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EFFECTS OF PARA-DICHLOROBENZENE AND SODIUM AZIDE ON GERMINATION AND SEEDLING GROWTH OF SESAME (*Sesamum indicum* L.)

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ABSTRACT

Sesame is an important source of income and edible oil particularly in sub-saharan Africa. Its cultivation is constrains by many factors including lack of improved varietiess, pests and disease and abiotic stresses. Creating genetic variability could provide a base upon which improvement could made. This research was carried out to evaluate the effect of Sodium Azide and Para-dichlorobenzene on Germination and Seedlings growth of three sesame varieties (Ex-sudan, E-8 and JAN-IRI). Pot experiments were conducted during 2017 and 2018 seasons using completely randomized design (CRD). The treated seeds were planted and resulting plants (M_0) were allowed to produce seeds. The seeds of the M_0 were used in generating M_1 plants which were used to evaluate the effect of the mutagens. Few days to germination were recorded in seeds treated with 3.0 mM So dium azide (3.0 days) or 3.0 mM Para-dichlorobenzene (3.0 days) in EX-SUDAN as well as seeds treated with 3.0 mM Sodium azide (3.0 days) in E-8. Percentage germination was significantly increased The highest shoot length was obtained when EX-SUDAN was treated with 2.0 mM (19.3cm) or 3.0 mM Sodium azide (18.2cm while highest root length was obtained when JAN-IRI and EX-SUDAN were treated with either 1.0 mM Para-dichlorobenzene (4.2cm) or 3.0 mM Sodium azide (3.8cm). The mutagens (Sodium azide and Para-dichlorobenzene) could be used to create variability for genetic improvement of Sesame.

Keywords*:* Sesame, Chemical Mutagens, Germination, Vegetative Characters, Genetic variability.

INTRODUCTION

The Sesame (*Sesamum indicum* L.) belongs to the *Pedaliaceae* family and is one of the most ancient oil seed known to mankind and plays a major role in human nutrition (komivi *et al.*, 2017). Sesame is widely grown by small-holder farmers in Nigeria and is a major cash crop in northern part of the country (NAERLS, 2010). Nigeria is the third producer of sesame in Africa after Ethiopia and Sudan with annual production of 450,000 metric tones (Benshoshan *et al.*2010). Sesame *(Sesamum indicum L.)* is an important source of high quality edible oil and protein food for poor farmers of major sesame growing countries (Reckha and Lannger, 2007).

Shattering ability, pests, diseases, abiotic stresses and lack of improved varietiess constitute major challenges to Sesame production in sub-saharan Africa. Crop improvement in Sesame is constrained by narrow genetic base of the crop. This necessitate the need for creating genetic variability upon which improved varieties could be made. Mutagenesis is the process causing changes in the genetic information of an organism not caused by genetic segregation but induced by chemical and physical agents (Abou *et al*., 2017). Chemical mutagens cause changes in DNA of an organism by increasing the frequency of mutations above the natural background level (Ashish *et al*., 2011) and has been utilized in creating genetic variability in many crop plants. Induce mutation have played a pivotal role in enhancing world food security, as new food crop varieties with various induced mutation have brought about a significant increase in crop production at locations people could directly access (Kharlkwal and Shu, 2010).

Sodium azide is a chemical mutagen and considers as one of the most powerful mutagen in plant breeding which induces chromosomal aberrations through the action of an organic metabolite - L-azidoalanine synthesize by O-acetylserine sulfhydrylase, Domain *et al*.,(2012). Paradichlorobenzene is a colourless solid compound consisting of a benzene ring with two chlorine atoms (replacing hydrogen atoms) on opposing sites of the ring (Hunger and Herbst, 2012). Its importance in polyploidy was first realized by Carey & McDonough (2014). With the realization of this important property of this chemical, it has been successfully applied for chromosome studies in various groups of plants (Ahloowalia, and Maluszynski, 2001). This study was carried out to evaluated the effects of Sodium Azide growth in Sesame.

MATERIALS AND METHODS

Pot trials were conducted during 2017and 2018 dry seasons in the screenhouse of the Department of Plant Biology, Bayero University, Kano which lies on latitude 11^{0} 58N and longitude 8^{0} 30E with altitude of 440m above sea level. The site is a typical Sudan Savanna ecological zone of Nigeria. The seeds of the two improved and one Local variety were collected from Kano State Agricultural and Rural Development Authority (KNARDA). The two improved sesame varieties were Ex-Sudan and E-8 and the Local variety was Jan-Iri. A total of hundred (100) seeds of each variety were sorted out and surface sterilized in 1.0% Sodium hypochlorite for one minute. The seeds were then rinsed three (3) times in sterile distilled water. Ten (10) seeds were placed in Nylon net bags for easy handling. The seeds were soaked in different concentrations (0.00mM, 1.0mM, 2.0mM, 3.0mM and 4.0mM) of Sodium azide or Para-dichlorobenzene for three (3) hours. The seeds were then rinsed with distilled water to remove the excess chemicals from the seeds. The treated seeds were planted in polypots (30 x 30 x 40cm) containing 7kg of top soil and compost manure (3:1). The resulting plants (M_0) were allowed to produce seeds of three different sesame varieties. The seeds of the M_0 were planted in a Complete Randomized Design (CRD) to generate M_1 plants which were used to evaluate the effect of the mutagens. Data was collected on days to germination, percentage germination, shoot length and root length of three different sesame varieties. The data was subjected to analysis of variance (ANOVA) using SPSS software version 23 and means were separated using least significant difference (LSD).

RESULTS AND DISCUSSIONS

In this study, the interaction involving variety and concentrations of mutagens were significant ($P \leq 0.05$) for days to germination, percentage germination, shoot length and root length. The lowest days to germination was recorded in EX-SUDAN when seeds were treated with 1.0mM Sodium azide, 2.0mM, 3.0mM or 4.0mM Para-dichlorobenzene, indicating that these mutagenic agents increase the rate of germination by increasing the expression of genes that control germination in Sesame. In E-8, seeds treated with 3.0mM or 4.0mM Sodium azide or 3.0mM paradichlorobenzene demonstrated significantly ($P \leq 0.05$) lower days to germination when compared with the control and other treatments used in this experiment. In the case of JAN-IRI however, treatment with Sodium azide or Paradichlorobenzene did not significantly ($P \leq 0.05$) reduce days to germination, indicating that the concentrations used in this study are not optimum for germination in this variety. This study also indicated that days to germination can significantly be reduced when seeds of EX-SUDAN and E-8 are treated with Sodium azide or para-dichlorobenzene. This finding is in agreement with work of Adelanwa *et al,* (2011) and Olaolorun *et al.* (2019), who reported significant reduction in days to emergence in wheat when seeds were treated with various dosages of ethylmethane sulphonate. Aparna *et al*, (2013) also reported decrease in days to germination with increase in dose of gamma rays in Groundnut.The decrease in days to germination could be due to the ability of the mutagens to cause genetic changes that stimulate increase the rate metabolism and respiration in living cell thereby creating favorable condition for energy transformation during germination processes, Abraham *et al.* (2013). Similarly, Vijayata and Navnath (2015), reported that the decrease in days to germination when seeds were treated with mutagen could be attributed to physiological changes at cellular level.

Table 1. Effect of Variety versus Mutagen Interaction on Days to Germination

		Days to Germination		
Mutagen	**Concentration (mM)**	**JAN-IRI Mean±SE**	**Ex-sudan Mean±SE**	**E-8 Mean±SE**
Control	0.0	$7.0^{de} \pm 1.7$	$6.0^{cd} \pm 2.1$	$5.0^{bc} \pm 1.8$
S.Azide	1.0	$6.0^{cd} \pm 2.0$	$6.0^{cd} \pm 1.8$	$6.0^{cd} \pm 2.5$
S.Azide	2.0	$5.0^{bc} \pm 2.7$	$5.0^{bc} \pm 2.5$	$6.0^{cd} \pm 1.7$
S.Azide	3.0	$7.0^{de} \pm 2.2$	$3.0^{a} \pm 2.6$	$3.0^{a} \pm 2.3$
S.Azide	4.0	$6.0^{cd} \pm 1.2$	$5.0^{bc} \pm 1.4$	$4.0^{ab} \pm 2.2$
P. Benzene	1.0	$8.0^{e} \pm 1.2$	$5.0^{bc} \pm 1.5$	$6.0^{cd} \pm 1.5$
P. Benzene	2.0	$6.0^{cd} \pm 1.5$	$4.0^{ab} \pm 2.2$	$5.0^{bc} \pm 1.6$
P. Benzene	3.0	$6.0^{cd} \pm 1.7$	$3.0^{a} \pm 2.5$	$4.0^{ab} \pm 2.4$
P. Benzene	4.0	$7.0^{de} \pm 1.4$	4.0^{ab}c 1.4	$5.0^{bc} \pm 2.2$

Means followed by the same letter superscripts are not significantly different using least significant difference (LSD) $P \leq 0.05$ Key : S.Azide ═Sodium azide, P. Benzene═ Para-dichlorobenzene, SE ═ Standard Error.

The results also showed a significant ($p \leq 0.05$) interaction between the mutagens and the Sesame genotypes (Table 2). The highest percentage germination was recorded in EX-SUDAN treated with 3.0 mM Sodium azide (80%) and the untreated

JAN-IRI (97%), indicating that this concentration promotes germination in EX-SUDAN and not optimal in JAN-IRI Sesame varieties. The lowest percentage germination was recorded when EX-SUDAN was treated with 4.0 mM of either Sodium azide (43%) or Para-dichlorobenzene (43%). Our results was in line with finding of Mensah *et al*, (2005) in Grapple plant and Udensi *et al*, (2012) in Pterodiscus plant, who attributed the reduction in germination to genetic changes which might have probably affected some biological pathway necessary for seed germination in the treated seeds. Mensah and Obadoni, (2007) reported that chemical mutagens affect quantitative traits which control physiological processes involved in germination resulting in reduction of germination rate and survival of the seedling.

Table 2. Variety Versus Mutagen Interaction on Germination percentage (%) in sesame

Mutagen	Concentration (mM)	Germination percentage (%) JAN-IRI Mean±SE	Ex-sudan Mean±SE	E-8 Mean±SE
Control	0.0	79a ± 0.0	57^{g} ± 0.2	57^{g} ± 0.2
S.Azide	1.0	60^{f}± 0.0	57^{g} ±0.2	77^{b}± 0.2
S.Azide	2.0	50^{i}± 0.3	77^{b}± 0.2	53^{h}± 0.2
S.Azide	3.0	70^{d} ± 0.2	80^{a} ± 0.0	70^{d} ± 0.0
S.Azide	4.0	60^{f}± 0.0	43^{k}± 0.2	67^{e}± 0.2
P. Benzene	1.0	50^{i}± 0.0	47^{j}± 0.2	47^{j}± 0.2
P. Benzene	2.0	60^{f} ± 0.2	67^{e} ± 0.2	50^{i} ± 0.0
P. Benzene	3.0	60^{f} ± 0.2	77^{b} ± 0.7	73^{c} ± 0.2
P. Benzene	4.0	70^{d} ± 0.2	43^{k}± 0.2	67^{e}± 0.2

Means followed by the same letter superscripts are not significantly different using Least Significant Difference (LSD) at P≤0.05. Key : S.Azide ═ Sodium azide, P. Benzene═ Para-dichlorobenzene, SE ═ Standard Error.

The Sesame genotype versus mutagens interaction with respect to root length is shown in table 3. Root length was significantly increased in JAN-IRI and EX-SUDAN when seeds were treated with 1.0 mM Para-dichlorobenzene. Similarly, treating seeds with 3.0 or 4.0 mM Sodium azide significantly (p≤ 0.05) increased root length in EX-SUDAN. On the other hand, while at 1.0 mM Para-dichlorobenzene significantly increase roots in JAN-IRI and EX-SUDAN, it proved to have adverse effect on root development in E-8 variety producing the lowest root length (2.3cm). Sodium Azide and Paradichlorobenzene at optimum concentrations promote cell division and elongation probably by changing the hormonal balance in plants. This study is in line with finding of Ashish *et al*, (2011) who reported decrease in shoot and root length in *Abelmoschus moschatus,* when seeds were treated with chemical mutagens (Sodium Azide or Para-dichlorobezene). It is however, contrary to the report of Adamu, (2004) who reported significant increase in root and shoot length when seeds were irradiated with gamma rays and thermal neutron.

Table3. Variety Versus Mutagen Interaction on Root Length (cm) in Sesame Three Weeks After Sowing (3WAS)

Mutagen	Concentration (mM)	Root Length (cm) JAN-IRI Mean±SE	Ex-sudan Mean±SE	E-8 Mean±SE
Control	0.0	2.7^{f-i} ± 0.15	2.7^{f-i} ± 0.02	2.5^{ghi} ± 0.02
S.Azide	1.0	2.7^{f-i} ± 0.50	2.8^{e-i} ± 0.30	2.5^{ghi}± 0.13
S.Azide	2.0	2.9^{d-h} ± 0.71	3.4^{bc} ± 0.50	2.8^{e-i} ± 0.02
S.Azide	3.0	3.2^{b-e} ± 0.62	3.8^{ab} ± 0.30	2.4^{hi} ± 0.70
S.Azide	4.0	2.7^{f-I} ± 0.80	3.7^{abc} ± 0.60	2.4^{hi} ± 0.30
P. Benzene	1.0	4.2^{a} ± 0.02	4.2^{a} ± 0.80	2.3^{i} ± 0.30
P. Benzene	2.0	2.7^{f-i}± 0.52	2.7^{f-i} ± 0.70	3.3^{bcd}± 0.50
P. Benzene	3.0	2.9^{d-g} ± 0.8	2.9^{d-h} ± 0.02	2.7^{f-i}± 0.50
P. Benzene	4.0	3.0^{d-g} ±0.13	3.0^{d-g} ± 0.02	2.7^{f-i}± 0.50

Means followed by the same letter superscripts are not significantly different using least significant difference (LSD) P≤ 0.05 probability level. Key : S.Azide ═ Sodium azide, P. Benzene═ Para-dichlorobenzene, SE ± ═ Standard Error.

Shoot length was also significantly influence by the different concentrations of the mutagens (table 4). The results revealed that shoot length was significantly increased in EX-SUDAN when seeds were treated with 2.0mM, 3.0mM Sodium azide or

3.0mM Para-dichlorobezene. However, the different concentration of the mutagens evaluated in this study failed to significantly increase shoot length in the seedlings of JAN-IRI and E-8 varieties. Adamu, (2004) reported significant increase in shoot length when seeds of popcorn where irradiated with gamma rays and thermal neutron. Mutagens were reported to influence shoot or root length by creating genetic mutations that influence hormonal balance in plants. Therefore, increase in shoot length recorded in EX-SUDAN variety might be as result of mutations which changes hormonal balance in favor of shoot elongation.

Table 4. Variety Versus Mutagen Interaction on Shoot Length (cm) at Three Weeks After Sowing (3WAS)

Mutagen	**Concentration** (mM)	**Shoot Length (cm)** **JAN-IRI** **Mean±SE**	**Ex-sudan** **Mean±SE**	**E-8** **Mean±SE**
Control	0.0	$8.8^{L} \pm 0.14$	$14.7^{d-g} \pm 0.13$	$10.8^{ijk} \pm 0.24$
S.Azide	1.0	$8.8^{L} \pm 0.24$	$14.8^{d-g} \pm 0.22$	$15.7^{cde} \pm 0.31$
S.Azide	2.0	$16.9^{bcd} \pm 0.50$	$19.3^{a} \pm 0.30$	$17.2^{bc} \pm 0.54$
S.Azide	3.0	$11.5^{ij} \pm 0.20$	$18.2^{a} \pm 0.80$	$13.2^{ghi} \pm 0.22$
S.Azide	4.0	$11.4^{ijk} \pm 0.50$	$13.6^{fg} \pm 0.31$	$15.7^{cde} \pm 0.25$
P. Benzene	1.0	$13.4^{fgh} \pm 0.30$	$13.8^{efg} \pm 0.23$	$9.4^{kl} \pm 0.13$
P. Benzene	2.0	$10.0^{jkl} \pm 0.21$	$14.1^{efg} \pm 0.33$	$13.9^{efg} \pm 0.21$
P. Benzene	3.0	$11.3^{ijk} \pm 0.22$	$17.4^{abc} \pm 0.31$	$15.0^{def} \pm 0.15$
P. Benzene	4.0	$10.7^{jkl} \pm 0.18$	$14.9^{def} \pm 0.24$	$11.8^{hij} \pm 0.81$

Means followed by the same letter superscripts are not significantly different using least significant difference (LSD) $P \leq 0.05$ probability level. Key : S.Azide ═ Sodium azide, P. Benzene═ Para-dichlorobenzene, SE ± ═ Standard Error.

CONCLUSION

3.0mM Sodium azide could be used to create genetic mutations that favor both germination and seedlings development in Sesame. The procedure could be adopted in creating genetic variability upon which selection could be made at seedling stage in Sesame.

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