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Genetic Variant Detected by RAPD-PCR and ISSR in *Catharanthus roseus* (L.) Cells Exposed to Low Doses of Gamma Rays

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CATHARANTHUS ROSEUS (L.) (*C. roseus*) 10 samples, genetically different of irradiated and control cell suspension culture were detected by both random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) and inter simple sequence repeat (ISSR). The RAPD-PCR and ISSR-PCR profiles were used for building phenetic trees by using Totallab Quant software, showing similarity in the topology of the trees. Both dendograms presented three major clusters that 10 samples irradiated and control, according to genetic similarity.

The control and the irradiated samples at 2.5 and 4Gy, which are highly in the similarity index recorded as 0.101, while the lowest similarity index recorded was 0.058, which was observed between 3.5 and 4.5Gy.

A dendrogram RAPD-PCR for the genetic relationships among the 10 samples irradiated and control of *C. roseus* the cell suspension culture taxa was carried out. The 10 samples irradiated and control from *C. roseus* cell suspension culture taxa were separated into three clusters; cluster one included 1, 1.5, 2 & 3Gy, while the cluster two included control, 2, 2.5, 3.5, 4 & 4.5Gy. The cluster three included 4.5 & 5Gy.

The best doses of gamma rays were from 0.5 to 5Gy in order to *C. roseus* cells genome manipulation and induced mutations. The genome modification was the stimulation of the gene expression changes in order to changes of physiological cell and production of cell lines. The aim of the production cell lines to elicit cells enjoys the attributes of high productivity, secondary products, which are used in widely used in the pharmaceutical industry.

Keyword: *Catharanthus roseus* (L.), genetic variant, RAPD, ISSR, PCR, γ rays, cell culture.

C. roseus is an ornamental and a medicinal plant species. This species, including the family Apocynaceae, consists of 411 genera and 4650 species, many of them are of ornamental and medicinal values (Simpson, 2006). The genus *Catharanthus* content on seven species, other than *C. roseus*, namely *C. coriaceus, C. lanceus, C. longifolius, C. ovalis, C. pusillus, C. scitulus* and *C. trichophyllus*, of which *C. pusillus* is endemic to India whereas others are endemic to Madagascar. The species *C. roseus* and *C. trichophyllus* are crosshybridizable. Several scores of ornamental cultivars of *C. roseus*, bred for differing shoot habit, time of blooming, petal coloration and suitability for cultivation in homes and gardens are in vogue worldwide (van der Heijden *et al*. 2004).

A variety of DNA markers, including restriction fragment length polymorphism RFLP, random amplified polymorphic DNA RAPD, inter simple sequence repeats ISSR, simple sequence repeats SSR, amplified fragment length polymorphism (AFLP) and their variants have been proven useful for studying segregation in mapping populations and help in the genetic linkage determination for map construction (Lorz and Wenzel, 2005).

C. roseus, 2n= 2x= 16 is the very important species in the genus with a genome size of 1500Mbp. It is an ever blooming, perennial, a tropical plant with reports of frequent out crossing (Sreevalli *et al.*, 2000). Even though many aspects of the alkaloid biosynthesis have been investigated, attempts need to be made to identify high alkaloid yielding accessions out of the vast resources of naturally occurring *C. roseus* germplasm. The genetic resources remain largely uncharacterized and unexploited and so does the structure and organization of the *C. roseus* genome (van der Heijden *et al*. (2004).

C. roseus G. Don. produces more than one hundred terpenoid indole alkaloids (TIAs) and research on TIAs biosynthesis is focused on the pharmaceutical applications of several of its final compounds such vincristine, vinblastine, ajmalicine and serpentine, which are used as anticancer and antihypertensive drugs (Pasquali *et al.*, 1992). Many of the genes involved in the TIAS biosynthetic pathway of *C. roseus* have been cloned and sequenced for the analysis of their expression in various plant organs (Mahroug *et al*., 2006).

A large number of proteins and enzymes involved in the primary metabolism of *C. roseus* have been shown to be highly homologous to corresponding ones already characterized and sequenced in one or more other plant species (Jacobs *et al*., 2005).

In the present work we studied the segregation of RAPD and ISSR markers in the cell suspension of the gamma irradiated by gamma rays and genetically different uncover lines to construct a relationship of genetically dendrogram (similarity) of *C. roseus*.

Materials and Methods

Irradiation source

C. roseus cell suspensions were exposed to gamma ray. The source used was ^{137}Cs with a dose rate of 0.43 Gy/ min, located at the NCRRT, Nasr City, Egypt. The doses used were 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 & 5Gy.

Media

C. roseus plant seeds were washed with tap water, and then the seed surface sterilized by soaking for 15 min in 30% Clorox, after that thoroughly washed to assure that any residues of Clorox were removed. The seeds were placed in jars containing water agar free hormones. The cultured jars were incubated in a growth chamber with temperatures of (26± 2°C) at day and 22°C at night under a photoperiod of 16 h/ day of 2000LUX intensity (Constable *et al.,* 1981).

After three weeks in culture, leaves, buds 1.5 - 2.0mm in length of seedling *C. roseus*, were selected from seedlings and placed on the Murashige and Skoog media (MS) major and minor salts (M5524-1L Sigma-Aldrich) Murashige and Skoog (1962) supplemented with 1mg α-naphthalene acetic acid (NAA)+ 1mg N6-Benzyle adenine (BA)/ L. with 0.8% agar, with 8% sucrose and pH 5.8 in jars, cultures were incubated at the same conditions, within 4weeks were 75% of the callus was induced. After 7weeks individual calli was serially sub cultured by transferring to maintenance medium consisting of Gamborg's B5 medium (G5893-1L), Sigma-Aldrich **(**Gamborg *et al.,* 1968), supplemented with 1mg/ L 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.1 mg/L with 2% sucrose, pH 5.8 for 4weeks on gyratory shakers at 130rpm (Morris *et al.,* 1989).

Then cell suspensions were irradiated to, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 & 5Gy with non-irradiated sample as a control.

Cells extraction

Cells were harvested after irradiation to the above doses for Random Amplified Polymorphic DNA RAPD and Inter simple sequence repeat ISSR analysis. The cells in the media were harvested by immediately frozen in liquid N_2 and the cells of the plant were collected in clean tubes (50ml) and frozen in 20°C freezer until use (Ramani *et al.*, 2010).

Analysis

DNA isolation

Five grams of cells were collected from *C. roseus* cell suspension culture. Then bulked DNA extraction was performed using the DNeasy-plant-Mini Kit (QIAGEN).

RAPD and ISSR procedure

RAPD and ISSR were used for the identification of markers associated with 10samples of irradiated and control from *C. roseus* cell suspension culture.

PCR reactions were conducted (a) using 4arbitrary 10-mer primers in RAPD analysis, their names and sequences are shown in Table 1. on the other hand, ISSR analysis was conducted using 4arbitrary 11 & 14-mer primers, their names and sequences are shown in Table 2, the annealing temperature was calculated as follows equation:

$$\text{Anneal. °C= Ta= } 2(A+T)+ 4(G+C)- 2.$$

TABLE 1. List of primer names and their nucleotide sequences used in RAPD study.

Primer codes	Sequences (5'→3')	GC %	Anneal. °C
OP-B01	5' – GTTTCGCTCC-3'	60%	30
OP-B02	5' – TGATCCCTGG-3'	60%	30
OP-B05	5' – TGCGCCCTTC-3'	70%	32
OP-B07	5'– GGTGACGCAG-3'	70%	32

RAPD and ISSR analysis were performed with four primers synthesized by (Table 1&2) using the Ready-to-Go™ RAPD & ISSR Analysis Beads kit (Amersham Pharmacia Biotech, Uppsala, Sweden), following the protocol recommended by the manufacturers. The reactions were performed in a final volume of 25µl. Using 0.80µl (10pmol) of each primer and 2.5µl (50ng) of the

extracted template DNA, 3µl (RAPD & ISSR) master mix, analysis kit, overlaid with 25µl mineral oil. Amplification was programmed for 45cycles of 5second at 94°C, 15second at 36°C and 1min at 72°C followed by 45cycles of 5second at 94°C, 15second at 36°C and 1min at 72°C. A final cycle of 7min at 72°C on a thermal cycler (PTC-100TM, MJ Research Inc.) for DNA denaturing, annealing and primer extension, respectively, as described by Gupta *et al.* (2007) the extension step was extended to 5 min in the final cycle. And a final cycle of 7min at 72°C. The ISSR amplifications were carried out with a preliminary cycle of 2min at 94°C, followed by 35cycles of 20 second at 94°C, 50 second at 50°C and 90second at 72°C and a final cycle of 7min at 72°C.

Anneal. °C= Ta= 2(A+T)+ 4(G+C)- 2.

TABLE 2. List of primer names and their nucleotide sequences used in (ISSR) study.

Primer codes	Sequences (5'→3')	GC %	Anneal. °C
HA – 98	5' CACACACACACAGT 3'	50%	28
HA – 99	5' CACACACACACAAG 3'	50%	28
HB – 12	5' CACCACCACGC 3'	72%	32
HB – 13	5' GAGGAGGAGGC 3'	72%	32

PCR-amplified fragments were separated by electrophoresis on 2% (w/v) agarose gel (Bio-Rad's Certified™ molecular biology agarose) in 5× Tris-borate, pH 8 (TBE) buffer, visualized by ethidium bromide staining and photographed under UV-light. The ladder used was ϕx174 DNA/ BsuRI (HaeIII) marker, 9catalog number: SM 0251 thermo fisher scientific with range (72-1353) bp.

Data analysis

DNA bands were treated as dominant markers and were scored in terms of a binary code as presence (1) or absence (0). The genetic similarity among samples was calculated using the Totallab Quant software and the dendrogram tree was constructed using the Totallab Quant software program.

Results and Discussion

DNA RAPD assay

Primer OP-B01

Primer OP-B01 gave 7monomorphic fragments with molecular sizes ranging from 603-1353bp and from 42-60bp with 8detected polymorphic fragments (53%) with numbers 5, 6, 7, 8, 9, 10, 11 & 12 with corresponding molecular sizes of 315,

281, 234, 194, 118, 99, 72 & 60bp were observed, whereas the other bands were monomorphic. The samples were irradiated with 4.5 & 5Gy exhibited the maximum number (6) of fragments, while the lowest number (3) appeared in the samples were irradiated with 2 & 3.5Gy.

Primer OP-B02

Primer OP-B02 pattern resulted in 15DNA fragments ranging in molecular sizes between 42-1353bp. Eight polymorphic fragments (53%) with numbers 5, 6, 7, 8, 9, 10, 11 & 12 with corresponding molecular sizes of 315, 281, 234, 194, 118, 99, 72 & 60bp were observed, whereas the other bands were monomorphic. The 3 & 3.5Gy rootstocks exhibited the maximum number (6) of fragments, while the lowest number (1) appeared in the control and the 4.5Gy.

Primer OP-B05

Primer OP-B05 resulted in 6DNA fragments ranging in molecular sizes from 72-281bp as shown in Fig. 1. Six polymorphic fragments (40 %) with numbers 6, 7, 8, 9, 10 & 11 with corresponding molecular sizes of 281, 234, 194, 118, 99 and 72 bp were observed, whereas, the other bands were monomorphic. In the control, 1.5 & 3Gy rootstocks exhibited the maximum number (3) of fragments, while the lowest number (1) appeared in the 2.5, 4, 4.5 and 5 Gy samples.

Primer OP-B07

Primer OP-B07 exhibited 8DNA fragments ranging in molecular sizes from 72-603bp as shown Fig. 1. Eight polymorphic fragments (53.3 %) with numbers 4, 5, 6, 7, 8, 9, 10 & 11 with corresponding molecular sizes of 603, 315, 281, 234, 194, 118, 99 & 72bp, respectively were observed, whereas the remaining bands were monomorphic. In the 1.5Gy rootstock exhibited the maximum number (6) of fragments, while the lowest number (2) appeared in the 1, 2.5 & 3Gy samples.

RAPD markers of the 10samples irradiated and control from C. roseus cell suspension culture with 4RAPD primers.

Data of the amplified fragments using the aforementioned four, 10-mer arbitrary primers for the 10irradiated samples and the control from *C. roseus* cell suspension cultures indicated successful amplification of PCR products. In Table 3. polymorphism levels differed from one primer to the other. The main results were the only one primer OP-B05 (40%) showed exhibited low polymorphism

differences among the samples, while other primers exhibited medium levels of polymorphism such as OP-B01 (53%), OP-B2 (53%) and OP- B07 (53.3%).

Genetic similarity and cluster analysis based on RAPDs markers

The RAPD data were used to estimate the genetic similarity among the 10irradiated samples and the control of *C. roseus* cell suspension culture taxa by using Totallab Quant software. The highest similarity index recorded was 0.101, which was observed between the two taxa 4.5 & 5Gy while the lowest similarity index recorded was 0.058, which was observed between the 3.5 & 45Gy.

TABLE 3. RAPD markers of the control and 10 irradiated cell suspensions (by Gy) from *C. roseus* with 4RAPD primers.

Treatments by Gy		Primers				TAF
		OP-B01.	OP-B02.	OP-B05.	OP-B07.	
Con.	AF	4	1	3	4	12
	SM	0	0	1	1	2
0.5	AF	6	3	2	4	15
	SM	1	1	1	0	3
1	AF	5	2	2	2	11
	SM	0	1	1	1	3
1.5	AF	6	3	3	5	17
	SM	1	0	1	2	4
2	AF	3	4	2	3	12
	SM	1	0	0	1	2
2.5	AF	6	4	1	2	13
	SM	0	2	0	0	2
3	AF	6	6	3	2	17
	SM	0	2	3	0	5
3.5	AF	3	6	2	3	14
	SM	0	2	0	0	2
4	AF	6	0	1	3	10
	SM	0	0	1	1	2
4.5	AF	7	1	1	3	12
	SM	1	1	0	0	2
5	AF	7	4	1	4	16
	SM	0	3	0	1	4

TAF: Total amplified fragment. AF: Amplified fragment. SM: Specific marker.

A dendrogram for the genetic relationships among the 10irradiated samples and the control of *C. roseus* cell suspension culture taxa was carried out as in Fig. 1.

The 10samples irradiated and control from *C. roseus* cell suspension culture taxa were separated into three clusters; cluster one included 1, 1.5, 2 & 3Gy, while the cluster two included control, 2, 2.5, 3.5, 4 & 4.5Gy. The cluster three included 4.5 & 5Gy.

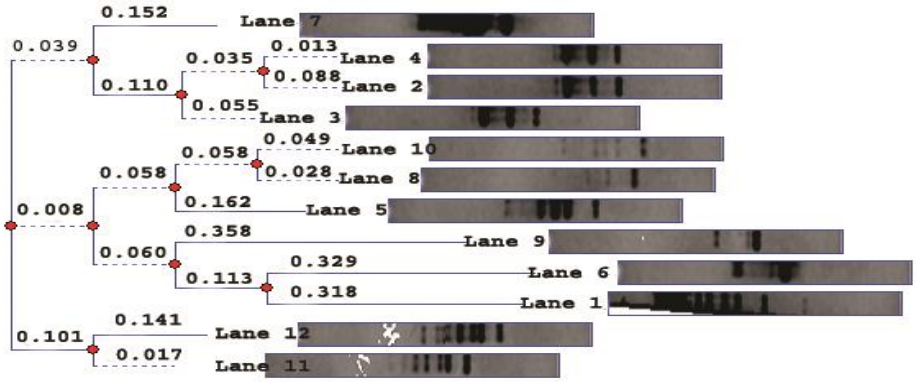

Fig. 1. Dendrogram for the genetic distances combining four primers among the control and the 10irradiated cell suspensions (by Gy) of *C. roseus* genotypes based on similarity index data of RAPD analysis.

A dendrogram for the genetic relationships among the control and 10irradiated cell suspensions from *C. roseus* taxa was carried out as in fig. 1. Within the cluster one, two sub clusters, the first one contained 2.5Gy sample (0.152), while the second divided into two sub-sub clusters, as 0.5Gy (0.055) (in the first sub-sub clusters) and the second sub-sub clusters was contain control and 1Gy (0.013). The second cluster divided into two sub clusters the first one contained two sub-sub clusters, the first one in sub-sub clusters, as 3 & 4Gy treated samples, while the second sub-sub cluster contained on 1.5Gy sample; the second sub clusters was contain 1.5Gy treated. the second sub clusters in clusters two divided into two sub-sub clusters, as 3.5Gy (in the first sub clusters), the second sub-sub clusters was contained on 2Gy sample, while the cluster three were contained on 4.5 & 5Gy sample.

ISSR assay

The ISSR are ideal as markers for genetic mapping and population studies because of their abundance and the high degree of polymorphism between individuals within a population of closely related genotypes. Those properties indicate their potential role as good supplements for RAPD based genome analysis (Korbin *et al.*, 2002).

Primer HA-98

Primer HA-98 resulted in 10fragments with molecular sizes ranging from 42-281bp, 10polymorphic fragments (59%). A bands corresponding to molecular sizes of 281, 234, 200, 149, 118, 105, 99, 72, 60 & 42bp, respectively, were observed whereas the remaining bands were monomorphic.

This primer showed that, the fragment with 281bp appeared only in the 5Gy, and absent in all other taxa. So, it could be considered as a molecular marker for the 5Gy. The fragments with 234, 200 & 72bp appeared only at the 0.5Gy treatment, but it was absent in all other taxa. So, it could be considered as a molecular marker for the 0.5Gy, the fragment with 200bp, appeared only in 4Gy, while it was absent in all other taxa. So, it could be considered as a molecular marker for the 4Gy treatment.

The fragments with 105, 99 & 42bp appear only in the control exclusively, while it was absent in all other taxa therefore, it could be considered as a molecular marker for the control**.**

Primer HA-99

The results of primer HA-99 showed 9fragments with molecular sizes ranging from 72-603 bp, 9polymorphic fragments (53%) with numbers 4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 & 13with corresponding molecular sizes of 603, 315, 281, 234, 200, 194, 118, 105 & 72bp, respectively were observed, whereas the remaining band was monomorphic.

This primer showed that, the fragment with 315bp appeared only in the 2, 4.5 & 5Gy samples, while it was absent in all other taxa therefore, it could be considered as a molecular marker for the 2, 4.5 & 5Gy treatments. The fragment

with 105bp appeared in 4.5 & 5Gy, while it was absent in all other taxa, therefore, it could be considered as a molecular marker for 4.5 & 5Gy samples.

Primer HB-12

Primer HB-12 resulted in 15fragments with molecular sizes ranging from 38-872 bp, 15polymorphic fragments (88.24%) with numbers 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 & 17 with corresponding molecular sizes of 872, 603, 315, 281, 234, 200, 194, 118, 105, 99, 72, 60, 55, 40 & 38bp, respectively were observed, whereas the remaining band were monomorphic.

In this primers, the fragment with 872 bp appeared only in the control, therefore it could be considered as a molecular marker for the above taxa. The fragment with 42 bp. appeared only in the control 0, 300 & 3.5Gy, therefore, it could be considered as a molecular marker for the control, 3 & 3.5Gy.

Primer HB-13

This primer showed 14fragments with molecular sizes ranged from 38-603bp, 14polymorphic fragments (82.35%) with numbers 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 & 17 with corresponding molecular sizes of 603, 315, 281, 234, 200, 194, 118, 105, 99, 72, 60, 55, 40 & 38bp, respectively were observed, whereas the remaining band were monomorphic.

The fragment with 603bp appeared only in the control, 0.5 & 1Gy, therefore, it could be considered as molecular markers for the control, 0.5 & 1Gy. The fragment with 315bp appeared only in the 1.5 & 2Gy therefore, it could be considered as molecular markers for 1.5 & 2Gy.

Specific ISSR markers of the 10irradiated treatments and the control from C. roseus with four ISSR primers.

Data of the amplified fragments using the aforementioned four ISSR primers for the ten irradiated treatments and control samples of *C. roseus* indicated successful amplification of PCR products. Polymorphism levels differed from one primer to the other. The main results were as follows:

Only two primers HA-98 (59%) and HA-99 (53%) showed exhibited low polymorphism differences among the samples (Table 4), while HB-12 (88.24%) and HB-13 (82.35%) primers exhibited highly polymorphism.

TABLE 4. RAPD markers of the control and 10irradiated cell suspension (by Gy) from C. roseus with 4ISSR primers.

Treatments by Gy		Primers				TAF
		HA-98.	HA-99.	HB-12.	HB-13.	
Con.	AF	3	6	9	10	28
	SM	3	0	2	1	6
0.5	AF	3	4	7	11	25
	SM	3	0	0	1	4
1	AF	1	5	8	8	22
	SM	0	0	0	1	1
1.5	AF	1	2	9	11	23
	SM	0	0	0	1	1
2	AF	4	5	8	10	27
	SM	0	1	0	1	2
2.5	AF	3	3	9	8	23
	SM	0	0	0	0	0
3	AF	3	2	9	9	23
	SM	0	2	1	0	1
3.5	AF	6	3	8	8	25
	SM	0	2	1	0	1
4	AF	5	5	8	10	28
	SM	1	0	0	0	1
4.5	AF	2	8	5	8	23
	SM	0	2	0	0	2
5	AF	6	8	12	6	32
	SM	1	2	0	0	3

TAF: Total amplified fragment. AF: Amplified fragment. SM: Specific marker.

Genetic similarity and cluster analysis based on ISSRs markers.

The ISSR data were used to estimate the genetic similarity among the 10irradiated treatments and control samples of *C. roseus* taxa by using SPSS computer analysis. The highest similarity index recorded was 0.270, which was observed among the three taxa 0.5, 1 & 2 Gy; while the lowest similarity index recorded was 0.017, which was observed between 3.5 & 5Gy.

A dendrogram for the genetic relationships among the 10irradiated treatments and control samples of *C. roseus* taxa was carried out as in Fig. 2. The 10irradiated treatments and control samples taxa were separated into three clusters; the first cluster included samples of 2.5Gy, while the second cluster included control, 0.5, 1.5, 2 & 4Gy. The third cluster included 1, 3, 3.5, 4.5 & 5Gy.

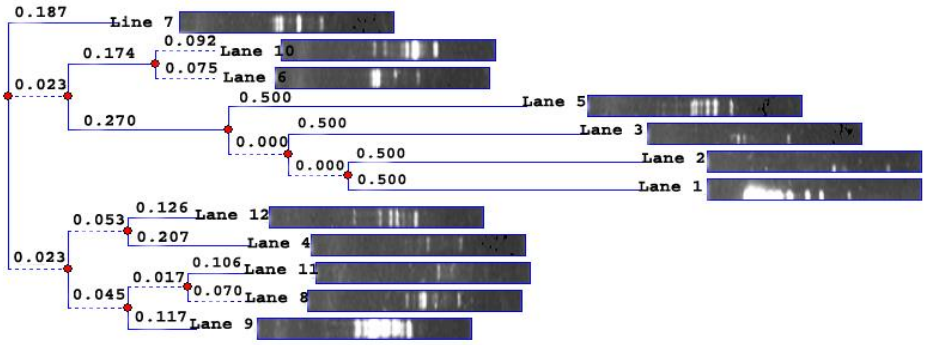

Fig. 2. Dendrogram for the genetic distances combined four primers among the control and the 10irradiated cell suspensions (by Gy) of *C. roseus* genotypes based on similarity index data of ISSR analysis.

The first cluster contained 2.5Gy treatment only. Within The second clusters two sub clusters were observed, the first one contained 2 & 4Gy (in one division). The second one contained divided into two sub-sub clusters, the first one contained the control & 5Gy; in other sub-sub clusters contained 1.5 Gy. The third clusters were divided into two sub clusters, the first one contained 3.5Gy, while the second sub cluster was divided into two sub-sub clusters, as 1 & 5Gy, the third sub-sub cluster contained 3 & 4.5Gy.

In our study, chemical or physical parameters may explain differences found among the plants of *C. roseus* and the genetic relationships. The studies indicated that RAPD and ISSR techniques are useful in the establishment of the genetic fingerprinting and estimation of relationships between *C. roseus* genotypes. The percentage (%) of polymorphic in RAPD and ISSR were 97 & 100, respectively, the percentage (%) of Specific markers in RAPD & ISSR were 34.3 & 28, respectively. Our RAPD results are in disagreement with the findings by Gupta *et al.* (2007) in RAPD, who studied a genetic linkage map of the *C. roseus* by RAPD, ISSR, SSR techniques and morphological markers in order to assess their genetic relationships. They stated that RAPD technique, through discriminating among all the second generation F2 population of plants and distinguishing among the RAPD, ISSR, SSR and morphological markers in order to construct the genetic linking map. The results of RAPD were 69.1% specific markers. On the other hand, our ISSR results were in agreement with Gupta *et al.* (2007) with 47.6% of specific markers.

Also, our results are in disagreements with Prasad (2014) how studied the genetic diversity in *C. species* by RAPD-PCR using two primers namely OP D20 & OP U17. The results were analyzed based on the principle that a band was considered to be 'polymorphic' if it is present in some samples and absent in others, and 'monomorphic' if present in all the samples. In this study, both the primers OP D20 & OP U17 produced a total of 45 & 37bands, respectively.

Among the bands obtained using the primer OP D20, 88.88% were polymorphic and only 11.11% were monomorphic. Among the bands obtained using OP U17, 94.59 % were polymorphic and 5.4% were monomorphic. This RAPD data was further used for dendrogram analysis. The dendrogram obtained using primer OP D20 showed that 66.67% exhibiting maximum similarity whereas the least similarity exhibit was 20% indicating the diversity among them. Similarly, dendrogram using primer OP U17 showed that maximum similarity 80% and the least similarity was observed was (20%). Similar diversity study among *Catharanthus sp.* has been reported by Vardhan *et al.* (2012).

DNA fingerprinting technique has the major advancement in identification of species or measurement of genetic distance. In the present study DNA fingerprinting was used to produce unique fingerprinting, which would differentiate among closely related species. RAPD-PCR. Welsh and McClelland (1990), stated that, it was efficient in generating a large number of markers for genomic mapping without any previous knowledge about the organism genetics, it requires small amounts of DNA, the quickness, simplicity and reproducibility in the data acquisition, the low cost and accessibility of this technology and the potential automation, with the possibility of being used. RAPD-PCR is discriminatory because it analyzes the whole genome and reveals the possible relationship between host origin, mutation and genetic variations. In the present study DNA fingerprinting was used to produce unique fingerprinting, which would differentiate among closely related species.

The dendrogram obtained using the RAPD data separated the *Catharanthus* samples into different clusters. The primary clusters were subdivided into sub clusters as seen in the dendrogram. Sometimes, morphological based grouping did not match molecular/ genomic relationship

among the species. The RAPD technique has also been successfully used to distinguish other genera of cultivated *Catharanthus*, such as for the discrimination of different species of *Catharanthus* (Leal *et al.*, 2010). This results were refer to the relationship between the cell culture conditions and changes in cell genome, these changes among the above samples in genome characters, going back to the irradiated treatments. These results were in agreement with results by Dutta *et al*. (2007) how studied the genome of *C. roseus* through microarray, semi quantitative RT-PCR and northern analysis on a rotation culture system, then, comparative profiling of genes catalyzing the important steps of 2-C methyl-D-erythritol-4- phosphate (MEP), shikimate and TIAS biosynthetic pathways, their activator and repressors comprising differentiated and proliferated cells. The results obtained, demonstrate that the TIAS biosynthetic pathway genes and their activators show a variable expression pattern, which was correlated with the changes in the cellular conditions in these systems.

On the other hands Shokeen *et al.* (2007) developed the microsatellite markers were developed and utilized them for analysis of genetic diversity in *C. roseus* in order to isolate microsatellite sequences, they used to 24STMS markers, evaluated the genetic polymorphism in 37genotypes, which comprised of 32accessions of *C. roseus*, a single accession, each of two related species *C. trichophyllus* and *C. pusillus* and one accession, each of three related genera *Vinca minor*, *Thevetia peruviana* and *Nerium indicum*. The 24STMS markers detected 26 loci with two markers amplifying more than one locus. A total of 124 alleles were amplified in the 37genotypes ranging from 2-10 alleles with an average of 4.76 alleles per locus. The high average expected heterozygosity value of 0.56 and observed heterozygosity value of 0.52 established the efficiency of the STMS markers for discriminating the *C. roseus* genotypes.

Conclusion

The TIAs biosynthesis pathway regulation in response to different abiotic stresses in *vivo* such as, gamma rays are not known and the regulatory components governing these processes are not yet identified. This study provides to our knowledge the first report of gamma rays effect on *C. roseus* genome. The possible relationship of DNA modifications and TIAs biosynthetic

pathway have not been tested here and hence need to be deciphered in future for better understanding the mechanism, involved in abiotic stress gamma rays irradiated in *C. roseus*.

Irradiation of *C. Roses* cell suspensions with a dose level higher than 5Gy of gamma rays resulted in a significant mortality of these cells ranged from 60-90% accordingly, no data were obtained after exposure cell suspension to a dose level exceeding 5Gy; on the other hand, the best doses from gamma rays for genome manipulation in *C. roseus* were from 0.5-5Gy.

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الاختلافات الوراثية المتحقق منها بواسطة إختبارات التعريف العشوائي و تكرار التتابعات داخل العينة في مزارع خلايا نبات الونكا المعرضة لجرعات صغيرة من الإشعاع الجامي

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قسم بحوث المنتجات الطبيعية ، المركز القومي لبحوث و تكنولوجيا الإشعاع ، هيئة الطاقة الذرية المصرية ، ص. ب. ٢٩ مدينة نصر ، مصر.

تم تعريف الاختلافات الوراثية بين ١٠ عينات مشعة بالإضافة الى العينة الضابطة (الغير مشعة) في مزارع معلقات خلايا نبات الونكا و التحقق منها من خلال كلا من: إختبار التعريف العشوائي للتفاعل المتسلسل و إختبار تكرار التتابعات داخل العينة للتفاعل المتسلسل ، و ذلك لبناء نموذج تقارب شجيري و بيان مدي التشابة. و قد تم بناء الشكل التعريفي بواسطة برنامج (Totallab Quant) ، لبيان مدي التقارب في طبيعة الشكل الشجيري و قد ظهر أن كلا من: الشكلين الاعلى في نسبة التشابة ، حيث كانت قيمة التشابة ٠.١٠١ ، بينما كانت نسبة الشجيريين أن هناك ثلاث مجاميع رئيسية و ذلك للعينات المشعة (١٠) بالاضافة للعيتة الضابطة و ذلك طبقا للتقارب الشجيري. و قد ظهر أن العيتة الضابطة و العينات التي تعرضت لجرعة ٢.٥ و ٤ جراي كانت التشابة الاقل قد سجلت بقيمة ٠.٠٥٨ و ذلك بين العينات المشعة بجرعات ٣.٥ و ٤.٥ جراي.

أظهر الشكل الشجيري الذي تم بنائه لاظهار العلاقة بين العشرة عينات المشعة بالاضافة للعيتة الضابطة و ذلك في معلق الخلايا لنبات الونكا ان الـ ١٠ عينات بالاضافة للعيتة الضابطة قد أنفصلت الى ثلاث مجاميع و كانت المجموعة الاولى تضم العينات المشعة بجرعات ١و٠.١و٢و٣ جراي بينما كانت المجموعة الاخرى تضم الكونترول و العينات المشعة بجرعات ٢.٥ و ٣.٥ و ٤ و ٤.٥ جراي أما المجموعة الثالثة فكانت تضم العينات المشعة على جرعات ٤.٥ و ٥ جراي.

كانت أفضل الجرعات المستخدمة من أشعة جاما تتراوح من ٠.٥ الى ٥ جراي و ذلك بهدف التلاعب في المحتوى الجينومي لخلايا نبات الونكا و إستحداث الطفرات ، و يعتبر التلاعب الجيني محرك التغيير في التعبير الجيني و ذلك بهدف إستحداث تغيير في فسيولوجية الخلايا و أنتاج خطوط خلوية ، و يهدف إنتاج جطوط خلوية الى إستنباط خليا ذات إنتاجية عالية من المنتجات الثانوية و التي تستخدم في نطاق واسع في الصناعات الدوائية.