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Molecular Evaluation of *Cordyline fruticosa* (L.) A.Chev. Behavior Affected by Different Chemical Mutagens

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Abstract

Cordyline fruticosa is a medicinal plant that is also ornamental due to its variegated coloured leaves. Some traits benefit from mutation. Guanidine hydrochloride and sodium azide were the chemical mutagens used in this study. For each, concentrations of 30, 40, and 50 mM were used. The plantlets were exposed to these mutagens, and the explants were cultured in vitro using micropropagation. To estimate the effect of mutagens in comparison to non-mutant (control), different 8 morphological characters were measured. In the case of guanidine hydrochloride, all of the treatments are nearly identical to control, with no significant differences except for 30 mM, which is lower than the others. *Cordyline fruticosa* genetic variation resulted in a wide range of polymorphism percentages. In the case of sodium azide, it was 74.66 %, while in the case of guanidine hydrochloride, it was 37.77 %. All morphological parameters decreased in sodium azide comparing to control, with the exception of the number of branches, which increased more at 50 mM than at other concentrations and control. All morphological parameters increased in mutants rather than controls, unlike guanidine hydrochloride. For photosynthetic pigmentations, all pigments decreased with sodium azide treatments. As a result, chemical mutagens cause genetic instability and variation, which manifests itself in morphological and physiological characteristics.

Keywords: *Cordyline fruticose*; Guanidine hydrochloride; Sodium azide; photosynthetic pigmentations; RAPD-PCR.

1. Introduction

Cordyline fruticosa is a medicinal, ornamental, and woody plant native to the southern hemisphere and tropical areas of the world. It is a monocotyledonous ornamental plant with evergreen flowers in family Asparagaceae. It is also known for its medicinal treatments for human diseases such as hemostatic, toothache, sore throat, and neck pain. *C. fruticosa* has antioxidant activity and nutritional value in its leaves and roots, among other organs. It also has cytotoxic and antimicrobial properties against microorganisms due to the presence of steroidal saponins. In some traditions and cultures, *C. fruticosa* was thought to ward off evil spirits and bring good luck (Fouedjou et al., 2014, 2016).

Induced chemical mutation produces genetic diversity, which results in new varieties with improved traits. Induced mutations also have a mechanism for abiotic and biotic stress resistance, allowing the creation of a new resistant strain (El-Mokadem and Mostafa, 2013; Suprasanna et al., 2015).

The chemical mutagen sodium azide (NaN_3) is a potent mutagen in plants. The application of a mutagen to a plant is simple and cheap, and it allows for mutation to enhance the plant's characteristics. The efficiency of mutant production is influenced by a number of factors, including azide concentration and treatment time. This causes point mutations, damages chromosomes, and results in plant tolerance to various conditions (El-Mokadem and Mostafa, 2013). These changes lead to increase in seed germination percentage, seedling height and root length (El Kaaby et al., 2015).

Chemical mutagens known as guanidine hydrochlorides alkylate the structure of DNA nitrogen bases. Three categories of guanidine hydrochlorides were identified: Phenylethylbi-guanidide, even at relatively high concentrations, causes only partial inhibition. About 70 % of malate respiration is inhibited by Decamethylenedi-guanidide (e.g., succinate respiration and ascorbate-tetramethyl-p-phenylenediamine). Finally, all three phosphorylation sites and cyanide-insensitive respiration are inhibited by octyl-guanidine hydrochloride, but to varying degrees and concentrations (Wilson and Bonner, 1970).

The goal of that study was to see how two chemical mutagens (sodium azide and guanidine hydrochloride) affected *Cordyline fruticosa* growth. It also aimed to generate genetic diversity in the content of morphology and chlorophyll detected by RAPD-PCR.

2. Materials and Methods

2.1. Plant Materials

Cordyline fruticosa is a medicinal plant found in the cultivated gardens of Ain Shams University's Faculty of Agriculture.

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2.2. Mutagens

Two chemical mutagens were used in this study, guanidine hydrochloride and sodium azide. They were prepared in concentrations of (0, 30, 40, and 50 mM).

2.3. In Vitro Culture (Micropropagation)

The standard sterilization conditions were the elimination of stacked dust/soil practices. The explants were de-foliated and washed thoroughly in tap water. 20% Clorox + 0.1% $HgCl_2$ was used for 20 minutes and washed 4 to 5 times with sterile ddH_2O with surface sterilization of the explants. Under the sterilized condition, excision and culture procedures for stem nodal ex-plants were performed. Nodal explants of this ornamental plant were sterilized to be cultured on full power MS-free media, according to (Murashige and Skoog. 1962). After that, the resulting plantlets were twice regenerated for multiplication on ¾ MS-free media. The plantlets of both ornamental plants were then cut into nodal segments and incubated for 3 hours in various mutagenic concentrations. The cells were cleared in sterile dH_2O and moved to clear MS jars then incubated for five weeks at 25ºC (16 h light/8 h dark) with light intensity of 2000 lux.

2.4. Morphological Measurements

Compared with control, the measured parameters were estimated for the mutant plants. The parameters were fresh weight, shoot length, length of roots, number of lateral roots, number of branches, number of leaves, leaf length and leaf width.

2.5. Photosynthetic Pigments Estimation

Chlorophyll pigmentation is an essential physiological parameter that determines and controls most of the plant cell pathways. Chlorophylls compared with control plants were estimated for both mutants. Chlorophyll a, b, and carotenoids were measured in the plantlets' fresh leaves (Metzener et al., 1965). A solution of 85 % acetone was used to homogenize 0.5 g of fresh leaves. The supernatant, which contained the pigments, reached to 7 ml of 85 % acetone after centrifugation. The extract was compared to a blank of pure 85 % aqueous acetone using a colorimeter at different 3 wavelengths (452, 645, and 664 nm). The concentrations of chlorophyll a, b, and carotenoid were calculated in g/ml as follow:

$$\text{Chl. a} = 10.3\ A664 - 0.918\ A645 \quad (1)$$

$$\text{Chl. b} = 19.7\ A645 - 3.87\ A664 \quad (2)$$

$$\text{Carot.} = 4.3\ A452\ (0.0265\ \text{Chl. a} + 0.426\ \text{Chl. b}) \quad (3)$$

After that, the fractions were used as mg/g fresh weight:

$$\frac{fraction \times dilution}{1000}\ mg/g \quad (4)$$

2.6. Molecular marker

2.6.1. DNA isolation and RAPD-PCR bioassay

Cordyline fruticosa's total genomic DNA (mutagens and control) was extracted by CTAB protocol (Doyle and Doyle, 1990). With 800 µl of 2 % CTAB buffer, half g of leaves was ground and incubated for 30 min at water bath (65ºC) with vortex 10 min intervals. The Eppendorf tubes were centrifuged for 10 minutes at 12,000 rpm, with the supernatant transferred to new tubes. Each tube was filled with an equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged for 10 minutes at 4ºC at 12,000 rpm. Move the upper aqueous layer to another Eppendorf's with addition of 900 µl of isopropanol and left 2h at -20ºC. DNA pellets were formed after centrifugation and wash using 70% ice-cold ethanol, then the air-dried pellets were resuspended in 50 µl TE buffer and stored at -20ºC.

Only five of eight RAPD primers performed (Table 3), gave reproducible clear bands. Biometra thermocycler machine was applied for the RAPD-PCR reaction. A total volume of 25 µl was used, which included 12.5 µl of master mix (COSMO PCR RED M. Mix), 3 µl of DNA (50ng), 1 µl of each primer (100 nanomole) (Willowfort), and 8.5 µl of ddH_2O. A 35 cycles reaction program was run and included of 30-second denaturation at 94ºC, 30-sec annealing (Table 3), and 1-min extension at 72ºC. Then a final extension step at 72°C for 10 min, followed by cooling at 4°C. The amplified products were checked on 1.4 % agarose gel.

2.7. Statistical Analysis

Electrophoresis of the gel resulted in analyzed images. A band's presence was scored as 1, while the absence of the band was coded as 0 using Bio-Rad Quantity one (4.6.2). Jaccard's coefficient of similarity, a similarity matrix was generated. Cluster analysis was applied to obtain a dendrogram by using the unweighted pair group method with the arithmetic averaging algorithm (UPGMA). These computations were carried out (Shuaib et al., 2997).

The data collected was analyzed for variance tests in SPSS 21. Estimated mean average and standard deviations. Using Tukey test multivariate analysis, significant means have been separated. For the 25 different treatments, Tukey homogeneous Subsets were used with one-way ANOVA, where Tukey homogeneous Subsets were used with multivariate analysis for the two factors.

3. Results

Different concentrations of guanidine hydrochloride and sodium azide were prepared (0, 30, 40, 50 mM). They were used to treat the explants of *Cordyline fruticosa* plantlets. The following parameters were recorded.

3.1. Morphological Parameters

Eight morphological parameters were estimated to respond to these plants to the different mutagens' concentrations (Fig. 1). The data in Table (1) showed significant differences compared to control for Cordyline fruticosa affected by sodium azide and guanidine hydrochloride. The high concentration of sodium azide (50 mM) showed branching in both *C. fruticosa*. In the case of guanidine hydrochloride mutagen, it causes stimulation in growth and morphology.

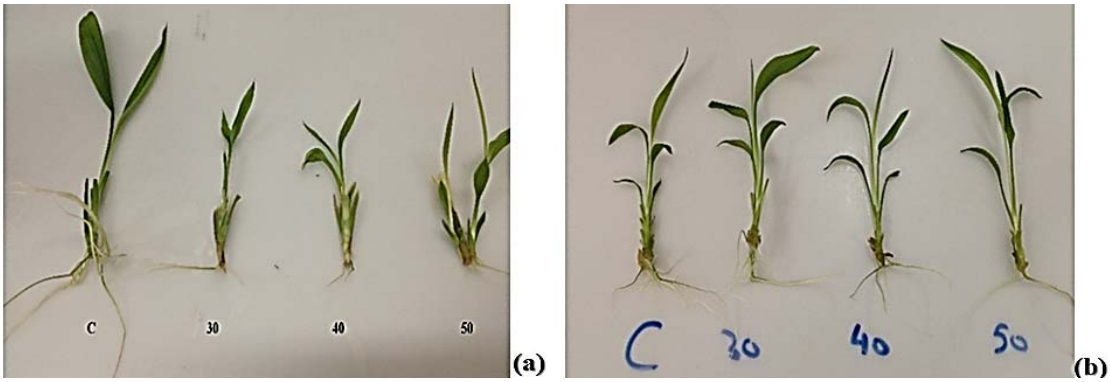

Figure 1. Phenological responses of *Cordyline fruticosa* to different mutagens; a) with sodium azide, b) with guanidine

Table 1. Phenological means estimation of *Cordyline fruticosa* in response to different mutagens

Species	*Cordyline fruticosa*							
Mutagens	Sod. azide				Guanidine hydrochloride			
Conc. mM	0	30	40	50	0	30	40	50
Fresh wt. (g)	0.5143	0.2731	0.2852	0.2604	0.4223	0.6184	0.4195	0.5412
Shoot length cm	8.9000	6.1333	4.4667	4.6000	8.1333	9.5000	8.4000	7.6667
Root length cm	5.2333	1.9667	1.7000	1.7000	5.1500	6.8833	16.9000	4.4083
No. of roots	4.6667	2.3333	2.0000	2.3333	3.0000	5.0000	4.0000	4.6667
No. of leaves	2.6667	2.6667	2.3333	2.6667	4.3333	5.6667	4.3333	4.3333
No. of branches	2.0000	1.0000	1.6667	2.3333	1.0000	1.3333	1.0000	1.3333
Leaf length cm	5.2200	3.4200	2.5800	3.0000	4.1000	5.1875	4.9813	5.2063
Leaf width cm	0.7300	0.4800	0.3200	0.3500	0.6375	0.7250	0.6625	0.7063
F-value	16.059**	53.364**	12.904**	15.698**	25.576**	36.760**	1.083**	14.937**

** highly significant

3.2. Photosynthetic Pigments

Chlorophyll content (chlorophyll a, b and carotenoids) was measured for response of *C. fruticosa* to the mutagens' concentrations. These were shown in Table (2) for sod. azide and guanidine. In the case of sodium azide mutagen, compared to control, the concentrations of the mutants' pigments decrease. Still, at a 50 mM concentration, the pigmentations are significantly increasing more than the other concentrations (30 and 40 mM). In the case of guanidine hydrochloride mutagen, the pigmentations are less affected than sodium azide. At a concentration of 30 mM guanidine hydrochloride, the pigmentation is inhibited more than others, where the control, 40, and 50 mM are not significantly different.

Table 2. Means of chlorophyll content estimation of *Cordyline fruticosa* with different mutagens

Species	*Cordyline fruticosa*							
Mutagens	Sod. azide				Guanidine hydrochloride			
Conc. mM	0	30	40	50	0	30	40	50
Chl. a	0.30618	0.13515	0.18170	0.19692	0.27834	0.20023	0.24840	0.24245
Chl. b	0.43028	0.08340	0.11298	0.30852	0.39116	0.31673	0.38181	0.37985
Cart.	1.39506	0.12159	0.11936	0.99555	0.12682	0.10472	0.12230	0.12257
F-value	198265.83**	1149.23**	771.199**	26653.80**	13941.31**	26820.43**	48600.6**	61297.2**

** highly significant

3.3. Molecular Markers

In order to measure the genetic diversity resulting from treated mutagens, RAPD-PCR was performed after total genomic DNA was extracted. Results on agarose gel (Fig. 2 and 3) for sodium azide and guanidine hydrochloride were shown for selected oligonucleotide decamers. The table (3) below shows the polymorphism data from these

primers. *Cordyline fruticosa's* total polymorphism percentage affected by sodium azide was 74.66 percent, with 37.77 percent of the polymorphism percentage resulting from the treatment of guanidine hydrochloride. Table 4 and 5 showed a matrix of similarities between the various concentrations of sod. azide and guanidine, respectively. Figure (4) illustrated a dendrogram of the sodium azide impact on *Cordyline fruticose* on genetic variation. Control has been shown to be separated on its own from other mutants, where 40 and 50 mM are genetically similar. The effect of hydrochloride guanidine on genetics was demonstrated in (Fig. 5). This revealed that control is distinct, with 30 and 40 mM being genetically equivalent.

Table 3 List of primers used in the RAPD-PCR of *Cordyline fruticosa* with both mutagens, including their names (ID), nucleotide sequences, and polymorphism percentage

No.	Primer name	Primers sequence	GC %	Tm	Sodium azide		Guanidine hydrochloride	
					Total bands	Polymorphism %	Total bands	Polymorphism %
1	OPA-12	5'-TCGGCGATAG-3'	60	34	5	40	6	50
2	OPB-17	5'-AGGGAACGAG-3'	60	33.1	4	75	4	0
3	OPZ-07	5'-CCAGGAGGAC-3'	70	34.6	4	75	4	50
4	RFu-25	5'-CCGGCTGGAA-3'	70	39.8	6	83.33	6	33.33
5	Deca-11	5'-ATCGGCTGGG-3'	70	39.3	5	100	9	55.55
Total					24	74.66	29	37.77

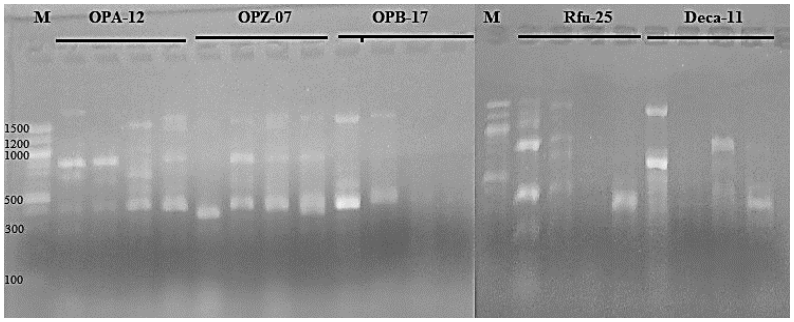

Figure 2. RAPD-PCR product pattern of *Corydaline fruticosa* with sodium azide

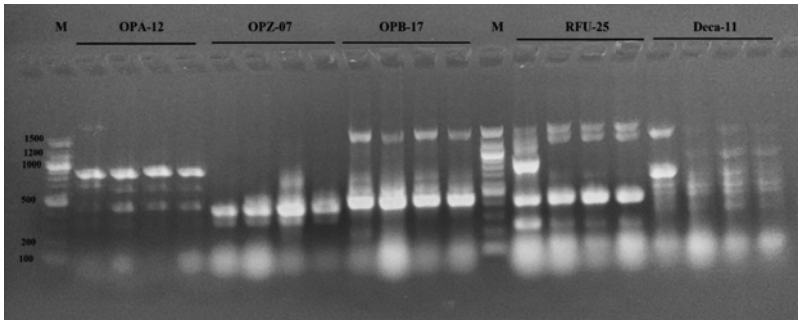

Figure 3. RAPD-PCR product pattern of *Corydaline fruticosa* with guanidine hydrochloride

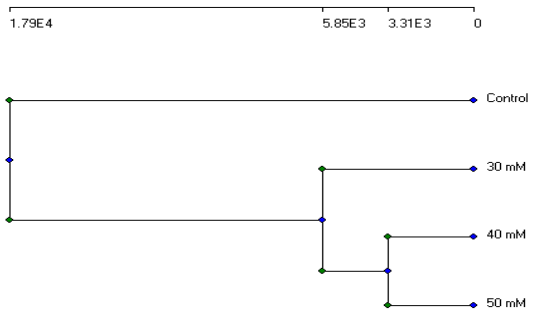

Figure 4. Dendogram based on RAPD patterns for *Cordyline fruticosa* treated with sodium azide

Table 4 Similarity matrix based on RAPD-PCR for *Cordyline fruticosa* treated with sodium azide mutagen

	Control	30 mM	40 mM	50 mM
Control	100	32.86	21.98	10.06
30 mM	32.86	100	49.96	48.9
40 mM	21.98	49.96	100	60.18
50 mM	10.06	48.9	60.18	100

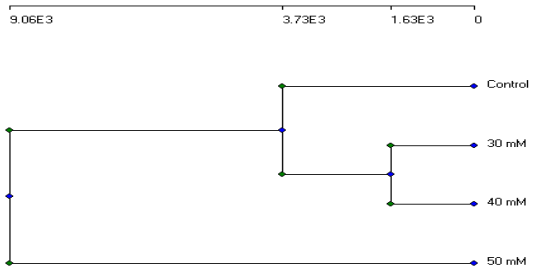

Figure 5. Dendogram based on RAPD pattern for *Cordyline fruticosa* treated with guanidine hydrochloride mutagen

Table 5 Similarity matrix based on RAPD-PCR for *Cordyline fruticosa* treated with guanidine hydrochloride mutagen

	Control	30 mM	40 mM	50 mM
Control	100	57.06	67.74	36.04
30 mM	57.06	100	72.56	40.30
40 mM	67.74	72.56	100	43.3
50 mM	36.04	40.30	43.3	100

4. Discussion

Cordyline fruticosa is a medicinal and ornamental plant. We can make some changes by mutations to enhance these ornamental characters and increase their medicinal value. Two chemical mutagens were used in this study: sodium azide and guanidine hydrochloride to cause these mutants' changes compared to control plant individuals. Many mutagens were used for the improvement of several ornamental plants. Research on the enhancement of ornamental and medicinal plants using induced chemical or physical mutations has been started by the Malaysian Nuclear Agency. The new varieties were moved to different users and private government agencies (Ahmad et al., 2012). As found in *Amaranthus caudatus* (El-Nashar, 2006), *Mirabilis jalapa* (Al-Gawwad, and Makka, 2009), and *Helianthus annuus* (Mostafa, 2011), sodium azide has been used to induce mutations in many types of research. *Eruca sativa* was mutated by sodium azide by Al-Qurainy (2009) and nine morphological traits and photosynthetic pigmentation were estimated. Also, proved that important differences are caused by sodium azide. El-Nashar and Asrar (2016) applied two mutagens for diversity estimation in *Calendula officinalis*. Many morphological and physiological parameters were calculated, and significant mutant differences comparing to control parameters were discovered. El Kaaby et al. (2015) mentioned that application of sodium azide to tomato lead to increase in seed germination percentage, seedling height and root length. Also, Eze and Dambo (2015) mentioned that sod. Azide treatment in maize enhance growth rate and seed size. The application of guanidine hydrochloride was confirmed by Tawfik and Fathy (2020) to enhance several morphological parameters comparing to control.

Several molecular markers have been applied to evaluate genetic diversity in many plants responded to different mutagens. Here, this work introduced RAPD technique to estimate genetic polymorphism in *Cordyline fruticosa* from to the action of guanidine and sod. azide. Wannajindaporn et al. (2014) used tissue culture to estimate the effect of sodium azide on *Dendrobium*, using ISSR molecular markers to compare mutants with controls. This is consistent with numerous studies that have discovered genetic variation in plants as a result of chemical mutagen treatments.

5. Conclusion

Cordyline fruticosa is a medicinal and ornamental monocot plant. Two chemical mutagens were used in this study: guanidine hydrochloride and sodium azide. They were used in concentrations of 30, 40, and 50 mM for each one. Eight morphological traits were measured. They were fresh weight, roots and shoot lengths, number of branches, lateral roots and leaves, leaf length and leaf width. Sodium azide inhibited all of the morphological parameters (except the branching and leaves number). So, we can use sodium azide to obtain *C. fruticosa* individuals with more branching and leaves. Unlike, guanidine hydrochloride which promotes morphological behavior except in 30 mM, which is less than others. The genetic polymorphism percentage resulted from genetic variation of *Cordyline fruticosa* varied. It was 74.66% in sodium azide, 37.77% in the case of guanidine hydrochloride.

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