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Effects of Sodium Azide and Nitrous Acid on the Morphology and Leaf Anatomy of *Jatropha curcas* L. (Euphorbiaceae)

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ABSTRACT

Morphological and anatomical effects of two chemomutagens namely sodium azide and nitrous acid were studied on *Jatropha curcas*. Seeds were soaked in the two mutagens at various concentrations (1mM, 2mM, 3mM and 4mM) for 4 hours and later rinsed in the distilled water to remove excess mutagens. The seeds were later air dried and planted in plastic pots for observations for 12 weeks. Results shown increase in the seedling height, number of leaves, high frequency of paracytic stomata, higher stomatal index and density on the abaxial leaf surface and large stomata in seedlings induced with sodium azide (1mMNaN_3 and 2mMNaN_3). Also nitrous acid along with the sodium azide enhanced higher stomatal index and density, and large stomata. The effects of sodium azide especially 1mMNaN_3 and 2mMNaN_3 induced beneficial traits in morphology and anatomy of *J. curcas* than nitrous acid.

Keywords: *Jatropha curcas*, nitrous acid, sodium azide, morphology, leaf anatomy

INTRODUCTION

Mutagenesis has been used effectively for genetic studies as well as for selective breeding in many plants. Successful mutant isolation largely relies on the use of efficient mutagens. However, mutations may take place in the genetic information causing a cell or living creature to be different from the other. Mutation methodology has been used to produce many cultivars with improved economic value and study of genetics and plant developmental phenomena (Van *et al.*, 1990; Bertagne-Sagnard and Chupeau,

1996). It has been demonstrated that genetic variability for several desired characters can be induced successfully through mutations and its practical value in plant improvement programmes has been well established. The main advantage of mutation breeding is the possibility of improving one or two characters without changing the rest of the genotype. Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al.*, 2001). A number of workers (Mashenkov, 1986; Ricardo and Ando, 1998; Adamu and Aliyu, 2007) have reported the role of chemical mutagens in enhancing genetic variability in higher plants. Genetic variability is fundamental to successful breeding programmes in vegetatively and sexually propagated plants (Kleinhofs *et al.*, 1978). This variation can occur naturally or can be artificially induced through mutations, using physical, biological or chemical mutagens and this has attracted the interest of plant breeders for many decades (Ahloowalia and Maluszynski, 2001).

Due to the economic importance of *Jatropha curcas* especially as biofuel plant, the need to improve its yield is on increase. The plant originated from Central America but has spread to other tropical and subtropical countries and mainly grows in Asia and Africa. The leaves are usually green to pale green in colour; the flowers are unisexual but occasionally hermaphrodite. The fruits are produced mainly during the rainy season and the seeds are mature if the capsule changes from green to yellow (Dehyghan and Webseter, 1997).

Meanwhile, this work elucidate the mutagenic effects of two chemical mutagens (sodium azide and nitrous acid) on *Jatropha curcas* with the aim to ascertain the effects of these mutagenic agents on the morphology and leaf anatomy of the plant.

MATERIALS AND METHODS

Acquisition, treatment and planting of seeds

The seeds of *Jatropha curcas* were obtained from Sabo area in Oyo town, Oyo State, Nigeria. 1mM, 2mM and 4mM of each mutagenic agent were prepared in 1.0M phosphate buffer. The seeds were then soaked in the solutions of the mutagens separately for 4 hours. The containers were periodically agitated to ensure that the seeds evenly take up the chemical mutagens. The control seeds were also soaked in 0.1M phosphate buffer. Treated and control seeds were thereafter air-dried and planted in the pots for 10 weeks. Each treatment was replicated three

times using completely randomized design (CRD). The treated and control seeds were planted on 5th of January, 2013.

Morphological data collection

Data were collected on the following growth parameters: germination percentage, seedling survival, seedling height, number of leaves per seedling, number of leaves per plant and root length. These data were collected every two weeks.

Leaf anatomical data collection

Prepared slides of macerated cuticles from the leaves of the species were observed using 35 fields of view at x40 objectives as quadrats. The trichome occurrence, distribution and type were counted and recorded. The number of subsidiary cells per stomata was noted and recorded to determine the frequency of the different stomatal complex types present in each specimen. The frequency of each complex type is expressed as percentage occurrence of such complex types based on all occurrences (Obiremi and Oladele, 2001). The terminologies for naming stomatal complex types followed those of Dilcher (1974).

The stomatal index was determined using the formulae described by:

$$SI = \frac{S}{E+S} \times 100$$

Where S= number of stomatal per square millimeter

E= number of ordinary epidermal cell per square millimeter

The stomatal density was determined as number of stomata per square millimeter, while the mean stomatal size or area was determined using the formulae described by Baderinwa and Morakinyo (2002).

$$l \times b \times k$$

Where L= length, B= breadth and K= Franco's constant = 0.79 (Franco constant).

RESULTS

Germination of seeds

Germination percentage which is an estimate of the viability of a population of seeds was 100%. Each of the seeds planted in the pots survived throughout the experimental period making the seedling survival rate 100%. Both the treated and control seeds germinated on the same day.

Morphological effects

Measurement of some quantitative and qualitative effects of the mutagens on the seedlings started at 4WAP and terminated at the 10WAP. At 4WAP, tallest seedling height was recorded in 2mMNaN_3 (18.23cm±0.72) followed by the control while shortest height was in 1mMHNO_2 (11.97cm±1.57). At the 10WAP where the tallest seedling (36.33cm±1.11) was also in 2mMNaN_3 seedlings and shortest seedling (26.77cm±0.68) was still in 1mMHNO_2 seedlings. Generally, seedlings treated with sodium azide were taller than those of nitrous acid. There were significant differences between sodium azide and nitrous acid in the effects impacted on seedling height but no such difference was observed between the seedlings in the control and sodium azide-induced seedlings. Similarly, leaf production is more in sodium azide seedlings at 4WAP and 10WAP in 1mM NaN_3 and small number of leaves was produced in 2mM HNO_2 and 1mM HNO_2 at 4WAP and 10WAP respectively (Table 1). From 4WAP through 10WAP, there were significant differences in leaf production in control and sodium azide and nitrous acid seedlings but no difference between control and 1mMNaN_3 seedlings. At 10WAP, roots of seedling were longer in the control (22.17cm±4.63) but shorter in 1mMHNO_2 (16.43cm±0.60). There was a significant difference between the root length of seedling in 1mMHNO_2 and those of other seedlings (Table 1).

Table 1: Effects of mutagens on seedling height, number of leaf per seedling and root length of *Jatropha curcas*

	4WAP*		6WAP		8WAP		10WAP		
Treatments	Seedling height (cm)	Number of leaves per seedling	Seedling height (cm)	Number of leaves per seedling	Seedling height (cm)	Number of leaves per seedling	Seedling height (cm)	Number of leaves per seedling	Root length (cm)
Control	17.53^{a} ± 1.26	10.00a±1.73	23.40^{ab}± 0.89	16.00^{a}± 3.00	28.77^{a}± 2.87	21.00^{ab}± 4.51	33.73^{a}±3.36	28.00^{a}± 3.00	22.17^{a}± 4.63
1mM NaN_3	17.10^{a}± 0.89	11.00a±3.06	24.43^{a}± 1.24	16.00a± 1.00	29.10^{a}± 0.90	26.00^{b}± 5.03	35.53^{a}±1.55	32.00^{ab}±5.29	19.67^{ab}±1.52
2mM NaN_3	18.23^{a}± 0.72	7.00b± 0.58	23.43^{ab}± 2.64	17.00^{a}± 5.57	29.60^{a}± 3.14	18.00^{c}± 2.89	36.33^{a}±1.11	23.00^{ab}±5.20	19.50^{ab}±1.14
4mM NaN_3	17.60^{a}± 0.36	6.00b± 0.80	23.23^{ab}± 1.33	8.00^{b}± 2.00	27.63^{ab}± 1.10	14.00^{c}± 1.53	35.00^{a}±2.21	18.00^{ab}±1.15	19.33^{ab}±2.21
1mMHNO_2	11.97^{c}± 1.57	6.00b± 1.15	18.80^{c}± 1.30	9.00^{b}± 1.15	21.40^{c}± 1.77	13.00^{c}± 1.15	26.77^{b}±0.68	16.00^{b}± 1.53	16.43^{b}± 0.60
2mM HNO_2	12.37^{c}± 2.01b	5.00b± 0.55	20.80^{c}± 1.65	10.00^{b}± 1.00	23.87^{c}± 2.45	13.00^{c}± 2.65	27.20^{b}±1.35	18.00^{ab}±2.65	19.30^{ab}±1.84
4mM HNO_2	14.70^{b}± 1.81	6.00b± 1.15	20.90^{c}± 2.19	10.00^{b}± 1.53	26.87^{ab}± 1.40	14.00^{c}± 2.65	29.03^{b}±3.25	18.00^{ab}±4.62	19.10^{ab}±3.32

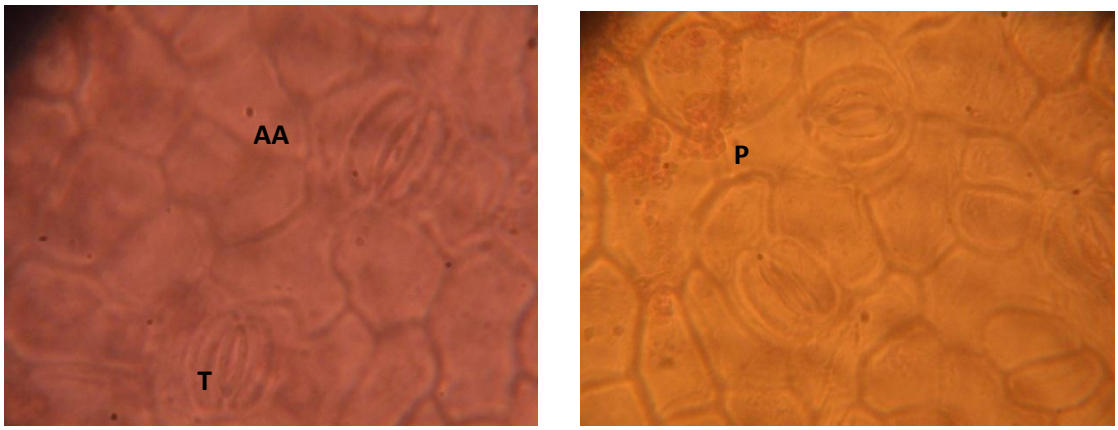
*WAP denotes week(s) after planting.

Values with same letter(s) are not significantly different at $p \leq 0.05$

Leaf Anatomical effects

Leaf of *J. curcas* is an amphistomatic (i.e. having stomata on both abaxial and adaxial surfaces of the leaf). The stomatal complex types present are paracytic, tetracytic and anomocytic. Paracytic stomatal complex occur more frequently in the seedlings of the control and sodium azide followed by anomocytic and tetracytic types on both leaf surfaces. In nitrous acid induced-seedlings, both paracytic and anomocytic stomata were the most frequent, followed by tetracytic stomata. Higher stomatal index (77.50%) and density (62.00 mm^2±1.22) on the adaxial surface was observed in 2mMHNO_2 respectively while lower index (55.41%) and density (38.60mm^2±3.97) on the adaxial surface in 4mMNaN_3 and control seedlings respectively. Generally, stomatal index and

density are higher on the abaxial surface than on the adaxial surface in all seedlings across the treatments and in the control except in $2mMHNO_2$, $4HNO_2$, and $2mMHNO_2$ respectively. Larger stomata (69.52µm) on the adaxial surface and smaller stomata (54.04µm) on the abaxial surface are in the seedlings of $1mMNaN_3$. There are significant differences in the stomatal size of the seedlings (Fig. 1; Table 2). The anticlinal wall patterns are straights and epidermal cell shape is polygonal on both leaf surfaces for both the treatments and the control (Fig 1; Table 3).



(a) (b)

Figure 1: Leaf epidermis of *Jatropha curcas* showing tetracytic (T), paracytic and anomocytic (AA) stomata on the adaxial (a) and abaxial (b) surfaces X600

Table 2: Quantitative and qualitative stomata characteristics of *Jatropha curcas* as affected by mutagenic treatments at 10WAP

Treatments	Surface of leaf	Stomatal complex type	Stomatal frequency (%)	Stomatal index (%)	Stomatal density (mm^2)	Stomatal size (µm)
Control	Adaxial	Paracytic Tetracytic Anomocytic	40.93 21.76 37.31	58.84	38.60 ± 3.97	63.20[a]
	Abaxial	Paracytic Tetracytic Anomocytic	47.02 8.77 44.21	61.43	57.00 ± 6.36	56.98[ab]
1mMNaN$_3$	Adaxial	Paracytic Tetracytic Anomocytic	47.98 8.07 43.95	61.43	44.06 ± 3.58	69.52[a]
	Abaxial	Paracytic Tetracytic Anomocytic	39.78 12.37 47.85	72.94	56.60 ± 6.50	54.04[ab]
2mMNaN$_3$	Adaxial	Paracytic Tetracytic Anomocytic	39.42 17.70 42.88	59.04	41.80 ± 7.69	59.40[ab]
	Abaxial	Paracytic Tetracytic Anomocytic	37.69 22.31 40.00	63.41	52.00 ± 5.15	65.98[a]
4mMNaN$_3$	Adaxial	Paracytic Tetracytic Anomocytic	42.06 9.14 48.80	55.41	43.75 ± 3.67	66.36[a]
	Abaxial	Paracytic Tetracytic Anomocytic	39.46 21.43 39.11	64.76	58.80± 5.17	61.62[a]
1mMHNO$_2$	Adaxial	Paracytic Tetracytic Anomocytic	39.02 14.22 46.76	62.12	49.20 ± 2.77	62.28[a]
	Abaxial	Paracytic Tetracytic Anomocytic	50.39 7.54 42.07	62.69	50.49 ± 4.10	60.02[a]
2mMHNO$_2$	Adaxial	Paracytic Tetracytic Anomocytic	41.61 14.52 43.87	77.50	62.00 ± 1.22	58.87[ab]
	Abaxial	Paracytic Tetracytic Anomocytic	38.53 13.23 47.74	57.69	43.60 ± 5.55	62.86[a]
4mMHNO$_2$	Adaxial	Paracytic Tetracytic Anomocytic	43.75 17.08 39.17	62.34	48.00 ± 2.35	67.12[a]
	Abaxial	Paracytic Tetracytic Anomocytic	43.39 16.61 40.00	62.11	59.00 ± 6.59	63.20[a]

Means with same letter(s) are not significantly different at $p<0.05$

Table 3: Anticlinal cell wall patterns of the epidermal cells of *Jatropha curcas*

Treatments	Leaf surface	Anticlinal wall	Epidermal cell shape
Control	Adaxial	Straight	Polygonal
	Abaxial	Straight	Polygonal
1mMNaN_3	Adaxial	Straight	Polygonal
	Abaxial	Straight	Polygonal
2mMNaN_3	Adaxial	Straight	Polygonal
	Abaxial	Straight	Polygonal
4mMNaN_3	Adaxial	Straight	Polygonal
	Abaxial	Straight	Polygonal
1mMHNO_2	Adaxial	Straight	Polygonal
	Abaxial	Straight	Polygonal
2mMHNO_2	Adaxial	Straight	Polygonal
	Abaxial	Straight	Polygonal
4mHNO_2	Adaxial	Straight	Polygonal
	Abaxial	Straight	Polygonal

DISCUSSION

The choice of mutagens and seed has been used for improving plants yield has been demonstrated by many workers (Maluszynski *et al*., 1955; Mashenkov, 1986; Mahandjiev *et al*., 2001; Adamu and Aliyu, 2007; Nehvi *et al*., 2010).

Seed germination and survival of the seedlings were 100% in both mutagenic treatments. The results depicted that with the increase in concentration of mutagens there was no decrease or increase in germination percentage of the studied species. The seedling survival of 100% indicates that the concentration of the mutagens did not affect the survival of the studied species. The closer view of the data collected on the seedling height illustrated that the potency of the mutagens at such a low concentration induced a very distinct and obvious variation into the shoot length. Also, sodium azide significantly enhanced the number of leaf produced than the nitrous acid throughout the period of the study. In a similar work, Al-Qurainy (2009) reported that in *Eruca sativa* (salad rocket or colewort), plant height, leaf area, fresh and dry weight per plant were increased as compared to the control after 60 days of sowing except dry root weight of plants of

treated seeds at 1mM of sodium azide and was observed maximum at 3mM of sodium azide. Earlier, Adamu and Aliyu (2007) in their work on tomato (*Lycopersicon esculenta*) concluded that sodium azide is a strong mutagen and that impact of sodium azide has been observed effectiveness in inducing mutations with respect to germination percentage, root length, seedling height, seedling survival, number of branches per plant and yield per plant. In the present study, the length of root in control seedlings were longer than in the seedlings treated with sodium and azide and nitrous acid.

Anatomically, the mutagens increased some stomatal parameters such as stomatal density, index and size. A similar observation was made in *Crocus sativus* (saffron crocus) by Nehvi *et al*. (2010). The higher concentration of the stomata density and index on the abaxial leaf surface than on the adaxial surface is an adptational advantage to the plant. This will ensure conservation of water due to the fact that the abaxial surface is far away from the direct impact of the sun rays (Obiremi and Oladele, 2001; Oyeleke *et al*., 2004; AbdulRahaman and Oladele, 2009; AbdulRahaman *et al.*, 2010). Coupled with this, is the higher frequency of paracytic stomata with small number of subsidiary cells than the tetracytic and anomocytic stomata with three and more than four subsidiary cells respectively. The latter open more frequently and subsequently transpire more than the former (Carr and Carr, 1990; Obiremi and Oladele, 2001; Oyeleke *et al*., 2004; AbdulRahaman and Oladele, 2008; AbdulRahaman and Oladele, 2009; Saadu *et al*., 2009; AbdulRahaman *et al.*, 2010).

In general, the increase in the stomatal size of the studied species as a result of the mutagenic treatment is advantageous to the physiology of food crop, trees or ornamental plants inhabiting the tropical zone or temperate region. This is because increase in the stomatal size will increase the transpirational rate and this will lead to the increase in the 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. Meanwhile, if this increment in the stomatal size occurred in the dry season and it is not check, this may lead to the physiological disorder such as wilting. It however can be said that physiological process called transpiration is a necessary evil. Succinctly, large stomata will increase the size of the guard cells and since the photosynthetic organelle (chloroplast) is present on the mesophyll cells and as well as the guard cells, this will incessantly increase the chloroplast cells which contain larger amount of chlorophyll

molecules on the leaf surfaces (Microsoft Encarta, 2009). This will efficiently contribute to the photosynthetic production of ATP, production of osmotically active sugars by photosynthetically carbon assimilation (Talbott and Zeiger, 1998). The guard cells could also accumulate starch in the dark and hydrolyze it in the light and could also store starch, either from carbon assimilated in the guard cell chloroplasts or imported from the mesophyll of the leaf.

The effect and prospect of sodium azide (1mMNaN_3 and 2mMNaN_3) to induce favourable mutation into the morphology (seedling height and leaf production) and anatomy (higher frequency of paracytic stomata, higher stomatal index and density on the abaxial leaf surface and large stomata) of the test plant has been clearly shown to be of greater advantage since it increased the seedling height and several other anatomical features that promote the growth, seed production and yield of the plant. Also nitrous acid along with the sodium azide enhances higher stomatal index and density, and large stomata. Sodium azide is therefore recommended for effective usage by the farmer to improve the traits in plants and ultimately increase the possibility of isolating beneficial mutants for improvement of the economic crops such as *Jatropha curcas*.

The observed variation in the treated plants (especially those induced with sodium azide) was more than those of control plants and it was the expected result because the control plants are supposed to be genetically similar and any kind of difference observed in the control plants is only due to the environment factors. The results of the present study suggest that particular dose of sodium azide (i.e. 1mM and 2mM for 4 h treatment) can be used to create genetic variability in *Jatropha curcas*, which would be the basis for quality improvement in this species.

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