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FIELD PERFORMANCE AND MOLECULAR DIVERSITY OF SEVENTEEN QUINOA GENOTYPES IN EGYPT

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ABSTRACT

A field experiment was carried out in Ismailia Agricultural Research Station, Agricultural Research Center, Egypt during, 2016/2017 and 2017/2018 winter seasons to assess field performance and molecular diversity among 17 quinoa genotypes: six cultivars (Giza-1, Giza-2, Sajama, Santa-Maria, Misr-1 and Regalona-Baer) and 11 accessions (Q12, Q21, Q22, Q26, Q27, Q29, Q31, QS14, QS16, QS17-1, QS18) were used. The seventeen quinoa genotypes were arranged in a randomized complete blocks design (RCBD) with three replicates. The results clearly indicated that the earliest mature and shortest duration genotypes were Giza-1, QS17-1 and QS18, which stayed only 119 days in the field, whereas QS14 and QS16 accessions, stayed up to 147 days from planting date till harvest as a moderate maturity accessions in their growth duration. The rest of the genotypes stayed between 120 and 140 days from planting to harvest as a short to moderate duration. The highest values of plant height and the number of branches per plant were recorded for QS16 accession, whereas the lowest values were recorded for QS17-1 accession in both seasons. The heaviest 1000-grains weights were recorded for QS18, QS17-1 accessions and Giza-2 cultivar. Meanwhile the lightest 1000-grain weight were recorded for QS16 and QS14 accessions, in both seasons, regarding grain yield per plant and per ha, Misr-1 cultivar recorded the highest values, while QS17-1 recorded the lowest values in both seasons. The study concluded that Misr-1 cultivar gave a high yield potential under sandy soil conditions, while Q27, Q21, Q26, Q18 and Q22 are promising accessions for developing new varieties. Molecular diversity was measured using inter simple sequence repeat (ISSR) and random amplified polymorphism DNA (RAPD). The results revealed that the polymorphism level differs from one ISSR primer to another, which reflects the primers ability to detect diversity among quinoa genotypes. On the other hand,the data revealed the ability of RAPD to discriminate among the seventeen quinoa genotypes. Combined data across ISSR and RAPD systems explored similarity indices among the seventeen quinoa genotypes. The highest value was 94% among genotypes Q27, Q29, Q12 and Q29 and genotypes Q18 and QS16. While, the lowest values were recorded among genotypes Q26 and QS17-1 followed by genotypes Giza-2 and Misr-1 and also genotypes Giza-2 and Q29. The combined dendrogram had two main clusters; cluster number 1 had genotype Giza-2 in the first sub-cluster, while genotypes QS18 and QS17-1 were in the second sub-cluster. Cluster number 2 was divided into two main sub-clusters. Sub-cluster number one had genotypes Q12 and Q29 in the one group. Furthermore, genotype Sajama was found in the second group, while genotype Q26 was in the third group alone. In addition, genotype Q22 is located in group 3 alone in the sub-cluster two.

Key words: *Chenopodium quinoa Willd., ISSR and RAPD.*

1. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a staple food for Andean countries in South America from 3,000 to 5,000 years BC **(**Tapia, 1979). It is a herbaceous annual plant grown as a grain crop primarily for its edible seeds. It is not a grass, it is a pseudo-cereal rather than a true cereal due to its uses

as a grain (NRC, 1989). Quinoa is closely related to the edible plants beetroot, spinach, and amaranth (*Amaranthus* spp.): Amaranthaceae is a family of flowering plants commonly known as the amaranth family (Martinez *et al*., 2015).

Recently quinoa crop attracted attention because of its high nutritional value and its strong growth potential under extreme harsh conditions of drought and soil salinity (Shams, 2011). FAO (2003) chose quinoa as one of the main crops to play an important role in ensuring food security in the 21st century. Quinoa crop contains high protein content, rich in amino acids, minerals, mono-saturated fatty acids and vitamins, which meet or exceed human requirements especially for children and people who also suffer from celiac disease (allergic to gluten), as a gluten-free crop (FAO, 2011, 2013 and Shams and Galal, 2014). The grains have not been found to contain anti-nutritional factors and ideal candidate crop for NASA Controlled Ecological Life Support System (CELSS) (NASA, 1993). Quinoa crop is recommended to replenish part of cereals gap, where it can grow successfully and competitively with high profitability to the small-scale farmers under sandy soil conditions (Shams, 2012 and 2018).

Quinoa is an allotetraploid (2n=4×=36) and shows disomic inheritance for most qualitative traits (Ward 2000 and Zurita-Silva *et al*., 2014).

Genetic markers are particularly important for germplasm conservation and core-collection development (Diwan *et al.*, 1995; Staub *et al.*, 1996 and Tanksley and McCouch 1997). The discovery of genetic markers for quinoa was the creation by Maughan *et al*. (2004), Mason *et al*. (2005) and Christensen *et al*. (2007) of a genetic linkage map. Furthermore, Jarvis *et al*., (2008) studied the allotetraploid quinoa genome, and mentioned that it may be useful in cytological analyses and genome evolutionary studies. They made a comparison between SSRs and other marker techniques and concluded that SSRs are relatively inexpensive once they have been developed, highly polymorphic, and easy to use.

Del Castillo *et al.* (2007) reported that quinoa has a strong population structure and a high intra-population variation with using RAPD markers. An effect of geographical structure of the populations was highlighted, due to population isolation, not simply linked to distance but more probably to climatic and orographic barriers present in the studied zone. They also found that intra-population genetic diversity was higher than that expected for a mainly autogamous species, and higher than that reported in anterior studies based on germplasm collections. Moreover, Ruas *et al*. (1999) reported a low level of intraspecific variation among germplasm accessions of quinoa based on RAPD markers. The main objective of this study was to assess field performance and molecular diversity among new quinoa genotypes.

2. MATERIALS AND METHODS

2.1. Field performance

A field experiment was carried out in Ismailia Agricultural Research Station, Agricultural Research Center (Lat. 30° 35' 30" N, Long. 32° 14' 50" E, 10 m above the sea level), Egypt during 2016/2017 and 2017/2018 winter seasons to evaluate 17 quinoa genotypes under sandy soil conditions for identifying their agronomic performance and molecular diversity. Table (1) shows code, name, origin and breeding state of the seventeen quinoa genotypes.

Three soil samples were taken from 0-30 cm depth before planting quinoa and mixed together and sent to Water, Soil and Environment Research Institute, ARC for mechanical and chemical analysis (Table 2) according to Jackson (1958) and Chapman and Pratt (1961).

The seventeen quinoa genotypes were arranged in a randomized complete blocks design (RCBD) with three replications. The area of each plot was 10.8 m^2, 2.4 m in width (4 lines 60 cm apart) and 4.5 m in length. Quinoa was drilled in lines on the 15*th* of November in both seasons and thinned to one plant at a distance of 15 cm between hills after a month from sowing date. Plots were kept free of weeds through hoeing four times. Sprinkler was the irrigation system. The field was finely prepared and calcium super phosphate (15.5% P_2O_5) was applied during soil preparation at the rate of 74 kg P_2O_5 ha^{-1}. Ammonium nitrate (33.5% N) was applied

Table (1): Quinoa code, name, origin and breeding state.

Code	Name	Origin	Breeding state
1	Giza-1	Egypt	Selection
2	Giza-2	Egypt	Selection
3	Santa-Maria	Bolivia	Introduction
4	Sajama	Bolivia	Introduction
5	Q12	USA	Introduction
6	Q18	Chile	Introduction
7	Misr-1	Egypt	Selection
8	Q21	Chile	Introduction
9	Q22	Chile	Introduction
10	Q26	Chile	Introduction
11	Q27	Chile	Introduction
12	Q29	Chile	Introduction
13	Regalona-Baer	Chile	Introduction
14	QS14	Denmark	Introduction
15	QS16	Denmark	Introduction
16	QS17-1	Peru	Introduction
17	QS18	Peru	Introduction

Table (2). Chemical analysis of the experimental soil before growing quinoa genotypes.

	Growing season	
	2016/17	2017/18
Mechanical analysis		
Clay %	5.12	5.12
Silt %	2.00	0.00
Sand %	92.88	94.88
Soil texture	Sandy	Sandy
Chemical analysis		
pH	7.40	7.70
EC (dS/m)	0.18	0.18
N (ppm)	10.00	5.00
P (ppm)	4.00	1.00
K (ppm)	64.00	96.00
Organic matter (%)	0.54	0.49

at the rate of 214.2 kg N ha^{-1} in five equal doses; the first after two weeks from planting date and the other doses were every two weeks. Potassium sulphate (48% K_2O) at the rate of 57 Kg k_2O ha^{-1} was applied in two equal doses with the third and fourth doses of nitrogen.

2.2. Data recorded

At full growth and prior to harvest, traits of plant height and number of branches per plant were recorded from samples of 10 plants from inner rows of each plot. Growth duration was recorded by estimating number of days from sowing to harvest.

At harvest, samples of 10 plants from inner rows were randomly taken from each plot to measure 1000-grain weight and grain yield per plant. Grain yield per ha (t) was recorded on the basis of plot area by harvesting all plants of each plot and converted to yield per ha.

2.3. Statistical analysis

Data were analyzed using ANOVA in Randomized Complete Blocks Design with three replications. MSTAT-C (1988) was used for statistical computations.

2.4. Molecular diversity

2.4.1.Genomic DNA extraction and purification: Fresh tissue parts (0.25 g) were collected separately from seedling after 15 days from germination. Extraction of total DNA was performed using methods for medicinal and aromatic plants according to Anna *et al.* (2001).

2.4.2.Inter Simple Sequence Repeat –PCR (ISSR –PCR) Analysis: The DNA amplifications were performed in an automated thermal cycle (model Techni512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 57º C, and 2 min at 72º C. The reaction was finally stored at 72º C for 10 min.

The primer names and sequences of ISSR and RAPD markers are presented in Table (3).

2.4.3.Statistical analysis: The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the

Table(3): List of the primer names and their nucleotide sequences used in the study for ISSR and RAPD procedures.

ISSR			RAPD		
	Name	**Sequence of primer (5´ → 3`)**		**Name**	**Sequence of primer (5´ → 3`)**
1	14A	$(CT)_8TG$	**1**	OP-A18	GTA GAC CCG T
2	44B	$(CT)_8$ GC	**2**	OP-B04	CCC TGT CGC A
3	HB-10	$(GA)_6$ CC	**3**	OP- B11	GGC TGT CCG T
4	HB-11	$(GT)_6$ TGT CC	**4**	OP-E15	GAT GAC CGC C
5	HB-12	$(CAC)_3$ GC	**5**	OP- Q18	GTT GCC AGC C

presence or absence of each DNA band was treated as a binary character in data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct a dendrogram tree among the studied 17 quinoa genotypes. Calculation was achieved using Dice similarity coefficients (Dice, 1945) as implemented in the computer program SPSS-10. Gel preparation procedure: Agarose (1.50 g) was mixed with (100ml) l x TBE buffer and boiled in microwave. Ethidium bromide (2.5µl) was added to the melted gel after the temperature became 55°C. The melted gel was poured in the tray of mini-gel apparatus and comb was inserted immediately, then comb was removed when the gel becomes hardened. The gel was covered by the electrophoretic buffer (1 x TBE). DNA amplified product (15 µl) was loaded in each well. DNA ladder (100bp) mix was used as standard DNA with known molecular weights of 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 .

2.5.Random amplified polymorphism DNA (RAPD –PCR) Analysis: Polymerase chain reaction (PCR) condition stock solutions: 5X Tris-borate (TBE), pH 8.0 (Tris-base 5.40 g; Boric acid 2.75 g; 500 mM EDTA, Ph 8.0 0.29; H_2O (d.w) up to 100.00 ml. Ethidium bromide: The stock solution was prepared by dissolving 1 g of ethidium bromide in 100 ml distilled water and mixed well with magnetic stirrer; transferred to a dark bottle and stored at room temperature. Sample loading dye (5x) [Na-EDTA, pH 8.0, 2.00 ml; Glycerol (100%) 5.00 ml; Bromophenol blue 0.75 ml; H_2O (d.w.) 1.50 ml]. PCR was performed in 30-µl volume tubes according to Williams *et al.* (1990) that contained the following: dNTPs (2.5 mM) 3.00 µl; MgCl2 (25 mM) 3.00 µl; Buffer (10 x) 3.00 µl; Primer (10 pmol) 2.00 µl; Taq DNA polymerse 0.20 µl; Template DNA (17) 2.00 µl and H2O (d.w.) 16.80 µl. Randomly amplified polymorphic DNA-PCR (RAPD-PCR): The DNA amplifications were performed in an automated thermal cycle (model Techni 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 37º C, and 2 min at 72º C. The reaction was finally stored at 72º C for 10 min.

3. RESULTS AND DISCUSSION

3.1. Field performance

Results in Fig. (1) indicated clearly that the earliest mature and shortest duration genotypes are Giza-1, QS17-1 and QS18 which stayed only 119 days in the field, whereas QS14 and QS16 accessions, stayed up to 147 days from planting date as a moderate accessions in their growth duration. The rest of the genotypes stayed between 120 and 140 days from planting to harvest as a short to moderate duration. These results are in accordance with those obtained by Shams (2018) who tested nine quinoa genotypes including six Peruvian varieties (Amarilla Marangani, Amarilla Sacaca, Blanca de Junin, Kancolla, Salcedo INIA and Rosada de Huancayo) and three new accessions (QS14, QS16 and QS17-2) and reported that QS14 and QS16

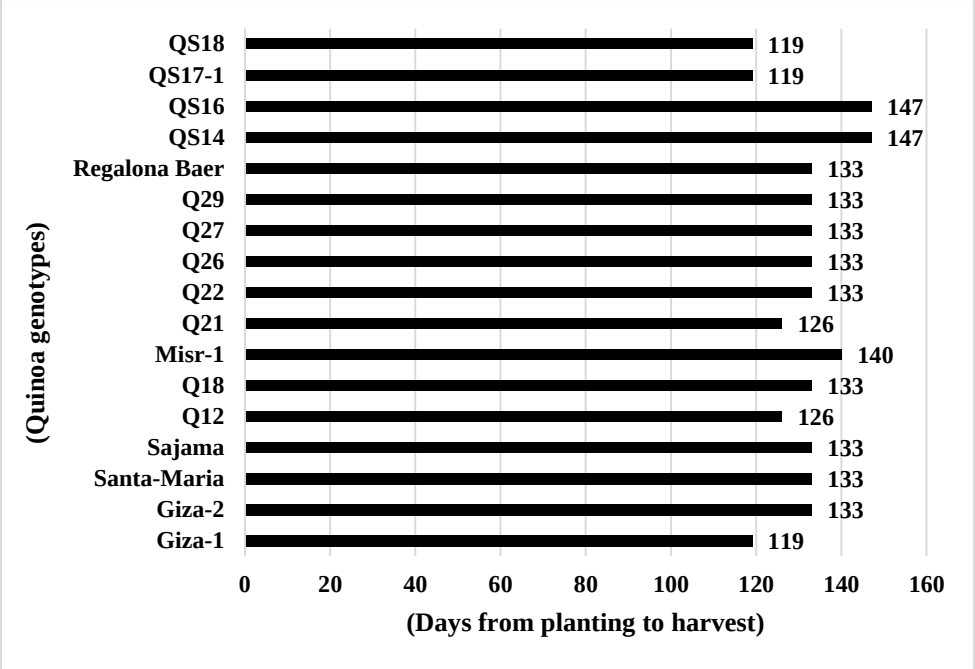

Fig. (1): Growth duration of the tested quinoa genotypes across two seasons.

accessions were moderate in their growth duration, while varieties of Amarilla Marangani, Amarilla Sacaca stayed up to160 days which treated as long duration genotypes.

Results in Table (4) revealed that QS16 accession was the tallest genotype, whereas QS17-1 accession was the shortest in the first and second season, respectively. The maximum number of branches per plant was obtained with QS16 accession, while the lowest number of branches was obtained with QS17-1 accession in the first and second season, respectively.

These results are in agreement with Shams (2018), who reported that QS16 was the tallest and most branched genotype among the nine genotypes tested in the evaluation trial.

The heaviest 1000-grain weight were recorded for QS18, QS17-1 accessions and Giza-2 cultivar, meanwhile the lightest 1000-grains weight were recorded by QS16 and QS14 accessions. In case of grain yield per plant and per ha, results indicated that Misr-1 cultivar recorded the highest values while QS17-1 recorded the lowest value in both seasons. Didier *et al.* (2016) tested a set of 21 different quinoa genotypes in nine sowing sites and concluded that genotypes Q12, Q18, Q21 and Q26 gave good yield stability across sites, meanwhile Q27 accession gave a high yield potential under Egyptian conditions.

3.2. Molecular diversity

3.2.1. Inter simple Sequence repeats (ISSR) analysis

Fig. (2) depicts the DNA banding patterns obtained with five ISSR primers (Table 3) for the seventeen genotypes, (Table 1). The total number of bands from five primers was 29 bands distributed as 14 polymorphic bands and 15 monomorphic bands. The polymorphism level differed from one primer to another that reflects the primers ability to detect diversity among quinoa genotypes as shown in Table (5) and Fig. (2).

The ISSR Primers gave polymorphism percentage ranged from 40% with 44B to 60% with 14A (Table, 5 and Fig. 2). The total bands differed according to the kind of primers; HB10 and HB11 were better than all primes in total bands. The average polymorphism percentage by ISSR-PCR system was 48.28% from 29 bands (Table 5 and Fig. 2).

The data in Table (6) explored the genetic similarity indices among the 17 quinoa genotypes. The highest value was 97% between genotypes Sajama and Q29 also Q12 and Q29. While the lowest value

Table (4): Agronomic evaluation of quinoa genotypes under sandy soil conditions in (2016/2017 and 2017/2018) growing seasons.

Trait / Genotype	Plant height (cm)		No. of branches plant^{-1}		1000-grain weight (g)		Grain yield plant^{-1} (g)		Grain yield ha^{-1} (t)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Giza-1	96.0	94.3	15.3	14.7	2.93	2.68	24.0	23.1	2.078	1.975
Giza-2	85.2	79.7	15.0	14.0	4.62	4.55	23.1	21.1	1.944	1.690
Santa-Maria	109.2	103.3	12.3	12.0	3.51	3.49	16.9	15.9	1.705	1.468
Sajama	103.8	100.3	15.3	14.1	3.66	3.62	18.0	16.7	1.837	1.640
Q12	115.4	110.3	16.3	15.2	3.01	2.83	28.3	26.7	2.382	2.154
Q18	111.3	104.7	16.7	16.0	3.26	2.94	34.9	34.3	3.046	2.813
Misr-1	114.3	108.7	18.3	17.1	2.84	2.66	42.0	38.0	3.363	3.186
Q21	111.6	106.0	16.3	14.8	3.38	3.11	36.7	34.5	3.069	2.910
Q22	113.9	108.3	17.7	16.3	3.03	2.87	33.7	30.7	3.033	2.749
Q26	122.1	120.0	17.3	16.0	3.34	3.06	36.0	34.3	3.053	2.824
Q27	112.7	108.3	18.0	16.7	3.33	3.02	36.9	35.2	3.126	3.019
Q29	117.9	112.0	16.7	15.5	3.13	2.93	30.7	29.0	2.662	2.447
Regalona-Baer	124.7	124.0	19.0	17.6	2.70	2.57	32.0	30.1	2.936	2.638
QS14	146.1	140.0	20.3	19.4	2.39	2.36	22.1	20.8	1.889	1.667
QS16	148.8	142.3	21.0	19.9	2.31	2.29	26.0	24.7	2.357	2.144
QS17-1	63.3	56.0	11.0	10.7	4.13	4.12	13.2	11.2	1.097	1.088
QS18	90.3	89.3	13.7	13.3	4.63	4.58	15.7	13.0	1.405	1.233
LSD at 0.05%	**13.8**	**13.9**	**3.1**	**2.5**	**0.37**	**0.42**	**3.1**	**4.1**	**0.139**	**0.190**

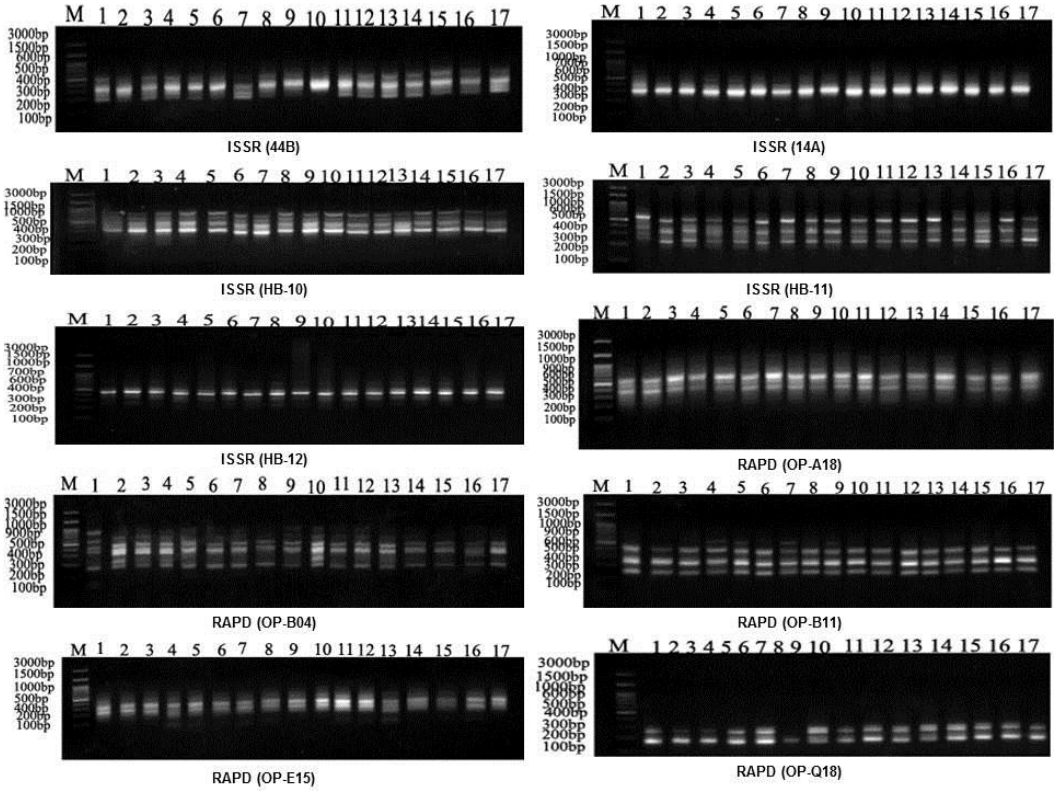

Fig. (2): Illustration of ISSR–PCR and RAPD–PCR reactions with 17 genotypes of quinoa. The names of the genotypes from one to 17 are presented in Table (1).

Table (5): Primer name, monomorphic bands, polymorphic bands, total number of bands and polymorphism (%).

Primer name		Monomorphic band	Polymorphic band	Total band	Polymorphism %
ISSR	**44B**	3	2	5	40
	14A	2	3	5	60
	HB-10	4	3	7	42.86
	HB-11	3	4	7	57.14
	HB-12	3	2	5	40
	Total	**15**	**14**	**29**	**48.28**
RAPD	**OP-A18**	3	1	4	25
	OP-B04	2	4	6	66.67
	OP-B11	3	1	4	25
	OP-E15	3	4	7	57.14
	OP-Q18	2	2	4	50
	Total	**13**	**12**	**25**	**48**
Total		**28**	**26**	**54**	**48.15**

was 80% between genotype Giza-2 and genotype Santa-Maria.

The dendrogram of genetic distance among the 17 quinoa genotypes based on five ISSR primers located genotypes in two main clusters (Fig. 3); the cluster number one had sub cluster one and sub cluster two. The sub cluster one has genotypes Giza-2 and Q22. The sub cluster two gave three groups; group number one had genotype QS17-1 only. Group number two included Regalona-Baer and Giza-1. Group number three had QS18 and QS14 genotypes. Cluster number two had three sub clusters; sub cluster number one gave genotype Santa-Maria only. Sub cluster number two included two groups. Genotypes number Q21 and Q27 in one group, while genotypes

Table (6): Similarity indices among the 17 imported quinoa genotypes based on ISSR analysis.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	100																
2	82	100															
3	90	80	100														
4	92	81	90	100													
5	92	81	90	100	100												
6	85	82	87	93	93	100											
7	85	83	92	89	89	90	100										
8	90	88	92	89	89	86	91	100									
9	82	95	80	86	86	87	83	88	100								
10	87	90	85	91	91	93	88	89	85	100							
11	87	90	85	91	91	88	93	93	90	90	100						
12	89	83	88	97	97	90	86	91	88	88	93	100					
13	93	88	88	89	89	86	86	91	88	88	88	86	100				
14	89	83	84	89	89	86	82	87	83	88	84	86	91	100			
15	92	81	90	91	91	93	93	89	86	86	91	89	89	89	100		
16	89	82	83	88	88	85	81	82	87	83	83	86	90	86	84	100	
17	89	88	88	89	89	86	86	91	88	88	88	86	95	95	89	90	100

QS16 and MISR-1 in the second group. Sub cluster number three had two groups; the first group included Q18 and Q29, while Sajama and Q12 were in the same group. There was a strong genetic relationship among the quinoa genotypes and strong similarities among genotypes (Table, 6). This indicates the strength of ISSR marker in detecting relationships and diversity among the 17 genotypes studied.

Molecular marker approaches are considered efficient in fingerprinting of plant genome. This study investigated the usefulness and effectiveness of two PCR-based molecular techniques, ISSR and RAPD in detecting polymorphism in quinoa. ISSR revealed high polymorphism in quinoa. Similar results were reported by Tautz *et al.* (1986), Pejic *et al.* (1998), Xu and Sun (2001) and Ray and Roy (2007).

3.2.2. Random amplified polymorphism DNA (RAPD)

Analysis of 17 quinoa genotypes (Table, 1) with five primers (OP-A18, OP-B04, OP-B11, OP-E15 and OP-Q18) revealed 25 different bands ranging from141.945 to 982.947bp (Table 3 and Fig. 2). The total number of bands was 25 band; 12 polymorphic and 13 monomorphic. The highest primer produced fragment OP-E15 seven bands (Table 5 and Fig. 2). Data revealed the ability of RAPD to discriminate among the seventeen genotypes.

In the dendrogram based on the average cluster analysis (Fig. 3): two main clusters could be identified at 63% Jaccard level. The first cluster had three subclusters; the first subcluster included genotypes Giza-2 and Sajama in the same subcluster and genotypes Giza-1 and Santa-Maria in different group in the same subcluster. Subcluster two included genotypes QS17-1 and QS18 in the same subcluster. Subcluster number three included two groups, genotype Q26 only in the first group, and genotypes Q27 and Q29 in the second group. Cluster number two had three subclusters, subcluster one gave two groups; the first group had genotype Q18 only. While, genotypes QS14 and QS16 were located in the second group. The second subcluster showed genotype Q21 in one group, while genotypes Misr-1 and Regalona-Baer were in the second group. Subcluster three included genotypes Q12 and Q22.

The data in Table (7) revealed that the lowest similarity was between genotypes Giza-2 and Q12 (74%) and the highest similarity (97%) was between genotypes Giza-1 and Santa-Maria.

Del Castillo *et al.* (2007), revealed that the proportion of polymorphic sites per population varied from 24.0% to 60.5% with an average at the population level of 45.4%. Moreover genetic diversity of quinoa populations was more comparable to the mean diversity observed with RAPD in

Table (7): Similarity indices among the seventeen imported quinoa genotypes based on RAPD analysis.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	100																
2	89	100															
3	97	91	100														
4	90	89	87	100													
5	85	74	82	86	100												
6	91	91	94	81	81	100											
7	79	78	81	80	95	86	100										
8	83	82	86	79	89	91	94	100									
9	87	76	84	83	98	83	92	86	100								
10	85	84	82	86	86	81	80	79	88	100							
11	85	79	87	81	90	86	90	89	88	90	100						
12	85	79	82	86	90	81	85	84	88	95	95	100					
13	84	78	86	80	95	85	95	94	92	85	95	90	100				
14	86	86	89	77	87	94	92	91	89	87	92	87	92	100			
15	86	85	88	76	86	94	91	91	89	81	86	81	91	94	100		
16	84	83	86	80	85	86	89	89	82	75	85	80	89	86	91	100	
17	86	91	88	81	76	88	80	85	78	76	76	76	80	82	87	91	100

allogamous species or species with mixed reproduction system (0.22–0.26) according to Nybom and Bartish (2000).

3.2.3. Combined data across ISSR and RAPD systems

Data combined across ISSR and RAPD systems are presented in Fig. (3) and Table (8). These systems produced 54 bands as combined total bands; 26 fragments were polymorphic with ratio 48.15% and 28 monomorphic bands.

The combined data did not give any unique bands (Fig. 3 and Table 5). A comparison between RAPD and ISSR lacks predecessors in the literature. RAPD analyses have been adopted excessively for genetic diversity studies of various tropical crops (Youssef *et al.,* 2014) and comparison with other methods like RFLP has proved the value of this method (Moniruzzaman *et al.,* 2019), whereas ISSR has so far only been applied sporadically. RAPD has also been used to assess the interspecific relationships of the genus *Manihotaiming* in tracing wild relatives (Herzberg *et al*., 2004). To put sampling strategies and the management of germplasm collections on a rationale basis is best achieved by the establishment of molecular marker technology; PCR-based methods are particular and useful tools, not only to characterize the genetic diversity, but also to develop marker assisted breeding strategies.

The data in the Table (8) of similarity indices among 17 imported quinoa genotypes. The highest value (94%) was among genotypes Q27 and Q29, Q12 and Q29 and genotypes Q18 and QS16, while the lowest value was among genotypes Q26 and QS17-1 followed by genotypes Giza-2 and Misr-1, also genotypes Giza-2 and Q29. These genotypes were considered the highest similar genotypes in the field performance, while the lowest similar genotypes were different genotypes in the field performance.

The dendrogram (Fig. 3) had two main clusters; cluster number one had genotype Giza-2 in the first sub-cluster, while genotypes QS18 and QS17-1 were in the second sub-cluster. Cluster number two was divided into two main sub-clusters. Sub-cluster number one had genotypes Q12 and Q29 in the one group. Furthermore, genotype Sajama was found in the second group, while genotype Q26 was in the third group alone.

In addition, genotype Q22 located in group 3 alone in the sub-cluster two. Genotypes Giza-1 and Santa-Maria located in the first group, while genotypes Q21 and Regalona-Baer located in the second group. Furthermore, genotypes Q27 and Misr-1 were located in the third group.

A conclusion could not have been reached based on information from germplasm collections. Indeed, Ruas *et al.*

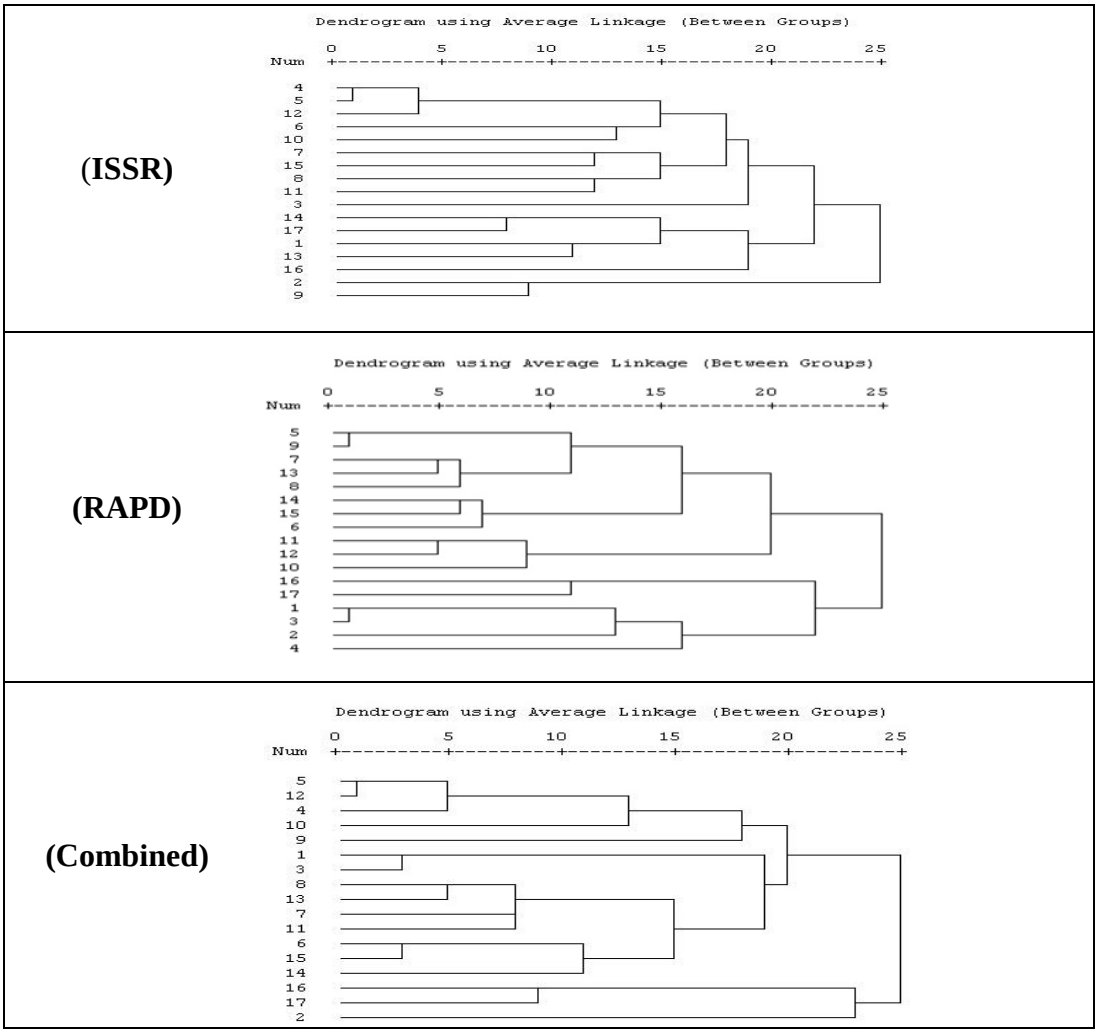

Fig. (3): Dendrogram of the genetic distances among 17 genotypes of quinoa based on five primers ISSR-PCR, five RAPD-PCR and combined data. The names of the genotypes from one to seventeen are founded in Table (1).

Table (8): Similarity indices among the seventeen imported quinoa genotypes based on combined analysis.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	100																
2	85	100															
3	93	85	100														
4	91	85	89	100													
5	89	78	87	93	100												
6	88	86	90	88	88	100											
7	83	81	87	85	92	89	100										
8	87	86	90	85	90	89	93	100									
9	85	86	82	85	92	86	88	88	100								
10	86	88	84	89	89	88	85	85	87	100							
11	86	85	86	86	91	88	92	92	89	91	100						
12	88	81	85	92	94	86	86	88	88	92	94	100					
13	90	84	87	85	92	86	90	93	90	87	92	88	100				
14	88	85	86	84	88	90	87	89	86	88	88	87	92	100			
15	89	83	89	85	89	94	93	90	88	84	89	85	90	91	100		
16	87	83	85	85	87	86	85	85	85	80	84	83	90	86	88	100	
17	88	89	88	86	83	87	84	89	84	83	83	82	89	90	89	91	100

(1999) reported a low level of intraspecific variation among germplasm accessions of quinoa (originating mainly from Bolivia) based on RAPD markers. According to Doebley *et al.* (1985), Morden *et al*. (1989) and Dje *et al*. (1999), this may result from different factors: (1) small sample size at the accession level, (2) genetic bottleneck during sampling and reproduction procedure in germplasm collection, and (3) different geographic scale between genebank and *in situ* studies.

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الأداء الحقلي والتنوع الجزيئى لسبعة عشرة تركيبا وراثيا من الكينوا فى مصر

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ملخص

اقيمت تجربة حقلية بمحطة البحوث الزراعية بالإسماعيلية، مركز البحوث الزراعية، مصر ، خلال الموسمين الشتويين 2016 / 2017، 2017 / 2018 لتقدير الأداء الحقلى والتنوع الجزيئى بين سبعة عشر تركيبا وراثيا من الكينوا هى : 6 أصناف (جيزة-1، جيزة-2، Sajama ، Santa-Maria ، مصر-1، Regalona-Baer) و11 تركيبا وراثيا (Q12, Q21, Q22, Q26, Q27, Q29, Q31, QS14, QS16, QS17-1, QS18). استخدم تصميم القطاعات الكاملة العشوائية فى ثلاث مكررات. أظهرت النتائج بوضوح ان التراكيب الوراثية جيزة-1، QS17-1، QS18 هى تراكيب مبكرة النضج حيث تمكث فى الحقل فقط مدة 119 يوم، بينما تمكث التراكيب الوراثية QS14، QS16 حتى 147 يوم من الزراعة وحتى الحصاد كتراكيب متوسطة فى مدة بقائها فى الأرض. تمكث باقى التراكيب الوراثية مابين 120-140 يوم من الزراعة وحتى الحصاد كتراكيب مبكره الى متوسطة مدة البقاء. سجلت أكبر قيمة لطول النبات وعدد الأفرع للنبات مع التركيب الوراثى QS16 بينما سجلت أقل القيم مع التركيب الوراثى QS17-1 خلال موسمى الزراعة. سجلت اكبر قيم لوزن الـ 1000 حبة مع التراكيب الوراثية QS18 ، QS17-1 ، جيزة-2 وفى ذات الوقت سجلت اقل القيم مع التراكيب الوراثية QS16، QS17-1 فى كلا الموسمين. وفى كلا من صفتى محصول حبوب النبات ومحصول حبوب الهكتار سجل الصنف مصر-1 أعلى القيم بينما سجل التركيب الوراثى QS17-1 اقل القيم فى الموسمين. خلصت الدراسة الى ان صنف الكينوا مصر-1 أعطى أفضل اداء محصولى تحت ظروف الأراضى الرملية مع مدة مكث فى الأرض لا تتجاوز الـ 140 يوم (صنف قصير الى متوسط). كانت التراكيب الوراثية Q27، Q21، Q26، Q18 ، Q22 واعده لإنتخاب أصناف جديدة. تم قياس التنوع الجزيئى بإستخدام تقنيتى الـ ISSR و الـ RAPD وقد أظهرت النتائج ان التباينات الجزيئية تختلف من بادئ جزيئى لأخر فى تقنية الـ ISSR والتى تعكس قدرة البادئات الجزيئية لتقنية الـ ISSR على قياس التنوع الجزيئى بين التراكيب الوراثية وقد أظهرت نتائج تقنية الـ RAPD القدرة على التفريق بين التراكيب الوراثية للكينوا. استعرض التحليل المجمع بين تقنيتى الـ ISSR و الـ RAPD نسبة التشابه بين للكينوا حيث كانت أعلى قيمة 94% بين Q27 و Q12 وأيضا Q29 . كما ظهرت أعلى قيمة تشابه بين Q29 Q18 و QS16 . بينما كانت أقل قيمة تشابه بين Q26 QS17-1 اتبع ذلك النسبة مابين جيزة-2 و مصر-1 كذلك كانت أقل قيمة تشابه بين جيزة-2 و Q29. اوضحت النتائج المجمعة للـ dendrogram ظهور مجموعتين رئيسيتين، احتوت المجموعة الأولى على التركيب الوراثى جيزة-2 في المجموعة الفرعية الأولى، بينما كانت QS18 ، QS17-1 في المجموعة الفرعية الثانية. وتم تقسيم المجموعة الثانية الى مجموعتين فرعيتين رئيسيتين. احتوت المجموعة الفرعية الأولى على Q12، Q29 في مجموعة واحدة. علاوة على ذلك يظهر Sajama في المجموعة الثانية، في حين ان Q26 ظهر في المجموعة الثالثة بمفرده. بالإضافة الى ذلك، ظهر Q22 في المجموعة الفرعية الثانية منفردا ضمن المجموعة الثالثة.

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