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Mutagenic Effects of Sodium Azide (Nan_3) On Morphological Characteristics Of Tomato (Lycopersicum Esculentum)

Matthew Omoniyi Adebola, Department of Biological Sciences. Lecturer I,
Ibrahim Badamasi Babangida University, Lapai Nigeria

INTRODUCTION

Tomato *(Lycopersicum esculentum)* is native to South America. It is now grown worldwide for its edible fruits, with thousands of cultivars having been selected with varying fruit types, and for optimum growth in differing growing conditions.Tomatoes contain carotene lycopene, one of the most powerful natural antioxidants.Lycopne had been reported to prevents prostate cancer, breast cancer, head and neck cancers, improve the skin's ability to protect against harmful UV rays and protect against oxidative damage in many epidemiological and experimental studies. (Freedman*et al.,* 2008; Zderika *et al* .,2010).

Tomatoes suffer from several problems that include high diseases incident, pest infections and adverse effect of environment stressthat greatly affects its production (Encarter, 2005; Adamu and Aliyu, 2007). Development of pathogen resistant and high yielding tomato is necessary to reduce the environmental pollution caused by application of pesticides. Current protection measures rely heavily on chemical control measure for pathogen vectors, which have undesirable environmental consequences. A more effective approach to protect plants, from pathogen attack is to create plants that are endogenously resistant to pathogens. This can now be achieved using mutation tools, by providing the plant with genetic information required for attacking the pathogens and for being resistant to the disease cause by the pathogen. In recent times, chemical mutagens have become important tools in crop improvement. These mutagens are being used to produce resistant in various susceptible crops to improve their yield and quality traits against harmful pathogens. Sodium azide (NaN_3)is a chemical mutagen that creates point mutation in the genome of plant by producing metabolites and thus produced protein in mutant plant that has different function from the normal plant. The mutant plants produced by the treatment of NaN_3 are capable of surviving under various adverse conditions and have important yields, increase stress tolerance, longer self and reduced agronomic input in comparison to normal plants. The decrease in seedling emergence, seedling height, root length and seedling survival, height at maturity and fruit yield per plant with increasing mutagen concentration has been reported in mutagenesis studies (Adamu *et al*., 2002 Sheeba et al.,2005 ; Goyal and Khan, 2009). Keeping these facts in view, this research intends to find the effectiveness of low concentration of sodium azide at inducing mutations with respect to germination percentage, root length, seedling height, seedling survival, number of branches per plant, and yield per plant in tomato.

MATERIALS AND METHODS

Collection of Seeds

Air dried seeds of tomato (*Lycopersicum esculentum)* variety T106 was obtained from the Institute for AgriculturalResearch, Ahamdu Bello University Zaria.

Preparation of Seeds

The seeds of tomato (T106) presoaked in distilled water for six hours were treated with equal volume (20ml) of the chemical mutagen; Sodium Azide (NaN_3) at 1.0, 0.5, 0.1, and 0.05 percent in Phosphate buffer of PH7 for 4hrs. For each treatment, 30seeds were used. The treatments were periodically agitated at room temperature. The seeds were then thoroughly rinsed in tap water to remove excess mutagen. The control seeds were soaked in Buffer solution PH7 (Adamu and Aliyu, 2007 ; Goyal and Khan, 2009).

Planting of seeds

The treated and untreated seeds were sown in wooden box containing soil with three replicates in a complete randomized block design. Each replicate consisting 20seeds and kept at the Laboratory.

Raising of M1 generation

Four week-old seedlings were transplanted to the field in the Biological garden at IBB University Lapai, Nigeria in the month of May, 2011. The spacing was 80cm between rows and 50cm between plants. Each treatment was replicated three times in a complete randomized design. Recommended fertilizer levels of 50KgN/ha, 150KgP_2O_5/ha and 80 Kg K_2O/ha were applied at appropriate time. Stacking of plants was done twenty days after transplanting. Weeding and irrigation were carried out as required. Three rows of control (untreated) seedlings were planted at both ends of each plot. Various preliminary observations from seedling to maturity were made and recorded (Chidambaram *et al*., 2009 ; Khan and Goyal, 2009).

Data collection and Statistical analysis

Data were collected on germination percentage, seedling height, seedling survival, number of leaves per seedling, height at maturity, number of leaves per plant and yield per plant.

All data obtained were analyzed using Analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT)

RESULTS

Germination Percentage of Tomato Seedling

The germination percentage was observed to decrease with increased in concentration of sodium azide (NaN_3). The first germination in treated seeds was observed at 7-day as against the untreated at 5-day. However, there was significance difference ($P<0.05$) in germination percentage observed between the treated plots. The lowest germination percentage (Table 1) was recorded at the highest percentage concentration of (1.0%). At 8-day,

Table 1: Germination percentage of tomato seeds pretreated in different concentrations of Sodium azide

%concentration of Sodium azide	* Germination percentage of pretreated seeds	
	8days	15days
0.00 (control)	12a	70a
0.05	10a	68a
0.10	7b	64b
0.50	4c	62b
1.00	1d	58b

*Mean of the three replicates

Mean in a row followed by different letters differ significantly at $P<0.05$

germination percentage was 1% and 58% at 15-day in 1% NaN_3 concentration. The untreated plots had the highest germination percentage of 12% at 8-day and 70% at 15-day closely followed by plot treated with 0.05% which gave 10% and 68% germination at 8-day and 15-day respectively.

Percentage Seedling Survival at 14 -day and Maturity

A steady decrease in percentage seedling survival was observed with increased concentration of NaN_3 (Table 2).The untreated plot had the highest percentage seedling survival of 90% followed closely by 0.05% NaN_3 concentration (50%). There was no significant difference ($P<0.05$) between the percentage seedling survival in plots treated with 0.010, 0.50, and 1.00percent concentration. However, there was significance difference ($P<0.05$) between result obtained from treated and control plots. The trend in percentage survival at maturity was not different from that of the14-day.

Table 2: Percentage survival of tomato seedling at 14day and maturity

%concentration of Sodium azide	* Percentage seedling survival at 14day	*Percentage plant survival at maturity
0.00(control)	90a	89a
0.05	58b	67b
0.10	55b	58c
0.50	49c	56c
1.00	48c	53c

*Mean of the three replicates
Mean in a row followed by different letters differ significantly at $P<0.05$

Seedling Height at Day 10 and at Maturity

Seedling height decreased linearly as the concentration of NaN_3 increased from 0.05 to1.0% (Table 3). The shortest height (8.22cm) was obtained at 1.00% concentration and the highest height(12.00cm) at 0.05% which was very close to what was obtained with control (12.20cm).

Table 3: The height of tomato seedlings taken at 10day- old and at maturity

%concentration	*Seedling height at 10day-old(cm)	*Seedling height at maturity (cm)
0.00 (control)	12.20a	50.00a
0.05	12.00a	46.13b
0.10	10.30ab	47.00b
0.50	9.00b	46.35b
1.00	8.22b	40.31bc

*Mean of the three replicates
Mean in a row followed by different letters differ significantly at $P<0.05$

At maturity, the result obtained showed that with increased in concentration of NaN_3 there was a steady decreased in the height of the plants. The average shortest and highest plant height (40.31cm and 46.13cm) were obtained at 1.0% and 0.05% respectively. These were lowered than what was obtained in control. The result showed that there was no significant difference ($P<0.05$) in height of the seedlings at 10day- old between the treated and control plots, and also between the plants at maturity in all the treatments.

Root length, number of branches per plant and number of leaves per seedling

The result revealed that the root length at 20 day old in plots treated and control was not significantly different ($P<0.05$). The longest length (7.22cm) was obtained in control and 6.17cm among the treated plots (0.05%)(Table 4). The number of branches per plant was not significantly different ($P<0.05$)in all the plots. The branches per plant was observed to range from five to eight. The number of leaves per plant at 20day- old was highest in the control (10 leaves) and lowest (6 leaves)in plots treated with 1.00% NaN_3 concentration. However, there was no significantly different ($P<0.05$)in all the plots.

Table 4: Root length, number of branches per plant and number of leaves/seedling of 20day – old tomato seedlings

%concentration	*Root length (cm)	* Number of branches/plant	*Number of leaves/Seedling
0.00 (control)	7.22a	8a	10.00a
0.05	6.17a	8a	9.00a
0.10	5.50ab	7a	8.00a
0.50	5.62ab	5b	8.00a
1.00	4.60b	5b	6.00a

*Mean of the three replicates
Mean in a row followed by different letters differ significantly at $P<0.05$

Days to 50% flowering and number of fruit per plant

The time frame that took all the treated and control plants to reach 50% flowering was observed not to be significantly different ($P<0.05$)(Table 5). The most delayed among the treated plants was 1.0% concentration which took an average of 85.22days to reach 50%flowering as against 78.5days observed in control. The fruit yield per plant dropped gradually with increased concentration of NaN_3. 0.05%produced the highest fruits per plant (18) which was very closed to what was produced in control(21). However, there was no significant difference ($P<0.05$) in fruit produced per plant in all the treated plants.

Table 5: Days taken to reach 50% flowering and number of fruit of Tomato

%concentration	*Days to 50% flowering	*Number of fruit/plant
0.00 (control)	79b	21a
0.05	80a	18a
0.10	86a	17a
0.50	87a	17a
1.00	85a	15a

*Mean of the three replicates
Mean in a row followed by different letters differ significantly at $P<0.05$

DISCUSSION

In this study, the different concentrations of sodium azide showed various forms of morphological changes. The decreases in seedling emergence, seedling height, root length, seedling survival, height at maturity and fruit yield per plant with increasing in mutagen concentrations were observed. Sodium azide is a strong mutagen and growth of plant parts were strongly inhibited with increasing concentration. The impact of sodium azide had been reported on tomato and it was very effective at inducing mutations with respect to germination percentage, root length, seedling height, seedling survival number of branches and fruit per plant (Adamu and Aliyu, 2007).

The extent of injury caused by sodium azide was seen as a delay in the number of days it took to germinate in this study. The reduction in seed germination in treated seed with mutagen had been explained to be due to delayed or inhibition in physiological and biological process necessary for seed germination which include enzyme activity, hormonal imbalance and inhibition of mitotic process. These effects may hamper ATP biosynthesis resulting in decreased availability of ATP molecules which may slow the germination rate and reduce the germination percentage. Also seed may have probably developed tolerance to the inhibitory effect of NaN_3 on germination and had improved their physiological conditions on additional days with respect to seed germination. Geng and Gao, (1988)

reported a significant decrease in the percentage germination in barley seed treated NaN_3. Pearson et al.,(1975) reported that seeds treated with azide produced had a delayed in the initiation of growth.

It was also observed that as concentration increased, there was decrease in plant height. This was in contrary to Bohmova *et al*.,(1992) who reported that there was no significant different or change in plant height regardless of the mutagenic treatment. But was in support of Adamu *et al*.,(2007) who reported that the inhibitory effect of radiation on plant height is dose dependent. Plant treated with 1.0% concentration of NaN_3 gave a reduction of 20% in height which was considered to be the best for induction of reduction in tomato height by the earlier workers (Krishna *et al*., 1984). The root lengths observed to decreased with increase in NaN_3 concentrationwas in agreement with Ilbas*et al.,* (2005) who reported that the effect of different concentration of NaN_3 treatment on root length was least in crop exposed to 2.5mM for 3h in barley. Seedling emergence at 14day and seedling survival at maturity decreased in treated plots. The data indicated that the injury induced by mutagen treatment was generally not severe. The reduction in seedling survival may be attributed to cytogenetic damage and physiological disturbance as reported by Fahad and Salim, (2009).The greater sensitivity at higher concentration level was attributed to various factors such as changes in the metabolic activity of the cells, inhibitory effects of mutagens and disturbance of balance between promoter and inhibitors of growth regulators (Kishna *et al*., 1984) .

CONCLUSION AND RECOMMENDATION

In this study, lower concentrations of sodium azide was found to be very effective in inducing mutations with respect to germination percentage, root length, seedling height, seedling survival, number of branches per plant, and yieldper plant in tomato. The application was easy and in expensive. Therefore, Sodium azide at lower concentration could be utilized to increase variability in tomato that would ultimately increases the possibility of isolating beneficial mutants for improvement of tomato production.