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Evaluation of Biological Control of Sorghum Strains Using *Bacillus Thuringiensis* and *Pseudomonas Aeruginosa* Under Drought Stress

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Abstract

Background: *Sorghum is an economically significant staple food crop for more than half a billion people in developing nations, especially in arid and semi- arid locations where drought stress is a significant limiting factor. Despite usually being regarded as tolerant, sorghum suffers severely from drought stress, which lowers its productivity and nutritional quality throughout its principal cultivation areas.* ***Objective:*** *Improvements in DNA fingerprinting by ISSRs, SSRs, and RAPD markers have also been employed in sorghum genetic modification (GMOs) to enhance the economic characteristics of this crop.* ***Materials and methods:*** *To provide a natural defence against pests, the most tolerant plants among the seven varieties of sorghum bicolour were selected and planted in the second season of 2020–2021 under treatment with two microorganisms, B. thuringiensis and P. aeruginosa. This study considered seven varieties of sorghum bicolour planted under 50% water deficiency in 2019–2020. Genetic variability analysis of sorghum genotypes was performed using seven Inter-Simple Sequence Repeat (ISSR) primers, six Simple Sequence Repeat (SSR) primers, and five Random Amplified Polymorphic DNA (RAPD) primers. Seven Sorghum bicolour accessions were collected from various regions of Egypt and their phylogenetic relationships were evaluated. Additionally, DNA fingerprinting and analyses of the genetic diversity and evolutionary linkages in the sorghum germplasm employed the (ISSR) molecular marker technique.* ***Results and conclusion:*** *The Fisher Least Significant difference test (LSD) at $P < 0.05$, based on RAPD, ISSR, and SSR markers demonstrated a significant connection. The findings demonstrated that 51 bands with a size range of 100–1500 bp and polymorphism percentage of 72.5% were created using five RAPD primers. Seven ISSR primers generated 45 bands With a 57.8(%) polymorphism percentage, ranging in size from 100 to 3000 bp. six SSR primers generated 28 bands with (67.86%) polymorphism percentage of 67.86 %, ranging in size from 100 to 1500 bp. Morphological characteristics and ISSR, SSR, and RAPD analyses were used to group the UPGMA Dendrogram into groups. Jaccard's coefficient was used to analyse the genetic similarity matrix. The maximum*

similarity was observed for ISSR between Hybrid Sh1 and Hybrid Sh306 (0.984%), SSR between Hybrid Sh306 and Sudan grass (0.964%), and RAPD between Giza 15 and Indian Millet (0.706%). The classification of sorghum germplasm, breeding initiatives, and conservation efforts rely heavily on the determination of the genetic diversity among sorghum species. Identification of genetic variants, morphological features, and genetic analysis of ISSR, SSR, and RAPD are useful techniques. These findings demonstrate a large ratio of variation in sorghum. This work could serve as a guide for future research on sorghum and aid in the understanding of species and breeding initiatives.

Keywords: *Sorghum, Bacillus Thuringienese, Pseudomonas Aeruginosa, RAPD, ISSR, SSR, biological control*

1. Introduction

Cereal sorghum is produced in large quantities worldwide. While syrup is used as a sweetener, its entire grain is frequently utilized in baking. It was then utilized as a natural fuel source. Sorghum is one of the few hardy plants that can adapt well to the effects of climate change, particularly intensifying drought, soil salinity, and high temperatures. Because it is adaptable to harsh settings, the crop is extensively grown in hot locations with low water availability. Sorghum has specialized morphological and physiological traits that enable it to grow in unfavorable environments [1].

Any plant breeding project must begin with genetic diversity evaluation, and understanding the genetic links among various accessions is crucial for creating effective breeding and germplasm management methods. The degree of genetic variation in crop species essentially determines how effectively a trait can be genetically enhanced. Sorghum production is constrained by with many biotic and abiotic factors in farmers' fields. Major obstacles to sorghum production have been recognized, and efforts for genetic improvement have been launched to breed greater resistance [2].

Poor emergence, plant mortality, and decreased plant stands are frequent consequences of drought and/or heat stress during the seedling stage. When plants maintain their green colour and fill grains regularly after flowering, this indicates drought stress resistance. Additionally, stay-green genotypes have been reported to be resistant to lodging and charcoal rot [3].

A regulatory gene that activates a crucial gene family found in a wide range of plants was present in four copies of sorghum. Furthermore, it includes 328 cytochrome P450 genes as opposed to rice 228; these genes may aid plants in responding to drought stress [4].

The genetic similarity determined by the Dice coefficient utilising combined RAPD and SSR data. The dendrogram created using UPGMA showed similarities between the hybrids and their male parents, indicating that they were all authentic hybrids [5].

Utilizing phenotypic traits, inter simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) markers, researchers were able to evaluate sorghum genotype performance, as well as the degree of polymorphism and marker trait relationships [6].

Analysis of population structure using ISSR, RAPD, and directed amplification of minisatellites DNA (DAMD) markers and research on genetic diversity to show the genetic links of the mid-season drought-tolerant (MDT), mid-season drought-susceptible (MDS), stay green (SG), terminal drought-tolerant (TDT), saline-tolerant (ST), saline-susceptible (SS) and high Fe–Zn containinglines (HFL) populations of sorghum. The study has demonstrated that "within populations" rather than "among populations" of the chosen genotypes, there exists a relatively significant admixture, a high degree of genetic diversity, and low levels of genetic differentiation. These findings contribute to our understanding of the genetic makeup of sorghum collections, which is important for crop development strategies and rapid plant breeding applications. It offers recommendations for species conservation and is crucial for future adaptive changes or evolution to assess genetic variation at the inter-species level [7].

Twelve variants of Sorghum bicolour L. were molecularly characterized using 11 RAPD and three ISSR primers. The study also emphasized the fact that while RAPD may not be as accurate as PCR-based markers such as SSRs and AFLPs or hybridization-based markers such as RFLPs because of their randomness, they can work well with ISSRs to assess the inherent genetic diversity present in various crop varieties [8].

Sorghum fingerprinting using RAPD and ISSR is a potent tool for cultivar analyses. Additionally,

phylogenetic analysis based on dendrograms 1 and 2 produced from RAPD and ISSR supports the existence of region-specific variants as a result of several generations of selection that occurred after their introduction [9].

The adaptable microbe *Pseudomonas aeruginosa* can survive under various conditions. Worms and insects can die from *P. aeruginosa* [10, 11]. The large proportion of transcriptional regulators that enable cells to quickly adjust to changing environmental conditions contributes to *P. aeruginosa's* capacity to adapt to various settings [12]. By producing a variety of extracellular polysaccharides, *P. aeruginosa* can provide the host organism with additional survival advantages in shifting environmental conditions. Polysaccharides increase the resistance of cells to oxidizing agents, desiccation, and host defence mechanisms [13, 14].

Many plants carry cry genes that defend them from insect diseases, and GMPs based on BT toxin genes account for approximately 19% of all transgenic acreage worldwide [15, 16].

The purpose of this study Evaluation and selection seven genotypes from sorghum under drought stress and under the protective effect of the plant pathogenic microorganism against fungus to reduce the over usage of chemical inputs and to minimize broad use of fungicides, which leads to resistance in plant pathogens. In sustainable agriculture, plant growth promoting (PGP), and biocontrol agents (BCA's) have emerged as eco-friendly alternatives to most of the chemical pesticides. Two bio-agent *Bacillus thuringienese* as a bio-control and *Pseudomonas aeruginosa* as inducer plant growth. Different concentrations of biological control were made for different varieties of sorghum and they were injected into the roots of cultivated plants to enhance the economic characteristics of this crop- attached file- that need to be clarified. I am afraid that without addressing these points carefully, the manuscript cannot be published on its current form.

2. Materials And Methods Plant materials

Seven Sorghum bicolour genotypes, with varied genetic backgrounds, were used in this study. The experiment had two levels: level 1 examined the effects of drought on different varieties of the forage plant Sorghum, and level 2 examined the biological protective effects of two microorganisms acting as natural fungicides on the same stressed plant types.

Drought stress experiment

Seven sorghum varieties (Giza 15, Dorado, Hybrid Sh1, Hybrid Sh306, Sudan grass, Saudi Millet, and Indian Millet) were planted in the field soil using a random complete design (RCD) in three replicates with 50% water scarcity.

The experimental cultivars were planted in the field

One of the seven varieties of sorghum Giza 15, Dorado, Hybrid Sh1, Hybrid Sh306, Sudan grass, Saudi Millet, and Indian Millet had 50 sorghum seeds per line. To provide a natural defence against pests, this study considered seven varieties of sorghum bicolour that were planted in the first season of 2020–2021 while experiencing a 50% water shortage. The most tolerant plants among the varieties were chosen and planted in the second season under treatment with two microorganisms, *B. thuringiensis and P. aeruginosa*. The data were then processed using gel analysis programs to identify novel markers and sequencing was used to identify the genes involved in drought tolerance. The goal of the current study was to breed populations using novel genetic drought tolerance markers.

Bacterial strains

Pseudomonas aeruginosa (Pa) (GenBank accession number (LC215048) and *Bacillus thuringiensis* I977 (BT) are the two bacterial strains. The National Research Centre in Giza, Egypt's Microbial Genetic Department, provided the bacterial strains used in this study. Different strains were created at two concentrations (1.5, 3 ml) and were injected into the surface roots of growing plants.

Growth Media *P. aeruginosa and B. thuringiensis* strains were incubated. According to Davis et al. [17], Luria Broth medium was prepared by mixing tryptone (10 g), yeast extract (5 g), sodium chloride (5 g), and agar (20 g) in 1000 ml of distilled water at 30 °C for 24 h with shaking at 120 rpm.

DNA Markers study

Following a biokit technique, DNA was isolated from seedlings that were 6 days old and weighed approximately 1 g fresh weight to identify genetic markers for drought stress. Seven ISSR primers (Table 2), six SSR markers (Table 3), and five RAPD primers (Table 4) were used. The DNA was stained with ethidium bromide (0.1 g/ml) to determine its quality after electrophoreses for an hour at 100 volts in a 1% agarose gel with 1xTBE buffer.

ISSR-PCR analysis
According to Zietkiewicz *et al.* [18], PCR reactions were performed using ISSR primers. Isolated DNA was used in ISSR-PCR reactions of several samples. 2 l of genomic DNA, 1 l of the primer, 2.5 l of 10X Taq DNA polymerase reaction buffer, 1.5 units of Taq DNA polymerase, and 200 mM of each dNTP make up the reaction mixture in a 25 l container. A thermocycler for DNA amplification (PTC- 100 PCR version 9.0, M J Research-USA) was used. The device was set up to operate under the following conditions: denaturation at 94 °C for 5 min, followed by 35 cycles of 30 s at 94°C, 90 s at the annealing temperature (Table 4: primer sequences and primer code), and 90 s at 72°C. Only reproducible results will be considered for further data analysis once the amplifications have been carried out at least twice.

SSR-PCR analysis
The PCR reactions were optimized, and mixtures according to Litt. and Luty [19] consisted of dNTPs (200 M), Mg Cl2 (1.5 mM), 1x buffer, primer (0.2 M), DNA (50 ng), and Taq DNA polymerase (2 units) in a total volume of 25 µL. Amplification was performed in a thermo cycler set to 94 °C for 3 min (one cycle), then 60 °C for 1 min, 72 °C for 2 min (36 cycles), 72 °C for 10 min (one cycle), and finally 4 °C (infinitive). Amplification products (15 mL) were combined with 3 ml of loading buffer, separated on a 1.5% agarose gel, stained with 0.5 mg/ml ethidium bromide, and visualized under an ultraviolet light source before being captured on a camera. Comparisons with DNA markers were used to calculate the sizes of DNA.

RAPD analysis
Using five optimized RAPD primers, sorghum germplasm DNA was amplified in described method with [20, 21]. RAPDs were used to determine the genetic diversity among the sorghum varieties. Denaturation at 95°C for one minute, annealing at 36°C for one minute, and extension at 72°C for two minutes for 35 cycles comprised the three phases of PCR. The PCR program was set up to maintain the outcomes at 4°C. Six microliters of loading dye (0.50% xylene cyanol and 0.50% bromophenol blue) were added to the goods and spun in a mini centrifuge. The PCR product was electrophoresed on a 1.5% agarose gel, at a voltage of 100 V, and amplified bands were detected using a gel documentation system (Bio-Rad, Hercules) [22, 23].
Gel electrophoresis on 1.5% agarose gels in 1X TAE solution, the ISSR, SSR, and RAPD amplification products were separated using DNA ladders (1Kb for analysis). The polymers were subsequently detectable upon ethidium bromide staining [24]. The PCR products were photographed and documented using a Biometra Bio Doc. Analyse gel documentation technique.
Data analysis Only distinct, clear, and reproducible bands were used for data processing. Each band was regarded as a distinct area. Data will be evaluated as (1) for existence and (0) for absence for each cultivar to distinguish between positive and negative markers. The similarity coefficients generated by SPSS version 10 [25] were used to generate a dendrogram using the unweighted pair group approach with an arithmetical average.

Statistical Analyses
The data were statistically analysed in accordance with Gomez and Gomez [26]. The least significant differences (LSD) were used to compare treatment mean differences at the 5% and 1% levels of probability.
The bands of the ISSR and SSR types were assigned a present (1) or absent (0) number. The scores were then entered into a binary matrix using the PAST (free programes on the web) software [26]. The similarity between the quantitative morphological data and the ISSR and SSR molecular markers was assessed using the Nei and Li/Dice similarity index, and similarity estimations were evaluated using the unweighted pair group method using the arithmetic averages (UPGMA) clustering algorithm [27]. The groupings are shown in the dendrogram. The polymorphic information content (PIC) of each marker was calculated using the formula PIC = 1 Pi 2, where Pi is the band frequency of the gene [28, 29].

3. Results and Discussion Morphological traits

Agro-morphological variation
Table 1 lists the morphological characteristics of seven sorghum accessions to investigate their biodiversity. Some productivity-related morphological features, such the weight of 100 grains, which typically affect the varieties under study as a result of biological therapy while under the influence of drought, have improved. Our results show that all quantitative morphological traits are extremely polymorphic. The research showed that the highest value for Grain weight was in Accession Saudi Millet (76 g), while the lowest value was in Accession Giza 15 (32 g).

The genetic diversity between sunflower genotypes collected in Egypt was evaluated using quantitative morphological traits and molecular marker data, which are helpful tools in varietal development [30]. According to the characterisation, almost all quantitative morphological features investigated revealed considerable variation between sorghum genotypes, as indicated in (Table 1). Analysis of variance (ANOVA) reveals significant differences in many traits, including grain weight. In conclusion, the concentrations of *B. thuringiensis* and *P. aeruginosain* utilised in the study have an impact on the morphological features, with a lower concentration of *B. thuringiensis* and *P. aeruginosain* producing a direct improvement. The adaptable microbe Pseudomonas aeruginosa can live in a variety of conditions. Worms and insects can die from *P. aeruginosa* [32].

Table 1 lists the morphological characteristics of seven different barley accessions

Characters / Genotypes	pH cm))	LS (m^2))	100-GW(g)	pH (cm)	LS (m^2)	100-GW(g)	pH cm))	LS (m^2)	100-GW(g)	Main PH cm))	Main LS (m^2)	Main 100-GW (g)
	Under control			Under biological stress 1.5m/l			Under biological stress 3m/l					
Giza 15	1.56**b	0.89*b	32*c	1.62**a	0.84*b	34*c	1.63**a	0.86*b	37*	±163.33	±0.863	±34.33
Dorado	1.54**b	0.95*a	52*b	1.51**b	0.92*a	55**b	1.53**b	0.94*a	57**b	±1.52.67	±0.937	±54.67
Hybrid Sh1	1.87**a	0.84*b	54**b	1.77**a	0.83*b	55**b	1.82**a	0.83*b	62**b	±1.82	±0.833	±57
Hybrid Sh306	1.60**a	0.74*c	43*b	1.55**b	0.71*c	45*c	1.57**b	0.73*c	47*c	±1.57.33	±0.727	±45
Sudan grass	1.15**c	0.87*b	35*c	1.11**c	0.85*b	35*c	1.14**c	0.86*b	38*c	±1.133	±0.86	±36
Saudi Millet	1.32**c	0.96*a	76**a	1.30**c	0.93*a	80**a	1.31**c	0.95*a	82**a	±1.31	±0.947	±79.33
Indian Millet	1.47**c	0.76*c	48*b	1.42**b	0.74*c	52**b	1.40**b	0.75*c	55**b	±1.43	±0.75	±51.67
LSD							7.86*					

Mean ± Standard deviation, Means with the same litters was not significant difference, *, ** Significant at 0.05 and 0.01 levels, respectively.

Four growth phases can be used to categorise the stages of sorghum development: germination and seedling emergence, post-emergence or early seedling stage, midseason or pre-flowering, and terminal or post-flowering [33]. Poor emergence, plant mortality, and decreased plant stands are frequently consequences of drought and/or heat stress at the seedling stage. When plants maintain their green colour and fill grain regularly after flowering, this indicates drought stress resistance. Additionally, it has been noted that stay-green genotypes are resistant to lodging and charcoal rot [34].

Molecular results

Assessment of ISSR markers

To identify molecular markers for drought tolerance, seven oligonucleotide primers were utilised to create ISSR-PCR fingerprints of the seven sorghum genotypes seeded under biological control with *B. thuringiensis & P. aeruginosa*. These primers were HB, HB11, HB18, p1, p2, p4, and p5. With the various primers, there were significant differences in the quantity and size of the amplified products. Table (2) contains the ISSR-PCR findings for the genotypes of sorghum under investigation. This table makes it evident that these primers produced 26 polymorphic bands in the study samples, with a polymorphism proportion of 57.8%.

The primers P1, P2 and P4 identified 7 amplified DNA (3 of them were monomorphic and 4 polymorphic) with 57.1% polymorphism for each primer which includes sizes from 300 to 2200 bp, while the primers P5 and HB18 generate 5 portions (two were monomorphic and three had been polymorphic) with 62.5% polymorphism of P4 primer and 60% polymorphism of HB18 primer which includes with sizes from 200 to 2500 bp. Primer HB produced 8 amplicons with sizes ranging from 350 to 2400 bp, 3 monomorphic, 5 polymorphic, including one with 62.5% polymorphism. Primer HB11 revealed 6 fragments with sizes ranging from 150 to 1800 bp, 3 of which were monomorphic and 3 of which were polymorphic, including one with 50% polymorphism.

Genetic diversity and relationships

As shown in Fig. 2, the dendrogram created using the Dice coefficient indicated the genetic relationships between the seven sorghum cultivars with various linkage distances. The seven cultivars were divided into two clusters using the dendrogram, with the first grouping being genotype Indian Milt. The second cluster contained four sub-clusters, the first of which was separated by the genotype Sudan grass with 0.04 cm, and the second by the genotype Giza 15 by 0.1 cm. The genotype Hybrid Sh306 caused the third sub-cluster to be split by 0.2 cm. The four sub-clusters, had three genotypes: Saudi Milt, Dorado and Hybrid

Sh1 separated in 0.65 cm.
The high similarity matrix between Hybrid sh1, Dorado and Saudi milt on 0.8cm. The 0.7cm similarity matrix between Saudi milt and Indian Milt; however, the Sudan grass and Hybrid Sh306 on similarity matrix with 0.233; moreover, Indian milt and Hybrid Sh306 with similarity matrix 0.175. The similarity matrix between Saudi milt and Giza15 on 0.09; however, he low similarity matrix between Hybrid sh1 and Sudan grass with 0.075.

Table 2 lists the primers used, their order, and the number and size of amplified fragments (bands) produced in the sorghum by ISSR primers.

Primer codes	Sequence (5' to 3')	Monomorhic bands	Polymorphic bands	Total bands	Polymorphism%
P1	5'-CAGGCCCTTCCC-3'	3	4	7	57.1%
P2	5'-GGTCCCTGACCG-3'	3	4	7	57.1%
P4	5'-GTGACGTAGGAC-3'	3	4	7	57.1%
P5	5'-CCTGGGCTTCGGC-3'	2	3	5	62.5%
HB	5'-CCTGCTCATCC-3'	3	5	8	62.5%
HB11	5'-TGTGTGTGTCC-3'	3	3	6	50%
HB18	5'-CACCACCACGC-3'	2	3	5	60%
Total		19	26	45	57.8%

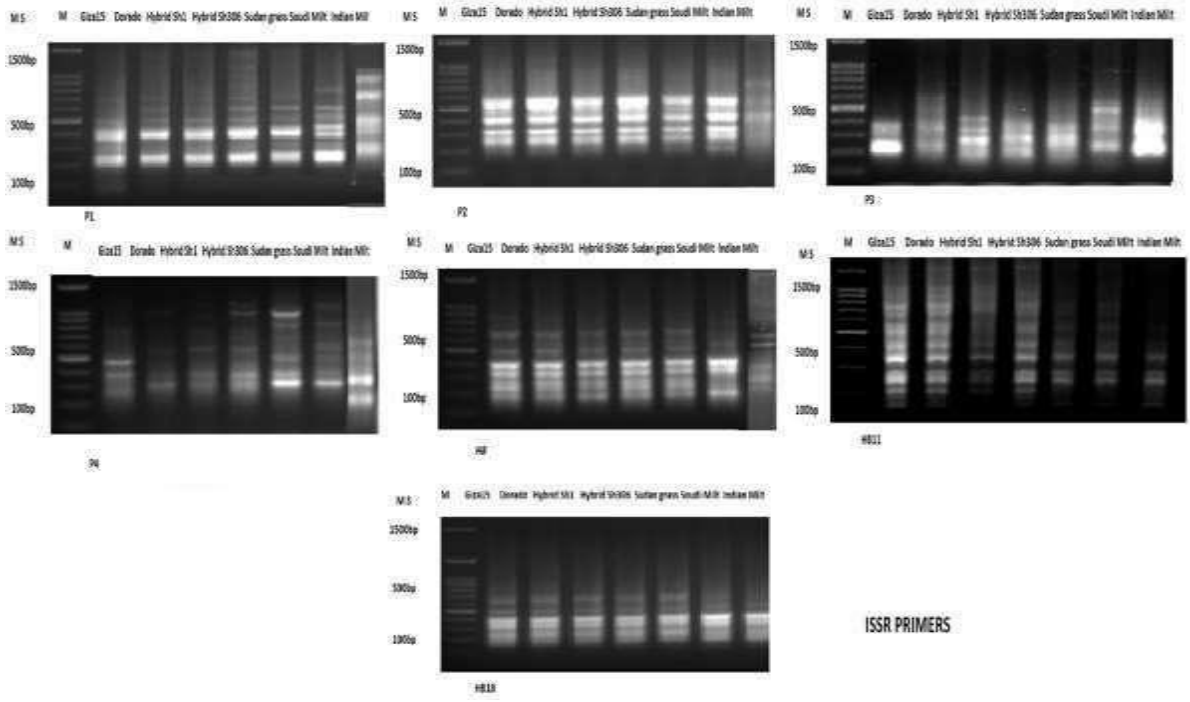

Figure 1 DNA polymorphisms in seven sorghum genotypes (Giza 15, Dorado, Hybrid Sh1, Hybrid Sh306, Sudan grass, Saudi Millet, and Indian Millet) with primer P1, P2, P3, P4, HB, HB11 and HB18.

The information in Table 3 showed that seven ISSR primers were used to create positive and negative specific markers that were used to distinguish between seven different sorghum genotypes. Seven of them were successful in locating seven markers using specialized ISSR primers, four of which were positive markers and three of which were negative for drought stress in seven sorghum varieties (table 3).
The primer P1 generated one marker with size 280 bp showed in the tolerant the genotype (Hybrid sh306) and this band might be used as marker assisted selection (MAS) for this genotype. The P4 primer generated one marker with size 650 bp, HB displayed one specific marker detected in the tolerant genotype (Sud), and this band would be used as marker assisted selection (MAS) for this genotype., One negative marker was found for P2 with a size of 610 bp, while P5 with a size of 512 bp, and HB18

with a size of 240 bp, respectively. This band could be used as marker assisted selection (MAS) for this genotype. HB exhibited one specific marker that was recorded in the tolerant genotype (Giza15).

Table 3 lists seven ISSR primers' positive and negative markers for drought stress in seven sorghum cultivars.

Primer's code	MS	Giza15	Hybrid sh1	Hybrid sh306	Dorado	Sudan grass	Saudi milt	Indian milt	P and N markers
P1	350	0	1	0	0	0	0	0	P
P2	610	1	1	1	1	0	1	1	N
P4	280	0	0	1	0	0	0	0	P
P5	512	1	1	1	1	1	0	1	N
HB	650	0	0	0	0	1	0	0	P
HB11	240	1	0	1	1	1	1	1	N
HB18	450	1	0	0	0	0	0	0	P

- P= positive for biological control and drought tolerance.
- N= negative for biological control and drought stress.

The seven sorghum genotypes have been successfully differentiated by molecular markers using ISSR analysis; it should be noted that these particular markers were regarded as a taxonomic basis among the recent entries and are an important cause of bearing these genotypes for drought-related conditions (Tables 2 and 3). These findings came from [35, 36].

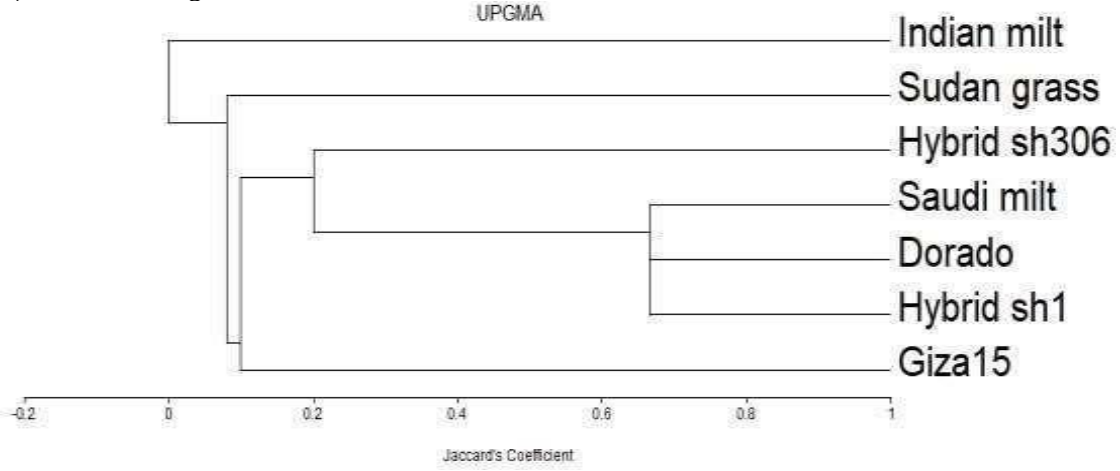

Figure 2 Dendrogram represents the average genetic relationships linkage among seven sorghum using the Minimum Viable Secure Product generated (MVSP) by variety of ordination and cluster analyses program.

Assessment of SSR markers

The seven sorghum genotypes seeded under biological control with *B. thuringiensis & P. aeruginosain* and drought stress were utilised to create SSR-PCR fingerprints with to identify molecular markers for drought resistance. Gpsb123A, mSbCIR238, mSbCIR246, mSbCIR262, mSbCIR283, and mSbCIR300 were the primers used. With the various primers, there were significant differences in the quantity and size of the amplified products. Results of SSR-PCR for the genotypes of sorghum under investigation are shown in (Table 4 & Fig. 2). This table makes it evident that these primers produced 19 polymorphic bands in the study samples, with a polymorphism percentage of 67.86%.

The primers gpsb123A detected five amplicons, two of which were monomorphic and three of which were polymorphic, with a polymorphism level of 60%. In contrast, the primers mSbCIR238, mSbCIR246 and mSbCIR283 produced four fragments, one of which was monomorphic and three of which were polymorphic, with a polymorphism level of 75% for each primer, while the primer.

Table 4: Six SSR Markers with Global Sorghum Characteristics and Genetic Diversity

SSR marker	Forward and Rev. primer sequence (5'-3') & (3'-5')	Chr.	Total loci	Monomorphic loci	Polymorphic loci	Polymorphism%
gpsb123 A	F.ATAGATGTTGACGAAGCA R.GTGGTATGGGACTGGA	8	5	2	3	60
mSbCIR238	F.AGAAGAAAAGGGGTAAGAGC	2	4	1	3	75
	R.CGAGAAACAATTACATGAACC					
mSbCIR246	F.TTTTGTTGCACTTTTGAGC R.GATGATAGCGACCACAAATC	8	4	1	3	75
mSbCIR262	F.GCACCAAAATCAGCGTCT R.CCATTTACCCGTGGATTAGT	5	6	2	4	66.67
mSbCIR283	F.TCCCTTCTGAGCTTGTAAAT R.CAAGTCACTACCAAATGCAC	3	4	1	3	75
mSbCIR300	F.TTGAGAGCGGCGAGGTAA R.AAAAGCCCAAGTCTCAGTGCTA	1	**5**	**2**	3	60
---------			**28**	**9**	19	67.86%

Seven genotypes of sorghum were tested using six primers, and four positive and two negative indicators for drought stress were found. Data in Table (5) and Fig. (2) showed positive and negative specific marker made from seven SSR primers that were used to distinguish between seven sorghum genotypes. Seven of them were successful in locating six markers using specialised ISSR primers, of which four were positive markers and two were negative ones for biological control and drought stress in seven sorghum varieties (Table 5 and Fig. 2).

The mSbCIR238 primer generated one marker with size 450 bp documented in the tolerant genotype (Hybrid sh306) and this band could be used as a marker-assisted selection (MAS) target for this genotype, while 680 bp, mSbCIR262 showed one specific marker was recorded in the tolerant genotype (Hybrid sh1). The primer gpsb123 A showed one particular marker with size 422 bp, which was reported in the results. For mSbCIR246 with a size of 710 bp and mSbCIR283 with a size of 350 bp, respectively, one negative marker was found.

Biological control and drought stress of a specific collection of sorghum germplasm were assessed using SSR markers as part of an internal project at the National Research Centre. This collection of accessions was thought to be representative of the global germplasm that is available to improve this crop. This collection was utilised to enhance and finish prior understanding of the sorghum's evolutionary history and domestication pattern [37]. A representative subset of this collection was selected using this data, and it was of a more manageable size for the full characterization of features important for plant breeding programmes and for the evaluation of allelic diversity in genes involved with variation in such traits [38].

Genetic diversity and relationships

As shown in Fig. 4, the dendrogram created using the Dice coefficient indicated the genetic relationships between the seven sorghum cultivars with various linkage distances. The seven cultivars were divided into two clusters using the dendrogram, with the first grouping being genotype Giza 15. The second cluster contained four sub-clusters, the first of which was separated by the genotype Sudan grass by 0.08 cm, and the second by the genotype Indian Milt by 0.1 cm. The genotype Hybrid Sh306 caused the third sub-cluster to be split by 0.15 cm.The four sub-clusters, however, had three genotypes: Hybrid Sh1 separated in 0.25 cm, whereas Dorado and Saudi Milt did so in 0.8 cm. The barley cultivars could not be distinguished and identified using SDS-PAGE markers. The conservation and use of plant genetic resources in breeding programmes may therefore be aided by expanding our knowledge of them [39, 40].

The high similarity matrix between Dorado and Saudi milt on 0.8cm. The 0.225cm similarity matrix between Hybrid sh1 and Indian Milt; however, the Sudan grass and Hybrid Sh306 on similarity matrix with 0.15; moreover, Saudi milt and Hybrid Sh306 with similarity matrix 0.112. The similarity matrix between Saudi milt and Sudan grass on 0.04; however, he low similarity matrix between Giza15 and Dorado with zero.

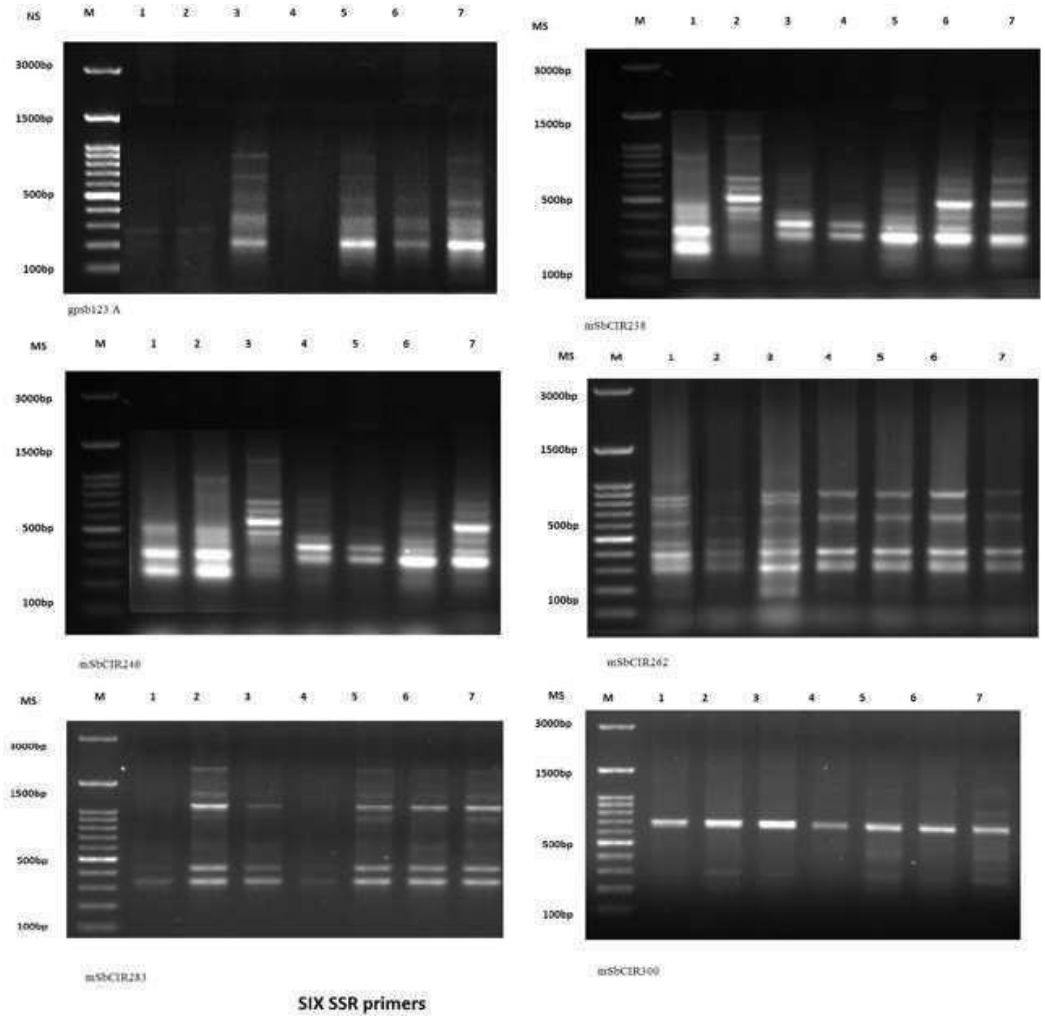

Figure 3 Amplify DNA using six SSR primers for seven sorghum cultivars (Giza 15, Dorado, Hybrid Sh1, Hybrid Sh306, Sudan grass, Saudi Millet, and Indian Millet) with primer gpsb123 A, mSbCIR238, mSbCIR246, mSbCIR262, mSbCIR283 and mSbCIR300.

Table 5 lists six SSR markers for the seven genotypes of sorghum.

Primer's code	MS	Giza15	Hybrid sh1	Hybrid sh306	Dorado	Sudan grass	Saudi milt	Indian milt	P and N markers
gpsb123 A	422	0	1	0	0	0	0	0	P
mSbCIR238	450	0	0	1	0	0	0	0	P
mSbCIR246	710	1	1	1	0	1	1	1	N
mSbCIR262	680	0	0	0	1	0	0	0	P
mSbCIR283	350	1	1	1	1	1	0	1	N
mSbCIR300	210	1	0	0	0	0	0	0	P

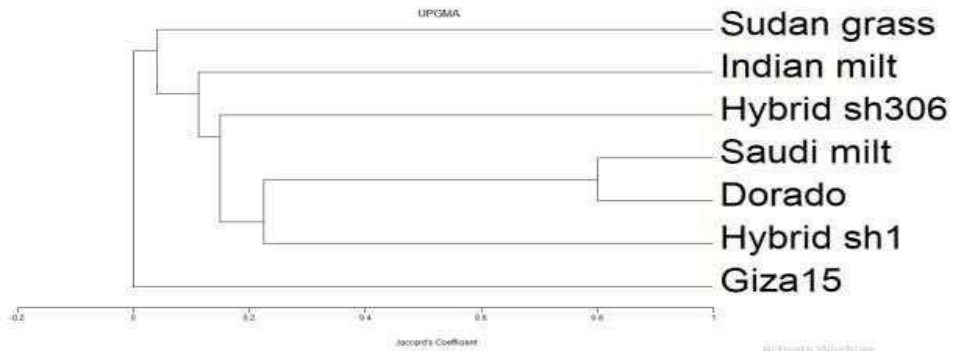

Figure 4 Dendrogram for seven sorghum represents the average genetic relationships linkage using the Minimum Viable Secure Product generated (MVSP) by variety of ordination and cluster analyses program.

Assessment of RAPD markers

By using RAPD-pcr to amplify the seven sorghum accessions, 51 unique bands with a 72.5% polymorphism rate were produced. 37 of these traits were polymorphic. The PCR-amplified fragments' total sizes ranged from 100 bp to 2000 bp (Table 6). For the primers OPB01, OPB05, OPB10, OPB12, and OPB18, the banding patterns of various sorghum genotypes are displayed (Figs. 3). Marker OPB01 generated ten bands, of which three were monomorphic and seven were polymorphic, resulting in a polymorphism rate of 70%. The ten bands, however, were created by OPB05, and of these bands, two had monomorphic and eight were polymorphic, with a polymorphism proportion of 80%. Utilising the OPB10 primer, eleven bands were produced, eight of which were polymorphic (72.7%) and three of which were monomorphic. OPB12 primer generated thirteen bands, including four monomorphic, nine polymorphic, and 69.2% polymorphism. Seven bands were produced by the OPB18 primer, of which two were monomorphic and five were polymorphic, representing a polymorphism percentage of 71.4%.

The ability of RAPD markers to pinpoint connections between various genotypes and show how molecular level yield parameters are related. It is crucial to identify the diversity of barley with to make sure that it can be produced sustainably and used to its maximum potential by barley breeders working with gene banks. This can be achieved through thorough phenotyping and genotyping of the barley collections using state-of-the-art molecular, biochemical, and physiological approaches [41]. figuring out how different media compositions affect different barley genotypes' embryogenic responses and regrowth. Using biochemical and molecular genetics analyses of protein, isozymes, and RAPD-PCR, the somaclonal variation in the three barley genotypes El-kasr, G126, and G130 was successfully identified [42].

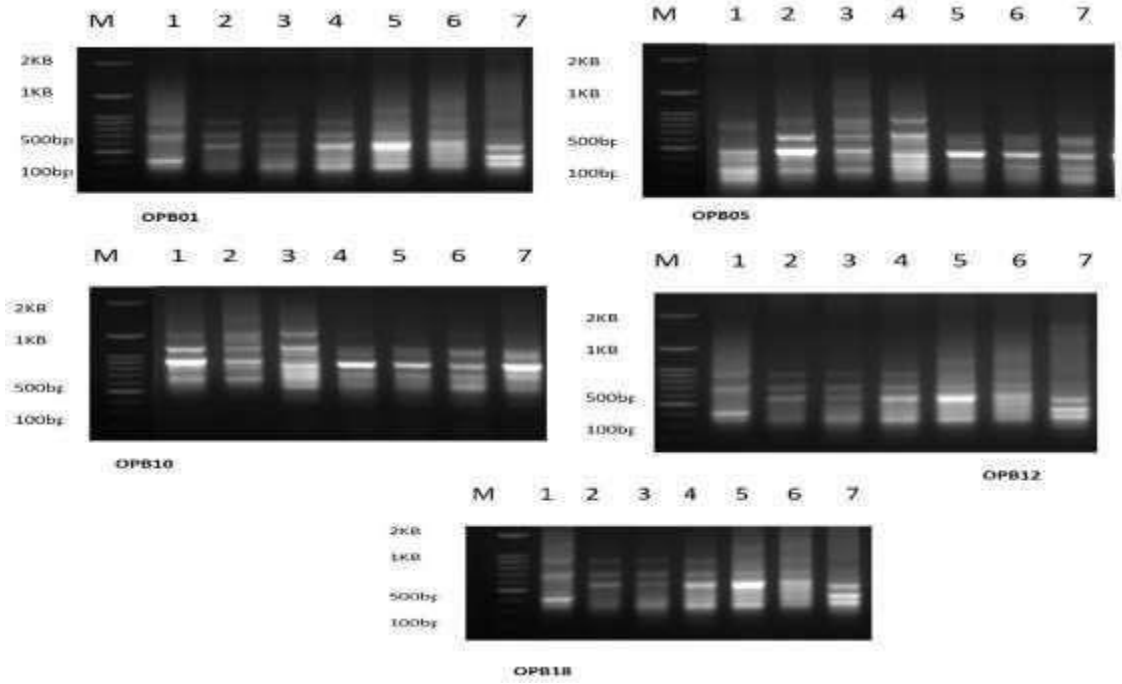

Figure 3 using the OPB01, OPB05, OPB10, OPB12, and OPB18 primer, shows the RAPD profiles of seven genotypes of Sorghum under biological control and drought stress.

Table 6 lists the sequences and RAPD primers employed for sorghum genotypes

No.	Primer code	Sequences	Monomorphic bands	Polymorphic bands	Total bands	Polymorphism%
1	OPB01	5´ GTTTCGCTCC 3´	3	7	10	70%
2	OPB05	5´ TGCGCCCTTC 3´	2	8	10	80%
3	OPB10	5´CTGCTGGGAC 3´	3	8	11	72.7%
4	OPB12	5´CCTTGACGCA 3´	4	9	13	69.2%
5	OPB18	5´CCACAGCAGT 3	2	5	7	71.4%
Total	-	-	14	37	51	72.5%

Cluster analysis of sorghum varieties under biological control and drought stress

(Fig. 4) displays the Dendrogram from the UPGMA clustering study of five RAPD markers from seven different sorghum types. The phenogram showed three separate groups with genetic similarity values ranging from 0.2 to 1.00. Two genotypes of Indian millet and Saudi millet have been combined at a taxonomic distance of 0.5, whereas one genotype of Sudan grass was isolated at a phylogenetic distance of 0.15 from the first Cluster. Dorado and Giza were in the second group. At a taxonomic distance of 0.25, 15 genotypes were clustered together and separated from the other genotypes. The third cluster consists of two genotypes: Hybrid Sh1 and Hybrid Sh306, which were separated from one another by
0.20 taxonomic units.

The Saudi Millet accession had the largest grain weights of 76g under control, 80g under biological stress 1.5m/l, and 82g under biological stress 3m/l, while the Giza 15 accession had the fewest. The findings showed that the Accession Saudi Millet had the highest grain yield (0.947 cm), while the Accession Hybrid Sh306 had the lowest (0.727 cm). Analysis of variance (ANOVA) results reveal significant variations in a many of attributes.

The accessions Hybrid Sh306 and Sudan grass, both two rowed, had the lowest similarity ratio of 0.243%, but Indian Millet and Giza15, both two rowed, had the highest similarity ratio of 0.706%. This can be explained by looking at how these genotypes have evolved in different agroclimatic zones, which implies considerable levels of variation in response to selection pressure as stated by several authors [43, 44]. The morphological dendrogram (Fig. 4) illustrates how cluster analysis revealed a slight link between the regional origin of genotypes and their separation.

Because it has several advantages over utilising simply conventional markers, the use of PCR-based molecular marker technology in breeding programmes and cultivar identification is thus gaining favour. In this case, genetic diversity was investigated using the Inter-simple sequence repeat (ISSR) and simple sequence repeat (SSR) methods, and phylogenetic connections between the sorghum species were determined using DNA fingerprinting [45, 46, 47].

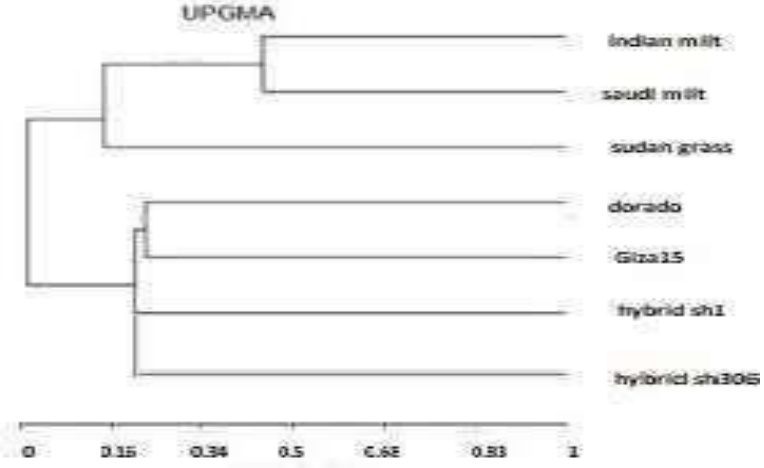

Figure 4 Dendrogram of different kinds of sorghum with RAPD primers.

Table 7 Similarities and differences among seven sorghum genotypes under drought stress.

Genotypes	Giza 15	Dorado	Hybrid Sh1	Hybrid Sh306	Sudan grass	Saudi Millet	Indian Millet
Giza 15	1.000	-	-	-	-	-	-
Dorado	0.422	1.000	-	-	-	-	-
Hybrid Sh1	0.590	0.464	1.000	-	-	-	-
Hybrid Sh306	0.627	0.542	0.475	1.000	-	-	-
Sudan grass	0.497	0.602	0.382	0.243	1.000	-	-
Saudi Millet	0.578	0.590	0.590	0.324	0.443	1.000	-
Indian Millet	0.706	0.566	0.519	0.343	0.486	0.640	1.000

4. Conclusion

Seven sorghum entries were used in the current study, which was carried out on a farm in Egypt's el Sharkia Governorate using 50% less water than usual when it was planted in the first season of 2019- 2020. In order to provide a natural defence against pests, the two-bacteria B. *thuringiensis & P. aeruginosa* were then

planted with the most tolerant plant species in the second season of 2020–2021. The aim of this study is to determine the processes underlying the tolerance of water deficiencies in sorghum plants, in addition to producing plants that are very resistant to this stress. These plants were created using *P. aeruginosa* and *B.* thuringiensis as biological controllers. In addition to high yield, resistance genes will now be transmitted to sensitive indigenous types in plant breeding schemes. In addition, based on the results of all physiological aspects, the best of the seven sorghum parents and their seven crosses were chosen using seven ISSR, six SSR, and five RAPD analysis methodologies. As a measure of drought tolerance, some physiological characteristics were used. The final results showed that genotypes (Giza 15, Dorado, Hybrid Sh1, Hybrid Sh306, Sudan grass, Saudi Millet, and Indian Millet) had high levels of resistance to water stress and biological control by B. thuringiensis and P. aeruginosa, produced the most desirable results for all traits under study for both treatments of irrigation, and were biologically controlled by these organisms. 45 amplicons with 57.8% polymorphism, 19 monomorphic, 26 polymorphic, and 4 different bands were generated using seven ISSR primers. With 67.86% polymorphism and 19 amplicons, including 4 different bands, six SSR primers generated 19 amplicons. Nine of them were monomorphic. Five RAPD primers with a polymorphism of 72.5% generated 51 amplicons, of which 14 were monomorphic and 37 polymorphic, including 4 different bands [48].

The results of the aforementioned study show how reliable and consistent ISSR, SSR, and RAPD analysis is for sorghum genetic diversity studies. A significant degree of variance in the grain sorghum was evident from the clustering pattern. Consequently, ISSR, SSR, and RAPD data were used to reveal the genetic diversity among the sorghum accessions that were under investigation, paving the way for the development of distinct cultivar groups for this crop.

Ethics declaration

The authors attest that all procedures and experiments were completed in accordance with all applicable rules and regulations.

Author contributions

SER, SAAH, EAE design the work, SER, SAAH conducted the experimental work and the manuscript was prepared by SAAH and SER. Each author contributed to the serious debate, data analysis, and text revision.

Conflicts of interest

The authors declare there are no conflicts of interest.

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