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Scientific Research

Effect of Gamma Irradiation on Morpho-Agronomic Characteristics of Groundnut (*Arachis hypogaea* L.)

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ABSTRACT

Induced mutation in plant improvement has been used in several crops to generate new sources of genetic variations. A study was conducted to determine the effect of different doses of gamma irradiation on different morpho-agronomic characteristics. Agronomic traits that were analyzed included: grain yield, number of pods/plant, number of seeds/plant and weight of 100 seeds and numbers of days to 50% flowering. Morphometric characterisation of the descriptive data included plant height, stem diameter, number of leaves/plant, leaflet length, leaflet width and number of ramification/ plant. Groundnut seeds were treated with various doses of gamma rays (100, 200, 400 and 600 Gy). Among the various dose treatments, gamma rays treatment at 100 Gy resulted in a higher increase of grain yield and other morpho-agronomic parameters especially for the JL24 variety. In fact the gamma irradiation at 100 Gy increased significantly grain yield by 14% for JL24, and 4 % for JL12. The number of pods per plant was increased by 2% for JL12 and 37% for JL24. For the number of seeds per plant, there was a significant increase of 8% for JL12, and 62% for JL24 at 100 Gy. A similar trend was observed for the JL24 at 200 Gy dose. Higher doses of gamma rays (400 and 600 Gy) reduced significantly plant growth and grain yield. The usefulness of the mutants identified in a groundnut breeding program is discussed.

Keywords: Gamma Ray Radiation; Groundnut; *Arachis hypogea*; Grain Yield; DR-Congo

1. Introduction

Groundnut (*Arachis hypogaea* L.) is an important oil seed crop and grain legume worldwide. However, it is self pollinating and possesses limited variability. Consequently, the extent to which groundnut cultivars may be improved through conventional breeding methods is limited. Mutation breeding supplements conventional plant breeding as a source of increasing variability and could confer specific improvement without significantly altering its phenotype [1]. The successful utilization of gamma rays to generate genetic variability in plant breeding has been reported in soybean [2-4] and other crops [5-10].

It has been demonstrated in many studies that genetic variability for several desired characters can be induced successfully through mutations and its practical value in plant improvement programmes has been well established [11]. The main advantage of mutation breeding is the possibility of improving one or two characters without changing the rest of the genotype. Groundnut breeding in Central Africa has been limited and the majorities of varieties that are available in national gene pools are from international programs and they are not always adapted to local growing conditions.

The main objective of the present study was to determine the effect of different doses of gamma irradiation on different morpho-agronomic characteristics and to identify mutant lines with some potential of high grain yield.

2. Materials and Methods

2.1. Gamma Radiation

The study was carried out in the DR-Congo. Groundnut

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seeds were provided by the Mvuazi and Gandajika research stations of the national institute for agronomic and research studies (INERA). Some characteristics of the three groundnut varieties analyzed in the present study are described in **Table 1**. To determine the effects of gamma radiations on morphometric and agronomic traits, seeds from Kimpese, JL24 and JL12 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 include 0 Gy, 100 Gy, 200 Gy, 400 Gy, and 600 Gy of gamma-rays. Irradiated seeds were grown and M1 and M2 generations were produced for field trials.

2.2. Field Trials

Field experiments were conducted over 2 years (2010-2012) at one site in Kinshasa (Mont-Amba) in the DR-Congo. The trials were carried out at the Experimental garden of the Regional Nuclear Energy Center, Kinshasa (CREN-K) (15˚30'E, 04˚41'S and 330 m altitude). The region falls within the Aw4 climate type according to Köppen classification characterized with 4 months of dry season (from mid-May to August) coupled with 8 months of rainy season, sometimes interrupted by a short dry season in January/February. Daily temperature averages 25˚C and annual rainfall is close to 1500 mm.

The main plot sizes were 11 m long and 5.6 m wide for M1and M2 populations. The sub-plots were 3 m × 1.2 m for both M1 and M2 generations. Two seeds were sown at every 30 cm to a depth of about 2 cm. Weeding was performed manually.

The experiment was a split plot design with three replicates. The varieties represented the main plot and the irradiation dose treatments were the sub-plots. The trial was conducted with no fertilizer or pesticide applications.

In total 11 characters were selected for germplasm characterization. The descriptive data included plant height, stem diameter, number of leaves/plant, leaflet length, leaflet width, number of ramifications/plant. Plant height was measured as the length of the main stem from the soil surface to the terminal node at maturity. Agronomic data include grain yield/ha, number of pods/plant, number of seeds/plant, weight of 100 seeds, numbers of days to 50% flowering.

Data were subjected to analysis of variance (ANOVA) using Statistix Edition 8 and R software. Main effects were separated by least significant differences (LSD) at P = 0.05 level.

3. Results

Mutation breeding in crop plants is an effective tool in hands of plant breeders especially in crops having narrow genetic base such as groundnut. In the present study, four main components of yields were analyzed in details. They include, number of pods per plant, number of seed per plant, grain yield per hectare, and weight of 100 seeds. **Tables 2** and **3** describe data for the M1 generation and **Tables 4** and **5** for M2 progenies.

No progenies from grains irradiated at 600 Gy were produced since all the plants died. Grain yield varied from 734 Kg/ha (for Kimpese at 400 Gy) to 2337 Kg/ha (for Kimpese at 0 Gy) in M1 and from 821 Kg/ha (for Kimpese at 400 Gy) to 2358 Kg/ha (for JL24 at 100 Gy) in M2 (**Tables 2** and **4**). In M1, gamma irradiation at any dose decreased significantly grain yield compared to control for the three varieties that were evaluated (**Table 2**).

There was a high level of variability in the M2 generation for all characters evaluated. In general, gamma irradiation increased significantly grain yield, number of pods/plant and number of seeds/plant compared to control without irradiation. The highest changes for agronomic grain yield, number of pods and seeds per plant were observed in JL24 variety. In fact the gamma irradiation at 100 Gy increased significantly grain yield by 14% for JL24, and 4% for JL12. By cons for variety Kimpese, an unexpected lack of germination was observed at 100 Gy. The number of pods per plant was increased by 2% for JL12 and 37% for JL24. For the number of seeds per plant, there was a significant increase of 8% for JL12, and 62% for JL24 at 100 Gy. A similar trend was observed for the JL24 at 200 Gy dose. For this treatment, there was a 6% and 10% increase over the control for grain yield and the number of pods per plants, respectively. The number of seeds per plant was also

Table 1. Principal characteristics of groundnut varieties used in the present study.

Varieties	Type	Source	Days to Color Maturity	Reaction to leaf spot	Disease
JL12	Spanish (INERA)	DR-Congo	90	Creamy white	Tolerant
JL24	Spanish (INERA)	India	90	Creamy white	Tolerant
Kimpese	Spanish	DR-Congo	90	Creamy white	Tolerant

Table 2. Grain yield, number of pods and seeds per plant, weight of 100 seeds and days to 50% flowering in M-1 generation of three groundnut accessions subjected to different doses of gamma irradiation.

Accessions	Irradiation Doses (Gy)	Grain yield/ha	Number of pods/plant	Number of seeds/plant	Weight of 100 seeds	Numbers of days to 50% flowering
		Kg	Mean number	Mean number	Gram	Mean number
JL12	0	2002 ± 122.6	19.25 ± 13.3	44.8 ± 25.7	50.2 ± 2.09	34.5 ± 4.04
	100	1782 ± 239.7	13.5 ± 3.8	34.5 ± 11.09	48.02 ± 2.05	42.75 ± 7.8
	200	1081 ± 166.2	14.75 ± 5.9	33.8 ± 15.6	49.42 ± 3.3	45.5 ± 3.3
	400	891 ± 130.3	10 ± 2.9	24.7 ± 6.65	42.16 ± 3.4	50.75 ± 3.3
	Mean	**1439**	**14.3**	**34.4**	**47.4**	**43.3**
KIMPESE	0	2337 ± 221.8	12.75 ± 7.4	27.25 ± 14.8	50.94 ± 2.3	33.75 ± 4.6
	100	1213 ± 199.7	11.75 ± 4.9	28.8 ± 7.4	48.99 ± 1.7	39 ± 4.1
	200	1004 ± 66.5	9.25 ± 3.5	21.62 ± 8.1	35.84 ± 0.8	45 ± 4.3
	400	734 ± 143.9	2.5 ± 1.2	4.7 ± 2.06	-	46.2 ± 6.7
	Mean	**1322**	**9.06**	**20.5**	**45.2**	**40.9**
JL24	0	2219 ± 402.8	11.5 ± 7.8	23.25 ± 17.6	49.34 ± 2.8	32 ± 3.1
	100	1546 ± 176.3	21.5 ± 12.01	45.6 ± 20.9	47.99 ± 1.7	38.75 ± 3.4
	200	1213 ± 162.7	12.25 ± 9.1	28 ± 22.4	46.99 ± 2.1	45.25 ± 5.8
	400	987 ± 20.2	4.5 ± 4.7	10.25 ± 10.2	39.27 ± 2.2	47.7 ± 6.8
	Mean	**1491.2**	**12.4**	**26.7**	**45.8**	**40.9**
LSD (p = 0.05)		299	9.9	4.9	13.2	1.04

Table 3. Plant height, stem diameter, number of leaves per plant, leaflet width, number of ramification per plant, pod length and width in M-1 generation of three groundnut accessions irradiated with different doses of gamma radiations.

Accessions	Irradiation Doses (Gy)	Plant height	Stem diameter	Number of leaves/plant	Leaflet length	Leaflet width	Number of ramifications/plant
		cm	mm	Mean number	mm	mm	Mean number
JL12	0	40.5 ± 10.5	4.6 ± 0.3	32.5 ± 13.3	5.7 ± 0.4	2.8 ± 0.4	8.75 ± 2.5
	100	23.5 ± 3.6	4.1 ± 1.1	25.5 ± 12.1	5.6 ± 0.4	2.9 ± 0.1	7 ± 0.8
	200	29.2 ± 6.02	4 ± 0.9	33.4 ± 3.8	5.3 ± 0.3	2.8 ± 0.3	7 ± 0.0
	400	29.2 ± 4.9	4.3 ± 0.5	27.7 ± 11.5	4.4 ± 0.3	2.6 ± 0.4	8.75 ± 2.7
	Mean	**30.6**	**4.2**	**29.7**	**5.2**	**2.7**	**7.8**
KIMPESE	0	33.2 ± 7.6	4.2 ± 0.4	31.5 ± 11.2	5.6 ± 0.8	3.1 ± 0.3	5 ± 1.6
	100	29.5 ± 3.1	4 ± 0.3	27 ± 7.8	5.2 ± 0.6	2.7 ± 0.5	6 ± 1.4
	200	27.2 ± 3.5	4.2 ± 0.9	32 ± 11.3	5.4 ± 0.2	3.1 ± 0.4	5.5 ± 1.2
	400	16.5 ± 3.8	3.6 ± 0.3	15.7 ± 2.6	4.9 ± 0.9	3.1 ± 0.4	4 ± 0.8
	Mean	**26.6**	**4**	**26.5**	**5.2**	**3**	**5.1**
JL24	0	27.7 ± 7.6	4.3 ± 0.5	27 ± 6.8	5.02 ± 0.7	2.7 ± 0.4	8 ± 3.5
	100	32.7 ± 5.7	4.2 ± 0.4	34.5 ± 7.4	4.9 ± 0.5	2.7 ± 0.5	13 ± 1.7
	200	31.2 ± 11.4	4.2 ± 0.4	28.2 ± 5.6	5.4 ± 0.3	3.2 ± 0.1	7.25 ± 1.9
	400	19.5 ± 1.2	3.4 ± 0.3	26.2 ± 4.3	4.6 ± 0.6	2.8 ± 0.4	6.5 ± 4.6
	Mean	**27.7**	**4.02**	**28.9**	**4.9**	**2.8**	**8.6**
LSD (p = 0.05)		12.3	0.7	2.6	0.5	0.3	3.2

increased over by 11%.

The effect of gamma irradiation on plant height, stem diameter, number of leaves/plant, leaflet length, leaflet width and number of ramification/plant varied between treatments and varieties (**Tables 3** and **5**). Using the non-irradiated seeds as control or reference, the effects of different doses were determined. In general 200 Gy and 400 Gy treatments resulted in reduction or no significant

Table 4. Grain yield, number of pods and seeds per plant, weight of 100 seeds and days to 50% flowering in M-2 generation of three groundnut accessions subjected to different doses of gamma irradiation.

Accessions	Irradiation Doses (Gy)	Grain yield/ha	Number of pods/plant	Number of seeds/plant	Weight of 100 seeds	Numbers of days to 50% flowering
		Kg	Mean number	Mean number	Gram	Mean number
JL12	0	2097 ± 26.1	10.2 ± 1.2	24.25 ± 6.01	44 ± 2.8	34 ± 5.6
	100	2174 ± 21.2	10.4 ± 3.1	26.25 ± 5.3	52.3 ± 2.05	39 ± 1.4
	200	1834 ± 121.62	9.7 ± 2.8	22.5 ± 9.1	48.02 ± 5.1	37.5 ± 4.9
	400	1346 ± 16.9	8 ± 2.6	10.5 ± 3.5	43.8 ± 1.03	49.5 ± 0.7
	Mean	**1862.7**	**9.5**	**20.8**	**47.03**	**40**
KIMPESE	0	2162 ± 241	9.6 ± 1.4	33 ± 7.07	48.1 ± 6.4	35.5 ± 3.5
	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	200	2036 ± 67.8	11.8 ± 1.6	32 ± 5.6	53.7 ± 1.4	42 ± 1.4
	400	821 ± 58.05	4.7 ± 3.3	12.5 ± 2.03	54.0 ± 1.4	47 ± 33.2
	Mean	**1673**	**8.7**	**25.8**	**51.9**	**41.5**
JL24	0	2073 ± 38.8	14.5 ± 2.1	24.25 ± 7.4	47 ± 1.8	30 ± 2.8
	100	2358 ± 289.9	20 ± 5.6	39.5 ± 12.7	55.5 ± 1.2	39 ± 5.6
	200	2190 ± 62.2	16 ± 2.8	27 ± 4.2	48.05 ± 1.9	46.5 ± 2.1
	400	1650 ± 76.3	9 ± 2.8	21.75 ± 9.5	48.5 ± 5.5	48.5 ± 3.5
	Mean	**2005.2**	**14.8**	**28.1**	**49.7**	**41**
LSD (p = 0.05)		187	6.07	14.8	5.6	11.4

Table 5. Plant height, stem diameter, number of leaves per plant, leaflet width, number of ramification per plant, pod length and width in M-2 generation of three groundnut accessions irradiated with different doses of gamma radiations.

Accessions	Irradiation Doses (Gy)	Plant height	Stem diameter	Number of leaves/plant	Leaflet length	Leaflet width	Number of ramifications/plant
		cm	Mm	Mean number	mm	mm	Mean number
JL12	0	16.0 ± 1.06	4.6 ± 0.2	30.8 ± 13.9	5.7 ± 0.01	2.9 ± 0.05	6.2 ± 1.7
	100	11.7 ± 6.3	4.1 ± 0.6	24.5 ± 14.1	5.5 ± 0.3	2.9 ± 0.01	6.5 ± 2.1
	200	14.7 ± 2.8	3.9 ± 0.5	30.3 ± 1.9	5.09 ± 0.3	2.8 ± 0.2	9.1 ± 1.2
	400	10.1 ± 1.5	4.2 ± 0.1	27 ± 15.9	4.2 ± 0.2	2.6 ± 0.3	5.1 ± 1.2
	Mean	**13.1**	**4.2**	**28.1**	**5.1**	**2.8**	**6.7**
KIMPESE	0	13.8 ± 8.6	4.3 ± 0.5	29.2 ± 8.8	5.3 ± 0.4	2.9 ± 0.1	6.6 ± 2.2
	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	200	18.1 ± 0.1	4.2 ± 0.6	30.1 ± 9.01	5.3 ± 0.1	3.0 ± 0.1	6.0 ± 0.0
	400	8.7 ± 6.1	3.8 ± 2.6	22.7 ± 16.08	4.6 ± 3.2	2.7 ± 1.9	5.5 ± 3.8
	Mean	**13.5**	**4.1**	**27.3**	**5.06**	**2.8**	**6.03**
JL24	0	17.7 ± 0.4	4.07 ± 0.6	30.5 ± 8.3	6.3 ± 0.4	2.9 ± 0.1	8.7 ± 3.8
	100	21.2 ± 4.5	4.5 ± 0.4	44.4 ± 2.8	5.9 ± 0.6	2.7 ± 0.3	8.3 ± 1.3
	200	20.7 ± 6.01	4.4 ± 0.6	27.3 ± 12.8	5.8 ± 0.2	3.1 ± 0.4	9.9 ± 2.2
	400	14.0 ± 1.6	3.6 ± 1.4	27.7 ± 0.3	4.2 ± 0.2	2.7 ± 0.1	6.1 ± 0.6
	Mean	**18.4**	**4.1**	**32.4**	**5.5**	**2.8**	**8.2**
LSD (p = 0.05)		7.6	0.9	16.2	1.1	0.9	3.1

effect for targeted descriptive traits. In the M1 generation, there was a 30% reduction of height for JL12, 18% for Kimpese irradiated at 200 Gy dose. An unexpected increase of 11% was observed for JL24 at the same dose (200 Gy). The levels of reduction were 13% for JL12 and 2% for Kimpese and JL24 for stem diameter. A similar level of reduction was observed for the 400 Gy treatment with 28%, 50% and 30% decrease of plant height for

JL12, Kimpese and JL24, respectively. Reduction of stem diameter was 6.5% for JL12, 14% for Kimpese and 30% for JL24. Significant decrease of the number of leaves per plant, leaflet length, and number of ramifications per plant was observed only for the 400 Gy treatment. In fact, for this treatment, 15%, 50% and 3% decrease of the number of leaves per plant were observed for JL12, Kimpese and JL24, respectively. Leaflet length change was observed at 400 Gy treatment for JL12 (−23%), Kimpese (−12.5%) and JL24 (−8%). For the number of ramifications per plant, the change was noted with 20% and 19% reductions in Kimpese and JL24, respectively.

There were significant differences in plant height and other morphological traits in the M2 generation derived from seeds treated with 100 Gy for the three varieties (**Table 5**). The level of reduction was 27% for JL12. By cons for JL24, the gamma irradiation at 100 Gy increased significantly plant height by 20%. The 200 Gy irradiation induced a significant reduction of plant height in JL12 (8%), an increase (31%) in Kimpese and JL24 (17%). A decrease of the leaflet length (11%) in JL12 and JL24 (8%), and an increase of number of ramifications per plant for JL12 (46%) and JL24 (14%) were observed.

4. Discussion

The results of the present study illustrated that gamma ray radiation is an efficient tool for increasing genetic variability and grain yield in groundnut varieties. The positive effect of low doses of gamma rays irradiation (100 Gy) on plant growth may be due to the stimulation of cell division or elongation, or the alteration of metabolic processes that affect the synthesis of phytohormones or nucleic acids [12,13]. In addition, high doses of gamma irradiation were reported to be harmful in several studies like that of Ramachandran and Goud [14], who reported that higher doses of gamma irradiation reduced plant height, number of leaves and branching capacity of safflower.

By comparison of the different treatments (different radiation doses) and the control (not irradiated), it was observed that there were significant alterations in the plant development and production in the M1 generation for all the gamma rays doses. Significant reduction of all the agro-morphometric characteristics was observed at that stage. Seeds treated at irradiation dose of 600 Gy did not survive to produce progenies for evaluation.

Higher exposures of gamma rays caused injury to seeds and affected seedling development. These data are consistent with other reports by Devi and Mullainathan [10] in blackgram, and Yakoob and Ahamad [15] in mungbeans. Many studies have shown that treatment with higher dose of gamma rays were inhibitory, whereas lower exposures were sometimes stimulatory. Gamma rays produce radicals that can damage and affect differentially plant morphology, anatomy, biochemistry, and physiology depending on the irradiation level.

On the other hand, the results of the M2 generation reported in the present study showed that it is possible to increase grain yields components to a dose of 100 Gy. Improvement of agronomic characteristics by using gamma radiation has been reported in several studies. Khan *et al*. [16] reported a significant increase of chickpea grain yield using gamma irradiation at 600 Gy. Gustafson *et al.* [17] developed a high yielding and early maturing barley by mutation breeding methods. Mudibu *et al.* [4] reported the highest grain yield increase in soybean irradiated with 200 Gy of gamma rays.

In the present study, an increase of number of pods per plant was observed in the varieties JL12 and JL24 for gamma irradiation at 100 Gy dose. Khan *et al.* [16] reported a decrease of pod number at 400 Gy treatment and an increase at 500 Gy without a change in the number of seed per pod. Similar results have been reported by Shakoor *et al.* [18] in mungbean, Devi and Mullianathan [10] in blackgram, and Kumar and Ratnam [19] in sunflower.

There were no significant changes of plant height between different irradiation treatments compared to the control in M2 generation. This is consistent with report by Shakoor *et al.*, Rao, and Khan *et al.* [16,18,20] in mungbean, and Mudibu *et al.* [4] in soybean. The reports of Rao [20] in pigeon pea and Khan *et al*. [21] in sorghum did not agree with these results. They observed that plant height increased with the application of gamma irradiation. This may be due to the different genetic material and environmental conditions.

The dose of gamma rays radiation is important for inducing genetic variation that can lead to positive effect in mutants. In fact, molecular analysis of similar set of groundnut using ISSR markers in a previous study has shown that gamma ray irradiation at a dose of 100 Gy gamma rays increased the level of genetic variability [22]. In fact the level of polymorphic loci observed was significantly increased by more than 37% when 100 Gy gamma rays treatment was compared with the control for the JL24 variety [22]. Bensliman and Khelifi [8] and, Ramani and Jadon [23] also reported that the highest genetic variation in M2 generation of groundnut was observed with the 100 Gy treatment. The analysis of M1 and M2 generations indicate that the grain yield gain and other beneficial effects generated with gamma ray radiation are heritable.

5. Conclusion

Mutation induction has proven to be a workable, sustainable, highly efficient, environmentally acceptable, flexible, unregulated, non-hazardous and a low-cost technology in the breeder's toolbox to enhance crop improvement. In the present study, the agronomic and morphological characteristics were improved by gamma ray treatments. Among the various dose treatments, 100 Gy of gamma rays treatment resulted in higher genetic variability and grain yield increase. This genetic gain can be stabilized over few generations through selfing. Overall, the results of the present study indicate that groundnut mutant lines will increase the variability of the current groundnut genepool in the DR-Congo. Additional agronomic, nutritional, and organoleptic analyses of the selected groundnut mutants are underway before multilocation evaluation.

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