ISSN: 2754-477X

Journal of Food Technology & Nutrition Sciences

SCIENTIFIC Research and Community

Research Article

Open Access

Effects of Hydrogen Peroxide and Benzene Treatments on Morphological Traits of Sesame (*Sesamum indicum L.*)

Ablaku AB[1], Abimiku SE[1] and Ablaku BE[2]*

[1]Nasarawa State University, Keffi, PMB. 1022, Nasarawa State, Nigeria

[2]Lower Benue River Basin Development Authority, Otukpo Road, Makurdi, Benue State, Nigeria

ABSTRACT

A study was carried out on the effects of hydrogen peroxide and benzene treatments on morphological traits of sesame (*Sesamum indicum L.*) in Keffi. NCRIBEN-02M variety of sesame was exposed to varying concentration (100, 75, 50, 25 and 0%), of benzene, hydrogen peroxide, and the mixture of the two chemical mutagens. The M2 generation of the genotypes were assessed for plant height, number of leaves, leaves area. Benzene treatment, at all levels, have the highest plant height. The increase in number of leaves and leaf area are significant at $p \leq 0.05$ and 95% confidence. The optimum concentrations of the mutagens used for mutation in sesame, were effective at 100 and 75% of all the treatments. The most effective mutagen for inducing mutation in sesame under Keffi environment is benzene, followed by Hydrogen Peroxide. Benzene and hydrogen peroxide have proven themselves as chemical mutagens, in mutation breeding. They have unlocked several agronomic traits in sesame. Further, study on these mutagens will enhance the genetic variability in the growing of sesame for higher performance.

***Corresponding author**

Ablaku BE, Lower Benue River Basin Development Authority, Otukpo Road, Makurdi, Benue State, Nigeria. E-mail: ablaku.barnabas@gmail.com

Received: September 01, 2021; **Accepted:** September 10, 2021; **Published:** September 23, 2021

Introduction and Literature Review

Sesame (*Sesamum indicum L.*), otherwise known as Sesame or benniseed is a member of the family Pedaliaceae. It is one of the most ancient oilseeds crop known to humanity. Sesame plays an important role in human nutrition [1]. Narrow gene pool in the available germplasms demands the need of crop restructuring for higher productivity. On the other hand, development of superior varieties may foster higher production of sesame in the country both through horizontal and vertical expansion and can obviously narrow down the huge demand-supply gap of oilseeds. Creation of variability transpires to be primary step to get desirable types. Mutation breeding has long been known as a potential technique to unlock additional genetic variability for supplementing conventional crop breeding methodology [2-4]. Mutagenesis offers a unique scope for creating variation, as it may alter even those genes that are common to all the varieties of a species. Induced mutation has been extensively and successfully used for the improvement of many crops including oilseed crop like sesame [5-14].

Sesame indicum L. produces seeds that are rich in both protein and oil. Attention has recently been focused on this crop in Nigeria because of its potential as an export commodity for its seeds. The seed is used for the production of high quality odourless oil. To increase production of the crop there is need to have a better understanding of its genetic background. The present study is designed to fill the gap in knowledge of the genetic background by creating variation using through mutagenic treatment like, hydrogen peroxide and benzene. Other mutagens like colchicine, sodium azide, ethidium bromide have been used to improve agronomic traits of sesame in the past years. This study will increase the narrow genetic base, variability in the genotype of the plant. Chemical mutagens like colchicine, sodium azide and ethidium bromide have been used to improve agronomic traits of sesame in the past years, but are very expensive. Mutagens like benzene and hydrogen peroxide are less expensive. They could replace the expensive mutagens if found to unlock the same variability. The present study was aimed at evaluating effects of hydrogen peroxide and benzene treatments on the morphological traits of sesame in Keffi, Nasarawa State.

Materials and Methods

Study Area

This study was carried out in the Department Plant Science and Biotechnology Research Garden Nasarawa State University, Keffi. Keffi Local Government Area is located 8°50'22.8"N 7°54'16.9"E about 68KM from Abuja the Federal capital and 128KM from Lafia the State capital [15].

Sample Collection

The material consisted of one variety of sesame (*Sesamum indicum L.*), NCRIBEN 002, collected from National Cereal Reseach Institude (NCRI), Badeggi. Niger State.

Sample Preparation

Seed viability test was carried out using the floating method. Genetically pure and uniform dry seeds (10 - 12% moisture content) were soaked in 100, 75, 50, and 25% concentration of hydrogen peroxide in water for 12hrs, and thus referred as A. Another set of seeds were soaked in 100, 75, 50, and 25%

concentration of benzene in hexane. After 12hrs, distilled water was used to rinse the seeds. The seeds, after rinsing, referred to as B. Finally, a set of seeds were soaked in mixture of hydrogen peroxide in water and benzene in hexane in the ratio of 50:50, 37.5: 37.5, 25:25, and 12.5:12.5% for 12h. The seeds was rinsed with distilled water and thus referred to as C [16]. The final set of seeds were soaked in water for 12h. The seeds were thus referred to as D.

Experimental Design

The treated seeds were washed in running water to remove excess chemicals and exudates from the seeds and sown directly in a spilt plot design. Germination was observed daily until maximum germination was attained on the 7th day after sowing (DAS) [16].

Data Generation and Collection

The treated seeds were grown from September to December 2015 at Department of Plant Science and Biotechnology Research Garden of Nasarawa State University Keffi, Nasarawa State, to rise M1 generation. The M1 generation was harvested and M2 generation was grown from February to May 2016 at the same Department Plant Science and Biotechnology Research Garden. In each generation, data was recorded for morphological traits.

Result and Discussion

Plant Height

The plant height of sesame treated with Benzene, Hydrogen Peroxide and the mixture of Benzene and Hydrogen Peroxide at levels 100%, 75%, 50% and 25% are presented in Table 1. Water was used as control at all levels.

From Table 1 it is evident that the total mean value of Benzene treatment is the highest with 56.18cm whereas the Hydrogen Peroxide treatment have the least mean value as 34.94cm all at 100% level. It can be deduced from the foregoing that Benzene treatment observe an increase in height, whereas Hydrogen Peroxide treatment deteriorated in height when compared to the control i.e. the water treatment which has the mean value of 44.38±0.92.

However, as the level of Benzene treatment is decreasing from 100% to 25%, its effect on plant height became less significant, resulting in shorter plant when compared to the 100% level.

This phenomenon is contrary to the Hydrogen Peroxide treatment in which the plant appreciate in height at lower levels. Implication of this is that, 100% level of hydrogen peroxide are shorter than the 25% level of the same treatment. This can be seen in Table 1.

Table 1: Plant Height After Treatment with Four Levels Between Two Weeks After Planting (2 WAP) and Eight Weeks After Planting (8 WAP)

Level	Plant Height	Benzene		Hydrogen peroxide		Benzene & Hydrogen peroxide		Water/Control	
		Mean (cm)	Std. D.	Mean (cm)	Std. D.	Mean (cm)	Std. D.	Mean (cm)	Std. D.
100%	Two WAP	5.73	0.07	4.57	0.09	5.65	0.18	4.27	0.32
	Three WAP	11.2	0.13	8.54	0.43	9.81	0.29	9.38	0.55
	Four WAP	38.31	0.57	21.48	1.02	27.12	0.65	20.06	0.49
	Six WAP	70.35	1.33	52.68	2.50	64.30	0.69	50.43	0.81
	Eight WAP	155.30	5.49	92.44	1.68	149.83	1.52	137.76	2.43
	Total	**56.18**	**1.52**	**35.94**	**1.14**	**51.34**	**0.67**	**44.38**	**0.92**
75%	Two WAP	5.45	0.07	4.77	0.04	5.52	0.08	4.30	0.28
	Three WAP	10.22	0.34	9.44	0.11	9.48	0.06	9.05	0.40
	Four WAP	34.33	0.48	23.79	0.46	25.30	0.99	22.45	2.25
	Six WAP	67.23	0.92	58.19	1.63	60.27	0.74	52.99	1.44
	Eight WAP	148.72	1.77	92.66	3.15	144.51	1.77	134.42	4.08
	Total	**53.19**	**0.72**	**37.77**	**1.08**	**49.02**	**0.73**	**44.64**	**1.69**
50%	Two WAP	5.02	0.1	4.93	0.10	5.21	0.10	4.23	0.26
	Three WAP	9.15	0.14	9.87	0.13	9.15	0.16	9.29	0.68
	Four WAP	31.21	0.39	26.57	0.53	23.04	0.67	20.99	1.45
	Six WAP	64.34	1.12	64.41	0.77	56.81	1.22	51.16	3.22
	Eight WAP	143.06	2.41	102.37	3.23	140.09	0.90	135.78	4.91
	Total	**50.56**	**0.83**	**41.63**	**0.95**	**46.86**	**0.61**	**44.29**	**2.104**
25%	Two WAP	4.50	0.37	5.34	0.20	4.97	0.14	4.04	0.20
	Three WAP	8.33	0.27	10.37	0.15	8.87	0.16	9.57	0.19
	Four WAP	27.95	0.81	28.51	1.05	20.65	0.31	21.21	2.16
	Six WAP	58.81	3.71	67.46	0.58	50.18	0.20	50.05	1.59
	Eight WAP	134.32	2.65	125.19	1.02	137.27	1.39	137.85	0.18
	Total	**46.78**	**1.56**	**47.374**	**0.60**	**44.39**	**0.44**	**44.54**	**0.864**

The result obtained from the M2 difference between mean value of plant height (Table 2) indicated a significant increase in plant height between 2WAP to 8WAP at p≤0.5 level with 95% confidence interval. Based on the observed means, the mean square (error) is 1.75. In all levels, plant growth were observed to be slow in the first four weeks and rapid in the next four weeks. The mean difference also confirm the growth rate from 2WAP to 8WAP. However, it was observed that hydrogen peroxide treatment were shorter when compared to other treatment, especially at levels 100% and 75% (Table 1).

Table 2: Differences Between mean values of plant height from 2 Weeks to 8 Weeks

Mean Plant Height WAP (I)	Mean Plant Height WAP (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
4.9069	9.4846	-4.5777*	1.07383	0.002	-6.9704	-2.1851
	25.8133	-20.9065*	1.07383	0.000	-23.2991	-18.5138
	58.4783	-53.5715*	1.07383	0.000	-55.9641	-51.1788
	129.8490	-124.9421*	1.07383	0.000	-127.3347	-122.5494
9.4846	4.9069	4.5777*	1.07383	0.002	2.1851	6.9704
	25.8133	-16.3288*	1.07383	0.000	-18.7214	-13.9361
	58.4783	-48.9938*	1.07383	0.000	-51.3864	-46.6011
	129.8490	-120.3644*	1.07383	0.000	-122.7570	-117.9717
25.8133	4.9069	20.9065*	1.07383	0.000	18.5138	23.2991
	9.4846	16.3288*	1.07383	0.000	13.9361	18.7214
	58.4783	-32.6650*	1.07383	0.000	-35.0577	-30.2723
	129.8490	-104.0356*	1.07383	0.000	-106.4283	-101.6430
58.4783	4.9069	53.5715*	1.07383	0.000	51.1788	55.9641
	9.4846	48.9938*	1.07383	0.000	46.6011	51.3864
	25.8133	32.6650*	1.07383	0.000	30.2723	35.0577
	129.8490	-71.3706*	1.07383	0.000	-73.7633	-68.9780
129.8490	4.9069	124.9421*	1.07383	0.000	122.5494	127.3347
	9.4846	120.3644*	1.07383	0.000	117.9717	122.7570
	25.8133	104.0356*	1.07383	0.000	101.6430	106.4283
	58.4783	71.3706*	1.07383	0.000	68.9780	73.7633

WAP: Weeks after Planting. Two WAP = 4.9069, Three WAP = 9.4846, Four WAP = 25.8133, Six WAP = 58.4783, Eight WAP = 129.8490. Based on observed means. The error term is Mean Square (Error) = 1.730. * The mean difference is significant at the 0.05 level.

The result obtained in Table 2 showed that the treatment with increase in plant height is the benzene and mixture of benzene and hydrogen peroxide treatment. Whereas hydrogen peroxide treatment, reveal decrease in height when compared with the control. The test of within subject effects shows significant increase for plant height at all treatment and levels except for hydrogen peroxide all levels. Plant height was also significant for test within subject contrast. The Leven's test of equality of error variances test the null hypothesis. The error variance of the dependent variable is equal across groups.

The characteristic slow growth (in the first 4WAP), altered phenology, and prolonged flowering of polyploids might have result from slow mitotic divisions and cell divisions of larger cells with more chromosomes [17,18]. Plant height was lower in hydrogen peroxide treatment compared to the control. The reduced height was partly due to shorter internodal length. Colchicine treatment had decreased sesame height [19-22]. The decrease in plant height is in agreement with the results of our study for hydrogen peroxide treatment only, for benzene and mixture of benzene and hydrogen peroxide, they are contrary to the same reports in the forgoing sentence.

Number of leaves

Table 3 shows the mean and standard deviation of number of leave at M2 generation after the treatment with Benzene, Hydrogen Peroxide and the mixture of Benzene and Hydrogen Peroxide. The number of leaves were uniform for the first three weeks after planting. At 2 WAP, all the M2 plants have six leaves, irrespective of treatment or level. At 3 WAP, the number of leaves increase by two leaves bringing the total number of leaves to eight, irrespective of treatment or level. As the number of weeks after planting increases to four, the effects of treatment and level begin to manifest in little amounts.

From Table 3, it can be deduced that the treatment improves the number of leaves at levels 100%, 75%, 50% and 25%. The dependency of the numbers of leaves is a direct linear relationship. The higher the level, the higher the number of leaves, whereas the lower the level, the lower the number of leaves. Hydrogen Peroxide treatments produces more leaves with about 66.71±1.63 number of leaves, than the Benzene treatment with 48.69±1.34 number of leaves.

Table 3 is in agreement with the result from analysis of variance on the number of leaves as presented in Table 3. The number of leaves in the first three weeks were uniform. Disparity sets in at the fourth week. The control group were lagging with 2-3 number of leaves. At six weeks after planting, Hydrogen Peroxide treatment at all levels shows an unbelievable boom in number of leaves. There was no equal variances among the groups and levels tested.

The tested null hypothesis reveal that the error variance of the dependent variable is equal across groups. The Leven's test of equality of error variances for this study is invalid or rejected. The grand mean for number of leaves is 47.13, while the standard error is 0.231 at 95% confidence interval at $p \leq 0.05$ level.

Table 3: Number of Leaves After Treatment at Four Levels Between Two Weeks After Planting (2 WAP) and Eight Weeks After Planting (8 WAP)

Level	Number of Leaves	Benzene		Hydrogen peroxide		Benzene & Hydrogen peroxide		Water/Control	
		Mean	Std. D.	Mean	Std. D.	Mean	Std. D.	Mean	Std. D.
100%	Two WAP	6.00	0.00	6.00	0.00	6.00	0.00	6.00	0.00
	Three WAP	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00
	Four WAP	13.20	0.35	14.59	0.36	12.80	0.35	10.73	1.10
	Six WAP	54.17	3.37	90.11	2.13	75.73	2.77	55.78	2.56
	Eight WAP	162.08	2.89	214.85	5.68	188.62	3.32	110.73	6.29
	Total	**48.69**	**1.32**	**66.71**	**1.63**	**58.23**	**1.29**	**38.25**	**1.99**
75%	Two WAP	6.00	0.00	6.00	0.00	6.00	0.00	6.00	0.00
	Three WAP	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00
	Four WAP	11.93	0.31	14.26	0.29	12.23	0.21	12.20	0.35
	Six WAP	52.99	1.91	84.33	1.17	63.59	2.55	55.43	3.96
	Eight WAP	146.04	2.74	192.09	9.36	177.63	3.58	109.53	8.97
	Total	**44.99**	**0.99**	**60.94**	**2.164**	**53.49**	**1.27**	**38.23**	**2.66**
50%	Two WAP	6.00	0.00	6.00	0.00	6.00	0.00	6.00	0.00
	Three WAP	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00
	Four WAP	12.53	0.75	14.2	0.35	11.53	0.81	11.20	1.25
	Six WAP	46.40	1.99	76.27	3.64	52.96	3.24	51.49	0.36
	Eight WAP	122.65	5.53	178.75	6.66	166.95	3.79	74.62	54.83
	Total	**39.12**	**1.65**	**56.64**	**2.13**	**49.09**	**1.568**	**30.26**	**11.29**
25%	Two WAP	6.00	0.00	6.00	0.00	6.00	0.00	6.00	0.00
	Three WAP	8.00	0.00	8.00	0.00	8.00	0.00	8.00	0.00
	Four WAP	12.13	0.23	14.07	0.12	12.60	1.04	10.80	1.39
	Six WAP	41.25	1.74	70.98	1.48	46.48	2.46	54.24	3.36
	Eight WAP	109.09	7.23	156.72	7.90	156.26	3.88	106.56	4.94
	Total	**35.29**	**1.84**	**51.15**	**1.90**	**45.87**	**1.48**	**37.12**	**1.94**

Number of leaves in hydrogen peroxide treatment at all levels triple between six weeks and eight weeks after planting. Contrary to this vein, the benzene, benzene and hydrogen peroxide mixture, and water treatment at all levels double their number of leaves between six weeks after planting and eight weeks after planting. Despite the doubling in number of leaves in Benzene and the mixture of benzene and hydrogen peroxide, they could not compare to the number of leaves in Hydrogen Peroxide treatment at all levels. A modified gene controlling leaves growth and shooting of new branches could have attributed the number of leaves in Hydrogen Peroxide treatment. The Hydrogen Peroxide treatment have more branches in a network that is dissimilar to sesame. The Hydrogen Peroxide treatment double the number of leaves in water treatment, which serves as control (Table 3 and Table 4).

Table 4.4: Difference Between Number of Leaves at 2Weeks to 8 Weeks after planting

Mean Number of Leaves WAP (I)	Mean Number of Leaves WAP (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
6.00	8.00	-2.00*	0.73	0.02	-3.62	-0.37
	12.66	-6.56*	0.73	0.00	-8.19	-4.93
	61.67	-54.76*	0.73	0.00	-56.38	-53.13
	150.11	-142.32*	0.73	0.00	-143.95	-140.69
8.00	6.00	2.00*	0.73	0.02	0.37	3.62
	12.66	-4.56*	0.73	0.00	-6.19	-2.93
	61.67	-52.76*	0.73	0.00	-54.38	-51.13
	150.11	-140.32*	0.73	0.00	-141.95	-138.66
12.66	6.00	6.56*	0.73	0.00	4.93	8.19
	8.00	4.56*	0.73	0.00	2.93	6.19
	61.67	-48.19*	0.73	0.00	-49.82	-46.57
	150.11	-135.75*	0.73	0.00	-137.38	-134.13
61.67	6.00	54.76*	0.73	0.00	53.13	56.38
	8.00	52.76*	0.73	0.00	51.13	54.38
	12.66	48.19*	0.73	0.00	46.57	49.82
	150.11	-87.56*	0.73	0.00	-89.18	-85.93
150.11	6.00	142.32*	0.73	0.00	140.69	143.95
	8.00	140.32*	0.73	0.00	138.69	141.95
	12.66	135.75*	0.73	0.00	134.13	137.38
	61.67	87.56*	0.73	0.00	85.93	89.18

WAP: Weeks after Planting. Two WAP = 6.0000, Three WAP = 8.0000, Four WAP = 12.6600, Six WAP = 61.6751, Eight WAP = 150.1131. Based on observed means. The error term is Mean Square (Error) = 0.800. * The mean difference is significant at the 0.05 level.

The increases in dimensions and leaf area were possibly due to the fact that cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasm to nuclear volume. The increase in size may translate to an increase in plant parts such as leaf area, diameter of the stem, size of roots, bud and capsule size [22]. The increase in the leaf area of the sesame variety under investigation implies that there is chromosomal increase in size. Polyploidy usually leads to thicker leaves, a deeper green colour, increased width-to-length ratio of leaves, larger and heavily textured flowers, and a more compact growth habit and hence in agreement with other studies [23-26].

M2 generation usually have larger leaves when compared with main plants or M1 [27]. In other hand, polyploid plants usually have thicker roots and stems [28-30,22]. In this study plants under ploidy inducer agents (mutagens) differed from diploid plants (controls plant) in growth rate and morphology with broader leaves, more branches, more number of leaves, among others. Similar phenotypic variations, such as larger plant parts in polyploid compared with diploid plants, as reported in plants of alfalfa, potato and barley [31,32].

Liu & Bao, (2007) stated that the number of branches in the tetraploid plants are lower (was reduced) and the average branch length was shorter while increased branching has been reported with colchicines treatment in other studies [33-36] reports that colchicine treatments enhanced the number of branches in sesame as well. Which agrees with this study. It was also observed, that the number of branches increased with higher concentrations of mutation inducer agents. Especially in the Hydrogen Peroxide and the mixture of benzene and hydrogen peroxide.

Leaf Area

The leaves area during the M2 generation after treatment with Benzene, Hydrogen Peroxide and the mixture of Benzene, and Hydrogen Peroxide at all levels is presented in Table 5. A close look at Table 5 will reveal that the leaves area in the Hydrogen Peroxide treatment is broad. When compared to the Benzene treatment at all levels. The leaves area in Hydrogen Peroxide treatment is significant at $p \leq 0.05$ when compared to control. Benzene treatment alone at all levels do not show any significant increase in leave area at $P \leq 0.05$ when compared to the control. However, the leaves appear dark green in colour.

The mixture of Benzene and Hydrogen peroxide at level 50% and 25% produce plants with smaller leaves than the same treatment at level 100% and 75%.

The leaves area of sesame continue to increase throughout from two weeks after planting to eight weeks after planting to eight weeks after planting. This can be observed in the mean values of the leaves area at all treatments and levels in Table 5.

Table 4.5: Leaves Area After Treatment at Four Levels Between Two Weeks After Planting (2 WAP) and Eight Weeks After Planting (8 WAP)

Level	Leaves Area	Benzene		Hydrogen peroxide		Benzene & Hydrogen peroxide		Water/Control	
		Mean (cm^2)	Std. D.	Mean (cm^2)	Std. D.	Mean (cm^2)	Std. D.	Mean (cm^2)	Std. D.
100%	Two WAP	9.48	0.07	6.16	0.16	5.30	0.09	4.20	0.10
	Three WAP	32.17	0.43	25.98	0.61	31.17	0.67	19.68	1.00
	Four WAP	61.56	1.21	104.92	2.06	82.03	1.74	60.51	1.80
	Six WAP	175.89	13.22	285.27	3.82	248.29	1.59	201.82	8.57
	Eight WAP	288.13	8.32	408.49	15.92	298.68	69.75	254.27	11.50
	Total	**113.44**	**4.65**	**166.16**	**4.51**	**133.09**	**14.77**	**108.09**	**4.59**
75%	Two WAP	8.77	0.41	5.80	0.13	5.38	0.21	4.38	0.25
	Three WAP	26.95	0.749	24.33	0.60	30.06	0.41	18.95	0.74
	Four WAP	79.98	0.20	101.94	1.15	68.55	0.87	59.84	1.16
	Six WAP	206.77	5.59	277.38	1.92	237.79	3.96	200.64	8.64
	Eight WAP	225.53	7.84	346.31	36.89	265.24	19.61	243.94	12.18
	Total	**109.59**	**2.96**	**151.15**	**8.14**	**121.40**	**5.01**	**105.55**	**4.59**
50%	Two WAP	8.45	0.05	5.14	0.06	4.85	0.05	4.22	0.36
	Three WAP	25.71	0.84	23.34	1.01	25.27	1.00	19.18	1.78
	Four WAP	88.70	0.78	94.77	1.02	72.10	1.39	62.30	2.79
	Six WAP	229.15	7.45	267.20	2.93	164.99	13.07	199.60	9.84
	Eight WAP	186.32	12.19	261.95	17.80	218.70	4.480	222.27	12.19
	Total	**107.67**	**4.27**	**130.48**	**4.57**	**97.18**	**4.00**	**101.52**	**5.38**
25%	Two WAP	8.10	0.09	4.96	0.15	4.53	0.29	4.472	0.37
	Three WAP	24.58	0.64	21.32	1.04	25.30	0.97	19.596	0.57
	Four WAP	54.61	0.65	87.42	1.73	63.75	1.16	65.3467	3.05
	Six WAP	231.95	3.52	256.11	1.27	134.20	7.08	199.4067	14.56
	Eight WAP	177.43	6.97	231.17	8.54	209.01	11.58	223.7307	38.13
	Total	**99.34**	**2.37**	**120.19**	**2.55**	**87.36**	**4.22**	**102.51042**	**11.34**

How much a plant use sunlight to manufacture its own food directly depend on the surface area of the plant's leaf area, number of leaves, height and size. In this study, the leaf area differs with respect to treatment and levels respectively. Mauchly's test of sphericity shows that, there is no equal variances among the test groups and levels. This implies that, the mutagenic effect of benzene and hydrogen peroxide affect the leaves area of the tested variety of sesame. Two weeks after planting, the leaf area of hydrogen peroxide treatment was between 8.1 and 9.5. This figure is high compare to the values obtained for benzene, mixture of benzene and hydrogen peroxide and control treatments. Benzene treatment later show increase in leave area between three weeks to four weeks after planting. The leaf area were larger than the hydrogen peroxide treatment after four weeks of planting.

Table 6: Mean value Comparisons of Leaf Area after 2 Weeks to 8 Weeks of planting

Mean Leaves Area WAP (I)	Mean Leaves Area WAP (J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
5.88	24.60	-18.71*	2.77	0.00	-24.88	-12.53
	75.52	-69.63*	2.77	0.00	-75.80	-63.46
	219.27	-213.39*	2.77	0.00	-219.56	-207.21
	253.20	-247.31*	2.77	0.00	-253.48	-241.13
24.60	5.88	18.71*	2.77	0.00	12.53	24.88
	75.52	-50.92*	2.77	0.00	-57.09	-44.74
	219.27	-194.67*	2.77	0.00	-200.85	-188.50
	253.20	-228.59*	2.77	0.00	-234.77	-222.42
75.52	5.88	69.63*	2.77	0.00	63.46	75.80
	24.60	50.92*	2.77	0.00	44.74	57.09
	219.27	-143.75*	2.77	0.00	-149.93	-137.58
	253.20	-177.67*	2.77	0.00	-183.85	-171.50
219.27	5.88	213.39*	2.77	0.00	207.21	219.56
	24.60	194.67*	2.77	0.00	188.50	200.85
	75.52	143.75*	2.77	0.00	137.58	149.93
	253.20	-33.92*	2.77	0.00	-40.09	-27.74
253.2009	5.88	247.31*	2.77	0.00	241.13	253.48
	24.60	228.59*	2.77	0.00	222.42	234.77
	75.52	177.67*	2.77	0.00	171.50	183.85
	219.27	33.92*	2.77	0.00	27.74	40.09

WAP: Weeks after Planting. Two WAP = 5.8893, Three WAP = 24.6012, Four WAP = 75.5228, Six WAP = 219.2798, Eight WAP = 253.2009.
Measure: LSD
Based on observed means
The error term is Mean Square (Error) = 11.514
* The mean difference is significant at the 0.05 level.

Equality of error variance was measured by Leven's test. The test shows that the error variances are significant at $p \leq 0.05$. Within subject contrast, there is significant difference between treatment and levels, giving rise to increase in leaf area.

From Table 5, the leaf area were growing at steady rate between two weeks after planting and three weeks after planting. After three weeks of planting, the leaf area were no longer uniform. Benzene treatment have the largest plot at all levels as against the control, which has the least plot. The hydrogen peroxide treatment also experience a low plot area, signifying no significant effect of the treatment at all levels. However, between four and six weeks after planting, the leaf area were larger. This could be the period where activities within the plant is at its peak and hence need more source of light from the sunlight and expelling waste through transpiration.

Kirhara (1951) also reported that the leaves of mutant plants were large, thick and dark green than the diploid plants [37]. Similarly, broader leaves in Citrus, higher ratio of leaf width to length in Alocasia, and broader and thicker leaves in B. globosa have been reported in tetraploid genotypes [30,38,39]. Variation in the leaf size is observed among controls and plant under ploidy inducer agents' plants under the same growing conditions. From this study, the increase in leaves area and darker green leaves shows that the mutagens (benzene and hydrogen peroxide) might have induced polyploidy in sesame [24,26].

Summary

This study was carried out to evaluate the effects of hydrogen peroxide and benzene treatments on morphological traits of sesame in Keffi. NCRIBEN 02 Variety of sesame was exposed to varying concentration of benzene, hydrogen peroxide, and the mixture of the two chemical mutagens. The M2 generation of the genotypes were assessed for agronomic traits. Benzene treatment, at all levels, have the highest plant height. In M2 generation, Hydrogen Peroxide increases in number of leaves and leaf area. The increase in number of leaves and leaf area are significant at $p \leq 0.05$. The most effective mutagen for inducing mutation in sesame under Keffi environment is benzene, followed by Hydrogen Peroxide.

Conclusion

Benzene and hydrogen peroxide have proven themselves as chemical mutagens, in mutation breeding. They have unlocked several agronomic traits in sesame. Benzene treatments at all levels, show increase in plant height, while Hydrogen Peroxide shows increase in number of leaves and broad leaf area as against the control. Benzene treatment also show agronomic traits improvement at all levels.

Recommendations

The investigation designed in this study; induce variability through mutagenic treatments of existing genotype of sesame. The mutagenic effects of hydrogen peroxide, benzene and combination of the two mutagens (hydrogen peroxide and benzene) on sesame should be investigated further using other phenotypes. They may likely replace other chemical mutagens that are expensive, if further investigations confirm these findings.

APPENDIX 1: Randomize Complete Block Design

		BLOCK 1								BLOCK 2								BLOCK 3							
		100%		75%		50%		25%		100%		75%		50%		25%		100%		75%		50%		25%	
		2 m	1 m	2 m	1 m	2 m	1 m	2 m	2 m	2 m	1 m	2 m	1 m	2 m	1 m	2 m	2 m	2 m	1 m	2 m	1 m	2 m	1 m	2 m	
11 Meters	2 m	B		C		A		D		A		B		C		D		C		B		A		D	2m
	1 m																								1 m
	2 m	C		D		B		A		C		D		A		B		D		A		C		B	2m
	1m																								1m
	2 m	D		A		C		B		B		C		D		A		B		C		D		A	2m
	1m																								1m
	2m	A		B		D		C		D		A		B		C		A		D		B		C	2m
		2 m	1 m	2 m	1 m	2 m	1 m	2 m	2 m	2 m	1 m	2 m	1 m	2 m	1 m	2 m	2 m	2 m	1 m	2 m	1 m	2 m	1 m	2 m	
		36 meters																							

Figure 1: Spilt Plot Design; Plot area = Length x breath = 37 x 11 = 407 m2, A= Hydrogen Peroxide, B= Benzene, C= Mixture of Hydrogen Peroxide and Benzene, D= Water.

References

1. Kumar R, N Shunmugavalli (2016) Gamma rays and EMS induced flower colour and seed mutants in sesame Sesamum indicum l.
2. Brown JR, Thornton JL (1957) Percivall Pott (1714-1788) and Chimney Sweepers' Cancer of the Scrotum. British journal of industrial medicine 14: 68-70.
3. Hollarender A (1971) Chemical mutagens, Principles and Methods for their detection. Plenum Press, New York. 2: 552.
4. Nazarenko M (2016) Identification and characterization of mutants induced by gamma radiation in winter wheat (Triticum aestivum L.). Scientific Papers. Series A. Agronomy, 59: 350-353.
5. Das ML, Haque MM (1997) Response of sesame seeds to gamma ray and EMS in M1 generation Bangladesh J Bot 26: 75-78.
6. Li Y, Chen QM (1998) Induction of male sterility mutation in sesame (Sesamum indicum L.) and a preliminary study of the genetics of the mutation. Chinese J Oil Crop Sci 20: 24-27.
7. Mehta AK, Singh CB (1998) A gamma irradiated dichotomously branched plant type in Sesamum. Plant Sci 11: 233-235.
8. Sorour WAI, Hussein MH, Ei-imam MA (1999) Gamma-ray induced mutations in sesame (Sesamum indicum L.) I. Selection of useful mutants. Bull Faculty Agric University of Cairo 50: 516-531.
9. Govindarasu R, Ramamoorthi N (2000) Increased genetic variability following hybridization and mutagenesis in sesame. Indian J Genet 60: 251-253.
10. Sheeba A, Anbumalarmathi J, Babu S, Ibrahim SM (2005) Mutagenic effect of gamma rays and EMS in M1 generation in sesame (Sesamum indicum L.). Res on Crops 6: 303-306.
11. Diouf M, Boureima S, Diop T, Çağirgan M (2010) Gamma rays-induced mutant spectrum and frequency in sesame. Turk J Field Crops 15: 99-105.
12. Begum T, Dasgupta T (2010) A comparison on the effect of physical and chemical mutagenic treatments in sesame (Sesamum indicum L.). Genet Mol Biol 33: 761-766.
13. Begum T, Dasgupta T (2011) Effect of mutagens on character association in sesame (Sesamum indicum L.). Pak J Bot 43: 243-251.
14. Begum T, Dasgupta T (2014) Induced genetic variability, heritability and genetic advance in sesame (Sesamum indicum L.). SABRAO J Breed Genet 46: 21-33.
15. Binbol NL, Marcus ND (2010) Geography of Nasarawa State: A study of flora and fauna, Root books and Journals Ltd.
16. AOAC (1990) Official methods of Analysis. Association of official chemists, 15th Edn., Washington DC, Association of official analytical chemists, pp10-30.
17. Noggle GR (1946) The physiology of polyploidy in plants. I. Review of the literature. Lloydia 9: 153-73.
18. Stebbins GL (1971) Chromosomal Evolution in Higher Plants. Addison-Wesley, London.
19. Liu G, Li Z, Bao MO (2007) Colchicineinduced chromosome doubling in Platanus acerifolia and its effect on plant morphology. Euphytica 157: 145-154.
20. Kerr A (2001) Tetraploidy conversions: An easy and effective method of colchicine method.
21. Wright JW (1976) Introduction to forest genetics. Academic Press, New York.
22. Raufe S, Khan IA, Khan FA (2006) Colchicine-Induced Tetraploidy and Changes in Allele Frequencies in Colchicine-Treated Populations of Diploids Assessed with RAPD Markers in Gossypium arboreum L. Turk J Biol 30: 93-100.
23. Gao SL, Zhu DN, Cai ZH, Xu DR (1996) Autotetraploid plants from colchicine treated bud culture of Salvia miltiorrhiza Bge. Plant Cell Tissue Org Cult 47: 73-77.
24. Kehr AE (1996) Woody Plant Polyploidy. Am Nurseryman 183: 38-47.
25. Kermani MJ, Sarasan V, Roberts AV, Yokoya K, Wentworth J, at al. (2003) Oryzalin-induced chromosome doubling in Rosa and its eVect on plant morphology and pollen viability. Theor Appl Genet 107: 1195–1200.
26. Shao J, Chen C, Deng X (2003) In vitro induction of tetraploid in pomegranate (Punica granatum). Plant Cell Tissue & Organ Culture 75: 241-246.
27. Vandenhout H, Ortiz R, Vuylsteke D, Swennen R, Bail KV (1995) Effect of ploidy on stomatal and other quantitative traits in plantain and banana hybrids. Euphytica 83: 117-122.
28. Pal M, Khoshoo TN (1977) Evolution and improvement of

cultivated amaranths. VIII. Induced autotetra ploidy in grain types. Z.P flanzenzuecht 78: 135-48.

29. Hahn SK, Bai KV, Asiedu R (1990) Tetraploids, triploids, and 2n pollen from diploid interspecific crosses with cassava. Theor. Appl.Genet 79: 433-39.
30. Rose JB, Kubba, J, Tobutt KR (2000) Chromosome doubling in sterile Syringa vulgaris × S. pinnatifolia hybrids by in vitro culture of nodal explants. Plant Cell Tissue Org Cult 63: 127-132.
31. Lee M, Phillips RL (1988) The chromosomal basis of somaclonal variation. Annu Rev Plant Physiol Plant Mol Biol 39: 413-437.
32. Choi HW, Lemaux PG, Cho MJ (2000) Increased chromosomal variation in transgenic versus nontransgenic barley (Hordeumvulgare L.) Plants Crop Sci 40: 524-533.
33. Blakeslee AF, Avery AG (1976) Methods of inducing doubling of chromosomes in plants by treatment with colchicines. J Hered 28: 393-411.
34. Hewawasam WD, Bandara DC, Aberathne WM (2004) New Phenotypes of Crossandra infundibuliformis Var. Damica through in-vitro culture and induced mutations. Trop Agric Res 16: 253-270.
35. Ndukwu BC, Obute GC (2006) Chromosome manipulation in Black Nightshade (Solanum nigrum L. Solanaceae). Niger Delta Biologia 5: 35-40.
36. Mensah JK, Obadoni BO, Akomeah PA., Ikhajiagbe B, Ajibolu J (2007) The effects of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (Sesame indicum L.). African Journal of Biotechnology 6: 534-538.
37. Kirhara H (1951) Triploid watermelons. Proc Am Soc Hort Sci 58: 217-230.
38. Jaskani MJ, Khan IA, Husnain S (1996) Morphological description of citrus colchiploids. Proc Intl Soc Citricult 1: 130-132.
39. Thao NTP, Ureshino K, Miyajima I, Ozaki Y, Okubo H (2003) Induction of tetraploids in ornamental Alocasia through colchicine and oryzalin treatments. Plant Cell Tissue Org Cult 72: 19-25.