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ALKYLATING EFFICIENCY OF SODIUM AZIDE ON POD YIELD, NUT SIZE AND NUTRITION COMPOSITION OF SAMNUT 10 AND SAMNUT 20 VARIETIES OF GROUNDNUT (*ARACHIS HYPOGEA* L.)

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ABSTRACT

Mutation has been utilised to improve growth and yield of many food crops, but only little effort has been made to ascertain the nutritional advantages in such improved crops. The present study evaluates the alkylating efficiency of sodium azide of different concentrations on pod yield, nut size and nutritional composition of two groundnut varieties. Dry seeds of groundnut (*Arachis hypogea* L.); Samnut 10 and Samnut 20 varieties obtained from the Ministry of Agriculture, Ilorin, Kwara State of Nigeria were treated with different sodium azide concentrations (10, 20, 30, 40 and 50mM) for 12 hours. Seeds soaked in distilled water for 12 hours were used as control for each variety. The treated seeds and control were sown in planting bags in randomized complete block design with three replicates. Alkylating effects of the treatments were studied on pod parameters and nut size while proximate analysis was carried out on the nuts to determine their nutritional composition. The results showed that sodium azide treatment of 50 mM produced significantly taller plants with more branches and leaves. Analysis of collected data revealed that sodium azide is effective in achieving significantly earlier- maturing plants with higher pod yield, bigger nut size and heavier nuts. All concentrations of Sodium azide applied induced significant higher crude protein and fat with respect to control in samnut 10 while 50 mM yielded highest protein and fat in samnut 20 variety. Generally, nutritional values of the studied groundnut were improved by sodium azide treatments with respect to protein and fat content which are the most important constituents in groundnut utilization as food or raw material for edible oil. The study concluded that the alkylating effect of sodium azide was effective on pod yield, nut size and other nut characteristics of groundnut and could be employed to improve protein, crude fat and other nutrition contents of the nuts for human and animal consumption as well as industrial applications.

Key words: Alkylating effect, nutrition composition, sodium-azide

INTRODUCTION

One of the most profound multifaceted challenges which the global population is facing in the present millennium is the need to ensure not just adequate quantity but also high quality food. Groundnut (*Arachis hypogea*) is one of the food and cash crops with potential to provide cheap plant protein and agro-based raw material for the ever increasing global population. Groundnut is an oilseed that occupies a prime place in the global economy and plays an important role as a valuable source of energy, both for humans and livestock [1]. It is also a source of edible oil and provides raw materials for a wide range of industrial products [2]. Utilization of groundnut for food and its other economic purposes have been reported [3, 4]. The value and utilization of groundnut as a crop for both nutritional and industrial purpose primarily depends upon the quality of its composition.

Several efforts have been made to manipulate economic crops' quality through production of new cultivars with relatively improved nutritional and valuable chemical or physiochemical composition targeted towards a specific end use. Plant breeding is concerned with the creation, identification, isolation, multiplication and management of genetic variability towards the development of improved cultivars. Mutation breeding has been identified as a tool in plant breeding and also as a method for the creation of genetic variability for further selection and hybridization. Mutation breeding has attained importance in recent years and has produced an increasing number of desirable cultivars in different crops by generating variability which is an important requirement for success in plant breeding programmes [5].

In nature, mutations are main source of variability, although the occurrence of natural mutations is less. Ionizing radiations and chemical mutagens provide opportunity to the breeder to enhance the mutation frequency [6]. However, the mutations are random events over which the breeder has no direct control. The effectiveness of obtaining a novel genetic variation for a given trait with mutagenesis depends on the mutagenic treatment, efficient screening techniques and also very probably, genetic background of the original phenotype.

Mutagenesis has been used to introduce novel genetic variability in ornamental, medicinal, cash and major food crops which are propagated by seeds. [6, 7]. Similarly, the role of chemical mutagen in enhancing variability in higher plants has been reported [8, 9]. The induction of a wide spectrum of genetic variability with physical and chemical mutagens enhances the available spontaneous variability for crop improvement. In addition, it also helps in analyzing and understanding of genes, their regulation and organization. The use of mutation techniques has expanded beyond application in breeding to gene discovery and reverses genetics which requires high-throughput applications of specific mutations that are induced with high efficiency over entire crop plant genomes. Consequently, the knowledge of the precise nature of induced mutation is becoming expansive in understanding specific effect of mutagens on crop plants.

Groundnut has been exposed variously to mutagenic treatments for induction of variability and improvement using both physical and chemical mutagens. Mutants have been obtained in groundnut either spontaneously or induced by physical or chemical mutagens for characteristics like lethal, chlorophyll deficiencies, plant stature, growth habit, branching pattern, pod and nuts yields[10, 11, 12]. However, there is little or no information on the mutagenic effects on the seed quality and nutritional values of the groundnut.

The present study is aimed at investigating the alkylating efficiency of sodium azide of different concentrations on fruit yield, nut size and nutritional quality of groundnut seeds of two varieties grown for commercial purposes in Nigeria.

MATERIALS AND METHODS

Materials: Healthy dry seeds of two varieties of *Arachis hypogea*; Samnut10 and Samnut 20 were obtained from Kwara State Ministry of Agriculture (KSMoA), Ilorin, Nigeria for the study. Planting bags of 30 x 36 cm were also purchased from KSMoA. Sodium azide was obtained from the Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Nigeria.

Methods

Chemical treatment: Sodium azide (NaN_3) of 10, 20, 30, 40 and 50 mM concentrations were freshly prepared in different beakers. Groundnut seeds (Samnut 10 and Samnut 20) were treated with different concentrations at room temperature (25^0C) with intermittent shaking for 12 hours. A control was set up for each of the variety by soaking seeds in distilled water for 12 hours. After 12 hours, the chemical treated seeds were removed and washed in running tap water to remove excess chemical residue. Also, the control seeds were removed from distilled water and all the seeds (including sodium azide treated seeds) were air dried before sowing.

Pot Experiment: The pot experiment was carried out in the Botanical garden, University of Ilorin (N 08^0 28' 53.3'', E 04^0 40' 28.9"), Ilorin Nigeria. The treated and control seeds were sown directly into 30 x 36 cm planting bags filled with mixture of garden soil (clay sand in ratio 2:1), laid out at 0.5 x 0.5 m spacing in a net house. Three seeds were sown in each planting bag and the experiment was in Randomized Complete Block Design (RCBD) with three replicates. The bags were moderately watered every other day, weeding and other cultural practices were carried out as at when required. At maturity, alkylating effects of sodium azide were studied with respect to morphological parameters such as plant height, number of leaves per plant, leaf length and breadth, and number of branches per plant. In addition, quantitative characters of fruit and seed related parameters, number of days of emergence of flowers, number of flowers, number of pod per plant, number of seeds per pod, seeds per plant, nut size and weight of 100 nuts were evaluated, and data were collected in replicates of three. Determination of physical seed properties like seed size dimensions were carried out following a procedure described [13]. Ten randomly selected seeds were evaluated for dimension

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(using electronic venire caliper *Titan-23175* model and Micrometer screw gauge) and the measurements were carried out in triplicates.

Proximate analysis: Determination of proximate components was carried out in the Food Analysis Laboratory of Nigerian Institute for Store Product Research Ilorin (NISPRI), Kwara State Nigeria. The percentage dry matter (moisture) was determined by drying the nuts in an oven at 103ºC – 105ºC for 24 hours and the amount of moisture was determined based on standard reports [14]**.** The micro-Kjeldahl method was employed to determine the total nitrogen and crude protein (N x 5.95) by standard methods of [14]. Crude lipids were extracted with petroleum ether using Soxhlet apparatus HT-extraction technique, percentage ash (% minerals) and crude fibre percentage was determined based on published method outlined [14.] Total carbohydrate was estimated by difference method.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using SPSS statistical software for Microsoft Window Operating System version 16.0. Significance of the differences was defined as $p < 0.05$ for ANOVA. The difference in means was compared and separated by Duncan's Multiple Range Test (DMRT) [15].

RESULTS

The alkylating effect of sodium azide on two varieties (Samnut 10 and Samnut 20) of groundnut was studied on the nut size and the nutrient composition. The results of quantitative vegetative and seed related characters evaluated at maturity showed that there was significant difference in the response of the two groundnut varieties to sodium azide concentrations. With respect to control, plant height was significantly higher in both varieties at 50 mM treatment. Also, 40 mM concentration of sodium azide produced taller plants than control in Samnut 20 variety. Leaf production was higher than control plant for all the tested concentrations in Samnut20, while in Samnut 10, higher concentrations of 30 – 50 mM yielded more leaves than control, but fewer leaves were recorded among the 10 and 20 mM plants in Samnut 10 than control (Table 1).

Generally, early maturing plants were obtained with all treatment concentrations in relation to control, nonetheless, higher doses of 40 – 50 mM plants were significantly faster in maturity for both varieties as shown in Table 1. The number of pods per plant varied among the varieties and treatment concentrations. For instance, 50 mM treated plant had an average of 51.17 pods per plant and significantly different from control plants which produced average of 47.36 pods per plant in Samnut 20 (table 1). In Samnut10, the trend was slightly different because, 40 and 50 mM treated plants produced mean pod per plant of 69.66 and 72.25 respectively to stay ahead of control (57.33). The effect of sodium azide concentrations on weight of hundred pods with nuts, number of nuts in hundred pods, weight of nuts in hundred pods are summarized in Table 1. The weight of 100 nuts was significantly higher for all treatment concentrations than control in Samnut 10, with 10 -50mM having weight that ranged from 50.67 – 55.44 g while control was 49.87 g. But, in Samnut 20, only higher concentration of 30, 40, and 50 mM (86.12, 88.10 and 88.40 g) weighed more than control (75.24) which produced heavier nut than 10 and 20 mM (74.96 an 73.17 g respectively.) as shown in Table 1.

Table 2 showed the correlation coefficient of the relationship between leaf characters evaluated and number of days to flowering for the two varieties studied. In both varieties, there was significant correlation between plant height and number of leaves. Also, leaf area correlated with number of leaves and plant height and number of leaflets. However, there was no significant correlation between leaf area and number of leaflets in Samnut 20. In the same way, no positive correlation existed between the vegetative character considered and days to flowering.

Proximate analysis showed that Samnut10 seeds treated with 10mM contained highest moisture content of 21.55% highest crude fat (43.49%) and the least carbohydrate (2.81%). The 30 mM treatment of Samnut 10 groundnut variety yielded the highest crude protein (28.10%), all mutagenic treatment in Samnut 10 yielded higher protein than the control (Table 3). Among Samnut 20 plants, highest protein was obtained in 50 mM treated plant (23.61%) which is slightly higher than that of 40 mM treated plant (23.25%) and the control (21.94%). However, maximum yield of crude fat was obtained for 10 mM treated Samnut 20 (34.95%). Generally, with respect to crude protein and fat, higher yield percentage were obtained in Samnut 10 than Samnut 20. The percentage nutrient compositions of the two varieties are summarized in Table 3.

DISCUSSION

The results of vegetative, seed (nut) characters and nutritional analyses showed significant differences in both varieties of groundnut treated with sodium azide concentrations when compared with control. Significant correlation exists between plant height and leaf parameters evaluated. Statistical analysis of data revealed significant difference in plant height and number of branches in comparison with control. Taller plants with highest number of branches were induced by 50 mM concentration of sodium azide in the two varieties studied. This finding is in agreement with report of improvement in plant height at maturity in pepper (*Capsicum annum* and *C. frutescens*) treated with Fast Neutron Irradiation for varying times [16]. In addition, it has been reported that increase in irradiation doses from 300-500 Gy favoured increases in certain morphological traits such as plant height in okra exposed to gamma irradiation [17]. However, these findings are at variance with Poornananda and Hosakatee [18], who reported a decrease in plant height of *Guizotia abyssinica* treated with sodium azide and gamma rays. Similarly, negative effects of sodium azide and Colchicine were observed on growth parameters of *Sesame indicum* [19]. The contrast in these findings suggested that different plants would respond differently to mutagenic treatments of specific dosage or concentration; it may therefore be imperative to compare the effects of different mutagens on growth parameters of crops to arrive at a valid conclusion.

The significant correlations found between plant height, number of branches and leaf characters suggested linkage among these characters. Positive relationship has been reported between plant height and foliage parameters in other plants exposed to mutagens [5, 20]. Breeding for improved plant height in groundnut would result in improvement in number of branches and leaf characters. Whereas, poor correlation of plant height, leaf parameters and number of days to flowering indicated that flowering is likely independent of these characteristics, therefore improvement in plant height and leaf characteristics may not necessarily translate into early flowering. Sodium azide treatment of all concentrations in the study possibly affected the genes that are responsible for switching from vegetative to reproductive phase in the crop by stimulating the flower and fruit related hormones which consequently resulted in early fruiting and maturity. Early flowering and fruiting process in crops treated with mutagenic agents have been reported by previous workers [19, 21].

The results obtained from the analysis of various nut (seed) parameters indicated that sodium azide could be utilised to improve nut yield, size and other nut-related characters in groundnut. In this study, it was observed that 50 mM concentration of sodium azide significantly increased the number of nuts per pod and nut dimension. Daudu and Falusi [22] also reported higher number of fruits, higher number of seed per fruit and larger fruit dimension in pepper species at high irradiation treatment with Neutron irradiation from Americium-Beryllium source. High heritability coupled with high pod yield in groundnut treated with sodium azide was also reported [10], the authors opined that effects of mutagen could have caused an additive gene effect which plays an important role in the expression of such traits.

Furthermore, 40–50 mM sodium azide significantly increased the weight of 100 nuts both in Samnut 10 and 20. Besides producing higher number of pod per plant, these concentrations produced heavier nuts with 50 mM consistently performing optimally in fruit and nut related characters. Traditionally, improved seed yield has been achieved in crop plants by mutagenesis. While studying the effect of mutagens on quantitative characters of M_2 and M_3 generations of horsegram (*Macrotyloma uniflorum*) Bolbhat and Dhumal [21] reported that combination of gamma irradiation and ethyl-methane sulphurnate (EMS) increased 1000 seed weight.

The proximate analysis revealed that protein which is the most valuable nutritive component of groundnut was improved by sodium azide of all concentrations studied in Samnut 10. While control nuts yielded 25.42% of crude protein, sodium azide treated nuts crude protein yield ranged from 25.62 to 27.78%. Though sodium azide reduced the ash content with respect to control in Samnut 10, however, its application of 10-40 mM concentrations considerably increased crude fat content of the nuts. Whereas, all applied concentrations improved crude protein composition in Samnut 10, only concentrations of 40 and 50 mM increased protein contents in Samnut 20. Moreover, the percent crude protein in Samnut 20 was generally lower than Samnut 10. Highest fat content was obtained in low concentration of 10 mM in Samnut 20. The results from nutritional analysis indicated that the two variety response to concentrations of sodium azide varied with regard to nutrient composition which concurred with the report of

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Adamu *et al.*[5] who opined that, the trend in some characters observed in their studies were mutagenic dosage or concentration dependent. The two most important nutritional components (protein and fat) for human or animal diets and industrial utilizations were higher in Samnut 10. As such, sodium azide (10–50 mM) has considerably improved the two components in Samnut 10. Although Samnut 20 contained lower amounts of these two major nutritional components, it yielded higher amount of carbohydrate, crude fibre and moisture content. The concentration for optimal effects in both varieties (Samnut 10 and 20) was obtained at 50 mM.

CONCLUSION

The present study considered the alkylating effects of sodium azide on nut size and nutrition compositions of two groundnut varieties (Samnut 10 and 20). The results showed that sodium azide is effective in achieving early maturing mutants of groundnut. The treatments also influenced nut size and increased the weight of the nuts. All concentrations of sodium azide applied in this study produced higher crude protein and fat with respect to control in Samnut 10 while 50 mM yielded highest protein and fat in Samnut 20. The study concluded that sodium azide could be employed to improve pod yield, nut size, protein, fat and other nutritional contents of the nuts.

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Table 1: Vegetative and fruit characteristics (at maturity) of groundnut varieties Samnut 10 and Samnut 20 treated with different concentrations of sodium azide

Trt	PH (cm)	NL	NDM	NPP	NNP	NNHP	WHP (g)	WNHP (g)	ND (cm)	NL (cm)	WHN (g)
						Samnut 10					
10 mM	38.96^{b}	47.33^{c}	65.33^{a}	56.10^{c}	1.75^{c}	168.12^{e}	115.30^{b}	101.12^{b}	0.68^{bc}	1.12^{cd}	50.67^{b}
20 mM	39.63^{b}	49.00^{bc}	59.33^{b}	45.33^{e}	2.38^{b}	241.45^{c}	103.84^{c}	92.65^{c}	0.64^{c}	1.09^{d}	51.36^{b}
30 mM	35.57^{c}	52.00^{ab}	58.10^{bc}	49.50^{d}	1.78^{c}	180.10^{d}	117.81^{ab}	102.79^{ab}	0.69^{b}	1.16^{c}	53.15^{ab}
40 mM	38.60^{b}	52.00^{ab}	57.42^{c}	69.66^{b}	2.61^{ab}	258.90^{b}	120.12^{a}	103.36^{a}	0.74^{b}	1.21^{bc}	55.39^{a}
50 mM	42.51^{a}	53.67^{a}	53.06^{d}	72.25^{a}	2.87^{a}	262.56^{ab}	118.72^{a}	104.10^{a}	0.77^{a}	1.43^{a}	55.44^{a}
Control	38.93^{b}	50.97^{ab}	65.67^{a}	57.33^{c}	2.85^{a}	264.03^{a}	104.32^{c}	89.38^{d}	0.69^{b}	1.23^{b}	49.87^{c}
						Samnut 20					

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10 mM	34.10^{b}	47.33^{b}	63.33^{a}	45.33^{c}	2.20^{cd}	198.65^{e}	233.75^{bc}	217.07^{bc}	0.72^{cd}	1.43^{b}	74.96^{cd}
20 mM	34.63^{b}	48.67^{b}	62.18^{a}	44.98^{c}	1.87^{d}	171.27^{f}	241.72^{ab}	222.28^{b}	0.69^{d}	1.27^{d}	73.17^{d}
30 mM	37.57^{ab}	49.67^{a}	56.00^{b}	41.66^{d}	2.38^{c}	214.96^{d}	244.57^{a}	229.02^{a}	0.73^{c}	1.32^{c}	86.12^{b}
40 mM	38.60^{a}	49.33^{a}	52.08^{ab}	47.21^{b}	2.52^{b}	227.43^{c}	224.99^{c}	209.46^{d}	0.77^{ab}	1.41^{bc}	88.10^{a}
50 mM	39.00^{a}	50.00^{a}	50.17^{c}	51.17^{a}	2.81^{a}	258.61^{a}	225.27^{c}	208.27^{d}	0.79^{a}	1.52^{a}	88.40^{a}
Control	34.93^{b}	47.00^{b}	63.67^{a}	47.36^{b}	2.70^{ab}	248.92^{b}	235.44^{b}	215.46^{c}	0.76^{b}	1.25^{e}	75.24^{c}

Values bearing the same letter(s) along the same column are not significantly different at $p \leq 0.05$.

Keys: Trt = Treatment, PH = Plant height, NL = Number of leaf, NDM = Number of day to maturity, NPP = Number of pods/plant, NNP = Number of nut.pod, NNHP = Number of nuts in hundred pods, WHP = Weight of hundred pods with nuts, WNHP = Weight of nuts in hundred pods, ND = Nut diameter, NL = Nut length, WHN = Weight of hundred nuts.

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Table 2: Showing co-efficient correlations among the vegetative character and number of days to flowering in groundnut varieties Samnut 10 and Samnut 20 treated with different concentrations of sodium azide

	Samnut 10					
	No of leaf	Plant height (cm)	No of branches	Leaf area (cm^2)	No of leaflets	Days to flowering
No of leaf	1.000					
Plant height (cm)	0.762**	1.000				
No of branches	0.773**	0.067	1.000			
Leaf area (cm^2)	0.777**	0.810**	0.178	1.000		
No of leaflets	0.995**	0.777**	0.658*	0.767**	1.000	
Days to flowering	0.216	0.271	-0.112	0.122	0.234	1.000
	Samnut 20					
	No of leaf	Plant height (cm)	No of branches	Leaf area (cm^2)	No of leaflets	Days to Flowering
No of leaf	1.000					
Plant height (cm)	0.695**	1.000				
No of branches	0.733**	0.245	1.000			
Leaf area (cm^2)	0.617*	0.812**	0.211	1.000		
No of leaflets	0.997**	0.695**	0.653*	0.457	1.000	
Days to flowering	0.178	-0.044	0.213	-0.034	0.178	1.00

** Values significantly correlate at $p \leq 0.01$
*Values significantly correlate at $p \leq 0.05$

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Table 3: Proximate analysis of Samnut 10 and Samnut 20 varieties of groundnut treated with varying concentrations of sodium azide

Treatment	Moisture content (%)	Crude Protein (%)	Ash content (%)	Crude Fat (%)	Fibre (%)	Carbohydrate (%)
			Samnut 10			
10 mM	21.55	25.62	2.40	43.49	4.13	2.81
20 mM	20.77	26.11	2.25	43.05	4.34	3.48
30 mM	19.60	28.10	2.01	42.86	4.02	3.41
40 mM	20.26	27.85	2.58	41.45	4.34	3.79
50 mM	21.38	27.98	2.51	41.01	4.02	3.10
Control	20.58	25.42	2.99	41.01	4.02	3.310
			Samnut 20			
10 mM	23.10	20.83	2.56	34.95	11.96	6.60
20 mM	31.59	20.11	2.51	30.01	10.86	4.92
30 mM	24.80	22.15	2.48	32.68	11.74	6.20
40 mM	22.62	23.25	2.43	33.35	11.69	6.66
50 mM	27.44	23.61	2.26	30.48	11.01	5.20
Control	24.28	21.94	2.26	30.48	11.01	5.20

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