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Genetic Variation of Three BBTV-Infected Banana Cultivars Based on SCoT DNA Marker Technique

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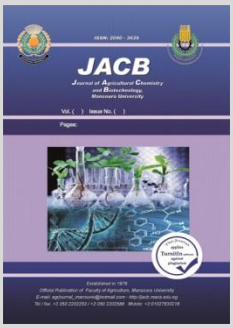

ABSTRACT

Several banana cultivars are grown in all types of farming systems, which Egyptians use in a mature way due to their nutritional value, and banana bunchy top virus (BBTV) contributive pathogen of Banana Bunchy Top Disease (BBTD) remains to be the furthermost devastating pathogen, which is threatening production in major banana growing areas. The current study was planned to determine the genetic variations of some banana cultivars using some molecular tools. Genetic variations of three BBTV-infected banana cultivars (Edward Cavendish, Maghrabi and Williams) collected from Qalyubia Governorate compared to healthy banana ones determined by using two molecular techniques, *i.e.*, start codon targeted (SCoT) and/or SDS-PAGE. SCoT technique was also used to determine DNA fingerprinting of Maghrabi plant cultivar grown under different climate conditions. Results showed that, quantum of genetic variation in genome of similar variety whatsoever healthy or infected with BBTV was highest probable in Maghrabi cultivar (6-9%) followed by Williams cultivar (3-6%) and the lowest was Edward Cavendish cultivar (3-4%). It was proved that identities percentage between samples of two regions of Qalyubia Governorate exceeds 70% and that they both found in same cluster, while identities percentage between samples of both Qena and Aswan increased more than 80%. Results of SDS-PAGE technique showed twelve protein bands (five monomorphic and seven polymorphic bands) and there aren't any protein bands found that might be reflected as a distinctive marker protein. It was also noted that both healthy and BBTV-infected samples from the same region were found together in a separate cluster.

Keywords: BBTV-Infected Banana, genetic variation, DNA and protein fingerprinting, SDS-PAGE, ScoT technique.

INTRODUCTION

The banana plant belongs to the family of Musaceae which is the most significant economic crop of nutritional value in worldwide (Dale 1987). In Egypt banana cultivars (Maghrabi, Indian, Williams, Leady Finger, Basari, and Edward Cavendish etc.) are grown in all types of farming systems, which Egyptians use in a mature way (Allam et al 2000). BBTV remains to be the most devastating pathogen, which is threatening the production in all major banana growing areas of the world (Jones 2000, Sila et al., 2020) as well as in Egypt (Allam et al 2000).

Currently plant genome variation including bananas was successfully studied by several DNA molecular tools, *i.e.*, Amplified Fragment Length Polymorphism (AFLP) technique (Dziechciarkova et al 2004, Rao et al, 2002, Gupta et al 1999) , Random Amplified Polymorphic DNA (RAPD) technique (Dessoky et al 2020, Attia et al 2017, Dessoky et al 2017, Lamare and Rao 2015, Mukunthakumar et al 2013, Gorji et al 2011, Kelly and Miklas 1998), Microsatellite Markers (Creste et al 2004, Creste et al 2003) and Inter Simple Sequence Repeat (ISSR) technique (Dessoky et al 2020, Attia et al 2017, Dessoky et al 2017, Lamare and Rao 2015, Gorji et al 2011, Rahayuniati, 2021) have been used for determination of relationship or genetic diversity between different plant types.

Start codon targeted (SCoT) technique conventional according to Collard and Mackill (2009) depends on short, conserved region in genes of plants surrounding the ATG translation start codon. This technique is used for amplifying multiple regions of the genome which could be used for refined between different plant species (Moawed and Ibrahim 2016, Satya et al 2015, Zhang et al 2015, Shahlaei et al 2014, Amirmoradi et al 2012, Xiong et al 2011). SCoT polymorphism was achieving reputation for its advantage over other dominant DNA marker techniques (Mulpuri et al 2013, Gorji et al 2011). In Egypt, Moawed and Ibrahim (2016) characterized and distinguished a number of 17 taxa of 13 species and five genera belonging to family Zygophyllaceae collected from Saudi Arabia and Egypt by SEM and SCoT technique.

This work aimed to determine the genetic variation of three banana cultivars (Edward Cavendish, Maghrabi and Williams) collected from Qalyubia Governorate and infected with BBTV, compared to healthy using SCoT technique. In addition, the effect of climate conditions on the genome of BBTV-infected Maghrabi banana plants cultivated in Qalyubia, Qena and Aswan was also conducted via SCoT technique and SDS-PAGE.

MATERIALS AND METHODS

It is attraction to declare that this part of the study was carried out at Laboratory of MGGM at the AGERI, ARC, Giza, Egypt during season 2019.

Source of banana samples:

Both of healthy and exhibiting BBTD-like symptoms banana leaf samples were collected from different open field farms at Qalyubia Governorate in 2018. Three

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different cultivars, *i.e.*, Williams Grand Nain, Maghrabi and Edward Cavendish were gotten. On year of 2019, samples of Maghrabi banana cultivar were also collected from Qena and Aswan. All leaves of banana from different cultivars were placed in plastic bags then stored at 20°C until used.

DNA fingerprinting of BBTD-infected banana cultivars using SCoT technique:

To accomplish such an aim, the BBTV-infected banana plants belonging to three cultivars (Williams Grand Nain, Maghrabi and Edward Cavendish) were determined compared to the healthy ones of the same cultivars. Extraction of DNA was done according to the modified CTAB (hexadecyl trimethyl ammonium bromide) method reported by Porebski et al (1997).

Estimation for the DNA concentration

The quantity of DNA extracts prepared from BBTV-infected banana plants was estimated by using the NanoDrop protocol.

SCoT-PCR

A number of five SCoT primers called SCoT-02 (5'ACC ATG GCT ACC ACC GG C3'), SCoT-03 (5'AC GAC ATG GCG ACC CAC A3'), SCoT-20 (5' CA ACA ATG GCT ACC ACG C3'), SCoT-22 (5'CC ATG GCT ACC ACC GCA C3') and SCoT-23 (5'C ATG GCT ACC ACC GGC CC3') were used for studying the genetic differences of three infected BBTV banana plant cultivars likened to the healthy one. The amplification reaction was conducted according to the protocol of Ibrahim et al (2016) among a volume of 25 µL having (2 µL $MgCl_2$ (25 mM), 0.5 µL dNTPs (10 mM), 5 µL 5X-PCR buffer, 2.5 µL SCoT primer (10 pmol), 3 µL DNA template (50 ng), and 0.2 µL *Taq* DNA Polymerase (5 U/µL)). To complete the volume up to µL sterile $d.H_2O$ was used. PCR amplification was accomplished in a Perkin-Elmer/GeneAmp®PCR System 9700 (PE Applied Biosystems) programmed to do 35 cycles after an initial denaturation cycle at 94ºC for five min. Each cycle consisted of a denaturation for 1 minute at 94ºC, annealing for 1 minute at 50ºC, also elongation for 1.5 minute at 72ºC. The primer extension fragment was extended at 72ºC for 7 min. in the last cycle.

Detection of the PCR products

The amplified PCR products (DNA polymorphisms) were resoluted using electrophoresis in a 1.5% agarose gel stained with (0.5 µg/mL) ethidium bromide in 1X TBE buffer at 95 volts for 1.5 hr. PCR products were envisioned and photographed using UV light and Gel Documentation System (BIO-RAD 2000).

Fingerprinting of BBTD-infected banana cultivars collected from different regions in Egypt:

Using SCoT technique

SCoT PCR amplification

A number of eight SCoT primers (SCoT-1:5'ACGACATGGCGACCACGC3'; SCoT-2:5'ACCATGGCTACCACCGGC3'; SCoT-3:5'ACGACATGGCGACCCACA3'; SCoT-4:5'ACCATGGCTACCACCGCA3'; SCoT-7:5'ACAATGGCTACCACTGAC3'; SCoT-9:5'ACAATGGCTACCACTGCC3'; SCoT-11:5'ACAATGGCTACCACTACC3'and SCoT-12:5'CAACAATGGCTACCACCG3') was applied for determination of genetic variation of BBTD-infected Maghrabi banana cultivar collected from Qena, Aswan and Qalyubia Governorates in Egypt. The amplification reaction was done in a volume of 25 µL according to Ibrahim et al (2019) as mentioned above.

Using SDS-PAGE

Protein fingerprinting of BBTV-infected banana plants compared to healthy ones was conducted *via* 12% SDS-PAGE as described by Laemmli (1970). A weight of 0.5 g of frozen banana tissues was minced in 1.0 mL 2X treatment buffer (4% SDS, 0.25 M Tris-HCl pH 6.8, 10% β-mercaptoethanol and 20% glycerol) then boiled for 90 seconds in a water bath and after that rapidly transferred into ice and reserved until loading into the gel. Discontinuous polyacrylamide gels consisted of a stacking gel (upper) and resolving or separating gel (lower) was carried out based on the protocol **of** Laemmli (1970).

Analyses of SCoT and SDS-PAGE markers

The fragments of DNA produced by SCoT markers were matched to evaluate the genetic relationships between the banana cultivars. For all samples, clear and dissimilar amplification products were keep score as (0) and (1) for absent and present bands, respectively. The binary statistic matrix was formerly created. Then calculation of Dice's similarity matrix coefficients was done among genotypes using the technique of Unweighted Pair Group with Mrithmetic Averages (UPGMA). This matrix was used to concept a phylogenetic tree (Dendrogram). Principal coordinate analysis (PCA) was done according to method of Euclidean similarity index using means of the PAST software Version 1.91 (Hammer et al 2001). Calculation of The DNA polymorphisms information content was done using means of the Power Marker software (Liu and Muse 2005).

RESULTS AND DISCUSSION

Banana (*Musa* spp.) is one of the supreme economic significant fruit plants in Egypt (Allam et al 2000). BBTV the contributing agent of Banana BBTD (Dale 1987), and there is no doubt that this harmful effect of BBTV infection could be resulted from the effect of the BBTV on the genome of the banana plant which carries all the codes for all the genes of the banana plant.

During the past two decades, global production of banana crops has grown to a compound annual rate of about 3.2%, producing 114 million tons of bananas at the end of the second decade of the twenty-first century, compared to a productivity of 67 million tons (Dale 1987).

It is well known that the viral infection of plants often results in a decrease in the productivity of the crop or gave useless fruits and a great economic loss at the governmental and private levels, whether for companies or individuals will occur. There is no doubt that this harmful effect of BBTV infection will result from the effect of the BBTV on the genome of the banana plant which carries all the codes for all the genes of the banana plant as the main host for BBTV. Thus, there are undoubtedly some changes in the banana plant genome because of infection with the BBTV, which can be estimated by some modern molecular biological tools, including estimating the genetic DNA-fingerprint of healthy and BBTV-infected plants to reach the effect of viral infection on the banana plant genome, which is reflected in different external symptoms.

In banana, genetic diversity amongst 25 genotypes of wild *M. acuminata* from Meghalaya region of northeast

India were recorded by Lamare and Rao (2015) by three single primer-based techniques of DNA marker (RAPD, ISSR and directed amplification of minisatellites DNA). Genetic analysis was also done in some Brazilian banana cultivars by microsatellite markers (Creste et al. 2003, Creste et al. 2004), and amid wild backgrounds of banana (*Musa acuminata* Colla) by RAPD markers (Mukunthakumar et al. 2013).

In this study, genetic differences in BBTV genome of the infected banana plants likened to the healthy plants were determined using SCoT technique to estimate the DNA fingerprinting of three cultivars of banana (Williams, Edward Cavedish and Maghrabi) full-grown in the similar governorate (Qalyubia) as well as one cultivar (Maghrabi) full-grown in dissimilar regions (Qena, Aswan and Qalyubia) infected with BBTV compared to healthy ones. Results in Fig (1) showed that total DNAs were successfully extracted from Maghrabi, Williams and Edward Cavendish banana cultivars whatever healthy or BBTV-infected. Data also showed that the concentration of DNA extract and its purity were more suitable for using as templates for PCR to determine the DNA fingerprinting of banana cultivars.

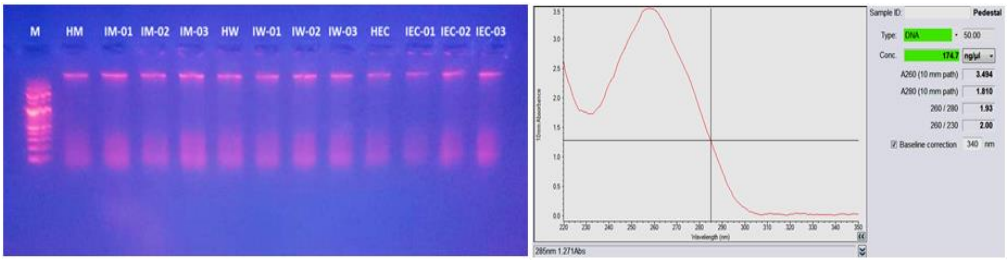

Fig. 1. 1.5% of Agarose gel stained by ethidium bromide illustrations DNA extracts prepared from three cultivars of banana (M: Maghrabi, W: Williams and EC: Edward Cavendish). I: BBTV-infected. H: Healthy. M: 1 kb DNA Ladder. Note: DNA concentration (174 ng/µL) of one DNA extract (HM) as example

Results of genetic variation of healthy and BBTV-infected banana plants obtained of three banana cultivars grown in Qalyubia Governorate were estimated by five primers of SCoT (SCoT-2, SCoT-3, SCoT-20, SCoT-22 and SCoT-23) recorded in Tables (1-3) and illustrated by Figs (2-3). Results presented that 70 of DNA fragments achieved and dispersed as follows: 13, 15, 18, 15 and 14 using the five SCoT primers, respectively. Results revealed that when the extracted DNA from three banana cultivars were used as DNA templates, numbers of 52,51 and 64 of DNA fragments were amplified from healthy banana plants, respectively. Whereas the average number of amplified DNA fragments for the same viral infected cultivars was 52,50 and 58 respectively.

Table 1. Analysis of DNA polymorphisms of healthy (H) and BBTV-infected (I) banana cultivars (Maghrabi (M), Williams (W) and Edward Cavendish (EC)) amplified by five SCoT primers.

Primers used	TAF	Maghrabi banana samples collected from different Governorates											
		HM	IM-01	IM-02	IM-03	HW	IW-01	IW-02	IW-03	HEC	IEC-01	IEC-02	IEC-03
SCoT-02	13	13	13	13	13	12	12	12	12	12	12	12	12
SCoT-03	10	10	9	9	9	7	7	7	7	7	7	7	7
SCoT-20	18	15	11	12	11	15	13	15	12	16	15	15	16
SCoT-22	15	13	12	12	14	5	6	6	6	6	6	6	6
SCoT-23	14	13	12	13	13	12	12	13	10	11	11	12	12
Total	70	64	57	59	60	51	50	53	47	52	51	52	53

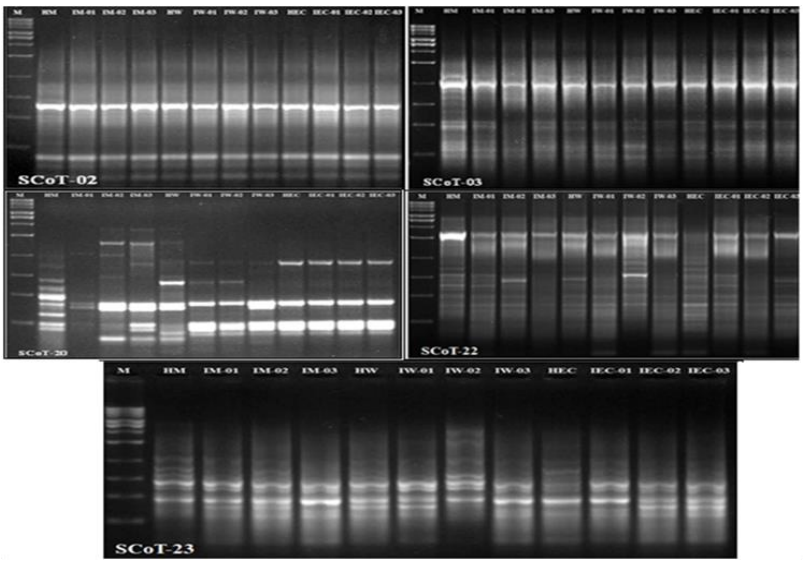

Fig. 2. 1.5% of Agarose gels stained by ethidium bromide indications that DNA polymorphisms amplified using SCoT-2, SCoT-3, SCoT-20, SCoT-22 and SCoT-23 primers and DNA templates prepared from three banana cultivars (M: Maghrabi, W: Williams and EC: Edward Cavendish) infected with BBTV (I). M: 1 kb DNA Ladder.

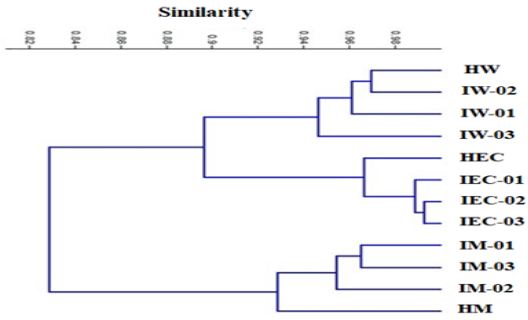

Fig. 3. Genetic dendrogram of DNA polymorphisms of healthy (H) and BBTV-infected (I) banana cultivars (Maghrabi (M), Williams (W) and Edward Cavendish (EC)) amplified by five SCoT primers.

Concerning the type of the seventy amplified DNA fragments, data revealed that amplified DNA fragments were dispersed as follows: 37 monomorphic, 33 polymorphic. Two out of the 33 polymorphic fragments were considered as unique DNA markers. These only two were amplified by SCoT-3 and SCoT-22 primers as shown in Table (2). The other three primers didn't form any unique DNA markers. It was also distinguished that 12 out of the 13 DNA fragments amplified by SCoT-2 primer were monomorphic. On the other hand, the number of polymorphic fragments was 13 compared to two monomorphic fragments for the similar primer, when the SCoT-22 primer was used. It was moreover noted that the number of monomorphic fragments was greater than the number of polymorphic fragments, when SCoT-3 and SCoT-23 primers were used (Table 2).

Table 2. Types of DNA polymorphisms of healthy and BBTV-infected banana cultivars amplified by five SCoT primers.

Primers used		Types of DNA fragments		
Names	TAF	Polymorphic	Monomorphic	Unique marker
SCoT-02	13	1	12	0
SCoT-03	10	4	6	1
SCoT-20	18	10	8	0
SCoT-22	15	13	2	1
SCoT-23	14	5	9	0
Total	70	33	37	2

Data in Table (3) showed that the percentage identities of DNA polymorphisms of healthy and BBTV-infected banana cultivars (Maghrabi, Williams and Edward Cavendish) amplified by the five SCoT primers. Results in Table (3) revealed that the identities percentage between healthy and viral infected plants of the equal variety ranged among, 94-97 and 96-97, 94-97 and 91-94 % Edward Cavendish, Williams and Maghrabi cultivars, respectively. The quantities of genetic difference in the genome of the same variety, whatsoever was healthy or infected were the highest possible in the Maghrabi cultivar (6-9%), followed by Williams cultivar (3-6%) and the lowest possible was in Edward Cavendish cultivar (3-4%).

Table 3. Percentage (%) identities of DNA polymorphisms of healthy (H) and BBTV-infected (I) banana cultivars (Maghrabi (M), Williams (W) and Edward Cavendish (EC)) amplified by five SCoT primers.

Banana samples	Banana samples											
	HM	IM-01	IM-02	IM-03	HW	IW-01	IW-02	IW-03	HEC	IEC-01	IEC-02	IEC-03
HM	100											
IM-01	91	100										
IM-02	94	96	100									
IM-03	93	96	94	100								
HW	83	81	84	82	100							
IW-01	83	82	84	83	97	100						
IW-02	83	79	83	80	97	95	100					
IW-03	81	78	79	79	94	95	95	100				
HEC	83	80	83	81	91	89	91	87	100			
IEC-01	85	83	84	84	91	90	89	85	97	100		
IEC-02	86	84	85	85	91	91	90	86	96	99	100	
IEC-03	86	84	86	85	92	92	91	87	97	98	99	100

After comparing the percentage of genetic variation of the three healthy banana cultivars, it was verified that they are certainly different cultivars, and this could be seen from the fact that the identities percentages among them was 83% between Maghrabi cultivar and both of Williams and Cavendish cultivars, whereas it was 91% between the cultivars of Williams and Edward Cavendish. In the case of BBTV-infected plants, the percentage of identity between cultivars of Williams and Maghrabi was 83%. But it was 82% between Edward Cavendish and Maghrabi cultivars. Although the percentage of identity between cultivars of Edward Cavendish and Williams was 90%. Hence, one can accomplish that the Edward Cavendish cultivar is closer in its genetic origin to the Williams cultivar compared to the Maghrabi cultivar. Genetic dendrogram of DNA polymorphisms presented in Fig(3) exposed that the BBTV-infected and healthy banana samples fell together in the same cluster.

Kakati and Nath (2018) collected a number of 12 banana samples belonging to different cultivars infected with BBTV from India and determined the genetic variation in between based on bioinformatics analysis. Banana samples of Assama revealed percentage of similarity ranged from 86 to 99% matched with the Pacific Indian Ocean BBTV group. SCoT technique was established as reported by Collard and Mackill (2009) and used for amplifying multiple regions of the genome which could be used for discriminating between different plant species (Zhang et al 2015, Satya et al., 2015, Shahlaei et al., 2014, Amirmoradi et al., 2012, Xiong et al 2011). SCoT polymorphism was achievement reputation for its advantage over other dominant DNA marker systems (Mulpuri *et al* 2013, Gorji *et al* 2011).

The impact of climate on Maghrabi banana cultivar cultivated in three different Governorates (Qalyubia, Qena and Aswan), representing three different environmental conditions was determined using SCoT technique. The effect of environmental conditions on the genome of Maghrabi banana plant cultivar grown in three different Governorates, *i.e.*, Qalyubia, Qena and Aswan, was studied by using eight SCoT primers. Results in Table (4) revealed that 72 amplified DNA fragments were recorded as follows:

11, 8, 5, 8, 14, 9, 10 and 7 belonging to 8 SCoT primers; 1, 2, 3, 4, 7 , 9 and 12 respectively as shown in Fig 4. Likewise, the number of 29 and 34 fragments) was in BBTV-infected plants but it recorded 30 and 44 fragments in the healthy plants (Qalyubia Governorate samples). On the other hand, the number of both viral infected samples compared to healthy plants (Aswan and Qena Governorates) was recorded as follows: 38 and 45 in Aswan samples but recorded 48 and 50 in Qena samples. On conclusion, at all Governorates, the number of DNA fragments from viral infected plants was less than in healthy plants.

Table 4. Analysis of DNA polymorphisms of healthy (H) and BBTV-infected (I) Maghrabi banana cultivar collected from three different locations amplified by eight SCoT primers.

Primers used						Maghrabi banana samples collected from three different Governorates							
						Qalyubia R01		Qalyubia R02		Qena		Aswan	
Names					TAF	I	H	I	H	I	H	I	H
SCoT-01					11	7	7	7	7	5	5	10	10
SCoT-02					8	6	6	4	6	2	5	4	4
SCoT-03					5	1	1	1	4	4	4	2	3
SCoT-04					8	5	5	5	6	8	4	4	5
SCoT-07					14	3	3	4	5	13	12	5	7
SCoT-09					9	1	1	6	6	6	6	7	8
SCoT-11	10	2	2	4	5	6		7		3		4	
SCoT-12	7	4	5	3	5	4		7		3		4	
Total	72	29	30	34	44	48		50		38		45	

TAF: Total amplified fragments. H: Healthy. I: BBTV-Infected.

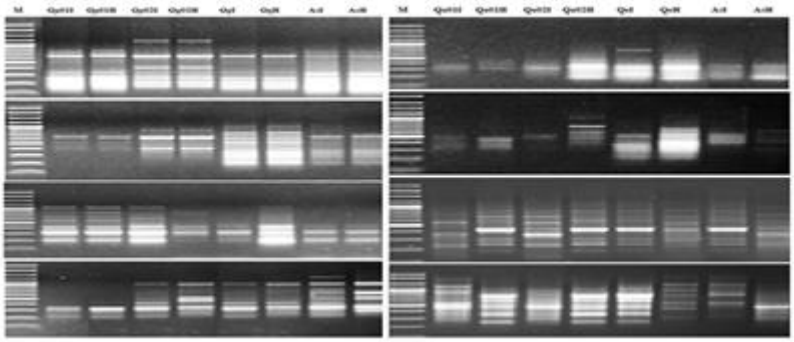

Fig. 4. 1.5% of Agarose gels stained by ethidium bromide shows DNA polymorphisms amplified using eight SCoT primers and DNA templates prepared from Maghrabi banana cultivar collected from different Governorates Qa-1 I, Qa-1 H, Qa-2 I, Qa-2 H, QeI, QeH, AsI and AsH). M: 1 kb DNA Ladder.

Regarding the type of DNA fragments in the obtained polymorphisms, it was revealed that from 72 fragments, a number of 63 pieces were polymorphic fragments. Whereas 9 fragments belonged to the monomorphic fragments. Surrounded by the polymorphic fragments, it was concluded that there were five unique DNA markers were amplified by SCoT-3 primer (one fragment), SCoT-7 primer (one fragment) and SCoT-11 primer (three fragments) as presented in Table (5). Results in Fig (5) show the percentage of identities between the DNA polymorphisms obtained from samples of healthy and BBTV-infected plants and the degree of genetic affinity between them. It was proved that the percentage of identities between the samples of the two regions of Qalyubia Governorate exceeds 70% and that they both found in the same cluster, while the percentage of identities between the samples of both Qena and Aswan increased to more than 80%.

Data paid attention to that the infected plants lengthwise with the healthy plants for each of the Governorate were present in a separate cluster together. Also the plants were genetically closer to each other in samples from Aswan and Qena. Based on the achieved data, it was initiated that the Banana Bunchy Top Virus has affected on the DNA of the Maghrabi cultivar in all areas, and this is apparent from the low identities percentage between infected and healthy plant samples.

Currently plant genome variation was successfully studied by several DNA molecular tools (Collard and Mackill 2008, Gostimsky *et al.* 2005, Botha and Venter 2000, Mueller and Wolfenbarger 1999).

Table 5. Types of DNA polymorphisms of healthy and BBTV-infected Maghrabi banana cultivar collected from three different locations amplified by eight SCoT primers.

Primers used		Types of DNA fragments		
Names	TAF	Polymorphic	Monomorphic	Unique marker
SCoT-01	11	7	4	0
SCoT-02	8	7	1	0
SCoT-03	5	5	0	1
SCoT-04	8	7	1	0
SCoT-07	14	12	2	1
SCoT-09	9	8	1	0
SCoT-11	10	10	0	3
SCoT-12	7	7	0	0
Total	72	63	9	5

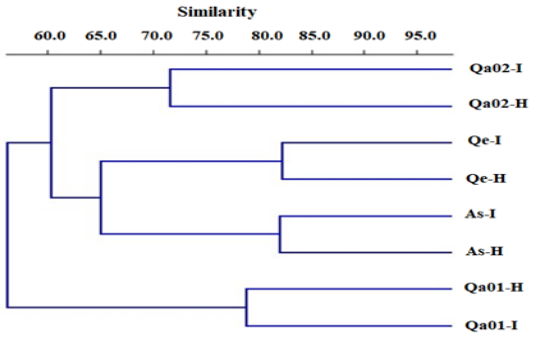

Fig. 5. Genetic dendrogram of DNA polymorphisms of healthy (H) and BBTV-infected (I) Maghrabi banana cultivar collected from three different locations amplified by eight SCoT primers.

One of these methods are RAPD, ISSR and AFLP markers have been used for determination of genetic diversity or relationship between different plant types, such as wheat (Gupta et al 1999), Lactuca (Dziechciarkova et al 2004), common bean (Kelly and Miklas 1998), rice (Rao et al 2002), Acacia (Attia et al 2017), and pomegranate (Dessoky et al. 2020, Dessoky et al. 2017).

Results in Table (6) and illustrated in Fig (6 & 7) showed that SDS-PAGE (12%) analysis of the Maghrabi banana healthy and infected plants grown-up in three dissimilar areas (Aswan, Qena and Qalyubia) diverse in their climatic environments. Data were presented that, a number of twelve protein bands were achieved and dispersed from all samples(Qa-1- I, Qa-1- H, Qa-2- I, Qa-2- H and Qe- I and Qe- H, As- I, and As- H)as follows: 10, 11, 10, 10, 11, 12, 7 and 8,respectively. A similarity was perceived in the number of protein bands achieved in healthy and BBTV-infected plants. In most tested samples, it was found that BBTV-infected samples were lower than healthy samples in the number of protein bands.

Data also showed that only five from twelve fragments were belonged to the monomorphic type. Whereas seven bands were polymorphic fragments. No protein bands were considered as a unique marker protein. Finally, the bands were equal in number, even if they varied in their molecular weight in all Qalyubia Governorate samples (Qa-2). Results in Table (7) showed that the percentage of similarity between the protein patterns obtained from Maghrabi cultivar samples, whether were viral infected or healthy and grown in 3 dissimilar climatic areas belonging to Qalyubia Governorate (Delta area) and 2 areas belonging to Qena and Aswan Governorates (Upper Egypt area).

Table 6. Percentage identities of protein patterns of healthy and BBTV-infected Maghrabi banana cultivar collected from three different locations based on SDS-PAGE.

Maghrabi banana samples	Maghrabi banana samples							
	Qa-1-I	Qa-1-H	Qa-2-I	Qa-2-H	Qe-I	Qe-H	As-I	As-H
Qa-1-I	100							
Qa-1-H	54.55	100						
Qa-2-I	72.73	60.00	100					
Qa-2-H	76.92	66.67	66.67	100				
Qe-I	85.71	76.93	76.93	93.33	100			
Qe-H	92.31	66.67	83.33	85.71	93.33	100		
As-I	85.71	76.92	76.92	93.33	100.0	93.33	100	
As-H	90.91	60.00	80.00	66.67	76.93	83.33	76.92	100

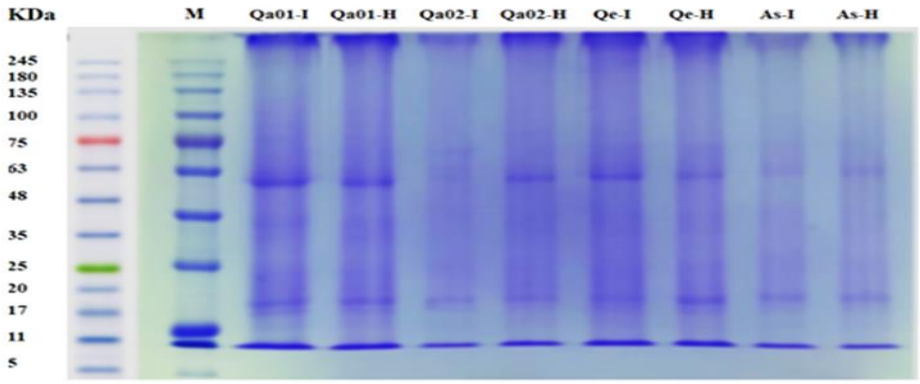

Fig. 6. SDSPAGE (12%) stained with Coomassie brilliant blue shows protein patterns of Maghrabi banana cultivar collected from different Governorates (Qa-1-I, Qa-1-H, Qa-2-I, Qa-2-H, Qe-I, Qe-H, As-I and As-H).

Data also showed that the highest percentage (100%) was between infected samples from Qena (Qe- I) and Aswan (As- I) Governorates. But the lowest similarity percentage (54.55%) was between infected samples (Qa-1- I) and the healthy plants of Qalyubia Governorate region-1 (Qa-1- H). Results in Fig (6) exposed that the genetic relationship based on similarity percentage data of protein patterns were slightly dissimilar from those that were recorded among the same samples.

Qena (Qe- H) and Aswan (As- H) samples showed that the healthy samples were fell in the same cluster encloses viral infected sample of Qalyubia Governorate (Qa-1- I). Whereas the Qa-2- H sample was fell with the infected samples of Qena and Aswan Governorates (Qe- I & As- I). Though the BBTV-infected Qalyubia Governorate area (Qa-1- I) sample transpired in a separate cluster, and it was genetically related at the protein level with the BBTV-sample (Qa-2- I) from Qalyubia Governorate area . Nady et al 2023 revealed that the diagnostic host of BBTV was restricted and the alignment exhibited that EGY-Behira had an extreme similarity of 99.7% with India BBTV isolate.

Finally, it can concluded that the different climatic environments predisposed the plant genome of the matching variety, whether it was viral infected or healthy, and SCoT procedure was efficaciously used to determine the genetic variation of the tested samples.

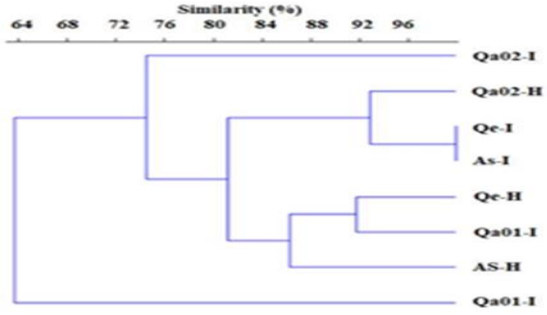

Fig. 7. Genetic dendrogram of protein patterns of healthy and BBTV-infected Maghrabi banana cultivar collected from three different locations based on SDS-PAGE.

CONCLUSION

BBTV has precious the genome of the Maghrabi banana plant, and this is unmistakable from the identities

among BBTV-infected plants after compared to healthy plants grouped from dissimilar areas. Edward Cavendish cultivar is nearer in its genetic origin to the Williams cultivar compared to the Maghrabi cultivar. Genetic dendrogram of DNA polymorphisms exposed that the healthy and BBTV-infected banana plant samples fell together in the same cluster. In conclusion, the different climatic conditions inclined the plant genome of the same variety, whether it was virus-infected or healthy, and SCoT method was efficaciously used to determine the genetic variation of tested samples. It was also distinguished that BBTV-infected samples as well as the healthy ones from the same region were present together in a separate cluster.

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التباين الجيني لثلاثة أصناف موز مصابة بفيروس تورد القمة في الموز بناء على تقنية علامة الحمض النووي الـ SC0T المميز

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الملخص

تُزرع العديد من أصناف الموز في جميع أنواع النظم الزراعية، والتي يستخدمها المصريون بطريقة ناضجة نظرًا لقيمتها الغذائية، ولا يزال فيروس تورد القمة في الموز (BBTV) العامل المسبب لمرض تورد القمة في الموز (BBTD) الأكثر تدميرا حيث يهدد إنتاجية محصول الموز في جميع مناطق زراعته الرئيسية. تم تصميم هذا العمل لتحديد الاختلافات الجينية لبعض أصناف الموز (إدوارد كافنديش، مغربي، ويليامز) والتي تم جمعها من محافظة القليوبية والمصابة بهذا الفيروس مقارنة بالسليمة من نفس الأصناف. وقد تم تقديرها باستخدام أدوات جزيئية مختلفة معتمدة على حمض الدنا مثل تقنية الـ SCoT وأو والبروتين مثل تكنيك الـ SDS-PAGE. وبالنسبة لصنف المغربي المنزرع تحت ظروف مناخية تمتقدير البصمة الوراثية له باستخدام تقنية الـ SCoT . أظهرت النتائج أن مقدار التباين الوراثي في جينوم الصنف نفسه سواء كان مصابًا بالـ BBTV أو سليما كان أقل ما يمكن في صنف إدوارد كافنديش بنسبة (3-4٪)، يليه الصنف ويليامز بنسبة (3-6٪) والصنف المغربي بنسبة (6-9٪). كما ثبت أن نسبة التشابه بين عينات منطقتي محافظة القليوبية تتجاوز 70٪ وأن كلاهما وجد في نفس العنقود بينما ارتفعت نسبة التشابه بين عينات كل من قنا وأسوان إلى أكثر من 80٪. وأظهرت نتائج SDS-PAGE أنه تم الحصول على عدد من اثني عشر حزمة بروتينة (خمسة منها صنفت كمونومورفيك وسبعة حزم صنفت كبولى مورفيك)، ولم يتم الحصول على أي حزم بروتينية يمكن اعتبارها بروتينًا مميزًا فريدًا كماركر. ولوحظ أيضًا أن العينات المصابة بالـ BBTV وكذلك العينات السليمة من نفس المنطقة كانت موجودة معًا في مجموعة منفصلة.