EFFECTS OF SODIUM AZIDE ON GROWTH, MORPHOLGY AND CYTOLOGY OF SOYBEAN (*GLYCINE MAX* (L) Merr)

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ABSTRACT

Seeds of three varieties of Soybean (*Glycine max*) (GTX 1448, GTX 1019, and SAMSOY II) were subjected to mutagenic treatment using Sodium Azide at concentrations of 0.5mM, 1.0mM, 1.5mM, 2.0mM, 3.0mM, and 4.0mM. Another set of seeds were soaked in distilled water and buffer solution of pH 3 and used as control treatment. These were planted in polythene bags in rows of five by five columns in a Completely Randomized Design (CRD) with three replications of each treatment. The plant attributes measured were germination percentage, seedling survival, seedling root length, seedling height, height at maturity, number of days to 50% flowering, number of fruits per plant and percentage lethality. Germination was found to be significantly ($p = 0.05$) higher in the control and decreased with increasing mutagen concentration for all three varieties. Seedling survival, seedling height, seedling root length and height at maturity also followed similar pattern. Number of days to 50% flowering was lowest at 2.0mM concentration for the three varieties and highest at 4.0mM concentration. 2.0mM concentration also produced the highest number of pods while 4.0mM concentration produced the lowest for the three varieties. Sterile mutants were also observed at higher concentration of 4.0mM. Cytological studies revealed no chromosomal aberration in all concentrations and varieties studied. The increased yield and early maturity recorded at 2.0mM concentration for the three varieties are important agronomic traits that could be utilized for subsequent improvement of Soyabean.

Keywords; Cytology, Morphology,Mutation, Sodium Azide, Soybean

INTRODUCTION

Soybean is an annual legume crop of the family Fabaceae and the genus *Glycine*. The name Soybean was derived from the Japanese word 'Shoyu', which means sauce. The plant is believed to have its origin in South East Asia and records suggest that it was first domesticated in North East China at about the 7th century BC (Cobley and Steele, 1976). It has been under cultivation for a very long time in India, Korea and Japan where it is regarded as one of the five sacred plants.

Soybean is grown for both its relatively high protein content of about 39-45% (the highest obtainable in any crop plant) and high oil content of about 20-30%. It is one of the best sources of protein as it possesses 'A' class protein, which can only be found in meat (Cobley and Steele, 1976). The oil gotten from soybean is edible and contains low level of cholesterol.

The genus *Glycine* has over forty species of which *Glycine max* is the most important. Other species in the genus include *Glycine soja, Glycine hispida* and *Glycine gracilis* (Cobley and Steele, 1976). The United States of America has been the world largest producer of Soybean since 1965 with about 33% of the world output which is about 82.3 million metric tons per annum (FAO, 2011).

The seeds occur in several seed coat colors, which include black, brown, blue, yellow and mottled. The testa color plays an important role in determining the chemical composition of the seed for example yellow testa color indicates high oil content while brown, black or blue indicate high protein content (Cobley and Steele, 1976).

Soybean production in Nigeria is faced with several problems such as attack by pests and diseases,adverse environmental conditions, late maturity, low yield e.t.c, hence there is need for research on Soybean to be focused on improving yield, enhancing maturity, enhancing resistance to diseases and pest, develop varieties that have less lodging and

free of shattering at maturity. While this can be achieved through conventional breeding methods, the genetic variability can be enhanced by utilizing novel and hopefully beneficial mutations that can be obtained by use of chemical mutagens. The present investigation was therefore undertaken to obtain information on the effect of achemical mutagen, Sodium Azide (SA) on the cytological and morphological traits of M_1 generation of three local Soybean varieties: TGX-1448-ZE, TGX-1019-ZE and SAMSOY II.

MATERIALS AND METHODS

Seeds of three varieties of *Glycine max* L (marr): TGX1448-ZE, TGX1019-ZE and SAMSOY II were obtained from the Institute of Agricultural Research (IAR) Ahmadu Bello University, Zaria.

MUTAGENIC TREATMENT

Seeds of the soybean varieties were presoaked in water for four hours and air-dried. The air-dried seeds were soaked with Sodium Azide at concentrations of 0.5mM, 1.0mM, 1.5mM, 2.0mM, 3.0mM and 4.0mM for four hours. The Sodium Azide solution was prepared using 0.1m phosphate buffer at a pH of 3. Another set of seeds were soaked in distilled water and buffer solution of pH of 3 and used as control treatment. The treatments were periodically agitated and the entire procedure conducted at room temperature. After treatment, the seeds were thoroughly rinsed with tap water.

SOWING OF SEEDS

Immediately after mutagenic treatment, the seeds were planted both in the garden and laboratory. In the laboratory, sowing involved planting treated seeds in lunch boxes on vermiculite at 30 seeds per box. Each treatment was replicated three times in a Completely Randomized Design (CRD). Planting in garden was done using polythene bags filled with loamy soil. The seeds were planted 3 per bag in rows of 5 by columns of 5.

DATA COLLECTION

i. Germination percentage: Germination percentage of each treatment was taken 14 days after planting.

ii. Seedling height: This was measured 20 days after planting to detect injury caused by the mutagen. This parameter was measured using a 30cm ruler and averages were taken and compared among treatments.

iii. Seedling survival: the number of seedlings that survived to maturity was counted at the onset of flowering. The percentage survival was then calculated per treatment to be:

$$\frac{\text{Number of plants at maturity}}{\text{Number of plants that germinated}} \times 100$$

Average of these values were then taken and compared among treatments.

iv. Height at maturity: this was measured from the soil level to the tip of the longest branch or apical bud at maturity. The measurement was done using a meter rule.

v. Yield/number of pods produced per plant: This was observed by counting the number of fruits produced per plant. The averages were then taken for the various treatments.

vi. Root length: The root lengths for the various treatments were measured using a 30cm ruler. This measurement was done using the plants in the laboratory 5 days after germination.

vii. Chlorophyll deficient mutants: no chlorophyll deficient mutant was observed in the course of the study.

viii. Number of days to 50% flowering: this was calculated from when the seeds were planted to the day they show 50% flowering. The days were then recorded for each treatment and compared.

ix. Percentage lethality: this parameter was determined to observe the extent of mutation injuries. Percentage lethality was calculated by subtracting the number of plants that survived from the number of plants that germinated and expressing the difference as a percentage. This value was also obtained for all the treatments and compared.

CYTOLOGICAL STUDY

Cytological study was carried out to check the effect(s) if any of the mutagen on the morphology and structure of the chromosomes. Cytological study was carried out in two phases i.e. mitotic and meiotic using root tips and flower buds respectively.

I. Mitotic study

Four (4) mm long root tips were cut from the laboratory seedling in each of the treatments. These were treated with Cholchicine in water for 4 hours at room temperature. The tips were then fixed in Carnoy's fluid (3:3:1) for 24 hours after which they were then transferred to 70% alcohol and stored in a refrigerator at 4°C till needed for

observation.

Before observation, the root tips were washed in distilled water, rinsed in cold 1M Ammonium chloride (NH_4Cl) and then hydrolyzed in 1M hydrogen chloride (HCl) at 60^0c for 10 minutes. Smears were then made and stained with aceto-orcein and covered with cover slip. This was pressed gently with small blotting paper, and observed under the microscope at x40 magnification.

ii. Meiotic study

Young flower buds were fixed in Carnoy's fluid for 24 hours then transferred to 70% alcohol and stored at 4^0C until needed for observation. Prior to observation, the anthers were dissected out on a clean glass slide and carefully teased out using a needle. Smears were made using the end of a clean glass rod and stained with aceto-orcein. The preparation was covered with a cover slip and viewed under a microscope at x40 magnification.

STATISTICALANALYSIS

Data collected were subjected to Analysis of variance (ANOVA) according to Gomez and Gomez (1984) to test significance among the treatments and varieties. Least Significant Different (LSD) at P=0.05 according to Scheff (1953) was used to separate the means where there was significant difference.

RESULTS

Results obtained from the treatment of *Glycine max* with Sodium Azide at concentrations of 0.5mM, 1.0mM, 1.5mM, 2.0mM, 3.0mM and 4.0mM were as follows;

PERCENTAGE GERMINATION

In all three varieties of soybean i.e. TGX 1448-ZE, TGX 1019-ZE and SAMSOY II there was a delay in germination days, which varied with increasing concentrations of the mutagen. The mean percentage germination also decreased with increased mutagen concentration. The response to mutagenic treatment was evident in the difference in the germination period of the control and treated plants. For the control plants, they germinated after seven (7) days but treated seeds germinated after eleven (11) days and at the highest concentration of 4.0mM, germination in two varieties (TGX 1448-ZE and TGX1019-ZE) took thirteen (13) days while SAMSOY II took fourteen (14) days to germinate. At concentration of 3.0mM TGX1019-ZE showed no germination at all in all three replicates. The percentage germination was highest in the control plants for the 3 varieties and declined gradually with increasing mutagen concentration except for TGX 1448 where concentration of 2.0mM had 73.33% as against 71.11% in both 1.0mM and 1.5mM. (Table 1) ANOVA showed that results obtained were significantly different ($p < 0.05$) significance level for both varieties and treatment.

Table 1: Performance of three varieties of soybean (*Glycine max*) in response to various concentrations of Sodium Azide

Variety	Treatment	% lethality	Seedling root length	% seedling survival	Number of pods per plant	Height at maturity	No. of days to 50% flowering	Seedling height	% germination
GX 1448	Control	0.00g	10.43ab	100.00a	71.00c	62.87ab	70.00b	23.33ab	82.22a
	0.5mM	14.33f	11.43a	85.67b	69.00c	64.13a	69.00bc	24.33a	73.33a
	1.0mM	21.67d	11.87a	78.37bc	74.00c	44.33c	67.00c	21.83abc	71.11a
	1.5mM	18.67e	12.57a	73.33c	90.00b	44.33c	69.00bc	21.17bc	71.11a
	2.0mM	27.00c	9.20b	73.00c	121.00a	47.27bc	58.00d	24.17a	73.33a
	3.0mM	55.00b	7.03c	45.00d	64.00c	53.53abc	66.00c	21.13bc	44.44b
	4.0mM	81.00a	5.03c	19.00e	52.00d	52.23abc	75.00a	19.83c	48.88b
TGX 1019	Control	0.00e	12.30a	98.00a	62.00c	46.90b	72.00b	25.50a	77.78a
	0.5mM	14.00d	12.43a	84.67b	63.00c	46.67b	68.00c	25.20a	77.78a
	1.0mM	13.00d	12.33a	87.00b	74.00bc	46.17b	68.00c	24.00b	51.11b
	1.5mM	23.50c	11.03b	78.00b	86.00b	56.53a	66.00d	23.17b	57.78b
	2.0mM	39.07b	9.97c	78.00b	132.00a	50.33ab	62.00e	22.17c	53.33b
	3.0mM	0.00e	0.00e	0.00d	0.00e	38.00b	0.00f	0.00e	0.00d
	4.0mM	82.53a	7.93d	18.00e	36.00d	50.33ab	78.00a	19.87d	20.00c
SAMSO	Control	0.00f	13.17a	100.00a	50.00c	68.00a	66.00bc	20.67a	80.00a
	0.5mM	25.33e	12.47a	72.00b	58.00bc	68.33a	66.00bc	20.33a	64.45ab
	1.0mM	27.67e	10.37b	74.00b	59.00bc	64.87a	62.00cd	17.50b	62.22b
	1.5mM	33.67d	8.53c	66.00bc	62.00b	62.67a	60.00d	17.37b	60.00b
	2.0mM	42.00c	6.20d	58.00c	77.00a	63.00a	51.00e	16.53b	53.33b
	3.0mM	62.67b	6.63d	32.00d	35.00d	43.67b	69.00b	13.00c	51.11bc
	4.0mM	76.33a	5.00d	18.00e	8.00e	35.67b	78.00a	9.00d	35.56c

Note: Values with the same letters in each column for the varieties are not significantly different ($p < 0.05$)

SEEDLING HEIGHT

The mean seedling height gave an indication of the extent of mutagenic injury to the plant. For all three varieties, there was steady decline in this parameter as the mutagen concentration increased except for TGX1448 where the seedling height at 2.0mM was higher (24.17cm) than 21.17cm and 21.83cm for 1.0mM and 1.5mM respectively (Table 1). The seedling heights in all control plants were not significantly different from the heights at the lower mutagenic concentrations of 0.5mM 1.5mM. SAMSOY II showed the lowest seedling height of 9.00cm at the highest mutagen concentration of 4.0mM (Table 1). Across varieties however, combined ANOVA showed significant differences ($p < 0.05$) in seedling height with TGX 1448 having the highest and SAMSOY II the lowest.

SEEDLING ROOT LENGTH

The seedling root length also gave an indication of the severity of the effect of Sodium Azide on *Glycine max* seedlings. Each of the three varieties showed different responses for this parameter. In TGX 1448, the root length in the control plant increased gradually with increasing concentration of the mutagen up till 1.5mM. At 2.0mM, the mean seedling root length started declining and continued till the highest concentration (4.0mM) which had the lowest root length of 5.27cm as against 12.60cm in 1.5mM (Table 1). For TGX 1019, there was no any significant difference ($p < 0.05$) for the concentrations from control to 1.5mM. At 2.0mM, the parameter began to decline and at 4.0mM, the lowest was observed to be 7.90cm. SAMSOY II, seedling root length decreased gradually from 13.20cm in the control to 6.20cm at 2.0mM, increased to 6.60cm at 3.0mM and reduced to 5.00cm at 4.0mM. In all varieties, SAMSOY II had the shortest mean root length at the concentration of 4.0mM. Across varieties combined ANOVA showed significant differences ($p < 0.05$) between TGX 1448 and TGX 1019 and SAMSOY II but no significant difference ($p < 0.05$) between TGX 1019 and SAMSOY II (Table 4).

PERCENTAGE LETHALITY

In contrast to seedling survival, lethality showed an increase with increasing mutagen concentration in all varieties. For, TGX 1448, the highest lethality of 81.00% was obtained at 4.0mM concentration and the lowest of 14.33% at 0.5mM. TGX 1019 also had the highest percentage lethality of 82.53% at 4.0mM and the lowest of 14.00% at 0.5mM while SAMSOY II had the lowest of 25.33% at 0.5mM and highest of 76.33% at 4.0mM. Generally, TGX 1019 had the highest lethality of 82.53% and also lowest of 14.00%. These results show that with increase in the mutagen concentration there is a corresponding increase in percentage lethality, which was significantly different ($p < 0.05$) across treatments and varieties.

Table 4: Mean performance of three varieties of soybean treated with Sodium Azide

Variety	% lethality	Seedling root length	% seedling survival	Number of pods per plant	Height at maturity	No. of days to 50% flowering	Seedling height	% germination
TGX 1448	31.10b	9.69a	67.77a	77a	52.67b	66.67a	22.26a	66.35a
TGX 1019	24.59c	9.42ab	63.38b	65b	41.09c	64.57b	19.99b	48.25c
SAMSOY II	38.24a	8.91b	60.00b	49c	58.03a	59.10c	16.34c	58.10b

Note: means with the same letters in each column are not significantly different ($p < 0.05$)

HEIGHT AT MATURITY

For all the varieties studied, results obtained from height at maturity of the plants did not follow any linear pattern of increase or decrease (Table 1). TGX 1448, the tallest plants were 64.13cm at 0.5mM concentration and the shortest was 44.33cm at 1.0mM and 1.5mM concentrations. For TGX 1019; the tallest plant was 56.53cm at 1.5mM and the shortest 38.00cm at 3.0mM concentration while SAMSOY II had 68.33cm at 0.5mM concentration and 35.67cm at 4.0mM concentration of Sodium Azide. ANOVA showed that there was a significant difference ($p < 0.05$) for both varieties and concentrations (Tables 2 & 3). DMRT also showed that the means were also significantly different ($p < 0.05$).

Table 2: Combined mean square values of effects of Sodium Azide on soybean (*Glycine max* (l) merr.)

Source of variation	Df	% lethality	Seedling root length (cm)	% seedling survival	Number of pods per plant	Height at maturity	No. of days to 50% flowering	Seedling height (cm)	% germination
Replication	2	1.09^{NS}	1.02^{*}	22.33^{NS}	44.59^{*}	41.75^{*}	0.59^{NS}	14.00^{**}	352.17^{*}
Variety	2	979.23^{**}	3.27^{**}	318.05^{*}	3930.73^{**}	1574.01^{**}	395.64^{**}	186.93^{**}	1723.20^{*}
Treatment	6	5638.42^{**}	83.52^{**}	8154.92^{**}	6541.07^{**}	779.84^{*}	923.78^{**}	165.43^{**}	2905.13^{*}
Treatment/variety	12	535.14^{**}	14.27^{*}	339.38^{*}	746.82^{*}	458.93^{*}	735.86^{**}	69.23^{**}	371.17^{*}
Error	40	5.31	1.91	41.45	46.52	45.46	3.84	1.91	107.61

Note
** = Highly significant ($p < 0.05$)
*= significant ($p < 0.05$)
NS = not significant ($p < 0.05$)

Table 3: Mean response of all three varieties of Soybean to various concentrations of Sodium Azide

Treatment	% lethality	Seedling root length (cm)	% seedling survival	Number of pods per plant	Height at maturity	No. of days to 50% flowering	Seedling height (cm)	% germination
Control	0.00g	11.97a	93.33a	61d	59.26a	69b	23.17a	80.00a
0.5mM	17.89f	12.11a	80.78b	63c	59.71a	68b	23.29a	71.85ab
1.0mM	20.78e	11.52ab	79.78b	69c	51.79bc	66c	21.11b	61.48bc
1.5mM	25.28d	10.71b	72.44c	79b	51.51bc	65c	20.57b	62.96bc
2.0mM	36.02c	8.46c	69.67c	110a	53.43ab	57d	20.96b	60.00c
3.0mM	39.22b	4.56e	25.67d	33e	32.40d	45e	11.38c	31.81d
4.0mM	79.69a	6.06d	18.33e	32e	46.08c	77a	16.23c	34.81d

Note: Figures with the same letter(s) in each column are not significantly different using DMRT ($p < 0.05$)

NUMBER OF DAYS TO 50% FLOWERING

For all the varieties, there was a decrease in the number of days to 50% flowering with increasing concentration up to 2.0mM which had the least number of days to 50% flowering for all varieties. At higher concentration of 3.0mM and 4.0mM, there was an increase in the number of days to 50% for all varieties (Table 1). SAMSOY II had the least of 51 days at 2.0mM, followed by TGX 1448 with 58 days then TGX 1019 with 62 days. The highest number of days to 50% flowering was observed at 4.0mM which was 78 days for both SAMSOY II and TGX 1019 and 75 days for TGX 1448.

For the control plants, TGX 1019 had the highest number of days to 50% flowering of 72 days while TGX 1448 had 70 days and SAMSOY II had 66 days. ANOVA and DMRT showed significant difference for this parameter (Table 2). Across treatment, the least was observed at 2.0mM while the varieties SAMSOY II had the lowest (Table 4).

Table 4: Mean performance of three varieties of soybean treated with Sodium Azide

Variety	% lethality	Seedling root length	% seedling survival	Number of pods per plant	Height at maturity (cm)	No. of days to 50% flowering	Seedling height (cm)	% germination
TGX 1448	31.10b	9.69a	67.77a	77a	52.67b	66.67a	22.26a	66.35a
TGX 1019	24.59c	9.42ab	63.38b	65b	41.09c	64.57b	19.99b	48.25c
SAMSOY II	38.24a	8.91b	60.00b	49c	58.03a	59.10c	16.34c	58.10b

Note: means with the same letters in each column are not significantly different ($p < 0.05$)

NUMBER OF PODS PER PLANT/YIELD

This serves as a measure of fertility in plants. In all the varieties, there was a gradual increase in the number of pods produced per plant as the concentration increases up to 2.0mM (Table 1).The highest overall yield was 132 in TGX 1019 at 2.0mM and the lowest was 8 in SAMSOY II at 4.0mM concentration. ANOVA showed significant difference ($p < 0.05$) across treatments with 2.0mM concentration having the greatest (110) (Table 3) and across varieties TGX 1448 having the highest number (77) while SAMSOY II had the least (49) (Table 4).

CHLOROPHYLL DEFICIENT MUTANTS

No chlorophyll deficient mutants were observed in the course of this study.

OTHER MUTANTS OBSERVED

Other forms of mutants were observed and these include;

i. A very tall mutant plant of 73cm was observed in SAMSOY II at 0.05mM concentration

ii. A sterile mutant was observed in TGX 1019 at 4.0mM concentration which produced flowers but no fruits

iii. A sterile mutant was observed in TGX 1448 at 4.0mM which produced pods with no seeds

iv. A sterile mutant was observed in SAMSOY II at 3.0mM which bore no flower at all.

DISCUSSION

Generally all the treatment concentrations reduced germination percentage in all three varieties (TGX 1448, TGX 1019 and SAMSOY II) of *Glycine max*. There was significant difference in their response to Sodium Azide and this is in conformity to other studies (Bohmova *et al.*, 1999; Waghmare and Mehra, 2000) which reported that mutagenic treatment showed a reduction in germination with increasing concentration of mutagen and that dose dependent increase of biological damages were observed.

Increase in mutagen concentration also showed a corresponding increase in the percentage lethality in all the three varieties and a corresponding decrease in the percentage seedling survival which was seen to be highly dependent on the dose of Sodium Azide used to treat the three varieties. At the highest dose, seedling survival was between 18 19% in the three varieties and the highest lethality of 76 82% was recorded. This conforms to studies of Bohmova *et al.* (1999) who reported that seeds treated with Sodium Azide showed lethality in the M_2 generation at a lesser degree.

Height at maturity of the three varieties did not show significant variation across treatments. This contradicts studies of Ashri, (1988) who reported corresponding decrease in height due to increased dose rate. This also contradicts Adamu *et al.* (2002) who reported that inhibitory effect on plant height by radiation is dose dependent but agrees with findings of Bohmova *et al.* (1999) who reported no significant difference in plant height regardless of mutagenic treatment.

The treated plants at lower concentrations matured earlier than the control plants; this is contrary to findings of Ramani and Jadon, (1991) who reported a delay in flowering of treated populations. At higher doses of 3.0 and 4.0mM, maturity was delayed. The failure of some plants to produce flowers or fruits at the highest concentration agrees with findings of Bohmova *et al.* (1999) on *Glycine max* treated with gamma radiation and Sodium Azide.

The induction of beneficial mutants in terms of plant yield was also observed in this study. High yielding mutants were observed at lower concentrations and exceptionally high yielding mutants at 2.0mM concentration with 132 in TGX 1019, 121 in TGX 1448 and 77 in SAMSOY II. This contrasts with studies of Sasi *et al.* (2005) who reported that all plant type mutants registered lower yield when compared to their parents in Okra.

The sterile mutants proved that there was genetic effect of the chemical treatment, which affected the reproductive pathways (Adamu *et al.*, 2002).

The lack of visible chromosomal aberrations in the three varieties treated with Sodium Azide conformed to the finding which indicated no chromosomal breakage in Barley (Nilan and Pearson, 1975; Kleinhofs *et al.*, 1975).

CONCLUSION

The study showed that Sodium Azide is effective in inducing mutation in Soybean. The different varieties of Soybean showed different responses to treatment concentration. For two important agronomic traits of early maturity and increased yield, the concentration of 2.0mM elicited the best response in all three varieties. The varieties, TGX 1448 showed the best response with regards to increased yield while SAMSOY II showed the best response with regards to early maturity.

REFERENCES:

Adamu, A. K., Olorunju, P. E., Bate, J. A. and Ogunlade, O. T. E. (2002): Radiosensitivity and effective dose determination in Groundnut (*Arachis hypogeal* L.) Irradiation with gamma rays. *Journal of Agriculture and Environment*, 3(1): pp71 84.

Ashri A. (1988). Mutagenic studies and breeding of groundnut (*Arachis hypogeal* L.) In: *Improvement of grain and legumes production*, proceedings of a workshop, Ollowaro, Washington, U.S.A., 1 5 July 1988.

Bohmova, B., Anna, P., Hajduch, M. and Debre, F. (1999). Effects of different mutagenic treatments on morphological traits of M_2 generation of Soya bean. *Soya bean Genetics newsletter* 26. URL http:/www.Soygenetics. org/article s/sign 1999.

Cobley, S. and Steel, L. (1976). Introduction to Botany of Tropical crops. 2nd ed. Oxford University press, London U.Kp118

FAO (2011). www.FAO.org: quarterly bulletin of statistics

Gomez, K. A. and Gomez A. A. (1984). *Statistical procedures for Agricultural Research* (2nd ed). John Wiley and Sons, p. 680

Kleinhofs, A, Sander, C.N, Lan, R.A, and Konzak, C. (1975). Azide mutagenesis mechanism and nature of mutants induced in polyploidy and induced mutation in plant breeding (proceedings of meeting, FAO (IAEA) Viena, pp195-199

Nilan, R.A. and Pearson, O. W. (1975). Lack of chromosome breakage by Azide in embryonic shoot of Barley BGN 5:33

Ramani, G. M. and Jadon, B. S. (1991). Induced variability in Groundnut in M_2 generation. *Gujarat Agriculture University Research Journal* 16(12): pp23 26

Sasi, A., Dhanavel, D. and Paradai, P. (2005). Effects of chemical mutagenesis on *Abelmoschus esculentus*. Rs On crops. 6(2) 300 306

Scheff, H. (1953). A method of judging all contrast in the Analysis of Variance. *Biometrica*. 40,pp104-107

Waghmare, V. N. and Mehra, R. B. (2002). Induced mutation in grass pea (*Lathyrus sativus*), *Newsletter*. 1 (2000), India