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GENETIC VARIATION ANALYSIS OF TAIF'S GRAPEVINE PLANTS USING THREE DIFFERENT TYPES OF GENE –TARGETED MOLECULAR MARKERS

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Abstract

The grapevine (*Vitis vinifera* L.) is a valuable commercial fruit crop in Taif Province, Saudi Arabia. Three different molecular markers, start codon targeted (SCoT), conserved DNA-derived polymorphism (CDDP) and inter-simple sequence repeats (ISSR), were used. The aim of this analysis was to examine the genetic relationships between 29 individual grape samples collected from three different locations in the Taif governorate. Firstly, all individual samples were confirmed to be *Vitis vinifera* L. by sequencing the chloroplast barcoding gene *rbcL*. Six primers of each molecular marker were used and successfully amplified 74, 70 and 101 loci for ISSR, SCot and CDDP markers, correspondingly, of which all were polymorphic. The acquired average polymorphism information content was for ISSR, 0.16; for SCoT, 0.48; and for CDDP, 0.33, while average band informativeness was: ISSR, 12.2; SCoT, 34.1; and CDDP, 33.8. The results indicated that all used techniques were effectual for assessing the genetic diversity. The cluster partitions in the neighbour-joining dendrogram based on ISSR, SCoT and CDDP markers were semi-similar and grouped all individuals into two major groups. However, the dendrogram generated based on SCoT and CDDP can separate sample R12 into a third major group. Thus, these results indicate that SCoT and CDDP markers could be utilized as a consistent technique for the evaluation of genetic diversity and correlations among grapevine species, and DNA barcoding is necessary for authentication.

Keywords: Molecular marker, *Vitis vinifera*, Genetic diversity, Saudi Arabia.

Introduction

Grapes (*Vitis vinifera* L.) are an important crop that is ranked second after the cultivation of palm trees in the Kingdom of Saudi Arabia. About 100 thousand acres are under cultivation of grapes, and the most important areas cultivated are the city of Medina, Taif and Qassim. About 43% of all grape production comes from Medina and about 31% from Taif (Nagaty & El-Assal, 2011; Dessoky *et al*., 2017). The grapevine (*Vitis vinifera* L.) is among the most important fruit crops in Taif city, where the grapes yield in 2016 was about 89,789 tons (Dessoky *et al.*, 2017). The study of genetic diversity in *Vitis vinifera* of Taif cultivars is important not only to check the origin of various cultivars but also those cultivars are important for the government's improvement and preservation germplasm fund for this important species (Nagaty & El-Assal, 2011).

Classical methods of identification based on morphological characters are not always the most accurate way to check this due to instability of morphological characteristics under different environmental conditions (Nagaty & El-Assal, 2011). Molecular markers such as RAPD, RFLP and AFLP play an important role in identification and characterisation of grapevine culverins (Resta *et al.*, 1995; Adam-Blondon *et al*., 2001). SSR and SCAR markers have been developed in grapes (Bodea *et al*., 2009; Nagaty & El-Assal, 2011). In recent years, researchers have widely developed PCR-based marker techniques that provide valuable tools for genetic variation of plants. These include the start codon targeted (SCoT) and conserved DNA-derived polymorphism (CDDP) techniques (Collard & Mackill, 2009a; Salayeva *et al*., 2016). These markers have been found to deliver an adequate level of polymorphism with reproducible fingerprinting profiles for assessing genetic diversity (Collard & Mackill, 2009a; Mulpuri *et al.*, 2013; Saidi *et al.*, 2017). ISSR-PCR molecular marker technique was documented in several reports as a quick, reproducible, and cheap method (Zhang *et al*., 2006; Golkar *et al*., 2011; Nadeem *et al*., 2014). On other hand, DNA barcoding is a fundamental indicative technique that utilizes a short uniform genetic marker in an organism's DNA to help recognizable proof at a specific taxonomic level. The specific selected DNA region should uncover the group classification of the target species. Subsequently, this marker region would offer a high inter-specific changeability and low intraspecific difference. Therefore it should permit the distinguishing proof of whatever number species as could reasonably be expected having a place with a common or higher taxonomical level such as genus, family or order (Lahaye *et al*., 2008; Amirmoradi *et al*., 2012).

In the present study, three PCR-based molecular markers (SCoT, CDDP and ISSR) were used to compare the genetic diversity among 29 individual grape samples collected from three different locations in the Taif governorate. This was to get a better understanding of the genetic diversity of these genotypes. It is believed that this study is the first molecular comparison analysis of the locally grown Taif grapevine varieties (*Vitis vinifera*) using these three unique primer sets.

Material and Methods

DNA isolation: Total of 29 individual plant samples of Taif grapevine plants collected from different regions (Hada, Shafa & Wady Qurish) were used in the current work. From each genotype, total genomic DNA was isolated from young leaves using the FavorPrep™ Plant Genomic DNA Extraction Mini Kit (Favorgen, Taiwan) according to the manufacturer's instructions.

ISSR–PCR amplification: PCR amplification of the ISSR technique was carried out as in Hassan *et al*., (2014). Amplified DNA products were investigated by electrophoresis in a 1.5% agarose gel run in TAE buffer. The gels were stained with and Stained with Ethedium bromide then were visualized by UV illumination and then photographed using a Bio-Rad Gel Doc 2000 device.

SCoT and CDDP–PCR amplification: Six primers were used for each marker. Primers Sequences are given in Table 1. PCR amplification of SCoT and CDDP techniques was carried out as stated by Collard & Mackill (2009a, 2009b). Amplification was run as previously described and PCR amplicons were then photographed by a Bio-Rad Gel Documentation 2000 tool.

Table 1. Primers sequence used in ISSR, SCoT, and CDDP marker systems for study of genetic variations among 29 Taif grapevine plants.

Serial number	Primers name	Primers sequences 5' → 3'
1	ISSR-2	GAG AGA GAG AGA GAG AA
2	ISSR-3	AGA GAG AGA GAG AGA GTG
3	ISSR-4	GAG AGA GAG AGA GAG ATT
4	ISSR-9	TAG CAC ACA CAC ACA CA
5	ISSR-12	AGA GAG AGA GAG AGA GAG T
6	ISSR-18	ACA CAC ACA CAC ACA CG
7	SCoT-2	ACC ATG GCT ACC ACC GGC
8	SCoT-3	CAA TGG CTA CCA CTA GCG
9	SCoT-8	ACA ATG GCT ACC ACT GCC
10	SCoT-11	AAG CAA TGG CTA CCA CCA
11	SCoT-12	ACG ACA TGG CGA CCA ACG
12	SCoT-14	ACG ACA TGG CGA CCA CGC
13	WRKY-R1	GTG GTT GTG CTT GCC
14	WRKY-R2	GCC CTC GTA SGT SGT
15	WRKY-R3	GCA SGT GTG CTC GCC
16	WRKY-R2B	TGS TGSA TGC TCC CG
17	WRKY-R3B	CCG CTC GTG TGS ACG
18	Myb2	GGC AAG GGC TGC CGG

Amplification and sequencing of the *rbcL* gene: Amplification of the *rbcL* gene was done using the forward primer (5`-ATG TCA CCA CAA ACA GAG ACT AAA GC-3`) and reverse primer (5`-GTA AAA TCA AGT CCA CCR CG-3`). The specific 560-bp *rbcL* fragments were isolated from the gel using a purification kit (QIAquick PCR,, QIAGEN, USA). The cleaned-fragments were then passed through sequencing procedure with the same primers using the sequencer Gene Analyser 3121 (Macrogen Co, South Korea).

Sequence Alignment and Phylogenetic Analyses: The sequenced *rbcL* gene fragment of all individual samples of grapevine (*Vitis vinifera*) were all aligned with recognized *rbcL* sequences in GenBank operating the BLAST-NCBI database, and the percentages of homology scores were produced to identify the strains.

Data analysis: The genetic relationship between plants was evaluated by calculating the Jaccard's similarity coefficient using the neighbour-joining approach and a dendrogram was constructed. The calculations were completed using the NTSys-PC version 2.01 (Rohlf, 2000).

Results

Grapevine samples identification using *rbcL* gene: Unambiguous nucleotides of about 560 bp of *rbc*L gene were sequenced. A clear 537 bp from all samples were chosen and the base compositions of this fragment were A = 140; T = 155; G = 122 and C = 120. Three different grapevine species (*Vitis vinifera*, *V. betulifolia* and *V. californica*) were chosen to represent the high diversity of grapevine species and to test the utility of *rbc*L for barcoding such ornamental plants. The *rbc*L DNA sequences of the all 29 samples were completely identical, and 100% similar to *Vitis vinifera*, *V. betulifolia* and *V. californica* (Fig. 1). BLAST search resulted in 100% identity with 0 gaps.

Polymorphism of ISSR markers: Total six primers of each ISSR. SCoT and CDDP markers were tested in the genome of 29 individual grapevine plants (Table 1). For ISSR, the number of loci amplified per primer varied from 9 to 16. The primers were selected because they had better amplification than others. By evaluating the amplification standard of these primers using genomic DNA of 29 individuals (Table 2 and Fig. 2), we found that ISSR-9 primer gave the highest number of loci (16 loci), while the primer ISSR-18 gave the lowest number of loci (9 loci). The ISSR-2 primer showed the highest polymorphism with about 23.1%, and the ISSR-9 primer had the lowest polymorphism with about 6.2%. The results of the ISSR-PCR analysis of the 29 local grapevine samples revealed approximately 74 different banding patterns, nine of them were assessed as polymorphic bands (12.2%) and remaining 65 fragments were assessed as monomorphic bands (87.8%). The genetic similarity among 29 individuals, using the neighbour joining method, ranged from 0. 00 to 0.24 (Fig. 3). The grapevine plants were classified into two main clusters with about 76% genetic similarity. The first cluster consisted of the individual R-22 and R24 plants only, and the second main cluster was separated into two sub-clusters. The first sub-cluster contained R-6 plants only, and the second sub-cluster consisted of all other samples. The lowest genetic distance (0.00) was estimated between samples such as R-15 and R-28 or R-17 and R-18.

SCoT assay: The SCoT primers were good for PCR amplification, as the number of loci amplified per primer varied from 9 to 16 loci. By evaluating the amplification standard of these primers using the genomic DNA of 29 individuals (Table 2 and Fig. 4), we found that the SCoT-12 primer gave the highest number of loci (16 loci), while the primer SCoT-8 and SCoT-11 generated the lowest number of loci (9 loci). The SCoT-12 primer showed the highest polymorphism (about 62.5%), while the SCoT-8 primer showed the lowest polymorphism (about 11.1%). The results of the SCoT marker analysis for all individuals showed approximately 70 different banding pattern; 27 of them were polymorphic bands (38.6%) and remaining 43 fragments were monomorphic bands (61.4%). This rate of polymorphism was higher than the values obtained with ISSR primers. The genetic similarity among 29 individuals using the neighbour-joining method

ranged from 0.07 to 0.83 (Fig. 5). The phylogenetic analysis showed a genetic distance between tested grapevine plants from different geographical areas.

The grapevine plants were arranged in two large clusters with about 24% genetic similarity. The first cluster included R-12 samples only, and the second cluster included all other grapevine samples. The second main cluster was then divided into two sub-clusters; the first of these comprised R-21 only and the second consisted of all other samples. These results suggested that generally, the genetic distance between individual grapevines samples was higher than that obtained from the ISSR marker for each individual grapevine sample. The smallest genetic distance (0.07) was estimated between samples R-17 and R-19.

Polymorphism of CDDP markers: The total number of CDDP marker bands varied from 12 bands with primer WRKY-R3B to 19 bands with primers WRKY-R1, WRKY-R2B and Myb2 (Table 2). There were 64 monomorphic amplicons and 37 polymorphic amplicons. The band sizes of all individual samples using CDDP primers ranged from 250 to 1750 base pairs. Eight similar bands were detected in all samples that revealed about 42.1% monomorphism. On other hand, eleven bands were found as a polymorphism with 57.9% (Table 2; Fig. 6). Overall, 101 bands produced using CDDP marker were sufficient for the differentiation and identification of genetic variation and thus constructing a phylogenetic tree by using the neighbour-joining method. The genetic similarity ranged from 0.04 to 0.56 (Fig. 7). The grapevine plants were classified in two large clusters with around 0.48 genetic similarities. The first cluster consisted of R-12 sample only. The second main cluster was divided into two sub-clusters; the first one containing R-21, R-22 and R-23, while the second sub-cluster contained all other samples (Fig. 7). The lowest genetic distance (0.04) was assessed between samples such as R-6 and R-8.

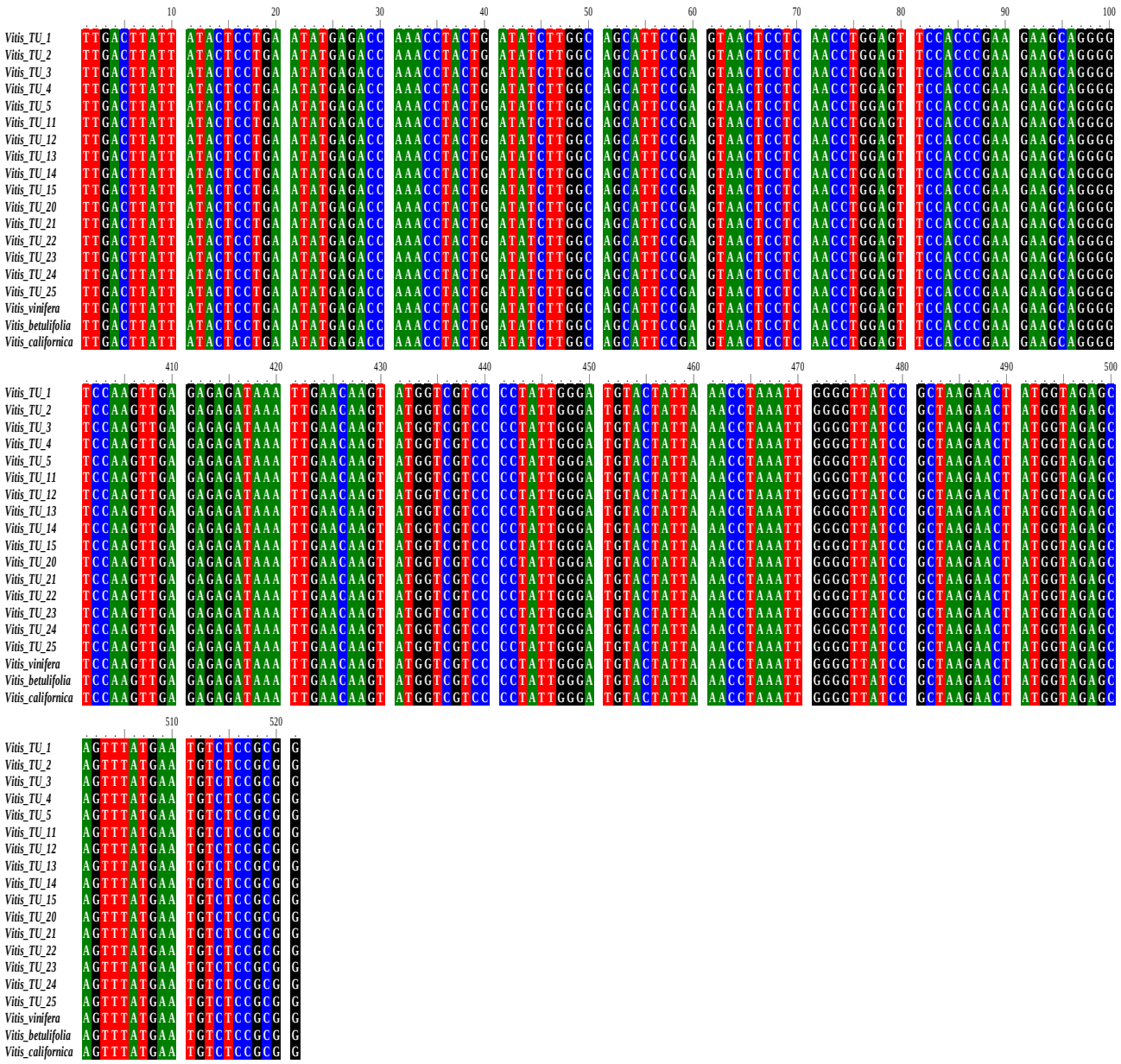

Fig. 1. The selected nucleotide sequence alignment of *rbc*L gene grapevine plants collected from Taif compared to 3 selected *Vitis* species obtained from a BLAST search of the NCBI.

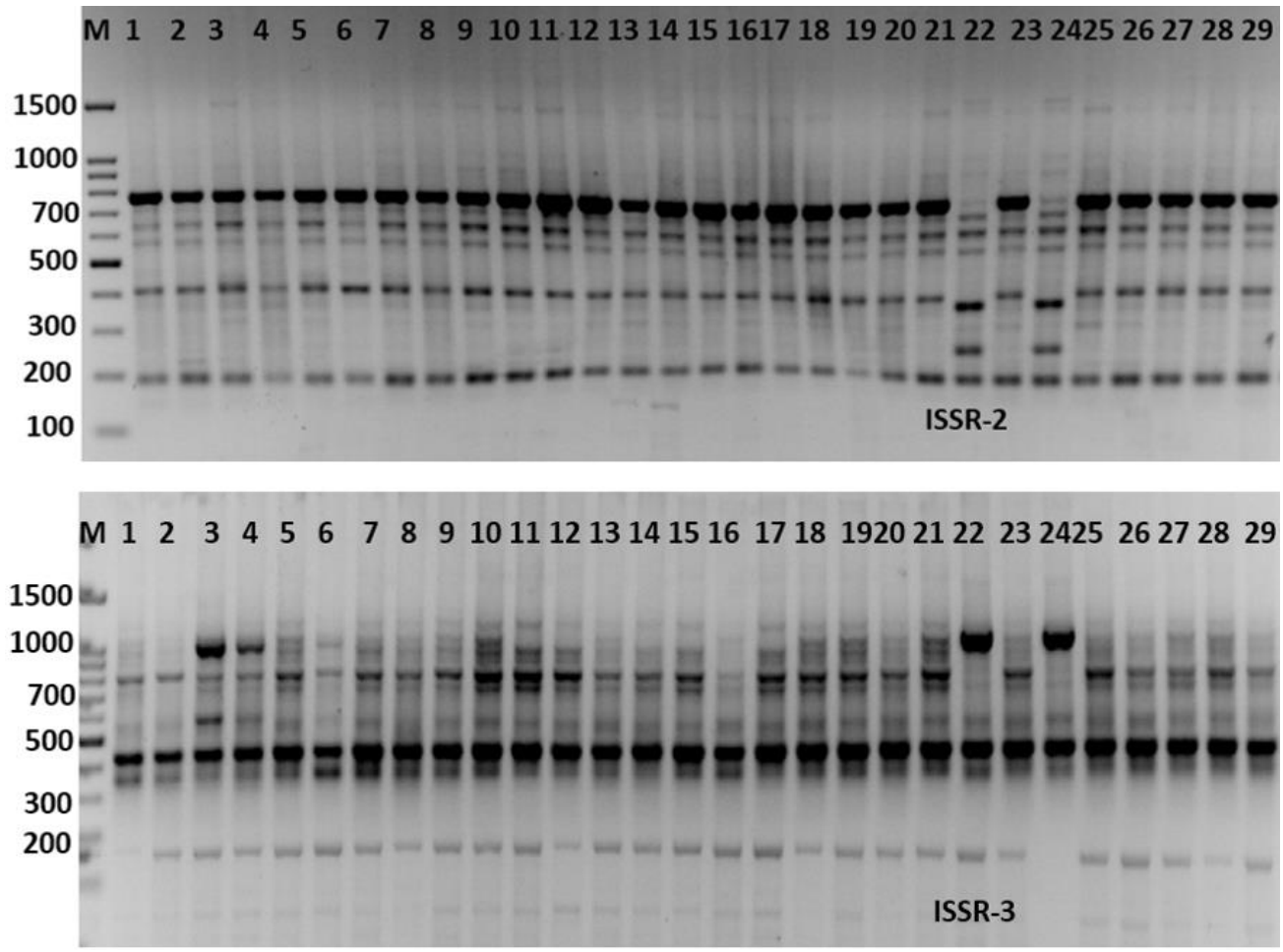

Fig. 2. ISSR-PCR profile among 29 individual grapevine plant samples generated by ISSR-2 and ISSR-3 primers. M= 100 bp. DNA Ladder.

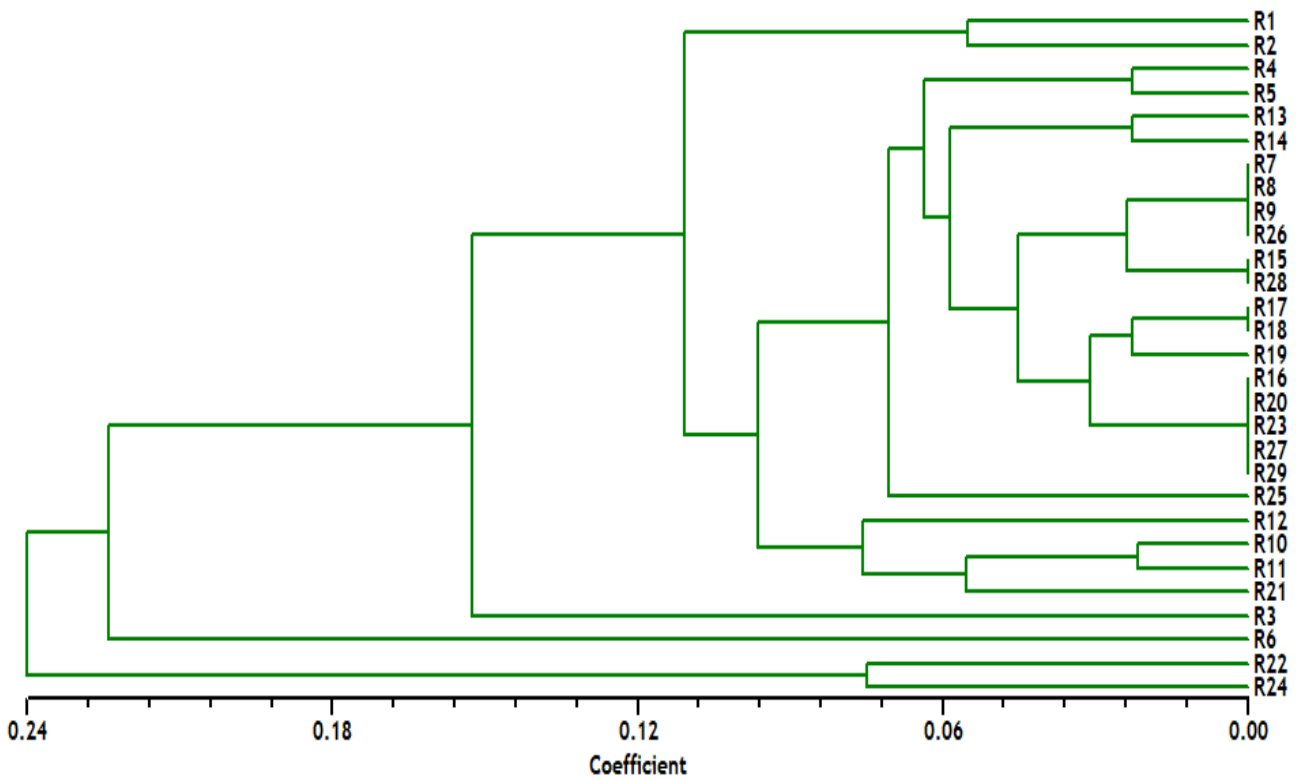

Fig. 3. Dendrogram analysis showing the relationship of 29 individual plant samples of Taif grapevine based on ISSR marker using Jaccard's similarity coefficient.

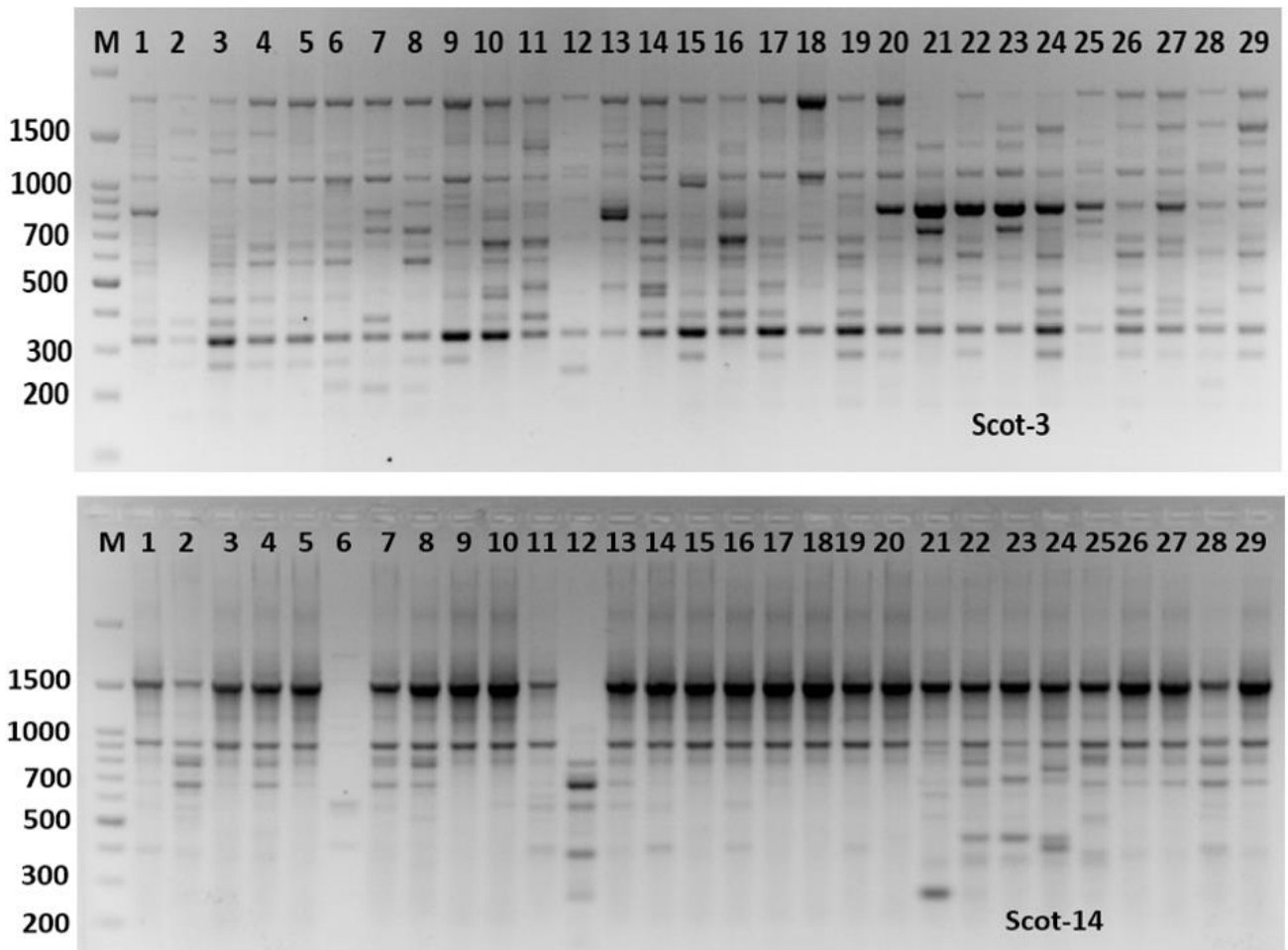

Fig. 4. SCoT marker profile among 29 individual grapevine plant samples generated by SCoT-3 and SCoT-14 primers. M= 100 bp DNA Ladder.

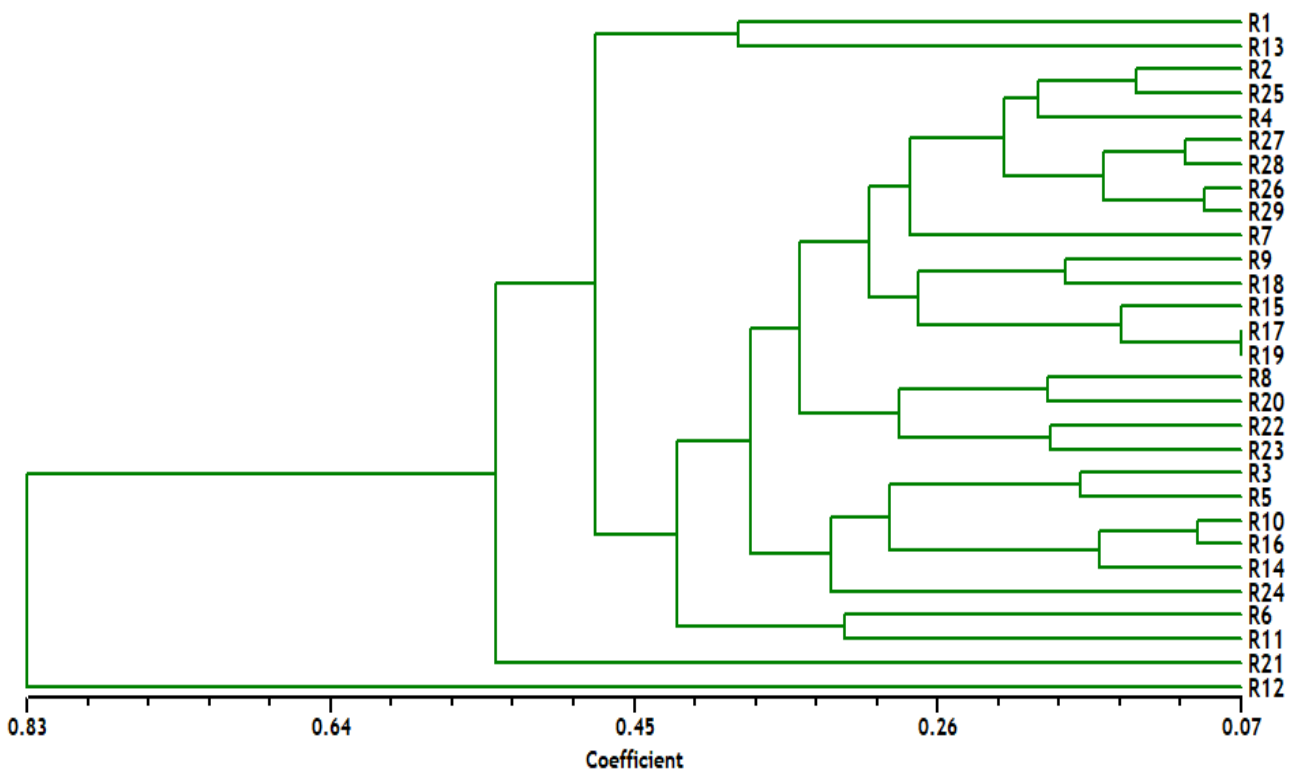

Fig. 5. Dendrogram analysis showing the relationship of 29 individual plant samples of Taif grapevine based on SCoT marker using Jaccard's similarity coefficient.

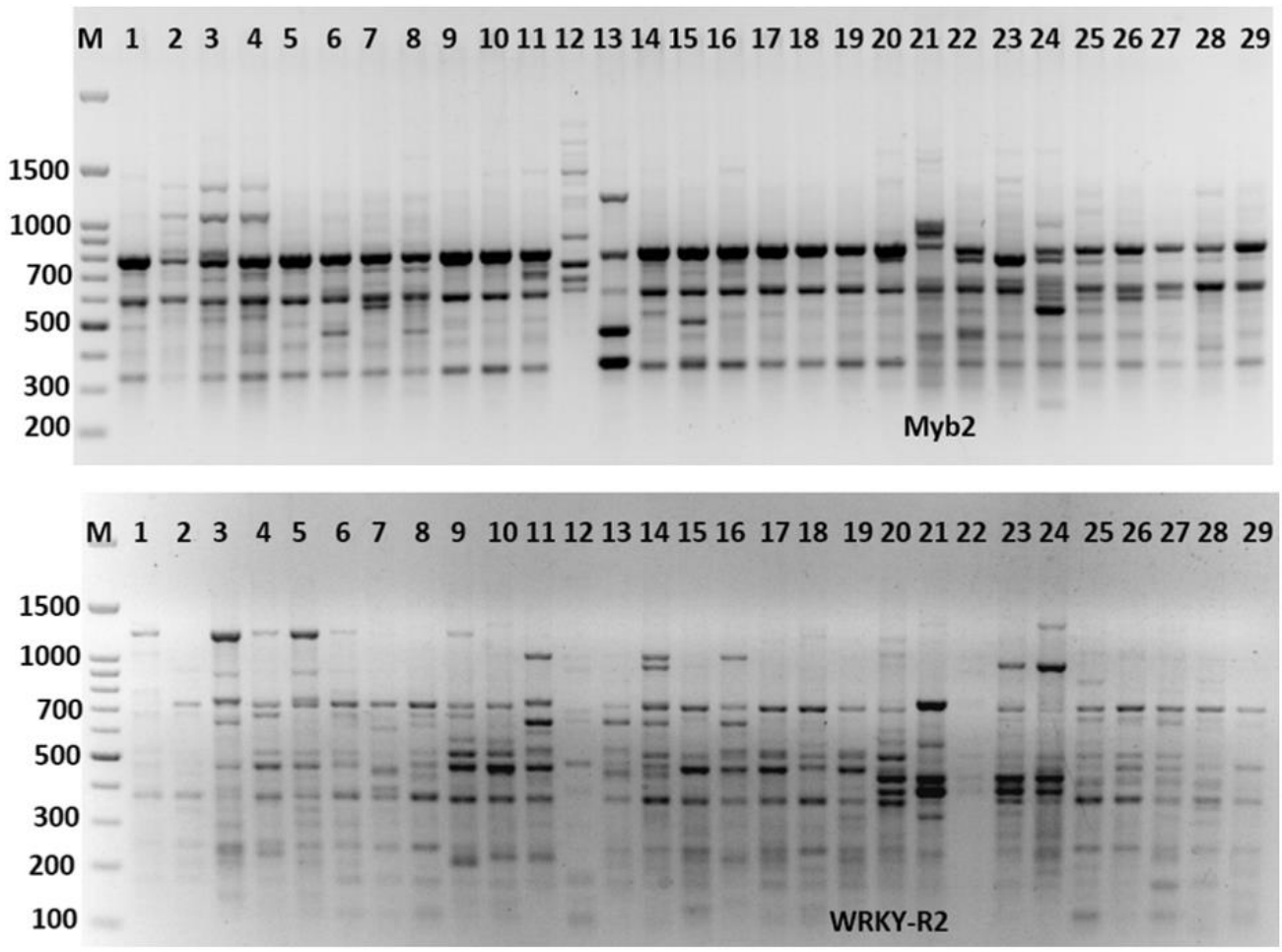

Fig. 6. CDDP marker profile among 29 individual grapevine plant samples generated by Myb2 and WRKY-R2 primers. M= 100 bp DNA Ladder.

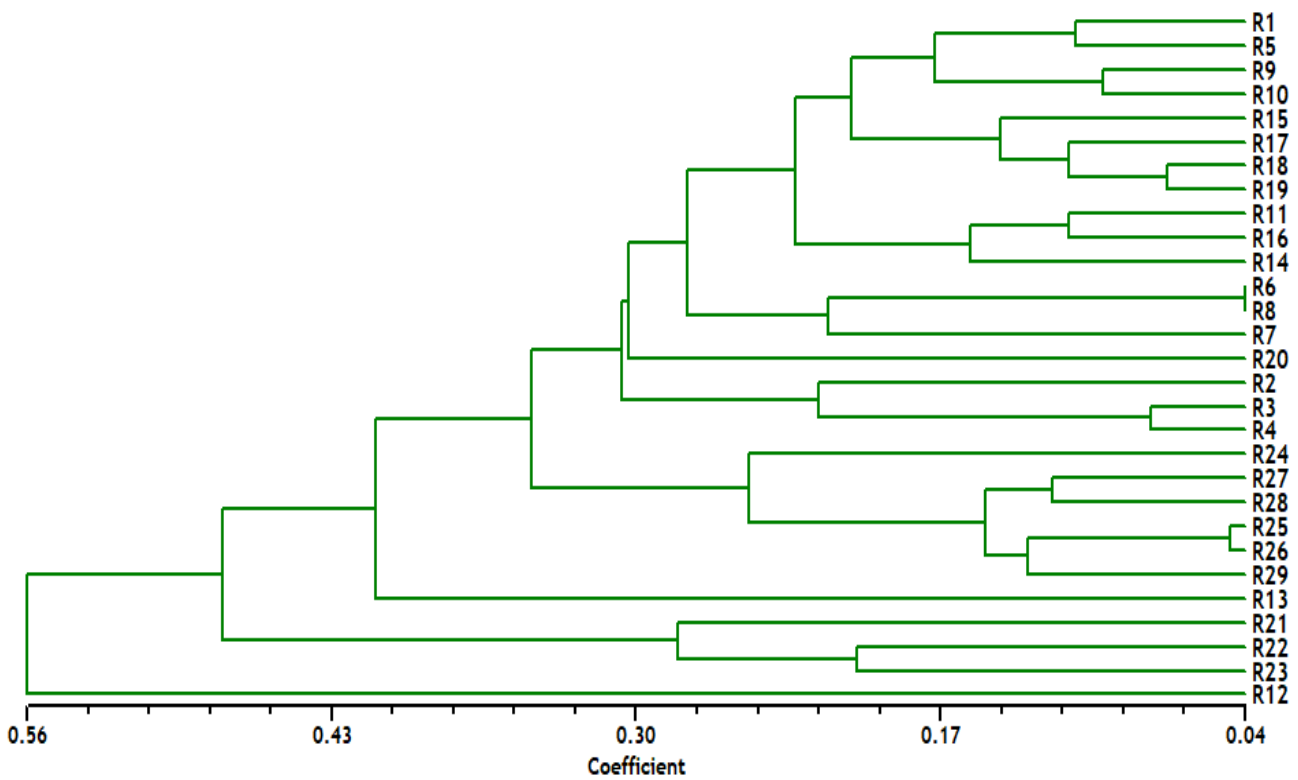

Fig. 7. Dendrogram analysis showing relationship of 29 individual plant samples of Taif grapevine based on CDDP marker using Jaccard's similarity coefficient.

Table 2. Six primers of each ISSR, ScoT and CDDP marker used to genotype grapevine plant samples, the total bands (TB), polymorphic bands (PB), monomorphic bands (MB), percentage of polymorphic bands (PPB) number of specific bands, (NSB) and specific band ratio (PSB).

Primers name	TB	PB	MB	PPB (%)	NSB	PSB
ISSR-2	13	3	10	23.1	2.00	15.3
ISSR-3	10	1	9	10.0	0.00	0.00
ISSR-4	12	1	11	8.30	0.00	0.00
ISSR-9	16	1	15	6.20	0.00	0.00
ISSR-12	14	2	12	14.2	1.00	7.10
ISSR-18	9	1	8	11.1	0.00	0.00
Total/mean	74	9	65	12.2[m]	3.00	3.73[m]
SCoT-2	10	2	8	20.0	1.00	10.0
SCoT-3	14	9	5	64.3	1.00	7.10
SCoT-8	9	1	8	11.1	1.00	11.1
SCoT-11	9	2	7	22.2	2.00	22.2
SCoT-12	16	10	6	62.5	1.00	6.25
SCoT-14	12	3	9	25.0	2.00	16.7
Total/mean	70	27	43	34.1[m]	8	12.2[m]
WRKY-R1	19	9	10	47.3	0.00	0.00
WRKY-R2	18	10	8	55.6	1.00	5.60
WRKY-R3	14	3	11	21.4	2.00	14.2
WRKY-R2B	19	4	15	21.1	1.00	5.20
WRKY-R3B	12	0	12	0.00	0.00	0.00
Myb2	19	11	8	57.9	1.00	5.20
Total	101	37	64	33.8[m]	5	5.03[m]

Discussion

Genetic diversity is vital for the preservation of Taif grapevines in their natural habitat as it defends the plants from an assortment of environmental conditions. The influence of these environmental conditions may differ on the same or diverse plant species depending on the numerous morphological and/or physiological characteristics of the plant. Several molecular markers systems have been established for the identification of genetic diversity in grapevines. However, these markers have both advantages and disadvantages (Jahnke *et al*., 2009; Carimi *et al*., 2011). In the current study, the more reproducible SCoT molecular marker named was used to assess genetic diversity in Taif grapevine plants. The SCoT marker method utilizes single primers intended to anneal to the flanking areas of the conserved region localized at the initiation codon (ATG) on both DNA strands. Crosswise over the functional domains of well-characterized plant genes, these short labels would then be able to produce useful banding patterns that have numerous utilizations; for instance, germplasm variety evaluation or mapping; trait association studies and QTL mapping and bulked segregate analysis (Collard & Mackill, 2009). Dissimilar to RAPD, AFLP and ISSR marker procedures, SCoT is a quality-focused marker with a few locos nature, and it can deliver extra data related with biological traits and is stable in high genetic polymorphism. Evaluation of SCoT markers in molecular variety examination and characteristic fingerprinting has just been recognized in several plants such as citrus plant (Han *et al*., 2011), *Arachis* plants (Xiong *et al*., 2010, 2011), *Dimocarpus longan* (Chen *et al*., 2010), *Mangifera indica* L., plant (Luo *et al*., 2014), and date palm (Al-Qurainy *et al*., 2015). In the present study, the SCot marker produced about 38.6% polymorphism percentage, while ISSR and CDDP markers produced 12.2% and 33.8%, respectively (Table 2). Previous studies reported that the polymorphism detected by the SCot marker in date palm cultivars was low (AL-Qurainy *et al*., 2015). Finally, we can conclude that SCot marker used in this study was sufficient to evaluate the genetic diversity within and amongst the grapevine cultivars. A conceivable explanation for differences in the resolution of SCoTs, CDDP and ISSR markers is that the target regions on the genome of the three-markers are different. Also, some differences may be as a result of banding errors and/or the percentage polymorphism distinguished by the three markers. Thus, these facts support the importance of the number of loci producing from each marker and their coverage of the overall genome in acquiring dependable assessments of genetic relationships among cultivars (Gajeraa *et al*., 2010).

More genetic diversity was found among the samples from different locations than between samples from the same location. Thus, assessing the genetic diversity of the samples from different location (rather than from the same location) would be beneficial for the conservation of the Taif grapevine cultivars. The identification, collection and preservation of grapevine plants from various topographical regions of Saudi Arabia, would consequently be of extraordinary significance in the detailing support techniques for different species of this genus. Predictable with these outcomes, a few reports have discovered elevated levels of genetic diversity in numerous varieties of *Vitis vinifera* (Dessoky *et al*., 2017; Collard & Mackill, 2009a; Salayeva *et al*., 2016). The capability of CDDP markers for examining the genetic diversity and relationships among *Vitis* species is another key outcome with practical significance. This study supports the accessible proof of using molecular techniques either independently or in grouping with other marker techniques to assess genetic variation and acquire solid data about genetic relationships (Poczai *et al*., 2013). Therefore, this would help strategies for powerful accumulation of the *Vitis* germplasm and its conservation. Our outcomes can help in isolating populations for genetic investigations and in distinguishing germplasm material for the introgression of desirable genes from varied sources. Nevertheless, investigations of an increasingly broad gathering of the Taif grapevine germplasm is needed to characterize the genetical

theory of more-closely-related species as maternal parents of these lines to additionally clarify their relationships and placement in the *Vitis* gene-pool. Given the strict maternal inheritance of plastid DNA in *Vitis*, investigations of the nuclear genome should likewise be considered by future investigations to follow the paternal ancestry of Taif grapevine lines. Here, we have initiated such experiments dependent on microsatellite markers and nuclear DNA sequences to give further bits of knowledge into the origin of the economically significant Taif grapevine lines.

Conclusion

The present research is the first report of genetic polymorphism on 29 grapevine individuals from three different locations in the Taif governorate using ISSR, SCot and CDDP marker techniques. All of the primers used in this study for the three markers were suitable and produced polymorphic bands. The present report found that the SCot and CDDP markers are better than the ISSR technique, as they have the characteristics of (for example) higher polymorphism, more reproducibility, low cost, ease of handling, and also time saving. This research will be therefore be helpful for the development of conservation strategies and for further grapevine studies, especially in marker assisted selection.

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