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Leaf Anatomical Characteristics and Its Heritability of Different Quinoa (*Chenopodium quinoa* Willd.) Genotypes as Influenced by Moderate and Severe 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 and AEEB 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 can avoid the adverse effects of drought by developing special epidermal cell bladders which may serve as external water reservoirs and having small and thick-walled cells. The present investigation aimed at: (*i*) studying the effect of drought stress on quinoa leaf anatomical traits and their heritability, genetic advance from selection and (*ii*) describing differences among drought tolerant and susceptible 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)], moderate water stress (WS) [65% FC] and severe water stress (SWS) [35% FC]

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and sub plots to five genotypes. Mean squares due to genotypes, irrigation regimes and their interaction were significant for all studied leaf 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 genotype) was the first in thickness for upper epidermis, and leaf and second in lower epidermis, palisade and spongy layers. On the other hand, the genotypes Ollague (sensitive) had the thinnest layers in upper and lower epidermis. Broad-sense heritability estimates for anatomical traits were very high in magnitude (>87.0%), except for lower epidermis (41.18, 59.41 and 33.33%) under WW, WS and SWS, respectively. Genetic advance from selection ranged from 15.40% for upper epidermis to 72.97% for palisade layer under SWS, from 52.66% for leaf thickness to 82.72% for palisade layer under WS and from 30.40% for leaf thickness to 87.12% for spongy layer under WW.

Keywords: Chenopodium quinoa; drought; epidermis; palisade; spongy layer; leaf thickness.

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, it was domesticated by people living in the Andes, particularly in Peru and Bolivia, thousands of years ago. 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].

Quinoa appears to employ a wide variety of drought resistance mechanisms; these include drought escape, tolerance and avoidance [5]. The escape appears as a faster development of the vegetative growth and early maturing. Drought tolerance is mainly achieved through quinoa's tissue elasticity and putative low osmotic potential [6,7]. The accumulation of both inorganic and organic osmolytes has been found in quinoa under drought and saline conditions [5,8-10].

Additionally, quinoa can avoid the adverse effects of drought by growing a deep and dense root system along with the reduction of leaf area, leaf dropping, developing special epidermal cell bladders which may serve as external water reservoirs and having vesicular glands, small and thick-walled cells [6,11,12]. The knowledge gained by exploring those differences could be used in breeding program aimed at developing more suitable quinoa genotypes for specific environments.

The increasing population in Egypt requires an increase in food production along with 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 that require minimal water requirements. Drought-tolerant Quinoa is qualified to be cultivated in such region. Information on leaf anatomy of tolerant and susceptible quinoa genotypes in response to water stress are limited. The delay of plant breeders to incorporate drought stress tolerance into breeding programs is related to the lack of understanding the genetic behavior of such a complicated character and especially biochemical constituents and anatomical attributes responsible for drought tolerance [13,14]. Reports on heritability and genetic advance from selection for leaf anatomical traits of quinoa imposed to drought stress are scarce. The present investigation aimed at: (*i*) studying the effect of drought stress on quinoa leaf anatomical traits and their heritability, genetic advance from selection and (*ii*) describing differences among drought tolerant and susceptible 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 83,300 plants/ha. Each experimental plot included three 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 arrangement in randomized complete block (RCB) design 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

The following three different water regimes were used:

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 205 m^3 water/ha; thus, the total quantity of water given in the whole season for WW treatment was 8200 m^3 per ha.
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 250 m^3 water/ha; thus, the total quantity of water given in the whole season for WS treatment was 5000 m^3 per ha.
3. **Severe water stress (SWS)**, where the field capacity (FC) was about 35%. Irrigation in this treatment (WW) was given each twelve days; with ten irrigations during the whole season. The water meter recorded at the end of each irrigation about 236.8 m^3 water/ha; thus, the total quantity of water given in the whole season for WW treatment was 2368 m^3 per ha.

2.6 Fertilization Regimes

2.6.1 First: organic fertilizer

A Compost locally made of plant and animal wastes of the farm at New Salhiya was added to the soil at rate of 28 tons/ha and was well mixed with the soil two weeks before sowing at a depth of 10-15 cm.

2.6.2 Second: mineral fertilizers

Nitrogen fertilizer at the rate of 166 kg N/ha 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 70 kg P_2O_5/ha was added as soil application in two equals doses, the first (35 kg P_2O_5/ha)

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

before sowing during preparing the soil for planting and the second (35 kg P_2O_5/ha) after 25 days from sowing. Potassium fertilizer at the rate of 60 kg K_2O/ha was added as soil application in two doses; before planting (35 kg K_2O/ha) and after 25 day from sowing (25 kg K_2O/ha) as Potassium Sulfate (48% K_2O). Calcium Sulfate or Gypsum (22% Ca, 17% S) at the rate of 50 kg/ha 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.

2.6.3 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 type was sandy and consist 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). 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 Leaf Anatomy Laboratory 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 the five quinoa genotypes at 70 days from emergence at the 3rd node from the top of the 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 under the room temperature. Leaves were transferred through different levels of Ethyl Alcohol to get the leaves dried, i.e. Ethyl alcohol 70% 2h, Ethyl alcohol 85% 2 h, Ethyl alcohol 95% 2h, Ethyl alcohol absolute 24 h, Ethyl alcohol 3:1 chloroform 2 h, Ethyl alcohol 2:2 chloroform 2 h, Ethyl alcohol 1:3 chloroform 24h. 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 (one gram gelatin in 100 ml warm 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 thin 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 for the split plot was performed on the basis of individual plot observation using the MIXED procedure of MSTAT ®. Moreover, an analysis of variance for randomized complete block design (RCBD) was performed for each environment separately (WW, WS or SWS). Least significant difference (LSD) values were calculated to test the significance of differences between means according to Steel et al. [15]. Expected mean squares at separate environments were estimated from ANOVA table (Table 2) according to Hallauer et al. [16].

Table 2. Analysis of variance of RCBD and expected mean squares (EMS) of separate treatment

SOV	df	MS	EMS
Replications (R)	2	-	-
Genotypes (G)	4	M_2	$\delta^2_e + r\,\delta^2_g$
Error	8	M_1	δ^2_e

Genotypic (σ^2_g), phenotypic (σ^2_{ph}), and error variances were computed as follows: $\sigma^2_g = (M_2 - M_1)/r$ and $\sigma^2_{ph} = \sigma^2_g + \sigma^2_e / r$. Where r = number of replications.

2.9 Heritability in the Broad Sense

Heritability in the broad sense (h^2_b %) for a trait in a separate environment and combined across

environments was estimated according to Singh and Narayanan (2000) using the following formula:

$$h^2_b\ \% = 100 \times (\delta^2_g / \delta^2_{ph})$$

Where: σ^2_g = genetic variance, and δ^2_{ph} = phenotypic variance.

2.10 Expected Genetic Advance from Selection

Expected genetic advance from selection for all studied traits as a percent of the mean was calculated according to Singh and Narayanan [17] as follows: GA (%) = 100 K h^2_b σ_{ph} / $\overline{x}$, Where: $\overline{x}$ = General mean, σ_{ph} = Square root of the denominator of the appropriate heritability, h^2_b = The applied heritability, K = Selection differential (K = 1.76, for 10% selection intensity used in this study).

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Analysis of variance (Table 3) of leaf anatomical traits for five quinoa genotypes evaluated in 2015/2016 season under three soil moisture regimes (WW, WS and SWS), revealed significant ($p \leq 0.01$) 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 regime interaction were significant ($p \leq 0.01$or $p \leq 0.05$) for all studied anatomical traits, suggesting that thickness of leaf and different leaf layers of quinoa varies with water supply. A similar conclusion was reported by several investigators [18-20].

Analysis of variance of randomized complete blocks design for studied leaf anatomical traits of five quinoa genotypes under three environments (WW, WS and SWS) is presented in Table 4. Mean squares due to genotypes were significant ($P \leq 0.01$or $p \leq 0.05$) for all leaf anatomical traits, indicating 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.

3.2 Effect of Water Stress on Leaf Anatomical Traits

The effects of soil moisture stress levels on the means of leaf anatomical traits across all quinoa genotypes are presented in Table 5. 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. Consistent to these results, some investigators reported increases in thickness of tissue layers of quinoa [21,22], but others [18-20,23] 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. Quinoa appears to employ a wide variety of drought resistance mechanisms; these include drought escape, tolerance and avoidance. Drought tolerance is mainly achieved through quinoa's tissue elasticity and putative low osmotic potential [6,7], Additionally, quinoa can avoid the negative effects of drought by developing special epidermal cell bladders which may serve as external water reservoirs [6,11,12] and having vesicular glands, small and thick-walled cells.

Table 3. 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 4. Analysis of variance of randomized complete blocks design for leaf anatomical traits of five quinoa genotypes under well watering (95% FC), water stress (65% FC) and severe water stress (35% FC)

SOV	df	Mean squares				
		Leaf thickness	Upper epidermis	Lower epidermis	Palisade layer	Spongy layer
Well watering (95% FC)						
Genotype (G)	4	0.322**	0.012**	0.05*	0.06**	0.20**
Error	16	0.002	0.001	0.03	0.003	0.002
Water stress (65% FC)						
Genotype (G)	4	0.894**	0.008**	0.033**	0.415**	0.203**
Error	16	0.002	0.001	0.004	0.002	0.002
Severe water stress (35% FC)						
Genotype (G)	4	0.295**	0.14**	0. 43**	0.201**	0.052**
Error	16	0.001	0.021	0.039	0.001	0.002

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

Table 5. 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				

3.3 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 6. 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.

It is observed from Table 5 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-407 followed 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.

Table 6. 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				

From the abovementioned results presented in Table 5, it could be concluded that the genotype CICA-17 (the most tolerant genotype) was the first in thickness for upper epidermis, and leaf and second 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 2 (upper and lower epidermis), 1 (spongy layer), 1 (leaf) and 1 (palisade layer) cases, respectively.

3.4 Description of Leaf Transverse Sections of Quinoa Genotypes

3.4.1 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 5 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 5 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 5 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.

3.4.2 Chipaya genotype

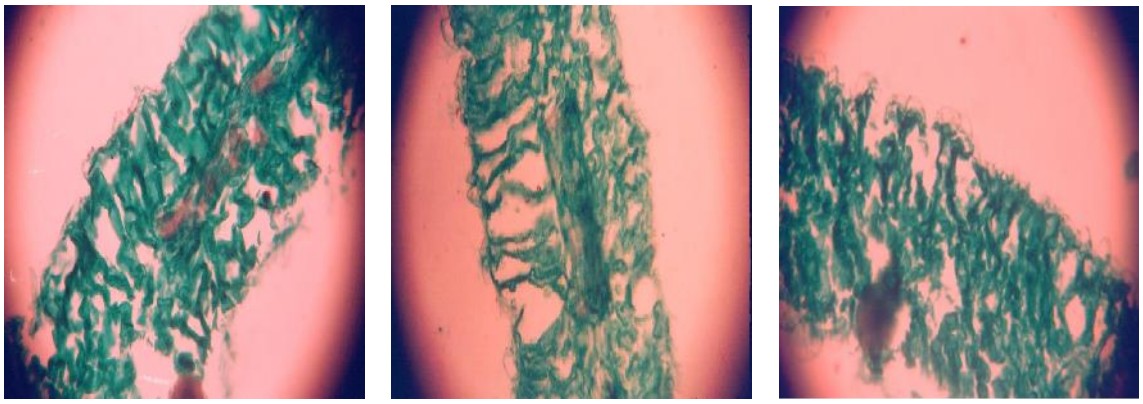
Under the well moisture conditions (WW), the cells of Chipaya were full of water which led to large air spaces that found all over the leaf and the leaf thickness was 1.44 µ (Table 5 and Fig. 2). The palisade cells were rupture in the upper epidermis while no spongy layer cells were found in the lower epidermis. 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 5 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 5 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.

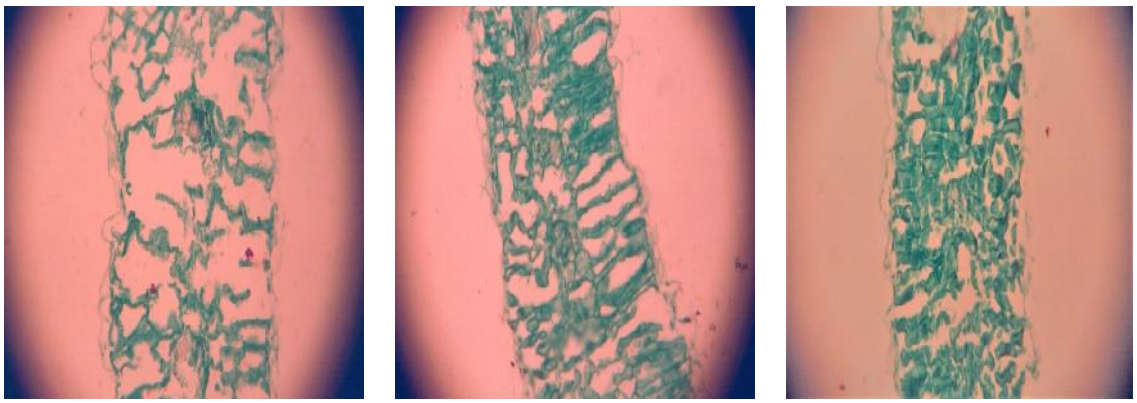
3.4.3 CIC A-17 genotype

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 5 and Fig. 3). The palisade cells were not organized in the upper epidermis and the spongy layer cells showed disarrangement in the lower epidermis.



95% FC **65% FC** **35% FC**

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 rupture in the lower epidermis, under soil moisture 65% FC showing that the air spaces are large, chloroplasts are less and there is a rupture in the lower epidermis and under soil moisture of 35% FC showing that the air spaces were small, and there is a rupture in the upper epidermis and it was swollen (X. 80)



95% FC **65% FC** **35% FC**

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 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 5 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).

Also for CICA-17 genotype under severe drought conditions (35% FC), the air spaces were found in the lower epidermis of the leaf and the thickness of the layer was 1.70 µ (Table 5 and Fig. 3). The palisade cells were organized in the upper epidermis and the spongy layer cells showed disarrangement in the lower epidermis. Thus, CICA-17 genotype is considered tolerant to severe water stress.

3.4.4 CO-407 genotype

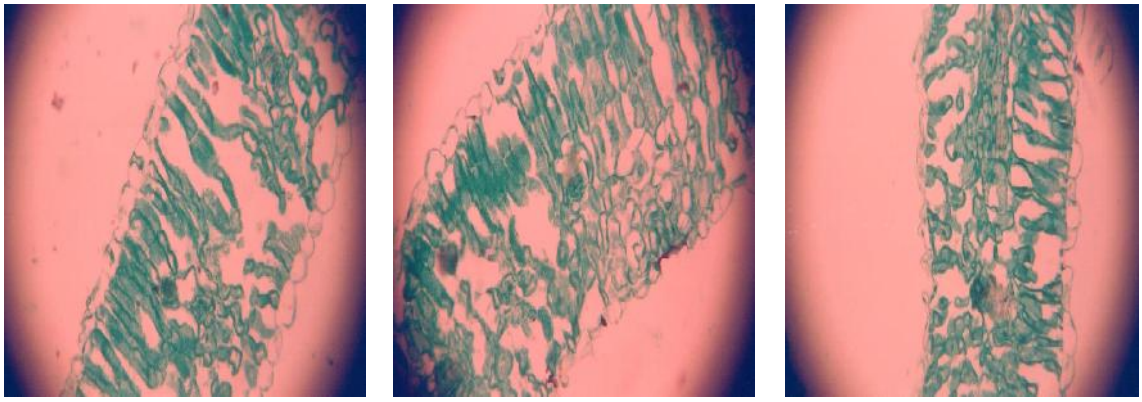
Under the optimum moisture conditions, the air spaces of genotype C0-407 were found in the lower epidermis and the thickness of the layer was 1.39 µ (Table 5 and Fig. 4). The palisade cells were found organized in the upper epidermis and the spongy layer cells had disarrangement in the lower epidermis.

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 5 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 in size, the thickness of the layer was 1.14.5 µ (Table 5 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.

3.4.5 Ollague genotype

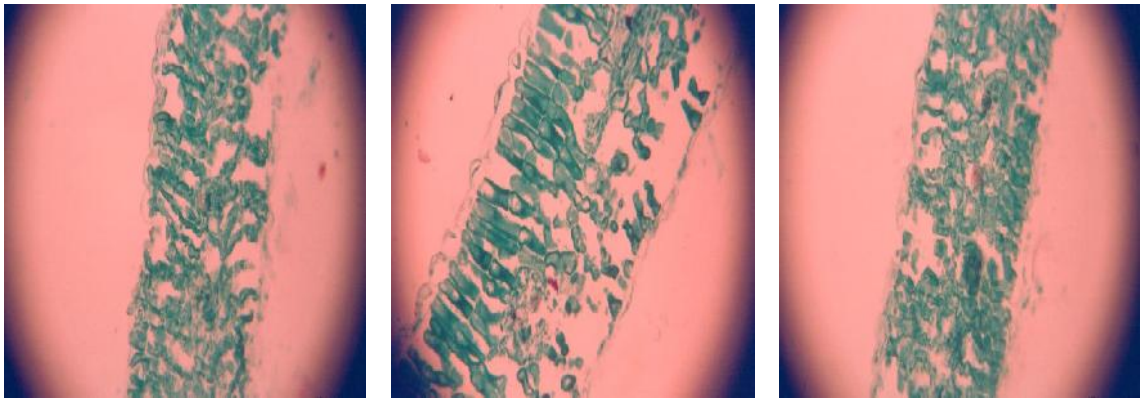
For Ollague genotype under well watering conditions (95% FC), the air spaces found in the lower epidermis became of small size and the thickness of the layer was 1.39 µ (Table 5 and Fig. 5). The palisade cells were found organized in the upper epidermis and the spongy layer cells showed disarrangement 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 5 and Fig. 5). The palisade and the spongy layer cells showed disarrangement and were damaged. Under the severe drought conditions (35% FC), the genotype Ollague was not tolerant. The air spaces were found all over the leaf and the thickness of the layer was 1.13 µ (Table 5 and Fig. 5). The palisade cells were damaged in the upper epidermis and the spongy layer cells were damaged in the lower epidermis.



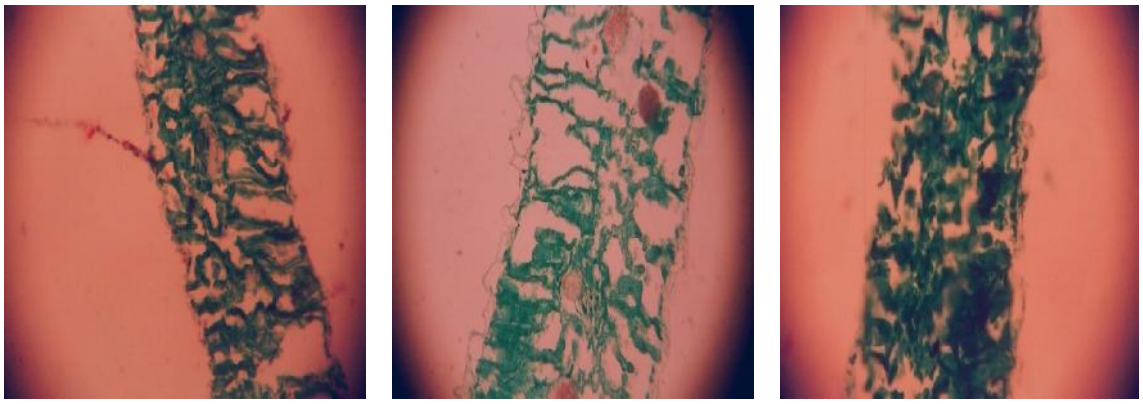
95% FC **65% FC** **35% FC**

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)



95% FC **65% FC** **35% FC**

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)



95% FC **65% FC** **35% FC**

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)

It is observed from Table 5 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 5 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.

From the above mentioned 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 to moderate and severe water stress conditions. Drought tolerant genotypes had thicker layers than sensitive ones under drought stress.

Our results are in agreement with those reported by several investigators [18-20,24-26], who 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.

Drought tolerance is mainly achieved through quinoa's tissue elasticity and putative low osmotic potential [6,7]. Quinoa can avoid the negative effects of drought by having thick-walled cells and developing special epidermal cell bladders which may serve as external water reservoirs and having vesicular glands, small and thick-walled cells [6,11,12]. 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 [27]. 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 also greater production of carbohydrates [28]. 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 [29].

4. HERITABILITY AND GENETIC ADVANCE FOR LEAF ANATOMICAL TRAITS IN QUINOA

Estimates of heritability in the broad sense (h^2_b) and expected genetic advance from selection as a percentage of the mean (GA%) for leaf anatomical traits under well watering (WW), water stress (WS) and severe water stress (SWS) conditions are presented in Table 7. In general, heritability estimates in the broad sense for anatomical traits were very high in magnitude (>87.5%), except for lower epidermis (41.18, 59.41 and 33.33) under WW, WS and SWS, respectively, indicating that environment had very small effect on the phenotype of most studied anatomical traits in leaves of quinoa. The highest h^2_b estimate (100%) was shown by upper epidermis under severe water stress.

The genetic advance (GA%) from selection was generally higher under moderate water stress (WS) for 3 anatomical traits, namely leaf thickness, lower epidermis and palisade layer and under well watering for two traits, namely upper epidermis and spongy layer (Table 7). GA ranged from 15.40% for upper epidermis to 72.97% for palisade layer under SWS, from 52.66% for leaf thickness to 82.72% for palisade layer under water stress and from 30.40% for leaf thickness to 87.12% for spongy layer under well watering (WW).

Table 7. Heritability in broad sense (h^2_b) and genetic advance from selection (GA) for leaf anatomical traits of quinoa under WW, WS and SWS environments in 2015/2016 season

Anatomical traits	h^2_b %			GA%		
	WW	WS	SWS	WW	WS	SWS
Leaf thickness	99.38	99.78	99.66	30.40	52.66	31.10
Upper epidermis	91.67	87.50	100.00	60.80	56.00	15.40
Lower epidermis	41.18	59.41	33.33	56.30	59.44	40.17
Palisade layer	94.55	99.52	99.50	30.62	82.72	72.97
Spongy layer	99.00	99.51	98.08	87.12	70.58	46.32

Since the efficiency of selection would depend upon the magnitude of heritable variability, higher heritability accompanied with high expected genetic advance for the leaf anatomical traits studied should be quite valuable. It is obvious from the results of this study, that palisade and spongy layers under all environments were characterized by having high heritability accompanied by high values of expected genetic advance, especially under WS and SWS.

Two groups of researchers reported two contrasting conclusions; the first group of investigators reported that heritability and expected genetic advance is higher under stress than non-stress conditions, and that selection should be practiced in the target (stressed) environment to obtain higher genetic advance [30-37]. The second group of researchers found that heritability and GA from selection for grain yield is higher under non-stress than those under stress [38-41]. Our results are in agreement with the second group for upper epidermis and spongy layer and with the first group for palisade layer and leaf thickness.

5. CONCLUSIONS

Significance of variances due to the two factors (irrigation regimes and quinoa genotypes) and their interaction for studied leaf anatomical traits suggested that these traits in quinoa varies with water supply and selection would be efficient under a specific water stressed environment. Results concluded that the drought tolerant quinoa genotypes in this study had thicker layers of leaf anatomy than the sensitive ones under drought conditions. This conclusion might be explained by the increase in thickness of leaf layers by the drought tolerant genotypes as a result of drought stress imposed on their plants as a mechanism of drought tolerance. Leaf anatomy revealed that the variety CICA-17 (the most drought tolerant in this study) showed the thickest layers of most studied anatomical traits, especially upper and lower epidermis, palisade and spongy layers, which confirms the role of these layers in drought tolerance. It is obvious from the results of this study, that most of studied anatomical traits were characterized by having high heritability accompanied by high values of expected genetic advance, especially under moderate water stress conditions. To the best of our knowledge these results on genotypic variability, heritability and genetic advance on anatomical traits of quinoa under water stress environments are believed to be the first record in the literature and need further investigation on the type of gene action controlling the inheritance of these traits to help plant breeders in tackling the physiologically and biochemically complex drought tolerance trait.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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