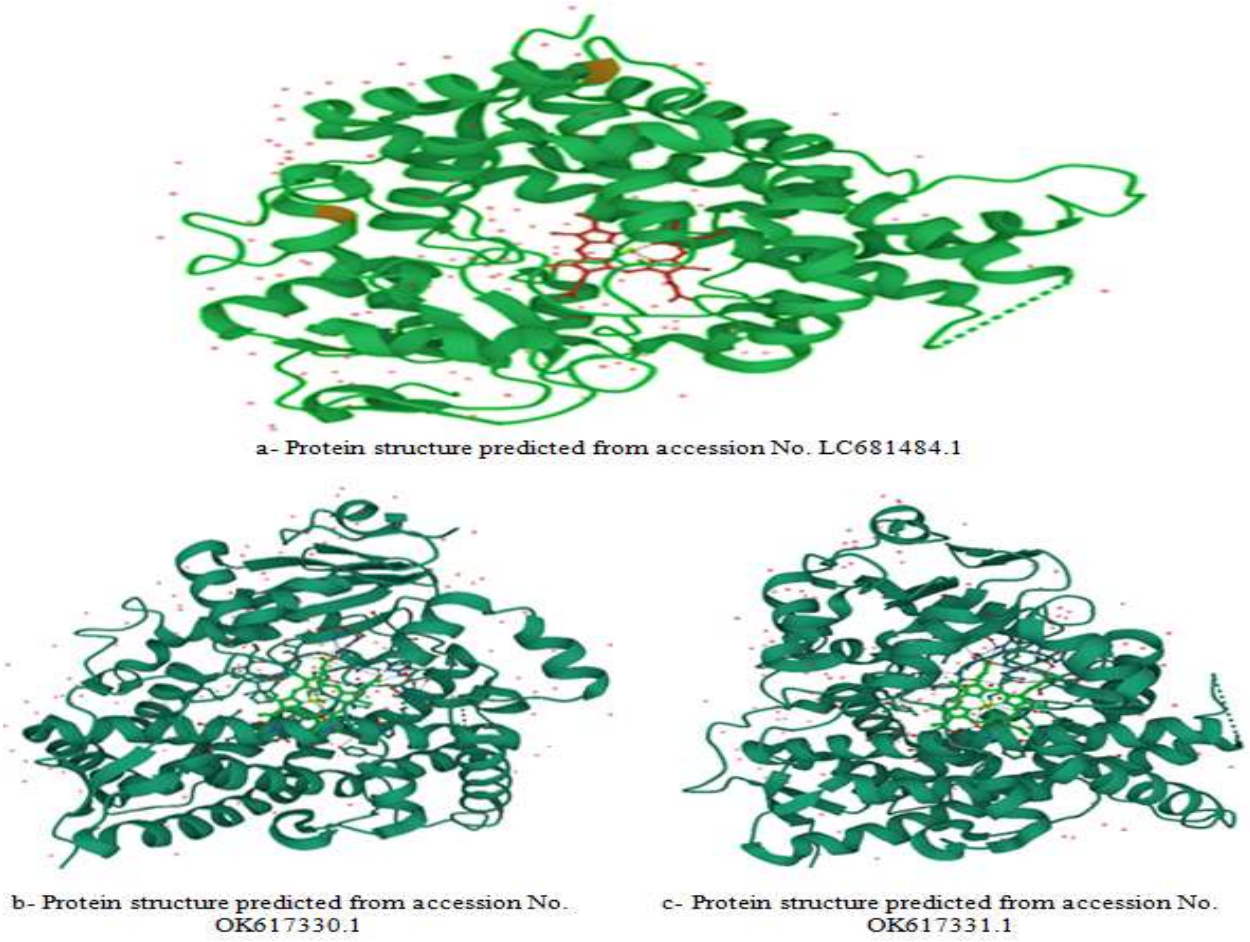

Figure 3. The 3D structure of protein translated from the sequences of *Cyamopsis tetragonoloba* possibly responsive genes, (a) plant height, (b) crude protein sequence1 and (c) crude protein sequence 2**.**

The LC681484.1 sequence is similar to the sequence with accession No. KJ400017.1 refers to *Cyamopsis tetragonoloba* clone RGC1003 (3) SCAR marker genomic sequence. This result is in harmony with Sharma *et al*. (2014), who observed the KJ400017.1 using the SCAR marker. The translated protein for the LC681484.1 sequence belongs to the FHY3/FAR1 gene family. The proteins are homologous and essential for controlling responses to far-red light in plants through phytochrome A (phyA). Depending on the analysis of the sequence, the fragment of 921 bp contains a 302 bp open reading frame (ORF) and encodes 302 amino acids.

Based on the analysis of OK617330.1 that linked to crude protein, the fragment of 1178 bp contains 433 bp ORF and encodes 433 amino acids. The structure is illustrated in Figure 3. The FAR1 and FHY3 have been identified individually as the major signaling vectors for responses of phyA-mediated FR-HIR (Wang and Deng, 2002; Hudson *et al*., 2003). The protein formed from OK617330.1 linked to crude protein was similar to the sequence with accession No. KHN05952.1 refers to putative membrane protein ycf1 (*Glycine soja*). Many developmental and physiological changes in the plant essentially combined with cell wall adjustments, and these changes are entirely identified by cell wall proteins (CWPs) (Cramer *et al*., 2011; Zhu *et al*., 2014; Hoehenwarter *et al*., 2016; Zhu *et al*., 2016; Zhu *et al*., 2018).

Also, OK617330.1 linked to crude protein (Figure 3) showed similar to the sequence with accession No. of YP_009556542.1, which refers to ribosomal protein L2 (Rhabdodendronamazonicum). Ribosomal protein L2 (RPL2) is one of the 49 proteins that belong to the large 60S subunit of the eukaryotic ribosome. It is obligatory for the

combination of the 30S and 50S subunits and involved in the binding of tRNA at the A- and P-sites (Rippa *et al*., 2010). The protein of OK617331.1 is linked to crude protein (Figure 3). The produced protein is highly similar to *Cyamopsis tetragonoloba* chloroplast, complete genome (NC_037714.1).

CONCLUSIONS

Gamma-ray (i.e., 150 and 250 Gy), individually or with 900 W microwave irradiation applied for various times (1, 2, 3, and 4 min), significantly influenced guar plant height and crude protein. Fragments with 925, 1427, and 2145 bp linked to plant height and crude protein were eluted from gel, sequenced, and then registered on GenBank. The fragment linked to plant height received accession No. LC681484.1 and fragments linked to crude protein received accession No. OK617330.1 and OK617331.1. Based on sequence analysis, the PCR fragment of 925 bp consisted of a 302 bp open reading frame (ORF) and encoded 302 amino acids. However, the fragment of 1427 bp has 433 ORF, and the fragment of 2145 has 705 ORF. Proteome analysis revealed the stability of protein produced from guar, which is responsible for plant elongation and cell wall characterizations.

List of abbreviations

M_1: the first mutated generation, M_2: the second mutated generation, γ-rays: gamma rays, Gy: gray unit is the absorption of one joule of radiation energy per kilogram of matter (Gray = 100 rad), kR = kilo rad = 1000 rad, CP: crude protein, LSD: the least significant difference, SCoT: start codon targeted marker, PIC: polymorphism information content, RP: resolving power, EMR: effective multiplex ratio, MI: marker index, phyA: phytochrome A, ORF: open reading frame, Tm(°C): annealing temperature, GC%: the number of G's and C's in the primer as a percentage of the total bases, BLAST: Basic Local Alignment Search Tool, BLASTn: Nucleotide BLAST, BLASTp: Protein BLAST

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