



EFFECT OF GAMMA RAYS ON THE VARIABILITY OF YIELD ATTRIBUTING TRAITS AND GENETIC PARAMETERS IN FONIO (*DIGITARIA EXILIS* STAPF.)

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ABSTRACT

A research was conducted to investigate the extent of genetic variability induced by different doses of gamma rays with the aim of identifying the suitable dose to be employed in fonio (*Digitaria exilis*) improvement program. Seeds of five different accessions of fonio were irradiated with five different doses of gamma rays (100 Gy, 200 Gy, 400 Gy, 500 Gy and 0 Gy as control). The experiment was conducted during 2014 and 2015 wet seasons and was laid out in Randomized Completely Block Design with three replications. The result obtained from the M₂ generation revealed highly significant difference ($P \le 0.01$) in the effects of different doses of gamma rays in the five fonio accessions were found to be genoype dependents. The results for the estimation of genetic parameters revealed higher PCV and GCV values and that PCV values were slightly greater than GCV values for all the traits studied. However, moderate to high heritability among the mutants' traits indicates that the traits are primarily under genetic control. While, the predominance of moderate heritability estimates and high GAM in most of the traits revealed additive gene effects induced by the mutagen in controlling such traits indicating that selection for improvement might be effective. Thus, 100 Gy is found to be suitable for fonio improvement.

Keywords: Doses, Fonio, Gamma Rays, Genetic Estimates.

INTRODUCTION

Fonio (*Digitaria exilis* Stapf.) is known as Acha or hungry rice (Purseglove, 1972). It is traditionally grown in the savannah zone of West-Africa. Due to its hardy nature, this traditional millet is regarded as priority crop in West-Africa, where it is essential to the diets of millions of people in Africa and has high value in their cultural traditions. This native African grain crop fits perfectly into the low-input farming systems of resource-poor African farmers because of its unique ability to withstand diseases, drought and tolerate poor soils (Aslafy, 2003). Fonio improvement requires understanding of the genetic mechanism governing yield and yield components. The grain yield is basically a complex trait being the consequence of several genes and their interaction as stressed by Rashid *et al.* (2012).

Despite all the tremendous benefits of fonio to human livelihood, it is generally unimproved and its cultivation is not beyond subsistence level in Nigeria. There is neglect from the authorities concerned as there is no single fonio variety registered in the country. Farmers grow traditional landraces with poor yields and there is paucity of information on the inheritance of agronomic traits in fonio which makes its germplasm analysis to depend on phenotypic traits that can easily be influenced by the environment. More so, the miniature size of its grains and floral organs make hybridization very difficult to practice. Researches (Irving and Jideani, 1997; Kwon-Ndung and Misari, 1999) have previously reported fonio facing the problems of low yield and narrow genetic variability; yet its genetic improvement is not well explored. Mutations

(changes in the genetic materials of an organism) are known to enhance the genetic variability of crop plants. Induced mutations have been used to generate genetic variability and have been successfully utilized to improve yield components of various crops (Naik and Murthy, 2009). It provides raw materials for the genetic improvement of economic crops (Adamu *et al.*, 2004) by facilitating the isolation, identification and cloning of genes which would ultimately help in designing crops with improved yield, increased stress tolerance and longer life span with reduced agronomic in-puts (Ahloowalia and Maluszynski, 2001). Gamma irradiations have been known to produce changes in plants by improving growth and yield components. This research therefore aimed at creating variability in fonio using different doses of gamma rays that could be utilized in the genetic improvement of the crop.

MATERIALS AND METHODS

Experimental site

The research was conducted at the Botanical Garden of the Department of Botany, Ahmadu Bello University, Zaria, located within the northern guinea savanna zone of Nigeria (Lat 11° 11' N; Long 7°N 38'E and on alt 660m above sea level).

Sources of the Seeds

Seeds of five different accessions of fonio used for this research were obtained from the National Cereal Research Institute, Badeggi, Niger state.

Treatment and Experimental Design

The seeds of five fonio accessions (Dinat, Jakah, Jiw 1, Jiw 2 and Nkpowas) were exposed to 60 Co gamma rays at five different doses (100 Gy, 200 Gy, 400 Gy, and 500 Gy) at the Plant Breeding and Molecular Biology Laboratory, International Atomic Energy Agency (IAEA), Vienna, Austria. The treated seeds of the accessions were sown along with respective controls to raise the M₁ generation. The seeds were planted in a Randomized Completely Block Design (RCBD) with three replications. All the recommended agronomic and cultural practices such as planting, fertilizer application, weeding and thinning as well as harvesting methodologies were done according to the procedures described by NAERLS (2012) to raise a good crop. Ten competitive plants were selected from each accession in each replication of the M₂ generation.

DATA ANALYSES

Multivariate analysis of variance was carried out using SAS (2008) version 9.0 with DMRT to separate the means. Probit analysis was used to determine the LD_{50} of different mutagenic treatment in each accession of fonio. The variability present in the population was estimated from combined analysis of variance by their mean performance. Phenotypic variance (PV), genotypic variance (GV), phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated based on the formula described by Syukur *et al.* (2012) as follow:

 $\sigma^{2}_{G} = \left[(MSG) - (MSE) \right] / r$

 $\sigma^2_{\rm P} = [\sigma^2_{\rm G} + (\sigma^2 E/r)].$

Where: σ^2_G = Genotypic variance; σ^2_P = Phenotypic variance; $\sigma^2 E$ = environmental variance (error mean square from the analysis of variance); MSG = mean square of genotypes; MSE = error mean square; r = number of replications. GCV (%) = $\sqrt{(\sigma^2_G)} \times 100$

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PCV (%) = $\frac{\sqrt{(\sigma^2 P)}}{x} \times 100$

Where: σ^2_G = Genotypic variance; σ^2_P = Phenotypic variance; x is grand mean of a character. The Broad Sense Heritability (H²), Genetic Advance (GA) and Genetic Advance as percent of the Mean (GAM) were estimated according to the formula described by Singh and Choudhury (1985).

$$H^{2}(\%) = \frac{\sigma^{2}G}{\sigma^{2}P} \times 100$$

 $GA = k\sigma_P H^2$

Where k is the selection differential in standard units in the present study and it was 2.06 at 5% level of selection, σ_P is standard deviation of the phenotypic variance and H² is Broad sense heritability. Genetic advance expressed as percentage of mean (GAM) was measured by the following formula: GAM (%) = GA x 100

PCV, GCV, heritability, G,A and GAM were classified as follows:

PCV and GCV: 0 - 10 % =Low, 10 - 20 % = Moderate and >20 % = High

 $H^2: 0 - 30 \% = Low, 30 - 60 \% = Moderate and >60 \% = High.$ GA: 0 - 10 % = Low, 10 - 20 % = Moderate and >20 % = High.

RESULTS

The result for the effect of gamma rays on growth and yield attributing traits of fonio is shown in Table 1. The result revealed highly significant difference ($P \le 0.01$) in the effect of the mutagen on all the selected traits of fonio. It revealed that, gamma irradiations produced mutants that displayed germination percents of 46.74-71.50% after one week of planting. The result showed that the mutants attained heights of 3.32-3.60 cm at seedlings stage and 42.54-55.73 cm at matured stage. The mutants produced 3-7 leaves that were 4.19-7.53 cm² in cross sectional area and which produced 11-18 tillers. More so, the mutants were found to produce 3-6 spikes that were 8.27-10.86 cm long and which bear 63-113 seeds/spike. Furthermore, 1000 seeds weights of the mutants were measured to be 0.55-0.61 g and produce 6.51-10.15 g of dry matter contents.

Table 1: Means for the Effect of Gamma Ra	vs on Agronomic Traits of Fonio
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Dose	%Germinatio	Seedling	Height	No. of	Leaf	No.	No.	Spikes	No. of	1000	Dry
(Gy)	n (1 WAP)	s Height	at	Leave	Area	of	of	Lengt	Seeds/spik	Seeds	Weigh
		(cm)	Maturit	S	(cm ²	Tiller	Spike	h (cm)	e	Weigh	t (g)
			y (cm))	S	S			t (g)	
0	73.43 ^{a*1}	4.16 ^a	71.27 ^a	3.33 ^d	5.21°	11.87 d	3.40°	9.10 ^b	77.60 ^d	0.57 ^d	7.11 ^{dc}
100	71.50 ^a	3.60 ^b	55.73 ^b	7.27 ^a	7.53 ^a	17.80 a	5.86 ^a	10.86 ^a	112.80 ^a	0.61 ^a	10.15 ^a
200	62.90 ^b	3.57 ^b	52.25°	5.60 ^b	6.35 ^b	15.73 b	4.80 ^b	11.18 ^a	104.13 ^b	0.60 ^b	8.98 ^b
400	56.37°	3.41°	47.80 ^d	5.00 ^c	5.03°	13.53 c	3.53°	10.64 ^a	89.93°	0.59°	7.42 ^c
500	46.74 ^d	3.32 ^d	42.54 ^e	3.33 ^d	4.19 ^d	10.66 d	2.53 ^d	8.27 ^c	63.40 ^e	0.55 ^e	6.51 ^d
Mean s	62.19	3.61	53.91	4.91	5.66	13.92	4.02	10.01	89.57	0.59	8.03
S.E	5.84	0.12	1.86	0.70	0.75	1.78	0.76	0.88	6.45	0.01	0.83

N.B: *1 Means within the columns with the same superscript letter(s) are not significantly different (P≤0.05)

However, significant variation was found in the LD_{50} among the accessions (Table 2). The LD_{50} values of gamma rays in the five fonio accessions were found to range from 180 Gy (in accession Jiw 1) to 228 Gy (in accession Dinat and Jiw 2). This result showed that the LD_{50} values are genotype dependents.

Table 2: Lethal Doses	Values of Different	Gamma Rays in	Different Fonio Accessions
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Accession	%Germination	% of	% Reduction	Observed	% LD ₅₀
	(2 WAP)	Control	over Control	Mortality	
Dinat	49.66	59.29	20.71	43	228Gy
Jakah	43.17	61.40	18.56	47	204Gy
Jiw 1	43.98	55.83	24.17	48	180Gy
Jiw 2	40.91	59.80	20.20	49	228Gy
Nkpowas	48.77	63.90	16.09	43	204Gy

More so, the estimates of phenotypic and genotypic coefficient of variation among fonio mutants induced by different doses of gamma rays are presented in Table 3. The PCV values were found to be slightly higher than the GCV values in all the different gamma rays induced mutants of fonio (except in seedlings heights). The PCV and GCV values expressed in terms of percentages were higher at 500 Gy doses of gamma rays for all the quantitative traits of gamma-induced fonio mutants. The GCV and PCV values were high at some of the growth and yield parameters of 500 Gy induced mutants of fonio such as number of leaves (47.19%; 65.59%), leaf area (34.67%; 47.97%), number of tillers (54.97%; 77.15%), number of spikes (34.46%; 45.58%) and spikes length (24.33%; 33.86%).

Furthermore, high heritability estimates (Table 3) were induced by different doses of gamma rays in most of the fonio traits except in germination percents (1 WAP) and dry weights where moderate values were found and survival percents where low values were found (Table 20). The genetic advance (GA) and genetic advance as percent of the means (GAM) values were high in almost all doses of gamma rays treatment. However, 100 Gy of gamma rays revealed highest values of heritability in terms of number of leaves/plant (71.97%), number of seeds/spike (75.61%) and number of days to maturity (73.04%). Similarly, high Genetic Advance (GA) and Genetic Advance as percent of the Means (GAM) estimates were recorded to be induced by all the gamma ray doses in fonio. However, the highest values of GA and GAM were found among 500 Gy induced mutants of fonio. The GA and GAM values were found to increase with increased in gamma rays doses.

Table 3: Estimates Genetic Param	eters Induced by Different Do	ses of Gamma Rays on A	Agronomic Traits of Fonio
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Dees	Demander	%Germ	SH	HM	NI	LA	NT	NC	SL	NG/C	1000 SW	$\mathbf{DW}(\mathbf{z})$
Dose	Parameter	(1WAP)	(cm)	(cm)	NL	(cm ²)	IN I	INS	(cm)	N2/2	(g)	Dw (g)
Control	GCV	1.66	6.79	6.28	47.19	27.88	49.37	25.64	22.11	2.13	1.47	1.99
	PCV	3.02	4.81	8.76	65.10	38.57	69.28	33.92	30.76	3.04	1.75	4.87
	H^2	54.97	99.70	71.69	72.49	72.28	71.26	175.59	71.88	70.07	84.00	40.86
	GA	196.77	535.99	437.09	1204.83	924.77	1221.87	906.69	821.23	122.16	228.91	185.76
	GAM	267.97	12884.38	613.29	36181.08	17749.90	10293.77	26667.35	9024.51	157.42	40159.65	2612.66
100 Gy	GCV	1.71	7.86	8.04	21.62	19.29	32.92	14.88	18.53	1.46	1.37	1.39
	PCV	3.11	5.56	11.20	30.04	26.69	46.20	19.68	25.78	2.09	1.64	3.41
	H^2	54.98	141.36	71.78	71.97	72.27	71.26	75.61	71.87	69.86	83.54	40.76
	GA	199.75	565.43	494.89	817.45	769.19	997.71	690.96	751.79	208.04	220.38	155.06
	GAM	279.37	15706.39	888.01	11244.15	10215.01	5605.11	11791.13	6922.56	184.43	36127.87	1527.68
200 Gy	GCV	1.94	7.92	8.57	20.07	22.88	37.25	18.16	18.00	1.59	1.39	1.58
	PCV	3.53	5.60	11.87	39.00	31.65	52.28	24.03	25.04	2.27	1.67	3.86
	H^2	54.96	141.42	72.19	51.46	72.29	71.25	75.57	71.88	70.04	83.23	40.95
	GA	212.71	689.44	248.75	662.13	837.79	1061.26	763.14	746.32	217.39	221.57	165.66
	GAM	338.17	19312.05	476.08	11823.75	13193.54	6746.73	15898.75	6675.49	208.77	36928.33	1844.77
400 Gy	GCV	2.17	8.29	9.37	31.43	28.88	43.31	24.69	18.91	1.84	1.42	1.91
	PCV	3.94	5.87	13.06	43.68	39.96	60.78	32.67	26.32	2.62	1.69	4.67
	H^2	55.08	141.23	71.75	71.96	72.27	71.26	75.57	71.85	70.23	84.02	40.89
	GA	225.21	704.86	534.12	979.65	941.13	1144.39	889.84	759.30	234.17	225.02	182.03
	GAM	399.52	20670.38	1117.41	19593	18710.34	8458.17	25207.93	7136.28	260.39	4240.68	2453.23
500 Gy	GCV	2.61	8.52	10.53	47.19	34.67	54.97	34.46	24.33	2.61	1.52	2.17
	PCV	4.76	6.02	14.67	65.59	47.97	77.15	45.58	33.86	3.72	1.82	5.32
	H^2	54.83	141.53	71.78	71.95	72.27	71.25	75.60	71.85	70.16	83.52	40.79
	GA	246.44	715.33	566.34	1200.32	1031.18	1289.21	1051.47	861.32	278.76	232.09	193.81
	GAM	527.26	21546.08	1331.31	36045.65	24610.50	12093.90	41560.08	10414.99	439.68	42198.18	2977.11

N.B: PCV and GCV: 0 - 10 % = Low, 10 - 20 % = Moderate and >20 % = High.

 $H^2: 0 - 30 \% = Low, 30 - 60 \% = Moderate and >60 \% = High.$

GA: 0 - 10 % = Low, 10 - 20 % = Moderate and >20 % = High.

% Germ = % germination SH= Seedlings heightHM= Height at MaturityNL= Number of LeavesLA=LeafAreaNT= Number of TillersNS= Number of SpikesSL= Spikes LengthNS/S= Number of Seeds/SpikeSD= SeedDiameter 1000SW=1000 Seeds WeightDW= Dry WeightNDM= Number of Days to Maturity

DISCUSSION

Seed germination is an important parameter to estimate the effect of mutagen on plants. Inhibition in seed germination, after the treatment of seed with different mutagen is a convenient technique for studying their effects of mutagens on plants as reported by Wani et al. (2013). The decreased in seed germination with increasing dose of gamma rays doses in fonio indicates that the mutagens have exerted an inhibitory effect on seed germination at higher doses. Such a dose dependant inhibition of germination was reported earlier by Kumar and Mishra (2004) in Okra. Reduction in germination in mutagenic treatments has been explained due to delay or inhibition of physiological and biological processes necessary for seed germination, which include enzyme activity, hormonal imbalances and inhibition of mitotic process as reported by Ananthaswamy et al. (1971). Similar results of decrease in the level of germination via induced mutagenesis were reported by Talebi et al. (2012).

Reduction in plant heights in fonio due to gamma irradiation was similar to the previous findings by Toker *et al.* (2005) who reported decrease in shoot length at high dose. However, disparity in the effects of gamma radiation on plant growth was reported by Melki and Marouani (2009). The induced reduction in plant height caused by gamma rays could be attributed to the ability of gamma rays to cause DNA damage and interrupt the metabolic pathway as stressed by Esnault *et al.* (2010) thereby slowing growth process. However, Marcu *et al.* (2013) reported a contrary finding reporting growth stimulation by the effect of a low dose of gamma rays.

The significant improvement in fonio yield due to low gamma rays exposure reported by the present study is in conformity with the work of Tshilenge-Lukanda et al. (2013) who reported similar observation among gamma rays induced mutants of groundnut. It therefore implies that it is possible to improve yield components of economic plants using a gamma dose of 100 Gy. This finding is in conformity with the work of Kara et al. (2016) in Glycine max and Suresh et al. (2017) in Phaseolus lunatus. More so, Mudibu et al. (2010) reported a result that was slight different from the finding of this research as they recorded highest grain yield increase in soy- bean among 200 Gy gamma irradiated mutants. However, the findings of this study were almost similar to that of Animasaun et al. (2014) who reported 80 Gy gamma ray dose as low dosage form of gamma irradiation using cobalt (60) that could be utilized to increase variability and yield in Digitaria exilis but is contrary to the findings of Khan et al. (2005) who reported a significant increase of chickpea grain yield using gamma irradiation at 600 Gy. The 100 Gy dose of gamma rays radiation is important for inducing genetic variation that can lead to genetic improvement among fonio mutants.

The LD₅₀ values of gamma rays obtained by this research were fixed between 180 Gy (in accession Jiw 1) to 228 Gy (Dinat and Jiw 2). These values were almost similar to that of Rajarajann *et al.* (2016) who reported LD₅₀ values of gamma rays in rice to be 229Gy *in vitro* and 235Gy *in vivo*. Similarly, the LC₅₀ values of colchicine concentrations in the present study were fixed between 0.35 mM (Nkpowas) to 0.90 (Jakah). These values were within the range obtained by Jency *et al.* (2017) who reported the LC₅₀ concentration for EMS to be fixed at 0.42% *in vitro* and 0.49% *in vivo* in Kodomillet and is in line with the findings of Ramchander *et al.* (2015) in some rice varieties.

The slight differences between PCV values above GCV values indicate the presence of high contribution of genotypic effect for phenotypic expression of the selected traits and that there is high genetic variability for the traits. This finding is in conformity with that of Yohannes et al. (2015) who reported similar finding in sorghum. More so, the low differences between PCV and GCV implies that the characters studied were influenced by environment to lesser extent, thus the selection based on phenotypic performance will be reliable as reported by Islam et al. (2009). The presence of moderate to high heritability in most of the traits was similar to the work of Chee and Campbell (2009) and Liu et al. (2010) who reported similar findings. Heritability values are helpful in predicting the expected progress to be achieved through selection process. Moderate to high heritability, G.A and GCV estimates coupled with high GAM explained the influence of additive gene effect as reported by Ibrahim and Hussein (2006) and that heritability is a property of a character in the population, environment and the conditions of the genotypes as stressed by Yadav et al. (2011). This also conforms to the earlier findings of Vashistha et al. (2013) in maize, Wolie et al. (2013) in finger millet, Ogunbayo et al. (2014) in rice, Mishra et al. (2014) in gladiolus and Shrimali et al. (2017) in barley that these parameters were important for selection. Induction of mutations for factors which govern the heredity of quantitative characters are therefore promising tools for creating new genotypes as reported by El-Degwy (2013) thereby causing changes in major genes at loci governing quantitative characters. Thus, mutants with such characters need to be selected for improvement. Broad sense heritability (H²) therefore represents the relative strength of the traits and indicates the efficiency of selection systems as reported by Hugar et al. (2015).

CONCLUSION

It was concluded that lower doses of gamma rays (100 Gy) induced high genetic variability in fonio and the LD_{50} fixed between 180 Gy (in accession Jiw 1) to 228 Gy (Dinat and Jiw 2). It was found that moderate to high heritability, GA and GAM estimates were found in most of the traits studied indicating that selection for improvement might be effective.

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