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SRAP and IRAP revealed molecular characterization and genetic relationships among cowpea (*Vigna unguiculata* L.) irradiated by gamma-ray

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Abstract

Background Cowpea is a high-protein legume that can be grown in many environments. Gamma radiation can modify plant metabolism and growth. An experiment was conducted to determine the effect of different gamma-ray doses on cowpea yield and growth. The results showed that gamma radiation had a significant effect on cowpea yield and growth, with the highest yields and growth rates observed at lower doses. Higher doses had a negative effect on cowpea yield and growth. The study suggests that gamma radiation can be used to improve cowpea yield and growth, but that the optimal dose must be determined for each specific cultivar and environment.

Results Results specified that gamma ray significantly affected growth characters and forage yield. Gamma-ray dose of 50 Gy in M_1 and M_2 generations gave the highest values for all studied characteristics compared to the control and all other irradiation doses, except the crude fiber, followed by 75 and 100 Gy doses for all characters, except number of tillers plant^{-1} and crude fiber. Seven combinations of SRAP produced 227 loci with an average polymorphism percentage of 85%. The allele frequency of target loci ranged between 0.29 and 0.60, and the PIC was 0.41–0.50 in range, while the GTS% of SRAP combinations ranged from 36 to 63%. The genetic similarities ranged between −4 and 30%. Ten primers produced 450 loci with a polymorphism percentage of 88%. The allele frequency of target loci ranged between 0.28 and 0.46. The PIC was 0.40–0.50 in range, and the GTS% of IRAP primers ranged from 29 to 71%. The genetic similarities ranged from 8 to 37%.

Conclusions SRAP and IRAP analysis revealed more than two clusters of treated plants. In silico analysis showed that some SRAP primers could align with genes in cowpea and related genomes. The SRAP-F12R9 primer is unique to Phaseolus vulgaris, while the SRAP-F13R15 and SRAP-F13em1 primers align with genes on different chromosomes in cowpea cultivar Xiabao 2.

Key message

Gamma ray significantly affected growth characters and forage yield of Cowpew. In silico analysis revealed three SRAP combination primers that could align with some genes along cowpea and related family members' genomes.

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1 Background

Cowpea is an annual herbaceous legume under the genus *Vigna* that belongs to Fabaceae. It is a member of excellent substantial legumes for food and feed in Sub-Saharan Africa. Cowpea is an important natural source of fiber, vitamins, carbohydrates, proteins, and minerals. Also, cowpea, a self-pollinating crop, has a limited genetic diversity that has hampered the enhancement of breeding programs. Cowpea production is impeded by abiotic and biotic stresses, necessitating the need to widen its diverse genetically. An irradiation process with no change in nutritional value is a way to avoid these inconveniences [1, 2]. To overcome the problem of limited genetic diversity and develop new superior cultivars with high yield capacity and stress tolerance, a systematic breeding program is necessary to reduce the current yield gap between producers and consumers in cowpeas [3].

Mutations are required to evolve plants and animals and their speciation and domestication. As a result, induced mutagenesis employing varying radiation dosages has been regarded as a powerful method for creating differences in features of interest. Among the traditional and current breeding ways of plants, breeding by mutation is a quick, low-cost, and consistent way to produce and screen genotypes of crops with promising and enhanced agronomic traits [4]. X-rays, fast neutrons, neutrons–alpha–beta particles, UV light, gamma rays, and thermal neutrons (physical mutagens) are more commonly utilized than ethyl methane sulfonate (EMS) (chemical mutagens) because of their accuracy, safety, and low costs [5–9]. To enhance the genetic diversity rate, breeders expand the application of mutagenesis agents, either chemical or physical. In the mutation breeding program, gamma rays are the most popular utilized radiation [10]. Several researches have been conducted on the role of radiation on genetic, morphological, and biological alterations, besides the various uses that result in disciplines such as agriculture, pharmacy, and medicine [5, 11–31]. Because it is hard to depend on spontaneous mutations for improvement to limit their development, these differences aid breeders in agricultural improvements and the acquisition of new types [32, 33]. Although most of the mutations are recessive and have a detrimental influence, they have proved significant and successful in improving plants globally. Their effect is explicit and noteworthy on the growth in specific crop production. Several articles have previously assessed the impact of mutation approaches on crop development [5, 34].

Cowpea has a genome size of 613 Mb with diploidy ($2n=22$) [35, 36]. Molecular markers give helpful information since they detect mostly selectively neutral variations at the DNA levels [20, 26]. Additionally, they are well-known, and their advantages and disadvantages have been described by [37]. Furthermore, the molecular markers' genome abundance, polymorphism, locus specificity, repeatability, dominance or co-dominance, technological needs, and financial investment all differ. Molecular markers are classed as either non-polymerase chain reaction (PCR)-based markers, such as RFLPs, or PCR-based markers, such as RAPDs, AFLPs, SSRs, ISSRs, and SNPs, overall [38].

Molecular techniques are applied to determine gene expression and identify genotypes affected by abiotic stress [39]. The gene expression and QTLs in plants were identified using the start codon-targeted marker (SCoT), simple sequence repeats (SSRs), and amplified fragment length (AFLP) [40–43]. cDNA-SCoT (cDNA starts codon-targeted) molecular technique has been proposed to be an appropriate and powerful tool for identifying variations in gene expression, stress tolerance and genetic stability in plants [41, 44]. cDNA-SCoT markers were used to determine the gene expression in *Saccharum officinarum*, *Phoenix dactylifera*, *Mangifera indica, Olea europaea*tree, and *Dendrobium officinales* [45–47]. Plant cells develop many molecular pathways to recognize and face the environment quickly. Proteins are essential because (1) regulate the physiological processes to adapt to environmental fluctuations and form new phenotypes. (2) 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 these complicated signals network and biological regulations that influence plant response toward environmental changes. Therefore, several proteins are together involved in response against environmental stress. Consequently, it is essential to know proteins and their role when stress exposure.

Inter-retrotransposon amplified polymorphism (IRAP) is created based upon amplifying target fragments on the genome located between two loci that retrotransposon insert [48]. Additionally, which is applied to detect retrotransposon insertional polymorphism [49]. The IRAP is considered a promising marker for identifying polymorphisms that are inserted by amplifying the fragment between retrotransposons [50, 51]. In comparison, sequence-related amplified polymorphisms (SRAP) are PCR-based markers that generate several co-dominant markers per each amplification of open reading frames. SRAPs target open reading frames in genomic sequences using forward and reverse primers with 17 or 18 nucleotides containing a core sequence of 13 or 14 bases [52]. Furthermore, SRAP markers are exposed to be as powerful and varied as AFLP, but a far less technically challenging approach obtains them. SRAP markers are mainly employed in horticultural and agronomic research,

assessing the genetic diversity of enormous germplasm collections and improving quantitative trait loci in advanced hybrids [53].

This investigation aimed to induce variation in the genetic contents of cowpea irradiated with different gamma-ray doses depending on some traits, then identify and evaluate the genetic variants using the IRAP and SRAP genetic markers and select a promising genotype to use in the breeding programs.

2 Methods

2.1 Irradiation with gamma-ray doses

Seeds of Giza 18 cowpea variety were irradiated at the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Cairo, Egypt. It was exposed to 0, 25, 50, 75, and 100 Gy of gamma-ray doses of Cobalt-60 source at a rate of 7.03 Gy min^{-1}.

2.2 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 the Agricultural Research Station, ARC, Giza governorate, Egypt. The preceding crop in the two summer seasons was barley. The experiment with three replications was designed in a split-plot. Plot size was $12m^2$ (4×3 m) and consisted of 20 rows. The irradiated and non-irradiated cowpea seeds were hand-drilled in rows 20 cm apart at the seeding rate of 20 kg fed^{-1}. The chemical fertilizer was applied in the form of 150 kg fed^{-1} calcium superphosphate (15.5% P_2O_5), and 100 kg fed^{-1} potassium sulfate (48% K_2O) was applied before sowing. After seed germination and before irrigation as a motivating dose, nitrogen fertilizer in the form of urea (46 percent N) was added as an activation dose of 15 kg N fed^{-1}, and seeds were inoculated with the proper Rhizobia (Okadin) prior to planting. Cowpea's seed was sown on the 7th May, 2019, and on the 12th May, 2020, in M_1 and M_2 generations. The other agronomic practices were done as recommended up to harvest.

The experimental plots were divided into two equal parts; the 1st was for estimating growth, yield components, fresh, and dry yields (ton fed^{-1}). At the same time, the 2nd was left to estimate seed yield (kg fed^{-1}). Other cultural practices were followed as recommended. Ten guarded plants of each plot were taken for measuring individual plant characters. One cut was taken 60 days after sowing then cowpea plants were left for seed production.

2.3 Data recorded

The data of vegetative growth (forage yield) were recorded as follows: fresh and dry forage yield (ton fed^{-1}), plant height (cm), and the number of tillers $plant^{-1}$. Data included in the 2nd sample were about seed yield at harvest time, i.e., number of pods $plant^{-1}$, the weight of pods $plant^{-1}$ (g), length of pods (cm), number of seeds pod^{-1}, 100 seeds weight (g), and seeds yield (Kg fed^{-1}).

Fresh forage yield (ton fed^{-1}) was estimated according to [54] as follows: plants were hand-clipped and weighed in kg $plot^{-1}$, then converted to ton fed^{-1}, then were calculated as an average of ten normal seedlings from each replication. While dry forage yield (ton fed^{-1}) was estimated as follows: 100 g plant samples from each plot were dried at 105 °C until constant weight and dry matter percentage (DM%) were evaluated. The dry forage yield (ton fed^{-1}) was calculated by multiplying the fresh forage yield (ton fed^{-1}) with the DM%.

Chemical analysis was followed in the conventional method recommended by the Association of the Official Agricultural Chemists [55] on the dried forage sample at 70 °C for the two seasons to determine crude protein (CP%), crude fiber (CF%), and ash (%).

2.4 Statistical analysis

The data were statistically analyzed according to the procedures outlined by [56]. A combined analysis of the two experimental seasons was carried out based upon the results of Bartlett's test. Means were compared using the least significant difference (LSD) at 5% probability levels.

2.5 Molecular investigation

2.5.1 Extraction of genomic DNA

Ten leaf samples from irradiated and non-irradiated Giza 18 cowpea varieties were collected, then DNA was extracted and purified [39]. The extracted DNA concentration and quality were estimated by running on 1% agarose gel electrophoresis, using a DNA size marker (100 bp DNA ladder) and ND-1000 spectrophotometer (Nano-Drop Technologies, Thermo Fisher Scientific Inc.).

2.5.2 SRAP combinations and IRAP primers amplification

Seven SRAP primer combinations and ten IRAP primers were used to detect polymorphism among the treated samples (Tables 1, 2). The amplification reaction was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (0), 1.5 µl forward primer, 1.5 µl reverse primer (10pcmol), 2.5 µl template DNA (10 ng), and 7 µl dH_2O. PCR technique was executed

Table 1 SRAP combinations used in the PCR reaction and their sequences, GC%, and annealing temperature

No.	Primer			GC%	Ann. temp
1	SRAP-ME3EM1	F	ME3-5′-TGAGTCCAAACCGGAAT-3′	47	45
		R	EM1-5′-GACTGCGTACGAATTAAT-3	39	45
2	SRAP-ME4EM1	F	ME4-5′-TGAGTCCAAACCGGACC-3′	59	49
		R	EM1-5′-GACTGCGTACGAATTAAT-3	39	45
3	SRAP-F12R9	F	F12-5′-CGAATCTTAGCCGGAGC-3′	59	49
		R	R9-5′-GACACCGTACGAATTTGA-3′	44	47
4	SRAP-ME1EM2	F	ME1-5′-TGAGTCCAAACCGGATA-3′	47	45
		R	EM2-5′-GACTGCGTACGAATTTGC-3′	50	49
5	SRAP-F13R15	F	F13-5′-CGAATCTTAGCCGGCAC-3′	59	49
		R	R15-5′-CGCACGTCCGTAATTCCA-3′	56	51
6	SRAP-ME5EM2	F	ME5-5′-TGAGTCCAAACCGGAAG-3′	53	47
		R	EM2-5′-GACTGCGTACGAATTTGC-3′	50	49
7	SRAP-F13em1	F	F13-5′-CGAATCTTAGCCGGCAC-3′	59	49
		R	EM1-5′-GACTGCGTACGAATTAAT-3	39	45

Table 2 IRAP combinations used in the PCR reaction and their sequences, GC%, and annealing temperature

No.	Primer name		Sequence	GC%	Ann. temp
1	IRAP 2175		5′-TTAGACCCGGAACCGCCGTG-3′	65	61
2	IRAP 2198		5′-ATCCTTCGCGTAGATCAAGCGCCA-3′	54	69
3	IRAP 2197		5′-GAAGTACCGATTTACTTCCGTGTA-3′	42	63
4	IRAP 2200		5′-ATGTGACAGTCGACTAACCAC-3′	48	57
5	IRAP 2202		5′-TGGCGCTTGATCTACGCGAAGGA-3′	57	67
6	IRAP 2204		5′-AACTTGATCCAGATCATCTCC-3′	43	55
7	LTRM	F	5′-GCGCTTAGCGTTAGGCTAACT-3′	52	59
		R	5′-CGTGTAGCCTCTTTGGCCCTA-3′	57	61
8	LTRG3	F	5′-CGAGTAGTAGGAAGGAACCGG-3′	57	61
		R	5′-GGCGGCTAGCTTATAGGACTT-3′	52	59
9	CL22	F	5′-TGATCAGAGAAGAAAGGGGA-3′	45	53
		R	5′-CACGCAGAGAGATTGACACG-3′	55	57
10	CL34	F	5′-GAACGATTACCTCACAGACA-3	45	53
		R	5′-GAGCAATAAAGAGAAGCCCG-3	50	55

using Perkin-Elmer/GeneAmp® PCR System 9700 (*PE* Applied Biosystems). The amplification of SRAP markers was done for 40 cycles: an initial denaturation cycle at 94 °C for 1 min, annealing at 48 °C for 1 min, elongation at 72ºC for 2 min, and then finally 5 min for the extension. Conversely, IRAP amplification was done for 35 cycles as follows: 5 min at 94 °C denaturations, 7 min annealing at 59 °C, and elongation in the final cycle at 72 °C. The PCR amplification products of SRAP and IRAP were run on agarose 1.5% supplemented by ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 V. PCR products were visualized on UV light and photographed using a gel documentation system (BIO-RAD 2000).

2.5.3 Data analysis based upon SRAP and IRAP profiles

The similarity among irradiated plants and control was assessed based upon the Dice similarity coefficient using TotalLab ID 14.3 software. The banding patterns obtained from the 7 SRAP combinations and 10 IRAP primers were scored to (1) and (0) as bands' presence and absence. Pairwise comparisons of SRAP and IRAP profiles were used to construct a phylogenetic tree using the UPGMA module.

The polymorphic and monomorphic bands; the total bands' number and polymorphism% were recorded, then the polymorphism information content (PIC) was calculated. Polymorphic information content (PIC) for dominant markers was calculated using 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 ith allele). PIC has a maximum of 0.5 for '$f = 0.5$ (in the case of dominant markers) [57]. The equation: GTS $(\%) = (1 - a/n) \times 100$ was used to calculate the GTS, where 'a' is the number of detected polymorphic bands/treated sample, and 'n' is the total control bands. Principal component analysis (PCA) and heatmap were constructed using XLSTAT-Excel 365 v2019.2.2.

3 Result

3.1 Cowpea characteristics as influenced by gamma-ray doses

Gamma-ray doses significantly influenced fresh and dry forage yield ($P < 0.05$). The heaviest fresh forage yield of cowpea plants appeared in plants irradiated with 50 Gy of gamma ray in both M_1 (11.72 ton fed^{-1}) and M_2 (11.36 ton fed^{-1}), which could be estimated by an increase of 54.2 and 56.47%, respectively, compared with the control, followed by 75 Gy then 100 Gy of gamma ray (Fig. 1). The heaviest dry forage yield of cowpea plants appeared in plants irradiated with 50 Gy of gamma-ray doses in both M_1 (1.56 ton fed^{-1}) and M_2 (1.53 ton fed^{-1}), which could be estimated by an increase of 90.24 and 91.25%, respectively, compared with the control in M_1 and M_2 generations, followed by 75 Gy then 100 Gy of gamma ray (Fig. 1).

Gamma-ray doses significantly influenced the number of tillers plant^{-1} and plant height ($P < 0.05$). The tallest cowpea plants appeared in plants irradiated with 50 Gy of gamma-ray dose in both M_1 (116.58 cm) and M_2 (115.38), which could be estimated by an increase of 82.87 and 83.29%, respectively, compared with the control in M_1 and M_2 generations, followed by 75 Gy then 100 Gy of gamma ray (Fig. 2). The highest number of plant tillers was observed in plants irradiated with 50 Gy of gamma-ray dose in both M_1 (6.67 tillers) and M_2 (6.30 tillers), which could be estimated by an increase of 54.04 and 57.5%, respectively, compared with the control in M_1 and M_2 generations, followed by 100 Gy then 75 Gy of gamma ray in M_1. In contrast, it is followed by 75 and then 100 Gy in M_2 (Fig. 2).

Gamma-ray doses significantly influenced number of pods plant-1, weight of pods plant^{-1}, and pod length ($P < 0.05$). The greatest pod number plant^{-1} was counted

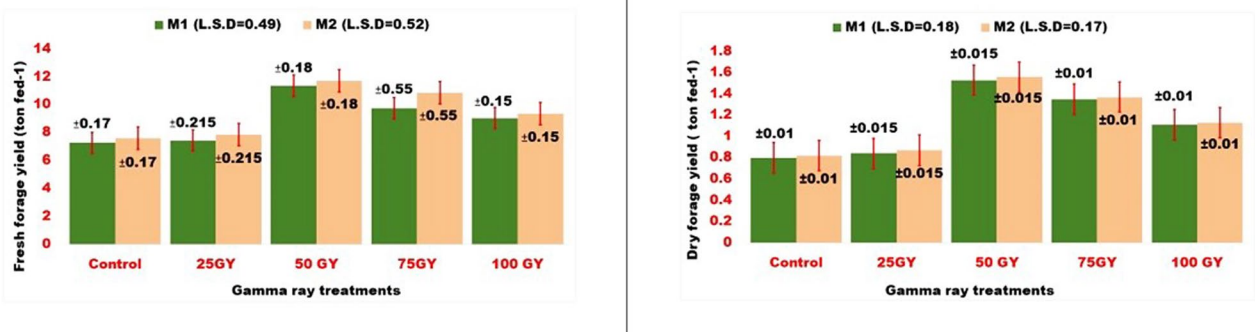

Fig. 1 The effect of gamma-ray doses on fresh and dry forage yield of cowpea in M_1 and M_2 generations (2019, 2020)

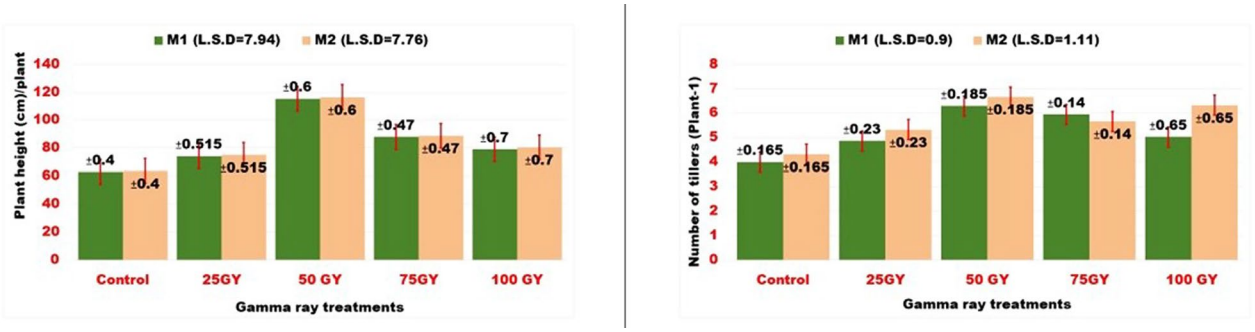

Fig. 2 The effect of gamma-ray doses on plant height, and number of tiller plant^{-1} of cowpea in M_1 and M_2 generations (2019, 2020)

in plants irradiated with 50 Gy of gamma ray in both M_1 (65.91 pods plant^{-1}) and M_2 (64.24 pods plant^{-1}), which could be estimated by an increase of 157.16 and 168.11%, respectively compared with the control, followed by 75 Gy then 100 Gy of gamma ray (Fig. 3). The heaviest pod weight plant^{-1} was found in plants irradiated with 50 Gy gamma ray in both M_1 (124.89 g) and M_2 (122.22 g), followed by 75 Gy and then 100 Gy of gamma ray. Also, the tallest pods were found in cowpea plants irradiated with 50 Gy, followed by 75 Gy and then 100 Gy of gamma ray (Fig. 3).

The number of seeds pod^{-1}, 100- seed weight, and seed yield were significantly affected ($P < 0.05$) by irradiation with all used gamma-ray doses. The largest number of seeds pod^{-1} of cowpea appeared in plants irradiated with 50 Gy of gamma ray in both M_1 (14.77 seeds pod^{-1}) and M_2 (14.48 seeds pod^{-1}), which could be estimated by an increase of 52.43 and 54.21%, respectively, compared with the control in M_1 and M_2 generations, followed by 75 Gy then 100 Gy of gamma ray (Fig. 4). The heaviest 100- seed weight was observed in plants irradiated with 50 Gy gamma ray in both M_1 (11.57 g) and M_2 (11.30 g), which could be estimated by an increase of 62.5 and 63.53%, respectively, compared with the control, followed by 75 Gy then 100 Gy. Also, the highest seed yield was noticed in cowpea plants irradiated with 50 Gy, followed by 75 Gy and then 100 Gy of gamma ray. The 50 Gy gamma ray increased seed yield by 83.86 and 90.09, respectively, compared with the control in M_1 and M_2 generations, followed by 75 Gy then 100 Gy (Fig. 4).

Crude protein% and ash% of cowpea plants were significantly ($P < 0.05$) increased by irradiation with all used gamma-ray doses in both M_1 and M_2 generations. The highest crude protein values appeared in plants irradiated with 50 Gy of gamma-ray dose, followed by 75 and 100 Gy, respectively. The 50 Gy of gamma ray increased the crude protein by 11.38 and 16.98%, respectively, in M_1 and M_2 compared with the control. As well, the highest ash% values appeared in plants irradiated with 50 Gy of gamma ray, followed by 75 and 100 Gy, respectively. The 50 Gy of gamma-ray dose increased the ash % by 14.70 and 17.49%, respectively, in M_1 and M_2 compared with the control. In contrast, 50 Gy of gamma ray gave the lowest crude fiber value, while the control and the 25 Gy dose gave higher values in both generations (Fig. 5).

3.2 Molecular analysis

3.2.1 Genetic variability revealed by SRAP markers

Reproducible informative amplification of SRAP fragments was produced using seven combinations of SRAP primers (Fig. 6 and Table 3). The seven combinations that produced 227 loci among the M_1 and M_2 generations ranged between 33 and 4131 bp, and 193 are polymorphic with an average of 85% polymorphism in both generations (Table 3). The M_1 generation exhibited 92 polymorphic loci out of 111 loci, with an average of 13

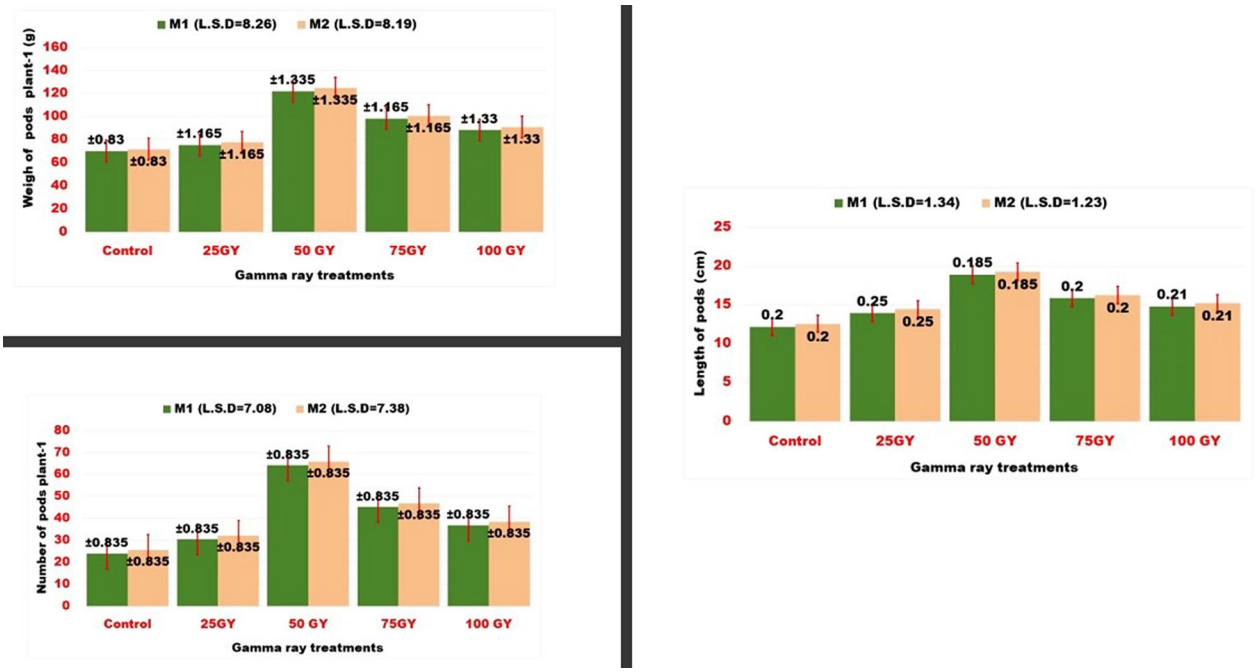

Fig. 3 The effect of gamma-ray doses on number of pods plant^{-1}, weight of pods plant^{-1}, and length of pods of cowpea in M_1 and M_2 generations (2019, 2020)

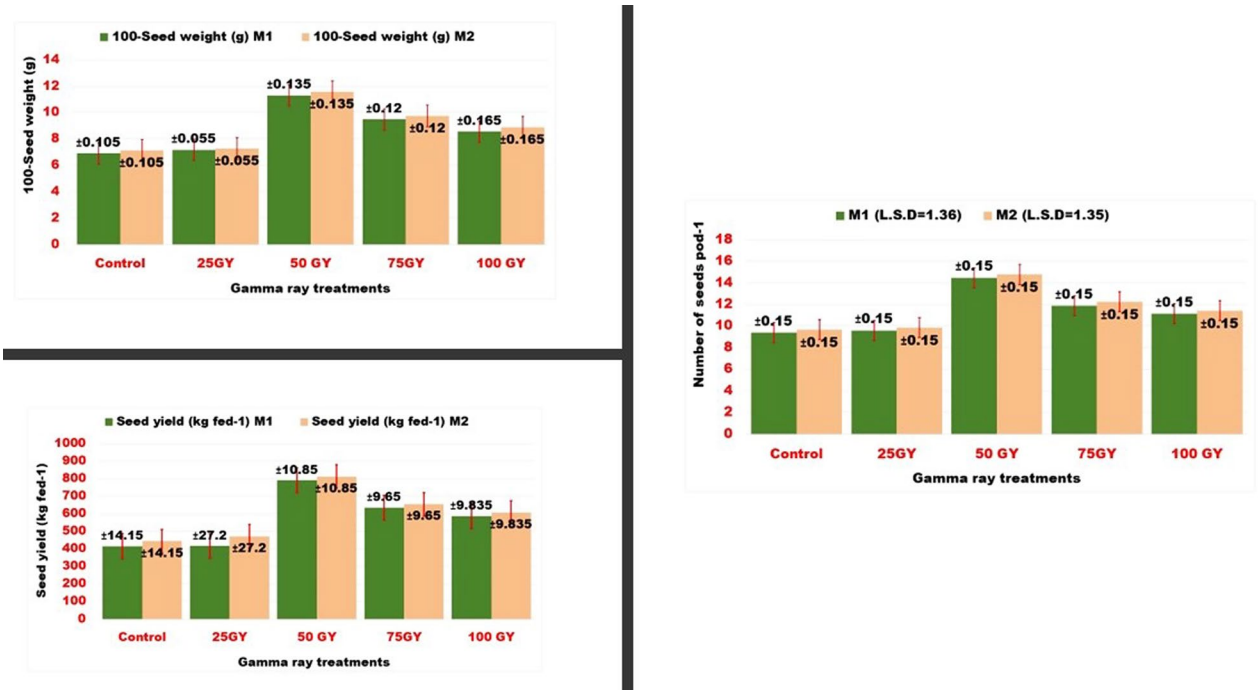

Fig. 4 The effect of gamma-ray doses on the number of seeds pod^{-1}, 100 seeds weight, and seed yield (Kg fed^{-1} of cowpea in M_1 and M_2 generations (2019, 2020)

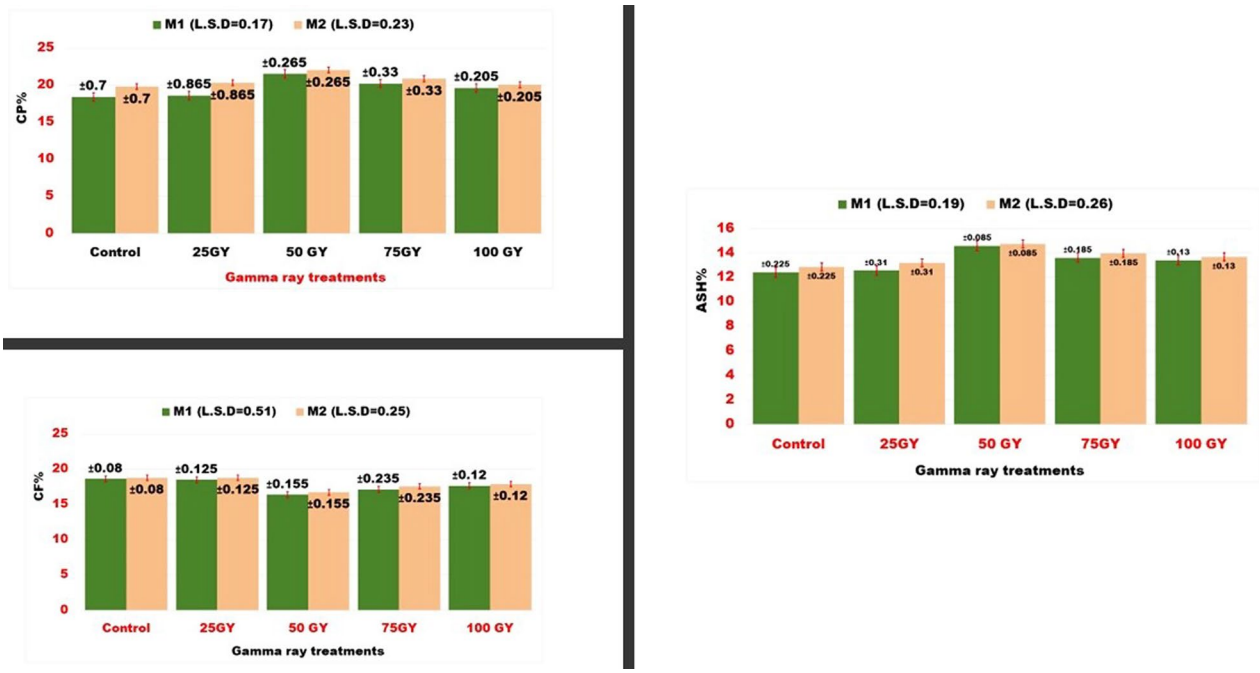

Fig. 5 The effect of gamma-ray doses on crude protein (CP%), crude fiber (CF%), and ash (%) in M_1 and M_2 generations (2019, 2020)

loci/combination and 83% polymorphism, while M_2 exhibited 101 polymorphic loci out of 116 loci, with an average of 14 loci/combination. The allele frequency of target loci ranged between 0.29 and 0.60, with an average of 0.46 in M_1 and 0.39 in M_2 generation (Table 3). The PIC were 0.41–0.50 in range with an average of 0.48 for

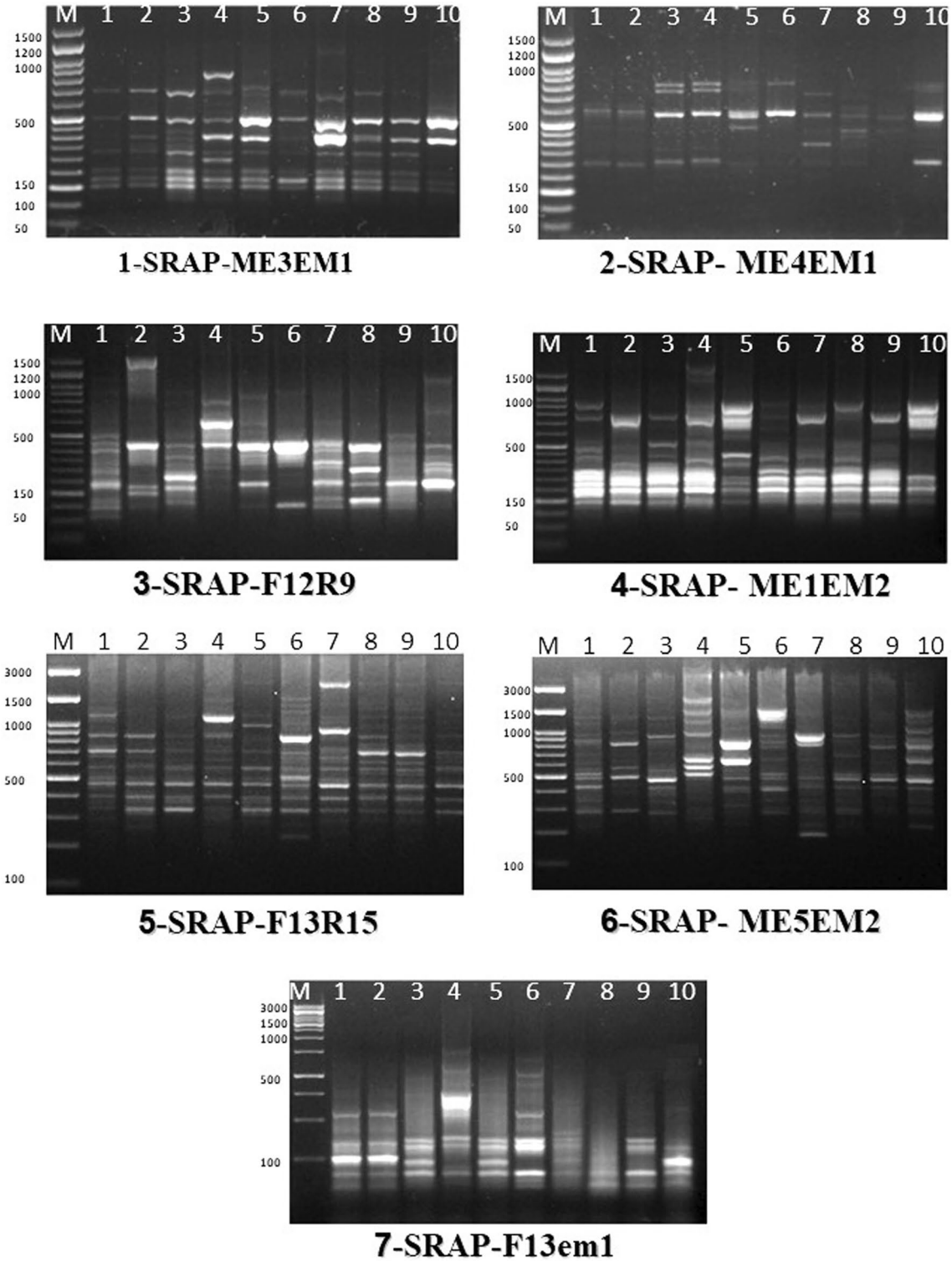

Fig. 6 Pattern of cowpea plants treated with gamma irradiation (25, 50, 75, and 100 Gy) revealed by SRAP combinations, M: DNA ladder, 1 and 10 untreated plants (control), 2–5 in the M_1 generation, and 6–9 in the M_2 generation

Table 3 Band size, polymorphism percentage, allele frequency, and the polymorphism information contents of cowpea revealed by 7 SRAP combinations

Ser	Primer	Size (bp)	Total bands	PB	MB	PM%	*f*	PIC
1	SRAP-ME3EM1	143–1807	13	8	5	62	0.60	0.48
2	SRAP-ME4EM1	257–862	7	7	0	100	0.49	0.50
3	SRAP-F12R9	69–1394	18	17	1	94	0.29	0.41
4	SRAP-ME1EM2	147–1617	18	14	4	78	0.43	0.49
5	SRAP-F13R15	33–496	14	13	1	93	0.43	0.49
6	SRAP-ME5EM2	324–3750	15	10	5	67	0.55	0.50
7	SRAP-F13em1	194–4131	26	23	3	88	0.42	0.49
	Total M1		111	92	19			
	Average		16	13	3	83	0.46	0.48
1	SRAP-ME3EM1	143–1807	17	16	1	94	0.39	0.48
2	SRAP-ME4EM1	257–862	10	9	1	90	0.30	0.42
3	SRAP-F12R9	69–1394	12	11	1	92	0.40	0.48
4	SRAP-ME1EM2	147–1617	15	12	3	80	0.40	0.48
5	SRAP-F13R15	33–496	13	12	1	92	0.38	0.47
6	SRAP-ME5EM2	324–3750	23	17	6	74	0.46	0.50
7	SRAP-F13em1	194–4131	26	24	2	92	0.40	0.48
	Total M2		116	101	15			
	Average		17	14	2	88	0.39	0.47
	Total		227	193	34			

PB Polymorphic bands, *MB* Monomorphic bands, *PM*% Polymorphism percentage, *f* Allele frequency, *PIC* Polymorphism information contents

Table 4 Change in the number of produced bands in SRAP analysis of plant sample raised with gamma radiation treatment 25 Gy, 50 Gy, 75 Gy, and 100 Gy dose and the control and genomic template stability (GTS%) in cowpeas

Primer	C	25 Gy		50 Gy		75 Gy		100 Gy	
	n	*p*	*d*	*P*	*d*	*p*	*D*	*p*	*d*
M_1									
SRAP-ME3EM1	7	0	2	0	1	2	1	2	0
SRAP-ME4EM1	5	0	3	0	3	1	1	1	1
SRAP-F12R9	8	3	2	1	2	3	3	1	3
SRAP-ME1EM2	12	0	1	0	2	1	3	1	0
SRAP-F13R15	6	4	3	4	3	0	0	6	3
SRAP-ME5EM2	11	0	8	0	4	0	2	0	2
SRAP-F13em1	11	1	1	0	4	1	4	0	7
Total	60	8	20	5	19	8	14	11	16
GTS%		53		60		63		55	
M_2									
SRAP-ME3EM1	7	2	2	2	0	1	0	1	1
SRAP-ME4EM1	4	0	2	1	1	1	2	0	3
SRAP-F12R9	8	1	7	1	2	0	4	1	4
SRAP-ME1EM2	9	1	1	1	0	1	0	0	1
SRAP-F13R15	4	4	0	1	1	0	3	1	1
SRAP-ME5EM2	8	0	3	1	2	0	3	2	2
SRAP-F13em1	10	5	4	1	5	3	3	3	3
Total	50	13	19	8	11	6	15	8	15
GTS%		36		62		58		54	

M_1 and 0.47 for M_2 generation (Table 3). The GTS% of SRAP combinations ranged from 53 to 63% in M_1 and 36–62% in M_2 generation (Table 4).

The genetic similarities and phylogenetic tree are presented in Fig. 7 and Table 5. In M_1 generation, the highest similarity (30%) was identified between the control and treatment (25 Gy). In contrast, the lowest similarity (−4%) was determined between the control and treatment (100 Gy). In M_2 generation, the highest similarity (35%) was noticed between the control and treatment (100 Gy), while the lowest similarity (−6%) was discovered between the treatment (25 Gy) and treatment (75 Gy) (Table 5).

The dendrogram in M_1 generation revealed three clusters; the 1st divided into two subclusters containing control and 25 Gy, the 2nd cluster grouped 50 Gy and 100 Gy, and the third contains 75 Gy only. Conversely, M_2 generation exhibited four clusters; the 1st one grouped 75 Gy and 100 Gy, while the other three clusters contain one for each control, 25 Gy, and 50 Gy, respectively.

3.2.2 Genetic variability revealed by IRAP markers

Reproducible informative amplification of IRAP fragments was generated by 10 IRAP primers (Fig. 8 and Table 6). The ten primers that produced 450 loci among the M_1 and M_2 generations ranged between 186 and 2525 bp; 398 are polymorphic with an average polymorphism percentage of 88% in both generations (Table 6). The M_1 generation exhibited 209 polymorphic loci out of 239 loci, with an average of 21 loci/primer and 87.2% polymorphism, while M_2 exhibited 189 polymorphic loci out of 211 loci, with an average of 22 loci/primer. The allele frequency of target loci ranged between 0.28 and 0.46, with an average of 0.40 in M_1 and 0.36 in M_2 generation. The PIC ranged between 0.40 and 0.50, with an average of 0.48 for M_1 and 0.45 for M_2 generation (Table 6). The GTS% of IRAP primers ranged from 29 to 41% in M_1 and 55–71% in M_2 generation (Table 7).

The genetic similarities and phylogenetic tree are presented in Fig. 9 and Table 8. In M_1 generation, the highest similarity (37%) was found between the control and treatment (25 Gy). The lowest similarity (8%) was noticed between the control and treatments of 75 and 100 Gy. In M_2 generation, the highest similarity (28%) was identified between the treatment (75 Gy) and treatment (100 Gy). While the lowest similarity (−1%) was noticed between the control and treatment (25 Gy) (Table 8).

The dendrogram in M1 generation revealed three clusters; the 1st one is grouped the control and 25 Gy, the 2nd cluster contains the 50 Gy, and the third contains the 75 Gy and 100 Gy. Conversely, M2 generation exhibited four clusters; the 1st one is grouped the control and 100 Gy, while the other three clusters contain one for each 25 Gy, 50 Gy, and 75 Gy, respectively.

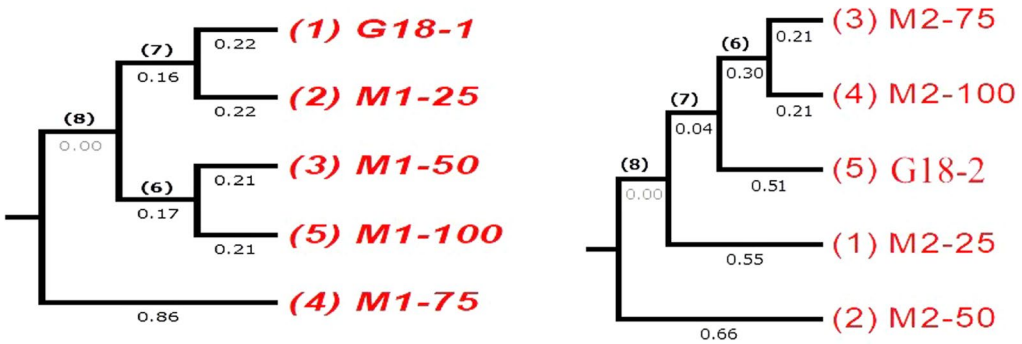

Fig. 7 Dendrogram revealed phylogenetic neighbor-joining tree based upon SRAP marker analysis in M_1 and M_2

Table 5 Genetic similarities among the irradiated and non-irradiated cowpea plants (control, 25, 50, 75, and 100 Gy) revealed by SRAP combinations

M_1					M_2				
	G18-1	**M_1-25**	**M_1-50**	**M_1-75**		**M_2-25**	**M_2-50**	**M_2-75**	**M_2-100**
M_1-25	0.30				M_2-50	0.12			
M_1-50	0.20	0.26			M_2-75	−0.06	0.29		
M_1-75	0.05	0.12	0.21		M_2-100	0.16	0.28	0.31	
M_1-100	−0.04	0.06	0.25	0.28	G18-2	0.12	0.27	0.22	0.35

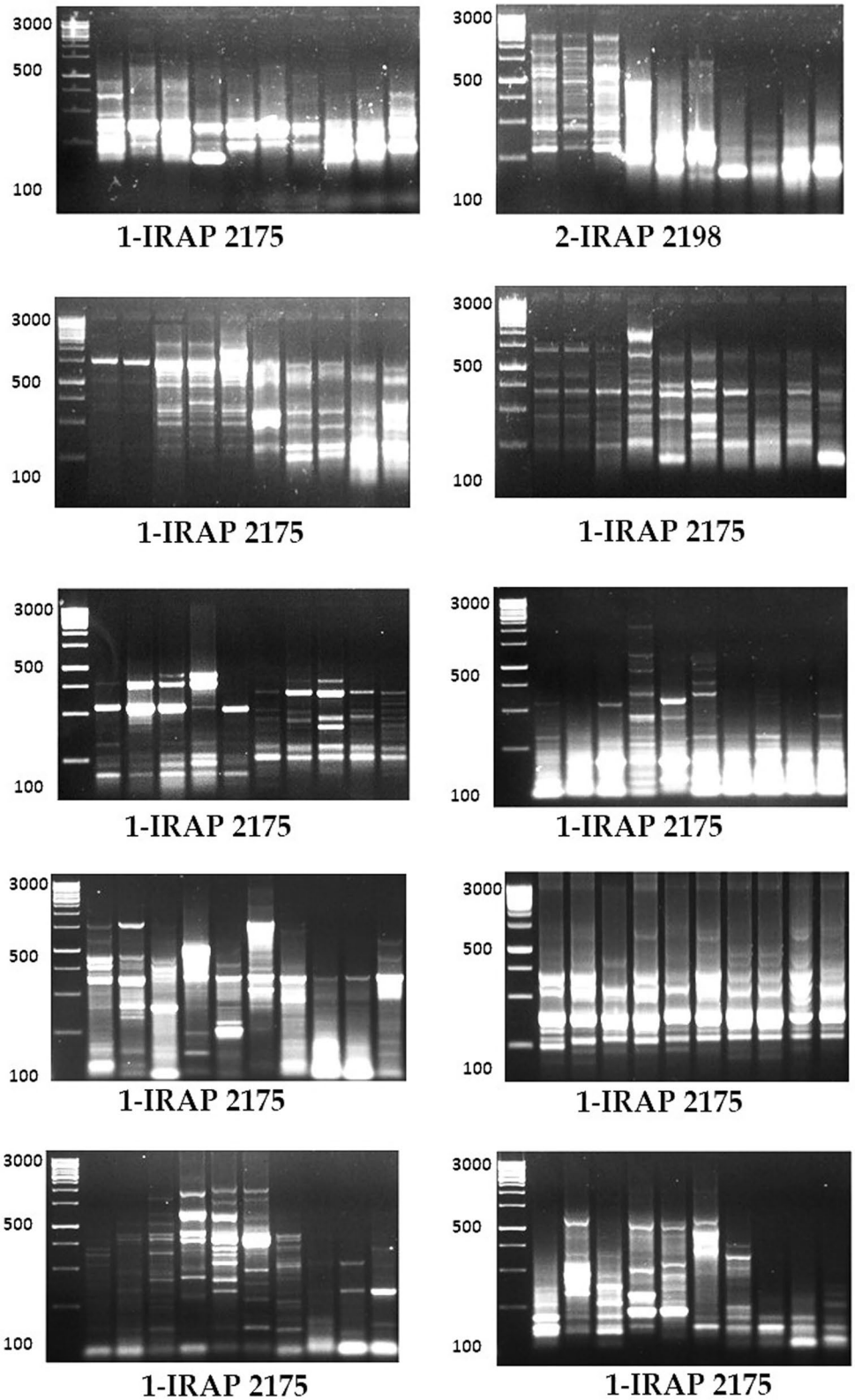

Fig. 8 Pattern of cowpea plants treated with gamma irradiation (25, 50, 75, and 100 Gy) revealed by IRAP primers, M: DNA ladder, 1 and 10 untreated plants (control), 2–5 in the M_1 generation, and 6–9 in the M_2 generation

Table 6 Band size, polymorphism percentage, allele frequency, and the polymorphism information contents of cowpea revealed by 10 IRAP primers

Ser	Primer	Size (bp)	Total bands	PB	MB	PM%	*f*	PIC
M_1								
1	IRAP 2175	186–2525	28	26	2	92.86	0.36	0.46
2	IRAP 2198	213–1021	27	23	4	85.19	0.45	0.50
3	IRAP 2197	200–2447	19	18	1	94.74	0.46	0.50
4	IRAP 2200	269–2120	28	25	3	89.29	0.33	0.44
5	IRAP 2202	213–517	21	18	3	85.71	0.44	0.49
6	IRAP 2204	224–641	20	16	4	80.00	0.43	0.49
7	LTRM	246–509	27	23	4	85.19	0.40	0.48
8	LTRG3	288–2335	19	15	4	78.95	0.40	0.48
9	CL22	241–1032	26	23	3	88.46	0.35	0.45
10	CL34	250–793	24	22	2	91.67	0.41	0.48
	Total M_1		239	209	30			
	Average		24	21	3	87.20	0.40	0.48
M_2								
1	IRAP 2175	186–2525	26	23	3	88.46	0.38	0.47
2	IRAP 2198	213–1021	16	15	1	93.75	0.29	0.41
3	IRAP 2197	200–2447	24	22	2	91.67	0.35	0.46
4	IRAP 2200	269–2120	18	18	0	100.00	0.41	0.48
5	IRAP 2202	213–517	20	15	5	75.00	0.46	0.50
6	IRAP 2204	224–641	21	16	5	76.19	0.40	0.48
7	LTRM	246–509	24	24	0	100.00	0.30	0.42
8	LTRG3	288–2335	19	16	3	84.21	0.42	0.49
9	CL22	241–1032	25	24	1	96.00	0.28	0.40
10	CL34	250–793	18	16	2	88.89	0.32	0.44
	Total M_2		211	189	22			
	Average		21	19	2	89.42	0.36	0.45
	Total		450	398	52			

PB Polymorphic bands, *MB* Monomorphic bands, *PM*% Polymorphism percentage, *f* Allele frequency, *PIC* Polymorphism information contents

3.2.3 Molecular characterization and genetic relationships as revealed via SRAP and IRAP markers

By plotting PC1 and PC2 clearly discriminating Giza 18 cowpea variety with different irradiation doses of gamma ray (Fig. 10) in M_1 and M_2 generations, the principal component analysis (PCA) reflects the strength of the IRAP, and SRAP markers to classify Giza 18 cowpea variety in a PCA scatter plot, the results of M_1 revealed the characteristic grouping of the control and 25 Gy dose. The 25 Gy, 50 Gy, and 100 Gy were grouped together in this regard. In contrast, the results of M_2 indicated the characteristic grouping of the control. Conversely, 50 Gy, 75 Gy, and 100 Gy were grouped together.

Heat map analysis was also utilized to create a heat map with Excel 365. As shown by the columns, the four doses of gamma ray clustered and control into two clusters of two or three doses of gamma ray in the M_1 generation (Fig. 11). The 1st cluster grouped the control and 25 Gy doses together. Meanwhile, the 50 Gy and 75 Gy were grouped together adjacent to 100 Gy. In the same context, the thirteen morphological traits clustered, as shown by the rows, for exploration of the effect of the different doses of gamma ray on these morphological traits, and the results revealed that a positive correlation between entirely morphological traits at 50 Gy except crude fiber that detects strong negative correlation, no correlation was recorded for entirely morphological traits at 75, and 100 Gy. While the results indicated a positive correlation for crude fiber at 25 Gy, no correlation was between the number of tillers and plant height.

In contrast, ten morphological traits recorded a negative correlation, as well in M_2 generation, the four doses of gamma ray, and the control, as shown in the columns in (Fig. 12). The first cluster combined the control and 25 Gy doses, while the 50 Gy and 75 Gy doses were two adjacent pairs of doses next to the 100 Gy dose. The thirteen morphological features clustered as shown in the rows, demonstrating the effect of the different

Table 7 Change in the number of produced bands in IRAP analysis of plant sample raised with gamma radiation treatment 25 Gy, 50 Gy, 75 Gy, and 100 Gy dose and the control and genomic template stability (GTS%) in cowpeas

Primer	C	25 Gy		50 Gy		75 Gy		100 Gy	
	n	*p*	*d*	*P*	*d*	*p*	*D*	*p*	*d*
M_1									
IRAP 2175	6	7	2	6	3	6	2	2	3
IRAP 2198	6	10	3	11	3	6	3	4	3
IRAP 2197	15	2	8	2	9	2	8	6	2
IRAP 2200	8	2	5	4	3	5	2	6	2
IRAP 2202	13	2	8	2	5	2	7	4	7
IRAP 2204	10	3	3	2	4	2	3	5	2
LTRM	10	5	2	4	2	2	2	4	2
LTRG3	18	2	8	2	8	2	5	2	7
CL22	14	3	8	5	5	3	5	3	7
CL34	10	6	2	2	3	6	2	3	2
Total	83	23	36	21	30	22	26	27	29
GTS%		29		39		42		33	
M_2									
IRAP 2175	4	5	0	4	1	4	0	0	1
IRAP 2198	4	8	1	9	1	4	1	2	1
IRAP 2197	13	0	6	0	7	0	6	4	0
IRAP 2200	6	0	3	2	1	3	0	4	0
IRAP 2202	11	0	6	0	3	0	5	2	5
IRAP 2204	8	1	1	0	2	0	1	3	0
LTRM	8	3	0	2	0	0	0	2	0
LTRG3	16	0	6	0	6	0	3	0	5
CL22	12	1	6	3	3	1	3	1	5
CL34	8	4	0	0	1	4	0	1	0
Total	69	9	22	7	16	8	12	13	15
GTS%		55		67		71		59	

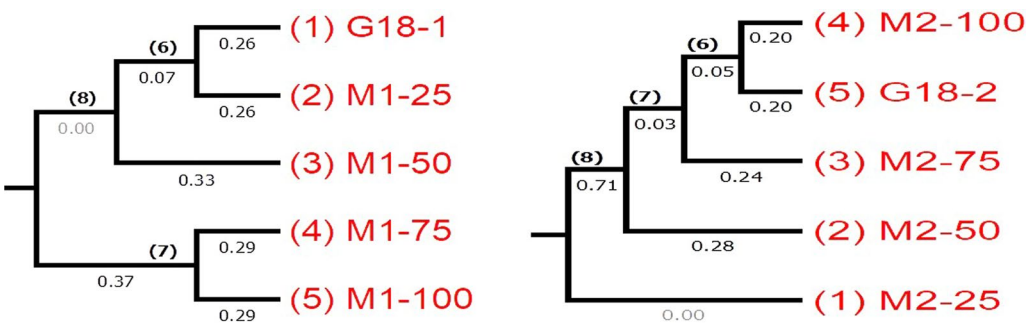

Fig. 9 Dendrogram revealed phylogenetic neighbor-joining tree based upon SRAP marker analysis in M_1 and M_2

gamma-ray doses on these traits. At 50 Gy, there was a negative correlation in the crude fiber trait and also in the number of tillers at the control. No correlation between any traits was observed at 100 Gy and 75 Gy. While a positive correlation was found between crude fiber and a negative correlation among eight traits except for three characteristics: number of tillers, pod length, and pod number at 25 Gy. At 50 Gy, a positive correlation among three traits included (number of tillers, fresh yield, and dry yield), and also a strong positive correlation was found between nine traits such as (pods of length, plant height, pod number, 100 seed

Table 8 Genetic similarities among the irradiated and non-irradiated cowpea plants (control, 25, 50, 75, and 100 Gy) revealed by IRAP primers

	G18-1	M_1-25	M_1-50	M_1-75		M_2-25	M_2-50	M_2-75	M_2-100
M_1-25	0.37				M_2-50	−0.03			
M_1-50	0.25	0.31			M_2-75	−0.11	0.13		
M_1-75	0.08	0.14	0.23		M_2-100	0.05	0.12	0.28	
M_1-100	0.08	0.16	0.35	0.26	G18-2	−0.01	0.06	0.05	0.26

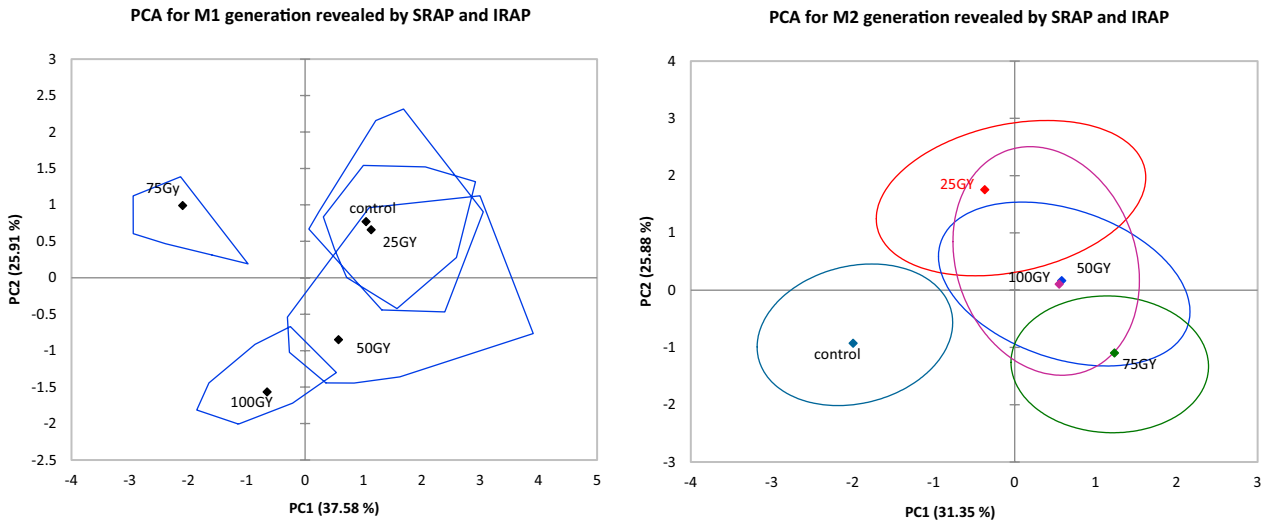

Fig. 10 Principal component analysis (PCA) scatter diagram demonstrating the genetic diversity expressed by grouping Giza 18 cowpea variety at different doses of gamma-ray doses established on the analysis of SRAP and IRAP marker polymorphism and by blotting the 1st two principal components

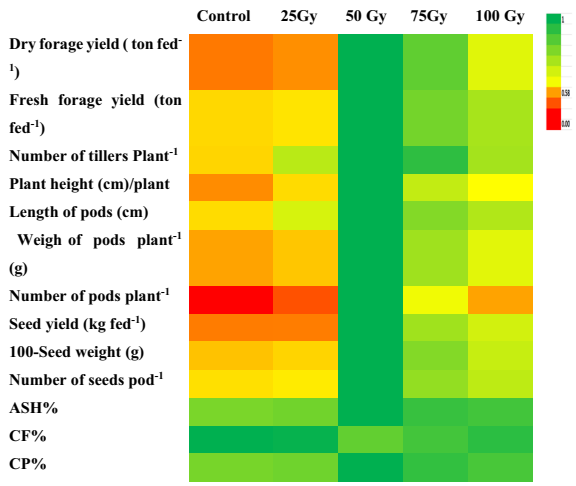

Fig. 11 Heat map illustrating the genetic diversity of Giza 18 cowpea variety at different doses of gamma ray in M_1 generation based upon the SRAP and IRAP

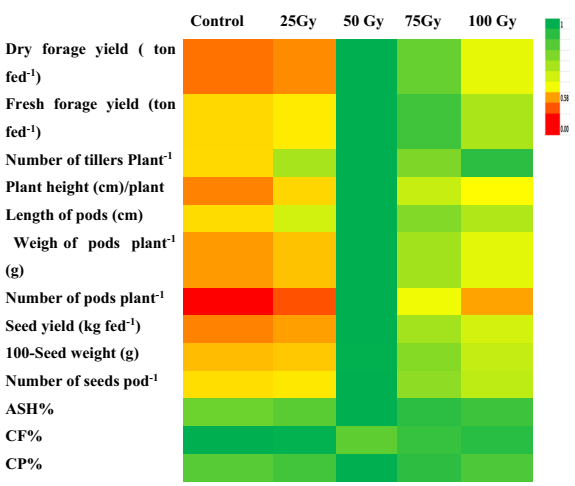

Fig. 12 Heat map illustrating the genetic diversity of Giza 18 cowpea variety at different doses of gamma ray in M_1 generation based upon the SRAP and IRAP

weight, crude protein, seed yield, % ash, pods weight, and seeds number).

3.2.4 In silico analysis of the cowpea Vigna unguiculata L. genome based upon the SRAP and IRAP finding

In silico analyses were carried out for a *Vigna unguiculata* L. genome and related family members, including the primer combination and amplicon size (Additional file 1: (S1)).

According to the findings, the SRAP and IRAP approaches generated 227 and 450 amplicons, respectively. The In Silico analysis revealed 3 SRAP combination primers that could align with some genes along cowpea and related family members' genomes. The three combinations were SRAP-F12R9, SRAP-F13R15, and SRAP-F13em1. The SRAP combinations, their fragment, fragment alignment, and the gene accession on the database exist in Table 9.

The accessions obtained were aligned using BLAST tools to determine their similarity with the cowpea genome. The fragment with 229 bp, similar to accession FJ748896.1, has no resemblance with any part of the cowpea genome. Still, it is a unique sequence for *Phaseolus vulgaris* that belongs to the same family (Fabaceae). The fragment with 635 bp, similar to accession FJ748893.1, has been identified in the cowpea genome and aligns with *Vigna unguiculata* cultivar Xiabao 2 chromosome Vu02 (GenBank: CP039348.1). The fragment with 532 bp, similar to accession FJ748897.1, was found on the cowpea genome and aligned with *Vigna unguiculata* cultivar Xiabao 2 chromosome Vu01 (GenBank: CP039350.1).

4 Discussion

Cowpea, a self-pollinating crop, has a limited genetic background, which hampered crop improvement. To overcome the problem of limited genetic diversity and to develop new high-yielding-stress tolerant genotypes, a systematic breeding program is necessary to reduce the current yield gap between producer and consumer in cowpea [3, 19]. Induced mutagenesis as a powerful method for creating differences in features of interest in plant breeding is a quick, low-cost, and consistent way for producing and screening the promising and enhanced genotypes [4]. Gamma rays, X-rays, neutrons–alpha–beta particles, UV light, and fast thermal neutrons (physical mutagens) are more commonly utilized than ethyl methanesulfonate (EMS-chemical mutagens) because they are safer, more accurate, and less expensive [5–7]. To increase the rate of genetic diversity, breeders buckle down to the utilization of physical or chemical mutagenesis. In the mutation breeding program, gamma rays are the most popular and utilized radiation [10].

Irradiated cowpeas with 25, 50, 75, and 100 Gy gamma-ray doses significantly affected growth characters and forage yields (i.e., fresh and dry forage yield, plant height, the number of tillers plant^{-1}, number of pods plant^{-1}, the weight of pods plant^{-1}, length of pods, number of seeds pod^{-1}, 100 seeds weight, seeds yield and crude protein, crude fiber, and ash). Gamma-ray dose of 50 Gy in M_1 and M_2 generations gave the highest values for all studied characteristics compared to the control and all other irradiation doses, except the crude fiber, followed by the dose rate of 75 and 100 Gy for all characters, except number of tiller plant^{-1} and crude fiber. Beneficial mutations are changes to the genotypic structure that increase the variability of the species and favor their adaptation to various selection stresses. These can be induced by physical mutagenic agents such as ionizing radiation (gamma rays). Ionizing radiation (IR) induces the change from neutral molecules or atoms to their ionized forms; this change requires ionization energy, which is the minimum amount of energy that separates the electron from a free atom in its lower energy state, through two effects: Compton and photoelectric.

IR can directly induce physical, biological, and chemical changes in the cells, altering the chemical nature of the molecules. It can induce specific changes in the genome and indirectly induce an alteration of free radicals generated mainly by the ionization of water molecules.

Efficient induction of mutagenesis by gamma radiation (GR) requires the determination of the optimal radiation dose; that is, the dose that reduces 50% of the population (median lethal dose, LD50). It also requires variables such as survival, mass, or number of germinated specimens, among others, or the radiation dose that reduces growth in 50% of the population (median growth reduction, GR50). Both doses depend on the plant tissue (seed, meristem, callus, etc.), stage of development and moisture

Table 9 The SRAP combinations, their fragment, fragment alignment, and the gene accession on the database

No.	SRAP combination	Size (bp)	Fragment on the database (bp)	Accession No	Description
1	SRAP-F12R9	229	241	FJ748896.1	Phaseolus vulgaris SRAP marker F12R9.280 genomic sequence
2	SRAP-F13R15	635	600	FJ748893.1	Phaseolus vulgaris SRAP marker F13Em1.600 genomic sequence
3	SRAP-F13em1	532	537	FJ748897.1	Phaseolus vulgaris SRAP marker F13R15.600 genomic sequence

content, among other parameters. High radiation doses can induce radio inhibition by affecting growth promoters and, eventually, tissue destruction. It can also cause loss of regenerative capacity and malformation of plant tissues, as well as tissue destruction. Radio sensitivity assays allow determining the appropriate radiation dose to induce the highest mutation rate with negligible effects on the gene complex. Radiation stimulation can be obtained with low radiation doses that favor the induction of metabolites and biochemical changes involved in plant regeneration, that is 50 Gy in our study. The effective dose and the lethal one differs from one plant to another, as many scientific manuscripts confirm. Similarly, [58] also reported that the effect of gamma radiation improving plant growth, seed quality, and physiological processes is highly related to the dose use level. In agreement with our results, [59] reported that pre-sowing seed treatments such as gamma radiation may improve seed performance under field conditions. And [60] confirmed that gamma rays had been proven economical and effective as compared to other ionizing radiations such as electric field, magnetic field, laser radiation, and microwave radiation because of their easy availability and the power of penetration, which helps in their broader application for the improvement of various plant species. As well as in Namibia [61], irradiated seeds with different doses of gamma rays and selected the desirable mutants through generations. After exposing cowpea to mutants, ten new mutants that are stable across generations, including maturity, flowering capacity, grain yield, flower, and seed color, were isolated for large-scale production or breeding. In the same regard [3], mentioned that increasing the genetic variability by gamma rays was detected using biochemical, physiological, and molecular profiling. The most genetically diverged with high yielding has also a significant increase in micronutrient and protein content [62, 63] found a considerable increase in vitro protein digestibility in pigeon pea flour irradiated at 20 kGy. Irradiation apparently did not cause fissures or splitting in cowpea starch granules up to 50 kGy [7, 64].

Several researches have been conducted on the effects of radiation on genetic, morphological, and biological alterations, as well as the various uses that result in disciplines such as agriculture, pharmacy, and medicine. Because it is difficult to rely on spontaneous mutations to limit their development, these differences aid breeders in agricultural improvements and acquiring new types [32, 33]. Although most of these mutations are recessive and have a detrimental influence, they have proved a significant and successful part of the development of plants globally. Their effect has been evident and noticeable in the growth in the production of specific crops. Several articles have previously assessed the impact of mutation approaches on crop development [5, 11–31].

Seven combinations of SRAP primers generated reproducible informative amplification of SRAP fragments. SRAP markers are applied to amplify coding regions of DNA through primers targeting open reading frames. These markers have proven to be robust and highly variable [53]. The seven SRAP combinations produced 227 loci that showed 85% polymorphism among M_1 and M_2. SRAP markers generated a relatively higher level of polymorphism in cowpea [65] used 25 SRAP combinations to assess cowpea genotypes' genetic diversity. The combinations produced 250 bands, 245 of which were polymorphic. (98%) [66] used 34 SRAP primer pair combinations for cowpea's genetic diversity cowpea 1003 amplicons were generated, and 100% polymorphism was shown with an average of PIC of about 0.93.

The PIC were 0.41–0.50 in range with an average of 0.48 for M_1 and 0.47 for M_2 generation (Table 3). The GTS% of SRAP combinations ranged from 53 to 63% in the M_1 and 36–62% in the M_2 generation. The PIC value of SRAP primers varied from 0.97 (the highest value) to 0.71 (the lowest value), with an average PIC of 0.87.

The dendrogram in M_1 generation revealed three clusters; the 1st gathered the control and 25 Gy, the 2nd cluster grouped 50 Gy and 100 Gy, and the third contained the 75 Gy only. Conversely, M_2 generation exhibited four clusters; the 1st one grouped 75 Gy and 100 Gy, while the other three clusters contain one for each (control, 25 Gy, and 50 Gy, respectively), like our results [65] constructed dendrogram based upon morpho-agronomic and molecular genetics in 7 cowpea genotypes enabled grouping into 2 clusters. The 1st cluster comprised two cowpea genotypes, whereas the 2nd one formed five. Also [66], based upon cluster analysis of UPGMA, revealed two distinct clusters comprised of 7 cowpeas with high bootstrap values. The 1st cluster consisted of three determinate landraces, while the 2nd encompassed the four landraces.

The 10 IRAP primers produced 450 loci among the M_1 and M_2 generations ranging between 186 and 2525 bp; 398 are polymorphic with an average of 88% polymorphism in both generations (Table 5). The allele frequency of target loci ranged between 0.28 and 0.46. The PIC was 0.40–0.50 in the range. The GTS% of IRAP primers ranged from 29 to 41% in the M_1 and 55–71% in the M_2 generation (Table 6).

Similarly, nine IRAPs were used by [67], producing 90 distinguishable and scorable loci, out of which 74 loci (81.78%). Also [68] revealed 16 IRAP markers with consistently well-resolved and reproducible amplicon patterns among all the 58 Asian bamboo accessions. Two

hundred fifteen scorable amplicons were produced, of which 214 were polymorphic (99.5%). Polymorphic alleles were created with an average of 13.3 alleles per marker.

In this respect ([69]) used eight IRAP groups to detect polymorphisms in *Bletilla striata*. The eight groups produced 50 clear and 47 polymorphic bands with an average of 94% polymorphism and PIC ranging between 0.85 and 0.98.

ISSR and SRAP markers were applied to determine the genetic fidelity [70]. Additionally, genetic stability is now regularly assessed using a variety of molecular approaches. RAPD, SSR, AFLP, RFLP, and ISSR analyses are among the polymerase chain reaction (PCR)-based molecular markers employed by [71].

Our results agree with [72], who reported that in comparison to the control and the dose of 30 Gy, gamma-ray irradiation at doses of 10 Gy and 20 Gy demonstrates good growth in all parameters. Although there is no statistically significant difference between the control plant and the plants created by irradiation, the plants with the greatest dosage treatment appear to develop slower. High dosages of gamma-ray radiation have been shown to destroy cellular components of macromolecules such as cell walls, membranes, and DNA [73]. Principal component analysis (PCA) helps replicate total variation among many variables with a considerably smaller number of primary components. To evaluate molecular data, a heat map is used. PCA algorithms have been used to identify subgroups based upon a measure of similarity. Because the PCA tools stress their contribution to variability, they can be valuable tools for accelerating the breeding program [74]. Multivariate statistical analysis of morphological data, such as PCA and HCA, assessed genetic diversity and defined and classified a collection of 19 Egyptian maize cultivars and populations [75]. The newly gathered genetic resources were subjected to principal component analysis (PCA) and hierarchical clustering analysis (HCA) to assess phenotypic diversity and relationships [76].

In silico analysis revealed three SRAP combination primers that could align with some genes along cowpea and related family members' genomes. The three combinations were SRAP-F12R9, SRAP-F13R15, and SRAP-F13em1.

SRAP-F13R15 combination exhibited a fragment with 635 bp similar to accession FJ748893.1 (600 bp) and aligned with *Vigna unguiculata* cultivar Xiabao 2 chromosome Vu02 (GenBank: CP039348.1). The obtained sequence has two domains. The 1st one is a part of the sequence of Glycosyltransferase family 92 (GenBank: QCD90542.1). The characterization of the protein expressed from this gene is presented Additional file 1: S1. The 2nd domain is part of HEAT repeat associated with sister chromatid cohesion protein (GenBank: QCD90543.1), that characterized in Additional file 1: S1.

SRAP-F13em1 combination exhibited a fragment with 532 bp similar to accession FJ748897.1 (537 bp) and aligned with *Vigna unguiculata* cultivar Xiabao 2 chromosome Vu01 (GenBank: CP039350.1). The obtained sequence has two domains. The 1st one is a part of the sequence of aspartyl protease family protein (GenBank: QCD96127.1). The characterization of the protein expressed from this gene is presented in supplemented file (S1). The 2nd domain is part of ATP-dependent RNA helicase DeaD (GenBank: QCD96125.1), that characterized in S1.

The coefficient of extinction demonstrates the amount of light that a protein retains at a specific wavelength. The coefficient of extinction is gainful for determining this coefficient by a spectrophotometer during protein purification [77]. Two values are produced by ProtParam, both for proteins measured in water at 280 nm. The 1st value displays the calculated value assuming that all cysteine remains act as half cystines (viz, all pairs of the Cys residues form cystines). In contrast, the 2nd value assumes that no cysteine appears as half cystine (viz assuming all the Cys residues are reduced). Based upon previous experience, the computation is quite reliable for Trp-containing proteins. However, for proteins lacking Trp residues, there may be more than a 10% error. These values are a good approximation for denatured protein extinction coefficients; however, they could also be used to calculate native protein extinction coefficients [77]. The conclusion was reached after calculating the molar extinction coefficients of 18 globular proteins (44 total values). The agreement between measured and calculated values was generally good (to 5% in most cases, as stated in the abstract), but six of the values deviated by more than 10%. The instability index calculates the protein's stability in a test tube. A statistical analysis revealed that certain dipeptides in the unstable proteins significantly differed from those in the stable proteins. Proteins with an instability index of less than 40 are thought to be stable [78], confirming that our protein is stable. It has an instability index of 39.7.

Meanwhile [79], reported that gamma-ray induction of DNA polymorphism in this plant genome may result in the emergence of a desired phenotype and genetic traits that may be applied in yardlong bean development initiatives. Also, similarity with chloroplastic ATP synthase CF1 alpha chain and gypsy-like retrotransposon element was found by nucleotide sequencing and in silico analysis of two polymorphic amplicons produced using SRAP markers. According to the in silico study, the

retrotransposon sequence and the transposable element flanking regions surrounding the serine hydroxymethyltransferase (SHMT) coding area are similar in this context. Ultimately, the findings indicate that, for the first time, TRAP and SRAP-based markers may be applied to characterize the mutant germplasm produced by radiation-induced mutagenesis in the sugar crops [80]. This is the first publication on the genetic characterization of gamma-irradiation-induced mutagenesis in purple carrots and determining effective mutagen dosages. Variations between different levels of mutagen dosages were successfully detected by ISSR and SRAP markers [81].

5 Conclusion

In mutation breeding studies, determining the optimal dose of a mutagen is essential to develop lines with the desired agronomic traits. Thus, we applied gamma radiation doses range of 25–100 Gy to cowpea. This study revealed that exposure of cowpea seeds to 50 Gy is best for plant height, number of tiller plant^{-1} of cowpea in M_1 and M_2 generations, fresh and dry forage yield of cowpea in M_1 and M_2 generations, and number of pods Plant^{-1}, weight of pods plant^{-1}, and length of pods of cowpea in M_1 and M_2 generations, number of seeds pod^{-1}, 100 seeds weight, and seed yield (Kg fed^{-1}) of cowpea in M_1 and M_2 generations, and finally, crude protein (CP%), crude fiber (CF%), and ash (%) in M_1 and M_2 generations. This is the first report on using SRAP and IRAP markers to characterize induced mutagenesis through gamma irradiation in cowpea. Our results indicate that IRAP and SRAP markers successfully detected DNA polymorphism among the non-irradiated and irradiated cowpea individuals. Finally, the analysis In Silico revealed some SRAP combination primers that could align with some genes along cowpea and related family members' genomes. The SRAP-F12R9 is a unique sequence for *Phaseolus vulgaris* that belongs to the same family (Fabaceae). The SRAP F13R15 is found in the cowpea genome and aligns with *Vigna unguiculata* cultivar Xiabao 2 chromosome Vu02 (GenBank: CP039348.1). In contrast, the SRAP-F13em1 was found on the cowpea genome and aligned with *Vigna unguiculata* cultivar Xiabao 2 chromosome Vu01 (GenBank: CP039350.1). Our results could be useful for cowpea breeding programs. The resulting genotypes need to be monitored in the next generations to select high-yielding genotypes, as well as adapted ones for harsh environmental conditions. We recommend using gamma-ray irradiation as a vital tool to improve cowpea genotypes, especially when conjugated with molecular markers.

Abbreviations

M1	The first mutant generation
M2	The second mutant 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	Kilorad = 1000 rad
CP	Crude protein
LSD	The least significant difference
IRAP	Inter-retrotransposon amplified polymorphism
SRAP	Sequence-related amplified polymorphism
PIC	Polymorphism information content
RP	Resolving power
EMR	Effective multiplex ratio
MI	Marker index
phyA	Phytochrome A
ORF	Open reading frame
T_m (°C)	Melting 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

Supplementary Information

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Additional file 1. S1.

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Author contributions

Conceptualization was performed by CRA, FMS, MSR, MZSA, SDI, and KAMK; methodology was conducted by CRA, FMS, MRIS, EFA, AN, HD, and KAMK; software was given by KAMK; formal analysis was done by CRA and KAMK; investigation was analyzed by CRA and KAMK; resources was provided by AN, HD, CRA, FMS, and KAMK; data curation was conducted by EFA, AN, HD, CRA, and KAMK; writing—original draft preparation was revised by CRA, FMS, MSR, MZSA, ShDI, EFA, AN, HD, and KAMK; writing—review and editing was prepared by CRA, EFA, and KAMK; visualization was showed by CRA, FMS, MSR, MZSA, ShDI, AN, and KAMK; supervision was carried out by CRA, FMS, MSR, MZSA, ShDI, and KAMK. All authors have read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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