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Combined Effects of Gamma Rays and Sodium Azide on Yield Components of Selected Varieties of Guinea Corn (*Sorghum Bicolor L*)

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Abstract: *The seeds of some varieties of guinea corn were treated with a combination of gamma rays and sodium azide at 20, 30, 40 and 100 Kilorads at the centre for energy research and development, Obafemi Awolowo University (OAU) Ile Ife, Oyo state, Nigeria. The treated seeds were later soaked in 3mM and 4mM respectively with the aim of assessing the desirable effect of these mutagenes on the yield components of the varieties used. The difference was significant for all traits studied. However, there was generally an inverse relationship between the mutagens and the varieties. As the dose/concentration of the mutagens increased, there was decrease in height at maturity, number of grains per spikelet and leaf length. The variety samsorg 4 was most tolerant to the mutagenic treatments while a combination of 20Krad/3mM was the most effective dose for inducing desirable mutants such as plant survival, grain number and weight with low level of damage.*
Keywords*: Gamma rays, sodium azide, mutagenesis, dose, sorghum, variety and yield.*

I. Introduction

Mutation has been shown to be advantageous when propagating crop plants using their genetic materials (Auld, 1998). This is against the backdrop that the genetic variability for desirable traits/characters in crop plants determines different plant breeding purposes (Obilana, 1981). It is important to note that the primary source of this variability is natural mutation. The rate is however too slow to meet the yearnings and aspirations of crop breeders (Nwasike, 1987; Odeje *et al*., 2012). Several efforts have been made via hybridization in an attempt to meet this challenge. In spite of these efforts, breeders require even greater variability due to the ever increasing demand by farmers for improved varieties that possess good yield qualities as well as resistance (Obilana, 1995). In the light of this, the employment of induced mutation to provide viable additional option to plant breeders to create useful genetic variability to meet this demand is welcome.

The use of mutagens to induce desirable traits in some plant species has been reported. Odeje and Adamu,(2012) reported that low concentrations of sodium azide have induced significant variations in all the traits of *Sorghum bicolor* studied. Similarly, Kiruki *et al.* (2006) reported resistance of maize to striga attack in maize varieties. A combination of gamma rays, ethylmethane sulphonate and sodium azide resulted in the induction of resistance against two harmful fungal diseases. These two fungal diseases (early leaf spot- (*Cercospora arachidicola*) and late spot- *Phaeoisariopsis personata* individually or combined can cause yield losses up to 50% (McDonald *et al*., 1985).

In common bean (*Phaseolus vulgaris* L.), a white seed bean mutant derived as a result of treatment with ethylmethane sulphonate and gamma rays of black-seeded bean mutant has led to the production of upright short vine architecture (Nichterlein, 1999). In the same way, 200 rads of gamma irradiation and two concentrations of sodium azide (0.1mM and 1.0mM) treatments induced significant improvement in pods per plant in both IT846-1246 and Vita-7 when compared to parent plant (Odeigah *et al*., 1998).
Furthermore, in *Lycopersicon esculentum* Mill, high-yielding mutants were induced when it was treated with methyl-nitroso-urethane and followed by gamma irradiation. Additionally, Akhtar *et al*.(2012) reported that EMS is more effective in inducing variability than sodium azide when the two mutagens were employed to treat *Linum usitattissimum* L while, treating wheat variety- HD-2733 with sodium azide (0.02-0.05%), Srivastava *et al.* (2011) induced better yield components especially at 0.02 % than the control.

`In a country like Nigeria where population is increasing astronomically (170 million people), there is a greater need to increase the level of sorghum production as 80% of the sorghum produced is eaten. Furthermore, if the desire to look inwards for most raw materials in agro-based sector in Nigeria is to be met, then the production level must be increased considerably in order to meet the industrial demands of this crop.
It has been reported that waxy sorghum is used as adhesives and for sizing paper and fabric and is an ingredient in oil-drilling mud (Sistino, 2003). Similarly, Dogget, (2000) reported that cellulose, hemicelluloses and lignin which are components of sorghum biomass can be employed as alternative to petroleum and other fossil resources.

In view of this potential and the enormous uses of this crop, it is imperative that breeders take advantage of every available methodology to further increase the yield potential of this crop. The present study was therefore undertaken to assess the effectiveness of gamma rays and sodium azide combined on yield components of guinea corn (*Sorghum bicolor* L.).

II. Materials And Methods

Air-dried seeds of three varieties of guinea corn (*Sorghum bicolor* l. Moench) Samsorg 4, Samsorg 40 and Samsorg 41 obtained from the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria were irradiated with gamma cell 220 cobalt 60(^{60}Co) source at the doses 0,20,30,40 and 100kilorads (Krads) at the Centre for Energy Research and Development, Obafemi Awolowo University (OAU), Ile Ife, Osun State, Nigeria. The irradiated seeds were soaked in 3millimole and 4millimoles (mM) of sodium azide respectively for three hours (3hrs)

Experimental Layout and Seed Planting

Treatment and the control seeds were planted in the botanical garden, A.B.U., Zaria using the principle of randomization. There were twenty-four plots comprising of five ridges each and 4m long. Inter- and intra-row spacing was 75cm and 20cm respectively. Each ridge was 4m long with seeding depth of 2cm. Each treatment was replicated twice and the design and analysis followed a Completely Randomized Block Design.

Data collection

Germination Count and Percentage: The total number of seeds that emerged per plot was recorded 15 days after planting and the percentage calculated using the formula:

$$\% \text{ Germination} = \frac{\text{No of germinated seeds}}{\text{Total treated seeds}} \times \frac{100}{1}$$

Number of Grains per Spikelet: grains from a spikelet were threshed and the number of grains were counted and recorded

Spikelet Length: The length of the spikelet was measured from the spike to the end of the spikelet using a metre rule calibrated in "cm"

Weight of 1000 Grains: The grains on the panicle of sorghum were threshed and weight was taken with an electric weighing balance in grams.

Seedling Survival: The plants that survived were estimated 30 days after planting through visual assessment of dead plant tissues.

Survival to Maturity: Estimates of the number of plants in each treatment at maturity was taken and the percentage of surviving plants was calculated using the formula:

$$\% \text{ Germination} = \frac{\text{No of surviving plants}}{\text{Total treated seeds}} \times \frac{100}{1}$$

Height of Plant at Maturity: The height of plant at maturity was taken in cm by measuring the whole plant in cm from the ground level to the highest point of the panicle.

Leaf Length at maturity: Each leaf was measured from the leaf sheath to the tip of the leaf using a metre rule. The measurements were taken when the crops were considered matured.

Data Analysis:

Statistical analysis was carried out using the Genstat release 4.03 Package. The data collected were subjected to analysis of variance (ANOVA). Significance in the treatments was further analyzed using New Duncan's Multiple Range Test (NDMRT) to separate and compare individual means of the varieties. Correlation coefficient (r) analysis was used to determine relationship among the sorghum parameters.

III. Results

Table 1 shows the mean square values of the characters of the Sorghum varieties studied. The results indicated a highly significant difference ($P \leq 0.01$) for survival to maturity, height at maturity and leaf length. Similarly, grains per spikelet and weight of 1000 grains were observed to be significant. The result however revealed a non-significant difference for percentage germination and spikelet length. The interaction between the dose and variety indicated a non-significant difference for all the traits studied except height at maturity which was highly significant (Table 1).

Table 1: Mean Square estimate of the performance of Sorghum varieties treated with gamma rays and sodium azide combined

Source of Variation	DF	Germ percent (%)	Seedling Survival (%)	Survival to Maturity (%)	Height at Maturity (cm)	Spikelet Length (cm)	Grains/ spikelet (no.)	Leaf Length (cm)	Wt of 1000 grains(g)
Dose	4	404.2^{ns}	493.2*	1101.7**	1892.4**	1.6^{ns}	355.1*	919.9**	94.0*
Variety	2	585.0*	640.3**	232.9*	259.5*	1.7^{ns}	50.4^{ns}	38.4^{ns}	34.3^{ns}
Dose vs Variety	8	5.74^{ns}	21.7^{ns}	37.0^{ns}	487.3**	3.0^{ns}	97.3^{ns}	9.5^{ns}	2.0^{ns}
Error	16	415.5	140.1	189.2	166.8	3.1	207.8	93.3	68.6

** = Highly significant ($P \leq 0.01$)
* = Significant ($P \leq 0.05$)
ns = Non significant

There was a gradual decrease in all the traits studied with increase in the dose of the gamma rays in the combination of mutagens (Table 2). When the effect of the mutagens respectively were compared, the highest percentage germination, seedling survival, height at maturity and number of grains per spikelet were observed at 20Krad of gamma rays combined with 3mM of sodium azide and 20 Krad with 4 mM. It was even observed that seeds treated with 20 Krads/4mM weighed more than the control (Table 2). A moderate effect was obtained when 30Krad was combined with 3mM.

Table 2: The effects of the combined doses of gamma rays and sodium azide on some sorghum parameters

Treatment Variation	Germ percent (%)	Seedling Survival (%)	Survival to Maturity (%)	Height at Maturity (cm)	Spikelet Length (cm)	No Grains/ Spikelet (no)	Leaf Length (cm)	Wt of 1000 grains(g)
3mM vs 0 Krad	55.0a	45.0a	73.3a	120.7a	4.7a	36.0a	65.9a	74.0b
3mM vs 20Krad	45.0ab	17.8ab	46.7c	81.0b	3.7ab	14.7b	30.5b	66.0c
3mM vs 30 Krad	30.0bc	15.7b	21.7d	69.5c	2.7b	11.7bc	28.4bc	65.0c
4mM vs 20 Krad	43.3ab	17.3ab	60.0b	68.3c	3.8ab	12.0bc	27.5bc	76.0a
4mM vs 30 Krad	26.7c	16.3	54.2bc	55.0d	3.2ab	9.7c	22.2c	66.0c

Means with the same letter within a column are not significantly different (P □ 0.05)

The different varieties performed differently as indicated in Table 3. The variety Samsorg 4 performed better than the other two varieties for all the traits studied except for number of grains per spikelet and leaf length for which Samsorg 40 showed the highest values. The least response to the mutagenic treatments was observed in Samsorg 41 for all the traits.

Table 3: Mean Performance of three varieties of Sorghum treated with gamma rays and sodium azide combined

Variety	Germ percent (%)	Seedling Survival (%)	Survival to Maturity (%)	Height at Maturity (cm)	Spikelet Length (cm)	Grains/ spikelet (no.)	Leaf Length (cm)	Wt of 1000 grains(g)
Samsorg 4	49.0a	63.5a	62.5a	86.2a	4.3a	18.0a	34.4a	21.8a
Samsorg40	43.0b	62.7a	51.6ab	78.0b	3.4a	19.2a	38.4a	23.0a
Samsorg41	28.0c	43.5b	40.0c	72.8c	3.2a	13.2b	33.1a	22.0a

Means with the same letter within a column are not significantly different (P 0.05)

Furthermore, Table 4 reveals the mean square values of the performance of the Sorghum varieties in relation to the mutagenic treatments. The dose, 20Kr/3mM gave the highest Percentage germination (65%), height at maturity(90) and leaf length(38.4).While the combination of 20Kr/4mm gave the highest survivability in all the varieties. However among the varieties, number of grains per spikelet was observed to be highest in samsorg 40 at the treatment combination of 20KR/4mM while, the lowest number of grains per spikelet as revealed in Table, 4 was observed in variety Samsorg 41 (8.0). Similarly, this combination (20KR/4mM) was most effective on survival to maturity in all varieties. Variety Samsorg 4 performed better than varieties 40 and 41 in all the traits except for leaf length. Variety Samsorg 40 had the longest leaf (38.4 cm) while the variety samsorg 41 had the least performance for this trait (21.0 cm). Additionally, there was an inverse proportionality with the combined effects of the mutagens. Thus, as the dose of the gamma rays increased in the combination with sodium azide, there was a gradual decrease in all the traits among the three varieties (Table, 4).

Table 4: Mean Square estimate of the performance of Sorghum varieties treated with gamma rays and sodium azide combined

Variety	Concen tration	Germ percent (%)	Seedling Survival (%)	Survival to Maturity (%)	Height at Maturity (cm)	Spikelet Length (cm)	Grains/ spikelet (no.)	Leaf Length (cm)	Wt of 1000 grains(g)
Samsorg 4	0KRvs3mM	60.0	83.3	80.0	130.0	6.0	38.0	59.0	56.0
	20kRvs3mM	65.0	61.5	65.0	90.0	4.0	18.0	33.7	48.0
	30KRvs3mM	40.0	62.5	35.0	76.0	3.0	12.0	28.6	32.0
	40KRvs3mM	_	_	_	_	_	_	_	_
	20KRvs4mM	50.0	60.0	70.0	75.0	4.5	13.0	28.0	32.0
	30KRvs 4mM	49.0	63.5	62.5	86.2	4.3	18.0	34.4	42.8
	Mean	53.8	66.8	62.5	92.8	4.4	20.3	37.3	50.0
	S.E ±	1.75	2.26	0.46	4.14	0.15	1.57	1.93	8.8
Samsorg 40	0KRvs3mM	65.0	76.9	80.0	120.0	6.0	40.0	75.0	46.0
	20kRvs3mM	43.0	62.8	51.0	78.6	4.7	19.2	38.4	40.0
	30KRvs3mM	30.0	66.7	20.0	68.0	3.0	13.0	30.9	40.0
	40KRvs3mM	_	_	_	_	_	_	_	_
	20KRvs4mM	40.0	50.0	60.0	70.0	4.5	12.0	29.4	52.0
	30KRvs4Mm	30.0	50.0	50.0	55.0	3.9	20.0	23.1	50.0
	Mean	41.6	61.3	52.2	78.3	4.4	20.8	38.4	46.0
	S.E ±	2.0	1.4	2.95	3.4	0.1	1.6	2.8	1.75
Samsorg 41	0KRvs 3mM	40.0	62.5	60.0	112.0	5.0	30.0	63.8	46.0
	20kRvs3mM	20.0	50.0	30.0	73.0	3.0	10.0	30.0	44.0
	30KRvs3mM	20.0	50.0	10.0	64.0	2.0	10.0	25.8	34.0
	40KRvs3Mm	_	_	_	_	_	_	_	_
	20KRvs4mM	40.0	30.0	50.0	60.0	3.0	8.0	25.0	48.0
	30KRvs4Mm	20.0	25.0	50.0	65.0	2.8	8.0	21.0	50.0
	Mean	28.0	43.5	40.0	71.8	3.2	13.2	33.1	44.2
	S.E ±	1.5	2.1	2.69	3.2	0.14	1.3	2.4	1.74

IV. Discussion

Variability in crop plants is brought about by different factors, which may include spontaneous and/or induced mutation. The effects are observed morphologically or cytologically. The results from this study revealed that lower doses/concentrations were effective in causing polygenic variability in the various quantitative traits of the sorghum genotypes studied. It also showed however that there was an inverse relationship between the doses of the mutagens and the traits. As the dose increased, there was a progressive decrease in the characters of the varieties. A similar trend had been reported by Thilagavati and Mullainathan (2009) in black gram and Tamina and Tapash (2010) who reported that lower doses of chemical mutagens produced higher variability than the higher doses. It may be worthy of note that in this study, as the dose of gamma rays increased in relation to the sodium azide, there was a gradual decrease in the values of the traits studied (Table, 4) It was therefore observed that the gamma rays seems to have greater effect on the treatment materials than the sodium azide. At 40Krad and above, there was no germination recorded. This may be due to the lethal effect of such a high dose on biological materials. These results are in line with the results of Al Qurainy (2009) who performed his experiment on *Eruca sativa*. The reduction in germination percentages may be attributed to the delay or inhibition of physiological and biological processes necessary for seed germination which include enzyme activity and hormonal imbalance (Khan and Al-Qurainy, 2009). In addition, it is a potent inhibitor of the proton pump and alters the mitochondrial membrane potential (Zhang 2000). These effects together may hamper ATP biosynthesis resulting in the decreased availability of ATP, which may slow down the germination rate and reduce the germination percentage.

Various doses of treatment affected the survivability of seedlings in a dose dependent manner as shown in table 4. The reduction in seedling survival can be attributed to cytogenetic damage and physiological disturbances caused by mutagen treatment. Thus the probable reason of the drop in seedling survivability may be the hindrance caused by the sodium azide and gamma rays on different metabolic pathways of the cells. Similar findings have also been reported by Rachovska and Dimova (2000) in wheat, Ilbas *et al.* (2005) in barley, Adamu and Aliyu (2000) in tomato, Khan *et al.* (2004) in mungbean, and Mostafa (2011) in sunflower.

Furthermore, the result revealed that there was no direct proportionality between the increase in grain weight and the dose/concentration of the mutagens. The results demonstrated that the grain weight was higher than their respective controls at the dose combination of 20KR/4mM in varieties Samsorg 40 and Samsorg 41 respectively. This increase in grain weight may be ascribed to the duplication of chromosomes. This was also reported by Said *et al.* (1988). However, high doses/concentrations were reported to have decreased grain weight and number of grains per spikelet (Odeje *et al.*, 2012; Benamer, 1990) when they carried out their studies on Sorghum and barley respectively.

Among the treatment materials, height of plants at maturity was similarly observed to decrease considerably as dose combinations increased with the highest plant being observed in samsorg 4 at the dose combination of 20Kr/3mM. However, the longer leaf observed in samsorg 40 may be due to the proper adaptability of the variety to the mutagenic treatment compared to samsorg 4 and 41 respectively. This observation was also reported by Odeje and Adamu (2012)

Effect on spikelet length revealed that 20Krad combined with3mM and 4mM had greater effect than 30Krad combined with 3mM. This suggested that the higher the concentration of gamma rays in the combination of the mutagens, the lower the trait. This reduction in spikelet length may be due to the lack of proper adaptability of the varieties to the mutagenic treatment. A similar result was reported by Lal *et al* (2009) on black gram. Correlation analysis revealed that the number of grains correlated with spikelet length as well as weight of 1000 grains. All these are indications of the potential for desirable traits.

V. Conclusion

In view of the aforementioned, it can be concluded that low doses of gamma rays/sodium azide can generate desirable polygenic traits such as grain weight and number of grain per spikelet, seedling height, survival as well as leaf length with minimal level of damage.. It can also be concluded that samsorg 4 performed better than samsorgs 40 and 41 in almost all the traits. Hence a combination of gamma rays and sodium azide can be a veritable tool that can be used for inducing desirable mutants in S*orghum bicolor*

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