International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Effects of Gamma Radiation And Colchicine on Crude Protein Content of Soy-Bean [*Glycine Max* (L.) Merrill] Varieties

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ABSTRACT

A pot experiments was conducted to determine the effects of gamma radiation and colchicine on Crude protein content of soybean varieties. The seeds of two varieties of Soybean (TGX 1448-2E and SAMSOY-2) were subjected to five treatments (0, 5, 10, 15, and 20Krad) of gamma rays and five colchicine concentrations (0, 0.5, 1.0, 1.5 and 2.0mM). Combinations of these two mutagens were also used to treat the two soybean varieties. The treatments were laid out in a complete randomized design. The plants were sampled for flower production, number of pods, 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. The higher doses of gamma rays (15Krad and 20Krad) were particularly lethal, as there was no seedling emergence in all the two varieties used in this research. Similarly, The combination of the two mutagens gave a significant decrease in pod dry weight. At higher doses of gamma ray or higher concentrations of colchicine (20Krad and 2mM) the results revealed an increase in grain yield and 100 seed weight. The data showed that, variety TGX 1448-2E showed higher values in flower production, pod weight, 100seed weight, grain yield and crude protein content than the other variety. The results also revealed that the treatment doses in the gamma rays were effective. In conclusion, the impact of mutagens on 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: Yield, Crude Protein Content, TGX 1448-2E and SAMSOY-2.

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Introduction

Soybean is botanically called [*Glycine max* (L.) Merrill] belongs to the Order Fabales, Family Fabaceae, Sub-family *Faboideae*, Genus *Glycine* and Species *max*. (Singh *et al*., 2006). 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).

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.

This research will provide baseline information on the effects of gamma radiation and colchicine on crude protein content of soybean plant.

The aim of this research was to assess the mutagenic effects of Gamma radiation and Colchicine on the crude protein content 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

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 2.0mM) for two hours, respectively. The treated seeds were then rinsed thoroughly with distilled water, partly dried and sown in soil in polythene bags.

In the second sets of treatment, another four set of seeds were also irradiated with Gamma rays (0, 5, 10, 15 and 20Krad).These seeds were also sown in soil in polythene bags.

In the third set of treatment, combined treatment where the seeds were irradiated with Gamma rays (0, 5, 10, 15 and 20Krad) and the irradiated seeds were also soaked in all colchicine concentration for one hour, respectively. The seeds were washed with distilled water, partly dried and sown 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 900 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; Nine hundred (900) polythene bags were filled with top soil and arranged randomly with three (3) replication per treatment and the treatment was represented six times having (150) 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 (0, 5, 10, 15, and 20Krad) of gamma rays and five colchicine concentrations (0, 0.5, 1.0, 1.5 and 2.0mM). Combinations of these two mutagens were also used to treat the two soybean varieties. The experiment was replicated thrice and each replicate was represented with 6 bags. A total of 900 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: yield parameters

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

Days to flowering

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

Number of pods

Number of pod per plant was determined.

Pod dry weight

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

Colchicine Concentration

Days to flowering

The highest day to flowering was under 2.0 mM colchicine treatment (46.03). Generally, all the treatments resulted in comparable days to flowering in soybean varieties. The lowest day to flowering was due to 1.5 mM colchicine treatment (44.20) (Table 1).

Number of pods

The highest number of pods was obtained from 2.0 mM colchicine treatment (8.13). Generally, all the treatments produced comparable number of pods. The lowest number of pods was due to 0.5 mM (7.70) and 1.5 mM colchicine treatments (7.70) (Table 1).

Pod weight

The highest pod weight was produced under 2.0 mM colchicine treatment (3.41 g). The control 0 mM (2.89 g), 1.0 mM (2.86 g) and 1.5 mM colchicine treatments (3.01 g) produced comparable pod weight. The lowest pod weight was due to 0.5 mM (2.66

g) and 1.5 mM colchicine treatments (7.70) (Table 1).

100 Seeds weight

The highest 100 Seeds weight resulted from 2.0 mM colchicine treatment (11.42 g). The control (10.82 g), 0.5 mM (10.97 g) and 1.5 mM colchicine treatments (10.74 g) produced comparable 100 seed weight. The lowest 100 Seeds weight was due to 1.0 mM colchicine treatment (10.22 g) (Table 1).

Grain Yield

The highest grain yield resulted from 2.0 mM colchicine treatment (2.11 g). All the treatments resulted in comparable grain yield except 2.0 mM colchicine treatment (2.11 g). The lowest grain yield was due to 1.0 mM colchicine treatment (1.68 g) (Table 1).

Crude protein content

The highest crude protein in soybean seed was due to 1.0 mM colchicine treatment (35.51 %). All the treatments resulted in comparable seed crude protein content. The lowest crude protein was due to 2.0 mM colchicine treatment (35.14 %) (Table 1).

Gamma Radiation

Days to flowering

The highest day to flowering resulted from 20 Krad (46.20) treatment. Generally, all the treatments resulted in comparable number of days to flowering. The lowest day to flowering was due to 10 Krad (44.73) (Table 1).

Number of pods

The highest pods number was produced from 5 Krad (8.13). Generally, all the treatments produced comparable number of pods in soybean varieties. The lowest pod number was due to the control (0 Krad) (7.43) (Table 1).

Pod weight

The highest pod weight resulted from the control 0 Krad (3.15 g). Generally, all the treatments produced comparable pod weight except 15 Krad (2.77 g). The lowest pod weight was due to 15 Krad (2.77 g) (Table 1).

100 Seeds weight

The highest 100 seeds weight resulted from 20 Krad (10.96 g). Generally, all the treatments produced comparable 100 seeds weight. The lowest 100 seeds weight was due to 15 Krad (10.68 g) (Table 1).

Grain yield

The highest grain yield resulted from the control 0 Krad (1.87 g), 5 Krad (1.88 g) and 20 Krad (1.86 g) treatments. Generally, the control (1.87 g), 5 Krad (1.88 g) and 20 Krad (1.86 g) produced comparable grain yield with each other. The lowest grain yield was due to 10 krad (1.72 g) and 15 Krad (1.69 g) treatments (Table 1).

Crude protein content

The highest crude protein content resulted from 5 Krad (35.46 %) treatment. Generally, all the treatments produced comparable crude protein content. The lowest content of protein was due to 20 Krad (35.19 %) treatment (Table 1).

Varieties

Days to flowering was significantly ($p \leq 0.05$) higher in SAMSOY-2 than other variety, SAMSOY-2 variety produced significant ($p<0.05$) number of pods than the TGX 1448-2E (7.52). The pod weight, 100 seeds weight, grain yield and crude protein were significantly ($p<0.05$) higher in TGX 1448-2E variety than the other variety (Table 1).

Interaction effects of Gamma Radiation and Colchicine Concentrations

Days to flowering was significantly ($p<0.05$) interacted only at G×V, the number of pods were controlled significantly ($p<0.05$) by the effect of C×G. Pod weight, 100 seeds weight and crude protein were not control by the variables. There was high significant ($p \leq 0.01$) influence of C×G and C×V on the grain yield (Table 1).

p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Table 1: Effects of Gamma Radiation, Colchicine Concentration and their Interaction on Yield Parameters and Crude Protein Content of two Soybean Varieties.

Treatment	Days to Flowering	Number of Pods	Pod weight (g)	100 Seeds weight (g)	Grain yield (g)	Crude protein (%)
Colchicine (C)						
Control	45.50^{a}	7.93^{a}	2.89^{bc}	10.82^{b}	1.76^{b}	35.29^{a}
C1 (0.5mM)	45.03^{a}	7.70^{a}	2.66^{c}	10.97^{ab}	1.74^{b}	35.38^{a}
C2 (1.0mM)	45.60^{a}	7.96^{a}	2.86^{bc}	10.22^{c}	1.68^{b}	35.51^{a}
C3 (1.5mM)	44.20^{a}	7.70^{a}	3.01^{b}	10.74^{bc}	1.74^{b}	35.31^{a}
C4 (2.0mM)	46.03^{a}	8.13^{a}	3.41^{a}	11.42^{a}	2.11^{a}	35.14^{a}
SE±	0.89	0.31	0.18	0.27	0.12	0.26
Gamma Radiation (G)						
Control	45.03^{a}	7.43^{a}	3.15^{a}	10.92^{a}	1.87^{a}	35.31^{a}
G1 (5Krad)	45.53^{a}	8.13^{a}	3.04^{ab}	10.75^{a}	1.88^{a}	35.46^{a}
G2 (10Krad)	44.73^{a}	7.80^{a}	2.94^{ab}	10.86^{a}	1.72^{b}	35.29^{a}
G3 (15Krad)	44.86^{a}	8.03^{a}	2.77^{b}	10.68^{a}	1.69^{b}	35.38^{a}
G4 (20Krad)	46.20^{a}	8.03^{a}	2.92^{ab}	10.96^{a}	1.86^{a}	35.19^{a}
SE±	0.89	0.39	0.12	0.28	0.05	0.27
Variety						
TGX	43.81^{b}	7.52^{b}	3.15^{a}	12.91^{a}	1.93^{a}	35.63^{a}
SAM	46.73^{a}	8.25^{a}	2.77^{b}	8.75^{b}	1.68^{b}	35.02^{b}
SE±	0.56	0.24	0.08	0.18	0.03	0.17
Interaction						
C×G	NS	*	NS	NS	**	NS
C×V	NS	NS	NS	NS	**	NS
G×V	*	NS	NS	NS	NS	NS
CV (%)	7.65	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.

Discussion

Variability in crop plants is brought about by different factors, which may include spontaneous and manifest mutations. The effects are observable morphologically or cytologically. The present study took into cognizance, such traits induced by some mutagens (gamma ray, colchicine concentration and a combination of the two). 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 significant in combination of V×G, whereas, leaf number is significant at variety level. The application of gamma radiation and colchicine increased days to flowering 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. The increase in days to flowering 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 doses and concentration of gamma radiation and 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 gamma radiation and colchicine generally had no significant effect on numbers of pod but highly significant in variety at ($P \leq 0.01$) were in combination of C×G is significant at ($P \leq 0.05$) and 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 ($P \leq 0.01$) were gamma radiation at ($P \leq 0.05$). The Increase in colchicine concentration and gamma radiation 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 gamma radiation and colchicine is highly significant on all the treatment ($P \leq 0.01$), in interaction of V×G and C×G is not significant on the total grain yield 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 gamma rays and colchicine concentration in inducing mutants that are both beneficial and deleterious or even lethal on the crude protein content of soybean. The conclusions drawn from this study are: low doses or concentrations of the mutagens (0.5 and1.0mM; 5 and 10Krad) were highly effective in inducing mutant improvements in traits such as grain yield, percentage germination and survival rate

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