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ASSESSING THE GENETIC RELATIONSHIP OF TAIF ROSE WITH SOME ROSE GENOTYPES (*ROSA SP.*) BASED ON RANDOM AMPLIFIED POLYMORPHIC DNA, INTER SIMPLE SEQUENCE REPEAT AND SIMPLE SEQUENCE REPEAT MARKERS

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ABSTRACT

Taif-roses are a famous rose type that cultivated in Taif region and well known with their deep and intensive fragrance in the Arabian World. Despite of the great economical importance of Taif-roses for the kingdom of Saudi Arabia, their genetic origin has not been yet elucidated. The present study was mainly aimed to assess the genetic relationship between Taif-roses and some rose genotypes that grown in some kingdom neighboring countries using molecular markers and aromatic amino acids contents. Three Taif-roses genotypes namely Hada, Shafa-1 and Shafa-2 were compared to nine different rose genotypes that are grown in Egypt and Syria. Out of 12 RAPD, 8 ISSR and 8 SSR primers used, clear and repeatable band profile of 8, 6 and 7 primers was obtained from the three markers, respectively. Total of 111, 64 and 15 bands with polymorohism of 96.4, 90.6 and 93.3% were obtained using RAPD, ISSR and SSR, respectively. The discriminating power of the three markers has led to efficient grouping of the 12 rose genotypes using Unweighted Pair Group Method (UPGMA). Among the 12 genotypes, Syrian-Gory rose shown the highest genetic similarity of 75, 92 and 65% with the three Taif-roses genotypes Hada, Shafa-1 and Shafa-2, respectively. The established dendrogram was clearly separated the 12 rose genotypes into four major groups in which the three Taif-roses genotypes were clustered in the same group with the Gory rose-Syrian genotype. Moreover, the data revealed that among the studied rose genotypes, the contents of aromatic amino acids in Syrian-Gory rose and the Taif rose-Hada was the highest and followed by the Egyptian Balady rose 1. While Dutch rose 1, 2, 3 and Dutch tulip 1, 2 were recorded to be the lowest. Together, these results indicate that Taif-rose has closed genetic relations to the Gory rose-Syrian cultivated in Syria. Additionally, a reproducible protocol for *In vitro* propagation, of Taif-rose genotype (Hada) was developed.

Keywords: Taif Roses, Genetic Relationship, RAPD, ISSR, SSR, Amino Acids Analysis and Regeneration

1. INTRODUCTION

The roses of Al-Taif are well known with their deep and intensive fragrance in the Arabian region. These oil-rich, 30-petal rose has been cultivated in Taif for three centuries. Taif city is characterized with its favorable temperatures (as West of the Taif city rises above 2000 meters over the see level), plentiful groundwater, well-established irrigation systems and fine topsoil. These advantages have combined to earn the region the name

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of "Arabia's Rose," ever since roses began to be cultivated there in the Ottoman era. Taif-roses have an important role in the national economy for their great value in exportation of their oil to the Arab countries, ornamentation, medicinal use as well as manufacture of perfumes (Bazaid, 2004). The flowers, whose fragrance is even more intensive than fragrance of the Damask Rose, are harvested in April. Despite of the likely origin of El Taif-Roses as a Damask Rose (Rosa x *Damascena trigintipetala*), it has never been completely cleared how it appeared in Taif. However, due to their close resemblance famous Bulgarian "kazanlik" strain, it has been suggested that Taif-Roses were brought from Balkans by Turks, who occupied this area in the 14th century. The genetic relations of the Taif-Roses with other rose genotypes have never been studies before. In addition, production of Taif-Roses is modest when compared to the quantities produced by larger and export-oriented operations countries such as Turkey, Bulgaria, Russia, China, India, Morocco and Iran.

DNA-based molecular marker systems are efficient and informative for genetic analysis of roses because DNA polymorphism indicated by these markers is not affected by environmental conditions. Several molecular marker systems, i.e., Restriction Fragment Length Polymorphism (RFLP) (Rajapakse *et al*., 2001), Random Amplified Polymorphic DNA (RAPD) (Atienza *et al*., 2005; Kaur *et al*., 2007) Simple Sequence Repeat (SSR) (Esselink *et al*., 2003) and Amplified Fragment Length Polymorphism (AFLP) (Baydar *et al*., 2004) have been used for genetic analysis of rose species, cultivars and rootstocks. RAPD markers have been widely used in many plant species such as anthurium (Nowbuth *et al*., 2005), strawberry (Gaafar and Saker, 2006), polyscias and schefflera (Rout *et al*., 2007), banana (Brown *et al*., 2009), olive (Sesli and Yeğenoğlu, 2009) for identification, varietal analysis, population studies and genetic linkage mapping. RAPD markers are used to detect DNA polymorphism without requirement of previous knowledge of the target genome (Williams *et al*., 1990). Microsatellite markers are also markers of choice (Esselink *et al*., 2003) because they are abundant, uniformly distributed, highly polymorphic, codominant, rapidly produced by PCR and easily accessed through published primer sequences (Gupta and Varshney, 2000).

The major objective of this study was to use the molecular markers RAPD, ISSR and SSR to investigate the genetic relations of Taif-Roses with some rose genotypes that are cultivating in some kingdom-borders countries. Also the total amino acid and aromatic components of these genotypes were measured and used for comparison. Moreover, the development of simple micropropagation system for mass production of Taif-roses was also addressed.

2. MATERIALS AND METHODS

2.1. Materials

Three genotypes of Taif-Roses grown in Taif city in the Kingdome of Saudi Arabia namely Hada, Shafa-1 and Shafa-2 were collected in addition to other nine different rose genotypes that are cultivating in Egypt and Syria and used in this study. The nine rose genotypes are namely, Balady rose 1, 2 and 3 (from Egypt), Dutch rose 1, 2 and 3, Dutch Tulips 1 and 2 (cultivated in Egypt) and Gory rose-Syrian (cultivated in Syria). The stem nodes of rose plants (genotype Hada) were collected and were used for developing a micropropagation system for mass production of Taif- Roses.

2.2. Methods

2.2.1. DNA Isolation

The DNA was extracted from the 12 rose genotypes and was used as templates for 12 RAPD, 8 ISSR and 8 SSR reactions. DNA extraction at sufficient amount and quality is very difficult from rose leaves because the presence of the secondary compounds. To get rid of the phenolic compounds, we applied the protocol of Doyle and Doyle (1988) for DNA isolation using Cationic hexadecyl Trimethyl Ammonium Bromide (CTAB) with slight modifications. Two times extraction with 2-mercaptoethanol was performed after the addition of an antioxidant and PVP to bound the phenolic compounds.

2.3. RAPD Analysis

2.3.1. PCR Reaction and Condition

A total of 12 random primers were used to detect the polymorphism among the 12 different rose genotypes. The sequence of the 8 primers that produce a clear and repeatable banding pattern is shown in (**Table 1**). The amplification was performed in a 25 μL reaction volume containing about 3 μL (10 ng μL) genomic DNA, 3 μL primer (Operon Technologies Inc.) and 19 μL master mix (Promega). The PCR temperature profile was applied through a Gene Amp® PCR System 9700 (Perkin Elmer, England). The thermal cycler was programmed with an initial step of 5 min at 94°C that was followed by 40 repeated cycles of 60 s at 94°C, an annealing step of 1 min at 37°C and an elongation step of 1 min at 72°C; and finally a 7 min extension at 72°C. The amplification

products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 µg mL) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000).

2.4. ISSR Analysis

Eight ISSR primers were tested using a specific and optimal annealing temperature for each one. Only 6 ISSR primers showed repeatable products (**Table 2**). PCR reactions were performed in a volume of 20 µL in Eppendorf thermocycler. The reaction mixture contained 0.6 µL of each primer, 100 µM of each deoxinucleotide, 0.5 units of Go Taq polymerase (Promega), 10x Taq buffer containing 2.5 mM MgCl2 and 10 ng of template DNA. Amplification reaction was 94°C/5 min, followed by 30 cycles of 94°C/1 min, 48-60°C (specific for each primer)/1 min and 72°C/2 min and ending with an extension step of 72°C/7 min. PCR products were analyzed using agarose (2% w/v) electrophoresis gels stained with ethidium bromide and only bands with high intensity and well separated were selected.

2.5. SSR Analysis

Out of 8 pair of primers that were tested to detect the polymorphism, seven pairs have recorded clear reproducible amplifications among the 12 rose genotypes (**Table 3**). The amplification was performed in a 25 µL final volume of a reaction mixture, which included reaction buffer 1x, 0.2 mM each deoxinucleotide, 0.25 mM each primer, 1.5 Mm MgCl2, 1U of enzyme Taq polymerase (Promega) and 50-100 ng genomic DNA matrix. The PCR reaction was performed using a thermo-cycler (Applied Biosystem GeneAmp 9600) programmed for: 3 min initial denaturation at 94°C, followed by 35 cycles, each consisting of: 1 min at 94°C, 1 min at 61°C, 2 min at 72°C and a final extension of 10 min at 72°C. PCR products were evaluated by electrophoresis, on 2% agarose gels in 0.5x TBE buffer, stained with ethidium bromide and were recorded as BioPrint images.

2.6. Data Analysis

PCR reactions were repeated three times and only reproducible bands were scored. Each polymorphic amplified fragment was scored as presence (1) and absence (0) and data were transcribed into binary format. Based on the matrix of genetic similarity, cluster analysis was done. The Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method was used for clustering employing the NTSYS pc (Numerical Taxonomy and Multivariate Analysis System, version 1.8) program (Rholf, 1994).

2.7. Amino Acid Analysis

Different amino acids were extracted from rose petioles and analyzed using the LC-6A HPLC system (LC-6A HPLC, Shimadzu, Kyoto, Japan). The amino acidswas extracted from 1 gm of dried rose petioles as described by (Yamanaka *et al*., 1994; Helsen *et al*., 2008).

Table 1: List of random primers that have been used for RAPD analysis

Primer name	Primer sequence	No. of amplicons	No. of polymorphic bands	Polymorphism (%)
OPA-04	5'-AATCGGGCTG-3'	12	11	92
OPA-09	5'-GGGTAACGCC-3'	14	13	93
OPA-15	5'-TTCCGAACCC-3'	12	12	100
OPA-20	5'-GTTGCGATCC-3'	19	19	100
OPB-03	5'-CATCCCCCTG-3'	11	10	91
OPB-16	5'-TTTGCCCGGA-3'	21	21	100
OPC-02	5'-GTGAGGCGTC-3'	10	09	100
OPC-07	5-'GTCCCGACGA-3'-	12	12	100
Total		111	107	96.4

Table 2: List of primers that have been used for ISSR analysis (Jabbarzadeh *et al*., 2010)

Primer name	Sequence	No. of Amplicons	No. of polymorphic bands	Polymorphism (%)
ISSR 1	5-CCA(CT)8-3	11	11	100
ISSR 2	5-(GTG)3GC-3	11	11	100
ISSR 3	5-(CT)8AC-3	11	11	100
ISSR 4	5-(GA)8ACC-3	12	10	83
ISSR 5	5-(GT)6CC-3	09	08	89
ISSR 6	5-CCA(AG)8T-3	10	07	70
Total		64	58	90.6

Table 3. SSR markers and corresponding primers used in this study (Zhang *et al*., 2006)

Marker name		Primer	No. of amplicons	No. of polymorphic bands	Polymorphism %
Rw10J19	F	GCGAGTTGACGACGAGTT			
	R	GGGTGGGCTTCCTTAGTTA	3	3	100
Rw4E22	F	ATGGGAGACAGAGGTGTAAG			
	R	TCCTAACTCTCGGTGGAGAT	3	3	100
Rw3N19	F	CTGGCTGGTTCTCTTTCTG			
	R	ATGGGTCGTCGTCGATATG	2	2	100
Rw17I7	F	CAGGTAATTTGCGGATGAAG			
	R	GATCCGCCGTTTCCAGT	2	2	100
Rw14A5	F	CCCTCAAAACCCCTCTTA			
	R	CGTAATAACGGTAATAAAATC	2	2	100
Rw22A3	F	AGAGAATTGAAAAGGGCAAG			
	R	GAGCAAGCAAGACACTGTAA	2	2	100
Rw22B6	F	ACAGTGAGTTGTTCGCTTCT			
	R	TTCATTGCTAGGAAGCAGTA	1	0	0
Total			15	14	93.3

2.8. *In vitro* Propagation of Taif-Roses

The stem nods of the Taif-Rose genotype Hada were used for the development of micropropagation system. Nodal explants containing lateral buds of actively field-grown were cut and sterilized with 0.1% solution of mercuric chloride for 10 min. The disinfected explants were inoculated aseptically on culture medium (Murashige and Skoog, 1962) supplemented with different concentration of 2,4, D (1.0 to 4.0 mg L^{-1}) singly or in combination with Kinetin (1 mg L^{-1}). The pH of medium was adjusted to 5.7 and solidified by 0.6 g L^{-1} gelan gum. It was autoclaved at 121°C and pressure of 1 kg/cm^2 for 20 min. For subsequent subcultures, BAP (1.0 to 4.0 mg L^{-1}) were added to the basal medium for induction of multiple shoots. Each treatment was consisted of 3 replications in which 10 to 12 explants were used. The LSD analysis was performed using the Analyse-it software LTD (PO box 77, Leeds, LS125XA, UK) according to Maxwell and Delaney (1989). All cultures were grown under a photoperiod of 16 h light from white fluorescent tubes at a temperature of 25±1°C.

3. RESULTS

3.1. RAPD-PCR Fingerprinting of Rose Genotypes

Out of tested 12 RAPD primers only eight repeatable reactions were reproducible. The RAPD profiles obtained by primers OPA-04, OPA-09 and OPC-02 are illustrated in **Fig. 1**. PCR products of these 8 primers were used for analyzing genetic relations of Taif-Roses with other 9 rose genotypes. Amplified bands information related to each primer is giving in **Table 1**. Total number of amplified products was 111 bands with molecular weight ranging from 100 to 2500 bp with 96.4% of total polymorphism. The RAPD-PCR reaction with the primer OPA-16 produced the highest numbers of band (21), while the lowest (10) was obtained with the primer OPC-02 (**Fig. 1**). Three primers namely OPA-O4, OPA-O9 and OPB-03 showed 92, 93 and 91% of polymorphism, respectively, while the other six primers showed 100% polymorphism. The average of amplified and polymorphic bands per each primer was 13.8 and 13.4, respectively.

3.2. ISSR-PCR Fingerprinting of Rose Genotypes

Six ISSR reactions revealed 64 amplified products ranging from 450 to 2500 bp in which 66 (90.6) were polymorphic. The ISSR profiles obtained by some ISSR primers are shown in **Fig. 2**. Maximum numbers of bands was generated by ISSR 4 (12 bands with 83% polymorphic), while, the minimum numbers of bands was generated by ISSR 5 (9 bands with 89% polymorphic), respectively. The average of amplified and polymorphic bands per each primer was 10.7 and 9.6, respectively. Amplified bands information related to each primer is giving in **Table 2**. The studied primer sequences were composed of di and tri-nucleotide repeat sequences. The highest polymorphism observed in the case of tri-nucleotide repeat primers ISSR 2.

3.3. SSR Analysis Fingerprinting of Rose Genotypes

In the present study, out of the eight primer pairs flanking simple sequence repeats that were employed to investigate the level of polymorphism among the 12 rose genotypes, seven primers showed clear and reproducible products.

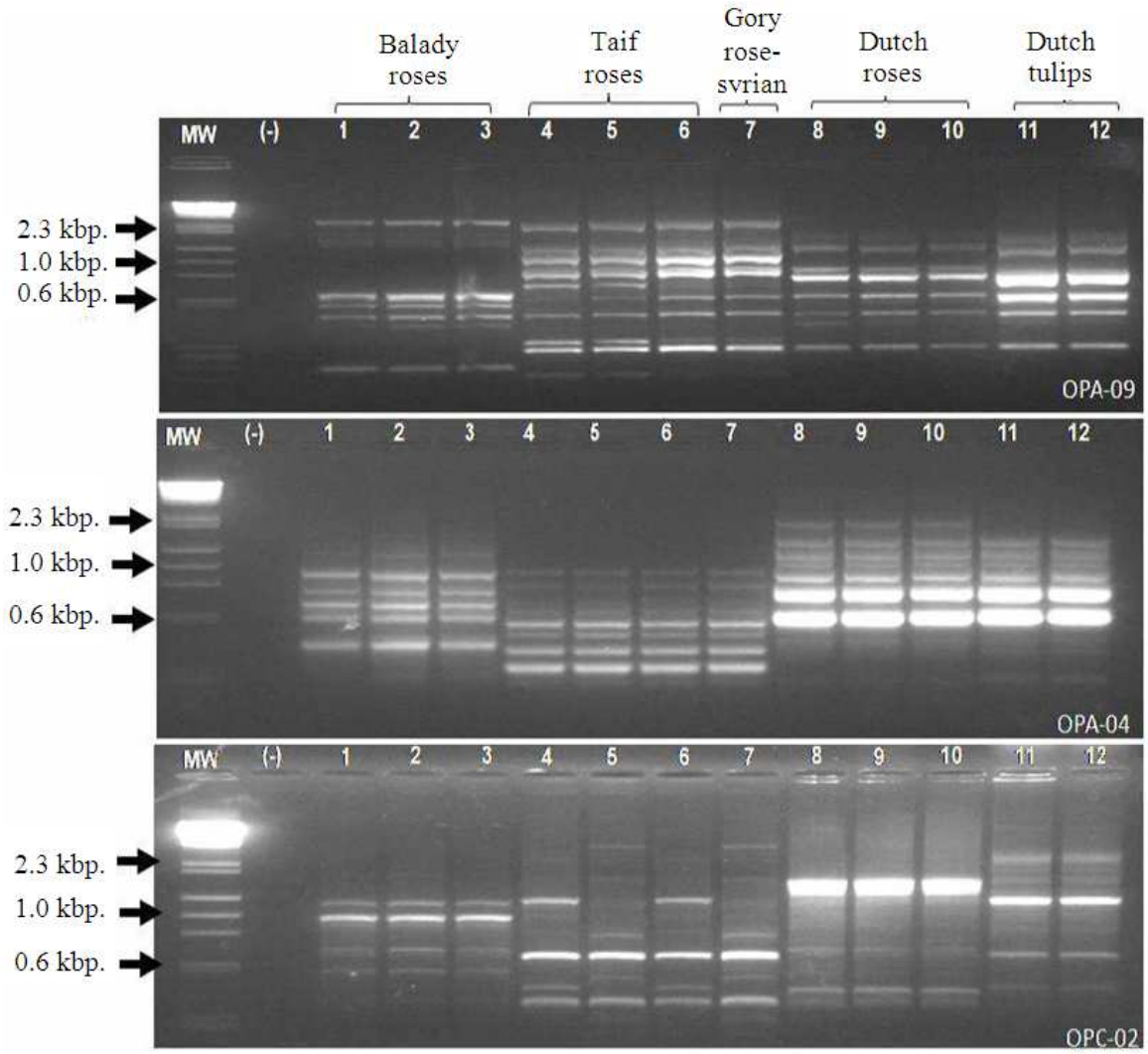

Fig. 1. RAPD profile of 12 different rose genotypes using the three primers OPA-09, OPA-04 and OPC-02. MW: is 1 kb. Ladder; (-) is a negative PCR sample. (1-3) Balady rose1, 2 and 3 ;(4-6) Taif rose (Hada, Shafa 1 and Shafa 2; (7) Gory rose-Syrian; (8-10) Dutch rose1, 2 and 3 and (11-12) Dutch Tulips 1 and 2

The reproducible (true) PCR product obtained using the primers pair for the markers Rw10J19, Rw4E22 and Rw3N19 are depicted in **Fig. 3**. The number of alleles per marker ranged from 1 to 3 with an average of 2.1 alleles per locus (**Table 3**).

3.4. Genetic Relationships as Revealed by RAPD, ISSR and SSR Markers and Cluster Analysis

To examine the genetic relationships of Taif-Roses with other rose genotypes, the data scored from the three markers were compiled and analyzed using the Dice similarity coefficient. The genetic similarity matrices based on the Dice coefficients was illustrated in **Table 4**. The Genetic Similarity (GS) was ranged between 3 and 98.5%. The highest GS value was 98.5% between Dutch Tulips-1 and Dutch Tulips-2 while the lowest was 3% between Balady Rose-1 and Dutch Rose-1. The highest GS values between Taif-Roses and other rose genotypes were 92.3, 74.1 and 65.7% between the three genotypes Hada, Shafa-1, Shafa-2 and Gory rose-Syrian, respectively. The GS values between Taif-Roses and the Egyptian rose genotypes (Balady-1, 2, 3) were the lowest and ranged between 7.4 to 31%.

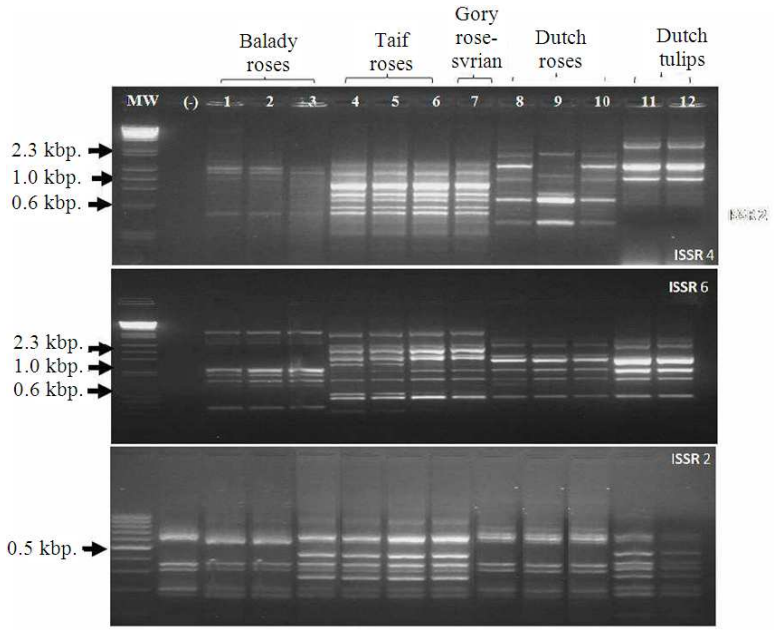

Fig. 2. ISSR profiles of 12 different rose collections using the ISSR primers 2, 4 and 6. (1-3) Balady rose1, 2 and 3; (4-6) Taif rose (Hada, Shafa 1 and Shafa 2; (7) Gory rose-Syrian; (8-10) Dutch rose1, 2 and 3 and (11-12) Dutch Tulips 1 and 2. MW: is 1 kb. Ladder for ISSR 4 and 6 and is 100 bp. Ladder for ISSR 2; (-) is a negative PCR sample

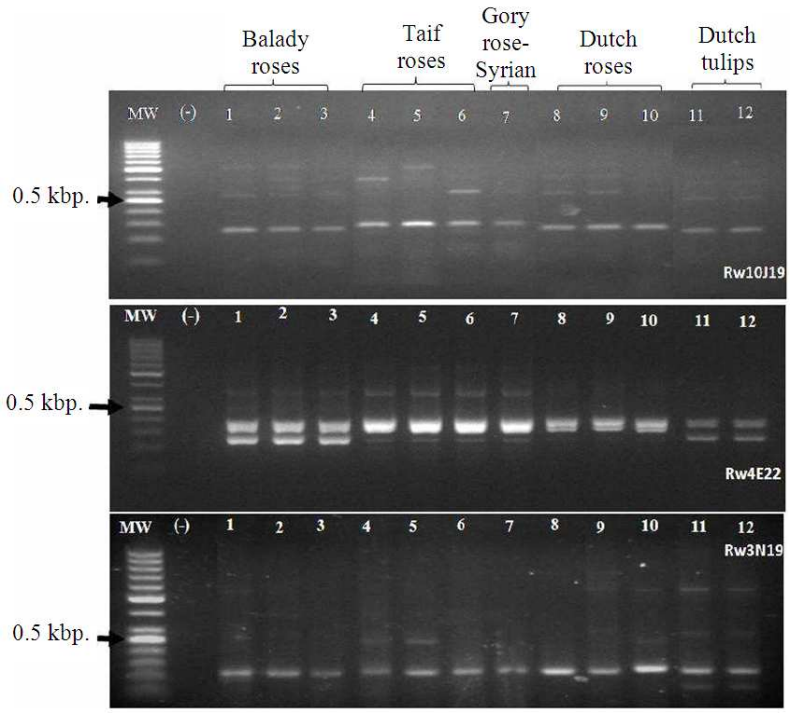

Fig. 3. SSR fingerprinting of 12 different rose collections using three SSR markers. (1-3) Balady rose1, 2 and 3; (4-6) Taif rose (Hada, Shafa 1 and Shafa 2; (7) Gory rose-Syrian; (8-10) Dutch rose1, 2 and 3 and (11-12) Dutch Tulips 1 and 2. MW: is 100 bp.; (-is a negative PCR sample

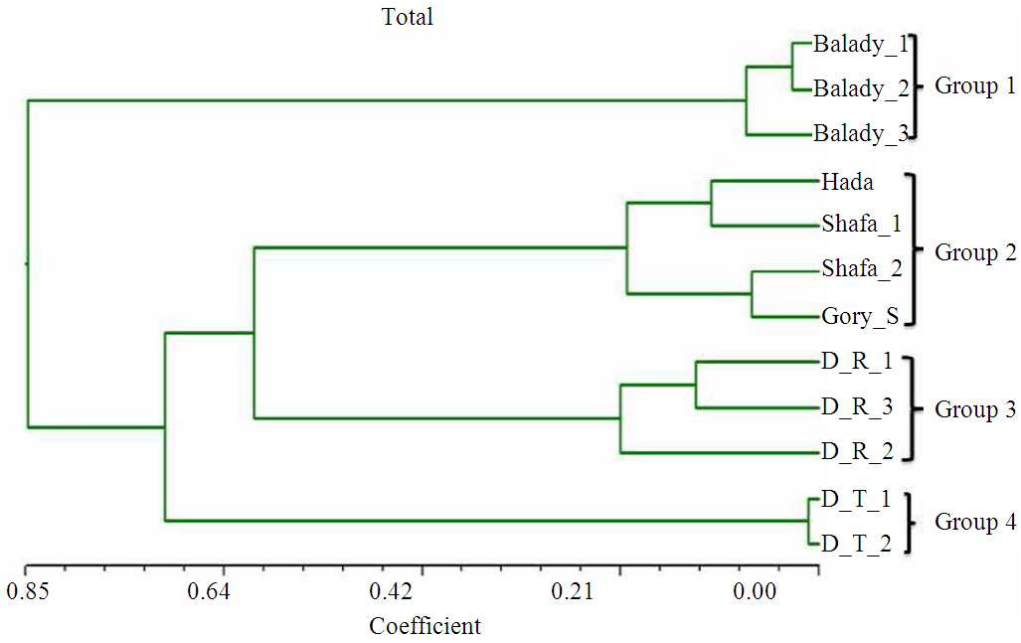

Fig. 4. RAPD's, ISSR's and SSR, s phylogenetic analysis among 12 rose collections. Dutch rose 1, 2, 3; Balady rose 1, 2, 3; Taif rose-Hada; Taif rose-Shafa 1, 2; Gody rose-Syrian and Dutch tulip 1, 2

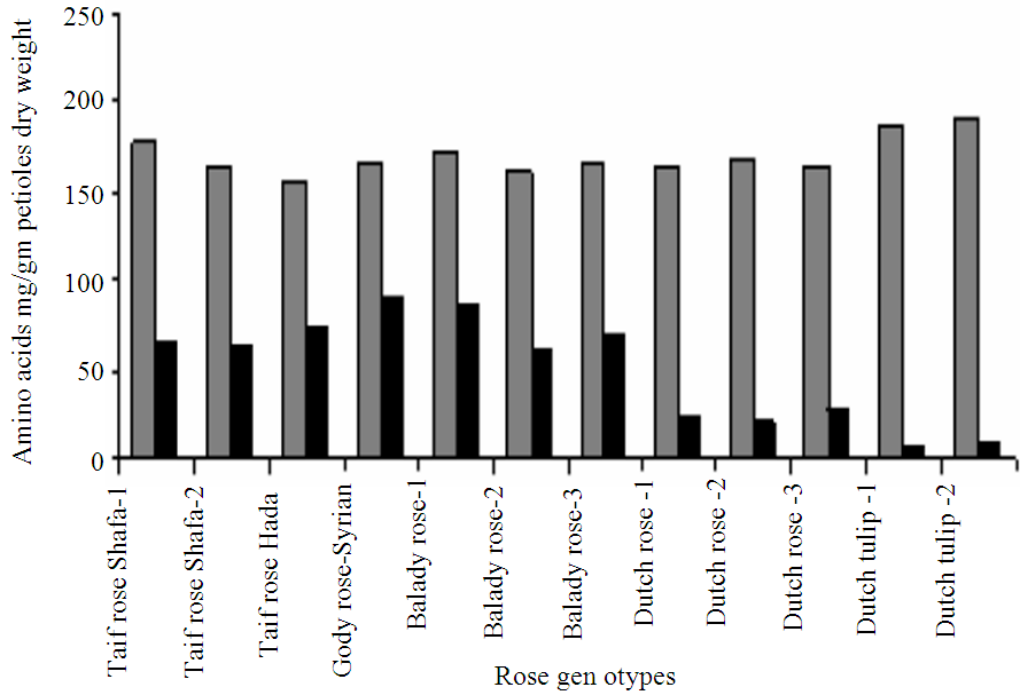

Fig. 5. Amino acid contents analysis using HPLC technology of twelve rose genotypes. Dutch rose 1, 2, 3; Balady rose 1, 2, 3; Taif rose-Hada; Taif rose-Shafa 1, 2; Gody rose-Syrian and Dutch tulip 1, 2. Gray bars are presenting the total amino acids in mg/gm of rose petioles. Black bars are presenting the aromatic amino acids in mg/gm of rose petioles

Table 4. Genetic dissimilarity (GS) matrices computed according to Dice coefficient from the combined RAPD's, ISSR's and SSR's markers of the twelve rose genotypes, Taif rose-Hada; Taif rose-Shafa 1, 2; Dutch rose 1, 2, 3; Gody rose-Syrian and Dutch tulip 1, 2

	Bala.-1	Bala.-2	Bala. -3	Hada	Shafa-1	Shafa-2	Gory-S	Du-R1	Du-R2	Du-R3	Du-T1	Du-T2
Bala.1	0.000											
Bala.-2	0.0270	0.000										
Bala.-3	0.0892	0.1162	0.000									
Hada	0.9237	0.9268	0.9211	0.000								
Shafa-1	0.6931	0.7202	0.7824	0.3466	0.000							
Shafa-2	0.6931	0.7202	0.9159	0.1234	0.1823	0.000						
Gory-S	0.6649	0.6919	0.6363	0.3436	0.0771	0.2594	0.000					
Du-R1	0.9721	0.9542	0.8350	0.8047	0.8636	0.7458	0.6222	0.000				
Du-R2	0.8880	0.9150	0.7416	0.7113	0.7702	0.7702	0.6466	0.2475	0.000			
Du-R-3	0.7458	0.7729	0.7172	0.8047	0.7458	0.7458	0.7175	0.2231	0.1298	0.000		
Du-T1	0.9159	0.8094	0.8873	0.8570	0.6646	0.6646	0.7416	0.9686	0.9371	0.9427	0.000	
Du-T2	0.9277	0.9126	0.9311	0.8267	0.7520	0.7520	0.8291	0.9324	0.9491	0.9447	0.0103	0.000

While, the genetic similarity values between Taif-Roses Hada, Shafa-1, Shafa-2 and the Dutch roses and Dutch Tulips genotypes (Dutch Rose 1, 2, 3 and Dutch tulips 1, 2) were ranged between 14 and 34%.

UPGMA clustering dendrogram based on DICE similarity index was obtained (**Fig. 4**). The dendrogram cluster diagram classified the evaluated genotypes in two major clusters and that formed four clear groups. The first cluster contains the three Egyptian Rose genotypes (Balady1, 2 and 3) and represented the first group. The second main cluster was divided into two subclusters in which the first contained the two Dutch Tulips and represented Group 4. The second subcluster was further divided into two branches, the first contained the three Dutch rose genotypes and formed Group 3 and the second contained the three Taif-roses genotypes Hada, Shafa-1 and Shafa-2 in addition to the Gory rose-Syrian and formed Group 2.

3.5. Amino Acid Analysis of Rose Genotypes using High-Performance Liquid Chromatography (HPLC) Technique

The concentration of the total amino acids and the aromatic amino acids were measured and compared among the twelve rose genotypes (**Fig. 5**). While, no significant difference in the amount of total amino acid of the twelve genotypes was shown. The ration of the amount of aromatic amino acids to the amount of total amino acid in the three genotypes Balady rose 1, Gody rose-Syrian and Taif rose-Hada was the highest comparing to the rest of the tested genotypes. While, Dutch rose 1, 2, 3 and Dutch tulip 1, 2 genotypes were showed the lowest ratio.

3.6. *In vitro* Propagation of Taif Rose

After four weeks of culture, nodal segment explants showed different responses in production and development of multiple shoots when cultured on Murashige and Skoog medium with different concentrations of 2,4, D and kiniten. Among the various phytohormones concentrations and combinations used in this study, a combination of 1 mg L^{-1} 2,4,D and 1 mg L^{-1} kinetin was found to be the best to yield the highest frequency of proliferation of axillary buds and formation of axillary shoots. While the concentration of 1 mg L^{-1} of BAP was found to be the best to yield the highest number of multiple shoots (**Fig. 1c**). The maximum length of shoots was also obtained using the same concentration. The multiple shoots were obtained after 2 months and the highest number of multiple shoots per explant recorded was 2.7.

4. DISCUSSION

4.1. Molecular Characterization

The use of molecular markers was aiming to show fast and reliable discrimination of genetic relations of Taif-Roses with other rose genotypes. The three molecular markers have confirmed each other and the combined results were realistic. The discriminating power of the three markers has led to efficient grouping of the 12 rose genotypes and successfully has reveled the genetic relations of Taif-Roses with other rose genotypes using Unweighted Pair Group method (UPGMA). The RAPD's, ISSR's and SSR's have confirmed each other and the combined results were realistic, when the dendrogram clearly separates the analyzed rose genotypes into four major groups. The

separation of the groups was mainly in correlation to the country of origin and rose-type. The three Taif roses showed highest GS with the Syrian-Gory rose and they were clustered together in the group 2 that further divided into two subclusters. Taif-roses genotypes Hada and Shafa-2 were existed together in the first subcluster with GS exceeded 88%. While, the second sub-cluster contained Shafa-1 and the Gory Rose-Syrian with GS of 92.3%.

Since rose is one of the most important ornamental and aromatic crops and represents a major commodity in the commercial market of the floriculture as well as the essential oil industry, the aromatic amino acids measurements was important in order to characterize the different rose genotypes and to select the best genotypes for the commercial usage. Among the 12 tested genotypes, the ration of the amount of aromatic amino acids to the amount of total amino acid in the three genotypes Balady rose 1, Gody rose-Syrian and Taif rose-Hada was the highest. These results may be confirmed the closed genetics relations between Gody rose-Syrian and Taif rose-Hada where these two genotypes were falling in the same cluster (**Fig. 4**). However, the comparison using the amount of each araomatic amino acid could give better indications than the using of total aromatic amino acids.

Together, these results indicate that the Taif-roses have closed genetic relations to the Gory rose-Syrian that are cultivating in Syria.

Additionally, a simple protocol for mass production of the Taif-rose genotype Hada using the nodal explants was developed (**Fig. 6**). Horn (1992) reported that the explants with vegetative meristems are often suitable for enhanced axillary branching and the performance of nodal segments is much better than the shoot tips. The most commonly used explants in shoot proliferation of roses are the nodal stem segments, wherein the axillary bud is made to proliferate and form multiple shoots (Khosravi *et al*., 2007). It was found that the combination of the auxin 2,4, D and the cytokinin, with the concentrations of 1 mg L^{-1} for each resulted in the best frequency of proliferation of axillary buds and formation of axillary shoots. Caboni and Tonelli (1999; George, 1993) explained that the presence of auxin in defined combinations with cytokinins in the culture medium is necessary to obtain adventitious shoot formation and the optimum rates of shoot initiation generally occur with combinations of auxin and cytokinin. In addition, the concentration of 1 mg L^{-1} of BAP was found to be the best to yield the highest number of multiple shoots (**Fig. 1c**), the maximum length of shoots was also obtained using the same concentration.

Fig. 6. Micropropagation stages of Taif rose using nodal segments. A: is proliferation of axillary buds and formation of axillary shoots after 3-4 weeks on culture medium. B: is shoots elongation stage (2 months) and C: is the multiplication stage (2 months). Each treatment was repeated three times

Although several different cytokinins have been used in rose proliferation, the best proliferation rate was obtained by using BA. Thi *et al*. (2008) demonstrated that the most suitable concentration for shoot multiplication was found on MS medium supplemented with 3 mg L^{-1} BAP.

The long term objective of this work was to use these fingerprints to identify molecular markers that co-segregate and could be used in isolating gene(s) which controlling some important traits. Moreover, the developed *In vitro* propagation system for Taif-roses could be used for mass production of this economically important crop at any time in the year. Moreover, the improved system can help in the improvement of several desirable traits in Taif-roses such as oil content, increase of flower yield, plant vigour and resistance to fungal diseases through genetic engineering.

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