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Impact of Environmental Diversity in Egypt on *Catharanthus roseus* Cultivars Genome and Assessment that by Different DNA Markers

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THE GENETIC diversity relationship was studied among ten cultivars of *Catharanthus roseus* depending on geographic locations in Egypt (five governorates: Marsa Matruh, Kafr ash-Shaykh, Port Said, Cairo, and Bur-Safajah) and two colors of flowers (white and pink) using RAPD and ISSR assay. In the RAPD assay, four primers amplified gave a low polymorphism (30%) and (18%) in ISSR markers.

The highest value of similarity was 1, while the lowest value of similarity indexed was 0.539. In ISSR analysis, 17.6% polymorphism was observed, the highest value of similarity was 1 and the lowest value of similarity index recorded was 0.106. The combined RAPD and ISSR analysis data revealed the highest similarity observed (0.984) but, the least similarity was 0.448. The primer OP-L13 only was given two specific markers in RAPD assay. The present study revealed that RAPD would be a useful assay compared to the ISSR phylogenetic assay for studies to identify the genetic diversity of the genome. The Principle component analysis (PCA) of RAPD and ISSR assays using 4 primers (both of them) was studied among ten genotypes cultivars of *Catharanthus roseus* plant. The results showed that the RAPD assay has given three clusters with 72.66 and 25.35% of the variance, the ISSR assay has given two clusters with 75.55 and 24.26% of the variance. On the other hand, the combined RAPD and ISSR assays have given the three clusters with 67.01 and 20.74% of the variance.

Keywords: *Catharanthus roseus*, Environmental impact, ISSR, RAPD.

Introduction

Catharanthus roseus (L.) G. Don belongs to the Apocynaceae family, is an ornamental and medicinal plant. The english name is Vinca rosea and common name is Periwinkle. *C. roseus* is regional to Madagascar and endemic in the hot and semi-warm regions (Shaw et al., 2009).

The family Apocynaceae, includes 411 genus and 4650 species, and almost all of them have a medicinal value (Simpson, 2006). The genus *Catharanthus* consists of eight species; *pusillus, roseus*, *coriaceus, lanceus, longifolius, ovalis, scitulus,* and *trichophyllus*. The first species, *pusillus* is regional to India, but the other species are regional in Madagascar. The species *C. roseus* and *C. trichophyllus* are originated through crosshybridizable. The ornamental cultivars of *C. roseus* appeared as a results of a bred shoot, blooming time, the coloration of petal and appropriateness for cultivation in gardens and homes, the *C. roseus* plant has approximately 130 different types of alkaloids such as catharanthine, ajmalicine, vincrystine, vinblastine, etc. These alkaloids are used as antitumor and anti-diabetic drugs and as traditional medicine for treating the blood pressure (Snoeijer, 2001; van der Heijden, et al., 2004).

Catharanthus roseus, 2n= 2x = 16 is the highly significant species in the genus and the genome size is 1500 Mbp. (Sreevalli et al., 2000). Alkaloid biosynthesis has been studied hard but, what has

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been done is not enough and needs to be developed to take advantage of the enormous potential of naturally occurring *C. roseus* germplasm. The genetic resources remain unknown, the structure and organization of the *C. roseus* genome (Van Der Heijden et al., 2004).

Genetic diversity of crops in species is useful to breeding programs. Genetic diversity study can be used in plant improvement. The relationship between wild lines and pure lines and understanding it can be used in planning crosses (Hallauer & Miranda Filho, 1988). Studying germplasm by Genetic diversity analysis is important to classify and discover subspecies which can be important in plant breeding.

Many marker assays are important in genetic studies and description studying. These contain; studding morphological, cytological analysis, biochemical assay and DNA markers protocols. DNA markers are dependent on the Complex and sophisticated tools for analysis of genetic relationships and revealed unlimited numbers, so that they are of high polymorphism and considerd of environmental affects (Singh et al., 2004).

Markers of DNA include Inter Simple Sequence Repeats (ISSR) (Zietkiewicz et al., 1994) and Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990).

PCA can be used in yield and quality components, taxonomic similarities, and association between either genetic or environmental attributes in horticultural crops (Iezzoni & Pritts, 1991). PCA is often applied to standardize data because the results are sensitive to the choice of units of measurement. The choice of units is often arbitrary (Rohlf, 2005).

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 to be easily visualized (Wold et al., 1984). However, the Biology identification system may have a limited usefulness for characterizing microbial communities since it is inherently based on culture ability of the microorganisms. Whole cell methods have also been used to characterize microbial communities, based on respiration studies with different soils after adding a variety of substrates (Degens & Harris, 1997). In addition, White et al. (1996) have described an approach in which microbial communities are characterized by signature lipid biomarkers. PCA was performed on these profiles and score plots revealed differences between the different microbial communities.

The present investigation was conducted for the purpose of studying the diversity of *C. roseus* cultivars genetic using RAPD and ISSR markers. Also, the current study was performed to obtain unbiased and easily visualized profiles of the ten cultivars of *Catharanthus roseus* communities. This was accomplished by PCA evaluation of DNA fingerprints (FPs) derived from Egyptian environment.

Materials and Methods

This study included *Catharanthus roseus* cultivars plants of both types cultivars due to their two colors of flowers (white and pink) and endemic in five Egyptian governorates (Marsa Matruh, Kafr ash-Shaykh, Port Said, Cairo, and Bur-Safajah). Young and fresh leaves were collected early in the morning before sunrise, then the samples were frozen by liquid nitrogen until experiments. Therefore, it can be considered that the present study included ten cultivars.

DNA isolation

Young and fresh leaves samples were extracted to give the bulked of DNA performed using GeneJET™ Plant Genomic DNA Purification Mini Kit, Thermofisher catalog number: #K0791, #K0792.

Randomly amplified polymorphic DNA (RAPD) and Inter-simple sequence repeats (ISSRs) analysis

In this study, RAPD and ISSR assays were used for the identification of markers associated with the ten cultivars of *C. roseus* plants taxa genotypes according to Williams et al. (1990).

The RAPD and ISSR analysis using polymerase chain reaction (PCR) were performed using DreamTaq™ Green PCR Master Mix (2X) Thermofisher catalog number: #K1081, 1.5 µM of primer in (RAPD analysis and ISSR analysis) from (Operon Technology USA) their codes, sequences and GC % are shown in Table 1 and 25ng of template DNA. The reaction was carried out using thermocycler (PTC-100, Perkin Elmer-USA). The ladder used was O'RangeRuler™ 200 bp DNA Ladder, ready-to-use, 0.05 µg/µl.

TABLE 1. List of primers names and their nucleotide sequences used in this assays (RAPD) and (ISSR).

No.	Name	Sequence	GC %	Annealing temperature (˚C) °C= At = 2(A+T) +4(G+C) - 2
RAPD primers				
1	OP-B11	5' CAGCACTGCT 3`	60%	30˚C
2	OP-C10	5` TGTCTGGGTG 3`	60%	30˚C
3	OP-D07	5' CAATCCGTCC 3`	60%	30˚C
4	OP-L13	5` ACCGCCTGCT 3`	70%	32˚C
ISSR primers				
5	HA – 99	5' CACACACACACAAG 3'	50%	40˚C
6	HB – 12	5' CACCACCACGC 3'	73%	36˚C
7	HB – 13	5' GAGGAGGAGGC 3'	73%	36˚C
8	HB - 14	5' CTCCTCCTCGC 3'	73%	36˚C

Gel and data analysis

Gels were photographed and scanned at a wavelength of 577 nm. The similarity matrices were conducted, and the relationships among rootstock genotypes as revealed by dendrograms were performed using the SPSS windows (Version 22) program. The Principle Component Analysis (PCA) was performed to determine eigenvalues, percentage and cumulative variance. The PCA was performed using the SPSS windows (Version 22) program.

Result and Discussion

RAPD and ISSR assay

The genomic analysis using RAPD technique and four primers were used to detect the genetic diversity in ten cultivars due to their two colors of flowers (white and pink) of *Catharanthus roseus* from five locations. All the primers revealed duplicate bands. The banding pattern of the RAPD assay are shown in Fig. 1. The data of the banding pattern are shown in Table 2. All primers revealed amplifications in all ten cultivars, and total bands observed (342; 102) were a polymorphic while, 240 bands were monomorphic. The primer OP-B11 gave the maximum number (150) of bands, but the minimum number of bands (59) with the 15.3% polymorphism was observed in primer OP-D07. The amplicons in all primers were observed ranging from 400 to 2400 bp. The biggest amplicon (2400 bp) was amplified with OP-B11 primer, while the smaller amplicon (400 bp) was amplified by the same primer above OP-B11. The primer OP-L13 only was given two specific markers.

The fragment with 400 and 500 bp revealed exclusively in cairo cultivars with pink flower, but was absent in Cairo cultivars with white flower and all other cultivars. So, it could be used as a molecular marker for Cairo cultivars with pink flower.

In the ISSR assay, four primers were used. All the primers gave duplicate bands. The information are given in Table 2. All primers gave amplifications in all ten cultivars. ISSR primers generate 313 bands. The polymorphic bands were 55 bands while 254 bands were monomorphic. The polymorphism percentage was 18%. The maximum number of bands(94) was observed in (HB-14) primer, while the minimum number of bands (60) was observed in (HB-12) primer. The amplicon were detected ranging from 200-2000bp. The largest amplicon was amplified by (HB-13) primer, but the smallest amplicon was amplified by (HB-12) primer.

All primers in the ISSR assay did not give any special bands so, no specific markers revealed in ISSR assay.

The amplified fragments Data using the eight 10-mer arbitrary primers for the ten *Catharanthus roseus* cultivars revealed successful amplification of PCR products. The main results were as in Table (2): the primers OP-C10 in RAPD assay and the primers HB-12 & HB-13 in ISSR assay showed no polymorphic differences among the cultivars, while the primers showed low polymorphism such as OP-D07 (15.3%) in RAPD assay and the primer HA-99 (13%) & HB-14 (49%) in ISSR assay.

TABLE 2. List of arbitrary primers with total polymorphic amplicon and polymorphism reproducible for 10 *C. roseus* cultivars

No.	Primer	Range of amplicon (bp)	Total bands (a)	Total polymorphic bands (b)	Polymorphism (%) b/a × 100	Total monomorphic bands (c)	Monomorphic (%) c/a × 100	SM
RAPD analysis data								
1	OP-B11	400-2400	150	0	0	150	100	0
2	OP-C10	600-1400	66	46	70	20	30	0
3	OP-D07	500-1400	59	9	15.3	50	84.7	0
4	OP-L13	400-1000	67	47	70	20	30	2
-	Total	-	342	102	30	240	70	2
ISSR analysis data								
1	HA-99	600-1600	69	9	13	56	87	0
2	HB-12	1000-1500	60	0	0	60	100	0
3	HB-13	600-2000	90	0	0	90	100	0
4	HB-14	200-1800	94	46	49	48	51	0
-	Total	-	313	55	18	254	82	0

SM: Specific markers

On the other hand, the primers gave high levels of polymorphism such as OP-C10 (70%) & OP-L13 (70%) in RAPD assay, but in ISSR assay the primers did not give any high levels of polymorphism. The primer OP-B11 in RAPD assay and the primers HP-12 & HP-13 in ISSR assay did not give bands polymorphism. the current results revealed the lower polymorphism (18%) in ISSR markers compared to RAPD markers (30%).

The similarity of RAPD and ISSR assay

The dendrogram constructed was explained successfully differentiatting ten *Catharanthus roseus* cultivars using RAPD and ISSR assay.

The RAPD and ISSR data were used to appraise the genetic similarity among 10 *Catharanthus roseus* cultivars taxa using SPSS 22 computer analysis as shown in Tables 3 and 4. In the RAPD assay, the highest similarity index documented was 1, which was observed between the taxa as follows: Cairo Pink flower cultivars & Bur-Safajah white flower cultivars, Cairo Pink flower cultivars & Bur-Safajah pink flower cultivars and Bur-Safajah white flower cultivars & Bur-Safajah pink flower cultivars, while the lowest similarity index documented was 0.539 which was observed between Port Said white flower cultivars and Cairo white flower cultivars.

The genetic relationships dendrogram among the ten *Catharanthus roseus* taxa was carried out as in Fig. 1. The 10 *Catharanthus roseus* taxa were separated into two clusters; cluster one included Port Said pink flower cultivars, Marsa Matruh pink flower cultivars, Marsa Matruh white flower cultivars, Kafr ash-Shaykh white flower cultivars, Port Said white flower cultivars and Kafr ash-Shaykh pink flower cultivars. However, cluster two was included Cairo white flower cultivars, Cairo pink flower cultivars, Bur-Safajah pink flower cultivars, and Bur-Safajah white flower cultivars.

Within cluster one, two sub-clusters were observed, the first one was divided into two sub sub-clusters, the first one of sub-sub-cluster contained Port Said pink flower cultivars only. The second sub-sub-cluster of first one contained Marsa Matruh pink flower cultivars, Marsa Matruh white flower cultivars. In the first one, the second sub-cluster contained Kafr ash-Shaykh white flower cultivars, Port Said white flower cultivars and Kafr ash-Shaykh pink flower cultivars.

The second cluster was divided into two sub-clusters, the first sub-cluster contained Cairo white flower cultivars only, while the second sub clusters contained Cairo pink flower cultivars, Bur-Safajah pink flower cultivars, and Bur-Safajah white flower cultivars.

The DNA fingerprint (FPs) generated by RAPD assay was evaluated and mapped by PCA. Each point in Fig. 3 represents a FP projected down to a two-dimensional PCA score plot from a 1000-dimensional space. Principal components (PCs) 1 and 2 in Fig. 2 explained 72.66 and 25.35% of the variance, respectively. Ten *C.*

roseus Egyptian cultivars were identified (Fig. 2). Furthermore, at the three clusters, the first one included (Marsa Matruh white flower cultivars, Marsa Matruh pink flower cultivars, Kafr ash-Shaykh pink flower cultivars, Port Said white flower cultivars and Kafr ash-Shaykh white flower cultivas). The second clusters contained Port Said pink flower cultivars only. The second clusters contained Bur-Safajah pink flower cultivars, Bur-Safajah white flower cultivars, Cairo pink flower cultivars and Cairo white flower cultivars,representing FPs for genome similarity which were significantly separated from each other (P= 0.05, Coomans plot (Wold et al., 1984). The dispersion within each cluster, as shown in Fig. 2 represents variations in the parallel DNA extractions and RAPD reactions.

There is a significant match between the results of the genetic similarity of ten genotypes from *Catharanthus roseus* cultivars through RAPD assay by 4 primers and the results of Principal components analysis (PCA) of the same above *Catharanthus roseus* cultivars genotypes.

TABLE 3. Similarity indices among the ten *Catharanthus roseus* cultivars Taxa based on RAPD-PCR using 4 primers.

Taxa	Marsa Matruh W	Marsa Matruh P	Kafr ash-Shayk W	Kafr ash-Shayk P	Port Said W	Port Said P	Cairo W	Cairo P	Bur-Safajah W	Bur-Safajah P
Marsa Matruh W	1									
Marsa Matruh P	.984	1								
Kafr ash-Shayk W	.898	.953	1							
Kafr ash-Shayk P	.930	.958	.986	1						
Port Said W	.936	.952	.971	.997	1					
Port Said P	.946	.980	.919	.897	.879	1				
Cairo W	.740	.770	.623	.569	.539	.873	1			
Cairo P	.896	.891	.732	.723	.712	.938	.953	1		
Bur-Safajah W	.896	.891	.732	.723	.712	.938	.953	1.000	1	
Bur-Safajah P	.896	.891	.732	.723	.712	.938	.953	1.000	1.000	1

W: White flower, P: Pink flower

TABLE 4. Similarity indices among the ten *Catharanthus roseus* cultivars Taxa based on ISSR-PCR using 4 primers.

Taxa	Marsa Ma-truh W	Marsa Ma-truh P	Kafr ash-Shayk W	Kafr ash-Shayk P	Port Said W	Port Said P	Cairo W	Cairo P	Bur-Safajah W	Bur-Safajah P
Marsa Matruh W	1									
Marsa Matruh P	.531	1								
Kafr ash-Shayk W	.531	1.000	1							
Kafr ash-Shayk P	.645	.988	.988	1						
Port Said W	.913	.757	.757	.849	1					
Port Said P	.531	1.000	1.000	.988	.757	1				
Cairo W	.531	1.000	1.000	.988	.757	1.000	1			
Cairo P	.531	1.000	1.000	.988	.757	1.000	1.000	1		
Bur-Safajah W	.913	.757	.757	.849	1.000	.757	.757	.757	1	
Bur-Safajah P	.833	.106	.106	.258	.730	.106	.106	.106	.730	1

W: White flower P: Pink flower.

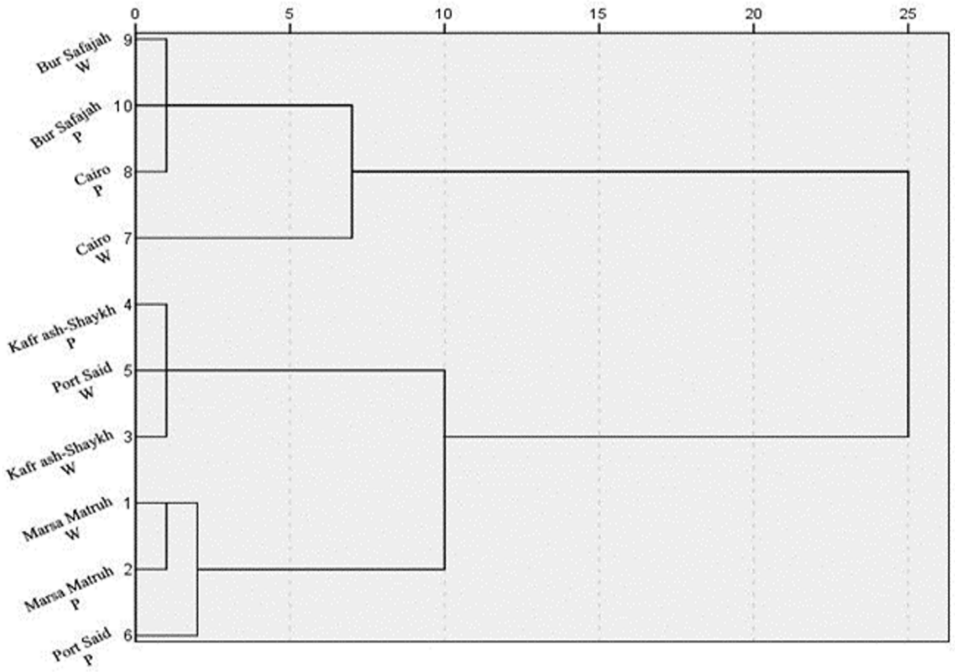

Fig. 1. Dendrogram for the genetic relationships among the ten *Catharanthus roseus* cultivars Taxa based on similarity indices RAPD analysis data.

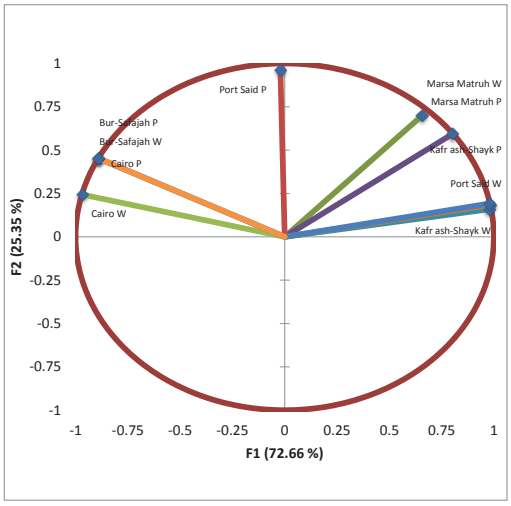

Fig. 2. Basic Analysis Components Diagram (PCA) showing the different fingerprints derived from DNA extraction from ten genotypes from *Catharanthus roseus* cultivars through RAPD assay by 4 primers.

On the other hand, the ISSR assay shows that the highest similarity index documented was 1, which was observed (Table 4) among the taxa as follows: Marsa Matruh pink flower cultivars & Kafr ash-Shayk white flower cultivars, Marsa Matruh pink flower cultivars & Port Said pink flower cultivars, Marsa Matruh pink flower cultivars & Cairo white flower cultivars, Marsa Matruh pink flower cultivars & Cairo pink flower cultivars, Kafr ash-Shayk white flower cultivars & Port Said pink flower cultivars, Kafr ash-Shayk white flower cultivars & Cairo white flower cultivars, Kafr ash-Shayk white flower cultivars & Cairo pink flower cultivars, Port Said white flower cultivars & Bur-Safajah white flower cultivars, Port Said pink flower cultivars & Cairo white flower cultivars, Port Said pink flower cultivars & Cairo pink flower cultivars and Cairo white flower cultivars & Cairo pink flower cultivars.

The lowest similarity index documented was 0.106 which was observed among Bur-Safajah pink flower cultivars and both of them; Marsa Matruh pink flower cultivars & Kafr ash-Shaykh white flower cultivars & Port Said pink flower cultivars & Cairo white flower cultivars and Cairo pink flower cultivars.

The genetic relationships dendrogram among the ten *Catharanthus roseus* taxa was carried out as in Fig. 3. The 10 *Catharanthus roseus* taxa were separated into two clusters; cluster one included Bur-Safajah pink flower cultivars only. However,cluster two included. Kafr ash-Shayk pink flowers cultivar, Port Said pink flower cultivars, Kafr ash-Shayk white flower cultivars, Marsa Matruh pink flower cultivars, Cairo pink flower cultivars, Cairo white flower cultivars, Marsa Matruh white flower cultivars, Port Said white flower cultivars and Bur-Safajah white flower cultivars.

The first cluster contained Bur-Safajah pink flowers cultivars only. Cluster two was divided into two sub-clusters, the first one sub-clusters in the cluster two contained Kafr ash-Shayk pink flower cultivars, Port Said pink flower cultivars, Kafr ash-Shayk white flower cultivars, Marsa Matruh pink flower cultivars, Cairo pink flower cultivars, Cairo white flower cultivars. The second sub cluster was divided into two sub-sub-clusters, the first sub-sub-cluster contained the Marsa Matruh white flower cultivars, the second sub-sub-cluster contained Bur-Safajah white flower cultivars and Port Said white flower cultivars.

The results shown in Fig. 4 revealed that there was among the DNA fingerprint (FPs), this was generated by ISSR assay variation. Principal components (PCs) 1 and 2 in Fig. 4 explained 75.55 and 24.26% of the variance, respectively. Ten *C. roseus* Egyptian cultivars were identified in Fig. 4. Concerning the two clusters, the first one contains Kafr ash-Shayk pink flower cultivars, Cairo pink flower cultivars, Cairo white flower cultivars, Port Said pink flower cultivars, Marsa Matruh pink flower cultivars and Kafr ash-Shayk white flower cultivars. The second cluster contains Bur-Safajah white flower cultivars, Port Said white flower cultivars, Marsa Matruh white flower cultivars and Bur-Safajah pink flowers cultivars. Representing FPs for genome similarity was significantly separated from each other (P= 0.05, Coomans plot, Wold et al., 1984). The dispersion within each cluster, as shown in Fig. 4, represents variations introduced in the parallel DNA extractions and ISSR reactions.

There is a significant match between the results of the genetic similarity of ten genotypes from *Catharanthus roseus* cultivars through ISSR assay by 4 primers and the results of Principal components analysis (PCA) of the same above *Catharanthus roseus* cultivars genotypes.

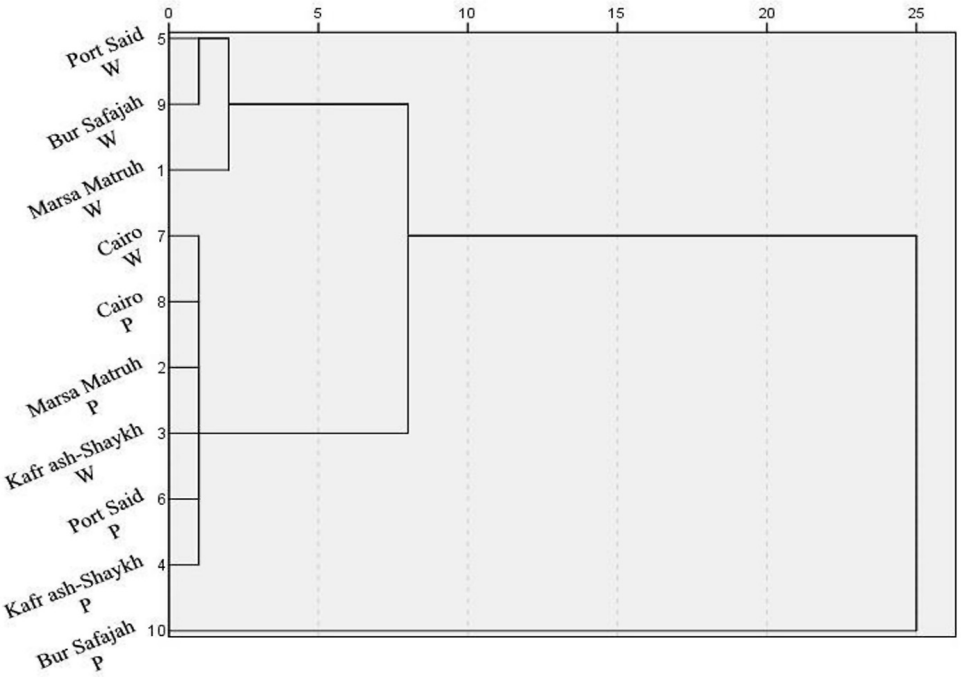

Fig. 3. Dendrogram for the genetic relationships among the ten *Catharanthus roseus* cultivars Taxa based on similarity indices ISSR analysis data.

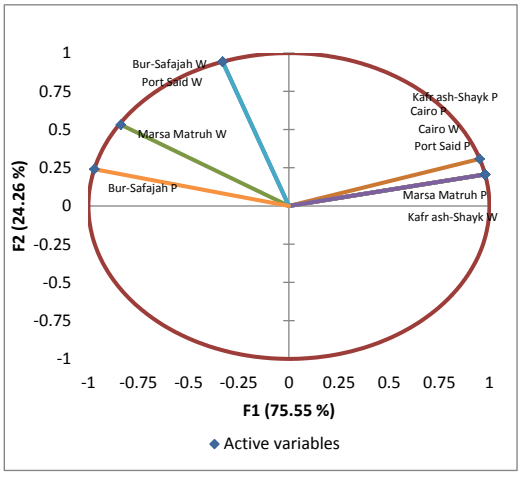

Fig. 4. Basic Analysis Components Diagram (PCA) showing the different fingerprints derived from DNA extraction from ten genotypes from *Catharanthus roseus* through ISSR assay by 4 primers.

The RAPD and ISSR combined analysis markers and relationships detected

The analysis of the combined RAPD and ISSR data as shown in Table 5 revealed a high level of genetic variations. The highest similarity recorded (0.984) was observed between Kafr ash-Shayk pink flower cultivars and Port Said white flower cultivars but, the least similarity recorded (0.448) was observed between Port Said white flowers cultivars and Cairo white flower cultivars.

The genetic relationships dendrogram among the ten *C. roseus* cultivars taxa depend on RAPD and ISSR markers assay by using 8 primers Fig. 5. The 10 *Catharanthus roseus* taxa were separated into two clusters; cluster one, contained on Bur-Safajah pink flower cultivars, Cairo white flower cultivars, Cairo pink flower cultivars and Bur-Safajah white flower cultivars. However, cluster two included Marsa Matruh white flower cultivars, Port Said pink flower cultivars, Marsa Matruh pink flower cultivars, Port Said white flower cultivars, Kafr ash-Shayk pink flower cultivars, and Kafr ash-Shayk white flower cultivars.

Cluster one was divided into two sub-clusters, the first one sub-clusters divided into two sub-sub-clusters, the first sub-sub-cluster included the Marsa Matruh white flower cultivars only. The second sub-sub-cluster of the first one contained Port Said pink flower cultivars, and Marsa Matruh pink flower cultivars.

The second sub-cluster of first one cluster contained Port Said white flower cultivars, Kafr ash-Shayk pink flower cultivars and Kafr ash-Shayk white flower cultivars.

TABLE 5. Similarity indices among the ten *Catharanthus roseus* cultivars Taxa based on combined RAPD and ISSR using 8 primers.

Taxa	Marsa Matruh W	Marsa Matruh P	Kafr ash-Shayk W	Kafr ash-Shayk P	Port Said W	Port Said P	Cairo W	Cairo P	Bur-Safajah W	Bur-Safajah P
Marsa Matruh W	1									
Marsa Matruh P	.893	1								
Kafr ash-Shayk W	.790	.944	1							
Kafr ash-Shayk P	.828	.932	.984	1						
Port Said W	.865	.892	.943	.984	1					
Port Said P	.870	.975	.884	.842	.791	1				
Cairo W	.712	.772	.582	.507	.448	.887	1			
Cairo P	.824	.867	.672	.632	.586	.938	.967	1		
Bur-Safajah W	.875	.768	.567	.551	.563	.851	.915	.936	1	
Bur-Safajah P	.849	.652	.460	.462	.509	.736	.818	.831	.973	1

W: White flowers P: Pink flowers.

On the other hand, cluster number two was divided into two sub-clusters, the first sub-clusterswas divided into two sub-sub-clusters, the first sub-sub-cluster was divided into two sub-sub-sub-clusters, and the first sub-sub-sub includes the Marsa Matruh white flower cultivar only. The second sub-sub-sub-cluster of the sub-sub-clusters of two contained Port Said pink flower cultivars, and Marsa Matruh pink flower cultivars.

The second sub cluster contained the Port Said white flowers cultivars, Kafr ash-Shayk pink flowers cultivars, and Kafr ash-Shayk white flowers cultivars.

The results shown in Fig. 6 revealed that there was among the DNA fingerprint (FPs), this was generated by the combined RAPD and ISSR assays variation. Principal components (PCs) 1 and 2 in Fig. 6 explained 67.01 and 20.74% of the variance, respectively. Ten *C. roseus* Egyptian cultivars were identified (Fig. 6). Furthermore, at the three clusters, the first one contains Marsa Matruh pink flower cultivars, Marsa Matruh white flower cultivars, Kafr ash-Shayk white flower cultivars, Kafr ash-Shayk pink flower cultivars and Port Said white flower cultivars. The second cluster contains the Port Said pink flower cultivars only. The third cluster contains the Cairo pink flower cultivars, Cairo white flower cultivars, Bur-Safajah white flower cultivars and Bur-Safajah pink flowers cultivars. Representing FPs for genome similarity was significantly separated from each other (P= 0.05, Coomans plot, Wold et al., 1984). The dispersion within each cluster, as shown in Fig. 7, represents variations introduced in the parallel DNA extractions for RAPD and ISSR reactions.

There is a significant match between the results of the genetic similarity of ten genotypes from *Catharanthus roseus* cultivars through using the combined RAPD and ISSR assays by 4 primers from each of them and the results of Principal components analysis (PCA) of the same *Catharanthus roseus* cultivars genotypes.

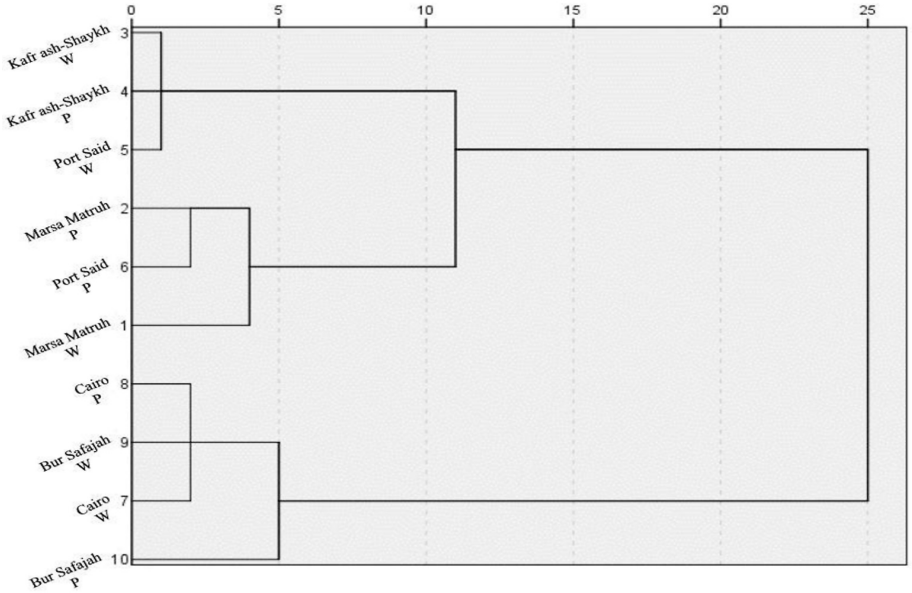

Fig. 5. The genetic relationships dendrogram among the ten *Catharanthus roseus* cultivars Taxa dependent on similarity indices data of combined RAPD and ISSR assay.

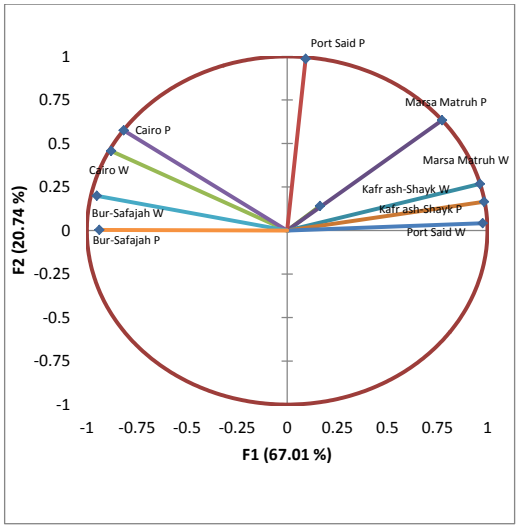

Fig. 6. Basic Analysis Components Diagram (PCA) showing the different fingerprints derived from DNA extraction from ten genotypes from *Catharanthus roseus* through combined RAPD and ISSR assays by 4 primers each.

The current results do not agree with those of the Ibrahim et al. (2013) who studied the molecular description of six *Catharanthus roseus* cultivars endemic in Egypt using RAPD and ISSR markers. RAPD markers results, giving 41 amplified fragments, with 18 of them were polymorphic (44%) using five primers. However, ISSR markers assay has given 50 amplified fragments, with 30 of them were polymorphic (60%) using five primers.

The genetic variation was studied by Lalhruaitluanga & Prasad (2009) in *Melocanna baccifera* (Roxb.) using RAPD and ISSR markers assays endemic in India. Their study yielded results different from the current one, both of whom acknowledged that the RAPD markers yielded results high polymorphism (98.02%) was observed. On the other hand, the ISSR markers polymorphism was (84.1%) although in the present study it was found that lower polymorphism (18%) was observed in ISSR assay compared to RAPD assay (30%).

The genetic diversity in rice observed by using different marker assay was studied by Parsons et al. (1997), the same results revealed 56% polymorphism in ISSR assay although 50% polymorphism was revealed in RAPD markers. Datta et al. (2010) studied the RAPD and ISSR markers assay in *Cicer arietinum* and *Cajanus cajan* and revealed a high polymorphism (95%) in ISSR markers compared the RAPD polymorphism markers assay (87%).

Ajibade et al. (2000) and Galvan et al. (2003) reported that the ISSR marker assay was more accurate than the RAPD marker assay and gives clearer and more comprehensive phylogenetic results. Nagaoka & Ogihara (1997) showed similar results, the ISSR marker assay was more valuable and important than the RAPD markers assay in wheat. The present study showed that the ISSR

assay has given more accurate and important results than the RAPD assay for phylogenetic investigation.

Shaw et al. (2009) studied comparative analysis of data of 18 RAPD and ISSR assay of genetic variation in *C. roseus* cultivars. The genetic variability was observed. Both markers were equal in ability to distinguish among *C. roseus* cultivars. The present study confirmed the same results revealed in the polymorphism assay, the polymorphism data were equal in RAPD and ISSR assays among *C. roseus* Egyptian cultivars. In the present study, the combined RAPD and ISSR assays were successfully used in detecting differences among ten *Catharanthus roseus* cultivars from each other.

Conclusion

There are reports available regarding the genetic diversity analysis in *C. roseus* by using two markers.

The data revealed throw DNA markers assay such as polymorphism data can be useful in plant breeding programs, improvement of crop researches and also might be useful in future strategic programs for the evolution of plant genotypes and devising of new genotypes capable of producing desired medical compounds in economical quantities in *C. roseus* cultivars. This is in addition to discovering genotypes that can be used in the future to produce cellular lines needed to produce drugs in bioreactors.

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