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Research Article

Using of DNA-Barcoding, SCoT and SDS-PAGE Protein to Assess Soma-Clonal Variation in Micro-Propagated Fig (*Ficus carica* L.) Plant

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Abstract

Background and Objective: *In vitro* propagation of fig (*Ficus carica* L.) is one of the possible approaches that may be used to maximize the diversity of plant species. The current work was carried out to evaluate genetic stability of micropropagated fig plantlets and to determine the effect of *in vitro* propagation on genomic content of Saudi fig. **Materials and Methods:** The start codon-targeted (SCoT), DNA-barcoding chloroplast gene RNA polymerase1 (*rpoC1* sequencing) and total protein profiling assays (SDS-PAGE) techniques were used to detect genetic stability in micropropagated fig plantlets. **Results:** The Scorable PCR bands were produced with 10 SCoT primers used, where the total number of bands was 135 bands. Twenty polymorphic bands were generated with 18.4% of a polymorphism percentage. According to the result, no visual unique bands were generated which confirmed the genetic homogeneity of micropropagated plantlets samples compared to the control sample (mother plant). Sequence analysis and phylogenetic tree generated using fig *rpoC1* sequence showed high similarity between control and plantlets samples of fig plant. The protein profiling results revealed no remarkable changes between micropropagated plantlets and the mother plant. **Conclusion:** The results indicate that using SCoT, DNA barcoding and protein profiling have demonstrated their utility to detect genetic homogeneity in micropropagated fig plantlets, which suggests using of micropropagation protocol of plants applied on the plantlets in the current study as a reliable protocol for *in vitro* culture and conservation of fig plant.

Key words: Fig (*Ficus carica* L.), Micro-propagation, soma-clonal variation, SCoT, DNA-barcoding, SDS-PAGE

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The fig tree (*Ficus carica* L.) is a deciduous perennial tree belonging to the Moraceae family. Fig has been a traditional part of the Mediterranean diet for millennia, because of its rich nutritional content¹. Fig fruits contain seventeen groups of amino acids at least, the main of them are aspartic acid as well as glutamine². Dried figs often produce relatively high amounts of fibres, more than all other common fruits, which have been used in weight loss, control of blood cholesterol and blood sugar³. In folk medicine, fig tree roots are almost used as a cure for ringworms, sweet leucoderma and their fruits have purgative, antipyretic, aphrodisiac characteristics⁴. According to FAO, global figs production in 2018 was roughly a million tons. Countries including Afghanistan, Albania and Algeria were among the top fig fruit producers⁵.

Saudi Arabia (KSA) flora is one of the richest ecosystems areas and is a significant genetic resource for exotic plants⁶. The KSA is categorized by its vast territory, exhibiting climatic variation attributed to differing in height, leading to wide variability in flora. Figs are cultivated in KSA, where the diversity of some local varieties has been documented through nutritional studies⁷. Wild fig trees have been reported in KSA's Al-Baha region, where their crude extract antimicrobial activity has been documented⁸. Such studies show KSA's possible importance as an origin for figs and its significance for diversity studies.

To encourage local production and maintenance of national figs resources, a high-performance and efficient procedure for the propagation of figs is needed. Using tissue culture for *in vitro* propagation of the varieties of Saudi figs is a possible method to produce plant clones from single cells using controlled dietary and for plant resources conservation^{9,10}. It is one of the possible approaches that may be used to maximize the diversity of plant species.

Furthermore, tissue culture can be used to induce soma-clonal variations ranging from easy to assess differences, especially morphological traits, to significant variances in bunching, fruit size and chemical content¹¹. Such variation requires an effective and rapid method of detection that can be used at the industrial propagation level.

Molecular marker assays are reliable, cheap and could be used effectively to detect the soma-clonal variation in plants¹². Start Codon-Targeted (SCoT), DNA barcoding, RAPD, AFLP and ISSR are the most commonly utilized molecular marker assays in the assessment of soma-clonal variation^{10,13-15}. The SCoT was constructed based on a short standard area flanking ATG start codon in the plant genome. The SCoT markers should be more efficient than other random marker assays in particular due to

high annealing temperatures and longer primers length¹⁶. It does not involve comprehensive genomic information so that it can be applied to plants without a genomic reference¹⁷. The implementations of SCoT molecular assays were reported in different plant species including coconut¹⁸, jojoba¹⁹, olive²⁰ and tomato²¹.

One of the most successful molecular marker assays is DNA-barcoding, in which a standardized DNA region is sequenced as a tool of identification of species and it can help in plant documentation²²⁻²⁴. Numerous plastid, mitochondrial and nuclear genome regions including *ropC1*, *matK*, *rpoB*, *rbcl* and *trnH-psbA*, which were used extensively to assess diversity and identification of different plant species, *ropC1* gene has been utilized to screen genetic variability of different species i.e., *Calluna*²⁵, *Apocynaceae*²⁶ and *Gongora*²⁷. It has also been successfully applied in some plant species to study soma-clonal variations²⁸. The study of protein profiles of micropropagated plants could be used to identify any genomic changes. Protein assays using SDS-PAGE was used to assess stability and soma-clonal variation of grass pea plants which regenerated *in vitro*²⁹, *Orthosiphon stamineus*³⁰ and oil palm³¹.

The current work was carried out to evaluate the genetic stability of micropropagated fig plantlets. The SCoT, DNA-barcoding, total protein profiling assays were used to evaluate its ability in identifying possible soma-clonal variation in the micropropagated plantlets. The DNA-barcoding was used to determine the biodiversity of Saudi fig variety relative to known species of plants.

MATERIALS AND METHODS

Study area: This research study was conducted from June, 2019 to October, 2021.

Plant material: Ten samples of micropropagated plantlets were obtained from *in vitro* propagated fig plant according to El-Dessoky *et al.*¹⁰, control sample was collected from mother plant grown in greenhouse of Taif University main campus.

Isolation of DNA: Total genomic DNA was isolated by DNA easy Plant Mini Kit, about 2 g of 11 fig tissue samples, obtained from 10 micropropagated plantlets and one control, were used for DNA extraction. The quality and quantity of DNA were evaluated by comparing DNA samples (2 µL) to DNA marker on 1% agarose gel. The amount and consistency of the DNA samples were determined relative to the fluorescence strength of the DNA marker bands.

Molecular marker assay: Ten SCoT primers and the *rpoC1* gene region were used in the present investigation in Table 1 and 2. The *rpoC1* PCR program (40 cycles) and gene sequencing protocol was applied as reported by Phong *et al.*³². The SCoT assay reaction content and the PCR program (40 cycles) were conducted according to Awad *et al.*³³. Final products of PCR were stored at 4°C. Agarose gel (8%) stained with ethidium bromide was utilized to separate the PCR fragments compared to 1kb DNA Ladder (4 µL) (NEB NEBNext® Ultra™). The documentation of gel images was conducted using the Gel Doc XR system (Bio-Rad, Hercules, CA, USA).

The NCBI BLASTn program has been used to identify related species according to fig's *rpoC1* gene³⁴. ClustalW software was used to study ornithological genes using phylogenetic analysis³⁵. Interactive Tree of Life (ITOL) online tool was used to construct phylogenetic trees that reflect gene relationships³⁶. Moreover, the PCR fragments were counted as absent (0) or present (1) and utilized for the coefficients of similarity matrix between different samples and illustrated using phylogenetic dendrograms.

Protein profiling: In this study to obtain protein profiles, with different molecular weights of each sample, SDS-PAGE was used. Extraction of protein was performed by grinding 2 g of micropropagated plantlets and control samples into a powder. The total protein was isolated as reported by Sahara *et al.*³¹, SDS-PAGE electrophoresis using 12% acrylamide gel was applied to resolve protein samples. Electrophoresis at 100 V was used for 1.5-2.5 hrs. Coomassie blue was used to stain the gel and it was washed using distilled water d.H₂O and stored at room temperature for 24 hrs.

Table 1: The *rpoC1* gene primer sequence

Primer code	Sequence	Product size
<i>rpoC1</i> -F	5'-GGCAAAGAGGGAAGATTTCG-3'	500 bp
<i>rpoC1</i> -R	5'-CCATAAGCATATCTTGAGTTGG-3'	500 bp

Table 2: Sequence information of SCoT primers used in this study

Name	Sequence 5'-3'
SCoT-2	CAACAATGGCTACCACCC
SCoT-3	CAACAATGGCTACCACCG
SCoT-4	CAACAATGGCTACCACCT
SCoT-11	AAGCAATGGCTACCACCA
SCoT-12	ACGACATGGCGACCAACG
SCoT-13	ACGACATGGCGACCATCG
SCoT-14	ACGACATGGCGACCACGC
SCoT-16	ACCATGGCTACCACCGAC
SCoT-20	ACCATGGCTACCACCGCG
SCoT-22	AACCATGGCTACCACCAC

RESULTS

SCoT marker assay: In this study, Scorable PCR bands were produced with 10 SCoT primers used, SCoT-02, SCoT-03, SCoT-04, SCoT-11, SCoT-12, SCoT-13, SCoT-14, SCoT-16, SCoT-20 and SCoT-22 as shown in Fig. 1 and Table 3. The number of total bands was 135 with a mean of 13.5 bands/primer in Fig. 1a-j and Table 3. Where SCoT bands ranged from 6 (SCoT-11) to 19 (SCoT-03). Twenty polymorphic bands were generated using SCoT-PCR assay, of which primer SCoT-16 produced the highest number of 4 polymorphic bands, with 24% of polymorphism percentage. The results indicated that the lowest Polymorphic bands (0) were detected using primers SCoT-11 and SCoT-22, with 0% of polymorphism percentage. The phylogenetic relationship constructed using binary SCoT data revealed genetic variation among the investigated plant samples in Fig. 2. The phylogenetic tree was separated into three clusters, separating sample 5 into one branch. Control, 1 and 2 samples were Hollings worthed together, while 10, 7, 8 and 9 samples were Hollings worthed in a different cluster.

DNA-barcoding analysis using *rpoC1* gene: Analysis of DNA-barcode using *rpoC1* gene sequencing was employed to evaluate the soma-clonal variability between micropropagated plantlets and control (mother plant) of fig plants. The NCBI-BLAST results indicate high sequence similarity with a mean of 99.5% of both sequences to *Ficus carica* (common fig) plant species in Fig. 3a-d and 4a-d. The phylogenetic tree was generated using the fig *rpoC1* sequence and the most related sequences acquired from other species in Fig. 5. The sequence alignment of the two sequences of control and micropropagated plants shows low single nucleotide mutation in Fig. 6.

Protein profiling: The protein profiling of control and 10 plantlets samples of fig plant revealed several protein bands in Fig. 7. None of these bands was successful to differentiate between control and micropropagated plants.

Table 3: Polymorphism percentage of the SCoT primers used in this study

PN	TB	MB	PB	PP
SCoT-02	15	14	1	0.07
SCoT-04	11	8	3	0.27
SCoT-03	19	16	3	0.16
SCoT-11	6	6	0	0
SCoT-12	14	12	2	0.14
SCoT-13	17	15	2	0.12
SCoT-14	13	10	3	0.23
SCoT-16	17	13	4	0.24
SCoT-20	11	9	2	0.18
SCoT-22	12	12	0	0
Total	135	115	20	

PN: Primer name, TB: Total number of PCR bands, MB: Monomorphic bands, PB: Polymorphic bands and PP: Polymorphism percentage

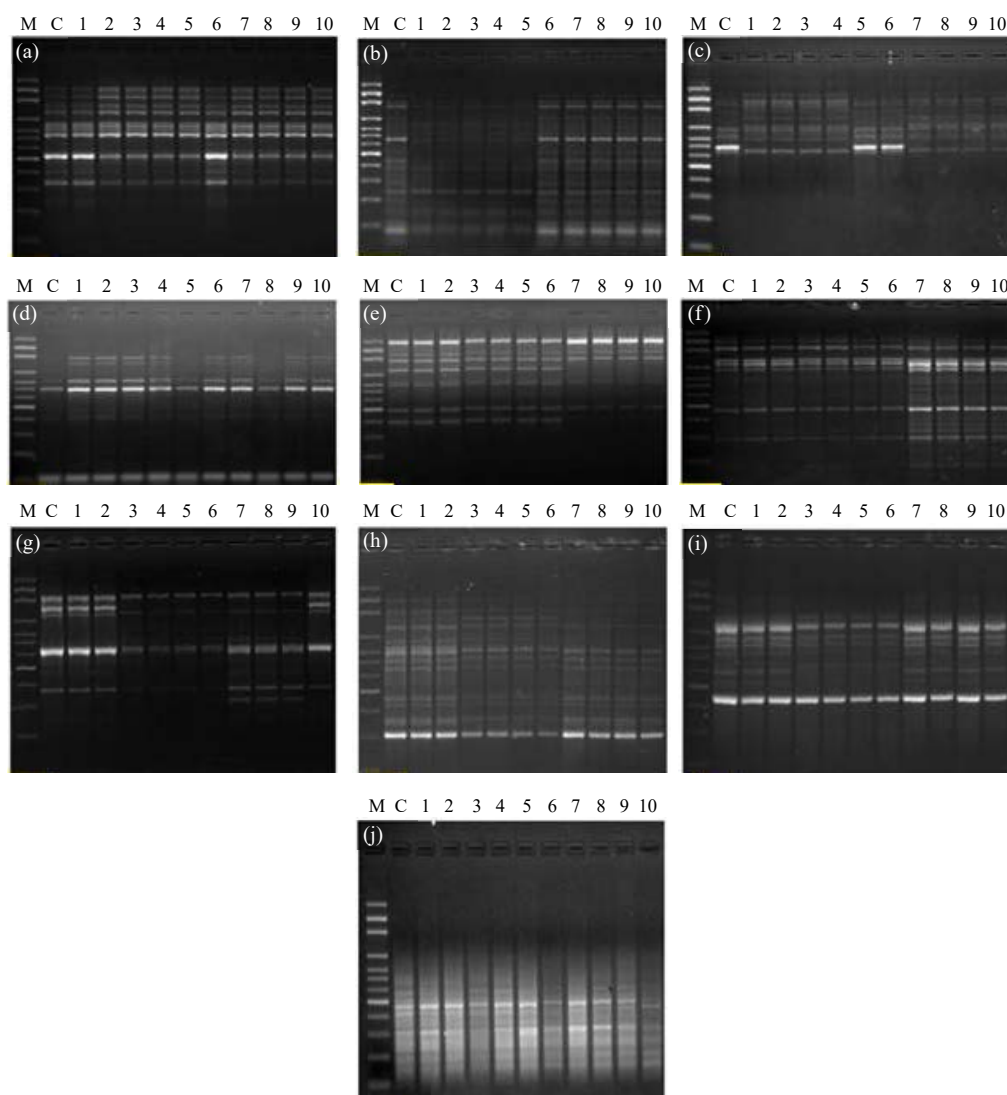


Fig. 1(a-j): PCR profiles of SCoT primers used to study genetic homogeneity between control and ten plantlets samples of plant (a) SCoT-02, (b) SCoT-03, (c) SCoT-04, (d) SCoT-11, (e) SCoT-12, (f) SCoT-13, (g) SCoT-14, (h) SCoT-16, (i) SCoT-20 and (j) SCoT-22

M: DNA marker (1 kb DNA ladder), C: Control (mother plant and (1-10): Micropropagated plantlets samples

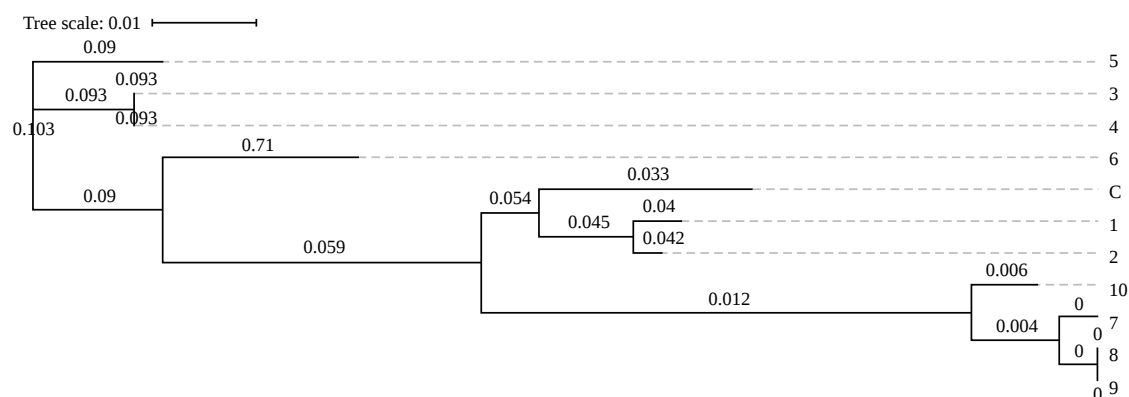


Fig. 2: Phylogenetic tree constructed using SCoT molecular assay data control (mother plant) and 10 micropropagated plantlets samples of plant

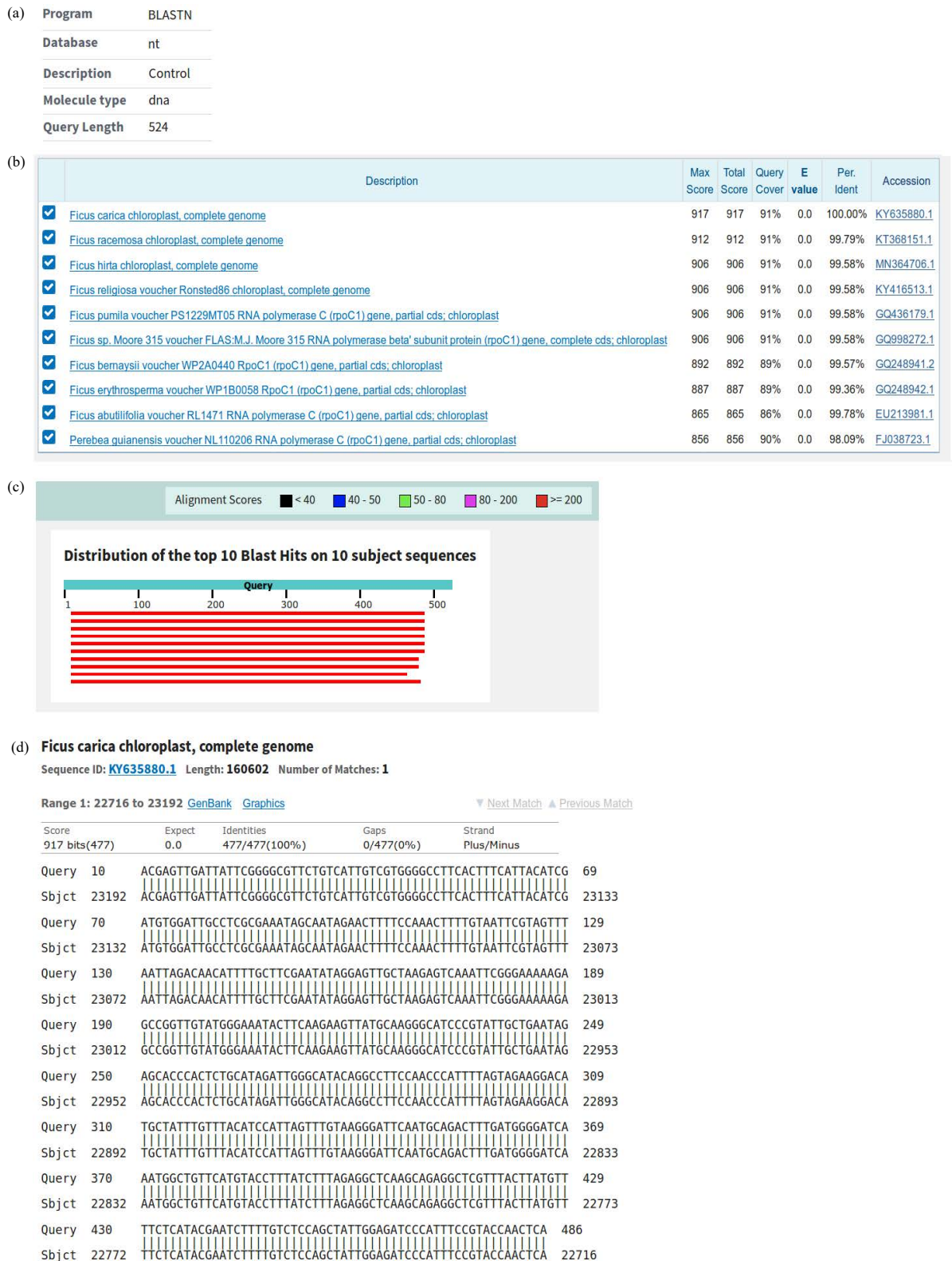


Fig.3(a-d): NCBI-BLAST result of the *rpoC1* gene sequence recovered from the control (mother plant), (a) Sequence information, (b) Similar species and blast result, (c) Shared sequences region and (d) Sample sequence alignment between fig and similar species

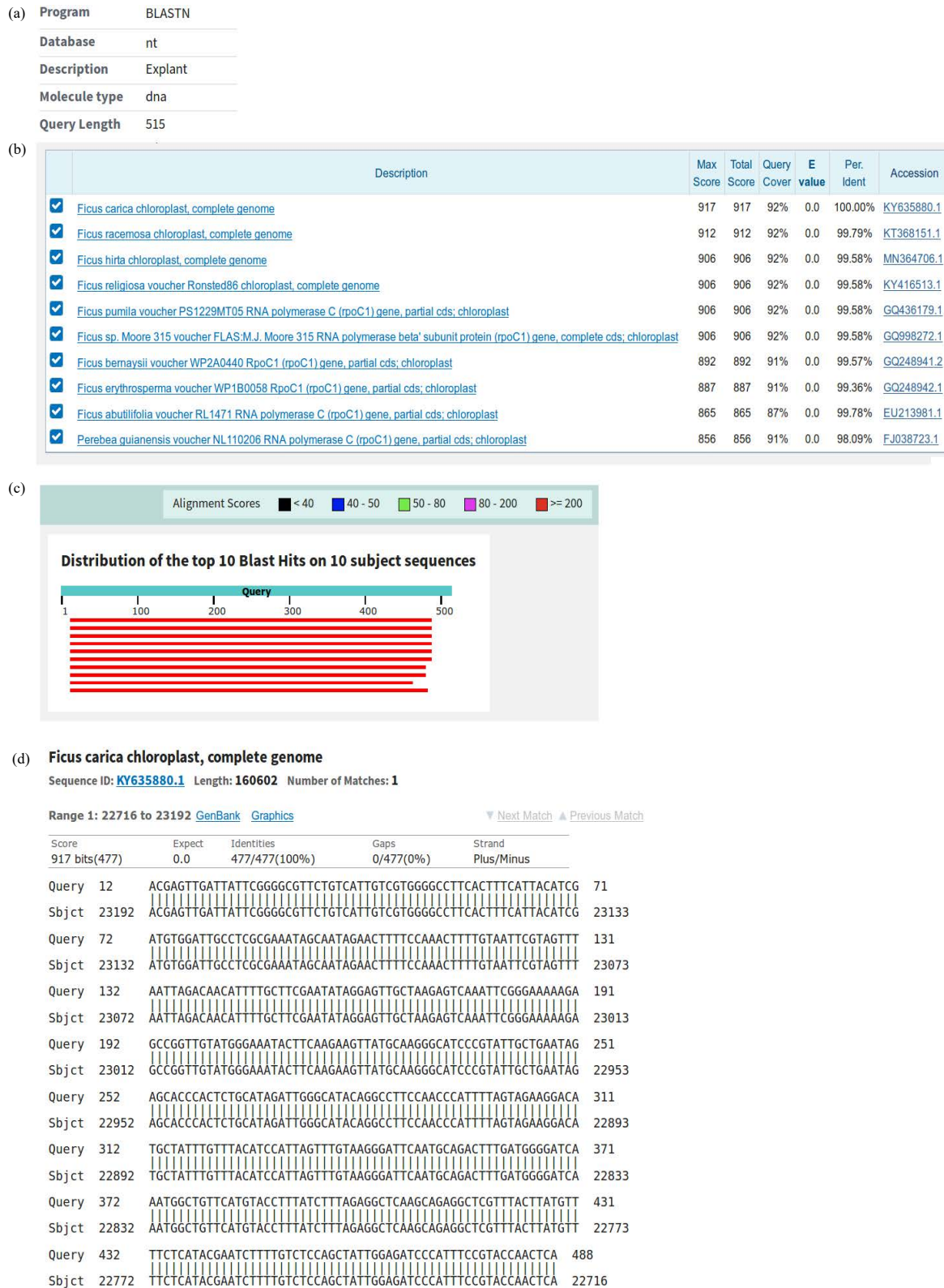


Fig.4(a-d): NCBI-BLAST result of the *rpoC1* gene sequence recovered from explant (micropropagated plantlets), (a) Sequence information, (b) Similar species and blast result, (c) Shared sequences region and (d) Sample sequence alignment

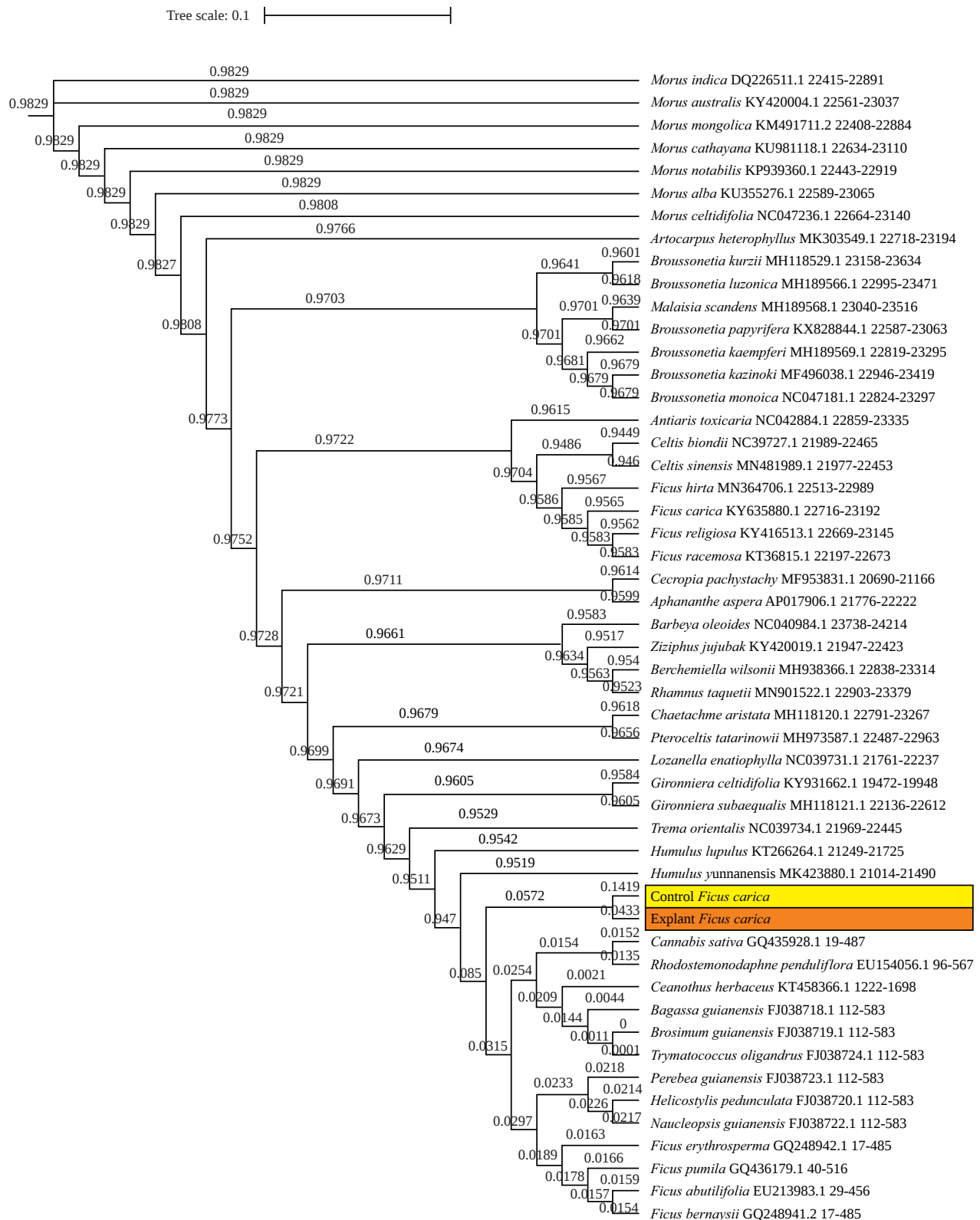


Fig. 5: Phylogenetic tree constructed using sequences of *rpoC1* gene of control (mother plant) and micropropagated plantlets and the most similar sequences generated from other species

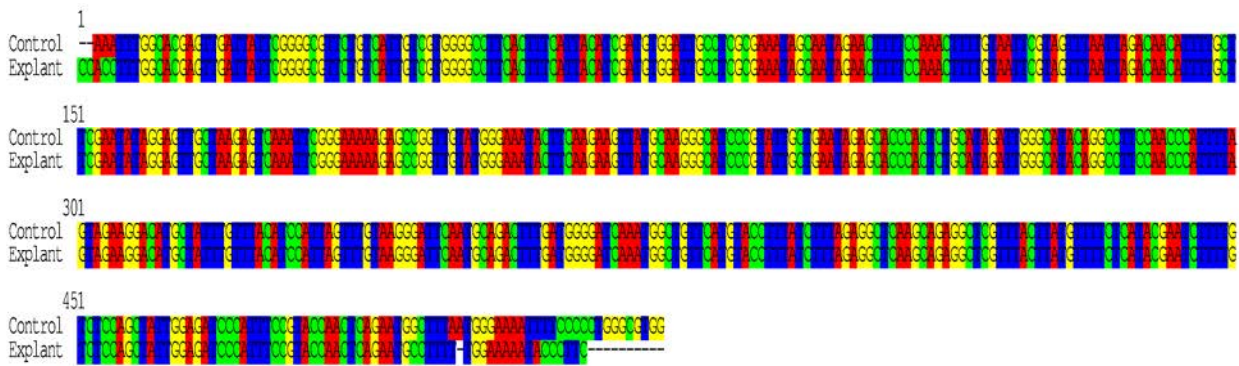


Fig. 6: Sequence alignment constructed using *rpoC1* sequences of control (mother plant) and micropropagated plantlets sequences

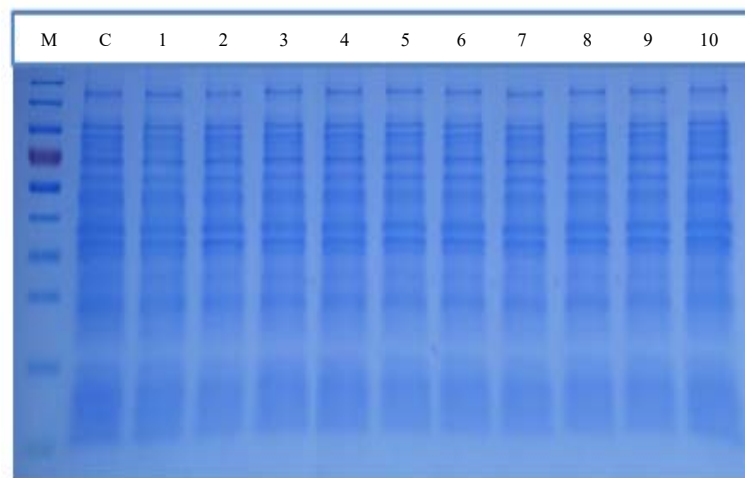


Fig. 7: The proteins profile of Fig plant samples
M: protein marker, C: control (mother plant) and ten samples (1-10) of micropropagated plantlets

DISCUSSION

Soma-clonal variation between the sub clones is common during plant micropropagation. Plantlets, that propagated *in vitro*, were reported to have soma-clonal variations that are sometimes inheritable^{37,38}. The SCoT, *rpoC1*-sequencing and protein profiling assays were used to evaluate the genetic impacts of soma-clonal variability generated during *in vitro* propagation of fig plant. These molecular assays were used to detect genetic stability of *in vitro* propagated some fig species located across Saudi Arabia.

In the present study, the SCoT-PCR assay generated twenty polymorphic bands with 27% of polymorphism percentage (Table 3). The SCoT assay could not generate any unique bands that distinguish between plantlets and control of fig plant samples. The analysis of genetic instability using SCoT was reported in previous work i.e., Rathore *et al.*¹²

demonstrated that analysis of micropropagated *Cleome gynandra* produced a total of 65 bands with a mean of 4.3 ranging from 2-7 per primer. In contrast, there were no polymorphic bands distinguish between the regenerated and control, suggesting the genetic integrity of *in vitro* grown plantlets. Similarly, employing SCoT assay in evaluating the genetic homogeneity of micropropagated *Alhagi maurorum* generated monomorphic PCR products through all investigated micropropagated plants³⁹. While the analysis of genetic homogeneity of *in vitro* propagated *Ansellia africana*, a total of 70 PCR bands were revealed through using 16 SCoT primers, five of which were polymorphic with 7.14% of a polymorphism percentage⁴⁰.

In the current investigation, the phylogenetic relationship constructed using binary SCoT data revealed genetic variation among the investigated plant samples (Fig. 2). According to SCoT assay low variation was observed among studied

samples, which indicates the low impact of micropropagation on fig's genetic content. During the evaluation of genetic fidelity no polymorphism was recorded between the mother plant and *in vitro* propagated *Cleome gynandra* plants, indicating the genetic stability of the *in vitro* raised plantlets¹². Monomorphic band patterns were resulted by ISSR and SCoT assays, during the assessment of genetic stability between micropropagated plants and mother plant of *Helicteres isora* L.⁴¹, Agarwal *et al.*⁴² demonstrated that SCoT assay showed high polymorphism (100%) during detection of genetic variation 29 different *Rosa* germplasm.

In the current study, evaluation of soma-clonal variability between micropropagated and control fig plants by the analysis of DNA-barcode using *rpoC1* gene sequencing showed that high sequence similarity of both sequences to *Ficus carica* (common fig) plant species was indicated by NCBI-BLAST results indicate (Fig. 3 and 4). The phylogenetic tree was not successful in differentiating between control and plantlets samples of fig plant (Fig. 5). Such results could indicate the low impact of the micropropagation protocol on the genetic background of fig plants or the poor efficacy of DNA barcoding using *rpoC1* in the differentiation between control and micro-propagated explants. Cristina-Mirela *et al.*²⁵, according to their results on the identification of *Calluna vulgaris* (L.) Hull species by few barcode markers, they found that barcode markers matK and *rpoC1* are suitable markers in the identification of *Calluna vulgaris* species. Guardado *et al.*²⁷ reported that similar phylogenetic trees were provided by the sequences of *rpoB* and *rpoC1* which confirms the present classification of *Gongora* as individual species.

According to protein profiling of control and 10 plantlets samples of fig plant by SDS-PAGE (Fig. 7). None of these bands was successful to differentiate between control and micropropagated plantlet. This result could indicate the low impact of soma-clonal variation on functional genes of micropropagated fig plantlets samples. In oil palm callus culture, early detection of soma-clonal variation was carried out by using SDS-PAGE protein analysis³¹. The SDS-PAGE exhibits multiple molecular protein weights between the rooted callus and the friable nodular aggregate. A specific protein was detected in the nodular friable callus, which distinguishes embryonic and non-embryogenic calluses³¹.

CONCLUSION

The main advantage of the *in vitro* propagation process is producing true to type plants. The use of efficient and more sophisticated molecular assays is needed to identify soma-clonal variation between *in vitro* raised plants. According to the results of current research using SCoT, DNA

barcoding and protein profiling have demonstrated their utility to detect genetic homogeneity in micropropagated fig plantlets, which suggests using of micropropagation protocol of fig plants applied on the plantlets in the current study as a reliable protocol for *in vitro* culture and conservation of fig plant.

SIGNIFICANCE STATEMENT

The results indicate that using SCoT, DNA barcoding and protein profiling have demonstrated their utility to detect genetic homogeneity in micropropagated fig plantlets, that suggests using micropropagation of fig plants as a reliable method for *in vitro* culture and conservatron of fig plant.

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