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RESEARCH ARTICLE

Effects of gamma radiation on quantitative traits and genetic variation of three successive generations of cowpea (*Vigna unguiculata* (L.) Walp.)

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ABSTRACT

An annual pulse crop cowpea (*Vigna unguiculata* (L.) Walp.), commonly named southern pea, is a nourishing constituent for the human diet and fodder. Gamma rays are a potent mutagenic agent to stimulate genetic variation with better characteristics, improving the yield relating traits in crops. Hence, the present study focused on exploring genetic variation between three generations in the mutant populations of cowpea through SCoT markers. The mutant populations of three successive generations, M_1, M_2 and M_3, were induced by different doses [200, 400, 600, 800, 1000 and 1200 Gray (Gy)] gamma irradiation. The results depict that the quantitative characters were reduced by increasing the dosage of gamma irradiation in the M_1 generation. In contrast, the second and third generation of plants showed a significant increase in yield and yield contributing traits than control and the maximum increase was noticed at 200 Gy and 400 Gy. Days to first flowering was delayed in irradiated plants than control of M_1 generation. In contrast, in consecutive generations (M_2 and M_3), the early first flowering was noticed at 400 Gy and late first flowering was observed at 800 Gy compared respectively to control and other doses. Seed yield per plant mean value was increased at 200 Gy in both generations (M_2 and M_3); it may produce new genotypes to desirable traits such as yield and quality. SCoT markers were used to explore genetic variation at the genomic level of mutant populations and screened with eight primers. Among them, seven primers showed amplification of 222 bands, in which 133 bands showed polymorphism. The polymorphic bands varied from 3.03–96.07%. The genetic variation, such as the number of different alleles (*Na*), effective number of alleles (*Ne*), Shannon's information index (*I*), expected heterozygosity (*He*) and unbiased expected heterozygosity (*uHe*) showed an average value of 1.352 ± 0.092, 1.278 ± 0.027, 0.293 ± 0.023, 0.184 ± 0.016, and 0.194 ± 0.016, respectively. AMOVA depicted significant genetic variation between all generations and indicated a total of 95% within populations and 5% among population variation by the marker used. The present investigations prominently showed that the variations induced by gamma irradiation were inherited from successive generations of the improvement in cowpea quantitative traits. This investigation gives acceptable proof that the SCoT markers are a valuable tool to identify the genetic variation among the three generations of cowpea.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is the most cultivated and consumed tropical grain legume, especially in Africa and Asian countries, which contain essential protein sources and rich in amino acids (1). The grain of cowpea contains protein (23%) and carbohydrate (57%) as a major component and leaves contain 27–34% of proteins (2). Cowpea production was hindered by quality, low grain yields, and lack of improved cultivars (3). On the other hand, cowpea's crop improvement through hybridisation and recombination is complicated because of the cleistogamous nature of flowers. Therefore, inducted

mutation plays a vital role in creating variability for enhancing crop yield with desired traits.

The physical and chemical mutagenesis creates genetic variation, produces new varieties with enhancing characteristics (4). Mutation breeding can be a valuable technique to traditional breeding methods and have been successful in the improvement of qualitative and quantitative traits in many crops such as cowpea (5–7), soybean (8, 9), black gram (10–12). Moreover, mutation breeding takes less time, easy to handle and is highly useful in producing crop cultivars than traditional breeding methods (13, 14). An updated database of IAEA/MVD (International Atomic Energy

Agency)/(Mutant Variety Database) maintained a list of 3308 varieties released through artificial mutations; 16 mutant varieties of cowpea have been registered, in which ten mutant varieties released by physical mutagen - gamma rays (15). The mentioned 16 mutant varieties have different specific agronomic characteristics in various geographical zones based on the needs they developed.

Different types of mutagenic agents, irradiations (ionising and non-ionising), have been effectively applied for the induced mutation in different crops (16). Among the physical mutagens, gamma radiation is probably the best to induce hereditary changes in crops. The physical mutagens, like ionising radiation, produced 89% of mutant varieties worldwide (17). Among these, 70% of genetic changes have been created by gamma irradiation alone (18). Many mutant varieties, such as crops resistant to biotic, abiotic factors and plants with desired traits, have been produced through gamma irradiation (19). Genetic variability is an essential trait for plant breeding, contributing to significant genetic variability through increasing DNA polymorphism (20). Genetic markers like the marker-based selection is a remarkable tool to plant breeders for selecting and evaluating specific traits (21). The gamma radiation has induced changes in the morphological and quantitative traits in cowpea and black oats (22, 23). The start codon targeted (SCoT) marker was developing based on the short conserved region flanking the start codon (ATG) in the plant gene (24). It correlated with functional genes with their response of corresponding traits. The SCoT marker has extended primer (18-mer) length and develop high polymorphisms than dominant and co-dominant markers (25, 26). The present study focused on the effect of gamma irradiation in the yield and yielded contributing traits of three successive generations of cowpea and the genetic variability analysis using SCoT marker for M_1, M_2 and M_3 generations of cowpea.

Materials and Methods

Seed material and Mutagenic treatment

The healthy and well-matured cowpea seeds (*V. unguiculata*) CO-7 was procured from the National Pulse Research Centre (NPRC), Vamban, Pudukkottai, Tamil Nadu, India. A hundred seeds per set were packed for each dose in a paper cover to subject irradiation such as 200, 400, 600, 800, 1000 and 1200 Gy and non-irradiated seeds used as control. The source of gamma irradiation ^{60}Co (Cobalt) was emitted 28 Gy/min dose rate in every seed and the facility was used in Radiological Safety Division, Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam, Chennai, Tamil Nadu, India. In the present study, the raising of M_1, M_2 and M_3 generations was carried out from 2016–2019 with respective seasons in Botanical Garden, Department of Botany, Periyar University, Salem, Tamil Nadu, India.

Field preparation and Experimental Design

The organic fertiliser (Cow Dung) was impregnated in the cultivated field and laid out into three plots, which consists of 6 rows. The experiment was designed in a randomised block design (RBD) in 2016–2017 for M_1 generation and the seeds cultivated along with control in three replication. The plant distance was maintained at 10–15 cm interval with spacing at 45 cm between the rows. Weeds controlled at regular intervals and cultivation practices such as irrigation, manures, pesticides and insecticides were done for proper growth. Among different irradiation doses, the plants were survived at 200 Gy, 400 Gy, 600 Gy and 800 Gy, along with control. However, the higher doses, such as 1000 Gy and 1200 Gy, showed the least survival rate; hence those seeds were discarded without further investigation. All the biometrical characteristics were scored in the M_1 generation at the harvest stage.

The M_1 seeds were collected separately based on different doses and raised M_2 generation. Seeds harvested from M_1 plants were bulked for each dose and raised in the field with triplicates in RBD during 2017–2018. After reap, the M_2 plants packed in individual bags based on irradiation doses and raised in the field for M_3 generation with triplicates followed by RBD. After maturation, the plants were harvested, and seeds were stored separately in polythene cover with respective doses marked. The quantitative traits of M_1, M_2 and M_3 generations were evaluated during 2016–2017, 2017–2018 and 2018–2019 with respective seasons (Fig. 1).

DNA isolation and SCoT amplification

The treated and control plant leaves (200 mg) of *V. unguiculata* from three successive generations were extracted for genomic DNA based on the HipurA™ SuperPlant DNA purification kit (Himedia, Code: MB571; Mumbai, India). The SCoT primer (8 sets) was custom synthesised by Sigma Aldrich (Bangalore, India), and it consists of GC content between 50–61% (Table 1). The optimised PCR reaction was performed in PCR Thermal Cycler (Cyberlab, Smart PCR) with

Table 1. List of SCoT primers used for the study.

S. No.	Name of primer	Primer sequence (5'-3')
1.	SCoT1	CAACAATGGCTACCACCA
2.	SCoT2	CAACAATGGCTACCACCG
3.	SCoT3	CAACAATGGCTACCACGC
4.	SCoT6	ACGACATGGCGACCAACG
5.	SCoT7	ACGACATGGCGACCATCG
6.	SCoT8	ACCATGGCTACCACCGAC
7.	SCoT09	ACCATGGCTACCACCGAG
8.	SCoT10	ACGACATGGCGACCCACA

GoTaq G2 Green PCR master mix (Promega, Cat: M7822; Madison, USA) contains 1x concentration of 12µl reaction mixture with the following PCR program: 3 minutes 94 °C, 40 cycles of 1 min at 94 °C, 1 min at 50 °C, 2 min 72 °C, followed by 5 minutes of final extension at 72°C. The PCR reactions (12 µl) were checked on 1.5 % agarose (Himedia, Mumbai) in 1x TAE buffer gel with 50 V applied current. The amplified profiles were visualised under a UV Transilluminator (Medox-Bio™, UV Transilluminator Dual).

Gamma
irradiation

Gamma irradiated
seeds of cowpea

M1 Generation
(2016-2017)

Sowing of irradiated
seeds and harvest
with respective
doses of M1 seeds

M2 Generation
(2017-2018)

Sowing of M1 seeds
and harvesting
individual doses of
M2 seeds

M3 Generation
(2018-2019)

Sowing of M2 seeds
and harvesting
individual doses
of M3 seeds

Fig. 1. Field experiments: Cowpea with the effect of gamma irradiation.

Data analysis of DNA amplicons

The unambiguous SCoT amplicons were manually converted into a binary matrix as presence (1) or absence (0) of the resulting band. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) construction method, similarity coefficient (27), and pairwise genetic similarity of Jaccard's similarity coefficient (28) were assessed in FreeTree software, ver. 9.1. The original pattern was resampled with 1000 times bootstrap, and a consent tree was developed. All tree was laid out using Tree View ver. 1.6 (29). The percentage of polymorphic variation was calculated by using the following formula:

$$Percentage\ of\ Polymorphism = \frac{Number\ of\ polymorphic\ bands}{Total\ number\ of\ bands} \times 100$$

Genetic diversity

Estimation of genetic diversity like number of different alleles (N_a), Effective alleles (N_e), Shanon's information index (*I*), Expected heterozygosity (*He*) and Nei's expected heterozygosity (*uHe*), Principal Coordinate Analysis (PCoA) and Analysis of molecular variance (AMOVA) were performed in GenAlEx v.6.1 (30).

Data Collection and Statistical Analysis

Observations of various morphological and quantitative traits were recorded for M_1, M_2 and M_3 generations (Table 2). One-way ANOVA and Pearson Correlation were employed to understand better trait-associated characters in M_1, M_2 and M_3 generations using a statistical package (SPSS 21 for Windows).

Results

Different doses of gamma rays were induced, and the changes of various morphological and quantitative (yield) traits were recorded for M_1, M_2, and M_3 generations.

Quantitative traits in M_1 generation

Morphological and quantitative characters were reduced with increasing gamma irradiation doses except for days to first flowering (Table 3). The treated plants were taken more days to flower than per plant, the number of fruit cluster per plant, the number of pods per plant, the number of seeds per pod, pod length (cm), a hundred seed weight (g) and seed yield per plant (g) were gradually reduced with increasing doses of gamma irradiation than control.

All quantitative characters were reduced at 200 Gy, and the maximum reduction was noticed at 800 Gy than control. In this study, statistical analysis showed that day to first flowering depicts a significant negative correlation (*p<0.01*) among yield characters; hence, the yield was reduced at M_1 generation (Table 6).

Quantitative traits in M_2 and M_3 generations

A significant improvement was noted in the quantitative traits such as plant height, number of leaves per plant, days to first flowering, number of fruit cluster per plant, number of pods per plant, number of seeds per pod, a hundred seed weight and seed yield per plant in M_2 and M_3 generations (Table 4 and 5). The present result showed that the plant was heightened at 200 Gy than control. The correlation coefficient confirmed that the plant height was significantly positive correlated to seed yield per plant (*p<0.01*) (Table 7). The plant height was substantially increased at 200 Gy in both M_2 and M_3 generations compared to other doses and control. The decrease in the number of branches per plant was recorded at 600 Gy (3.9 ± 0.16) in M_2 and 400 Gy (4.2 ± 0.26) in M_3 generations. However, a significant increase was observed in the number of leaves per plant at 200 Gy (126.86 ± 2.65) in M_2 generation.

In contrast, the M_3 generation decreased the number of leaves per plant in the irradiation doses than control plants. Gamma irradiation induces a stimulatory effect in early flowering in 400 Gy (34.06 ± 0.26) in the second generation. Similarly, in the third generation, the same traits were observed at 400 Gy (41.7 ± 0.50), but in higher doses, 800 Gy induced late-flowering on M_2 and M_3 generations. The present result proved that gamma radiation is creating early flowering in consecutive generations. Gamma irradiation can be inducing early flowering in lower doses, while higher doses induced late flowering.

In M_2 generation, the gradual increase in the

Table 2. List of morphology and quantitative traits and their description of the measurement

Growth/Yield trait	Denotation	Method of evaluation
Plant height	PH	The height from the base of the plant to the tip of the last leaf
Number of Branches per plant	NBP	Count the total number of primary branches per plant
Number of leaves per plant	NLP	Count the total number of leaves per plant
Days to first flowering	DFF	Count the number of days from seed sown to first flowering
Number of fruit cluster per plant	NCP	Count the total number of cluster per plant
Number of pods per plant	NPP	Count the number of pods per plant
Pod Length	PL	Measure the base to the tip of the pod
Number of seeds per pod	NSP	Count the total number of seeds per pod
Hundred seed weight	HSW	Count the one hundred seeds randomly and the weighed
Seed yield per plant	SYP	Weighing the total number of seeds produced in a plant

control plants. Plants treated with 800 Gy gamma-irradiated doses took more days to first flowering (41.76 ± 0.15) than in control (34.16 ± 0.11). The quantitative characters like plant height (cm), the number of branches per plant, the number of leaves

number of fruit clusters per plant was noted in 200 Gy (23.96 ± 1.25). In this study, the number of fruit clusters was significantly positively correlated with plant height (*p<0.05*), number of branches per plant, number of leaves per plant (*p<0.01*), and negative

Table 3. Effects of gamma radiation on quantitative traits of cowpea in M_1 generation

Doses (Gy)	PH	NBP	NLP	DFF	NCP	NPP	PL	NSP	HSW (g)	SYP (g)
Control	60.60 ± 1.08	6.5 ± 0.24	107.90 ± 1.19	34.16 ± 0.11	19.26 ± 0.66	39.53 ± 1.12	15.76 ± 0.20	16.22 ± 0.23	10.82 ± 0.03	35.22 ± 0.85
200 Gy	57.86 ± 1.21	5.53 ± 0.22	103.3 ± 0.87	35.53 ± 0.17	18.33 ± 0.58	35.77 ± 1.35	14.87 ± 0.24	14.59 ± 0.19	9.84 ± 0.05	31.61 ± 1.76
400 Gy	53.35 ± 1.04	5.43 ± 0.18	98.2 ± 3.09	36.13 ± 0.19	18.03 ± 0.566	31.46 ± 1.71	14.03 ± 0.18	14.97 ± 0.28	9.64 ± 0.14	26.36 ± 1.47
600 Gy	51.10 ± 0.79	4.73 ± 0.14	95.4 ± 3.61	38.3 ± 0.12	14.9 ± 0.66	24.33 ± 0.97	12.43 ± 0.25	13.31 ± 0.17	8.74 ± 0.04	20.74 ± 0.60
800 Gy	45.74 ± 0.68	3.36 ± 0.17	90.83 ± 2.47	41.76 ± 0.15	11.9 ± 0.53	18.13 ± 0.84	8.1 ± 0.26	10.03 ± 0.26	8.07 ± 0.04	14.32 ± 0.38

Data were expressed as mean ± SE (Standard Error), PH-Plant height, NBP-Number of branches per plant, NLP-Number of leaves per plant, DFF- Days to first flowering, NCP-Number of fruit cluster per plant, NPP-Number of pods per, NSP-Number of seeds per pod plant, PL- Pod length, HSW-Hundred seed weight, SYP- Seed yield per plant.

Table 4. Effects of gamma radiation on quantitative traits of cowpea in M_2 generation

Doses (Gy)	PH	NBP	NLP	DFF	NCP	NPP	PL	NSP	HSW (g)	SYP (g)
Control	60.52 ± 1.63	4.50 ± 0.11	119.8 ± 2.39	36.2 ± 0.37	20.93 ± 0.96	41.9 ± 1.51	14.67 ± 0.15	14.7 ± 0.18	10.16 ± 0.15	36.07 ± 1.46
200 Gy	64.64 ± 0.62	4.53 ± 0.14	126.86 ± 2.65	35.1 ± 0.32	23.96 ± 1.25	45.6 ± 2.04	15.63 ± 0.18	14.63 ± 0.27	10.43 ± 0.15	40.13 ± 0.93
400 Gy	57.97 ± 1.13	4.2 ± 0.25	110.2 ± 4.04	34.06 ± 0.26	21.833 ± 1.55	36.3 ± 2.19	13.65 ± 0.20	13.26 ± 0.33	9.86 ± 0.16	30.40 ± 2.04
600 Gy	55.83 ± 1.91	3.9 ± 0.16	101.56 ± 1.45	35.8 ± 0.35	20.733 ± 1.42	32.166 ± 1.25	13.93 ± 0.27	13.1 ± 0.40	9.39 ± 0.17	27.64 ± 1.07
800 Gy	55.64 ± 1.75	4.1 ± 0.16	95.66 ± 2.64	38.43 ± 0.40	17.6 ± 0.77	22.06 ± 1.17	13.49 ± 0.22	13.00 ± 0.38	9.91 ± 0.12	19.68 ± 0.96

Data were expressed as mean ± SE (Standard Error), PH-Plant height, NBP-Number of branches per plant, NLP-Number of leaves per plant, DFF- Days to first flowering, NCP-Number of fruit cluster per plant, NPP-Number of pods per, NSP-Number of seeds per pod plant, PL- Pod length, HSW-Hundred seed weight, SYP- Seed yield per plant.

Table 5. Effects of gamma radiation on quantitative traits of cowpea in M_3 generation

Doses (Gy)	PH	NBP	NLP	DFF	NCP	NPP	PL	NSP	HSW (g)	SYP (g)
Control	59.02 ± 1.52	5.16 ± 0.22	261.16 ± 17.24	43.83 ± 0.33	39.3 ± 2.63	48.4 ± 2.13	13.05 ± 0.23	12.1 ± 0.24	11.29 ± 0.16	41.25 ± 1.85
200 Gy	62.21 ± 2.60	4.8 ± 0.29	260.33 ± 18.74	43.53 ± 0.20	37.36 ± 3.25	55.3 ± 3.27	13.61 ± 0.29	12.46 ± 0.37	11.91 ± 0.11	48.56 ± 2.60
400 Gy	54.46 ± 2.25	4.2 ± 0.26	166.76 ± 12.11	41.7 ± 0.50	26.5 ± 2.36	50.03 ± 4.03	13.52 ± 0.15	13.23 ± 0.32	12.71 ± 0.11	44.23 ± 3.11
600 Gy	52.07 ± 1.79	4.43 ± 0.20	170.23 ± 9.41	41.96 ± 0.60	28.7 ± 1.64	54.16 ± 2.92	12.72 ± 0.21	12.00 ± 0.34	11.90 ± 0.12	38.31 ± 2.25
800 Gy	50.73 ± 1.78	4.5 ± 0.19	170.3 ± 12.92	46.73 ± 0.51	24.66 ± 1.59	38.53 ± 2.50	11.58 ± 0.26	9.83 ± 0.39	11.65 ± 0.11	29.49 ± 2.03

Data were expressed as mean ± SE (Standard Error), PH-Plant height, NBP-Number of branches per plant, NLP-Number of leaves per plant, DFF- Days to first flowering, NCP-Number of fruit cluster per plant, NPP-Number of pods per, NSP-Number of seeds per pod plant, PL- Pod length, HSW-Hundred seed weight, SYP- Seed yield per plant.

correlation obtained in days to first flowering (Table 7). On the contrary, the M_3 generation showed decreased fruit clusters in all the doses compared to control. A positive mean value was shifted in the number of pods per plant in both M_2 and M_3 generations. The increasing number of pods per plant was observed at 200 Gy in M_2 (45.6 ± 2.04) and M_3 (55.3 ± 3.27) generations. The pod length was also significantly increased in the second mutagenic generation compared to the third generation. The number of seeds per pod decreased in the second generation, with increasing doses of gamma irradiation and maximum decreased at 800 Gy (13 ± 0.38). The result confirmed that the number of seeds per pod had a strong positive correlation ($p<0.01$) to pod length (Table 6). Whereas in M_3 generation, a substantial increase in the number of seeds per plant observed at 400 Gy (13.23 ± 0.32) when compared to control and other doses respectively. Gamma irradiation revealed a considerable enhancement in yield traits of both second and third mutagenic generations of cowpea. In M_2 generation, the

Table 6. Pearson's correlation coefficients of different quantitative traits of M_1 generation

	PH	NBP	NLP	DFF	NCP	NPP	PL	NSP	HSW	SYP
PH	1	.424**	.248**	-.634**	.373**	.528**	.545**	.594**	.612**	.646**
NBP		1	.376**	-.655**	.452**	.514**	.644**	.648**	.525**	.590**
NLP			1	-.342**	.316**	.283**	.284**	.276**	.312**	.403**
DFF				1	-.630**	-.722**	-.794**	-.872**	-.733**	-.843**
NCP					1	.708**	.506**	.543**	.491**	.549**
NPP						1	.614**	.660**	.754**	.725**
PL							1	.894**	.625**	.724**
NSP								1	.676**	.767**
HSW									1	.744**
SYP										1

** indicate significance of p values of *< 0.001* level

PH-Plant height, NBP-Number of branches per plant, NLP-Number of leaves per plant, DFF- Days to first flowering, NCP-Number of fruit cluster per plant, NPP-Number of pods per, NSP-Number of seeds per pod plant, PL- Pod length, HSW-Hundred seed weight, SYP- Seed yield per plant.

Table 7. Pearson's correlation coefficients of different quantitative traits of M_2 generation

	PH	NBP	NLP	DFF	NCP	NPP	PL	NSP	HSW	SYP
PH	1									
NBP	.189*	1								
NLP	.314**	.537**	1							
DFF	-.070	-.009	-.213**	1						
NCP	.195*	.648**	.480**	-.096	1					
NPP	.341**	.570**	.583**	-.230**	.651**	1				
PL	.256**	.174*	.303**	-.194*	.270**	.331**	1			
NSP	.098	.160	.223**	-.059	.165*	.294**	.668**	1		
HSW	.130	.259**	.261**	.041	.188*	.278**	.181*	.193*	1	
SYP	.325**	.518**	.583**	-.205*	.546**	.874**	.367**	.303**	.271**	1

* and ** indicate significant at the *p < 0.05* and *0.01* level

PH-Plant height, NBP-Number of branches per plant, NLP-Number of leaves per plant, DFF- Days to first flowering, NCP-Number of fruit cluster per plant, NPP-Number of pods per, NSP-Number of seeds per pod plant, PL- Pod length, HSW-Hundred seed weight, SYP- Seed yield per plant.

enhancement of the 100 seed weight was noted in 200 Gy (10.43 ± 0.15), whereas other doses showed decreased level compared to control. In M_3 generation, 100 seed weight was higher in all mutagenic treatments than control. The increase of 100 seed weight was observed at 400 Gy (12.71 ± 0.11), followed by 200 Gy (11.91 ± 0.11), 600 Gy (11.90 ± 0.12), and 800 Gy (11.65 ± 0.11). In the M_3 generation, the 100 seed weight was enhanced than the M_2 generation. In the second generation, a lower dose of gamma rays increased the seed yield at 200 Gy (40.13 ± 0.93) and decreased in other doses compared to control. Irradiation doses of 200 Gy (48.56 ± 2.60) and 400 Gy (44.23 ± 3.11) increased the seed yield while other doses were decreased compared to control of the third generation. Seed yield revealed a significant positive correlation to the plant height, number of branches per plant, number of leaves per plant, number of fruit cluster per plant, number of pods per plant, and pod length. Furthermore, a significant negative correlation obtained for days to first flowering, so the yield was increased (Table 8).

SCoT marker

In the present investigation, the genetic variation among M_1, M_2, and M_3 generations of cowpea tested with varied doses of gamma irradiation and control were analysed by the SCoT marker. Among the eight primers tested, seven primers were amplified and found reproducible, whereas no amplification was detected in the primer SCoT09 for all three generations. In all generations, 222 bands were scored, of which 133 were polymorphic (Table 9). The % of polymorphic bands in each primer differed from 3.03–96.07%, with an average of 67.10%. The polymorphic bands per primer range between 2 (SCoT01) to 49 (SCoT06), within an average of 19%, and the number of bands varied between generations. The SCoT01 primer obtained more bands than other primers, whereas a minimum number of amplicons were observed at SCoT10. The highest polymorphic bands were observed in SCoT06 (49), whilst the lowest polymorphic band was noted in SCoT01 (2) primer. The SCoT03 and SCoT08 primers detected the absence of bands for M_3 generation, whereas it was amplified for M_1 and M_2 generations. In M_2 generation, all the primers were amplified; however SCoT08 primer was not amplified in M_3 generation because the primer site (ATG) does not bind DNA samples, degradation of chemicals, buffer solutions, polymerase enzymes etc.

Genetic diversity

Table 10 showed the genetic variation of cowpea among M_1, M_2, M_3 generations. In M_1 generation, the number of different alleles (*Na*) varies between 1.143 ± 0.404 to 2 ± 0.001, the number of effective alleles (*Ne*) varied from 1.270 ± 0.117 to 1.443 ± 0.124. The Shannon's Information Index (*I*) were ranged from 0.268 ± 0.106 to 0.434 ± 0.073, Expected heterozygosity (*He*) ranged from 0.173 ± 0.072 to 0.277 ± 0.059, and unbiased Expected Heterozygosity (*uHe*) between 0.182 ± 0.075 to 0.291 ± 0.063. The higher dose (800 Gy) of gamma irradiation showed high genetic diversity than other doses and control. In M_2 generation, *Na* value ranged from 1.429 ± 0.369 to 2.000 ± 0.001, *Ne* lowest to highest ranged from 1.133 ± 0.050 to 1.386 ± 0.063, *I* minimum to maximum value between 0.198 ± 0.062 to 0.433 ± 0.047, *He* ranged from 0.108 ± 0.037 to 0.269 ± 0.036 and *uHe* minimum to

Table 8. Pearson's correlation coefficients of different quantitative traits of M_3 generation

	PH	NBP	NLP	DFF	NCP	NPP	PL	NSP	HSW	SYP
PH	1									
NBP	.384**	1								
NLP	.427**	.724**	1							
DFF	-.061	-.032	-.101	1						
NCP	.254**	.444**	.599**	-.183*	1					
NPP	.254**	.354**	.315**	-.357**	.576**	1				
PL	.221**	-.047	.127	-.143	.028	.104	1			
NSP	.169*	-.108	.045	-.189*	.005	.059	.858**	1		
HSW	-.048	-.097	-.124	-.169*	-.186*	.054	.152	.248**	1	
SYP	.286**	.276**	.314**	-.230**	.502**	.798**	.182*	.111	.100	1

* and ** indicate significance at the $p < 0.05$ and *0.01* level respectively

PH-Plant height, NBP-Number of branches per plant, NLP-Number of leaves per plant, DFF- Days to first flowering, NCP-Number of fruit cluster per plant, NPP-Number of pods per, NSP-Number of seeds per pod plant, PL- Pod length, HSW-Hundred seed weight, SYP- Seed yield per plant.

Table 9. SCoT primers used in M_1, M_2, and M_3 generations of cowpea under the effects of gamma irradiation

No. of primers	Generations															Total no. of bands	No. of polymorphic bands	Percentage (%) of polymorphism
	M₁					M₂					M₃							
	CPCM₁	CP2M₁	CP4M₁	CP6M₁	CP8M₁	CPCM₂	CP2M₂	CP4M₂	CP6M₂	CP8M₂	CPCM₃	CP2 M₃	CP4 M₃	CP6 M₃	CP8 M₃			
SCOT01	5	4	5	5	6	1	4	4	6	3	0	5	6	6	6	66	2	3.03
SCOT02	1	1	3	5	4	1	3	2	2	2	0	0	0	0	4	28	21	75.00
SCOT03	0	3	1	1	2	0	1	1	1	0	0	0	0	0	0	10	8	80.00
SCOT06	4	5	4	5	6	3	4	0	3	4	3	3	3	0	4	51	49	96.07
SCOT07	4	4	2	3	3	2	2	3	3	2	2	2	2	2	2	38	33	86.84
SCOT08	0	0	0	0	1	0	4	2	3	1	0	0	0	0	0	11	5	45.45
SCOT10	0	0	0	0	1	1	3	0	0	0	0	4	4	0	5	18	15	83.33
Total	14	17	15	19	23	8	21	12	18	12	5	14	15	8	21	222	133	67.10
Average																31.71	19.00	

CPC-Cowpea Control, CP2-Cowpea 200Gy, CP4-Cowpea 400 Gy, CP6-Cowpea 600 Gy, CP8-Cowpea 800 Gy

maximum value from 0.112 ± 0.039 to 0.283 ± 0.038 were observed control and gamma irradiated doses. However, 200 Gy irradiation doses induced more genetic variability compared to control. Whereas in M_3 generation, genetic diversity showed lowest to highest *Na* value from 0.571 ± 0.369 to 1.429 ± 0.369, *Ne* lowest to highest value range from 1.087 ± 0.058 to 1.412 ± 0.129, minimum to maximum *I* ranged from 0.112 ± 0.073 to 0.381 ± 0.105, *He* value ranged from 0.066 ± 0.044 to 0.182 ± 0.072 and *uHe* minimum to maximum value between 0.069 ± 0.046 to 0.266 ± 0.076 were noted in control and gamma treated doses. Here, in all three generations, irradiated doses are induced high genetic variability compared to control.

Genetic distance

Table 11 showed Nei's unbiased pairwise genetic distance of cowpea genotypes calculated in three successive generations treated by different gamma irradiation doses. In M_1 generation, the higher genetic distance was observed in 400 Gy and 600 Gy (0.705) and low genetic distance obtained in control and 800 Gy (0.377). Whereas in M_2 generation, the high distance was observed between 400 Gy and 600 Gy (0.758), 600 Gy and 800 Gy (0.758) showed the same distance value, and the low genetic distance was observed at the control and 400 Gy (0.4). In M_3 generation, a high genetic distance was observed at 200 Gy and 400 Gy (0.896), a low distance value at the control and 800 Gy (0.2307). Compared to all the three generations, M_2 generations showed more genetic distance.

Analysis of Molecular Variance (AMOVA) and Dendrogram

Analysis of molecular variance (AMOVA) results showed that genetic difference (5%) was observed among the populations, whereas 95% variation was observed within populations (Table 12) for all three generations. The phylogenic tree was used to visualise the genetic variations in three generations of the different doses of gamma irradiation. The dendrogram of three generations was divided into four clusters (Fig. 2). Cluster I consists of M_2 generation alone, 400 Gy and 600 Gy shared a common sister group, and 200 Gy showed a common ancestor for both doses of gamma irradiation (400 Gy and 600 Gy). Cluster II and III shared a common internode consisting of M_1 and M_3 generations. Besides, Cluster II consists of 200 Gy, 400 Gy, 600 Gy and 800 Gy, along with control. The 200 Gy and control shared a common branch and 400 Gy and 800 Gy shared a common node. Cluster III was showed proximity with 800 Gy, 200 Gy and 400 Gy. Cluster IV consists of M_3 control, and these are the common ancestor for all the Operational Taxonomic Units (OTU).

Principal Coordinates Analysis (PCoA)

PCoA analysis showed the genetic relationships between the generations of cowpea by the effects of gamma irradiation (Fig. 3). The cowpea genotypes were divided into 5 groups. It consists of group I (6 doses), group II (7 doses), group III (5 doses), group IV (10 doses) and group V (10 doses) were grouped and individual doses were scattered from the grouped.

The first, second and third axes represented 31.90%, 53.14%, and 69.09% of the cumulative variation.

horse gram (42). Whereas the days to first flowering were delayed in all the irradiated seeds, particularly

Table 10. Genetic diversity analysis in M_1, M_2, M_3 generations of cowpea revealed by SCoT primers

Samples/Populations	Na±SE	Ne±SE	I±SE	He±SE	uHe±SE
CPCM$_1$	1.143±0.404	1.270±0.117	0.268±0.106	0.173±0.072	0.182±0.075
CP2 M$_1$	1.429 ±0.369	1.323±0.109	0.331±0.098	0.212±0.066	0.223±0.070
CP4 M$_1$	1.429±0.369	1.280±0.103	0.303±0.092	0.189±0.062	0.199±0.066
CP6 M$_1$	1.429±0.369	1.372±0.128	0.352±0.106	0.230±0.074	0.243±0.077
CP8 M$_1$	2.000±0.001	1.443±0.124	0.434±0.073	0.277±0.059	0.291±0.063
CPCM$_2$	1.429±0.369	1.133±0.050	0.198±0.062	0.108±0.037	0.112±0.039
CP2 M$_2$	2.000±0.001	1.386±0.063	0.433±0.047	0.269±0.036	0.283±0.038
CP4 M$_2$	1.429±0.369	1.212±0.075	0.265±0.079	0.157±0.050	0.165±0.053
CP6 M$_2$	1.714±0.286	1.334±0.105	0.362±0.079	0.224±0.056	0.236±0.059
CP8 M$_2$	1.429±0.369	1.212±0.075	0.265±0.079	0.157±0.050	0.165±0.053
CPCM$_3$	0.571±0.369	1.087±0.058	0.112±0.073	0.066±0.044	0.069±0.046
CP2 M$_3$	1.143±0.404	1.265±0.108	0.274±0.102	0.175±0.067	0.184±0.071
CP4 M$_3$	1.143±0.404	1.288±0.125	0.282±0.106	0.182±0.072	0.192±0.075
CP6 M$_3$	0.571±0.369	1.157±0.123	0.142±0.098	0.093±0.067	0.098±0.071
CP8 M$_3$	1.429±0.369	1.412±0.129	0.381±0.105	0.252±0.073	0.266±0.076
Total	1.352±0.092	1.278±0.027	0.293±0.023	0.184±0.016	0.194±0.016

Na-Number of different alleles, Ne-Number of effective alleles, I-Shannon's information Index, He-Expected heterozygosity, uHe-unbiased Expected heterozygosity. CPC-Cowpea Control, CP2-Cowpea 200 Gy, CP4-Cowpea 400 Gy, CP6-Cowpea 600 Gy, CP8-Cowpea 800 Gy

Table 11. Nei's unbiased pair wise genetic distance values recorded in M_1, M_2, M_3 generations of cowpea treated with different doses of gamma irradiation

Doses	CPCM$_1$	CP2M$_1$	CP4M$_1$	CP6M$_1$	CP8M$_1$	CPCM$_2$	CP2M$_2$	CP4M$_2$	CP6M$_2$	CP8M$_2$	CPCM$_3$	CP2M$_3$	CP4M$_3$	CP6M$_3$	CP8M$_3$
CPCM$_1$	***	0.6451	0.5517	0.4242	0.3778	0.1818	0.3428	0.3846	0.4516	0.4615	0.2105	0.5000	0.6206	0.3636	0.5714
CP2 M$_1$		***	0.6875	0.5555	0.5000	0.3200	0.4210	0.4137	0.4705	0.4827	0.1818	0.4516	0.3750	0.3200	0.5263
CP4 M$_1$			***	0.7058	0.6842	0.3478	0.3333	0.3703	0.5000	0.5185	0.2000	0.5517	0.4666	0.4347	0.6666
CP6 M$_1$				***	0.6666	0.3703	0.4000	0.3225	0.4444	0.5161	0.1666	0.4242	0.4117	0.3703	0.5500
CP8 M$_1$					***	0.3871	0.4545	0.4571	0.6000	0.5714	0.2857	0.3783	0.3684	0.3871	0.5454
CPCM$_2$						***	0.4827	0.4000	0.4800	0.7000	0.4615	0.2727	0.2608	0.2500	0.3448
CP2 M$_2$							***	0.5454	0.7368	0.6060	0.1538	0.2857	0.3888	0.2069	0.5238
CP4 M$_2$								***	0.7586	0.5833	0.1176	0.1538	0.2222	0.4000	0.3636
CP6 M$_2$									***	0.7586	0.1818	0.3225	0.3750	0.4000	0.4736
CP8 M$_2$										***	0.3529	0.3076	0.3703	0.3000	0.4242
CPCM$_3$											***	0.3157	0.3000	0.3076	0.2307
CP2 M$_3$												***	0.8965	0.5454	0.7428
CP4 M$_3$													***	0.5217	0.7222
CP6 M$_3$														***	0.4137
CP8 M$_3$															***

CPC-Cowpea Control, CP2-Cowpea 200Gy, CP4-Cowpea 400 Gy, CP6-Cowpea 600 Gy, CP8-Cowpea 800 Gy

Table 12. Analysis of molecular variance (AMOVA) in M_1, M_2, M_3 generations of cowpea using SCoT primer

Source	df	SS	MS	Est.Var	Percentage of variation (%)
Among populations	14	21.133	1.510	0.050	5%
Within populations	135	136.000	1.007	1.007	95%
Total	149	157.133		1.058	100%

df- Degree of freedom, SS-Sum of squares deviation, MS- Mean of squared deviation, Est. Var.- Estimates of variance, %-Percentage of variation.

Discussion

Mutation breeding is a promising tool to induce genetic variability in quantitative and qualitative traits in crops. Gamma irradiation is the best physical mutagen in plant breeding technique, and it has been beneficial for the breed in several pulse crops such as mung bean (31), peanut (32), chickpea (33), cowpea (34) and pigeon pea (35). In this study, the M_1 results showed that the increase of gamma radiation dose gradually decreased morphological and quantitative traits compared to control (Table 3). The present results reveal that gamma irradiation might induce physiological activity disturbances, arrest the cell division, change or inhibit enzyme activity. Similar results were observed in many crops such as cowpea (36–38), black gram (11, 39, 40), soybean (41) and

in 800 Gy, which took more days (41.76 ± 0.15) compared to control. Besides, gamma irradiation-induced the delay of days to the first flower in the M_1 generation of cowpea (38). The higher dose of gamma rays affected the physiological process, which leads to induce late flowering. Physiological disproportion and different forms of chromosomal distortion could be the leading causes of the decrease in plant survival (43).

The present results depict that the M_2 and M_3 generations obtained enhancement of yield and yield-related components of cowpea under gamma radiation treatment. In M_2 and M_3 generations, the plant height was increased at 200 Gy than control. The lower doses of gamma rays induced the plant height due to the expansion of cell division, altering metabolic growth that influences the
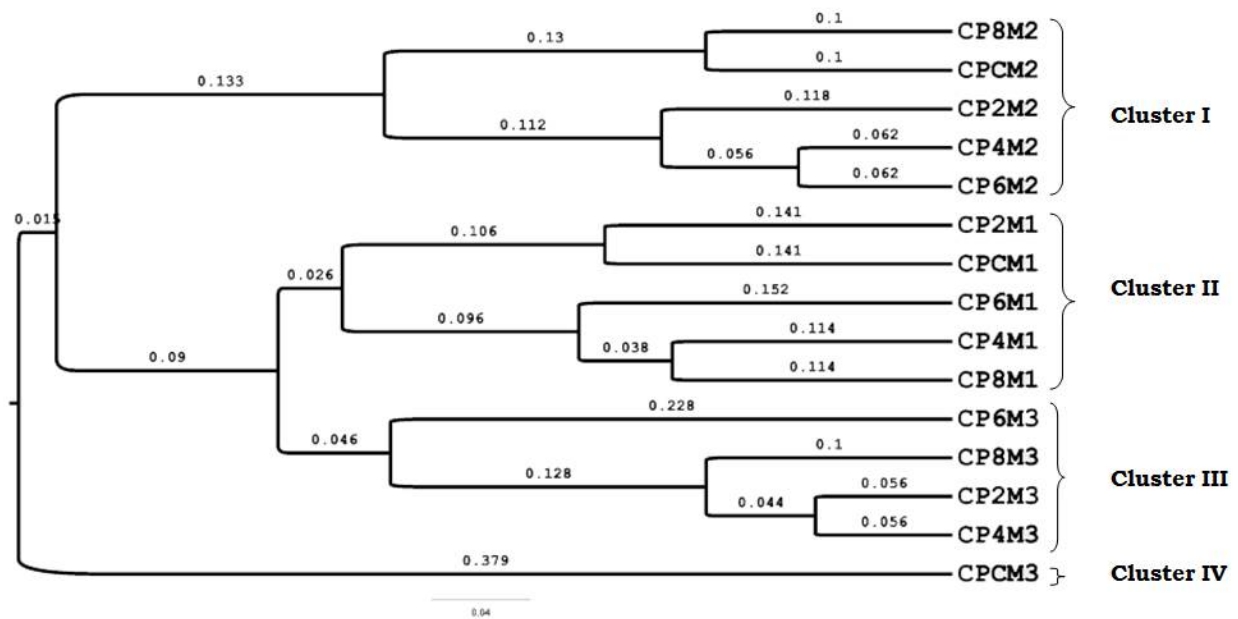
Fig. 2. Dendrogram of cowpea analysis by using UPGMA cluster analysis based on the genetic similarities coefficient.
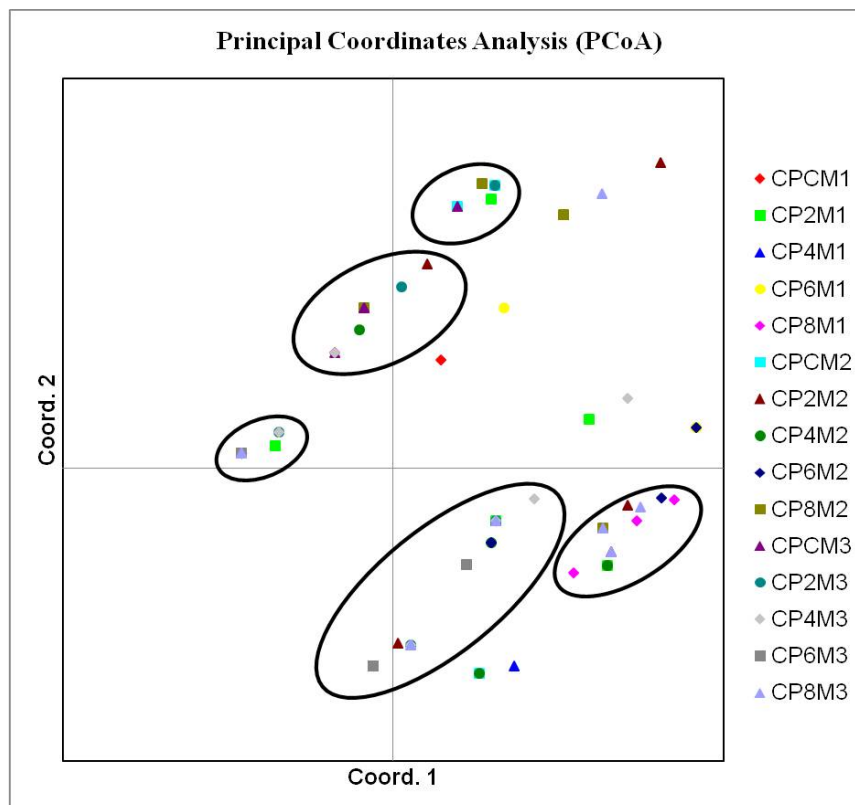CPC-Cowpea Control, CP2-Cowpea 200Gy, CP4-Cowpea 400 Gy, CP6-Cowpea 600 Gy, CP8-Cowpea 800 Gy

Fig. 3. PCoA analysis of cowpea genotypes under the treated with gamma irradiation
CPC-Cowpea Control, CP2-Cowpea 200Gy, CP4-Cowpea 400 Gy, CP6-Cowpea 600 Gy, CP8-Cowpea 800 Gy

synthesis of phytohormones and nucleic acids (44). The same results were observed in enhancing plant height in black gram (45) and chickpea (46, 47). In M_2 and M_3 generations, the significant reduction of days for early flowering was recorded at 400 Gy than other doses and control. Early flowering indicates that gamma radiation-induced a short lifetime and positively impacted cowpea improvement. The present results agree with the influence of gamma irradiation in green gram (48) and pigeon pea (49).

In the present study, the increase of quantitative characters including the number of fruit cluster per plant, the number of pods per plant, pod length and hundred seed weight in second and third generations of the mutagenic population, whereas in the number of seeds per pod decreased in M_2 generations but increased in M_3 generation of cowpea. The present results depict the significant development of yield-related characters in second and third generations. These indicate that the different doses of gamma irradiation might alter the different quantitative

characters. Similar results were found in urdbean (50) and cowpea (51). A lower dose of gamma radiation may induce yield characters by stimulating plant hormone signalling and cell components. The increasing number of seeds per pod was noted at a lower mutagen dose; similar results were observed in grass pea (52) and cowpea (53). It is noteworthy that the increasing mean values of quantitative traits are due to the incidence of polygenic mutation with cumulative effects (54). After the mutagenic treatment, the mean values changed in legumes such as urdbean (55), mungbean (56–58), lentil (59) and sesame (60).

In M_2 generations, the seed yield per plant was increased at 200 Gy, whereas in M_3 generation, it was observed in both doses of 200 Gy and 400 Gy. Early flowering and high yield characters were observed in the M_3 generation of *Lathyrus sativus* (61) under radiation treatment. A positive value in seed yield in chickpea by gamma irradiation at 600 Gy (47). Among the doses, 400 Gy induced beneficial traits especially high seed yield compared to other doses and control plants. In the present study, the lower dose of gamma irradiation-induced stimulation growth in quantitative traits due to enhanced signalling networks. Seed yield is an important trait in breeding programmes to develop better varieties. Similar findings reported that diverge genetic resources are essential for plant breeding programmes focused on developing new cultivars with desirable characters (62).

Induced mutagenesis has been effectively serviced to improve productivity and generate variability in morphological and physiological characters by inducing new plant genotypes (63, 64). In the present investigation, the M_3 population was more superior in all traits than the M_2 population due to mutation-induced at the genomic level. Gamma irradiation changes the morphological traits such as pod colour with leaflet changes, early maturity with dwarf plant and pigmented pods at 196 and 245 Gy of cowpea (22). These changes or variations can occur in plants due to irradiation treatment and are also caused by the environment.

Results herein present that the eight SCoT primers were tested for the genetic variability of three generations. Among the eight primers, seven primers were amplified and showed reproducible results. The percentage of polymorphic profiles in each primer range from 3.03–96.07%, with an average of 67.10%. The present study indicates that SCoT primers induced the appearance and disappearance of bands in three generations. Similar results findings reported that changes of DNA like single or double strands break, modified nucleotide bases, oxidized bases and bulky adducts by gamma irradiation (65). SCoT which obviously reveal the how genetic content has modified in the doses of gamma ray treated populations. Hence, gamma-ray has proved a potential mutagen for improving cowpea with desired traits based on the selection of agronomic characters. The present results are supported by earlier reports (66), that the

polymorphic variations were obtained in cowpea by start codon target markers.

On the other hand, the SSR and RAPD markers showed the genetic relationship with cowpea breeding lines and wild varieties in Senegal (67). RAPD and ISSR study revealed that gamma irradiation might induce the appearance of new bands and as well as the absence in mutants than control samples of strawflower at two generations (68). The influence of gamma irradiation on corn, soybean, wheat and their result showed that the long strands of DNA broken were into small strands at a low dose (69). In contrast, short and long DNA strands are damaged at a higher dose of gamma irradiation. Similarly, the RAPD analysis showed the presence and absence of new bands in M_1 and M_2 generation of fenugreek tested with varied gamma irradiation doses (70).

Conclusion

A low range of gamma irradiation changed the growth and yield traits of cowpea. The M_1 generation showed that the morphological and quantitative traits proportionally decreased with increasing concentrations of gamma radiation. Whereas in M_2 and M_3 generations showed increased quantitative traits at 200 Gy and 400 Gy and maximum variability was observed in these two doses, and it may be recommended for the beneficial traits. However, in past years, there was no much attention to improve this variety, hence, we take into consideration to induced mutation for improving the traits. The genetic study and information of this crop is poor due to we attempt to find the genetic variability by SCoT marker which one of the robust methods for assessing genetic variation. SCoT marker is a simple technique to analyse the genetic variability induced by gamma radiation. This investigation gives acceptable proof that the SCoT markers are a valuable tool to identify the genetic variation in the three generations of cowpea. The presence of a new band observed in treated samples and showed the novel mutation might occur effects of gamma irradiation. This variability may change the growth and yield traits, leads to the improvement of the crop. Hence, gamma irradiation plays a vital role in crop breeding, in which 200 Gy or 400 Gy doses of gamma rays were optimum doses to obtain the desired characters (high yield traits) for the next successive generations.

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Authors' contributions

Designing of Experiments (DAB); Fieldwork and data collection (SV and VS); Laboratory experiment, analysis of data, interpretation and statistical

analysis (DAB and SV); Preparation of manuscript (DAB and SV).

Conflict of interests

The authors declare no conflict of interest.

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