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Effect of Long Period Cooling Storage on the Nucleic Acid of Harvested Cowpea Seeds (*Vigna Sinensis* L.) Treated by Gamma Irradiation and Micro Elements

O.I. Saleh and I.M. Salama

Natural Products Research Department, National Center for Radiation Research and Technology (NCRRT), Atomic energy authority, Cairo, Egypt.

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ABSTRACT

Cowpea seeds (*Vigna sinensis* L.) were exposed to 40 & 80 Gy gamma radiation, in order to study the effect of long period under cooling storage by using RAPD and ISSR PCR facilities.

The obtained results indicated that RAPD protocol gave 65% monomorphic and 56% polymorphic fragments between the samples as compared to storage and non-storage controls. While, ISSR protocol gave 83% monomorphic and 85% polymorphic fragments. It should be mentioned that other percentages 86% and 91% were found among samples in case of using another primers.

The results could be summarized as follow:

1- Primer OP-B01 gave 7 monomorphic and 13 polymorphic fragments (65%).

2- The Primer OP-B02 and Primer OP-B05 gave 4 monomorphic fragments with 14 polymorphic fragments (79%).

3- The Primer HA-98 gave 4 monomorphic fragments with 19 detected polymorphic 83%.

4- The Primer HA-99 and HB-12 gave 3 monomorphic fragments and 17 polymorphic 85 and 86%, respectively.

5- The Primer HB-13 gave 2 monomorphic fragments with 21 detected polymorphic fragments 91%.

6- The primer HA-98 gave 83% while the primer HA-99 gave 85%.

The previous results showed some polymorphism differences among the samples, while the primer HB-12 gave 86% and the primer HB-13 91% exhibited high levels of polymorphism. The DNA of stored cowpea seeds which were exposed to 80 Gy in the presence of zinc showed the highest differentiation, while radiation dose 40 Gy treated with zinc or boron, 80 Gy with boron and 40 or 80 Gy treatment alone compared to the two controls (storage and non storage).

Key words: Cowpea, Vigna sinensis, γ-rays, nucleic acids, DNA.

INTRODUCTION

Genetic changes controlled by environmental factors and exposing to physical and chemical factors. The effect of γ-ray and variation in the media surrounding plants has an influence on the genetic material. The present study deals with the variation in cowpea seeds under cooling and variation in the presence of micro elements. The cowpea seeds are important food for human beings and animals allover the world [(1)]. Two cowpea cultivates were exposed to three mutagens; sodium azide (NaN_3), ethyl methane sulphonate (EMS) and gamma rays showing differences in number and intensity of bands [(2)], as well as generating non-transmissible mutations via complicated chromosomal alterations at high dose levels [(3)]. Gamma radiation is often used to develop varieties that are agriculturally and economically important and have high productivity potential [(4)]. However, low doses of gamma irradiation have been used for mutant isolation in conventional plant breeding [(5)]. Many mutant crop varieties including plants having resistance to diseases, cold, salt and plants with desired qualities have been developed by using gamma rays [(6)].

Authors Emails: omarslh3@gmail.com, genetech49@yahoo.com

Changes in seed storage protein markers as revealed by electrophoresis have been successfully used to resolve and characterize species and varieties in several legumes such as *Vigna* [7] and *Lupinus*[8].

The random amplified polymorphic DNA (RAPD) by arbitrary primers was reported by [9]. Markers of RAPD have been widely used for the identification of genetic relationships among cultivars [10] and inter-simple sequence repeats (ISSRs) which reveal regions that lay within the microsatellite repeats [11]. Also, **Ba *et al.*,** [12] used RAPD to characterize genetic variation in domesticated cowpea and its wild progenitor, as well as their relationships. **Fana *et al.*,** [13] observed (using RAPD analysis) the genetic characterization variation in domesticated cowpea and its wild progenitor, as well as their relationships. A total of 28 primers generated 202 RAPD bands. 108 bands were polymorphic among the domesticated compared to 181 among wild/ weedy cowpea accessions. Although the variabilty of domesticated cowpea was the highest ever recorded, cultivar-groups were poorly resolved with a larger number of markers, RAPD data confirmed the single domestication hypothesis, between wild and domesticated cowpea. **Diouf and Hilu** [14] applied RAPD and ISSR markers to determine the genetic diversity among cowpea breeding lines and local varieties in Senegal. **Feleke, *et al.*** [15] mentioned a very important mutation for studying cowpea evolution and domestication. A loss of a BamHI restriction site in chloroplast DNA characterized all domesticated accessions and a few wild (*Vigna unguiculata* ssp. *unguiculata* var. *spontanea*) accessions by using PCR RFLP (Restriction Fragment Length Polymorphism) or direct PCR methods. RAPD markers were also used to evaluate the genetic diversity in a representative population of cowpea from different eco-geographical regions in India [16].

The genotype is the genetic structure of organism which represents a composition of effects on DNA molecules [17]. The mutation could be explained as spontaneous heritable changes in DNA sequences that induce changes in gene structure, which happened during cell replication and / or due to exposure to mutagenesis or radiation leading to extreme phenotype change. This resulted in a reduction in genetic information. However, beneficial mutation could be occurred. Moreover, accumulation of mutation represents effective stress factor as mutation load.

Heba [18] reported that toxic element and γ-rays induced changes in the genomic pattern of DNA represented in appearance or disappearance of DNA morphic bands. The radiation levels were found to cause an increment in the DNA break.

Cowpea is considered a main crop in Egypt which has more than one genotype. Changes in DNA structure will be estimated in both storage and non-storage control. Results indicated that among samples, about 65% was monomorphic and 95% polymorphic.

The genotype is the genetic structure of an organism which represents a combination of alleles on RNA molecule [19]. The organism development depends on both genotype and its environment for genotype except in twins developed from one egg.

Mutation could be explained as spontaneous heritable changes in DNA sequences that induce changes in gene structure. This could result during cell replication and or due to exposure to mutagenesis. Mutation can induce extreme phenotype changes which lead to reduction in genetic information and less genetic information. However, beneficial mutation could be occurred, at the same time accumulation of mutations represents stress factor as mutation load on morphological criteria and random amplification of polymorphic DNA. Polymerase chain reaction of investigated seeds can affect the morphological criteria and cause random amplification of polymorphic DNA. Polymerase chain reaction RAPD PCR of investigated seeds using 4 primers indicated that the appearance of DNA polymerase at extremely low dose and fewer concentrations of elements induced more changes in genomic DNA pattern of cowpea seeds.

The current work aims to study the effect of long period storage under cooling on the differences of DNA among samples of harvested cowpea seeds, as a result of seeds treated by gamma irradiation doses (40 & 80 Gy) and micro elements (Boron & Zinc).

MATERIALS AND METHODS

A cowpea cultivar (*Vigna sinensis* L.), Kafr El Sheikh was obtained from the Horticulture Research Institute, Agriculture Research Center, Dokki, Giza. The cowpea seeds were irradiated at dose levels of (40 and 80 Gy) at the National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt. Irradiation facility used was Indian Gamma Cell Research Irradiator (Co^{60}); the dose rate was 1.8 Gy/ sec. At time of experiment, it was carried out in a wire house at (NCRRT). The soil used for cultivation was clay- loamy soil with pH 7.7. Irradiated and un-irradiated seeds were planted at depth of a 2-5cm and a space 30 Cm, and four replicates from each treatment in complete randomized blocks. After one month from planting date, plants were sprayed by microelements, of zinc (Z) [300 ppm / L] and boron (B) [50 ppm / L], monthly till harvest date in May 2006.

The treated cowpea seeds samples, from control, 40 & 80 Gy, symbolled as follows; Fresh control (A), storage control (a), 40 Gy (b), 80 Gy (c), 40 Gy sprayed by Boron micro element (bB), 80 Gy sprayed by Boron micro element (cB) (B = 50 ppm) and 40 Gy sprayed by Zinc micro element (bZ), 80 Gy sprayed by Zinc micro element (cZ) (Z = 300 ppm). Samples were collected and stored for 8 years under cooling at 7 ˚C, then the DNA collected from cowpea seeds from all treatment and determination by using RAPD and ISSR PCR protocols.

Analyses

a- DNA Isolation

10 g of seeds powder from collected samples of cowpea, ***Vigna sinensis* L.** DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN).

b- Randomly Amplified Polymorphic DNA (RAPD) Procedure

For RAPD finger-printing as outlined by Williams et al [9]. RAPD was used for the identification of markers associated with 8 samples; two controls (fresh & stored) and six treatments (irradiation & micro elements) of *V. sinensis*. PCR reactions were conducted using 4 arbitrary 10-mer primers. Their names and sequences are shown in Table (1).

Table (1): List of primer names and their nucleotide sequences used (RAPD).

Primer codes	Sequences (5'→3')	GC %
OP-B01	5' – GTTTCGCTCC-3'	60%
OP-B02	5' – TGATCCCTGG-3'	60%
OP-B05	5' – TGCGCCCTTC-3'	70%
OP-B07	5' – GGTGACGCAG-3'	70%

RAPD analysis was performed with 4 primers (Table 1) using the Ready-To-GoTM RAPD Analysis Beads kit (Amersham Pharmacia Biotech, Uppsala, Sweden), following the protocol recommended by the manufacturers. The reactions were performed in a final volume of 20 µl, using 6.4 pmol of each primer and 1 ng of template DNA, overlaid with 20 µl mineral oil. Amplification was programmed for 45 cycles in 1 min at 94 ˚C, 1 min at 36 ˚C and 2 min at 72 ˚C on a thermal cycler (PTC-100TM, MJ Research Inc.), for DNA denaturing, annealing and primer extension, respectively, the extension step was extended to 5 min in the final cycle.

c- Inter Simple Sequence Repeat (ISSR)

PCR reactions were conducted using 4 arbitrary 14 and 11-mer primers. Their names and sequences are shown in Table (2).

A set of four ISSR primers were used for screening in this study (Table 2) for 11 samples. PCR amplification was performed in 25 µl reaction volume (100 ng template DNA, 0.8 mM ISSR primers, 1 U Taq DNA polymerase, 0.4 mM dNTPs, 1× PCR buffer, and 1 mM $MgCl_2$).

Table (2): List of primer names and their nucleotide sequences used (ISSR).

Primer codes	Sequences (5'→3')	GC %
HA – 98	5' CACACACACACAGT 3'	50%
HA – 99	5' CACACACACACAAG 3'	50%
HB – 12	5' CACCACCACGC 3'	72%
HB – 13	5' GAGGAGGAGGC 3'	72%

The PCR program was as follows: the initial denaturation at 94 ˚C for 5 min; followed by 35 cycles at 94 ˚C for 1 min, 40-50 ˚C (vary with primers) for 1 min and 72 ˚C for 1 min; and a final extension at 72 ˚C for 15 min. PCR-amplified fragments were separated by electrophoresis on 2% (w/v) agarose gel in 0.5× TBE buffer, visualized by ethidium bromide staining and photographed under UV light.

d- Data Analysis

The genetic similarity among cowpea was calculated using Jaccard genetic similarity coefficient and the dendrogram was constructed using Unweighted Pair Group Method with Total lab Quant program.

The Lab image program version 2.7 produced by [20] Bio-Imaging GmbH was used for DNA size determination. The presence or absence of protein, RAPD and ISSR bands was scored as 1 for presence or 0 for absence of markers respectively for estimating genetic variation. Euclidian distance [21] was calculated and used for measuring the similarity between the parent varieties and the M2 genotypes using the software program, Community Analysis Package 4.0 (CAP). The CAP software was used for to clustering the M2 genotypes based on distance estimates using the agglomerative clustering analysis and the average linkage tree building method.

RESULTS AND DISCUSSION

1- Random Amplified Polymorphic DNA (RAPD)

Data of the amplified fragments used 10-mer arbitrary primers for eight samples; two controls (fresh & stored) and six treatments (irradiation & micro elements) of *V. sinensis* L. with four RAPD primers successful amplification of PCR products have been observed. Polymorphism levels differed from one to another primer. The main results were as recorded in Tables (3 & 4).

Table (3): RAPD markers of *V. sinensis* with 4 RAPD primers.

Treatments		Primers				TAF
		OP-B01	OP-B02	OP-B05	OP-B07	
Lan 2 (A) Fresh control	AF	9	5	7	12	33
	SM	0	0	0	0	0
Lan 3 (a) Storage control	AF	10	9	10	14	43
	SM	0	0	0	0	0
Lan 4 (b) 40 Gy	AF	5	8	9	13	35
	SM	0	0	0	0	0
Lan 7 (c) 80 Gy	AF	9	8	6	9	32
	SM	0	0	0	0	0
Lan 5 (bB) 40 Gy B	AF	9	9	12	12	42
	SM	0	0	0	1	1
Lan 8 (cB) 80 Gy B	AF	9	8	7	7	31
	SM	1	0	0	0	1
Lan 6 (bZ) 40 Gy Z	AF	7	11	7	13	38
	SM	0	0	0	0	0
Lan 9 (cZ) 80 Gy Z	AF	7	10	8	13	38
	SM	0	2	0	0	2

TAF: total amplified fragment AF: amplified fragment SM: specific marker

Results of randomly amplified polymorphic DNA (RAPD) protocol of the *V. sinensis* L. eight samples with 4 primers used, showed one specific marker; in the samples of 40 Gy and sprayed by Boron micro element (bB) at primer OP-B07 had polymorphic fragments (91%) at band number 4, with molecular size (758 bp) . The samples of 80 Gy sprayed by Boron micro element (cB) at primer OP-B01 had polymorphic fragments (65%) at band number 20, with molecular size (111 bp). While two specific marker in sample of 80 Gy and sprayed by Zinc micro element (cZ) at primer OP-B02 had polymorphic fragments (79%) at band numbers 2 and 4; with molecular sizes (1023 and 881 bp, respectively). The quantification of total DNA showed significant variation among seeds treated with gamma-irradiation, depending on the absorbed dose.

Table (4): Number and type of the amplified DNA bands as well as the percentage of the total polymorphism generated by four primers RAPD among treatments of *V. sinensis* seeds.

Primer code	Treatment numbers	Monomorphic bands	Polymorphic band		Total bands	Polymorphism %
			Shared bands	Unique bands		
OP-B01	1, 2,3,4,5,7 and 8	7	13	-	20	65.0 %
	6	7	12	1	20	
OP-B02	1, 2,3,4,5,6 and 7	4	15	-	19	78.9 %
	8	4	13	2	19	
OP-B05	1, 2,3,4,5, 6,7 and 8	4	14	-	18	77.7 %
OP-B07	1, 2,4,5, 6,7 and 8	2	20	-	22	90.9 %
	3	2	19	1	22	
Total		30	107	4	140	79.3 %

[1= Fresh control (A)], [2= Storage control (a)], [3= 40 Gy (b)], [4= 80 Gy (c)], [5= 40 Gy B (bB)], [6=80 Gy B (cB)], [7= 40 Gy Zn (bZ)] and [8= 80 Gy Zn (cZ)].

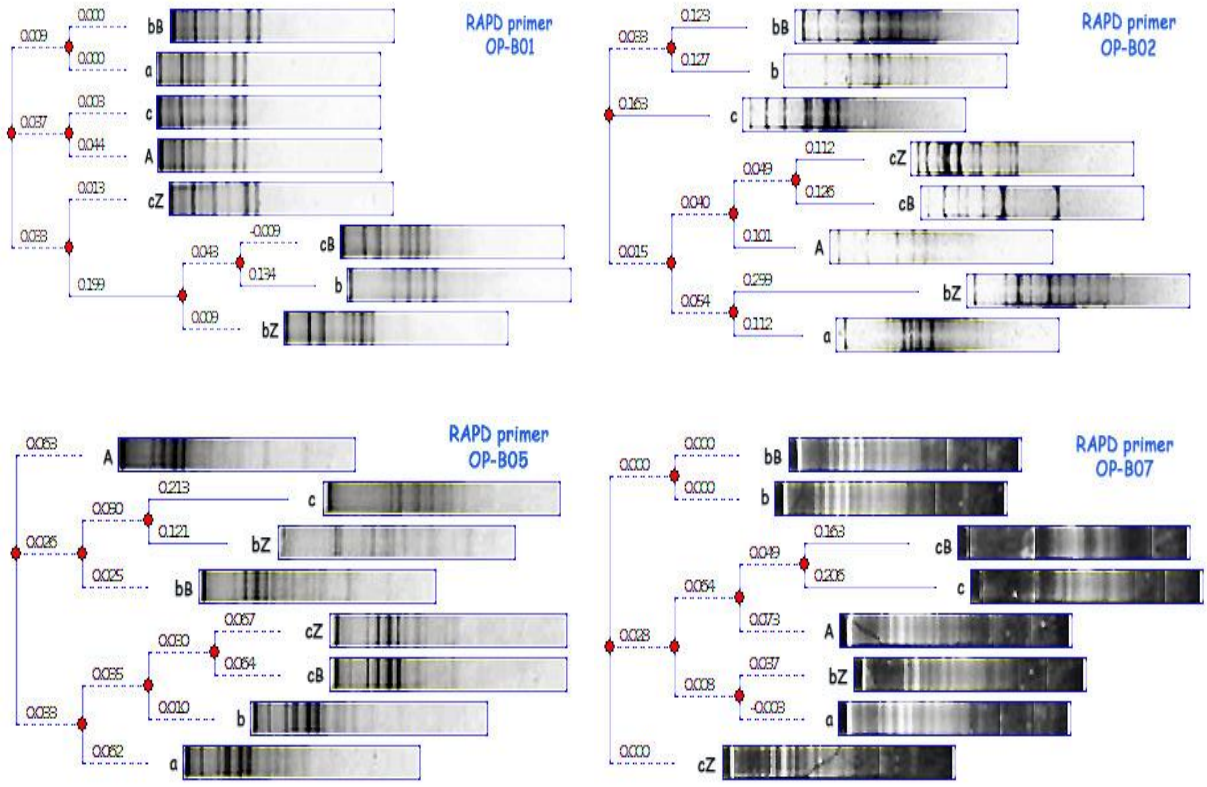


Fig. (1): Dendrogram of DNA polymorphism using RAPD amplified with 4 primers.

Primer OP-B01 gave 20 band number (7) monomorphic fragments (35%), band numbers (1, 9, 10, 13, 15, 16 and 17) with molecular sizes at 1118, 404, 331, 242, 190, 162 and 147 bp; and the other, 13 detected polymorphic fragments (65%) with molecular sizes of 1016, 980, 881, 730, 564, 501, 489, 309, 274, 216, 132, 129 and 111 bp were observed in Figure 1. The sample irradiated with 80 Gy exhibited band number 9 of fragments. Samples that had the same effects were (A, bB and cB) and least of them in the band number 7 of samples (bZ and cZ). While the lowest band number 5 appeared in the sample (b) treated by 40 Gy, the maximum band (10) appeared in the stored control sample (a). In this primer the dendogram showed 3 groups; the first (bB and a), the second (c and A) and the third (cZ, cB, b and bZ).

Primer OP-B02 gave 4 monomorphic fragments (21%) at band numbers 1, 11, 18 and 19 with corresponding 1118, 331, 67 and 34 bp. While 15 detected polymorphic fragments (79%) with, molecular sizes range from 1023 to 404 bp and from 286 to 110 bp as in Figure 1. The samples were treated with 40 Gy and spread by zinc micro elements (bZ) exhibited the maximum band number (11) of fragments, while the lowest band number (5) appeared in the samples of fresh control. In this primer the dendogram showed 3 groups; the first (bB and b), the second (c) only and the third (cZ, cB, A, bZ and a).

Primer OP-B05 gave 4 monomorphic fragments (22%) with molecular sizes 1118, 242, 67 and 34 bp. While 14 detected polymorphic fragments (78%) with molecular size range from 989 to 277 bp and from 190 to 92 bp were observed in Figuer 1. The samples had treated by 40 Gy and spared by Boron micro element (bB) exhibited the maximum band number (12) of fragments, while the lowest band number (6) of fragments appeared in 80 Gy (c). On the other hand, all the samples [fresh control (A), treated with 80 Gy and sprayed by Boron micro elements (cB) and 40 Gy and sprayed by Zinc micro element (bZ) appeared the same band number (7) of fragments. In this primer the dendogram showed 3 groups; the first (A) only, the second [(c, bZ) and (bB)] and the third (cZ, cB, b, and a).

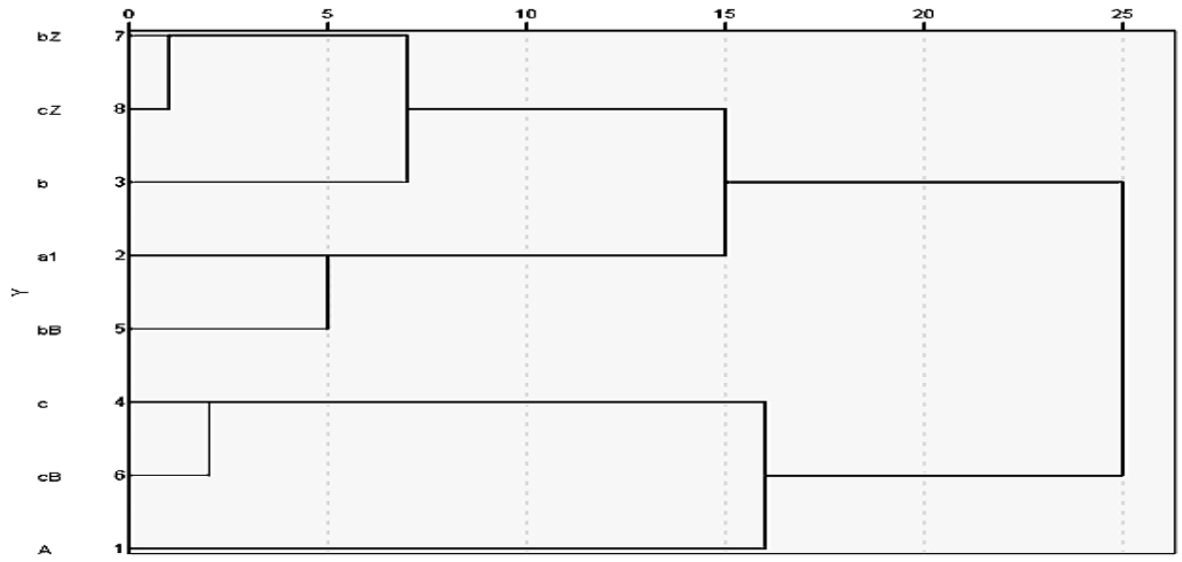


Fig.(2) Agglomerative clustering dendrogram of the genetic of the four primers among control and treatments of *V. sinensis* based on similarity index data of RAPD analysis.

Primer OP-B07 gave 2 monomorphic fragments (9%) with molecular sizes; 1118 and 34 bp. While, 20 detected polymorphic fragments (91%) with molecular sizes ranging from 823 to 44 bp were observed in Figure 1. The samples of storage control (a) exhibited the maximum band number (14) fragments. On the other hand, the lowest band number (7) appeared in the samples treated with 80 Gy and sprayed by Boron micro elements (cB). In this primer the dendogram showed 3 groups; the first (bB and b), the second (cB, c, A, bZ and a) and the third (cZ) only.

Only one primer OP-B07 (91%) exhibited high polymorphism differences among the samples, while other primers exhibited polymorphism; eg. OP-B01, OP-B5 and OP- B02 and their percentages 65, 78 and 79% respectively.

Genetic Similarity and Cluster Analysis Based on RAPDs Markers

RAPD data were used to estimate the genetic similarity among 8 samples; two controls and treatments of irradiation & micro elements of *V. sinensis*. The highest similarity index was observed between two taxa (A and c) while the lowest similarity index was observed between (cZ and bZ). A dendrogram for the genetic relationships among aforementioned 8 samples taxa were carried out as shown in Figure (2). The 8 samples were separated into three clusters; cluster one included (b, cZ and bZ). While cluster two included (bB and a). Cluster three included (A, cB and c).

Laity *et al.*, [22] studied the Genetic diversity in cowpea, by using RAPD techniques with 44 primers were screened with DNA samples from seven cowpea varieties, and found that, 10 primers did not amplify the DNA; 9 amplified, but did not show any discrimination, while 25 gave polymorphic RAPD patterns. 11 of the 25 polymorphic primers have given clearly bands. In total, 331 bands (amplification products) were produced by the PCR reactions. 61 of the bands were polymorphic. **Munir, *et al.*** [23] studied different doses of gamma irradiation (5 to 10 Gy) as well as chemical mutagenesis by sodium azide on grape crop. The RAPD analyses indicated that the plantlets subjected to gamma radiation had a great genetic diversity as compared to the control.

2- Inter Simple Sequence Repeat (ISSR)

ISSR markers of *V. sinensis* samples of with 4 ISSR primers are shown in Tables (5 & 6). Data of amplified fragments using the aforementioned specific primers for the 8 samples indicated successful amplification of PCR products. Polymorphism levels differed from one primer to another. The main results are given in Tables (5 & 6).

Inter Simple Sequence Repeat (ISSR) protocol of 8 samples (*V. sinensis*) with 4 primers used in this study, reviled that six specific markers in samples fresh control and 80 Gy sprayed with Zinc micro element (cZ) at primer HB-12 that gave Primer HA-98 gave 4 monomorphic fragments (17%) with molecular sizes 1118, 111, 67 and 34 bp polymorphic fragments (90%) at band numbers ranging from 1 to 10 with corresponding molecular sizes ranging from 1118 to 385 bp, also ranging band numbers from 12 to 19 with corresponding molecular sizes ranging from 242 to 88 bp.

Table (5): ISSR markers of *V. sinensis* samples with 4 ISSR primers.

Treatments		Primers				TAF
		HA-98	HA-99	HB-12	HB-13	
Lan 2 (A) Fresh control	AF	11	5	7	10	33
	SM	1	3	4	3	11
Lan 3 (a) Storage control	AF	13	7	8	7	31
	SM	1	0	0	1	2
Lan 4 (b) 40 Gy	AF	7	8	8	11	34
	SM	0	1	0	0	1
Lan 7 (c) 80 Gy	AF	7	8	9	9	33
	SM	0	0	0	1	1
Lan 5 (bB) 40 Gy B	AF	9	8	8	8	33
	SM	0	0	0	0	0
Lan 8 (cB) 80 Gy B	AF	7	9	9	9	34
	SM	1	0	0	0	1
Lan 6 (bZ) 40 Gy Z	AF	10	9	9	10	38
	SM	0	0	0	1	1
Lan 9 (cZ) 80 Gy Z	AF	8	6	6	7	27
	SM	0	0	2	0	2

TAF: total amplified fragment AF: amplified fragment SM: specific marker

The samples of 80 Gy (c), 80 Gy & sprayed by Boron micro elements (cB) and 40 Gy & sprayed by Zinc micro elements (bZ) exhibited the maximum band number (9) of fragments, while the lowest band number (6) appeared in the samples of 80 Gy & sprayed by Zinc micro

elements (cZ). At this primer, the dendogram showed 3 groups; the first (bB) only, the second (bZ, b and a) and the third (cZ, cB, c and A).

Table (6): Number and type of the amplified DNA bands as well as the percentage of the total polymorphism generated by four primers ISSR among treatments of *V. sinensis* seeds.

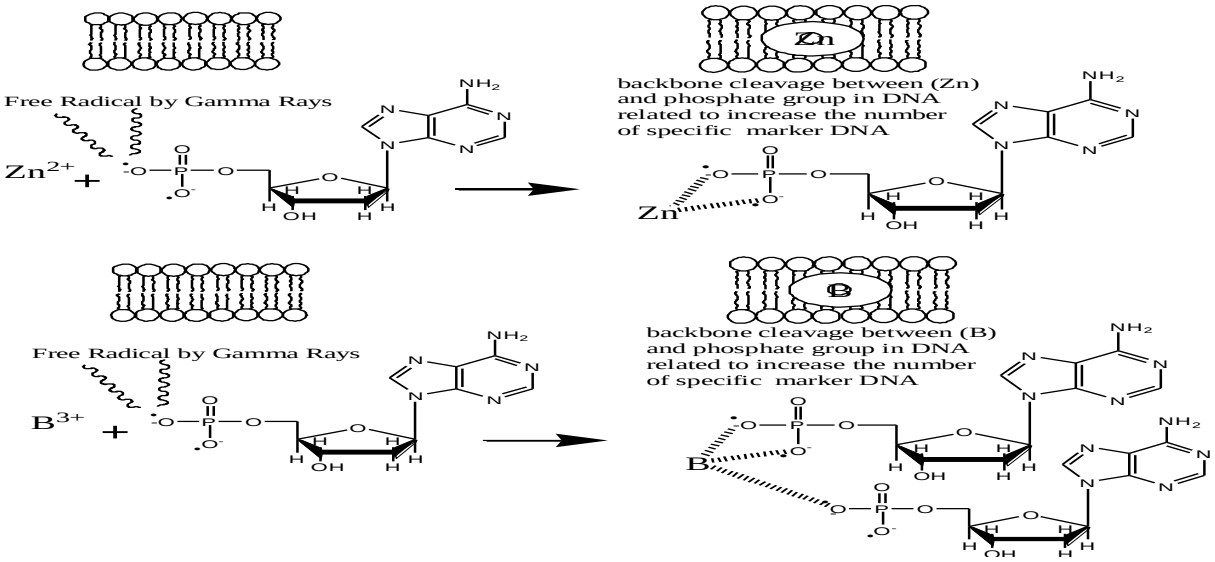
Primer code	Treatment numbers	Monomorphic bands	Polymorphic band		Total bands	Polymorphism %
			Shared bands	Unique bands		
HA-98	3,4,5,7 and 8	4	19	-	23	82.6%
	1,2 and 6	4	18	1	23	
HA-99	2,4,5,6,7 and 8	3	17	-	20	85.0%
	1	3	14	3	20	
	3	3	16	1	20	
HB-12	2,3,4,5, 6 and 7	3	18	-	21	85.7%
	1	3	14	4	21	
	8	3	16	2	21	
HB-13	3,5,6 and 8	2	21	-	23	91.3%
	1	2	18	3	23	
	2,4 and 7	2	20	1	23	
Total		32	191	15	238	86.5%

[1= Fresh control (A)], [2= Storage control (a)], [3= 40 Gy (b)], [4= 80 Gy (c)], [5= 40 Gy B (bB)], [6=80 Gy B (cB)], [7= 40 Gy Zn (bZ)] and [8= 80 Gy Zn (cZ)].

Primer HB-13 gave 2 monomorphic fragments (9%) with molecular sizes 1118 and 34 bp with 21 detected polymorphic fragments (91%) with band numbers ranging from 2 to 22 with corresponding molecular sizes ranging from 881 to 67 bp were observed in Figure 3. The samples treated with 40 Gy (b) exhibited the maximum band number (11) of fragments, while the lowest band number (7) appeared in the samples of storage control (a) and 80 Gy & speared by Zinc micro elements (cZ). At this primer the dendogram showed 3 groups; the first (a) only, the second (c, b, a, bZ and bB) and the third (cZ and cB).

While three specific markers, in samples [fresh control (A), storage control (c) and 80 Gy & sprayed with Boron micro elements (cB)] at primer HA-98 that gave polymorphic fragments (83%) with band numbers ranging from 2 to 17 with corresponding molecular sizes ranging from 1050 to 123 bp. The molecular sizes are: 110, 86 and 56 bp at band numbers 19, 20 and 22, respectively. Four specific markers; in samples of fresh control (A) and 40 Gy (b) at primer HA-99 that gave polymorphic fragments (85%) at band numbers; 6, 11 and 12 with corresponding molecular sizes; 768, 404 and 257 bp and band number 18 with corresponding molecular size 88 bp.

Also, Tables (5 & 6), illustrated an increase in specific marker (SM) for samples sprayed with micro-elements (Boron & Zinc) and irradiated by gamma irradiation [(bB), (cB), (bZ) and (cZ)]. This increase is probably due to backbone cleavage to kation (+) of boron and zinc with phosphate group in DNA affected by free radical from gamma rays, which generates new fragmentations from DNA associated with boron and zinc ions, this leads to an increase in the number of specific marker (SM), compared to samples that have been actively irradiated by gamma doses only, except for control. This conclusion was mentioned by Piekarczyk et al [24].



Schiedam: Screening of micro-elements (Boron & Zinc) and irradiated by gamma irradiation are related to backbone cleavage between kation (+) of boron & zinc and phosphate group in DNA, which labeled and related to increase specific marker DNA.

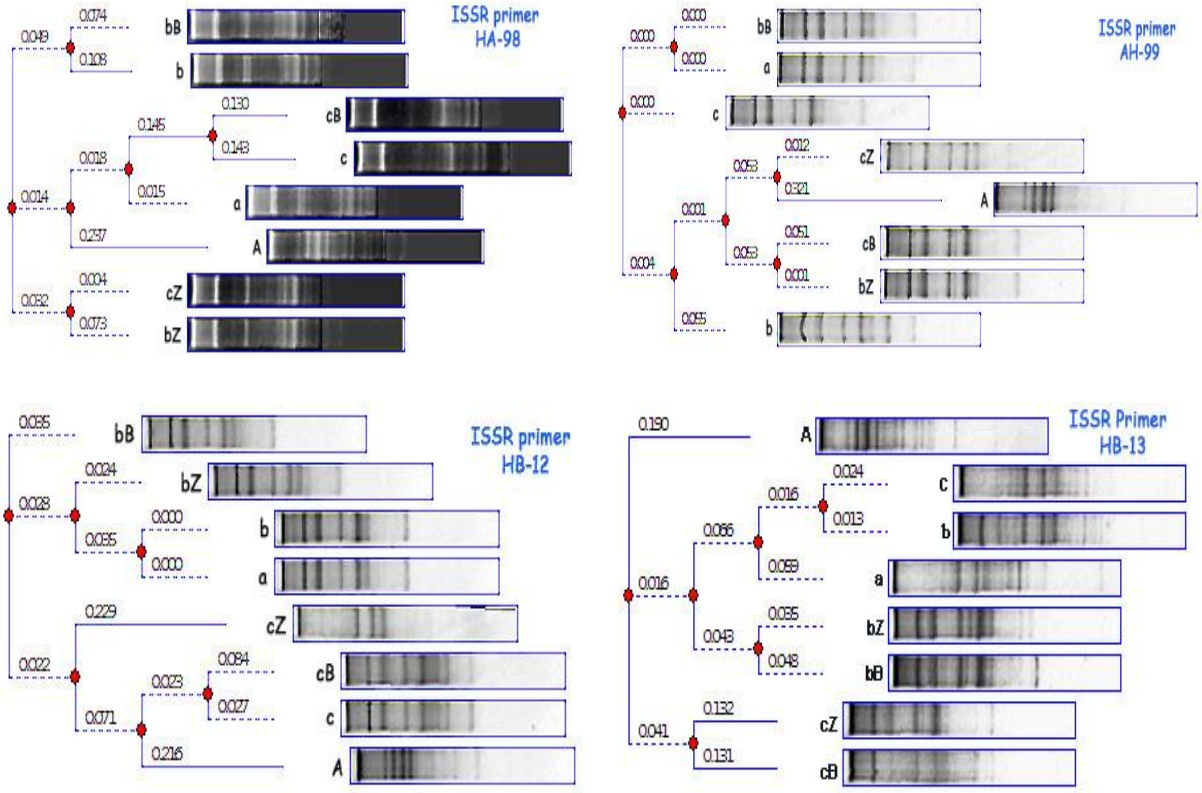


Fig. (3): Agglomerative clustering dendrogram of the genetic of the four primers among *V. sinensis* samples based on similarity index data of ISSR analysis.

Primer HA-98 gave 4 monomorphic fragments (17%) with molecular sizes 1118, 111, 67 and 34 bp and with 19 detected polymorphic fragments (83%), with band numbers ranging from 2 to 17 with corresponding molecular sizes ranging from 1050 to 123 bp were observed in Figure 3; also with molecular size 110, 86 and 56 bp at band numbers 19, 20 and 22. The samples were storage control (a) exhibited the maximum band number (13) of fragments, while the lowest band number (7) appeared in

the samples that were treated with [40 Gy (b), 80 Gy (c) and 80 Gy & sprayed by Boron micro elements (cB)].

Figure (3) showed that 3 groups apperd in the dndogram; the first (bB and b), the second (cB, c, a and A) and the third (cZ and bZ).

Primer HA-99 gave 3 monomorphic fragments (15%) with molecular sizes 1118, 489 and 34 bp and detected 17 polymorphic fragments (85%) with numbers ranging from 2 to 9 with corresponding molecular sizes ranging from 989 to 491 bp were observed in Figure 3. In addition to this, from 11 to 19 with corresponding molecular sizes ranging from 404 to 58 bp. The samples were treated [80 Gy & spared by Boron micro element (cB) and 40 Gy & sprayed by Zinc micro elements (bZ)] exhibited the maximum band number (9) of fragments, while the lowest band number (5) appeared in the samples of fresh control (A). In this primer the dendogram showed 3 groups; the first (bB and a), the second (c) only and the third (cZ, A, cB, bZ and b).

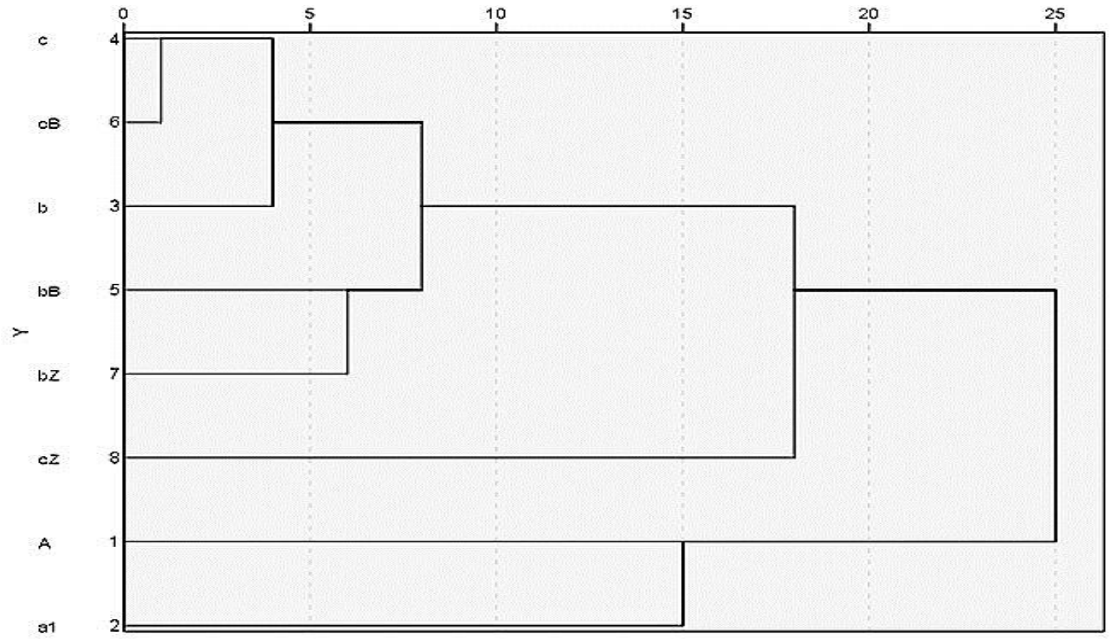


Fig. (4): Agglomerative clustering dendrogram of the genetic four primers among *V.sinensis* treatments based on similarity index data of ISSR analysis.

Genetic Similarity and Cluster Analysis Based on ISSR Markers

The ISSR data were used to estimate the genetic similarity among 8 samples of *V. sinensis*. The highest similarity index was observed between the two taxa (A and a), while the lowest similarity index was observed between (c and cB).

A dendrogram of genetic relationships among the 8 samples; two controls and treatments of irradiation & micro elements of *V. sinensis* taxa was carried out in Figure (4). The 8 samples were separated into three clusters; cluster one included (bZ, bB, b, cB and c), while cluster two included (cZ) and cluster three included (A and a).

Abdelfattah *et al.*, [25] exposed five cowpea seed varieties to different doses of gamma radiation at50, 100, 200 and 300Gy. Variation in seed protein electrophoretic pattern, RAPD and ISSR fingerprinting was scored to assess genetic variation among the M2 genotypes. The gamma dose of 50 Gy resulted in an increase of growth parameters and enhanced yield components in most of varieties. Gamma radiation induced more genetic variation in the genotypes of var. Kaha 1 and var. Dokki 331 compared to other varieties as estimated by the cluster analysis of seed protein, RAPD and ISSR markers.

The result is in agreement with that of McMurray et al [26] who confirmed that the large molecule of DNA is an easy target for ionizing radiation. Hence, the changes in DNA considered a detection method for irradiated food. In contrast, to the observed effects of gamma irradiation on the protein content of seeds. Also, similar decreases in DNA content, as a consequence of gamma irradiation, have been documented in pea seeds [27].

The present study showed that RAPD markers were effective in the detection of polymorphism in cowpea seeds. Pasquet [28] mentioned that total genetic diversity in var. *unguiculata* was much higher than those obtained with isozymes, from 0.018 to 0.061. Diversity levels in domesticated cowpea were similar in West Africa (H s 0.107) and north-eastern Africa (H s 0.109) [29].

At the molecular level, new protein bands appeared in the seed protein while other bands disappeared after exposure to the γ-radiation; these changes may be due to denaturation of protein or to protein association or deamination [30]. The appearance and disappearance of protein band may refer to environmental stresses that affect causes changes in gene expression [31]. On the other hand, **Kiong *et al.*** [32] reported no significant change in protein constituents after γ-irradiation. For most prominent mechanism, the production of different proteins entails a vast array of DNA binding proteins that act in various combinations to either activate or repress gene expression [33]. The RAPD markers provided some markers that are potentially useful in studies of genetic diversity and breeding of cowpea through mutations using the γ-radiation. In this respect, **Diouf and Hilu** [14] studied the potential application of RAPD and ISSR techniques in determining genetic diversity among cowpea breeding lines and local varieties in Senegal. Among the 61 RAPD primers used, 12 showed polymorphism; a much lower proportion compared to the proportion of RAPD polymorphism found in the 22 M2 genotypes produced by exposure to different doses of γ-radiation used. Primer 3 however, produced a number of makers that are specific for different varieties and may also be important markers for the identification of these varieties in future studies on the genetic diversity and breeding of new cowpea lines. **Yoko *et al.*** [34] studied the effect of gamma irradiation on the genomic DNA of corn, soybeans and wheat. They concluded that, large DNA strands were broken into small strands at low irradiation dose but small and large DNA strands were broken at higher irradiation doses. The RAPD method was also used by Raisheed et al [35] to detect the genetic variation induced by gamma rays. Also, **Mudibu, *et al.*** [36] indicated an increase in polymorphism after irradiation with gamma rays of soybean. The effects of ISSR finger printing might be connected to structural rearrangements in DNA caused by different types of DNA damages [37].

CONCLUSION

The study emphasized the importance of the detection of genetic changes in cowpea seeds under physical and chemical treatments. The present results indicated that the variation in the genetic material (DNA) of cowpea seeds was more pronounced in case of exposure of the seeds to 80 Gy with zinc treatment, but the changes were very low in case of boron treatment, while the changes after 40 Gy with zinc or boron treatments were less than the previous treatments. The case of exposure of the seeds to 40 or 80 Gy without mineral treatment recorded the least changes as compared with the storage and non-storage seeds.

The changes in DNA of cowpea seeds leads to presence of new phenotype and genetic characters. The obtained results could represent a further step for improving the chemical and physical trial of the seeds that may give seeds high vegetation percentage and plenty of crop harvest of healthier proteins. Important physical and chemical genetic trials of seed could be achieved. Moreover, to increase the resistance of the plants towards, salinity, drought, disease and insect infection in case of negative variation in the surrounding environmental conditions.

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