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Full Length Research Paper

Molecular characterization of groundnut (*Arachis hypogaea* L.) accessions from a gene pool: Application of gamma ray radiations

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Cultivated peanut or groundnut (*Arachis hypogaea* L.) native to South America is an important source of oil and protein. Knowledge of genetic diversity within and among varieties in a crop is prerequisite to its improvement. In the present study, the level of genetic variability among groundnut accessions from a gene pool was analyzed using inter-simple sequence repeat (ISSR) markers. The effect of gamma ray radiation on molecular variation was also determined in targeted accessions. The rate of polymorphic loci, Nei's gene diversity (h), and Shannon's index (I) among accessions were 54%, 0.188, and 0.28, respectively. The groundnut accessions were genetically closely related with the genetic distance values varying from 0.11 to 0.37. The amplitude of variation based on the level of polymorphic loci was increased by 37% with 0.10 KGy gamma-ray treatments compared to control for the JL 24 accession from Brazil. Gene diversity and Shannon index were increased by 84 and 57%, respectively, for the same treatment (0.1 KGy).

Key words: Groundnuts, *Arachis hypogaea* L., inter-simple sequence repeat (ISSR), genetic diversity, gamma ray radiations, gene pool, DR-Congo.

INTRODUCTION

The cultivated peanut or groundnut (*Arachis hypogaea* L.), which is the world's third most important grain legume crop, originated in South America and is now grown throughout the tropical and warm temperate regions of the world. Groundnut is an allotetraploid (2n = 4x = 40), belonging to the genus *Arachis* that includes 70 species (Kumazawa and Nishimura, 1953; Kraovickas and Gregory, 1994; Ferguson et al., 2004). Cultivated groundnut is divided in two subspecies: *Arachis hypogaea* spp. *Hypogaea* and *Arachis hypogaea* ssp. *Fastigiata* Waldr., which are further classified into fourbotanical varieties (Kumazawa and Nishimura, 1953; Kraovickas and Gregory, 1994). Groundnut productions in sub-Saharan Africa are less than half of those obtained in India and China, and a quarter of those obtained in USA. There is therefore a significant scope to improve groundnut productivity in the region by exploiting germplasm diversity.

Assessment of genetic diversity in a crop species is prerequisite to its improvement and helps to generate genetically diversified breeding populations. Considerable variation has been recorded for morphological, physiological, and agronomic traits in groundnut crop. Kumazawa and Nishimura (1953) classified peanut varieties into four

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market types (Spanish, Valencia, Virginia, and Southeast Runner). Valencia and Spanish types correspond to the subspecies *fastigiata* (Kraovickas and Gregory, 1994). Their flowers set on their main axis. Initially, there are erect, small seeded, and less branched types. Spanish types have smaller pods that contain two round seeds and Valencia is intermediate in size and shape with long pods that contain three to four seeds. Virginia and Southeast Runner correspond to subspecies *hypogaea* (Kraovickas and Gregory, 1994). They do not set seeds at their main axis and are highly branched. Southeast Runner and Virginia types have similar morphological characteristics and habitats, but Virginia has larger pods and elongated seeds while Southeast Runner has small pods.

Meanwhile, it is very difficult to classify accessions solely according to their morphological traits which can be affected by environmental conditions. DNA-based technologies are the most reliable tools for assessing genetic variability because they are not influenced by plant growing conditions. Different molecular markers have been used to study polymorphism in groundnut (Ferguson et al., 2004; Lang and Hang, 2007; Krishna et al., 2004; Milla et al., 2005; Tang et al., 2007; Naito et al. 2008). Very low to moderate levels of polymorphic variations in cultivated groundnut have been reported using Amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR) (Dwivedi et al., 2001; Raina et al., 2001; Herselman, 2003). Assessment genetic variability among groundnut accessions using inter-simple sequence repeat (ISSR) is very limited. The major advantage of the ISSR technique is higher reproducibility compared to RAPD and simplicity of its application compared to AFLP and SSR. Genetic variability can also be increased by inducing mutations with ionized radiations (Bhatnagar, 1991; Ramani and Jadon, 1991; Mudibu et al., 2011). Nevertheless, the application of gamma ray radiation in groundnut breeding has been so far very limited.

The main objective of the present study was to analyze the level of molecular variation and genetic relatedness among groundnut accessions from a gene-pool and to determine the effect of gamma radiation on genetic variability for targeted accessions.

MATERIALS AND METHODS

Genetic materials

A seed set of 40 groundnut accessions were provided by INERA Mvuazi and Gandajika research stations (DR-Congo). The sources and market types of these varieties are described in Table 1. Seeds were placed in clear polycarbonate germination boxes containing wet cellulose paper and kept in a germinator at 25°C. Ten-day-old seedlings were collected and roots and seed debris were discarded. Seedlings were weighed, frozen in liquid nitrogen, and stored at -80°C until use for DNA extraction. In total, 40 accessions were analyzed.

Gamma radiation

To determine the effects of gamma radiations on genetic variation, 180 seeds from JL12 and JL24 varieties were irradiated with different doses of gamma radiations with a cesium 137 source using “Lisa 1 conservatome” equipment at the Regional Nuclear Energy Center of Kinshasa (CRENK) in the DR-Congo. The treatments included 0, 0.1, 0.2, 0.4, and 0.8 KGy of gamma-rays. Irradiated seeds were grown and the progenies (third generation of seedlings) were collected for DNA analysis. In total, 80 to 110 seedlings from M_3 generations were stored for DNA extraction and analysis.

DNA extraction

The total genomic DNA was isolated from individual seedlings or bulks of several seedlings using the method described by Nkongolo et al. (2005), with some modifications. The concentration of each sample was determined using the DNA quantitation kit from Bio-Rad and the purity was determined using a spectrophotometer (Varian Cary 100 UV-VIS spectrophotometer).

Amplification of ISSR markers

Thirty-five ISSR primers synthesized by Invitrogen, were chosen for preliminary amplification. DNA amplification was performed following the procedure described by Nagaoka and Ogihara (1997) and Nkongolo et al. (2005) with some modifications. Each polymerase chain reaction (PCR) was performed in a 25-µl volume containing 5 ng of genomic plant DNA, 10 mM Tris-HCl, pH 8.3 (at 25°C); 50 mM KCl; Applied Biosystems, Foster City, CA), 3.5 mM $MgCl_2$, 200 µM of each dNTP (Applied Biosystems, Foster City, CA), 0.5 µM primer and 0.625 U of *Taq* DNA polymerase (Applied Biosystems, Foster City, Calif.). For each primer, a negative control reaction with double distilled water was included. A drop of mineral oil was added to each reaction and the samples were amplified on a DNA thermal cycler (Perkin Elmer, Foster City, CA). The cycles performed were as follows: an initial denaturation at 95°C for 5 min followed by a 2 min incubation at 85°C at which point the polymerase was added; 42 cycles of 90 s at 95°C, 2 min at 55°C and 60 s at 72°C were performed; a final extension at 72°C for 7 min and a subsequent incubation at 4°C followed. PCR products were loaded onto 1% agarose gels (Invitrogen) in 0.5 X Tris-borate-EDTA (TBE) buffers containing ethidium bromide and run at 2.8 V/cm for 90 min. The agarose gels were documented using the Bio-Rad ChemiDoc XRS system and analyzed with the Discovery Series Quantity One 1 D Analysis Software.

ISSR analysis

Only ISSR primers that gave consistent profiles across populations were selected. The presence and absence of bands were scored as 1 or 0, respectively. Faint bands were not recorded for analysis. The following parameters were generated using POPGENE 1.31 to describe genetic variation: the percentage of polymorphic loci (P%, 5% criterion), Nei’s gene diversity (h), Shannon’s information index (i), the observed number of alleles (Na) and the effective number of alleles (Ne) (Nei, 1973; Yeh and Boyle, 1997). Jaccard’s similarity coefficients were generated to determine the genetic distances among populations using the Free Tree Program. A dendrogram

Table 1. Principal characteristics of groundnut accessions analyzed in the present study.

Varieties	Type	Origin	Days to maturity (days)	Seed color	Reaction to leaf spot disease
A24	Spanish	Brazil	100 - 110	Red	Resistant
A65	Valencia	Brazil	90 - 100	Bright red	Susceptible
A1052	Spanish	DR - Congo	95 - 100	Creamy white	Susceptible
A1408	Spanish	DR-Congo	95	Red	Susceptible
A157313-60673	Spanish	India (ICRISAT)	100 - 110	Red	Resistant
CG7	Spanish	India (ICRISAT)	100 - 110	Red	Resistant
E246	Spanish	India (ICRISAT)	100 - 110	Creamy	Susceptible
G17	Valencia	DR-Congo	90	Red	Susceptible
HB91700 - 10	Spanish	India (ICRISAT)	100 - 110	Red	Resistant
HYQ(CES)14	Spanish	India (ICRISAT)	100 - 110	Red	Resistant
ICGF(DRS) - 16	Spanish	India (ICRISAT)	100 - 110	Creamy	Tolerant
ICGM281	Valencia	Bolivia	120	Red	Resistant
ICGV-SM7850-35	Spanish	India (ICRISAT)	100 - 110	Creamy	Resistant
ICGV-SM 95523	Spanish	India (ICRISAT)	106	Red	Susceptible
ICGV-SM 96722	Valencia	India (ICRISAT)	90	Creamy rose	Resistant
ICGV-SM 98541	Spanish	India (ICRISAT)	110	Creamy rose	Susceptible
ICGV-SM 99588	Spanish	India (ICRISAT)	105 - 110	Rose	Susceptible
ICGV-SM 86021	Spanish	India (ICRISAT)	100 - 110	Red	Susceptible
ICGV-SM 95530	Spanish	India (ICRISAT)	90	Red	Susceptible
ICGV-SM 99594	Spanish	India (ICRISAT)	95	Creamy rose	Susceptible
ICGV-SM 00571	Spanish	India (ICRISAT)	100 - 110	Creamy rose	Susceptible
ICGV-SM 99599	Spanish	India (ICRISAT)	125	Creamy rose	Susceptible
ICGV12991	Spanish	India (ICRISAT)	100 - 110	Creamy white	Susceptible
JL12	Spanish	DR-Congo	90	Creamy white	Tolerant
JL24	Spanish	India (ICRISAT)	90	Creamy white	Tolerant
Kimpese	Spanish	DR-Congo	90	Rose	Tolerant
K12	Spanish	DR-Congo	90	Creamy white	Tolerant
MCV4	Spanish	India (ICRISAT)	100 - 110	Red	Resistant
018/04/3	Spanish	Cameroun	105 -110	Rose	Tolerant
045/04/3	Spanish	Cameroun	105 - 110	Creamy rose	Tolerant
047/04/1	Spanish	Cameroun	105 - 110	Creamy rose	Tolerant
048/04/3	Spanish	Cameroun	105 - 110	Creamy rose	Resistant
055/04/2	Spanish	Cameroun	105 - 110	Creamy rose	Susceptible
064/04/2	Spanish	Cameroun	105 - 110	Creamy rose	Tolerant
077/04/4	Spanish	Cameroun	105 - 110	Rose	Tolerant
080/04/1	Spanish	Cameroun	105 - 110	Creamy rose	Resistant

was constructed using the neighbor-joining analysis (Figure 1). This method starts with a star-like tree with no hierarchical structure and in a stepwise fashion finds the two operational taxonomic units that minimize the total branch length at each cycle of clustering. The un-rooted tree generated by the neighbor-joining method is constructed under the principle of minimum evolution (Saitou and Nei, 1987).

RESULTS

Of the 35 primers tested, 8 were selected for further analysis. They included UBC 809 UBC 825, UBC 827, UBC 827, UBC 841, UBC 879, ISSR 17898B, ISSR 8, and ISSR 9. Specific characterizations of these primers are described in Table 2. They amplified bands with the levels of inter-accession polymorphism ranging from 25% for primer ISSR 17898B to 93% for ISSR 9 (Table 2). An example of ISSR amplified products is depicted in Figure 2a and 2b for UBC 841 and ISSR 8 primers, respectively. The rate of polymorphic loci among accessions was 54% for the eight primers screened. Similar level of polymorphic loci was observed within accessions. Nei's

Table 2. The nucleotide sequence, number of fragments generated, fragment size range and of percentage of polymorphic loci generated for the eight ISSR primers used to amplify DNA samples from groundnuts (*Arachis hypogeal* L.).

Primer identification	Nucleotide sequence (5'→3')	G + C content (%)	Number of fragments generated	Fragment size range (bp)	Polymorphic loci (%)
ISSR primers					
ISSR 8	AGA TAG ATA GAT AGA TAG ATG Y	27.27	25	230-2000	76.00
ISSR 9	GAT CGA TCG ATC GC	57.14	14	395-1507	92.86
17898B	CAC ACA CAC ACA GT	50.00	12	205-1308	25.00
UBC 809	AGA GAG AGA GAG AGA GG	52.94	8	265-780	37.50
UBC 825	ACA CAC ACA CAC ACA CT	47.06	12	410-1210	50.00
UBC 827	ACA CAC ACA CAC ACA CG	52.94	12	210-1460	41.67
UBC 841	GAA GGA GAG AGA GAG AYC	50.00	12	265-1240	50.00
UBC 879	CTT CAC TTC ACT CA	42.86	16	230-2700	43.75

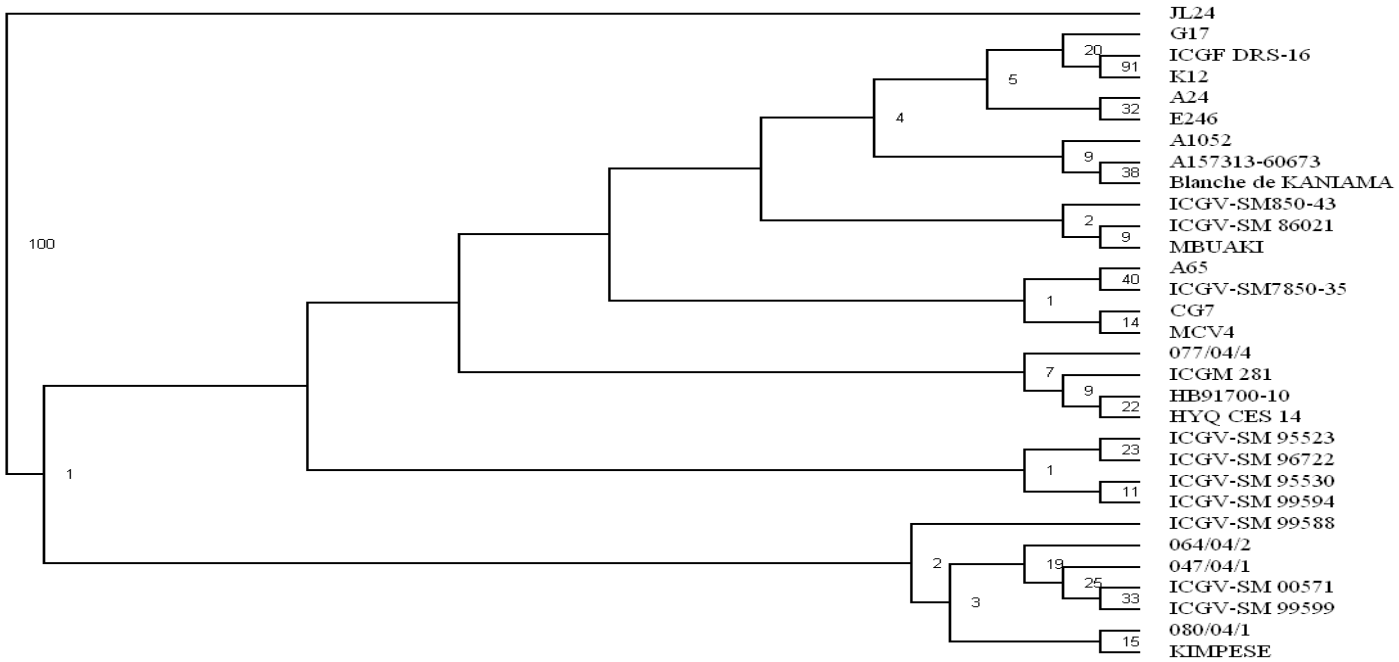

Figure 1. Dendrogram of the genetic relationship among 31 groundnut accessions based on the Jaccard similarity matrix using ISSR primers (Free Tree Program).

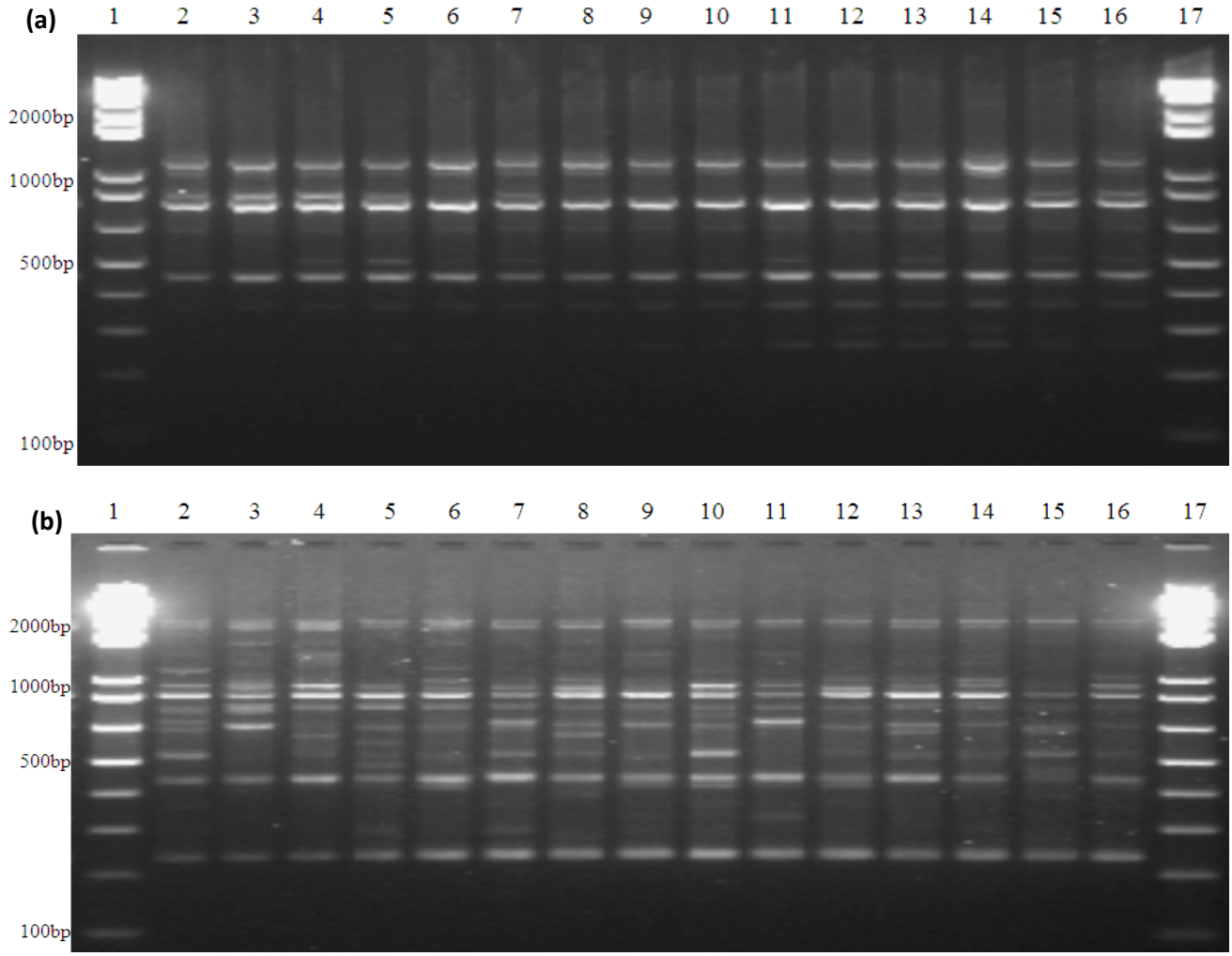

Figure 2. ISSR amplification of groundnut samples with (a) primer UBC841 and (b) primer ISSR 8. In each picture, lanes 1 and 17 contain 1Kb^{+} ladder, lanes 2 to 16 contain samples CG7, ICGV-SM7850-35, A65, HB91700-10, A1052, MCV4, A157313-60673, E246, K12, A24, ICGF (DRS)-16, ICGV-SM850-43, Blanche de KANIAMA, MBUAKI and G17.

gene diversity (h) and Shannon's index (I) among accessions were 0.188, and 0.28, respectively.

The genetic relatedness among accessions was determined using Jaccard's similarity coefficients. The scale used for the genetic distance runs from 0 (meaning no genetic difference) to 1 (different for all conditions - criteria). The genetic distance among the groundnut accessions varied from 0.11 to 0.37 (Table 3). No identical ISSR amplification profiles were identified. In general, 90% of genetic distance values were below 0.30 and 13% were below 0.20. Accessions K12 and ICGF (DRS)-16 from India were the most closely related and accessions A65 (from Brazil) and ICGV-SM00571 (from India) were the most distantly related. No distinctive main clusters were identified, but in general, accessions from the same origins had tendency to cluster together. Seven small clusters were identified but with weak bootstrap values. Only the accession JL24 from Brazil was separated from the rest of the collection with a bootstrap of 100. The accessions ICGF DRS-16 and K12 clustered together as documented by a strong bootstrap of 91 (Figure 1). There was no association between morphological similarity based on seed color and type or reaction to leaf spot disease and molecular relationships based on ISSR profiles.

Gamma radiation

All the plants from seeds irradiated at 0.6 KGy, and 0.8 KGy died few days after seeding. Progenies from seeds irradiated at 0.1 KGy, 0.2 KGy, and 0.4 KGy, were analyzed using ISSR markers as described above. The genetic diversity within each population was moderate to

Table 3. Distance matrix generated from ISSR data using the Jaccard's similarity coefficient analysis for 31 groundnuts accessions (Free Tree Program).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	0	0.16	0.17	0.23	0.23	0.25	0.24	0.27	0.29	0.28	0.27	0.20	0.19	0.25	0.25	0.23	0.19	0.23	0.28	0.31	0.26	0.22	0.26	0.28	0.30	0.23	0.27	0.29	0.32	0.27	0.25
2		0	0.18	0.15	0.19	0.18	0.23	0.22	0.21	0.20	0.23	0.19	0.20	0.24	0.24	0.20	0.28	0.24	0.27	0.26	0.22	0.25	0.22	0.24	0.33	0.21	0.26	0.22	0.27	0.18	0.26
3			0	0.18	0.23	0.23	0.24	0.20	0.18	0.17	0.22	0.15	0.19	0.23	0.22	0.16	0.19	0.26	0.26	0.31	0.25	0.23	0.27	0.27	0.27	0.24	0.25	0.21	0.31	0.24	0.28
4				0	0.18	0.18	0.21	0.19	0.17	0.18	0.27	0.21	0.20	0.22	0.19	0.24	0.22	0.31	0.25	0.28	0.24	0.25	0.22	0.26	0.27	0.24	0.26	0.18	0.25	0.18	0.26
5					0	0.19	0.27	0.24	0.24	0.27	0.25	0.25	0.31	0.22	0.19	0.24	0.20	0.31	0.21	0.30	0.28	0.21	0.28	0.30	0.34	0.24	0.32	0.26	0.31	0.26	0.28
6						0	0.23	0.19	0.19	0.23	0.27	0.21	0.20	0.24	0.24	0.20	0.26	0.31	0.31	0.30	0.20	0.27	0.20	0.22	0.29	0.19	0.28	0.24	0.29	0.28	0.29
7							0	0.16	0.18	0.22	0.29	0.20	0.22	0.29	0.33	0.23	0.29	0.33	0.37	0.31	0.26	0.28	0.31	0.33	0.32	0.31	0.35	0.31	0.34	0.31	0.33
8								0	0.12	0.14	0.23	0.14	0.20	0.26	0.27	0.15	0.26	0.29	0.27	0.34	0.22	0.26	0.24	0.26	0.30	0.23	0.33	0.22	0.30	0.26	0.28
9									0	0.16	0.20	0.21	0.20	0.26	0.25	0.15	0.24	0.29	0.26	0.31	0.18	0.24	0.26	0.26	0.26	0.27	0.29	0.23	0.28	0.21	0.29
10										0	0.24	0.19	0.17	0.27	0.28	0.21	0.23	0.26	0.29	0.33	0.21	0.29	0.21	0.19	0.26	0.26	0.27	0.21	0.26	0.22	0.28
11											0	0.20	0.24	0.23	0.23	0.21	0.28	0.31	0.28	0.28	0.26	0.29	0.29	0.28	0.30	0.25	0.31	0.31	0.32	0.27	0.31
12												0	0.15	0.21	0.27	0.15	0.24	0.23	0.28	0.33	0.22	0.26	0.24	0.29	0.29	0.29	0.29	0.19	0.26	0.27	0.31
13													0	0.23	0.22	0.21	0.21	0.20	0.29	0.33	0.23	0.25	0.17	0.25	0.27	0.28	0.27	0.21	0.26	0.24	0.28
14														0	0.17	0.26	0.20	0.26	0.25	0.22	0.22	0.21	0.22	0.26	0.31	0.26	0.24	0.26	0.23	0.20	0.22
15															0	0.24	0.19	0.25	0.24	0.24	0.28	0.22	0.24	0.28	0.24	0.21	0.19	0.19	0.28	0.21	0.21
16																0	0.22	0.22	0.21	0.30	0.18	0.19	0.22	0.26	0.27	0.26	0.26	0.20	0.23	0.28	0.29
17																	0	0.24	0.21	0.30	0.29	0.19	0.27	0.27	0.27	0.24	0.24	0.24	0.31	0.26	0.24
18																		0	0.20	0.33	0.24	0.26	0.22	0.26	0.30	0.33	0.24	0.25	0.24	0.27	0.25
19																			0	0.25	0.25	0.22	0.27	0.31	0.28	0.30	0.27	0.25	0.28	0.23	0.23
20																				0	0.30	0.25	0.32	0.30	0.31	0.29	0.26	0.29	0.33	0.26	0.24
21																					0	0.21	0.16	0.19	0.23	0.19	0.24	0.26	0.17	0.22	0.22
22																						0	0.19	0.25	0.26	0.24	0.25	0.19	0.22	0.26	0.22
23																							0	0.12	0.21	0.22	0.20	0.16	0.13	0.20	0.22
24																								0	0.19	0.15	0.20	0.20	0.19	0.24	0.22
25																									0	0.24	0.11	0.19	0.20	0.23	0.23
26																										0	0.22	0.23	0.26	0.25	0.19
27																											0	0.18	0.19	0.20	0.18
28																												0	0.19	0.20	0.24
29																													0	0.19	0.23
30																														0	0.19
31																															0

1 = IGCV-SM 95523; 2 = ICGV-SM 96722; 3 = ICGV-SM99588; 4 = ICGV-SM 86021; 5 = ICGV-SM 95530; 6 = ICGV-SM 99594; 7 = ICGV-SM 00571; 8 = ICGV-SM 99599; 9 = 047/04/1; 10 = 064/04/2; 11 = 077/04/4; 12 = 080/04/1; 13 = KIMPESE; 14 = HYQ (CES) 14; 15 = ICGM 281; 16 = JL24; 17 = CG7; 18 = ICGV-SM7850-35; 19 = A65; 20 = HB91700-10; 21 = A1052; 22 = MCV4; 23 = A157313-60673; 24 = E246; 25 = K12; 26 = A24; 27 = ICGF (DRS)-16; 28 = ICGV-SM850-43; 29 = Blanche de KANIAMA; 30 = MBUAKI; 31 = G17.

high. For the JL24 accession, the percentage of polymorphic loci (P %) was 54% for 0 Krad, 74% for 0.1 KGy, and 48% for 0.2 KGy treatments (Table 2). Shannon's index (I) was 0.123 for control population and 0.36 and 0.21 for 0.1 KGy and 0.2 KGy treatments, respectively. For JL12 accession, the level of polymorphic loci was similar (49%) for the control and the 0.10 KGy treatment and 54.8% for the 0.2 KGy treatment. This value was lower (43.8%) for the 0.4 KGy treatment. The Shannon's information index and the Nei's gene diversity were the same (0.15) for 0 KGy, 0.1 KGy, and 0.2 KGy treatments and lower (0.19) for the 0.4 KGy treatment (Table 4).

Overall, the amplitude of molecular variation

Table 4. Genetic diversity parameters of seven groundnut populations derived from JL24 and JL 12 accessions seeds irradiated with gamma rays at different doses.

Treatment	Na	Ne	h	I	P (%)
JL24 accession					
0.0 KGy (Control)	1.54	1.24	0.13	0.23	54.1
0.10 KGy	1.74	1.39	0.24	0.36	74.0
0.2 KGy	1.47	1.23	0.14	0.21	48.0
JL12 accession					
0.0 KGy	1.49	1.23	0.15	0.23	49.0
0.10 KGy	1.49	1.23	0.15	0.23	49.3
0.2 KGy	1.55	1.23	0.15	0.23	54.8
0.4 KGy	1.44	0.21	0.13	0.19	43.8

h = Nei's gene diversity; I = Shannon's information index; Na = Observed number of alleles; Ne = Expected number of alleles and P = Percentage of polymorphic loci.

measured by the level of polymorphic loci was significantly increased by 37% when 0.1 KGy (10 Krad) gamma-rays treatment was compared with the control for the JL24 accession. Strongly amplified ISSR products that appear to be induced by gamma ray radiation were identified in JL24 samples treated with gamma ray radiation at 0.1 Kgy dose (Figure 3). No significant differences were observed among treatments for the JL12 accession (data not shown).

DISCUSSION

The accessions analyzed in the present study derived were from four different countries. They included 89% Spanish type and 11% Valencia type. In general, 33% of accessions had red seeds, 31% creamy rose, 14% creamy white, 11% rose, 8% creamy, and 3% bright red. The molecular analysis results using ISSR primers revealed a level of 54% of polymorphic loci among accessions. This level of genetic variation is consistent with Raina et al. (2001) who reported 54% of polymorphism among 13 groundnut accessions using ISSR markers and 42% with RAPD markers. Dwivedi et al. (2001) analyzed a groundnut collection similar to the one described in the present study using 8 RAPD primers. They found a low level of polymorphism ranging from 8.7 to 33%. Other studies have reported a higher level of polymorphic loci in RAPD analysis compared to ISSR (Nkongolo et al., 2005). This could suggest that the primers used may represent a key factor for detecting molecular variability.

The level of genetic dissimilarity observed among accessions with the 8 ISSR primers varied between 11 and 33% suggesting that the accessions analyzed were different but genetically closely related. This is consistent with a previous RAPD analysis of similar genetic materials that revealed genetic dissimilarity values ranging from 41 to 1.2% (Dwivedi et al., 2001). The dendrogram constructed revealed with a high level of confidence that JL24 accession was an out-group that was separated from the rest of the collection. Other molecular studies detected up to 52% genetic dissimilarity with AFLPs (He and Prakash, 2001), and 56% with SSR (Mace et al., 2006) using different groundnut collections. The present study also revealed a moderate level of intra-accession variability of 50% polymorphic loci on average. This is relatively high for a self pollinated species such as groundnuts. The ISSR analysis used in the present study involved amplification of regions between adjacent, inversely oriented microsatellites using a SSR motif containing primers anchored at the 3' or 5' end by two or four arbitrary, often degenerate nucleotides (Zietkiewicz et al., 1994). ISSRs specifically target the di- and trinucleotide repeat types of microsatellite as these are characteristic of the nuclear genome (mononucleotide are found in chloroplast genome).

The ISSR method provides an alternative choice to obtain highly reproducible markers without any necessity for prior sequence information for various genetic analyses. It takes advantage of the ubiquitously distributed SSRs in the eukaryotic genomes. Because of these abundant and rapidly evolving SSR regions, ISSR amplification has the potential of revealing much larger numbers of polymorphic fragments per primer that any other marker system used such as RAPD or microsatellite (Godwin et al., 1997). The present study shows, however, that there is a great level of variability among ISSR primers for generating polymorphic loci. ISSRs are regions that lie within the microsatellite repeats and offer great potential to determine intra-genomic and inter-genomic diversity compared to other arbitrary primers, since they reveal variation within unique regions of the genome at several loci simultaneously.

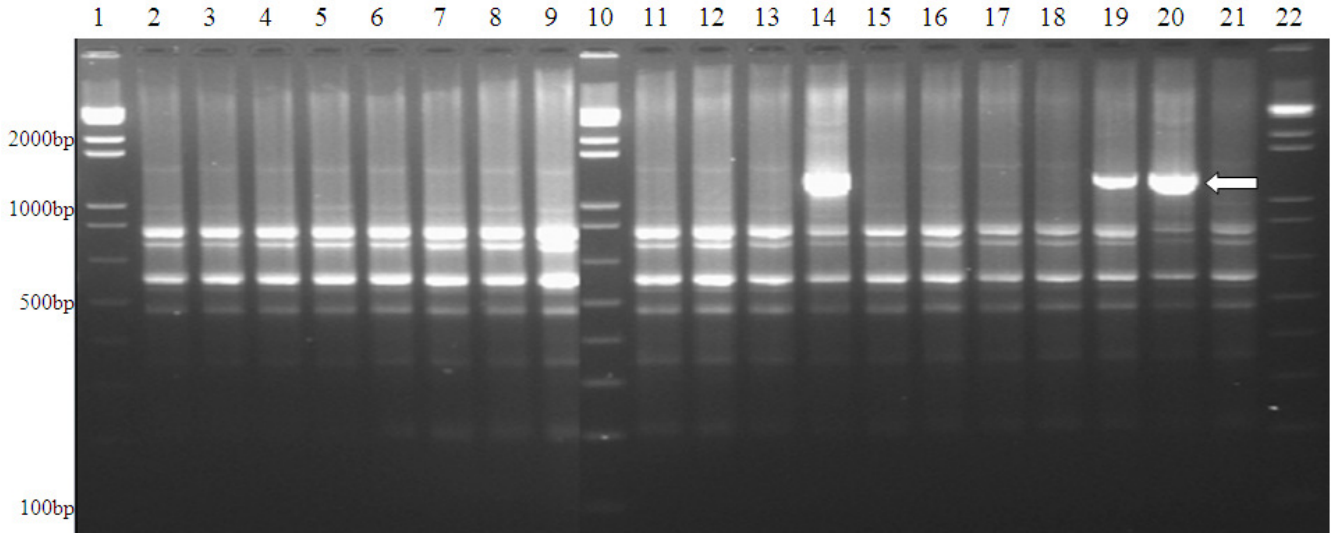

Figure 3. ISSR amplification of irradiated groundnut samples with primer UBC 827. Lanes 1, 10 and 22 contains 1Kb^{+} ladder, lanes 2 to 9 contain samples from JL24 control and lane 11 to 21 contain samples from JL24 100gy. Arrow shows amplified ISSR product identified in JL24 population subjected to 0.1 KGy gamma ray radiations.

Several properties of microsatellite such as high variability among taxa, ubiquitous occurrence and high copy number in eukaryotic genomes make ISSRs extremely useful marker (Morgante et al., 1996). They exhibit specificity of sequence tagged site markers, but need no sequence information for primer synthesis enjoying the advantage of random markers.

The multilocus profiles generated by ISSR primers are highly polymorphic and as such are ideal for studying genetic variation. Several studies have demonstrated the utility of the technique in a wide range of applications and plant families *(*Asteraceae, Brassicaceae, Hippocastanaceae, Orchidaceae, Poaceae, Scrophulariaceae, Violaceae, and Pinaceae) (Nkongolo et al., 2005; Danilova et al., 2003; Bornet and Branchard, 2004). ISSR marker accesses variation in the numerous microsatellite regions dispersed throughout the various genomes (particularly the nuclear genome) and circumvents the challenge of characterizing individual loci that other molecular approaches require (Semagn et al., 2006; Sharma et al., 2008). Compared to RAPD, ISSR assays are highly reproducible.

Mutations

The genetic distances among accessions in this gene pool were small, most of them below 0.3. Mutation induction through gamma radiation was a way of increasing novel genetic variability. In the present study, the percentage change in the mean of the treatments in relation to the control was significant for JL24 accession. The amplitude of variation based on the level of polymorphic loci was increased by 37% in 0.10 KGy gamma-ray treatments compared to control. The level of genetic diversity based on gene diversity and Shannon index was increased by 84 and 57%, respectively, in populations derived from seeds treated at 0.1 KGy (10 Krad) compared to the control (untreated) for the JL24 accession. Thus, these results suggest that gamma irradiation is an adequate tool for increasing the level of genetic variation in groundnut. This observation is in line with Montalván and Ando (1998) who observed an increase in variation for some quantitative traits in rice lines subjected to 0.2 KGy (20 Krad) gamma-ray treatment.

In other studies, an increase in genetic variation of quantitative characters has been induced by irradiating lentils (Sharma et al., 2008; Sharma and Sharma, 1984) and wheat (Kumar, 1977). Gamma-ray treatment at 0.2 KGy (20 Krad) have been found to increase variation in plant height, number of grains per plant, and grain yield in wheat (Jamil and Khan, 2002). Similar results were reported for plant growth and development and for molecular variation in soybeans (Ferguson et al., 2004; Hanafiah et al., 2010). Mudibu et al. (2011) also reported an increase of 10% of ISSR variation in soybeans. Some authors indicate that early generation (after irradiation) such as M_2 are not suitable for detecting the effect of physical or chemical mutagens on genetic variation. It has been suggested that data from more advanced generations are much more reliable for assessing genetic variability (Montalvan and Ando, 1998). This may explain the high level of increase in molecular variation in M_3 generation of JL24 accession analyzed compared to M_2 data from the soybean study conducted by Mudibu et al.

(2011). Field trials are underway to determine if this molecular change induced by gamma ray radiations translate in agronomic benefits.

Conclusion

The results of this study reveals that the groundnut accessions analyzed are genetically, closely related despite the high level of molecular variation detected using ISSR markers. The observed genetic diversity can be further increased with gamma-ray treatment at 0.1 KGy (10 Krad) dose. The groundnut JL24 accession from Brazil was genetically distant from the core collection analyzed and represents a key source to increasing variability in the DR-Congo groundnut breeding program.

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