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Morphological Variations of White Yam (*Dioscorea Rotundata)* Treated with Different Concentrations of Ethyl Methane Sulfonate

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Abstract
White yam (*Dioscorea rotundata*) is an essential staple crop in many tropical regions, contributing immensely to food security and livelihoods for millions of people. However, its cultivation is limited by factors such as low yield and lack of genetic diversity. To address these challenges, this study investigated the response of white yam to different concentrations and timings of Ethyl methane sulfonate (EMS), a chemical mutagen used to induce genetic variation. Minitubers obtained from single nodal vine cuttings with a leaf each previously treated with 0.5% and 1.0% concentrations with exposure durations of 1.0, 1.5, 2.0, and 2.5 hours were planted and allowed to grow into mother plants. Two months later, single vine nodes were carefully cut from the mother plants and planted directly into sterilized topsoil within a controlled environment, and morphological variations were evaluated using ANOVA (SAS 9.0 version), and differences in means were separated at $P \leq 0.05$ using LSD. At eight weeks, yam seedlings with EMS 1% treatment for 1 hour had the highest number of leaves (7.22 ± 0.53) and the longest vine (49.41 ± 3.60 cm) and produced the highest number of nodes count (7.19 ± 0.53), while the fewest leaves (3.88 ± 0.53) and the shortest vine (29.84 ± 3.60 cm) were recorded for the control. The highest leaf area (43.07 ± 1.84 cm^2) was recorded for the seedlings treated with 0.5% for 2 hrs., and the lowest leaf area (30.27 ± 1.84 cm^2) was recorded for 0.5% at 2.5 hrs. The largest tuber weight was obtained with EMS 1% for 1 hour (21.95 ± 4.89 g). The results suggest that EMS 1% treatment for 1 hour can significantly improve yam productivity and yield.

Keywords: Yam, Ethyl Methane-Sulfonate (EMS), Vine Cuttings, Mutation, Morphological Traits

Introduction

Yam (Dioscorea spp.) is an essential monocot herbaceous crawling vine plant, which are primarily cultivated for their starchy underground tubers grown in the tropical and sub-tropical regions globally. Of almost 600 species of yams, only eleven are edible and economically significant (Gatarira et al., 2021). Six of these, however, are significant because they offer dietary nutrients like starch, protein,fibre,vitamins and micronutrients for people who rely on them as a staple food, including *Dioscorea rotundata* (white yam), *D. alata* (water yam), *D. bulbifera* (aerial yam), and *D. dumetorum* (trifoliate yam) (Asiedu, 2010; Umber et al., 2020; Syombua et al., 2022). Yam besides being essential for maintaining food security, it also plays a substantial cultural, social, and economic significance and is found very useful in pharmaceutical industries (Asiedu et al., 2010; Ettien et al., 2013; Guo-Fu et al., 2022). According to Price et al. (2017), yams are a highly valued tuber crop in West Africa, accounting for over 32% of the region's overall farm revenue and 15% of the daily caloric intake of over 300 million people. The FAO (2020) reports that over 90% of the world's yam production came from West Africa. The gross economic value of yams in Africa is more than that of all other staple crops and equal to the sum of the values of the top three cereal crops, rice, sorghum, and maize, despite the fact that their output is 40% lower than that of cassava (FAO, 2020). Despite its high value, West African yam production has stagnated over the past 20 years due to a number of issues, including yam anthracnose, climate change, vulnerability to nematode and virus infections, low soil fertility, limited genetic diversity, a lack of high-quality planting materials, storage pests, and diseases (Asiedu, 2010; Balogun et al., 2014; Umber et al., 2020).

Challenges facing by yam producers continue to persist because of the varietal development in yam that has been so slow due to a number of biological constrains, including irregular flowering, dioecy, polypoidy, high heterozygosity, low multiplication ratio associated with the use of vegetative propagation method (Mignouna et al., 1998). In order to meet consumer preferences to ameliorate these challenges to yam cultivation,

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development of varieties and screening for the variety with high resistant to both biotic and abiotic stressors is highly imperative.

Traditionally, yams have been propagated vegetatively through underground or aerial tubers. However, this method of propagation is hindered by low rates of flowering, infrequent fruit production, and a high likelihood of producing infertile seeds. Moreover, yams are susceptible to various biotic and abiotic stresses, which constrain their genetic diversity. Consequently, the genetic resources available for selection are severely limited, resulting in a small number of cultivars that have been vegetatively reproduced for generations. The absence of hybridization and continual vegetative propagation has hindered the introduction of new genetic traits, resulting to a stagnation in yam improvement efforts (Sadik & Okereke, 1975).

Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Subuthi et al., 1991). Induced mutation using physical and chemical mutagen is a modern method to create genetic variation leading to new varieties with better characteristics. To overcome these constraints, mutation breeding emerges as a valuable tool for yam improvement. By introducing mutations into yam crops, new genetic variations can be generated and selected for desirable traits like disease resistance, increased yield, and improved quality. This strategy can serve to broaden the genetic diversity of yams and expedite the development of new and enhanced cultivars better suited to withstand the changing biophysical and socio-economic conditions in production areas. The application of induced mutation has been recognized as a critical and reliable approach for enhancing yam crops. As noted by Villamor and Cardinez (2008), the controlled use of induced mutation on yam planting materials significantly impacts the size and weight of the tubers. Furthermore, inducing mutations in yam has led to the development of high-yielding yam lines that do not necessitate staking (Nwachukwu et al., 2008).

Materials and Methods

Experimental Location

This experiment was conducted in the screen house at the rooftop garden, Department of Crop Protection and Environmental Biology, University of Ibadan situated on Latitude 7°27'.047"N, Longitude 3°53'.832"E; Elevation: 218m above sea level (ASL). However, initial treatment with EMS was done at the Cell Biology unit, Bioscience Center, IITA, situated on Latitude 7°30'.11"N, Longitude 3°54'.28"E; Elevation: 261m ASL.

Preparation of EMS solution and Source of planting materials

EMS was sterilized using filter sterilization. For the 1% solution, 1g of EMS was dissolved in 1ml of absolute ethanol and then brought to a final volume of 100ml using sterile distilled water. Similarly, for the 0.5% solution, 0.5g of EMS was dissolved in 0.5ml of absolute ethanol and then brought to a final volume of 100ml with sterile distilled water.

The clean, virus-free *Dioscorea rotundata* minitubers raised from single nodal vine cuttings with a leaf each previously treated with 0.5% and 1.0% concentrations EMS at different timing of 1.0, 1.5, 2.0 and 2.5 hours were planted and allowed to grow into mother plants. Two months after, single vine nodes were carefully cut from the mother plants and planted directly into sterilized topsoil within a controlled environment.

Topsoil was packed and sterilized at temperatures of 121°C for a duration of 4 hours. The following day, a 5 kg sterilized soil was distributed into 8 kg pots, and then arranged in the screen house.

Experimental design

The experiment was a 2 (EMS concentrations) by 4 (timing of exposure) factorial design laid in a completely randomized design and replicated three times.

Data Collection and Statistical Analysis

Bi-weekly, morphological data were taken on the number of leaves per plant, vine length, leaf area and number of nodes, while at harvest, the weight of minitubers and the number of tubers per pot were collected. Data collected were analyzed using R software, and means were separated at a significance level of $p<0.05$.

Results

Morphological Variations Following Treatment with Ems

Number of leaves at 2 weeks' interval after planting

Table 1 shows that there were significant differences among the mean number of leaves fortnightly after planting. At two weeks after planting, the group treated with EMS 1% for 1 hour had the highest number of leaves (1.55±0.21), while the control group had the lowest number of leaves (0.22±0.21) after planting. At four weeks after planting, the control group (0.22±0.26) and the group treated with EMS 1% for 2.5 hours (1.22±0.26) showed no significant differences and they both had the least number of leaves, unlike the group

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treated with 1% for 1 hour maintained the highest number of leaves (3.33±0.26) as was recorded two weeks after planting. Moreover, at 6 weeks after planting, the group treated with EMS 1% for 1 hour had the highest number of leaves (5.22±0.46) while no significant difference was observed among the group treated with 1% at 2 hours (3.00±0.46), 2.5 hours (3.00±0.46) and the control (3.00±0.46). At 8 weeks after planting, the group treated with 1% for 1 hour (7.22±0.53) remained significantly highest number of leaves than the control group (3.88 ±0.53), which had the least number. Same number of leaves were recorded for the group treated with 1% for 1.5 hours (6.00±0.53) and 0.5 % for 2 hours (6.00±0.53), hence no significant difference was observed.

Table 1: Effects of EMS concentrations and timing in the number of plantlets' leaves produced at the interval of 2 weeks after planting

EMS Concentration (%)	Timing (hours)	NOL 2 WAP	NOL 4 WAP	NOL 6 WAP	NOL 8 WAP
0.5%	1.0	0.77bcde	2.44b	4.11abcd	5.22bcd
	1.5	0.88bcd	2.11bc	3.44bcd	4.33cd
	2.0	1.22ab	2.66ab	4.55ab	6.00ab
	2.5	0.55cde	2.00bc	3.11cd	4.77bcd
1%	1.0	1.55a	3.33a	5.22a	7.22a
	1.5	1.00abc	2.44b	4.33abc	6.00ab
	2.0	0.44cde	1.67cd	3.00d	5.55bc
	2.5	0.33de	1.22d	3.00d	4.22cd
Control		0.22e	0.22d	3.00d	3.88d
S.E		0.21	0.26	0.46	0.53

*Means in the same column with the same letters are not significantly different at p > 0.05

Vine length (cm) at 2 weeks after planting

There were significant differences in the vine length between the two concentrations at different timing. The group treated with EMS 1% at 1 hour had the longest vine (22.61+2.06 cm), which was significantly lengthier than the control group (9.42+2.06 cm), EMS 0.5% at 1 hour (11.72+2.06 cm) and EMS 1% at 2.5 hours (8.97+2.06 cm) had the shortest vine at 4 weeks after planting. The group treated with EMS 1% at 1 hour (37.85+3.42 cm), which had the longest vine length, was the group treated with 1% at 1.5 hours (30.47+3.42 cm), and the shortest vine was recorded for 1% at 2.5 hours at 6 weeks after planting. Similar result was observed for the group treated with 1% at 1 hour, which significantly lengthier than (49.41+3.60 cm), both EMS 0.5% (29.33+3.60 cm) at 1.5 hours and the control group (29.84+3.60 cm), which reportedly had the shortest vine at 8 weeks after planting (Table 2).

Table 2: Effects of EMS concentrations and timing in the vine length of plantlets' leaves produced at the interval of 2 weeks after planting

EMS Concentration (%)	Timing (hours)	VL 4 WAP	VL 6 WAP	VL 8 WAP
0.5%	1.0	11.72c	28.95abc	36.58bc
	1.5	14.61bc	20.34cde	29.33c
	2.0	18.55ab	30.56ab	44.65ab
	2.5	9.44c	19.83cde	32.66c
1%	1.0	22.61a	37.85a	49.41a
	1.5	14.55bc	30.47ab	43.64ab
	2.0	13.73bc	25.27bcd	36.24bc
	2.5	8.97c	14.02e	27.11c
Control		9.42c	18.28de	29.84c
S.E		2.06	3.42	3.60

*Means in the same column with the same letters are not significantly different at p > 0.05

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Leaf Area (cm^2)

Table 3 shows the leaf area of plantlets treated with EMS at 4, 6 and 8 weeks after planting. EMS 0.5% at 2 hours had the highest leaf area all through while 0.5% at 2.5 hours had the smallest leaf area at 6 and 8 weeks after planting.

Number of Nodes

Table 4 shows the number of nodes of plantlets treated with EMS at 4, 6 and 8 weeks after planting. There was a significant difference with respect to the number of nodes. EMS 1% for 1hour had the highest number of nodes all through while at 4 weeks EMS 0.5% at 2.5 hours and the control had the smallest number of nodes, and also at 8 weeks the control had the lowest number of nodes.

Table 3: Effects of EMS concentrations and timing in the leaf area and the number of nodes of plantlets' leaves produced at the interval of 2 weeks after planting

EMS Concentrations(%)	Timing (hours)	4 WAP		6 WAP		8 WAP	
		LA	NND	LA	NND	LA	NND
0.5%	1.0	24.80b	2.33bc	29.73bcd	4.11abcd	33.13bcd	5.22bcd
	1.5	26.34ab	2.11bc	33.94ab	3.44bcd	35.92bc	4.33cd
	2.0	30.76a	2.66ab	35.83a	4.55ab	43.07a	6.0ab
	2.5	22.67b	2.00bc	25.98d	3.11cd	30.27d	4.77bcd
1.0 %	1.0	29.77ab	3.33a	33.18abc	5.22a	37.56b	7.22a
	1.5	25.74ab	2.44b	31.93abc	4.33abc	37.31b	6.0ab
	2.0	26.57ab	1.66cd	34.79ab	3.00d	36.47b	5.55bc
	2.5	22.24b	1.22d	25.07d	3.00d	30.91cd	4.22cd
Control	-	23.70b	1.22d	27.81cd	3.00d	35.17bcd	3.89d
S.E		1.98	0.26	1.92	0.46	1.84	0.53

*Means in the same column with the same letters are not significantly different at $p > 0.05$

Tuber weight (g):

There were significant differences among the group of plantlets treated with different concentration of EMS at varying time. The average tuber weight obtained from the group of plantlets treated with EMS 1% for 1 hour (21.95±4.98 g) was the highest followed by the control (12.68±4.89 g) which received no treatment and the least tuber weight was recorded for the group of plantlets that received EMS 0.5 % for 1. 5 hours (1. 05±4.89 g). Although, the group of plantlets that received EMS 0.5 % for 1.0 hour (9.31±4.98 g) and 2. 0 hours (10.91±4.98 g) statistically showed no significant differences as compared to the control group (12.68±4.98 g), but the latter was heavier than the two formers (Table 4).

Table 4: Effects of EMS concentrations and timing on the average tuber weight per tuber of yam plants

EMS concentration (%)	Timing (hours)	Tuber weight/plant (grams)
0.5 %	1.0	9.31abc
	1.5	1.05c
	2.0	10.91abc
	2.5	5.75bc
1.0 %	1.0	21.95a
	1.5	7.43bc
	2.0	2.36c
	2.5	3.14c
Control	-	12.68abc
S.E		4.89

*Means in the same column with the same letters are not significantly different at $p > 0.05$

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Discussion

Induced mutations are a mechanism of broadening genetic variations in any crop plants with a higher frequency than the occurrence of spontaneous mutations. This study showed that the quantitative traits of white yam respond significantly when subjected to different concentration and duration of Ethyl Methane Sulfonate, hence enhances genetic variations. This result was supported by Cvejić et al. (2015) who worked on sunflower plants, where it was reported that efficiency of Ethyl Methane Sulfonate is capable of creating variations in plants. The high survival rate of nodal cuttings of yam treated with EMS indicates that EMS, within the tested parameters, did not have lethal effects on yam nodal cuttings. This finding is in line with previous research on other tuberous plant species, such as potato callus treated with EMS where a higher percentage of plantlets regeneration was recovered after the treatment (Luan et al., 2007). The absence of lethality suggests that EMS can be potentially used as a mutagenic agent in yam breeding programs without adversely affecting the survival of nodal cuttings. Plantlets treated with EMS 1% for 1 hour exhibited the highest number of leaves over the 8-week period compared to the control group. This indicates that EMS treatment had a positive impact on leaf development, potentially enhancing the plant's overall growth and photosynthetic capacity. The control group consistently showed the lowest number of leaves at various time points, suggesting that EMS treatment stimulated leaf production in the treated plantlets.

Additionally, the vine length of plantlets treated with EMS 1% for 1 hour consistently exhibited the highest vine length throughout the observation period. This indicates that the EMS treatment at a concentration of 1% for a duration of 1 hour had a sustained positive effect on vine growth from the initial stages and maintained this growth advantage over time. The continuous superiority of EMS 1% at 1 hour in promoting vine length highlights the potential long-term benefits of this treatment in enhancing vine development and overall plant growth. In contrast, the control group and plantlets treated with EMS 1% for 2.5 hours had the shortest vines at 4 weeks, highlighting the potential negative effects of prolonged EMS exposure on vine length. It was evident that when the exposure timing of EMS concentration was increased, the vine length and number of leaves of the plantlets decreased, and had similar results to the control. The EMS 0.5% and 1.0 % levels at 2.5 hours exhibited similar results with the control for vine length at 8 weeks after planting. In agreement with our results, Behera et al. (2012) showed that induction of mutagenesis through EMS treatment in Asteracantha longifolia affected plant height, morphology, and even leaf size. Mutations in genes that control the development of leaves might have phenotypic effects that vary from lethality to the absence of visible alterations. Hence, a decreasing trend in the length of plantlets and number of leaves was observed with the increase in the timing exposure of EMS concentration. This result was similar to the previous study of capsicum annuum that seeds treated with 1.5% of EMS dose in M1 generations had the lowest growth indices (Hasan et al., 2022).

According to Table 3, plantlets treated with EMS exhibited varying leaf areas fortnightly after planting. EMS treatment at a concentration of 0.5% for 2 hours consistently resulted in the highest leaf area across all the time points measured. This suggests that the EMS treatment at this specific concentration and duration had a positive and sustained effect on leaf expansion and development, potentially enhancing the overall photosynthetic capacity of the plantlets. Saba and Mirza (2002) reported a similar finding by discovering that tomato plants treated with 0.5% EMS for three hours had the maximum chlorophyll content along with other photosynthetic attributes. The weight of tubers harvested from yam plants treated with EMS provides valuable insights into the potential effects of EMS treatment on tuber development and yield. Among the different treatment combinations, plantlets treated with EMS 1% for 1 hour produced the highest tuber weight per plant. This suggests that EMS treatment, particularly at a concentration of 1% for a duration of 1 hour, is ideal tuber growth and development, leading to increased tuber yield. Conversely, plantlets treated with EMS 1% for 2.5 hours exhibited the lowest weight of tubers. This output indicates that prolonged exposure to EMS at a higher concentration have adverse effects on tuber formation or yield, potentially inhibiting tuber growth. Maximum chlorophyll content was seen when Capsicum annuum was treated with 0.1% EMS for 3 hrs, according to Ahmad and Asif (2023). However, extremely prolong time exposure and higher doses could cause a significant change in the DNA of the rice plants, as has been reported for mutagenesis studies in rice (Abid et al., 2018; Viana et al., 2019). The treatments showing maximum variation in quantitative characters may show the stable gene mutations in subsequent generation.

Conclusion

All the quantitative and yield traits were proportionately decreased with increase time exposure to different concentrations of EMS mutagens. The decrease quantitative characters have been attributed to the physiological disturbance and or chromosomal damage caused to the cells of the plant by the mutagen. Chemical mutagens

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usually cause point mutation, sometimes loss of a chromosome segment or deletion can also occur. Studies suggested that the most important parameters for inducing chemical mutagen for growth and yield characters depend on the concentration and duration of treatment. In the investigation of different concentration and timing of Ethyl Methane Sulphonate effects on the characteristics of *Dioscorea rotundata* we observed morphological variations in vine length, number of leaves, leaf area and tuber weight. Moreover, the weight of tubers harvested from yam plants treated with EMS highlighted the influence of EMS concentration and duration on tuber development and yield. EMS 1% for 1 hour resulted in the highest tuber weight, emphasizing the importance of selecting appropriate EMS treatment regime to maximize crop productivity. These findings have implications for the development of new, high-yielding varieties of white yam, which can contribute to food security and improved livelihoods for smallholder farmers.

Recommendations

1. Further research into the underlying mechanisms of EMS action and the optimization of treatment protocols is essential for harnessing the full potential of EMS in agricultural practices to improve crop resilience and productivity in varying environmental conditions.
2. Also, anatomical structure of the treated leaves and tubers harvest should be taken into consideration.

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