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RESEARCH ARTICLE

Genetic diversity of *Sporisorium scitamineum* associated with Sugarcane smut disease in Luxor Governorate, Egypt using SCoT marker technique

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Abstract

Sugarcane smut disease caused by fungus *Sporisorium scitamineum* is a major disease and can cause significant yield losses worldwide. In Egypt, this disease is widespread in the commercial sugarcane variety G.T.54-9. To study the molecular differentiation of *S. scitamineum*, Five *S. scitamineum* isolates were collected from five sugarcane production areas in Luxor Governorate (Luxor Grb, EL-Hbeal, EL-Karaia, EL-Mres and Ahaly EL-Mres) in season 2022 and were assessed based on the start codon-targeted (SCoT) marker technique. Four selected SCoT primers amplified 56 DNA fragments, of which 48 (85.71 %) were polymorphic. Polymorphic data ranged from 0.240 to 0.393, Which show that there is a degree of genetic diversity among these Egyptian fungus *S. scitamineum* isolates. Cluster analysis using UPGMA method was divided the *S. scitamineum* isolates into two main groups, I and II. Group II included only one isolate (isolate 2). Group I was divided into two sub-groups included Sub-G1 (isolates 1 & 3) and Sub-G2 (isolates 4 & 5). The results indicated that diversity of S. scitamineum was partly associated to its geographical origin. These results are useful in breeding programs to obtain smut-resistant sugarcane genotypes. Our results demonstrated that SCoT markers can be used to evaluate the genetic diversity of *S. scitamineum*.

Keywords: Sugarcane smut disease; *S. scitamineum*; Start codon targeted (SCoT) polymorphism; Genetic diversity.

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Introduction

Sugarcane is high economic importance crop and the main ingredient in the production of sugar (Prabowo et al. 2014). and has been planted widely in tropical and subtropical regions of the world. It is one of the main cash and industrial crops in Egypt and source of raw material to sugar industry as well as sugarcane is source of white crystal sugar and other of sweeteners, molasses, bagasse, ethanol and bio compost preparation. Dried leaves and crop residues are directly used for mulching and compost preparation. The cultivated area of sugarcane production in season 2020 was 325,052/feddan yielded about 865,494 tons sugar represents 37.9% of the sugar productivity (Annual Report for Sugar Crops in Egypt, December 2020).

Sugarcane infected by various kinds of pathogens viz., fungi, bacteria, viruses and phytoplasma. Among them, smut, wilt, seedling rot, sett rot and mosaic are the major diseases that are affecting sugarcane production in Egypt. When sugarcane plants infect with diseases or pest's production of sugarcane dropped (Mehareb and El-Mansoub 2020). Smut disease is playing significant role in the yield reduction in sugarcane fields.

Sugarcane smut disease caused by a fungus *S. scitamineum* (Syd.) M. Peipenbr., M. Stoll and Oberw. (Previously called *Ustilago scitaminea)* Stoll et al. (2003). It's a major disease of sugarcane worldwide that causes considerable yield losses if susceptible varieties are grown (Comstock et al. 2000). Smut disease occurs worldwide, and causing epidemics and serious losses in sugarcane production and caused reduce the yield to a significant level in infected plants (Comstock et al. 2000; Que et al. 2012; McNeil et al 2018). In Egypt it was recorded presence a new races infected the sugarcane crop (Abd El- Fattah et al. 2010).The disease destroyed caused significant yield losses in the cultivar NCo-310 which made the authorities start to change the damaged cultivar with a new moderate resistant cultivar G.T.54-9 (El-Zayat et al. 1986; Esh et al. 2018).

However, smut disease a major disease to be considered for selection and development of new cultivars in Egypt. The disease is referred as "culmicolous smut" as it infects the stalk of the cane. The floral parts are converted into black smut sours which releases enormous quantities of spores in the latter stages and spreads to larger area. The most identifiable diagnostic characteristic of a smut infected plant is the emergence of a "smut whip". A smut whip is a curved, black in colour, pencil-thick growth, that emerges from the meristem part of the affected cane. The disease spreads through teliospores produced by the whip and that are disseminated by wind. In addition, infected setts they germinate in the presence of moisture and produces promycelium followed by sporidia. Sugarcane smut can be effectively controlled through host resistance (Martinez-Espinoza et al. 2002; Kavitha 2017). Reduction in yield and quality in sugarcane mostly dependent on mostly dependent on the races of the pathogen, genotypes resistance rate and the prevailing environmental conditions (Sundar et al. 2012).

SCoT polymorphism is a new and credible gene-targeted marker tool based on the translation start codon (Collard and Mackill 2009).

SCoT markers discover the polymorphism in the conserved regions flanking the start codon sequences of genes. Advantages of the technique are represented in high reproducibility of obtained results and low cost of analysis.

SCoT is earning in publicity for its notability over other DNA markers such as ISSR and RAPD for higher polymorphism (Gorji et al. 2011). The aim of this study was to assess the genetic diversity among *S. scitamineum* isolates from Luxor Governorate, Egypt using SCoT marker technique.

Materials and Methods

Collection of smut samples

Samples (5 samples) of sugarcane smut whips were collected from the commercial variety cultivated in sugarcane growing areas in Upper Egypt (Luxor governorate) in April 2022 (Table 1).

Table 1. Source of smut isolates obtained from the sugarcane varity G. T. 54/9 in Luxor Governorate.

No. of samples	Sugarcane variety	Locations	District
1		Luxor	Luxor Grb (Zemam Luxor)
2		Luxor	EL-Hbeal (EL-Tarbea El-kebly
3	G. T. 54/9	Esna	EL-Karaia (El-Berak)
4		Armnt	EL-Mres (EL-Tawel El-Grby)
5		Armnt	Ahaly EL-Mres (El-Bklla EL-Kebbly)

The collected whips were separated and left on a laboratory bench under room temperature for five days. After drying, the whips at 22°C crushed by hand in a big container to release the spores. The major plant depresses were removedandthe remains were screened through a fine mesh to collect the spores. Spores were stored in paper bags in the laboratory under room temperature and dry conditions.

Isolation of *S. scitamineum*

Teliospores were obtained by shaking the whip on a sterilized plastic sheet. Fungal spores were suspended for 24 hours in1.5% copper sulphate solution for sterilization Shuai et al (2023). A loop of fungal suspension was streaked on Petri plates containing (PDA) and then incubated at 30$^{\circ C}$ for 10days. Tips of fungal mycelium were taken and transferred to tubes containing PDA medium, incubated at 30$^{\circ C}$ for 10 days after that kept in a refrigerator at 4$^{\circ C}$ as stock cultures for further studies Abd El Fattah et al. (2010).

Molecular Characterization of *S. scitamineum*

DNA extraction

Mycelia derived from a single spore were used in DNA extraction as described by Shen et al. (2006). DNA purity was detected using the absorbances ratio at 260 nm to 280 nm.

SCoT amplification

Four SCoT primers were selected (Table 2). PCR reactions were performed in a volume of 25 µL as described by Abdullah et al. (2022). All PCR results were loaded into 2% agarose gels electrophoresis, stained with ethidium bromide and visualized under UV transilluminator.

Table 2. SCoT primers names and their sequences

Primers	Sequences (5′-3′)
SCoT 1	CAACAATGGCTACCACCA
SCoT 2	CCATGGCTACCACCGCAC
SCoT 3	CCATGGCTACCACCGCAG
SCoT 4	ACCATGGCTACCACCGCA

Data analysis

On the basis of the SCoT-PCR amplifications, the presence of a DNA band was marked as "1", while the absence of a DNA band was marked as "0". UPGMA cluster analysis based on the Jaccard similarity coefficient and principal component analysis (PCA) were conducted by NTSYS-pc2.10 software (Rohlf 2000). The polymorphic information content (PIC) was calculated using formulas described by Botstein et al. (1980).

Results and Discussion

Collection of smut samples and Isolation of *S. scitamineum* Five isolates were associated with diseased samples showing sugarcane smut disease symptoms, were obtained (Figs. 1 and 2).

Fig. 1. Symptoms of natural infection with sugarcane smut in fields on commercial variety G.T. 54-9.

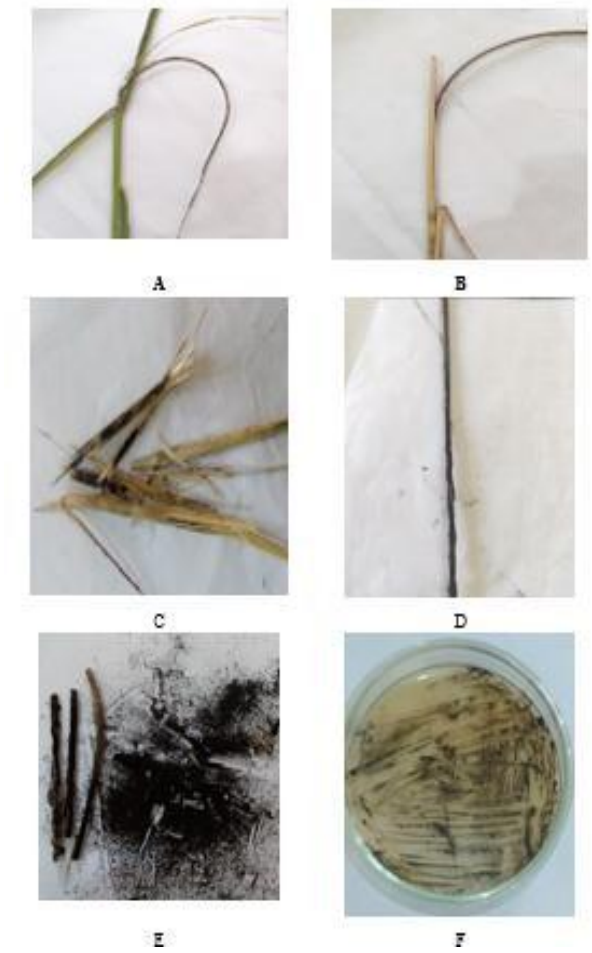

Fig. 2. Collected whip from infection fields, (A) Drying whip, (B), Major plant depresses were removed, (C) Separately whip after remove major plant depresses, (D) Whip crushed to release the spores. F. Spores streaked on Petri plates containing (PDA) medium (E).

Molecular Characterization of *S. scitamineum*

SCoT polymorphism analysis in *S. scitamineum*

Mycelia derived from a single spore (Fig. 3) were used in DNA extraction using the cetyltrimethylammonium bromide (CTAB) method.

A **B**

Fig. 3. Mycelia derived from a single spore on Petri plate (A) and Flask (B). .

A total of 56 SCoT bands were obtained, of which 48 (85.71 %) were polymorphic among the 5 *S. scitamineum* isolates using 4 SCoT primers. The profiles resulted by primers SCoT1and SCoT2 are shown in Fig. 4. bands amplified from the 5 *S. scitamineum* isolates listed in Table 1.

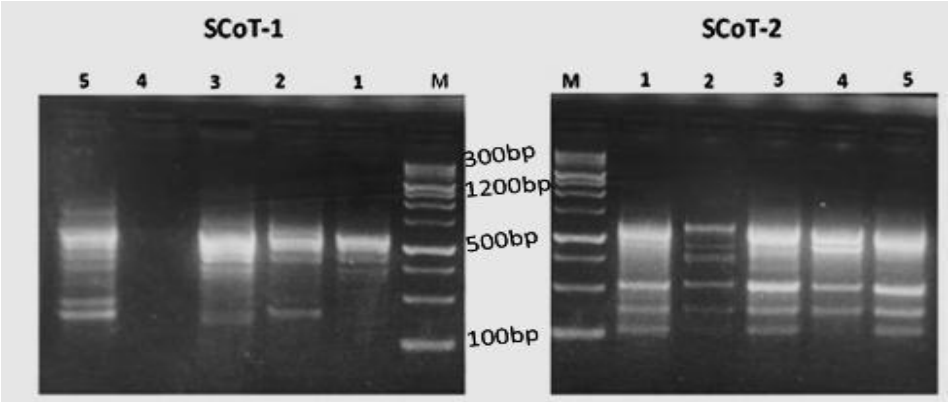

Fig. 4. SCoT analysis of *S. scitamineum* isolates with primers SCoT1 and SCoT2 . M: DNA marker; Lanes 1–5: SCoT-PCR

While profiles resulted by primers SCoT3and SCoT4 are shown in Fig. 5.

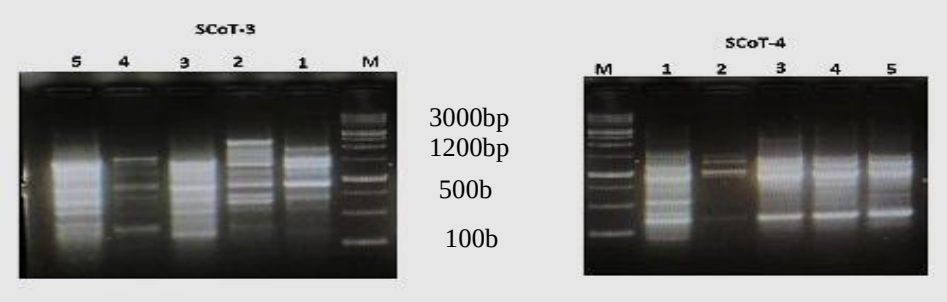

Fig. 5. SCoT analysis of *S. scitamineum* isolates with primers SCoT3 and SCoT4 . M: DNA marker; Lanes 1–5: SCoT-PCR bands amplified from the 5 *S. scitamineum* isolates listed in Table 1.

The number of bands and the degree of polymorphism detected by each primer are listed in Table 3.

Table 3. SCoT amp icons analysis.

Primers	Total no. amplicons	Polymorphic amplicons	Monomorphic amplicons	Polymorphism %	Polymorphism information content
SCoT 1	11	10	1	90.9	0.393
SCoT 2	10	6	4	60.0	0.240
SCoT 3	18	17	1	94.4	0.386
SCoT 4	17	15	2	88.2	0.376

The rate of polymorphism of each primer ranged from 60.0 to 94.4%. The number of bands generated by each primer was in the range from 10 (SCoT2) to 18 (SCoT3) with an average number of 14.

Among the primers used in this study, SCoT3 produced the highest number of polymorphic bands (17), while SCoT2 produced lower polymorphic bands (6). Overall, the PIC value of these 4 SCoT primers ranged from 0.240 to 0.393.

Primer SCoT11 was the most distinguishing with a PIC value of 0.393, while SCoT2 was the lowest PIC value of 0.240. SCoT has been shown to be an effective molecular technique for studying genetic diversity in plants (Collard and Mackill 2009; Gorji et al. 2011; Abdullah et. al. 2022) as well as fungus *S. scitamineum* associated with Sugarcane smut disease (Shen et al. 2016).

Genetic Similarity Matrix and Cluster Analysis

The results in Table 4, indicated that the lowest genetic similarity was shown between isolates No 2 and No 3 (33.0%), while the highest rate was noticed between isolates No1 and No3 (82.0%).

Table 4. Similarity matrix based on SCoT analysis

	1	2	3	4	5
1	1.00				
2	0.40	1.00			
3	0.82	0.33	1.00		
4	0.55	0.38	0.51	1.00	
5	0.61	0.37	0.73	0.75	1.00

Based on the dendrogram in Fig.6, the *S. scitamineum* isolates were divided into two main groups, I and II. Group II included only one isolate (isolate No. 2). While Group I was divided into two sub-groups included Sub-G1 (isolates No.1 & 3) and Sub-G2 (isolates No. 4 & 5).

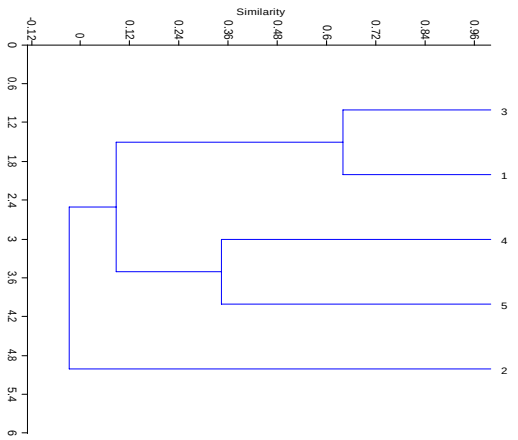

Fig.6. Cluster analysis based on jaccard's similarity index derived from the SCoT data.

Conclusions

This study demonstrated that the molecular diversity of *S. scitamineum* was partly related to its geographic origin, but this interpretation does not fit all isolates.

The fact that all isolates were collected from the same sugarcane variety (GT54-9) supported that the genetic variation is unrelated to the origin of the host.

From the previous reports, regarding the relationship between genetic diversity of the fungus *S. scitamineum* and geographical origin, there were three conclusions, one was related to geographic origin (Xu et al. 2003; Shen et al. 2012), another to host origin (Knapova et al. 2002), and the third neither to geographical nor to host origin (Cantone and Vandenberg 1998; Huang et al. 2001).

In conclusion, the results in this study indicated that diversity of *S. scitamineum* was partly associated to its geographical origin. These results are useful in breeding programs to obtain smut-resistant sugarcane genotypes. Our results demonstrated that SCoT markers can be used to evaluate the genetic diversity of *S. scitamineum*.

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