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(RESEARCH ARTICLE)

Effect of sodium azide on agro-morphological traits of four varieties of Kenaf (*Hibiscus cannabinus*)

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Abstract

Mutation induction and detection of desired mutants represents an accelerated means of reaching the desired superior Variety. In this work seeds of Kenaf varieties NG, HIB18, HIB24 and HIB42 collected from National Centre for Genetic Resources and Biotechnology (NAGRAB) Ibadan, Nigeria were subjected to mutagenesis using sodium azide at concentrations of 0.2%, 0.4%, and 0.6%. These treated seeds were planted in polythene bags in row of Four by Four columns in a completely Randomized Design (CRD) with three replications of each treatments. Data on germination percentage, plant height (cm), leaf number, fresh weight and dry weight were taken. Germination was found to be significantly higher in the control and decreased gradually with increasing mutagen concentration for all the four varieties. Among the treatments, NG at 0.6% SA showed better germination compared to other varieties of the same concentration. This indicated differential intervarietal response to the same dose of sodium azide under the same environmental condition. Analyses of data showed significant differences in all the varieties of *Hibiscus cannabinus L*. treated with sodium azide compared to control. Results indicate sodium azide improved yield performance of Kenaf. Also, negative effect of sodium azide was observed in plants height in variety NG. These finding suggests that plants Varieties would respond differently to mutagenic treatments of specific dosage or concentration. Since 0.6%SA appears to have negative effects on germination and seedling performance of the Varieties planted it is advisable to use lower concentration of sodium azide.

Keywords: Kenaf; Mutagen; Sodium azide; Concentration; Varieties; Breeding

1. Introduction

Kenaf (*Hibiscus cannabinus L.)* is an herbaceous annual crop of the *Malvaceae* family of which cotton and okra are also members [1]. Kenaf is a low-risk cash crop whose cultivation requires minimal chemical applications. It also helps to alleviate global warming by absorbing carbon dioxide gas due to its rapid growth rate [2]. Kenaf can be considered a dual non-food crop cultivated for its fiber and/ or its oil production [3]. Kenaf was highlighted in Malaysia in the late 1990's as an alternative and cheaper source of material for producing panel products such as fiberboard and particleboard, textiles, and fuel [4]. Recently, kenaf is being used as a valuable dual purpose crop for fiber and medicine. Its leaves contain large amounts of compounds including polyphenols [5]. Kenaf plant, which is native to Africa, can grow to maturity in 120 days in over 23 States in Nigeria (Raw materials Research and Development council statistics).

One of kenaf's advantages as a crop is that it can be successfully grown in a wide range of soil types, from high organic peat soils to sandy desert soils. Although kenaf grows better on well-drained, fertile soils with a neutral pH, the crop can withstand late season flooding, low soil fertility, and a wide range of soil pH values [6].

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Change in climate brings about changes in rain pattern, temperature, and negative effects on water and land resources, flood and droughts. Climate change is a global phenomenon; however, its impacts are more widely felt in the developing countries [7]. To cope with and mitigate the adverse effects of climate change, there is a need for development of new varieties that will adapt to environmental changes. Mutation is one of the means of developing new varieties.

Breeding selection of new kenaf cultivars is the most effective measure to increase biomass and seed yields per unit area [8]. Mutation breeding has the merits of creating new mutant characteristics and adding only very few traits without disturbing the other characteristics of a cultivar [9, 10]. Mutagenic agents, such as radiation, can be used to induce mutations and generate genetic variants from which desired mutants may be selected [8]. This process offers the possibility of inducing desirable attributes that either have not developed in nature or have been lost during evolution. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement [11]. Thus, this study was carried out to observe the effects of sodium azide on the agro-morphological parameters of four varieties of kenaf (*Hibiscus cannabinus. L*).

2. Material and methods

2.1. Apparatus used

The apparatus used during the course of this research work are; beakers, glass funnels, measuring cylinder, stirrers, conical flasks, masking tape and electronic weighing machine.

Chemical Mutagen: The chemical Mutagen used in the study was sodium azide (SA) by Central Arog House (p) Ltd, New Delhi, India.

2.2. Seed collection and treatment

Seeds of Four Kenaf varieties viz NG, HIB 24, HIB 42 and HIB18 were obtained from National Center for Genetic Resources and Biotechnology (NACGRAB), Ibadan. Nigeria. Fifteen seeds of each of the four kenaf varieties were selected into four beakers labeled with the varieties of kenaf. The seeds in the beakers were pre-soaked with distilled water for four hours after which the water was decanted. Sodium azide solution was added at concentrations of 0.2, 0.4 and 0.6% and left for four hours. A stirrer was used at intervals to stir the solution containing the seeds. For control, the seeds were soaked in distilled water. Pretreatment and treatment of the kenaf seeds were done at room temperature. The treated seeds were washed with tap water for 2 hours to remove residual mutagens and dried on filter paper.

2.3. Experimental site and design

The research work was carried out in the Botanical Garden of Biological Sciences Garden, Kogi State University, Anyigba (Latitude 7^0 6^1N and Longitiude 7^0 43^1), Kogi State Nigeria. A total of three viable un-treated (control) and treated seeds of each concentrations (0.2%, 0.4%, and 0.6%) were planted in experimental pots, filled with sandy-loamy soil. Each treatment were replicated three times and arranged in a Randomized Complete Block Design (RCBD). The bags were moderately watered every other day, hand weeding and other cultural practices were carried out as at when required.

2.4. Data collection and analysis

The parameters collected in this experiment were

2.4.1. Germination percentage

Numbers of germinated seedlings in each treatment were counted 10 days after sowing for the sprouting percentage. It was calculated using the formula below

$$\text{Germination percentage (\%)} = \frac{\text{Total number of germinated Seeds}}{\text{Total number of planted seeds}} \times 100$$

2.4.2. Plant height

Plant heights of randomly selected plants from all replicate pots were tagged and measured by scale from ground level to the top of the tallest leaf at 2WAP, 4WAP, 6WAP and 9WAP using measuring (cm) tape/meter.

2.4.3. Wet weight

The soils attached to the harvested plant roots were washed off with tap water and spread on filter paper for drying. The wet weight of the whole plant was determined using electronic weighing machine.

2.4.4. Dry weight

After taking the wet, the plants were spread in room temperature for two weeks before the dry weight of the whole plant was determined using electron weighing machine.

2.4.5. Number of leaves per plant

Plants were selected in replicate pots of control and each treatment for all the variety and their leaves were counted weekly. The average leave numbers were recorded.

The data generated were subjected to statistical analysis using Analysis of variance (ANOVA) to test for significant differences and Duncan's multiple range test (DMRT) was used to separate the means where there were differences. All data analyses were carried out using the Statistical Package for Social Science version 20 at 5% level of significance.

3. Results and discussion

3.1. Effect of sodium azide on percentage germination

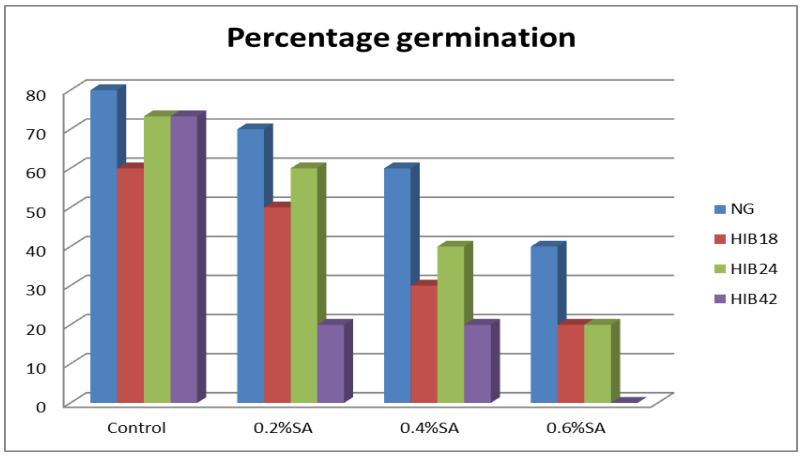

Figure 1 Percentage germination of treated and untreated seeds of four Kenaf Varieties

Figure 1 reveals that germination decreased with increased concentrations of sodium azide. The highest germination percentages were 80% in controls of variety NG, 73.3% in variety HIB24 and 60% in variety HIB18. The mutagenic effect of sodium azide at 0.6% proves to be lethal for HIB42 under field conditions as none of the treated seed germinated.

NG at 0.6% SA showed better germination percentage indicating differential inter varietal response to the same dose of SA under the same environment with 40% compared to HIB18 and HIB24 which both had 20% germination. NG at 0.2% SA showed a better germination percentage with 70%, followed by 60% in HIB24, 50% in HIB18 and 20% in HIB42. At 0.4%SA, NG showed 60% followed by HIB24 with 40%, 30% in HIB18 and 20% in HIB42. The reduction and inhibition in seed germination and seedling survival at M1 generation due to sodium azide has also been observed in *Vicia faba* [12] *Dianthus* [13], Wheat [14] and *Stevia rebaudiana* [15].

3.2. Effect of sodium azide on plant height

Table 1 Effects of sodium azide on plant height of four varieties of *Hibiscus cannabinus*

Varieties	Sodium azide Conc(%)	2WAP	4WAP	6WAP	9WAP
NG	Control(0)	16.00±1.00 g	26.03±1.05 gh	43.83±0.76 k	65.17±1.26efg
	0.2%	26.00±1.00 c	27.00±1.00g	43.83±0.76 k	65.07±1.01 efg
	0.4%	12.00±1.00 h	28.00±1.00g	56.00±1.00 j	68.33±0.58efg
	0.6%	21.00±1.00 e	23.83±0.76 h	48.00±1.00 i	48.00±1.00 g
HIB 18	Control(0)	26.00±1.00 c	50.00±1.04a	66.67±1.53 g	77.00±1.00 def
	0.2%	31.67±0.58 a	44.33±0.58 cd	80.50±0.50 d	84.67±0.58de
	0.4%	21.00±0.00 e	47.00±1.00 b	95.00±1.00 b	97.33±0.58cd
	0.6%	22.67±1.53d	47.00±1.00 b	60.40±1.53 h	61.67±0.58fg
HIB24	Control(0)	19.00±1.00f	45.00±1.00 bc	86.00±1.00 c	122.00±1.00b
	0.2%	28.00±1.00b	42.300±1.47 de	108.00±0.58 a	130.00±1.00ab
	0.4%	16.00±1.00g	46.00±1.00 bc	80.17±1.26 d	146.67±45. 33a
	0.6%	28.00±1.00b	35.17±1.04 f	76.83±0.76 e	80.00±0.58def
HIB 42	Control(0)	30.330.58a	34.67±1.53 f	77.33±1.53 e	84.67±1.53de
	0.2%	28.00±1.00 b	36.67±0.58 f	74.10±1.02 f	82.00±1.00def
	0.4%	25.33±0.58 c	40.77±1.00 e	86.80±0.70 c	111.00±1.00bc
	0.6%	0.00±0.00 i	0.00±0.00 i	0.00±0.00 l	0.00±0.00 h

Note: Values are means ±SD of three replicates. Different superscript in the same column indicate significant difference at P<0.05(DMRT)

The result presented in Table 1 Showed there were significant differences in the plant heights of varieties of kenaf treated with sodium azide when compared to plant heights of their controls. SA at 0.2% and 0.6% had significant effect on plant height (26.00 cm and 21.00 cm) compared to control (16.00 cm) in variety NG. SA at 0.2% in HIB18 was significantly different to the control as it showed increase in plant height (31.67 cm) compared to the control (26.00 cm) at 2WAP. There was no significant difference in plant height (28.00cm) of variety HIB24 at 0.2% SA and 0.6% SA, both were significantly different when compared to the plant height of the control which is (19.00 cm). This result agrees with result of [16] that SA improved growth of Amaranths seedlings. Plant having greater and faster growth could be as a result of stimulatory effect of sodium azide and may be attributed to the increase in the rate of cell division or cell elongation as reported by [17].

There were Negative effects of SA in NG Variety on plant height at 0.4%, in HIB 18 at 0.4% and 0.6% (21.00 cm and 22.67 cm), in HIB24 at 0.4% (16.00 cm) and all the treatments in HIB42 when compared to their respective controls. These findings are in compliance with [18] who reported a decrease in plant height of *Guizotia abyssinica* treated with sodium azide and gamma rays. Similarly, sodium azide was also reported *Sesame indicum* [19] and wheat [20] to reduce the plant height.At 6WAP, NG at 0.4% SA, HIB18 at 0.2% SA and 0.4% SA, HIB24 at 0.2% SA and HIB42 at 0.4% SA showed increased plant height compared to control, while NG at 0.4% SA was significantly the same with control and HIB18 at 0.6% SA, HIB24 at 0.4% SA and 0.6% SA, and HIB42 at 0.2% SA all showed decreased plant height compared to their respective controls.

At 9WAP, 0.2% SA and 0.4%SA showed no significant difference in plant height with the control, while 0.6% SA showed decreased plant height compared to others. Reduced plant height may be attributed to the increase in destruction on growth inhibitors, drop in the auxin level or inhibition of auxin synthesis [21].

The treatment of 0.4% SA and 0.6%SA in variety NG produced dwarfed plants. Joshi *et al* [17] explained the dwarfed growth to auxin depletion, changes in ascorbic acid content, physiological and biochemical disturbances.

3.3. Effect of sodium azide on leaf number

Table 2 Effects of sodium azide on leaf number of four varieties of *Hibiscus cannabinus*

Varieties	Sodium azide Conc. (%)	2WAP	4WAP	6WAP	9WAP
NG	Control(0)	5.333±0.58[a]	17.33±0.58[f]	23.00±1.00[d]	27.00±0.58[de]
	0.2%	3.667±0.58[bcd]	25.00±0.58[c]	28.00±1.00[c]	42.67±1.00[a]
	0.4%	3.333±0.58[cd]	27.00±1.00[b]	16.00±1.00[f]	13.00±1.00[i]
	0.6%	3.67±0.58[bcd]	20.00±1.00[e]	27.00±1.00[c]	25.00±1.00[f]
HIB 18	Control(0)	4.67±0.58[ab]	21.33±1.00[de]	16.00±1.00[f]	10.67±0.58[j]
	0.2%	4.33±0.58[abc]	26.67±1.00[bc]	18.33±0.58[e]	9.67±1.00[j]
	0.4%	3.00±1.00[d]	17.00±1.00[fg]	13.00±1.00[g]	10.00±1.0[j]
	0.6%	4.00±0.58[bcd]	21.67±1.00[d]	27.67±0.58[c]	22.67±0.58[g]
HIB24	Control(0)	4.33±0.58[abc]	25.67±1.00[bc]	32.00±1.00[b]	36.00±1.00[b]
	0.2%	3.67±0.58[bcd]	27.00±1.00[b]	27.00±1.00[c]	21.00±1.00[h]
	0.4%	3.33±0.58[cd]	15.67±1.00[g]	24.00±1.00[d]	28.00±1.00[d]
	0.6%	4.33±0.58[abc]	22.67±1.00[d]	35.00±1.00[a]	36.33±0.58[b]
HIB 42	Control(0)	4.67±0.58[ab]	26.33±1.00[bc]	33.00±1.00[b]	33.33±1.00[c]
	0.2%	4.67±1.00[ab]	26.00±1.00[bc]	32.33±1.00[b]	26.00±1.00[ef]
	0.4%	3.67±1.00[bcd]	16.00±1.00[fg]	24.00±1.00[d]	22.00±1.00[gh]
	0.6%	0.00±0.00[e]	0.00±0.00[h]	0.00±0.00[h]	0.00±0.00[k]
P value		**0.00**	**0.00**	**0.00**	**0.00**

Note: Values are means ±SD of three replicates. Different superscript in the same column indicate significant difference at P<0.05(DMRT)

The highest number of leaves was recorded for controls in all the varieties compared to treatments in week 2, and also in Variety HIB42 at week 6 and week 9 (Table 2). Mutagenic effect on leave reduction has been documented by [22] on black rice, [23] on tomato, [16] on *Amaranthus caudatus*, and [24] on *Helianthus annus*.

3.4. Effect of sodium azide on fresh and dry weights

Table 3 Effects of sodium azide on fresh and dry weights of seedlings of four varieties of *Hibiscus cannabinus* at 2WAP

Variety/Sodium azide Conc. (%)	Fresh weight Mean(g)	Std. Deviation	Dry weight Mean (g)	Std. Deviation
NG Control(0)	2.32	0.28	0.54	0.05
NG 0.2	2.17	0.01	0.26	0.01
NG 0.4	2.06	0.05	0.36	0.03
NG 0.6	3.48	0.13	0.41	0.01
HIB18 Control(0)	1.51	0.02	0.24	0.01
HIB18 0.2	1.41	0.01	0.34	0.02
HIB18 0.4	1.96	0.05	0.57	0.02
HIB18 0.6	2.06	0.05	0.24	0.01
HIB 24 Control(0)	1.84	0.03	0.24	0.01
HIB 24 0.2	2.47	0.06	0.60	0.01
HIB 24 0.4	2.20	0.17	0.33	0.01
HIB 24 0.6	3.25	0.06	0.64	0.02
HIB 42 Control(0)	1.71	0.01	0.56	0.04
HIB 42 0.2	1.81	0.39	0.22	0.01
HIB 42 0.4	1.58	0.05	0.55	0.20
HIB 42 0.6	0.00	0.00	0.00	0.00
Total	**1.9882**	**.76807**	**0.382**	**0.183**

The result of Fresh weight in Table 3 showed that the heaviest fresh weight (3.48g) was from NG Variety at 0.6%SA compared to control (2.32g). Variety HIB18 and HIB24, at 0.6%SA showed the heaviest weight compared to their respective controls.

The results of dry weight of seedlings of all the varieties taken 2WAP presented in Table 3 shows control of NG Variety had the heaviest dry weight compared to other treatments. Variety HIB18 at 0.4%SA had the heaviest dry weight (0.57g) compared to the control (0.24g). HIB24 at 0.6%SA had the heaviest dry weight (0.64g) compared to the control (0.24g) of the same Variety. The heaviest dry weight in HIB42 was shown in control (0.56g) compared to other treatments. The results presented in Table 3 indicates positive increase in dry weight of seedlings when seeds are treated with Sodium azide before planting which agrees with results of [16] and [25].

4. Conclusion

There were varying responses of kenaf (*Hibiscus cannabinus*) varieties to induced mutagenesis by sodium azide. Higher concentrations of sodium azide caused delay in emergence of seeds and decreased percentage germination. There was either increase or decrease in plant heights and leave numbers of treated Varieties compared to their control. These finding suggests that plants Varieties would respond differently to mutagenic treatments of specific dosage or concentration. Since 0.6%SA appears to have negative effects on germination and seedling performance of the Varieties planted it is advisable to use lower concentration or percentage of Sodium azide. The use of pesticide containing Sodium azide should be put into consideration as higher concentration could be harmful to the plant.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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