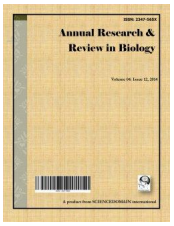

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Leaf Amino Acids and Anatomical Traits of Drought Tolerant vs Sensitive Genotypes of Quinoa (*Chenopodium quinoa* Willd.) under Elevated Levels of Water Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMMAN designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AMMAN, RMAES, AEEB and STB supervised the study and managed the literature searches. Author MMAEM managed the experimental process and performed data analyses. All authors read and approved the final manuscript.

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ABSTRACT

Many plants accumulate compatible osmolytes at high levels in plant cells such as amino acids and/or develop special epidermal cell bladders which may serve as external water reservoirs and having small and thick-walled cells in response to water deficit. The objectives of the present investigation were: (*i*) to study effects of water stress on the anatomical traits and accumulation of free amino acids in quinoa leaves and (*ii*) to describe differences among drought tolerant and sensitive genotypes in such traits following the imposition of water deficit. A field experiment was carried out in the growing season 2015/2016, using a split plot design with five replications. Main plots were allotted to three irrigation regimes, *i.e.* well watering (WW) [95% field capacity (FC)],

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moderate water stress (WS) [65% FC] and severe water stress (SWS) [35% FC] and sub plots to five genotypes. Mean squares due to genotypes, irrigation regimes and their interaction were significant ($p \leq 0.01$) for studied leaf free amino acids and anatomical traits. Water stress caused a significant decrease in leaf thickness under WS and SWS, upper and lower epidermis under WS, palisade and spongy layers under SWS, but caused a significant increase in palisade and spongy layers under WS and upper and lower epidermis under SWS. The genotype CICA-17 (tolerant) had the thickest leaf and upper epidermis and second thickest lower epidermis, palisade and spongy layers. Contents of each amino acid were significantly increased due to water stress, except Leucine. Increases in amino acid content increased by increasing severity of water stress. Maximum increase (109.6%) was shown by Threonine under SWS, but minimum (8.08%) was by Arginine under WS. Under SWS, the tolerant genotype CICA-17 showed the highest mean increase percentage (47.9%) in total amount of amino acids relative to WW; it showed the highest increase in all amino acids, especially Proline, Methionine and Phenylalanine.

Keywords: Quinoa; water deficit; epidermis; palisade; compatible osmolytes.

1. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) plant belongs to the *Chenopodiaceae* family, which also includes spinach and beet. There are approximately 250 species of this family all over the world and it is an endemic plant peculiar to South America. However, people living in the Andes, particularly in Peru and Bolivia, thousands of years ago, domesticated it. Interest in quinoa has recently spread to Europe, where it has been demonstrated to have the potential to become a promising environmentally friendly newcomer requiring few or no inputs of pesticides and inorganic fertilizers [1-3]. It draws attention with its high nutritional value, and more importantly, it is highly resistant to weather, climate, and soil conditions such as salinity and drought [4].

Many plants, including halophytes, accumulate compatible osmolytes at high levels in plant cells in response to water deficit such as amino acids (e.g. Proline), sugar alcohol (e.g. Pinitol), other sugars (e.g. Fructans) and quaternary ammonium compounds (e.g. Glycine Betaine) [5]. It has been suggested that compatible osmolytes do not interfere with normal biochemical reactions and act as osmoprotectants during osmotic stress [6,7]. The most striking change in amino acid composition following the imposition of water deficit was an approximately sixty-fold increase in Proline levels [8]. They reported that glutamate levels also increased, although the increase was not as dramatic as that observed for Proline. Results of investigations indicated that over production of Proline results in increased tolerance of transgenic tobacco plants to osmotic stress [6].

Quinoa appears to employ a wide variety of drought resistance mechanisms; these include drought escape, tolerance and avoidance [9]. Drought tolerance is mainly achieved through quinoa's tissue elasticity and putative low osmotic potential [10,11]. The accumulation of both inorganic and organic osmolytes has been found in quinoa under drought and saline conditions [9,12-14]. Additionally, quinoa can avoid the negative effects of drought by developing special epidermal cell bladders, which may serve as external water reservoirs, and having vesicular glands, small and thick-walled cells [10,15,16].

The increasing population in Egypt demands an increase in food production along with a shift towards environmentally sound sustainable agriculture. Expansion of agriculture is only available in the newly reclaimed lands in desert areas of Egypt. There is a need for cultivation of crops or varieties that require minimum inputs including soil moisture availability in these areas. Quinoa crop is qualified to be cultivated in such areas, especially the drought tolerant varieties of this crop. The knowledge gained by exploring those differences could be used in breeding program aimed at developing more suitable quinoa varieties for specific conditions, as well as potentially extrapolated to breeding other crops for drought tolerance. Information on leaf anatomy and amino acids of tolerant and susceptible quinoa genotypes in response to imposition of water stress are generally limited. The present investigation aimed at: (*i*) studying the effect of different soil moisture levels (95, 65 and 35% of field capacity; FC) on quinoa leaf anatomical traits and the accumulation of free amino acids and (*ii*) describing differences among drought tolerant and sensitive genotypes

in such traits following the imposition of water deficit.

2. MATERIALS AND METHODS

This study was carried out in the growing winter season 2015/2016 at New Salhiya station, Sharqiya Governorate, Egypt. The station is located at 30° 18' 24" N latitude and 31° 6' 47" E longitude with an altitude of 20 meters above sea level.

2.1 Plant Materials

Seeds of five quinoa (*Chenopodium quinoa* Willd.) genotypes differed in drought tolerance (three tolerant and two sensitive) were obtained from Madison University, Wisconsin, USA. The origin and some traits of these genotypes are presented in Table 1.

2.2 Field Experiment

On the 19th of November the seeds were planted along the irrigation pipes of drip irrigation system. Each pipe (row) length was 90 meter and keeping row to row distance of 60 cm and hill to hill of 60 cm. Seeds (7-10) were sown in each hill, thereafter (after 35 days) were thinned to three plants/hill to achieve a plant density of 35,000 plants/fed (83,300 plants/ha). Each experimental plot included 3 rows of 0.6 meter width and 12.0 meters long (plot size = 21.6 m^2) with a 1.0 meter ally between irrigation treatments.

2.3 Experimental Design

A split-plot design in randomized complete block (RCB) arrangement with five replications was used. Main plots were allotted to three irrigation regimes, *i.e.* well watering (WW), water stress (WS) and severe water stress (SWS). Sub plots were devoted to five quinoa genotypes.

2.4 Irrigation System

The irrigation method used in this study was drip irrigation system, which gives the chance to supply a specific amount of water for each plant separately. The main irrigation lines were allotted to the irrigation pipes, each main line is operated by a pressure reducing valve to control the water pressure in the irrigation system and to control the water regime application during the season.

2.5 Water Regimes

1. **Well watering (WW)**, where the field capacity (FC) was about 95%. Irrigation in this treatment (WW) was given each three days; with 40 irrigations during the whole season. The water meter recorded at the end of each irrigation about 86 m^3 water/feddan; thus, the total quantity of water given in the whole season for WW treatment was 3440 m^3 per feddan.
2. **Water stress (WS)**, where the field capacity (FC) was about 65%. Irrigation in this treatment (WS) was given each six days; with 20 irrigations during the whole season. The water meter recorded at the end of each irrigation about 105 m^3 water/feddan; thus, the total quantity of water given in the whole season for WS treatment was 2010 m^3 per feddan.
3. **Severe water stress (SWS)**, where the field capacity (FC) was about 35%. Irrigation in this treatment (WW) was given each nine days; with 10 irrigations during the whole season. The water meter recorded at the end of each irrigation about 99.5 m^3 water/feddan; thus, the total quantity of water given in the whole season for WW treatment was 995 m^3 per feddan.

Table 1. Name, origin, seed color and drought tolerance of quinoa genotypes under investigation

Name	Origin	Seed color	Drought tolerance*
QL-3	Bolivia	Light yellow	Sensitive
Chipaya	Altiplano Salares, Bolivia	Mixed (white & Paige color)	Tolerant
CICA-17	Peru	Yellow	Tolerant
CO-407	Colorado, USA	Mixed (light yellow & white)	Tolerant
Ollague	Altiplano Salares, Bolivia	Yellow	Sensitive

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2.6 Fertilization Regimes

First: Organic fertilizer: A Compost locally made of plant and animal wastes of the farm at New Salhiya was added to the soil with the rate of 12 tons/fed and was well mixed with the soil two weeks before sowing at a depth of 10-15 cm.

Second: Mineral fertilizers: The following mineral fertilizers were applied: Nitrogen fertilizer at the rate of 70 kg N / fed was applied through irrigation system after 25, 50 and 75 days from sowing in three equals doses as ammonium nitrate (33.5 % N). Triple Superphosphate Fertilizer (46% P_2O_5) at the rate of 30 kg P_2O_5/fed was added as soil application in two equals doses, the first (15 kg P_2O_5/fed) before sowing during preparing the soil for planting and the second (15 kg P_2O_5/fed) after 25 days from sowing. Potassium fertilizer at the rate of 25 kg K_2O/fed was added as soil application in two doses; before planting (15 kg K_2O/fed) and after 25 day from sowing (10 kg K_2O/fed) as Potassium Sulfate (48% K_2O). Calcium Sulfate or Gypsum (22% Ca, 17% S) at the rate of 20 kg /fed was added as soil application in two equal doses, the first time during preparing the soil for planting and the second time 75 days after sowing. Trace elements (Chelated iron 3%, Chelated zinc 2%, Boron 0.5%, Magnisium 3%) were added through irrigation system at a rate of half liter/month. Phosphoric acid (52:60% P_2O_5) at a rate of two Liters every 15 days was added through irrigation system when needed to open closed drippers.

Soil and water analysis: Full analyses for the soil and water were performed by Central Lab for Soil and Water Analysis, Desert Research Center, Cairo Egypt. The soil was sandy and consisted of silt (9.9%), fine sand (63.4%) and coarse sand (26.7%); soil pH was 8.1 and EC was 0.2 dSm^{-1}. Soluble cations of soil in mEqu/l were Ca (2.45), Mg (5.8), Na (8.5), K (6.8). Soluble anions of soil in mEqu/l were Cl (5.3), CO3 (0.0), SO4 (2.39) (Table 2). Irrigation water EC was 0.67 dSm^{-1}. Soluble cations of water in mEqu/l were Ca (1.4), Mg (0.4), Na (4.9), K (0.3). Soluble anions of water in mEqu/l were Cl (3.0), CO3 (0.0), SO4 (0.0).

2.7 Laboratory Work

2.7.1 Leaf free amino acids

Samples were taken from three replications of each irrigation treatment from the mature leaves of five quinoa genotypes at age of 50 days after emergence (leaf on the third node from the top of the main stem). The leaf free amino acids Asparagine, Therionine, Serine, Glutathione, Glycine, Alanine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Lysine, Arginine and Proline were determined in the laboratory as follows:

1. Principle: The acid hydrolyzed amino acids by amide bond breakage were determined according to Pellet and Young [19]. Ninhydrin is used for the detection of amino acids at λ 440 for proline and 570 nm for the other amino acids through an oxidative decarboxylation reaction of the amino acids with ninhydrin, to give ruhemann's purple compound, which could be detected by the spectrophotometer. Aliquot of 515.46 ml of 36% HCl (6N) was completed to 1000 ml distilled water. Sodium acetate buffer (0.1 N) of pH 2.2 was used as sample dilution buffer.

2. Acid hydrolysis: From each fresh sample of quinoa (leaves collected from plants of age 50 days after emergence from the 3rd node from the top of main stem), 1 g was hydrolyzed in sealed evacuated Pyrex test tube using 5 ml of 6 N HCl at 110°C for 24 h. At the end of this period, hydrolysate was transferred quantitatively to other containers and the hydrochloric acid was then evaporated to dryness at 50 – 60°C on water bath. Distilled water (5 ml) was added to the hydrolysate and then evaporated to dryness to remove the excess HCl. Further addition of distilled water was carried out until complete removal of excess HCl and samples were dried till the dry film was obtained. The obtained dry film was dissolved in a known volume of sample dilution buffer (0.1N sodium acetate buffer, pH 2.2) and the solution was filtered through a 0.45 mm membrane filter, and then stored frozen in sealed vials until fractionation of the amino acids by amino acid analyzer.

3. Separation of amino acids: Samples were injected into amino acid analyzer (SYKAM, S4300) Model: S 5200, Serial: 014513, Germany in the Central Lab of Desert Research Center (DRC) for analysis at the following fractionation conditions:

Column: Hydrolysate column SYKAM (S4300) – (150x4.6 mm) of a temperature of 57°C.

Sample: 100 µl

Table 2. Some physical and chemical properties of soil

Soil depth (cm)	Particle size distribution			Texture	
	Sand (%)	Silt (%)	Clay (%)		
0-30	80.1	9.9	0.0	Sandy	
Soil depth (cm)	**pH**	**$CaCO_3$ (%)**	**EC (dS m^{-1})**	**Potassium (ppm)**	**Mg (ppm)**
0-30	8.1	0.0	0.2	6.8	5.8

Buffer system: Sodium acetate, buffer A (pH 3.45), buffer B (pH 10.85) and buffer C (regeneration solution).

Flow rate: 0.25 ml/min for ninhydrin pump, 0.45 ml/min for quaternary pump.

Detection: Ninhydrin is used for the detection of amino acids spectrophotometrically at λ 440 for proline and 570 nm for the other amino acids through an oxidative decarboxylation reaction to give ruhemann's purple color.

4. Calculation of amino acids content: The peak area and percentage of each amino acid was calculated using an external standard by the computer software SYKAM (S4300).

2.7.2 Leaf anatomy work

The leaf samples were taken from five replications of control (95% FC) and drought at 65 and 35% FC treatments were taken from the field of quinoa genotypes at age of 70 days from emergence at the 3rd node from the top of main stem. Leaves were preserved in a solution of 1-5 ml formaldehyde acetic acid (FAA), 2-5 ml glacial acetic acid (GAA) and 90 ml Ethyl alcohol 70% and kept in vials. Leaves were transferred through different levels of Ethyl Alcohol to get the leaves dried, i.e. Ethyl alcohol 70% 2 h, Ethyl alcohol 85% 2 h, Ethyl alcohol 95% 2 h, Ethyl alcohol absolute 24 h, Ethyl alcohol 3:1 chloroform 2 h, Ethyl alcohol 2:2 chloroform 2 h, Ethyl alcohol 1:3 chloroform 24 h. Hot paraffin wax was poured to the sample and then kept in oven at 60°C with the ability to change the wax every 24 h, then wax was taken outside the oven to let it dry to be prepared for cutting by microtome to get transverse sections with a thickness of 8-12 micron. Glass slide was covered by adhesive solution (1 g gelatin in100 ml worm water) to prevent specimen from falling of the surface of the slide, then left it to dry. After the slide got dried it was ready to go to dying stage, consisting of 16 dye solution (Xylene 24 h, Xylene + Ethyl absolute (0.5:0.5) 2 min, Ethyl absolute 2 min, Ethyl alcohol 95% 2 min Ethyl alcohol 85% 2 min, Ethyl alcohol 70% 2 min, Safranin (overnight), Ethyl alcohol 70% 2 min, Ethyl alcohol 85% 2 min, Ethyl alcohol 95% 2 min, Ethyl absolute 2 min, Fast green, light green "sec", Ethyl absolute, Xylene + Ethyl absolute (0.5:0.5) 2 min and Xylene 1 min). The slides were covered by fine glass cover using Canada Balsam as adhesive before we examined it under the microscope (Lica, Germany) at 40x and 80x eye length. Finally, photographs were taken with a digital camera (Canon) attached to a microscope. Measurements were taken on leaf thickness and different types of layers, namely the upper epidermis, lower epidermis, the palisade and spongy layer.

2.8 Biometrical and Genetic Analyses

Analysis of variance of the split-split plot design in RCB arrangement was performed based on individual plot observation using the MIXED procedure of MSTAT ®. Moreover, analysis of variance for each environment separately was performed as randomized complete block design. Least significant difference (LSD) values were calculated to test the significance of differences between means according to Steel et al. [20].

3. RESULTS

3.1 Leaf Free Amino Acids

3.1.1 Analysis of variance for amino acids

Analysis of variance (Table 3) of 16 leaf free amino acids and their total content of five quinoa genotypes evaluated in 2015/2016 season under three soil moisture regimes (WW, WS and SWS), revealed significant ($p \leq 0.01$) differences among genotypes and among soil moisture regimes for the 16 amino acids and their total. Moreover, mean squares due to genotypes x irrigation regimes interaction were significant ($p \leq 0.01$or $p \leq 0.05$) for all free amino acids and their total content.

Table 3. Analysis of variance of split plot for leaf free amino acids of five quinoa genotypes (G) under three irrigation treatments (T) in 2014/2015 season

SOV	df	Mean squares					
		Asparagine	**Threonine**	**Serine**	**Glutathione**	**Glycine**	**Alanine**
Genotypes (G)	4	22.28**	7.91**	6.64**	29.61**	3.97**	8.61**
Irrigation (T)	2	31.42**	4.13**	4.98**	32.45**	2.94**	7.11**
G x T	8	1.68**	0.74**	0.87**	3.26**	0.51**	1.001**
Error	28	0.2	0.04	0.43	0.21	0.01	0.01
		Valine	**Methionine**	**Isoleucine**	**Leucine**	**Tyrosine**	**Phenylalanine**
Genotypes (G)	4	5.92**	0.20**	4.14**	12.943**	2.82**	3.9**
Irrigation (T)	2	4.67**	0.65**	4.04**	10.922**	2.33**	3.75**
G x T	8	0.68**	0.21**	0.63**	1.428**	0.38*	0.52**
Error	28	0.01	0	0.01	0	0.2	0.01
		Histidine	**Lysine**	**Arginine**	**Proline**	**Total**	
Genotypes (G)	4	1.47**	3.20**	3.36**	21.63**	1656.3**	
Irrigation (T)	2	2.77**	1.95**	2.47**	18.04**	1669.4**	
G x T	8	0.25**	0.11**	0.33**	1.46**	130.4**	
Error	28	0.02	0.03	0.01	0.04	2.67	

**and ** indicate significant at 0.05 and 0.01 probability levels, respectively*

Analysis of variance of RCBD for each free amino acid of quinoa genotypes under each environment (data not presented) revealed that mean squares due to genotypes were significant ($P \leq 0.01$ or $p \leq 0.05$) for all amino acids and their total content.

3.1.2 Effect of water stress on amino acids

The effects of soil moisture stress levels on the means of leaf free amino acids and their total across all quinoa genotypes are presented in Table 4. Contents of each and total content of the sixteen amino acids were significantly ($p \leq 0.01$ or $p \leq 0.05$) increased due to water stress, except Leucine which was decreased. The decrease shown by amino acid Leucine was amounted to 7.7 and 8.09% due to water stress and severe water stress, respectively.

Water stress caused an increase in total amount of amino acids by 13.9 and 17.7% under WS and SWS, respectively. Magnitude of the increase in amino acid content due to water stress differed from amino acid to another and from irrigation treatment to another. In general, increases in amino acid content due to water stress increased sharply by increasing severity of water stress. Maximum increase (109.6%) was shown by Threonine under severe water stress (35% of field capacity), but minimum increase (8.08%) was exhibited by Arginine under moderate water stress (65% of FC).

The increases in amino acid contents across all quinoa genotypes due to water stress ranged from 8.08% for Arginine to 17.14% for Histidine under moderate water stress (65% FC) and from 13.39% for Lysine to 109.6% for Threonine under severe water stress (35% FC).

3.1.3 Quinoa genotypic differences in free amino acids under water stress

The amount of each leaf free amino acid and their total content in mg/g dry matter for each of the five quinoa genotypes evaluated in the field in 2015/2016 season under well watering (WW), water stress (WS) and severe water stress (SWS) and combined across the three irrigation treatments are presented in Table 5. Genotypes of quinoa under investigation showed significant differences, expressed in ranges for all free amino acids (Table 4) and for each amino acid of each genotype under each of the three studied water treatments (Table 5). The ranges became wider as water stress increased for Glutathione, Valine, Methionine, Isoleucine, Histidin, Proline and total amino acids.

Combined across the three irrigation treatments, the highest mean content for all amino acids were shown by the quinoa variety CO-407, except for Methionine and Leucine, which were at maximum by the variety CICA-17. The second highest mean amino acids content was shown by the variety CICA-17. On the contrary, the lowest mean content for most amino acids under each irrigation regime and across them was shown by the variety Ollague (a Bolivian sensitive variety).

Table 4. Summary of means ± SE (standard error), reduction (Red%) from well watering (WW) to water stress (WS) and severe water stress (SWS), minimum (Min) and maximum (Max) values for amino acids across all quinoa genotypes in 2015/2016 winter season

Stress	Mean± SE	Red%	Max	Min	Mean± SE	Red%	Max	Min
	Asparagine				**Threonine**			
WW	14.85± 0.17	-	17.05	13.61	7.16 ± 0.1	-	8.55	6.26
WS	16.86±0.05	-13.5**	18.62	14.22	7.75±0.29	-8.2**	8.78	5.95
SWS	17.65±0.76	-18.9**	19.6	15.01	15.01±0.12	-109.6**	9.41	6.71
	Serine				**Glutathione**			
WW	6.51±0.76	-	7.83	5.41	15.49±0.79	-	17.01	13.85
WS	7.23±0.78	-11.1**	7.86	5.49	17.47±0.06	-12.8**	19.78	14.41
SWS	7.65±0.80	-17.5**	8.61	6.13	18.37±0.06	-18.6**	20.65	14.86
	Glycine				**Alanine**			
WW	5.42±0.06	-	6.5	4.89	9.59±0.10	-	11.39	8.55
WS	6.07±0.08	-12.0**	6.97	4.91	10.45±0.10	-9.0*	11.56	8.71
SWS	6.26±0.12	-15.5**	7.03	5.13	10.95±0.63	-14.2**	12.01	9.41
	Valine				**Methionine**			
WW	7.07±0.09	-	8.31	6.41	0.50±0.03	-	0.57	0.42
WS	7.77±0.00	-9.9*	8.84	6.62	0.56±0.03	-12**	0.61	0.48
SWS	8.18±0.06	-15.7**	9.26	6.48	0.89±0.05	-78**	1.61	0.57
	Isoleucine				**Leucine**			
WW	6.02±0.12	-	6.95	5.48	12.3±8.02	-	20.63	9.32
WS	6.61±0.06	-9.8*	7.6	5.53	11.35±0.09	7.7*	12.83	9.39
SWS	7.06±0.03	-16.6**	8.06	5.61	11.92±0.05	8.1*	13.61	9.41
	Tyrosine				**Phenylalanine**			
WW	4.13±0.09	-	4.8	3.48	5.56±0.063	-	6.57	4.98
WS	4.54±0.78	-9.9*	5.9	3.49	6.05±0.45	-8.8*	6.66	4.85
SWS	4.92±0.11	-19.1**	5.19	4.2	6.56±0.032	-18.0**	7.3	5.2
	Histidine				**Lysine**			
WW	3.50±0.077	-	3.97	3	5.30±0.07	-	6.3	4.68
WS	4.10±0.00	-17.1**	4.69	3.61	5.76±0.06	-8.7*	6.39	4.91
SWS	4.34±0.18	-24.0**	5.14	3.89	6.01±0.29	-13.4**	6.92	5.06
	Arginine				**Proline**			
WW	5.32±0.09	-	6.3	4.62	11.44±0.06	-	13.9	10.32
WS	5.75±0.10	-8.1*	6.48	4.72	12.66±0.33	-10.7**	14.49	10.33
SWS	6.13±0.10	-15.2**	6.94	5.05	13.63±0.45	-19.1**	15.26	11.26
	Total							
WW	117.9±0.77	-	137.9	105.6				
WS	131.3±2.62	-13.9**	146.1	108.2				
SWS	138.8±0.77	-17.7**	155.7	114.0				

**and ** indicate significant at 0.05 and 0.01 probability levels, respectively*

Means of total amino acid contents of each of the five quinoa genotypes under each environment and combined across environments (WW, WS and SWS) and genotypes are presented in Table 6. High means of all free amino acids were considered favorable, while low means were considered unfavorable. The total amount of leaf free amino acids combined across the three irrigation treatments was the highest in the genotype CO-407 (146.13 mg/g) followed by CICA-17 (137.76 mg/g), while the lowest was exhibited by the genotype Ollague (109.26 mg/g). Note that both CO-407 and CICA-17 are drought tolerant, while Ollague is drought sensitive. Under severe water stress (35% FC), the variety CICA-17 showed the highest total amount of amino acids (155.7 mg/g) followed by CO-407 (151.57 mg/g), but the lowest was exhibited by the genotype Ollague (114.0 mg/g). Under moderate water stress (65% FC), the variety CO-407 showed the highest total amount of amino acids (146.13 mg/g) followed by CICA-17 (140.83 mg/g), but the lowest was exhibited by the genotype Ollague (108.2 mg/g).Under well watering (95% FC), the variety CO-407 (drought tolerant) showed the highest total amount of amino acids (137.97 mg/g) followed by Chipaya (119.47 mg/g), but the lowest was exhibited by the genotype Ollague (drought sensitive) (105.57 mg/g).

Table 5. Amino acid contents of each quinoa genotype under well watering (WW), water stress (WS) and severe water stress (SWS) in 2015/2016 season

Genotype	WW	WS	SWS	Combined	WW	WS	SWS	Combined
			Asparagine				**Threonine**	
QL-3	13.61	16.88	17.05	15.85	6.8	7.95	8.13	7.63
Chipaya	15.08	15.98	17.88	16.31	7.18	7.41	7.86	7.48
CICA-17	14.89	18.6	18.73	17.41	6.99	8.67	9.41	8.36
CO-407	17.05	18.62	19.6	18.42	8.55	8.78	8.9	8.74
Ollague	13.61	14.22	15.01	14.28	6.26	6.29	6.71	6.42
LSD 0.05	0.18	0.05	0.83	0.43	0.11	0.33	0.13	0.18
			Serine				**Glutathione**	
QL-3	6.92	7.33	7.44	7.23	14.84	17.55	18.64	17.01
Chipaya	6.53	7.86	7.87	7.42	16.38	16.9	16.92	16.74
CICA-17	5.88	7.69	8.61	7.39	15.38	18.72	20.76	18.29
CO-407	7.83	7.8	8.21	7.95	17.01	19.78	20.65	19.14
Ollague	5.41	5.49	6.13	5.67	13.85	14.41	14.86	14.37
LSD 0.05	0.83	0.85	0.08	0.63	86	0.02	0.07	0.44
			Glycine				**Alanine**	
QL-3	5.04	6.06	6.36	5.82	9	10.75	10.68	10.14
Chipaya	5.44	5.67	6.03	5.72	9.71	9.92	11.07	10.23
CICA-17	5.23	6.97	7.03	6.41	9.31	11.32	12.01	10.88
CO-407	6.5	6.77	6.77	6.68	11.39	11.56	11.6	11.52
Ollague	4.89	4.91	5.13	4.98	8.55	8.71	9.41	8.89
LSD 0.05	0.07	0.09	0.13	0.09	0.11	0.03	0.07	0.09
			Valine				**Methionine**	
QL-3	6.51	8.07	8.12	7.57	0.57	0.55	0.62	7.57
Chipaya	7.08	7.55	8	7.54	0.51	0.55	1.03	7.54
CICA-17	7.07	7.77	9.26	8.03	0.44	0.59	1.61	8.03
CO-407	8.31	8.84	9.02	8.72	0.56	0.61	0.6	8.72
Ollague	6.41	6.62	6.48	6.5	0.42	0.48	0.57	6.5
LSD 0.05	0.11	0	0.06	0.68	0.03	0.03	0.05	0.03
			Isoleucine				**Leucine**	
QL-3	5.5	6.98	6.99	6.49	9.41	11.74	11.67	10.94
Chipaya	6.07	6.52	7	6.53	10.36	10.83	11.97	11.05
CICA-17	6.11	6.44	8.06	6.87	20.63	11.97	13.01	15.21
CO-407	6.95	7.6	7.63	7.39	11.79	12.83	12.92	12.51
Ollague	5.48	5.53	5.61	5.54	9.32	9.39	9.41	9.37
LSD 0.05	0.13	0.07	0.03	0.08	8.71	0.1	0.05	0.06
			Tyrosine				**Phenylalanine**	
QL-3	4.08	4.61	4.75	4.48	5.08	6.29	6.4	5.92
Chipaya	4.1	4.26	5.07	4.48	5.67	5.82	6.96	6.15
CICA-17	4.17	4.41	5.37	4.65	5.48	6.65	7.3	6.48
CO-407	4.8	5.9	5.19	5.3	6.57	6.66	6.91	6.71
Ollague	3.48	3.49	4.2	3.72	4.98	4.85	5.2	5.01
LSD 0.05	0.11	0.84	0.11	0.43	0.07	0.15	0.03	0.09
			Histidine				**Lysine**	
QL-3	3	3.99	3.85	3.61	4.8	5.67	5.9	5.45
Chipaya	3.73	3.83	4.07	3.88	5.45	5.72	5.91	5.69
CICA-17	3.52	4.69	5.14	4.45	5.36	6.1	6.26	5.91
CO-407	3.97	4.36	4.74	4.36	6.22	6.39	6.92	6.51
Ollague	3.29	3.61	3.89	3.6	4.68	4.91	5.06	4.88
LSD 0.05	0.08	0	0.19	0.12	0.08	0.07	0.32	0.17
			Arginine				**Proline**	
QL-3	5.19	5.93	6.14	5.75	10.69	13.05	13.4	12.38
Chipaya	5.43	5.58	5.92	5.65	10.8	11.26	12.95	11.67
CICA-17	5.07	6.03	6.94	6.01	11.48	14.15	15.26	13.63
CO-407	6.3	6.48	6.62	6.46	13.9	14.49	15.26	14.55
Ollague	4.62	4.72	5.05	4.8	10.32	10.33	11.26	10.64
LSD 0.05	0.1	0.1	0.11	0.09	0.06	0.36	0.05	0.19

Table 6. Total leaf free amino acid contents (mg/g dry matter) of five quinoa genotypes evaluated in the field under well watering (WW), water stress (WS), severe water stress (SWS) and combined across environments and genotypes in season 2015/2016

Irrigation treatment	QL-3	Chipaya	CICA-17
WW	110.07	119.47	116.73
WS	133.40	127.93	140.83
SWS	136.17	136.50	155.70
Combined	126.54	127.97	137.76
	CO-407	**Ollague**	**Combined**
WW	137.97	105.57	115.42
WS	146.13	108.20	134.05
SWS	151.57	114.00	142.79
Combined	145.22	109.26	130.76

Table 7. Percentage increase (%) of free amino acid contents in the leaf of each quinoa genotype and across genotypes from well watering (WW) to water stress (WS) and severe water stress (SWS) in season 2015/2016

Amino acid	WS	SWS	WS	SWS	WS	SWS
	QL3		Chipaya		CICA-17	
Aspragine	24.7	25.3	4.8	18.1	24.8	32.6
Therionine	19.5	21.6	2.5	10.3	25.4	35.3
Serine	23.8	25.3	5.3	22.1	31.8	47.3
Glutathione	17.8	25.6	3.7	3.8	22.9	35.8
Glycine	20.1	24.0	5.5	10.5	32.3	34.7
Alanine	17.4	18.4	2.1	12.9	22.8	29.4
Valine	24.4	25.3	4.7	10.5	9.3	30.3
Methionine	0.0	5.2	9.8	96.1	34.8	252.2
Isoleucine	25.0	25.9	6.9	14.6	5.5	31.6
Leuocine	24.0	25.0	4.7	15.7	17.7	32.6
Tyrosine	11.9	14.6	3.7	22.0	7.8	30.6
Phenyl alanine	22.8	24.5	3.2	24.6	20.9	32.0
Histidine	26.8	30.4	2.4	6.1	32.7	48.3
Lysine	19.0	22.5	5.5	10.1	14.3	23.7
Arginine	14.9	15.6	2.2	9.5	17.5	36.0
Proline	21.6	24.5	4.4	20.4	24.3	33.2
Mean	19.6	22.1	4.5	19.2	21.6	47.9
	CO-407		Ollague		Combined	
Aspragine	9.3	14.7	4.6	10.4	13.5	17.3
Therionine	3.9	5.2	0.8	8.2	9.9	15.5
Serine	0.5	6.5	0.9	13.7	11.4	21.8
Glutathione	9.5	14.5	3.8	7.3	11.5	17.3
Glycine	3.2	3.7	0.4	6.3	11.9	15.2
Alanine	1.5	1.8	2.1	10.2	8.9	14.0
Valine	7.5	9.2	3.3	0.6	9.6	14.9
Methionine	14.5	12.7	4.8	33.3	33.3	49.0
Isoleucine	10.0	10.6	1.5	2.9	10.0	16.7
Leuocine	8.6	9.8	0.2	0.7	11.2	16.3
Tyrosine	2.5	7.9	0.3	21.0	3.8	14.1
Phenyl alanine	2.0	6.1	1.4	5.1	10.6	17.3
Histidine	7.4	17.6	10.7	18.3	16.6	22.0
Lysine	1.6	10.5	5.6	7.9	8.9	14.9
Arginine	2.4	4.9	6.5	9.8	8.6	14.6
Proline	1.9	9.2	0.5	9.4	6.8	12.8
Mean	5.4	9.1	3.0	10.3	11.7	18.4

The percentage increase in leaf free amino acids due to the imposition of quinoa plants to drought stress (65 and 35% FC for WS and SWS, respectively) for the five quinoa genotypes and combined across genotypes are presented in Table 7. For combined data across all studied genotypes, percentage increase of all amino acids was 11.7% under moderate water stress

(WS) and 18.4% under severe water stress (SWS). It ranged from 33.3% (Methionine) to 3.8% (Tyrosine) under WS and from 49.0% (Methionine) to 12.8% (Proline) under SWS. Combined data across genotypes indicated that the severest the drought stress the more percentage increase in the total amount of amino acids. Under the severest water stress (SWS), the most drought tolerant genotype in the present study (CICA-17) showed the highest mean increase percentage (47.9%) in the total amount of amino acids relative to well watering (WW); it showed the highest increase in all amino acids, especially proline (33.2%), methionine (252.2%) and phenylalanine (32.4%). On the contrary, the lowest increase in all amino acids including proline was exhibited by CO-407 and Ollague genotypes.

Under the moderate water stress (WS), again the most drought tolerant genotype in the present study (CICA-17) showed the highest mean increase percentage (24.3%) in the total amount of amino acids compared to well watering (WW); it showed the highest increase in nine amino acids including proline. The genotype QL-3 showed the highest increase in seven amino acids.

3.2 Leaf Anatomy

3.2.1 Analysis of variance of anatomical traits

Analysis of variance (Table 8) of leaf anatomical traits for five quinoa genotypes evaluated in 2015/2016 season under three soil moisture regimes (WW, WS and SWS), revealed significant (p≤0.01) differences among genotypes and among irrigation regimes for the five anatomical traits, except irrigation treatments for lower epidermis, which were not significant. Moreover, mean squares due to genotype x irrigation regimes interaction were significant (p≤0.01or p≤0.05) for all studied anatomical traits.

Analysis of variance of RCBD for studied leaf anatomical traits of five quinoa genotypes under each environment (data not presented) showed that mean squares due to genotypes were significant (P ≤ 0.01or p≤0.05) for all leaf anatomical traits.

3.2.2 Effect of water stress on leaf anatomical traits

The effects of soil moisture levels on the means of leaf anatomical traits across all quinoa genotypes are presented in Table 9. Thickness of leaf was significantly decreased due to water stress by 3.42 and 6.16% under WS and SWS, respectively.

The decrease shown by leaf thickness due to water stress was associated with decrease in upper and lower epidermis (15.38%) under WS, palisade layer (15.79%) and spongy layer (5%) under SWS. On the contrary, water stress caused a significant increase in palisade layer (7.01%) and spongy layer (25.00%) under WS and upper epidermis (7.69%) and lower epidermis (76.92%) under SWS.

3.2.3 Genotypic differences in leaf anatomical traits under drought stress

Thickness measurements of upper and lower epidermis, palisade and spongy layers as well as leaf thickness for each genotype under WW, WS and SWS are presented in Table 10. The effect of soil moisture content on leaf tissues had shown significant differences among the studied genotypes of quinoa. The genotype CICA-17 (the most drought tolerant) had shown the thickest leaf under WW, WS, SWS and combined across all irrigation regimes, while the thinnest leaf was shown by the genotype CO-407 and Ollague (drought sensitive) under WS and combined across all irrigation regimes conditions.

Table 8. Analysis of variance of split plot for leaf anatomical traits of five quinoa genotypes (G) under three irrigation treatments (T) in 2014/2015 season

SOV	df	Mean squares				
		Leaf thickness	Upper epidermis	Lower epidermis	Palisade layer	Spongy layer
Genotypes(G)	4	0.662**	0.073**	0.056**	0.335**	0.184**
Treatments (T)	2	0.046**	0.036**	0.001	0.1**	0.105**
G x T	8	0.424*	0.044**	0.027**	0.167**	0.136**
Error	56	0.002	0.007	0.021	0.002	0.001

**and ** indicate significant at 0.05 and 0.01 probability levels, respectively*

Table 9. Summary of means ± SE (standard error), reduction (Red%) from well watering (WW) to water stress (WS) and severe water stress (SWS), minimum (Min) and maximum (Max) values for thickness of leaf and studied layers across all quinoa genotypes

Stress	Mean± SE	Red%	Max	Min	Mean± SE	Red%	Max	Min
	Leaf thickness				Upper epidermis			
WW	1.46±0.03	-	1.88	1.19	0.13±0.01	-	0.17	0.05
WS	1.41±0.04	3.42	1.67	0.66	0.11±0.01	15.38	0.16	0.05
SWS	1.37±0.03	6.16	1.70	1.13	0.14±0.02	-7.69	0.38	0.05
	Lower epidermis				Palisade layer			
WW	0.13±0.07	-	0.30	0.06	0.57±0.06	-	0.72	0.46
WS	0.11±0.06	15.38	0.26	0.06	0.61±0.05	-7.01	0.29	0.87
SWS	0.23±0.07	-76.92	0.024	0.05	0.48±0.01	15.79	0.68	0.19
	Spongy layer							
WW	0.40±0.05	-	0.63	0.13				
WS	0.50±0.03	-25.00	0.71	0.27				
SWS	0.38±0.01	5.00	0.48	0.26				

Table 10. Thickness (µ) of leaf, upper and lower epidermis, palisade and spongy layers of studied quinoa genotypes as affected by water stress (WS) and severe water stress (SWS) compared to well watering (WW)

Genotype	WW	WS	SWS	Combined	WW	WS	SWS	Combined
	Leaf thickness				Upper epidermis			
QL-3	1.19	1.62	1.46	1.42	0.17	0.11	0.18	0.15
Chipaya	1.44	0.66	1.44	1.18	0.17	0.10	0.08	0.12
CICA-17	1.88	1.67	1.70	1.75	0.15	0.16	0.17	0.16
CO-407	1.39	1.52	1.14	1.35	0.13	0.13	0.06	0.11
Ollague	1.39	1.57	1.13	1.36	0.05	0.05	0.19	0.10
LSD_{05}	0.03	0.04	0.03	0.03	0.02	0.03	0.05	0.03
	Lower epidermis				Palisade layer			
QL-3	0.13	0.20	0.50	0.28	0.48	0.3	0.19	0.32
Chipaya	0.07	0.13	0.15	0.12	0.58	0.29	0.57	0.48
CICA-17	0.32	0.12	0.16	0.20	0.72	0.87	0.38	0.66
CO-407	0.06	0.06	0.16	0.08	0.6	0.79	0.59	0.66
Ollague	0.06	0.06	0.17	0.10	0.46	0.79	0.68	0.64
LSD_{05}	0.13	0.06	0.03	0.11	0.04	0.04	0.03	0.03
	Sponge layer							
QL-3	0.47	0.27	0.28	0.34				
Chipaya	0.49	0.71	0.47	0.55				
CICA-17	0.63	0.7	0.26	0.53				
CO-407	0.13	0.34	0.46	0.31				
Ollague	0.25	0.48	0.44	0.39				
LSD_{05}	0.04	0.04	0.03	0.03				

It is observed from Table 10 that the thickest upper epidermis was shown by CICA-17 followed by QL-3 under all and across environments. On the contrary, the genotype Ollague (sensitive) had the thinnest upper epidermis under all and across environments. Regarding lower epidermis, the thickest genotype was QL-3 followed by CICA-17 (the most drought genotype) under WW, SWS and combined across all environments. The thinnest lower epidermis was shown by CO-407followed by Ollague (sensitive) under WW, WS and combined across environments. For palisade, the thickest layer was exhibited by CICA-17 and CO-407 (drought tolerant genotypes) under most studied irrigation regimes. On the contrary, the thinnest palisade layer was shown by the genotype QL-3 (sensitive). The genotypes Chipaya and CICA-17 (both are drought tolerant) had the thickest spongy layer under most environments, but the genotype CO-407 followed by QL-3 had the thinnest spongy layer under WW and WS, respectively.

3.2.4 Description of leaf transverse sections of quinoa genotypes

3.2.4.1 QL-3 Genotype

Under the optimum soil moisture conditions (WW), the cells of the tested leaf tissue of QL-3 were healthy, but the air spaces were found near

the lower epidermis and the thickness of the leaf was 1.19 µ (Table 10 and Fig. 1). The palisade cells were organized in the upper epidermis, while the spongy layer cells showed disarrangement in the lower epidermis due to the increase of water for the surrounded cells. Under the moderate soil moisture conditions (65% FC), the cells of QL-3 had large air spaces that found near the upper epidermis and the thickness of the leaf was 1.62 µ (Table 10 and Fig. 1). The palisade cells were in two layers not well organized in the upper epidermis, while the spongy layer cells were showing disarrangement in the lower epidermis. Cytoplasm existed in the wall due to the damage occurred to this leaf. Under the severe drought conditions (SWS), the cells of QL-3 were affected by the severe lower amount of water, the air spaces were found all over the leaf and the thickness of the leaf was 1.46 µ (Table 10 and Fig. 1). The palisade cells were not organized in the upper epidermis and the spongy layer cells showed disarrangement in the lower epidermis. Chloroplasts were attached to the wall of the epidermis due to the severe drought stress.

3.2.4.2 Chipaya genotype

Under the well moisture conditions (WW), the cells of Chipaya were full of water which led to large air spaces that found all over the leaf and the leaf thickness was 1.44 µ (Table 10 and Fig. 2). The palisade cells were rapture in the upper epidermis while no spongy layer cells were found in the lower epidermis.

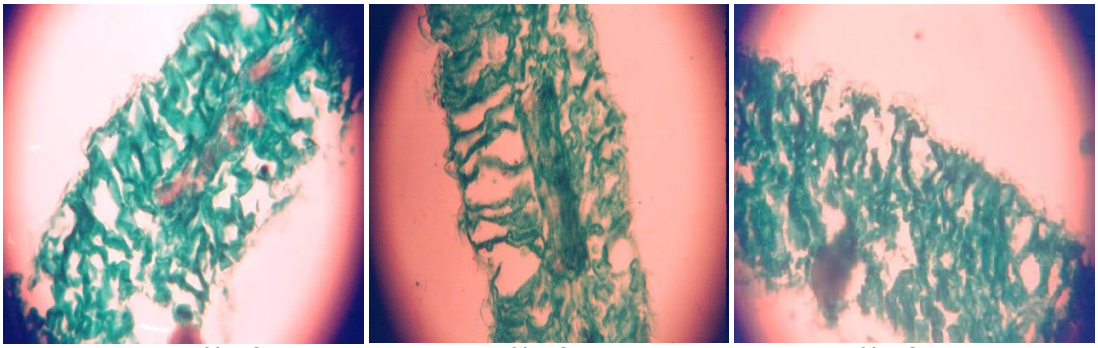

Fig. 1. Leaf transverse section for quinoa genotype QL-3 under the soil moisture 95% FC showing that the air spaces are large, chloroplasts are less and there is a rapture in the lower epidermis. Under soil moisture 65% FC showing that the air spaces are large, chloroplasts are less and there is a rapture in the lower epidermis and under soil moisture of 35% FC showing that the air spaces were small, and there is a rapture in the upper epidermis and it was swollen (X. 80)

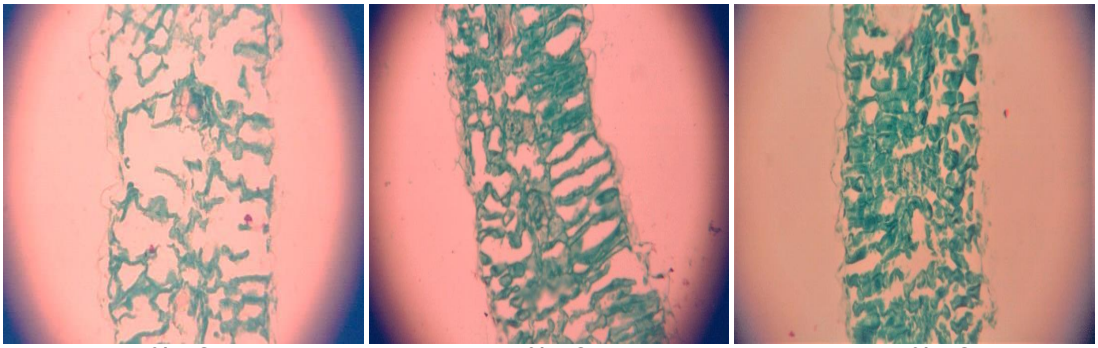

Fig. 2. Leaf transverse section for quinoa genotype Chipaya under the soil moisture 95% FC showing that the air spaces are large, upper and lower epidermis are not normal, under soil moisture of 65% FC showing that the air spaces are less and under soil moisture of 35% FC showing that the air spaces are less (X. 80)

Under the moderate moisture conditions (65% FC), the cells of Chipaya had small size of air spaces which found all over the leaf and the thickness of the leaf was 0.66 µ (Table 10 and Fig. 2). The palisade cells were arranged in the upper epidermis while no spongy layer cells were found in the lower epidermis, the genotype Chipaya is therefore considered moderately tolerant to this stress level. Under the drought conditions of 35% FC, the cells of Chipaya were affected by the very little amount of water, the air spaces were found all over the leaf and the thickness of the leaf was 1.44 µ (Table 10 and Fig. 2). The palisade cells were not organized in the upper epidermis and the spongy layer cells showed disarrangement in the lower epidermis. Chloroplasts were attached to the wall of the epidermis due to this severe drought stress.

3.2.4.3 CICA-17 genotype

Under the optimum soil moisture conditions (95% FC), the air spaces of CICA-17 genotype were found in the lower epidermis of the leaf and the leaf thickness was 1.88 µ (Table 10 and Fig. 3). The palisade cells were not organized in the upper epidermis and the spongy layer cells showed disarrangement in the lower epidermis. Under moderate water stress (65% FC), cells of CICA-17 genotype were healthy and the air spaces were small and the thickness of the leaf layer was 1.67 µ (Table 10 and Fig. 3). The three layers of palisade cells were well organized in the upper epidermis and the spongy layer cells showed disarrangement in the lower epidermis. The variety CICA-17 is therefore considered tolerant to this type of water stress (65% FC).

3.2.4.4 CO-407 genotype

Under the optimum moisture conditions, the air spaces of genotype C0-407 were found in the lower epidermis and the thickness of the layer was 1.39 µ (Table 10 and Fig. 4). The palisade cells were found organized in the upper epidermis and the spongy layer cells had disarrangement in the lower epidermis. Under the moderate stress (65% FC), the air spaces of genotype CO-407 were found in the lower epidermis of the leaf and the thickness of the layer was 1.52 µ (Table 10 and Fig. 4). The three layers of the palisade cells were organized in the upper epidermis and the spongy layer cells were damaged in the lower epidermis. Under the severe drought conditions (35% FC), CO-407 genotype had air spaces found in the lower epidermis were small, the thickness of the layer was 1.14.5 µ (Table 10 and Fig. 4). The palisade cells were well organized in the upper epidermis and the spongy layer cells showed disarrangement in the lower epidermis. This genotype is considered moderately tolerant.

3.2.4.5 Ollague genotype

For Ollague genotype under well watering conditions (95% FC), the air spaces found in the lower epidermis became of small size and the thickness of the layer was 1.39 µ (Table 10 and Fig. 5). The palisade cells were found organized in the upper epidermis and the spongy layer cells showed disarrangement in the lower epidermis. Under the moderate drought conditions (65% FC) for Ollague genotype, the air spaces were of small size, the thickness of the layer was 1.57 µ (Table 10 and Fig. 5). The palisade and the spongy layer cells showed disarrangement and were damaged. The air spaces were found all over the leaf and the thickness of the layer was 1.13 µ (Table 10 and Fig. 5). The palisade cells were damaged in the upper epidermis and the spongy layer cells were damaged in the lower epidermis.

Under the severe drought conditions (35% FC), the genotype Ollague was not tolerant. It is observed from Table 10 that increasing drought severity caused remarkable reduction in the thickness of the upper epidermis of Ollague, QL3 and Chipaya. However the variety CICA-17 showed an increase in this layer by increasing drought severity; which reached 2.5-3.0 fold under WS and SWS as compared to WW. It is interesting to mention that the variety CO-407 exhibited relative stability in upper epidermis thickness under WS and SWS. The varieties Ollague, Chipaya and QL3 under WS and SWS and CO-407 under SWS showed absence of the lower epidermis, on the contrary, the drought tolerant variety CICA-17 showed development of the lower epidermis layer under both water stress treatments (WS and SWS). Regarding palisade layer, it is obvious from Table 10 that varieties CICA-17 and Ollague showed an increase in thickness, but varieties QL3, Chipaya and CO-407 showed a remarkable decrease. For spongy layer the tolerant variety CICA-17 showed remarkable increase in the thickness of this layer under WS and SWS. The variety CO-407 showed an increase in spongy layer thickness under WS, but showed decrease in thickness of this layer under SWS. The variety Ollague showed a decrease in this layer thickness under

WS and increase under SWS. On the contrary, varieties QL3 and Chipaya showed remarkable decrease in the spongy layer thickness under WS and SWS conditions.

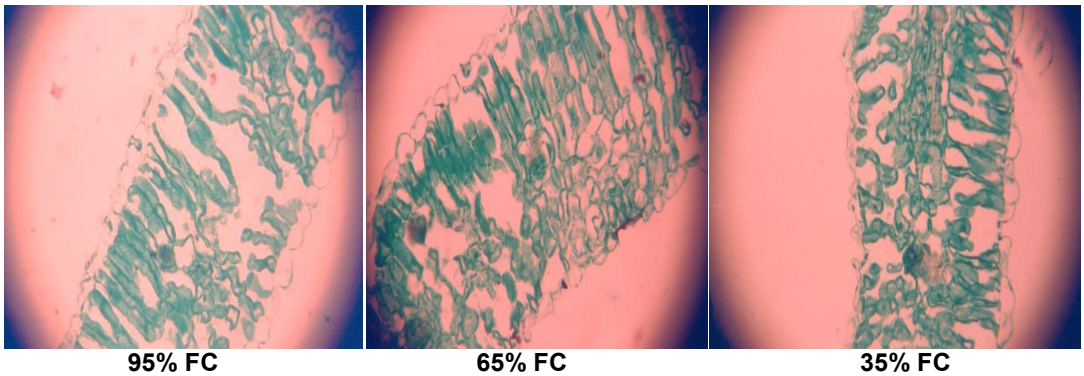

Fig. 3. Leaf transverse section for quinoa genotype CICA-17 at the soil moisture of 95% FC showing that the air spaces are large, upper and lower epidermis are normal. Moderate soil moisture stress (65% FC) showing the three layers of palisade cells are well organized in the upper epidermis; upper and lower epidermis are normal and soil moisture of 35% FC showing that the air spaces are large. Upper and lower epidermis are normal (X. 80)

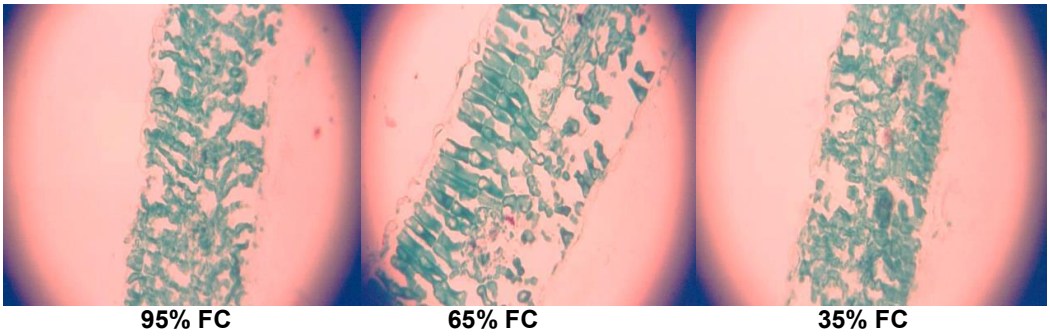

Fig. 4. Leaf transverse section for quinoa genotype CO-407 at the soil moisture of 95% FC showing that the air spaces are small size; upper and lower epidermis are not normal, soil moisture of 65% FC showing upper and lower epidermis are normal and soil moisture of 35% FC showing upper and lower epidermis are normal (X. 80)

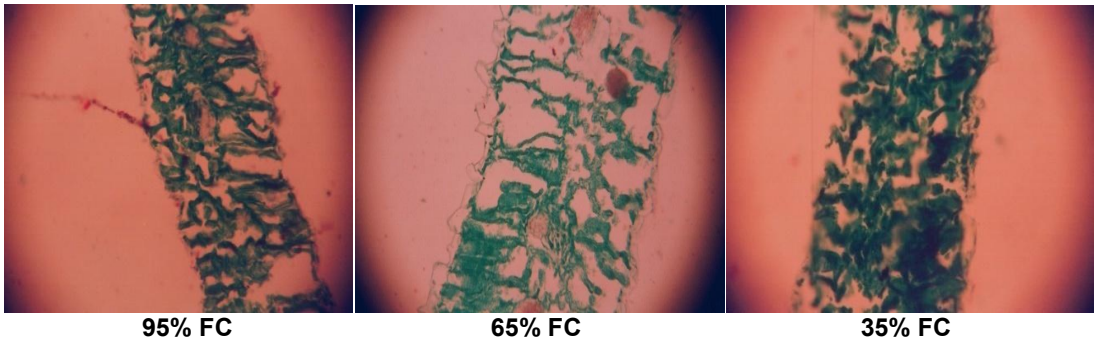

Fig. 5. Leaf transverse section for quinoa genotype Ollague at the moisture 95% F.C showing that the air spaces are small size, upper and lower epidermis are normal. Moisture 65% F.C showing that the air spaces are small size, upper and lower epidermis are not exist and moisture 35% F.C showing that the air spaces are large in size, upper and lower epidermis are not normal (X. 80)

4. DISCUSSION

Quinoa can employ different mechanisms to tolerate drought stress; among them accumulation of some amino acids in cells as osmoprotectants [7,9] and increased thickness of leaf layers [21-23]. For each amino acids and their total, analysis of variance in the present study indicated that both studied factors (genotypes and irrigation regimes) and their interaction were significant, suggesting that content of free amino acids in quinoa leaves varies with water supply. Al-Naggar et al. [24,25] reported a similar conclusion in sorghum. Analysis of variance of separate environments indicated the significance of differences among studied quinoa genotypes for all studied amino acids and their total content under all water stress environments and selection would be efficient under a specific water stress environment.

Water stress caused an increase in total amount of amino acids by 13.9 and 17.7% under WS and SWS, respectively. Magnitude of the increase in amino acid content due to water stress differed from amino acid to another and from irrigation treatment to another. In general, increases in amino acid content due to water stress increased sharply by increasing severity of water stress. Consistent to these results, several investigators reported increases in free amino acids due to drought stress [7,24,25]. The accumulation of organic (soluble sugars and proline) osmolytes has been found in quinoa under drought and saline conditions [9,13,14].

Wider ranges among genotypes of Glutathione, Valine, Methionine, Isoleucine, Histidin, Proline and total amino acids under water stress and severe water stress than well watering suggest that selection for high content of amino acids would be more efficient under water stressed than non-stressed environments.

It is observed from Table 6 that total free amino acids was increased by increasing water stress, i.e. from WW to WS and SWS for all quinoa genotypes. Results concluded that the drought tolerant quinoa genotypes in this study had high amounts of total amino acids under drought conditions and the *vice versa* for the sensitive genotypes. This conclusion might be explained by the increase in producing some amino acids by the drought tolerant genotypes because of drought stress imposed on their plants as a mechanism of drought tolerance. The highest increase in proline shown by the most drought tolerant genotype in this study (CICA-17) was reported by several investigators [24-26]. Many investigators [5,27,28] explained the role of proline in protection of plant cells against drought negative effects. It acts as osmoprotectant against many abiotic stresses. Possible role of proline may be (1) to neutralize toxic free ammonia produced in water stressed leaves [29], (2) to serve as a substrate for respiration and an energy source for the recovering plant [30] and (3) to reduce stress induced cellular acidification, *i.e*. to mediate osmotic adjustment, stabilize subcellular structures and scavenge free radicals [31]. The accumulation of proline appears to be an excellent means of storing energy since the oxidation of one molecule of proline can yield 30 ATP equivalents [32]. Proline synthesis has also been implicated as a mechanism of alleviating cytosolic acidosis, a condition often associated with stress [33]. A decrease in intracellular pH has been implicated as a factor capable of eliciting proline accumulation in plants [34] and removal of H+ excess due to proline synthesis may prevent a depression in respiration in salt-or water-stressed soybean seedlings [35]. Phenylalanine was observed to increase in higher tolerant than in sensitive varieties with drought stress. This result is similar to that noticed by Thompson et al. [36] in turnip leaves, Singh et al. [37] in barley leaves, Ashour [38] in soybean leaves and Al-Naggar et al. [24,25] in sorghum leaves. The exchange in the relative percentages of the individual free amino acids coincides with the speculation of Fallon and Phillips [39] and Ashour [38] that cells may primarily responded to drought stress by altering the rates of assimilation, synthesis, utilization and interconversion of amino acids.

For leaf anatomical traits, analysis of variance indicated that both studied factors (genotypes and irrigation regimes) and their interaction were significant, suggesting that thickness of leaf and different leaf layers of quinoa varies with water supply. Chartzoulakisa et al. [40], Dawood et al. [41], Faycal et al. [42] and Al-Naggar et al. [23] reported a similar conclusion. Analysis of variance of separate environments indicated the significance of differences among studied quinoa genotypes for all leaf anatomical traits under all irrigation treatments and selection would be efficient under a specific water stress environments.

Water stress caused a decrease in leaf thickness, upper and lower epidermis under WS,

palisade layer and spongy layer under SWS, but caused a significant increase in palisade and spongy layer under WS and upper epidermis and lower epidermis under SWS. Consistent to these results, some investigators reported increases in thickness of tissue layers of quinoa [21-23], but others [40-44] reported decreases in these layers due to drought stress. Differences in results may be attributed to differences in drought tolerance of genotypes used in different experiments. Drought tolerance is mainly achieved through quinoa's tissue elasticity and putative low osmotic potential [10,11]. Additionally, quinoa can avoid the negative effects of drought by developing special epidermal cell bladders, which may serve as external water reservoirs [10,15,16] and having vesicular glands, small and thick-walled cells.

The results (Table 10) concluded that the most tolerant genotype (CICA-17) had the thickest upper epidermis and leaf and was the second thickest in lower epidermis, palisade and spongy layers. On the other hand, the genotypes Ollague (sensitive), CO-407, Chipaya and QL-3 (sensitive) had the thinnest layers in two (upper and lower epidermis), one (spongy layer), one (leaf) and one (palisade layer) cases, respectively. From the results on the thickness of upper and lower epidermis, palisade and spongy layer, it could be concluded that the variety CICA-17 is considered as drought tolerant under moderate and severe water stresses, the variety CO-407 is considered as moderately tolerant, but the varieties QL3, Ollague and Chipaya could be considered sensitive under moderate and severe water stress conditions. Drought tolerant genotypes had thicker layers than sensitive ones under drought stress. Our results are in agreement with several investigators [40-46]. They found that abiotic stresses, such as salinity and drought caused remarkable decrease in the thickness of different tissue layers of the sensitive varieties but tolerant ones showed an increase in the thickness of these layers; as a mechanism of drought tolerance, under water stress conditions. Increased leaf thickness has been reported as a successful trait for plant species growing under saline conditions. Leaf thickening is considered as a mechanism to increase the water retention by mesophyll tissues in order to counteract salt toxicity [21,22]. On the other hand, thick palisade helps in more mesophyll conductance and hence enhances the CO_2 diffusion that may increase the photosynthesis rate [47]. Furthermore, the process of photosynthesis takes place mainly within palisade cells, and then an increased thickness of the palisade parenchyma allows higher photosynthetic activity and greater production of carbohydrates [48]. In agreement with these findings drought-treated CICA-17 leaves exhibit an increased number of palisade parenchyma cell layers compared with drought-untreated leaves. Palisade cells of CICA leaf also showed increased cell size. We assume that this feature could be related to greater sucrose synthesis occurring in these leaves. Our assumption agrees with previous results obtained in *Cucumis melo*, which suggested that an increase in the number of large cells promotes the sucrose accumulation [49].

5. CONCLUSIONS

Significance of variances due to the two studied factors (irrigation regimes and quinoa genotypes) and their interaction for leaf free amino acids and leaf anatomical traits suggested that content of free amino acids and leaf anatomical traits in quinoa varies with water supply and selection would be efficient under a specific water stressed environment. Results indicated that the drought tolerant quinoa genotypes in this study had higher amounts of amino acids and thicker layers of leaf anatomy than the sensitive ones under drought conditions. This behavior might be explained by the increase in producing some amino acids and increased thickness of leaf layers by the drought tolerant genotypes because of drought stress imposed on their plants as mechanisms of drought tolerance. The variety CICA-17 (the most drought tolerant in this study) showed the highest concentration and the highest increase percentage in most studied amino acids, especially proline, methionine and phenylalanine and the thickest upper and lower epidermis, palisade and spongy layers under severe water stress, which confirms the role of these amino acids and the thickness of these leaf layers in drought tolerance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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