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Article

Evaluation of smut resistance in selected sugarcane genotypes and their molecular characterization using SCoT, ISSR, and RAPD analysis

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Abstract:

Sporisorium scitamineum causes sugarcane smut, which is a global problem that seriously reduces yield and quality. The disease is most effectively managed through the use of resistant cultivars. Molecular markers can support breeding programs by helping to identify resistant genotypes at early stages. In this study, three sugarcane varieties G.2003-47 (G3), G.2004-27 (G4), and the commercial cultivar G.T.54-9 (C9) were evaluated for whip smut response under artificial inoculation and characterized using Start codon Targeted (SCoT), inter simple sequence repeats (ISSR), and random amplified polymorphic DNA (RAPD) markers. Disease incidence was lowest in G.2004-27 (4%), while G.2003-47 and G.T.54-9 showed higher infection levels (13.34% and 12.88%, respectively). SCoT primers generated 25 bands with 55.33% polymorphism, whereas ISSR and RAPD produced 37 and 32 bands with polymorphism levels of 46.3% and 49%, respectively. Several primers across the three marker systems distinguished the relatively resistant variety G.2004-27 from the more susceptible genotypes. These findings indicate that SCoT, ISSR, and RAPD markers can complement phenotypic screening and assist in the preliminary identification of whip smut–resistant sugarcane clones for further field evaluation. Consequently, the variety G.2004-27 is recommended as a promising source of resistance for Egyptian sugarcane breeding programs. Additionally, SCoT markers proved to be more informative than RAPD and ISSR in discriminating among the tested genotypes.

Keywords:

Sugarcane, whip smut, *Sporisorium scitamineum,* molecular markers, SCoT, ISSR, RAPD.

1. Introduction

Sugarcane (*Saccharum* spp.) is a major economic crop in many countries, and its productivity is constrained by numerous diseases, among which smut is one of the most destructive (Bhuiyan et al., 2021; Rott et al., 2000). The disease was first reported in Natal, South Africa, in 1877 (Braithwaite et al., 2004; Rott et al., 2000), and spread to other sugarcane-growing areas in Central, East, and West Africa, Indonesia, Central and South America, Brazil, and Australia. Sugarcane smut is caused by the biotrophic basidiomycete fungus *Sporisorium scitamineum* (synonym *Ustilago scitaminea*) and can cause severe losses in productivity and quality of cane (Comstock, 2000). In Egypt, the area cultivated with sugarcane during the 2023/24 season was approximately 132,762 hectares (equivalent to 316,099 feddans), producing about 642,166 tons of sugar, which accounted for nearly 29% of the country's total sugar production (Annual Report for Sugar Crops in Egypt, 2024). The characteristic feature of this disease is the appearance of a typical "whip"-like structure, known as a sorus, from the aerial part of a plant, consisting of a central vascular strand surrounded by massive colonies of dark teliospores enveloped in a thin membranous covering (Hoy et al., 1986). Smut propagules are primarily dispersed by wind, with additional spread via smut-infected planting material and agricultural equipment (Croft and Braithwaite, 2006; Rott et al., 2000). Globally, smut control depends mainly on growing resistant cultivars, supported by other practices within an integrated disease management (IDM) approach. Key IDM measures include using resistant varieties, removing diseased plants, planting disease-free seed-cane, and treating planting material with fungicides in infested fields. Together, these approaches have been proven effective in controlling smut outbreaks and associated yield losses (Bhuiyan et al., 2021). Recently, greenhouse experiments conducted in Egypt have demonstrated the efficacy of chemical fungicides in preventing sugarcane smut infection and whip formation caused by *S. scitamineum*, highlighting their potential role in managing this major sugarcane pathogen (Osman et al., 2025). Resistant varieties are generally considered the most efficient and cost-effective methods in controlling sugarcane smut. Resistance to *S. scitamineum* is complex and modulated by multiple host-pathogen interactions. Early in resistance work, it was found that resistance may be linked to morphological characteristics of sugarcane buds, making infection difficult, thereby confirming the importance of the host's genetic makeup in resistance (Fawcett, 1946). However, conventional resistance evaluation methods are time-consuming, require long crop cycles, and are highly dependent on environmental conditions (Bhuiyan et al., 2021). After five years of selective breeding for resistant parents, the percentage of susceptible seedlings in Hawaii decreased significantly from 64% at the time of the smut intrusion to 11% (Comstock et al., 1983). In Australia, awareness of the importance of smut as a serious biosecurity risk led to preventive screenings in Indonesia and Western Australia prior to the 2006 outbreak along the southeastern coast (Croft et al., 2008a). Initial screenings showed that over 70% of commercial and breeding seedlings were susceptible to the pathogen (Croft et al., 2008b), and, as a result, the strategy changed to use resistant parents. As a result, the percentage of biparental crosses resistant to smut increased from 0.4% to 52% from 2000 to 2007, almost doubling the number of resistant seedlings by 2011. Through this continuous selective breeding program, there was an impressive decrease in the percentage of seedlings that were susceptible to smut from over 70% in 2004 to below 10% by 2019. Molecular approaches also provide useful support to traditional breeding by enabling discrimination among different sugarcane varieties based on genotype. Marker-assisted selection (MAS) has become useful in crop breeding programs to accelerate the selection of favorable genotypes without relying solely on phenotypic selection (Collard and Mackill, 2009). In previous studies on sugarcane smut, molecular marker techniques have been used effectively to analyze genetic diversity and assist breeding efforts to improve resistance to the disease (Que et al., 2012; Wei et al., 2006). Therefore, the objective of the present study was to evaluate the response of the common sugarcane varieties in Egypt to whip smut disease and to discriminate between resistant and susceptible genotypes using five start codon-targeted (SCoT) primers, eleven inter-simple sequence repeat (ISSR) primers, and six random amplified polymorphic DNA (RAPD) primers, thereby supporting sugarcane breeding programs through the efficient identification of resistant clones.

2. Materials and Methods

2.1 Collection of smut samples

Samples of sugarcane smut whips were collected from the commercial cultivar G.T.54-9 (C9) grown in the sugarcane-growing regions of El-Minia governorate, Egypt.

2.2 Inoculum preparation

Collected smut whips were air-dried and stored at room temperature on a laboratory bench for five days. Teliospores were released by manually crushing the dried whips in a large container. Major plant debris was removed, and the remaining material was passed through a fine-mesh screener. Finally, the obtained spores were maintained in paper bags at room temperature until use for inoculum preparation (Gillaspie et al.,1983).

2.3 Varietal response

Three sugarcane cultivars were used: G.2003-47 (G3; CP 55-30 ♀ × EI 85-1696 ♂) and G.2004-27 (G4; CP 55-30 ♀ × ROC 22 ♂), both newly registered varieties developed by the Egyptian Sugarcane Breeding Program, and G.T.54-9 (C9; NCo 310 ♀ × F 73-925 ♂), which is widely used as a commercial cultivar in Egypt. Cuttings of the three sugarcane cultivars were obtained from the Sugar Crops Research Institute (SCRI), Agricultural Research Center, Giza, Egypt. The cuts of each variety, containing one bud, were soaked in a fungal spore suspension (4 g per 1 L of water) for 2 hours. Three cuts were sown in 35 cm diameter pots containing clay soil. The greenhouse experiment was arranged in a Randomized Complete Design (RCD) with five replicates for each variety. The experiment was repeated twice (2024 and 2025). The greenhouse of the Maize disease and Sugar crops Research section at the Plant Pathology Research Institute, ARC, Giza was used for the experiment.

2.4 Disease assessment

Disease symptoms, such as whip-like structures, assessed six months after sowing and used to estimate disease incidence (Firehun et al., 2009).

$$\text{Disease incidence (DI \%)} = \frac{\text{Number of infected tillers}}{\text{Number of total tillers}} \times 100$$

2.5 Molecular identification of the causal organism

After using liquid nitrogen to grind one gram of *S. scitamineum* fungus spores into a fine powder in a mortar, DNA was extracted using the Bio Basic DNA Extraction Kit.

2.5.1 PCR reactions and conditions

Specific primers bE4 (5-CGCTCTGGTTCATCAACG-3) and bE8(5-TGCTGTCGATGGAAGGTGT-3) (Albert and Schenck, 1996), the amplification reaction was carried out in 15 µl includes 2.4 µl master mix Solis Biodyne, 1 µl of each primer (10 µm concentration), 10 µl sterilized distilled water and 0.5 µl DNA. Applied Biosystems 2720 Thermal cycler was used in a PCR reaction at 96 °C for 6 minutes. As initial denaturation, then 35 cycles of 94 °C for 1 minute, 52 °C for 1 minute, and 72 °C for 1 minute. And final extension 72 °C for 7 minutes.

2.6 Molecular markers

Three types of molecular markers were used in this study: five SCoT primers, eleven ISSR primers, and six RAPD primers (Table 1).

2.6.1 DNA extraction

One gram of sugarcane leaves of the three varieties were collected after two months post inoculation, then washed with sterilized distilled water and ground with liquid nitrogen to obtain a fine powder. DNA extraction was carried out using the Biobasic DNA extraction kit. The quality and quantity of the extracted DNA were measured by running the DNA on a 1% agarose gel. Biobasic Inc., Canada, added 5 5 µL Ethidium bromide (10mg/mL) alongside a 100 bp DNA ladder (Solis Biodyne) for 1.5 hours.

2.6.2 PCR reactions and conditions

PCR reactions were performed in a total volume of 15 µL using an Applied Biosystems 2720 thermal cycler. Cycling conditions were optimized separately for each molecular marker system as follows: Start Codon Targeted (SCoT) markers. PCR amplification was carried out with an initial denaturation at 94 °C for 4 min, followed by 45 cycles of denaturation at 94 °C for 40 s, annealing at 45 °C for 1 min, and extension at 72 °C for 2 min, with a final extension at 72 °C for 7 min.

Table 1: List of SCoT, ISSR and RAPD primers and their sequence.

Molecular marker	Sequence
SCoT	
1	CAACAATGGCTACCACGC
2	CAACAATGGCTACCACGT
3	CAACAATGGCTACCAGCA
4	ACGACATGGCGACCAACG
5	CACCATGGCTACCACCAG
ISSR	
1	AG9C
2	AC9T
3	GA9A
4	GA9T
5	GA9C
6	CA9G
7	AC9C
8	AC9G
9	TA10T
10	CA9A
11	CA9T
RAPD	
1	CACGGCGAGT
2	GTCGATGTCG
3	AAGCCTCCCC
4	CGTCGCCCAT
5	GGGTTTGGCA
6	AGCGAGCAAG

2.6.2.1 Inter-Simple sequence repeat markers

PCR amplification consisted of an initial denaturation at 94 °C for 4 min, followed by 45 cycles of 94 °C for 40 s, 47 °C for 1 min, and 72 °C for 2 min, and a final extension at 72 °C for 7 min.

2.6.2.2 Random Amplified Polymorphic DNA markers

PCR amplification was performed with an initial denaturation at 94 °C for 4 min, followed by 45 cycles of denaturation at 94 °C for 40 s, annealing at 37 °C for 1 min, and extension at 72 °C for 2 min, with a final extension at 72°C for 7 min.

2.6.3 Gel electrophoresis

A 15µl of PCR product was loaded onto a 1.7% agarose gel (Biobasic Inc., Canada) containing 5µl of Ethidium bromide (10 mg /ml) in an electrophoresis tank (13cm x 16 cm) containing 1X TAE buffer. The PCR product was run for 1.5 hours at 60 °C. The first and end lanes of the comb were loaded with Thermo Scientific O Gene Ruler Ready-to-Use 100 bp plus DNA ladder, containing 14 discrete DNA fragments ranging from 100 bp to 3000 bp. After the DNA fragment ran on the gel, it was exposed to UV light using a Hero Lab UV-40 S/L transilluminator, and then the image was captured manually with a Sony Cyber-shot camera.

2.7 Statistical analysis

The percentage of plants exhibiting whip-like shape was used to calculate the disease incidence, and an analysis of variance (ANOVA) was calculated using Minitab 17 statistical software. In SCoT, ISSR, and RAPD markers, each scorable band was treated as a single locus; a data matrix was generated by scored as present (1) or absent (0). The percentage of polymorphic bands and the total number of bands were calculated based on Ng and Tan (2015). For every molecular marker, a cluster analysis dendrogram and a similarity matrix were calculated using the Dice coefficient, using the SPSS program version 16. Principal component analysis (PCA) was performed with the Past statistical package version 4.03.

3. Results

3.1 Morphological identification of the causal organism

Infected sugarcane plants exhibited typical smut symptoms characterized by the emergence of a whip-like sorus from the shoot apex. The sorus consisted of a central vascular core surrounded by abundant dark, powdery teliospores enclosed within a thin membranous sheath (Figure 1A). These symptoms were consistent with sugarcane smut caused by *S. scitamineum*. Microscopic examination of teliospores

collected from mature smut whips revealed dark brown to black, globose to subglobose spores with thick walls (Figure 1B). The observed teliospore morphology was consistent with published descriptions of *S. scitamineum*, supporting the morphological identification of the pathogen prior to molecular confirmation (Hoy et al., 1986; Rott et al., 2000).

Figure 1: Field symptoms on sugarcane plants showing characteristic whip-like, blackened structures emerging from the shoot apex (A). (B) Microscopic view of the pathogen showing numerous rounds to oval, thick-walled teliospores. The spores appear brownish and are densely aggregated, consistent with the teliospores of the sugarcane smut fungus.

3.2 Molecular identification of the causal organism

PCR amplification using the species-specific primers bE4 and bE8 produced a single amplicon of approximately 459 bp, confirming the identity of the causal organism as *S. scitamineum*. (Figure 2).

3.3 Varietal response to whip smut

Three sugarcane varieties were evaluated for their response to whip smut under artificial inoculation. Disease incidence differed among the tested varieties. The lowest disease incidence was recorded in G.2004-27 (G4), with 4% infected plants, whereas higher disease incidence was observed in G.T.54-9 (C9) and G.2003-47 (G3), with values of 12.88% and 13.34%, respectively. Disease incidence values represent the mean of two independent experiments.

3.4 Molecular diversity of sugarcane varieties

3.4.1 SCoT marker analysis

Five SCoT primers yielded 25 scorable bands, averaging 5.0 per primer. Of these, 55.33% were polymorphic, with an average of 2.8 polymorphic bands per primer (Table 2).

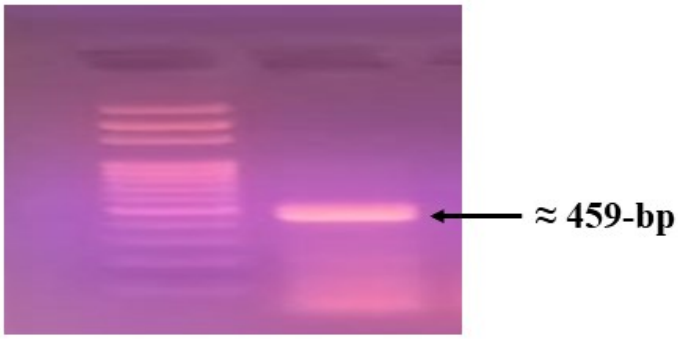


Figure 2: Agarose gel electrophoresis of PCR amplification using bE4/bE8 primers for detection of *Sporisorium scitamineum*. Lane L, 100-bp DNA ladder; lane S, fungal DNA sample showing the expected 459-bp fragment.

Polymorphic information content (PIC) values ranged from 0.15 to 0.37, with a mean value of 0.25. Banding patterns obtained with SCoT primers 2 and 5 differentiated G.2004-27 (G4) from the other two varieties (Figure 3). Primer 1 grouped G.2003-47 (G3) and G.2004-27 (G4) together, with 86% similarity (Figure 4). Cluster analysis based on combined SCoT data grouped the three varieties into two main clusters. G.2004-27 (G4) clustered with G.T.54-9 (C9), whereas G.2003-47 (G3) formed a separate cluster (Figure 5).

Table 2: Total number of bands, polymorphic bands, percentage of polymorphic bands, and PIC values for SCoT primers.

SCoT Primer	TNB	PB	PPB	PIC
1	4	2	50	0.22
2	4	2	50	0.22
3	6	2	33.33	0.15
4	6	5	88.33	0.37
5	5	3	60	0.33
Total	25	14	-	-
Average	5.0	2.8	55.33	0.25

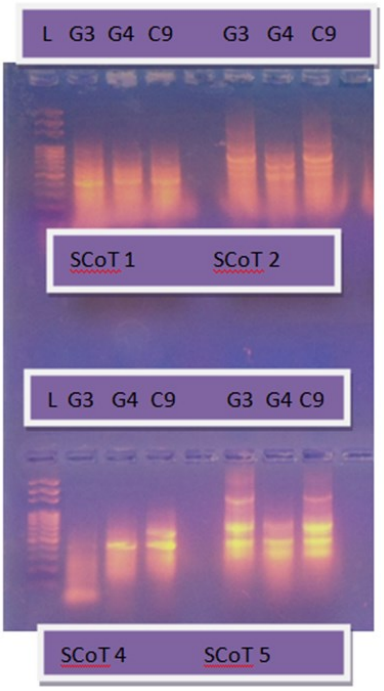


Figure 3: Agarose gel electrophoresis profiles generated by Start Codon Targeted (SCoT) markers in three sugarcane varieties. Lanes are labeled as follows: L, 100-bp DNA ladder; G3, sugarcane variety G.2003-47; G4, sugarcane variety G.2004-27; C9, commercial variety G.T.54-9. The upper panel shows amplification patterns obtained with SCoT primers 1 and 2, and the lower panel shows amplification patterns obtained with SCoT primers 4 and 5. Differences in banding patterns among the varieties indicate polymorphism detected by the SCoT markers.

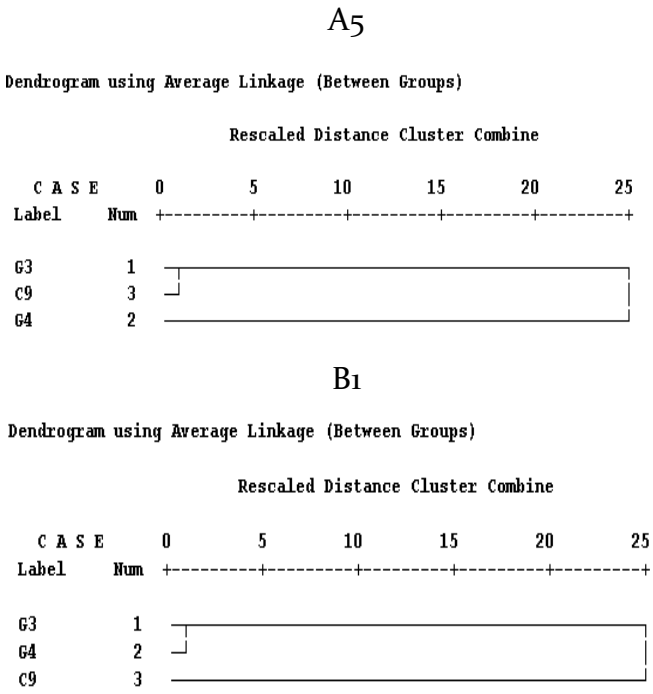


Figure 4: Dendrograms derived from SCoT marker analysis. (A) Cluster analysis based on SCoT primer 5 grouped sugarcane varieties G.2003-47 (G3) and G.T.54-9 (C9) into one cluster, whereas G.2004-27 (G4) formed a separate cluster. (B) Cluster analysis based on SCoT primer 1 grouped G.2003-47 (G3) and G.2004-27 (G4) together in the same cluster.

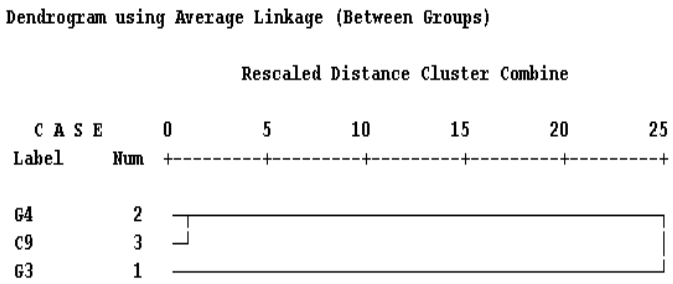


Figure 5: Dendrogram showing cluster analysis of sugarcane varieties G.2003-47 (G3), G.2004-27 (G4), and G.T.54-9 (C9) based on combined data from all SCoT primers.

3.4.2 ISSR marker analysis

Eleven ISSR primers produced 37 scorable bands, averaging 3.4 per primer. Sixteen bands (46.3%) were polymorphic, with an average of 1.8 polymorphic bands per primer (Table 3). PIC values ranged from 0.09 to 0.44, with a mean value of 0.21. Several ISSR primers differentiated G.2004-27 (G4) from the other two varieties (Figure 6). Primers 2 and 3 grouped G.2003-47 (G3) and G.2004-27 (G4) together with complete similarity (Figure 7). Cluster analysis based on combined ISSR data separated the three varieties into two clusters, with G.2003-47 (G3) and G.T.54-9 (C9) grouped together and G.2004-27 (G4) forming a separate cluster (Figure 8).

Table 3: Total number of bands, polymorphic bands, percentage of polymorphic bands, and PIC values for ISSR primers.

ISSR Primer	TNB	PB	PPB	PIC
1	5	1	20	0.09
2	2	1	50	0.22
3	3	1	33.33	0.15
4	6	4	66.66	0.33
5	5	3	60	0.27
6	3	1	33.33	0.15
7	1	0	0	0
8	1	0	0	0
9	3	3	100	0.44
10	5	1	20	0.09
11	3	1	33.33	0.15
Total	37	16	-	-
Average	3.4	1.8	46.3	0.21

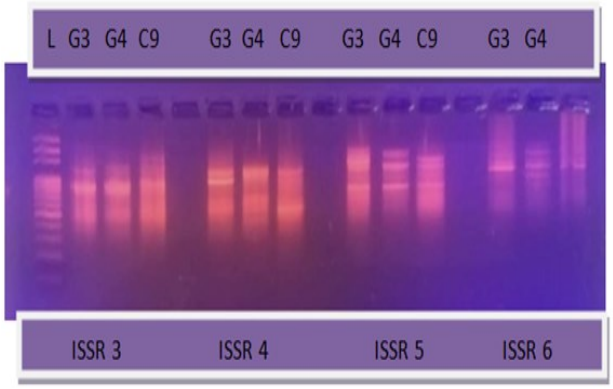


Figure 6: Agarose gel electrophoresis profiles generated by ISSR markers in three sugarcane varieties. Lanes are labeled as follows: G.2003-47 (G3), G.2004-27 (G4), and G.T.54-9 (C9). Banding patterns obtained with ISSR primers 3, 4, and 5 show polymorphic profiles among the tested varieties.

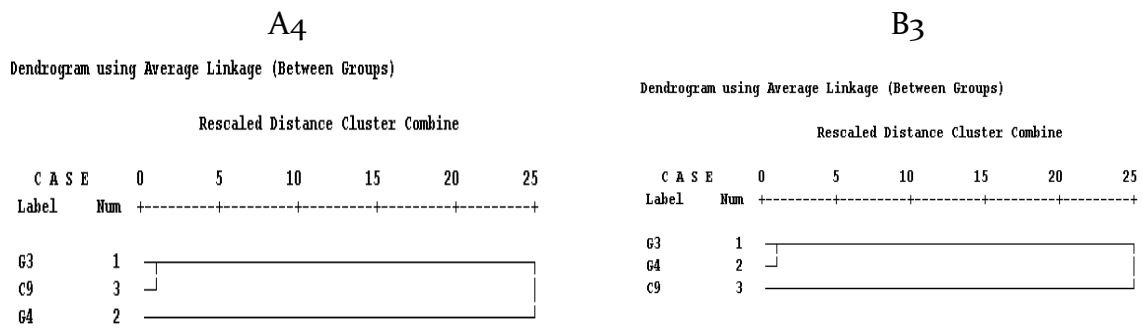


Figure 7: Dendrograms derived from ISSR marker analysis. (A) Cluster analysis based on ISSR primer 4 grouped sugarcane varieties G.2003-47 (G3) and G.T.54-9 (C9) into one cluster, whereas G.2004-27 (G4) formed a separate cluster. (B) Cluster analysis based on ISSR primer 3 grouped G.2003-47 (G3) and G.2004-27 (G4) together in the same cluster.

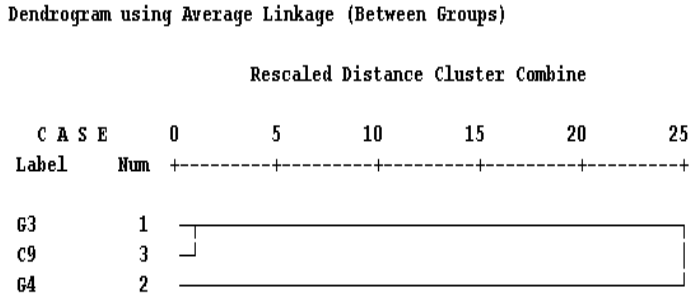


Figure 8: Dendrogram showing cluster analysis of sugarcane varieties G.2003-47 (G3), G.2004-27 (G4), and G.T.54-9 (C9) based on combined data from all ISSR primers.

3.4.3 RAPD marker analysis

Six RAPD primers yielded 32 scorable bands, averaging 5.33 per primer. Sixteen bands (49%) were polymorphic, with an average of 2.66 polymorphic bands per primer (Table 4). PIC values ranged from 0.07 to 0.30, with a mean value of 0.20. Banding patterns obtained with RAPD primers 4 and 5 differentiated G.2004-27 (G4) from the other varieties, whereas primer 2 grouped G.2003-47 (G3) and G.2004-27 (G4) together (Figures 9–10). Cluster analysis based on combined RAPD data grouped G.2003-47 (G3) and G.T.54-9 (C9) together, while G.2004-27 (G4) formed a separate cluster (Figure 11).

Table 4: Total number of bands, number of polymorphic bands, percentage of polymorphic bands, and polymorphic information content (PIC) for RAPD primers.

RAPD Primer	TNB	PB	PPB	PIC
1	8	5	62.5	0.30
2	6	3	50	0.07
3	4	1	25	0.11
4	5	2	40	0.20
5	3	2	66.6	0.30
6	6	3	50	0.22
Total	32	16	----	----
Average	5.33	2.66	49	0.20

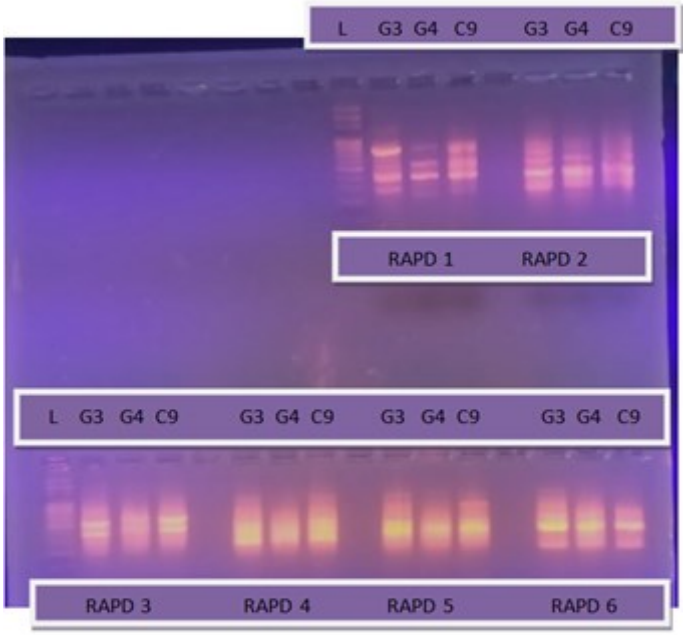
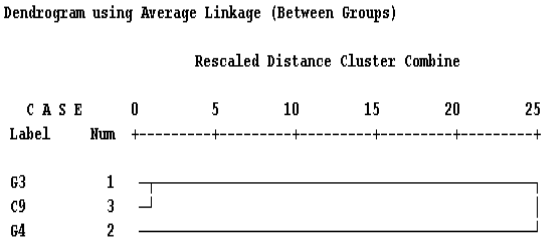


Figure 9: Agarose gel electrophoresis profiles generated by RAPD markers in three sugarcane varieties. Lanes are labeled as G.2003-47 (G3), G.2004-27 (G4), and G.T.54-9 (C9). Banding patterns obtained with RAPD primers 2, 4, and 5 show polymorphisms among the tested varieties.

A5



B2

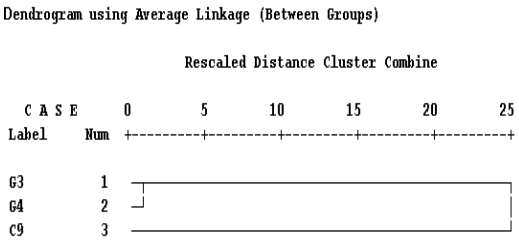


Figure 10: Dendrograms derived from RAPD marker analysis. (A) Cluster analysis based on RAPD primer 5 grouped sugarcane varieties G.2003-47 (G3) and G.T.54-9 (C9) into one cluster, whereas G.2004-27 (G4) formed a separate cluster. (B) Cluster analysis based on RAPD primer 2 grouped G.2003-47 (G3) and G.2004-27 (G4) together in the same cluster.

3.4.4 Combined marker analysis and principal component analysis

Combined analysis of SCoT, ISSR, and RAPD marker data revealed 82% similarity between G.2003-47 (G3) and G.T.54-9 (C9), whereas similarity between G.2004-27 (G4) and G.T.54-9 (C9) was 76%. Cluster analysis based on merged marker data grouped G.2003-47 (G3) and G.T.54-9 (C9) into one cluster, while G.2004-27 (G4) formed a separate cluster (Figure 12). Principal component analysis (PCA) combining varietal response and molecular marker data showed that the first principal component explained 91.34% of the total variation (eigenvalue = 148.60), while the second component explained 8.65% (eigenvalue = 14.08), as illustrated in the scree plot (Figure 13). The PCA ordination separated G.2004-27 (G4) from G.2003-47 (G3) and G.T.54-9 (C9), which clustered together (Figure 14).

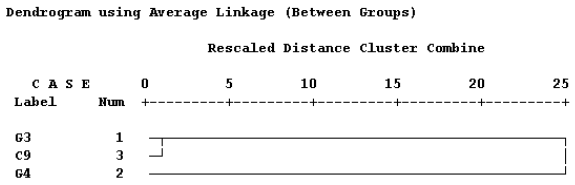


Figure 11: Dendrogram showing cluster analysis of sugarcane varieties G.2003-47 (G3), G.2004-27 (G4), and G.T.54-9 (C9) based on combined data from all RAPD primers.

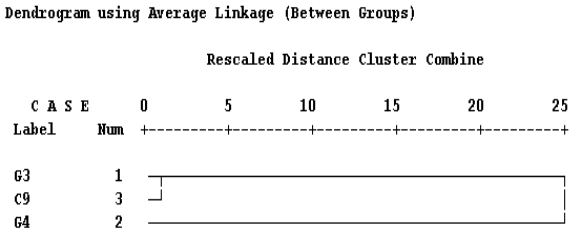


Figure 12: Dendrogram showing cluster analysis based on combined SCoT, ISSR, and RAPD marker data.

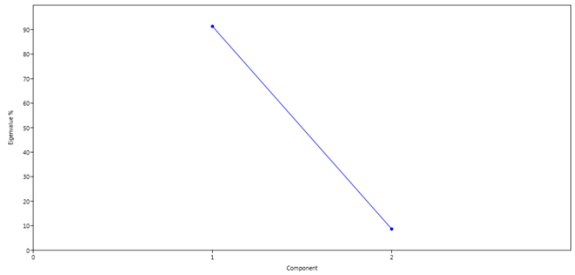


Figure 13: Scree plot derived from principal component analysis (PCA) of three sugarcane varieties, G.2003-47 (G3), G.2004-27 (G4), and G.T.54-9 (C9), based on combined varietal response and molecular marker data.

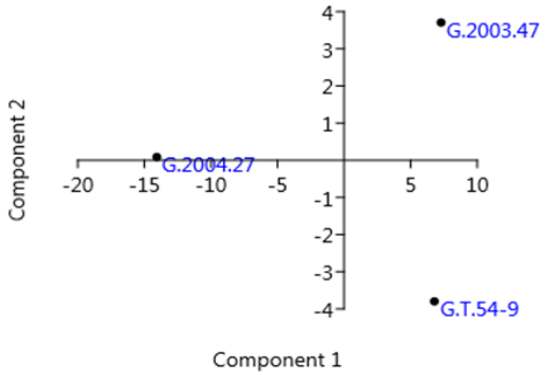


Figure 14: Principal component analysis (PCA) ordination of three sugarcane varieties, G.2003-47 (G3), G.2004-27 (G4), and G.T.54-9 (C9), based on combined varietal response and molecular marker data.

4. Discussion

Previous studies in Egypt have primarily focused on chemical and integrated management strategies for sugarcane smut under local conditions (Osman et al., 2025). In this context, the present study complements earlier work by emphasizing host varietal response and molecular discrimination among sugarcane genotypes. Morphological characterization of infected plants revealed the distinctive whip-like sorus emerging from the shoot apex, accompanied by abundant dark, powdery teliospores, which is diagnostic of smut caused by *S. scitamineum*. Microscopic results revealed abundant thick-walled teliospores that were globose to subglobose, in agreement with previously published morphological descriptions of the pathogen (Hoy et al., 1986; Rott et al., 2000). Based on these features, the pathogen was tentatively identified, and subsequently confirmed by molecular analyses. Molecular identification using species-specific primers targeting the bE gene complex yielded a single amplicon of the expected size (≈459 bp), confirming the presence of *S. scitamineum*. PCR assays targeting primers derived from the bE gene have been shown to provide a reliable and specific means of identifying *S. scitamineum*, particularly when morphological characteristics alone do not allow clear discrimination (Albert and Schenck, 1996). The agreement between morphological and molecular identification in this study supports the reliability of the pathogen confirmation approach used before resistance evaluation. Evaluation of varietal response under artificial inoculation revealed apparent differences in disease incidence among the tested sugarcane varieties. The variety G.2004-27 (G4) consistently recorded the lowest disease incidence, while G.T.54-9 (C9) and G.2003-47 (G3) exhibited higher, yet comparable, infection rates. Such variation in smut incidence among sugarcane genotypes has been widely reported and is largely attributed to differences in host

genetic background, which affect pathogen infection, colonization, and subsequent disease development (Bhuiyan et al., 2021; Fawcett, 1946). The relatively lower disease incidence observed in G4 suggests a higher level of tolerance or resistance under the conditions of this study. However, resistance expression in sugarcane is known to be influenced by environmental factors and the long crop cycle highlighting the need for further field validation. Molecular marker analysis provided additional resolution in differentiating the tested varieties. All three-marker systems SCoT, ISSR, and RAPD detected polymorphisms among the genotypes, demonstrating their suitability for assessing genetic variation in sugarcane. Our results indicate that SCoT markers yielded higher polymorphism (55.33%) compared to ISSR and RAPD, suggesting that gene-targeted markers (SCoT) are more effective in detecting genetic variation related to functional traits in sugarcane. SCoT markers target conserved regions flanking the start codon and have been reported to be informative for detecting functional genetic variation associated with agronomically important traits, including disease response (Collard and Mackill, 2009). The markers (ISSR and RAPD) showed clear genetic differentiation among the three tested varieties, although the resulting clustering patterns differed only slightly between marker systems. Such variations are expected, as each marker type targets distinct genomic regions and therefore captures different aspects of underlying genetic diversity. Similar marker-dependent clustering patterns have been reported previously in studies of sugarcane smut and other pathosystems, highlighting the value of using multiple marker systems to obtain a more comprehensive view of genetic relationships (Que et al., 2012). When marker data were combined, cluster analysis consistently separated G.2004-27 from G.2003-47 and G.T.54-9, which clustered together. This pattern was further supported by principal component analysis, in which the first principal component explained most of the total variation and clearly distinguished G.2004-27 from the other two varieties. The agreement among pathogenicity assessments, cluster analysis, and principal component analysis (PCA) reinforces the link between molecular marker profiles and disease response, indicating that the genetic variation detected by these markers is closely associated with smut susceptibility and resistance. The specific bands identified in G.2004-27 can be considered as candidate markers for differentiating resistant genotypes, pending further validation on larger populations. Overall, the results demonstrate that an integrated approach combining varietal response assay and molecular marker analysis offers a robust framework for assessing sugarcane responses to whip smut. While common markers such as SCoT, ISSR, and RAPD do not directly target resistance genes, they are effective in separating genotypes with different disease reactions and are therefore suitable for preliminary screening in breeding programs. These approaches facilitate the more efficient identification of sugarcane clones worthy of further assessment under field conditions when used alongside traditional resistance evaluation methods (Bhuiyan et al., 2021; Collard and Mackill, 2009).

5. Conclusion

Future studies should focus on validating these potential markers using a larger segregating population (e.g., F2 or RILs) derived from the cross between G.2004-27 and susceptible parents to establish tight linkage with the smut resistance gene.

Declarations

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