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MUTAGENIC EFFECTIVENESS AND EFFICIENCY OF GAMMA RAYS ON VARIABILITY FOR YIELD ATTRIBUTING TRAITS IN FONIO (*DIGITARIA EXILIS* [KIPPIST] STAPF.)

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ABSTRACT

A study was conducted to determine the mutagenic effectiveness and efficiency of different doses of gamma rays in inducing variability that could be exploited in the genetic improvement of fonio. Seeds of five 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 seeds were sown in a plot in a completely randomized design with three replications to rise the M_1 generation which was advanced to M_2 generation. The result obtained from the M_2 mutants revealed highly significant difference ($P \leq 0.01$) in the effects of different gamma rays doses on the growth and yield traits of fonio. This implies the presence of high genetic variability induced by the mutagen in fonio. The effect is concentration dependent, increase with decrease in irradiation dose. More so, the effectiveness of the mutagen decreases with increase in dose. Seven different chlorophyll deficient mutants were found in the form of: Albina, Chlorina, Lustescent, Striata, Viridis, Viriscent and Xantha, with Xantha, Chlorina and Albina as the most frequent chimeras, The result indicated that lower dose of gamma rays (100 Gy) is more effective and efficient as it induces favorable mutation that could be utilized in the genetic improvement of fonio. Accession Nkpowas was found to respond more to the mutagenic treatment.

Keywords: Accessions, Fonio, Gamma Rays, Mutation.

INTRODUCTION

Fonio (*Digitaria exilis* [Kippist] Stapf.) is known popularly as Acha, hungry rice, petit mil, fundi or findi (Jideani, 1999). The English name "Hungry Rice" attributed to fonio was believed to have been coined by Europeans (Kwon-Ndung and Misari, 1999; Ibrahim, 2001). Fonio is a minor cereal crop cultivated in the savanna of West Africa as a staple food or food for hunger period. The crop supplies food to 3-4 million people (Vodouhe and Achigan Dako, 2007). Besides these, fonio is one of the world's fastest maturing cereals producing grains just 6 to 8 weeks (42 to 56 days) after planting for the extra early varieties (Ibrahim, 2001). The late varieties take up to 150 days to grow (CIRAD, 2004). It is traditionally grown in the savannah zone of West-Africa where it has high socio-cultural and economic value. Fonio is also highly prized by nutritionists due to its rich contents of two vital amino acids (methionine and cysteine) that are vital to human health and deficient in today's major cereals like wheat, rice, maize, sorghum or barley (Barikmo and Ouattara, 2004).

Despite its tremendous nutraceutical and economic importance, fonio is underdeveloped, because of lack of attention by research and extension services. Although very important for its organoleptic qualities, the crop is neglected because of its low yielding capacity and its tedious work requirement at postharvest transformation (Vodouhe and Diallo, 2004). Thus the plant is threatened by genetic erosion due to neglect by the authorities concerned.

Mutations (changes in the genetic materials of an organism) are known to enhance the genetic variability of crop plants (Pathirana *et al*., 2002). Induced mutations facilitate the development of improved varieties (Maluszynski, 1990). 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 inputs (Ahloowalia and Maluszynski, 2001). Gamma rays are widely employed for mutation studies as they have shorter wave length and therefore, possess more energy per photon than X-rays and penetrate deep into the tissue (Khin, 2006; Zhu *et al*., 2006). Gamma radiation is used as a physical mutagen for fonio improvement due to the fact that its effects have been well studied and it is known to generate point mutations (Zhu *et al*., 2006). It is known to induce chlorophyll deficiency (Kumari *et al*., 2016) among mutants that served as markers in genetic, physiological and biochemical investigations. They are the most frequently observed and easily identified factorial mutations in M_2 generation serving as the most dependable index for determining the extent of induced genetic changes in the mutagenic treated population and effectiveness of mutagenic treatments (Gustafsson , 1940). Improvement in the frequency and spectrum of mutations in a predictable manner and thereby achieving desired plant characteristics for their either direct or indirect exploitation in the breeding programme is an important goal (Patial *et al.,* 2017). This research therefore aimed at investigating the frequency and spectrum of gamma rays-induced mutagenesis and variability in some agronomic traits of fonio that could be used in the genetic improvement of the crop.

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MATERIALS AND METHODS

Study Site

The research was conducted at the Botanical Garden of the Department of Biological Sciences, Ahmadu Bello University, Zaria, located within the tropical guinea savanna zone of Nigeria (Lat 11^0 11^1 N; Long 7^0N 38^1E and on alt 660m above sea level) (Abbas and Arigbede, 2012) during the 2015 wet season.

Sources of the Seeds

Seeds of five different accessions of fonio (Dinat, Jakah, Jiw 1, Jiw 2 and Nkpowas) used were obtained from the National Cereal Research Institute, Badeggi, Niger state.

Treatment and Experimental Design

1000 g of the seeds of five fonio accessions (200 g from each accession) were exposed to ^{60}Co gamma rays at four 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 irradiated seeds were kept sealed in air-tight container to prevent oxygen effect until the period of sowing. The control is pre-soaked in distilled water for four hours, allowed to dry over night at room temperature on Whatman No. 1 filter paper. The irradiated treated seeds were sown in the field plots along with untreated control at 20 cm and spacing between stands. The seeds soaked in distilled water served as control. The seeds were planted in a plot in a Completely Randomized Design (CRD) in a factorial arrangement with three replications to rise the first mutant generation (M_1 generation). The M_1 generation mutants showing desirable characters were selected and were advanced to M_2 generation. 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. The chlorophyll deficient mutants were categorized from the M_2 generation and mutation frequency was estimated as below:

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutants/plant}}{\text{Total number of plants scored}} \times 100$$

Mutation effectiveness and efficiency were estimated according to Sasikala and Kamala (1988) as follows:

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency}}{\text{Dose (Kr x time)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency}}{\text{Percentage lethality in } M_1} \times 100$$

Statistical Analysis

Based on the observations recorded on quantitative traits of both the five accessions of fonio were subjected to Multivariate Analysis of variance (MANOVA) using SAS (2008) Version 9.1. Duncan's multiple range tests (DMRT) was used to separate the means.

RESULTS

The result for the effects of different doses of gamma rays on the M_2 generation of fonio is presented in Table 1. The result showed highly significant difference ($P \leq 0.01$) in the effects of different doses of gamma rays on growth and yield traits of fonio; indicating the presence of high variability induced by the mutagen in fonio. The gamma treatments produced mutants with reduced germination rates that are shorter in stature with high number of leaves. Similarly, the gamma rays induced mutants produced high number of tillers and spikes that are longer and produce more number of seeds that weigh higher than the controls. Similarly, the number of days to maturity was significantly reduced among the gamma rays induced mutants of fonio. The effect is dose dependent, decreases with increase in radiation dose. Furthermore, the result of the responses of five accession of fonio to different doses of gamma rays was presented in Table 3. The result showed that accession Nkpowas showed the highest responses to the mutagen treatment.

Table 1: M_2 Generation Mean Effect of Gamma Irradiation on Yield Attributing Traits of Fonio Accessions

Dose (Gy)	% Germination (1 WAP)	Height at Maturity (cm)	No of Leaves	No of Tillers	No of Spikes	Spikes Length (cm)	No of Seeds/ spike	1000 Seeds Weight (g)	No of Days to Maturity
0	73.43^{a*1}	71.27^{a}	3.33^{d}	11.87^{d}	3.40^{c}	9.10^{b}	77.60^{d}	0.57^{d}	123.27^{a}
100	71.50^{a}	55.73^{b}	7.27^{a}	17.80^{a}	5.86^{a}	10.86^{a}	112.80^{a}	0.61^{a}	94.53^{d}
200	62.90^{b}	52.25^{c}	5.60^{b}	15.73^{b}	4.80^{b}	11.18^{a}	104.13^{b}	0.60^{b}	97.20^{c}
400	56.37^{c}	47.80^{d}	5.00^{c}	13.53^{c}	3.53^{c}	10.64^{a}	89.93^{c}	0.59^{c}	98.40^{c}
500	46.74^{d}	42.54^{e}	3.33^{d}	10.66^{d}	2.53^{d}	8.27^{c}	63.40^{e}	0.55^{e}	105.80^{b}
Means	62.19	53.91	4.91	13.92	4.02	10.01	89.57	0.59	103.84
S.E (±)	5.84	1.86	0.70	1.78	0.76	0.88	6.45	0.01	2.94

N.B: ^{*1} Means within the columns with the same superscript letter(s) are not significantly different ($P \leq 0.05$)

Table 2: M_2 Generation Mean Responses of Fonio Accessions to Gamma Irradiation

Accession	% Germination (1 WAP)	Height at Maturity (cm)	No of Leaves	No of Tillers	No of Spikes	Spikes Length (cm)	No of Seeds/ spike	1000 Seeds Weight (g)	No of Days to Maturity
DINAT	63.60^{a*1}	49.53^{c}	4.07^{c}	13.67^{cb}	3.87^{b}	10.21^{b}	89.73^{a}	0.59^{a}	102.00^{b}
JAKAH	61.60^{a}	55.93^{b}	6.60^{a}	19.20^{a}	3.47^{b}	10.29^{ba}	89.13^{a}	0.58^{ba}	101.53^{b}
JIW 1	61.81^{a}	52.67^{b}	4.33^{b}	12.86^{c}	3.80^{b}	7.81^{c}	91.33^{a}	0.57^{b}	106.40^{a}
JIW 2	60.33^{a}	56.32^{a}	4.80^{b}	8.93^{d}	4.07^{b}	10.83^{ba}	90.33^{a}	0.59^{a}	101.40^{b}
NKPOWAS	63.59^{a}	55.13^{a}	4.73^{b}	14.93^{b}	4.93^{a}	10.92^{a}	87.33^{a}	0.58^{ba}	107.87^{a}

N.B: ^{*1} Means within the columns with the same superscript letter(s) are not significantly different ($P \leq 0.05$)

The result obtained for the spectrum and frequency of chlorophyll deficient mutants induced by different doses of gamma irradiations in fonio was presented in Table 3. The result showed that seven different chlorophyll deficient mutants (albina, chlorina, lustescent, striata, viridis, viriscent and xantha) were induced by different doses of gamma rays in fonio; with Xantha, Chlorina and Albina as the most frequent chimeras and that the frequency increases with increase in irradiation doses. The frequency of chimeras showed that Xantha> Chlorina>Albina. Xantha was identified by its display of leaf color variation from orange-yellow to faint yellowish white. Chlorina was characterized by its possession of persistent green colored leaves and it is a viable mutation. Viridis was characterized by reduced height and showed a dark green foliage colour. Albina was white, lethal and survived for up to 10-20 days after germination. Striata was characterized by having longitudinal stripes of yellow color on the green colored part of a leaf or white stripes on the green leaf parts. Virescent was identified by yellow-green coloration of the leaves which later gradually changes to green. Lustescent was characterized by having green coloration of the leaves but later the pigments fade. The plants were slow growing and had low seed yield. Among the seven categories of chlorophyll deficient mutants observed, Xantha and Lustescent are lethal, while Chlorina, Striata and Virescent are viable. Albina and

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xantha mutants were dying within 10 to 20 days after emergence. In some instances chlorina mutants survived. Similarly, the efficiency of the gamma rays induced mutagenesis in fonio increases with increase in radiation dose. However, the effectiveness decreases with increase in the radiation dose

Table 3: Mutation Frequency, Effectiveness and Efficiency Induced by Gamma Rays on Fonio

Acceession	Dose (Gy)	No of Plants	Chlorina	Xantha	Albina	Viridis	Lustescent	Striata	Virescent	TOTAL	% Lethality	Mutation Frequency	Mutation Effectiveness	Mutation Efficiency
Dinat	100	46	2	3	2	1	0	0	1	9	34.33	19.57	1.96	57
	200	42	2	5	1	2	1	1	2	14	42.34	33.33	1.67	79
	400	44	4	6	3	3	2	3	3	24	54.67	54.55	1.36	99
	500	41	6	8	5	4	3	4	4	34	66.67	82.93	1.66	124
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	173	14	22	11	10	6	8	10	81	198.01	46.82		24
Jakah	100	38	2	4	2	0	0	2	0	10	38.67	26.32	2.63	68
	200	42	4	6	3	1	0	2	0	16	43.67	38.09	1.91	87
	400	47	5	6	4	3	1	2	0	21	52.34	44.68	1.12	85
	500	44	6	7	5	5	2	3	1	29	69.00	65.91	1.32	96
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	171	17	23	14	9	3	9	1	76		44.44		
Jiw 1	100	32	4	4	3	0	0	1	0	12	39.34	37.5	3.75	95
	200	38	4	5	4	1	0	2	2	18	40.67	47.37	2.37	117
	400	41	5	6	5	3	1	3	3	26	66.33	63.42	1.59	96
	500	40	5	6	5	4	2	4	5	31	69.00	77.5	1.55	112
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	151	18	21	17	8	3	10	10	87				
Jiw 2	100	38	2	3	1	0	0	1	0	7	40.00	18.42	1.84	46
	200	40	3	3	2	1	0	2	1	12	37.00	30	1.50	81
	400	37	4	4	3	2	1	3	1	18	52.67	48.65	1.22	92
	500	35	5	6	3	3	2	4	2	25	70.34	71.43	1.43	102
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	150	14	16	9	6	3	10	4	62			0	
Nkpowas	100	40	2	3	2	1	1	-	-	9	37.33	22.50	2.25	60
	200	44	3	4	3	2	1	-	1	14	42.34	31.82	1.59	75
	400	41	4	4	5	3	2	1	1	20	64.67	48.78	1.22	75
	500	43	5	6	5	4	3	2	2	27	70.67	62.79	1.26	89
	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	168	14	17	15	10	7	3	4	70				

DISCUSSION

Induced mutation was reported by Bartley *et al*. (1994) to be useful in the genetic control of carotenoid bio-synthesis in certain plants like *Arabidopsis thaliana, Lycopersicon esculentum, Zea mays* and other species of plants. The whole range of chlorophyll mutation occurred due to the deficiency in either: chlorophylls, carotenoids or combination of both; resulting from mutation in a number of loci as reported by Adamu (1992) or in plastid genes causing variegation as reported by Kirk and Tilmey Basset (1978). Monogenic inherited sub-lethal changes such as albina, xantha or alboviridis-chlorophyll mutations in barley, spectabilis in *Antirrhinum majus* or anthocyanin factor in pea when heterozygous was reported by Maluszynski *et al*. (2001) to surpass the original pure line and express significant hybrid vigor. However, the presence of high frequency of Xanthas is in conformity with the findings of Bara *et al*. (2017) who reported similar result in chickpea (*Cicer arietinum* L.) but contradicts the findings of Umavathi and Mullainathan (2016) who reported the highest frequency of Viridis among gamma rays induced mutants of chickpea. Similarly, Kumari *et al*. (2016) reported increased in the frequency of chlorophyll deficient mutants in sesame with increased in the dose of gamma rays but with Chlorina as the most predominant. Chlorophyll deficient mutants may have certain deficiencies emanating from alteration/suppression of photosynthesis pigments. This may include the survival rates of plants and their germination rates due to alteration in the quality and/or quantity of photosynthates to be formed. The higher mutagenic effectiveness in 100 Gy treated plants is in conformity with the findings of Sidhya and Pandit (2015) who reported similar result among gamma rays induced mutants of snake gourd (*Trichosanthes anguina* L.). The high mutagenic efficiency of higher gamma rays dose (500 Gy) could be attributed to the magnitude of damage induced in M_1 generation genes. This finding is in line with that of Sharma *et al*. (2006) in Urdbean.

Artificial induction of mutation using gamma rays in crop plants have displayed significant role in the improvement of their growth and yield attributes. The increased in growth and yield components of fonio due to low doses of gamma irradiation is in conformity with the findings of Yadav *et al*. (2016) who reported significant improvements in growth and yield attributes among low doses induced-mutants of *Zea mays*. The decreased in the germination percentages of fonio with increase in the dose of gamma rays is in line with the findings of Kiong *et al*. (2008) who reported decreased in seed germination with increasing radiation dose as high doses of gamma radiation induce chromosomal damage that interferes with germination process by probably reducing growth regulators such as cytokinins by breaking them down or inhibits their synthesis. Low doses of gamma rays therefore were found to

induce growth stimulation signals by increasing the antioxidative ability of cells or by changing the hormonal signaling in plants as stressed by Ali *et al*. (2016).
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 (2010). The induced reduction in plant height caused by gamma rays could be attributed to the ability of gamma rays to interrupt the metabolic pathway as stressed by Esnault *et al*. (2010) and Vandenhove *et al*. (2010) thereby slowing growth process. But Marcu *et al*. (2013) reported a contrary finding reporting growth stimulation by the effect of a low dose of gamma rays. However, 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 Mokobia *et al.* (2006) in *Zea mays* and *Arachis hypogaea,* Rahimi and Bahrani (2011) in *Brassica napus*, 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. Similar results have been reported by Kumar and Ratnam (2010) in sunflower. However, the findings of this study are 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 chick-pea 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 mutants.

Conclusion
It was concluded that seven spectrums of chlorophyll deficient mutants were induced by gamma irradiations and 100 Gy is the most effective producing higher yield. Similarly accession Nkpowas response to the mutagens treatments is better. It was also found that the effects of the mutagen were dose dependent, decrease with increase in the radiation. Thus, 100 Gy is found to be the best dose for fonio improvement by induced mutagenesis using gamma rays.

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