The detrimental impact of Drought on *Triticum astevium* plant using SCGE (Single Cell Gel Electrophoresis) and ISSR molecular Marker

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Abstract

Egypt is a good area to study related wheat species in order to improve bread wheat features because it is one of the most important genetic and distribution centers for wheat species. Agronomic parameters, ISSR (inter simple sequence repeats), and SCGE were used (Single Cell Gel Electrophoresis), to study abiotic challenges such as drought. Climate change has increased the impact of drought stress on wheat production, which affects plant growth and development, grain formation, grain quality, and eventually yield. Drought stress has been adapted to by plants in a variety of ways, including genetic adaptations. Four potential wheat lines were tested for genetic diversity and molecular marker identification. There was significant variation in most agronomic traits. Four wheat cultivars (Sakha-8, Gemmiza-7, Giza168, and Sids-1) were subjected to watering levels of 20, 60, and 100% in this study. The Comet assay results revealed that Gemmiza-7 produced low-tailed DNA (8 %), indicating its drought resistance when compared to other varieties. The ten ISSR primers used achieved a rate of polymorphism ranging from 62.5% to 100%, with an average of 83.3% for all primers. Our findings with ISSR molecular markers tested pointed to some distinguishing and unique bands that could be used as cultivar selection tools.

Keywords: *Tritium astevium*; Drought; ISSR; comet assay.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important staple crops, grown on 216.7 million hectares worldwide, which is the third largest crop in terms of area and production with a total production of 765.4 million tons (USDA, 2019). It is a rich source of protein among cereal crops, providing 20% of the total calories to feed 30% of the world's population **(**Igrejas and Branlard, 2020), additionally, it is a significant source of minerals, vitamins, including vitamins B1, B2, B3, and E. (e.g., Se, Mn, P, and Cu). The wheat crop is used to produce alcohol, starch, and straw as well as food, animal feed, and industrial raw materials (Nhemachena and Kirsten, 2017). In recent years increasing wheat production under abiotic stress conditions has become very important. The most common environmental stress is drought, which has negative effects on the growth and development of plants (EL-Hosary et al., 2019).

Many natural disasters, including drought (Thungo et., 2020), result in crop production and quality losses worldwide, and global climatic change makes the situation worse. Simply put, drought is defined as a prolonged period of time, which could be months, seasons, or years, when specific areas' water supplies are depleted which has serious consequences for cultivated crops, lowering productivity due to their response to water scarcity and changes in molecular and biochemical pathways (Kamble et al., 2020). Furthermore, drought stress causes several changes in plant behavior, such as: limiting root system growth affects water absorption,

and changes in nutrient uptake led to a change in chlorophyll content (Fathallah et al., 2020). When abiotic stressors like drought cause stress in wheat plants, H2O2 is created to make up for the lack of electrons in the respiratory and photosynthetic electron transport chains to oxygen. This damages DNA linear validity (Sallam et al., 2019).

Due to seasonal variation in environmental conditions, genetic identification of wheat cultivars/lines based solely on morphological measurements is insufficient, as morphological measurements frequently require extensive phenotypic data and cropping season repetition for screening and evaluation (Gazal et al., 2021).

A gene pool's genetic variation can be evaluated to help with genotype selection, encourage the best genetic advancement, and speed up breeding (Abdelhaliem et al., 2021, Zenda et al., 2021).

It forms the basis for choosing the right parental forms during cross-development and is crucial in the definition of breeding lines, cultivars, or species (Cieplak et al., 2021). Biochemical protein analysis (isozyme test), morphological and agronomic evaluation, and DNA analysis are typically employed to measure genetic variety (molecular markers) (George et al., 2022, Shaban et al., 2022).

The surroundings of plants are exposed to a variety of environmental stresses, such as drought stress, which results in genotoxic and oxidative stress, damages DNA and proteins, and decreases the stability, growth, and productivity of plant genomes (Dutta et al., 2018, George et al., 2021).

The comet assay, Single Cell Gel Electrophoresis (SCGE), which is deemed sensitive, adaptable, and quick for identifying chromosomal and nuclear abnormalities brought on by abiotic drought stress and DNA repair capability, is one of the methods now used to assess and quantify DNA damage (Abotaleb et al., 2021).

In studies on external exposures to both biotic and abiotic agents in the environment, DNA lesion estimated using the comet assay is commonly used as a biomarker of exposure (Azqueta et al., 2022). The comet assay uses a horizontal gel electrophoresis technique to help measure the degree of DNA migration from nuclei, identify double- and single-stranded DNA breaks in a single cell, and detect DNA damage quickly (Koppen et al., 2017).

Siddiqui et al. (2017) Said, analyzing comets was used to Identify DNA damage and detect genotoxicity in a variety of plant parts exposed to external stress, including leaves, roots, seeds, and fruits. As a result, a greater understanding of plant cell processes at the Plant bioassays' usefulness for genotoxicity monitoring and screening can be enhanced by DNA level.

Using ISSR markers, it is possible to evaluate the genetic diversity of wheat germplasm as well as the genetic similarity and dissimilarity of genotypes (George, 2015, Ahmed et al., 2022)**.** ISSR markers have high efficiency, and two primers were enough to differentiate several of the examined durum wheat varieties (George and Hussien 2014, Shaygan et al., 2022). Moreover, Kumar et al. (2020) Said, ISSR markers were discovered to be useful for classifying, identifying, and measuring genetic variation in a variety of wheat genotypes.

Many QTLs in wheat have already been identified. Using the SARP marker, five

markers were found to be connected to cell membrane stability, flag leaf senescence, and chlorophyll concentration (Maqsood et al., 2017).

Eleven SSR markers were discovered to be associated with wheat's relative water content, own length, grain weight per kilogram, coleoptile length, and shoot length (Hao et al., 2020). Water stress tolerance and photosynthetic rate QTLs have also been reported, however, due to the complexity of these qualities, there aren't enough of them to account for all the phenotypic variation in these variables (Maqsood et al., 2017). This research sought to determine whether there were any genetic variations in the germination of wheat grain until seedlings were under drought-stress circumstances.

MATERIAL AND METHODS

Plant kind and growth environment

The Wheat Research Department, Field Crops Research Institute, and Agricultural Research Center provided the wheat grains, Giza, Egypt (Sakha-8 (Sa); Sids-1 (Si); Giza-168 (Gi); and Gemmiza-7 (Ge). (Table 1). Under field circumstances, the grains were cultivated in the greenhouse. After the seedlings emerged, each pot received ten grains of each cultivar, which were then trimmed to five per pot after 14 days and treated twice a week with tap water. On the 16th day, each cultivar's pots were divided into three groups: One was left as a control and watered with one litre of tap water, while the others were forced to grow in arid conditions by receiving only 600 ml and 200 ml of water, respectively, after calculating the pot's water holding capacity. At (35 days). The experimental strategy. Samples from each variety were collected and used for genetic and cytogenetic determination, some leaves were collected and stored in a Deep freezer at -40 C for genetic analyses (Ali et al., 2013).

Comet assay

Isolation of nuclei and preparation suspensions

Untreated and treated (*T. astevium*) grains were used for the isolation of nuclei and their suspension, as described in the study of Mohamed et al. with some modifications, Individual wheat leaves were placed in a small Petri dish with 200 µl of cold 400 mM Tris-HCl buffer (pH 7.5) and 200 µl of water (on ice). Under yellow light, using a razor blade, the leaf was delicately cut into a "fringe" to release nuclei into the buffer. In control cells, a mixture was placed on each slide. of 55 µl of nuclear suspension and 55 µl of LMP agarose (low melting point (LMP) at 40°C After being previously coated with 1 percent agarose: normal melting point (NMP), dried, and the lid slipped. The slide was placed on ice for at least five minutes before the coverslip was removed. After that, 110 µl of LMP agarose (0.5 percent) was poured on the slide. After five minutes on ice, the coverslip was taken off

SCGE (Single Cell Gel Electrophoresis)

Slides containing plant cell nuclei were incubated in a horizontal gel electrophoresis tank with freshly made cold electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH>13) for 15 minutes after being exposed to the mutagen solutions for two hours at 26 degrees. After that, the slides were rinsed three times in ice-cold distilled water for five minutes each time. At 4°C, 30 minutes of

electrophoresis were performed at 16 V, 300 mA. The gels were then stained for 5 minutes with ethidium bromide (20 g/ml) and rinsed three times in 400 mM Tris-HCl, pH 7.5 after staining, in order to conduct an analysis, the gels were immediately immersed in ice-cold pure water. A 546 nm excitation filter and a fluorescence microscope were used to analyze each slide's 50 randomly chosen cells.

DNA extraction and ISSR finger-printing

Plant resources are used to extract DNA.

With certain adjustments, the Tian-gene plant genomic DNA extraction kit was used to recover the genomic DNAs from the analyzed wheat leaves in accordance with the procedures of Esfandani-Bozchaloyi et al. Spectrophotometry and agarose gel electrophoresis were used to evaluate the quantity and quality of the recovered DNAs.

Analysis of ISSR-PCR

The ISSR marker's ten primers were used to amplify the genomic DNA (Table 2) (Khaled et al., 2019).

The PCR reaction was carried out by equal volumes of 30 µl including 15 µl Master Mix (Gene Direx one PCR TM), 2 µl template DNA (10ng), and 2 µl primers. Ten primers were evaluated (Table 3). The amplification reaction, carried out with a Thermal Cycler Model (MG96+), began with a 5 minute denaturation stage at 94°C, followed by 30 cycles: Denaturation took place at 94 °C for 30 seconds, annealing took place at 46 to 52 °C depending on the GC content for 45 seconds, and extension took place at 72 °C for 2 minutes before being completed at 72 °C for 10 minutes. The amplification products were separated by electrophoresis on 1.5 percent agarose in 1x TBE buffer. The gels were photographed using a gel documentation system after being stained with ethidium bromide.

Separation of ISSR amplification products

The ISSR fingerprinting was photographed and visualized using a UV transilluminator. Each band's size was calculated using a 3 kbp DNA ladder as a standard marker. By electrophoresis in 1.5 percent agarose with ethidium bromide made with 100ml TAE buffer, the amplification products were separated.

RESULTS

Comet assay analysis

As seen in the current study's findings (Table 3 and Fig. 1), drought-induced DNA lesions Increased DNA mobility and considerable DNA loss as comet tails in wheat nuclei may weaken the integrity of the plant genome and impede the overall plant's growth and development. In the current investigation, the measure of the percentage of DNA in the tail was the most precise quantitative indication of DNA damage because it reflects the overall intensity of the tail and the total intensity of the comet is independent of the length of the tail. Under these experimental circumstances, DNA fragments moved from the comet's head to its tail, boosting the amount of DNA there. Four cultivars of wheat (Sakha-8, Gemmiza-7, Giza168, and Sids-1) were treated to irrigation levels of 20, 60, and 100% in this study. The findings showed that Gemmiza-7 had low-tailed DNA at a higher drought level (8%) indicating its resistance to drought, followed by drought resistance Sids-1, which had DNA tail (10 %), Giza168 (13%), and Sakha-8 (14%), confirmed that Gemmiza-7 and Sids-1 more resistant to drought than

Sakha-8 and Giza168.

ISSR-based DNA fingerprinting of wheat varieties and drought effect

With 10 primers used to determine the genetic divergence of the examined wheat plants, the Inter Simple Sequence Repeats (ISSRs) technique was used to assess the genetic linkages between control and stressed wheat varieties. The total amount of amplified fragments, monomorphic fragments, distinct bands, polymorphic fragments, and the proportion of polymorphism gained by ISSR primers are displayed in (Table 4). Except for the primer UBC 808, which failed to produce any distinct bands despite the studied wheat cultivars having a high level of polymorphism, other primers successfully produced high polymorphism and unique bands (Table 4 and Fig 2). The ISSR primers' high capacity to produce polymorphic bands allowed them to achieve a rate of polymorphism ranging from 62.5 % (ISSR 10) to 100 % (UBC 835, UBC 808), with an average of 83.3 % for all primers (Fig 3, Table 4).

There were 73 bands amplified in all, 61 (83.56%) of which were polymorphic, while 12 (16.43%) were monomorphic.

It was found that 9 of them, or 12.32 % of all amplified bands, were positive unique bands. In addition, it was discovered that 4 bands, or 5.47 percent of all amplified bands, were negative unique bands. Between 170 and 2050 bp, the average number of total amplified bands per primer was 7.3 bands, as well as 83.3 percent being the average ISSR polymorphism rate (Table 4).

There are unique bands to be found in addition to the high polymorphism bands recognized by all primers, especially UBC 825 and UBC 826, and UBC 827 (Fig.2,3,4, and Table 4).

The tested cultivars were divided into three major groups by the cluster analysis based on ISSR markers (Fig.6). Treatments from the first and second groups (Ge, con, 200, 600 and Gi con, 200, 600, respectively) as well as the third group treatments of (Sa con, 200, 600 and Si con, 200, and 600).

PCA scatter Based on the correlations between the tested wheat strains' ISSR fingerprinting polymorphism, a plot was created using the PAST-pc 4.2 software. The PCA scatter plot clearly distinguished the four subgroups of the tested accessions/species, which is the same as how the clustering analysis separated them.

DISCUSSION

The lack of readily available water is one of the main obstacles to wheat farming. Compared to other plant species, wheat is more susceptible to the climate, the type of soil, and the duration of the day.

Due to their sessile nature, plants are constantly confronted with environmental problems like drought, salinity, ionizing radiation (IR), ultraviolet (UV), and genotoxins, which cause reactive oxidative species (ROS) that can damage their DNA. Single (SSB) and double (DSB) strand breaks as well as clusters of oxidized DNA lesions are both brought on by radiomimetic bleomycin, which acts as a catalyst to create ROS (Cannan and Pederson, 2016). When altering the parameters of the commonly used technique with electrophoresis in 0.3 M NaOH, pH>13 solutions/A test), SSBs and DSBs are easily separated and assessed by comet assay. Under "neutral" conditions,

DSBs are discovered using the N/N test in the conventional electrophoretic buffer (Moore et al., 2019), A/N assays are used to detect SSBs, and an alkali-unwinding step in 0.3 M NaOH is performed before electrophoresis (Alhumaydhi, 2016). Better resolution in DSBs and SSBs assays is observed when "neutral" conditions are set between pH 9-10, still well below DNA denaturing Ph. The kinetics of comet tail generation during the alkaline A/A operation is linked to ssDNA fragments, the ends of which are pushed away from the comet head by the electric force. In contrast, the comet tail in the N/N technique is produced by larger DNA loops. Without a shadow of a doubt, the topological state of DNA influences how rapidly DNA is released (Wang et al., 2017).

By estimating the effect of drought on the nucleic acid of wheat plants using SCGE, discovered that the percentage of tailed (damaged DNA) increases as the level of drought increases. Furthermore, this cytogenetic approach allows us to investigate or distinguish between sensitive and resistant strains (Heikal and Şuţan, 2021).

Alkharabsheh et al. his studies have revealed that the photosynthetic system, membrane lipids, and nucleic acids of plants are all impacted by the stress of drought**.** The mobility of DNA in neutral conditions is related to other analytical limitations of the neutral comet test in plants. The size of plant genomes and protein content are frequently the determinants of variation (Lovecká et al., 2021), which need for adjustments to lysis temperature, duration, and electrophoresis procedures. According to earlier studies, this necessitates a change in the electrophoresis procedures, lysis duration, and temperature (Azqueta et al., 2019). Additionally, found a relationship between the DNA migration's length and the strength of the electric field. These circumstances also have an impact on the degree of DNA damage as measured by the average percentage of DNA in comet tails in control samples of wheat nuclei. According to the survey results revealed in (Table 4, fig.1), Gemmiza-7 and Sids-1 have the highest value of untailed tail (healthy DNA), confirming the fact that prior strains are more tolerant than others in the face of biotic stress. A crop species' genetic diversity must be assessed in order to improve it and create breeding populations that are genetically diverse. In population genetics studies, molecular markers have been employed to distinguish between different genotypes (Dar et al., 2019). Due to their high repetition, low cost, and delivery of genetic information, ISSRs are advantageous among DNA markers. Due to the important information, they offer for comprehending the relationships between species, ISSRs have been frequently used in plants. Consequently, the research's findings might help in the creation of fresh breeding strategies (Liu et al., 2022).

Shaban et al, in their studies under both normal and drought conditions, the genetic diversity of ten prospective wheat genotypes was investigated. Significant agronomic characteristics, as well as a low drought susceptibility index, have been observed by seven SCoT and seven ISSR primers were used to create 112 amplified DNA fragments (EL-Hosary et al., 2019). twelve wheat genotypes were evaluated for their genetic diversity and resistance to drought under two

different irrigation regimes. Flag leaf area, relative water content, and grain yield all decreased as a result of the drought. The five polymorphic ISSR primers generated 25 of the 48 amplicons, (Nesr genotype) and showed the highest total number of amplicons, which indicated greater stress tolerance mechanisms in this genotype, showing the maximum stress tolerance (TOL), stress susceptibility index (SSI), and yield stability index (YSI).

El-Hosary et al, (2019) in another study about grain yield under normal and water-stressed circumstances was notably different. Genotypes of wheat differed in their differences. Gemmiza 11 had the highest grain production, followed by Giza 168. The Yakora Rojo genotype had the lowest yield but was extremely resilient to water stress. The five polymorphic ISSR primers generated 25 of the 48 amplicons. While the primer HB 8 showed 6 amplicons, the primer 844A produced 14 amplicons. Sids 13 and Yakora Rojo, who had the largest total number of amplicons (36 and 35, respectively), may have significant drought resistance. The highest grain yield and stress tolerance index (STI) were produced by cultivars Sids 12, Giza 168, line 127, and Giza 171, whereas Line 145 and Yakora Rojo had the lowest STI.

Our findings showed that all wheat cultivars under drought stress exhibited considerable genetic diversity, and these cultivars could be successfully identified using ISSR primers. DNA polymorphism was estimated to be 83.3 percent on average for all primers based on the number of polymorphic bands and distinct bands found in this investigation. In the current investigation, the ISSRs with the highest percentages of polymorphism were ISSR1, ISSR2, ISSR5, and ISSR7. Haque et al Investigated greater polymorphic band primers are superior at identifying crops and studying genetic diversity. Stress from the drought had an impact on the qualities that contributed to yield and greatly decreased grain output. Sonalika, Sourav, and BARI gom-28 showed the least damage and developed a tolerance for drought. Durum, Pavon-76, BARI Gom-25, and BINA gom-1 were particularly vulnerable and harmed.

Tawfik and El-Mouhamady discovered that the 10 sorghum genotypes indicated 151 pieces as taxonomic divides in the ISSR profile study of the fifteenth sorghum genotypes (38 of them were monomorphic and 113 polymorphic with 74.83 percent polymorphism).

These results are consistent with the finding of El-Moneim, (2020) Studied Cluster analysis employing ISSR and SCoT combine data between examined genotypes were used to identify phylogenetic relationships between Misr, Misr 3, Sids 12, Bani Seuf 7, Sohag 4, Shandaweel 1, Giza 168, and Sakha 95. Using the UPGMA algorithm and Jaccard's similarity coefficients, dendrograms were generated. In this study, the clustering analysis revealed good relationships between drought treatments.

14 wheat (Triticum aestivum L.) cultivars from diverse regions of North Africa were distinguished and identified using the SCoT marker by Ibrahim, M., et al., (2017). These cultivars were then divided into multiple groups based on the generated dendrogram. Although they were occasionally assigned to separate clusters, the cluster analysis demonstrates with certainty that genotypes

from the same origin grouped together (Figure 6).

Finally, our work showed the genetic diversity among important Egyptian wheat cultivars using ISSR markers. More polymorphic ISSR markers have been used to identify certain genetic connections. When examining wheat germplasm for breeding programs, breeders must find innovative specific markers.

CONCLUSION

Global warming and significant climate change are currently harming agriculture. It is urgently necessary to develop high-yielding, stress-tolerant crops in order to maintain the equilibrium between food production and rising human demand because, in the modern world, it is not possible to meet rising needs with existing resources. Drought has a deleterious impact on wheat productivity and grain quality, necessitating the development of drought-tolerant wheat varieties. Sids-1 and Gemmiza-7 are the most drought-resistant types, and they withstand drought without much effort when compared to other varieties, according to our analysis of the drought effect using the Comet test cytogenetic tool and the ISSR molecular marker.

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I want to thanks my supervisiors, my husband, My Son and my mother

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FIGURES

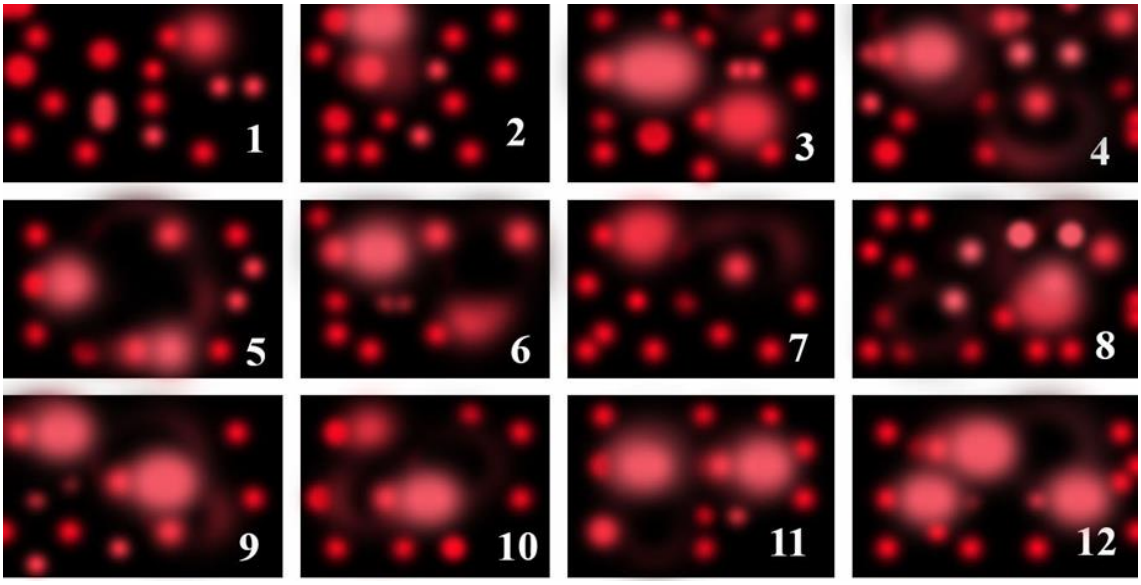

Fig. 1. Comet assay Based on single-cell gel electrophoresis (SCGE), wheat nuclei with and without treatment exhibit varying degrees of nuclear DNA damage (from 1-12 were mentioned before in Table 3).

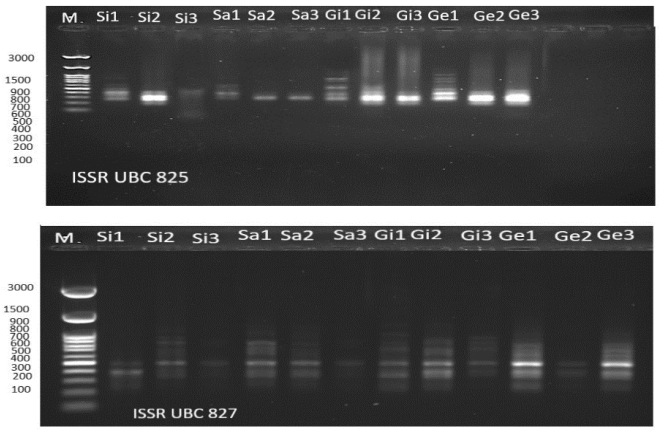

Fig. 2. The Inter Simple Sequence Repeats (ISSR) products of genomic DNA extracted from wheat strain leaves using p-UBC primers (825 and 827). Lane M =3 Kb DNA marker, where Si1 mean Sids-1 control, Si2 (Sids-1 600), Si3 (Sids-1 200), Sa1 refer to (Sakha-8 control), Sa2 (Sakha-8 600), Sa3 (Sakha-8 200), Gi 1 (Giza168 control), Gi2 (Giza168 600), Gi3 (Giza 168 200), while Ge1 (Gemmiza-7 control), Ge2 (Gemmiza-7 600) and Ge3 (Gemmiza-7 200).

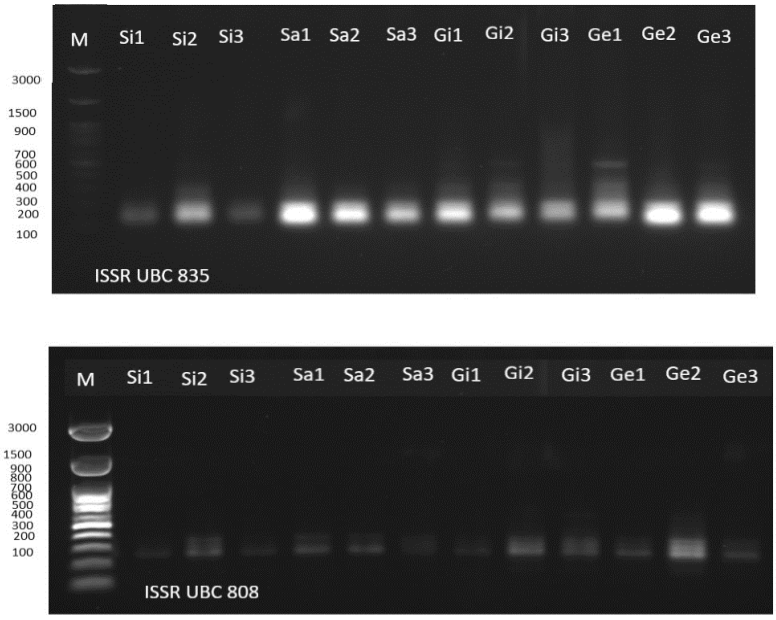

Fig. 3: The Inter Simple Sequence Repeats (ISSR) products of wheat strain leaf genomic DNA obtained using p-UBC primers are shown in Figure 3. (835 and 808). Lane M is a 3 Kb DNA marker, where Si1 refers to the Sids-1 control, Si2 to the Sids-1 600, Si3 to the Sids-1 200, Sa1 to the Sakha-8 control, Sa2 to the Sakha-8 600, Sa3 to the Sakha-8 200, Gi1 to the Giza168 control, Gi2 to the Giza168 600, and Gi3 to the Giza168 200, while Ge1 to the Gemmiza-7 control, Ge2 to (Gemmiza-7 200).

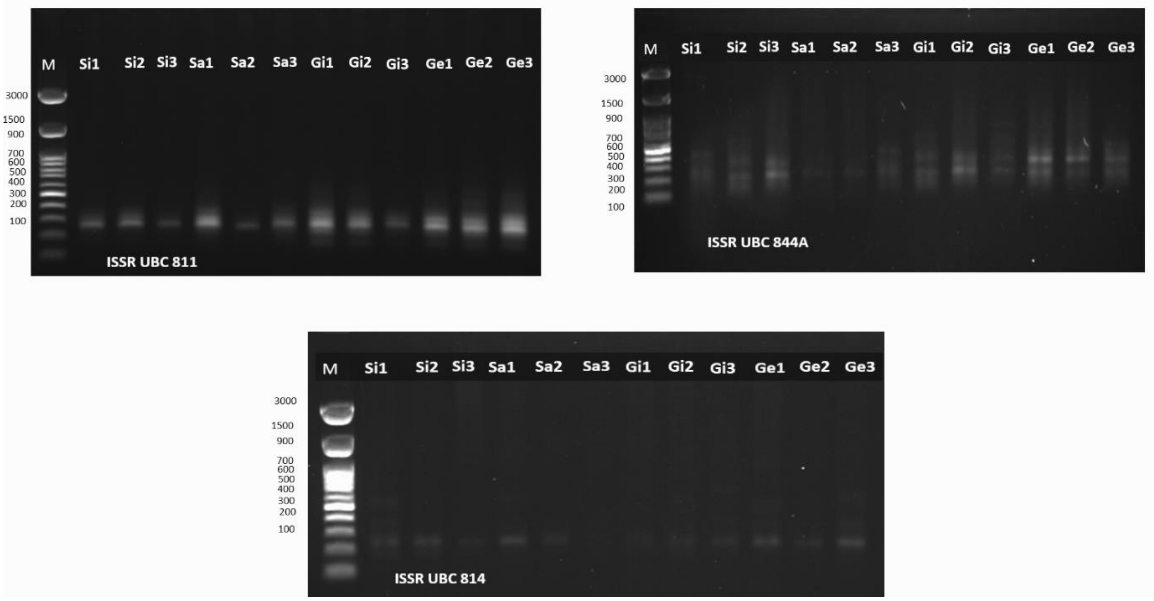

Fig. 4. This figure displays the wheat strain leaf genomic DNA Inter Simple Sequence Repeats (ISSR) products produced using p-UBC primers (811,844 and 814). Lane M is a 3 Kb DNA marker, where Si1 denotes the Sids-1 control, Si2 denotes the Sids-1 600, Si3 denotes the Sids-1 200, Sa1 denotes the Sakha-8 control, Sa2 denotes the Sakha-8 600, Sa3 denotes the Sakha-8 200, Gi1 denotes the Giza168 control, Gi2 denotes the Giza168 600, and Gi3 denotes the Giza168 (Gemmiza-7 200).

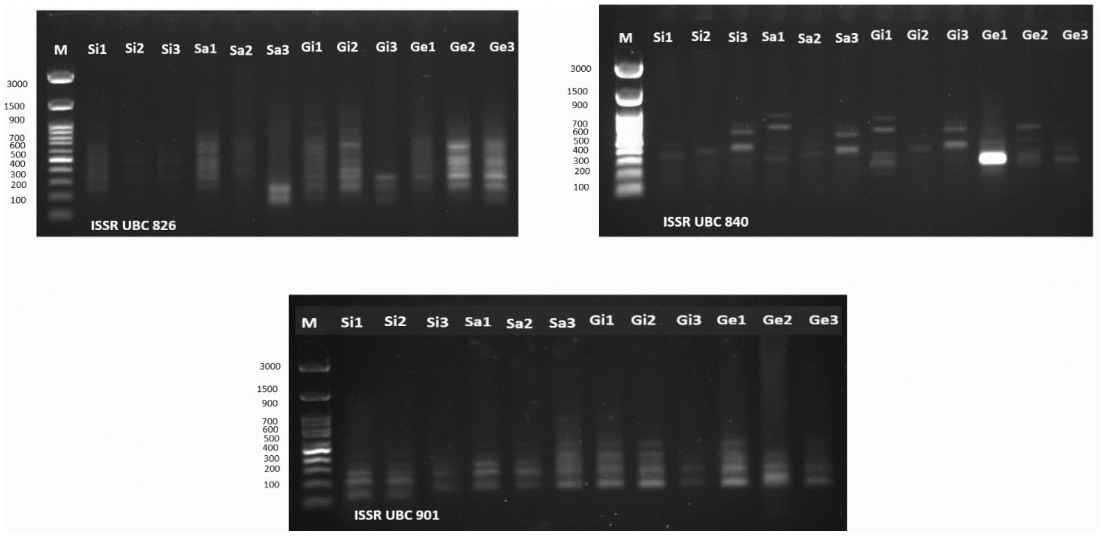

Fig. 5: The wheat strain's leaf genomic DNA Inter Simple Sequence Repeats (ISSR) products are shown in Figure 5 and were generated using p-UBC primers (826,840 and 901). In Lane M, a 3 Kb DNA marker, Si1 stands for the Sids-1 control, Si2 for the Sids-1 600, Si3 for the Sids-1 200, Sa1 for the Sakha-8 control, Sa2 for the Sakha-8 600, Sa3 for the Sakha-8 200, and Gi1 for the Giza168 control, Gi2 for the Giza168 600, and Gi3 for the Giza168 200 (Gemmiza-7 200).

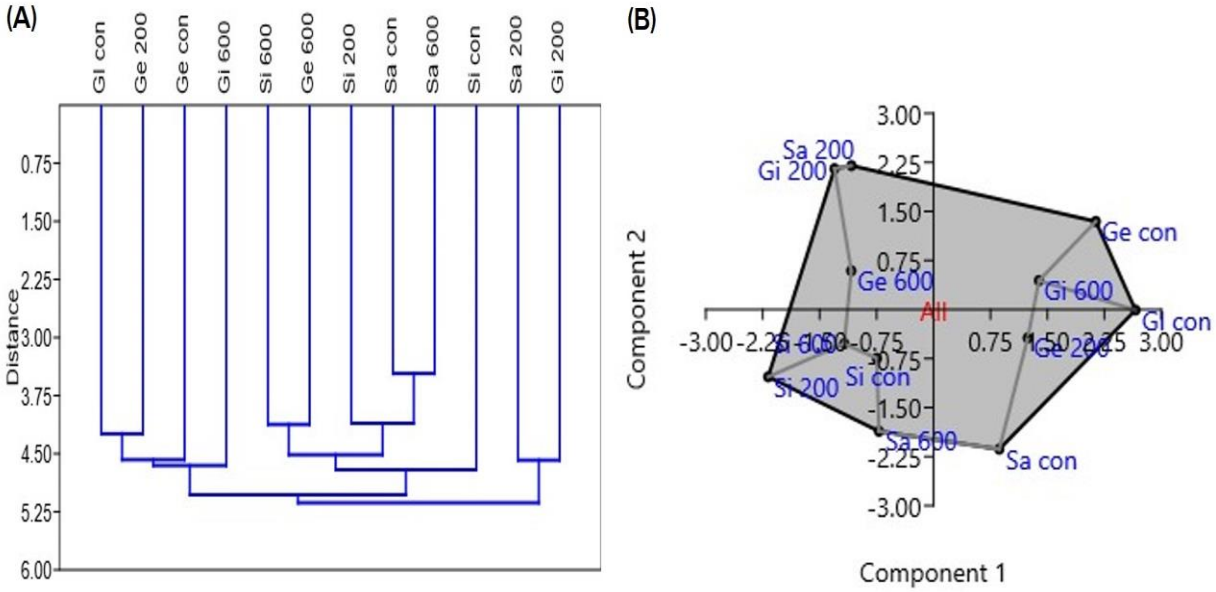

Fig. 6: depicts the phylogenetic tree (Linkage dendrogram) of the researched wheat cultivars based on the ISSR DNA banding patterns, the Clustering analysis (a), and the PCA scatter plot created using the PAST-pc 4.2 software, illustrating the genetic relationships between the researched wheat strains based on the ISSR fingerprinting polymorphism database.

TABLES

Table 1. Origin, pedigree, and selection history of the four varieties of wheat and lines used in the present study.

No.	Genotype	Origin	Pedigree and/or selection history
1	**Sakha-8 (Sa)**	Egypt	INDS/NORTENO PK3418-65-0S-0S
2	**Sids-1 (Si)**	Egypt	HD2172/PAVON//1158.57/MAYA74 SD46-4SD-2SD-1SD-0SD
3	**Giza-168 (Gi)**	Egypt	MRL/BUC//SERI CM93046-8M-0Y-0M-2Y-0B-0GZ
4	**Gemmiza-7 (Ge)**	Egypt	CMH74A.630/5x//Seri82/3/Agent CGM 4611-2GM-3GM-1GM-OGM

Table 2. PCR reaction components were used in the amplification of ISSR primers for the two examined *Triticum aestevium*.

NO.	Primer code	Primer sequence
1	ISSR UBC 825	ACACACACACACACT
2	ISSR UBC 835	AGAGAGAGAGAGAGAYC
3	ISSR UBC 814	CTCTCTCTCTCTCTCAT
4	ISSR UBC 826	ACACACACACACACACC
5	ISSR UBC 827	ACACACACACACACACG
6	ISSR UBC 840	GAGAGAGAGAGAGAGATT
7	ISSR UBC 808	AGAGAGAGAGAGAGAGC
8	ISSR UBC 811	GAGAGAGAGAGAGAGGC
9	ISSR UBC 844A	CTCTCTCTCTCTCTCT AC
10	ISSR UBC 901	CACACACACACACACARY

R: (A or C or T), Y: (C or T)

Table 3. the degree of nuclear DNA damage caused by single-cell gel electrophoresis (SCGE) in wheat nuclei that had been both untreated and treated.

Treatment	Serial	Wheat varieties	Tailed%	Untailed %	(µm) Tail length	Tail DNA %	UNIT Tail moment
1000	1	Sids-1	4	96	1.33	1.02	1.3566
600	2	Sids-1	7	93	1.95	1.66	3.237
200	3	Sids-1	10	90	2.55	2.11	5.3805
1000	4	Gemmiza-7	3	97	1.05	1.26	1.323
600	5	Gemmiza-7	6	94	2.51	2.33	5.8483
200	6	Gemmiza-7	8	92	2.91	3.16	9.1956
1000	7	Giza-168	4	96	1.11	1.27	1.4097
600	8	Giza-168	7	93	1.93	1.53	2.9529
200	9	Giza-168	13	87	2.27	2.06	4.6762
1000	10	Sakha-8	4	96	1.37	1.13	1.5481
600	11	Sakha-8	9	91	2.01	1.88	3.7788
200	12	Sakha-8	14	86	2.33	1.95	4.5435

Table 4. The code and sequences of the ten ISSR primers, Numerous monomorphic and polymorphic (unique and non-unique bands) bands were produced by the ISSR analysis of four wheat genotypes.

No	Primes and the number of their amplification DNA bands					Types of amplified bands					%Of Polymorphism	Genome stability %
	Primer code	Primer sequence	Amplicon lengths (Kbps)	Total no. of DNA bands	% Of amplified bands	Monomorphic bands	Polymorphic bands			Total no. of mono- and polymorphic bands		
							Unique band	Non-unique	Polymorphic bands			
1	ISSR UBC 825	$(AC)_7$ T	2.57:9.90	51	13.9	1	1	1	9	10	92.4	90.0
2	ISSR UBC 835	$(AG)_8$ YC	1.90:6.11	42	13.6	0	0	1	6	6	83.3	100
3	ISSR UBC 814	$(CT)_7$ CAT	2.50:7.55	30	12.9	1	0	0	4	5	86.7	80
4	ISSR UBC 826	$(AC)_8$ C	1.80:9.80	96	7.07	2	4	0	11	13	100	84.6
5	ISSR UBC 827	$(AC)_8$ G	2.10:20.50	83	9.3	1	1	1	10	11	98.6	90.9
6	ISSR UBC 840	$(GA)_8$ TT	250:940	41	9.6	1	1	1	5	6	100	83.3
7	ISSR UBC 808	$(AG)_8$ C	210-580	29	7.06	0	1	0	4	4	83.3	100

8	ISSR UBC 811	$(GA)_7$ GC	170 - 389	28	7.09	1	0	0	3	4	66.2	75.0
9	ISSR UBC 844A	$(CT)_8$ AC	245 - 760	43	12.9	2	0	0	5	7	87.5	66.7
10	ISSR UBC 901	$(CA)_8$ RY	230 - 980	55	12.4	3	1	0	4	7	87.5	62.5
Total DNA bands				498	100	12	9	4	61	73	83.56	-