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Full Length Research Paper

The effects of sodium azide and colchicine treatments on morphological and yield traits of sesame seed (*Sesame indicum L.*)

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Seeds of Sesame (*Sesame indicum L.*) were exposed to varying concentrations of sodium azide and colchicine solutions ranging from 0 - 0.250% (w/v). Variations in the percentage germination and survival, number of days to maturity, plant heights, total leaf area/plant, chlorophyll content, pollen sterility, dry matter and fruit size were recorded in the C_1 and C_2 generations. The frequency of mutation/injury increased with increasing concentrations of the mutagens. The LC_{50} values based on survival percentages in the M_1 generation were fixed at 0.0776 and 0.0473% for sodium azide and colchicine respectively. There were dose related effects of the mutagenic treatments on quantitative traits resulting in reductions in traits such as germination and survival percentages, plant height, number of fruit/plant, but increases in leaf area, maturity time and fruit size. Colchicine treatment produced shortened internodes, deformed leaves, and chlorophyll mutants. Low doses of both mutagens (<0.125%) produced early maturing variants and robust/high yields and can be imposed to obtain beneficial mutants in sesame.

Key words: Sodium azide, Colchicine, Sesame, Seeds, Mutagenic effects.

INTRODUCTION

The use of mutagens in crop improvement helps to understand the mechanism of mutation induction and to quantify the frequency as well as the pattern of changes in different selected plants by mutagens. Mutation breeding generates a knowledge base that guides future users of mutation technology for crop improvement.

The mutagenic effects of sodium azide have been documented in previous reports. Kleinhofs et al. (1978) reported that sodium azide is a very potent mutagen in barley and induced chlorophyll deficiency as well as a wide range of morphological and physiological mutants. The chemical induces genetic sterility in rice without changes in vigour (Mensah et al., 2005). On the other hand colchicine is both a polyploidising and mutagenic agent (Bragal, 1955). This chemical has been used for a long time to produce polyploid plants. The mutagenic effects on plant morphology, chlorophyll, sterility and yield have earlier been confirmed by Ahoowalia (1967), Mensah (1977), and Castro et al. (2003). Balkanjieva (1980) has reported the influence of genotype on mutagenic variability in barley following colchicine treatment.

Sesame indicum L. produces seeds, which 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. Sesame oil is in high demand because of its importance in the confectionary industry worldwide. 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. However, there is paucity of information on the locally cultivated varieties, which lack variability because of their self-pollination status. The present study was therefore undertaken to fill the gap in knowledge of the genetic background of the crop and assess the effect of the two chemicals on the plant. It is our hope that the two mutagens would induce variability and thus provide information on the pattern of variation in mutagenic experiments using these mutagens on sesame plant.

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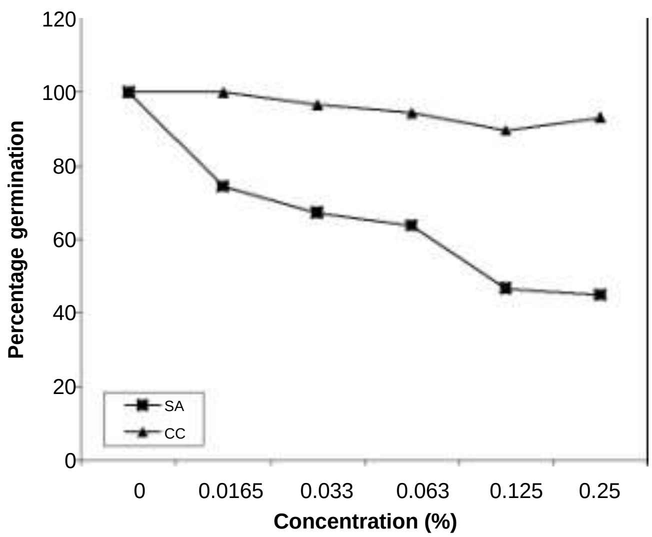

Figure 1. Effect of sodiuim azide and colchicine on germination percentage of sesame in the C_1 generation.

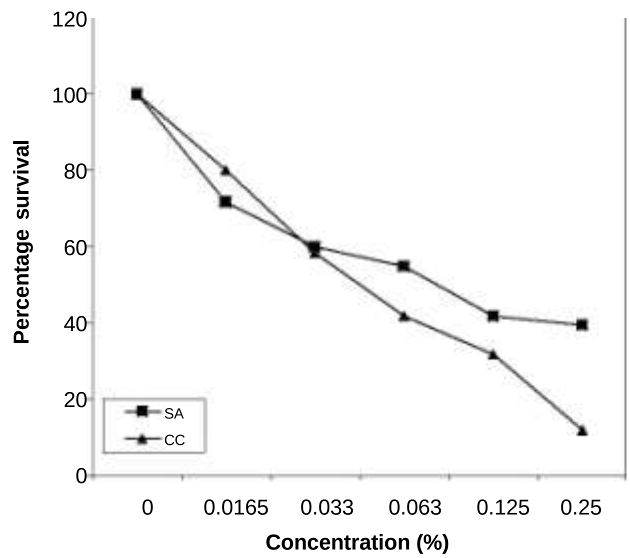

Figure 2. Effect of sodium azide and colchicine of survival percentage on sesame in the C_1 genertion.

MATERIAL AND METHODS

Germination

Seeds of *S. indicum L.* obtained from local farmers at Jattu in Edo State, Nigeria, were subjected to varying concentrations of sodium azide and colchicine (0 - 0.250% solutions) for 24 h. The treated seeds were washed in running water to remove excess chemicals and exudates from the seeds and sown in Petri dishes containing distilled water to observe germination. The seeds were observed daily until maximum germination was attained on the 7th day after sowing (DAS). The seedlings were then transferred into pots containing sandy loam soils.

Pollen studies

Estimation of pollen sterility in the treated and control material was made after Ojomo (1970). Ten flower buds of the same age were harvested randomly from five plants of each treatment, fixed together in 70% ethyl alcohol and stored in a cooler controlled by a thermostat at 4°C. Pollen that stained blue-black in iodine was considered viable while non-viable ones did not take up the stain. The ratio of viable pollen to the total number counted for each treatment was expressed as percentage pollen viability. The procedure was repeated for all buds in a sample belonging to a particular treatment. Pollen sterility was recorded as 100-pollen viability.

Pot experiments

Pot studies were undertaken to determine the effects of the two chemicals on survival (21 DAS); plant height, leaf area, number of leaves/plant; and number of fruits per plant. Fruit size and dry weights of the shoot and roots were determined to evaluate the effects of the two chemicals on yield. The effects of the chemicals on the chlorophyll content, lethality, morphological injury and the spectrum of mutations from the two mutagens were also investigated.

Chlorophyll determinations

The chlorophyll content of the leaves was carried out by following the methods of Arnon (1949). Four leaf discs of the third youngest leaf from each plant of 21 days of age from the five treatments were grounded, extracted with acetone and used for chlorophyll determinations.

RESULTS AND DISCUSSION

Laboratory studies

There were reductions in the germination and survival percentages with increasing concentrations for both chemicals in the C_1 generation (Figures 1 and 2). Reductions in germination and survival percentages due to the effects of mutagens on various crop plants have earlier been documented (Mensah, 1977; Mensah and Akomeah, 1997; Mensah et al., 2005). However the effects were more pronounced in the C_1 than C_2 generation.

To estimate the mutagenic effects of environmental factors, experiments with pollen grains (containing the male gametes) are useful because the pollen grains are relatively simple, are produced in large numbers per individual plant and can express both dominant and recessive mutations since they are haploid (Hollarender, 1971). From the present study, the effect of the chemicals on pollen sterility (Tables 1 and 2) is uniform, in that, higher concentrations led to high pollen sterility.

It is reasonable to suspect that various physiological and chromosomal damages resulting from the chemicals are responsible for the production of the large quantities of non- viable pollen and hence pollen sterility. It is interesting to note that despite the occurrence of the high level of pollen sterility at the higher dosages in the C_1 generation, seed formation was apparently not affected, because the plants are self-fertilising and hence only a minimum amount of pollen was required to effect seed

Table 1. Effects of sodium azide (SA) and colchicines (CC) on some agronomic characters of sesame in the C_1 generation.

Parameters		Concentrations (%)					
		0	0.0165	0.033	0.0625	0.125	0.250
Germination (%)	SA	100	65	65	60	45	45
	CC	100	95	85	95	85	80
Survival (%)	SA	100	81.7	50	54.8	51.7	43.3
	CC	100	80	48.3	41.7	31.7	11.7
No. of days to flowering	SA	60.8	56.5	56	62	68	68
	CC	60.8	54	53	63	73	84
Plant height of 21 DAS (cm)	SA	44.8	39.5	32.3	32.5	31.6	32.8
	CC	44.8	41.9	21.3	29.6	12.6	28.8
Dry weight of root (g)	SA	3.5	2.6	2.3	1.5	0.8	0.6
	CC	3.5	3.8	4.9	4.0	3.0	1.5
Dry wt of shoot (g)	SA	10	11.5	11.8	10.9	10.9	11.0
	CC	10	12.7	14.7	15.3	13.1	14.0
Number of fruits/plant	SA	32	30.5	22.0	19.5	15.0	12.5
	CC	32	18.4	14.0	7.5	6.5	1.5
Fruit size (g)	SA	2.36	2.49	2.3	3.0	2.2	2.4
	CC	2.7	2.7	3.2	2.3	2.3	2.0
Total Leaf area (cm)	SA	23.5	34.5	27.6	24.0	23.5	37.1
	CC	23.5	40.0	75.7	60.3	55.2	50.2
Chlorophyll content (mg/g leaf)	SA	0.52	0.597	0.664	0.510	0.450	0.230
	CC	0.52	0.52	0.52	0.521	0.573	0.560
Pollen sterility (%)	SA	0	10	16	33	40	60
	CC	0	10	20	45	50	68

DAS = Days after sowing.

formation. Similar observations have been reported by Fautrier (1976) in Lucerne, Sapra et al. (1976) in wheat and Mensah (1981) in Cowpea.

Pot studies

The number of surviving plants on the 21st day followed similar trends as that of germination but were only slightly lower in values. It has been observed from the present study that colchicine is more lethal and caused more gross morphological changes or higher mutation frequency than sodium azide. Based on the survival of the plants on the 21st day, the LC_{50} was fixed at 0.0776% for sodium azide and 0.0473% for colchicine. Thus fewer plants survived the effects of higher concentrations of colchicine compared to that of sodium azide. Both sodium azide and colchicine are known not to cause chromosomal breaks (Kleinhofs et al., 1978). The ability of these mutagens to enter the cell of living organisms to interact with the DNA produces the general toxic effects associated with their mutagenic properties. Thus, their effects are mainly due to the direct interaction between the mutagen and the DNA molecules.

The effects of sodium azide and colchicine on root or shoot length are presented in Figures 3 and 4; and Tables 1 and 2. The results revealed that increasing the concentrations of the mutagens led to decreases shoot and root lengths and hence dry weights. However, there was slight stimulatory effect on root length due to 0.033 - 0625% colchicine treatments in the C_1 and C_2 generations. The implication of this is that drought resistant variants, which are known to have long roots, could be isolated after colchicine treatment. The two chemicals induced early flowering variants at low doses (<0.125%). At higher doses (>0.125%) of mutagen treatment, days required for maturity were significantly increased. The maximum prolongation of maturity was 23 days under 0.250% colchicine treatment in the C_1 generation. In fact late as well as early maturing variants have been isolated in various crops after chemical treatment. Due to reduced vigour and prolongation of maturity period the number of pods/plants was significantly affected in these treatments. Thus reductions in number of fruits/plant over control rose from 4.4 to 95.3 % at 0.250% of the mutagens in the C_1 generation.

However, the number of leaves/plant and seed size in different treatments indicated significant reductions in the

Table 2. Effects of sodium azide (SA) and colchicines (CC) on some agronomic characters of sesame in the C_2 generation.

Parameters		Concentration (%)					
		0	0.0165	0.033	0.0625	0.1250	0.2500
Germination (%)	SA	100	100	100	98	98	90
	CC	100	100	100	96	95	92
Survival (%)	SA	100	100	98	95	94	96
	CC	100	98	96	98	95	90
No. of days to flowering	SA	61.3	58.8	56.7	55.9	62.4	62.4
	CC	61.3	54.6	53.4	58.3	60.5	61.0
Plant height at 30 DAS (cm)	SA	45.3	45.3	44.7	44.9	44.7	44.8
	CC	45.3	45.6	45.6	49.3	40.3	45.2
Dry wt of Root (g)	SA	2.5	2.5	2.6	2.6	2.5	2.5
	CC	2.5	2.8	3.3	4.2	3.5	3.4
Dry wt of shoot (g)	SA	11.1	12.3	10.9	10.9	11.4	12.1
	CC	11.1	12.3	10.5	14.6	14.7	11.5
Number of fruits/plant	SA	33.5	32.4	32.5	34.3	33.6	34.0
	CC	33.5	34.5	38.3	35.6	31.2	31.5
Fruit size (g)	SA	2.40	2.40	2.50	3.1	3.2	2.8
	CC	2.40	2.30	3.60	4.7	3.0	3.0
Total leaf area (cm^2)	SA	24.6	33.7	27.5	25.6	27.1	24.8
	CC	24.6	40.1	50.3	55.3	60.1	25.0
Chlorophyll content (mg/g leaf)	SA	0.52	0.60	0.60	0.58	0.55	0.53
	CC	0.52	0.52	0.52	0.56	0.53	0.54
Pollen sterility (%)	SA	0	0	1.6	1.8	5.0	6.9
	CC	0	0	2.2	3.7	31.7	29.9

DAS = Days after sowing.

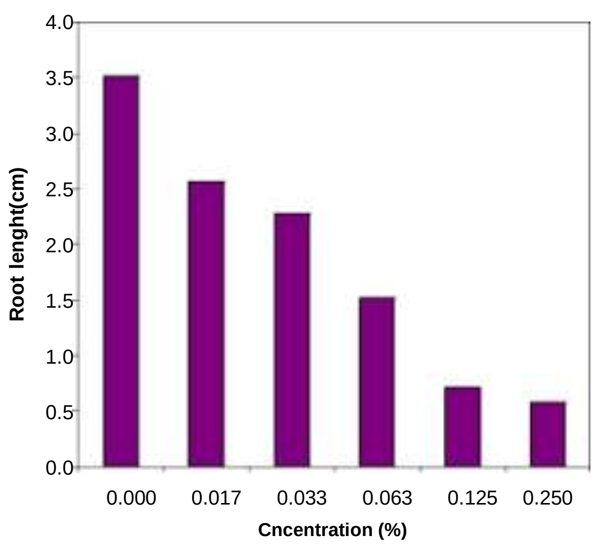

Figure 3. Effect of sodium azide on root length of sesame.

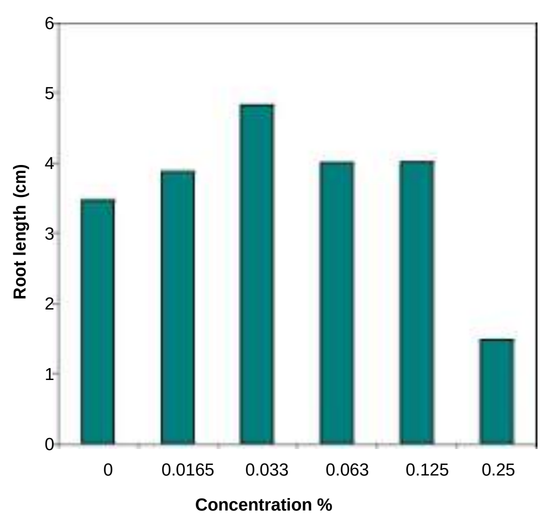

Figure 4. Effect of colchicine on root length of sesame.

higher doses of the mutagen only. Chlorophyll, which is the green pigments in leaves, is very important in plant life through the process of photosynthesis. The amount of chlorophyll produced per gram leaf tissue is affected by environmental conditions and genetic composition of the plant. In the present

Table 3. Comparison of some growth parameters of control and C_3 variant derived from C_2 generation plants.

Character	Control	C_2 variant	C_3 variant
No. of days to maturity	73.4	62.1	64.5
Plant height (cm)	44.8	55.6	50.40
No. of branches /plant	6.2	8.7	8.7
No. of fruits/plant	32.1	44.5	45.3
Yield/plant (g)	2.7	7.3	6.8
Chlorophyll content (mg/g leaf)	0.52	0.720	0.66
Dry wt of plant (g)	11.3	15.9	14.3

studies, the total chlorophyll content in the plants treated with colchicine increased at higher concentrations while sodium azide treated plants led to increases in total chlorophyll at lower concentrations only**.**

The results revealed that the most efficient concentration for inducing mutations in sesame using sodium azide is 0.0625% while 0.0125% is most efficient concentration for colchicine. Leaf abnormalities normally associated with C_l generation of mutagenic treatment were observed; yellow – green (xantha) colour was apparent on some of the leaves on some branches, under 0.0625, in the C_1 generation, while in others the deficiency was made up of irregular yellow/white spots (Viridis) of colchicine treated plants in the C_1 generation. Other leaf abnormalities recor-ded included changes in shape of leaves and chlorophyll deficiencies. The abnormalities were mainly chimera in natu-re and therefore absent in the C_2 generation. The high inci-dence of abnormalities in the experiments might have been due to diplontic selection (Gaul, 1961) or disturbances at some stage in the chlorophyll apparatus, which becomes corrected as growth progressed. The mutant cells resulting from the mutagenic treatment apparently die and non-mutants ones seem to be the precursors of the new parts. In addition morphological changes due to the mutagens observed included shortened and swollen internodes, and deformed leaves. The viable mutants recorded in the C_2 generations were however greater than those of C_1 gene-ration. Thus there was a general recovery of the adverse effects recorded during the C_1 generation compared to the C_2 generation.

A comparison of the control plants with a mutant isolated from C_2 generation of 0.125% colchicine treatment is shown in Table 3. The variant significantly produced more branches and fruit per plants than the control ($P <0.05\%$). Similar increases in number of branches and pods per plant have been reported by Biswas and Datta (1988) in *Trigonella foenumfraeum* using 0.25% E.M.S. High yielding variants using colchicine in various crops has also been developed (Bragal, 1955).

Conclusion

Generally, reduced fruit yield and vegetative matter partitioned into shoot and root dry weights were recorded. However, plants with higher yield were observed under 0.125% colchicine and given special consideration/further studies (Table 3). The useful mutant isolated through the present study need to be tested further on a wider scale to establish any changes in chromosome or allele frequency and also to assess its performance in later generations.

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