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GENTIC EVALUSATION OF SOME QUINOA GENOTYPES UNDER RAS SUDER CONDITIONS

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ABSTRACT

Genetic diversity of quinoa around the world is narrow which may threaten the ability of breeders to improve and increase the crop yield. A field experiment was carried out at the experimental farm at Ras Sudr, South Sinai Governorate during growing season 2012/2013. Yield, its components and the genetic variation among five quinoa genotypes viz: Kvlsra 2, Kvlsra 3, Regalona, Q-37 and Q-52 were evaluated under Ras Sudr conditions. The results indicated that the genotype Q-37 followed by Regalona cultivar recorded the highest mean values for seed yield. The correlation for mean performances were positive and high significant among number of heads and each of heads yield, 1000- seed weight, seed yield and straw yield, such traits may be taken in account in quinoa breeding programs.

Biochemical and molecular markers were used to identify the level of polymorphism and to study the genetic relationships among the five quinoa genotypes. Seventeen polymorphic protein bands produced (59%) of polymorphism. Regalona cultivar and Q-37 revealed that highest number of protein unique bands which could be considered as marker for salinity tolerance. Five isozymes systems including POD, ACPH, β-EST, α-EST and ADH revealed six polymorphic bands and produced moderate polymorphism (53%) and two unique bands for Q-37 as markers for salinity tolerance. Five RAPD primers produced fifteen polymorphic bands, and produced the highest polymorphism (66%). Q-37 genotype produced the highest number of unique bands as specific band for salinity tolerance. The dendrogram for the genetic relationships of the five quinoa genotypes based on overall markers separated them into two major groups. The first group included Q-37 genotype and Regalona cultivar and the second group were included Kvlsra 2, Kvlsra 3 and Q-52 genotypes.

From the previous results, a considerable level of variations were detected among five quinoa genotypes by biochemical and molecular markers which can help to select the most suitable genotypes Q-37 and Regalona cultivar for stress tolerance, good yield, presented considerable interest for the genetic studies, plant improvement and accepted by farmer to enter breeding programs and for reclamation salt affected lands. The mean squares for all traits were significant among the five quinoa genotypes.

Keywords: Quinoa genotypes, salinity, Biochemical markers, molecular markers and Isozymes**.**

INTRODUCTION

Chenopodium quinoa is one of the most important food crops in the Andean highland of South America (Kadereit *et. al*., 2003).The seeds contain an excellent balance of carbohydrates, lipids and protein, it provides an ideal balance of all 20 essential amino acids making it an excellent food source (Chauhan *et. al*., 1999). Quinoa is one of the only few crop plants adapted to the extreme conditions of salinity and drought that characterize in different region (Prado *et. al*., 2000). In spite of the importance that has been

attributed to the quinoa crop to face different stresses conditions, only few lines of investigation exist to establish applied genetics and molecular characteristics of this crop. Up to now, only a few researchers have reported the development and use of breeding, biochemical and molecular markers in quinoa. Proteins and isozymes as simple cheap techniques have been successfully used to identify wild Chenopodiaceae species (Bhargava *et. al*., 2012 and Wilson, 1988). Maugham *et. al*., 2004 was used the data of isozymes in quinoa for confirming the genetic difference between ecotypes of the plateau and valleys for help to made a genetic map in order to establish genotypic differences between quinoa from the North and the South of Chile. In the most cases, studies regarding quinoa are only focused on morphology with high genetic diversity, physiological and biochemical comparison between wild or weedy forms and local cultivated or domesticated varieties. RAPD is a stable unaffected with a marker for different environmental conditions and have proven to be useful for the analysis of genetic structure in crop species. This technique has the advantages of being fast and easy, requiring little plant material, having high resolution without previous knowledge of DNA sequences (Nybom, 2004). Therefore, the main objectives of the present study were to investigate the molecular diversity and relationships among five quinoa genotypes and to compare the morphological characters such as yield components to use this information in future breeding programs.

MATERIALS AND METHODS

Field experiment

One field experiment was carried out at Ras Sudr Experimental farm Station of Desert Research Center, South Sinai governorate during 2012/2013 to evaluate the genetic variations, yield and its components for five quinoa genotypes namely**:** Kvlsra 2, Kvlsra 3**,** Regalona cultivar, Q-37 and Q-52 under saline soil and water of Ras Sudr conditions. Quinoa seeds were sown in November 18th in a complete randomized block design in three replication. The plot size was 8.0 × 3.5 m, quinoa seeds were sown in rows and two plants per hill (40 cm. spaces). The agricultural practices and recommended fertilization was applied. Plants were harvested after 150 days from sowing date. Data of yield and yield components were recorded i.e.: plant height (cm), number of lateral branches/plant, number of heads /m^2, heads yield (g/m^2), seed yield (g/m^2) and straw yield (g/plant). Data were analyzed for homogeneity of variances using a Bartlett test in one season and subjected to Analysis of Variance (ANOVA) using MSTAT-C computer software, Michigan State University, (1988). Physical and chemical properties of the soil and irrigated water are presented in Table (1).

Table 1: Physical and chemical properties of soil & chemical analysis of irrigation water at Ras Sudr

a) Physical analysis of soil										
Total sand (%)		**Clay (%)**		**Silt (%)**			**Texture class**			
85.14		9.01		5.85			Sandy loam			
b) Chemical analysis of soil and irrigation water										
EC dS/m	**ppm**	**pH**	**Cations (meq/L)**				**Anions (meq/L)**			
			Ca^{++}	**Mg^{++}**	**Na^{+}**	**K^{+}**	**$CO_3^{=}$**	**HCO_3^{-}**	**Cl^{-}**	**$SO_4^{=}$**
Chemical analysis of soil										
10.23	6547.2	7.64	36.42	14.32	60.87	0.651	------	3.061	58.65	50.55
Chemical analysis of irrigation water										
12.34	7897.6	7.51	34.85	15.89	80.23	0.387	------	2.98	71.05	57.327

Biochemical markers:

Protein electrophoresis:

One dimensional sodium dodecyl sulfate Polyacrylamide gel electrophoresis (SDS-PAGE) was conducted according to the method of Laemmli (1970) and modified by Studier (1973) to discriminate and fingerprint of the five quinoa genotypes i.e., Kvlsra2, Kvlsra3, Regalona cultivar, Q-37 and Q-52) under Ras Sudr stress conditions whereas the soil and water are saline. The gel was photographed scanned and analyzed using Gel Documentation 2000, Bio-Rad System.

Isozymes analysis:

Five isozymes systems including peroxidase (POD), acid phosphatase (ACPH), beta esterase (β-EST), alpha esterase (α-EST) and Alcohol dehydrogenase (ADH) were separated according to (Stegemann *et. al*., 1985). After electrophoresis, the gels were stained according to their enzyme systems with the appropriate substrate and chemical solutions then incubated at room temperature in dark for complete staining. In most cases incubation for about 1 to 2 hours is enough. After the appearance of the isozymes bands, the reaction was stopped by washing the gel two or three times with tap water, followed by adding the fixing solution. The gel was kept in the fixing solution for 24 hours and rinsed with tap water two times then the banding profile was photographed.

Molecular markers:

Extraction of DNA

Samples of fresh leaf were collected from the five quinoa genotypes and were ground under liquid nitrogen to a fine powder, then bulked of DNA extraction was performed using DNeasy plant mini kit (Qiagen). DNA was quantities by spectrophotometer (Unicam UV 300) at 260 nm before gel electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 µg mL-1) in 1X TBE buffer at 100 volts for one hour.

RAPD-PCR analysis

PCR amplification was performed using five random 10-mer arbitrary primers synthesized by Operon biotechnologies, Inc. Germany, Table (2). The PCR amplification was performed in a 25 µl reaction volume containing the following: 2.5 µl of dNTPs (2.5 mM), 1.5µl of Mg Cl_2 (25 mM), 2.5 µl of

10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH_2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. The reaction was subjected to one cycle at 95 ºC for 5 minutes, followed by 35 cycles at 94 ºC for 30 seconds, 37 ºC for 30 seconds, and 72 ºC for 30 seconds, then a final cycle of 72 ºC for 12 minutes. PCR products were run at 100 V for one hour on 1.4 % agarose gels to detect the polymorphism between the five quinoa genotypes. The amplified DNA fragments were separated and stained. Fragments sizes were estimated with 100bp ladder marker. The amplified pattern was visualized on an ultraviolet light transilluminator and photographed by gel documentation system (Bio-Rad® Gel Doc-2001) (Germany).

Table 2: List of random primers and their nucleotide sequences

No.	Primers	Sequences
1	OPA4	5'-AATCGGGCTG-3'
2	OPA17	5'-GACCGCTTGT-3'
3	OPD5	5'-TGAGCGGACA-3'
4	OPC1	5'-TTCGAGCCAG-3'
5	OPC16	5'-CACACTCCAG-3'

Statistical analysis

Protein, isozymes and DNA bands generated were counted and their molecular sizes were compared with protein and DNA markers. The presence or absence of protein, isozymes and DNA bands were entered into computer program SPSS-10.

RESULTS AND DISCUSSION

Yield and its components

The analyses of variance for grain yield and other related traits are given in Table (3). There was a highly significant difference (P<0.05) between genotypes for all studied traits revealing the existence of substantial amount of variation among the genotypes. Such diversity of genotypes population might be due to factors as heterogeneity, genetic architecture of population, history of selection and/or developmental traits. These results agree with (Ghafoor *et. al*., 2001 and Bhargava *et. al*., 2007) they reported that there was a significant in 0.05 and 0.01 percent probability level differences for grain yield and its components in different crop species.

Table 3:Observed mean squares of quinoa genotypes for different studied traits

S.O.V	d.f	Plant height (cm)	No. of branches/ plant	Number of heads /m^2	Heads yield (g/m^2)	1000-Seed weight (g.)	seed yield (g/m^2)	straw yield (g/plant)
Replication	2	8.38*	0.02	1.54	1007.00**	0.01	5.77	362.8**
Genotypes	4	105.95**	20.40**	70.79**	15501.33**	3.86**	6828.82**	5579.57**
Error	8	0.17	0.01	0.03	3.12	0.03	11.3677	1.12
Total	14							

***and**significance at the 0.05and 0.01 levels probability, respectively.**

The mean performances of different five quinoa genotypes were shown in Table (4). Significant differences were detected among all genotypes for all recorded traits. The genotype Q-37 followed by Regalona cultivar recorded the highest means for seed yield/m2 which had values 185.58 and 166.01 g/m^2, respectively. This superiority in yielding ability attributed to heads yield (g/m^2), number of heads /m^2 and straw yield (g/ m^2). While, for 1000 seed weight, Kvlsra 3 and Regalona cultivar which had values 4.87 and 4.00 g. respectively.

Table 4:Mean performance of five quinoa genotypes for different studied traits.

Genotypes	Plant height (cm.)	No. of branches/plant	No. of heads /m^2	Heads yield (g/m^2)	1000-seed weight (g.)	Seed yield (g/m^2)	Straw yield (g/ m^2)
Kvlsra2	100.55	7.54	41.48	531.34	1.81	86.35	318.81
Kvlsra 3	97.48	10.83	50.30	571.61	4.87	157.65	342.97
Regalona	98.20	4.68	46.29	574.98	4.00	166.01	344.99
Q-37	100.66	10.66	52.11	696.33	3.56	185.58	417.80
Q-52	87.68	7.01	42.44	511.36	3.09	83.47	306.82
L.S.D.	0.25	0.12	01.46	030.31	3.33	01.69	002.20

Correlation coefficient between yield and its components

Correlation coefficient values are presented in Table (5). The interrelationship between mean performance were positive and significant between number of branches/plant and number of heads/m^2 and were positive and highly significant between number of heads /m^2 and each of; heads yield (g/m^2), 1000-seed weight, seed yield/$m^2$and straw yield/ m^2; heads yield/m^2 and each of seed yield/m^2 and straw yield/m^2; 1000-seed weight and seed yield/m^2 and seed yield/m^2 and straw yield/m^2. Such traits may be taken in account in quinoa screening programs for salinity stress tolerance. Other traits showed low significant correlation, suggesting the independent of mean genotypic performance under control treatment and reduction percentage occurring under water stress conditions

Table 5: Simple phenotypic correlation coefficients between genotypes mean performance

Traits	Plant height (cm.)	No. of branches/plant	No. of heads /m^2	Heads yield (g/m^2)	1000-Seed weight (g.)	Seed yield (g/m^2)
No. of branches/ plant	0.264					
No. of heads /m^2	0.421	0.669*				
Heads yield (g/m^2)	0.574	0.526	0.851**			
1000- Seed weight (g.)	0.030	0.303	0.734**	0.316		
Seed yield (g/m^2)	0.532	0.339	0.917**	0.837**	0.709**	
Straw yield (g/ m^2)	0.583	0.526	0.851**	0.942**	0.316	0.837**

Biochemical markers:-

Protein marker

Data of biochemical markers are used to assess genetic variability within and among populations. (Cooke 1995 and Chauhan *et. al.,* 1999). Figure 1 shows the protein electrophoretic banding patterns of leaf protein analysis for the five quinoa genotypes under Ras Sudr conditions. It is produced 29 bands distributed in all genotypes with molecular weights ranging from 8 kDa to 103 kDa. The distribution of these bands in the studied genotypes and their molecular weights are illustrated in tables 6 and 7.The results showed twelve common bands among the five quinoa genotypes at the molecular weights; 49, 42, 41, 40, 38, 32, 27, 22, 21, 20, 10 and 8 kDa. In addition, Kvlsra2, Kvlsra3, Regalona cultivar and Q-37 produced positive marker at the molecular weight 67 kDa. On the other hand, Kvlsra2 and Kvlsra 3 revealed unique bands at the molecular weights 103 and 98 kDa, respectively. While, Regalona cultivar and Q-37 revealed six and seven unique bands at different molecular weights, respectively. Moreover, Q-52 genotype produced two unique bands at the molecular weights 19 and 18 kDa. Finally, seventeen polymorphic bands produced (59%) of polymorphism which indicated the genetic variations of five quinoa genotypes under Ras Sudr conditions. These results were in agreement with Bhargava *et. al*., (2012) who separated forty cultivated and wild taxa of *Chenopodium* by SDS-PAGE and the results showed that the total protein electrophoresis was useful for genetic identification of genotypes.

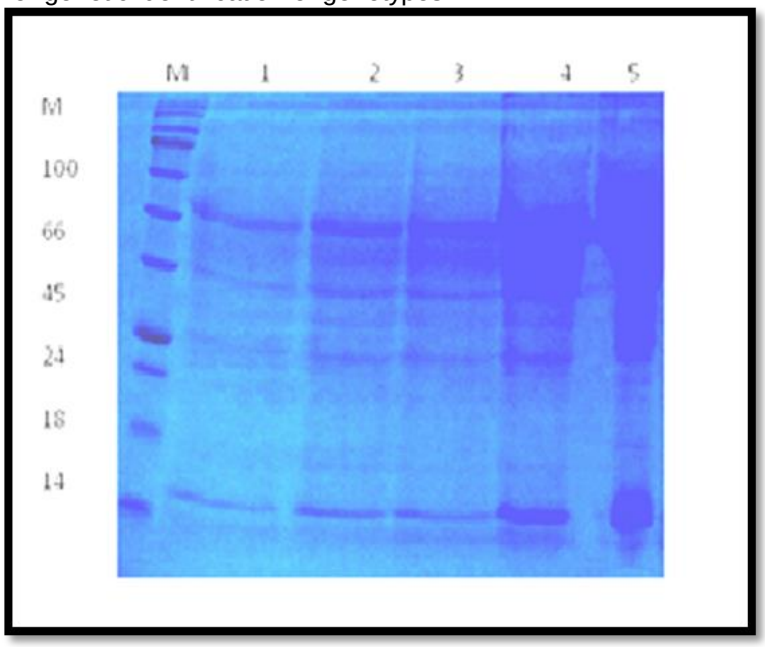

Fig. 1: SDS- PAGE protein banding pattern for the five quinoa genotypes viz; Kvlsra2, Kvlsra3, Regalona, Q-37 and Q-52.

Table 6: SDS-PAGE protein analysis for the five quinoa genotypes.

Band No.	MW	Kvlsra2	Kvlsra3	Regalona	Q-37	Q-52	Polymorphism
1	103	1	0	0	0	0	Unique
2	100	0	0	0	1	0	Unique
3	98	0	1	1	0	0	Poymorphic
4	92	0	0	1	0	0	Unique
5	87	0	0	1	0	0	Unique
6	80	0	0	0	1	0	Unique
7	74	0	0	0	1	0	Unique
8	69	0	0	0	1	0	Unique
9	67	1	1	1	1	0	Positive marker
10	63	0	0	0	1	0	Unique
11	52	0	0	0	1	0	Unique
12	49	1	1	1	1	1	Monomorphic
13	42	1	1	1	1	1	Monomorphic
14	41	1	1	1	1	1	Monomorphic
15	40	1	1	1	1	1	Monomorphic
16	38	1	1	1	1	1	Monomorphic
17	32	1	1	1	1	1	Monomorphic
18	27	1	1	1	1	1	Monomorphic
19	22	1	1	1	1	1	Monomorphic
20	21	1	1	1	1	1	Monomorphic
21	20	1	1	1	1	1	Monomorphic
22	19	0	0	0	0	1	Unique
23	18	0	0	0	0	1	Unique
24	13	0	0	1	0	0	Unique
25	12	0	0	1	0	0	Unique
26	10	1	1	1	1	1	Monomorphic
27	9	0	0	0	1	0	Unique
28	8.5	0	0	1	0	0	Unique
29	8	1	1	1	1	1	Monomorphic
Total		**14**	**14**	**19**	**20**	**14**	

***1= band present and 0 = band absent**

Table 7: Number, types and polymorphism percentage of leaf soluble protein of five quinoa genotypes.

Monomorphic bands	Polymorphic bands		Total bands	Polymorphism %
	Non-unique bands	Unique bands		
12	3	14	29	59%

Genetic similarity and cluster analysis based on protein:

Similarity matrix based on protein was developed by SPSS computer package system as shown in Table 8 and Fig 2. The highest relationship (73%) was scored between Regalona cultivar and Q-37 genotypes. While, the lowest relationship (13%) was scored between Kvlsra3 and Regalona cultivar. The dendogram based on protein, separated the five quinoa genotypes into two main clusters. Moreover, Kvlsra2, Regalona, Q-37 and Q-

52 were separated in the first main cluster, while Kvlsra3 was separated in the second cluster.

Table 8:Similarity matrix of the five quinoa genotypes based on protein marker.

Genotypes	Kvlsra2	Kvlsra 3	Regalona	Q-37	Q-52
Kvlsra2	100				
Kvlsra 3	15	100			
Regalona	60	13	100		
Q-37	63	30	73	100	
Q-52	45	40	57	57	100

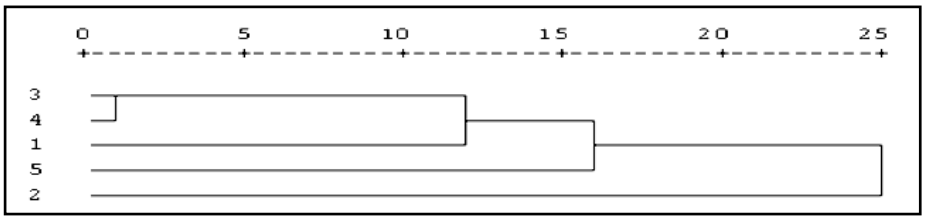

Fig. 2: Dendrogram based on protein marker of the five quinoa genotypes under study

Isozymes marker

Five isozymes systems included POD, ACPH, β-EST, α-EST and ADH were used for the five quinoa genotypes as shown in Fig (3) and Tables 9 and 10. The results showed that there were fifteen total bands, seven monomorphic bands and two unique bands for Q-37 genotype. While, six polymorphic bands revealed (53%) of polymorphism. The highest polymorphism (80%) was revealed by α- EST and the lowest polymorphism (33%) was revealed by ACPH and β-EST. The obtained results were in agreement with (Abd El- Maboud and Khalil, 2013)) who used isozymes to detect genetic diversity and relationships in some species of the genus *Suaeda* (Chenopodiaceae) from different sites in Egypt along the Mediterranean Sea. Eight isozymes systems including acid phosphatase, alcohol dehydrogenase, α- esterase, β-esterase, aldehyde oxidase, malic acid, malate dehydrogenase and peroxidase produced twenty one total bands with (76%) of polymorphism.

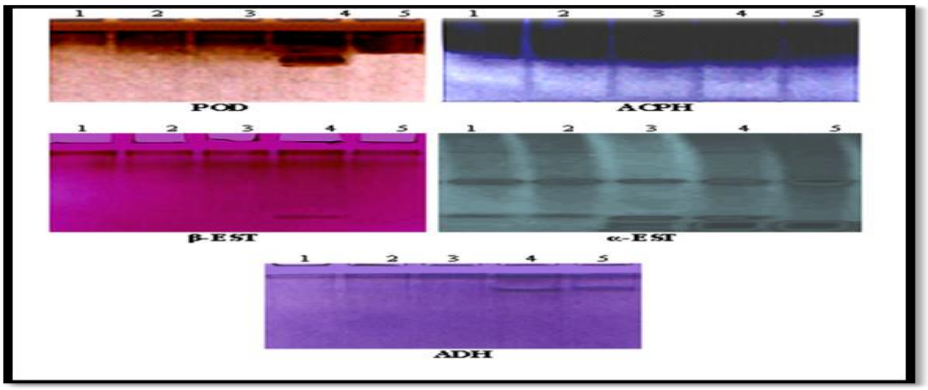

Fig 3: POD, ACPH, β-EST, α-EST and ADH isozymes banding patterns among the five quinoa genotypes viz; Kvlsra 2, Kvlsra 3, Regalona, Q-37and Q-52.

Table 9: Polymorphism percentages generated by the five isozymes systems among the five quinoa genotypes

Isozymes types	No. bands	Kvlsra 2	Kvlsra 3	Regalona	Q-37	Q-52	Polymorphism
POD	1	1.00	1.00	1.00	1.00	1.00	Monomorphic
	2	0.00	0.00	0.00	1.00	0.00	Unique
ACPH	1	1.00	1.00	1.00	1.00	1.00	Monomorphic
	2	1.00	1.00	1.00	1.00	1.00	Monomorphic
	3	0.00	1.00	0.00	1.00	1.00	Polymorphic
β-EST	1	1.00	1.00	1.00	1.00	1.00	Monomorphic
	2	1.00	1.00	1.00	1.00	1.00	Monomorphic
	3	0.00	0.00	0.00	1.00	0.00	Unique
α-EST	1	0.00	0.00	0.00	1.00	1.00	Polymorphic
	2	1.00	1.00	1.00	1.00	1.00	Monomorphic
	3	1.00	0.00	1.00	1.00	0.00	Polymorphic
	4	0.00	0.00	1.00	1.00	0.00	Polymorphic
	5	0.00	0.00	1.00	1.00	0.00	Polymorphic
ADH	1	1.00	1.00	1.00	1.00	1.00	Monomorphic
	2	0.00	0.00	1.00	1.00	1.00	Polymorphic

Table 10: Isozymes types, monomorphic bands, polymorphic bands, unique bands, total amplified bands and polymorphism percentages generated by the five isozymes systems among the five quinoa genotypes

Isozymes types	Monomorphic bands	Polymorphic bands	Unique bands	Total bands	Polymorphism (%)
POD	1	0	1	2	50%
ACPH	2	1	0	3	33%
β-EST	2	0	1	3	33%
α -EST	1	4	0	5	80%
ADH	1	1	0	2	50%
Total	7	6	2	15	53%

Genetic similarity and cluster analysis based on isozymes

Based on isozymes markers, similarity matrix was developed by SPSS computer package system as presented in Table (11) and Fig (4). The highest relationship (88%) was scored between Q-37 and Regalona cultivar and the lowest relationship (10%) was scored between Kvlsra2 and Q-52 genotypes. The dendrogram based on isozymes, separated the five quinoa genotypes into two main clusters. Moreover, Kvlsra 2, Regalona cultivar, Q-37 and Q-52 genotypes were separated in the first cluster, while Kvlsra 3genotype was separated in the second cluster.

Table 11:Similarity matrix for five quinoa genotypes based on isozymes under Ras Sudr conditions

Genotypes	Kvlsra2	Kvlsra3	Regalona	Q-37	Q-52
Kvlsra2	100				
Kvlsra3	68	100			
Regalona	13	87	100		
Q-37	51	39	88	100	
Q-52	10	40	30	80	100

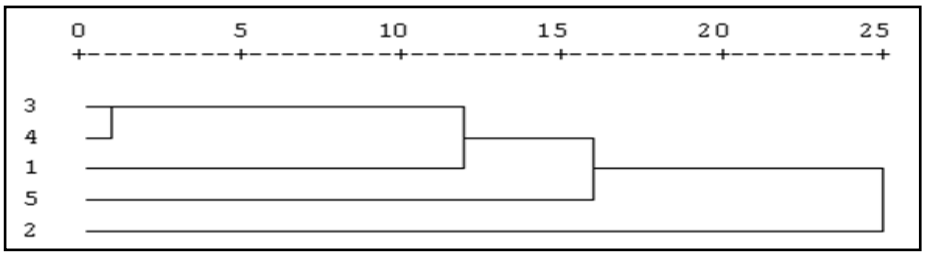

Fig 4: Dendrogram based on isozymes markers of five quinoa genotypes viz; Kvlsra2, Kvlsra3, Regalona cultivar, Q37 and Q-52 genotypes.

Molecular markers

RAPD-PCR: Five RAPD primers amplified DNA fragments for the five quinoa genotypes as illustrated in Fig (5) and Table (12 &13). A total of forty four bands, fifteen bands were monomorphic and the fifteen bands were polymorphic (66% of polymorphism) revealed for the five quinoa genotypes. Fourteen bands were unique and could be considered as specific bands for species and salinity tolerance. Kvlsra2 produced unique band of the molecular weight 400bp used as specific band. While, Regalona cultivar produced three unique bands of the molecular weights; 1200, 1000 and 550 bp, respectively. So, Q-37genotype produced ten unique bands of different molecular weights used as species specific. Polymorphism levels differed from one primer to another. OPC1 primer exhibited the lowest level of polymorphism (40%) among five quinoa genotypes, while, OPC16, OPD5 OPA4, and OPA17 primer exhibited the highest levels of polymorphism (82%, 80%, 72% and 55%, respectively). These results agreed with **(Leonardo and** Max 2009) who coincided that molecular marker separated quinoa into two

types: a coastal type (Chile) and an Andean plateau type a complement to the work of (Ruas *et. al*., 1999) who used RAPD marker to detect the degree of polymorphism between cultivated and wild species of quinoa. They showed that wild and crop populations of *Chenopodium quinoa* share a low level of molecular variation, without differentiation between sympatric domesticated and weedy populations, and low levels of intraspecific variation within accessions because of the same genetic pool with limited seed exchange among geographically isolated regions is also probably a factor of differentiation as well as the cultivation practices of farmers.

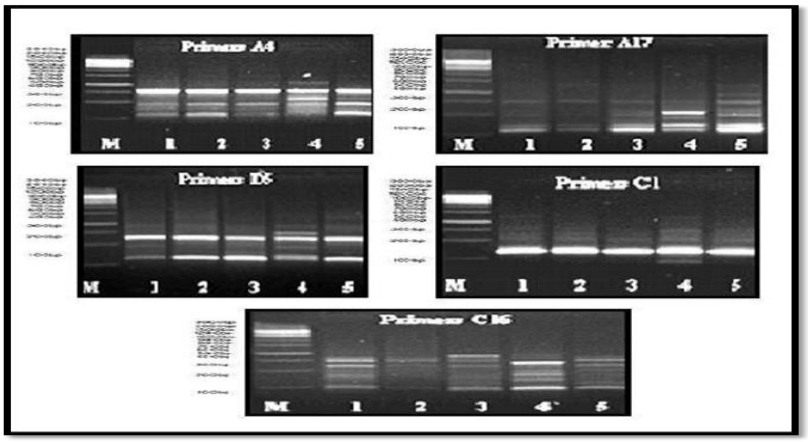

Fig 5: RAPD primers viz; OPA1, OPA17, OPD5, OPC1 and OPC16 among the five quinoa genotypes viz; Kvlsra2, Kvlsra3, Regalona cultivar, Q-37and Q-52

Table 12: Amplified fragments obtained from the DNAs of five quinoa genotypes

RAPD Primers	Kvlsra 2	Kvlsra 3	Regalona	Q-37	Q-52	Pb	Polymorphism
Primer A4	1	1	0	0	0	270	Polymorphic
	1	1	1	1	1	300	Monomorphic
	0	0	0	1	1	330	
	1	1	1	1	1	500	Monomorphic
	0	0	0	1	1	600	Polymorphic
	0	0	0	1	0	650	Unique
	1	1	1	0	1	700	Polymorphic
	1	1	1	1	1	750	Monomorphic
	0	0	0	1	0	1000	Unique
	0	0	1	0	0	1200	Unique
Primer A17	1	1	1	1	1	270	Monomorphic
	1	1	1	1	1	300	Monomorphic
	0	0	0	1	0	430	Unique
	1	1	1	0	1	470	Polymorphic
	1	1	1	1	1	520	Monomorphic
	0	0	1	0	0	550	Unique
	1	1	1	1	1	750	Monomorphic
	0	0	1	0	0	1000	Unique
	0	1	0	0	1	1200	Polymorphic
Primer D5	1	1	1	1	1	300	Monomorphic
	0	0	0	1	0	350	Unique
	1	0	0	1	0	450	Unique
	0	0	0	1	0	550	Unique
	1	1	1	1	1	630	Monomorphic
	0	0	0	1	0	750	Unique
	1	1	1	1	0	800	Polymorphic
Primer C1	0	0	0	1	0	200	Unique
	1	1	1	1	1	300	Monomorphic
	1	1	1	1	1	350	Monomorphic
	1	1	1	1	1	500	Monomorphic
	1	1	1	1	1	650	Monomorphic
	1	1	1	1	1	750	Monomorphic
	1	1	0	1	0	1150	Polymorphic
Primer C16	1	1	1	1	1	250	Monomorphic
	0	0	1	0	1	300	Polymorphic
	0	0	0	1	0	350	Unique
	1	0	0	0	0	400	Unique
	1	0	0	1	0	450	Polymorphic
	1	0	0	1	1	500	Polymorphic
	1	1	1	1	1	650	Polymorphic
	0	0	0	1	0	680	Unique
	1	1	1	0	1	700	Polymorphic
	1	1	1	1	1	750	Monomorphic
	0	0	1	1	0	800	Polymorphic

Table 13: Primer codes, length range (bp), monomorphic bands, polymorphic bands, unique bands, total amplified bands and polymorphism percentages of the five RAPD primers of five quinoa genotypes.

Primer codes	Length range (pb)	Monomorphic bands	Polymorphic bands	Unique bands	Total amplified bands	Polymorphism percentages
OPA 4	1200-270	2	5	3	10	80%
OPA17	1200-270	4	2	3	9	55%
OPD 5	800-300	2	1	4	7	72%
OPC 1	1500-200	5	1	1	7	40%
OPC16	800-250	2	6	3	11	82%
Total		15	15	14	44	66%

Genetic similarity and cluster analysis based on RAPD markers

The RAPD data were used to estimate the genetic similarity among the five quinoa genotypes by using SPSS computer analysis as shown in Table 14 and Fig 6. The highest similarity (95%) was recorded between Q-37 and Regalona cultivar, while the lowest similarity (12%) was detected between Q-37 and Q-52genotypes.The dendrogram for the genetic relationships of the five quinoa genotypes were separated into two major groups. The first group included two subgroups, the first subgroup included Q-37 and Regalona cultivar, and the second subgroup included Kvlsra 2.While, the second group included Kvlsra 3 and Q-52 genotype.

Table 14: Similarity matrix based on RAPD marker for five quinoa genotypes

Genotypes	Kvlsra2	Kvlsra3	Regalona	Q-37	Q-52
Kvlsra2	100				
Kvlsra3	36	100			
Regalona	63	15	100		
Q-37	66	16	95	100	
Q-52	31	93	20	12	100

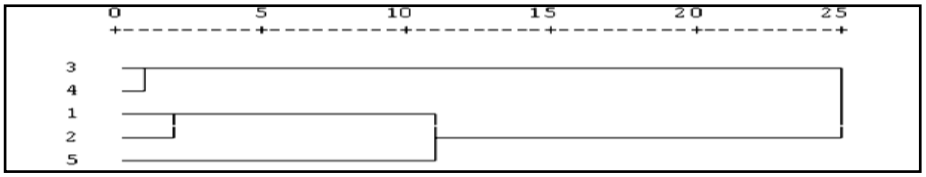

Fig. 6:Dendrogram based total analysis RAPD markers of the five quinoa genotypes, Kvlsra2, Kvlsra3, Regalona, Q-37 and Q-52.

Genetic similarity and cluster analysis based on protein, isozymes and RAPD markers

The obtained data across protein, isozymes and RAPD profiles in this work were pooled together to estimate the genetic similarity of the five quinoa genotypes under salinity conditions by using SPSS computer analysis as shown in Table (15) and Fig (7). The highest similarity (89%) was recorded between Q-37 and Regalona. While the lowest similarity (60%) was detected between Q-37 and KVLSRA3. The dendrogram for the genetic relationships of the five quinoa genotypes exhibited in Fig. (7), which separated them into

two major groups. The first group included Q-37 and Regalona. While the second group included Kvlsra2, Kvlsra3 and Q-52.

Table 15: Similarity matrix of total analysis (protein, isozymes and RAPD) markers of the five genotypes, Kvlsra2, Kvlsra3, Regalona, Q-37 and Q-52 genotype.

Genotypes	Kvlsra 2	Kvlsra 3	Regalona	Q-37	Q-52
Kvlsra2	100				
Kvlsra3	65	100			
Regalona	68	68	100		
Q-37	67	60	89	100	
Q-52	78	83	67	66	100

The dendrogram for the genetic relationships of the five quinoa genotypes base on overall markers

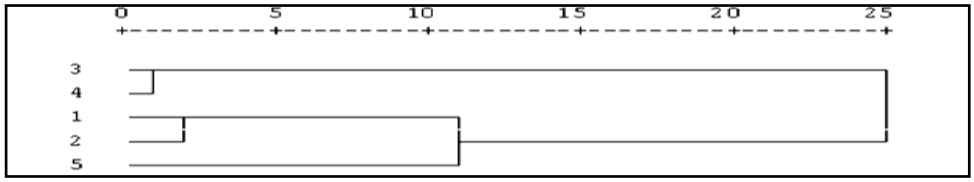

Fig 7: Dendrogram based on total analysis (protein, isozymes and RAPD) of the five quinoa genotypes.

CONCLUSION

The results showed that significant differences were detected among all genotypes for all traits. The genotype Q-37 followed by Regalona cultivar for mean seed yield. The interrelationship between mean performance were positive and high significant among number of heads and each of heads yield, 1000- seed weight, seed yield and straw yield, Such traits may be taken in account in quinoa genotypes screening programs.

The results of the study can be used as a starting point for future researches with the aims of defining the level of genetic diversity of five quinoa genotypes under Ras Sudr conditions.

The results of biochemical and molecular markers showed that Q-37 followed by Regalona produced highest number of total and unique bands as specific bands for species and salinity tolerance which confirm breeding results with superior morphological characters.. This study have given the important clues in understanding the relationships of quinoa genotypes, which may further assist in developing and planning breeding strategies to select the most promising genotypes.

REFERENCES

Abd El- Maboud M. M, and M. A. R. Khalil, (2013). Ecophysiological and genetic studies on some species of the genus *Suaeda* Forssk ex. Scop. in the Mediterranean Sea Coast. World Applied Sciences Journal, 27 (7): 811-825.

Bhargava, A., T. S. ,Rana, S. Shukla, and D.Ohri, (2012). Seed protein electrophoresis of some cultivated and wild species of Chenopodium. Biologica Palantarum, 49, 4: 505-511.

Bhargava, A., S., Shukla, D., Ohri, (2007). Genetic variability and interrelationship among various morphological and quality traits in quinoa (*Chenopodium quinoa* Willd.). Field Crop. Res. 101, 104–116.

Chauhan, G.S.; , N. Eskin and; R. Tkachuk, (1999). Effect of saponin extraction on the nutritional quality of quinoa (Chenopodium quinoa Willd.). J. Food Sci. Technol, 36: 123–126.

Cooke, R . J. (1995). Varietal identification of crop plants. *In:* JH Skerrit and R Appels (editors). New Diagnostics in Crop Science. CAB International, Wallingford, UK. pp. 33–63.

Ghafoor, A., A. Sharif, Z. Ahmad, M.A. Aahid and M.A. Rabbani. (2001). Genetic diversity in black gram (*Vigna mungo* (L.) Hepper). Field Crop Research, 69: 183-190.

Kadereit, G.; T. Borsch; K. Welsing, and H. Freitag, (2003). Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C4 photosynthesis. Int. J. Plant Sci, 164: 959–986.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of head bacteriophage T_4. Nature, 227: 680- 685.

Leonardo A.R. and T.I. Max (2009). Comparative analysis of genetic and morphologic diversity among quinoa accessions (*Chenopodium quinoa* Willd.) of the South of Chile and highland accessions. Journal of Plant Breeding and Crop Science. 1 (5). 210-216.

Maughan, P.; Bonifácio, A.; Jellen E; Stevens, M.; Coleman, C.; Ricks, M.; Mason, S.; Jarvis, D.; Gardunia, B. and Fairbanks, D. (2004). A genetic linkage map of quinoa (*Chenopodium quinoa* Willdenow) based on AFLP, RAPD, and SSR markers. Theory Applicated Genetic,109: 1188-1195.

Nybom, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants, Mol Ecol., 13:1143–1155.

Prado, R.; Boero,; Gallard, M.; Gonzalez, J. (2000). Effect of NaCl on germination, growth, and soluble sugar content in Chenopodium quinoa Willd. seeds. Bot. Bull. Acad. Sci,41: 27–34.

Ruas, P.M.; C. Bonifacio, A.; Ruas, C. F.; Fairbanks, D. J. and W. R., Andersen, (1999). Genetic relationship among 19 accessions of six species of Chenopodium L., by Random Amplified Polymorphic DNA fragments (RAPD). Euphytica, 105:25–32.

Stegemann, H., A. M. R. Afifiy and K. R. F. Hussein (1985). Cultivar identification of dates (Phoenix dectylifera) by protein patterns. 2nd international Symposium of Biochemical Approaches to Identification of Cultivars. Braunschweig, West Germany, 44 pp.

Studier, F. W., (1973). Analysis of bacteriophage T7 early RNAs and proteins of slab gels. J. Mol. Biol, 79: 237-248.
Wilson, H. D., (1988). Quinoa biosystematics I: domesticated populations. Econ. Bot. 42: 461–477.

التقيـيم الـوراثي لـبعض التراكيـب الوراثيـة مـن الكينـوا تحـت ظـروف رأس سـدر

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يعتمد تحديد أصناف الكينوا على السلوك المظهري للتركيب الوراثى تحت تأثير الظروف البيئة المختلفة. ونظرا لقلة التنوع الجيني للكينوا في معظم أنحاء العالم مما يهدد قدرة المربين على تحسين أصناف الكينوا والعمل على زيادة إنتاجيتها،

أجريت تجربة حقلية في محطة رأس سدر، جنوب سيناء خلال الموسم الشتوى 2012/2013 وذلك لتقييم خمسة تراكيب وراثية من الكينوا : Kvlsra 2، Kvlsra 3، Regalona، 37- Q و Q-52 تحت ظروف التربة المتأثرة بالأملاح والرى بالمياه المالحة. وقد أظهرت النتائج وجود فروق معنوية بين جميع التراكيب الوراثية المستخدمة لجميع الصفات تحت الدراسة. وقد سجل التركيب الوراثى Q-37 يليه الصنف Regalona أعلى متوسط لقيم محصول البذور. وكانت العلاقة بين متوسط الأداء موجبة وعالية ذات دلالة إحصائية بين عدد النورات، ووزن ال ١٠٠٠بذرة ووزن البذور، محصول البذور ومحصول القش، وعلى ذلك يمكن أن تؤخذ هذه الصفات في الإعتبار في برامج تربية الكينوا.

إستخدمت المعلمات البيوكيماوية والجزيئية لتحديد نسب تعدد الأشكال المظهرية ودراسة العلاقات الوراثية بين الخمسة تراكيب الوراثية للكينوا.وقد أعطت سبعة حزم بروتين متعددة الأشكال المظهرية نسبة إختلافات (٥٩%). كما أعطى أعطى الصنف Regalona والتركيب الوراثى Q- 37 أعلى عدد من الحزم البروتينية المتخصصة لتحمل للملوحة. أنتجت خمسة أنظمة انزيمية تشمل البيروكسيديز، الأسيد فوسفاتيز، ألبيتا أستيريز، ألفا أستيريز، الكحول ديهيدروجينيز ستة حزم متعددة الأشكال المظهرية ونسبة إختلافات متوسطة (٥٣%) وإثنان من الحزم المتخصصة للتركيب الوراثى Q- 37 لتحمل للملوحة، كما أظهرت خمسة بادئات عشوائية RAPD خمسة عشرة حزمة متعددة الأشكال المظهرية بأعلى نسبة إختلافات (٦٦%). وأعطى التركيب الوراثى Q- 37 أعلى عدد من الحزم البروتينية المتخصصة لتحمل للملوحة، فصلت شجرة القرابة الوراثية (الدندروجرام) بناء على كل المعلمات الوراثية المستخدمة فى الدراسة الخمسة تراكيب وراثية للكينوا إلى مجموعتين رئيسيتين. الأولى ضمت الصنف Regalona و37- Q. فى حين ضمت المجموعة الثانية Kvlsra 3, Q-52.

بناء على النتائج السابقة، وجدت إختلافات وراثية بين التراكيب الوراثية الخمسة للكينوا بإستخدام الدلائل البيوكيميائية والجزيئية والتى تساهم فى إختيار أفضل التراكيب وهما الصنف Regalona والتركيب الوراثى37- Q لتحمل الملوحة وجودة المحصول، كما أعطت أهمية خاصة للدراسات الوراثية لتحسين النبات المتوقعة من المزارعين لإدخال الكينوا فى برامج التربية وإستصلاح الأراضى الملحية.