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MORPHO-STOMATA EFFECT OF SODIUM AZIDE ON TWO VARIETIES OF GROUNDNUT (*Arachis hypogaea* L.)

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ABSTRACT

Dry seeds of groundnut (*Arachis hypogaea* L.) varieties Samnut 23 and Samnut 24 were obtained from Kashere market and duly identified at the Ministry of Agriculture, Gombe State, Nigeria. The seed were treated with five (5) different sodium azide concentrations (0.01, 0.02, 0.03, 0.04 and 0.05 M) to evaluate the plant growth parameters and stomata cell size of the varieties in response to treatment. Data obtained were subjected to Analysis of variance using Statistical Package for the Social Sciences SPSS, version 23 and the means were separated using Duncan Multiple Range Test (DMRT) at 0.05 probability level. The plant vegetative traits evaluated showed significance responses to different treatment concentrations. The highest vegetative growth (Plant height, number of leaves, and number of branches) was induced by 0.01M concentration at 10 week after sowing (WAS). Generally, the performances of the two varieties were optimal in response to 0.01M concentration and both varieties (Samnut 23 and Samnut 24) responded differently to sodium azide treatment of different concentrations. In both varieties, the anatomical feature (stomatal size) is significantly influenced by the sodium azide. These findings suggest that sodium azide can be utilized to create variability among existing germplasm for improving morphological and anatomical trait of groundnut.

Keywords: *Arachis hypogaea*, sodium azide, morpho – stomata effect.

INTRODUCTION

The peanuts or groundnut (*Arachis hypogaea* L.) is a species in the legume family (Fabaceae). It is an important oil seed legume grown worldwide and is known by many other local names such as earth nuts, pea nuts, goober peas, monkey nuts, and pig nuts (McDonald, 1968). It is grown in nearly 100 countries on six continents between 40^{o} N and S of the equator on nearly 24.6 m ha^{-1}, with a production of 41.3 million metric tons and productivity of 1676 kg ha^{-1} during 2012. China, India, Nigeria, USA and Myanmar are the leading groundnut producing countries in the world. Asia, with 11.6 m ha^{-1} (47.15%), and Africa, with 11.7 m ha^{-1} (47.56%), holds maximum global area under groundnut. Developing countries in Asia, Africa and South America account for over 97% of world groundnut area and 95% of total production. However, the productivity of Asia (2217 kg ha^{-1}) and Africa (929 kg ha^{-1}) is very poor as compared to Americas (3632 kg ha^{-1}) (FAOSTAT 2014). Groundnut is usually grown as a smallholder crop in the semi-arid tropics under rain fed conditions. It is an important crop in many countries, where it is a good source of protein (25%-34%), cooking oil (48%-50%) and vitamins. The skin of groundnut is rich in vitamin B, which is used as a base ingredient for cosmetics. It also provides important ingredients in numerous industries for sweet, ice-cream, coating, peanut butter and bakery products. Groundnut protein contents is of high biological value as it contains more protein than meat, about two and half times more than eggs and far more than any other vegetable foods except soya bean and yeast. The residue of the extraction process is used as commercial groundnut cake which is a concentrate feed for livestock and poultry. The nuts are eaten raw or after roasting as snacks. The green leaves or shoot makes excellent fodder and hay for animals (NPC, 2008). Crop improvement through conventional method of bleeding may not be to create desire variability on which a robust breeding programme could be built. Also, groundnut as a self-pollinating crop naturally would have less variability in its gene pool and thus limiting the number of natural varieties for which breeders could screen and exploit for improvement purposes (Animasaun *et al.*, 2014). Crop improvement by mutagenesis has been applied in a number of crops for yield improvement, creation of new cultivars, stress and drought tolerance, disease resistance and for horticultural or floriculture purposes (Ali *et al.*, 2007; Mensah and Obadoni, 2007). Induced mutations have been used to improve major crops that are mainly propagated by seeds (Adamu and Aliyu, 2007). Effects of Sodium azide on crop plants has earlier been reported some decades ago (Mostafa, 2011). The present study is aimed at evaluating the responses of the two varieties of *Arachis hypogaea* L. to sodium azide concentrations based on the plant growth and to also evaluate the effect of different concentrations of sodium azide on the stomatic cell sizes of the two varieties of groundnut.

MATERIALS AND METHODS

Healthy dry seeds of two varieties of groundnut (SAMNUT 23 and SAMNUT 24) were collected from Kashere market and were duly identified at the Ministry of Agriculture, Gombe State. The seed (nuts) were exposed to different concentrations of freshly prepared Sodium azide solution (0.01, 0.02, 0.03, 0.04, 0.05 M) for 6 hours. The control seeds for each of the varieties were soaked in distilled water for 6 hours. Thereafter, the seeds treated with Sodium azide were thoroughly washed in running tap water for ten minutes to remove excess erudite and chemicals from the seeds. Also, the control seeds were removed from distilled water and air dried for about 20minutes. The treated seeds along with the control were plated in a petri-dish padded with filter papers soaked in distilled water (6 seeds per plate) with labels corresponding to the treatment for studies. The pot experiments were conducted at the Botanical Garden, Federal University of Kashere, Gombe State, Nigeria. Pot studies were undertaken with one seed sown in a pot (21 cm x 18 cm planting bags) filled with sandy loam soil. Three replicates were made for each of the treatment concentrations and control which were arranged along in a Randomized Complete Block Design (RCBD) layout with 0.6 x 0.6m spacing. Effects of the mutagenic chemical were evaluated on number of leaves, number of branches, leaf area, and plant height and petiole length and stem girth. These data were collected every fortnight.The preparation of leaf samples for permanent slides to enhance epidermal morphology follow the method of Wilkinson's (1979) with slight modifications to accommodate the peculiarity of the plant specimens. The leaf samples was soaked in 70% Sodium Hypochlorite for three to five hours in order to remove the colouring pigments and surface debris follow by washing in several changes of water to remove excess reagents. Using fine grade camel hair brush, epidermal peels was carefully removed from the leaf sample surfaces. The slides of both the adaxial and abaxial surfaces of the leaves were prepared, labelled, viewed for micro morphological characters. Data obtained were subjected to one-way Analysis of variance using Statistical Package for the Social Sciences SPSS, version 23 and the means were separated using Duncan Multiple Range Test (DMRT) at 0.05 probability level.

RESULTS

The result shows different responses of the two varieties studied to different concentrations of sodium azide in growth parameters and stomatal size evaluated. The seed colour, shape and texture were the same before and after the treatments were applied. Table 1 shows the effect of sodium azide concentration on the grow parameters studied 10 weeks after sowing. With respect to plant height, the control treatment produced the tallest plants in both varieties. In Samnut 23, there was significant difference in the response of the variety to sodium azide concentrations. The plant with the treatment concentration of 0.01 M, 0.02 M and 0.03 M were statistically similar but taller than plant of higher concentrations. In Samnut 24, plant height shows no significant difference with respect to the concentrations. The number of leaves differed significantly with the treatments concentrations in Samnut 23 but exhibit no significant differences in Samnut 24. In Samnut 23, 0.01 M treated plants produced more number of leaves (472.62) which differed significantly from control (381.33), 0.02 M (383.33), 0.04 M (357.67) and 0.05 M treatment (373.33) that was statistically similar. The effect of sodium azide concentration on number of branches is showed in Table1. The concentrations of sodium azide significantly influence number of branches in Samnut 23 and do not influence Samnut 24 significantly. Numbers of branches were higher with 0.01 M concentration which produced 135.33 branches and differed significantly from other concentrations. The least number of branches were produced by 0.03M concentration which had 81.33 branches. Leaf area differed significantly to the varying concentration of sodium azide in both varieties. The concentration of 0.03 M produced the largest leaf area (19.58 cm^2 and 22.99 cm^2) in Samnut 23 and Samnut 24 respectively. While the least leaf area (11.60 cm^2) was observed with the concentration of 0.02 M in samnut 23. The control treatment produced the least leaf area (12.99 cm^2) in samnut24. In both varieties studied, the effected of sodium azide had no significant difference on the petiole length. The petiole lengths were comparable among concentrations and between varieties. There were varietal differences with respect to stem girth. The concentration of sodium azide does not influence samnut 23 significantly but does influence samnut 24 significantly. In samnut 24, the concentration of 0.05 M produced the largest stem girth (2.63 cm) while the concentration of 0.01M produced the least (2.20).

Table 1: Effect of sodium azide on morphological parameter of Groundnut at 10 weeks after sowing (WAS) in Kashere, Gombe State

Treatment	PH	NL	NB	LA	PL	SG
Samnut 23						
Control	27.48^{b}	381.33^{b}	103.33^{b}	13.88^{b}	6.37	2.23
0.01	26.90^{b}	472.67^{c}	135.33^{c}	16.00^{c}	6.57	2.26
0.02	27.47^{b}	383.33^{b}	110.33^{b}	11.60^{a}	6.37	2.27
0.03	27.07^{b}	279.33^{a}	81.33^{a}	19.58^{d}	6.63	2.27
0.04	22.37^{a}	357.67^{b}	102.67^{b}	13.15^{b}	6.73	2.27
0.05	22.53^{a}	373.33^{b}	107.67^{b}	15.00^{b}	6.67	2.24
Level of Sig.	×	××	××	××	NS	NS
Samnut 24						
Control	32.33	412.67	118.67	12.99^{a}	5.83	2.27^{ab}
0.01	31.17	420.00	121.67	14.33^{b}	6.73	2.20^{a}
0.02	31.53	436.00	125.67	16.52^{c}	6.77	2.60^{bc}
0.03	31.17	398.70	111.67	22.99^{e}	7.60	2.50^{abc}
0.04	30.03	412.00	133.00	21.25^{d}	6.77	2.47^{abc}
0.05	26.97	390.00	111.67	20.64^{d}	6.13	2.63^{c}
Level of Sig.	NS	NS	NS	××	NS	××

Means followed by different letter(s) within a treatment group are significantly different following DMRT.WAS: Weeks after sowing. × = Significant at 5% ($P \leq 0.05$). ×× = Significant at 5% ($P \leq 0.01$). Ns = Not Significant

Table 2 shows the effect of mutagen (Sodium azide) on the stomata size of groundnut. There was a significant difference in the stomata size between the control and the treatments on the adaxial and abaxial surface of Samnut 24. The size of the stomata for the control of the adaxial surface is smaller than that of the treatments. The situation applies to the abaxial surface, but with an exception in Samnut 23 where stomata size is smaller than that of the control in treatment 0.04M.

The stomata size is larger at 0.02M NaN_3 and smaller at 0.4M of NaN_3 at the Adaxial surface. Similarly the smallest size was observed at 0.4M of NaN_3 and the largest size was observed at 0.3M of NaN_3 of the Abaxial surface which were both significantly different from the control.

Table 2: Effect of Sodium azide on Stomata size of Groundnut in Kashere, Gombe State, Nigeria

Variety	Treatment conc. (M)	Epidermal measurement (mean±S.E)	
		Adaxial	Abaxial
Samnut 23	Control	2.32±0.12[cd]	1.88±0.06[a]
	0.1	1.89±0.74[a]	2.08±0.14[ab]
	0.2	2.48±0.11[d]	2.48±0.17[bc]
	0.3	1.86±0.08[b]	2.79±0.42[c]
	0.4	1.52±0.69[a]	1.86±0.26[a]
	0.5	2.03±0.16[b]	2.36±0.23[abc]
	Level of Sig.	×	×
Samnut 24	Control	1.52±0.39[a]	1.65±0.61[a]
	0.1	2.11±0.15[a]	2.01±0.20[a]
	0.2	2.37±0.23[a]	2.43±0.27[a]
	0.3	2.40±0.13[a]	2.29±0.20[a]
	0.4	2.45±0.46[a]	2.58±0.16[a]
	0.5	1.93±0.0[4a]	1.62±0.13[a]
	Level of Sig.	NS	NS

Means followed by different letter(s) within a treatment group are significantly different following DMRT. × = Significant at 5% ($P \leq 0.05$). NS = Not Significant

DISCUSSION

The experiment showed that in most of the growth parameter studied, Samnut 23, was most influenced by the various concentrations of sodium azide used, having significantly influenced in 4 out of 6 growth attributes assessed (plant height, number of leaf, number of branches and leaf area). In Samnut 24, only leaf area and stem girth were influenced significantly by the various concentration of sodium azide. Dissimilarity in performance may be ascribed primarily to genotypic variability among the varieties. Animasaun *et al*., 2014 reported significant differences in plant height, number of leaves and number of branches for Samnut10 and Samnut 20 which is in conformity with the evaluation carried out in this study with regard to Samnut 23. The lowest concentration of 0.01M positively and consistently produced significant effect on Samnut 23. Taller plants with the highest number of leaves and the highest number of branches were produced by the concentration of 0.01M in comparison with control. The concentration of 0.04 M and 0.05 M produced the least plant height. This finding is in conformity with report of Mensah and Obadoni (2007) reported that plant height decreases as mutagenic concentration increases while working on Sasame seed. Poornananda and Hosakatee (2009) reported a decrease in plant height of Guizotia abyssinica treated with sodium azide and gamma rays. The finding of this study was at variance with that of Animasaun *et al*., 2014 who reported that taller plants with highest number of branches were induced by 0.05M concentration of sodium azide in Samnut 10 and Samnut 20. In this study, the various concentrations did not significantly affect the plant height, number of leaves, number of branches and petiole length in Samnut 24. The finding suggests that different varieties would respond differently to mutagenic treatment of specific concentration. The mutagenic effects of the chemical mutagens successfully induced obvious change to the stomata size.

Stomata are present on both the leaf surfaces and are called Amphistomatic as it is on groundnut. The stomata size is big and varies due to the effect of the treatments. The increase in the stomatal size of the studied species is as a result of the mutagenic treatment which is advantageous to the physiology of food crop, trees or ornamental plants inhabiting the tropical zone or the template region. This is because the increase in stomatal size will increase the transpiration rate and this will lead to the increase in photosynthesis of the plant, thereby promote and facilitate the plant growth at a very faster rate during wet season and invariably increase the production of farm products.

CONCLUSION

This study reveals that Sodium azide was effective at low concentrations. Low concentration of 0.01M - 0.02M had optimal effects on vegetative parameters considered in this study. High doses of 0.03 - 0.05 M were probably too high to induce desirable morpho- stomata characters in the crop. Sodium azide could be used at low concentration for breeding programme targeted at improving varieties and expanding the existing germplasm.

RECOMMENDATION

From the above research, Sodium azide at a concentration of 0.01-0.02M is therefore recommended for effective usage by farmers to improve the traits in plants and ultimately increase the possibility of isolating beneficial mutants for improvement of the economic crops such as the test plants.

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APPENDIX

Figure 1a: Effect of Sodium azide on Somata Cells of Samnut 23(Adaxial surface)

Figure 1b: Effect of Sodium azide on Somata Cells of Samnut 23(Abaxial surface)

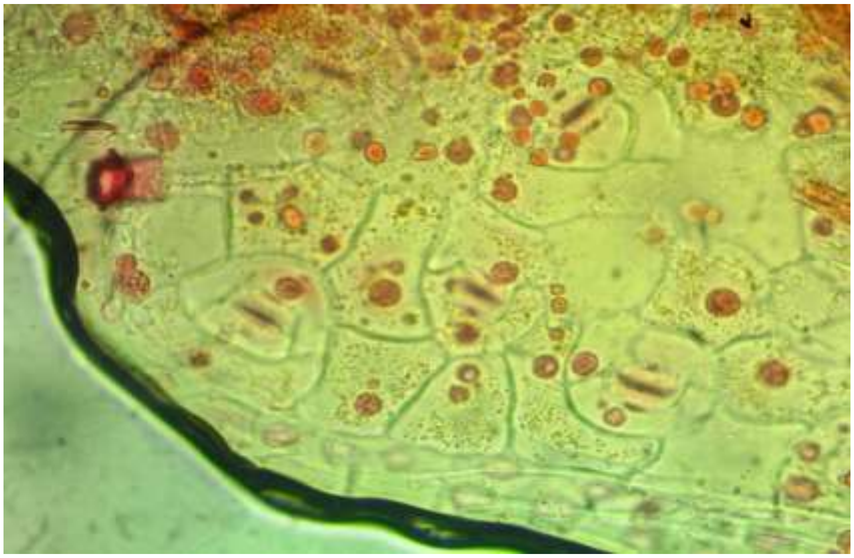

Figure 2a: Effect of Sodium azide on Somata Cells of Samnut 24(Adaxial surface)

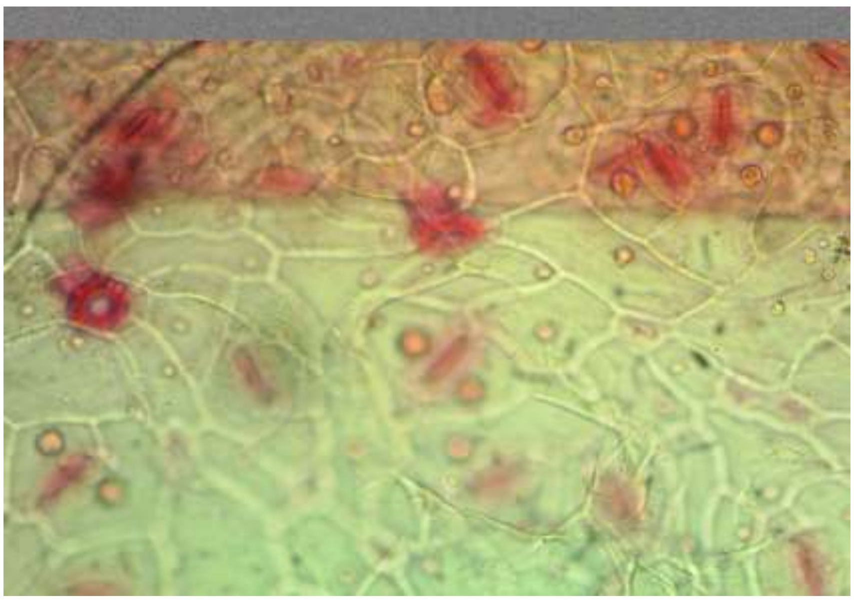

Figure 2b: Effect of Sodium azide on Somata Cells of Samnut 24(Abaxial surface)