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PHYLOGENETIC RELATIONSHIP AMONG DIFFERENT CULTIVARS OF CROTON (*CODIAEM VARIEGATUM* (L.) A. JUSS.) USING MOLECULAR MARKERS

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ABSTRACT: Croton (*Codiaeum variegatum* (L.) A. Juss.) is one of the Euphorbiaceae family, and represents a group of ornamental plants. This species possesses a wide range of variations in colour and leaf shape. In the present study, six cultivars of croton were used to assess the genetic relationship among them by using ISSR and RAPD techniques. The results generated 53 total bands from three ISSR and RAPD primers. Also, it ranged from 200 to 2000 bp. with 92.4% polymorphism. The relationship between the used cultivars showed that (5.48) considered the highest relationship between *Codiaeum variegatum* 'Ptra' (C3) and *Codiaeum variegatum* 'Victoria Gold Bell' (C1). However, the lowest similarity (1.57) was recorded between *Codiaeum variegatum* 'Victoria Gold Bell' and 'Yellow Lecton' (C6). The dendrogram depended on the achieved results which were generated from ISSR and RAPD primers. It divided the plants according to the leaf color with ignorance of leaf shape and size. It grouped 'Ptra' (C3) and 'Victoria Gold Bell' (C1) together in one group, but in the second group, the primers grouped 'Sunny Star' (C2), 'Gold Dust' (C5), var. *pictum* (C4) and 'Yellow Lecton' (C6) together. It should be mentioned that it can obtain different results in the dendrogram when using another molecular marker system.

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INTRODUCTION

Croton (*Codiaeum variegatum* (L). A. Juss.) is famous of its varied leaf colour and shape. *Codiaeum* is one of the genera in the Euphorbiaceae family. There are many species in this genus. Malaccan Island is the mother nation for this plant, and also it extends widely to several areas of Indonesia, India, Philippines, Thailand, Malaysia, Sri Lanka and other countries in the Pacific Islands (Magdalite *et al.*, 2014; Deng *et al*., 2010). There are over 300 croton cultivars according to Mollick and Yamasaki (2012). Croton can be used in landscape designs. It can also be considered a medicinal plant for gastric ulcers (Rahman and Akter, 2013). It was known that these leaf shape variations and coloration have interested growers, horticulturists and gardeners, and landscape designers, also many cultivars were chosen for advertising production (Chen and Stamps, 2006). Some scientists have classified croton according to its leaf shape and color. So that the diverseness in croton's leaf shape is wide. Leaves are different in shape including linear to ovate-shaped or appendiculate which are connected by midrib in the middle, entire to deeply lobed. Also, a prominent characteristic in croton is coloration and pattern of color on the leaves which distiniguish each cultivar.

On the other side, the diversity of phenotypic that noticed in the leaves of croton is very exciting in plant science, due to many types

of leaf morphology can be found in the same species, also leaf phenotype is excessive of the plasticity (Shimoji *et al*., 2006). It is still obscure for these high leaf diversity that genetic unreliability is affiliated with the diversity of leaf phenotype. Bron (1995) mentioned that cross pollination by ants and somatic mutations may help to explain the mechanism of the occurrence of this wide range of diversity. Others mentioned that the variability in chromosomes might be described as the clear variation in morphology of *C. variegatum* (Ogunwenmo *et al.*, 2007). Furthermore, the diversity in morphology among the different cultivars of this species may be ascribed to a wide range of variation in karyotypes and chromosome number (Deng *et al*. 2010 a). Newly, it was noticed the genetic kinship of *C. variegatum* cultivars by using AFLP markers, and it was concluded that there was genetically highly polymorphic between croton cultivars (Deng *et al*., 2010 b). There were horticultural investigations to describe the flexibility, because of these manifestations of basic biology, and also, the response of fertilizers' effectiveness on growth (Chase and Polle, 1989), micropropagation processes (Marconi and Radice, 1997). Furthermore, the effect of the growth hormones on axillary shoot production (Orlikowska *et al*., 2000). Finally, the *in vitro* propagation (Nasib *et al*., 2007).

It is essential to establish a systematic classification for distinguishing the cultivars to clarify the mechanism for high phenotypic diversity. For the identification and classification of croton cultivars, parameters of the leaf have been used as a traditional method. Not exclusively but one scientist (Brown, 1995) categorized cultivars of croton into 9 groups depending on leaf types: (1) small leaf, (2) spiral leaf, (3) oak leaf, (4) semi-oak leaf, (5) narrow leaf, (6) very narrow leaf, (7) broad leaf, (8) recurved leaf, and (9) interrupted leaf. It was found that classification depending on leaf types is insufficient. Also, one parameter is not enough to give back all characterization of phenotype dependens on the numeric basis, and it has situsual instrument to cultivars classification and identification (Revilla and Tracy, 1995; Rakonjac *et al* ., 2010).

Falcioni *et al.* (2023) demonstrated the advantage of combining magnification and fluorescence procedures which identify with vegetation indexes, and pigment profiling for appreciating the complex biochemical, physiological and biophysical changes of *Codiaeum variegatum* (L.) A. Juss. in variegated leaves. Their discoveries detect new attitudes in this field, and also, they can help to classify the dissimilar phases of colored leaves by using the JIP test, which may support them with vegetative indicators.

They also mentioned that the combining of hyperspectral and fluorescence instruments has improved their information for physical parameters of the plant and contracts remarkable potency for plant ecology research in future, and visual spectroscopic analysis(Falcioni *et al*., 2023).

Furthermore, morphological markers are tried of the observed number, also some characteristics of plant growth are expressed late. On the other hand, morphological markers can be influeneced by other morphological marks or characteristics of attentiveness ascribed to pleiotrophic the activity of genes (Andersen and Lubberstedt, 2003). A stable marker is needed with these limitations. In addition, molecular markers are more fixed than morphological markers. It might be used to examine plant genetics, permitting to obtain genes which control target characters quickly and also exactly.

William *et al*. (1990) reported that in genetic mapping, DNA fingerprinting, RAPD markers can be used, especially in population genetics studies.

So, there were different methods to classify croton (*Codiaeum variegatum* L.). This research was carried out to assess the genetic diversity of some croton cultivars (*Codiaeum variegatum*) in Egypt using RAPD and ISSR primers. So, the target of this investigation was to use a scientific basis for reporting the diverseness of the high leaf phenotype in croton.

MATERIALS AND METHODS

Morphological analysis:

Six different varieties of Croton were collected from a nursery in Alexandria (Egypt). The demonstration was executed through only one season of 2023 at the Faculty of Agriculture, Alexandria University, Egypt.

The six croton cultivars which examined in this investigation were:

Codiaeum variegatum 'Victoria Gold Bell' (C1), *Codiaeum variegatum* 'Sunny Star' (C2), *Codiaeum variegatum* 'Ptra' (C3), *Codiaeum variegatum* var. *pictum* (C4), *Codiaeum variegatum* 'Gold Dust' (C5) and *Codiaeum variegatum* 'Yellow Lecton' (C6) as shown in Figs. (1 and 2).

The experiment layout was designed as a complete randomized design (CRD) with 3 replicates. Three plants were analyzed in each replicate. Morphological markers were observed to distinguish the different croton cultivars. The shape and the color of the leaf and also the size of the leaf were the main measurements.

Molecular diversity studies:

This study was executed in the laboratory of molecular biology in the Faculty of Agriculture, Alexandria University, Egypt.

It used ISSR and RAPD PCR as molecular markers to conduct the genetic study. Three primers of ISSR and three of RAPD (Table, 1), were used to differentiate between the 6 croton cultivars used in this study.

Plant materials:

Three samples of young leaves for each cultivar were analysed, moist tissue papers were used to wrap these leaves between them and then kept in the refrigerator at 50 ˚C till the extract of DNA. To determine the DNA purity and concentration, a NanoDrop spectrophotometer was used. The ratio of 260/280 nm was found 108-109, indicating that the DNA extract was pure enough for RAPD analysis.

Each polymerase chain reaction consisted of 2 ml of primer, 10 ml master mix and 50 ng of genomic DNA. In a Bimetra, PCR amplification was performed, T1 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 bp DNA ladder. Also, by using a gel analyzer program, RAPD fragments were scored as present/absent. Clustering and similarity coefficients were estimated according to Rohlf (1998).

For fingerprinting data examination, each polymorphic band was considered as bring character and was scored as (0) for absence, but (1) for presence to each sample. It is collected in data matrix. According to the mathematical tools of Nie (1972) the similarity of matrix was caluclated to examine the genetic relationship among the above cultivars. To generate the dendrograms, UPGMA was used to express the similarity. In addition, the popgene 32 software package was used in the analysis (Yeh *et al.*, 1997). Furthermore, the correspondence analysis of the right vector from the paired data was outright by reason of graphically outlined relations on the interior of cultivars. This scanning was executed through a batch file by using the software package NTSYS pc (version 20) Rohlf (1998).

RESULTS AND DISCUSSION

The observed morphology data recorded on the different croton cultivars are shown in Table (2).

Because of the deficiency of details in the sector of molecular characterization of genetic variability in cultivated *Codiaeum variegatum* in Egypt, this investigation was initiated implying six different cultivars of croton. Among these 6 cultivars, molecular markers such as ISSR and RAPD primers

***Codiaeum variegatum* 'Victoria Gold Bell' (C1)**

***Codiaeum variegatum* 'Sunny Star' (C2)**

***Codiaeum variegatum* 'Ptra' (C3)**

***Codiaeum variegatum* var. *pictum* (C4)**

***Codiaeum variegatum* 'Gold Dust' (C5)**

Codiaeum variegatum 'Yellow Lecton' (C6).

Fig. 1. *Codiaeum variegatum* varieties used in this study.

Fig. 2. Leaf diversity of *Codiaeum variegatum* varieties used in this study.

Table 1. The used ISSR and RAPD, sequences and annealing temperature, also guanine and cytosine content percentage.

Primer number	Primer code	Sequences	CG%	Tm (°C) Value
1. RAPD	OPS 11	5´- AAA GTC GC GG-3´	60.00	32.00
2. RAPD	OPS 12	5´- TGT CAT CCCC-3´	60.00	32.00
3. RAPD	OPS 13	5´- AAGCCTCGTC-3´	60.00	32.00
4. ISSR	HB- 12	5´- CACCACCACGC-3´	72.73	38.00
5. ISSR	HA- 99	5´- CACACACACAAG-3´	50.00	42.00
6. ISSR	OPO 20	5´- AACCCGGTC-3´	60.00	32.00

Tm= temperature of melting
GC= guanine and cytosine ration (percentage)

Table 2. Morphological observation of different vegetative parameters of the used cultivars of croton.

Cultivars	Leaf color and shape	
***Codiaeum variegatum* Victoria 'Gold Bell' (C1)**	Long thin green leaf with red color in the middle	
***Codiaeum variegatum* 'Sunny Star' (C2)**	Wide green leaf with linear yellow color and sharp edge	
***Codiaeum variegatum* 'Ptra' (C3)**	long wide red green leaf with shape edge	
***Codiaeum variegatum var. pictum* (C4)**	Small green leaf spotted with yeallo color	
***Codiaeum variegatum* 'Gold Dust' (C5)**	Long thin green spotted with yellow	
***Codiaeum variegatum* 'Yellow Lecton' (C6)**	Long wide green leaf with yellow eadges	

were used to estimate the genome relationship between them.

In this investigation, DNA molecular analyses (ISSR and RAPD) were used to evaluate the genetic diversity and the relationship within the six different cultivars of croton.

Fig. (3) showed the obtained results by using OPS11, with 60% G+C (Table, 1). It was observed that the amplified DNA fragments length generated from this primer ranged from 200-2000 bp. C6 'Yellow Lecton' has no amplified bands. It was recorded that C1 'Victoria Gold Bell' has the highest number of the amplified bands which were obtained in C5 'Gold Dust'. It was obtained eleven polymorphic bands as a total number of amplified bands generated from their genomic. As a result, the polymorphism percentage was 100% (Table, 3) so all cultivars were polymorphic.

Fig. (4) represents the phylogenic tree of OPS11 primer, it is cleared that the 6 used cultivars of *Codieaum variegatum* were split into two great clusters A and B. The cluster A included 'Yellow Lecton' (C6) only, while the cluster B was split into two sub-clusters C and D. Sub-cluster C holds C2 'Sunny Star', C4 var. *pictum* and C5 'Gold Dust. It noticed that C4 var. *pictum* and C5 ' Gold Dust' were in a close relationship with each other. In addition, sub-cluster D contained C3 'Ptra' and C1 'Victoria Gold Bell' which were joined together.

The existing results in Fig. (5), disclosed that the random primer OPS12 with 60% G+C (Table, 1). It was found that, the amplified DNA fragments ranged from 200-900 bp by using this primer C1 'Victroia Gold Bell' has recorded the lowest number of amplified bounds which were two. While C5 'Gold Dust' recorded five amplified bands which were the highest ones. Table (3) concluded that the total number of amplified bands generated from the used cultivars was 6. Five polymorphic bands were found and one monomorphic, giving 83.3% polymorphism. Fig. (6) shows the obtained results of phylogenic tree of OPS12. It was noticed that phylogenic tree was split into A and B as major clusters. In addition, cluster A split into two sub-clusters C and D. Sub-cluster C contained four cultivars of *C. variegatum* every 2 cultivars joined with each other such as, C2 'Sunnay Star' and C4 var. *pictum* joined together and also, C3 'Ptra' and C5 'Gold Dust' joined together. These results mean that all of the four cultivars are in polymorphic relationship but every two cultivars joined together has a nearest kindship. On the other hand, culster D contained only C6 'Yellow Lecton'. Furthermore, cluster B contained only C1 'Victoria Gold Bell'.

Fig. (7) cleared the obtained results by using OPS13 primer with 60% G + C (Table, 1).

Amplified DNA fragment length which resulted from OPS13 primer ranged from 200-1000 bp. It was observed that cultivars C1 'Victoria Gold Bell' and C2 'Sunny Star' have no bands at all with this primer. Also, C3 'Ptra' recorded two amplified bands which are considered the lowest, but the highest number was five which was recorded in both C5 'Gold Dust' and C6 'Yellow Lecton'.

Table (3) shows that the total amplified bands number which results from the tested cultivars was 6, all of them were polymorphic giving 100% polymorphism. Fig. (8) shows the phylogenic tree of OPS13 primer, it was necessary to mention that there was a complete absence of C1 'Victoria Gold Bell' in the tree which means that this primer cannot join with C1 'Victoria Gold Bell'. It was noticed that the tree was split into A and B as great two clusters. Cluster A was split into two sub-culsters C and D. Sub-cluster C contained C3 'Ptra' but sub-cluster D contained C4 var. *pictum*, C5 'Gold Dust' and C6 'Yellow Lecton'. It was found that C5 and C6 are joined together which revealed that they are in close kinship. On the other side, cluster B contained C2 'Sunny Star'. The absence or presence of bands might specify the incident of genetic changes in the hybrids'

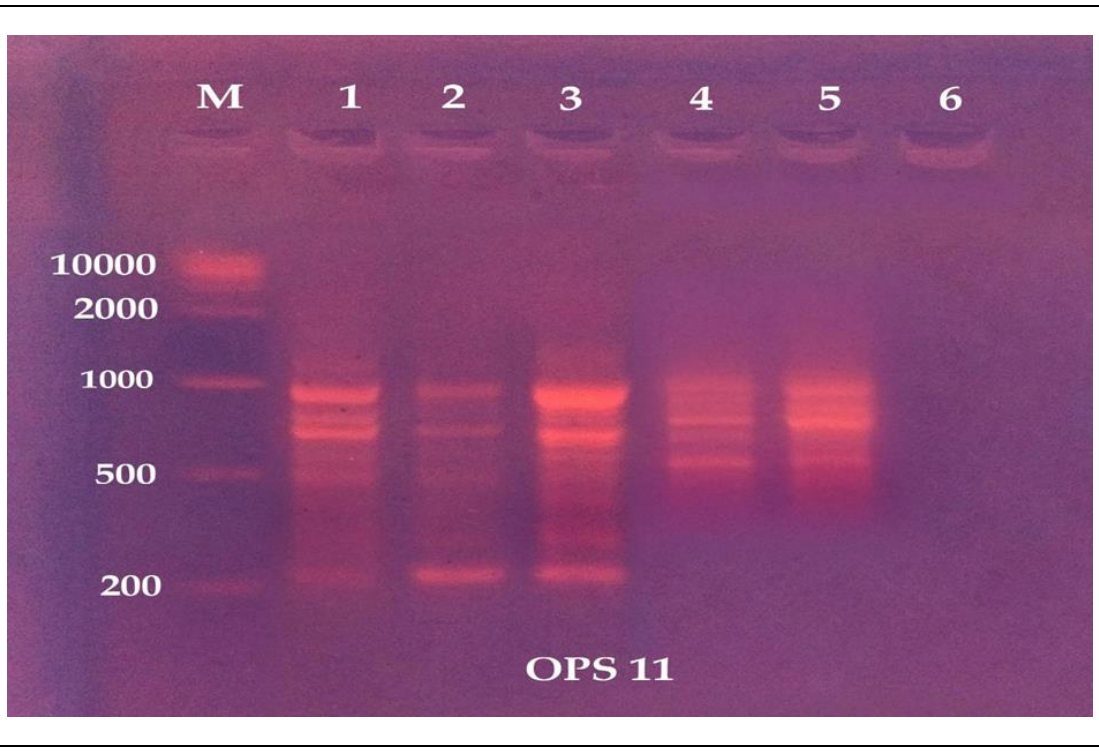

Fig. 3. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six croton cultivars by using the random primer OPS11. Where (M) marker, (1) *Codiaeum variegatum* 'Victoria Gold Bell', (2) *Codiaeum variegatum* 'Sunny Star', (3) *Codiaeum variegatum* 'Ptra', (4) *Codiaeum variegatum* var. *pictum*, (5) *Codiaeum variegatum* 'Gold Dust', (6) *Codiaeum variegatum* 'Yellow Lecton'.

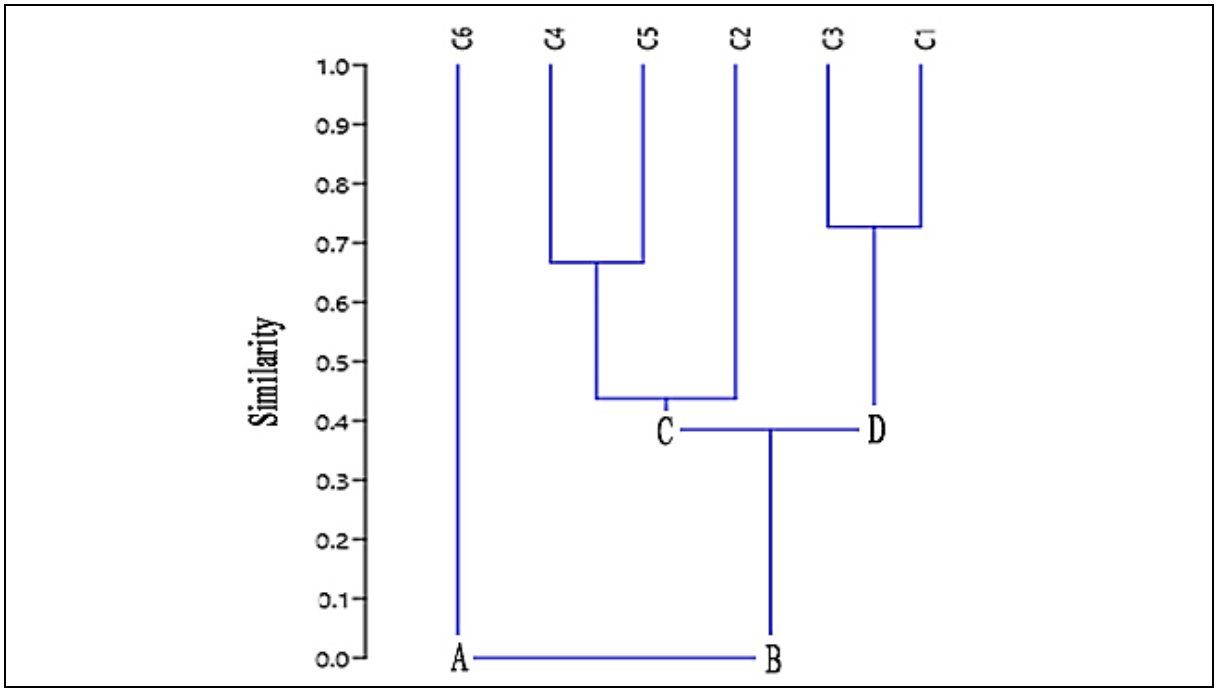

Fig. 4. Phylogenetic tree diagram cleared the relationships between the different used cultivars (OPS11).

Table 3. Total amplified fragments number and polymorphic fragments number 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 %
OPS11	**11**	**200-2000**	0	11	0	100%
OPS12	**6**	**200-900**	1	5	0	83.3%
OPS13	**6**	**200-1000**	0	6	0	100%
HB-12	**8**	**300-1000**	0	8	0	100%
HA-99	**8**	**400-1500**	0	8	0	100%
OPO20	**14**	**200-2000**	3	11	0	78.5%
Total	**53**	**-**	4	49	0	92.4%

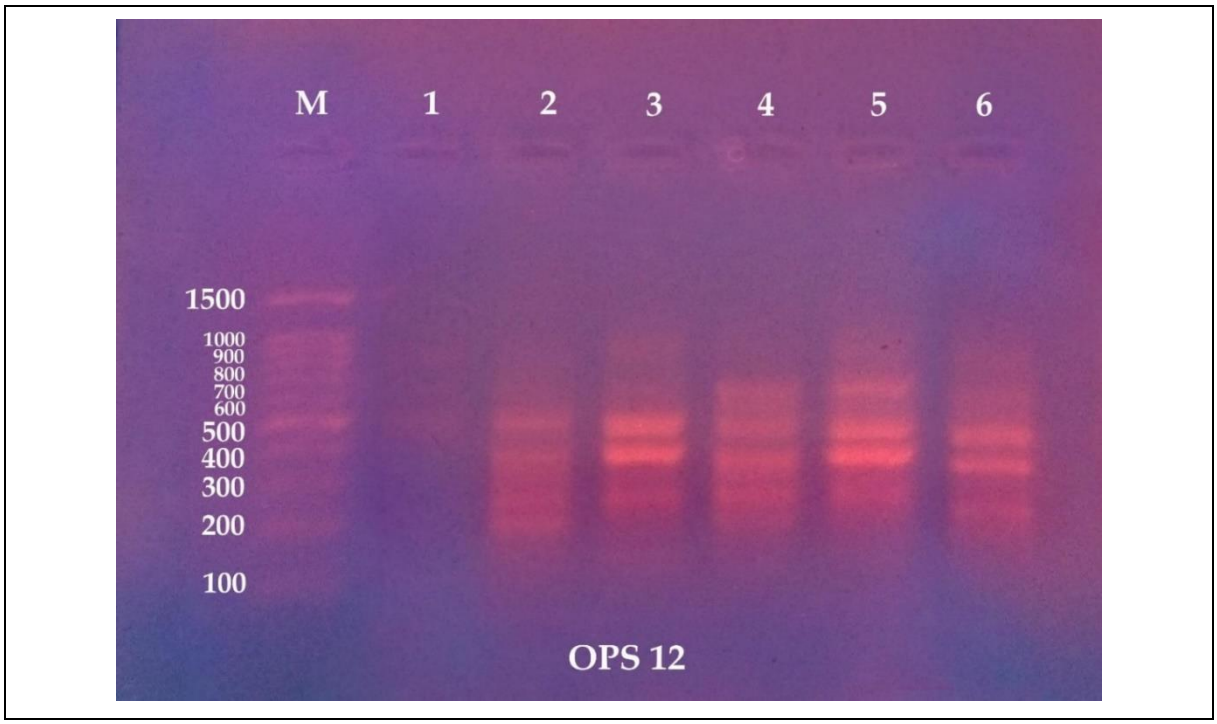

Fig. 5. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six croton cultivars by using the random primer OPS12. Where (M) marker, (1) *Codiaeum variegatum* 'Victoria Gold Bell', (2) *Codiaeum variegatum* 'Sunny Star', (3) *Codiaeum variegatum* 'Ptra', (4) *Codiaeum variegatum* var. *pictum*, (5) *Codiaeum variegatum* 'Gold Dust', (6) *Codiaeum variegatum* 'Yellow Lecton'.

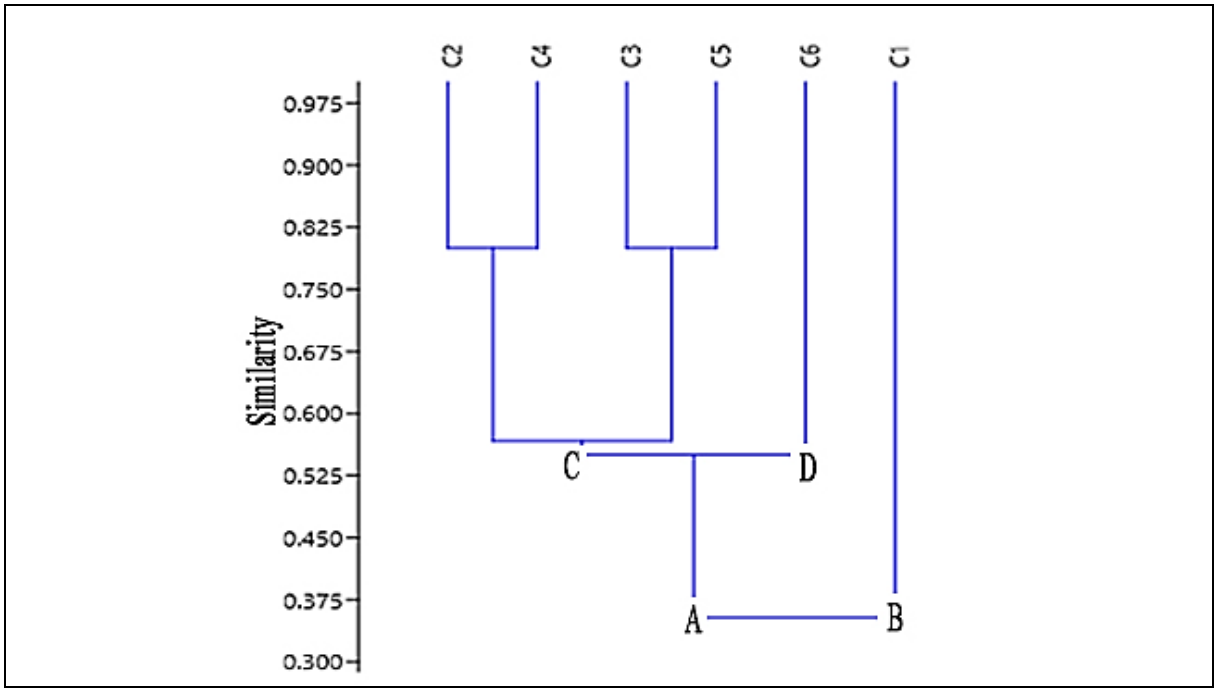

Fig. 6. Phylogenetic tree diagram cleared the relationships between the different used cultivars (OPS12).

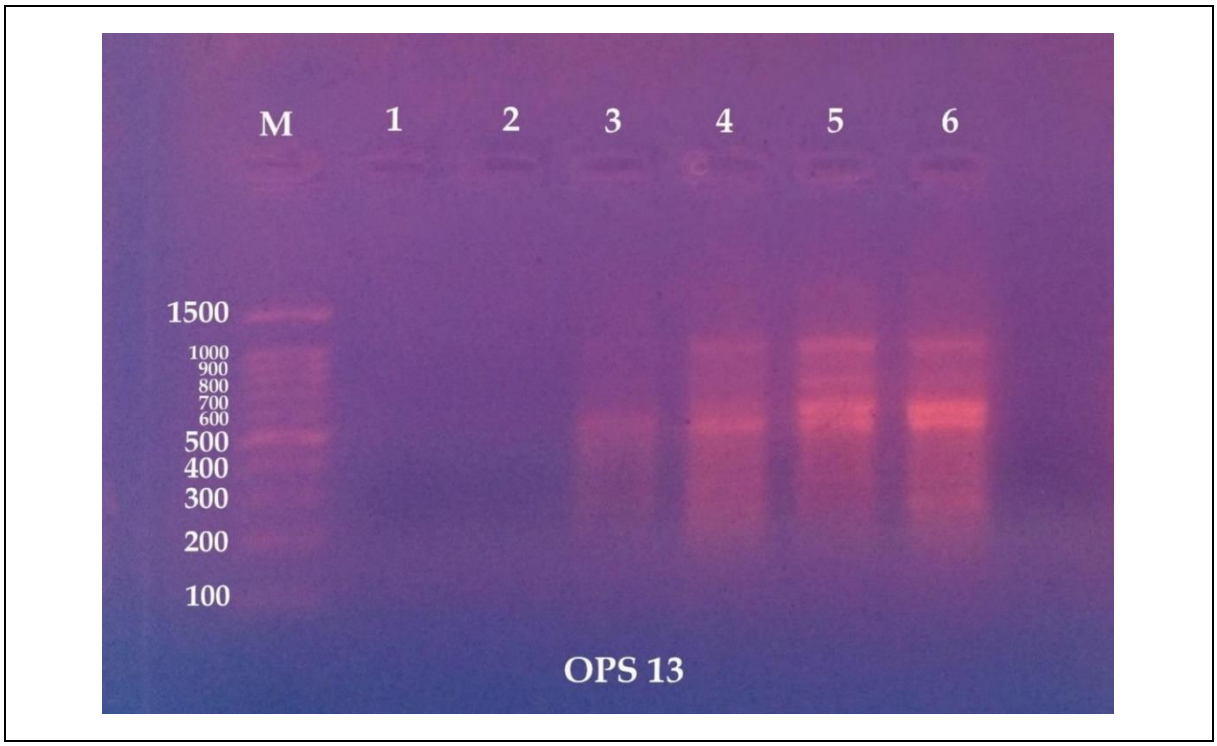

Fig. 7. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six croton cultivars by using the random primer OPS13. Where (M) marker, (1) *Codiaeum variegatum* 'Victoria Gold Bell', (2) *Codiaeum variegatum* 'Sunny Star', (3) *Codiaeum variegatum* 'Ptra', (4) *Codiaeum variegatum* var. *pictum*, (5) *Codiaeum variegatum* 'Gold Dust', (6) *Codiaeum variegatum* 'Yellow Lecton'.

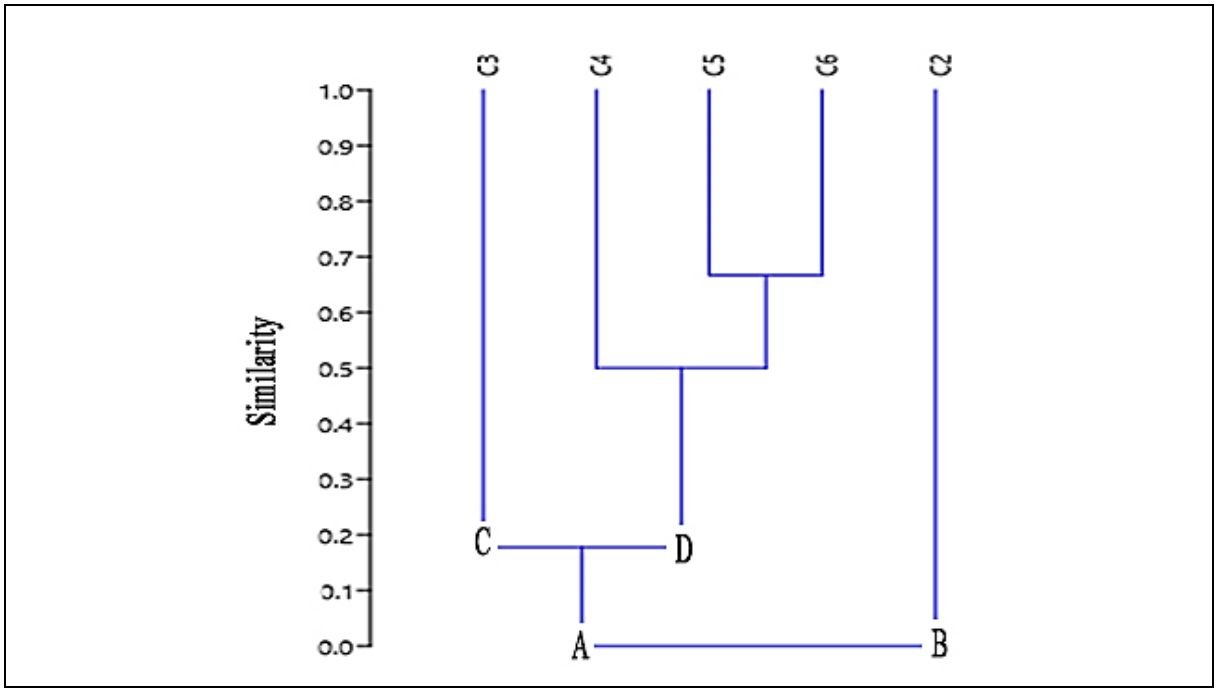

Fig. 8. Phylogenetic tree diagram cleared the relationships between the different used cultivars (OPS13).

genome either through readjusting or loss of some of their nucleotides (Li and Quiros, 2011; Farzaneh *et al*., 2010).

Results in Fig. (9) indicated that the random primer HB12 contains 73.73% G+C (Table, 1).

Amplified DNA fragment length which was produced from PCR by using this primer ranged from 300-1000 bp. C1 'Victroia Gold Bell' has no amplified bands. On the other side, C5 'Gold Dust' has the highest amplified band number which was seven. C6 'Yellow Lecton' recorded only five bands which were the lowest. It was observed eight bands as a total number resulted from the genomic of the previous cultivars, and all of them were polymorphic and also gave 100% polymorphism (Table, 3).

Fig. (10) cleared phylogenic tree constructed from HB12 primer. It introduced that the tree split into two major clusters A and B. Cluster A divided also into sub-clusters C and D. It was recorded that, sub-cluster C included C2 'Sunny Star' and C6 'Yellow Lecton' which were joined together and sub-cluster D contained C3 ' Ptra', and C5 'Gold Dust' which were joined together and also contained C4 var. *pictum* on the other hand cluster B contained only C1 'Victoria Gold Bell'.

Fig. (11) cleared the results procured by using HA99 primer with 50% G+C (Table, 1). Amplified DNA fragments length which produced from HA99 primer, ranged from 400-1500 bp. It was noticed that C1 'Victroia Gold Bell' has no amplified bands and also C3 'Ptra'. On the other hand, C5 'Gold Dust' recorded the highest amplified bands number which was five, while C4 var. *pictum* recorded the lowest one which was three amplified bands. It was noticed eight polymorphic bands as a total number of amplified bands for these cultivars with 100% polymorphism (Table, 3).

Phylogenic tree concluded HA99 primer results (Fig., 12). It was observed split in the tree into three big clusters A, B and C. Cluster A divided into 2 subclusters D and E. Sub-cluster D contained only C5 'Gold Dust', but also, sub-cluster E included C6 'Yellow Lecton', C2 'Sunny Star' and C4 var. *pictum.* It should be mentioned that C2 and C4 are

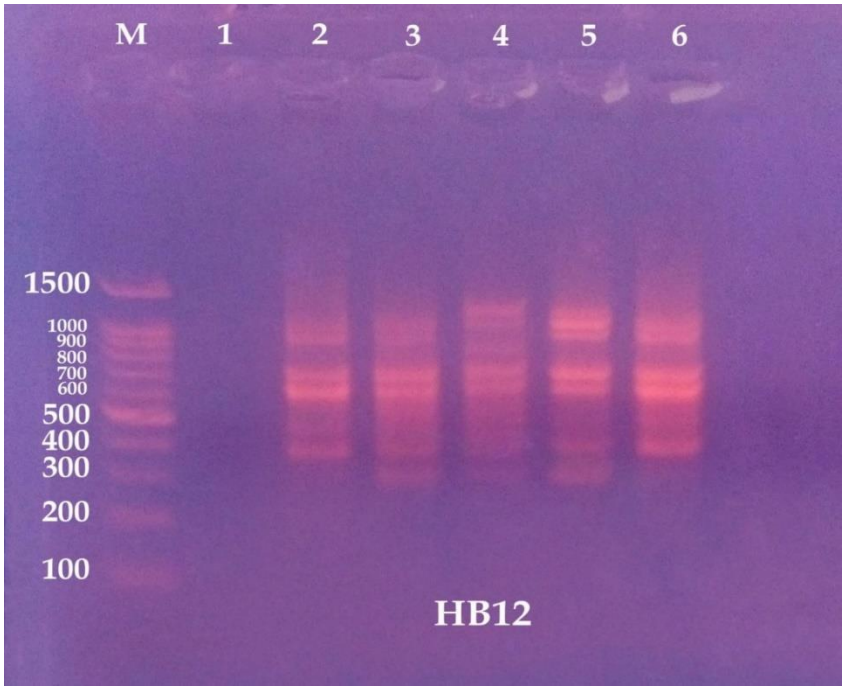

Fig. 9. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six croton cultivars by using the random primer HB12. Where (M) marker, (1) *Codiaeum variegatum* 'Victoria Gold Bell', (2) *Codiaeum variegatum* 'Sunny Star', (3) *Codiaeum variegatum* 'Ptra', (4) *Codiaeum variegatum* var. *pictum,* (5) *Codiaeum variegatum* 'Gold Dust', (6) *Codiaeum variegatum* 'Yellow Lecton'.

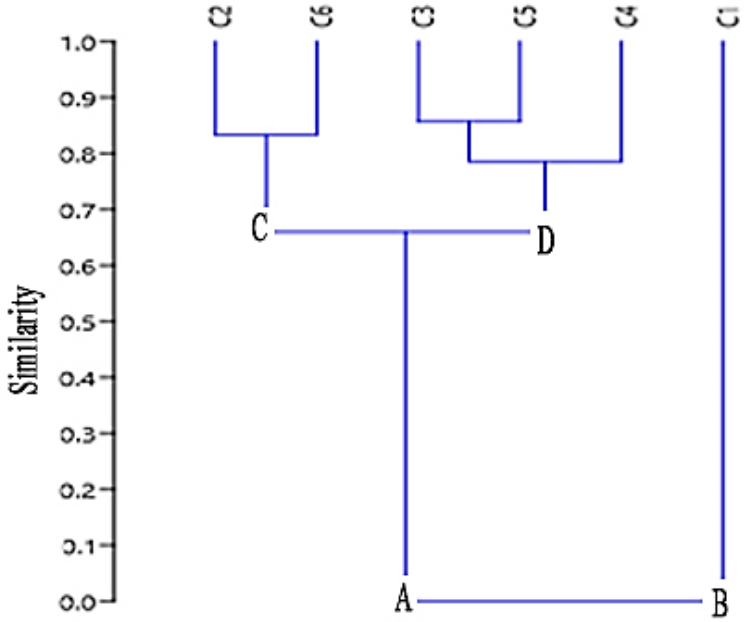

Fig. 10. Phylogenetic tree diagram cleared the relationships between the different used cultivars (HB12).

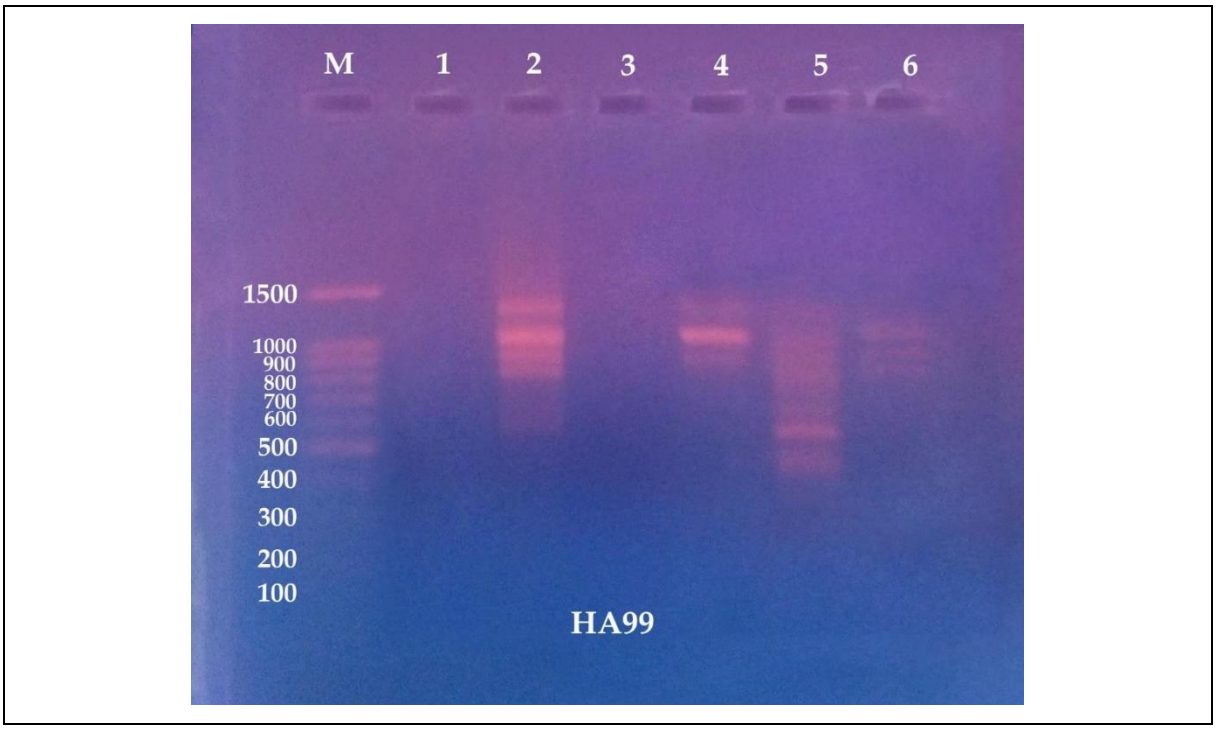

Fig. 11. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six croton cultivars by using the random primer HA99. Where (M) marker, (1) *Codiaeum variegatum* 'Victoria Gold Bell', (2) *Codiaeum variegatum* 'Sunny Star', (3) *Codiaeum variegatum* 'Ptra', (4) *Codiaeum variegatum* var. *pictum*, (5) *Codiaeum variegatum* 'Gold Dust', (6) *Codiaeum variegatum* 'Yellow Lecton'.

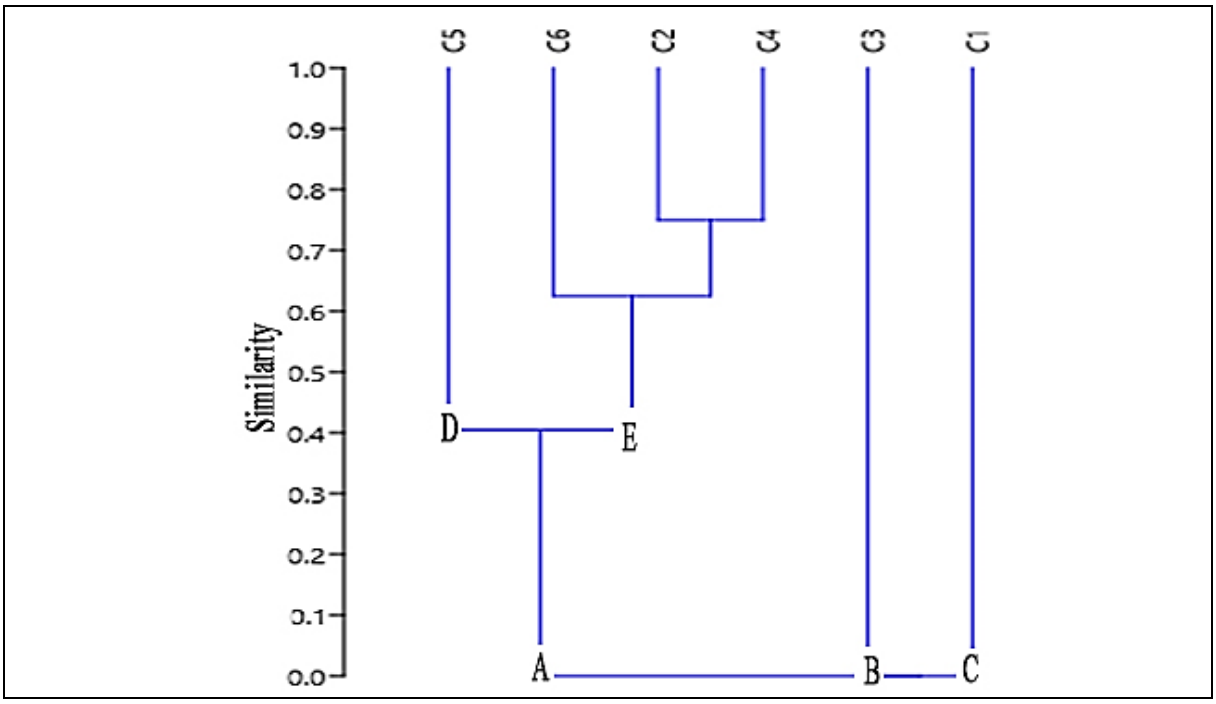

Fig. 12. Phylogenetic tree diagram cleared the relationships between the different used cultivars (HA99).

joined together which revealed that they are in a close ship. Cluster B had only C3 'Ptra'. Finally, cluster C had also only C1 'Victoria Gold Bell'.

Fig. (13) represents the obtained results using OPO20 primer with 60% G+C (Tabl, 1). It was noticed that amplified DNA fragments' length ranged from 200-2000 bp. C4 var. *pictum* was recorded ten amplified bands which was the highest, while C5 'Gold Dust' and C6 'Yellow Lecton' recorded six amplified bands which considered the lowest. it was recorded that this primer OPO20 has the highest total number of amplified bands which were fourteen (14), three of them were monomorphic bands and eleven were polymorphic giving 78.5% polymorphism percentage. These results indicated that OPO20 had the lowest percentage of polymorphism among the used primers in this investigation (Table, 3).

Fig. (14) concludes the phylogenic tree of OPO20 primer, the tree showed two great clusters A and B. Moreover, cluster A was split into two sub-clusters C assembled C6 'Yellow Lecton', C2 'Sunny Star' and C5 'Gold Dust', also C2 and C5 were joined together. The other sub-cluster D assembled C3 'Ptra' and C1 'Victoria Gold Bell' which were joined together. On the other hand cluster B contained only C4 var. *pictum*.

Table (3) condensed the total number of amplified bands, monomorphic, polymorphic and also the percentage of polymorphism by using three ISSR and three RAPD primers. The recorded results showed that the selected primers produced a total of 53 amplified bands. Most of these bands were polymorphic with 92.4% polymorphism. In addition, the obtained results revealed that there were no unique bands. RAPD and ISSR analysis using selected primers as shown in Table (1) produced a total of 53 major bands ranging from 200 to 2000 bp with 92.4% of polymorphism. Furthermore, the obtained results revealed that the amplified bands varied from 6 bands for OPS12 primer and 14 bands for OPO20 primer, with a total bands of 53. It was noticed that monomorphic bands were not present in all studied cultivars of *Codiaeum variegatum* which was used in this study, while polymorphic bands were present in all studied cultivars. Similar results were achieved by Asniawati and Purwantoro (2019). However, DNA length ranged from 200 to 2000 bp for the studied cultivars in this investigation. The greatest number of polymorphic bands achieved was 14 with OPO20 primer, it has 78.5% polymorphism which is considered the lowest. On the other side, OPS13 primer has the highest percentage of polymorphism (100%) but it has the lowest polymorphic band number (6). Many studies have proved the effectiveness ISSR primer on genetic diversity and characterization, such as those with *Croton heliotropiifolius* (Roch *et al*., 2016). It should be mentioned that polymorphic bands can be useful particularly in mapping genes or in the segregation pattern of a population. William *et al.* (1990) cleared that the deletions of priming sites may lead to polymorphism and also cause priming so far to support amplification, and may make differences in the size of DNA segments without preventing amplification. This may explain the different ranges in DNA length in the present study. The obtained result was in agreement with Penner *et al.* (1993) who mentioned that the variable in RAPD fragments is demonstrated in two ways, for example, the variable in size range amplified and inbred differences in the reproducibility of the involved primers.

Table (4) cleared the similarity coefficient of the six *Codiaeum variegatum* cultivars. It was concluded that the similarity coefficient ranged from 1.57 to 5.48. Also, it was mentioned that 1.57 was the lowest degree of similarity which between C6 'Yellow Lecton' and C1 'Victoria Gold Bell'. While the highest similarity degree (5.48) was recorded between C3 'Ptra'and C1 'Victoria Gold Bell'. Sayed *et al*. (2009) concluded that high similarity leads to propose that individuals have a nearer genetic kinship

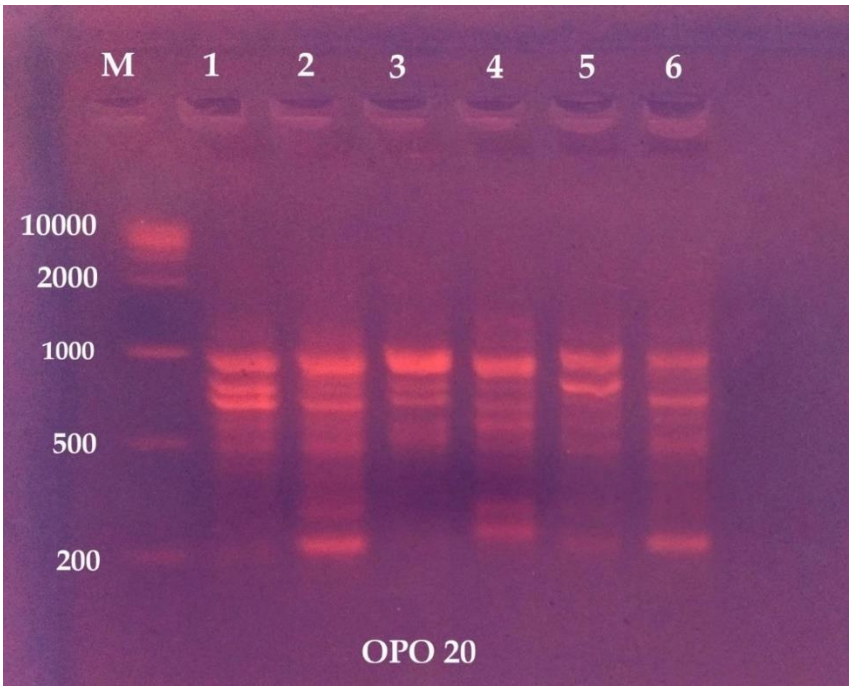

Fig. 13. Photograph of 1.5% agarose gel electrophoresis of the amplified PCR product of the isolated genomic DNA from leaves of the different six croton cultivars by using the random primer OPO20. Where (M) marker, (1) *Codiaeum variegatum* 'Victoria Gold Bell', (2) *Codiaeum variegatum* 'Sunny Star', (3) *Codiaeum variegatum* 'Ptra', (4) *Codiaeum variegatum* var. *pictum*, (5) *Codiaeum variegatum* 'Gold Dust', (6) *Codiaeum variegatum* 'Yellow Lecton'.

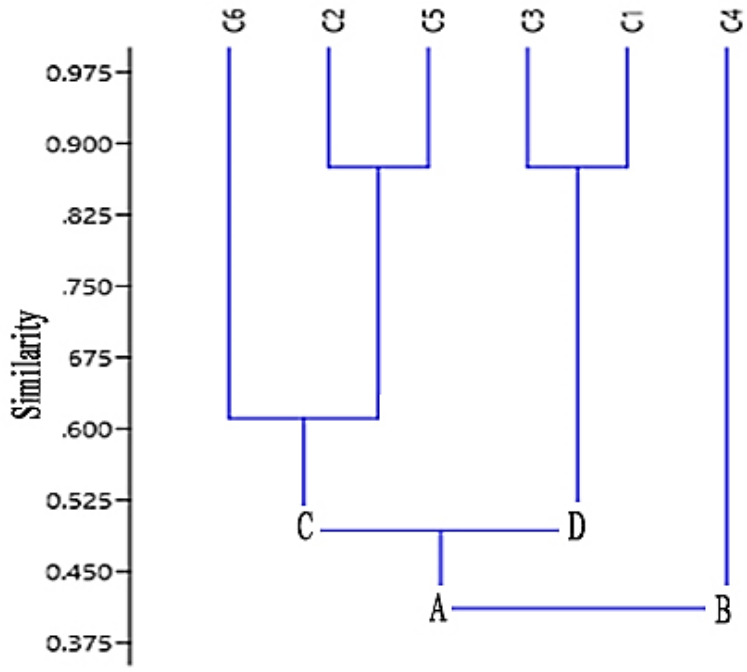

Fig. 14. Phylogenetic tree diagram cleared the relationships between the different used cultivars (OPO20).

Table 4. Similarity build on the gentic distance among the used cultivars of *codieaum varieagatum* by Euclidean for 6 random primers of ISSR and RAPD.

Cultivars	C1	C2	C3	C4	C5	C6
***Codiaeum variegatum* 'VictoriaGold Bell' (C1)**	0					
***Codiaeum variegatum* 'Sunny Star' (C2)**	3.05	0				
***Codiaeum variegatum* 'Ptra' (C3)**	5.48	4.47	0			
***Codiaeum variegatum var. pictum* (C4)**	2.55	4.87	3.77	0		
***Codiaeum variegatum* 'Gold Dust' (C5)**	2.85	5.25	4.09	5.45	0	
***Codiaeum variegatum* 'Yellow Lecton' (C6)**	1.57	5.00	3.00	4.50	5.26	0

between each other, however, the lowest leads to disclose significant genetic distance.

In this investigation, UPGMA analyses was assembled, and dendrograms were established. Two similar clusters with changeable percentages of similarity were disclosed depending on ISSR and RAPD and combined data.

Asniawati and Purwantoro (2019) analyzed genetic diversity of some cultivars of croton and their offspring. They recorded the genetic distance between the parents and their offspring which mentioned that all of the offspring were nearier to the male parent. Furthermore, Rajaseger *et al.* (1997) studied the genetic diversity among 22 cultivars of *Ixora* by using RAPD. They discovered that the markers of DNA were beneficial in distincty collected in 2 clusters. They added that all 22 cultivars collected in two cultivar groups: *Ixora coccinea* and *I. javanica*. And also, besides using RAPD markers for spotting particular *Ixora* cultivars, the relationships of phylogenetics obtained by using RAPD can be benefit in improvement programs.

Fig. (15) shows a dendrogram tree built based on the existing data from both ISSR and RAPD analysis.

In addition, the dendrogram tree concluded two different clusters with variable similarity percentages. It can be mentioned that cluster (A) was presumed C1 ' Victoria Gold Bell' and C3 'Ptra'. It was observed a big difference in leaf shape size and colour but they can be grouped although of the difference in morphological characters. It was proposed that the group of the used cultivars were collected together from a somaclone complex, which means that these cultivars might have originated from somatic mutation (Ud *et al.*, 2003).

On the other side, cluster (B) was split into two sub-clusters which were (C) and (D). Sub-cluster (C) included only C6 'Yellow Lecton'. Therefore sub-cluster (D) included C2 'Sunny Star' in one arm, and with a small distance the other arm included C4 var. *pictum* and C5 'Gold Dust' which were joined together. It observed that all of these cultivars share a green color of leaves with yellow. But the yellow color is different in shapes which ranged from small spots like in C4 var. *pictum*, big spots in C5 'Gold Dust' and linear shapes in C2 'Sunny Star' and C6 'Yellow Lecton'. These results were in agreement with Patra *et al.* (2008), which concluded that morphological disposition was concentrated in debating the variability in molecular of different cultivars of canna. The present investigation deduced that most of the obtained results of phylogenetic tree were in accordance with Asniawati and Purwantoro (2019), who cleared that there was very limited relationship between parents and their croton offspring. They added also that the genetic distance between Walet (a cultivar of *Codiaeum variegatum*) and the offspring had different range. They performed that the male parent and all their offspring were in close sheap. It is important to mention that, RAPD technique is rapid and also simple, it can be used to associate the hybridization of croton offspring. Therefore, the main problem of this technique is reproducibility and reliability, so

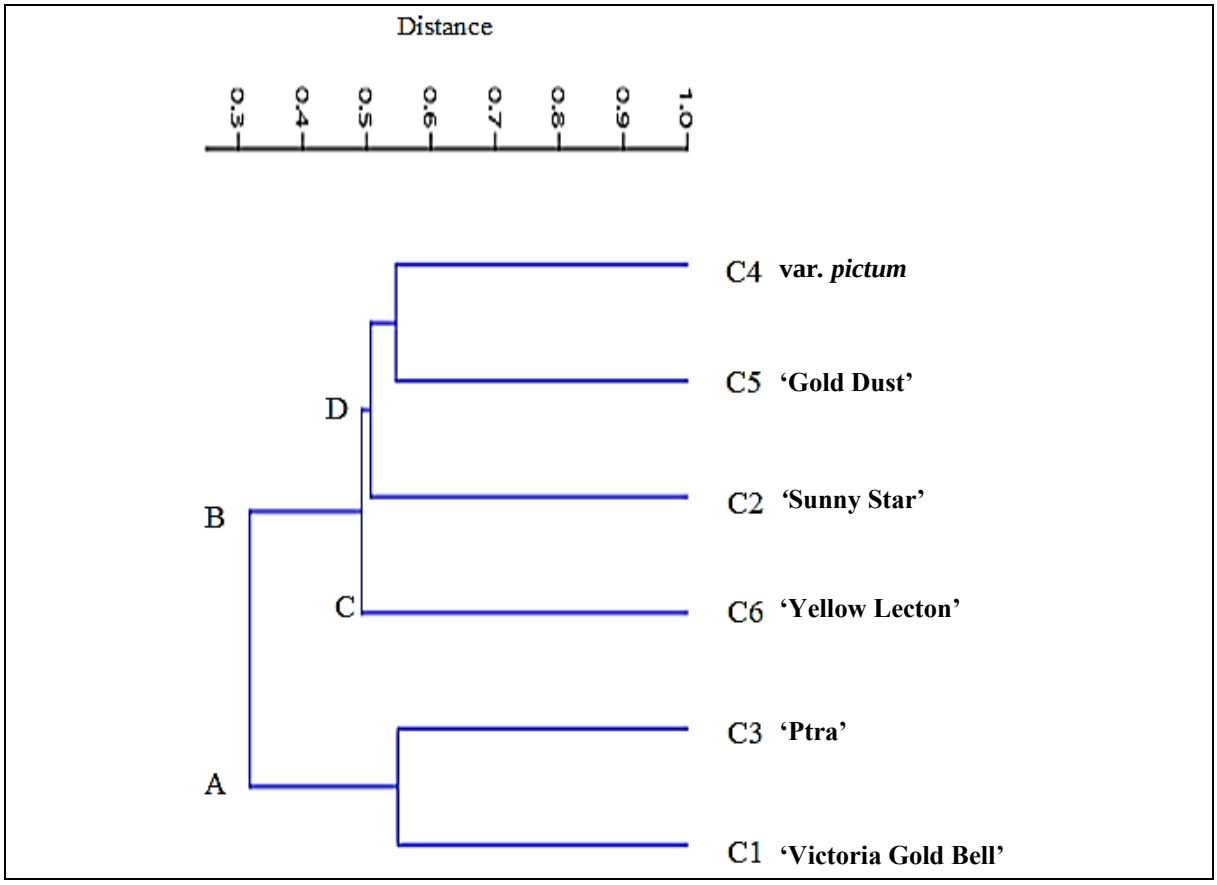

Fig. 15. Phylogenetic tree to show the relationships between the used cultivars: (C1) *Codiaeum variegatum* 'Victoria Gold Bell', (C2) *Codiaeum variegatum* 'Sunny Star', (C3) *Codiaeum variegatum* 'Ptra', (C4) *Codiaeum variegatum* var. *pictum*, (C5) *Codiaeum variegatum* 'Gold Dust', (C6) *Codiaeum variegatum* 'Yellow Lecton' according to Jacard (1908).

it is better to use more specific RAPD primers in sequences (Rajesh *et al.*, 2014).

On the other side, ISSR markers were efficient enough, that they can be permit the molecular profile formation in croton. Also, ISSR markers used on croton, had enough polymorphism to approximate the variable in genetics between the populations which had been studied (Almedia-Pereira *et al.*, 2017). These variabilities of different cultivars in this investigation may be due to that the plant genetic structure demand interactions of some definite procedures, like habitat fragmentation, and also, isolation changes in distribution of populations, reproductive, mutation, gene flow, genetic drift, (Schaal *et al.*,1998; Thendral Hepsibha *et al.*, 2010). Furthermore, many investigations have demonstrated the effectiveness of ISSR markers in genetic diversity and characterization of elevation between populations, such as *Croton heliotropiifolius* (Roch *et al.*, 2016). In addition, Lira-Neto (2011) evaluated 6 ISSR primers in 27 species of croton, they achieved 186 bands, and all were polymorphic. Also, Roha *et al.* (2016), performed that 18 RAPD primers were used to evaluate 41 *Croton heliotropiifolius* individuals, which is also a controlling marker, moreover, 15 ISSR primers, which gave 137 bands total. They found 73 bands generated from RAPD markers, however, 64 bands were generated from ISSR markers. They also added that there were a high relative of diversity. It can be deduced that the cultivars of croton cannot be separated from

somaclone complex which depends on leaf shape, color, size and plant height. The intraspecific genome relationship should be examined (Mishr *et al.,* 2018).

In addition, the inner relationship generated from the ISSR and RAPD markers used in the present study was following the results of Loh *et al*. (1999) who used AFLP analysis on seven cultivars and two species of *caladium*. They deduced that species which is closely related might be different, moreover, it can confirm the genetic difference between cultivars. Also, the results in this investigation were in agreement with Loh *et al.* (1999) who concluded that, at an intrshied, intraspecific level, it can be identified two preliminary clusters in *Caladium bicolor*, one group consisting of the 'Strap Leaf' cultivars, however, the other one has 'Fancy Leaf' cultivars. For these findings, the supposed simplification was that in caladium, the difference in leaf shape might be because of allelic differences indicative of the fact that the dichotomy between strap leaf and fancy leaf, may be not a very strong taxonomic character. As some, in this investigation, the reason for croton cultivars nearly have the same colour with different leaf shapes grouped in the same cluster. It can be deduced that the cultivars share leaf colour regardless of leaf shape grouped together in one cluster.

According to the achieved results in the present study. It can be suggested that the tree dendrogram is divided into two major clusters depending on the leaf color with regardless of leaf shape and size for the six different cultivars of croton. Also, this study demonstrated that both ISSR and RAPD primers offer an acceptableresons for detecting genetic diversity in *Codiaum variegatum* (L.).

CONCLUSION

It can be concluded that 53 major scorable as a total bands ranging from 200 to 2000 bp were produced by using three ISSR and three RAPD primers showing 92.4% polymorphism. It also noticed that *Codiaum variegatum* 'Ptra' (C3) had the highest correlation (5.48). While the lowest similarity (1.57) was reorded between C6 'Yellow Lecton' and C1 'Victoria Gold Bell'. The dendrogram tree cleared that, the used primers in this investigation split the present cultivars of croton into two main groups based on the leaf color.

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العلاقة الفيلوجينية بين أصناف مختلفة من نبات الكروتون بإستخدام الدلائل الجزيئية

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تم إجراء هذا البحث على ٦ أصناف من نبات الكروتون (*Codiaeum variegatum*) لمعرفة صلة القرابة بينهم وذلك بإستخدام تكنيك RAPD و ISSR. تم إختيار الأصناف على أساس الإختلاف في شكل وحجم ولون الأوراق مظهرياً، وكانت الستة أصناف المستخدمة في هذه الدراسة هي: (١) Victoria Gold Bell، (٢) Sunny Star، (٣) Ptra، (٤) var. *pictum*، (٥) Gold Dust، (٦) Yellow Lecton. تم استخدام ستة بادئات جزيئية (ثلاثة من نظام RAPD وثلاثة من نظام ISSR) على الأصناف الستة وكانت النتائج كما يلى: وُجد أن إجمالى مجموع الحزم ٥٣ حزمة تراوحت بين ٢٠٠ إلى ٢٠٠٠ قاعدة زوجية (bp) بإجمالى نسبة تباين وراثي بلغت ٩٢,٤٪ وقد سُجلت أعلى نسبة قرابة (٥,٤٨) بين الصنفين

(٣) Ptra و(١) Victoria Gold Bell. فى حين أن أقل قرابة كانت بين (٦) Yellow Lecton و (١) Victoria Gold Bellوقد سجلت ١,٥٧. أوضحت شجرة القرابة أن البادئات المستخدمة قد قسمت الأصناف إلى مجموعتين رئسيتين: أحدهما تضم الأصناف ذات لون الورقة الأخضر وبه صبغة حمراء، والمجموعة الثانية تضم الأصناف ذات لون الورقة الأخضر وبها صبغة صفراء بغض النظر عن شكل وحجم الورقة. هذا ويجب الإشارة إلى أن هذه النتائج تم الحصول عليها مع البادئات المستخدمة فى هذه الدراسة وربما تكون هناك نتائج أخرى بإستخدام بادئات أخرى أو تكنيكات مختلفة.