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Effects of streptomycin on the chemo-sensitivity and agronomic parameters of bambara groundnut (*Vigna* subterranean (L.) Verde)

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ABSTRACT

Dry seeds of Vigna subterranean (L.) Verde were exposed to varying concentrations (0%-1%) of streptomycin (ST) for 24 hours and their effects on chemo-sensitivity and agronomic parameters reported. Streptomycin was effective for inducing vegetative abnormalities (reduced plant height, swollen nodes) and different leaf colourations. However, 0.05% ST induced increases in the number of leaves, branches and root nodules per plant. The streptomycin treatment resulted in the reduction in number of days to flower at low concentration levels (0.005 and 0.05%ST) and could be utilized for improvement in the maturity date of the crop. The mutagenic frequency ranged from 2.75 to 42.7% of the surviving plants and the values increased with increases in concentrations of the chemical. The mutagenic effectiveness of streptomycin decreased with increases in concentrations. This made the lower concentrations of 0.005 and 0.05 % ST the most effective in inducing mutants in Bambara ground nut. The results indicated that the higher the concentration, the higher the level of lethality. Consequently, the higher concentrations recorded higher efficiency in inducing lethality.

Key words: Chemo- sensitivity, streptomycin, mutagenic effectiveness, Bambara groundnut.

INTRODUCTION

A good part of the study on chemical mutagenesis is based on the experience with radiation as a mutagenic agent. The broad experience obtained in that area over the past 80 years has put to good use in the chemical field [1-6]. Currently, a wide range of chemicals have been tested for their mutagenicity in various biological systems. Prominent among these are ethylenimine, N-ethyl-N-nitrisomourea, diethyl sulphonate and ethyl methane sulphonate [7-9]. Others include sodium azide, hydroxylamine, colchicin and streptomycin [10-12]

Streptomycin an antibiotic acts by reacting with non-chromosomal elements of the cell Cytoplasmic male sterility has been induced in maize through the use of streptomycin [13] and in pearl millet with a combination of streptomycin and mitomycin – C.[14]. Other workers were also able to induce both cytoplasmic and nuclear male sterility in cultivated sunflower using streptomycin and mitomycin-C[15]; barely seeds germinated in streptomycin solution contain neither chlorophyll nor carotenoids [16]. The effects of streptomycin on the structure of algal chloroplast and leaf chloroplast of higher plants have also been reported [17]. Streptomycin mimics the cool temperature reponse in rice plants. Exogenous application of streptomycin to etiolated seedlings of rice (*Oryza sativa* L) during growth in darkness at moderate temperatures induced the same type of chlorosis as that elicited by cool temperatures. A comparison of sensitive (*Indica*) and tolerant (*Japonica*) cultivars indicated a close relationship between sensitivity to streptomycin and cool temperatures. It has been observed that on exposure to streptomycin, *Euglena gracilis* var. *bacillaris* became colourless[18]. The effects of streptomycin on germinating seeds of barley, rye and cress have earlier been report. Streptomycin was observed to have two effects: a general or non-specific growth inhibiting effect resulting in stunting and thickening of the roots and thickening of the coleoptiles, and a bleaching effect [19]. Exposure to 0.2 percent (%) streptomycin was sufficient to cause the first and second leaves of the germinating seedlings to be green only at the tips. Higher concentrations resulted in completely white leaves. These earlier reports indicated that streptomycin does not destroy chlorophyll already formed but that further formation is inhibited [19].

To improve on the production of Bambara groundnut, strategies like genetic recombination and selection, induced mutation and appropriate biotechnological approaches are some of the techniques that could be used. Novel approaches aimed at shortening the generation cycle for faster breeding of protein legumes such as pea and bambara groundnut have been developed *in vitro* [20,21]. The objective of this study is to enhance genetic variability in Bambara groundnut (*Vigna subterranean (*L) Verde) using streptomycin (ST) to identify new genetic factors which would improve the breeding programe in the crop. This investigation was also undertaken to assess the frequency, effectiveness and efficiency of streptomycin as a mutagen in Bambara groundnut for the first time.

MATERIALS AND METHODS

Seeds of Bambara groundnut (*Vigna subterranean* (L.) Verde), an indigenous African legume belonging to the family Fabaceae were obtained from local farmers from Auchi, Edo State. Seeds uniform in size, cream colouration and free from pest and disease were selected and used during the present study. The seeds were treated with 0.005%, 0.05%, 0.5%, 0.75% and 1.0% (weight/volume) solutions of streptomycin (ST) in Petri-dishes at room temperature (25°C) with intermittent shaking. A set of 50 seeds were kept in distilled water to serve as control. After 24h treatment, all seeds were washed in distilled water to remove the excess chemical and toxic products, and sown in seed pans for a week and then transferred to field plots prepared in the experimental garden of Botany Department, Ambrose Alli University, Nigeria . Normal cultural practices were followed until harvest. The following parameters were studied: germination, emergence/survival rate percentage, number of leaves, number of days to flowering, dry weight, number of nodules per plant, number of branches per plant, vegetative abnormalities, and leaf

spot infestation. Mutagenic effectiveness and efficiency was determined following the methods of [12] using lethality as the basis for calculating the mutagenic efficiency.

RESULTS AND DISCUSSION

The effects of streptomycin on the parameters studied are presented below.

Germination, survival, chemo-sensitivity and mutagenic effectiveness.

The germination studies showed reductions in the germination percentages with increasing concentration of streptomycin treatment (Table 1).

Table 1: Effect of streptomycin on mean germination survival percentages, mutagenic efficiency and effectiveness on Bambara groundnut during the C1 generation

Mutagen Conc. (%)	Gernination (%)	Survival %	Lethality %	Mutagenic Frequency (M)	Mutagenic Effectiveness M/CxT	Mutagenic Efficiency M/L
0.005%	85.0	72.5	27.5	2.75	22.9	0.10
0.05%	82.0	69.5	30.5	5.8	4.83	0.19
0.5%	68.0	50.0	50	16.0	1.33	0.32
0.75%	65.0	42.5	57.5	28.2	0.60	0.49
1.0%	60.0	37.15	62.5	42.7	0.35	0.68

It is known in mutagenic experiments that the germination is inversely proportional to the dosage. The higher the mutagenic dosage, the lower the germination (Chandra and Tarar, 1988).. Survival studies were based on the number of surviving plants on the 21st day after planting (DAP) and the results followed similar trends as that of the germination (Table 1). It is apparent that the control plants had the highest survival percentage when compared with other concentrations of the mutagen. Generally, the number of survival plants decreased with increasing concentrations of the chemical mutagen under investigation. Based on the survival percentage, the LC_{50} of streptomycin was established at 0.50 % w/v exposure for 24hrs for Bambara groundnut. Previous reports [10,23,24,] have shown that survival trends are similar to that of germination but only a little lower. The present results are in line with these earlier reports It has been observed in the present investigation that higher concentrations 0.5% and 1.0% ST showed a remarkable decrease in germination. Based on the survival percentage the LC_{50} of streptomycin was established at 0.5%w/v. According to [25] the decrease in germination and survival could be due to physiological disturbance or chromosomal dosage caused to the cell by the mutagen.

The mutagenic frequency recorded in the present study ranged from 2.75to 42.7% and the frequency increased with increases in concentrations of the chemical hence dose dependent in the C1generation. The morphological aberrations used in the determination of the mutation frequencies included distorted leaf forms/ shapes, swollen /shortened internodes as well as various chlorophyll mutants (viridis, xantha and chlorina). The mutagenic effectiveness of streptomycin decreased with increases in concentrations. This made the lower concentrations of 0.005 and 0.05 % ST the most effective in inducing mutants in Bambara grountnut. The efficiency of the chemical was calculated based on its ability to cause the death of 21 day old seedlings. The results generally indicated that the higher the concentration, the higher the level of lethality. Consequently, the higher concentrations recorded higher efficiency. This result is

contrary to the previous report [12] which recorded higher mutagenic efficiency at lower concentrations in mung beans using pollen sterility as basis for the calculations.

Agronomic Studies

Increasing concentration of streptomycin led to gradual reductions in the number of leaves in 0.005%, 0.5% and 0.75%. Maximum number of leaves was recorded at 0.05% ST.

Table 2: Effect of streptomycin on the mean number of leaves per plant

Treatment	Mean Number of Leaves				
	Week 3	Week 4	Week 5	Week 6	Week 7
Control (0%)	10.00	12.66	28.21	36.00	38.36
0.005%	9.17	15.44	20.39	29.00	32.15
0.05%	10.67	20.83	30.72	42.00	45.20
0.5%	11.21	11.86	15.33	19.67	22.67
0.75%	9.15	10.13	14.00	20.19	25.00
1.0%	8.81	9.37	13.33	25.42	33.67

It was observed that increasing concentration of streptomycin showed no remarkable effect on the number of branches developed on each plant. Thus, there was no observed trend in the effects of the chemical on number of branches per plant in Bambara groundnut.

Increasing concentrations of streptomycin led to gradual reductions in the number of days to flowering at both 0.005% and 0.05% treatment by stimulating early initiation of flower bud. However, higher concentrations of 0.5%, 0.75% and 1.0% streptomycin caused delays in number of days to flowering (Table 3)

Table 3: Effect of streptomycin on the mean number of days to flowering

Treatment	Number of days to flowering
Control (0%)	39
0.005%	33
0.05%	32
0.5%	40
0.75%	44
1.0%	41

The results indicate that streptomycin could be used to produce early maturity variants. Earlier reports on the effect of streptomycin on the life cycle of plants have been documented by Mensah and Obadoni (2005).

Table 4: Effect of streptomycin on mean number of root nodules per plant

Concentration	Week 3	Week 4	Week 5	Week 6	Week 7
Control (0%)	26.67	27.00	27.50	28.00	28.00
0.05%	43.67	46.38	38.44	25.67	20.33
0.5%	36.60	30.92	26.50	26.50	26.33
0.075%	30.50	31.33	26.50	28.10	29.00
1.0%	28.11	38.90	33.33	36.17	28.33

A concentration of 0.05% ST treatment led to remarkable increases in number of nodules between the third and fifth week. This indicated that the streptomycin enhanced the development of nodules in Bambara beans. By the seventh week , the number of nodules had reduced progressively under each concentration , indicating the ineffectiveness of the root nodule system with age since the nodules have short life span in terms of age and functionality.

Vegetative yield

Increasing concentration of the chemical, led to gradual reduction in dry weight per plant but at 0.05%, the dry weight was remarkably higher than others (Table 5).

Table 5: Effect of streptomycin on mean dry weight per plant

Concentration	Week 3	Week 4	Week 5	Week 6	Week 7
Control (0%)	1.33	2.62	3.28	6.43	6.52
0.005%	1.23	1.55	1.89	3.78	4.33
0.05%	1.43	2.32	2.51	5.39	5.98
0.5%	1.32	1.39	1.47	2.00	2.03
0.75%	1.11	1.16	1.18	2.90	3.16
1.0%	1.05	1.09	1.29	2.77	3.19.

Leaf spot infestation

At higher concentrations, there appeared to be decreases in susceptibility of the plant to the leaf spot diseases. Thus, as the concentration of streptomycin increases, the number of leaves per plant infested decreases except under 0.005% treatment, where there was increase in the number of leaves infested per plant. This indicates that 0.005% ST is too low to effectively reduce cercospora leaf spot infestation.

Table 6: Effect of streptomycin on number of leaves per plant infected with leaf spot at 42 DAP

Concentration	Number of leaves per plant
Control (0%)	23.5
0.005%	23.0
0.05%	18.0
0.5%	16.5
0.75%	16.5
1.0%	13.5

It was observed in this study that 1.0 % treatment produced plants which were least susceptible to leaf spot disease (Table 6) compared to other treatments. It has previously been reported that the mutagenic treatment of Valencia 1 with Ethyl Methane Sulfonate (EMS) results in induction of large numbers of varieties to resistance to foliar disease[27].

CONCLUSION

On the basis of the observations made on the effects of streptomycin on Bambara groundnut (*Vigna subterranean* (L.) Verde), it can be asserted that the streptomycin induced variability in all the parameters under study. The useful traits observed in the present work are resistance to leaf spot disease as the concentration increases, early flowering and an increased number of leaves, branches and nodules under 0.05%. Leaf spot diseases are major causes of reduced yield in Bambara beans. Thus, plants treated with high doses of streptomycin or sprayed with streptomycin (0.05-1.0%) may serve as a source of resistance in breeding for resistant varieties in this crop.

Further studies would assist to have better understanding of inheritance pattern of these agronomic characters which were positively enhanced by the streptomycin treatment in Bambara groundnut so as to improve its genetic quality for the local farmers and hence improve on the present level of productivity of the crop.

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