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EFFECT OF SODIUM AZIDE INDUCTION ON MORPHOLOGICAL TRAITS OF *SHOMBO* AND *TATASE* (*Capsicum annuum* L.)

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Abstract
The present study was carried out to determine the mutagenic effect of sodium azide on the morphological features of *Shombo* and *Tatase* (*Capsicum annuum* L.) in M_1 generation. Seeds were pre-soaked in distilled water for 6hrs and later treated with different concentrations (0.00, 0.01, 0.02, 0.03 and 0.04 W/V%) of sodium azide mutagen for 6hrs. The experiment was planted using a 2×5 factorial in completely randomized design (CRD), replicated three times. Observation on the growth and fruit parameters of M_1 mutants of Shombo and Tatase showed significant variations ($P \leq 0.05$) on the number of leaves, total number of branches, internode length, fruit length, fruit diameter, number of seeds, pericarp thickness, fruit stalk length and fruit weight. Most of the parameters especially plant height decreased with increase in concentrations of sodium azide. Positive effect of the sodium azide induction on Tatase was observed on the fruit diameter at 0.04% and number of seeds at 0.02%. The results of this study showed that sodium azide had significant effect on plant height, fruit diameter and number of seeds. Therefore, induced mutation through sodium azide would be efficient in creating genetic variability of desirable traits in pepper without compromising its agronomic characteristics.

Keywords: Mutagen, Mutants, Pepper, Sodium azide, and Variability

Introduction

Pepper (*Capsicum* spp) is an important vegetable grown in tropical, sub tropical and temperate regions. It is a common spice used all over the world (Mohammed *et al*., 2013). Pepper is an important agricultural crop with an enormous economic importance (Abu *et al*., 2019a). Its fruit has both nutritional and medicinal value and an excellent source of natural colours, flavour and antioxidant compounds (Howard *et al*., 2000). Pepper is ranked as the world's third most important vegetable and the fruits contain essential vitamins and minerals which can help in maintaining healthy growth and development (Abu *et al*., 2019b). Despite the high nutritional value of pepper and its importance, its method of production needs to be improved to meet the demand required. There is growing interest in the manipulation of economic crops' quality through production of new cultivars with relatively improved morphological features and nutritional composition targeted towards a specific end use.

Mutations are major source of genetic variability in crops, though the occurrence of spontaneous mutations is slow. Induced mutation has been widely used for the improvement of plant characters in different crops (Elangovan and Pavadai, 2015; Abu *et al.,* 2019b). The role of mutagenesis is to increase genetic variability of desired traits in crops (Tah, 2006; Adamu and Aliyu, 2007; Khan and Goyal, 2009; Kozgar *et al*., 2011; Mostafa, 2011). Mutation breeding provides the possibility of inducing desired characters that either cannot be seen in nature or have been lost during evolution (Ahloowalia *et al*., 2004; Maluszynski and Szarejko, 2005). Several factors such as clear objective, efficient screening method, proper choice of mutagen and method of treatment, dose and rate and proper experimental conditions affect the success of mutation breeding (Acquaah, 2012).

Lately, numerous mutants of pepper have been reported among which are mutation induced change in shoot architecture and development of orange mature pepper fruits with increased β-carotene (pro-vitamin), which is currently used for molecular marker assisted selection (Paran *et al*., 2007; Tomlecova *et al*., 2009). Honda *et al*. (2006) reported the development of some induced mutants of sweet pepper (*C. annuum* L.). Sodium azide has been used in plant breeding for several biotic and abiotic stress such as *Zea mays* resistant against pathogen Striga (Kiruki *et al*., 2006), *Triticum aestivum* (durum wheat) for salt tolerance (Agata *et al*., 2001), *Oryza sativa* for reduced amylase content (Jeng *et al*., 2003) and *Oryza sativa* for enhanced yield (Jeng *et al*., 2006). The successful exploitation of sodium azide to induce genetic variability in plant breeding has been reported in barley and other crops (Jaya and Selva, 2003). Sodium azide is well-known to affect seed germination, shoot length, and root length and also induces high frequency of chlorophyll deficient mutations (Khan and Tyagi, 2009). Hence, this study aimed at identifying induced variations in characters and yield among the induced mutants of two pepper varieties in Nigeria (Shombo and Tatase) with a view of identifying traits that could be useful for breeding improved varieties of the plant.

Materials and Methods

The study was conducted from June-December 2016 in the Botanic Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Seeds of Shombo and Tatase (*Capsicum annuum* L.) were subjected to different treatment levels of sodium azide (NaN_3). Treatment parameters were five concentrations (0-control, 0.01, 0.02, 0.03 and 0.04% W/V). Sodium azide solution was prepared by dissolving 0.01, 0.02, 0.03, 0.04 g of sodium azide powder in 100ml of distilled water and shaken to form 0.01, 0.02, 0.03 and 0.04 % W/V concentrations of sodium azide respectively. Before treatment, seeds were pre-soaked in four different Petri dishes containing distilled water for 6hrs at room temperature to enhance the effect of the mutagen. Thereafter, four groups of Shombo and Tatase varieties were subjected to four (4) concentrations (0.01 %, 0.02 %, 0.03 % and 0.04 %) of sodium azide (NaN_3) solutions, and the control at room temperature (25ºC) for six (6) hours. After treatment, these seeds were washed thoroughly with distilled water to remove the residual amount of mutagens, air-dried on filter paper, and 100 seeds of each treatment were sown immediately in germination basket containing amended soil (top soil: poultry droppings: run-off soil in the ratio of 3:2:1) (Ojua *et al*., 2019). Germination started in about 7-14 days. Nursery was ready after two months and each plant was transplanted into a polypot containing the amended soil. The plants were laid out using a 2×5 factorial in completely randomized design (CRD), and replicated three times. Plants were irrigated properly (spray method) on alternate days, and management practices were done to evaluate the morphological and yield traits of Shombo and Tatase induced with sodium azide mutagen. The morphological traits studied include: plant height, number of leaves, internode length, stem circumference, number of branches, fruit length, fruit diameter, number of seeds, pericarp thickness, number of fruits, single fruit weight and fruit stalk length. Among the morphological parameters, plant height, stem circumference, internode length, fruit length and fruit stalk length were measured using measuring tape. Number of leaves, branches and seeds were counted manually. Fruit diameter and pericarp thickness were determined using vernier callipers, while the single fruit weight was determined using a sensitive weighing balance (FEJ-600 Model). Data collected were subjected to analysis of variance (ANOVA) using GenStat Discovery Edition 4 and significant means were separated using Fisher's least significant difference (F - LSD) test.

Results and Discussion

Sodium azide is a strong chemical mutagen which inhibited the growth of plant parts with increasing concentration. Field performance of the M_1 generation revealed significant variations in some parameters evaluated. The untreated pepper plant (0% NaN_3) had the tallest plant with mean values of 42.72 ± 2.6 cm, 78.56 ± 3.89 cm and 86.17 ± 5.98 cm at 12, 16 and 20 weeks after planting respectively (Table 1). The shortest plant height was observed in the mutants treated with 0.04% of sodium azide in both Shombo and Tatase at 20 weeks after planting. Increase in concentrations of sodium azide caused a gradual reduction in the plant height of the two pepper varieties. The result here may be due to the effect of the chemical mutagens which suppressed early development of the pepper crops. This result is in agreement with the findings of Ashish *et al.* (2011) and Jagajanantham *et al.* (2013). Reduction in plant height as exhibited by the mutants will facilitate mechanical harvesting in large scale farming. The mutants similarly exhibited significant decrease in number of leaves per plant from the control in all the treatments at 20 weeks after planting with highest reduction values, 52.67 ± 10.6 leaves for Shombo and 62.89 ± 3.92 for Tatase with 0.04% W/V of NaN_3 (Table 1). Higher levels of mutagens might have stopped the enzymes that are necessary for leaves initiation, thereby affecting the rate of photosynthesis, and also stimulating abscission in the mutants. Another reason could be that higher concentrations of sodium azide blocked cell division by decreasing the rate of physiological processes, and thus causing damage to the genetic material. Similar results were also reported in crops like tomato (Adamu and Aliyu, 2007) and rice (Ali *et al*., 2012). Data regarding inter-nodal length showed significant decrease from the control. Among different treatments, inter-nodal length was higher in the control for Shombo (1.99 ± 0.07 cm). The mutants with high levels of sodium azide produced plants with shorter internode length. This can be attributed to the deleterious effect of the mutagens on the plant physiology. This follows the findings of Motilal *et al*. (2012). They reported a decrease in internode length with the increase in dose level of ethyl methane sulfonate in *Asteracantha longifolia* L.

Generally, the stem circumference was significantly reduced by sodium azide mutagen. Among the different levels of sodium azide treatments, 0.03% W/V of NaN_3 had the lowest value at 12 weeks after planting for both Shombo and Tatase. Also, high dose of sodium azide mutagen (0.04% W/V) produced plants with the lowest stem circumference at 16 weeks after planting (Fig. 1). The results of the stem circumference could be attributed to destruction of genetic material and reduction of cell division, which ultimately retarded the growth of the stem girth. Data analysis for number of branches showed significant differences between various mutagenic treatments at 20 weeks after planting. The highest number of branches was observed in the control for Shombo. Results also revealed that higher concentrations of sodium azide reduced the number of branches on plants. Naheed (2014) reported a gradual decrease in the average number of branches in tomato with higher doses of mutagen.

Table 1: Mean effect of different concentrations of sodium azide on plant height, number of leaves and internode length of two pepper varieties at different weeks after planting (WAP)

Pepper varieties	Concentrations of sodium azide (%W/V)	12 WAP	16 WAP	20 WAP
			(A) Plant height (cm)	
Shombo	Control	42.72^{a} ± 2.60	78.56^{a} ± 3.89	86.17^{a} ± 5.98
	0.01	24.58^{bc} ± 2.89	51.72^{b} ± 5.65	76.56^{ab} ± 3.85
	0.02	24.10^{bc} ± 2.67	48.30^{bc} ± 3.52	71.06^{abc} ± 6.80
	0.03	18.24^{c} ± 1.64	41.17^{c} ± 2.55	64.33^{bc} ± 4.50
	0.04	23.56^{bc} ± 1.31	46.17^{bc} ± 2.21	58.50^{c} ± 6.44
Tatase	Control	29.06^{b} ± 2.57	45.92^{bc} ± 4.16	53.00^{c} ± 4.63
	0.01	24.24^{bc} ± 1.82	39.50^{c} ± 3.27	48.67^{c} ± 5.52
	0.02	23.22^{bc} ± 3.18	42.22^{b} ± 4.56	50.39^{c} ± 6.89
	0.03	22.68^{bc} ± 1.17	41.67^{c} ± 2.64	58.94^{c} ± 4.80
	0.04	22.12^{c} ± 1.95	44.00^{c} ± 3.45	56.94^{c} ± 5.75
LSD $_{(0.05)}$		6.408	10.47	15.52
		(B) Number of leaves		
Shombo	Control	56.00 ± 9.74	95.44 ± 17.55	181.22^{a} ± 14.03
	0.01	25.33 ± 3.83	77.00 ± 9.31	164.67^{a} ± 17.23
	0.02	27.67 ± 5.17	127.11 ± 27.52	110.00^{b} ± 20.55
	0.03	15.33 ± 1.71	111.89 ± 18.61	87.67^{bc} ± 10.09
	0.04	23.11 ± 3.17	96.11 ± 12.24	52.67^{d} ± 10.60
Tatase	Control	54.00 ± 9.06	58.44 ± 7.91	109.33^{b} ± 9.82
	0.01	35.89 ± 6.77	54.67 ± 6.63	92.44^{bc} ± 10.83
	0.02	38.89 ± 9.51	72.22 ± 10.88	86.67^{bcd} ± 11.19
	0.03	18.56 ± 1.49	97.89 ± 9.71	63.89^{cd} ± 7.06
	0.04	20.33 ± 3.49	113.33 ± 13.01	62.89^{cd} ± 3.92
LSD $_{(0.05)}$		NS	NS	34.86
		(C) Internode length (cm)		
Shombo	Control	1.50 ± 0.08	1.91 ± 0.10	1.99^{a} ± 0.07
	0.01	0.79 ± 0.14	1.73 ± 0.16	1.69^{abc} ± 0.13
	0.02	0.72 ± 0.09	1.88 ± 0.18	1.63^{abc} ± 0.12
	0.03	0.57 ± 0.07	1.66 ± 0.20	1.56^{bc} ± 0.16
	0.04	0.61 ± 0.10	1.80 ± 0.23	1.53^{bc} ± 0.14
Tatase	Control	1.20 ± 0.15	1.54 ± 0.11	1.59^{bc} ± 0.14
	0.01	0.98 ± 0.12	1.88 ± 0.12	1.39^{c} ± 0.10
	0.02	0.80 ± 0.08	1.46 ± 0.15	1.86^{ab} ± 0.17
	0.03	0.70 ± 0.09	1.59 ± 0.18	1.72^{abc} ± 0.11
	0.04	0.90 ± 0.10	1.73 ± 0.20	1.63^{abc} ± 0.13
LSD $_{(0.05)}$		NS	NS	0.36

Values with similar case letters along the column are not significantly different (P > 0.05)

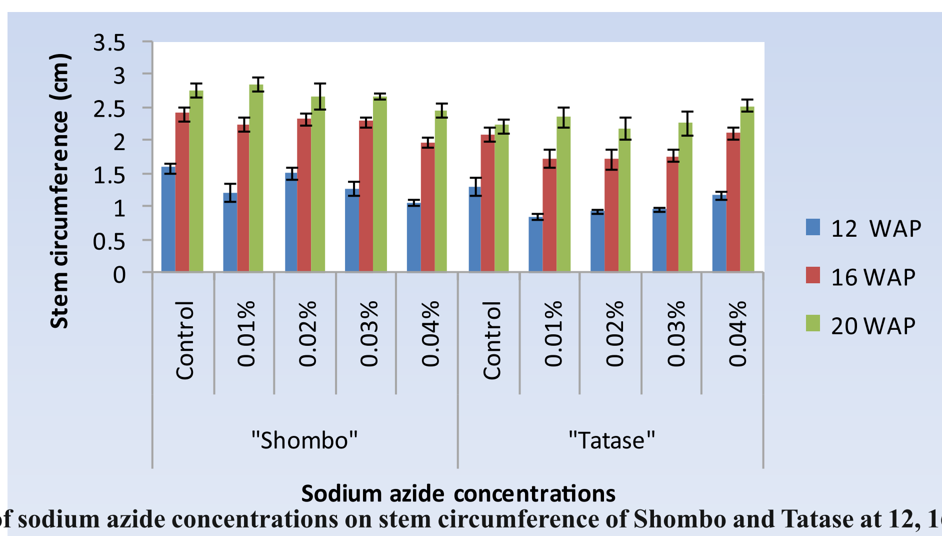

Fig. 1: Effect of sodium azide concentrations on stem circumference of Shombo and Tatase at 12, 16 and 20 WAP

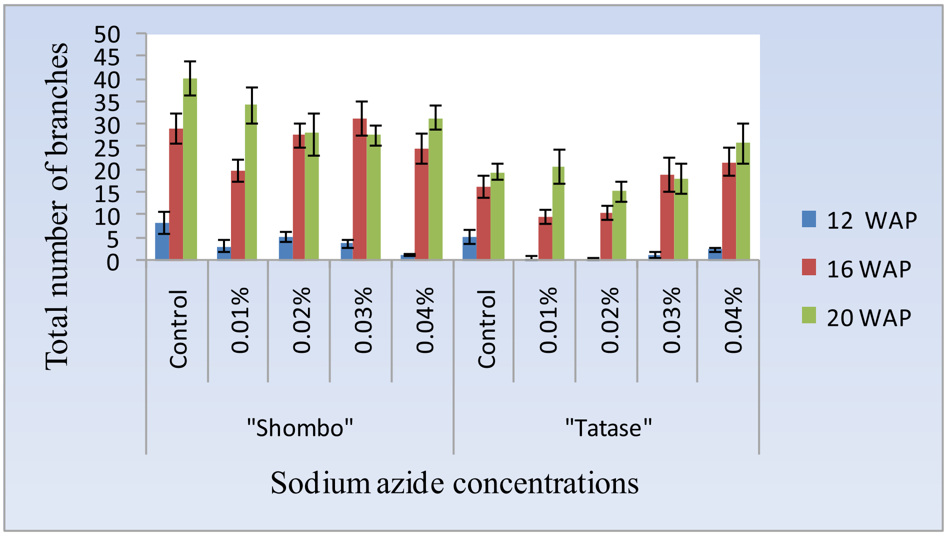

Fig. 2: Effect of sodium azide concentrations on number of branches in Shombo and Tatase at 12, 16 and 20 WAP

Data for fruit characteristics revealed that Tatase induced with 0.04 % of sodium azide produced the longest fruit with a mean value of 6.30 ± 0.44 cm, which did not differ significantly from the control. The shortest fruit length was recorded in Shombo induced with 0.02 % of sodium azide. Similarly, Tatase induced with 0.04 % of sodium had the widest fruit diameter with a mean of 2.96 ± 00 cm, while Shombo exposed to 0.03 % of sodium azide had the smallest fruit diameter (Table 2). The larger fruit diameter in some treated plants could be due to the favourable mutations caused by the mutagen. Sodium azide induced significant variation in the number of seeds found in the fruits of the two pepper varieties. Tatase with 0.02 % of sodium azide produced significantly the highest number of seeds per fruit with a mean value of 166.67 ± 5.46 (Table 2). The least number of seeds per fruit was recorded in Tatase induced with 0.01% of sodium azide (50.00 ± 0.00). The number of seeds per fruit revealed that some treatments showed comparable increase of seeds over the control, thus indicating that sodium azide could promote seed production at certain level. The enhancing effect may be due to sudden increase in the metabolic status of seedlings and increase in the activity of growth promoter's effect on seed production. Similar observation of increased number of seeds per pod in pigeon pea was made by Patil (2009) in cowpea plant.

Data regarding pericarp thickness of the pepper fruits showed that Tatase induced with 0.01 % of sodium azide produced pepper fruit with the thickest pericarp (0.21 ± 00 cm). The least pericarp thickness was recorded in Tatase induced with 0.02 and 0.03% of sodium azide. The number of fruits per plant was greatly affected by the mutagen. The control for Shombo produced the maximum number of fruits, while the minimum number of fruits was produced by Tatase induced with 0.01 and 0.02% of sodium azide. The highest fruit weight was recorded in the control for Tatase, which differed significantly from other treatments. The lowest fruit weight was recorded in Shombo induced with 0.04 % of sodium azide. In similar vein, the control for Shombo had the longest fruit stalk length, while Shombo induced with 0.04% of sodium azide produced the shortest fruit stalk length. There were gradual reductions in the fruit weight and fruit stalk length of the two pepper varieties as the concentrations of sodium azide increased. This could be due to the effect of sodium azide concentrations on the mutants which negatively affected the pepper plants, thereby affecting plant growth and fruit characteristics. This is in agreement with Lal *et al.* (2009), who reported that an increase in sodium azide concentrations led to decrease in morphological traits of the M_1 mutants of black gram.

Table 2: Mean effects of different concentrations of sodium azide on fruit characteristics

Pepper varieties	Sodium azide conc. (W/V%)	Fruit characteristics: Fruit length (cm)	Fruit diameter (cm)	Number of seeds	Pericarp thickness (cm)	Number of fruits	Single fruit weight (g)	Fruit stalk length (cm)
Shombo	Control	$6.17^{a} \pm 0.44$	$1.89^{c} \pm 0.12$	$56.67^{c} \pm 0.67$	$0.15^{bc} \pm 0.02$	21.67 ± 2.89	$7.23^{bc} \pm 0.63$	$4.30^{a} \pm 0.74$
	0.01	$5.60^{ab} \pm 0.46$	$1.75^{cd} \pm 0.03$	$67.00^{bc} \pm 7.81$	$0.12^{cd} \pm 0.01$	12.67 ± 2.89	$5.74^{cd} \pm 0.02$	$3.17^{b} \pm 0.09$
	0.02	$3.73^{c} \pm 0.19$	$1.49^{d} \pm 0.02$	$61.00^{c} \pm 7.51$	$0.17^{b} \pm 0.01$	15.00 ± 2.89	$2.74^{d} \pm 0.27$	$2.23^{c} \pm 0.15$
	0.03	$5.40^{ab} \pm 1.01$	$1.46^{d} \pm 0.12$	$60.67^{c} \pm 7.54$	$0.14^{bcd} \pm 0.01$	9.00 ± 2.89	$2.98^{d} \pm 0.52$	$2.47^{c} \pm 0.23$
	0.04	$4.30^{bc} \pm 0.53$	$1.70^{cd} \pm 0.05$	$53.33^{c} \pm 2.85$	$0.13^{cd} \pm 0.01$	12.67 ± 2.89	$2.50^{d} \pm 0.31$	$2.17^{c} \pm 0.38$
Tatase	Control	$4.43^{bc} \pm 0.28$	$2.50^{b} \pm 0.19$	$71.33^{bc} \pm 20.93$	$0.12^{cd} \pm 0.01$	5.00 ± 2.89	$16.63^{a} \pm 2.75$	$3.47^{ab} \pm 0.12$
	0.01	$3.70^{c} \pm 0.00$	$2.95^{a} \pm 0.00$	$50.00^{c} \pm 0.00$	$0.21^{a} \pm 0.00$	3.00 ± 2.89	$6.95^{c} \pm 0.00$	$4.00^{ab} \pm 0.00$
	0.02	$5.97^{a} \pm 0.59$	$2.53^{b} \pm 0.16$	$166.67^{a} \pm 5.46$	$0.11^{d} \pm 0.00$	3.00 ± 2.89	$10.64^{b} \pm 2.4$	$2.30^{c} \pm 0.00$
	0.03	$3.57^{c} \pm 0.13$	$1.88^{c} \pm 0.00$	$85.00^{b} \pm 0.00$	$0.11^{d} \pm 0.00$	3.33 ± 2.89	$6.10^{c} \pm 0.40$	$2.47^{c} \pm 0.27$
	0.04	$6.30^{a} \pm 0.00$	$2.96^{a} \pm 0.00$	$58.00^{c} \pm 0.00$	$0.17^{b} \pm 0.00$	6.00 ± 2.89	$7.83^{bc} \pm 0.00$	$3.77^{ab} \pm 0.22$
$LSD_{(0.05)}$		1.381	0.29	23.79	0.026	NS	3.532	0.887

Values with similar case letters along the column are not statistically different ($P > 0.05$)

Conclusion

The variation in morphological features of M_1 mutant lines of Shombo and Tatase is an indication that sodium azide was effective in inducing significant genetic variability in some pepper traits. Generally, differences in concentrations of the mutagen significantly affected most of the parameters evaluated in the two pepper varieties. The results of this study have shown that sodium azide had a significant and positive effect on plant height, fruit diameter and number of seeds. Therefore, induced mutation through sodium azide is efficient in creating genetic variability of desirable traits in pepper without compromising its agronomic characteristics.

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