

PHYLOGENETIC RELATIONSHIP AMONG DIFFERENT VARIETIES AND SPECIES OF CANNA USING MOLECULAR MARKERS

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ABSTRACT: *Canna*, the solitary genus of family Cannaceae, represents a group of ornamental plants species. In this investigation an attempt was made to access the genetic relationship among six species and varieties of *Canna* using RAPD and ISSR techniques. A total of 49 major scorable bands ranging from 200 to 1500 bp were generated from two RAPD and three ISSR primers showing 83.6% polymorphism. Generally low relationship between tested cultivars/species; and the highest correlation (5.09) was found between *Canna* lily (C3) and *Canna indica* red (C2) and between *Canna flaccida* (C5) and *Canna indica* red (C2). However, the lowest (3.316) similarity was observed between *Canna* lily red brilliant (C4) and *Canna flaccida* (C5). The dendrogram based on the contained data of both RAPD and ISSR which showed that *Canna paniculata* (C6) was always in a far correlation with the other species and varieties in this study. It was observed that the used primers of both RAPD and ISSR divided the plants according to the flower colour with ignorance to the leaves colour and grouped (*C. variegata*, *C. indica* red and *C. paniculata*) together in one cluster, but in the other cluster, the primers grouped them according to the leaves cluster with ignorance of the flower colour (*C. flaccida*, *Canna* lily red brilliant and *Canna* lily). Finally, it might be the same cultivars grouped in other way in case of using other primers or other molecular marker system.

Key words: *Canna*, varieties, species, ISSR, RAPD, molecular marker.

INTRODUCTION

The genus *Canna* comprises of about 51 species of flowering plants having flashy, brilliantly-colored flowered and large tropical foliage. The native of *Canna* species are South and North America, in addition they have been introduced to Asia and Europe. With the passage of time, they evolved into native varieties (Prince, 2010). Distribution of *Canna* species is most possibly due to the effect of human dispersal.

Transportation of *Canna* from their native place might have been the reason for occurrence of beautiful ornamental plant in Europe, Asia and Africa (Maas Van and

Maas, 2008). Most of *Canna* hybrids are used as cultivated garden ornamentals around the world. Some of wild *Canna* species are popularly known as elemental species of *Canna* those like *C. glauca*, *C. indica*, *C. iridiflora*, *C. warszewiczii* and *C. flaccida*.

The taxonomy of Cannaceae is disputed as it relies on morphological features. Also, the distinguishing features between *Canna* varieties are poor. No work has been reported on the studies of genetic diversity of *Canna* cultivars in India and world, except for Piyachomkwan *et al.* (2002); Patra *et al.* (2008) and Gupta *et al.* (2013), who had

done some experiments with very limited way. The present study was carried out to study the genetic diversity of some cultivated *Canna* (species and varieties) in Egypt using RAPD (Random amplified polymorphic DNA) primers and ISSR (inter specific sequence repeat).

MATERIALS AND METHODS

Morphological Analysis:

Some plants of different species and varieties of *Canna* were collected from a nursery in King Marriott, Alexandria (A.R.E.). A pot experiment was carried out during one season of 2019 at Faculty of Agriculture, Alexandria University.

Six *Canna* species and varieties were used in this study which were: *Canna variegata* "Pretoria" (C1) with orange flower and variegated leaves, *Canna indica* red (C2) with orange flower and red leaves, *Canna* lily (C3) with white flower and green leaves, *Canna* lily red brilliant (C4) with red flower and green leaves, *Canna flaccida* (golden canna) (C5) with yellow flower and green leaves and *Canna paniculata* (C6) with single orange flower and green leaves (Fig., 1).

The experimental layout was designed to provide a completely randomized design (CRD) containing three replicates. Each replicate had 3 plants. Some morphological characters of *Canna* were used for characterization and identification of the different *Canna* species and varieties. The main of these measurements were: plant height, petal color, flower shape, flower form (single or double), leaf color, leaf shape, width of adult leaf. All measurements were taken on three plants of each species or varieties. These measurements were replicated three times to minimize error where possible.

Molecular diversity studies:

These molecular studies were carried out in the molecular biology laboratory of the Faculty of Agriculture at the Alexandria University.

RAPD and ISSR PCR based molecular methods were used for the genetic study. Two primers of RAPD and three others of ISSR were used to distinguish among the six *Canna* species and varieties used in this investigation.

Plant Materials:

Young fresh leaf material representing six endemic varieties; *Canna* species were collected from nursery in King Marriott, Alexandria, Egypt. The collected leaves were wrapped between moist tissue papers and kept in the refrigerator at -50°C until DNA extraction.

Purification of *Canna* DNA (DNA Extraction):

Major contaminants in crude DNA preparation are RNA, polysaccharides and protein and it is essential to remove them because it will hamper further downstream processing. Also, it was found that *Canna* DNA extract always remain contaminated with starch when isolated through conventional procedure of Doyle and Doyle (1987). Because of these mentioned before, Kit protocol was used in this study (Biotech Kit, Biovision, Korai) for isolated genomic DNA from the young leaves (the third leaf from the top of plant) of each variety.

Determination of DNA purity and concentration:

Nanodrop spectrophotometer was used to determine the isolated DNA. The ratio 260/280 was found 1.8 – 1.99, this indicated that DNA extract was pure enough for RAPD analysis.

Primers:

In this study, two RAPD primers and three ISSR primers were used as shown in the Table (1).

Random amplified polymorphism DNA (RAPD):

Each polymerase chain reaction consisted of 2 μl of primer, 10 μl master mix and 50 ng of genomic DNA. In a Biometra, PCR amplification was performed, T1



Canna variegata (C1)



Canna indica red (C2)



Canna lily (C3)



Canna lily red brilliant (C4)



Canna flaccida (C5)



Canna paniculata (C6)

Fig. 1. Different 6 species and varieties of *Canna* which used in this study.

Table 1. Represents 5 random primers name, sequences and annealing temperature as well as guanine and cytosine content percentage; two RAPD primers and three ISSR primers.

Primer number	Primer code	Sequences	GC%	Tm(c) Value
1	HB-08 (ISSR)	5' – GAG AGA GAG AGA GG -3'	57.14	44
2	HA-98 (ISSR)	5' – CAC ACA CAC ACA AGT -3'	46.67	43.4
3	OPS-11 (RAPD)	5' – AAA GTC GCG G -3'	60.00	32
4	HB-13 (ISSR)	5' – GAG GAG GAG GC -3'	72.73	38
5	OPO-20 (RAPD)	5' – AAC CCG GTC -3'	60.00	32.0

Tm = Temperature of melting.

GC = Guanine and Cytosine ratio (percentage).

gradient thermal cycler for 45 cycles after initial denaturation for 2 min at 94 °C. Each cycle consisted of denaturation at 95 °C for 1 min; annealing at 37 °C for 1.30 min; extension at 72 °C for 2 min and final extension at 72 °C for 2 min. The PCR products were separated on 1.5% (w/v) agarose gel. The lengths of the different DNA fragments were determined by using 100 pb DNA ladder. Also, by using gel analyzer program, RAPD fragments were scored as present/absent. Clustering and similarity coefficients were estimated according to Rohlf (1998).

Fingerprinting Data Analysis:

Each polymorphic band was regard as a bring character and was scored as (0) absence and (1) presence for each sample. It assembled in a data matrix. A similarity matrix was calculated to analyze the genetic relationship among the previous varieties according to the mathematical tools of Nei (1972). In addition, similarities were graphically expressed by using the UPGMA to generate dendrograms. Popgene 32 software package was used in the analysis (Yeh *et al.*, 1997).

On the other hand, correspondence analysis of right vectors from the binary data was performed on the basis of graphically summarized associations within the varieties. This analysis was performed through a batch file by using the software package NTSYS pc (version 20) (Rohlf, 1998).

RESULTS AND DISCUSSION

The obtained morphological data on the different *Canna* species and varieties were recorded and shown in Tables 2 and 3 as follow:

In view of lack of information in the area of molecular characterization of genetic variability in cultivated cannas in Egypt, the present study was initiated involving 6 common varieties of *Canna* (from different *Canna* species). Molecular markers i.e. RAPD and ISSR were used to assess the genome relationship among these 6 varieties.

In the present study, DNA molecular analyses (RAPD and ISSR) were applied to estimate the genetic diversity and the relationships among the six of the *Canna* different varieties as shown (Figs., 2-11).

Fig. (2) showed the results which obtained by using HB-O8 add which contains 57.14% G+C (Table, 1). The length of amplified DNA fragments of the PCR products generated by this primer ranged from 200-1500 bp. The number of total bands varied among the varieties, where the lowest number was three amplified bands in *Canna flaccida* (C5), and the highest number was seven amplified bands in the *Canna indica* red (C2) variety. The total number of amplified bands from the genomic of them is polymorphic bands were five and three monomorphic which presented in varieties. Consequently, the polymorphism percentage was 62.5%.

Table 2. Morphological information of different vegetative parameters of different species and varieties of *Canna*.

Species and varieties	Vegetative parameters				
	Leaf colour	Plant height (cm)	No. of leaves/plant	Leaf (cm)	
				Length	Width
C1: <i>Canna variegata</i>	Variegated green with white	75.31 ^d	4.28 ^a	39.16 ^c	21.16 ^a
C2: <i>Canna indica</i> red	Red	130.47 ^b	6.29 ^a	35.39 ^d	13.18 ^c
C3: <i>Canna</i> lily	Green	72.26 ^e	5.14 ^a	32.47 ^c	15.28 ^b
C4: <i>Canna</i> lily red brilliant	Green	104.27 ^c	4.52 ^a	45.32 ^a	20.26 ^a
C5: <i>Canna flaccida</i>	Green	103.36 ^c	4.33 ^a	41.23 ^b	15.29 ^b
C6: <i>Canna paniculata</i>	Green	134.18 ^a	6.32 ^a	34.24 ^{de}	14.42 ^b

Table 3. Morphological information of different flowering parameters of different species and varieties of *Canna*.

Species and varieties	Flowering parameters				
	Flower colour	Spike length (cm)	Spike diameter (cm)	No. of flowers/spike	Petals No./spike
C1: <i>Canna variegata</i>	Orange	28.17 ^e	2.6 ^c	7.33 ^c	5+3 ^a
C2: <i>Canna indica</i> red	Orange	31.12 ^d	2.3 ^d	13.26 ^a	5+3 ^a
C3: <i>Canna</i> lily	White	43.58 ^a	2.7 ^{bc}	7.36 ^c	5+3 ^a
C4: <i>Canna</i> lily red brilliant	Red	29.24 ^e	3.0 ^a	4.33 ^d	5+3 ^a
C5: <i>Canna flaccida</i>	Yellow	40.11 ^b	2.8 ^b	5.56 ^{cd}	5+2 ^a
C6: <i>Canna paniculata</i>	Orange	34.25 ^c	1.3 ^e	11.26 ^b	3+2 ^a

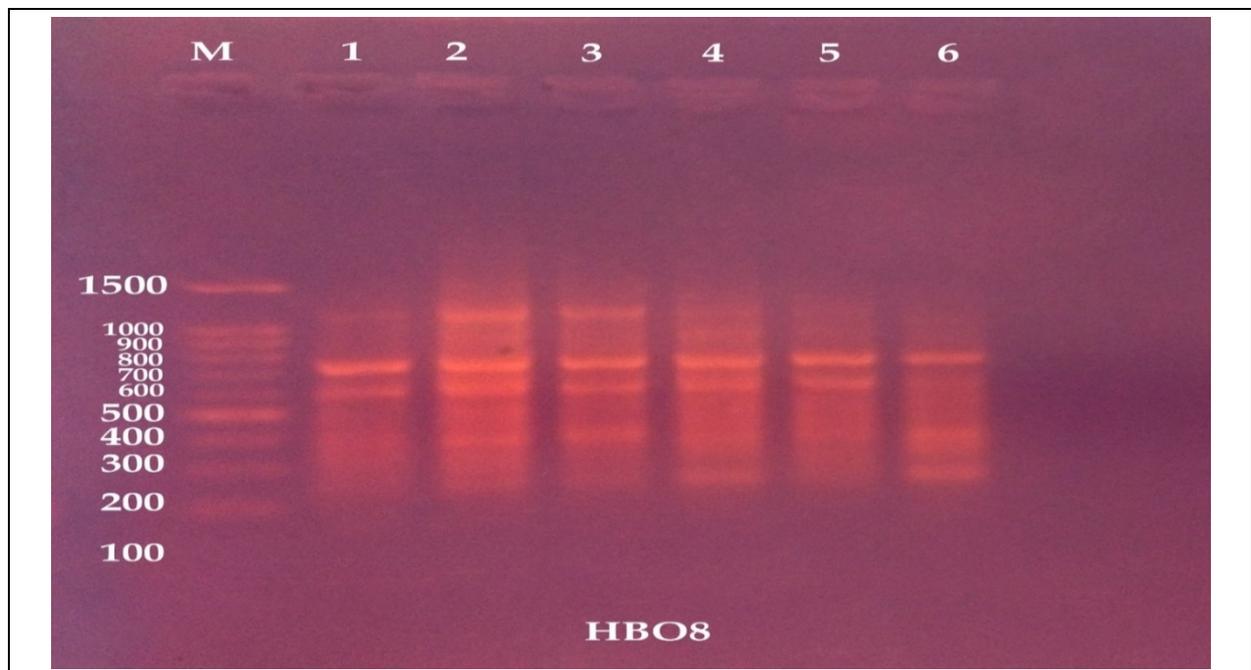


Fig. 2. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six *Canna* varieties by using the random primer HB-O8 where: lane M= marker, 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

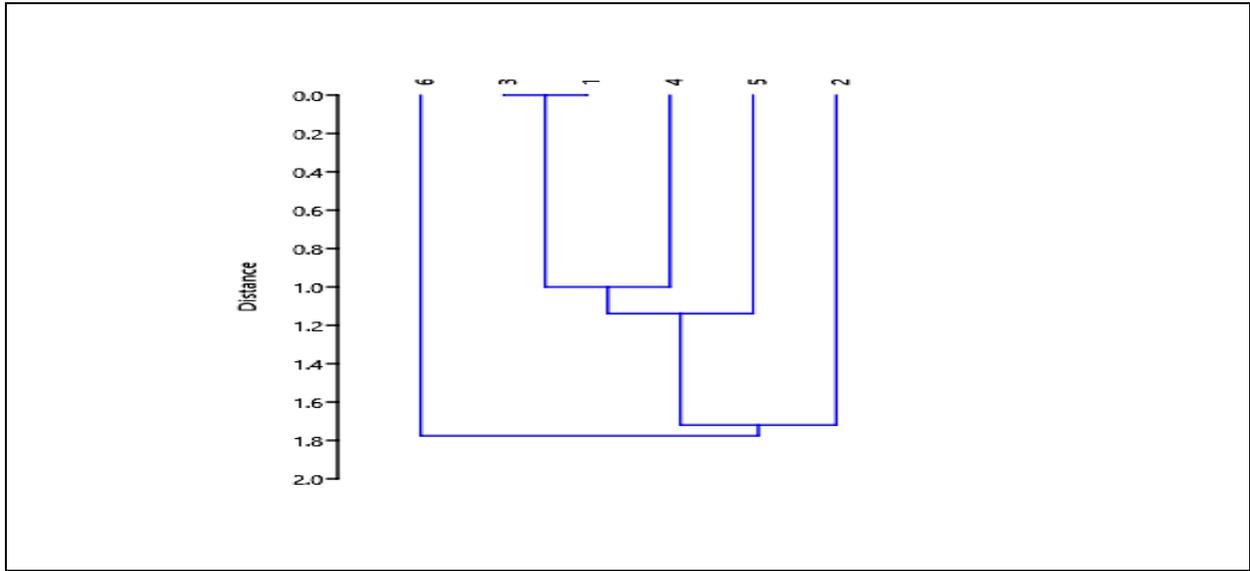


Fig. 3. Phylogenetic tree is a branching diagram used to show relationships between members of a group; a family tree. 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

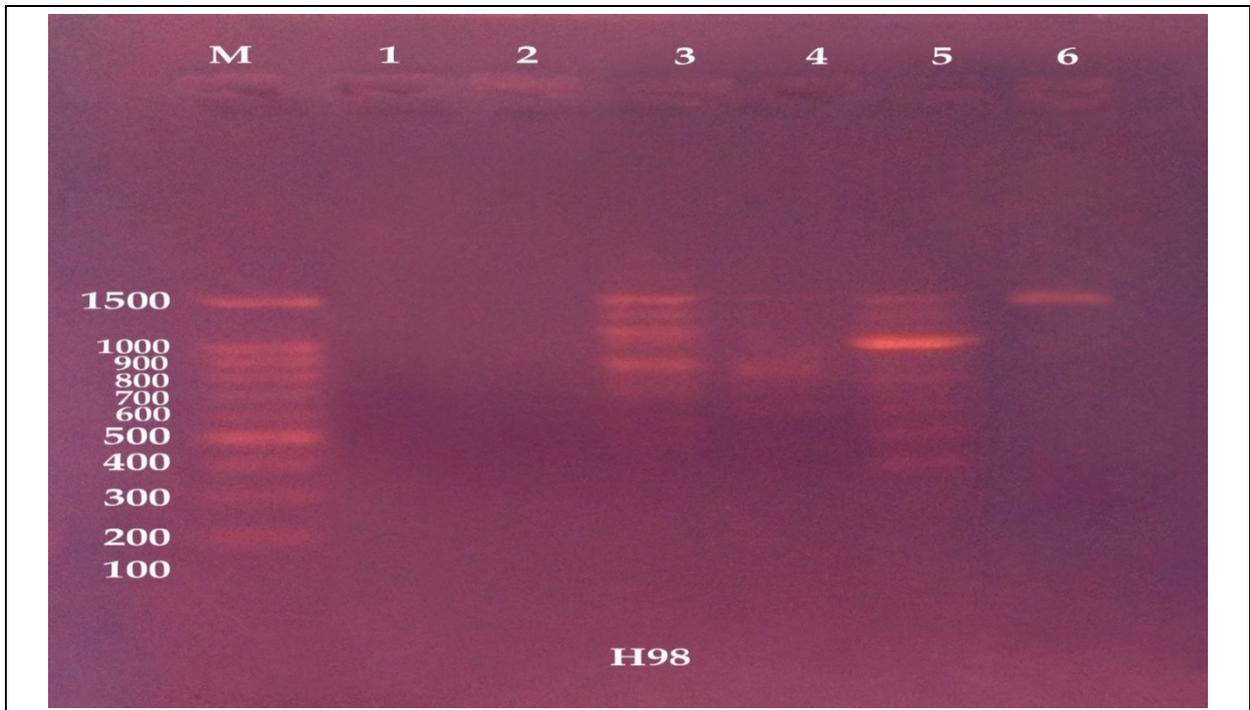


Fig. 4. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six *Canna* varieties by using the random primer HA-98 where: lane M= marker, 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

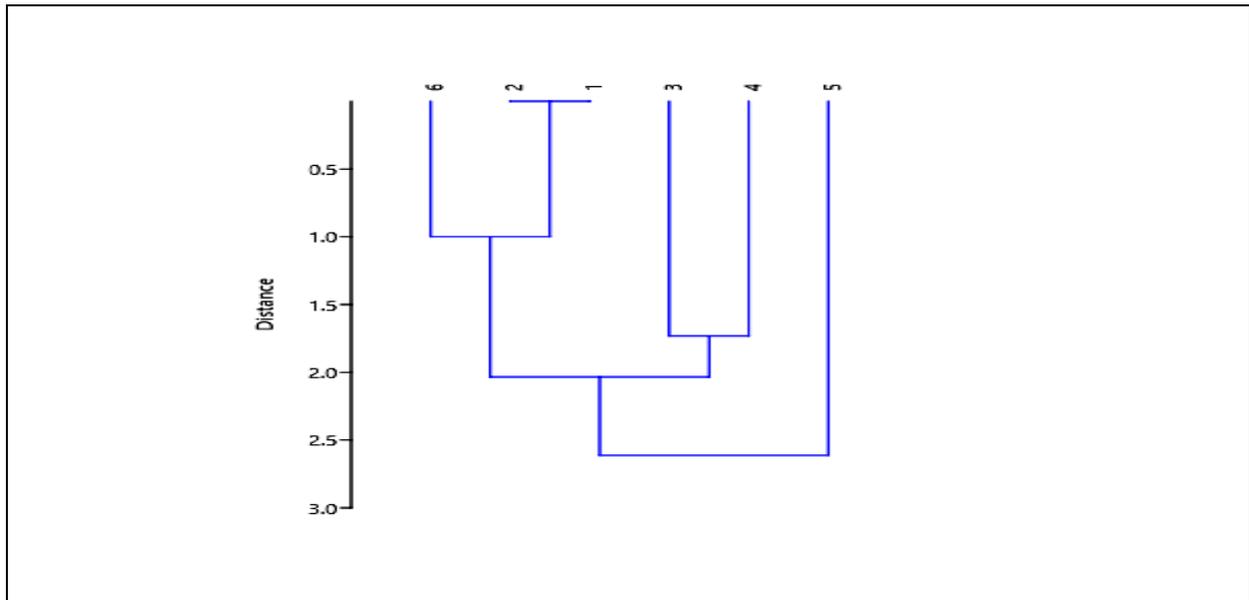


Fig. 5. Phylogenetic tree is a branching diagram used to show relationships between members of a group; a family tree. 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

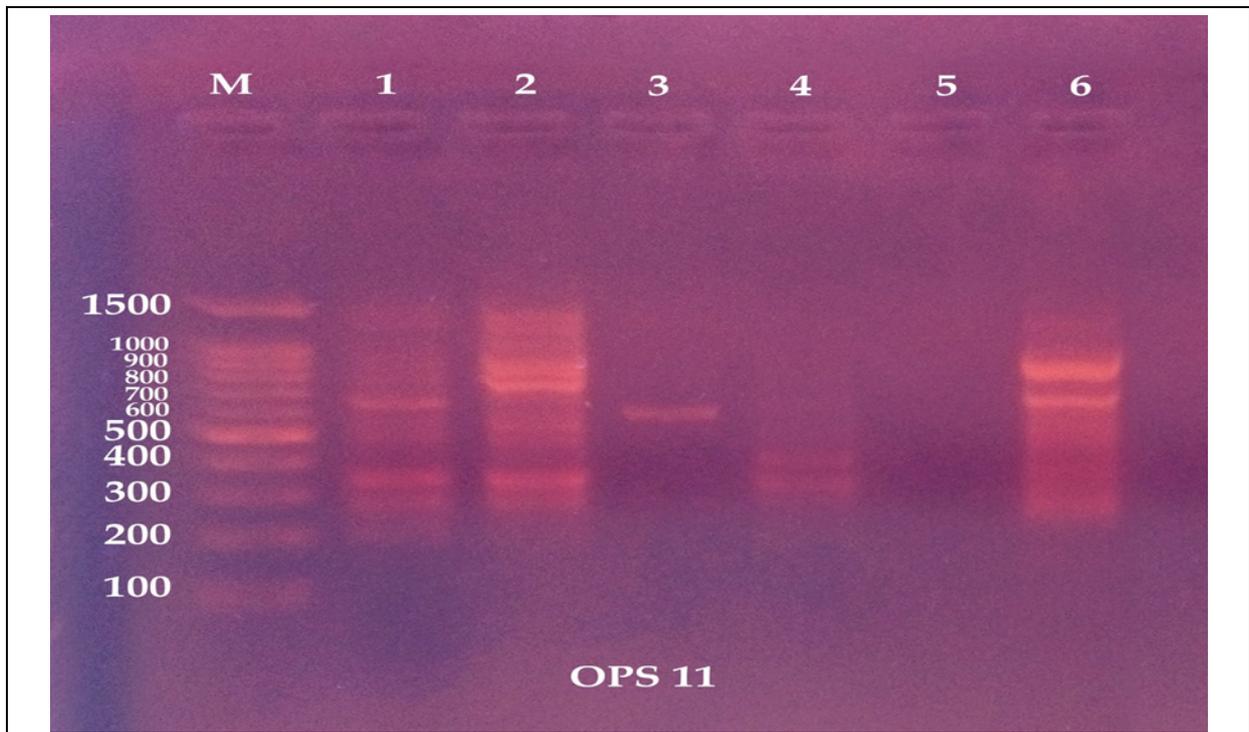


Fig. 6. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six *Canna* varieties by using the random primer OPS-11 where: lane M= marker, 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

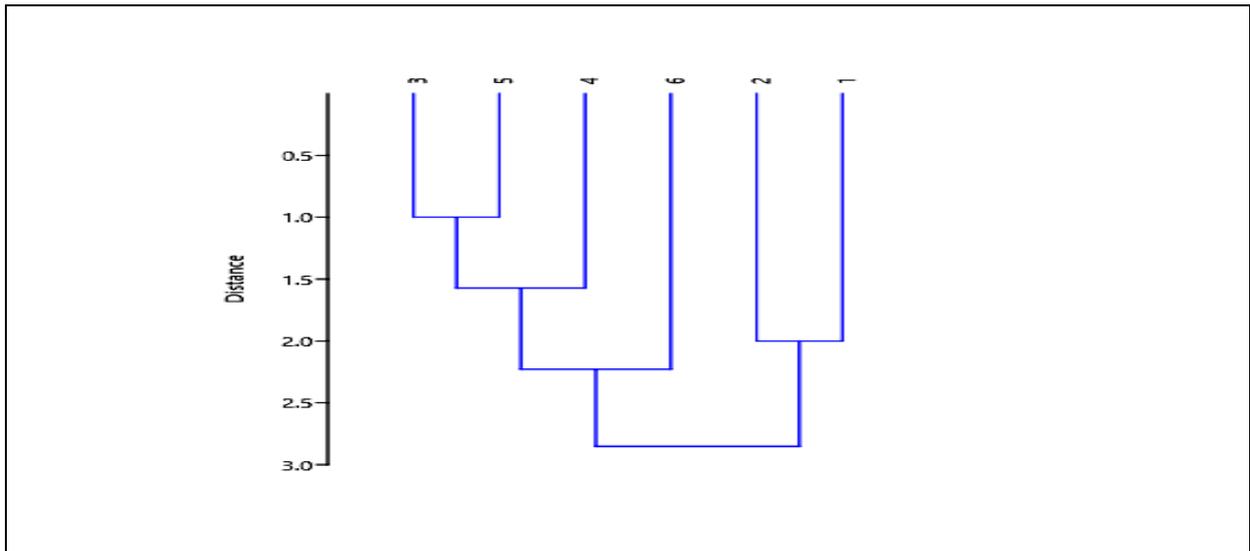


Fig. 7. Phylogenetic tree is a branching diagram used to show relationships between members of a group; a family tree. 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

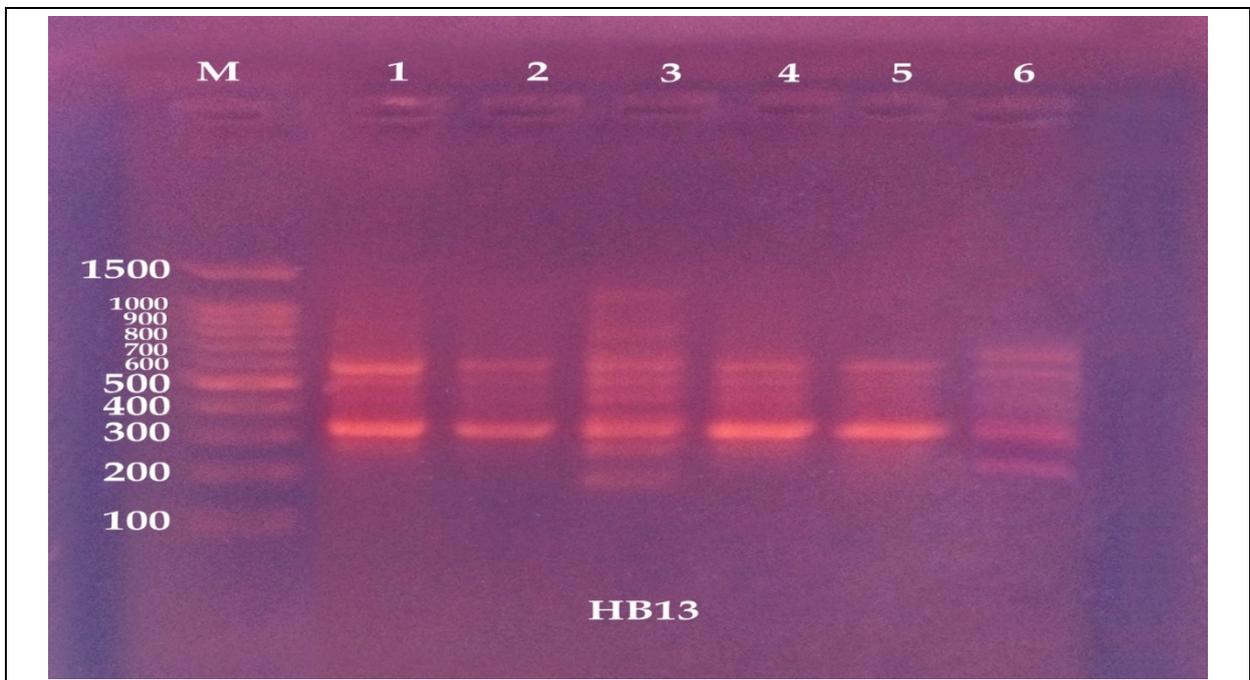


Fig. 8. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six *Canna* varieties by using the random primer HB-13 where: lane M= marker, 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

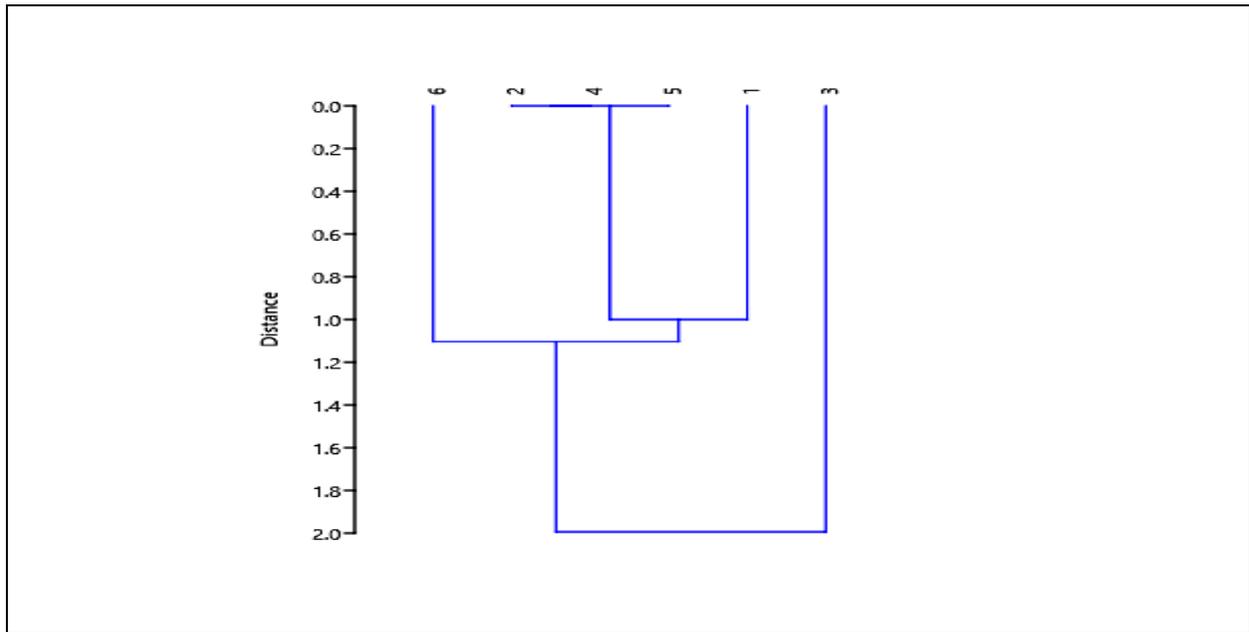


Fig. 9. Phylogenetic tree is a branching diagram used to show relationships between members of a group; a family tree. 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

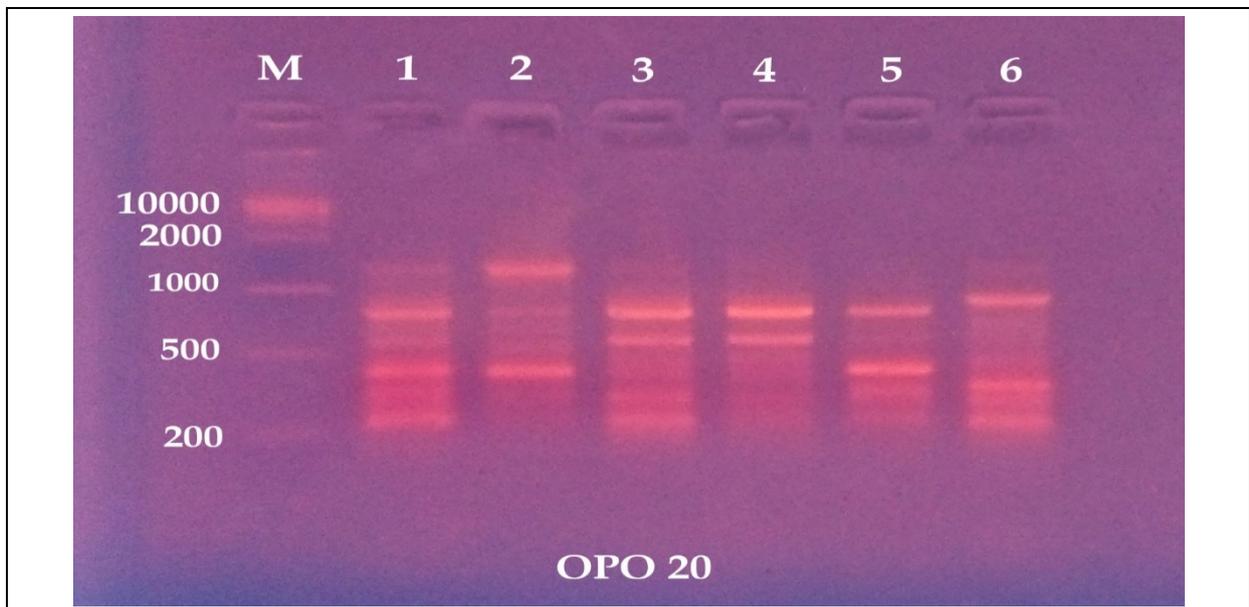


Fig. 10. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six *Canna* varieties by using the random primer OPO-20 where: lane M= marker, 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

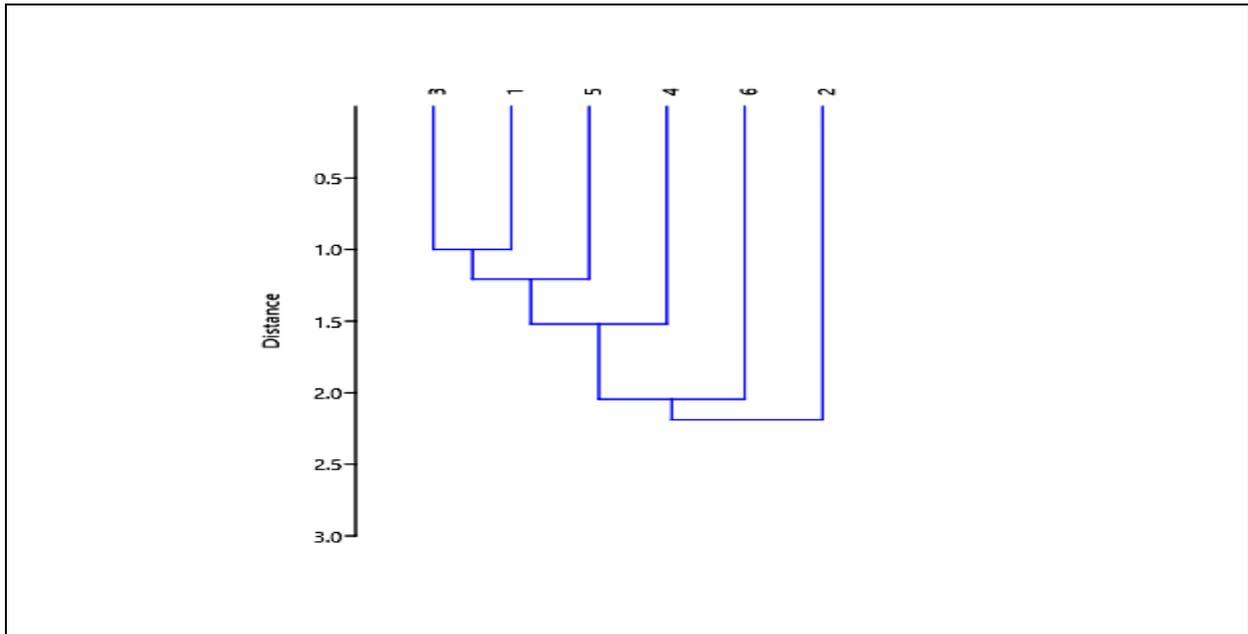


Fig. 11. Phylogenetic tree is a branching diagram used to show relationships between members of a group; a family tree. 1- C1: *Canna variegata*, 2- C2: *Canna indica* red, 3- C3: *Canna* lily, 4- C4: *Canna* lily red brilliant, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

Polygenic tree based on this primer (HB-O8) as shown in Fig. (3) revealed that the different six *Canna* varieties were divided into two major clusters. Cluster (A) included varieties *Canna variegata* (C1), *Canna indica* red (C2), *Canna* lily (C3), *Canna* lily red brilliant (C4), and *Canna flaccida* (C5). On the other side (B) included *Canna paniculata* (C6). Furthermore, cluster (A) divided into two sub clusters as shown in this Fig. from the previous results in this primer we can concluded that *Canna paniculata* (C6) was too far from the other varieties in its polygenetic relationship. Also, we can deduce that varieties *Canna variegata* (C1) and *Canna* lily (C3) in a high degree of polygenetic relationship, while the rest of varieties were in a close relationship.

Fig. (4) showed the results of HA-98 primer which contains 47.67% G+C (Table, 1), it reacted with all six different *Canna* varieties used in the present study. The length of amplified DNA fragments of the PCR products generated by this primer ranged from 400-1500 bp as shown in this figure. The number of total amplified bands, where the lowest number is zero amplified

bands in *Canna variegata* (C1) and *Canna indica* red (C2), while the highest number is nine in *Canna flaccida* (C5). The number of total amplified bands from the genomic of the tested six varieties was eleven which all were polymorphic giving 100% polymorphism.

In Fig. (5) phylogenetic tree for this primer (HA-98) divided into two major clusters A and B. The cluster A included *Canna flaccida* (C5) and the cluster B included two sub clusters (C and D), as (C) assembled *Canna* lily (C3) and *Canna* lily red brilliant (C4), while the other cluster (D) assembled varieties (C1) *Canna variegata* and (C2) *Canna indica* red and also included (C6) *Canna paniculata*.

On the other side, Fig. (6) showed the obtained results from OPS-11 primer which contains 60% G+C (Table, 1). The length of amplified DNA fragments of the PCR products generated by this primer ranged from 200-1500 bp. The total number of bands varied among all varieties except *Canna flaccida* (C5). In addition, the lowest number was one amplified band in *Canna* lily (C3), while the highest number nine in

Canna indica red (C2). The total number of amplified bands the genomic of these six varieties and 13 polymorphic bands and no monomorphic bands were obtained. So, the polymorphism percentage was 100%, and indicated that OPS-11 this primer had the highest percent of polymorphism.

Fig (7) showed the phylogenetic tree of OPS-11 primer, it revealed that the six different varieties of *Canna* were divided into two major clusters A and B. The cluster A included *Canna variegata* (C1) and *Canna indica* red (C2), while the cluster (B) was divided into two sub-clusters C and D. the sub-cluster (C) contained only the *Canna paniculata* (C6). However, the sub-cluster (D) contained varieties *Canna lily* (C3) *Canna lily* red brilliant (C4) and *Canna flaccida* (C5). It can be observed that (C3) *Canna lily* and *Canna flaccida* (C5) was in a close kinship with each other.

Results in Fig. (8), indicated that the random primer HB-13 contains 72.73% G+C (Table, 1). The length of amplified DNA fragments of the PCR products generated by this primer ranged from 200-1500 bp. The number of total bands varied among varieties, where the lowest number was four amplified bands in *Canna indica* red (C2), *Canna lily* red brilliant (C4) and *Canna flaccida* (C5), while the highest number were eight amplified bands in the *Canna lily* (C3) variety. The total numbers of amplified bands from the genomic of the previous six varieties were nine, with 5 polymorphic bands and 4 monomorphic bands giving 55.5% polymorphism percentage, which indicated that this primer had the lowest percent of polymorphism.

Phylogenetic tree represents the results of HB-13 primer (Fig., 9). It was observed that, the tree was divided into two major clusters. The cluster (A) contained (C3) *Canna lily* only. While the cluster (B) assumed the other varieties, which also divided into two sub-clusters. The sub-cluster (C) contained the following four varieties *Canna variegata* (C1), *Canna indica* red (C2), *Canna lily* red brilliant (C4) and *Canna flaccida* (C5).

These results revealed that the varieties (C2) *Canna indica* red, *Canna lily* red (C4) and *Canna flaccida* (C5) were closed together.

The obtained results in Fig. (10), revealed that the random primer OPO-20 contains 60% G+C (Table, 1). The length of amplified DNA fragments of the PCR products generated by this primer ranged from 200 bp to 1500 bp. The total number of bands varied among all examined varieties. On the other hand, lowest number were five amplified bands in these varieties *Canna lily* (C3), *Canna lily* red brilliant (C4), *Canna flaccida* (C5) and *Canna paniculata* (C6), while the highest number were six amplified bands in the *Canna variegata* (C1) and *Canna indica* red (C2). The total numbers of amplified bands from the genomic of the previous six varieties were eight bands, where seven bands of them were polymorphic and only one monomorphic band.

Fig. (11) showed phylogenetic tree based on OPO-20 primer, it presented that the tree divided into two major cluster. Cluster A included only *Canna indica* red (C2), while the other varieties were found in the two sub clusters of cluster B as shown in the upward. One of this sub clusters *Canna variegata* (C1) and *Canna lily* (C3) were joined together in a cluster which means that they are the nearest varieties in polymorphic relationship. The first one was *Canna indica* red (C2).

Table (4) summarized the total number of amplified bands, polymorphic bands and monomorphic bands as well as the percentage of polymorphism by using two RAPD and three ISSR primers. As shown in this Table, the obtained results revealed that all used primers produced a total of 49 amplified bands, which most of them were polymorphic bands with 83.6% percentage of polymorphism. These results also demonstrated that the presence of three different amplified unique bands in the genome *Canna* used as molecular markers to identify each one of these *Canna* varieties. On the other hand, a total of 49 major

scorable bands ranging from 200 to 1500 bp were generated from two RAPD and three ISSR primers showing 83.6% polymorphism.

As shown in Table (4), the numbers of the amplified bands varied for 8 HB-O8 and 13 OPS-11 bands primer, respectively, with total of 49 bands. On the other side, DNA lengths ranged from 200 to 1500 bp for all varieties of *Canna*. It was recorded that the maximum number of the polymorphic bands (13) for primer OPS-11 with percentage polymorphism 100%. On the contrary, the primer HB-13 exhibited the minimum number of the polymorphic bands (five) and also the minimum percent of polymorphism (55.5) similar results was reported by Piyachomkwan *et al.* (2002).

Similarity coefficient of the six *Canna* varieties ranged from 3.316 to 5.099. It was found that, the lowest similarity degree (3.31) was between *Canna flaccida* (C5) and *Canna lily red brilliant* (C4) brilliant, while the highest 5.099 correlation was recorded between *Canna lily* (C3) and *Canna indica red* (C2) and also between *Canna flaccida* (C5) and *Canna indica red* (C2) (Table 5).

In the present study, UPGMA analyses were performed, and dendrograms were constructed. Based on RAPD, ISSR and combined data, two similar clusters with variable percentages of similarity were detected.

Rajaseger *et al.* (1997) analyzed the genetic diversity among 22 cultivars of *Ixora* through RAPD and found DNA markers useful in distinctly grouped under two clusters. All 22 cultivars distinctly grouped under two cultivars groups, viz., *Ixora coccinea* and *I. javanica*. The study indicated that besides the use of RAPD makers for identification of particular *Ixora* cultivars, the phylogenetic relationships generated by RAPD analysis might be useful for varietal improvement programs. It was observed in the present study that it was possible to segregate the used varieties of *Canna* through RAPD and ISSR markers using 5 random primers.

Five random decamer oligonucleotide primers were used for RAPD and ISSR analysis. The dendrogram constructed on the basis of the obtained data from both RAPD and ISSR analysis is represented in Fig. (12).

Table 4. Total number of amplified fragments and number of polymorphic fragments generated by PCR using selected RAPD and ISSR primers.

Primer	Total number of bands	The length of amplified DNA	Monomorphic bands	Polymorphic bands	Unique bands	polymorphism %
HB-O8	8	200-1500	3	5	0	62.5
HA-98	11	400-1500	0	11	1	100
OPS-11	13	200-1500	0	13	0	100
HB-13	9	200-1500	4	5	2	55.5
OPO-20	8	200-1500	1	7	0	87.5
Total	49	-	8	41	3	83.6

Table 5. Similarity based upon the genetic distance among the different six *Canna* varieties by Euclidean for 5 random Primers of RAPD and ISSR.

<i>Canna</i> varieties	C1	C2	C3	C4	C5	C6
C1: <i>Canna variegata</i>	0					
C2: <i>Canna indica red</i>	3.464	0				
C3: <i>Canna lily</i>	4.242	5.099	0			
C4: <i>Canna lily red brilliant</i>	4.358	4.582	3.605	0		
C5: <i>Canna flaccida</i>	4.472	5.099	3.464	3.316	0	
C6: <i>Canna paniculata</i>	3.605	4.358	4.582	4.472	4.358	0

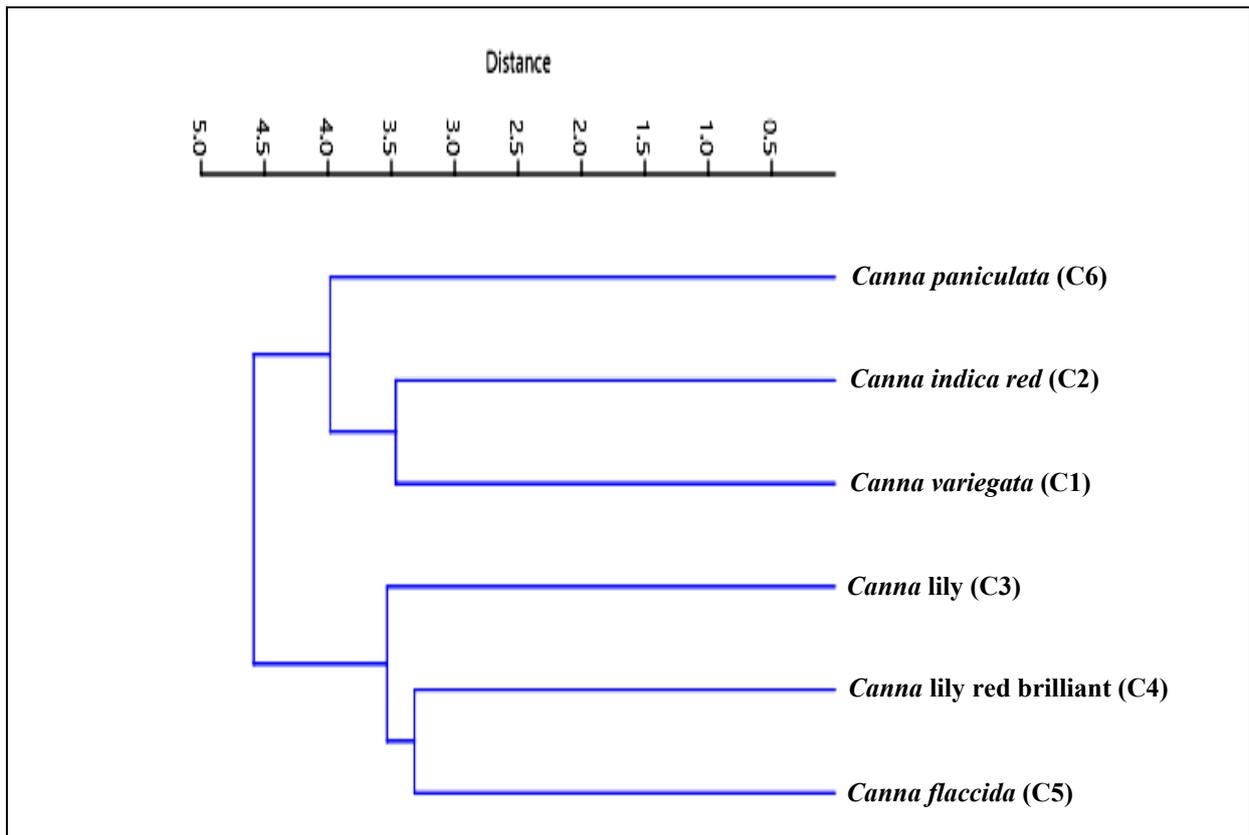


Fig. 12. Phylogenetic tree is a branching diagram used to show relationships between members of a group; a family tree. 1- C1: *Canna variegata*, 2- C2: *Canna indica red*, 3- C3: *Canna lily*, 4- C4: *Canna lily red brilliant*, 5- C5: *Canna flaccida*, and 6- C6: *Canna paniculata*.

Furthermore, dendrogram showed two similar clusters with variable percentage of similarity. It can be concluded that, cluster (A) was assumed *Canna variegata* (C1) and *Canna indica red* (C2) in sub cluster (C), in addition, this cluster had *Canna paniculata* (C6) in subcluster (D). It was observed that all these cultivars had same flower colour which were orange. However, they were completely different in leaves colours. So, it can be detected that varieties of *Canna* with orange flowers can be grouped together in large cluster. It was hypothesized that the group of these cultivars were clustered together to form a somaclone complex, which might have originated from somatic mutation of cultivars (Ude *et al.*, 2003). These findings were in accordance with Patra *et al.* (2008), where morphologic characters were focused in discussing the

molecular variability of different cultivars of *Canna*. In this study, morphological character was considered for analyzing the genetic variability.

On the other side, cluster (B) was assumed *Canna flaccida* (C5) and *Canna lily red brilliant* (C4) in sub cluster (E) with green leaves, yellow flower and red flower respectively. It also concluded *Canna lily* with green leaves and white flower which was represented in subcluster (F). It can be observed that, in this cluster (B), all varieties shared with green leaves with different flower colours.

Most of these obtained results of phylogenetic tree were corresponded with Piyachomkwan *et al.* (2002), who mentioned that high level of genetic similarity was found between indigenous of *Canna edulis*.

It can be concluded that the varieties cannot be segregated from somaclone complex which based on plant height, flower colour and shape, but also some intra-specific genome relationship might be considered (Mishra *et al.*, 2012).

The inter-relationship obtained from RAPD and ISSR analysis in *Canna* were in agreement with the findings of Loh *et al.* (1999) who made AFLP analysis of two species and 7 cultivars of *Caladium* and inferred that closely related species can clearly be differentiated and the genetic difference between cultivars can be also established. Furthermore, the findings in the present study were in agreement with the results of Loh *et al.* (1999) who indicated that, at intra-specific level, two preliminary clusters could be identified in *Caladium bicolor*, one group contains the “fancy leaf” cultivars and the other group with “Strap leaf” cultivars. The apparent explanation offered was that in *Caladium*, difference in leaf shape might be due to allelic difference indicative of the fact that the dichotomy between fancy leaf and at Strap leaf may not be a very strong taxonomic character. Same inference may be

drawn as to the flower colour in *Canna* and the reason for varieties having same flower colour came together in the same cluster, and also the varieties having same leaves colour came together in the other cluster.

In nature, *Canna* seems to have been evolved essentially by gene mutation and re-patterning of chromosomes. Man’s interference led to recombination emanating from inter-specific hybridization, somatic mutation and triploid has speeded up the process of evolution (Khoshoo, 1979; Khoshoo and Mukherjee, 1970). The natural variability was also widening by spontaneously occurring mutations although the rate of such occurrence is very low (Patra *et al.*, 2008).

This investigation demonstrated that both of RAPD and ISSR offers a suitable means for detecting genetic diversity in *Canna*.

From the previous figure, it can be represented that *Canna indica* red (C2) and *Canna lily* (C3) were in a high polymorphic relationship (Fig., 13). Also, *Canna paniculata* (C6) had the lowest relationship compared with the other used varieties.

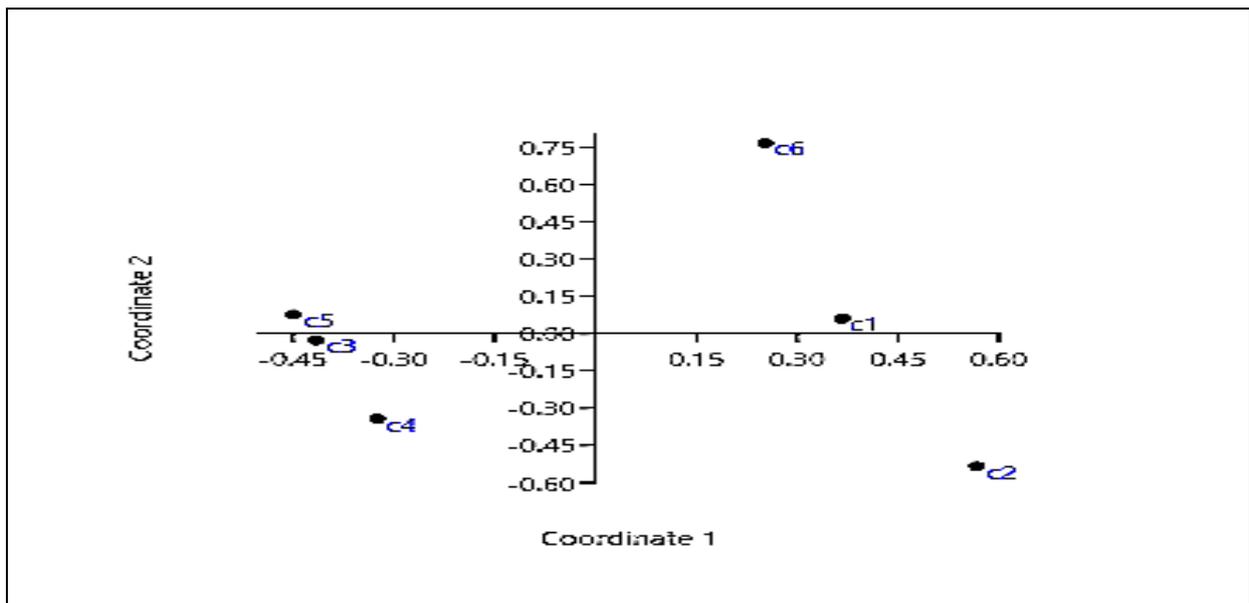


Fig. 13. Principal coordinate analysis (2-dimensional plot) of six varieties of *Canna* based on combined RAPD and ISSR analysis data.

CONCLUSION

It can be deduced that a total of 49 major scorable bands ranging from 200 to 1500 bp were generated from two RAPD and three ISSR primers showing 83.6% polymorphism. In addition, it was observed that *Canna paniculata* (C6) always in a far correlation with the other varieties used in this study. On the other hand, *Canna lily* (C3) and *Canna indica* red brilliant (C2) had the highest correlation (5.09), also *Canna flaccida* (C5) and *Canna indica* (C2) gave the same results. While, the lowest similarity (3.316) was observed between *Canna lily* red brilliant (C2) and *Canna flaccida* (C5). The dendrogram showed that the used primers divided the used *Canna* varieties into two groups according to the leaves colours and the flowers colours.

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القرابة الوراثية بين أصناف وأنواع مختلفة من نبات الكنا باستخدام الواسمات الجزيئية

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تم إجراء هذا البحث على ٦ أصناف من نبات الكنا (*Canna*) تتبع ٦ أنواع مختلفة لمعرفة صلة القرابة بينهم وذلك باستخدام تكتيك RAPD وISSR. تم اختيار الأصناف (الأنواع) بناء على الاختلاف في الشكل المظهري لألوان الأزهار وكذلك ألوان الأوراق. وكانت الأصناف الستة المستخدمة في الدراسة هي *Canna variegata*, *Canna indica red*, *Canna paniculata* و *Canna flaccida*, *Canna lily*, *Canna lily red brilliant* جزيئية (اثنتان من نظام RAPD وثلاثة من نظام ISSR) علي الأصناف الستة. وكانت النتائج كما يلي: وجد مجموع الحزم (Bands) ٤٩ حزمة تراوحت بين ٢٠٠ الي ١٥٠٠ bp بإجمالي نسبة تباين وراثي بلغت ٨٣,٦٪. وقد سجلت أعلى صلة قرابة (٥,٠٩) بين الصنفين (*C2*) و *C. indica red* (*C3*) و *Canna lily* أيضاً بين (*C2*) و *Canna indica red* (*C5*) و *Canna flaccida*. في حين أن أقل تشابه أو قرابة كانت بين (*C4*) و *Canna lily red brilliant* و *Canna flaccida* (*C5*) وقد سجلت (٣,٣١٦). أوضحت شجرة صلة القرابة أن صنف *Canna paniculata* كان دائماً بعيداً جداً عن باقي الأصناف المختبرة. وقد لوحظ أن البادئات الجزيئية المستخدمة قسمت الأصناف إلى مجموعتين رئيسيتين لشجرة القرابة الوراثية احدهما تضم مجموعة الأصناف ذات لون الورقة الواحد والمجموعة الثانية من الشجرة تضم مجموعة الأصناف ذات لون الزهرة الواحد. وتقسيم تلك الأصناف تم طبقاً للنظامين ولبادئاتهم الجزيئية المستخدمة في تلك الدراسة، والتي ربما تعطى نتائج مختلفة لو استخدمت أنظمة أخرى وبادئات متباينة.