Study of genetic differences of carnation cultivars using RAPD indicators

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Abstract

The experiment was conducted in the Biotechnology Laboratory in the Department of Horticulture and Landscaping, College of Agriculture, Al-Qasim Green University. The technique of random replication polymorphism of DNA sequence (RAPD) with seven primers was adopted to detect genetic differences between ten carnation cultivars (local,Bizet,Shridea,Orange,Amprose,Mariposa,Grand,White,Viana,Liberty). The work steps included isolating and purifying the DNA of the newly-grown leaves of the cultivars included in the research and detecting the discrepancies between the replicated pieces of each cultivar after the samples were migrated by the electrophoresis device in the RAPD reactions.The results of genetic analysis with RAPD indicators showed a clear difference in the number of DNA replication bands and their molecular weights, depending on the primer used. The total number of differentiated bands reached 368 bundles for all the primers used. The studied varieties were divided into two main groups A and B, the similarity ratio between the two groups was 350., included Group A had eight varieties, while group B included the two cultivars Viana and Liberty, which indicates that the two cultivars are genetically farthest from the rest of the cultivars, and the similarity ratio between the two cultivars was 850. As for the two plant cultivars, Orange and Mariposa, the highest percentage of similarity between the cultivars was 940.After calculating the percentage of the genetic dimension between the cultivars, genetic variations were found in them. The high genetic differences between the carnation cultivars may be due to the geographical locations to which they belong, in addition to the breeding and improvement processes that were conducted on them.

دراسة الاختلافات الوراثية لأصناف القرنفل باستخدام مؤشرات RAPD

المستخلص

نفذت التجربة في مختبر التقانات الأحيائية في قسم البستنة وهندسة الحدائق ـــ كلية الزراعة ـــ جامعة القاسم الخضراء. اعتمدت تقانة التضاعف العشوائي المتعدد الأشكال لسلسة الدنا (RAPD) وبسبعة بادئات للكشف عن الاختلافات الوراثية بين عشرة أصناف للقرنفل (Bizet,Shridea,Orange,Amprose,Mariposa,Grand,White,Viana,Liberty) و الصنف المحلي) . تضمنت خطوات العمل عزل وتنقية DNAالاوراق الحديثة النمو للأصناف الداخلة بالبحث والكشف عن التباينات بين القطع المتضاعفة لكل صنف بعد ترحيل العينات بجهاز الترحيل الكهربائي في تفاعلاتRAPD. بينت نتائج التحليل الوراثي بمؤشرات RAPD اختلافا واضحا في عدد حزم الـDNA المتضاعفة وأوزانها الجزيئية وذلك تبعا للبادئ المستخدم إذ بلغ العدد الكلي للحزم المتباينة 368 حزمة لكافة البادئات المستخدمة, إن الاصناف المدروسة قسمت على مجموعتين رئيستين A وB ، بلغت نسبة التشابه بين المجموعتين 350. ، ضمت المجموعة A ثمانية أصناف أما المجموعة B فضمت الصنفين Viana و Liberty مما يدل على إن الصنفين الابعد وراثيا عن باقي الاصناف وكانت نسبة التشابه بين الصنفين 850., اما الصنفين النباتين Orange و Mariposa أعطت أعلى نسبة تشابه بين الاصناف بلغت 940. . وبعد حساب النسبة المئوية للبعد الوراثي بين الاصناف وجد تغايرات وراثية فيها ربما تعود الاختلافات الوراثية العالية بين اصناف القرنفل إلى المواقع الجغرافية التي تنتمي إليها إضافة إلى عمليات التربية والتحسين التي أجريت عليها.

Introduction

The carnation plant *Dianthus caryophyllus* L. is one of the plants of the caryophyllaceae family, whose plants grow in the temperate zone of the northern hemisphere. This family includes 2100 species and 89 genera, one of which is the genus Dianthus, which includes about 300 species, most of which are annual or perennial herbaceous plants, and a few of them are shrubs (Jurgens et al., 2003). It is a perennial herbaceous plant with special breeding, where it needs high technical expertise in order to produce its flowers with good commercial quality. The carnation is one of the most important global and economic cut flowers of high coordination and aesthetic value.Molecular indicators are among the most recent and widely used in the characterization of plant cultivars. They are defined as special DNA sequences that can be inferred to a specific site on a chromosome or gene (Zaid et al., 1999).These indicators were used to study the genetic relationships between individuals and to find genetic fingerprints because they reflect the differences in the distinctive genetic information stored in those individuals (Paterson et al., 1991).It was possible to apply these indicators in the classification of many plant cultivars, whether alone or with other technologies such as AFLP and SSR, in a study conducted by Frederrick et al. (2002) using

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RAPD technology based on the PCR indicator in the genetic characterization of nine cultivars of Roses and using five primers revealed All of them were about genetic variations between the studied cultivars, as they gave different packet lengths (0.50-1.90 kbp),Debener and Mattiesch (2006) by studying the genetic relationship of two types of rose, Rosa multiflora and Rosa canina using 24 primers of different lengths (10, 15, 20 base pairs) using RAPD technology found that the number of new DNA pieces and their frequency depends on the length of the primer used.Hussein et al. (2008) invested in RAPD indicators to detect genetic variations caused by electrocution in Antirrhinum majus. The highest percentage of genetic dimension was 42% when the cultivars were treated with 8 amp × 6 min treatment. In a similar experiment on the plant Mathiol incana, the results of the RAPD technology recorded the presence of genetic differences between plants treated with electrocution, as the genetic dimension reached 35% when treated with 10 amps x 4 minutes (Al-Jubouri et al., 2009).Hussein (2011) was able to find the genetic fingerprint and the percentage of the genetic dimension for six cultivars of Rosa spp. It belonged to six species (tea rose, musk rose, hybrid perennial rose, climbing rose, dwarf rose, French rose) and using ten primers for RAPD indicators and found a clear difference in the number of DNA replication packets and their molecular weights. The total number of divergent Bands was 68 Bands, and the highest percentage of genetic dimension between hybrid perennial rose and climbing rose was 40.3%, and the lowest was 18.9% between tea rose and climbing rose. This research aims to study the genetic diversity and determine the genetic relationship of ten cultivars of carnation suitable for commercial cut flower based on the degree of genetic similarity between them and to determine genetic identity through genetic fingerprint analysis using the technique of random amplification of polymorphic DNA (RAPD).

Materials and methods

The experiment was conducted in the Biotechnology Laboratory in the Department of Horticulture and Landscaping, College of Agriculture, Al-Qasim Green University. The technique of random replication DNA sequence polymorphism (RAPD) was adopted to detect genetic differences between the plant cultivars under study.In order to facilitate the partial study of plant cultivars, they are symbolized by the symbols shown in Table (1). The leaves were collected by cutting the fourth pair of upper leaves of all plant cultivars after making sure that they were free of diseased infections on 1/20/2019. They were placed in unsealed plastic bags and taken directly to the laboratory. Then it was washed with running water to clean it of dust and suspended impurities and wiped

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with ethanol alcohol to remove contaminants.Isolation of total DNA from the leaves of carnation cultivars using a Genomic DNA Mini kit (Plant) and following the method described by Geneaid. The DNA concentration of the samples under experiment was measured using a nano-drop-spectrophotometer supplied by Bio Drop company at a wavelength of 260 nm.The tree diagram of genetic relationship based on the results of RAPD indicators was shown using the modified version of MEGA statistical program Version 10.0.5 and based on the similarity index Euclidean scale (Fig. 2). The studied samples were divided into two main groups A and B, the similarity ratio between the two groups was 0.35, Group A included eight cultivars, while group B included the two cultivars Viana and Liberty, which indicates that the two cultivars are genetically farthest from the rest of the cultivars and the similarity ratio between the two cultivars was 0.85. The main group A was divided into two subgroups a1 and a2, the similarity ratio between the two subgroups was 0.65, group a1 included five plant cultivars that were divided into two subgroups sub-secondary, namely C and D, and the similarity ratio between the two sub-secondary sub-groups was approximately 0.74.Group C was divided into two other groups, c1 and c2, and the similarity ratio between the two groups was approximately 0.86. Group c1 was divided into two other groups, E1, which included the two plant cultivars Orange and Mariposa, which gave the highest similarity rate of approximately 0.94 compared to the similarity ratio for the rest of the cultivars and group E2, which included the cultivars Vegetarian Amprose .The similarity ratio between the two groups was approximately 0.89, while c2 included the plant variety Shridea, while the sub-secondary group D was unique to the local plant variety. The secondary group a1 was also divided into two groups F1 and F2, with a similarity ratio of approximately 0.69. The F1 group included the plant cultivar White, and the F2 group included the two cultivars Bizet and Grand, which gave a percentage of similarities are approximately 0.84. As for the purity, it depends on the ratio between the two readings of the device on the wavelengths 260 and 280 nm. The device was filtered by placing 2 microliters of TE solution, then 2 microliters of DNA samples were placed in the focus of the device. under consideration. Seven random primers of RAPD technology obtained from Korea Bioneer Corporation were adopted to know which of them reflect polymorphism in the DNA of the studied samples. The primers s were dissolved and diluted with ionic distilled water (D.D.W) and according to each concentration fixed on the primers . Withdraw 10 µL of the starter solution and dilute by adding 90 µL of deionized distilled water

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(D.D.W)Table (3) shows the sequence and number of primers bases used in the RAPD technology, and the volume of ionic distilled water (D.D.W) needed to be added to dissolve and dilute these primers.

All random amplification reactions were performed according to Williams et al. (1990). The reaction products were passed through a 1.5% agarose gel in an electrophoresis in the presence of 1X TBE standard solution. DNA was stained in agarose gel with 10 mg ethidium bromide. 1 ml- distilled water for about an hour and a half, then examine the gel using an imaging device that contains a UV-transilumentor at the wavelength of 365 nm to see the DNA bands ,Images of the gel using an imaging device Gel documentation station ATTO Japans, sections are clarified and molecular weights are estimated when compared to the firmness and thickness of the volume guide on the gel side (Lewis, 2011).The results of the electrical migration were summed in the characterization table by giving the presence and absence of beam 1 and making a binary matrix whose columns are equal to the number of samples studied and the number of lines equal to the number of indices of variation (polymorphism).The statistical program MEGA with its modified version 10.0.5 and based on the similarity Euclidean index scale was used for the purpose of finding the genetic dimension and drawing the genetic relationship tree Dandrogram for the samples.

The following indicators were calculated, which were taken from the gel images:

1- The number of total packages produced from each starter.

2- The number of monomorphic bands.

3- The number of polymorphic bands.

4- The number of unique bands.

5- Percentage of band(similar, different and distinct) %, calculated from the law:

$$\text{Bands percentage} = \frac{The\ number\ of\ bands\ (similar, different, or\ distinct)\ produced\ from\ the\ primer}{Total\ number\ of\ bands\ produced\ from\ the\ primer} \times 100$$

$$\text{primer efficiency} = \frac{Total\ number\ of\ bands\ produced\ by\ primer}{Total\ number\ of\ bands\ for\ all\ primer} \times 100$$

7- Discrimination power (%) was calculated from the law:

$$\text{Discrimination power (\%)} = \frac{The\ number\ of\ polymorphic\ bands\ produced\ from\ the\ primer}{Number\ of\ polymorphic\ bands\ produced\ from\ all\ primer} \times 100$$

8- Genetic distance (%)

The genetic distance was found between the two individuals based on the results of

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genetic similarity values using the equation of Nei and Lei (1979).

Genetic Similarity (GS)= 2nxy/nx+ny

As:

(G.S) Genetic Similarity

nxy = the number of bands shared between the two (individual) models x and y whose similarity is to be studied

nx = number of total bands of the model x

ny = the number of total bands of the y model.

Then the percentage of genetic distance between the two individuals was calculated according to the equation:

Genetic distance =1- G.S × 100

Table (1) Plant cultivars under molecular study

vegetable variety	**Liberty**	**Viana**	**White**	**Grand**	**Mariposa**	**Amprose**	**Orange**	**Shridea**	**Bizet**	**local**
code	**Var.10**	**Var. 9**	**Var.8**	**Var.7**	**Var.6**	**Var.5**	**Var.4**	**Var.3**	**Var. 2**	**Var. 1**

Table (2) shows the concentration and average of DNA purity of the samples under study

purity average	Concentration Iμ /ng	code	Sample
1.98	33.3	1	Local
2.00	38.4	2	Bizet
2.01	80.9	3	Shridea
1.99	195.7	4	Orange
1.99	113.5	5	Amprose
1.91	7.9	6	Mariposa
1.97	143.9	7	Grand
1.97	326.1	8	White
1.79	59.2	9	Viana
2.00	114.5	10	Liberty

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Table (3) Random primer used in RAPD technology

The base sequence (5-3)	primer	Volume of ionic distilled water added (microliters)	base number
AGGCTGGGTC	Q18	203.5	10
GGGACGATGG	Q01	193.6	10
TCGCCCAGTC	Q20	230.7	10
GTGACGTAGG	A08	193.6	10
ACGTAGCGTC	O02	205.5	10
CCGCGTCTTG	Q05	237.8	10
CCGAATTCCC	F05	226.5	10

Results and discussion

The results of the electrophoresis showed a clear difference in the number of DNA replication bands, and a clear difference in their molecular weight, according to the primers used (Q18, Q01, Q20, A08, O02, Q05, F05) (Fig. 1).It is noted from Table (4) that the random primers that were used in the study produced 368 bands with an average of 52.57 bands, where the primer Q18 gave the most number of bands reached 91 bands, while the primer Q20 gave the least number of bands which amounted to 31 bands and the number of similar bands reached (Monomorphic bands).) 21 bands with an average of 3 bands for all the studied cultivars and the primers Q18 and Q01 gave the most number of similar bands which reached 5. While the primers Q20, A08, O02 and Q05 gave the lowest number of similar packets, amounting to 2 bands for each, and the highest percentage of similar bands was for F05 primer 7.31%, while the primers Q05 gave the lowest percentage of 4.34%. The number of polymorphic bands was 35 bands with an average of 5 bands for all plant cultivars under study. The primer Q18 gave the most number of differentiated bands, which amounted to 9 bands, while the primers Q20 and F05 gave the least number of bands, which was 2 bands for each, and the primers achieved O02 gave the highest percentage of the different bands amounted to 15.90%, while the primers F05 gave the lowest percentage of these bands amounted to 4.87%. As for the total number of distinct bands (Unique), it was 8 bands, with an average of 1.14 bands. The primers Q18 and A08 gave the most number of distinct bands,

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amounting to 3 bands, while the primers O02, Q05 and F05 did not give any distinct bands on the gel, and the primer A08 achieved the highest percentage of distinct bands reached 7.69%.While the lowest percentage of 0% was recorded for the primers that did not show any distinct bands. The efficiency of the primers was the highest at 24.73% for the primer Q18, while the primers Q20 gave the lowest efficiency of the primers , which amounted to 8.42%, and the primers Q18 achieved the highest percentage of diagnostic power, amounting to 27.27%, while the primers Q20 and F05 gave the lowest percentage of diagnostic power, amounting to 6.82% for each.The variation in the number of bands resulting from the primers used depends on the compatibility of the link of the primers with the plant genome, as well as the components of each of the nitrogenous bases. The number of primers generated from the prefixes used varied (Vos et al., 1995). Therefore, any change in the nucleotide sequence as a result of insertion, deletion, or rearrangement of nucleotides in the genome of the studied individuals for any reason, such as chromosomal karyotypic change or point mutations.Somatic Crossing Over, DNA Amplification, DNA Methylation, Transposable Elements, Change in organelles DNA,It will cause a change in the primer binding sites, which leads to a change in the size of the duplicative piece while leading to the appearance of differentiated bands, or their absence in specific sites on the gel (Brar and Jain, 1998). The resulting packages for all plant cultivars under study.

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Table (4) Numbers of the total, similar, distinct, Special bands, their percentage, primer efficiency, and Discrimination power resulting from the polymerization reaction of 7 primers and using random complete duplication of the DNA sequencing (RAPD) to distinguish between 10 plant cultivar of carnation

No.	Primer	Percentage of discrimination power of primer	primer Efficiency Percentage	Percentage of Special bands	Number of Special bands	Percentage of dissimilar bands	The number of polymorphic (differentiated) bands	Percentage for similar band	Number of similar bands	Total number of bands
1	Primer Q18	27.27	24.73	3.29	3	9.89	9	5.49	5	91
2	Primer Q01	18.18	20.65	1.31	1	9.21	7	6.57	5	76
3	Primer Q20	6.82	8.42	3.22	1	6.45	2	6.45	2	31
4	Primer A08	13.64	10.60	7.69	3	7.69	3	5.12	2	39
5	Primer O02	15.91	11.96	0	0	15.90	7	4.54	2	44
6	Primer Q05	11.36	12.50	0	0	10.86	5	4.34	2	46
7	Primer F05	6.82	11.14	0	0	4.87	2	7.31	3	41
Total		100	100		8		35		21	368
Average					1.14		5		3	52.57

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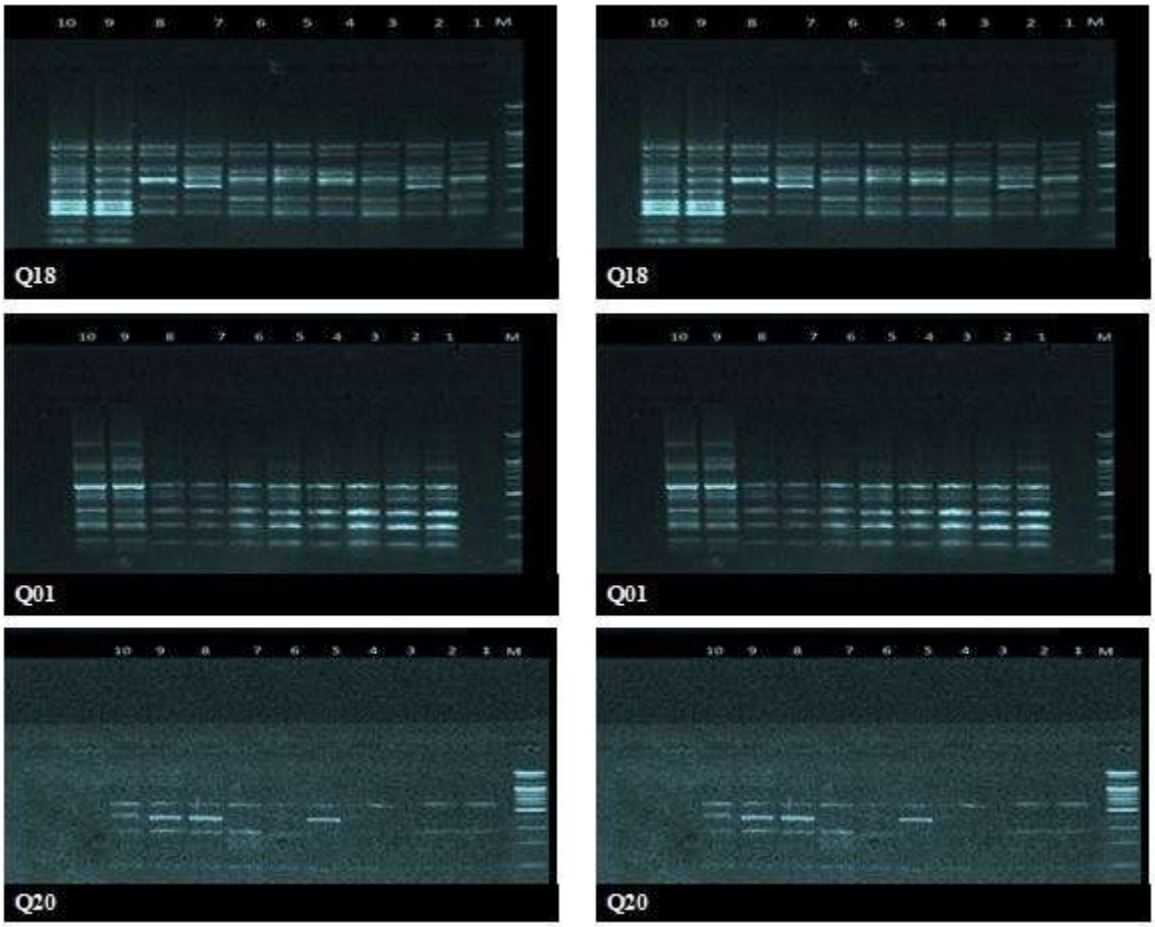

Figure (1) Electrophoresis of the PCR polymerase chain reaction on agarose gel at a concentration of 1.5% using the primers Q18, Q01, Q20, A08, O02, Q05, F05 for ten cultivars of carnation 1 = local, 2 = Bizet, 3 = Shridea, 4 = Orange , 5 = Amprose , 6 = Mariposa , 7 = Grand , 8 = White , 9 = Viana , 10 = Liberty , M = Molecular Weight Index 100 bp (DNA Ladder).

The method of analyzing the results of the genetic relationship study depended on the presence or absence of the bands resulting from the replication of certain pieces of the genome of the plants used and on the molecular weights of those bands that depend on the number and complementary positions of the primer sequences on the template DNA strand. The very light bands were neglected (Swoboda, 1997, Barone et al., 1999).As for the variation that depends on the difference in the intensity of the fluorescence of the bands (Intensity), which is usually caused by the appearance of some doubled bands together in the same molecular weight, it appears in the form of one thick band (which is in fact more than one (comigrating bands) may be caused by the homozygote state.In which the same

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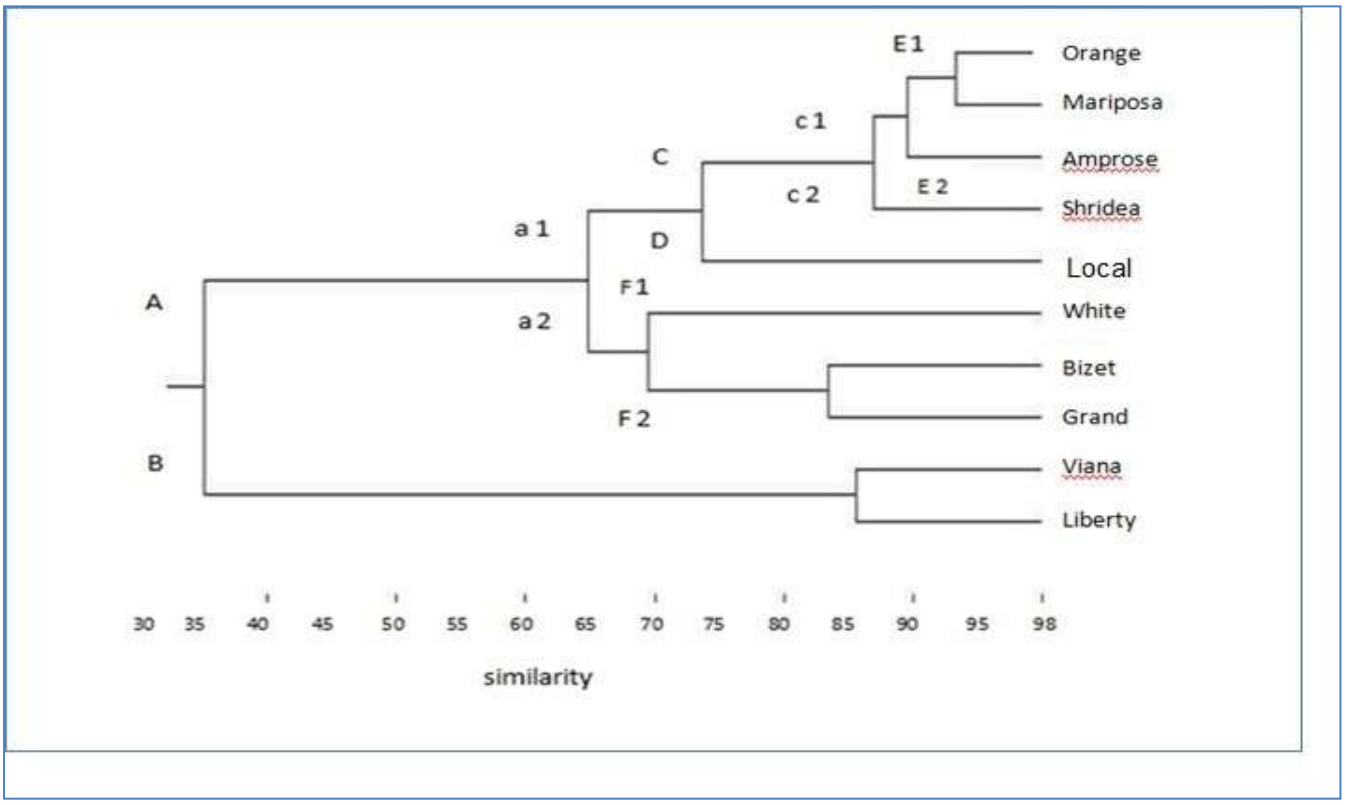
site is duplicated on the other allele, and since they are of the same molecular weight, the duplicating pieces are clustered in those sites together, and sometimes an increase in the concentration of template DNA leads to the repetition of the number of copies of the target DNA, which leads to the doubling of the same site more than once.Since the exact concentration of DNA is difficult to determine because it is affected by several factors, therefore, the difference in the thickness of the resulting bands cannot be used as a measure of genetic variance, especially that RAPD indicators are among the indicators that follow complete dominance, and thus it is not possible to estimate the number of alleles for a single site (Al-Husni, 2002) and agrees This is with Vogt et al. (1997) not to rely on beam fluorescence intensity as a measure of variance because of the difficulty of fine-tuning the DNA concentration.

Figure (2) Genetic kinship tree of plant cultivars based on the results of the RAPD indicators analysis.



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