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# Identifying molecular differences using ISSR markers on three quinoa genotypes (*Chenopodiumm quinoa*) under Sinai conditions, Egypt

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## Abstract

This study aimed to evaluate the performance of three quinoa genotypes (Giza1, Danish KVL3704, and Misr1) under three irrigation intervals (every three, six, and nine days). Genetic diversity among nine quinoa accessions was assessed using eight ISSR primers, yielding robust amplification products and polymorphic fingerprint patterns. A total of 102 bands were generated, averaging 12.75 bands per primer. Among these, 52 fragments were polymorphic, resulting in an average of 6.5 polymorphic bands per primer and an overall polymorphism level of 40.9%. Primer ISSR-8 exhibited the highest polymorphic capacity with 17 polymorphic bands, while primer ISSR-15 displayed the highest frequency (0.9). In contrast, primers ISSR-8 and ISSR-10 exhibited the lowest frequency (0.5). The polymorphism percentage ranged from 20% for primer ISSR-15 to 88% for primer ISSR-10. The similarity index revealed a minimum value of 78% between treatments, clustering the nine accessions into eight groups across four similarity levels, from 80% to a maximum of 93%. Dendrogram analysis underscored the utility of ISSR-PCR in detecting genetic relationships among quinoa accessions. These findings highlight the potential of ISSR-PCR as a reliable tool for genetic diversity studies and its applicability in quinoa breeding programs.

**Keywords:** Quinoa, genotypes, ISSR primers, Similarity index, relationship.

## Introduction

Quinoa (*Chenopodium quinoa*) is a pseudo-grain that produces seed-like grains, commonly used as whole grains in bread and soups and as a versatile ingredient for people with celiac disease. It is considered one of the most nutritious crops, offering a rich source of protein, essential amino acids, and fiber, making it an excellent alternative for gluten-free diets. Quinoa demonstrates significant potential as a sustainable crop for cultivation in salt-affected areas [1]. Water scarcity, climate change, and rapid population growth are significant challenges in Egypt, putting significant pressure on the country's agricultural productivity and food security. Egypt has extensive marginal lands unsuitable for agriculture due to high soil salinity or low-quality groundwater. However, these lands are viewed as an opportunity to enhance agricultural production and ensure food security by cultivating salt-tolerant crops like quinoa, which is considered one of the strategic solutions to cope with the high food demand of the growing population. With quinoa's adaptability to harsh conditions, it could play a critical role in improving agricultural sustainability and helping meet the nutritional needs of the population [2; 3]. Molecular markers are powerful tools for cultivar identification, germplasm conservation, and detecting genetic variability in various species. ISSR (inter-simple sequence repeat) markers are inexpensive and are easily scored manually, and prior knowledge of the flanking sequences is not required, making them ideal for large-scale genetic studies. The development of polymerase chain reaction (PCR) has made PCR-based marker systems key tools in genetic analyses. ISSR-PCR generates reproducible, abundant, and polymorphic multilocus patterns in plant genomes [4; 5].

This study aims to evaluate the performance of various quinoa (*Chenopodium quinoa*) genotypes under different irrigation intervals and stress conditions. With a focus on identifying drought and salinity tolerant genotypes suitable for marginal lands in Egypt. Additionally, to assess the genetic diversity of these quinoa genotypes using ISSR markers to inform breeding programs aimed at developing high-yielding, salt-tolerant quinoa varieties [6; 7].

## Materials and methods

### Field Experiment

The planting and harvesting activities were conducted during the 2023/2024 agricultural season, and following the DNeasy kit technique (Qiagen) [8], DNA was extracted and processed to ensure high-quality DNA for further analysis. To maintain integrity and avoid freezing, fresh quinoa leaf tissue was crushed using a mortar and pestle in liquid nitrogen. After transferring the powdered tissue to a tube, up to 100 mg of wet tissue tissue were mixed with 400 $\mu$l of Buffer AP1 and 4 $\mu$l of RNase A (100 mg/ml). The mixture was vortexed thoroughly, it was incubated at 65°C for 10 minutes to lyse the cells, and then cooled on ice for 5 minutes with the addition of 130 $\mu$l buffer AP2 to precipitate contaminants. The lysate was transferred to a spin column of a QIA shredder and centrifuged at high speed to get rid of cell debris [9].

The flow-through was collected and combined with 1.5 ml of buffer AP3/E. After centrifuging the resultant solution and discarding the flow-through, 650 $\mu$l pieces of it were put to a DNeasy mini spin column. Washing steps were performed using 500 $\mu$l of buffer AW, It was centrifuged once for one minute and then again for two minutes to dry it. Lastly, 100 $\mu$l of preheated (65°C) buffer AE was applied to the DNeasy membrane, incubating for 5 minutes at room temperature, and centrifuging. The elution buffer was 50 $\mu$l for higher concentration DNA [10; 11; 12].

Agarose gel electrophoresis was used to estimate the isolated DNA concentration. A small portion of the DNA sample was loaded onto a 1% agarose gel alongside a DNA ladder with known fragment sizes. The concentration of the extracted DNA can be estimated by comparing the intensity of its bands to those of the DNA ladder [13].

### ISSR-PCR Reactions

A total of 8 ISSR primers **(Table 1)** were used to detect polymorphisms. The amplification reaction was conducted in a 25 $\mu$l reaction volume, consisting of 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 $\mu$M primer, 1 U *Taq* DNA polymerase, and 30 ng template DNA [14].

A Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) was used for the PCR amplification. It was set up to complete 35 cycles following a 5-minute initial denaturation cycle. The steps in each cycle were 94°C for 1 minute of denaturation, 50°C for 1 minute of annealing, and 72°C for 1.5 minutes of elongation. In the final cycle, the primer extension was extended to 7 minutes at 72°C [15; 16].

Electrophoresis was used to resolve the amplification products in a 1.5% agarose gel with 0.5 $\mu$g/ww ml of ethidium bromide in 1X TBE buffer at 95 volts. The criterion for molecular size was a 100 bp DNA ladder. The PCR products were visualized under UV light and photographed using a gel documentation system (BIO-RAD 2000) [17].

**Table 1.** ISSR primer names and sequence.

| No. | Name Primer | Sequence 5'-3' |
|---|---|---|
| 1 | ISSR- 1 | 5'-AGAGAGAGAGAGAGAGYC-3' |
| 2 | ISSR- 5 | 5'-GTGTGTGTGTGTGTGTYG-3' |
| 3 | ISSR- 8 | 5'-AGACAGACAGACAGACGC-3' |
| 4 | ISSR- 10 | 5'-GACAGACAGACAGACAAT-3' |
| 5 | ISSR- 13 | 5'-AGAGAGAGAGAGAGAGYT-3' |
| 6 | ISSR- 14 | 5'-CTCCTCCTCCTCCTCTT-3' |
| 7 | ISSR- 15 | 5'-CTCTCTCTCTCTCTCTRG-3' |
| 8 | ISSR- 16 | 5'-TCTCTCTCTCTCTCTCA-3' |

### Data Analysis

The genetic relatedness of the samples under investigation was ascertained by comparing the banding patterns produced by ISSR-PCR marker tests. Clear and distinct amplification products were scored as 1 for the presence and 0 for the absence of bands. Bands with the same mobility were considered identical. The genetic similarity coefficient (GS) between two genotypes was calculated using the dice coefficient [18; 19].

$$GS_{ij} = \frac{2a}{2a + b + c}$$

The genetic similarity coefficient $GS_{ij}$ represents the genetic similarity between individuals $i$ and $j$, $a$ is the number of bands shared by both $i$ and $j$, $b$ is the number of bands present in $i$ and absent in $j$, and $c$ is the number of bands present in $j$ and absent in $i$.

Cluster analysis was performed on the similarity matrix using the unweighted pair group method with arithmetic mean (UPGMA) [20].

## Results and Discussions

### Polymorphism Analysis Identified Using ISSR Markers

In this study, eight ISSR primers were used for the analysis of nine *Quinoa* accessions, producing amplification products that resulted in polymorphic fingerprint patterns as shown in **Figure 1** and **Table 2**. The eight PCR primers generated a total of 102 bands, with an average of 12.75 bands per primer. Of these 102 amplified fragments, 52 were polymorphic, yielding an average of 6.5 polymorphic bands per primer. This corresponds to a polymorphism rate of 40.9% across the eight primers. Primer ISSR-8 produced the highest number of polymorphic bands, with 17 polymorphic amplification products detected. In contrast, primer ISSR-15 yielded the fewest polymorphic fragments, with only 2 detected. The polymorphism percentage ranged from 20%

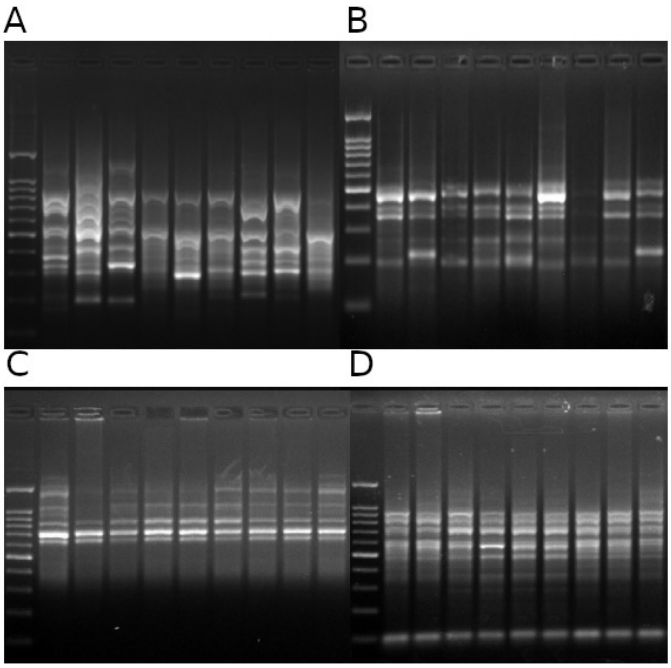


**Figure 1.** ISSR profiles of the using primer ((A) ISSR-8, (B) ISSR-13, (C) ISSR-14 and (D) ISSR- 16). 1 to 9 Samples accession, M: DNA molecular weight marker (100bp DNA ladder).

(for primer ISSR-15) to 88% (for primer ISSR-10). Primer ISSR-15 exhibited the highest frequency (0.9), while primers ISSR-8 and ISSR-10 showed the lowest frequency (0.5).

**Table 2.** Eight ISSR used in this study, the total bands (TB), monmorphic bands (MB), polymorphic bands (PB), percentage of polymorphic bands (%PB) and frequency (F).

| Primer | TB | MB | PB | %PB | F |
|---|---|---|---|---|---|
| ISSR- 1 | 9 | 6 | 3 | 33 | 0.8 |
| ISSR- 5 | 15 | 9 | 6 | 40 | 0.8 |
| ISSR- 8 | 22 | 5 | 17 | 77 | 0.5 |
| ISSR- 10 | 8 | 1 | 7 | 88 | 0.5 |
| ISSR- 13 | 11 | 5 | 6 | 55 | 0.6 |
| ISSR- 14 | 11 | 6 | 5 | 45 | 0.7 |
| ISSR- 15 | 10 | 8 | 2 | 20 | 0.9 |
| ISSR- 16 | 16 | 10 | 6 | 38 | 0.8 |
| Total | 102 | 50 | 52 | | |
| Average | 12.75 | 6.25 | 5.5 | 40.9 | 0.7 |

The results of primer ISSR-1 were illustrated in (**Table S1**). It gave 6 monomorphic and 3 polymorphic bands with different fragment sizes ranging from 160 to 958 bp for *Chenopodium quinoa*. Six common bands were observed across all sites at fragment sizes of 160, 196, 278, 323, 498, and 747 bp. A band with a fragment size of 248 bp was exclusively present in Giza 1 with irrigation every 3 days (I3), Giza 1 with irrigation every 6 days (I6), and Misr 1 across all treatments, but was absent in Giza 1 with irrigation every 9 days (I9) and Danish Kvl3704 in all treatments. A fragment size of 426 bp was exclusively present in Giza 1 (I3 and I6), Danish Kvl3704 (I3), and Misr 1 under all treatments, but it was absent in Giza 1 under I9 and in Danish Kvl3704 under I6 and I9. A fragment size of 958 bp was exclusively present in Danish Kvl3704 with I3 and Misr 1 under all treatments, but it was absent in Giza 1 under all treatments and in Danish Kvl3704 under I6 and I9.

The results of primer ISSR-5 were illustrated in (**Table S2**). It gave 9 monomorphic and 6 polymorphic bands with different fragment sizes ranging from 132 to 989 bp for *Chenopodium quinoa*. Nine common bands were observed across all sites at fragment sizes of 132, 158, 170, 263, 312, 372, 430, 538, and 637 bp. A band with a fragment size of 190 bp was exclusively present in Giza 1 across all treatments, but was not found in Danish Kvl3704 or Misr 1 across all treatments. A fragment size of 214 bp was exclusively present in Misr 1 across all treatments, but was not found in Giza 1 or Danish Kvl3704 across all treatments. A fragment size of 235 bp was exclusively present in Giza 1 and Misr 1 across all treatments, but was not found in Danish Kvl3704 across all treatments. In contrast, a fragment size of 703 bp was exclusively present in Giza 1 and Danish Kvl3704 across all treatments, but was absent in Misr 1 across all treatments. A fragment size of 772 bp was exclusively present in Misr 1 across all treatments, but was not found in Giza 1 or Danish Kvl3704 across all treatments. A fragment size of 989 bp was exclusively present in Giza 1 (I3), Danish Kvl3704 (I6), and Misr 1 across all treatments, but was absent in Giza 1 (I6 and I9) as well as Danish Kvl3704 (I3 and I9).

The results of primer ISSR-8 were illustrated in (**Table S3**). It gave 5 monomorphic and 17 polymorphic bands with different fragment sizes ranging from 180 to 1250 bp for *Chenopodium quinoa*. Five common bands were observed in all sites at fragment sizes of 350, 460, 500, 830, and 1100 bp. A band with a fragment size of 180 bp was exclusively present in Giza 1 in (I3), but it was not found in Giza 1 with (I6 and I9), Misr 1 under all treatments, and Danish Kvl3704 under all treatments. A fragment size of 200 bp was exclusively present in Giza 1 under all treatments, but it was not found in Misr 1 under all treatments and Danish Kvl3704 under all treatments. Respectively, a fragment size of 210 bp was exclusively present in Danish Kvl3704 with (I9), Misr 1 in (I3), as well as Misr 1 with (I6), but it was not found in Giza 1 under all treatments, Danish Kvl3704 in (I3 and I6), and Misr 1 with (I9). A fragment size of 240 bp was exclusively present in Danish Kvl3704 under all treatments and Misr 1 with (I6 and I9), but it was not found in Giza 1 under all treatments and Misr 1 under (I3). A fragment size of 260 bp was exclusively present in Giza 1 under (I3 and I6), Danish Kvl3704 in (I3 and I9), as well as Misr 1 with (I3 and I9), but it was not found in Giza 1 under (I9), Danish Kvl3704 with (I6), and Misr 1 under (I6). Respectively, a fragment size of 290 bp was exclusively present in Giza 1 in (I3 and I6), Danish Kvl3704 with (I6), and Misr 1 under (I3), but it was not found in Giza 1

with (I9), Danish Kvl3704 under (I3 and I9), as well as Misr 1 with (I6 and I9).

A fragment size of 300 bp was exclusively present in Giza 1 under (I9), Danish Kvl3704 with (I3 and I9), as well as Misr 1 under (I6 and I9), but it was not found in Giza 1 with (I3 and I6), Danish Kvl3704 under (I6), and Misr 1 with (I3). A fragment size of 320 bp was exclusively present in Giza 1 with all treatments and Danish Kvl3704 under (I6), but it was not found in Danish Kvl3704 under (I3 and I9) and Misr 1 with all treatments. Respectively, a fragment size of 390 bp was exclusively present in Giza 1 under all treatments and Danish Kvl3704 in (I3), but it was not found in Danish Kvl3704 with (I6 and I9) as well as Misr 1 under all treatments. A fragment size of 410 bp was exclusively present in Danish Kvl3704 under (I6 and I9), but it was not found in Giza 1 with all treatments, Danish Kvl3704 in (I3), and Misr 1 under all treatments. A fragment size of 610 bp was exclusively present in Giza 1 under (I6 and I9) as well as Misr 1 in (I6), but it was not found in Giza 1 under (I3), Danish Kvl3704 under all treatments, and Misr 1 with (I3 and I9). A fragment size of 650 bp was exclusively present in Giza 1 under (I3 and I9) as well as Misr 1 with (I3), but it was not found in Giza 1 under (I6), Danish Kvl3704 in all treatments, as well as Misr 1 with (I6 and I9). A fragment size of 730 bp was exclusively present in Giza 1 with all treatments, Danish Kvl3704 under (I9), and Misr 1 under (I3 and I6), but it was not found in Danish Kvl3704 with (I3 and I6) as well as Misr 1 with (I9). Respectively, a fragment size of 900 bp was exclusively present in Giza 1 with (I6), but it was not found in Giza 1 with (I3 and I9), Danish Kvl3704 under all treatments, and Misr 1 with all treatments. A fragment size of 1000 bp was exclusively present in Giza 1 with (I6), but it was not found in Giza 1 under (I3 and I9), Danish Kvl3704 with all treatments, and Misr 1 under all treatments. A fragment size of 1150 bp was exclusively present in Giza 1 under all treatments, but it was not found in Danish Kvl3704 with all treatments and Misr 1 in all treatments. A fragment size of 1250 bp was exclusively present in Giza 1 with (I9), but it was not found in Giza 1 with (I3 and I6), Danish Kvl3704 in all treatments, and Misr 1 under all treatments.

The results of primer ISSR-10 were illustrated in **(Table S3)**. It gave 1 monomorphic and 7 polymorphic bands with different fragment sizes ranging from 265 to 840 bp for *Chenopodium quinoa*. One common band was observed in all sites at a fragment size of 316 bp. A band with a fragment size of 265 bp was exclusively present in Giza 1 under (I6 and I9), Danish Kvl3704 in all treatments, and Misr 1 with all treatments, but it was not found in Giza 1 with (I3). A fragment size of 378 bp was exclusively present in Giza 1 under (I3 and I9), Danish Kvl3704 with all treatments, as well as Misr 1 under (I3 and I9), but it was not found in Giza 1 with (I6) and Misr 1 under (I6). A fragment size of 426 bp was exclusively present in Danish Kvl3704 under all treatments and Misr 1 in (I6), but it was not found in Giza 1 under all treatments as well as Misr 1 with (I3 and I9). A fragment size of 462 bp was exclusively present in Giza 1 with (I9) and Misr 1 under (I6), but it was not found in Giza 1 under (I3 and I6), Danish Kvl3704 with all treatments, as well as Misr 1 under (I3 and I9). A fragment size of 570 bp was exclusively present in Giza 1 under (I9), Danish Kvl3704 with (I6 and I9), as well as Misr 1 in (I9), but it was not found in Giza 1 under (I3 and I6), Danish Kvl3704 in (I3), and Misr 1 with (I3 and I6). A fragment size of 699 bp was exclusively present in Danish Kvl3704 under (I6 and I9) and Misr 1 with (I9), but it was not found in Giza 1 under all treatments, Danish Kvl3704 with (I3), and Misr 1 under (I3 and I6). A fragment size of 840 bp was exclusively present in Giza 1 with (I9), but it was not found in Giza 1 under (I3 and I6), Danish Kvl3704 with all treatments, and Misr 1 under all treatments.

The results of primer ISSR-13 were illustrated in (**Table S4**). It gave 5 monomorphic and 6 polymorphic bands with different fragment sizes ranging from 177 to 1074 bp for *Chenopodium quinoa*. Five common bands were observed in all sites at fragment sizes of 177, 264, 345, 397, and 472 bp. A band with a fragment size of 214 bp was exclusively present in Giza 1 under (I6) as well as Misr 1 with (I9), but it was not found in Giza 1 under (I3 and I9), Danish Kvl3704 with all treatments, as well as Misr 1 under (I3 and I6). A fragment size of 217 bp was exclusively present in Giza 1 with (I3), Danish Kvl3704 under (I3 and I9), and Misr 1 with (I6), but it was not found in Giza 1 under (I6 and I9), Danish Kvl3704 with (I6), and Misr 1 in (I3 and I9). A fragment size of 510 bp was exclusively present in Giza 1 under (I3) and Danish Kvl3704 with (I3 and I6), but it was not found in Giza 1 with (I6 and I9), Danish Kvl3704 in (I9), and Misr 1 under all treatments. Respectively, a fragment size of 550 bp was exclusively present in Giza 1 with (I9 and I6), Danish Kvl3704 in (I9), and Misr 1 under all treatments, but it was not found in Giza 1 under (I3) as well as Danish Kvl3704 with (I3 and I6). A fragment size of 666 bp was exclusively present in Giza 1 with (I3 and I6) as well as Danish Kvl3704 under (I9), but it was not found in Giza 1 under (I9), Danish Kvl3704 with (I3 and I6), and Misr 1 under all treatments. A fragment size of 1074 bp was exclusively present in Danish Kvl3704 with (I9), but it was not found in Giza 1 under all treatments, Danish Kvl3704 with (I3 and I6), and Misr 1 under all treatments.

The results of primer ISSR-14 were illustrated in **(Table S5)**. It gave 6 monomorphic and 5 polymorphic bands with different fragment sizes ranging from 720 to 2000 bp for *Chenopodium quinoa*. Six common bands were observed in all sites at fragment sizes of 720, 790, 860, 950, 1200, and 1500 bp. A band with a fragment size of 1050 bp was exclusively present in Giza 1 with (I3) and Misr 1 under (I9), but it was not found in Giza 1 with (I6 and I9), Danish Kvl3704 under all treatments, as well as Misr 1 under (I3 and I6). A fragment size of 1100 bp was exclusively present in Giza 1 under (I9) and Danish Kvl3704 with (I9), but it was not found in Giza 1 with (I3 and I6), Misr 1 under all treatments, and Danish Kvl3704 in (I3 and I6). A fragment size of 1350 bp was exclusively present in Giza 1 under (I9) and Misr 1 with (I9), but it was not found in Giza 1 under (I3

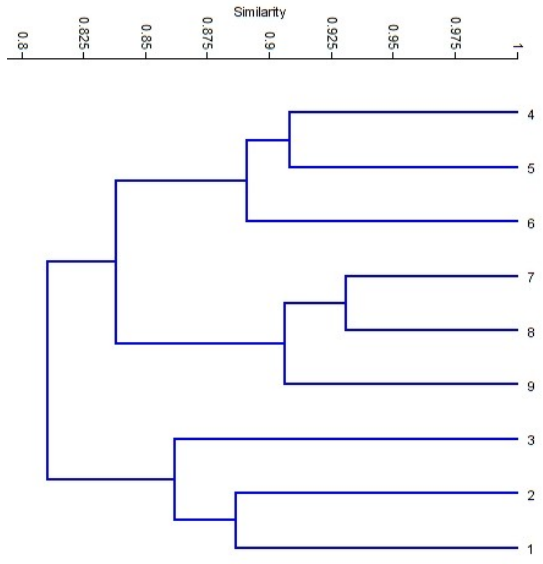


**Figure 2.** Dendogram of similarity index (SI) between the nine studied treatments.

and I6), Danish Kvl3704 in all treatments, as well as Misr 1 with (I3 and I6). A fragment size of 1650 bp was exclusively present in Giza 1 with (I3) and Misr 1 under (I9), but it was not found in Giza 1 under (I6 and I9), Danish Kvl3704 with all treatments, and Misr 1 under (I3 and I6). A fragment size of 2000 bp was exclusively present in Giza 1 with (I3), Danish Kvl3704 under (I9), and Misr 1 in (I9), but it was not found in Giza 1 under (I6 and I9), Danish Kvl3704 in (I3 and I6), and Misr 1 with (I3 and I6).

The results of primer ISSR-15 were illustrated in (**Table S6**). It gave 8 monomorphic and 2 polymorphic bands with different fragment sizes ranging from 310 to 1050 bp for *Chenopodium quinoa*. Eight common bands were observed in all sites at fragment sizes of 310, 490, 520, 630, 780, 850, 960, and 1050 bp. A band with a fragment size of 420 bp was exclusively present in Giza 1 with (I6), Danish Kvl3704 under all treatments, and Misr 1 with all treatments, but it was not found in Giza 1 under (I3 and I9). A fragment size of 720 bp was exclusively present in Giza 1 with (I3), but it was not found in Giza 1 with (I6 and I9), Danish Kvl3704 under all treatments, and Misr 1 with all treatments.

The results of primer ISSR-16 were illustrated in (**Table S7**). It gave 10 monomorphic and 6 polymorphic bands with different fragment sizes ranging from 136 to 944 bp for *Chenopodium quinoa*. Ten common bands were observed in all sites at fragment sizes of 136, 342, 144, 465, 390, 546, 702, 741, 814, and 862 bp. A band with a fragment size of 324 bp was exclusively present in Danish Kvl3704 with (I9) and Misr 1 under all treatments, but it was not found in Giza 1 with all treatments, Danish Kvl3704 under (I3 and I6), as well as Misr 1 in all treatments. A fragment size of 275 bp was exclusively present in Giza 1 with all treatments and Danish Kvl3704 under all treatments, but it was not found in Misr 1 under all treatments. A fragment size of 502 bp was exclusively present in Misr 1 with all treatments, but it was not found in Misr 1 under all treatments, Giza 1 in all treatments, and Danish Kvl3704 with all treatments. A fragment size of 591 bp was exclusively present in Giza 1 under all treatments and Misr 1 with all treatments, but it was not found in Danish Kvl3704 with all treatments. A fragment size of 908 bp was exclusively present in Danish Kvl3704 under all treatments and Misr 1 with all treatments, but it was not found in Giza 1 under all treatments. A fragment size of 944 bp was exclusively present in Giza 1 under all treatments, but it was not found in Danish Kvl3704 under all treatments and Misr 1 with all treatments.

**Table 3.** Similarity index (SI) of nine treatments based on ISSR PCR analysis.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 100 | | | | | | | | |
| 2 | 89 | 100 | | | | | | | |
| 3 | 85 | 88 | 100 | | | | | | |
| 4 | 82 | 83 | 81 | 100 | | | | | |
| 5 | 81 | 81 | 81 | 91 | 100 | | | | |
| 6 | 78 | 80 | 81 | 89 | 89 | 100 | | | |
| 7 | 83 | 85 | 81 | 85 | 82 | 83 | 100 | | |
| 8 | 78 | 82 | 81 | 87 | 81 | 84 | 93 | 100 | |
| 9 | 80 | 81 | 79 | 85 | 82 | 84 | 91 | 90 | 100 |

The similarity index (SI) based on ISSR–PCR is shown in **Table 3** as a cluster analysis in percentage. Among all studied treatments, the similarity index recorded the highest value between treatments (7 and 8), while the lowest value was recorded (78%) between treatments (6 and 1).

The dendrogram in (**Figure 2**) was based on the results of the similarity index. It was stratified into eight clusters indicating the relationship between the nine studied treatments, arranged in four levels of similarity from (80%) as the lowest level to (93%) as the highest recorded level.

In this respect, [21] have used seven ISSR markers to characterize and genetically compare five quinoa genotypes. The results revealed 85 loci, of which 37 were polymorphic, accounting for 43.53% of the total. The number of amplified loci varied from four (UBC-817) to 26 (UBC-845). The size of the amplified fragments ranged from 139 to 1,111 bp. Additionally, the overall PIC values ranged from 0.00 to 0.4. Likewise, [22] have used 10 ISSR primers to genetically differentiate among five quinoa genotypes. The results yielded 53 amplicons, 33 of which were polymorphic, resulting in an average polymorphism percentage of 61.83%. With an average of 5.3 pieces per primer, the range of amplicons per primer was three to ten. There were between one and six polymorphic amplicons, with an average of 3.3 pieces per primer. Finally, the size of the amplified fragments varied depending on the ISSR primer employed, ranging from 130 to 1,456 bp. Fifteen ISSR primers were employed in this study, producing a total of 172 amplified bands, with an average of 11.7 bands per primer. A total number of 90 amplified polymorphic

bands were obtained, with an average of 6.0 bands per primer; the average level of polymorphism was 49.7%. The effectiveness of ISSR markers in distinguishing the studied genotypes was assessed by calculating the PIC values, which ranged from 0.21 to 0.84, with an average value of 0.69 [23].

## Conclusions

The obtained deference's of the dendrogram indicated that ISSR–PCR was useful in detecting the similarity between the studied treatments. Also, it could be recommended as a suitable candidate method between the studied treatment in further breeding ISSR programs to evaluate some quinoa genotypes to different irrigation intervals on stress conditions.

## Supplementary

Table S1: Survey of olymorphic and monomorphic ISSR bands in Chenopodium Quinoa using primer ISSR-1. Table S2: Survey of polymorphic and monomorphic ISSR bands in Chenopodium Quinoa using primer ISSR-5. Table S3: Survey of polymorphic and monomorphic ISSR bands in Chenopodium Quinoa using primer ISSR-8. Table S4: Survey of polymorphic and Monomorphic ISSR bands in Chenopodium Quinoa using primer ISSR-10. Table S5: Survey of polymorphic and Monomorphic ISSR bands in Chenopodium Quinoa using primer ISSR-13. Table S6: Survey of polymorphic and Monomorphic ISSR bands in Chenopodium Quinoa using primer ISSR-14. Table S7: Survey of polymorphic and Monomorphic ISSR bands in Chenopodium Quinoa using primer ISSR-15. Table S8: Survey of polymorphic and Monomorphic ISSR bands in Chenopodium Quinoa using primer ISSR-16.

## Reference


1. Wali AM, Kenawey M, Ibrahim O, El Lateef EA. Productivity of Quinoa (*Chenopodium quinoa L.*) under new reclaimed soil conditions at the northwestern coast of Egypt. Bulletin of the National Research Centre. 2022;46(1):38.

2. ZOHRY AEH. Prospects of quinoa cultivation in marginal lands of Egypt. Moroccan Journal of Agricultural Sciences. 2020;1(3).

3. Mahmoud AH. Production of quinoa (*Chenopodium quinoa*) in the marginal environments of South Mediterranean region: Nile Delta, Egypt. Egyptian Journal of Soil Science. 2017;57(3):329-37.

4. Saad-Allah KM, Youssef MS. Phytochemical and genetic characterization of five quinoa (*Chenopodium quinoa Willd.*) genotypes introduced to Egypt. Physiology and Molecular Biology of Plants. 2018;24(4):617-29.

5. Kumar LD, Kathirvel M, Rao G, Nagaraju J. DNA profiling of disputed chilli samples (*Capsicum annum*) using ISSR-PCR and FISSR-PCR marker assays. Forensic Science International. 2001;116(1):63-8.

6. Adams RP, Schwarzbach AE, Pandey RN. The concordance of terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: an example from Juniperus. Biochemical Systematics and Ecology. 2003;31(4):375-87.

7. Belmonte C, Vasconcelos ES, Tsutsumi CY, Lorenzetti E, Hendges C, Coppo JC, et al. Agronomic and productivity performance for quinoa genotypes in an agroecological and conventional production system. American Journal of Plant Sciences. 2018;9:880-91.

8. Dellaporta SL, Wood J, Hicks JP. A plant DNA min Preparation, Version III. Plant Molecular Biology Report. 1983;1:19-21.

9. Joshi SP, Gupta VS, Aggarwal RK, Ranjekar PK, Brar DS. Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus Oryza. Theoretical and Applied Genetics. 2000;100(8):1311-20.

10. Iqbal H, Yaning C, Waqas M, Shareef M, Raza ST. Differential response of quinoa genotypes to drought and foliage-applied H2O2 in relation to oxidative damage, osmotic adjustment and antioxidant capacity. Ecotoxicology and Environmental Safety. 2018;164:344-54.

11. Castillo CD, Winkel T, Mahy G, Bizoux JP. Genetic structure of quinoa (*Chenopodium quinoa Willd.*) from the Bolivian altiplano as revealed by RAPD markers. Genetic Resources and Crop Evolution. 2007;54(4):897-905.

12. Ebrahim MEA. Evaluation of some (*Chenopodium quinoa*) cultivars as a new cash crop halophyte in Egypt. Ain Shams University; 2018. Thesis, pp. 4–5.

13. Ebrahim MEA, Hussin SA, Abdel-Ati AA, Ali SH, Eisa SS. Evaluation of some (*Chenopodium quinoa*) cultivars under saline soil conditions in Egypt. Arab University Journal of Agricultural Sciences, Ain Shams University, Cairo. 2018;26(1):337-47.

14. Gemmill CE, Grierson ER. Inter-simple sequence repeats (ISSR), microsatellite-primed genomic profiling using universal primers. Molecular Plant Taxonomy: Methods and Protocols. 2021:249-62.

15. Tan M, Temel S. Performance of some quinoa (*Chenopodium quinoa Willd.*) genotypes grown in different climate conditions. Turkish Journal of Field Crops. 2018;23(2):180-6.

16. Telahigue DC, Aljane F, Yahia LB, Belhouchett L, Toumi L. Grain yield, biomass productivity and water use efficiency in quinoa (*Chenopodium quinoa Willd.*) under drought stress. Journal of Scientific Agriculture. 2017;1:222-32.

17. Mahood A, Hama-Salih F. Characterization of genetic diversity and relationship in almond (Prunus dulcis [mill.] DA Webb.) genotypes by RAPD and ISSR markers in Sulaimani Governorate. Applied Ecology & Environmental Research. 2020;18(1).

18. Belle V, Papantonis I. Principles and practice of explainable machine learning. Frontiers in big Data. 2021;4:688969.

19. Bourrat P. Genetic Relatedness. In: Encyclopedia of Evolutionary Psychological Science. Springer; 2021. p. 3401-4.

20. Segura-Alabart N, Serratosa F, Gómez S, Fernández A. Nonunique UPGMA clusterings of microsatellite markers. Briefings in bioinformatics. 2022;23(5):bbac312.

21. Saad-Allah KM, Youssef MS. Phytochemical and genetic characterization of five quinoa (*Chenopodium quinoa Willd.*) genotypes introduced to Egypt. Physiology and Molecular Biology of Plants. 2018:1-13.

22. Al-Naggar AMM, Abd El-Salam RM, Badran AEE, El-Moghazi MMA. Molecular Differentiation of Five Quinoa (*Chenopodium quinoa Willd.*) Genotypes Using Inter-simple Sequence Repeat (ISSR) Markers. Biotechnology Journal International. 2017;20(1):1-12.

23. Ibrahim SD, Abd El-Hakim AF, Ali HE, Abd El-Maksoud RM. Genetic differentiation using ISSR, SCoT and DNA Barcoding for Quinoa genotypes. Arab Journal of Biotechnology. 2019;22(2).