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In vitro ultraviolet radiation effects on growth, chemical constituents and molecular aspects of *Spathiphyllum* plant

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Abstract

Background: *Spathiphyllum* is one of the best plants for indoor gardening as it is a rhizome and undemanding of light care. It is a member of lily family (*Araceae*). The plant itself should live for 10 years or more if not maltreated. *Spathiphyllum cannifolium* plants are used in clean air study for their ability to remove formaldehyde, benzene, and CO₂ from interior air. UV is divided into three wavelength ranges; UV radiation can have damaging effects on DNA and/or physiological processes and play central role in characterization of bioresponses. A large number of studies have been conducted to evaluate the potential consequences of an increase in UV-B radiation on many plants.

Results: In this investigation, plants treated with 45-min ultraviolet radiation (UV) recorded the highest values on growth parameter, anatomical structure, survival %, and proline %. Fifteen minutes of UV radiation exposure gave the second highest value of previous parameters. Conversely, exposure of plantlets to moderate dose (30 min of UV radiation exposure) recorded fewer values in previous mentioned parameters. Start codon targeted (SCoT) analyses exhibited 39 DNA bands which appeared by using seven primers. These bands were identified as 15 polymorphic and 24 monomorphic ones with 38.46% polymorphism. Twelve unique bands were identified in the resulted SCoT profile.

Conclusion: The results were nearly in an ascending order with increasing of UV-B radiation exposure time. Increasing exposure time to 45 min increased significantly the values of vegetative growth, anatomical parameters, and proline percentage in most cases compared with control and other UV-B radiation treatments. In the contrary, 30 min recorded decreased in previous mentioned treatments. The SCoT (Start codon targeted) marker is helpful for possible distinguishing, identifying, and characterizing of UV treatments.

Keywords: *Spathiphyllum*, UV radiation, *In vitro*, SCoT analyses, Anatomical structure

Introduction

Spathiphyllum is one of the best plants for indoor gardening as it is undemanding of light care. It is a member of lily family (*Araceae*) and has exceptionally attractive leaves as well as flowers (Sardoei 2014; El-Khateeb et al. 2018). The plant itself should live for 10 years or more if not maltreated. It is a rhizome. It can grow enormously in single season, doubling in size if given the correct conditions. The dark green leaves of this plant provide a perfect foil for pure white spaths, and these showy white spathes of *Spathiphyllum cannifolium* enhance popularity and

marketing as flowering foliage plants (Henny et al. 2004). This plant has a lovely cool feel and is best displayed on its own, growing it in a simple, modern interior away from distracting foliage or flowers of others plants. *Spathiphyllum* plants are used in a clean air study for their ability to remove formaldehyde, benzene, and CO₂ from interior air (McConnell 2003). UV constituted approximately 8–9% of total solar radiation and considers a part of non-ionizing rays of the electromagnetic spectrum region (Frederick 1993; Hollosy 2002). UV is divided into three wavelength ranges, UV-C (200–280 nm), UV-B (280–320 nm), and UV-A (320–400 nm) (Bernadeth et al. 2017). UV-B can induce a variety of damaging effects in plants while, UV-A considers approximately 6.3% of the incoming solar radiation and is the less hazardous part of UV rays. The absorption coefficient of ozone decreases

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rapidly at wavelengths longer than 280 nm and approaches zero at about 330 nm (Robberecht and Caldwell 1986). A small decrease in ozone levels may cause a large relative increase in biologically effective UV Rays (Madronich 1992; Madronich 1993). The effects of an increase in solar UV-B rays reaching to the earth surface have been investigated by numerous research groups; much of this research has focused on the effects of plant growth and physiology (Sinclair et al. 1990; Sullivan and Teramura 1990). UV radiation can have damaging effects on DNA and/or physiological processes and play a central role in the characterization of bioresponses (Coochill 1992; Caldwell et al. 1986).

A large number of studies have been conducted to evaluate the potential consequences of an increase in UV-B radiation on many plants (Zheng et al. 2003). However, most of these studies addressed the effects of UV-B on the whole plant level. Less information on the UV-induced damage at the DNA level are found. Owing to its high energy level, the impact of radiation on metabolic process can be very harmful. DNA, protein, and photosynthetic apparatus are considered primary potential targets of UV radiation on plants (Hollosoy 2002).

Recently, molecular markers have been used to investigate DNA sequence variation(s) in and among the crop species and create new sources of genetic variation by introducing new and favorable traits from landraces and related crop species. The SCoT (Start codon targeted) technique is a novel, simple, and reliable gene targeted marker technique based on the translation start codon (Collard and Mackill (2009). Primers for SCoT marker were designed based on the conserved region surrounding the translation initiation codon, ATG. Using a single 18-mer primer as a forward and reverse primer in the PCR, Collard and Mackill (2009) designed 36 primers that were used successfully for treatment identification and genetic diversity analysis in many crops. This study is aimed to investigate the *in vitro* basic effects of UV-B radiations on growth, physiological, anatomical, chemical constituents, and the genomic DNA of *Spathiphyllum cannifolium*.

Materials and methods

This work was carried out at tissue culture Laboratory of Ornamental Plants and Woody Trees Department, National Research Centre (NRC), Egypt, during 2016 and 2017 to establish *in vitro* micropropagation protocol and investigate the effect of ultra violet rays (UV-B (280–320 nm) on growth behavior as well as chemical composition of *Spathiphyllum cannifolium* plant.

Set up a tissue culture protocol

Explants of *Spathiphyllum cannifolium* were collected from mature plants, kept in polyethylene bags, and transferred directly to laboratory, where they were

immediately washed under running tap water for 1 h. Surface sterilization were in 70% ethanol for 30 s, then immersed in 15% sodium hypochlorite (Clorox+ 0.01% Tween 20) for 7 min, and finally rinsed with sterile water three times. The explants were then sterilized in 0.1% HgCl₂ solution for 5 min, rinsed five times in sterile water.

Explants were cultured after sterilization process on solid MS media-free hormones. The pH of the medium was adjusted to 5.7–5.8 to adding agar (8 g/l) and sucrose (25 g/l). Media was autoclaved at 121 °C and 1.5 kg. cm² for 20 min. Cultured explants were kept in growth chamber illumination of 16 h photoperiod, 2000 Lux at 25 ± 2 °C for 6 weeks to promote shootlet development. At the end of the 6-week intervals, in order to obtain sufficient number of shoots, the shoots were sub-cultured on MS medium supplemented with various concentrations (0.0, 0.5, 1.0, and 2.0 ppm) of BA (6-benzylaminopurine) individually or in combination with NAA at (0.0, 0.1, 0.2, and 0.3 ppm). The following parameters were recorded: number of shootlets/explant, number of leaves/shootlets, and length of shootlets.

Ultra violet rays (UV-B) treatments

The wavelength of UV-B rays was (280–320 nm). The plantlets were treated by UV-B at (0, 15, 30, and 45 min) exposure time. In the acclimatization of micro propagated plants, shoot number/plant, shoot length (cm), number of leaves/plant, number of roots/plant, root length (cm), shoot and root fresh weight (g), and plant dry weight (g) were recorded after 12 weeks of exposure time.

Anatomical structure

Leaf anatomy

Samples were taken from 30 leaves on the plant, then killed and fixed in FAA, solution (100 ml formalin, 50 ml acetic acid, and 500 ml ethyl alcohol 95% and 350 ml distiller water), according to Johansen (1940). Sections were examined microscopically and microphotography according to Corgen and Widmamayer (1971).

Chemical analysis

Determination of photosynthetic pigment content (mg per 100 g F.W) of chlorophyll a, b and carotenoids was carried out according to the method described by Saric et al. (1967). Total carbohydrate percentage in the dry samples after acclimatization was determined according to Dubois et al. (1956). Proline content was determined in fresh leaves using the method of Bates et al. (1973).

Molecular analysis

Leaves of the four treatments were collected and soaked in liquid nitrogen for DNA extraction; the DNA was extracted by cetyl trimethyl-ammonium bromide (CTAB)

Table 1 Effect of UV-B radiation on morphological micropropagated of *Spathiphyllum cannifolium* plant (mean of three subcultures)

Treatments	Characters			
	Shootles length (cm)	Leaves number/ shootlets	Root length (cm)	Survival%
0 min	4.83	4.00	6.83	17
15 min	8.00	7.00	13.33	100
30 min	4.83	5.00	10.33	100
45 min	9.16	7.00	14.33	100
L.S.D. at 0.05	1.88	0.998	2.02	28.54

method (Doyle and Doyle 1990). SCoT amplification was performed as described by Collard and Mackill (2009) and Xiong et al. (2011). Code and sequence of the seven SCoT primers used in the present investigation are showed in Table 5.

Statistical analysis

Data was statistically analyzed using one-way analysis of variance (ANOVA), and L.S.D. was performed for comparison of means at 0.05 probability, according to Snedecor and Cochran (1980) using COSTATV-63. The similarity matrices of SCoT markers were done using Gel works ID advanced software UVP-England Program.

Results

It can observed from Table 1 and Fig. 1 that the growth characters in terms of shootlet length, number of leaves, root length, and survival % were increased significantly in most cases with all doses of UV treatments.

The highest values of shootlet length, number of leaves, root length, and survival rate were obtained from 45-min exposure time followed with 15-min exposure time. It recorded 9.16, 7.00, 14.33, and 100 when compared with control samples 4.83, 4, 6.83, and 17, respectively.

As shown in Table 2 and Fig. 2, all doses of UV irradiation period have significantly influenced shootlet length, number of leaves, leaf area, shootlet number/explant, number of roots, rooting percentage, and fresh and dry weight of shoots in most cases of vegetative growth parameters as compared with control plants. Increasing the exposure time of UV (45 min) increased significantly the values of vegetative growth parameters in most cases compared to control and other UV radiation treatments.

It is clear from the data in Table 2 that the most effective treatment was the 45-min exposure time. It showed the highest significant value in shootlet length, number of leaves/shoots, leaf area, number of roots, and fresh and dry weight of shoots. It recorded 28.15, 42.85, 141.30, 264.80, 56.73, and 48.46%, respectively, over the

control plants. On the contrary, treated *Spythiphyllum cannifolium* plantlets with moderate dose (30-min exposure) recorded the lowest increasing values in all growth parameters compared with control and other UV radiation treatments.

Chemical constituents

It is clear from the data in Table 3 and Fig. 3 that the highest values of chlorophyll a, chlorophyll b, carotenoids, and carbohydrate content were obtained when plants were treated with 30 min of UV (0.2676, 0.1304, 0.4374, and 0.2463), respectively, compared with untreated plants, while the same dosage recorded lowest values of proline 66.9 compared to control 93.2. The highest significant values of proline content were obtained from 45-min exposure time of UV. It recorded 98.61 compared with control plants (93.2).

On the other hand, exposing plantlets of *Spathiphyllum cannifolium* to 45 min of UV-B radiation recorded decrement in chlorophyll a and b and carotenoids followed with 15-min exposure time. We can reveal from Table 3 that the higher dose of UV-B exhibited the lower significant decreased of 44.9, 27.79, and 46.475% lower than the control plants.

Anatomical structure

Data in Table 4 revealed that highest values of thickness of midvine, thickness of lamina, length and width of vascular bundle, and cuticle thickness were recorded when plantlets of *Spathiphyllum cannifolium* treated with higher dose of UV(45 min) followed by low dose (15 min) exposure time. It recorded 72.14, 23.62, 19.40, 16.75, and 8.76 compared to untreated plants which recorded 31.19, 8.22, 9.72, 9.40, and 3.10), respectively.

Conversely, exposing plantlets to UV radiation of moderate dose (30-min exposure time) recorded to be decreasing in all anatomical structure of leaves compared with control and other treatments.

Molecular analysis

The results of the amplified fragments using SCoT method with seven primers of the four treatments are presented in Table 5 and Fig. 4.

Seven SCoT primers were employed to investigate the genetic polymorphism among the three UV treatments and the control to *Spathiphyllum* plants. As shown in Table 5 and Fig. 4, the size of the amplified bands ranged from about 215 bp to 1500 bp. All of the seven primers detected polymorphic patterns and revealed a total number of 39 amplicons with 38.46% level of polymorphism. The number of amplicons per primer ranged from 3 to 9 with an average of 5.57



Fig. 1 *Spathyphyllum cannifolium* during multiplication stage (without radiation treatments)

amplicons per primer. The number of monomorphic amplicons varied from 1 to 6 with an average of 3.42 monomorphic amplicons per primer. However, the number of polymorphic amplicons varied from 0 to 4 with an average of 2.14 polymorphic amplicons per primer. Likewise, the number of unique fragments varied from 0 to 3 with an average of 1.71 unique fragments per primer.

Discussion

The results revealed significant increases in most of growth parameters, (shootlet length, number of leaves/shoots, leaf area, shootlet number, roots number, and fresh and dry weight of leaves) with low and higher doses of UV radiation treatments. Also, data showed that UV-B radiation treatments tended to decrease growth parameters. Similar results have been reported by Zu et al. (2010) and Rai et al. (2011). Therefore, it is important to understand how the plants respond to UV radiation stress. These increases in growth and anatomical parameters may be due to the protective mechanism of plants to UV stress that could include the formation of absorbing compounds like phenolic and flavonoids compounds in the outer epidermal tissue. Also, the plant produces antioxidant compounds and enzyme activity for repairing itself against UV radiation damage (Tegelberg et al. 2001; Donato et al. 2017). The absorbing compounds protect DNA and play a key role in plant defense system (Hagen et al. 2007; Agrawal et

al. 2009; Bernadeth et al. 2017). Exposing plants to non-ionize radiations enhancing growth parameters may be due to the role of GA (Gibberic acid) in cell elongation, where GA may cause cell elongation by induction of enzymes that weaken the cell wall and the radiations may promote growth hormones like GA and cytokinin which affect plant growth (Macleod 1962; Sami et al. 2010). The epidermal layer in *Pinus taeda* attenuated most of harmful UV radiation and the anatomical or chemical changes in epidermal cells which could be involved in the morphological changes. Plants subjected to UV radiation respond by altering the anatomy of leaves (Ruhland and Day 1996). Depending on species or genotype, the increase or decrease in the thickness of the epidermis of the leaf which modifies the response to high doses of UV radiation is considered as a defense strategy (Jansen 1998; Claudio et al. 2014). Enhanced UV-B may have influences on biological functions of plant in many aspects including inhibitions of photosynthesis, and because of these depressed reactions, the production and allocation of carbohydrates might be markedly affected, and therefore, the growth and development of plant are distinctly affected (Hyejin et al. 2014).

In this investigation, results also showed that UV-B radiation treatments led to a significant change in chlorophyll a and b, proline, and carbohydrate contents which depend on doses this may attributed to reduced photosynthesis process caused by a decline



Fig. 2 *Spathyphyllum cannifolium* plants during the acclimatization stage. (1) Control, (2) 15 min, (3) 30 min, and (4) 45 min

Table 2 Effect of UV-B radiation exposure time on growth parameters of *Spathiphyllum cannifolium* plant in the acclimatization stage (after 2 months)

Treatments	Characters								
	Shootles length (cm)	Number of leaves	Leaf area cm ²	Shootlet number explant	Number of roots	Root %	Fresh weight of shootlets (g)	Dry weight of shootlets (g)	Survival%
0 min	6.50	7.00	1.84	1.00	4.66	97.00	0.906	0.130	49.00
15 min	8.16	8.66	3.66	5.33	12.33	96.66	1.160	0.154	98.66
30 min	6.66	7.66	2.81	3.00	6.00	97.66	1.020	0.161	98.00
45 min	8.33	10.00	4.44	5.33	17.00	97.66	1.420	0.193	98.00
L.S.D. at 0.05	1.10	1.20	0.133	1200	2.28	4.46	0.042	0.028	1.912

in ribulose-1,5 biophosphate carboxylase enzyme protein which is responsible for CO₂ fixation in leaf tissue (Kadur et al. 2007).

The increase in photosynthesis activity was mirrored in an increase in carbohydrate contents. According to the data, there are remarkably increased carbohydrate contents. In this concern, Magdalena et al. (2014) found that UV-B radiation led to increase in the rate of photosynthesis, which reflected on carbohydrates content, whereas higher photosynthesis activity resulted an increase in soluble sugar which is required to provide cells by energy for protection, repair plant activity, and plant structure. Most studies have shown that the levels of carotenoids increase or decrease under UV radiation conditions (Sangtarash et al. 2009; Steel and Keller 2000; Zu et al. 2010). Those previous studies could be in analogy with our results. The results revealed that the carotenoid content decreased with increased UV radiation, perhaps because of the pathway that increased carotenoid reduction caused, UV may have already been saturated (Zu et al. 2010). It has also been

found in the results of this investigation that UV radiation treatments decreased or increase the proline content in leaves of *Spathiphyllum cannifolium* plantlets. This may be attributed to inhibition or enhancing of protein synthesis (Shahnaz 1998). The results hold true with finding Xue-RZ et al. (2017). They found that UV radiation significantly increased the level of proline in leaves of *Prunella vulgaris*. Also, plants respond to oxidative damage by activating their metabolism of antioxidant-like superoxide (SOD) and peroxidase (POD) which protect nucleic acid, lipids, and proteins (Zu et al. 2010).

The primer SCoT1 generated six monomorphic fragments and three polymorphic fragments. All treatments have fragment with molecular size 1340 bp, except treatment 4. Thus, it could be considered as a negative marker for treatment 4, while the fragments with molecular sizes 745 and 620 bp were absent in treatment 1 only. So, they considered as a negative marker for treatment 1.

One polymorphic fragment with molecular size 1230 bp was generated by primer SCoT2 which

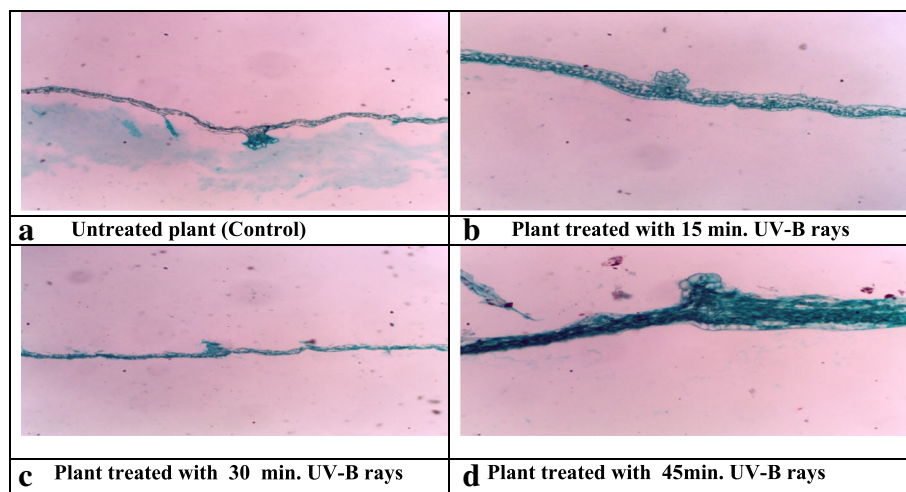


Fig. 3 Shows leaf anatomy of micropropagated *Spathiphyllum cannifolium* leaves under UV rays and time exposure where **a** control, **b** 15 min, **c** 30 min, and **d** 45 min. Light microphotograph showing transverse sections through the blade of the third in vitro micro propagated leaf developed on the main stem of *Spathiphyllum cannifolium* plantlets the sample was taken after the subculture stage (x10) (bar, 0.05 mm)

Table 3 Effect of UV-B radiation exposure time on chemical constituents of *Spathyphyllum cannifolium* plant

Treatments	Characters				
	Chlorophyll a (mg/100 g.F.W)	Chlorophyll b (mg/100 g.W)	Carotenoids (mg/100 g.F.W)	Carbohydrate (mg)	Proline %
0 min	0.2239	0.1126	0.3460	0.0854	93.20
15 min	0.1983	0.0974	0.3322	0.1628	75.90
30 min	0.2676	0.1304	0.4374	0.2463	66.49
45 min	0.1233	0.0813	0.1852	0.2250	98.61
L.S.D. at 0.05	0.0014	0.0008	0.0064	0.0065	0.047

appeared with treatments 2 and 3. Likewise, the primer SCoT 3 exhibited one polymorphic fragment with molecular size 720 bp which has been identified with all treatments except treatment 4; thus, it is considered as a negative marker for treatment 4.

Primer SCoT 6 scored three monomorphic bands with molecular sizes 570, 400, and 290 bp. At the same time, primer SCoT 8 exhibited one monomorphic fragment with molecular size 400 bp. The fragments with molecular sizes 500 bp and 280 bp appeared with treatment 4, and so these were considered as positive markers for treatment 4. A negative marker has been detected in treatment 3, by the fragment with molecular size 470 bp which appeared in all treatments except in treatment 3.

One positive marker with molecular size 1060 bp was identified only with treatment 4 by primer SCoT 10. On the contrary, the fragment with molecular size 860 bp was absent only with treatment 4, so it was considered as a negative marker for treatment 4, while the fragment with molecular size 970 bp appeared with treatments 2 and 3. Four monomorphic fragments with molecular sizes 600, 425, 375, and 215 bp were recognized.

The highest polymorphism (80%) was recorded by primer SCoT 12. The fragment with molecular size 835 bp was monomorphic, while the fragment with molecular size 420 bp was scored in treatments 1 and 2. On the other hand, the fragments with molecular sizes 540 bp and 500 bp were showed in all treatments except in treatments 4 and 3,

Table 4 Effect of UV-B radiation exposure time on leave anatomical structure of *Spathyphyllum cannifolium* plant

Treatment	Characters				
	Thickness of midvine	Thickness of lamina	Length of vascular bundle	Wide of vascular bundle	Cuticle thickness
0 min	31.14	8.22	9.72	9.40	3.10
15 min	39.18	21.84	14.28	10.93	3.91
30 min	27.00	7.65	4.21	4.46	1.56
45 min	72.14	23.62	19.40	16.75	8.76

respectively; these were considered as a negative marker for treatments 4 and 3, respectively. On the contrary, the fragment with molecular size 480 bp was exhibited only in treatment 3, so it was considered as a positive marker for treatment 3.

In this respect, since SCoT marker was recently innovated by Collard and Mackill (2009), till now, up to our knowledge, there is few published paper reporting the use of SCoT marker to assess the genetic changes that happened after plant irradiation. However, many studies employed SCoT as a novel molecular marker technique to evaluate and study the genetic relationships between closely related genotypes. In this manner, Xiong et al. (2011) used SCoT polymorphism technique to study genetic diversity and relatedness among 20 accessions of four major botanical varieties of peanut. A total of 157 fragments were generated by 18 primers. On the other hand, Luo et al. (2010) used two molecular marker systems (SCoT and ISSR) in order to identify and detect the genetic relatedness between 23 mango germplasms. Using 18 selected SCoT primers, 158 bands were generated, of which 104 (65.82%) were polymorphic. Mahjbi et al. (2015) used 12 SCoT primers for their

Table 5 Primer name, sequence, total number of bands, monomorphic bands, polymorphic bands, unique bands and percentage of polymorphism as revealed by SCoT markers among the three UV treatments and the control to *spathyphyllum* plants

Primer	Sequences 5'→3'	TB	MB	PB	UB	P%
SCoT 1	ACG ACA TGG CGA CCA CGC	9	6	3	3	33.33%
SCoT2	ACC ATG GCT ACC ACC GGC	6	5	1	0	16.66%
SCoT 3	ACG ACA TGG CGA CCC ACA	5	4	1	1	20%
SCoT 6	CAA TGG CTA CCA CTA CAG	3	3	0	0	0%
SCoT 8	ACA ATG GCT ACC ACT GAG	4	1	3	3	75%
SCoT 10	ACA ATG CTA CCA CCA AGC	7	4	3	2	42.85%
SCoT 12	CAA CAA TGG CTA CCA CCG	5	1	4	3	80%
Total		39	24	15	12	38.46%

TB Total bands, MB Monomorphic bands, PB Polymorphic bands, UB Unique bands, and P% Percentage of polymorphism

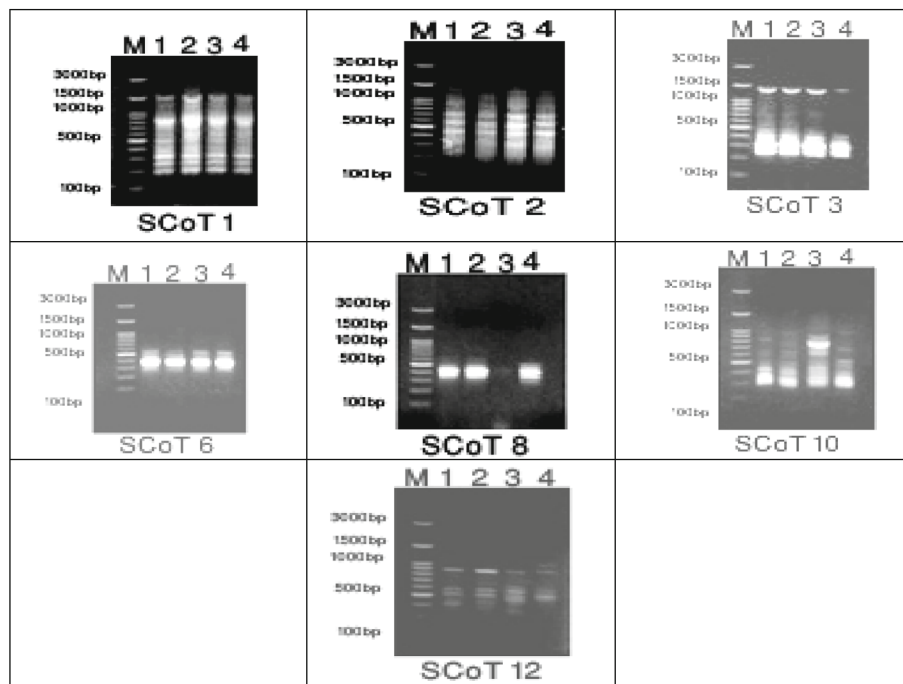


Fig. 4 The separation pattern of the SCoT products using seven primers. M: 100 bp ladder DNA marker. (1) control, (2) 15 min, (3) 30 min, and (4) 45 min. Exposure to UV, respectively

ability to reveal polymorphism of the targeted codon of initiation at the interspecies level in *Citrus* genus. Miró et al. (2017) described 12 SCoT primers with 14 cultivar Hungarian and international grape varieties and found one primer producing 17 polymorphic bands after data normalization, which was sufficient to separate the varieties. In potato, Gorji et al. (2011) employed 12 SCoT primers with 15 combinations generated 144 fragments. In both rice and Chinese grape varieties, 36 primers were tested (Collard and Mackill 2009; Guo et al. 2012), while in ramie 20 primers were evaluated (Satya et al. 2015). On the other hand, evaluated genetic variations on *Spathiphyllum* cultivars based on RAPD markers, and 14 random primers were selected in PCR analysis. A total of 243 RAPD markers were amplified among these cultivars. Khalifa (2016) used 6 RAPD primers to detect polymorphisms between the control and treated with different doses of gamma rays at *Spathiphyllum wallisii* Regel. and *Philodendron scandens* C. Koch and H. Sello plants.

Conclusion

The results are nearly in an ascending order with increasing of UV-B radiation exposure time. Increasing exposure time to 45 min increased significantly the values

of vegetative growth, anatomical parameters, and proline percentage in most cases compared with control and other UV-B radiation treatments. In the contrary, that in the 30 min recorded decreased in previous mentioned treatments. SCoT technique reveals a higher level of polymorphism which confirms the relevance and suggests the effectiveness of the SCoT markers for assessing genetic diversity, characterization, and identification of *Spathiphyllum canifolium* plant.

Abbreviations

BA: 6-Benzylaminopurine; bp: Base Pair; MB: Monomorphic bands; NAA: Naphthalene acetic acid; PB: Polymorphic bands; P%: Percentage of polymorphism; POD: Peroxidase; SCoT: Start codon targeted; SOD: Superoxide; TB: Total bands; UB: Unique bands; UV: Ultra violet rays

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Authors' contributions

The authors have participated and work on completing this manuscript and approved the final manuscript.

Ethics approval and consent to participate

The manuscript does not contain studies involving human participants, human or animal data, and animal or human tissue.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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