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Genetic Diversity Analysis of *Achillea fragrantissima* (Forskal) Schultz Bip Populations Collected From Different Regions of Jordan Using RAPD Markers

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Abstract

Medicinal plants are still used traditionally to cure some illnesses. *Achillea fragrantissima* is among medicinal species used by rural communities in Jordan. The genetic diversity of this species was studied using Random Amplified Polymorphic DNA (RAPD) markers of five populations sampled from five locations in Jordan. Fifteen polymorphic primers were used for the construction of the dendrogram and the similarity matrix. A total of 2599 bands were obtained, 420 of them were polymorphic. Similarity values between the populations samples ranged from 0.58 between the samples of Madaba (1 population) to 0.02 between Shoubak (3 populations) and Mwaqqar (2 populations). High similarity values were also recorded between Madaba (1population) and Mafraq (1 population), two samples of Ma'an (3), two samples of Madaba (1), two samples of Mwaqqar, Mafraq (1), and Madaba (1) populations with values from 0.54 to 0.49, respectively. Four main clusters were obtained based on UPGMA dendrogram. Both Mwaqqar2 and Ma'an 3 populations formed separate sub-clusters. This emphasizes the presence of genetic diversity among the studied populations. RAPD analysis has the ability to discriminate between *Achillea fragrantissima* populations.

Keywords: *Achillea fragrantissima*, genetic diversity and RAPD markers, polymorphism.

1. Introduction

Flora of Jordan is rich and diverse with medicinal and aromatic plants as well as herbs and spices. A total of 485 species of these plants belonging to 330 genera and 99 families were reported by Oran, 1994. These plants have been used by local people in folk medicine for curing human and animal illnesses. These species also contribute to increasing the income of poor people, especially women, who are involved in most of the fieldwork, processing and marketing of medicinal plants (Rawashdeh, 2007). *Achillea fragrantissima* is a medicinal shrub plant that belongs to the Asteraceae family (Sahin *et al*., 2006). *A. fragrantissima* species is diploid and contains this number of chromosomes: of 2n =18 (Rawashdeh *et al*., 2009 c; Rawashdeh, 2007). It is known as a sweet-smelling Lavender Cotton (Al-Esawi, 1998) and as one of the important medicinal and aromatic plant species in Jordan. Al-Esawi (1998) described *A. fragrantissima* as a perennial herb, 30-60 cm long, forming a hemispherical bushy growth, covered by minute, soft to woolly hairs, strongly aromatic. Leaves are simple, 0.5-1.5 cm long, with serrate margins, densely, woolly at lower surface. Flower heads are yellow, less than 1 cm in diameter, arranged in a flattened top, medium, compact inflorescence. It flowers from June to October. This species is mainly distributed in the desert and the dry areas of Jordan especially along runoff water places and is found in Eastern desert and Wadi Rum. *A. fragrantissima* is known by local communities as Qaisoum or Qisum (Mustafa, 1991) and is the only *Achillea* species used for medicinal purposes in Jordan. It is mainly used for treating diabetes, intestinal colic, lowering blood cholesterol level and as a carminative, dysmenorrheal and various infections (Atyat, 1993; Hammed, 1993; Mustafa, 1991; Kelly *et al*., 1988).

Several species of *Achillea* are grown for their flowers; their inflorescences are a rich source of active substances such as essential oils, sesquiterpene lactones, flavonoids and tannins (Špinarová and Petříková, 2003). *A. fragrantissima* is used for anti-inflammation, anti-nosebleeds, anti-excessive menstruation and hemohorrids (Atyat, 1993). Abu-Rabia (2005) reported that the boiled leaves of *A. fragrantissima* were used to treat smallpox (Jadra, Jadari) through using them in washing the whole body. Also, it was showed that the leaves had the most

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prominent cytotoxic activity (above 96%) against both types of melanoma cell lines (Sathiyamoorthy *et al*., 1999).

Many different molecular markers techniques were used for assessing genetic diversity (Karp and Edwards, 1997). Among these, the Amplified Fragment Length Polymorphism (AFLP) method has been successfully employed for fingerprinting varieties and cultivars (Vos *et al.,* 1995) and for studying the genetic diversity among and within different populations of *A. fragrantissima* (Rawashdeh *et al*., 2009 b**;** Rawashdeh, 2007).

Morphologic diversity in the field, genetic relatedness using AFLP markers, morphological diversity, chromosome counting, and essential oil composition were studied among and between *A. fragrantissima* populations collected from 15 monitoring areas in Jordan (Rawashdeh *et al*., 2009 a; Rawashdeh *et al*., 2009 b; Rawashdeh, *et al*., 2009 c; Rawashdeh, 2007). The results showed the existence of an association between morphology and molecular analysis, especially in the populations of Shoubak and Ma'an, which form separate groups in the analysis.

RAPD was used to detect the genetic variation among *A. fragrantissima, A. biebersteinii* and *A. santolina,* which have antimicrobial activity using four primers OPB01, OPB10, OPB15 and OPB18. The results showed 35% similarity between *A. fragrantissima,* and *A. santolina* (Kharma, 2004). RAPD was used for fingerprinting of five populations of *A. fragrantissima* in Egypt (Morsy, 2007), revealing that differences in locations were particularly reflected on DNA fingerprints.

This study aims at elucidating the genetic relationships among *A. fragrantissima* populations using RAPD technique.

2. Materials and methods

2.1. Plant material

The study evaluated a total of 50 samples of *A. fragrantissima* composed of 10 samples from each of the populations collected from different habitats in Ma'an (3), Shoubak (1), Madaba (1), Mwaqqar and Mafraq.

2.2. DNA isolation

Following the procedure described by Doyle and Doyle (1987) with minor modifications, total cellular DNA was extracted from the fresh leaves of *A. fragrantissima* populations when they were at the seedling stage. Approximately 20 mg of fresh leaves of "30-days-old" *A. fragrantissima* plants were grounded in liquid nitrogen and mixed with 600 µl of freshly and preheated 2x CTAB solution with 0.8g PVPP in 2ml tubes then placed at 65ºC for 30 min. The mixture was added to 600 µl of chlorophorm/isoamyl alcohol (24:1), vortexed for few seconds, and then centrifuged at 13,000g for 10 min. The supernatant was placed in 2ml tubes with 600ml isopropanol, and then shaken until the thread of DNA appeared, then centrifuged for 20 min at 13,000g. The solution is poured in tubes and left to dry, then 600 µl of cooled 70% ethanol was added to the solution and placed in the refrigerator (–20ºC) overnight. Next day, ethanol was poured in the dried tubes and 150µl of TE was added; the whole mixture was placed at 65ºC for 60 min. Four micolitters of RNAase (10mg/ml) were added per tube and left for 60 min at 37ºC. The DNA quantity was measured using S2100 UV/VIS DIODE-Array-Spectrophotometer, machine Version 1.7.

2.3. PCR amplification

The stored DNA at -20ºC was selected for PCR reaction. PCR reaction was performed as described by Williams *et al*. (1990) with 10-mer ologonucleotides synthesized by Operon technologies (Almeda, Calif.). The final volume of 25µl contained 10 x buffer with $MgCl_2$, 20ng of total genomic DNA, 0.25 mM dNTPs (Promega), 100 µM of primers, 1.5mM $MgCl_2$ and 1U of *Taq* polymerase. Amplification was carried out in thermocycler (MJ Research, USA, Model PCT-200), one cycle of 1 min at 94°C followed by 44 cycles, each consisting of a denaturation step for 1min at 94°C, followed by an annealing step for 1min at 36°C and an extension step for 2 min at 72°C, followed by a further extension step for 5 min at 72°C. After the final cycle, the samples were cooled at 4°C. Samples of 10 µl RAPD-PCR product were analyzed by electrophoresis on 1.4% a garose gel and the

amplified products were detected after staining by ethidium bromide. Forty-four RAPD primers, corresponding to kits A, B, C, D, N, T and Z (Table 1), were used to study the genetic variation and diversity among the *A. fragrantissima* populations. Fifteen RAPD primers which showed polymorphism were used to construct the dendrogram (Table 2).

2.4. Data analysis

RAPD bands were manually scored as present (1) or absent (0) for estimating the similarity among all the tested samples. The matrix of similarity, based on Jaccard's method, and similarity coefficients, based on Nei and Li (1979), were calculated and the dendrogram gained clustering according to the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) using SPSS, V. (11.0), software. The polymorphism percentage was estimated by dividing the number of polymorphic bands over the total number of bands.

3. Results

Forty-four RAPD primers (Table 1) were used for initial assessment of genetic variation in five populations of *Achillea fragrantissima*. A total of 50 samples (10 samples per population) collected from five regions in Jordan were amplified using 15 polymorphic RAPD oligonucleotides indicated in Table 2. The total number of bands, the number of polymorphic bands and the percent of polymorphism per primer are shown in Table 2. A total of 2599 RAPD fragments were consistently recognized and 420 (16%) of them were polymorphic for all population samples (Table 2). The number of polymorphic bands ranged from 34 for OPC03 to 21 for OPN14. The percent of polymorphism ranged from 13% for OPT16 to 21 for OPT15 with an average of 16.4 % polymorphism (Table 2). The total number of the bands amplified ranged from 233 bands (OPT16) to 120 bands (OPD18).

Table 1. Primers names and their sequences as used in this study

Primer name	Sequence 5'-3'	Primer name	Sequence 5'-3'
OPA09	GGGTAACGCC	23. OPC18	TGAGTGGGTG
OPA10	GTGATCGCAG	24. OPC20	ACTTCGCCAC
OPA13	CAGCACCCAC	25. OPD04	TCTGGTGAGG
OPA15	TTCCGAACCC	26. PD06	ACCTGAACGG
OPA16	AGCCAGCGAA	27. OPD10	GGTTCACACC
OPA18	AGGTGACCGT	28. OPD11	AGCGCCATTG
OPA20	GTTGCGATCC	29. OPD14	CTTCCCCAAG
OPB01	GTTTCGCTCC	30. OPD16	AGGGCGTAAG
OPB04	GGACTGGAGT	31. OPD18	GAGAGCCAAC
OPB05	TGCGCCCTTC	32. OPD20	ACCCGGTCAC
OPB06	TGCTCTGCCC	33. OPN14	TCGTGCGGGT
OPB08	GTCCACACGG	34. OPN16	AAGCGACCTG
OPB09	TGGGGGACTC	35. OPT03	TCCACTCCTG
OPB10	CTGCTGGGAC	36. OPT05	GGGTTTGGCA
OPB12	CCTTGACGCA	37. OPT10	CCTTCGGAAG
OPB13	TTCCCCCGCT	38. OPT12	GGGTGTGTAG
OPB14	TCCGCTCTGG	39. OPT15	GGATGCCACT
OPB17	AGGGAACGAG	40. OPT16	GGTGAACGCT
OPB19	ACCCCCGAAG	41. OPT19	GTCCGTATGG
OPC03	GGGGGTCTTT	42. OPZ07	CCAGGAGGAC
OPC09	CTCACCGTCC	43. OPZ10	CCGACAAACC
OPC10	TGTCTGGGTG	44. OPZ18	AGGGTCTGTG

Table 2. Total bands, number of polymorphic bands, and percent polymorphism per primer of 15 polymorphic RAPD primers as used in this study

Primer name	Total bands/primer	Number of polymorphic bands	% of polymorphism
OPA09	200	33	16
OPA10	173	25	14
OPB01	165	30	18
OPB06	175	26	15
OPB19	166	24	14
OPC03	206	34	17
OPD18	120	21	18
OPD20	184	24	13
OPN14	186	34	18
OPN16	164	25	15
OPT03	187	27	14
OPT05	129	22	17
OPT10	161	32	20
OPT16	233	31	13
OPT15	150	32	21
Total	**2599**	**420**	**243** **Mean: 16.4**

Similarity matrix values ranged from (0.58) between the samples of Madaba (1 population) to (0.02) between Shoubak (3 populations) and Mwaqqar (2 populations). High similarity values were also recorded between Madaba (1 population) and Mafraq (1 population), two samples of Ma´an (3 populations), two samples of Madaba (1 population), two samples of Mwaqqar population and Mafraq (1) and Madaba (1), which were 0.54, 0.51, 0.50, 0.49 and 0.49, respectively (Table 3). The dendrogram based on UPGMA analysis grouped the five populations into three main clusters (Figure 1) with Jaccard's similarity coefficients. The first main cluster included 10 samples of Madaba (1 population), 8 samples of Mafraq (1 population) and one sample of Ma´an (3 populations). The second cluster contained 10 samples of Shoubak (3 populations), 9 samples of Ma´an (3 populations) while the third cluster included 10 Mwaqqar (3 populations) and two samples of Mafraq (1 population) (Figure 1).

4. Discussion

High polymorphism that is obtained in this study indicates the presence of genetic variation among the *Achillea fragrantissima* populations collected from different districts in Jordan. This variation is attributed to the differences in the number of alleles per locus/or loci and their distribution within the population. The presence of a large range of similarity values (0.58-0.02) between the tested samples revealed that the differences in the ecosystems of *Achillea fragrantissima* might be reflected on RAPD-PCR. Similar results were obtained by Morsy (2007) when fingerprinting five populations of *Achillea fragrantissima* using RAPD-PCR. In this study, eight samples out of 10 of Shoubak populations formed a separate sub-cluster, which is in agreement with the findings by Rawashdeh *et al.*, (2009 b) using AFLP markers. This result indicates that a genetic diversity is found among the studied populations. RAPD analysis has a potential for studying the genetic diversity among and within *Achillea fragrantissima* populations. However, the application of biochemical and genetic systems play a great role in studying the status of wild plant species and in investigating their evolution and migration from their centers of distribution. Combining several of molecular patterns such as proteins, isoenzymes and AFLP, SSRs and ISSR profiling will allow discriminating between all populations and species of *Achillea* genus in the future.

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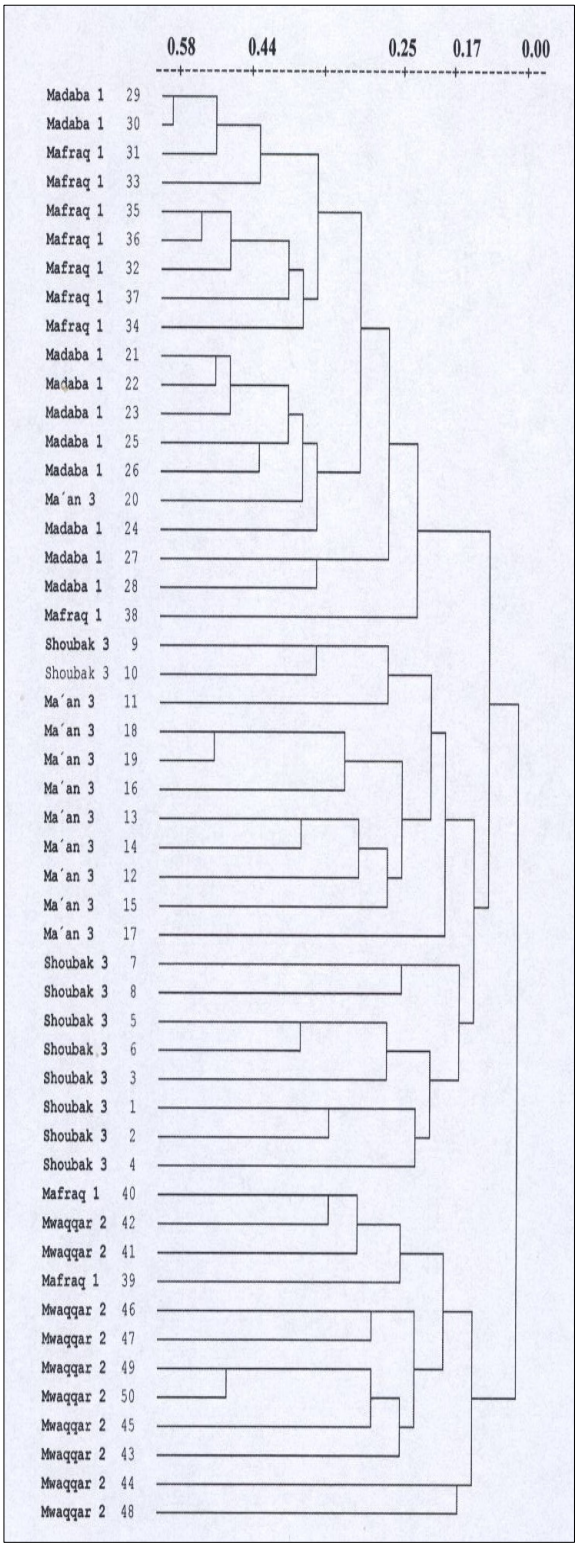

Figure 1. A dendrogram among five locations of medicinal plant species "*Achillea fragrantissima*" using fifteen polymorphic RAPD primers, based on Jaccards´ coefficient of similarity. The disconnected line is the point where the dendrogram is divided.

Table 3 continues next page……

Table 3: RAPD similarity matrix based on similarity coefficient of the amplified bands for *Achillea fragrantissima* species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1																													
2	0.35	1																												
3	0.22	0.28	1																											
4	0.22	0.24	0.21	1																										
5	0.16	0.19	0.30	0.25	1																									
6	0.18	0.21	0.26	0.22	039	1																								
7	0.16	0.15	0.16	0.13	0.16	0.16	1																							
8	0.20	0.15	0.20	0.19	0.17	0.22	0.25	1																						
9	0.16	0.14	0.21	0.18	0.24	0.23	0.19	0.30	1																					
10	0.11	0.12	0.15	0.15	0.20	0.14	0.14	0.20	0.36	1																				
11	0.18	0.17	0.22	0.21	0.21	0.21	0.18	0.20	0.26	0.27	1																			
12	0.15	0.13	0.11	0.20	0.15	0.14	0.09	0.11	0.21	0.23	0.24	1																		
13	0.16	0.19	0.15	0.16	0.18	0.17	0.16	0.21	0.28	0.31	0.25	0.33	1																	
14	0.19	0.19	0.12	0.16	0.13	0.15	0.14	0.16	0.20	0.21	0.27	0.31	0.40	1																
15	0.14	0.14	0.10	0.22	0.14	0.12	0.15	0.13	0.17	0.22	0.20	0.22	0.28	0.30	1															
16	0.11	0.10	0.13	0.14	0.13	0.14	0.14	0.11	0.19	0.16	0.22	0.24	0.28	0.23	030	1														
17	0.15	0.12	0.11	0.13	0.11	0.13	0.14	0.12	0.20	0.17	0.16	0.19	0.21	0.21	0.18	0.20	1													
18	0.14	0.15	0.11	0.14	0.11	0.08	0.13	0.11	0.22	0.18	0.17	0.25	0.32	0.27	0.19	0.34	0.22	1												
19	0.17	0.16	0.14	0.19	0.11	0.11	0.15	0.12	0.17	0.13	0.17	0.24	0.24	0.26	0.22	0.31	0.18	0.51	1											
20	0.14	0.10	0.09	0.10	0.09	0.12	0.04	0.07	0.11	0.12	0.18	0.20	0.11	0.17	0.17	0.18	0.16	0.17	0.19	1										
21	0.14	0.12	0.09	0.12	0.12	0.13	0.05	0.07	0.11	0.10	0.17	0.25	0.13	0.17	0.16	0.14	0.11	0.17	0.21	0.39	1									
22	0.18	0.13	0.10	0.13	0.10	0.13	0.05	0.07	0.12	0.07	0.14	0.18	0.10	0.17	0.14	0.15	0.11	0.19	0.20	0.42	0.50	1								
23	0.15	0.12	0.12	0.18	0.11	0.10	0.03	0.08	0.15	0.08	0.19	0.17	0.12	0.18	0.15	0.14	0.16	0.16	0.14	0.38	0.48	0.49	1							
24	0.15	0.13	0.16	0.13	0.11	0.14	0.09	0.11	0.15	0.09	0.20	0.18	0.14	0.16	0.12	0.15	0.15	0.16	0.16	0.32	0.32	0.41	0.41	1						
25	0.12	0.09	0.07	0.11	0.06	0.08	0.05	0.09	0.11	0.08	0.11	0.15	0.11	0.17	0.14	0.13	0.11	0.15	0.17	0.35	0.38	0.48	0.40	0.33	1					
26	0.11	0.10	0.09	0.11	0.08	0.11	0.08	0.10	0.10	0.07	0.16	0.16	0.13	0.17	0.13	0.15	0.10	0.17	0.21	0.37	0.45	0.42	0.39	0.42	0.44	1				
27	0.12	0.11	0.12	0.13	0.12	0.18	0.07	0.06	0.08	0.08	0.18	0.16	0.15	0.17	0.16	0.14	0.16	0.12	0.17	0.26	0.31	0.28	0.29	0.28	0.25	0.34	1			
28	0.18	0.10	0.10	0.12	0.08	0.12	0.11	0.06	0.12	0.11	0.16	0.18	0.12	0.14	0.16	0.13	0.20	0.15	0.18	0.29	0.28	0.26	0.25	0.31	0.24	0.33	0.36	1		
29	0.15	0.08	0.10	0.15	0.08	0.10	0.06	0.08	0.11	0.14	0.17	0.21	0.10	0.18	0.14	0.17	0.13	0.16	0.18	0.31	0.36	0.34	0.29	0.32	0.40	0.38	0.30	0.32	1	
30	0.17	0.12	0.14	0.15	0.09	0.15	0.05	0.06	0.10	0.11	0.20	0.17	0.15	0.16	0.17	0.18	0.11	0.13	0.19	0.36	0.38	0.35	0.30	0.32	0.32	0.40	0.38	0.35	0.58	1
31	0.13	0.15	0.14	0.16	0.10	0.11	0.07	0.07	0.10	0.12	0.14	0.20	0.14	0.16	0.16	0.18	0.14	0.20	0.22	0.32	0.44	0.37	0.35	0.29	0.33	0.38	0.29	0.33	0.49	0.54
32	0.22	0.15	0.17	0.16	0.16	0.14	0.06	0.09	0.14	0.18	0.17	0.22	0.17	0.21	0.19	0.22	0.14	0.19	0.21	0.34	0.34	0.37	0.36	0.27	0.32	0.28	0.24	0.29	0.41	0.49
33	0.12	0.10	0.13	0.17	0.11	0.16	0.06	0.09	0.12	0.12	0.18	0.17	0.14	0.14	0.13	0.15	0.17	0.14	0.17	0.29	0.36	0.39	0.35	0.26	0.41	0.35	0.38	0.30	0.42	0.46
34	0.16	0.13	0.09	0.21	0.15	0.12	0.06	0.09	0.12	0.14	0.17	0.16	0.14	009	0.08	0.10	0.08	0.15	0.13	0.23	0.32	0.29	0.33	0.20	0.26	0.27	0.18	0.21	0.28	0.34
35	0.15	0.13	0.10	0.19	0.15	0.15	0.6	0.09	0.12	0.16	0.12	0.19	0.15	0.15	0.12	0.15	0.12	0.19	0.15	0.25	0.34	0.33	0.34	0.21	0.28	0.26	0.19	0.18	0.37	0.41
36	018	0.13	0.15	0.15	0.15	0.14	0.06	0.11	0.13	0.15	0.16	0.23	0.14	0.16	0.13	015	0.17	0.17	0.19	0.32	0.34	0.41	0.32	0.25	0.32	0.31	0.23	0.22	0.32	0.36
37	0.14	0.14	0.15	0.18	0.14	0.14	0.09	0.11	0.14	0.10	0.15	0.14	0.11	0.14	0.13	0.12	0.10	0.13	0.12	0.28	0.24	0.29	0.27	0.18	0.25	0.24	0.16	0.18	0.35	0.32
38	0.17	0.16	0.14	0.21	0.11	0.13	0.06	0.08	0.09	0.13	0.12	0.17	0.10	0.14	0.11	0.12	0.14	0.11	0.15	0.21	0.25	0.25	0.23	0.14	0.21	0.19	0.16	0.16	0.19	0.23
39	0.07	0.047	0.07	0.07	0.07	0.10	0.05	0.07	0.12	0.13	0.12	0.09	0.05	0.06	0.07	0.07	0.08	0.13	0.08	0.10	0.09	0.08	0.07	0.12	0.04	0.11	0.10	0.14	0.09	0.09
40	0.09	0.09	0.07	0.10	0.08	0.14	0.07	0.07	0.08	0.17	0.10	0.08	0.06	0.13	0.09	0.09	0.09	0.12	0.11	0.15	0.13	0.14	0.14	0.10	0.10	0.14	0.14	0.13	0.14	0.14
41	0.08	0.06	0.07	0.11	0.06	0.10	0.09	0.08	0.12	0.14	0.12	0.19	0.12	0.16	0.16	0.13	0.16	0.16	0.15	0.13	0.11	0.12	0.09	0.13	0.10	0.11	0.11	0.14	0.16	0.12
42	0.08	0.06	0.04	0.08	0.05	0.10	0.06	0.06	0.11	0.12	0.12	0.11	0.09	0.12	0.12	0.10	0.12	0.11	0.10	0.12	0.10	0.11	0.10	0.09	0.08	0.10	0.11	0.16	0.11	0.10
43	0.06	0.06	0.07	0.09	0.07	0.06	0.08	0.05	0.09	0.13	0.10	0.15	0.07	0.08	0.10	0.11	0.06	0.09	0.08	0.12	0.11	0.09	0.09	0.07	0.06	0.09	0.10	0.11	0.12	0.13
44	0.08	0.07	0.09	0.10	0.07	0.09	0.07	0.09	0.11	0.08	0.10	0.16	0.09	0.18	0.12	0.08	0.12	0.10	0.14	0.11	0.08	0.06	0.07	0.06	0.07	0.07	0.11	0.14	0.09	0.06
45	0.13	0.08	0.09	0.09	0.11	0.14	0.08	0.09	0.14	0.12	0.11	0.11	0.04	0.11	0.09	0.07	0.11	0.07	0.09	0.10	0.12	0.13	0.13	0.08	0.09	0.09	0.13	0.11	0.12	0.08
46	0.06	0.05	0.03	0.09	0.07	0.11	0.09	0.08	0.07	0.09	0.06	0.09	0.07	0.07	0.07	0.04	0.08	0.06	0.06	00.6	0.07	0.05	0.07	0.06	0.05	0.10	0.07	0.06	0.07	0.05
47	0.04	0.02	0.20	0.06	0.04	0.05	0.06	0.05	0.05	0.09	0.04	0.05	0.03	0.02	0.07	0.02	0.03	0.03	0.03	0.05	0.04	0.07	0.04	0.07	0.08	0.05	0.07	0.07	0.06	0.07
48	0.06	0.07	0.08	00.8	0.10	0.08	0.07	0.10	0.06	0.09	0.10	0.10	0.08	0.08	0.11	0.05	0.09	0.11	0.10	0.20	0.06	0.04	0.04	0.04	0.02	0.07	0.10	0.09	0.06	0.07
49	0.07	0.04	0.05	0.08	0.09	0.10	0.07	0.05	0.06	0.07	0.08	0.08	0.03	0.06	0.07	0.04	0.09	0.06	0.03	0.04	0.07	0.06	0.06	0.06	0.05	0.06	0.08	0.11	0.11	0.09
50	0.07	0.4	0.05	0.09	0.08	0.11	0.05	0.05	0.07	0.11	0.11	0.10	0.05	0.07	0.07	0.03	0.07	0.05	0.05	0.06	0.09	0.09	0.07	0.08	0.06	0.07	0.08	0.09	0.12	0.11

Table 3: RAPD similarity matrix based on similarity coefficient of the amplified bands for *Achillea fragrantissima* species continues……..

	31	32	33	34	35	36	12	38	39	40	41	42	43	44	45	46	47	48	49	50
31	1																			
32	0.45	1																		
33	0.46	0.41	1																	
34	0.32	0.34	0.38	1																
35	0.41	0.48	0.41	0.49	1															
36	0.38	0.49	0.42	0.36	0.53	1														
37	0.27	0.38	0.31	0.35	0.44	0.41	1													
38	0.30	0.31	0.24	0.26	0.29	0.32	0.30	1												
39	0.09	0.12	0.07	0.12	0.07	0.08	0.07	0.13	1											
40	0.14	0.12	0.13	0.14	0.13	0.12	0.15	0.13	0.29	1										
41	0.15	0.13	0.13	0.09	0.09	0.11	0.10	0.14	0.25	0.33	1									
42	0.10	0.07	0.09	0.10	0.10	0.07	0.12	0.08	0.26	0.34	0.29	1								
43	0.11	0.13	0.11	0.09	0.10	0.09	0.14	0.09	0.16	0.22	0.23	0.25	1							
44	0.07	0.06	0.06	0.04	0.05	0.06	0.08	0.09	0.16	0.18	0.17	0.15	0.12	1						
45	0.08	0.13	0.14	0.10	0.14	0.15	0.16	0.12	0.15	0.16	0.23	0.19	0.24	0.23	1					
46	0.06	0.07	0.08	0.08	0.08	0.12	0.11	0.09	0.15	0.23	0.19	0.19	0.22	0.18	0.25	1				
47	0.03	0.05	0.08	0.07	0.09	0.06	0.15	0.05	0.18	0.15	0.14	0.24	0.27	0.16	0.16	0.29	1			
48	0.07	0.05	0.06	0.04	0.06	0.07	0.09	0.07	0.10	0.08	0.15	0.13	0.16	0.17	0.15	0.12	0.21	1		
49	0.08	0.07	0.08	0.10	0.12	0.12	0.13	0.12	0.12	0.13	0.11	0.21	0.17	0.11	0.25	0.23	0.18	0.10	1	
50	0.08	0.09	0.08	0.11	0.11	0.11	0.16	0.14	0.20	0.19	0.21	0.28	0.34	0.17	0.31	0.30	0.27	014	0.49	1

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