Volume 19, No 4 | International Journal of Radiation Research, October 2021

Effect of UV rays on the *Nerium oleander* genome and assessment by the inter simple sequence repeats (ISSR) markers

S. Islam*, E. Ragab, N. Eliwa

Natural Products Research Department, National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Egypt

► ***Original article***

Corresponding author:
Salama Islam, Ph.D.,
E-mail:
genetech49@yahoo.com

Revised: November 2020
Accepted: December 2020

Int. J. Radiat. Res., October 2021; 19(4): 1025-1033

DOI: 10.29242/ijrr.19.4.1025

ABSTRACT

Background: *Nerium oleander* L. plant comprises a large number of components that are beneficial to human health The DNA polymorphism is a useful molecular genetics marker assessment to describe and recognize new germplasm for employing in the crop breeding programs. This study aims at determining the best time durations used to expose *Nerium oleander* cell suspensions cultures to UV-A rays, for studying differences in the DNA to create new cell lines. ***Material and Methods:*** In this study, ISSR was used for the identification of markers associated with the seven treatments exposed to UV-A rays of *N. oleander* cell suspensions cultures using 10 primers. ***Results:*** In the ISSR marker assay, all the primers amplified gave bands, but three primers (UBC810, UBC819 and UBC814) generated low polymorphism, two of them (UBC810 and UBC819) had given low polymorphism percentages (43%) and (19%) respectively and the third primer UBC814 had given a medium polymorphism percentage (57%). The highest value of similarity was 0.950, while the lowest similarity indexed was 0.012. The present study revealed that the Inter-simple sequence repeats (ISSR) would be a useful assay to identify the genetic variations of the *Nerium oleander* genome under UV rays stress. ***Conclusion:*** This study revealed that the use of ultraviolet rays induces changes in the DNA of *N. oleander* cell suspensions cultures that produce new genotypes for use in cell lines programs.

Keywords: *Nerium oleander, cell suspensions cultures, ISSR marker and UV rays.*

INTRODUCTION

The *Nerium oleander* plant is a member of the family Apocynaceae, which is an evergreen shrub with a maximum height of 2-3 m and cultivated in the entire world, especially in temperate climates. Flowers grow in clusters in terminal branches, and appear in different colors such as red, pink, peach, yellow, and white [1]. *Nerium oleander* plant comprises a large number of components that are beneficial to human health. These benefits include antiviral and antibacterial effects, immune improving, skin care and anticancer products.

Yellow oleander plants contain important glycosides such as Thevetia [2]. In general, *Nerium oleander* plants contain cardiac glycosides such as oleandrin. Ultraviolet rays (UV) stimulate tryptophan and tyrosine induction [3]. The heme group is superior in absorbing ultraviolet radiation.UV rays lighting can, in some cases, induce enzyme inhibition [4].

Exposure of the plant to UV-B radiation causes multiple DNA photoproducts which may alter DNA sequence and cause some mutations during replication. Moreover, deletion, insertion of base pairs, DNA protein cross link and DNA stand breaks may be produced as a result of UV-B radiation. Consequently, this DNA variation can induce phenotype changes, but this may be a silent mutation [5]. Molecular marker can supply an amazing improvement in the

[Downloaded from ijrr.com on 2026-11-10]

[DOI: 10.52547/ijrr.19.4.32]

performance and evolution of plant breeding choice of a right marker system that depends on the kind of the study and easy availability of high polymorphic [6].

The DNA polymorphism is a useful molecular genetics marker assessment to describe and recognize new germplasm for employing in the crop breeding programs [7]. Advanced unlimited numbers of DNA markers were discovered through molecular biology techniques [8]. The genetic diversity determination can enhance the effective use of genetic variation in the crop breeding process [9]. In recent times, polymerase chain reaction (PCR) technology has become a common research technique and has led to the advancement of several new genetic assays, depending on precisely specified DNA amplification [10].

The ISSR molecular marker amplifies regions between microsatellite loci, this class of markers does not require any prior knowledge about the sequences to be amplified and shows a high polymorphism in the material, which is very useful in studies of genetic diversity, phylogeny, genomics and evolutionary biology [11]. This type of markers provides highly effective plant fingerprinting [12-13]. The PCA is a useful tool for visualizing information from large sets of data, enabling patterns and systematic changes in data tables that are difficult to comprehend from the raw data [14].

The aim of this study is to find out the genetic variations in the genomic DNA of *N. oleander* cell suspensions culture after exposure to UV rays, assessed by ISSR assay. The study also, shows the contrast and molecular markers, which help establishing new methods for breeding programs to select strains that can be used to produce high-yield cell lines from indole alkaloids.

MATERIALS AND METHODS

The seeds of local plant cultivar in Egypt, with pink and rose flowers of *Nerium oleander* were germinated in test tubes containing 20 ml of Murashige and Skoog Basal Salt Mixture (MS), basal medium (BM) [15].

Chemicals

Murashige and Skoog Basal Salt Mixture (MS), Sigma-Aldrich catalog number: #M5524-10L, 2, 4-Dichlorophenoxyacetic acid (2, 4-D) (Sigma-Aldrich catalog number: #D70724-5G), (Sigma-Aldrich catalog number: #K3378-1G), GeneJET™ Plant Genomic DNA Purification Mini Kit, Thermofisher catalog number: #K0791, #K0792, DreamTaq™ Green PCR Master Mix (2X) Thermofisher catalog number: #K1081, ladder used was O'RangeRuler™ 200 bp DNA Ladder, ready-to-use Thermo fisher catalog number: #SM0633. All reagents were obtained from Sigma-Aldrich and had a high degree of purity (purity > 97%) USA.

Callus induction

Peripheral buds of seedlings (15-day-old) were cultured in medium Murashige and Skoog Basal Salt Mixture (MS), containing with 2, 4-Dichlorophenoxyacetic acid (2, 4-D), 2.0 mg/l and Kineten 1.0 mg/l, then incubated in an incubator (Jencons Shell Lab 1916 Incubator + Wheaton Modular Cell Production Roller, UK) at (26 ± 2 ^{o}C) during the day and 22 ^{o}C at night under a photoperiod of 16 h/day of 2000 LUX intensity [16].

Cell suspension preparation and UV treatments

The induced callus was transferred to vessels containing the same callus induction media without agar. These vessels were groaned on gyratory shakers incubator (New Brunswick Scientific Incubator Gyratory Shaker, Model Innova 4200, USA) 130 rpm under the same conditions used in callus induction. After two weeks, cell suspension was irradiated by UV-A (365 nm) using Vilber Lourmat lamb model VL-4.LC, at different intervals (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50) minutes and compared to the control (zero time) [17].

DNA isolation

One gram of frozen callus was ground with liquid nitrogen in a pre-cooled mortar, and this was an extraction of bulked DNA using (GeneJET™ Plant Genomic DNA Purification Mini Kit. The DNA extraction and purification

[Downloaded from ijrr.com on 2026-11-10]

[DOI: 10.52547/ijrr.19.4.32]

were extracted according to the product catalog.

Inter-simple sequence repeats (ISSR) analysis

In this study, ISSR was used for the identification of markers associated with the seven treatments exposed to UV-A rays of *N. oleander* cell suspensions cultures taxa genotypes [18].

The ISSR analysis was performed using DreamTaq™ Green PCR Master Mix (2X), 1.5 µM of primer from (Sigma Technology USA), their codes, sequences and GC % were shown in table 1 and 25 µg of template DNA were added. The reaction was carried out using the thermo cycler (PTC-100, Perkin Elmer-USA). The reaction was carried in three steps.

The initial denaturation step was at 94 °C for 5 min for 45 cycles. Each cycle consisted of a denaturation step of 1 min at 92 °C. The annealing step was according to annealing temperatures in table1 and an extension step of 2 min at 72 °C. The last cycle was followed by a final extension step of 7 min at 72 °C. Products of the PCR were migrated on agarose (1%); the ladder used was O'RangeRuler™ 200 bp DNA Ladder, ready-to-use, 0.05 µg/µl.

Table 1. List of primer names and their nucleotide sequences used in the ISSR assay.

No.	Name	Sequence 5 – 3	GC %	Annealing temperature (°C) °C=At=2(A+T)+4(G+C)–2
1	UBC807	AGAGAGAGA-GAGAGAGT	0.47	48
2	UBC810	GAGAGAGA-GAGAGAGAT	0.47	48
3	UBC814	CTCTCTCTCTC TCTCTA	0.47	48
4	UBC819	GTGTGTGTGT GTGTGTA	0.47	48
5	UBC824	TCTCTCTCTCT CTCTCG	0.53	50
6	UBC834	AGAGAGAGA-GAGAGAGYT	0.45	50
7	UBC845	CTCTCTCTCTC TCTCTRG	0.50	50
8	UBC851	GTGTGTGTGT GTGTGTYG	0.50	50
9	UBC873	GACAGACAG-ACAGACA	0.50	50
10	UBC880	GGAGAGGA-GAGGAGA	0.60	50

Data and statistics analysis

Gels were photographed and scanned at a wavelength of 577nm by the Bio-Rad Gel Documentation System Gel Doc 2000, Germany. The similarity matrices and the relationships among rootstock genotypes as revealed were performed using (XLSTAT 2020) program in Microsoft Excel 2010. The principle component analysis (PCA) was performed to determine eigenvalues, percentages and cumulative variance. The PCA was conducted using the (XLSTAT 2020) program.

RESULTS

Cell survivor

Nerium oleander cell suspensions cultures were exposed to UV-A rays with an exposure time higher than 30 minutes has resulted in a significant mortality of 100 %. Thus, seven samples were irradiated at different time intervals (0, 5, 10, 15, 20, 25 and 30 minutes).

ISSR assay

In the ISSR assay, ten primers were used; all the primers gave reproducible bands. The banding pattern of ISSR is shown in figure 1 and the details are given in table 2. All primers generated amplifications in all seven UV treatments and ISSR primers produced 689 bands. The polymorphic bands were 56, while 633 bands were monomorphic. The maximum number of bands (126) was observed in (UBC824) primer, while the minimum number of bands (42) was observed in (UBC810) primer. The observed amplicons were ranging from 270 to 3680 bp. The largest amplicons were amplified by (UBC807) primer, but the smallest amplicons were amplified by (UBC851) primer. The primers (UBC810, IBC814, and UBC819) gave special bands (8, 4 and 4) respectively. Hence, these primers can be considered specific markers in the ISSR assay.

[Downloaded from ijrr.com on 2026-11-10]

[DOI: 10.52547/ijrr.19.4.32]

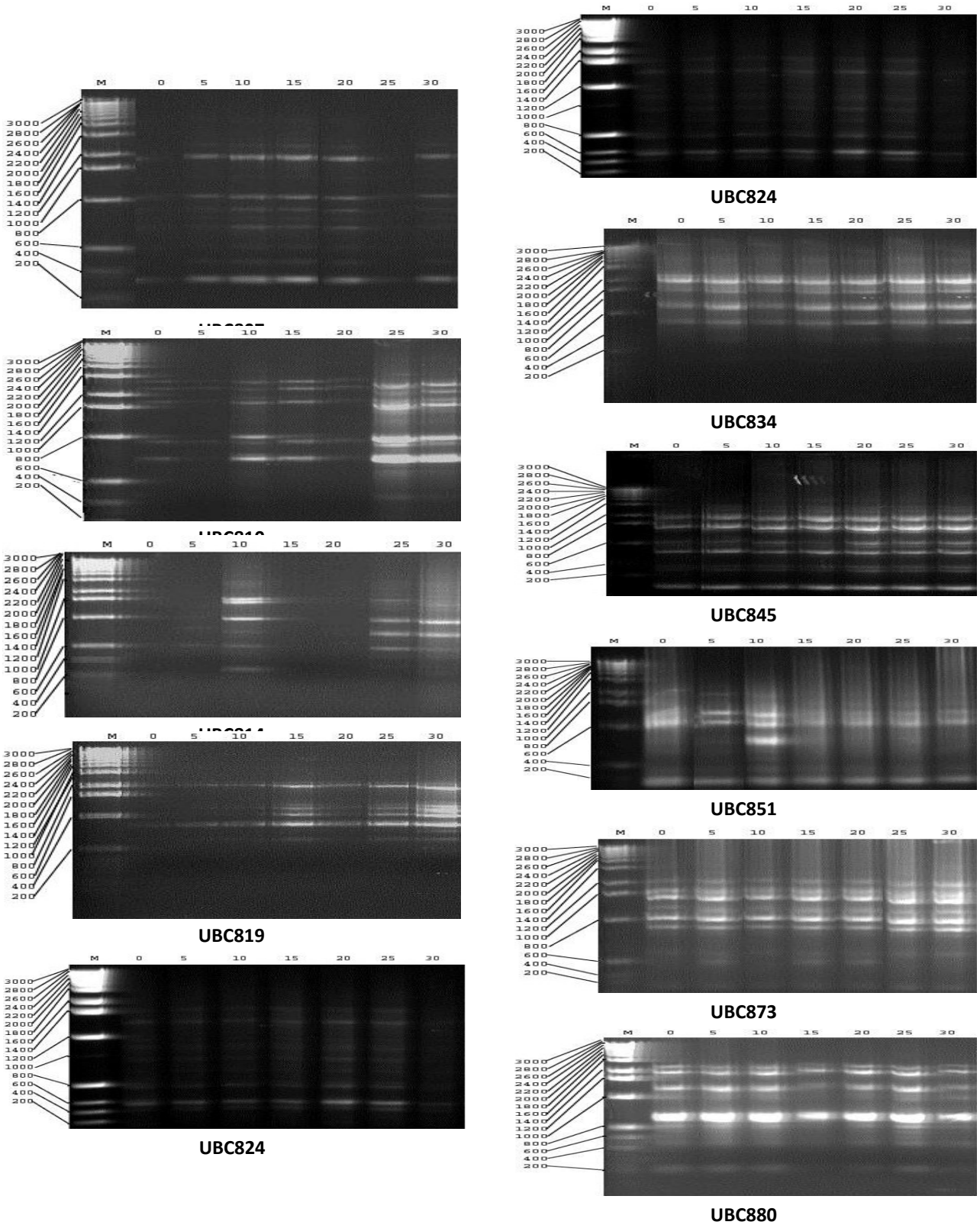


Figure 1. PCR-ISSR assay by using ten primers on the Nerium oleander cell suspensions culture which were exposed to UV-A ray intervals times 0, 5, 10, 15, 20, 25 and 30 minutes.

[Downloaded from ijrr.com on 2026-11-10]

[DOI: 10.52547/ijrr.19.4.32]

Table 2. List of arbitrary primers with total polymorphic amplicon and polymorphism generated by ISSR analysis for seven treatments of N. oleander cell suspensions culture exposed to UV-A rays at different intervals (0, 5, 10, 15, 20, 25 and 30 minutes).

No	Primer	RA (bp)	TB	TPB	P%	TMB	M%	SM	A. PIC
1	UBC807	420 - 3680	84	-	-	84	100	0	-
2	UBC810	790 - 2885	42	18	43	24	57	8	0.23
3	UBC814	580 - 2964	49	28	57	21	43	4	0.23
4	UBC819	740 - 2160	52	10	19	42	81	4	0.04
5	UBC824	565 - 2745	126	-	-	126	100	0	-
6	UBC834	760 - 2710	56	-	-	56	100	0	-
7	UBC845	400 - 1972	77	-	-	77	100	0	-
8	UBC851	640 - 1928	77	-	-	77	100	0	-
9	UBC873	530 - 2125	56	-	-	56	100	0	-
10	UBC880	270 - 2750	70	-	-	70	100	0	-
Total		-	689	56	-	633	-	16	0.5

RA: Range of amplicons. TB: Total bands. TPB: Total polymorphic bands. P%: Polymorphism (%) TPB/TB×100. TMB: Total monomorphic bands. M%: Monomorphic (%) TMB/TB×100. SM: specific markers. PIC: The polymorphism information content =1-ΣPi2; Pi is the band frequency of the ith allele. PIC: Average of PIC.

The amplified data fragments using ten Different-mer arbitrary primers for the seven intervals UV treatments of *N. oleander* cell suspensions culture revealed successful amplification of PCR-ISSR products. The main results were listed in table 2. The primers UBC807**,** UBC824, UBC834, UBC845, UBC851, UBC873 and UBC880 showed no polymorphic differences among the treatments using UV rays, while the primers UBC810 and UBC819 revealed low polymorphism (43%) and (19%) respectively. However, the primer UBC814 gave a medium polymorphism percentage (57%). Primers UBC810, UBC814 and UBC819 showed low PIC values 0.23, 0.23 and 0.04. On the other hand, all the remained primers did not give any PIC value.

The similarity of ISSR assay

The dendrogram constructed successfully explained the differentiated seven *Nerium oleander* cell suspensions cultures which were exposed to UV-A ray time intervals of 0, 5, 10, 15, 20, 25 and 30 minutes using ISSR assay.

The ISSR data were used to appraise the genetic similarity among the seven *Nerium oleander* UV treatments taxa using XLSTAT 6464 software analysis (table 3). In the ISSR assay, the highest similarity index documented was 0.950, and was observed between the taxa 15 minutes UV exposed and 20 minutes UV exposure, while the lowest similarity index documented was 0.012 which was observed between 5 minutes UV exposure and 30 minutes UV exposure.

Table 3. Similarity indices among the seven Nerium oleander cell suspensions culture which were exposed to UV-A ray time intervals of 0, 5, 10, 15, 20, 25 and 30 minutes - Taxa based on ISSR-PCR using 10 primers.

	Control	5	10	15	20	25	30
Control	1						
5	.934	1					
10	.774	.882	1				
15	.462	.720	.878	1			
20	.172	.470	.724	.950	1		
25	.156	.111	.499	.756	.907	1	
30	.308	.012	.353	.674	.862	.966	1

The genetic relationships dendrogram among the seven *Nerium oleander* cell suspensions cultures which were exposed to UV-A ray at 0, 5, 10, 15, 20, 25 and 30 minutes intervals, taxa were carried out as displayed in figure 2. The seven treatments of *Nerium oleander* taxa were separated into two main clusters A and B. The first cluster (A) included treatments that were exposed to UV rays at 10, 5 intervals in addition to the control (0 minutes). However, the second cluster (B) included treatments that were exposed to UV rays at 30, 25, 20 and 15 minutes intervals.

Cluster (A), is subdivided into two sub-clusters (I and II), the first one (I) contained treatments that were exposed to UV rays at 5 and 10 minutes. The second sub-cluster (II) contained intervals the control sample only.

The second cluster (B) was divided into two sub-clusters (I and II), the first one (I) was divided into two sub-sub-clusters (1 and 2), the first one (1) of sub-sub-cluster, contained the treatments which were exposed to UV rays at 25 and 30 minutes. The second sub-sub-cluster contained the treatment which was exposed to UV rays for 20 minutes only. While the second sub-cluster (II), contained treatment that was

[Downloaded from ijrr.com on 2026-11-10]

[DOI: 10.52547/ijrr.19.4.32]

exposed to UV rays for 15 minutes only.

The DNA fingerprint generated by ISSR assay was evaluated and mapped by Principal Components Analysis (PCA). Each point in figure 3 represents a fingerprint projected down to a two-dimensional (PCA) score plot from a 1000-dimensional space. PCA 1 and 2 in figure 3 explained 73.86 and 24.17% of the variance, respectively. The seven *Nerium oleander* cell suspensions cultures were identified (figure 3). Furthermore, at the four clusters, the first one included the treatment which was exposed to UV rays for 30 minutes only. The second cluster contained the treatment which was exposed to UV rays at 15, 20 and 25 minutes intervals. The third cluster contained the treatment which was exposed to UV rays at 5 and 10 minutes intervals. The forth cluster included the treatment which was exposed to UV rays interval control (0) minutes representing fingerprint for genome similarity that was significantly separated from each other (P=0.05, Coomans plot [13]. The dispersion within each cluster, as shown in figure 3 represents variations in the parallel DNA extractions and ISSR reactions. There is a significant matching between the results of the genetic similarity among the seven *Nerium oleander* UV treatments which were exposed to UV rays at 0, 5, 10, 15, 20, 25 and 30 minutes intervals and the results of the PCA for the same above-mentioned UV treatments.

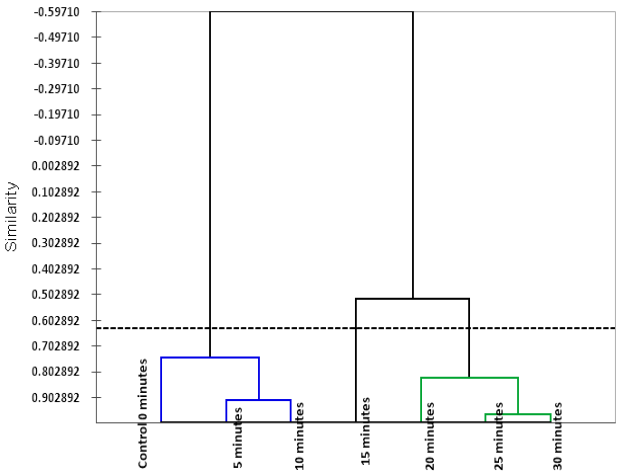


Figure 2. Dendrogram for the genetic distances relationships among the seven Nerium oleander cell suspensions cultures which were exposed to UV-A ray interval times 0, 5, 10, 15, 20, 25 and 30 minutes. Taxa based on similarity indices data of ISSR analysis.

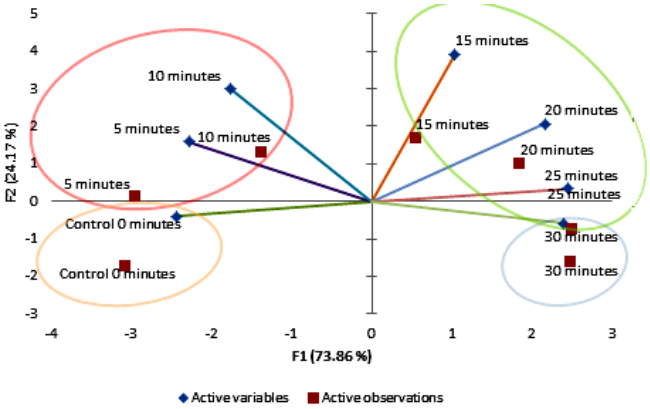


Figure 3. Basic Components Analysis Diagram (PCA) showing the different fingerprints derived from DNA extraction from the seven treatments from Nerium oleander cell suspensions cultures which were exposed to UV rays at 0, 5, 10, 15, 20, 25 and 30 minutes intervals through ISSR assay by the10 primers. The oval forms refer to the four clusters which were generated by PCA.

The RAPD marker was studied to employ the DNA change extent in yielded seeds from faba beans irradiated by the two mutagenic different irradiation doses especially fast neutron and UV-B on DNA damage by using 20 primers for the RAPD assay. The total amplified fragments were 248 with 76 polymorphic bands (30.65%), 27 unique bands (10.89%), and 8 monomorphic bands (3.23%). A maximum number of PCR products bands (60 bands =24.19%) were observed at fast neutron, whereas the minimum number of PCR products bands were 45 (18.15%) after being irradiated by UV-B for 3 hours.

DISCUSSION

Molecular biology is a good tool for detecting DNA variations in living organisms. Molecular markers such as ISSR, which are PCR based markers, are designed based on target microsatellite sites for genetics and molecular analysis [19].

As regards the effect of UV-B radiation on DNA, it can produce many changes such as: (1)

[Downloaded from ijrr.com on 2026-11-10]

[DOI: 10.52547/ijrr.19.4.32]

oxidative damage (pyrimidine hydrates) and cross link (both DNA – DNA and DNA protein. (2) cyclobutane pyrimidine dimers (CPDs) and other photoproducts, pyrimidine [4-6] pyrimidine dimers in nuclear, chloroplast and mitochondrial DNA. Plants possess different strategies to repair DNA damages. If DNA repair does not work well, the accumulation of DNA damage may change genetic stability due to the mutations happened [20]. Additionally, a major component of DNA damage is UV-B induced base dimerization. Also, UV-B could damage ribosomes by forming cross-links in ribosomal RNA or between m RNA, r RNA and proteins [21].

The genetic diversity of *Pelargonium graveolens* L. exposed to 4, 4.56. 4.6^0 and 4.7^2 W/ m2 of UV-B radiation was detected using 10 ISSR primers. The highest increase in polymorphism percentage (P %) was found at the highest UV-B radiation (0.38 W/m^2). The results, also, proved that biochemical changes are in line with genetic variations, so the most remarkable increase in biochemical and genetics variations was observed in (0.38 W/m2 treatment [22].

A study of *Gnaphalium luteoalbicans*e exposed to ultraviolet radiation showed an increase in polymorphism and genetic instability with ISSR primer [5].

In another study, RAPD techniques were employed to assess the genetic stability of *Nerium oleander*. RAPD patterns of 54 random primers were compared with those of another plant belonging to the same family (Apocynaceae). The RAPD primers gave 72 bands with 20 polymorphism bands. Comparing this to the present study, the ISSR gave 689 bands with 56 polymorphism bands.These results reinforced the belief that there is a direct effect of UV exposure to genetic material in plants [23].

Genetic instability in tobacco plants under ultra violet radiation is also detected [24] in 19 different lines of Arabidopsis. Genetic diversity has been detected after exposure to ultra violet radiation [25]. Otherwise, ISSR markers have been applied to grains of two bread wheat cultivars (*Triticum aestivum* L.), Sids-1, Sakha-93 which were irradiated with gamma rays at dose levels of 0.0, 100, 200 and 300 Gy, the results showed changes in un-irradiated and irradiated treatments. It is also clear that radiation is more effective on Sids-1 cultivar with polymorphism a percentage of (55.5%) compared to Sakha-93 cultivar with polymorphism (51.9%) [26].

DNA damages may affect the gene expression and modification in proteins that impact on metabolic processes such as plant growth, cell cycle, fertilization, and seed evolution [27].

On the other hand, the presence of new DNA bands is important to give structural changes in DNA (transpositions, breaks, canceling, etc.), while the absence of bands and band intensity changes may be significant with the polymerization or dimers among the pyrimidine -pyrimidine after irradiation in the DNA. This could decrease the number of binding locations for Taq polymerase and the opening copy of a specific DNA sequence in the DNA genome [28].

The results discovered through the ISSR marker assay represent an important resource for genetic analysis at the molecular level to distinguish the differences that occur in the *N. oleander* cell suspensions culture, after being exposed to UV rays with the intent of generating genetic differences that can be used in breeding programs to produce distinctive cell lines.

The presence of primers that have not amplified the DNA of all genotypes and that do not find complementary sequences on the genomic DNA is common. This phenomenon has been observed in many crop plants [29]**.** The possibility of discovering genetic differences in Safflower (*Carthamu stinctorius*) with an ISSR marker assay by using 13 primers were studied and it has been found that six primers did not give any bands. Seven primers gave polymorphic bands (39 polymorphic fragments) with a percentage of 63.38 [30]. Also the Genetic diversity in 23^{th} varieties of mulberry by using ISSR and SRAP markers assays was studied. A number of 100 primers were used in ISSR assay, only 12 primers gave amplifications and it has been found that the total numbers of bands were 116 and total numbers of polymorphic bands were 93. On the other hand, the remainder of the primers did not give amplifications [31]. The

[Downloaded from ijrr.com on 2026-11-10]

[DOI: 10.52547/ijrr.19.4.32]

Genetic diversity on Scrophulariaceae plant by ISSR assay through 35 primers was studied and it was found that the 10 primers were polymorphic and fragments generated [32].

CONCLUSION

Studies depended on genetic diversity assays were obtained by molecular markers such as AFLP, RAPD and ISSR assay, these assays need to be further studied in future works. Variables such as genetic mutations under stress, associated with plants may be useful to establish breeding programs among phylogenetically well -differentiated species to be used in the production of high-yield cell lines from secondary compounds utilized for medical purposes. The exposure of the *Nerium oleander* cell suspensions culture to ultraviolet radiation was successful in producing low level of changes at the DNA, and it is considered sufficient for attempting the production of new cell lines.

Conflicts of interest*: *Declared none.

REFERENCES

1. Dey P and Chaudhuri TK (2014) Pharmacological aspects of *Nerium indicum* Mill: a comprehensive review. *Pharmacogn Rev,* ***8(16)****: 156–162*.
2. Lin Y, Dubinsky WP, Ho DH, Felix E, Newman RA (2008) Determinants of human and mouse melanoma cell sensitivities to oleandrin. *J Exp Ther Oncol*, ***7(3)****: 195–205.*
3. Creed D (1984) The photophysics and photochemistry of the near-UV absorbing amino acids-II. Tyrosine and its simple derivatives. *Photochem. Photobiol,* ***4(39)****: 563–575*.
4. Saha A (1997) Photo-induced inactivation of dihydroorotate dehydrogenase in dilute aqueous solution. *Int J Radiat Biol*, ***72(1)****: 55–61.*
5. Cuadra P, Vargas D, Fajardo V, Herrera R (2010) Effects of UV-bradiation in morpho- genetic characters of *Gnaphalium luteoalbum*. *J Photochem Photobiol B,* ***101(1)****: 70–75*.
6. Weising K, Athkinsin RG, Gardner RC (1995) Genomic fingerprinting by microsatellite-primed PCR: a critical evaluation. *PCR Meth. Applications,* ***4****: 249-255.*
7. Neill R, Snowdon RJ, Kohler W (2003) Population genetics aspects of biodiversity. *Progress Bot,* ***64****: 115-137.*
8. Ahmad F, Khan AL, Awan FS, Sadia B (2010) Genetic diversity of chickpea (*Cicerarietinum* L.) germplasm in Pakistan as revealed by RAPD analysis. *Genet Mol Res,* ***9****: 1414-1420*.
9. Paterson AH, Damon S, Hewitt JD, Zamir D, Rabionowitch HD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. *Genet,* ***127(1)****: 181-197.*
10. Erlich HA (1989) PCR Technology Principles and Applications for DNA amplification. Stockton Press, New York.
11. Reddy PM, Sarla N, Siddiq EA (2002) Inter simple sequence re-peat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, ***128****: 9-17.*
12. Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS (2000) Genetic diversity and phy-logenetic relationship as revealed by inter-simplesequence repeat (ISSR) polymorphism in the genus *Oryza. Theor*. *Appl. Genet*, ***100****: 1311-1320*.
13. Adawy SS, Hussein EA, Saker MM, El-Itriby HA (2004) Intra -and inter-varietal variation of upper Egypt date palm cultivars (*Phoenix dactylifera* L.): I. As revealed by RAPD and ISSR markers. Sharm El-Sheikh, South Sinai, Egypt; *Proceed Int Conf Genet Eng Appl, 165-179.*
14. Wold S, Albano C, Dunn III, Edlund WJ , Esbensen U, Gelad, K, *et al.* (1984) Multivariate data analysis in chemistry. In: Chemometrics: Mathematics and Statistics in Chemistry (Kowalski, B.R, Ed.). Reidel, Dordrecht.
15. Niu GH, Rodriguez DS, Mackay W (2008) Growth and physiological responses to drought stress in four oleander clones. *J Amer Soc Hort Sci*, ***133(2):*** *188–196.*
16. Murashige T and Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum,* ***15(3)****: 473-497.*
17. Salama IM and Abd EL-Megid, MHM (2019) Reflex of oleandrin production and molecular changes on the *Nerium oleander* cell suspension culture under UV-A radiation stress effect. *Egypt J Rad Sci Applic,* ***32(1)****: 117 – 124.*
18. Williams JG, Kubelik AR, Livak KJ,Rafalski JA,Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res,* ***18(22)****: 6531-6535*.
19. Irshad M, Idrees M, Saeed A, Naeem M, Naeem R (2014) Genetic diversity among Asparagus species and cultivars of *Asparagus officinalis* L. using random amplified polymorphic DNA (RAPD) markers. *Int J Biodivers Conserv,* ***6(5)****: 392–399.*
20. Gill SS, Anjum NA, Gill R, Jha M, Tuteja N (2015) DNA damage andrepair in plants under ultraviolet and ionizing radiations. *Sci World J,* ***2015****: 250158*.
21. Noah JW, Shapkina T, Wollenzien P (2000) UV-induced crosslinks in the16 S rRNAs of *Escherichia coli*, *Bacillus subtilis* and *Thermus aquaticus* and their implications for ribosome structure and photochemistry. *Nucleic Acids Res,* ***28****: 3785–3792*.
22. Azarafshan M, Peyvandi M, Abbaspour H, Noormohammadi Z, Majd A (2020) The effects of UV-B radiation on genetic and biochemical changes of *Pelargonium graveolens* L´Her. *Physiol Mol Biol Plants,* ***26(3)****: 605–616*.
23. Zibbu G and Batra A (2014) *In-vivo* and *in-vitro* rapd analysis in apocynaceae family: population growing in different

[Downloaded from ijrr.com on 2026-11-10]

[DOI: 10.52547/ijrr.19.4.32]

regions of Rajasthan, INDIA. *J Pharm Sci Exp Pharmacol,* ***3 (3)****: 230-239*.

24. Ries G, Heller W, Puchta H, Sandermann H, Seidlitz HK, Hohn B (2000) Elevated UV-B radiation reduces genome stability in plants. *Nature,* ***406****: 98–101*.
25. Kravets EA, Zelena LB, Zabara EP, Blume YB (2012) Adaptationstrategy of barley plants to UV-B radiation. *Emir J Food Agric*, ***24(6)****: 632–645*.
26. Aly AA, Eliwa NE, Maraei RW (2019) Physiological and molecular studies on ISSR in two wheat cultivars after exposing to gamma radiation. *Sci Asia*, ***45(5)****: 436*.
27. Agrawal SB, Singh S, Agrawal M (2009) Chapter 3 ultraviolet B induced changes in gene expression and antioxidants in plants*. Adv Bot Res*, ***52****: 47–86*.
28. Danylchenko O and Sorochinsky B (2005) Use of RAPD assay for the detection of mutation changes in plant DNA induced by UV-B and R-rays. *BMC Plant Biol.*, ***5(1)****: 59*.
29. Bhagyawant SS and Srivastava N (2008) Genetic fingerprinting of chickpea (Cicerarietinum L). germplasm using ISSR markers and their relationships. *Afr J Biotechnol*, ***17***: *4428–4431*.
30. Houmanat K, Charafi J, Mazouz H, El-Fechtali M, Nabloussi A (2016) Genetic Diversity Analysis of Safflower (*Carthamus tinctorius*) Accessions from Different Geographic Origins using ISSR Markers *Int J Agric Biol,* ***18***: *1081–1087*.
31. Zhang N, Wang M, Hou Y, Zeng Y, Liu G, Ding X, Huang G (2019) Genetic diversity of mulberry cultivars using ISSR and SRAP molecular markers. *Int J Agric Biol*, ***22***: *427–434*.
32. Petrovaa G, Petrovb S, Banchevac S (2016) Molecular characterization of *Verbascum anisophyllum* (Scrophulariaceae) genetic resources through inter-simple sequence repeat (ISSR) Markers. *Nat Prod Commun*, ***12(2)****: 189-192*.

[Downloaded from ijrr.com on 2026-11-10]

[DOI: 10.52547/ijrr.19.4.32]

[DOI: 10.52547/ijrr.19.4.32] [Downloaded from ijrr.com on 2026-11-10]