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Agro-physiological and Genetic Characterization of Three Quinoa (*Chenopodium quinoa* Wild.) Cultivars to Drought Stress

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ABSTRACT

The present investigation aimed to study the impact of water stress on three quinoa cultivars namely; quinoa 1, rainbow and American cultivar. Where, the three quinoa cultivars rated as various reactions for water deficit tolerance and were evaluated under the control and water stress conditions during two growing seasons. Some agro-morphological and physiological traits associated with water stress tolerance were measured under both conditions during the two seasons. Also, heritability in broad sense, PCV %, GCV %, D^Z, GA and GAM % were the most important genetic parameters calculated for all studied traits under the same conditions during the two growing seasons. Water deficit tolerance indices were a fruitful and fertile test used to determine the various tolerance degrees of drought stress in the three quinoa cultivars for the traits; number of branches/plant, number of leaves/plant, 1000-seeds weight and seed yield/plant in both years. Five polymorphic protein bands out of six produced (83.3%) of polymorphism which indicated the genetic variations of three quinoa cultivars under water stress. The three quinoa cultivars were able to prove that they are varied tolerant to water stress, depending on the results of all studied traits, especially yield and its components, root and physiological traits namely; proline, glycine betaine and trehalose contents. Where, the cultivar quinoa 1 was coming at the first place as a tolerant, followed by rainbow and then followed by the American cultivar as a moderate to sensitive. In any case, the three quinoa cultivars recorded a good and satisfactory yield under water stress conditions compared to the standard experiment in parallel of the results of roots and physiological attributes represented in proline, glycine betaine and trehalose contents production which were excellent under stress compared to the control conditions. Using SCoT primers, there were high genetic diversity and generated 131 fragments where 89 of them were monomorphic besides, 42 polymorphic bands with 32.06 % polymorphism. In addition, detected 42 specific markers (17 positive and 25 negative) as genetic markers used at the molecular level to identify quinoa cultivars that are tolerant to water stress over those that are moderately to sensitive.

Keywords: Quinoa, Drought stress, Heritability in broad sense, genetic advance, protein pattern analysis and SCoT markers.

1. Introduction

Quinoa (*Chenopodium quinoa* Willd) is considering one of the most important food crops beneficial to human health, which was recently introduced into Egyptian agriculture. It has remarkable nutritional value and contains a high percentage of fiber, gluten free and rich in high-quality protein, its seeds could be used in the manufacture process of flour high nutritional value for manufacturing of bread (Repo-Carrasco *et al.,* 2003; Ogungbenle, 2003 and Shams, 2010) could be used as high-quality

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feed for animals (Bhargava *et al.,* 2007). It is worth mentioning that, quinoa seeds contain antioxidants and flavonoids making them one of the causes of resistance to a large number of viral diseases and the prevention of cancers, aging and long-term depression. Besides, it is a major reason for losing excess weight and preventing diabetes and a natural integrated source of important proteins for building damaged body cells. Egypt is considered one of the most world countries that exposed to the problem of water stress and the lack of water sources necessary for agriculture and its impact on the destruction of the final output of any crop in a large way. This is mainly related to both the salt and thermal stresses factors. In addition, the Egyptian agricultural belt, especially the valley and delta region is very close to sea water, which exacerbates the risk of exposure to high salinity and increases the problem of drought, (Ashraf, 2010). Also, water stress has bad effects on the processes of plant growth and elongation, as well as on metabolism and other biochemical processes besides, preventing access to the essential and important nutrients for the plant, (Ali and Ashraf, 2011), and has negative effects on photosynthesis and dry matter production, (Hasanuzzaman *et al.,* 2014). In the same context, environmental stresses, especially water stress, negatively effect on the growth, elongation and development of crops, and ultimately destroy the final output, (Dennis and Bruening, 2000). In order to achieve tolerance to water stress in plants, it is necessary to integrate a large number of quantitative and physiological traits that are related to endurance. Under water stress conditions, increase the production of proline and glycine betaine contents are considered one of the most importance and fruitful molecular responses for water deficit tolerance in plants, (Matysik *et al.,* 2002). Water deficit stress tolerance depends on several factors, including drought time, plant life cycle, the ability of the soil to retain water in the root zone, and a large number of morphological and physiological characteristics of the plant, (Mohsenzadeh *et al.,* 2006). After all the devastating damages of water stress on the growth and productivity of crops, especially quinoa plant, breeders must try to find actual solutions to reduce the risk of drought stress for this important crop through traditional plant breeding programs and the use of modern plant genetics methods such as biotechnology and genetic engineering to develop new quinoa lines that are highly tolerant to water deficit conditions and have high yielding at the same time. The following is a quick review of the results for the most important studies and research that discussed this topic in some detail in some regard. The genetic diversity of 8 quinoa cultivars performed from the Bolivian altiplano was detected through RAPD markers by Castillo *et al.* (2007) and divided studied quinoa accessions into three main groups in this regard. The genetic diversity in 28 Altiplano and 31 coastal Chilean accessions of quinoa using 20 SSR markers was studied by Fuentes *et al.* (2009) who reported that a total of 150 alleles were generated between quinoa plants ranging from 2 to 20 alleles per locus and an average 7.5 allele/locus. The physiological responses for drought tolerance in quinoa is based on water deficit stress avoidance mechanism, reduced transpiration and sustained water uptake besides, allowed root growth and water uptake to continue even in stress conditions, (Stikic *et al.,* 2015). Elewa *et al.* (2017) discussed the impact of various proline levels for enhancing the physiological reactions in quinoa under water deficit conditions through determining some agro-morphological, physiological and root traits such as shoot length, number of branches/plant, number of leaves/plant, fresh and dry weight/plant, 1000 seeds weight, seed yield/plant RWC%, root length, root fresh weight, root dry weight and proline content under drought treatment compared to the normal experiment. The final results confirmed that yield and its components were improved after treated with the proline doses 12.5 mM and 25.0 mM When preventing or withholding irrigation for two consecutive times in the water stress treatment. The tolerant quinoa entries gave much higher seed yield/ha than sensitive ones under moderate and severe water stress conditions, (Al-Naggar *et al.,* 2017 b). The two quinoa cultivars; Rainbow and altiplano have an fruitful mechanisms that enable it for avoiding the negative impact of water deficit conditions one of the most famous is stimulating the growth and deepening of the main root increased stomatal closure to reduce photosynthesis and transpiration during the time of drought stress, (Gamez *et al.,* 2019). Jamali *et al.* (2020) discussed the influence of water deficit conditions on yield and its components traits on NSRQC quinoa cultivar and they detected that with 50% reduction of water in vegetative and flowering stages and deficit irrigation in all growth stage compared to completely irrigation in all growth stage treatment, 1000 kernel weights were decreased by 19.0, 9.0, 4.5, and 26.6 % and grain yield was decreased by 19.3, 11.8, 7.5 and 21.2% respectively. Al-Naggar *et al.* (2017 a) described five quinoa accessions using 10 ISSR primers as trying to determine a unique marker for each genotype, ISSR markers generated 53 fragments, 33 of them were polymorphic with 61.83% polymorphism included 24 unique markers (11 positive and 13 negative). Mir *et al.* (2019)

studied the fruitful role of HIUS treatment for enhancing the gelling, functional and structural characteristics of quinoa protein isolates. El-Harty *et al.* (2021) revealed the morphological description in 32 quinoa entries through study 17 qualitative and 11 quantitative traits besides, molecular characterization using 21 SRAP markers and generate 75 fragments with a mean of 3.57 alleles per primer. The aims of this study to comparison among the three quinoa cultivars under study using agro-physiological, biochemical and SCoT markers to determine their reaction to water stress tolerance.

2. Materials and Methods

Two field experiments (The normal and water stress treatments) were conducted at Gimeaza Research Station, Field Crops Research Institute, Agricultural Research Center during two successive seasons of 2019/2020 and 2020/2021using three local quinoa cultivars namely; Quinoa 1, Rainbow and the American cultivar. The three quinoa cultivars were different response for drought stress tolerance where (Quinoa 1 was high tolerance, followed by the cultivar rainbow and the American cultivar was classified as moderate to sensitive). The quinoa cultivars were sowing under normal and water stress treatments in a randomized complete block design with three replicates for each experiment in both growing seasons. Under normal irrigation, the first irrigate at agriculture time, then irrigation every ten days and prevention of irrigation two weeks before harvest. For water stress treatment, the first irrigate at agriculture time, then irrigation every twenty one days, and prevention of irrigation two weeks before harvest. The recommended agricultural practices of growing quinoa were applied in both growing seasons. The physical and chemical analysis of Gimeaza soil during the two growing seasons is presented in Table (1).

Table 1: Chemical analysis in Gimeaza station during the two growing seasons (2019/2020 & 2020/2021).

Soil Properties	Season 2019/2020	Season 2020/2021
Sand	11.82	12.23
Silt	30.04	30.45
Clay	58.14	57.32
PH	7.93	8.07
EC ds m^{-1}	2.57	2.52
ESP	8.56	7.64
TDS mg/ litre (ppm)	289.13	311.06
Ca^{++}	1.82	2.15
Mg^{++}	1.06	1.08
Na^{+}	8.62	9.23
K^{+}	0.78	0.72
CO_3^{--}	0.03	0.05
HCO_3^{-}	1.74	1.59
Cl^{-}	10.72	10.36
SO_4^{-}	1.48	1.68
Texture	Clay	Clay

EC = Electrical conductivity, **TDS =** Total dissolved salts, ***** Measure of soil saturation, ****** Measure of soil water extract 1:5

Note: Each irrigation experiment was a completely independent experiment and completely isolated from the other experiment. As the isolation distance was 300 m^2 and this buffer distance was covered with linoleum on both sides to prevent water infiltration from the standard experiment to drought stress experiment.

2.1. Studied Traits

Twenty plants were selected randomly from each replicate of each experiment to evaluate the agro-morphological, yield and its components, root and physiological traits related to water stress tolerance as follow: - shoot length, fresh weight/plant and dry weight/plant for agro-morphological traits, number of branches/plant, number of leaves/plant, 1000 seeds weight and seed yield/plant for yield and its components traits and root length, root volume and root xylem vessel number for root traits.

Physiological traits related to drought stress tolerance were determined as follow:

Relative water content (RWC %) by the method of **Barrs and Weatherley (1962)**,

$$R.W.C. = \frac{FW\text{-}DW}{TW\text{-}DW} \times 100$$

Where:
FW = Fresh weight of leaf
DW = Dry weight.
TW = Full turgor.

Full turgor weight after the discs are floated on distilled water for 6 h, in Petri dishes under laboratory light and temperature conditions, and then blotted before weighing; and DW = dry weight of discs (a 105 ^{o}C for 48 hrs) were recorded. Proline content was determined from a standard curve and calculated on a fresh basis is as follows: [(µg proline / ml C m1 toluence) / 115.5 µg / µ mole] / [(g sample/5)] = µ moles proline / g of fresh weight material. The results related with proline content are average values at least 3-4 samples for each entry under both experiments, according to the method of (chinard, 1952) and modified method by (Bates *et al.,* 1973).
Glycine betaine and trehalose contents: They were determined according to the method of (Grieve and Grattan, 1983).

2.2. Genetic Parameters:

Variance components, heritability in broad sense, genetic coefficient of variability (GCV %), phenotypic coefficient of variability (PCV %), D^z or the difference between the phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %), expected genetic advance in addition, genetic advance as percentage of mean were the most important measurements calculated through the two seasons for normal and water stress conditions in this investigation as follows:

The genetic coefficient of variability (GCV %) and phenotypic coefficient of variability (PCV %) were estimates according to the method suggested by Burton and Devane, (1953) as follows:

Environmental Variance (σ^2e) = MS_e ……(1)

Genotypic Variance (G v) or (σ^2g) = $MS_g - MS_e / r$……..(2)

Phenotypic Variance (Ph v) or (σ^2ph) = (σ^2e) + (σ^2g) or $MS_e + MS_g$……(3)

Where:-
MSe = Mean Square of error.
MSg = Mean Square of cultivars.
r =Number of replicates
X = Mean of Trait

Genetic coefficient of variability (GCV %) = $\frac{\sqrt{Gv}}{X} x\ 100$……..(4)

Phenotypic coefficient of variability (PCV %) = $\frac{\sqrt{Ph\ v}}{X} x\ 100$……(5)

Estimation of heritability in broad sense: Broad sense heritability (h^2) expressed as the percentage of the ratio of the genotypic variance (g v) to the phenotypic variance (ph v) and was estimated on genotype mean basis as described by Burton and Devane, (1953) and Johnson *et al.* (1955) as:

$H^2B = (\sigma^2g) / (\sigma^2ph)$ X 100……(6)
D^z: The difference between the phenotypic coefficient of variation (PCV %) and genotypic coefficient of variation (GCV %) or (PCV %) - (GCV %).

Estimation of genetic advance: The expected genetic advance (GA) and percentage of the mean (GAM) assuming selection of superior 5% of the cultivars was estimated in accordance with the methods illustrated by Johnson *et al.* (1955) as:

(GA) = K X (σ^2g) **X** $\sqrt{\text{Ph v}}$ / Ph v……(7)

Where K = Standardized selection differential at 5% selection intensity (K = 2.068).
The genetic advance as percentage of mean (GAM) was computed as:

(GAM %) = (GA) / Mean x 100……(8)

2.3. Water stress tolerance indices:

All drought stress tolerance indices were estimated for the three quinoa cultivars in the four studied traits namely; number of branches/plant, number of leaves/plant, 1000-seeds weight and seed yield/plant according to (Fischer and Maurrer, 1978; Bouslama and Schapaugh, 1984; Lin *et al.,* 1986; Hossian *et al.,* 1990; Fernandez, 1992; Gavuzzi *et al.,* 1997 and Golestani and Assad, 1998).

2.4. Molecular Characterization

2.4.1. SDS–PAGE proteins profile

The six quinoa samples leaves (The three cultivars under normal and drought stress conditions) were analyzed for protein profile; total soluble protein was done as suggested by Larkindale and Huang, (2004) where the samples leaves from 1 to 3 were (under control conditions) and from 4 to 6 were (under drought stress treatment). Protein present in the supernatant was measured by a modification of the method using crystalline bovine albumin to establish a standard curve.

SDS PAGE was performed as described by Leammli *et al.* (1970) and the modified method by Studier, (1973). Changes in proteins having isoenzymic activity of the ROS scavenging enzymes were studied using PAGE under nonreduced, non-denatured conditions at 4 _C. Native PAGE analysis was performed for various enzymes involved in the ascorbate–glutathione cycle on a gel (10%) with protein load of 50 lg in each well. Specific procedures for running and staining of gels for different enzymes are given below. Staining of gels for SOD activity. Gels were soaked in NBT (2.45 mM) for 20 min followed by immersion in a solution containing TEMED (28 mM), riboflavin (3 lM), and potassium phosphate (50 mM, pH 7.8) for 15 min. Illumination was discontinued after maximum contrast between the achromatic zones and general blue colour was achieved. Gel was pre-run for 30 min using electrode buffer containing 2 mM Ascorbate before the samples were loaded. Gel showed dark brown bands and was photographed immediately. Staining for GR isoforms was performed.
Note: - the samples were as follows:
1: The cultivar quinoa 1 under control experiment, 2: The cultivar rainbow under control experiment, 3: The American cultivar under control experiment, 4: The cultivar quinoa 1 under drought stress treatment, 5: The cultivar rainbow under drought stress treatment and 6: The American cultivar under drought stress treatment.

2.4.2. SCoT "Start Codon Target"

2.4.2.1. SCoT-PCR Reactions

Total DNA was extracted from the fresh leaves of three quinoa cultivars by DNeasy Plant Kit (QIAGEN, Germany). The extracted DNA concentration and quality were estimated by NanoDrop. Eleven SCoT primers were used in the detection of polymorphism as shown in Table (6). The amplification reaction was carried out in 25 µl reaction volume containing 12.5 µl Master Mix (sigma), 2.5 µl primer (10pcmol), 3 µl template DNA (10ng) and 7 µl dH_2O.

PCR amplification was programmed to fulfill 45 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 45s, an annealing step at 50ºC for 50s, and an elongation step at 72ºC for 1min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 0.5X TBE buffer. PCR products were visualized on UV light and photographed using a Gel Documentation System.

The molecular weights of DNA ladder are 50 bp, 100bp, 150 bp, 200 bp, 250 bp, 300 bp, 350 bp, 400 bp, 450 bp, 500 bp, 600 bp, 700 bp, 800 bp, 900 bp, 1000 bp, 1200 bp and 1500 bp, respectively.

2.4.2.2. Data analysis

Each experiment was analyzed as a randomized complete blot design with six replicates independently for each year and all calculated data performed from all studied traits for the two experiments in two growing seasons were analyzed using the SPSS ver.17 and analysis of variance was detected as recorded by Gomez and Gomez, (1984). L.S.D. = t 5% or 1 % x $\sqrt{2MSe/r}$ where r: number of replicates. For SCoT analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples and final data sets included both polymorphic and monomorphic bands. Then, a binary statistic matrix was constructed. A similarity matrix coefficient was then calculated between cultivars using the un-weighted pair group method with arithmetic averages (UPGMA) through (Jaccard, 1908). This matrix was used to construct a phylogenetic tree (dendrogram) was performed according to Euclidean similarity index using the PAST software Version 1.91, (Hammer *et al.,* 2001).

3. Results and Discussion

3.1. Analysis of variance

Analysis of variance revealed highly significant differences among the three quinoa varieties for all studied traits under normal and drought stress conditions as shown in Table 2. Coefficient of variance percentage appeared low in some traits for example shoot length, fresh weight/plant, number of leaves/plant, RWC %, root xylem vessel number, proline, glycine betaine and trehalose contents under both conditions in the two seasons. While, the medium values were observed in the traits; dry weight/plant and root volume and were high in the two rest traits number of branches/plant and 1000 seeds-weight under normal and water stress conditions during the two growing seasons. Accordingly, the above-mentioned results presented in table (2) proved the weak environmental influence in inheriting of all traits under study and that these traits enjoyed great genetic stability over the two years under water stress conditions compared to the standard experiment. Also, this confirms that these three varieties were a fertile and excellent material for this investigation because of their genetic differences from each other and their genetic stability, as well, their remarkable tolerance for water stress although they were not equal in the tolerance degrees. These results were in agreement with those reported by (El-Mouhamady, 2003; Bhargava *et al.,* 2007; El-Mouhamady 2009; El-Mouhamady *et al.,* 2010 a & b; El-Mouhamady *et al.,* 2011; Abdel Sattar and El-Mouhamady, 2012; El-Seidy *et al.,* 2013; Abo-Hamid *et al.,* 2016; Elewa *et al.,* 2017; Khatab *et al.,* 2017; Khatab *et al.,* 2019; El-Mouhamady and Ibrahim, 2020; El-Mouhamady and El-Metwally, 2021 and El-Mouhamady *et al.,* 2021 a).

3.2. Mean Performance

Results obtained in table (3) confirmed that the three quinoa cultivars were exhibited highly rank of all studied traits under drought stress treatment compared to the control experiment during the two growing seasons. It is worth mentioning that the quinoa 1 cultivar was superior to the other two cultivars in all studied traits and achieved great tolerance to water stress in this regard. Where it recorded (4.17 & 3.73 and 3.25 & 2.69) for number of branches/plant trait, (27.14 & 25.39 and 21.24 & 19.36) for number of leaves/plant trait, (1.38 & 1.27 and 1.06 & 1.04) for 1000-seeds weight trait and (9.18 & 8.74 and 7.60 & 7.04) for seed yield/plant trait under normal and water stress conditions during the two years. The quinoa cultivar (rainbow) is coming in the second place for water stress tolerance measured on the data of all attributes under study and followed by the American cultivar as classified as moderate to sensitive in this context. The greatest evidence for drought tolerance of these three cultivars is that they were able to highlight a remarkable physiological and genetically changes by developing the performance of root traits through increasing root length to reach the water stored in the far and deep layers of the soil during water deficit conditions, (Al-Naggar *et al.,* 2017 b and Nguyen *et al.,* 2021). Also, these genetic materials succeed in development and increased root volume and root xylem vessel number to create an integrated and defensive circle to protect plants from water stress at the time of drought. Further, reducing transpiration and water loss during these difficult conditions and preserve it

Table 2: Analysis of variance for all studied traits of the three Quinoa cultivars under the control and drought stress conditions in the two growing seasons (2019/2020 and 2020/2021).

S.O.V	D.F	Seasons	Shoot length (cm)		Fresh weigh/plant (g)		Dry weight/plant (g)		Number of branches/plant		No. of leaves/plant		1000-Seeds weight (g)		Seed yield/plant (g)	
			N	S	N	S	N	S	N	S	N	S	N	S	N	S
Cultivars	2	2019/2020	23.18**	15.67**	33.15**	42.10**	7.26**	12.04**	18.45**	26.45**	13.48**	17.63**	8.42**	17.55**	10.07**	23.40**
		2020/2021	10.08**	7.23**	18.59**	26.22**	9.44**	10.05**	32.15**	14.55**	20.54**	29.06**	16.54**	38.07**	13.16**	25.77**
Replicates	5	2019/2020	14.87**	23.80**	9.12**	13.15**	39.56**	45.17**	107.05**	89.42**	6.34**	10.22**	47.39**	49.16**	79.32**	43.57**
		2020/2021	11.06**	10.44**	12.67**	21.05**	12.30**	16.42**	68.19**	47.03**	7.15**	8.47**	51.02**	54.67**	56.29**	64.01**
Error	10	2019/2020	1.55	1.38	0.95	1.27	1.69	1.74	1.20	1.31	1.07	1.40	1.52	1.69	1.04	1.16
		2020/2021	1.74	1.48	1.12	1.41	1.83	2.03	1.29	1.34	1.18	1.25	1.30	2.15	1.09	1.72
C.V. %		2019/2020	3.09	5.10	2.61	4.19	16.47	21.51	33.60	53.73	4.44	8.01	102.74	136.84	13.04	19.37
		2020/2021	3.24	5.0	2.84	4.72	16.22	25.71	36.63	60.29	4.81	7.67	101.80	164.75	3.31	24.33

S.O.V	D.F	Seasons	RWC %		Root length (cm)		Root volume		Root xylem vessel number		Proline Content		Glycine betaine Content		Trehalose content	
			N	S	N	S	N	S	N	S	N	S	N	S	N	S
Cultivars	2	2019/2020	19.88**	10.05**	25.41**	28.07**	43.55**	51.07**	54.19**	39.82**	37.11**	32.58**	124.32**	118.49**	206.08**	169.57**
		2020/2021	25.72**	8.44**	62.19**	55.03**	28.17**	30.28**	60.15**	73.10**	40.97**	43.05**	111.72**	126.29**	178.40**	174.05**
Replicates	5	2019/2020	32.12**	24.78**	14.35**	72.18**	51.09**	48.03**	8.23**	11.05**	8.93**	12.56**	65.17**	124.52**	49.26**	60.08**
		2020/2021	16.45**	21.05**	20.06**	14.29**	63.72**	69.20**	27.43**	24.81**	14.60**	18.42**	81.09**	113.65**	74.13**	83.45**
Error	10	2019/2020	1.18	1.29	1.62	1.47	1.55	1.68	1.28	1.22	1.74	1.56	1.46	1.21	1.65	1.73
		2020/2021	1.26	1.31	1.54	1.39	1.73	1.82	1.35	1.42	1.78	2.04	1.81	1.24	1.95	1.45
C.V. %		2019/2020	1.28	1.66	6.47	9.12	9.56	14.25	5.11	9.06	5.95	8.18	2.41	3.43	3.28	4.89
		2020/2021	1.33	1.69	6.66	9.67	9.50	16.90	5.50	8.66	6.41	9.78	2.75	3.56	3.85	4.97

* Significant at 5% probability level; ** Significant at 1% probability level, N: Normal treatment, S: Drought stress treatment

Table 3: Mean Performance of all studied attributes for the normal and drought stress conditions of the three quinoa cultivars in the two growing seasons (2019/2020 & 2020/2021).

Cultivars	Seasons	Shoot length (cm)		Fresh weigh/plant (g)		Dry weight/plant (g)		Number of branches/plant		No. of leaves /plant		1000-seeds weight (g)		Seed yield/plant (g)	
		N	S	N	S	N	S	N	S	N	S	N	S	N	S
Quinoa 1	2019/2020	38.42	31.04	40.51	33.02	9.24	8.44	4.17	3.25	27.14	21.24	1.38	1.06	9.18	7.60
	2020/2021	37.06	29.54	41.33	34.55	10.03	8.12	3.73	2.69	25.39	19.36	1.27	1.04	8.74	7.04
Rainbow	2019/2020	43.15	23.45	34.03	24.12	7.34	5.23	2.66	1.32	22.06	12.05	1.19	0.97	7.41	5.43
	2020/2021	44.76	18.37	33.17	21.09	7.57	4.61	2.40	1.49	21.31	10.18	1.08	0.92	7.69	4.87
American Cultivar	2019/2020	39.23	14.59	37.46	23.47	7.11	4.72	2.95	1.83	20.62	11.04	1.05	0.83	6.87	3.65
	2020/2021	40.03	25.02	36.94	19.76	7.43	3.91	3.18	1.58	21.04	14.17	1.03	0.71	7.11	4.28
Mean	2019/2020	40.26	23.02	37.33	26.87	7.89	6.13	3.26	2.13	23.27	14.77	1.20	0.95	7.82	5.56
	2020/2021	40.61	24.31	37.14	25.13	8.34	5.54	3.10	1.92	22.58	14.57	1.12	0.89	7.84	5.39
LSD at 5%	2019/2020	1.30	1.22	1.01	1.17	0.86	1.37	1.14	1.19	1.08	1.23	1.28	1.36	1.06	1.12
LSD at 1%		1.98	1.87	1.55	1.79	1.03	2.10	1.74	1.82	1.65	1.88	1.96	2.07	1.62	1.71
LSD at 5%	2020/2021	1.37	1.27	1.10	1.24	1.41	1.49	1.18	1.21	1.13	1.16	1.19	1.53	1.06	1.37
LSD at 1%		2.10	1.94	1.68	1.89	2.15	2.27	1.81	1.84	1.73	1.78	1.81	2.33	1.62	2.09

Cultivars	Seasons	RWC (%)		Root length (cm)		Root volume		Root xylem vessel number		Proline content		Glycine betaine Content		Trehalose content	
		N	S	N	S	N	S	N	S	N	S	N	S	N	S
Quinoa 1	2019/2020	89.13	73.41	23.17	18.46	16.52	13.45	28.37	19.41	26.77	22.45	64.88	42.15	55.79	47.16
	2020/2021	88.54	77.15	22.48	17.29	18.77	12.91	30.02	24.05	24.69	19.24	60.07	46.38	52.02	44.08
Rainbow	2019/2020	81.26	64.91	17.69	11.03	12.08	7.22	20.14	11.05	21.28	13.31	48.13	33.51	33.50	20.15
	2020/2021	80.68	63.22	15.32	8.17	11.72	5.86	18.71	9.18	20.07	11.85	46.45	29.06	31.66	17.42
American Cultivar	2019/2020	84.07	66.58	18.11	10.39	10.44	6.61	17.82	6.13	18.41	10.04	37.23	20.49	28.17	13.24
	2020/2021	83.92	62.04	18.04	11.10	11.03	5.18	14.63	8.04	17.63	12.72	40.11	18.23	25.09	11.09
Mean	2019/2020	84.82	68.30	19.65	13.29	13.01	9.09	22.11	12.19	22.15	15.26	50.08	32.05	39.15	26.85
	2020/2021	84.38	67.47	18.61	12.18	13.84	7.98	21.12	13.75	20.79	14.60	48.87	31.22	36.25	24.19
LSD at 5%	2019/2020	1.13	1.18	1.33	1.26	1.30	1.91	1.18	1.15	1.37	1.30	1.26	1.15	1.34	1.37
LSD at 1%		1.73	1.81	2.03	1.93	1.98	2.92	1.80	1.76	2.10	1.99	1.92	1.75	2.04	2.09
LSD at 5%	2020/2021	1.17	1.19	1.29	1.23	1.37	1.41	1.21	1.24	1.39	1.49	1.40	1.16	1.46	1.25
LSD at 1%		1.79	1.82	1.98	1.88	2.09	2.15	1.85	1.90	2.12	2.27	2.14	1.77	2.22	1.92

N: Normal treatment, S: Drought stress treatment

to complete the biochemical and physiological processes. Many papers discussed the fruitful role of root traits for enhancing drought stress tolerance in plants such as in barley by (El-Mouhamady *et al.,* 2012 b; and Ramadan *et al.,* 2016), in wheat by (El-Mouhamady *et al.,* 2014; El-Mouhamady *et al.,* 2016 and El-Mouhamady *et al.,* 2019) and in quinoa by (Elewa *et al.,* 2017). Also, maintaining a reasonable amount of water associated with cells (RWC %) during water stress and reducing its rate of loss during two growing seasons is considering one of the most important physiological indicators of plant resistance to drought stress, and this is what these three quinoa cultivars were enjoyed. This result is consistent with (Ramadan *et al.,* 2016 and Elewa *et al.,* 2017) whom reported that increasing the limit of RWC % succeeded in protecting barley and quinoa plants during water deficit conditions and reaching a good final yield. At the same time, these three quinoa varieties were able to enhance their high ability to water stress tolerance, especially the cultivar quinoa 1 through producing large quantities of organic acids associated with drought stress tolerance such as proline, glycine betaine and trehalose contents during the two growing seasons especially under water stress conditions compared to the standard experiments. A large number of studies have been conducted to discuss the role of these organic compounds in making and enhancing the water and salinity stresses tolerance mechanism in different crops, such as the one that was conducted in barley by (El-Mouhamady *et al.,* 2012 a & b), in quinoa by (Elewa *et al.,* 2017) and in flax by (El-Mouhamady *et al.,* 2021 b). After all this fruitful discuss of the most important results viewed in table (3), it can be said that the three quinoa cultivars have already succeeded in bearing water stress and giving a good yield under water deficit conditions compared to the natural treatment although they were not equal in their tolerance degree to this serious environmental obstacle, as quinoa 1 came in the first place, followed by rainbow and then the American cultivar was coming in the third place as it is moderate to sensitive in this context. On this basis, it represents a new and rich source for the production of both grains and flour highly protein to fill a large part of the nutritional gap which hits the world, especially Egypt. Because of, the high risks of water and salt stresses, especially after exposing Egypt to the dangers of water scarcity due to the construction of the Ethiopian Renaissance Dam. Based on this, the use of these promising quinoa cultivars that are superior in tolerating water stress in plant breeding programs to cope with high salinity and drought will be a very important step through hybridization with lines or other crops sensitive to this dangerous environmental factor by any means of traditional plant breeding or biotechnology programs and genetic engineering to transfer tolerance and resistance genes to it in this regard.

3.3. Genetic Parameters

Data obtained in table (4) confirmed that the values of genetic variance were higher than its counterpart in environmental variance in all studied traits under both conditions during the two growing seasons. In the same track, the values of phenotypic variance were higher than their counterparts in genetic variance for the same traits under study under normal and water stress conditions in both years. Note that, the great part of the phenotypic variance was in favor of genetic variance. This confirms the weakness of environmental variance on the inheritance of the above-mentioned traits under normal and drought stress conditions. As well, the large genetic stability that these three quinoa cultivars were enjoyed. Accordingly, the simple selection process for yield and its components and physiological traits associated with water stress tolerance will be feasible and very fruitful in the early segregation generations, (El-Mouhamady *et al.,* 2017; Al-Naggar *et al.,* 2017 b; El- Demardash *et al.,* 2017 and Tawfik and El-Mouhamady, 2019). Heritability in broad sense values were appeared high in the traits; fresh weight/plant, number of branches/plant, number of leaves/plant, root length, root volume, root xylem vessel number, proline, glycine betaine and trehalose contents under normal and water stress conditions during the two growing seasons. Also, it was high in the traits; shoot length for the first season, 1000-seeds weight, seed yield/plant and RWC % for the second season under both experiments. This result reflected the importance role of additive and additive X additive types of gene action for controlling and inheriting the previous studied traits especially focusing on genetic improvement of quantitative traits such as yield and its components, as well, on physiological traits namely; RWC %, proline, glycine betaine and trehalose contents and root traits in order to increase tolerance levels to drought stress of quinoa under Egyptian conditions, (El-Mouhamady *et al.,* 2015; Eldessouky *et al.,* 2016; Vasconcelos *et al.,* 2016; Al-Naggar *et al.,* 2017 b; El-Mouhamady and Habouh, 2019 and El-Mouhamady *et al.,* 2021 a & b & c). Further, the values of PCV % were higher than the values of GCV % for all traits under testing in both growing seasons. In the same context, D^z values were appeared low

Table 4: Estimation of all genetic parameters for all studied traits under normal and drought stress conditions in quinoa cultivars during the two growing season (2019/2020 & 2020/2021).

Genetic Parameters	Seasons	Shoot length (cm)		Fresh weigh/plant (g)		Dry weight/plant (g)		Number of branches/plant		No. of leaves/plant		1000-seeds weight (g)		Seed yield/plant (g)	
		N	S	N	S	N	S	N	S	N	S	N	S	N	S
Mean	**2019/2020**	40.26	23.02	37.33	26.87	7.89	6.13	3.26	2.13	23.27	14.77	1.20	0.95	7.82	5.56
	2020/2021	40.61	24.31	37.14	25.13	8.34	5.54	3.10	1.92	22.58	14.57	1.12	0.89	7.84	5.39
Genotypic Variance	**2019/2020**	3.60	2.38	5.36	6.80	0.92	1.71	2.87	4.19	2.06	2.70	1.15	2.64	1.50	3.70
	2020/2021	1.39	0.95	2.91	4.13	1.26	1.33	5.14	2.20	3.22	4.63	2.54	6.09	2.01	4.0
Environmental Variance	**2019/2020**	1.55	1.38	0.95	1.27	1.69	1.74	1.20	1.31	1.07	1.40	1.52	1.69	1.04	1.16
	2020/2021	1.74	1.48	1.12	1.41	1.83	2.03	1.29	1.34	1.18	1.25	1.30	2.15	1.09	1.72
Phenotypic Variance	**2019/2020**	5.15	3.76	6.31	8.07	2.61	3.45	4.07	5.50	3.13	4.10	2.67	4.33	2.54	4.86
	2020/2021	3.13	2.43	4.03	5.54	3.09	3.36	6.43	3.54	4.40	5.88	3.84	8.24	3.10	5.72
Heritability in Broad Sense	**2019/2020**	69.90	63.29	84.94	84.26	35.24	49.56	70.51	76.18	65.81	65.85	43.07	60.96	59.05	76.13
	2020/2021	44.40	39.09	72.20	74.54	40.77	39.58	79.93	62.14	73.18	78.74	66.14	73.90	64.83	69.93
(GCV %)	**2019/2020**	4.71	6.70	6.20	9.70	12.15	21.33	51.96	96.10	6.16	11.12	89.36	171.03	15.66	34.59
	2020/2021	2.90	4.0	4.59	8.08	13.45	20.81	73.13	77.25	7.94	14.76	142.29	277.28	18.08	37.10
(PCV %)	**2019/2020**	5.63	8.42	6.72	10.57	20.47	30.30	61.88	110.10	7.60	13.70	136.16	219.03	20.38	39.65
	2020/2021	4.35	6.41	5.40	9.36	21.07	33.08	81.79	97.99	9.28	16.64	174.96	322.53	22.45	44.37
D ^{z}	**2019/2020**	0.92	1.72	0.52	0.87	8.32	8.97	9.92	14.0	1.44	2.58	46.80	48.0	4.72	5.06
	2020/2021	1.45	2.41	0.81	1.28	7.62	12.27	8.66	20.74	1.34	1.88	32.67	45.25	4.37	7.27
GA or (Expected genetic advance)	**2019/2020**	3.28	2.53	4.41	4.95	1.17	1.90	2.94	3.69	2.40	2.75	1.45	2.62	1.94	3.47
	2020/2021	1.62	1.26	2.99	3.62	1.48	1.50	4.19	2.41	3.17	3.94	2.68	4.38	2.36	3.45
GAM or (Genetic advance as percentage of mean) %	**2019/2020**	8.14	10.99	11.81	18.42	14.82	30.99	90.18	173.23	10.31	18.61	120.83	275.78	24.80	62.41
	2020/2021	3.98	5.18	8.05	14.40	17.74	27.07	135.16	125.52	14.03	27.04	239.28	492.13	30.10	64.0

Table 4 Cont.

Genetic Parameters	Seasons	RWC %		Root length (cm)		Root volume		Root xylem vessel number		Proline Content		Glycine betaine Content		Trehalose content	
		N	S	N	S	N	S	N	S	N	S	N	S	N	S
Mean	**2019/2020**	84.82	68.30	19.65	13.29	13.01	9.09	22.11	12.19	22.15	15.26	50.08	32.05	39.15	26.85
	2020/2021	84.38	67.47	18.61	12.18	13.84	7.98	21.12	13.75	20.79	14.60	48.87	31.22	36.25	24.19
Genotypic Variance	**2019/2020**	3.11	1.46	3.96	4.43	3.97	4.39	8.81	6.43	5.89	5.17	20.47	19.54	34.07	27.97
	2020/2021	4.07	1.18	10.10	8.94	10.07	8.86	9.80	11.94	6.53	6.83	18.31	20.84	29.40	28.76
Environmental Variance	**2019/2020**	1.18	1.29	1.62	1.47	1.55	1.68	1.28	1.22	1.74	1.56	1.46	1.21	1.65	1.73
	2020/2021	1.26	1.31	1.54	1.39	1.73	1.82	1.35	1.42	1.78	2.04	1.81	1.24	1.95	1.45
Phenotypic Variance	**2019/2020**	4.29	2.75	5.58	5.90	5.52	6.07	10.09	7.65	7.63	6.73	21.93	20.75	35.72	29.70
	2020/2021	5.33	2.49	11.64	10.33	11.80	10.68	11.15	13.36	8.31	8.87	20.12	22.08	31.35	30.21
Heritability in Broad Sense	**2019/2020**	72.49	53.45	70.96	75.08	71.92	72.32	87.31	84.05	77.19	76.82	93.34	94.16	95.38	94.17
	2020/2021	76.36	47.38	86.76	86.54	85.33	82.95	87.89	89.37	78.58	77.0	91.0	94.38	93.77	95.20
(GCV %)	**2019/2020**	2.07	1.76	10.12	15.83	15.31	23.04	13.42	20.80	10.95	14.90	9.03	13.79	14.90	19.69
	2020/2021	2.39	1.61	17.07	24.54	22.92	37.30	14.82	25.13	12.29	17.90	8.75	14.62	14.95	22.16
(PCV %)	**2019/2020**	2.44	2.42	12.02	18.27	18.05	27.10	14.36	22.68	12.47	17.0	9.35	14.21	15.26	20.29
	2020/2021	2.73	2.33	18.33	26.38	24.82	40.95	15.81	26.58	13.86	20.39	9.17	15.05	15.44	22.72
D ^{z}	**2019/2020**	0.37	0.66	1.90	2.44	2.74	4.06	0.94	1.88	1.52	2.10	0.32	0.42	0.36	0.60
	2020/2021	0.34	0.72	1.26	1.84	1.90	3.65	0.99	1.45	1.57	2.49	0.42	0.43	0.49	0.56
GA or (Expected genetic advance)	**2019/2020**	3.10	1.82	3.46	3.77	3.49	3.68	5.73	4.80	4.40	4.12	9.03	8.87	11.78	10.61
	2020/2021	3.64	1.54	6.12	5.75	6.06	5.60	6.06	6.75	4.68	4.74	8.44	9.17	10.85	10.82
GAM or (Genetic advance as percentage of mean) %	**2019/2020**	3.65	2.66	17.60	28.36	26.82	40.48	25.91	39.37	19.86	26.99	18.03	27.67	30.08	39.51
	2020/2021	4.31	2.28	32.88	47.20	43.78	70.17	28.69	49.09	22.51	32.46	17.27	29.37	29.93	44.72

in the traits; shoot length, fresh weight/plant, number of leaves/plant, RWC %, root length, root volume, root xylem vessel number, proline, glycine betaine and trehalose contents under normal and drought stress conditions during the two years. While, the rest studied traits namely; dry weight/plant, number of branches/plant, 1000-seeds weight and seed yield/plant were exhibited medium to high values of this genetic parameter for the two experiments in both growing seasons. This confirms that the difference between phenotypic and genetic variance was very small, which also proves that the environmental variance was non-effective in bringing any change in the heritability of all the above-mentioned traits. Also, these studied traits confirmed beyond any doubt that the three quinoa varieties were different response for drought stress where quinoa 1 was tolerance followed by rainbow and American cultivar was classified as moderate and sensitive, respectively. In the same context, these results detected that the genetic development in these traits was not dependent on the genotype only, but also on the environment and the interaction between environmental X genotype. Thus, the simple selection processes for water stress tolerance traits especially in root traits, RWC %, proline, glycine betaine and trehalode contents through phenotype could be very importance in this investigation. These results are in agreement with (Eldessouky *et al.,* 2016; Vasconcelos *et al.,* 2016; Al-Naggar *et al.,* 2017 b, and El-Mouhamady *et al.,* 2021 a & b). Data assessment of expected genetic advance (GA) based on 5% selection recorded various results for all studied traits under both treatments in the two growing seasons and appeared low to medium in this regard. Further, the three physiological traits related to water stress tolerance namely; proline, glycine betaine and trehalose contents were exhibited the highly rank of (GA) under the same conditions. While that, the values of (GAM %) were appeared medium to high in all studied traits under normal and drought conditions during the two years except RWC % trait where it recorded the lowest level for this genetic parameter under the same conditions in this context. This fact indicated that additive and non-additive types of gene action were played fruitful and importance role for controlling the previous traits for drought stress tolerance in quinoa plants. Thus, the simple selection process for high level of RWC %, root length, root volume and root xylem vessel number besides, high rank of proline, glycine betaine and trehalose contents would be fruitful when the selection process is made on the basis of individual plant. This proves that the process of genetic improvement of water stress tolerance in quinoa cultivars has yielded a remarkable success in this regard, (Eldessouky *et al.,* 2016; Vasconcelos *et al.,* 2016; Al-Naggar *et al.,* 2017 b, and El-Mouhamady *et al.,* 2021 a & b).

3.4. Drought Stress Tolerance Indices

Data obtained in table (5) confirmed that the three quinoa cultivars were exhibited mean values lower than one for YSI parameter for the four traits; number of branches/plant, number of leaves/plant, 1000-seeds weight and seed yield/plant in both growing seasons. Further, the three quinoa cultivars were recorded the highest mean values in both years of the same four studied traits for MP and GMP parameters where the cultivar Quinoa1 was coming in the second rank, followed by rainbow and then followed by the American cultivar in this regard. Also, the cultivar (quinoa 1) only was recorded mean values higher than one for YI and DTI parameters for the four traits mentioned above in both seasons except number of leaves/plant trait for DTI in the second season where it was lower than one. On the other hand, the three quinoa entries were exhibited values lower than one for the previous four studied traits in the two growing seasons for YR parameter. For DSI parameter, the cultivar quinoa1for number of branches/plant in both seasons, quinoa 1only for the first season and quinoa 1 and American cultivar for number of leaves/plant in the second season, the three quinoa cultivars for 1000-seeds weight in both seasons besides, quinoa 1 in both seasons and quinoa 1 and rainbow in the second season for seed yield/plant were exhibited mean values lower than one in table (5), respectively. These results clearly reflect the different physiological responses of water stress tolerance in the three quinoa cultivars. The mechanisms of endurance have been proven by evaluating a large number of yield and its components, root and physiological traits which proved beyond any doubt about the ability of the three quinoa cultivars to reduce the risk of water stress especially quinoa 1, followed by rainbow and then followed by American cultivar. Because it was simply able to reduce the losing rate in the final yield during water stress conditions compared to the standard experiment and gave a good output in the end. This great development in water stress tolerance occurred through a series of genetic and physiological changes, which had a great credit for maintaining a large amount of water associated with cells (RWC %) and not losing it during drought stress and using it to complete biochemical and photosynthesis processes for producing dry matter. Also, the three quinoa varieties were able to make positive physiological

Table 5: Estimation of Drought stress tolerance indices for the three quinoa cultivars under the control and stress experiments especially for the traits; number of branches/plant, number of leaves/plant, 1000-seeds weight and seed yield/plant during the two growing season (2019/2020 & 2020/2021).

Cultivars	Number of branches/plant																	
	Season 2019/2020									Season 2020/2021								
	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI
Quinoa 1	4.17	3.25	0.77	1.52	3.71	1.27	3.68	0.23	0.67	3.73	2.69	0.72	1.40	3.21	1.04	3.16	0.28	0.73
Rainbow	2.66	1.32	0.49	0.61	1.99	0.33	1.87	0.51	1.5	2.40	1.49	0.62	0.77	1.94	0.37	1.89	0.38	1.0
American Cultivar	2.95	1.83	0.62	0.85	2.39	0.50	2.32	0.38	1.11	3.18	1.58	0.49	0.82	2.38	0.52	2.24	0.51	1.34
Cultivars	**Number of leaves/plant**																	
	Season 2019/2020									Season 2020/2021								
	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI
Quinoa 1	27.14	21.24	0.78	1.43	24.19	1.06	24.0	0.22	0.61	25.39	19.36	0.76	1.32	22.37	0.96	22.17	0.24	0.68
Rainbow	22.06	12.05	0.54	0.81	17.05	0.49	16.30	0.46	1.27	21.31	10.18	0.47	0.69	15.74	0.42	14.72	0.53	1.51
American Cultivar	20.62	11.04	0.53	0.74	15.83	0.42	15.08	0.47	1.30	21.04	14.17	0.67	0.97	17.60	0.58	17.26	0.33	0.94
Cultivars	**1000-seeds weight (g)**																	
	Season 2019/2020									Season 2020/2021								
	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI
Quinoa 1	1.38	1.06	0.76	1.11	1.22	1.01	1.20	0.24	0.96	1.27	1.04	0.81	1.16	1.15	1.05	1.14	0.19	0.95
Rainbow	1.19	0.97	0.81	1.02	1.08	0.80	1.07	0.19	0.76	1.08	0.92	0.85	1.03	1.0	0.79	0.99	0.15	0.75
American Cultivar	1.05	0.83	0.79	0.87	0.94	0.60	0.93	0.21	0.84	1.03	0.71	0.68	0.79	0.87	0.58	0.85	0.72	3.60
Cultivars	**Seed yield/plant (g)**																	
	Season 2019/2020									Season 2020/2021								
	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI	GYP	GYD	YSI	YI	MP	DTI	GMP	YR	DSI
Quinoa 1	9.18	7.60	0.82	1.36	8.39	1.14	8.35	0.18	0.64	8.74	7.04	0.80	1.30	7.89	1.0	7.84	0.20	0.66
Rainbow	7.41	5.43	0.73	0.73	6.42	0.65	6.34	0.27	0.96	7.69	4.87	0.63	0.90	6.28	0.60	6.11	0.37	1.23
American Cultivar	6.87	3.65	0.53	0.65	5.26	0.41	5.0	0.47	1.67	7.11	4.28	0.60	0.79	5.69	0.49	5.51	0.40	1.33

GYP: is meaning the grain yield/plant for the control experiment, GYS: is meaning the grain yield/plant for the drought stress experiment, YSI: is meaning yield stability index = YS/YP Where: - YS is the average of yield under stress and YP=the average of yield under the control experiment, YI: is meaning yield index (YS for each genotype/mean of YS for all cultivars), MP is means (Average yield for both trials): YS + YP/2, DTI: is meaning drought stress tolerance index (YP X YS/ (mean of YP) ^{2}, GMP: (GYP X GYD) $^{0.5}$, YR: is meaning yield reduction (1-YS/YP), DSI: is meaning drought susceptibility index = DSI = (1-YS/YW)/D where YS = mean yield under drought stress, Yw = mean yield under control condition, and D = environmental stress intensity = 1-(mean yield of all cultivars under stress/mean yield of all cultivars under irrigated conditions).

changes in all root attributes, which would have deepened the main root of the plant to reach the water stored in the deep soil layers during exposure to drought. As well, increasing the number, quality and efficiency of the rest of the other root traits such as root volume and root xylem vessel number. On the other hand, the production of some organic compounds that are closely related to water stress tolerance, such as proline, glycine betaine and trehalose contents is considering one of the most important mechanisms of tolerance in this context. As, these three varieties were able to increase the production of these organic compounds mentioned above under water stress conditions compared to the normal experiment where this strategy reach its peak in quinoa 1 , followed by rainbow and then followed by the American cultivar. All these results are consistent with (Elewa *et al.,* 2017; Esmail *et al.,* 2016; El-Mouhamady *et al.,* 2017; Al-Naggar *et al.,* 2017 b; El- Demardash *et al.,* 2017; Tawfik and El-Mouhamady, 2019; El-Mouhamady *et al.,* 2019; El-Mouhamady and Ibrahim, 2020, and El-Mouhamady *et al.,* 2021 b)

3.5. Biochemical and Molecular analysis

3.5.1. SDS–PAGE proteins profile

Data of SDS–PAGE protein markers are used to assess genetic variability. Figure 1 shows the protein electrophoretic banding patterns of protein analysis for the three quinoa cultivars under normal and water stress conditions. It is produced six bands distributed in all cultivars with molecular weights ranging from 11 kDa to 100 kDa.

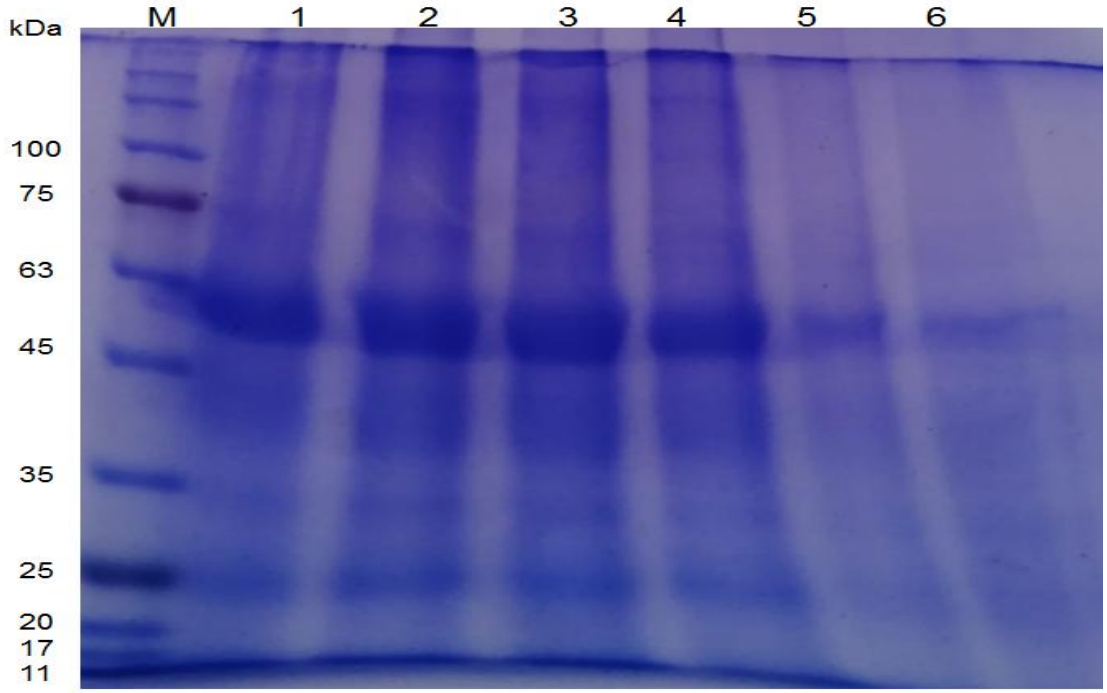


Fig. 1: SDS- PAGE protein banding pattern for the three quinoa cultivars M Marker, 1- Quinoa 1 , 2- Rainbow and 3- The American cultivar under normal condition; 4- Quinoa 1 , 5- Rainbow and 6- The American cultivar under water stress condition.

The distribution of these bands in the studied cultivars and their molecular weights showed one common bands among the three quinoa cultivars at the molecular weights around 60 kDa was induced at all levels of treatments. In addition, the protein of molecular weight 30 and 40 kDa is present only at control level and in Quinoa 1 and absent at other treatment levels for other cultivars Rainbow and the American cultivar. On the other hand, the protein of molecular weight 25 kDa is present only at control level and in Quinoa 1, but with different and low expression at other treatment levels for other cultivars Rainbow and the American cultivar which they could be moderate to sensitive to water stress. Finally, five polymorphic bands out of six produced (83.3%) of polymorphism which indicated the genetic variations of three quinoa cultivars under water stress. In plants tolerant to water stress, companionable solutes

are bound to protein thus stabilizing the native protein structure, while in sensitive plants; the proteins tend to be degraded (Hoekstra *et al.,* 2001). From these data quinoa 1 seems to be tolerant to water stress. Other two quinoa cultivars grown under water stress have change in protein pattern either with absent of bands or low gene expression (Omar *et al.,* 2014, Fischer *et al.,* 2017 and El-Mouhamady *et al.,* 2021 a).

3.5.2. Profile of SCoT analysis

The primers used for comparing among the studied quinoa cultivars gave polymorphic band except SCoT-10 gave only monomorphic bands and the primers exhibited a total of 131 bands, 89 of them were monomorphic. While that, 42 fragments were polymorphic with 32.06 % (polymorphism %) included 17 unique bands or positive specific markers as presented in (Table 6; Fig. 2). Polymorphic bands number ranged from 2 to 8 and molecular size ranging from 150 to 1700 bp as shown in table 6. The results revealed that the highest number of total bands were 17 obtained by SCoT-11 while the lowest number of fragments (9) were observed in primer SCoT-1. In the same regard, primer SCoT-11 exhibited the highest number of polymorphic fragments (8) while SCoT-2 primer recorded the lowest number of bands (2), respectively. Also, the primer SCoT-11 exhibited the highest number of unique band or positive specific markers (4) while the primers SCoT-1, 7, 9 and 12 were recorded the lowest number (1) in this context. Further, the highest polymorphism % was observed in the primer SCoT-5 (63.63 %) while SCoT-2 primer exhibited the lowest rank (20.0%) in this regard. Data viewed in (Table 7) confirmed that the highest number of amplicons were coming from quinoa cultivars; quinoa 1 and rainbow (112) for both of them and followed by the American cultivar (110). In the same track, primers SCoT-11, 9 and 3 recorded the highest number of amplified fragments (39, 37 and 33) for each one of them in all quinoa cultivars, respectively. While, the primers SCoT-1 and 5 generated the lowest number of bands (23 and 24) for both of them and the rest primers were exhibited a various number of amplified fragments.

Table 6: Band variation and polymorphism percentage in the three quinoa cultivars using 11 SCoT primers.

No.	SCoT primers	T.B	M.B	P.B	U.B or P.S.M	P %	R.S (bp)	Sequence
1	**SCoT-1**	9	6	3	1	33.33 %	180-1500	5'-ACGACATGGCGACCACGC-3'
2	**SCoT-2**	10	8	2	2	20.0 %	250-1500	5'-ACCATGGCTACCACCGGC-3'
3	**SCoT-3**	12	9	3	0	25.0 %	250-1600	5'-ACGACATGGCGACCCACA-3'
4	**SCoT-4**	12	7	5	3	41.66 %	250-1500	5'-ACCATGGCTACCACCGCA-3'
5	**SCoT-5**	11	4	7	2	63.63 %	200-1700	5'-CAATGGCTACCACTAGCG-3'
6	**SCoT-6**	12	9	3	2	25.0 %	250-1300	5'-CAATGGCTACCACTACAG-3'
7	**SCoT-7**	11	7	4	1	36.36 %	250-1500	5'-ACAATGGCTACCACTGAC-3'
8	**SCoT-9**	14	10	4	1	28.57 %	200-1800	5'-ACAATGGCTACCACTGCC-3'
9	**SCoT-10**	12	12	0	0	0	270-1500	5'-ACAATGGCTACCACCAGC-3'
10	**SCoT-11**	17	9	8	**4**	47.05 %	250-1700	5'-ACAATGGCTACCACTACC-3'
11	**SCoT-12**	11	8	3	1	27.27 %	150-1700	5'-CAACA**ATG**GCTACCACCG-3'
Total		131	89	42	17	32.06 %	150-1700	

T.B: Total bands, M.B: Monomorphic bands, **P.B:** Polymorphic bands, **U.B or P.S.M**: Unique bands or positive specific marker, **P%:** Polymorphism percentage and **R.S (bp):** Range size

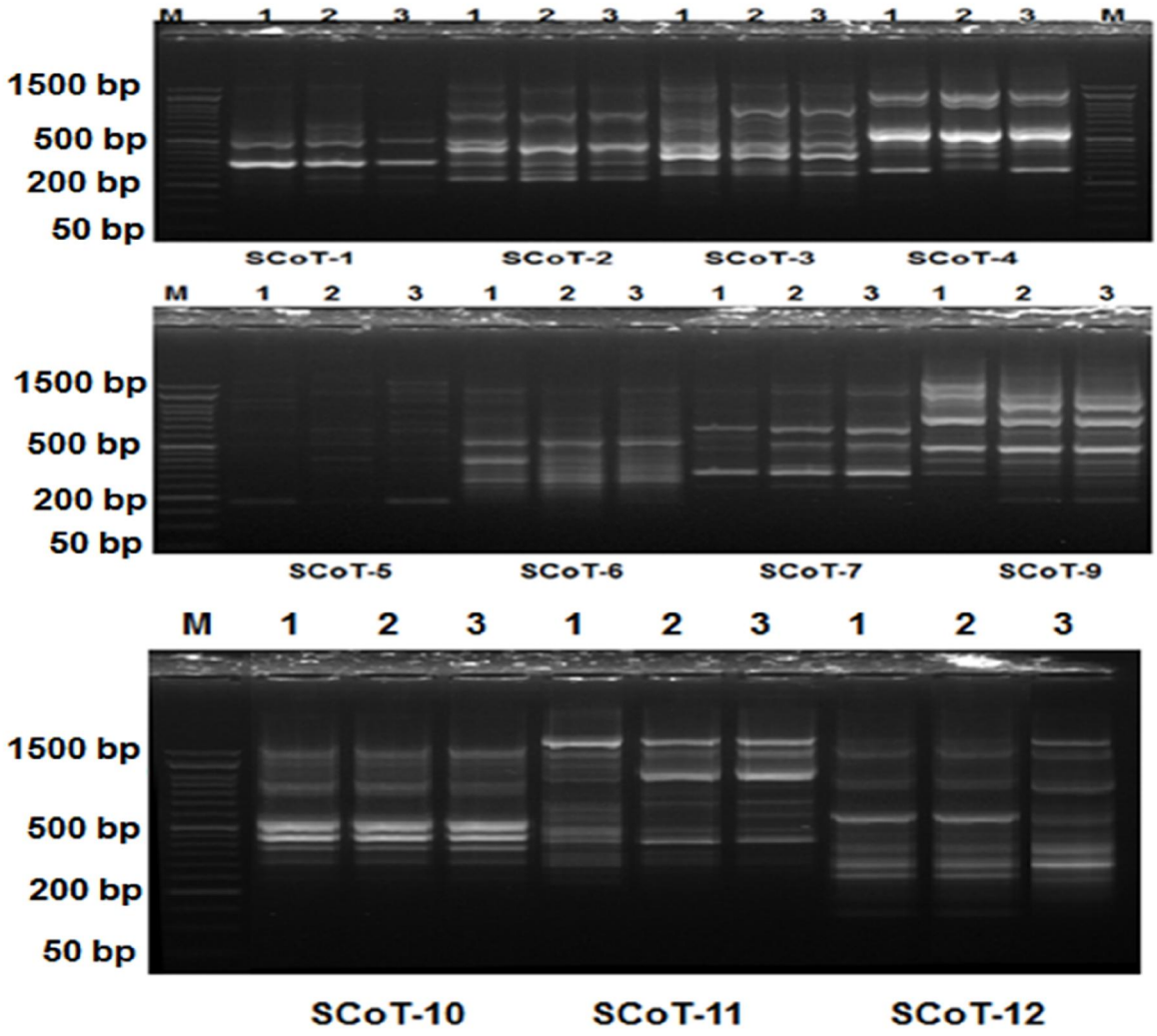


Fig. 2: PCR fragments using 11 SCoT primers in the three quinoa cultivars namely; 1: Quinoa 1, 2: Rainbow and 3: American cultivar, the molecular weights of DNA ladder.

Table 7: Total fragments obtained from the eleven SCoT primers of the three quinoa cultivars and all amplified fragments for each genotype.

Cultivars	Primers											
	SCoT-1	SCoT-2	SCoT-3	SCoT-4	SCoT-5	SCoT-6	SCoT-7	SCoT-9	SCoT-10	SCoT-11	SCoT-12	Total
Quinoa 1	8	10	11	10	6	10	8	11	12	16	10	112
Rainbow	8	8	11	11	8	9	10	13	12	12	10	112
American cultivar	7	8	11	7	10	12	10	13	12	11	9	110
Total Bands	23	26	33	28	24	31	28	37	36	39	29	334

Data presented in Table 8 exhibited 17 positives and 25 negative specific markers generated by eleven SCoT primers. These primers succeeded in determining the molecular genetic differences between the three quinoa cultivars. Further, these molecular genetic differences were very important in this regard and considered the taxonomic basic among the three quinoa cultivars.

Table 8: Mapping of positive and negative specific markers for the three quinoa cultivars using 11 SCoT primers.

SCoT Primers	MS(bp)	Quinoa 1	Rainbow	American Cultivar	Positive Marker
SCoT-1	1500	+	+	-	N (G3)
	250	+	-	-	P (G1)
	240	-	+	+	N (G1)
SCoT-2	1000	+	-	-	P (G1)
	500	+	-	-	P (G1)
SCoT-3	1200	+	-	+	N (G2)
	900	-	+	+	N (G1)
	300	+	+	-	N (G3)
SCoT-4	1500	+	-	-	P (G1)
	1000	+	+	-	N (G3)
	550	+	+	-	N (G3)
	340	-	+	-	P (G2)
	270	-	+	-	P (G2)
SCoT-5	1700	-	+	+	N (G1)
	1300	-	+	+	N (G1)
	1100	+	-	+	N (G2)
	1000	+	-	-	P (G1)
	900	-	-	+	P (G3)
	500	-	+	+	N (G1)
	340	-	+	+	N (G1)
SCoT-6	1000	+	-	+	N (G2)
	700	-	-	+	P (G3)
	450	-	-	+	P (G3)
SCoT-7	1500	-	+	+	N (G1)
	600	+	-	-	P (G1)
	550	-	+	+	N (G1)
	370	-	+	+	N (G1)
SCoT-9	1500	+	-	-	P (G1)
	1000	-	+	+	N (G1)
	250	-	+	+	N (G1)
	200	-	+	+	N (G1)
SCoT-11	1700	+	-	-	P (G1)
	1200	+	-	-	P (G1)
	800	-	+	+	N (G1)
	750	+	+	-	N (G3)
	550	+	-	+	N (G2)
	350	+	+	-	N (G3)
	300	+	-	-	P (G1)
	250	+	-	-	P (G1)
SCoT-12	320	+	+	-	N (G3)
	300	-	-	+	P (G3)
	150	+	+	-	N (G3)
Range	150-1700				
Total		11	2	4	17 (positive) + 25 (Negative)

G1: Genotype 1 (Quinoa 1), G2: Genotype 2 (Rainbow) and G3: Genotype 3 (American cultivar)

The following is a detailed explanation of SCoT primers that gave positive and negative markers in this track. SCoT-1 primer produced one positive specific marker in quinoa 1 with a molecular size of 250 bp and two negative markers in quinoa 1 and American cultivar with sizes of (240 bp & 1500 bp), respectively. While that, SCoT-2 primer generated 2 positive specific markers in quinoa 1 with sizes of (500 bp & 1000 bp). Further, SCoT-3 primer showed three negative markers for the three quinoa cultivars namely; quinoa 1, rainbow and the American cultivar with sizes of (900 bp, 1200 bp & 300 bp), respectively. Also, SCoT-4 primer recorded 5 markers where one of them was positive in quinoa 1 at size of 1500 bp and two positive markers in rainbow at sizes of 270 bp and 340 bp besides, two

negative specific markers in the American cultivar at sizes of 550 bp and 1000 bp, respectively. For SCoT-5 primer, there were seven markers distributors as follows; two positive in quinoa 1 and the American cultivar at sizes of (1000 bp & 900 bp) besides, five negative markers where four of them in quinoa 1 with sizes of (340 bp, 500 bp, 1300 bp & 1700 bp) and one negative marker in rainbow at size of 1100 bp, respectively. In the same regard, SCoT-6 primer recorded three markers where one of them was negative in rainbow at size of 1000 bp besides, two positive markers were obtained in the American cultivar with sizes of (450 bp & 700 bp). Also, SCoT-7 primer produced four markers in quinoa 1 where one of them was positive at size of 600 bp and three negative at sizes of (370 bp, 550 bp & 1500 bp), respectively. Four specific markers were generated by SCoT-9 primer and all observed in quinoa 1 where one of them was positive at size of 1500 bp in addition, three negative markers at sizes of (200 bp, 250 bp & 1000 bp), respectively. SCoT-11 primer exhibited eight markers where four of them were positive in quinoa 1 with sizes of (250 bp, 300 bp, 1200 bp & 1700 bp) besides, four negative markers distributors as follow; the first one in quinoa with size of 800 bp, the second marker in rainbow with size of 550 bp and two markers in the American cultivar with sizes of (350 bp & 750 bp), respectively. The last primer (SCoT-12) recorded three specific markers for the American cultivar only where one of them was positive at size of 300 bp besides, two negative markers with sizes of 150 bp and 320 bp.

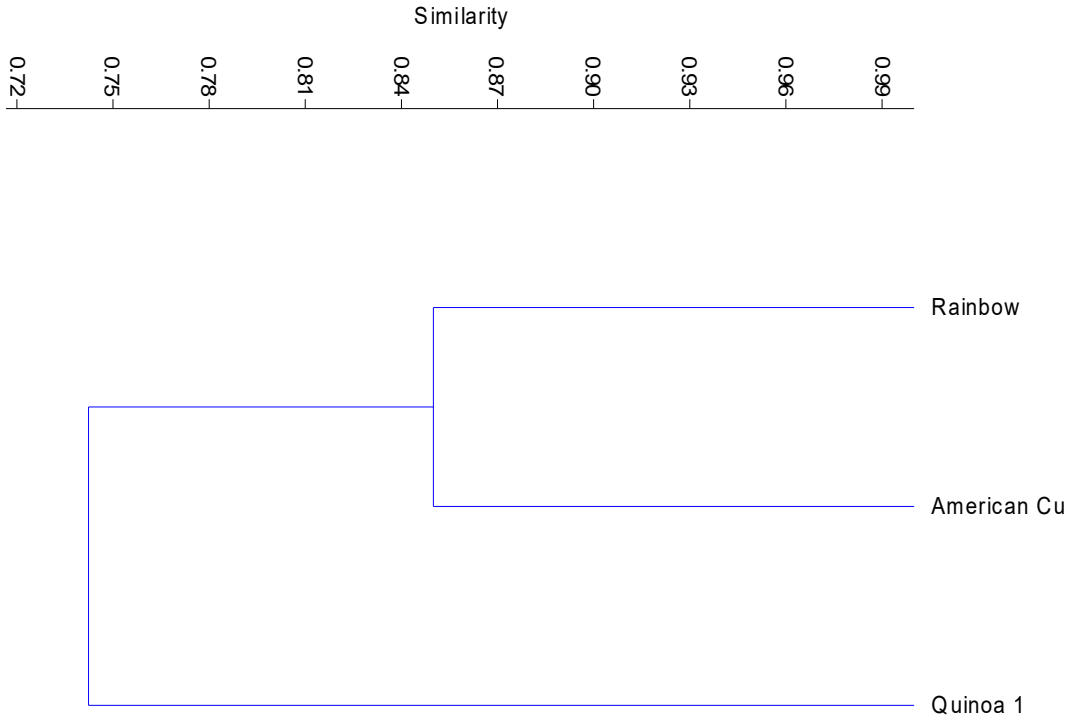
3.5.2.1. Proximity matrix and dendrogram analysis

Results showed in (Table 9) exhibited (3) pairwise comparisons to debate the genetic relationships between the three quinoa cultivars detected in terms of genetic similarity. The genetic similarity values ranged from (0.720 to 0.850) with an average of (0.785). Where, the highest limit of genetic similarity was (0.850) within (Rainbow & American cultivar) and followed by (0.763) among (Quinoa 1 & Rainbow). While that, the lowest level of similarity was (0.720) between (Quinoa 1 & American cultivar), respectively. Also, data of cluster analysis or phylogenetic tree viewed in (Fig. 3) divided all quinoa varieties into two main clusters. Where, the first one included quinoa 1 only. While, the cluster number two contained one sub-cluster (Rainbow & American cultivar), respectively.

Molecular genetics markers has succeeded in providing the complete knowledge for plant breeders to determine the mechanisms responsible for biotic and abiotic tolerance which plants are exposed, impeding their growth and affecting their final output especially water stress. This study dealt 11 SCoT primers for comparing among the three quinoa cultivars which gave 42 polymorphic amplicons with 32.06 % polymorphism confirms that these fragments were not only evidence for distinguishing between the three quinoa cultivars, but were also scientific evidence of their tolerance to water stress in this context, tables (6 & 7) and Fig.2. In the same track, SCoT markers were the most primers produced unique bands (17 positive and 25 negative specific markers) as molecular genetic markers for quinoa cultivars tolerant to water stress where the previous 11 SCoT primers were able to achieve this result carefully, table (8). These results are similar with (Heiba *et al.,* 2016 a) who detected 8 unique bands through using 7 RAPD primers for comparing among some rice entries and determining each of them resistance to heavy metal toxicity under Egyptian conditions. Also, (Heiba *et al.,* 2016 b) discovered 18 novel bands in three wheat new lines through using four ISSR primers after treated these lines with different doses of EMS. Further, (Khatab *et al.,* 2017) discussed water stress tolerance in 23 sorghum entries and confirmed that 50 and 32 bands were exhibited through using 6 SRAP and 6 RAPD whereas, 27 and 29 fragments of them were polymorphic, respectively. The influence of water stress on some rice entries was revealed by (Eldessouky *et al.,* 2016) through using 7 ISSR primers. Where, these primers gave 52 fragments (37 of them were polymorphic bands) with 71.15 % polymorphism besides, discovered 14 unique bands (one negative and 13 positive) as a taxonomic marker for drought stress tolerance in rice. Genetic stability in novel quinoa accessions was reported by (Lema-Rumińska *et al.,* 2018) through using RAPD and SCoT markers. They showed that RAPD and SCoT primers detected large genetic stability of the derivative quinoa cultivars (Faro' and 'Titicaca)' while variation was observed in plants representing original varieties: banding pattern various than predominant was present in three plants of 'Titicaca' (genetic distances from 7.5% to 55.9%) and in a single plant of 'Faro'(genetic distance 61.2% as indicated by SCoT technique). Further, Phytochemical depiction and genetic analysis of 5 quinoa accessions were done through using 4 RAPD and 7 ISSR primers by (Saad-Allah and Youssef, 2018).

Table 9: Genetic similarity % in the three quinoa cultivars using 11 SCoT Primers.

Genetic Similarity	Quinoa 1	Rainbow	American Cultivar
Quinoa 1	1.0		
Rainbow	0.763	1.0	
American Cultivar	0.720	0.850	1.0



UPGMA
Nei-Li's similarity coefficient

Fig. 3: Dendrogram representing the genetic relationship among the three quinoa cultivars namely; Quinoa 1, Rainbow and American Cultivar using UPGMA cluster analysis of Nei-Li's similarity coefficient generated from the 11 SCoT primers.

They confirmed that RAPD and ISSR markers could be used to recognize with excellent form among quinoa entries. In addition, the phytochemical and genetic depiction obtained hither will be a promising guide for breeding seed quality in quinoa. Also, (Khatab *et al.,* 2019) revealed drought stress tolerance in ten barley cultivars through exhibited 48 bands by 6 ISSR primers where 25 of them were polymorphic with 52.08 % polymorphism. In addition, (Tawfik and El-Mouhamady, 2019) detected drought stress tolerance in sorghum through produced 151 fragments through using 8 ISSR primers where 113 of them were polymorphic with 74.83 % polymorphism. Further, (El-Mouhamady *et al.,* 2021 a) discussed salt stress tolerance in three canola cultivars through using 11 SCOT primers which exhibited 32 unique bands besides, generated 50 polymorphic fragments with 38.16 % polymorphism. Also, (El-Mouhamady *et al.,* 2021 b) revealed the impact of salinity stress tolerance in 5 flax cultivars through using 6 ISSR primers and confirmed that ISSR markers succeed for producing a total of 29 bands, 11 of them were polymorphic with 37.93 % polymorphism. Further, (Khatab *et al.,* 2021) reported the importance of unique band or specific marker and its relation with salinity stress tolerance in barley entries through produced 15 polymorphic bands with 75.0 % polymorphism by 11 SSR primers. The analysis of UPGMA SCoT dendrogram obtained in (Table 9; Fig.3) and related with phylogenetic tree between the three quinoa cultivars revealed that the highly genetic similarity was observed among the two quinoa cultivars rainbow and American cultivar (0.850) and quinoa 1 and rainbow (0.763) which refers to the closely relation and the strong genetic compatibility between them. On the other hand,the similarity percentage between quinoa 1 and the American cultivar (0.720) and although it was not a bad result, but it is indicated that these were distantly related cultivars in this

context, (Saad-Allah and Youssef, 2018 and El-Mouhamady *et al.,* 2021 a). Based on these results, the three quinoa cultivars which are highly compatible with each other and proved to be varied in tolerant to drought stress. Thus, this study can be considered as a modest work to genetically and physiologically characterization for drought stress tolerance in three quinoa cultivar considering that this crop is important locally and globally source for providing food in light of its current scarcity.

4. Conclusion

This investigation focused on discussing the biological, physiological and biochemical impacts of drought stress on three Egyptian quinoa cultivars through evaluating some agro-morphological and physiological traits related to water deficit tolerance under normal and water stress conditions in two growing seasons. The results confirmed that the three quinoa cultivars were varied to their reaction to water stress tolerance of studied traits where the cultivar quinoa 1 was the most tolerant, followed by the cultivar Rainbow and then followed by American cultivar as a moderate to sensitive cultivars.

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