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Effects of Colchicine on Agronomic Performance of Soy-Bean [*Glycine Max* (L.) Merrill] Varieties

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ABSTRACT

The pot experiment was conducted to determine the effects of colchicine on Agronomic performance of soybean varieties. The seeds of two varieties of Soybean (TGX 1448-2E and SAMSOY-2) were subjected to five treatment colchicine concentrations (0, 0.5, 1.0, 1.5 and 2.0mM). The treatments were laid out in a complete randomized design. The plants were sampled for germination percentage two weeks after planting (2 WAP), plant survival (3 and 6 WAP), plant height (3 and 6 WAP), root nodule (6 WAP), leave number (6 WAP), root fresh weight (6 WAP), shoot fresh weight (6 WAP), fresh weight (6 WAP) and flower production for the assessment of growth parameters, the plants were also sampled for pod dry weight, 100 seed weight, grain yield and crude protein content at harvest. The results indicated a varying degree of effects in response to the mutagenic treatments. There was no seedling emergence in all the two varieties used in this research. Similarly, there was decrease in percentage germination with increase in colchicine

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concentrations observed in the treated seeds. At higher concentrations of colchicine (1.5 mM and 2 mM) the results revealed an increase in grain yield, crude protein and 100 seed weight. The data showed that, variety TGX 1448-2E showed higher values in germination percentage, plant survival, plant height, root nodule, leave number, shoot weight, flower production, fresh weight, pod weight, 100seed weight, grain yield and crude protein content than the other variety. In conclusion, the impact of mutagens on both vegetation and yield parameters of soybean varieties varied with the level of mutagens. TGX 1448-2E was shown to be the most responsive to the treatment doses than SAMSOY-2 variety.

Key words: Morphology, Growth, Yield, TGX 1448-2E and SAMSOY-2.

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Introduction

Soybean [*Glycine max* (L.) Merrill] belongs to the Order Fabales, Family Fabaceae, Sub-family *Faboideae*, Genus *Glycine* and Species *max*. Soybean is a legume that grows in tropical, subtropical and temperate climates. It has 40 chromosomes (2n = 40) and is a self-fertile species with less than 1% out-crossing (IITA, 2009). Soybean varies in growth and habit. The height of the plant varies from less than 0.2 to 2.0 m (0.66 to 6.56 ft). The pods, stems and leaves are covered with fine brown or gray hairs. The leaves are tri-foliate, having three to four leaflets per leaf, and the leaflets are 6–15 cm (2.4–5.9 in) long and 2–7 cm (0.79–2.76 in) broad (Lim, 2012). The leaves fall before the seeds are mature. The inconspicuous, self-fertile flowers are borne in the axial of the leaf and are white, pink or purple. The fruit is a hairy pod that grows in clusters of three to five; each pod is 3–8 cm long (1–3 in) and usually contains two to four (rarely more) seeds 5–11 mm in diameter (Lim, 2012).

Soybean occurs in various sizes, and in many hull or seed coat colours, including black, brown, blue, yellow, green and mottled. The hull of the mature bean is hard, water-resistant, and protects the cotyledon and hypocotyl from damage. If the seed coat is cracked, the seed will not germinate (Lim, 2012). Soybean is considered by many agencies to be a source of complete protein (Henkel, 2000). Complete protein is one that contains significant amounts of all the essential amino acids that must be provided to the human body because of the body's inability to synthesize them. For this reason, soybean is a good source of protein, amongst many others, for vegetarians and vegans or for people who want to reduce the amount of meat they eat (United States of America Food and Drug Administration, 2012).

Soy-protein is essentially identical to the protein of other legume seeds and pulses (Wolf, 2012). Moreover, soybean can produce at least twice as much protein per acre than any other major vegetable or grain crop besides hemp, 10 times more protein per acre than land set aside for grazing animals to make milk, and up to 15 times more protein per acre than land set aside for meat production (Wolf, 2012).

Lack of varieties tolerant to midseason drought stress and high yielding varieties tolerant to low phosphorus are among the abiotic constraints (Dugje *et al.*, 2009). Research on seed quality such as protein, oil, carbohydrate, and anti-nutritional factors is meager (Dugje *et al*., 2009). There is too much dependence on soybean [*Glycine max* (L.)Merill] which has led to tremendous increase in market price. In large parts of Sub-Saharan Africa particularly in Nigeria, smallholder agricultural production has remained consistently low and food security is catastrophically poor

This research will provide baseline information on the effects of colchicine on agronomic performance to soybean germination, morphology as well as the growth activity. The treatment of soybean with colchicine could possibly lead to attainment of beneficial genetic variability in the plant which might be useful to plant breeders.

The aim of this research was to assess the mutagenic effects of Colchicine on the morphology and growth performance of soybean (*Glycine max*).

Study Area

This research was carried out in the laboratory, Department of Biological Sciences and in the Botanical Garden, Ahmadu Bello University (A.B.U.), Zaria (Altitude 610 meters above sea level, latitude 11˚ 12'N and longitude 07˚33'E) (Osuhor *et al*., 2004), Nigeria. Samaru lies in the Northern Guinea Savanna agro ecological zone of Nigeria with a mean annual rainfall of about 1100 mm. Rainfall is essentially between May - September and dry season between October - May. Hottest month is around March - April, and the mean daily Temperature is about 27^0C. The coldest Month is between November – January (Osuhor *et al*., 2004).

Plant Source and Mutagen Treatment

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The seeds of two improved varieties of soybean *Glycine max* (L.) Merrill (*G. max* var.SAMSOY-2 and *G. max* var. TGX-1448-2E) were obtained from Department of Plant Science, Institute for Agricultural Research (I.A.R), Ahmadu Bello University, Zaria, and were taken to A.B.U Centre for Energy Research and Training, Samaru, Department of Radiology for irradiation with gamma ray (AmBe source) Fast Neutrons. Part of the seeds was treated with colchicine in the laboratory in Department of Biological Sciences, Ahmadu Bello University, Zaria (Altitude 667.88 meters above sea level, latitude 11˚ 04'N and longitude 7˚42'E).

Treatment of Seeds with the Mutagens

The seeds of two improved varieties of *Glycine max* (L.) Merrill (*G. max* var.Samsoy-2 and *G. max* var. TGX-1448-2E) were air –dried and divided into five sets, and were pre-soaked for 1 hour in distilled water. One set was the control and the remaining four sets of the seeds were soaked in various concentrations of colchicine (0, 0.5, 1.0, 1.5, and 2.0 mM) for two hours, respectively. The treated seeds were then rinsed thoroughly with distilled water, partly dried and sown in soil in polythene bags (Borkar and More., 2010).

Collection of Soil and Determination of its properties

Top soil was collected from uncultivated land within the Botanical Garden in Ahmadu Bello University, Zaria and used to fill the 60 polythene bags.

Sowing of the Seeds

Planting of the treated and the control seeds were done in Botanical garden, Ahmadu Bello University Zaria, using polythene bags; Ninety (90) polythene bags were filled with top soil and arranged randomly with

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three (3) replication per treatment and the treatment was represented six times having (30) treatment per replicate for the two varieties. Four seeds were sown per polythene bag and thinned to two plants in each polythene bag at 2WAP.

Experimental Design

The seeds of two varieties of Soybean (TGX 1448-2E and SAMSOY-2) were exposed to five treatments of colchicine concentrations (0, 0.5, 1.0, 1.5 and 2.0 mM). The experiment was replicated thrice and each replicate was represented with 6 bags. A total of 180 bags were used in this experiment. The bags were laid out in a complete randomized design.

Data Collection

One polythene bag was sampled per each replicate of treatment at 3 weeks interval starting from 3 WAP. One plant was selected from each polythene bag using simple random sampling method and the data was collected from the plant for the following parameters: germination percentage, plant height, survival at maturity, days to flowering, fresh weight and height at maturity.

Germination rate and percentage

The number of plants that came out was counted and divided by the number of plant sown, and multiplied by hundred. The estimate was carried out two weeks after planting (2 WAP).

Seedling height

Seedling heights were measured in centimeter at three weeks after planting (3 WAP). A ruler was used to measure seedlings from the ground level to the tip of the apical bud.

Seedling survival

The estimates of seedling survival were taken at three weeks after planting (3 WAP) to ascertain the relative emergence of seedling survival of the treatment population.

Survival to maturity

Plant were counted at six weeks after planting (6 WAP) and this was compared with the number of plant that germinated to determine whether there was lethality before maturity.

Days to flowering

This was determined by counting the number of days from planting to opening of flowers.

Heights at maturity

Plant height were measured six (6) weeks after planting (WAP) using a meter rule from the ground to the top of the apical bud on the main stem.

Fresh weight of the plant

Plants were uprooted six (6) weeks after planting (WAP), the bag of soil was carefully removed from the plant in order to recover most of the roots. The sampled plant was washed in water and then drained on clean table in the laboratory. The root nodules and leave number were counted then the shoot fresh weight, root fresh weight and the total fresh weight were measured using an electric weighing balance machine.

Yield Parameters

At harvest, the following yield components were determined: Number of pods, Pod weight, Total grain yield, 100 seed weight and crude protein content.

Number of pods

Number of pod per plant was determined.

Pod dry weight

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The harvested pods were dried in the laboratory after which the Pod dry weight in each treatment was measured using an electric weighing machine.

Total grain yield

Total grain yield per treatment were determined using an electric weighing machine.

100 seed weight

Seeds (100) were counted from each treatment and weighed using an electric weighing machine.

Statistical Analysis

The data obtained from the above parameters were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (2004). Duncan's multiple range test (DMRT) was used to compare significant differences between the treatment means.

Results

The results obtained from treating two varieties of Soybean with different mutagens were as follows:

Soil Properties

The properties of soil used in this experiment are as presented in Table 1. The soil contained 0.39 %N, 4.36 % organic carbon and the textural class was sandy-loam.

Colchicine Concentration

The findings on Soybean varieties with Colchicine concentration (Table, 2, 3 and 4) indicated a highly significant difference ($P \leq 0.01$) for germination percentage, plant survival 3 and 6 WAP, plant height 3 WAP, root nodules, root fresh weight, pod dry weight, 100seed weight and grain yield were significantly different ($P \leq 0.05$). There was however no significant difference for plant height 6 WAP, days to flowering, number of leave, shoot fresh weight and total fresh weight. When different concentrations of colchicine concentration were compared (Table, 2, 3 and 4), 2.0 millimole (mM) of colchicine concentration induced the greatest effect on germination. The least effect was however observed with 0.5 millimole while a moderate effect was observed with 1.0 and 1.5 mM. The lower effect produced by 0.5 and 1.0 mM were shown not to be statistically different ($P \leq 0.05$). The effect on plant survival 3 WAP followed similar pattern as the effect of plant survival 6 WAP. There was however no significant difference ($P \leq 0.05$) among the various concentrations on days to flowering, number of leaves, shoots fresh weight, total fresh weight, number of pods and crude protein. The height at maturity showed some variation in comparison with the control, 1.0 mM had the highest effect and 1.5mM had the least. A moderate effect was observed with 1.0 and 1.5 mM. On root nodules 0.5 and 1.0 produced the greatest effect compared to 2.0 mM. The effects on the concentrations on 100 seeds weight shows that 2.0 mM had the highest effect. A moderate effect was observed with 0.5 mM while the least effect was given by 1.0 and 1.5 mM (Table, 2, 3 and 4).

Varieties

The treatment of Soybean with different concentration of colchicine (Table, 2, 3 and 4) induced different responses for the two varieties. The mean estimate of the interaction obtained from the response of the two varieties indicated highly significant difference for germination percentage, plant survival 3 and 6 WAP, plant height 3 WAP, days to flowering, root nodules, shoots fresh weight, total fresh weight and all the yield parameters at ($P \leq 0.01$), were number of leave is significant at ($P \leq 0.05$) while plant height 6 WAP and root fresh weight are not

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significantly different. The mean performance of the two varieties also revealed that variety TGX 1448-2E showed the greatest response to the mutagenic treatment for all traits studied except plant height 3 and 6 WAP, days to flowering, root fresh weight and number of pods while variety SAMSOY-2 showed the least response to the mutagen for germination percentage. For plant height 6 WAP and root fresh weight moderate response was shown by variety SAMSOY-2 and showed the least response to the treatments. However, the response of the two varieties with respect to weight of 100 grains revealed that variety TGX 1448-2E gave the highest response to the mutagenic treatments while SAMSOY-2 gave the least response. There was no significant difference among the two varieties in terms of plant height 6 WAP and root fresh weight (Table, 2, 3 and 4). The number of seeds that germinated was highest at 0.5 mM in all varieties studied. This was observed in TGX 1448-2E while the least germination was observed in variety SAMSOY-2 at 2.0 mM. Furthermore, the results on height of plant at maturity revealed that the highest height attained by this crop plant at maturity was observed in variety TGX 1448-2E at 2.0 mM (23.30 cm). There was a gradual decrease in almost all the treatments among the two varieties (Table, 2, 3 and 4).

Discussion

Variability in crop plants is brought about by different factors, which may include spontaneous and manifest mutations. The effects are observable morphologically, the present study took into cognizance, such traits induced by colchicine concentration.

Percentage germination was highly significant in all the treatment studied ($P \leq 0.01$). Percentage germination was

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reduced by colchicine in the two varieties of Soybean (TGX 1448-2E and SAMSOY-2) used in this study. This reduction was found to be dose-dependent. As the dose increased, seed germination decreased. This may be attributed to high frequency of induced deletions or translocations (Adamu *et al*., 2004; Mensah *et al*., 2007; Adelanwa *et al.,* 2012 and Mathew, 2014). The result of the present study agrees with those of Khan and Wani (2005), who also reported adverse effects of radiation and chemical mutagens on seed germination in Mungbean.

Plant survival was highly significant in all the treatment studied ($P \leq 0.01$) except in the interaction of V×C was significant at ($P \leq 0.05$). Plant survivals were also found to decrease as the treatment dose increased in both varieties treated with colchicine. The result was in conformity with the work of Mensah *et al.* (2007), Shugufta (2012) and Mathew (2014) who reported that, there were dose related effects of mutagenic treatment on qualitative traits resulting in reductions of survival percentage. Adamu *et al.* (2002) made the same observation in groundnut.

Table 1: Physico-chemical parameters of the Soil used for the Experiment.

Soil Parameters	Value
pH in H_2O (1:2.5)	7.2
pH in 0.01m$CaCl_2$ (1:2.5)	6.65
Organic carbon (%)	4.36

Percentage Available Phosphorus in (ppm)	7.53
Total Nitrogen (%)	0.385
Exchange Base (Cmol/Kg)	
Ca	6.71
Mg	0.52
K	1.59
Na	0.63
CEC	12.5
Particle Size (%)	
Clay	20
Silt	26
Sand	54
Textural Class	Sandy-Loam.

Table 2: Effects of Colchicine Concentration and their Interaction on Growth Parameters of two Soybean Varieties.

Treatment	Germination Percentage (%)	Plant Survival (3 WAP) (%)	Plant Survival (6 WAP) (%)	Plant Height (3 WAP) (cm)	Plant Height (6 WAP) (cm)	Days to Flowering (No.)
Colchicine (C)						
Control	77.14^{a}	3.33^{a}	2.83^{ab}	10.15^{c}	23.15^{ab}	45.50^{a}
C1 (0.5mM)	66.46^{b}	3.16^{ab}	3.03^{a}	10.28^{bc}	23.03^{ab}	45.03^{a}
C2 (1.0mM)	60.56^{c}	2.86^{bc}	2.63^{b}	10.57^{ab}	23.51^{a}	45.60^{a}
C3 (1.5mM)	59.63^{c}	2.63^{c}	2.10^{c}	10.88^{a}	21.90^{b}	44.20^{a}
C4 (2.0mM)	52.92^{d}	2.26^{d}	1.86^{c}	10.73^{a}	23.30^{a}	46.03^{a}
SE±	1.97	0.15	0.12	0.19	0.60	0.89

Variety						
TGX	69.65^{a}	3.02^{a}	2.64^{a}	9.88^{b}	22.94^{a}	43.81^{b}
SAM	57.03^{b}	2.68^{b}	2.34^{b}	11.16^{a}	23.01^{a}	46.73^{a}
SE±	1.24	0.09	0.07	0.12	0.38	0.56
Interaction						
C×V	**	NS	NS	**	*	NS
CV $_{(\%)}$	12.07	20.79	19.35	7.17	10.25	7.65

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT (P≤ 0.05), * - Significant at (5%), **- Highly Significant at (1%).
Keys: TGX=TGX 1448-2E, SAM= SAMSOY=2, CV= Coefficient of Variance, SE±= Standard Error Mean.

Table 3: Effects of Colchicine Concentration and their Interaction on Growth Parameters of two Soybean Varieties.

Treatment	Root Nodules (No.)	No. of Leaves (No.)	Root weight (g)	Shoot weight (g)	Fresh weight (g)
Colchicine (C)					
Control	4.46^{c}	6.76^{a}	1.33^{bc}	2.96^{a}	4.29^{a}
C1 (0.5mM)	5.03^{c}	6.73^{a}	1.50^{ab}	3.00^{a}	4.50^{a}
C2 (1.0mM)	5.26^{c}	6.43^{a}	1.31^{bc}	3.03^{a}	4.34^{a}

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C3 (1.5mM)	7.93[b]	6.50[a]	1.63[a]	3.15[a]	4.78[a]
C4 (2.0mM)	13.83[a]	6.56[a]	1.15[c]	3.14[a]	4.29[a]
SE±	0.49	0.22	0.13	0.23	0.27
Variety					
TGX 1448-2E	10.12[a]	6.74[a]	1.30[a]	3.34[a]	4.65[a]
SAMSOY-2	4.49[b]	6.45[b]	1.46[a]	2.77[b]	4.23[b]
SE±	0.31	0.14	0.08	0.14	0.17
Interaction					
C×V	**	NS	**	NS	**
CV (%)	26.22	13.06	38.65	29.48	23.91

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), * - Significant at (5%), **- Highly Significant at (1%).

Keys: TGX=TGX 1448-2E, SAM= SAMSOY=2, CV= Coefficient of Variance, SE±= Standard Error Mean.

Table 4: Effects of Colchicine Concentration and their Interaction on Yield Parameters of two Soybean Varieties.

Treatment	Number of	Pod weight	100 Seeds	Grain yield	Crude protein

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	Pods (No.)	(g)	weight (g)	(g)	(%)
Colchicine (C)					
Control	7.93[a]	2.89[bc]	10.82[b]	1.76[b]	35.29[a]
C1 (0.5mM)	7.70[a]	2.66[c]	10.97[ab]	1.74[b]	35.38[a]
C2 (1.0mM)	7.96[a]	2.86[bc]	10.22[c]	1.68[b]	35.51[a]
C3 (1.5mM)	7.70[a]	3.01[b]	10.74[bc]	1.74[b]	35.31[a]
C4 (2.0mM)	8.13[a]	3.41[a]	11.42[a]	2.11[a]	35.14[a]
SE±	0.31	0.18	0.27	0.12	0.26
Variety					
TGX	7.52[b]	3.15[a]	12.91[a]	1.93[a]	35.63[a]
SAM	8.25[a]	2.77[b]	8.75[b]	1.68[b]	35.02[b]
SE±	0.24	0.08	0.18	0.03	0.17
Interaction					
C×V	NS	NS	NS	**	NS
CV (%)	19.18	16.88	10.25	12.42	3.06

Note: Means followed by the same letter(s) in each column, are not significantly different using DMRT ($P \leq 0.05$), * - Significant at (5%), **- Highly Significant at (1%).

Keys: TGX=TGX 1448-2E, SAM= SAMSOY=2, CV= Coefficient of Variance, SE±= Standard Error Mean.

The height of Soybean varieties is highly significant (P≤0.01) to all the treatments in plant height at 3 WAP and V×C in plant height at 6 WAP which is significant at (P≤0.05), the height of the plant increased considerably with dose of colchicine and their interaction. However, there were significant increases in the height of the treated plants than their respective controls. The increase in plant height in the present study might be due to the injurious effects of colchicine concentration combined with the gamma doses which led to increased cellular proliferation or cell division at cellular level. This is in conformity with the work of Lysenkov (1989) who induced a wide range of viable mutants and observed increase in wheat height. A similar result was reported by Adamu *et al*. (2002) when they irradiated the seeds of five varieties of groundnut (*Arachishypogaea*) with gamma rays Cobalt 60 (^{60}Co) source.

Days to flowering and leaf number are not significantly different to all the treatments except variety that is highly significant in days to flowering and leaf number is significant at variety level. The application of colchicine increased days to flowering and leaf number slightly higher in each variety compared with the control. This finding is in contrast to what Mensah *et al*. (2007) and Mathew (2014) reported when they observed that increase in colchicine concentration and para-dichlorobenzene decreased days to flowering and increase leaf number. The increase in days to flowering and leaf number in the present study might be due to inhibitory effects of the mutants and physiological changes in the plant as a result of delayed cellular division prior to flowering and reproductive stage especially at higher concentration of colchicine respectively. This type of contradiction might be due to either difference in the genetic material used or difference in agro-climatic condition under which the experiment was conducted

Applications of colchicine increased the number of root nodules slightly higher in each variety suggesting that this trait was also dose dependent. This may be as a result of the effectiveness of the radiation dose, which usually has adverse effect on biological materials as the radiation intensity increases (Khan and Wani, 2005).

Application of colchicine led to slight increase in root fresh weight, shoot fresh weight and fresh weight of soybean compared with the control. This gives an indication of proper adaptation to the effect of the mutagens, though the root fresh weight, shoot fresh weight and fresh weight was observed to be higher than the control. This finding is in consonan with earlier findings of Singh *et al.* (2001) who reported that increased in fresh weight was also dose dependent.

Applications of colchicine generally had no significant effect on numbers of pod but highly significant in variety at ($P \leq 0.01$) 100 seed weight is not significant but highly significant in variety and colchicine compared with the control. The pod dry weight was not significant in interaction but highly significant at variety and colchicines ($P \leq 0.01$). The Increase in colchicine concentration led to increase in pod number and 100 seed weight in Soybean varieties in most cases than their respective controls. This is in conformity with the work of Abdi *et al.* (2007), Mathew (2014), Wani and Khan (2006) who reported similar increase in number of pod, 100 seed weight, pod dry weight and also decrease in pod dry weight in *Vigna radiata* (L).

However, application of colchicine is highly significant on all the treatment ($P \leq 0.01$) compared with the control. The findings of Mathew (2014) and Wani and Khan (2006) were in agreement with those of this study when they stated that with increase in Sodium azide and gamma radiation on *Cajanus cajan* crude protein was not significantly effected, unlike grain yield.

Conclusions

The findings of this study gives an insight into the usefulness of colchicine concentration in inducing mutants that are both beneficial and deleterious or even lethal. The conclusions drawn from this study are: Seed germination in soybean varieties decreases with an increase in colchicine with 2.0 mM concentration as the most deleterious, low concentrations of the mutagens (0.5 and1.0 mM) were highly effective in inducing mutant improvements in traits such as grain yield, percentage germination and survival rate.

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