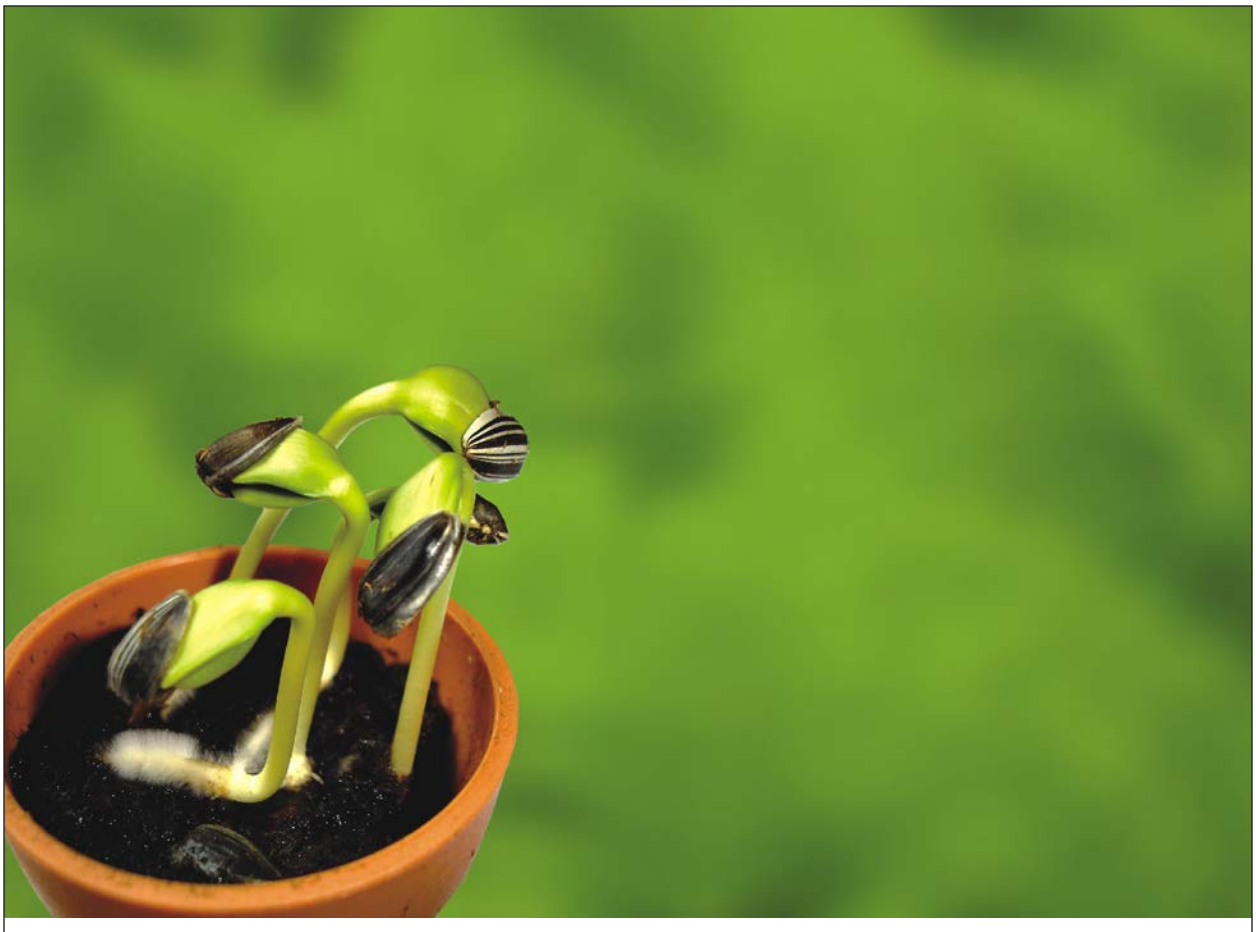

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Genetic Analysis of a Soybean Genetic Pool using ISSR Marker: Effect of Gamma Radiation on Genetic Variability

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ABSTRACT

Various studies showed that the genetic diversity in soybean (*Glycine max* L.) germplasm is limited. There is insufficient data on molecular analysis of soybean collections from Africa. The main objectives of the present study were (1) to analyze the genetic diversity and relationships among soybean accessions identified in the DR-Congo gene pool and (2) to determine the effect of gamma radiation on genetic variability. Genomic DNA from several cowpea accessions were analyzed using Inter-simple Sequence Repeat (ISSR) system. The level of polymorphic loci among the soybean varieties was high, varying from 70 to 90% based on ISSR primers used. The soybean varieties analyzed were genetically closely related with several accessions exhibiting similar ISSR amplification profiles. The genetic distance among the soybean accessions varied from 0.00 to 0.46. Some accessions from the International Institute of Tropical Agriculture (ITTA) revealed identical ISSR amplification profile. Seed treatment with gamma-rays at 0.2 KGy (20 Krad) increases the level of polymorphism in progenies by 10%. Low genetic diversity observed within varieties was increased with gamma-ray treatment at 0.1 KGy (10 Krad) and 0.2 KGy (20 Krad) dose.

Key words: *Glycine max*, DNA polymorphism, genetic distance, gamma-ray treatment, genetic diversity

INTRODUCTION

Soybean (*Glycine max* L.) was originally domesticated in China around 1700-1100 BC. It reached Africa through missionaries in the early 19th century and is now cultivated to a very limited extent in twenty-one sub-Saharan African countries. Some 17 tropical soybean varieties developed by the International Institute of Tropical Agriculture (ITTA) have been released by National Agricultural Research and Extension Systems (NARES) of several West and Central African countries (Nigeria, Benin, Ghana, DR-Congo, Togo) and Uganda. Since 2000, however, support for soybean research among NARES has declined. On-farm variety testing and releases is at standstill (Ogoke *et al*., 2003; Singh *et al*., 2002).

Various studies have demonstrated that current soybean cultivars are extremely uniform (Hiromoto and Vello, 1986; Brown-Guedira *et al*., 2000; Priolli *et al*., 2002; Bonato *et al*., 2006; Mulato *et al*., 2010). These studies showed that a few accessions have contributed to the majority of the genes in current cultivars and that the genetic diversity in soybean germplasm is limited. For example as few as five lines account for more than 55% of the genetic background of public

cultivars in North America (Brown-Guedira *et al.*, 2000). Only 35 ancestors contributed more than 95% of all alleles in soybean cultivars released in the United States between 1947 and 1988 (Gizlice *et al.*, 1994; Brown-Guedira *et al.*, 2000). Soybean gene pool in DR-Congo comprise less than 50 accessions of which half was developed at the International Institute of Tropical Agriculture (ITTA). Introducing novel germplasm sources and increasing genetic variation through wide hybridization or irradiation in breeding programs may provide the necessary genetic variability for the development and the adaptation of cultivars to biotic and abiotic factors.

Mutation breeding is an important supplementary approach to crop improvement as it increases unselected genetic variability for practical breeding application. Gamma irradiation is one of the tools used in mutation breeding to promote gene recombination and mutation frequency (Khundi *et al.*, 1997; Jamil and Khan, 2002; Majeed *et al.*, 2010). Low dose of gamma-ray treatment is recommended for the improvement of several plant species (Chen *et al.*, 2010). Ashraf *et al.* (2003) reported that seedling emergence, panicle fertility and grain yield declined with increasing dose level in all rice varieties that they studied.

To date, there are insufficient data on genetic diversity of soybean germplasm collections in African countries including DR- Congo. A research program aimed to enhance the capacity of national institutions in DR- Congo to use and develop participatory approaches to managing the agricultural biodiversity is being implemented. A more comprehensive assessment of genetic diversity would allow curators and users to manage and access *ex situ* collections more efficiently. Moreover, the knowledge of the genetic variation within accessions from germplasm collections is essential to the choice of strategy to incorporate useful diversity into the program and to facilitate the introgression of genes of interest into commercial cultivars. It will be also important to understand the evolutionary relations among accessions, to better sample germplasm diversity and to increase conservation efficiency using acquired knowledge (Fu, 2003).

Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular traits. Several DNA marker systems are now in common use in diversity studies of plants. Previous analyses of soybeans accessions have been based on Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), Amplified Fragment Lengths Polymorphisms (AFLPs) and Simple Sequence Repeats (SSR) (Brown-Guedira *et al.*, 2000; Dong *et al.*, 2001; Bonato *et al.*, 2006; Min *et al.*, 2010; Mulato *et al.*, 2010). Inter Simple Sequence Repeat (ISSR) markers are useful tools for genetic analysis of plant populations. Polymorphisms generated by ISSR assays have been used for assessment of variation, germplasm identification and genetic mapping in different species but not in soybean (Nkongolo *et al.*, 2005).

The objectives of the present study were to analyze the genetic diversity and relationships among soybean accessions identified in the DR-Congo gene pool and to determine the effect of gamma radiation on genetic variability detected with ISSR markers.

MATERIALS AND METHODS

Genetic material: Soybean seeds were provided by the Mvuazi and Gandajika research stations of the national Institute for agronomic and research studies (INERA) in 2008 and represent most of the varieties available in the DR- Congo soybean genepool. The sources and phenotypic characteristics 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

Table 1: Sources, leaf shape, pod color and seed color of 37 soybean varieties from the DR-Congo gene pool

Variety*	Sources/Origin	Leaf shape	Pod color	Seed color
AFYA	INERA Gandajika, DR-Congo	lanceolate	Light brown	Pink beige
AFYA Kiyaka	INERA Gandajika, DR-Congo	Rhomboid-lanceolate	Light beige	Lightbeige
BOTULA I	CREN-K/CGEA, DR-Congo	Rhomboid-lanceolate	Caramel	Lightbeige
DAVIS	California, USA	Lanceolate	Pink beige	Curry
KenSoyaI	Kenya	Rhomboid	Havana	Lightbeige
KenSoyaII	Kenya	Oval	Havana	Lightbeige
KITOKO	INERA, M'vuazi, DR-Congo	Rhomboid-lanceolate	Light beige	Lightbeige
MELOC	INERA Gandajika, DR-Congo	Rhomboid-lanceolate	Havana	Pink beige
NAMSOI 4m	INERA M'vuazi, DR-Congo	Lanceolate	Light beige	Curry
NI	INERA, Gandajika, DR-Congo	Lanceolate	Light beige	Lightbeige
No5	INERA Gandajika, DR-Congo	Rhomboid-lanceolate	Pink beige	Curry
ORIBI	INERA Mulungu, DR-Congo	Oval	Light beige	Lightbeige
PKO6	INERA Mulungu, DR-Congo	Oval	Light brown	Curry
SAPRO	INERA-Mulungu, DR-Congo	Rhomboid-lanceolate	Light beige	Curry
SB4	INERA M'vuazi, DR-Congo	Rhomboid	Ochre yellow	Curry
SB19	INERA Mulungu, RD-Congo	Rhomboid-lanceolate	Light beige	Lightbeige
SB24	INERA M'vuazi, DR-Congo	Oval	Caramel	Pink beige
TGM1196	INERA M'vuazi, DR-Congo	Lanceolate	Caramel	Curry
TGX BWAMANDA	Bwamanda, DR-Congo (IITA)	Lanceolate	Light brown	Lightbeige
TGX -198F-17F	IITA, Nigeria	-	-	-
TGX814-26D	IITA, Nigeria	Rhomboid-lanceolate	Light brown	Lightbeige
TGX814-49D	IITA, Nigeria	Rhomboid-lanceolate	Light beige	Curry
TGX1485-1D	ITTA, Nigeria	Rhomboid-lanceolate	Light beige	Lightbeige
TGX1485-1D Lub	ITTA, Nigeria	Rhomboid-lanceolate	Light beige	Lightbeige
TGX1740-7F	ITTA, Nigeria	Lanceolate	Light beige	Curry
TGX1879-9E	IITA, Nigeria	Lanceolate	Ochre yellow	Lightbeige
TGX1879-9F	ITTA, Nigeria	Rhomboid-lanceolate	Light beige	Lightbeige
TGX1879-13E	ITTA, Nigeria	Oval	Ochre yellow	Lightbeige
TGX1880-3E	ITTA, Nigeria	Lanceolate	Light beige	Lightbeige
TGX1888-29F	ITTA, Nigeria	Rhomboid-lanceolate	Light beige	Lightbeige
TGX1888-49F	ITTA, Nigeria	Lanceolate	Light beige	Lightbeige
TGX1895-33F	ITTA, Nigeria	Lanceolate	Creamy pink	Lightbeige
TGX1895-49F	ITTA, Nigeria	Lanceolate	Light beige	Pink beige
TGX1895 -50F	ITTA, Nigeria	-	-	-
TGX1904-2F	IITA, Nigeria	Rhomboid	Creamy pink	Lightbeige
TGX1908-6F	ITTA, Nigeria	Lanceolate	Light beige	Lightbeige
VUANGI	INERA M'vuazi, DR-Congo	Lanceolate	Pink beige	Lightbeige

*All the varieties from DR-Congo gene pool with the exception of Botula 1were provided in 2008 by INERA Gandajika and M'Vuazi.ITTA: International Institute of Tropical Agriculture, INERA: Institut National pour l'Etude et la Recherche Agronomiques'

in liquid nitrogen and stored at -80°C until use for DNA extraction. In total 37 accessions were analyzed. Leaf shape, pod and seed color were recorded based on observations of ten plants collected randomly in each population (accession).

Gamma radiation: To determine the effects of gamma radiations on genetic variation, 100 seeds from Kitoko variety were irradiated with different doses of gamma radiations with a cesium 137 source at the Regional Nuclear Energy Center of Kinshasa (CRENK) in the DR-Congo. The

treatments include 0 KGy (0 Krad), 0.1 KGy (10 Krad), 0.2 KGy (20 Krad), 0.4 KGy (40 Krad) and 0.8 KGy (80 Krad) of gamma rays. Irradiated seeds were grown and the progenies (second generation of seedlings) were collected for DNA analysis. In total 120 seedlings from M_2 generations were stored for DNA extraction and analysis.

DNA extraction: The total cellular 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: Twenty 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 PCR reaction 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 sec at 95°C, 2 min at 55°C and 60 sec 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 Vcm 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 RAPDistance Program version 1.04 as described by Nkongolo *et al.* (2005). Dendrograms were constructed using the neighbour-joining analysis. This method starts with a starlike 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 unrooted tree generated by the Neighbor joining method is constructed under the principle of minimum evolution (Saitou and Nei, 1987).

RESULTS

Genetic variation and relatedness: Of the 35 primers tested, eight were selected for further analysis. They included UBC 827 [$(AC)_8G$], UBC 829 [$(TG)_8C$], UBC 841 [GAAG $(GA)_6YC$], UBC 849 [$(GT)_8YA$], ISSR 17899 [$(CA)_6GT$], ISSR 1 [$(AG)_8RG$, ISSR 9 [$(GATC)_3GC$] and HB 13

$[(GAC)_3GC$. These primers amplified 82 bands with the levels of polymorphism among 37 varieties analyzed ranging from 71 to 90%. Figure 1 depicts an ISSR amplification profile using primer UBC 827. The rates of polymorphic loci within each variety were quite similar ranging from 37 to 38%. 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 soybean accessions varied from 0.00 to 0.46 (Table 2). Some accessions from IITA revealed identical ISSR amplification profile. For example, variety TGX 1888-49F was identical to TGX1879-9E, PK 06 (from INERA Mulungu), TGX 814-26D and TGX 814-49D. Likewise TGX 198F-17F was identical to TGX 1879-9E, PK 06, TGX 814-26D and TGX 814-49D. Accession PK06 was identical to TGX 814-26D and TGX 814-49D. Accession TGX 1879-9E was identical to PK06, TGX 814-26D and TGX 814-49D. Accession TGX 814-26D was identical to TGX 814-49D. Moreover 64 other genetic distance values were below 0.10. Accessions TGM 1196 and SB 24 both from INERA M'Vuazi were the most distantly related. A dendrogram was constructed from the similarity coefficients using RAPDistance program v. 10.4 (Fig. 2). The majority of soybean accessions in the DR-Congo gene pool were developed at IITA (Nigeria). No distinctive main clusters were identified. A general lack of agreement between clustering or genetic distance and morphological features (leaf shape, pod and seed colour) were observed. Accessions TGX 814-49F and TGX814-26D have similar ISSR amplification profile but possess different pod and seed color.

In general the genetic variation among the investigated accessions is high but the genetic distance among them is small. The genetic variation within accession was relatively high (40%) for a self pollinated species.

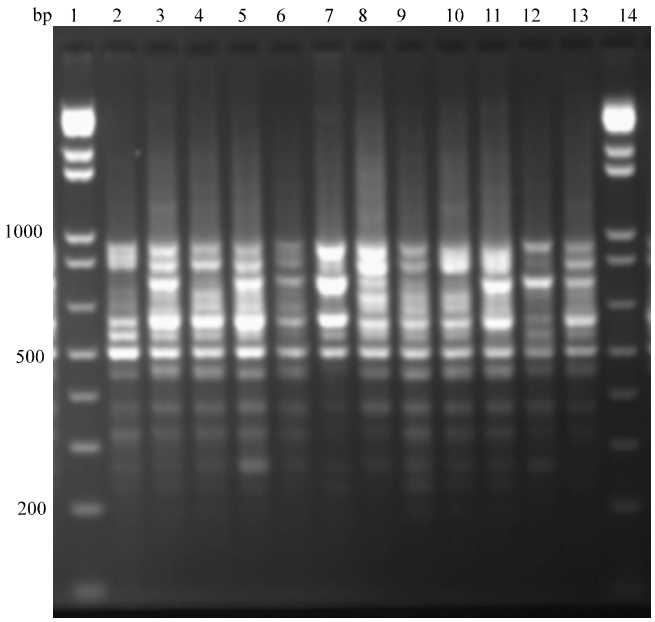

Fig. 1: ISSR profiles of genomic DNA from different soybean varieties amplified using primer UBC 827 in a 2% agarose gel. Lanes 1 and 14 contain 1-Kb + DNA ladder. Lanes 2 to 13 contain amplified soybean product (lane 2:BOTOLA I; lane 3: AFYA; lane 4: KEN SOYA I; lane 5: TGX 1888-29F; lane 6: No5; lane 7: SB 24; lane 8: KEN SOYA II; lane 9: ORIBI; lane 10: TGX 1904-2F; lane 11: TGX 1740-7F; lane 12: SB 4; lane 13: MELOC)

Table 2: Distance matrix for 37 soybean varieties using the Jaccard similarity coefficients

	v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24	v25	v26	v27	v28	v29	v30	v31	v32	v33	v34	v35	v36	v37
v1	0.00	0.18	0.09	0.38	0.22	0.19	0.17	0.14	0.22	0.16	0.14	0.17	0.30	0.09	0.25	0.14	0.34	0.34	0.17	0.19	0.22	0.24	0.22	0.25	0.22	0.14	0.11	0.16	0.11	0.19	0.23	0.11	0.11	0.11	0.19	0.11	0.17
v2		0.00	0.25	0.35	0.32	0.30	0.28	0.31	0.32	0.27	0.26	0.23	0.31	0.20	0.31	0.21	0.45	0.31	0.23	0.31	0.32	0.34	0.29	0.31	0.32	0.31	0.28	0.27	0.28	0.30	0.39	0.28	0.28	0.28	0.34	0.28	0.28
v3			0.00	0.35	0.19	0.16	0.14	0.11	0.19	0.14	0.17	0.14	0.22	0.16	0.27	0.17	0.27	0.41	0.19	0.22	0.19	0.21	0.19	0.22	0.14	0.11	0.09	0.08	0.09	0.11	0.20	0.09	0.09	0.09	0.11	0.09	0.19
v4				0.00	0.38	0.31	0.24	0.26	0.33	0.28	0.36	0.29	0.32	0.35	0.46	0.41	0.37	0.42	0.33	0.31	0.38	0.44	0.24	0.32	0.33	0.31	0.29	0.28	0.29	0.31	0.35	0.29	0.29	0.29	0.39	0.29	0.38
v5					0.00	0.09	0.17	0.19	0.22	0.16	0.24	0.17	0.30	0.24	0.30	0.19	0.30	0.43	0.22	0.19	0.17	0.24	0.27	0.30	0.22	0.24	0.22	0.16	0.22	0.19	0.32	0.22	0.22	0.22	0.28	0.22	0.22
v6						0.00	0.09	0.11	0.14	0.08	0.22	0.14	0.22	0.16	0.22	0.17	0.27	0.36	0.14	0.11	0.09	0.16	0.19	0.22	0.14	0.17	0.14	0.08	0.14	0.11	0.25	0.14	0.14	0.14	0.21	0.14	0.14
v7							0.00	0.03	0.11	0.06	0.14	0.11	0.20	0.14	0.25	0.19	0.25	0.34	0.17	0.14	0.17	0.24	0.12	0.15	0.11	0.09	0.06	0.06	0.06	0.19	0.18	0.06	0.06	0.06	0.19	0.06	0.17
v8								0.00	0.14	0.09	0.17	0.14	0.23	0.17	0.28	0.22	0.28	0.37	0.19	0.17	0.19	0.26	0.15	0.18	0.14	0.06	0.03	0.09	0.03	0.11	0.15	0.03	0.03	0.03	0.17	0.03	0.19
v9									0.00	0.06	0.14	0.11	0.25	0.14	0.20	0.19	0.20	0.39	0.17	0.14	0.22	0.19	0.22	0.20	0.17	0.19	0.17	0.11	0.17	0.14	0.23	0.17	0.17	0.17	0.24	0.17	0.06
v10										0.00	0.14	0.06	0.19	0.14	0.19	0.14	0.24	0.33	0.11	0.09	0.16	0.23	0.17	0.19	0.11	0.14	0.11	0.06	0.11	0.06	0.22	0.11	0.11	0.11	0.18	0.11	0.11
v11											0.00	0.14	0.28	0.11	0.18	0.12	0.23	0.32	0.19	0.22	0.19	0.22	0.15	0.12	0.14	0.12	0.14	0.14	0.14	0.17	0.21	0.14	0.14	0.14	0.22	0.14	0.14
v12												0.00	0.25	0.19	0.25	0.14	0.30	0.39	0.11	0.14	0.22	0.28	0.17	0.20	0.17	0.19	0.17	0.11	0.17	0.14	0.28	0.17	0.17	0.17	0.24	0.17	0.17
v13													0.00	0.27	0.24	0.28	0.33	0.28	0.20	0.23	0.20	0.32	0.26	0.24	0.15	0.23	0.20	0.14	0.20	0.12	0.31	0.20	0.20	0.20	0.27	0.20	0.25
v14														0.00	0.17	0.17	0.27	0.31	0.14	0.17	0.19	0.16	0.19	0.17	0.19	0.17	0.14	0.14	0.14	0.16	0.20	0.14	0.14	0.14	0.21	0.14	0.09
v15															0.00	0.12	0.29	0.28	0.15	0.18	0.15	0.22	0.26	0.18	0.15	0.23	0.25	0.19	0.25	0.17	0.31	0.25	0.25	0.25	0.27	0.25	0.15
v16																0.00	0.28	0.27	0.14	0.17	0.14	0.22	0.20	0.23	0.14	0.17	0.19	0.14	0.19	0.17	0.31	0.19	0.19	0.19	0.22	0.19	0.14
v17																	0.00	0.43	0.30	0.28	0.25	0.22	0.26	0.24	0.20	0.23	0.25	0.19	0.25	0.22	0.21	0.25	0.25	0.25	0.27	0.25	0.20
v18																		0.00	0.29	0.27	0.34	0.41	0.35	0.33	0.34	0.32	0.34	0.33	0.34	0.36	0.46	0.34	0.34	0.34	0.41	0.34	0.34
v19																			0.00	0.09	0.17	0.24	0.17	0.15	0.17	0.19	0.17	0.11	0.17	0.14	0.28	0.17	0.17	0.17	0.24	0.17	0.11
v20																				0.00	0.19	0.26	0.25	0.23	0.19	0.22	0.19	0.14	0.19	0.14	0.31	0.19	0.19	0.17	0.26	0.19	0.14
v21																					0.00	0.14	0.17	0.20	0.06	0.14	0.17	0.11	0.17	0.06	0.28	0.17	0.17	0.17	0.19	0.17	0.17
v22																						0.00	0.24	0.22	0.19	0.22	0.24	0.18	0.24	0.21	0.30	0.24	0.24	0.24	0.16	0.24	0.14
v23																							0.00	0.09	0.12	0.09	0.12	0.11	0.12	0.14	0.24	0.12	0.12	0.12	0.19	0.12	0.22
v24																								0.00	0.15	0.12	0.15	0.14	0.15	0.17	0.21	0.15	0.15	0.15	0.22	0.15	0.20
v25																									0.00	0.09	0.11	0.06	0.11	0.03	0.23	0.11	0.11	0.11	0.14	0.11	0.17
v26																										0.00	0.03	0.08	0.03	0.11	0.15	0.03	0.03	0.03	0.11	0.03	0.19
v27																											0.00	0.06	0.00	0.09	0.12	0.00	0.00	0.00	0.14	0.00	0.17
v28																												0.00	0.06	0.03	0.17	0.06	0.06	0.06	0.14	0.06	0.11
v29																													0.00	0.09	0.12	0.00	0.00	0.00	0.14	0.00	0.17
v30																														0.00	0.20	0.09	0.09	0.09	0.16	0.09	0.14
v31																															0.00	0.12	0.12	0.12	0.25	0.12	0.23
v32																																0.00	0.00	0.00	0.14	0.00	0.17
v33																																	0.00	0.00	0.14	0.00	0.17
v34																																		0.00	0.14	0.00	0.17
v35																																			0.00	0.14	0.24
v36																																				0.00	0.17
v37																																					0.00

V: Variety, 1: TGX BNA MAANDA, 2: NI, 3: TGX 1908-6F, 4: KITOKO, 5: AFYA KIAKA, 6: TGX 1895-50F, 7: DAVIS, 8: VUANGI, 9: TGX 1485-1D, 10: SB19, 11: SAPRO, 12: TGX 1880-3E, 13: BOTULA I, 14: AFYA, 15: KEN SOTA II 20: ORIBI, 21: TGX 1904-2F, 22: TGX 1740-7F, 23:SB4, 24: MELOC, 35:NTGX 1895-33F, 26: TGX 1895-49F, 27: TGX 888-49F, 28: NAMOSOI, 29: TGX 198F-17F, 30: TGX 1879-13E, 31: TGM 1196, 32: TGX 1879-9E, 33: PK 06, 34: TGX 814-26D, 35: TGX 1879-9F, 36: TGX 814-49D, 37: TGX 1485-1D Lubumbashi

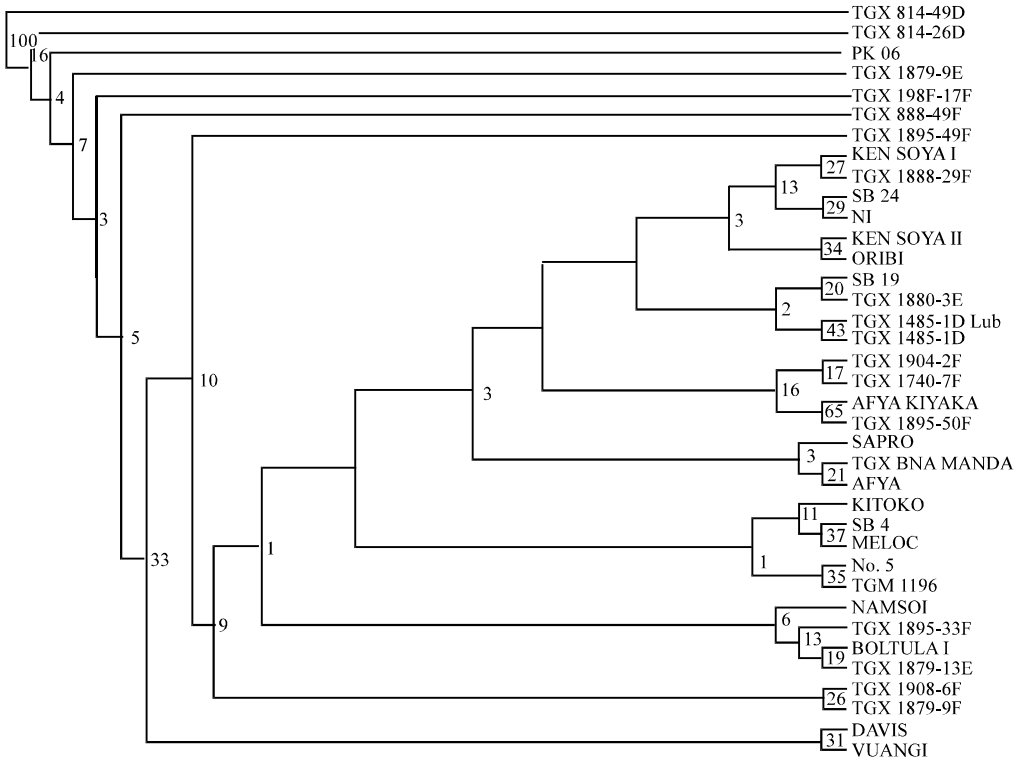

Fig. 2: Dendrogram of the genetic relationships between 37 soybean varieties using the data generated from the Jaccard similarity matrix from ISSR profiles. The bootstrapping values are indicated at the nodes

Table 3: Genetic diversity parameters of three soybean populations derived from *Kitoko* variety seeds irradiated with gamma rays at different doses

Treatment (Gamma-rays)	#PL	P%	na	ne	h	i
Kitoko variety						
0 KGy (0 Krads)	11	37.00	1.40	1.30	0.08	0.13
0.1 KGy (10 Krads)	11	38.00	1.38	1.21	0.13	0.19
0.2 KGy (20 Krads)	12	42.00	1.41	1.20	0.13	0.19

Genetic diversity descriptive statistics: #PL: No. of polymorphic loci; P%: Percentage of polymorphic loci; na: Observed No. of alleles; ne: Effective No. of alleles; h: Nei's gene diversity; I: Shannon's information index

Gamma radiation: All the plants from seeds irradiated at 0.4 KGy (40 Krad), 0.6 KGy (60 Krad) and 0.8 KGy (80 Krad) died few days after seeding. Progenies from seeds irradiated at 0.1 KGy (10 Krad) and 0.2 KGy (20 Krad) were analyzed using ISSR markers as described above. The genetic diversity within each population was moderate. The percentage of polymorphic loci (P%) was 37, 38 and 42% for populations from seeds treated with 0 KGy (0 Krad), 0.1 KGy (10 Krad) and 0.2 KGy (20 Krad) gamma-rays, respectively. The gene diversity index was 0.08 for the control and 0.13 for the populations derived from the 0.1 and 0.2 KGy treatments. Shannon's index (i) was 0.13 for the control population. This value was 0.19 for populations derived from 0.1 KGy (10 Krads) and 0.20 KGy (20 Krads) treatments. The mean observed number of alleles (Na) was quite similar for the three populations ranging from 1.38 to 1.4. The mean effective number of alleles (Ne) was 1.30 for the control (untreated) population and 1.20 for the populations derived from seeds treated at 0.1 KGy (10 Krad) and 0.2 KGy (20 Krad) gamma-rays (Table 3).

Overall, there was no difference in the means of polymorphic loci between the control (non-irradiated) and the 0.1 KGy (10 Krad) treatments. Interestingly, the amplitude of genetic variation measured by the level of polymorphic loci was significantly increased by more than 10% when 0.2 KGy (20 Krad) gamma-rays treatment was compared with the control.

DISCUSSION

ISSR marker and genetic diversity in soybean: In the present study, ISSR was used for the first time to assess genetic variation of a soybean gene pool. ISSR marker system accesses variation in the numerous microsatellite regions dispersed throughout the genome (Semagn *et al*., 2006) and circumvents the challenge of characterizing individual loci that other molecular approaches require. ISSR analysis involved amplification of regions between adjacent, inversely oriented microsatellites, using a Simple Sequence Repeat (SSR) motif containing primers anchored at the 3 or 5 end by two or four arbitrary, often degenerate nucleotides (Zietkiewicz *et al*., 1994). This method provides an alternative choice to other system for obtaining highly reproducible markers without any necessity for prior sequence information for various genetic analyses. Because of the abundant and rapidly evolving SSR regions, ISSR amplification has the potential of revealing much larger numbers of polymorphic fragments per primer than any other marker system used such as RFLP or microsatellite. 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. 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). The genetic information acquired from the present study can not be compared with previous studies since they used different marker systems. It should be pointed out however, that Mulato *et al*. (2010) reported a high genetic variation among five groups of soybean accessions assessed with microsatellite markers. Brown-Guedira *et al*. (2000) and Min *et al*. (2010) as high as 0.76 and 0.64 when wide and exotic soybean germplams were compared using RAPD and microsatellite analyses. In the ISSR analysis described in the present study, all the accessions used were cultivars and no wild soybean was analyzed.

The loss of genetic diversity, in part due to conventional breeding programs associated with modern agronomic and agricultural practices, has been dramatic for many crops including soybean. In fact several studies showed that domesticated soybeans have reduced genetic diversity, a changed distribution of alleles and in many cases, a change in allele frequencies (Gizlice *et al*., 1994; Dong *et al*., 2001; Min *et al*., 2010).

Many sub-Saharan African countries grow soybean varieties developed at IITA. The present study confirms using ISSR the close genetic relationship among soybean varieties. A variety developed in Davis, California was genetically closely related to all soybeans varieties developed in Africa that were analyzed. More than 10% of all the genetic values revealed that several accessions were very similar in their ISSR amplification profile. Recent studies report the monophyletic origin of all soybean cultivars. Phylogeny for wild and landrace soybeans showed that all landrace soybeans formed a single cluster supporting a monophyletic origin of all the cultivars (Guo *et al*., 2010). The authors used microsatellite analysis to determine that soybean (*Glycine max*) domestication is characterized by a single origin (Southern China) and moderate bottleneck.

The data described in the present study also indicate that the levels of polymorphic loci among varieties were high for all the primers tested. This is consistent with previous RAPD, SSR and AFLP analyses. A low level of association between ISSR amplification profile and qualitative traits analyzed (leaf shape, seed and pod color) was observed. In fact, despite the narrow genetic diversity among cultivated soybean cultivars, wide phenotypic variability in terms of seed shapes, size, color and chemical composition, plant morphology and maturity as well as resistance to broad range of biotic and abiotic stresses is characteristic of most soybean gene pools (Gregan, 2008).

Mutation: Mutation induction through gamma radiation was a way of increasing genetic variability. In the present study, the percentage change in the mean of the treatments in relation to the control was minimal. The amplitude of variation based on the level of polymorphic loci was significantly increased (10%) only in 20 Krad gamma-ray treatments compared to control. The level of genetic diversity based on gene diversity and Shannon index was increased by 40 and 50%, respectively in populations derived from seeds treated at 0.1 KGy (10 Krad) and 0.2 KGy (20 Krad) gamma dose compared to the control (untreated). The results of the present study suggest that gamma irradiation might be an adequate tool for increasing the level of genetic variation in soybeans. This observation is in line with Montalvan and Ando (1998) who observed in increased in variation for some quantitative traits in rice lines subject to 0.2 KGy (20 Krad) gamma-ray treatment. The data need to be confirmed with late generations derived from these treatments. In fact 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).

The present study represents the first documented report on the assessment of the effects of gamma-ray treatment on molecular variation in plant species, particularly in legumes. In other studies, an increase in genetic variation of quantitative characters has been induced by irradiating lentils (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).

CONCLUSION

The results of this study reveals that the bean varieties analyzed are genetically, closely related despite the high level of inter-varietal polymorphism detected using ISSR markers. The low genetic diversity observed within each variety can be increased with gamma-ray treatment at 0.2 KGy (20 Krad) dose.

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