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MOLECULAR GENETICAL STUDIES OF SOME QUINOA GENOTYPES UNDER DROUGHT STRESS

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ABSTRACT

This study was carried out at a pots under supervision of Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Governorate, Egypt (31° 08′ 40.3″ N, 33° 49′ 37.2″ E), during winter season (2021/2022) to analyze the growth and yield traits of the studied genotypes, analyze the molecular variation between the genotypes and investigate the relation between the genetic variation and the physiological performance as a phenotypic parameters. The experimental design was Randomized Complete Block Design (RCBD) in split- devoted plots with three replications. Results showed that all genotypes were drought tolerant, moreover the Regeolone 3 genotype showed the strongest resistance to stress conditions by exhibiting the best vegetative readings after 6 days of drought stress to investigate the relation between the genotypes genetic distinguish and this performance against water stress Irrigation intervals were applied after 30 days from sowing until three months had passed.The genetic diversity between the studied genotypes had been extended and analyzed molecular by using SCOT and ISSR markers. And translation output (SDS-PAGE) and results revealed that significant molecular differences had been observed among the studied genotypes.

INTRODUCTION

Quinoa is a pseudo-cereal with appealing nutritional properties, and its popularity has skyrocketed in recent years. This plant is thought to be one of the species capable of ensuring food security in the twenty-first century. Quinoa is high in gluten-free protein and contains essential amino acids and unsaturated fatty acids. It is also high in magnesium, iron, vitamins, and fibers. Quinoa has grown in popularity due to its high genetic variability. Quinoa can grow under harsh conditions due to its diversity and variability **(Gámez *et al.*, 2019).** Due to variation differential in various agro settings, quinoa grows well under a variety of drought stress. Quinoa also demonstrates a range of physiological and morphological responses to drought stress, including modifications to root and leaf growth and, in certain instances, a few oncogenic alterations **(Saddiq *et al.*, 2021).** Increased productivity with the least amount of water will be the main problem facing agriculture in the future decades, especially in nations with restricted water resources. Since the Mediterranean Basin is one of the driest locations in the world and only contains 1% of the world's freshwater resources, Northern Africa in particular has had a long-standing shortage of renewable freshwater resources **(Ali *et al.*, 2019).** Quinoa has been proposed as an alternative and promising crop for marginal environments due to its capacity to endure a variety of abiotic conditions, including drought **(Gaitan *et al.*, 2022).** Water stress, for instance, affect respiration and causes

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cell death in plants through photosynthetic processes **(Abd El-Moneim *et al.*, 2021)**. Accordingly, quinoa has been described as a drought-tolerant crop with an optimal growing range of 500 to 800 mm of precipitation, with water availability being crucial for crop establishment and seed filling **(Gaitan *et al.*, 2022)**. Quinoa is known as "the golden food" of the Incas because of its excellent nutritional qualities such as high energy, high quality, and gluten-free protein (**Nguyen *et al.*, 2021**).

Drought-resistant plants, on the other hand, have evolved a variety of adaptive strategies to deal with water scarcity. (I) morphological changes such as reduced leaf area and stomatal conductance allowing for reduced water loss and the development of profound root systems reaching deeper water sources; (ii) osmotic adjustment *via* the synthesis of compatible solutes and osmolytes such as proline, polyamines, and glutathione; increase in ROS scavenging capacity *via* increased antioxidant enzymes such as SOD, POD, CAT, APX **(Manaa *et al.*, 2021)**.

The current study aimed to:

1) Analyze the molecular variation between the genotypes.
2) Analyze the growth of the studied genotypes.
3) Investigate the relation between the genetic variation and the physiological performance as a phenotypic parameters.

MATERIALS AND METHODS

Agronomic Study

The experimental design was Randomized Complete Block Design (RCBD) in split – devoted plots with three replications. The main vouchers were assigned to three levels of dehydration (control (daily), after 3 days, after 6 days), while, the three genotypes (Regeolone-3, Q-37, Chipaya) were assigned to the sub-plots and 9 replicates for one treatment. 81 pots with a diameter of 30 cm were used with a mixture of 1: 1: 1: sand: clay: chick manure the planting dates were on November 25, 2021-2022. In each pot there were 5 seeds after germination in a week. The plants were diluted for one plant per the pot after 10 days of germination, imported NPK fertilizer was added 1 g /liter for the first week, then the fertilizer was increased to this amount by 5 grams every week.

After planting, the treatments for the drought irrigation intervals were applied after 30 days from sowing until three months had passed. The salinity of the water was 3000 ppm. The Agriculture Research Center in Cairo, Egypt, provided the seeds.

Vegetative readings

Growth traits

Plant height (cm), Number of spike branches, number of branches/plant^{-1}, number of leaves plant^{-1}, spike length and Leaf area (cm- dsm^{2}) LA = Length × Width × 0.74 was determined according to **Radford (1967).**

Chemical compositions

Vegetative growth samples were taken after 4 months of plant life

Protein content

For N analysis, seed meals were dried at 70ºC and stored. Protein percentage was determined according to the following formula.

Protein %=(T x 0.1 x 14 x 100 x 6.2)/ (Weight of sample x 1000)**.**

Where, T= Titration number.

Statistical Analysis

Data were statistical analyzed with analysis of variance (RCBD) procedure using the General Linear Models (GLMs) procedures using SAS (**SAS, 2004**). Differences between means were compared by using Duncan's multiple ranged tests, significant level 0.05 (**Duncan, 1955)**.

Table 1. The serial number, name and source of the genotype materials

No. of entries	Genotype name	Genotype source
1	Chipaya	Peruvian
2	Q-37	Chile
3	Regeolone-3	Chile

Molecular Study

DNA extraction

The DNA isolation from plant samples (after 90 DAS) was performed using a DNeasy Plant Mini Kit (QIAGEN). Isolation protocol of DNA was as follows:

1- Plant tissue was ground under liquid nitrogen to a fine powder using a mortar and pestle, then the powder was transferred to an appropriately sized tube and liquid nitrogen was allowed to dry.

2- A volume of 400 µl of buffer AP1 and 4 µl of RNase A stock solution (100 mg/ml) were added to a maximum of 20 mg of ground dried plant and then vortexed vigorously to remove any clumps.

3- The mixture was incubated at 65^{o}C for 10 min. and mixed 2-3 times during incubation by inverting tube to lysing the cells.

4- A volume of 130 µl of buffer AP2 was added to the lysate, mixed and incubated on ice for 5 min. Then the lysate was centrifuged at 10.000 rpm for 5 min.

5- The lysate was applied to the QIA shredder spin column sitting in a 2 ml collection tube and centrifuged at 10.000 rpm for 2 min.

6- A fraction of supernatant from step 5 was transferred to a new tube without disturbing the cell-debris pellet. Typically, 450 µl of lysate was recovered.

7- About 1.5 volume of buffer AP3/E and 1 volume of ethanol (96-100%) were added to the cleared lysate and mixed by pipetting immediately.

8- A volume of 650 µl of the mixture from step 7 was applied to the DNeasy mini spin column sitting in a 2 ml collection tube, then centrifuged at ≥8000 rpm for 1 min. and the flow-through was discarded. This step was repeated with the remaining sample and the flow-through and collection tube were discarded.

9- DNeasy column was placed in a new 2 ml collection tube, and then a volume of 500 µl of buffer AW was added to the DNeasy column and centrifuged at ≥8000 rpm for 1 min.

10- A volume of 500 µl of buffer AW was added to the DNeasy column and centrifuged at maximum speed (10.000 rpm) for 2 min. to dry the DNeasy column membrane. The flow-through and collection tube were then discarded.

11- The DNeasy column was then transferred to a 1.5 ml microcentrifuge tube, a volume of 100 µl of preheated (65^{o}C) buffer AE was directly pipetted onto the DNeasy column membrane, then incubated at room temperature for 5 min. and centrifuged at 8000 rpm for 1 min. to elute.

12- The elution was repeated once as described before.

ISSR Analysis

Inter simple sequence repeats (ISSR) analysis

The following stock solutions were used in polymerase chain reaction (PCR).

5 x Tris-borate (TBE) buffer (pH 8.0)

5.40 g	Tris-base
0.29 g	500 mM EDTA (pH 8.0)
Up to 100 ml	Distilled water
2.75 g	Boric acid

Ethidium bromide

1- Ethidium bromide stock solution was prepared by dissolving 1 g of ethidium bromide in 100 ml distilled water and then mixed well using magnetic stirrer.

2- The mixture was transferred to a dark bottle and stored at room temperature.

Polymerase chain reaction(PCR)

PCR was performed in 30-µl volume tubes according to **Williams *et al.* (1990)** with some modification. The PCR mixture consisted of the following:

dNTPs (2.5 mM)	3.00 µl
$MgCl_2$ (25 mM)	3.00 µl
Taq Buffer (10 x)	3.00 µl
Primer (10 pmol)	2.00 µl
Taq DNA polymerse (5U/ µl)	0.20 µl
Template DNA (25 ng)	2.00 µl
Distilled water	16.80 µl

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed as follows:

Initial denaturation (one cycle) at 94ºC for 4 min. followed by 45 cycles of 1 min. at 94ºC, 1 min. at 57ºC, and 2 min. at 72ºC. the reaction was finally stored at 72ºC for 10 min.

SCOT Analysis

Start codon targeted (SCoT) analysis

The following stock solutions were used in Polymerase chain reaction (PCR)

5x Tris-borate (TBE) buffer (pH 8.0)

5.40 g	Tris-base
2.75 g	Boric acid
0.29 g	500 mM EDTA (pH 8.0)
Up to 100 ml	Distilled water

Ethidium bromide

1- Ethidium bromide stock solution was prepared by dissolving 1 g of ethidium bromide in 100 ml distilled water and then mixed well using magnetic stirrer.

2- The mixture was transferred to a dark bottle and stored at room temperature.

Polymerase chain reaction

PCR was performed in 30-µl volume tubes according to **Williams *et al.* (1990)** with some modification. The PCR mixture consisted of the following:

dNTPs (2.5 mM)	3.00 µl
$MgCl_2$ (25 mM)	3.00 µl
Taq Buffer (10 x)	3.00 µl
Primer (10 pmol)	2.00 µl
Taq DNA polymerse (5U/ µl)	0.20 µl
Template DNA (25 ng)	2.00 µl
Distilled water	16.80 µl

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed as follows:

Initial denaturation (one cycle) at 94ºC for 4 min. followed by 45 cycles of 1 min. at 94ºC, 1 min. at 57ºC, and 2 min. at 72ºC. the reaction was finally stored at 72ºC for 10 min.

Gel preparation

1- About 1.50 g of Agarose was mixed with 100ml of l x TBE buffer and then boiled in microwave.

2- 5µl of ethidium bromide solution was added to the melted gel after the temperature reached 55°C.

3- The melted gel was poured in the tray of mini-gel apparatus and then the comb was inserted immediately. After the gel become hardened, the comb was removed.

Table 2. List of the primers names and their nucleotide sequences used in ISSR analysis

No.	Name	Sequence 5'-3'
1	14A	5' CTC TCT CTC TCT CTC TTG 3'
2	HB-9	5' GTG TGT GTG TGT GC 3'
3	HB-12	5' CAC CAC CAC GC 3'
4	HB-13	5' CAC CAC CAC CC 3'
5	HB-15	5' GTG GTG GTG GC 3'
6	44 B	$(CTC)_3(TCT)_2$ TGC
7	HB10	$(GAG)_2(AGA)_2$TGC CC
8	49 A	5`CAC ACA CAC ACA CAC AG 3`
9	UCB1	AGAGAGAGAGAGAGAGT
10	UCB 2	AGAGAGAGAGAGAGAGC

Table 3. List of the primers names and their nucleotide sequences used in SCoT analysis

No.	Name	Sequence
1	SCoT 6	5' CAA TGG CTA CCA CTA CAG 3'
2	SCoT 8	5' ACA ATG GCT ACC ACT ACC 3'
3	SCoT 9	5' ACA ATG GCT ACC ACT GCC 3'
4	SCoT 10	5' ACA ATG GCT ACC ACC AGC 3'
5	SCoT 11	5' ACA ATG GCT ACC ACT ACC 3'
6	SCoT 1	5´ CAA CAA TGG CTA CCA CCA 3`
7	SCoT 3	5´ CAA CAA TGG CTA CCA CCG 3`
8	SCoT 4	5´ CAA CAA TGG CTA CCA CCT 3`
9	SCoT 5	5´ CAA CAA TGG CTA CCACGT 3`
10	SCoT 2	5´ CAA CAA TGG CTA CCA GCA 3

4- The gel was covered by the electrophoretic buffer (1 x TBE).

5- 15 µl of the DNA amplified product was loaded in each well.

6- DNA ladder (1Kbp) mix was used as standard DNA with molecular weights of 3000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The run was performed for about 30 min. at 80 V in mini submarine gel BioRad.

RESULTS

Vegetative Growth

Results in Table 4 show that there were highly significant variation among the studied genotypes in all vegetative growth (plant height at 30, 60, 90 days after sowing DAS; Number of leaves at the same growing periods). Superiority for G_3 for the studied Traits (30.000, 57.000, 134.556 cm; 7.778, 37.778, 72.333) was recorded.

The lowest value for plant height at 30 DAS was found for G_1 25.222 and for number of leaves at 60 DAS 30.0. However, at 90 DAS G_2 gave the lowest number of leaves (63.333). There were no significant differences between G_1 and G_2 for plant height at 60 and 90 DAS and for number of leaves at 30 DAS.

In Table 4, results show that there were no significant differences in the leaves area at 30, 60, 90 DAS.

Results in Table 5 show that there were highly significant variation among the studied genotypes in all vegetative growth (number of branch at 60, 90 days after sowing DAS; number of spike branches at the same growing periods). Superiority for G_3 for the studied traits (12.111, 27.889; 6.778, 15.556) was noted. Also, G_2 recording the highest reading in number of branches at 60 DAS (12.444).

The lowest value in each of all vegetative growth (number of branch at 60, 90 days after sowing DAS; number of spike branches at the same growing periods) was recorded by G_1 for the studied traits (8.778, 24.333; 5.111, 11.667). There were no significant differences in spike length at 60 DAS, although. There were highly significant variation in spike length at 90 DAS the highest value were for G_3, G_2 (17.889, 17.000) and the lowest value was for G_1 (14.778).

Results in Table 6 show that there were highly significant effect of water stress treatments at 30, 60 and 90 DAS for plant height and number of leaves except number of leaves at 30 DAS. superiority for daily irrigation treatment (WS1) was recorded for plant height 30.000, 60.222 and 116.556 at 30, 60 and 90 DAS, respectively. Also, for number of leaves at 60, 90 DAS was superior at WS1 (40.333, 76.889).

There were lowest highly significant effect of water stress treatments at 30, 60 and 90 DAS for plant height and number of leaves except number of leaves at 30 DAS due to 6 days irrigation treatment (WS3) was recorded for plant height 25.667, 47.444 and 93.556 at 30, 60 and 90 DAS, respectively. Also, for number of leaves at 60, 90 DAS was at WS3 (28.667, 58.889).

There were non-significant effect for water stress treatments on number of leaves after 30 DAS, leaves area after 60 and 90 DAS.

In Table 6, results revealed that there are no significant differences in the leaves area at 30, 60, 90 DAS.

Results in Table 7 show that there were non-significant effect of water stress treatments among (spike length at 60 and 90 DAS; number of spike branches at the same growing periods). There were significant variation in number of branches after 60 and 90 DAS. Superiority for daily irrigation WS1 (12.889, 27.889) was noted.

Results in Table 8 show that there were significant variation among the studied interaction A× B in all of vegetative growth (plant height at 30, 60, 90 DAS; number of leaves at the same growing periods). Superiority for plant height at 30 DAS for

Table 4. Plant height, number of leaf and leaves area of some Quinoa genotypes

Genotype(A)	Plant height1 (cm) at 30DAS	Plant height 2 (cm) at 60DAS	Plant height 3 (cm)at 90DAS	Number of leaves at 30 DAS	Number of leaves at 60 DAS	Number of leaves at 90 DAS	leaf area at 30 DAS	Leaf area at 60 DAS	Leaf area at 90 DAS
G1 (chipaya)	25.222 c	50.556b	88.889b	6.889b	30.000c	68.111b	9.45	11.433	23.312
G2(Q-37)	28.000 b	51.778b	91.556b	6.556b	33.000b	63.333c	9.22	11.216	22.922
G3(Regeolone-3)	30.000 a	57.000a	134.556a	7.778a	37.778a	72.333a	8.22	10.402	21.956

Table 5. Number of branches, spike length and number of spike branches of some Quinoa genotypes

Genotype(A)	Number of branches at 60 DAS	Number of branches at 90 DAS	Spike length at 60 DAS	Spike length at 90 DAS	Number of spike branches at 60 DAS	Number of spike branches at 90 DAS
G1 (chipaya)	8.778b	24.333b	7.667	14.778b	5.111b	11.667b
G2(Q-37)	12.444a	25.333b	8.778	17.000a	6.556ab	14.000ab
G3(Regeolone-3)	12.111a	27.889a	8.889	17.889a	6.778a	15.556a

Table 6. Effect of irrigation intervals on plant height, number of leaves and leaf area of some Quinoa genotypes

Water Stress(B)	Plant height1 (cm) at 30DAS	Plant height 2 (cm) at 60DAS	Plant height 3 (cm) at 90DAS	Number of leaves at 30 DAS	Number of leaves at 60 DAS	Number of leaves at 90 DAS	Leaf area at 30 DAS	Leaf area at 60 DAS	Leaf area at 90 DAS
WS1 (level1)	30.000 a	60.222a	116.556a	7.444	40.333a	76.889a	9.156	11.356	23.434
WS2(level2)	27.556 b	51.667b	104.889b	6.889	31.778b	68.000b	9.024	11.224	22.858
WS3(level3)	25.667 c	47.444c	93.556c	6.889	28.667c	58.889c	8.271	10.471	21.898

Table 7. Effect of irrigation intervals on number of branch, spike length and Number of spike branches of some Quinoa genotypes

Water Stress (B)	Number of branches at 60 DAS	Number of branches at 90 DAS	Spike length at 60 DAS	Spike length at 90 DAS	Number of spike branches at 60 DAS	Number of spike branches at 90 DAS
WS1(level1)	12.889a	27.889a	8.556	16.667	6.222	14.222
WS2(level2)	10.111b	25.778b	8.778	17.333	6.444	14.556
WS3(level3)	10.333b	23.889b	8.000	15.667	5.778	12.444

Table 8. Effect of interaction (G× WS) on plant height, number of leaves and leaf area of some Quinoa genotypes

Interaction A x B	Plant height1 (cm) at 30DAS	Plant height2 (cm)at 60DAS	Plant height3 (cm)at 90DAS	number of leaves at 30 DAS	number of leaves at 60 das	number of leaves at 90 DAS	Leaves area at 60 DAS	Leaves area At 90 DAS
G_1 x WS_1	30.000 a	60.000ab	100.000d	7.667ab	33.667de	78.000a	11.933	24.370
G_1 x WS_2	25.667 c	47.333d	85.333ef	6.667bc	28.333fg	68.000c	11.800	23.630
G_1 x WS_3	20.000 d	44.333d	81.333f	6.333c	24.333h	58.333e	10.567	21.937
G_2 x WS_1	30.000 a	60.000ab	99.667d	6.667bc	39.333b	74.000b	10.533	22.053
G_2 x WS_2	27.000 b	51.667c	92.667de	6.333c	31.667ef	61.667d	11.867	23.853
G_2 x WS_3	27.000 b	43.667d	82.333f	6.667bc	28.000g	54.333f	11.247	22.860
G_3 x WS_1	30.000 a	60.667a	150.000a	8.000a	44.333a	78.667a	11.600	23.880
G_3 x WS_2	30.000 a	56.000bc	136.667b	7.667ab	35.333cd	74.333b	10.007	21.090
G_3 x WS_3	30.000 a	54.333c	117.000c	7.667ab	37.333bc	64.000d	9.600	20.897

G_1 x WS_1, G_2 x WS_1, G_3 x WS_1, G_3 x WS_2, G_3 x WS_3 (30.000 for all of the above); plant height at 60 and 90 DAS for G_3 x WS_1 for the studied traits (60.667, 150.000). The lowest value for plant height after 30 DAS at G_1 x WS_3 (20.000); plant height at 60 DAS for G_1 x WS_2, G_1 x WS_3, G_2 x WS_3 (47.333, 44.333, 43.667); plant height at 90 DAS for G_1xWS_3, G_2xWS_3 (81.333, 82.333). Also, superiority for number of leaves at 30 and 60 DAS for G_3 x WS_1 (8.000, 44.333); number of leaves at 90 DAS for G_1 x WS_1, G_3 x WS_1 (78.000, 78.667). The lowest value for number of leaves at 30 DAS for G_1 x WS_3, G_2 x WS_2 (6.333 for all of the above); number of leaves at 60 DAS for G_1 x WS_3 (24.333); number of leaves at 90 DAS for G_2 x WS_3 (54.333). There were no significant differences in the leaves area at 60, 90 DAS.

Results in Table 9 show that there were significant variation for number of branch at 60 and 90 DAS; number of spike branches at 90 DAS. Superiority for number of branches at 60 DAS for G_2 x WS_1, G_3 x WS_1 (14.333, 15.333); number of branch at 90 DAS for G_3 x WS_1 (30.333); number of spike branches at 90 DAS for G_3 x WS_1 (17.333). However, there were the lowest value for number of branch at 60 and 90

Table 9. Effect of interaction (G× WS) on number of branches, spike length and number of spike branches of some Quinoa genotypes

Interaction A x B	Number of branches at 60 DAS	Number of branches at 90 DAS	Spike length at 60 DAS	Spike length at 90 DAS	Number of spike branches at 60 DAS	Number of spike branches at 90 DAS
G_1 x WS_1	9.000bc	25.667bcd	7.333	14.000	4.333	11.333c
G_1 x WS_2	7.667c	23.333d	8.000	15.333	5.333	12.000bc
G_1 x WS_3	9.667bc	24.000cd	7.667	15.000	5.667	11.667bc
G_2 x WS_1	14.333a	27.667abc	8.667	17.000	6.333	14.000abc
G_2 x WS_2	11.333b	25.667bcd	9.000	18.000	7.333	15.333abc
G_2 x WS_3	10.667b	22.667d	8.667	16.000	6.000	12.667abc
G_3 x WS_1	15.333a	30.333a	9.667	19.000	8.000	17.333a
G_3 x WS_2	11.333b	28.333ab	9.333	18.667	6.667	16.333ab
G_3 x WS_3	10.667b	25.000bcd	7.667	16.000	5.667	13.000abc

DAS was recorded for G_1 x WS_2 (7.667, 23.333); number of spike branches at 90 DAS for G_1 x WS_1 (11.333). There were no significant differences in the spike length at 60, 90 DAS and number of spike branches at 60 DAS.

Chemical Analysis

The highest value of seed protein content was 14.44% for Q-37 genotype. while the lowest value was 10.59 for Regeolone-3.

Genetic Analyses

Scot analysis

The results of primer B1 are illustrated in Table 11, it gave 10 polymorphic bands and 9 monomorphic bands with different ranging from 225 to1288 bp. ten common bands were observed in all sites at fragment sizes of 1204, 854, 765, 731, 706, 667, 608, 388, 352 and 310 bp. and nine monomorphic bands of fragment sizes of 1288, 933, 914, 511, 466, 422, 283, 246 and 225 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among genotypes when using primer B1.

The results of primer B2 are illustrated in Table 12 it gave 2 polymorphic bands and 6 monomorphic bands with different ranging from 101 to752 bp. Two common bands were observed in all sites at fragment sizes of 430 and 346 bp and six monomorphic bands of fragment sizes of 752, 527, 472, 233, 152 and 101 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primer B2.

The results of primer B3 are illustrated in Table 13, It gave 3 polymorphic bands and 9 monomorphic bands with different ranging from 135 to 1638 bp, three common bands were observed in all sites at fragment sizes of 718, 202 and 135 bp and nine monomorphic bands of fragment sizes of 1638, 1098, 996, 794, 544, 464, 274, 266 and 241 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B3.

Table 10. Seed protein content

Genotype	Protein
Chipaya	12.10
Q-37	14.44
Regeolone-3	10.59

Table 11. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B1

MW	Chipaya	Q-37	Regeolone-3
1288.700	0.000	1.000	1.000
1204.881	1.000	1.000	1.000
933.819	0.000	1.000	1.000
914.196	1.000	0.000	0.000
854.735	1.000	1.000	1.000
765.908	1.000	1.000	1.000
731.464	1.000	1.000	1.000
706.025	1.000	1.000	1.000
667.151	1.000	1.000	1.000
608.494	1.000	1.000	1.000
511.601	1.000	0.000	0.000
466.619	1.000	0.000	1.000
422.591	1.000	0.000	1.000
388.174	1.000	1.000	1.000
352.793	1.000	1.000	1.000
310.584	1.000	1.000	1.000
283.277	1.000	0.000	0.000
246.751	1.000	0.000	0.000
225.14056	1.000	0.000	0.000
Total	17	12	14

Table 12. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B2

MW	Chipaya	Q-37	Regeolone-3
752.726	0.000	0.000	1.000
527.444	0.000	0.000	1.000
472.382	0.000	0.000	1.000
430.659	1.000	1.000	1.000
346.667	1.000	1.000	1.000
233.594	1.000	0.000	1.000
152.987	1.000	0.000	1.000
101.993	1.000	0.000	1.000
Total	5	2	8

Table 13. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B3

MW	Chipaya	Q-37	Regeolone-3
1638.983	0.000	1.000	1.000
1098.782	0.000	0.000	1.000
996.941	1.000	0.000	1.000
794.519	0.000	0.000	1.000
718.286	1.000	1.000	1.000
544.288	1.000	0.000	1.000
464.504	0.000	0.000	1.000
274.516	1.000	0.000	0.000
266.718	0.000	0.000	1.000
241.127	0.000	1.000	1.000
202.108	1.000	1.000	1.000
135.494	1.000	1.000	1.000
Total	6	5	11

The results of primer B4 are illustrated in Table 14, it gave 6 polymorphic bands and 5 monomorphic bands with different ranging from 193 to 680 bp. six common bands was observed in all sites at fragment sizes 631, 502, 444, 411, 377 and 274 bp. and five monomorphic bands of fragment sizes of 680, 583, 319, 295 and 193.with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among genotypes when using primes B4.

The results of primer B5 are illustrated in Table 15, it gave 3 polymorphic bands and 8 monomorphic bands with different ranging from 145 to 936 bp. three common bands was observed in all sites at fragment sizes 936, 512 and 437 bp. and eight monomorphic bands of fragment sizes of 884, 714, 636, 567, 465, 389, 185 and 145.with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among genotypes when using primes B5.

The results of primer B6 are illustrated in Table 16 it gave 3 polymorphic bands and 5 monomorphic bands with different ranging from 385 to 836 bp. three common bands was observed in all sites at fragment sizes 762, 445 and 385 bp. and five monomorphic bands of fragment sizes of 836, 636, 594, 541 and 488.with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among genotypes when using primes B6.

The results of primer B7 are illustrated in Table 17 it gave 3 polymorphic bands and 7 monomorphic bands with different ranging from 85 to 405 bp. three common bands was observed in all sites at fragment sizes 222, 199 and 85 bp. and seven monomorphic bands of fragment sizes of 405, 287, 243, 177, 167,165 and 109.with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among genotypes when using primes B7.

The results of primer B8 are illustrated in Table 18, it gave 3 polymorphic bands and 1 monomorphic bands with different ranging from 91 to 423 bp. three common bands was observed in all sites at fragment sizes 296, 225 and 91 bp. and one monomorphic bands of fragment sizes of 423 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among genotypes when using primes B8.

The results of primer B9 are illustrated in Table 19, it gave 4 polymorphic bands with different ranging from 231 to 97 bp. four common bands was observed in all sites at fragment sizes 231, 175, 144 and 97 bp. with the three quinoa analyzed genotypes. The recommendation of these results is that there was a genetic monomorphism among the genotypes when using primes B9.

The results of primer B10 are illustrated in Table 20, it gave 3 polymorphic bands and 6 monomorphic bands with different ranging from 303 to 1574 bp. three common bands were observed in all sites at fragment sizes 398, 363 and 303 bp. and six monomorphic bands of fragment sizes of 1574, 1390, 1177, 968, 847 and 610.with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B10.

The SCOT analysis on the three Quinoa seeds' DNA samples using ten primers composed of short random repeat sequences with or without anchor. Table 21 illustrating the SCOT profile of three Quinoa genotypes. A total of 96 amplicons-amplified fragments- (ranged from 101 to 1638 bp.) were generated by the tested primers with an average number of 10 amplicons per primer.

Table 14. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B4

MW	Chipaya	Q-37	Regeolone-3
680.096	0.000	1.000	0.000
631.265	1.000	1.000	1.000
583.762	1.000	0.000	1.000
502.945	1.000	1.000	1.000
444.764	1.000	1.000	1.000
411.295	1.000	1.000	1.000
377.522	1.000	1.000	1.000
319.254	0.000	1.000	1.000
295.230	0.000	0.000	1.000
274.033	1.000	1.000	1.000
193.073	0.000	1.000	0.000
Total	7	9	9

Table 15. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B5

MW	Chipaya	Q-37	Regeolone-3
936.991	1.000	1.000	1.000
884.240	1.000	0.000	0.000
714.987	1.000	0.000	1.000
636.749	1.000	0.000	1.000
567.072	1.000	0.000	1.000
512.884	1.000	1.000	1.000
465.669	1.000	0.000	0.000
437.758	1.000	1.000	1.000
389.856	1.000	0.000	0.000
185.691	1.000	0.000	0.000
145.018	1.000	0.000	0.000
Total	11	3	6

Table 16. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B6

MW	Chipaya	Q-37	Regeolone-3
836.759	0.000	0.000	1.000
762.338	1.000	1.000	1.000
636.049	1.000	1.000	0.000
594.668	1.000	1.000	0.000
541.779	1.000	1.000	0.000
488.512	0.000	0.000	1.000
445.064	1.000	1.000	1.000
385.032	1.000	1.000	1.000
Total	6	6	5

Table 17. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B7

MW	Chipaya	Q-37	Regeolone-3
405.337	0.000	0.000	1.000
287.186	0.000	1.000	0.000
243.174	1.000	1.000	0.000
222.440	1.000	1.000	1.000
199.880	1.000	1.000	1.000
177.486	0.000	0.000	1.000
167.248	1.000	0.000	0.000
165.273	0.000	1.000	0.000
109.040	0.000	1.000	1.000
85.467	1.000	1.000	1.000
Total	5	7	6

Table 18. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B8

MW	Chipaya	Q-37	Regeolone-3
423.405	0.000	0.000	1.000
296.373	1.000	1.000	1.000
225.459	1.000	1.000	1.000
91.874	1.000	1.000	1.000
Total	3	3	4

Table 19. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B9

MW	Chipaya	Q-37	Regeolone-3
144.399	1.000	1.000	1.000
97.337	1.000	1.000	1.000
175.877	1.000	1.000	1.000
231.798	1.000	1.000	1.000
Total	4	4	4

Table 20. Survey of polymorphic and monomorphic SCOT bands of the three quinoa genotype's seeds by using primer B10

MW	Chipaya	Q-37	Regeolone-3
1574.560	1.000	1.000	0.000
1390.042	1.000	1.000	0.000
1177.207	1.000	1.000	0.000
968.383	1.000	1.000	0.000
847.827	1.000	1.000	0.000
610.607	1.000	1.000	0.000
398.025	1.000	1.000	1.000
363.258	1.000	1.000	1.000
303.827	1.000	1.000	1.000
Total	9	9	3

Table 21. Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by SCOT markers

Primer	Total of amplicons	Polymorphic amplicons	(%) of Monomorphism	Monomorphic Amplicons	(%) of polymorphism
OPB-1	19	10	52.63	9	47.37
OPB-2	8	2	25.00	6	75.00
OPB-3	12	3	25.00	9	75.00
OPB-4	11	6	54.54	5	45.45
OPB-5	11	3	27.27	8	72.72
OPB-6	8	3	37.50	5	62.50
OPB-7	10	3	30.00	7	70.00
OPB-8	4	3	75.00	1	25.00
OPB-9	4	4	100.00	0	00.00
OPB-10	9	3	33.33	6	66.66
Total	96	40	41.67	56	58.33
Average	9.6	4,0	41.66	5.6	58.33

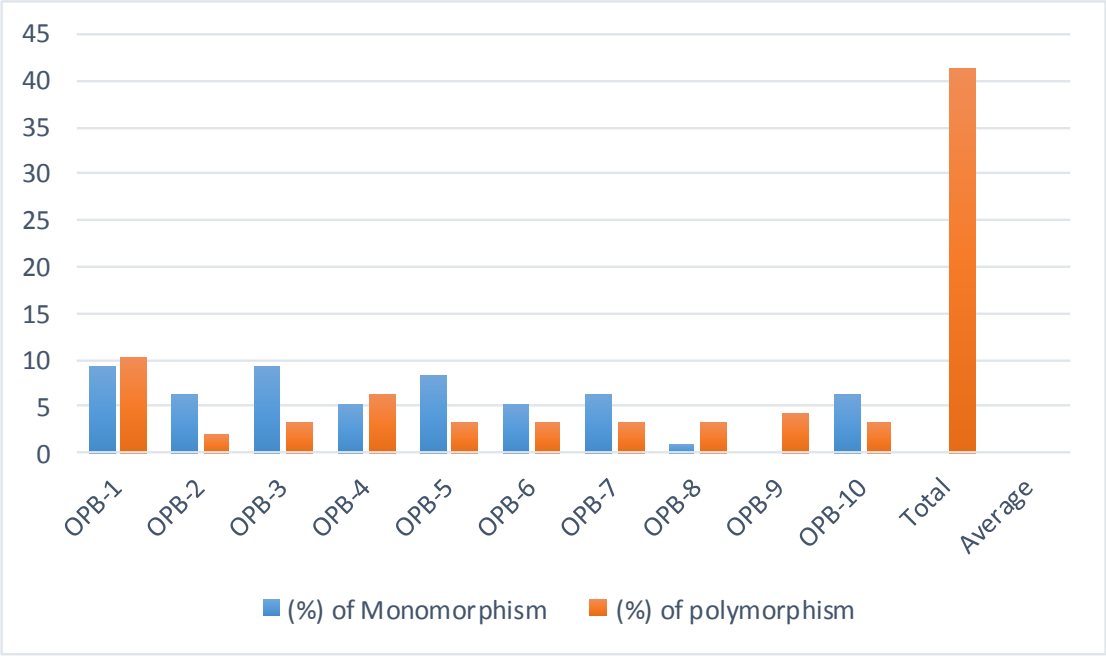

Fig. 1. Monomorphism and polymorphism as revealed by SCOT markers

SCOT primer OPB-1 exhibited the highest number of fragments as 19 amplicons, respectively followed by primer OPB-3 which generated 12 amplicons for each. Results in Table 21 show that the total number of polymorphic bands was 40 with an average of 4.0 polymorphic amplicons per primer. This represents a level of polymorphism about of 58.33%, while the total number of monomorphic bands was 56 with an average of 5.6 monomorphic amplicons per primer. This represents a level of monomorphism of about 41.67%. Primer OPB-1 and OPB-3 exhibited high monomorphic amplicons and monomorphism. On the other hand, primer OPB-1 and OPB- 4 exhibited high polymorphic and polymorphism differences and were useful in the three studied Quinoa genotypes identification. However, primers OPB-2 showed the lowest level of polymorphism but OPB-9 and OPB-8 showed the lowest level of monomorphism.

Similarity Index

Cluster analysis as percentage (similarity index) based on SCOT analysis is shown in Table 22. The highest recorded similarity index between studied genotypes was 221 between genotypes VAR0001 (Chipaya) and VAR0002 (Q-37). While the lowest recorded similarity index was .109 between genotypes VAR0002 (Q-37) and VAR0003 (Regeolone-3).

Genetic Relationship

A dendrogram for the genetic relationships between the studied three Quinoa genotypes were carried out as in Fig. 2. The three studied genotypes were separated into two clusters. Clusters 1 to 3 included genotypes chipaya , Q-Q37 and Regeolone-3 , respectively.

The dendrogram in Fig. 2 indicated that relationship between the 3 quinoa genotypes were classified in 2 levels. And these results could be useful for breeding purposes.

ISSR Analysis

The results of primer B1 are illustrated in Table 23, it gave 4 polymorphic bands and 8 monomorphic bands with different ranging from 140 to 1061 bp. four common bands were observed in all sites at fragment sizes 795, 413, 312 and 140 bp. and eight monomorphic Bands of fragment sizes of 1061, 998, 903, 891, 645, 556, 274 and 268 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B1.

The results of primer B2 are illustrated in Table 24, It gave 5 polymorphic bands and 9 monomorphic bands with different ranging from 139 to 749 bp. five common bands was observed in all sites at fragment sizes 749, 580, 545, 379 and 333 bp. and nine monomorphic bands of fragment sizes of 686, 618, 435, 431, 253, 216, 276, 301, 350, 182 and 139.with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B2.

The results of primer B3 are illustrated in Table 25, it gave 3 polymorphic bands and 10 monomorphic bands with different ranging from 122 to 1081 bp. three common bands was observed in all sites at fragment sizes 678, 506 and 301 bp. and ten monomorphic Bands of fragment sizes of 818, 457, 723, 895, 1081, 388, 346, 216, 198 and 122 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B3.

The results of primer B4 are illustrated in Table 26, it gave 2 polymorphic bands and 2 monomorphic bands with different ranging from 145 to 593 bp. two common bands was observed in all sites at fragment sizes 524 and 889 bp. and two monomorphic bands of fragment sizes of 889 and 145 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B4.

Table 22. Genetic similarity matrices among the 3 genotypes as computed according to Dice coefficient from SCOT

Proximity Matrix			
Correlation between Vectors of Values			
	VAR00001	**VAR00002**	**VAR00003**
VAR00001	1.000	0.221	- 0.122
VAR00002	0.221	1.000	0.109
VAR00003	- 0.122	0.109	1.000

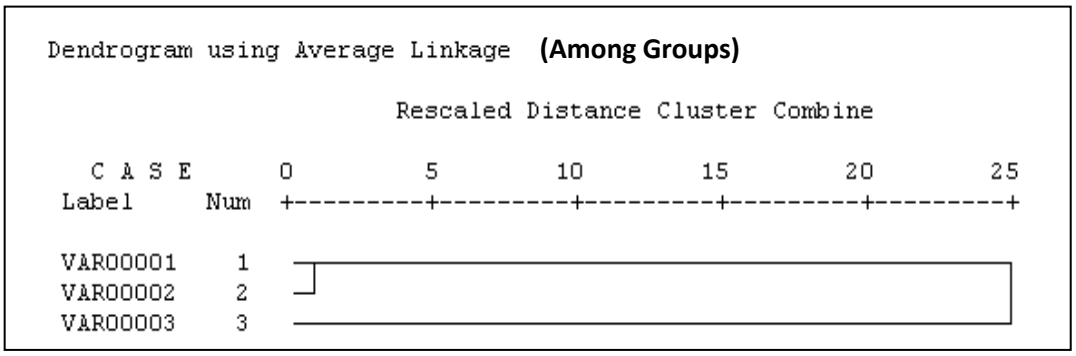

Fig. 2. Dendrogram for the three constructed from the SCOT data using unweighted pair-group arithmetic (UPGMA) and similarity matrices computed according to Dice coefficient

Table 23. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B1

MW	Chipaya	Q-37	Regeolone-3
1061.366	1.000	0.000	1.000
998.419	0.000	0.000	1.000
903.007	0.000	0.000	1.000
891.254	1.000	1.000	0.000
795.591	1.000	1.000	1.000
645.139	0.000	0.000	1.000
556.120	1.000	0.000	1.000
413.238	1.000	1.000	1.000
312.477	1.000	1.000	1.000
274.107	0.000	0.000	1.000
268.187	1.000	0.000	0.000
140.521	1.000	1.000	1.000
Total	8	5	10

Table 24. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B2

MW	Chipaya	Q-37	Regeolone-3
749.290	1.000	1.000	1.000
686.411	0.000	0.000	1.000
618.398	0.000	0.000	1.000
580.869	1.000	1.000	1.000
545.618	1.000	1.000	1.000
435.517	0.000	1.000	0.000
431.897	1.000	0.000	1.000
379.479	1.000	1.000	1.000
253.140	1.000	0.000	0.000
216.013	1.000	0.000	1.000
276.329	1.000	0.000	0.000
301.642	1.000	0.000	0.000
333.423	1.000	1.000	1.000
350.548	0.000	1.000	0.000
182.038	1.000	0.000	1.000
139.947	1.000	0.000	1.000
Total	12	7	11

Table 25. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B3

MW	Chipaya	Q-37	Regeolone-3
818.848	0.000	1.000	0.000
678.174	1.000	1.000	1.000
506.789	1.000	1.000	1.000
457.272	0.000	0.000	1.000
723.184	0.000	1.000	1.000
895.930	0.000	1.000	1.000
1081.774	0.000	1.000	0.000
388.575	0.000	1.000	0.000
346.132	0.000	1.000	1.000
301.790	1.000	1.000	1.000
216.993	0.000	1.000	0.000
198.324	1.000	0.000	1.000
122.744	1.000	0.000	1.000
Total	5	10	9

Table 26. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B4

MW	Chipaya	Q-37	Regeolone-3
524.602	1.000	1.000	1.000
593.917	1.000	1.000	1.000
889.860	0.000	0.000	1.000
145.127	1.000	0.000	1.000
Total	3	2	4

The results of primer B5 are illustrated in Table 27, it gave 1 polymorphic bands and 10 monomorphic bands with different ranging from 219 to 1269 bp. one common bands was observed in all sites at fragment sizes 219 bp. and ten monomorphic bands of fragment sizes of 1269, 1206, 929, 683, 558, 474, 434, 405, 300 and 266.with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B5.

The results of primer B6 are illustrated in Table 28, it gave 3 polymorphic bands and 5 monomorphic bands with different ranging from 121 to 842 bp. three common bands was observed in all sites at fragment sizes 842, 494 and 121 bp. and five monomorphic Bands of fragment sizes of 704, 622, 559, 464 and 452 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B6.

The results of primer B7 are illustrated in Table 29, it gave 1 polymorphic bands and 7 monomorphic bands with different ranging from 249 to 1023 bp. one common bands was observed in all sites at fragment sizes 747 bp. and seven monomorphic Bands of fragment sizes of 1023, 913, 617, 442, 305, 268 and 249 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B7.

The results of primer B8 are illustrated in Table 30, it gave 4 polymorphic bands and 4 monomorphic bands with different ranging from 191 to 1261 bp. four common bands was observed in all sites at fragment sizes 908, 665, 462 and 393 bp. and four monomorphic Bands of fragment sizes of 1261, 775, 239 and 191 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B8.

The results of primer B9 are illustrated in Table 31, it gave 1 polymorphic bands and 8 monomorphic bands with different ranging from 619 to 228 bp. one common bands was observed in all sites at fragment sizes 353 bp and eight monomorphic Bands of fragment sizes of 619, 478, 434,403, 318, 285, 246 and 228 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B9.

The results of primer B10 are illustrated in Table 32, It gave 9 monomorphic bands with different ranging from 1083 to 211 bp. nine monomorphic bands was observed in all sites at fragment sizes 375, 416, 1083, 335, 795, 635, 545, 492, 246, 294 and 211 bp. with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism among the genotypes when using primes B10.

Table 27. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B5

MW	Chipaya	Q-37	Regeolone-3
1269.908	0.000	0.000	1.000
1206.389	0.000	0.000	1.000
929.406	1.000	0.000	1.000
683.118	1.000	0.000	1.000
558.744	1.000	0.000	0.000
474.946	1.000	0.000	1.000
434.155	1.000	0.000	1.000
405.445	1.000	0.000	0.000
300.564	1.000	0.000	0.000
266.648	1.000	0.000	1.000
219.973	1.000	1.000	1.000
Total	9	1	8

Table 28. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B6

MW	Chipaya	Q-37	Regeolone-3
842.194	1.000	1.000	1.000
704.347	1.000	1.000	0.000
622.569	1.000	1.000	0.000
559.734	0.000	0.000	1.000
494.747	1.000	1.000	1.000
464.152	0.000	0.000	1.000
452.450	1.000	0.000	0.000
121.469	1.000	1.000	1.000
Total	6	5	5

Table 29. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B7

MW	Chipaya	Q-37	Regeolone-3
1023.344	0.000	1.000	0.000
913.030	1.000	0.000	1.000
747.818	1.000	1.000	1.000
617.511	1.000	0.000	1.000
442.153	1.000	0.000	1.000
305.196	1.000	0.000	1.000
268.989	1.000	0.000	1.000
249.971	1.000	0.000	1.000
Total	7	2	7

Table 30. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B8

MW	Chipaya	Q-37	Regeolone-3
1261.988	0.000	0.000	1.000
908.406	1.000	1.000	1.000
775.796	1.000	0.000	1.000
665.455	1.000	1.000	1.000
462.502	1.000	1.000	1.000
393.258	1.000	1.000	1.000
239.642	1.000	0.000	1.000
191.635	1.000	0.000	1.000
Total	7	4	8

Table 31. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B9

MW	Chipaya	Q-37	Regeolone-3
619.458	1.000	0.000	1.000
478.502	1.000	0.000	1.000
434.923	1.000	0.000	1.000
403.791	1.000	0.000	1.000
353.011	1.000	1.000	1.000
318.599	1.000	0.000	1.000
285.515	1.000	0.000	1.000
246.103	1.000	0.000	1.000
228.486	1.000	0.000	1.000
Total	9	1	9

Table 32. Survey of polymorphic and monomorphic ISSR bands of the three quinoa genotype's seeds by using primer B10

MW	Chipaya	Q-37	Regeolone-3
375.053	1.000	0.000	0.000
416.729	0.000	1.000	0.000
1083.576	0.000	1.000	1.000
335.101	0.000	1.000	1.000
795.669	0.000	1.000	0.000
635.182	0.000	1.000	0.000
545.285	0.000	1.000	0.000
492.539	0.000	1.000	0.000
246.064	0.000	1.000	1.000
211.239	0.000	1.000	1.000
294.015	0.000	1.000	1.000
Total	1	10	5

The ISSR analysis on the three Quinoa seeds' DNA samples using ten primers composed of short random repeat sequences with or without anchor. Table 33 illustrating the ISSR profile of three Quinoa genotypes. A total of 100 amplicons-amplified fragments (ranged from 121 to 1269 bp.) were generated by the tested primers with an average number of 10 amplicons per primer. ISSR primer OPB-2 exhibited the highest number of fragments as 16 amplicons, respectively followed by primer OPB-3 which generated 13 amplicons for each. Results in Table 33 show that the total number of polymorphic amplicons and polymorphism were 24 with an average of 2.8 polymorphic amplicons and polymorphism per primer while the total number of monomorphic bands and monomorphism were 72 with an average of 7.2 monomorphic amplicons and monomorphism per primer. Primer OPB-3 exhibited high monomorphic amplicons and monomorphism. On the other hand, primer OPB-2 exhibited high polymorphic and polymorphism differences and were useful in tree studied Quinoa genotypes identification. However, primers OPB-10 showed the lowest level of polymorphism but OPB-9 and OPB-4 showed the lowest level of monomorphism.

Similarity Index

Cluster analysis as percentage (similarity index) based on ISSR analysis is shown in Table 34. The highest recorded similarity index between studied genotypes was 0.755 between genotypes VAR0001 (Chipaya) and VAR0002 (Q37). While the lowest recorded similarity index was 0.710 between genotypes VAR0002 (Q-37) and VAR0003 (Regeolone-3).

Genetic Relationship

A dendrogram for the genetic relationships between the studied three Quinoa genotypes were carried out as in Fig. 4. The three studied genotypes were separated into two clusters. Clusters 1 to 3 included genotypes chipaya, Q-Q37and Regeolona, respectively.

The dendrogram in Fig. 4 indicated that relationship between the 3 quinoa genotypes were classified in 2 levels. And these results could be useful for breeding purposes.

SDS-PAGE

The results in Table 35, it gave 1 polymorphic bands and 5 monomorphic bands with different ranging from 700 to 375 bp. one common band was observed in all sites at fragment sizes 700 bp. and five monomorphic bands of fragment sizes of 600, 550, 400, 390 and 375 with the three quinoa analyzed genotypes. The recommendation of these results is that there were a genetic monomorphism and polymorphism among the genotypes when using primes B1.

The SDS-PAGE analysis on the three Quinoa seeds' DNA samples using ten primers composed of short random repeat sequences with or without anchor. Table (36) illustrating the SDS-PAGE profile of the three Quinoa genotypes. A total of 6 amplicons-amplified fragments (ranged from 375 to 700 bp). The total number of monomorphic amplicons was 5 and present age of monomorphism was 83.3%. On the other hand, polymorphic amplicons was 1 and present age of polymorphism was 16.6%.

Results in Fig. 6 show a variance in values polymorphism and monomorphism as a percentage. the highest value of polymorphism was 16.6 for SDS-PAGE. while the lowest value was 0 for ISSR. the highest value of monomorphism was 38.3 for SDS-PAGE while the lowest value was 0 for SCOT.

DISCUSSION

Quinoa is said to be exceptionally resistant to drought. In pure sand, it has been reported that it can grow with as little as 200 mm of yearly precipitation. In the Atacama Desert of northern Chile, yields

Table 33. Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by ISSR markers

Primer	Total of amplicons	Polymorphic amplicons	(%) of Monomorphism	Monomorphic Amplicons	(%) of polymorphism
OPB-1	12	4	33.33	8	66.67
OPB-2	16	5	43.75	9	56.25
OPB-3	13	3	23.07	10	76.92
OPB-4	4	2	50.00	2	50.00
OPB-5	11	1	9.09	10	90.90
OPB-6	8	3	37.50	5	62.50
OPB-7	8	1	12.50	7	87.50
OPB-8	8	4	50.00	4	50.00
OPB-9	9	1	11.11	8	88.88
OPB-10	11	0	18.18	9	81.81
Total	**100**	**24**	**28.00**	**72**	**72.00**
Average	**10**	**2.4**	**28.00**	**7,2**	**72.00**

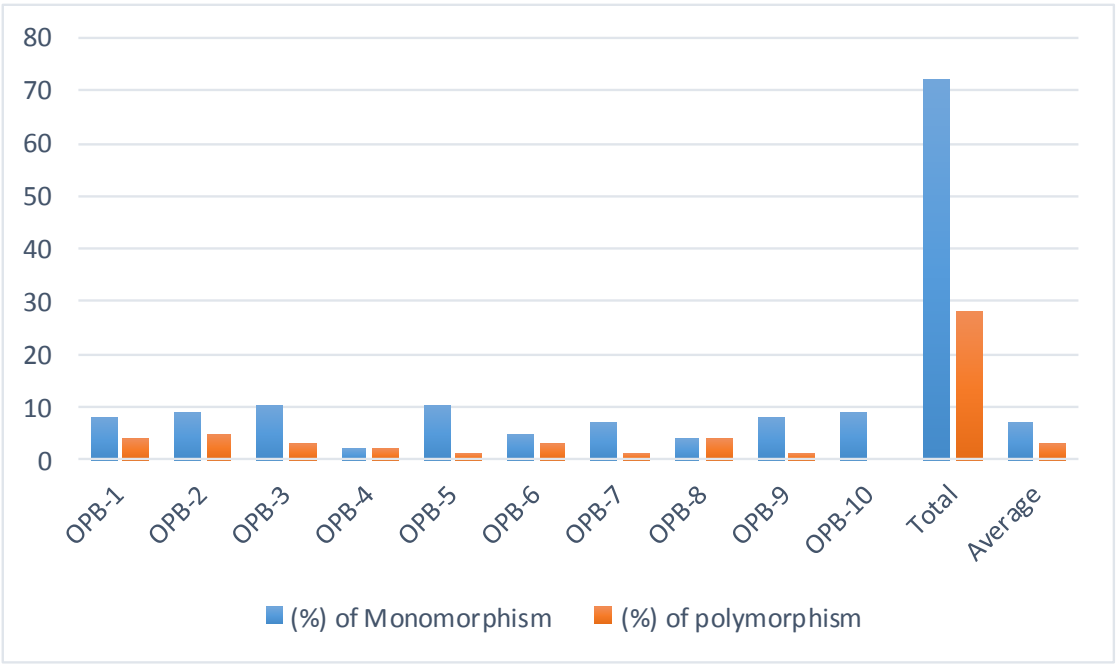

Fig. 3. monomorphism and polymorphism as revealed by ISSR markers

Table 34. Genetic similarity matrices among the 3 genotype as computed according to Dice coefficient from SCOT

Proximity Matrix			
	Cosine of Vectors of Values		
	VAR00001	VAR00002	VAR00003
VAR00001	1.000	0.755	0.713
VAR00002	0.755	1.000	0.710
VAR00003	0.713	0.710	1.000

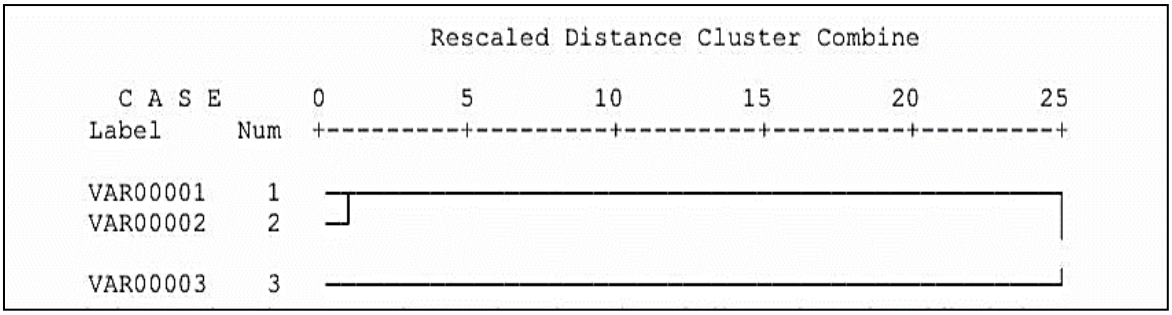

Fig. 4. Dendrogram for the tree constructed from the ISSR data using unweighted pair-group arithmetic (UPGMA) and similarity matrices computed according to Dice coefficient

Table 35. Survey of polymorphic and monomorphic SDS-PAGE bands of the three quinoa genotype's seeds by using primer B1

M	Chipaya	Q-37	Regeolone-3
700	1	1	1
600	0	1	0
550	0	0	1
400	1	0	1
390	1	0	1
375	0	1	0

Table 36. Total number of amplicons, Monomorphic amplicons, Polymorphic amplicons and percentage of polymorphism as revealed by SDS-PAGE markers.

Primer	Total of amplicons	Monomorphic Amplicons	(%) of Monomorphism	Polymorphic amplicons	(%) of Polymorphism
OPB-1	6	5	83.3	1	16.6

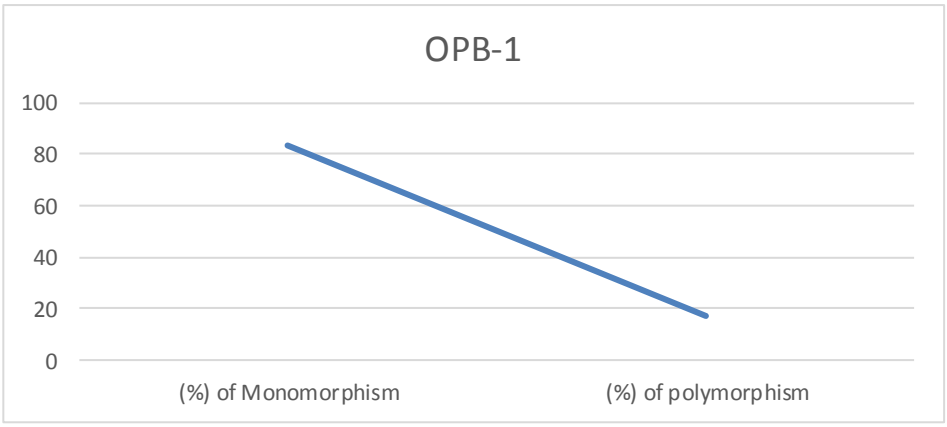

Fig. 5. Monomorphism and polymorphism as revealed by SDS-PAGE markers

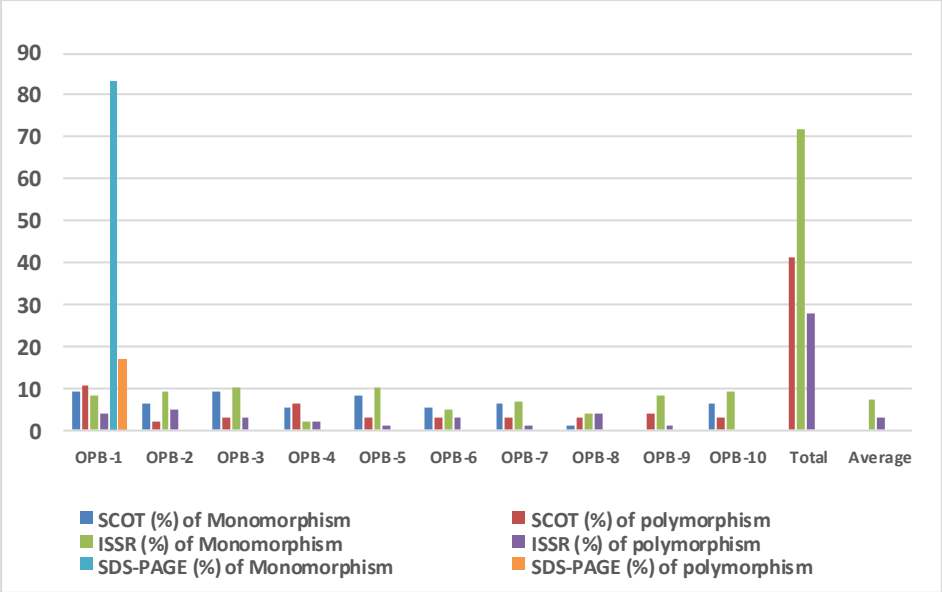

Fig. 6. Percentage of polymorphism and Monomorphism as revealed by ISSR, SCOT and SDS-PAGE markers

more than 1000 kg ha-1 have been observed with as little as 50 mm irrigation. However, irrigation in desert locations greatly improves harvests showed by **Al-Naggar *et al.* (2017). Oliveira *et al.* (2017)** found that Because of the rise in vegetative components (significant for plant height 32%, basal area 65%, and stem weight 120%, respectively), quinoa was one of the most responsive plants to water. This suggests that in order to increase water availability, planting should be anticipated.

A decrease in cell elongation and a slowdown in shoot and root growth are the first physiological changes to occur. The ramification of quinoa's roots and the growth of hygroscopic papillae on the leaf cuticle, which would lessen transpiration, are some of the reasons for the plant's resistance. Additionally, the plant escapes the detrimental impacts of dryness by reducing its leaf area through leaf drop. These results are obtained by **Fischer *et al.* (2013).**

Quinoa plants' leaf development and expansion were noticeably and similarly suppressed in response to deficit irrigation treatments due to a drought-induced

alteration in the leaf area index (LAI). Development of the leaf area was more responsive to soil dryness **(Fghire *et al.*, 2015).**

Under conditions of water shortage, combining deficit irrigation with organic matter may be the key to increasing quinoa yield because organic matter enhances soil water-holding capacity and boosts plant access of water and nutrients **(Hirich *et al.*, 2014)**.

Naz *et al.* (2022) reported that inadequate water availability has a negative impact on plants' metabolism, resulting in lower biomass.

It is important to choose and breed types that are tolerant to adverse environmental circumstances, like drought, salinity, high temperatures, and other stresses, in order to maintain the quinoa production under such conditions. Lack of suitable indicators that can be employed in breeding programs to resist environmental challenges hinders these attempts **(Badran, 2022)**.

According to research, quinoa plants may become shorter in response to drought stress due to decreased cell length, turbulence, volume, and eventually growth **(Mohammadi *et al.*, 2021)**.

Based on irrigation schedule of 295 mm, the average irrigation requirements for the four genotypes (Regalona, Q37, KVL-SR2, and Q21) were 295 mm, which dropped by 11 and 17% under water stress circumstances during drought tolerant stages. Although the average real water usage during the drought was 253 mm, it reduced by 17% (**Salim *et al.*, 2020).**

Abd El-Moneim *et al.* (2021) High polymorphism percentages of 90.91% and 85.26%, respectively, were found in quinoa ISSR and SCoT analyses. Furthermore, the most polymorphic amplicons were obtained by ISSR 1 and SCoT 7 (27 and 26). 11 bands with a polymorphism percentage of 27.27% were discovered by protein pattern analysis among the quinoa genotypes.

Quinoa-line Chenopodium (Willd.) While variation was observed in plants that represented original cultivars, SCoT technique revealed a high genetic stability of the derivative lines of "Faro" and "Titicaca": banding patterns other than the predominate pattern were present in three plants of "Titicaca" (genetic distances from 7.5% to 55.9%), and in one plant of "Faro" (genetic distance 61.2%), according to SCoT technique.

Molecular markers are crucial tools for identifying genetic variability among various species and are helpful for cultivar identification and the preservation of germplasm using ISSR and SCoT; similarity percentages ranged from 80% to 81% in this study for some quinoa type **(Abd El-Hakim *et al.* 2019).**

REFERENCES

Ali, D.; Fghire, R.; Anaya, F.; Benlhabib, O. and Wahbi, S. (2019). physiological and morphological responses of two quinoa cultivars (*Chenopodium quinoa willd.*) to drought stress, doi.org/10. 1007/s10343-019-00460-y.

Allah, A.; El-Murr, A. and Abd El-Hakim, Y. (2019). Dietary supplementation with low molecular weight sodium alginate improves growth, haematology, immune reactions and resistance against aeromonas hydrophila in *clarias gariepinus*, http:// www. wileyonline library.com /journal/are , 1547-1556.

Al-Naggar, A.; Abd El-salam, R.; Badran, A. and El-Moghazi, M. (2017). Drought tolerance of five quinoa (*Chenopodium quinoa* Willd.) genotypes and its association with other traits under moderate and severe drought stress, Asian J. Advances in Agric. Res., 3:1-13.

Badran, A.E. (2022). Assessment of variation and stability parameters of five quinoa genotypes under drought stress conditions, Egypt. J. Bot., 1: 21-30.

Duncan, D. (1955). Multiple Range and Multiple F Tests, International Biometric Society, 1:1-42.

Abd El-Moneim, D.; ELsarag, E.; Alouf, S.; El-Azraq, A.; ALshamrani, S.; Safhi, F. and Ibrahim, A.A. (2021). Quinoa (*Chenopodium quinoa* Willd.): Genetic Diversity According to ISSR and SCoT Markers, Relative Gene Expression, and Morpho-Physiol. Variation under Salinity Stress, doi.org/10.3390/plants10122802.

Fghire, R.; Anaya, F.; Ali, O.; Benlhabib, O.; Ragab, R. and Wahbi, S. (2015). Physiological and photosynthetic response of quinoa to drought stress, Chilan J. Agric. Res., 2: 174-183.

Fischer, S.; Wilckens, R.; Jara, J. and Aranda, M. (2013). Controlled water stress to improve functional and nutritional quality in quinoa seed, Boletín Latinoamericano y del Caribe de Plantas Medicinalesy Aromáticas, 5: 457 – 468.

Gaitan, I.; Rodriguez, S.; Orus, M.; Matias, J.; Cruz, V. and Bolanos, L. (2022). Genotype-dependent responses to long-term water stress reveal different water-saving strategies in *Chenopodium quinoa Willd*, Environ. and Exp. Bot., oi.org/10.1016/j.envexpbot.2022.10497.

Gámez, A.; Soba, D.; Zamarreño, A.; García-Mina, J.; Aranjuelo, I. and Morales, F. (2019). Effect of water stress during grain filling on yield, quality and physiological traits of illpa and rainbow quinoa (*Chenopodium quinoa Willd.)* Cultivars, Plants, doi: 10.3390/plants8060173.

Hirich, A.; Choukr-Allah, R. and Jacobsen, S.E. (2014). Deficit Irrigation and Organic Compost Improve Growth and Yield of Quinoa and Pea, J. Agron. and Crop Sci., 200: 390-398

Manaa, A.; Goussi, R.; Derbali, W.; Cantamessa, S.; Essemine, J. and Barbato, R. (2021). photosynthetic performance of quinoa (*Chenopodium quinoa* Willd.) after exposure to a gradual drought stress followed by a recovery period, elsevier, doi.org/ 10. 1016/j.bbabio.2021.148383.

Mohammadi, F.; Maleki, A. and Fathi, A. (2021). effects of drought stress and humic acid on plant growth, yield quality and its components of quinoa (*Chenopodium quinoa* Willd), J. Crop Nutr. Sci., 3: 11-23.

Naz, H.; Akram, N.; Ashraf, M.; Hefft, D. and Jan, B. (2022). Leaf extract of neem (*Azadirachta indica*) alleviates adverse effects of drought in quinoa (*Chenopodium quinoa* Willd.) plants through alterations in biochemical attributes and antioxidants, Saudi J. Biol. Sci., doi.org/10.1016/j.sjbs.2022.01.038.

Nguyen, L.; Bertero, D.; Hoang, D. and Long, N. (2021). Variation in quinoa roots growth responses to drought stresses, J. Agron. and Nutr. Sci., DOI: 10.1111/jac.12528.

Oliveira, A.; Júnior, W.; Ramos, M.; Zivian, A. and Jakelaitis, A. (2017). Amaranth, quinoa, and millet growth and development under different water regimes in the Brazilian Cerrado, Scielo Brazil Scientific Electronic Library Online, 8: 561-571.

Radford, P. (1967). Growth Analysis Formulae-Their Use and Abuse1, https:// doi.org/10. 2135.

Saddiq, M.; Wang, X.; Iqbal, S.; Hafeez, M.; Khan, S.; Raza, A.; Iqbal, J.; Maqbool, M.; Fiaz, S.; Qazi, M.; Bakhsh, A.; Jahanzai, B. and Allah, G.

(2021). Effect of water stress on grain yield and physiological characters of quinoa genotypes, doi.org/10.3390/ agronomy, 11101934.

Salim, S.; Hadeethi, I. and R. Al-hadithi, (2020). Water stress on different growing stages for quinoa (*Chenopodium quinoa* willd) and its infleunce on water requiriments and yield, Iraqi J. Agric. Sci., 3:953-966.

Ibrahim, S.; Abd El-Hakim, A.; Ali, H. and Abd El-Maksoud, R. (2019). Genetic differentiation using ISSR, SCoT and DNA Barcoding for Quinoa genotypes,https://www.researchgate.net.

Kamal, K.; Khodaeiaminjan, M.; Yahya, G.; El-Tantawy, A.; El-Moneim, D.; El-Esawi, M.; Abd-Elaziz, M. and Nassrallah, A. (2021). Modulation of cell cycle progression and chromatin dynamic as tolerance mechanisms to salinity and drought stress, DOI: 10.1111 / ppl. 13260, Physiologia Plantarum, 684:695.

Sas, A.; Kucharski, R.; Małkowski, E.; Pogrzeba, M.; Kuperberg, J. and Kryński (2004). Phytoextraction crop disposal—an unsolved problem, https:// www.sciencedirect.com, 373-379.

الملخص العربي

دراســـــات وراثيـــــة جـزيئيـة علـــى بعـض أصنـــاف من الكينـــوا تحت ظـروف الجفـــاف

بسمة محمد السباعي*، إيمان اسماعيل السراج، محمود إبراهيم محمود، معتز بالله أبو السعود

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أجريت هذه الدراسة في اصص تحت إشراف كلية العلوم الزراعية البيئية، جامعة العريش، محافظة شمال سيناء، مصر، (31°08′ 40.3″ N, 33°49′37.2″ E). خلال فصل الشتاء (2022) لتحليل صفات النمو والإنتاجية للأنماط الجينية المدروسة، وتحليل التباين الجزيئي بين الطرز الجينية، والتحقق من العلاقة بين التباين الجيني والأداء الفسيولوجي كعوامل نمطية. قسمت التجربة ثلاث مكررات. أظهرت النتائج أن جميع الأصناف تتحمل نقص المياه، كما أظهر الصنف Rignola أقوى مقاومة لظروف الإجهاد من خلال إظهار أفضل قراءات نباتية بعد 6 أيام من إجهاد الجفاف للتحقق من العلاقة بين التمايز الوراثي والأداء ضد ظروف الإجهاد المائي. بالنسبة للإجهاد المائي، تم استخدام ثلاثة مستويات للإجهاد المائي كل يوم ثم بعد ثلاث ايام ثم بعد سته ايام. تم تمييز التنوع الجيني بين الطرز الجينية المدروسة وتحليلها الجزيئي باستخدام علامات SCOT وISSR. وأظهرت نتائج الترجمة (SDS-PAGE) وجود فروق جزيئية معنوية بين الطرز الوراثية المدروسة.

الكلمات الإسترشادية: إجهاد الجفاف، الكينوا، الأنماط الجينية، RCBD، ISSR، SCOT، SDS-PAGE، التحليل الجزيئي.

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