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Polymorphism with in Different Six Forms of Urginea maritima (L.) Baker (Liliaceae) from the Mediterranean Coast, Egypt

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Abstract:

Urginea maritime (L.) Bakerisanonion-like monocotyledonous plant native to the Mediterranean area and belongs to family Liliaceae. It is use dinpharmacology for its well-known cardiotonic properties. In this study, we have analyzed the genetic variation and the relationships with in different six forms of Urginea maritime (L.) collected from different localities in Egypt using random amplified polymorphic DNA(RAPD)markers. For this purpose, we also analyzed different morphologicaltraits (The bulb weight, volume, length, diameter, leaf shape, length, width, area and number of leaves).Bulb weight ranged from679.8 to 140.9g,bulblengthrangedfrom16 to 9.06cm and leaf area ranged from102.1 to 29.7cm2.RAPD analysis was performed with 5primers chosen after a previous screening. Polymorphism percentage obtained from RAPD-PCR analysis was 87.26%. Significant genetic variability among those six forms was obtained bo that the morphological and molecular level. We obtained adendrogram for the morphologicaltraits and adendrogram for RAPD analysis and we compared them.

Keywords: *Urgineamaritima, RAPD analysis, dendrogram, geneticsimilarity, geneticpolymorphism,Liliaceae, Mediterranean flora.*

1 Introduction

Urgineamaritima(L.)Bakerisherbaceousperennial bulbousgeophytes(herbaceous plant with an underground storage organ)of the family Liliaceae, native to the Mediterranean basin and well-adapted to its type of climate(Kopp et al., 1996). It is apolymorphic species, and there were three distinctmorphological features according to color and size of the bulb (Batanouny and Khalifa, 1970). AbouTabl,(1966) described the genus Urgineaasit is characterized by the following: plants are bulbous herbs. Foliage leaves are radical, sometimes very narrowly linear, broadly strap-shape doralmos to belong, scape and simple. Flowers are found in a terminal raceme usually numerous, small or medium sized, whitish, roseorrarely paleyellowish. Ovary is sessile, three-celled; ovulesarenumerousin each cell. Fruit is capsule, loculicidally dehiscent, parchment like, globular or oblong. Urginea is closely related to the genus Scilla.Urginea maritima occurs in North-Eastern coast of Egypt (Rafah and El-Arish) and in Wadi El-Diagaat the in let from the north.

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Boulos (2005) reported that Urgineamaritima is belonging to family Hycinthaceae.The genusUrginea is found in Egypt with two species, U. undulate and U. maritime. The ancient Egyptians believed in its effect to get rid of devils.

Urgineamaritima has two major phono logical phases within a year; an active one, (autumn-spring) from leaf emergence to senescence of the aerial parts (photosynthetic period), and an inactive one(summer),which last still the leaves emerge(dormancy).The over-wintering bulb develops leaves from the shoot apex in the period between December to March and flowerings talks from August to September. The plant has ornamental value, flowering after several years when the bulb reaches a considerable size (Pascual-Villalobos& Fernandez, 1999).

Molecular markers analysis has greatly enhanced the DNA finger printing, genetic diversity and other plant genetic studies (Kosinski,etal., 2009;Samocha, etal.,2009; and Ikinci, 2010). In fact, classification of the various subgenera, species, and subspecies is based primarily on morphological attributes. However, the setra its may not be significantly distinct and usually require growing plants to maturity prior to identification. Moreover, morphological characters may be unstable due to environment alinfluences. Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular traits. Several DNA marker systems are now common use in diversity studies of plants. The most commonly used marker systems are random amplified polymorphic DNA(RAPD) which characterizes DNA variation patterns within species and among closely related taxa (Torkpo et al.,2006; Muthusamy et al.,2008;and kameshwari et al.,2012). RAPD remains one of the most extensively used molecular techniques due to its simplicity, low cost, high speed and require nopriorin formation of target genome. Thus, RAPD markers have been successfully used in many plants in providing a convenient and rapid assessment of genetic diversity among different genotypes. In general, RAPD can provide valuable data in the analysis of population genetic structure including genetic variation within and among populations, population subdivision, and degree of inbreeding and individual relatedness. Presence or absence of DNA bands in the gelisused as RAPD markers to study close genetic relationship (Laribietal., 2011; and Dwivedi and Sharma, 2011).

RAPD analysis had been proved as a valuable tool for estimating genetic diversity and particularly assists in the conservation of rarespecies and plant genetic resources.RAPD analysis is used to detect the genetic variation and polymorphism within these different populations(JohnDeBrittoetal.,2009; Hammad and Qari,2010; Noormohammadi,etal.,2012).

This work aims to find the polymorphism and the genetic diversity within different six forms collected from different localities in the Mediterranean coast of Egypt depending on morphologicaltra its and RAPD-PCR marker.

chronic radio dermatitis.

2 Materials and Methods:

Plant sampling: The different forms of Urginea maritime (L.) Baker were collected from different localities in the Northern Mediterranean coast of Egypt (Sidi-Barrani in Matrouh and El-Arish regions).Table (1) illustrates the citation, geographical distribution and number of collected samples. Morphological measurements: The measured parameters of U. maritime were as follow: bulb weight (g), length (cm), diameter (cm) and volume (cm3), leaves (number, length, width, and area)for samples from both Matrouh and El- Arish regions. Molecular procedures :DNA was extracted using the CTAB procedure by Doyle and Doyle,(1990)and modified by Edwards et al.(1991).

The RAPD analysis based on PCR reactions was performed according to the original protocol of William setal. (1990),modified by Harini et al. (2008) using 5RAPD primers (Table2).

2.1(Rohlf,1992).

Table (1) Location and citation of different studied forms of *Urginea maritime* in Egypt.

Species	Location	Coordinate	Sample size
1-*Urginea maritima*L. Red bulbs	MarsaMatrouh–Sidi-Barrani	31° 24" N 27° 14' 02" E	10
2-*Urginea maritima* L. Whitish-pinkbulbs	MarsaMatrouh–Sidi-Barrani	31° 24" N 27° 14' 02" E	3
3-*Urginea maritima*L. White bulbs	MarsaMatrouh–Sidi-Barrani	31° 30' 54" N 26° 14' 05" E	22
4-*Urginea maritima*L. Reddish, Whiteand red-white bulbs	El-Arish–Gebel-Ya'liq	30° 43' 35" N 34° 07' 36" E 13 mASL	20

Table (2) Codes, sequences and GC% for the five RAPD primersused in the present study

Primer	Oligonucleoti	GC
OPC-	GAACGGAC	60%
OPM-	GTTGGTGG	60%
OPZ	CAGCACCG	70%
OPAX-	AGGCATCG	60%
OPQ-	AGGCTGGG	70%

Statistical analysis: Analysis of the results was conducted by calculation of the mean, standard deviation, student T-test, analysis of variance(ANOVA) test and Duncan analysis using thesoftwareSPSS11;The other programs used in analyzing data in this work were: Quantity one(1D)analysis, Xn-View software and Community Analysis Package(CAP) program.

RAPD amplification products were scored using the software Quantity one (1D)analysis assuming that each band of different size reflects a single locus. Only unambiguously scored fragments were used for these timation of genetic similarity according to Nei and Li (1979).Based on these data UPGMA clustering (un weighted pair group method using arithmetic averages) was carried out using the software package NTSYS-pc

3 Results and Discussion:

Morphological measurements :Six different forms of U. maritime were noticed according to bulb color as follow: Matrouh region had three different forms (red, white and whitish-pink),and El-Arish region which had another three different colors (reddish-scaled, white-scaled and red-white scaled).

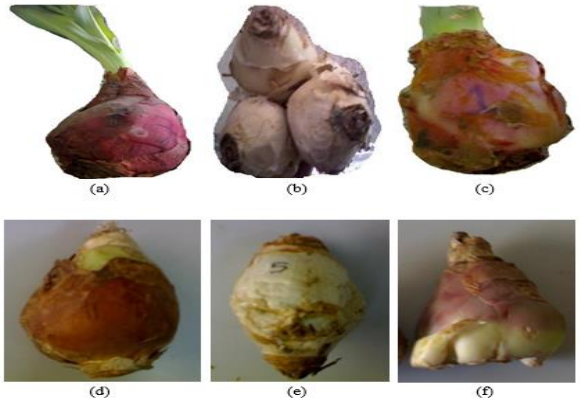
The measurements of U.maritima bulb sare represented in Table (3) as well as the different forms were represented .Generally, the bulbs of U. maritima from Matrouh were larger in all bulb parameters than those of El-Arish; where the bulbs from El-Arish region were more or less uniform in shape and size.

The measurements of U.maritima green leaves were represented in Table (4). Leaves of U.

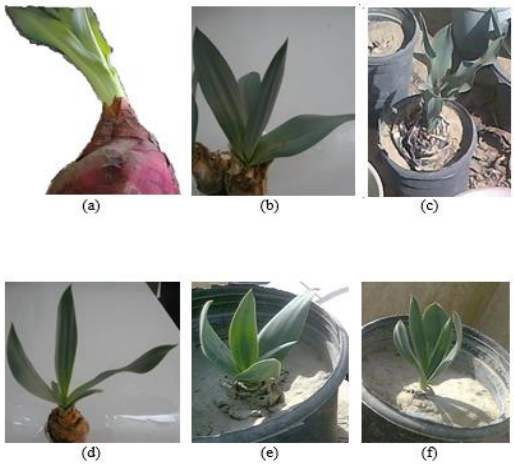
maritiman early grow randomly from the bulb. Leaves were grow ingirectly from the bulb without as talk, with lanceolate-to-undulate in shape. They had longitudinal parallel venation. Leaves had strap-shaped with entire outline. AbouTabl, (1966) described the genusUrginea as it is characterized by the following: plants are bulb ousherbs. Foliage leaves are radical, sometimes very narrowly linear ,broadly strap-shaped or almost oblong, scape and simple.

Speta (1980) also found the morphological differentiation within U. maritime collected from Germany. AbouTabl, (1966) collected white-colored bulb of U.maritima from Egypt(Matrouh)growing in clusters, where red-colored bulb of U.maritima var. pancratium grew frequently singly, sometimes in clusters. El-Sadek,et al. (1994) collected three different forms of U. maritime (red, white and whitish-pink bulbs) from Maruit region, where Batanouny and Khalifa (1970)collected only two forms of red and white blubs from Matrouh. These results indicate that the appearance of whitish-pink bulb as the intermediate format Matrouh region was from the time between 1970 and 1994. Batanouny and Khalifa (1970) collected only one form (intermediate; white tunics of reddish tinge)from El-Arish region were found. So it means that reddish and white one has been created after that date. U.maritima was collected from different localities around the world such as Spain, Italy and Algeria (Boscaiuetal.,2003),Turkey(Özhatay,2000),red and white forms from Greece (Touloupakis et al.,2006).It was also recorded in Mediterrane an coast, Lybia, Sinai, Palestine (Boulos, 2005).

Figure (1) illustrates the different forms of U.maritima collected from the two different localities in the Mediterranean region. From Matrouh (red, white and whitish-pink)and from El-Arish regions (reddish, white and red-white)were collected. The leafe mergence was indicated in fig.(2).The red and whitish-pink forms of U. maritime from Matrouh have undulate margin, where the other for msare entire Based on morphological traits measured on each form, we constructed a dendrogram (fig.3)that divided the all forms into two main clusters. One cluster contained U.maritima from Matrouh (red form);other cluster contained all the other forms and divided into two sub-clusters, one contained white and whitish-pink forms collected from Matrouh; where the other sub-cluster contained all forms collected from El-Arish region and divided into two clades, one contained red-white form and the other clade contained both red dish and white forms.



Figure(1) The different forms of wild *U.maritima* bulbs collected from Sidi-Barrani (Matrouh) at7/11/2008 and El-Arishat4/2009; forms of Matrouh are (a)red form,(b) white and(c) whitish-pink; forms of El-Arish are (d)reddish, (e) white and (f) red-white.



Figure(2) The leaf arrangement to different forms of *U.maritima* collected from Sidi-Barrani (Matrouh)andEl-Arish;formsofMatrouhare(a)redform,(b)whiteand(c)whitish-pink; forms of El-Arish are(d)reddish, (e)white and (f)red-white.

Table (3) Morphological measurements of bulb so different six forms of *U. maritime* collected from Sidi-Barrani and El-Arish regions.

LOCA	Forms	Parameter			
		Bulb weight (Fresh)(g)	Bulb length (cm)	Bulb diameter (cm)	Bulb volume (cm^3)
Matrouh	Red	679.80±561.20a	16.00±5.78a	14.70±5.30a	2284.80±2244.20a
	White	211.70±205b	9.20±2.72b	9.20±3.02b	535.30±473.70b
	Whitishpink	282.70±244.60b	12.50±0.92b	10.30±3.50b	732.90±725.30b
Arish	Reddish	150.00±78.53b	10.01±1.04b	9.70±1.82b	526.20±277.50b
	White	140.90±55.10b	9.06±1.07b	9.80±1.37b	513.90±224.40b
	Red-white	214.20±109.30b	11.01±2.30b	11.20±1.90b	793.50±455.20b
F- value		6.813**	6.835**	4.016**	4.600**

** : P value <0.01. The results are expressed as Mean+/- standard deviation (SD).

Table (4) Morphological measurements of leaves of different six forms of *U. maritime* collected from Sidi-Barrani and El-Arish

LOCALITY	Forms	Parameter			
		Number of leaves	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)
Matrouh	Red	8.62±4.30ab	25.90±9.10bc	5.2±0.97ab	102.1±44.2ab
	White	6.20±3.06ab	21.20±7.20c	4.4±1.04a	72.71±32.7a
	Whitish-pink	8.33±6.60a	29.40±2.90d	4.06±1.5ab	91.96±34.9b
Arish	Reddish	3.50±1.29ab	13.20±1.60cd	3.15±0.4b	34.08±15.7b
	White	3.25±0.50b	14.20±3.10bc	3.03±1.3b	32.8±11.29b
	Red-white	4.00±0.00ab	11.70±0.60cd	3.3±0.77b	29.7±7.57b
F–Value		1.834	8.917**	6.232**	8.974**

means P value <0.01, means P value <0.05. The results are expressed as Mean+/- standard deviation (SD)

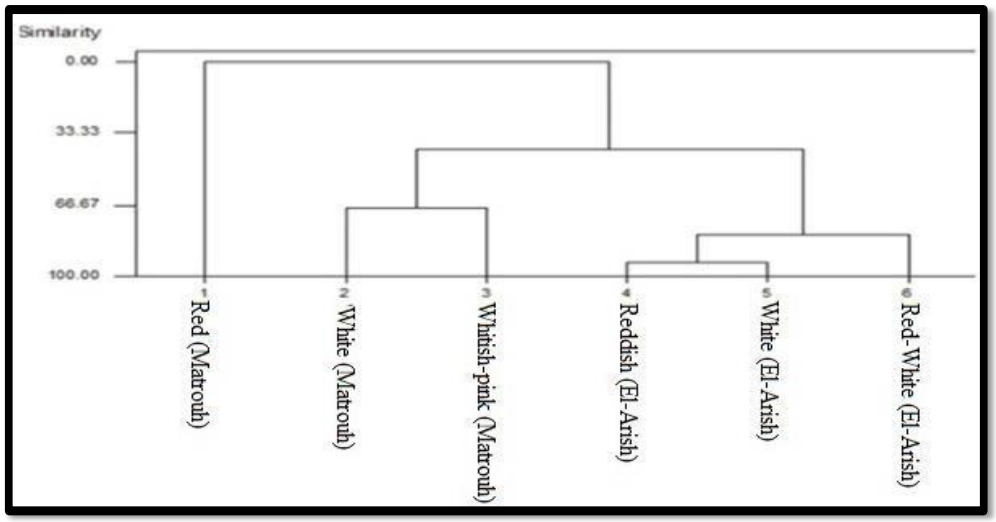


Figure (3) Dendrogram constructed for six forms of *Urginea maritima* based on morphological traits

Molecular polymorphism: RAPD analysis produced amplified fragments that can produce in for mative and polymorphic products resolvable by gel electrophoresis (1.4% Agarosegel) and revealed polymorphism among populations. A total number of 82 amplified fragments were obtained using the five RAPD primers ranged in molecular weight from 1649.484 to 80.341bp. Forty-three unique RAPD markers were identified to characterize the different six forms. The first primer Opax-06 showed8specific markers with the different forms of *U. maritime* collected from Matrouh region and only one from El-Arish region at 629bp ,the second primer(Opc-01) had three specific markers in forms of Matrouh region and also only one from El-Arishat1649 bp,Opm-01had only one specific marker in forms collected from Matrouh region at 250bp and four from El-Arish, Opq-18 is more informative primer and it produced six specific primers from Matrouh region and seven from El- Arish, finally, Opz-03 is also more informative primer as it produced eleven specific markers in the different forms of Matrouh region and three in El-Arish forms. These findings are illustrated in table (5) and fig.(4).

Ninemonomorphic bands common in all different six forms were recognized and thirty polymorphicb ands were detected. The monomorphicb ands were at 920bp with Opax-06primer;400bp with Opc-01;1000,800and433bp withOpm-01;733and618bpwithOpq-18 and finally at 41and312bpwith the last primer (Opz-03).Total polymorphism percentage was87.26%. Table(6) illustrates the similarity of all the different six forms of U.maritima collected from the different localities. The most relate forms were both white bulbs from Matrouh and El-Arish, where the forest related forms were both white and red-white forms collected from El-Arish.

Figure (5) explained the relativity and variation among the different forms of U. maritima based on RAPD-PCR analysis. The UPGMA dendrogram divided the forms into two main clusters. The first cluster contained two different forms of U. maritima from El-Arish (white form) and from Matrouh (red form) and the second cluster is divided into two sub-clusters. The first sub-cluster contained U. maritima from El-Arish (reddish form) and from Matrouh (white form) and the other sub- cluster contained the forms of Matrouh (whitish-pink form) and from El-Arish (red-white from). With respect to above results, Williams et al. (1990) approved that sources of polymorphism in RAPD assay may be due to deletion, addition or substitution of base within the priming site sequence. Also, Welsh and Mc Clell and (1990) found that simple and reproducible fingerprints of complex genomes can be generated using single primers and PCR. According to Lifante and Aguinagalde (1996); Samocha et al. (2009) and Singh et al. (2010) were agreed with our results and confirmed the importance of using RAPD analysis to characterize each form with the appearance of specific markers and produce informative bands that distinguished the different forms. Lifante and Aguinagalde, 1996 and Ikinci et al., (2010) stressed on utilization of cluster analysis to differentiate among the different species.

Looking at the two dendrograms based on the morphological traits and on the molecular markers we can observe that both dendrograms shows a genetic variation within the six forms of U. maritima. Both RAPD and morphological characters were sufficient to assess variability the forms. Despite the low correlation between those two dendrograms it can be observed that some forms were grouped in the same cluster in both dendrograms. For example, red form collected from Matrouh and red- white form collected from El-Arish appeared to be

grouped in the same cluster in both dendrograms. The same results were obtained by other authors in different studies (Maric et al., 2004). The low correlation between RAPD dendrogram and morphological dendrogram had been also reported in other studies in European barley varieties (Schut et al, 1997), synthetic hexaploid

wheat and their parents (Lage et al., 2003) and Squash (Ferriol et al., 2004). Normally until now, germplasm has been classified on the basis of morphological and agronomical traits, but recently the use of molecular markers to study variation and characterization of the plants has become more common. This difference between the two dendrograms can be due to the fact that the morphological traits can be influenced by factors such as: environmental conditions, the many sample size, the time of making the measures, etc. The present study identified that there are different six forms of U. maritima collected from two different localities. Also this study concluded that there are variations within these different forms depending on molecular structure by using RAPD-PCR technique.

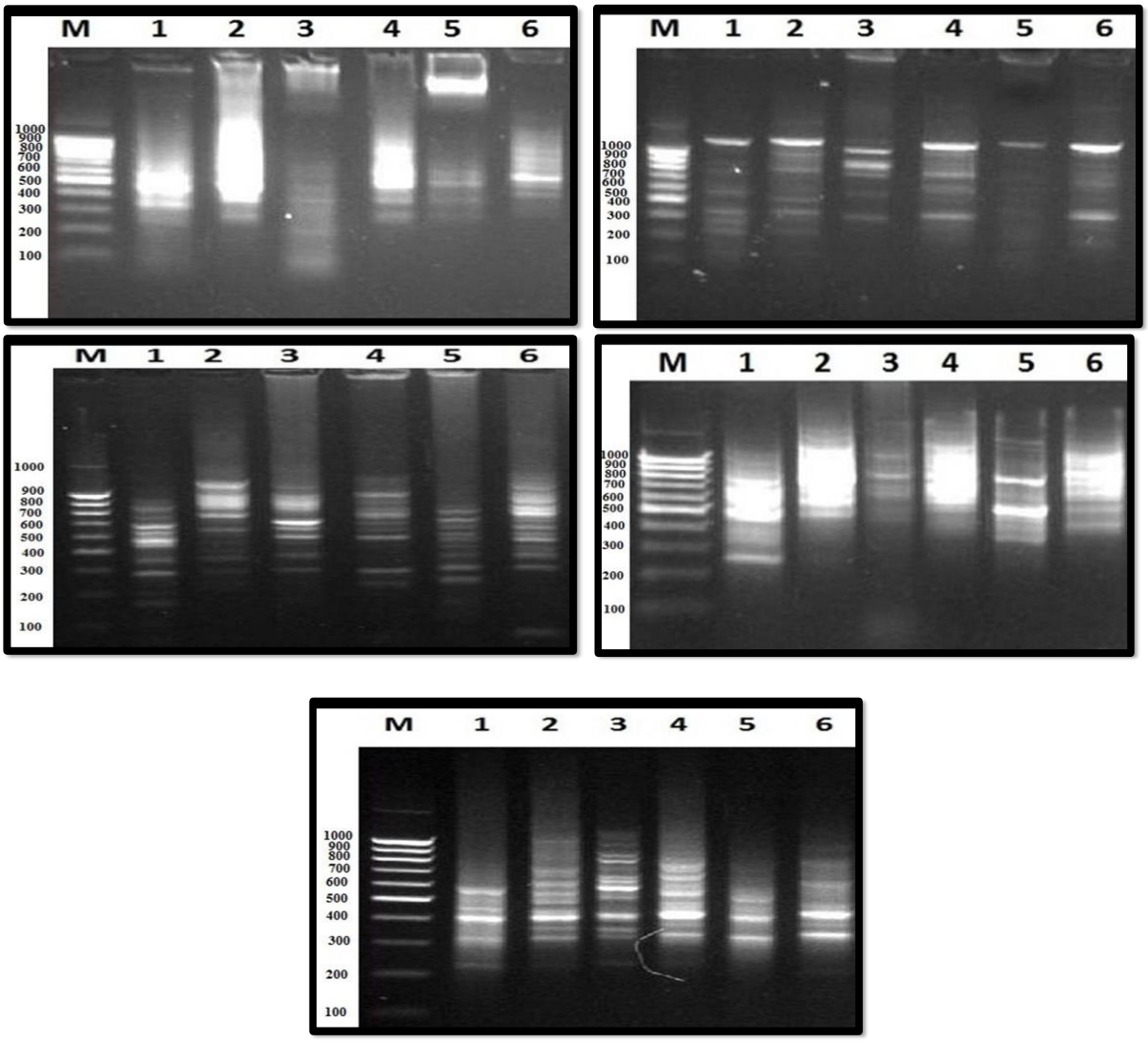


Figure (4) RAPD-PCR gel electrophoresis of different six forms of *U.maritima* using five different primers

Table (5) Number of amplified fragments and specific markers of the different six forms of *U. maritima* using RAPD analysis with five primers.

Primer	***U.maritima*bands**	
	AF	**SM**
Opax-06	13	7
Opc-06	15	4
Opm-01	11	5
Opq-18	23	13
Opz-03	20	14
Total	82	43
Total polymorphism percentage	87.26%	

AF= amplified fragments; SM= specific markers.

Table (6) The similarity matrix of the different six forms of *Urgineamaritima* with the five primers of RAPD-PCR analysis.

	1	2	3	4	5	6
1	100					
2	41	100				
3	28	35	100			
4	26	22	39	100		
5	44	49	24	28	100	
6	28	36	31	36	22	100

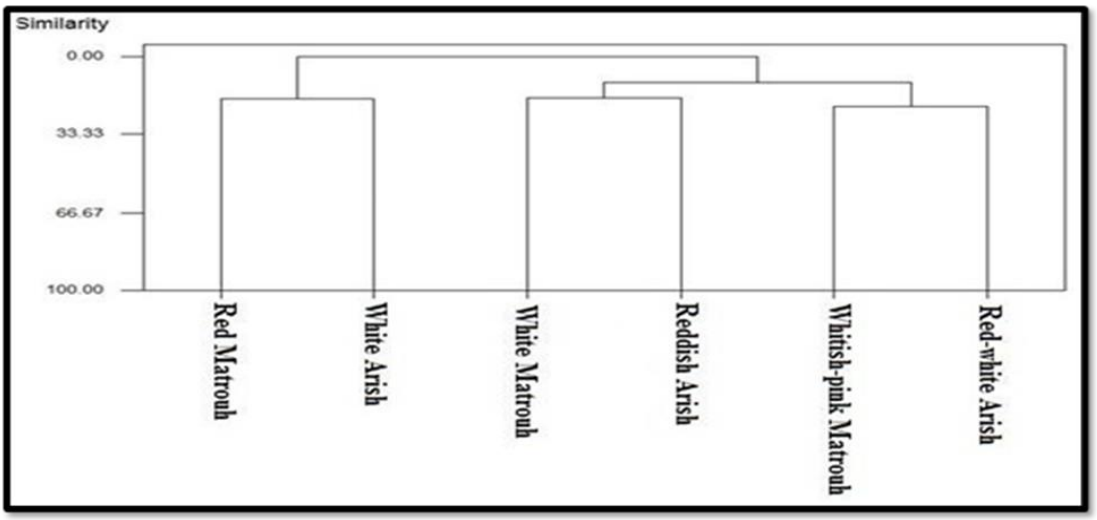


Figure (5) Dendrogram illustrating geneticrelation ships between six forms of *U. maretima* generated by UPGMA cluster analysis of polymorphic RAPD fragments.

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