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Creating Variability in Sunflower (*Helianthus annuus*L.) using Induced Mutation

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Abstract: The study was conducted to assess the effect of chemical mutagen (dimethyl sulphate) in creating variability in sunflower to improve the growth and yield productivity of the sunflower. Three different sunflower varieties (SAMSUN 1, SAMSUN 3, and SAMSUN 4) were collected from the Institute for Agricultural Research (IAR) Zaira, Kaduna State. They were exposed to T1/0.25%, T2/0.50%, T3/0.75% and T4/1.00% of dimethyl sulphate for 3 h. The treatments with control were laid out in randomized complete block design (RCBD) in three replications in the research garden of the Department of Botany Federal University of Lafia, during the cropping season of 2021/2022. Observation and data were collected for some growth and yield parameters such as days to 50% germination on 15th day, days to flowering, number of leaves, plant height(cm), stem diameter (cm), head diameter (cm), seed yield per plot (g), seed yield per plant (g), 100-seed weight (g), seed volume weight (g/100 ml), hull content (%). The data collected were subjected to a two way Analysis of Variance (ANOVA) using Genstat Discovery Edition Version 12.1 software and LSD (Least Significant Difference) was used to separate mean difference at $P \leq 0.05$ level of significance. The result showed that SAMSUN 1 variety had the highest plant height (171.7 cm) at T1, seed yield per plant (41.9 g) at T2 and hull content (34.71%) at T1. The SAMSUN 3 variety proved to be superior for number of leaves (18.93) at T4 and head diameter (24.91 cm) at T2 while SAMSUM 4 performed better for parameter like 50% germination at 15 [(DAS)(50.0%)] at T4, earliest days to first flowering (50.27) at T4, seed yield per plant (438.1kgha-) at T2, seed volume weight (33.4 g/100ml) at T3 and 100-seed weight (8.02 g) at T2. The correlation coefficient showed both positively and negatively significant correlation between the growth and yield parameters. The highest positive significant correlation was observed between stem girth and head diameter (0.816) and the highest negative significant correlation was between seed volume weight and head diameter (-0.484).

Keywords: Dimethyl sulphate, sunflower, SAMSUN

Introduction

According to [1] Sunflower (*Helianthus annuus* L.) belongs to the family Asteraceae. *Helianthus* genus contains 65 different species [1, 2]. It is an important oilseed crop native to South America that is currently cultivated in many countries around the world because of its nutritional and medicinal value [3]. The plant has a rough, hairy stem, broad, coarsely toothed, rough leaves and circular heads of flowers [4]. The heads consist of many individual flowers which mature into seeds on a receptacle base [5].

Sunflower is one of the world most important annual crops grown for edible oil together with soyabeans, peanut and rapeseed [6]. It is already been used as ornamental plant and was used in ancient ceremonies [7–9]. In addition, parts of this plant are used in making dyes for the textile industry, body painting, and other decorations. Sunflower oil is used in salad dressings, for cooking and in the manufacturing of margarine and shortening [10, 11]. It is used in industry for making paints and cosmetics.

Mutations are the tools used to study the nature and functions of genes which are the building blocks and the basis of plant development, thereby producing raw materials for the genetic improvement of economic crops [12]. The main aim of the study was to create variability in sunflower for improved yield using induced mutation.

Materials and Methods

Collection of planting materials

Three (3) different sunflower seed varieties were collected from the Department of Plant Science, Institute for Agricultural Research (IAR) Samaru, Ahmadu Bello University, Zaria. They include SAMSUN 1, SAMSUN 3 and SAMSUN 4.

Preparation of mutagenic solution

0.25, 0.50, 0.75, and 1.00% of dimethyl sulphate concentrations were prepared based on weight basis following methods by [13] and where designated as T1, T2, T3 and T4 respectively while distilled water was used as control (T0).

Pre-treatment of seed with dimethyl sulfate

Seeds were subjected to the various concentrations of DMS for 3 h and afterwards they were thoroughly rinsed under a running tap water to remove traces of mutagen sticking to the seed coat by [14, 15].

Germination study

Ten (10) of the treated seeds were sown in petri dishes in the laboratory. The seeds were laid on a regular pattern on moist cotton woolin the petri dishes. The germination percentage and radicle length were determined after germination.

Field study

The sets of pre-soaked seed were sown directly into the soil. Sowing was done in the evening, just beyond sunset following the methods of [16, 17]. Seed were sown at the rate of 3 seeds per hole and at a depth of 2 cm.

Experiment design

The Laboratory experiment was carried out in a Complete Randomized Design (CRD) while the field experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications.

Parameters considered

Data for growth and yield parameters was recorded from sowing date to maturity. Data such as days to 50% germination, days to flowering, number of leaves, plant height (cm), stem girth (cm), head diameter (cm), seed yield per plot (g), seed yield per plant (g), 100-seed weight (g), seed volume weight (g/100 ml) and hull content (%) were measured.

Data analysis

The data collected were subjected to two-way Analysis of Variance (ANOVA) using Genstat Discovery Edition Version 12.1 software and LSD (Least Significant Difference) was used to separate mean difference at $P \leq 0.05$ level of significance.

Results and Discussion

The results of creating variability in sunflower using induced mutation are represented in Tables 1-12.

The effectiveness of mutagenesis depends on appropriate mutagen dose (optimal dose) that will yield a reasonable number of desired mutations for traits of interest, while inflicting the least density of undesirable effect [18]. In this study the effect of DMS treatments on seed germination was assessed to determine the optimal dose of DMS that will produce a maximum mutation density without causing extensive lethality (Table 1). The low germination percentages observed in the present study could be due to the toxic nature of the mutagen which could affect the physiological and biological processes that regulates seedling growth, causing the embryos to become weak or die. It is in concurrence with [19–21, 14] who reported a decrease in seed germination with an increase in EMS concentration in wheat, sunflower and sodium azide on cowpea respectively. Similarly some researchers have reported such a dose/concentration dependent inhibition of seed germination in sunflower [22, 23]. Many works have also reported such a dose/concentration dependent inhibition of seed germination for different crops [24, 25] in soybean. A similar study by [26] reports that reduction in germination index of seeds treated with high doses or concentrations of mutagen could be due to delay or inhibition of physiological and biological processes necessary for seed germination including enzyme activities.

Table 1: Mean 50% germination of sunflower varieties exposed to dimethyl sulphate at 15 (DAS)

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN 1	31.5	40.7	42.6	29.6	37.0	36.29	6.18
SAMSUN 3	33.3	35.2	25.9	25.9	38.9	31.85	
SAMSUN 4	38.9	33.3	50.0	35.2	29.6	37.40	
Mean	34.6	36.4	39.5	30.2	35.2		
LSD_T	7.97						

Difference between the mean that are less than the LSD are not significant

T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Table 2: Mean number of leaves of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN 1	18.00	14.42	20.66	17.55	14.83	17.09	13.12
SAMSUN 3	17.39	18.17	12.61	23.61	22.89	18.93	
SAMSUN 4	15.55	18.39	17.94	13.55	22.17	17.52	
Mean	16.98	16.99	17.07	18.24	19.96		
LSD_T	4.75						

Difference between the mean that are less than the LSD are not significant

T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Table 3: Mean stem girth (cm) of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN 1	6.95	8.35	7.99	7.56	7.86	7.59	1.09
SAMSUN 3	7.87	8.52	8.01	8.61	9.19	7.75	
SAMSUN 4	7.51	8.10	8.11	6.55	7.65	8.44	
Mean	7.45	8.32	8.04	7.57	8.23		
LSD_T	1.41						

Difference between the mean that are less than the LSD are not significant

T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

The numbers of leaves were highest under T4 concentration exposed to dimethyl sulphate which shows that, optimal exposure resulted in increased vegetative character (Table 2). The difference in response of vegetative character to different levels of dimethyl sulphate suggests that it might have exerted an effect on the DNA and genetic structure of the plant. This corroborates with [27] who reported that appropriate application of dosage or concentration of mutagen have been found to improve vegetative yields in Cowpea (*Vigna unguiculata*). This is also supported by a similar study in cereal crop where it was reported that, mutagenic agents induced notable negative character in treated Sorghum bicolor [28, 29]. Stem girth is another important character in sunflower as thinner stem can break easily in adverse growing condition where 100% yield loss may occur. On the other hand, thicker stem had an improved crop establishment, as well as greater the stem thickness, lesser chance of lodging [30]. In this the Dimethyl sulphate have shown to stimulate greater stem girth in the various varieties compared to the control which can be used to develop lodging resistant mutant sunflower variety (Table 3). This is in agreement with a finding by

[31] who reported increased stem girth in soybean in M2 generation of gamma-radiation treatment.

The mutagen (DMS) stimulated diversity in plant height compared to control as shown in SAMSUN4 T2 (Table 4). Currently, an optimal plant height is a desirable agronomic trait that contributes to a high yield. This disagree with a previous study by [32] who reported that plant height was found to be significantly reduced by high doses of mutagenic treatments and in some cases plants responded positively to lower mutagen doses and recorded a slight increase in their heights. Similarly [33] in cowpea (*Vigna unguiculata* L.) also reported reduced plant height observed in the mutant population as compared with the checks may be attributed to inhibition of auxin synthesis by the chemical mutagen. This also agrees with the finding of [34] in which it was reported that mutagens DMS and DES treatments resulted in plant height changes in both *C. officinalis* cultivars, and other flowering plants including *Salvia splendens*. At the same time,itwas observed that variations in this important agronomic trait were dose-dependent, and they were same between the studied sunflower varieties this parameter was more than in the control. These differences are probably related to different levels of plasticity in plants of the studied varieties (sunflower), and in some cases, mutagen-induced damages of the plant cells could be repaired at the initial ontogenesis stages.

Table 4: Mean plant height (cm) of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN 1	177.2	170.3	176.7	169.6	164.6	171.7	13.12
SAMSUN 3	159.6	182.0	159.0	165.9	164.0	166.1	
SAMSUN 4	162.4	182.5	195.5	152.6	162.9	171.2	
Mean	166.4	178.3	177.1	162.7	163.8		
LSD_T	16.93						

Difference between the mean that are less than the LSD are not significant

T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Table 5: Mean number of days to first flowering of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN 1	52.00	52.00	53.67	50.33	48.00	51.20	1.74
SAMSUN 3	53.33	46.33	60.33	47.67	50.33	51.60	
SAMSUN 4	50.67	51.00	48.00	53.67	48.00	50.27	
Mean	52.00	49.78	54.00	50.56	48.78		
LSD_T	2.25						

Difference between the mean that are less than the LSD are not significant

T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Regarding days to flowering, the dimethyl sulphate triggered early flowering as seen in SAMSUN 4 T4 (Table 5, Plate 1). Plants may be triggered to flower earlier; this is similar to findings reported by [34, 35] who found that ethyl methanesulfonate concentrations resulted in a significant response towards earliness of flowering in *Salvia splendens*, maize [36, 37] and winter Swede rape [38]. In a similar study, [39] observed that certain concentration of sodium azide induced early maturity in groundnut while [40] demonstrated the utility of chemical mutagen in the development of early maturing mutants in tomato. [41] opined that EMS induced early flowering by activation of flowering hormones. This suggests that application of chemical mutagen at optimum concentration positively affect the gene switch mechanism from vegetative to reproductive phases of development. Early maturity is a desirable trait in plant breeding, more so in the recent time when the world climate trend is of concern. There is dire need for development of early maturing varieties if food security must be achieved. In addition low and intermediate concentrations of mutagens generally stimulate cell growth, increase growth rate, and produce earlier flowering in specific cases as reported by [42]. The alternative response is pushing plants towards a later flowering date as seen in SAMSUN 3 T2 (Table 5). This corroborates with findings by [43] who found that DES concentrations caused a slight delay in flowering of *Amaranthus caudatus* L.

Plate 1: SAMSUN 4 T1 at 11WAS (flowering stage)

Plate 2: SAMSUN 1 T3 at physiological maturity

From the result, chemical mutagen stimulates head diameter in the study compare to the control (Table 6, Plate 2). This corroborates with the earlier findings of [44] who observed increase in head diameter of sunflower EC 625693 variety treated with sodium azide. Furthermore, dimethyl sulphate have proven to stimulate seed yield per compared to the control (Table 7). This is in concert with [45] who reported increased seed yield in urdbean after mutagenic treatments with EMS, DMS and their combination with growth regulators Indole acetic acid (IAA) and gibberellin acid (GA). This agrees with [47] who reported increased grain yield in sorghum after treatment with gamma ray.

Table 6: Mean head diameter (cm) of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN 1	20.43	22.48	22.17	22.91	24.00	22.40	2.19
SAMSUN 3	25.05	24.50	25.27	24.55	25.16	24.91	
SAMSUN 4	23.18	23.39	24.68	21.67	22.94	23.17	
Mean	22.89	23.45	24.04	23.04	24.03		
LSD_T	3.05						

Difference between the mean that are less than the LSD are not significant
T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Table 7: Mean seed yield per plant (g) of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN1	30.7	56.3	47.1	33.1	42.3	41.9	10.45
SAMSUN3	40.3	44.5	38.2	42.9	41.3	41.5	
SAMSUN4	26.1	44.9	46.5	35.6	37.7	38.2	
Mean	32.4	48.6	44.0	37.2	40.4		
LSD_T	12.77						

Difference between the mean that are less than the LSD are not significant
T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Table 8: Mean seed yield per plot (kgha-) of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN1	215.2	336.3	483.2	329.1	445.2	361.5	123.8
SAMSUN3	191.6	263.2	199.3	267.5	352.3	254.4	
SAMSUN4	322.7	510.1	698.4	357.6	304.1	438.1	
Mean	243.4	370.2	460.1	317.2	367.3		
LSD_T	165.9						

Difference between the mean that are less than the LSD are not significant
T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Table 9: Mean seed vol. weight (g/100ml) of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN1	32.89	31.66	33.44	35.07	33.78	33.37	2.09
SAMSUN3	29.00	32.88	30.00	30.55	32.22	30.93	
SAMSUN4	33.22	34.66	34.11	34.55	30.44	33.40	
Mean	31.70	33.07	32.51	33.39	32.15		
LSD_T	2.71						

Difference between the mean that are less than the LSD are not significant
T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Table 10: Mean 100-seed weight (g) of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN 1	7.7	7.2	7.2	8.2	8.0	7.58	0.75
SAMSUN 3	7.4	8.0	8.2	7.2	7.0	7.66	
SAMSUN 4	7.6	7.7	7.8	8.2	8.9	8.02	
Mean	7.6	7.6	7.7	7.9	7.9		
LSD_T	0.97						

Difference between the mean that are less than the LSD are not significant
T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Table 11: Mean hull content (%) of sunflower varieties exposed to dimethyl sulphate

Variety	Treatment Control	T1	T2	T3	T4	Mean	LSD_V
SAMSUN1	38.23	35.31	34.44	33.00	32.57	34.71	2.82
SAMSUN3	30.76	32.47	31.61	28.89	35.93	31.93	
SAMSUN4	31.87	35.08	32.84	32.85	32.49	33.03	
Mean	33.62	34.29	32.96	31.58	33.66		
LSD_T	3.80						

Difference between the mean that are less than the LSD are not significant
T1= 0.25%; T2= 0.50%; T3= 0.75%; T4= 1.00%

Dimethylsulphate has proven to stimulate the weight of seed (100 seeds) as compared to the control (Table 10). Similar outcomes of significant increase in 100-seed weight, capitulum circumference and grain yield per plant over the checks were also recorded in the induced mutation study of [48] in sunflower.

As for the hull content, the mean value varied in both positive and negative direction compared to the control (Table 11). This corroborates with the earlier findings of [44] sunflower EC 318761 variety hull content.

Table 12: Correlation coefficient between the growth and yield parameters in sunflower as exposed to dimethyl sulphate

Parameter	Plant Height	Head Diameter	Seed Yield Per Plant	Hull Content	Seed Yield Per Plot	Seed Volume Weight	100 Seed Weight	Stem Girth	Days to First Flowering	50% Germin. at 15 days	No. of Leaves
Plant Height											
Head Diameter	0.458**										
Seed Yield Per Plant	0.309*	0.149									
Hull Content	0.266	-0.139	0.097								
Seed Yield Per Plot	0.408**	0.074	0.387**	-0.032							
Seed Volume Weight	-0.057	-0.484**	0.136	0.109	0.240						
One Hundred Seed Weight	0.104	0.193	-0.184	-0.026	0.034	-0.094					
Stem Girth	0.517**	0.816**	0.321*	0.036	0.078	-0.403**	0.111				
Days to First Flowering	-0.154	0.031	-0.118	0.046	-0.329*	-0.182	-0.001	-0.012			
Fifty Percent Germination at 15 day	0.098	-0.216	0.289	0.038	0.660**	0.255	-0.224	-0.085	-0.275		
Number of Leaves	0.226	0.083	0.085	0.108	0.164	-0.141	-0.121	0.206	-0.294*	0.050	

**Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed)

The significant and positive correlation between seed yield per plot and plant height corroborates with findings of [49] who evaluated 25 lines of sorghum during Rabi season 2015 and reported seed yield per plot showed significant positive correlation with plant height, ear head length, 100 seed weight, fodder yield and lodging percentage. The significant and positive correlation between seed yield per plant and plant height show that both parameters are affected by exposure to dimethyl sulphate. This is in conformity with the findings of [50, 51, 52] studies on sunflower correlation analysis that showed grain yield per plant was significantly and positively correlated with plant height, stem diameter, capitulum circumference and 100-seed weight. The positive and significant correlation between stem girth and head diameter concert with Kholghi *et al*. who reported significant positive correlation for head diameter, plant height, stem girth and number of filled seeds/head [53]. The negative and significant correlation between days to first flowering and seed yield per plot. The result is contrary with the result of [54, 55] who reported positive association of days to first flowering to the morpho-physiological and seed yield related traits.

Conclusion

Thus, findings on this research revealed that, there were significant and no significant differences in the growth and yield traits of sunflower as exposed to dimethyl sulphate. This suggests exposure to dimethyl sulphate might have effected some changes in the genetic material which promotes growth and yield traits of the plant. SAMSUN 1 have proven to be superior for seed yield per plant, plant height, hull content, SAMSUN 3 for head diameter, number of leaves while SAMSUN 4 was superior stem girth, 50% germination at 15 (DAS), early flowering, seed yield per plot, seed volume weight and 100-seed weight. This could also be indicative of genetic polymorphism among the sunflower varieties, which correlates with variability in the variety characteristics. This study revealed that dimethyl sulphate could be used to induce genetic variability for breeding improved crop and genetic studies in sunflower. Therefore, a careful testing of these mutagens at different concentrations is required for successive realization of plant breeding programs.

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