

Original Research Article

Leaf anatomical characteristics and its heritability of different quinoa (*Chenopodium quinoa* Willd.) genotypes as influenced by moderate and severe water stress

ABSTRACT

Many plants can avoid the **adversnegative** 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 its 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] 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.

Key words: *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

36 and inorganic fertilizers (Jacobsen, 1997; Jacobsen and Stolen, 1993 and Jacobsen *et al.*, 1996).
37 It draws attention with its high nutritional value, and more importantly, it is highly resistant to
38 weather, climate, and soil conditions such as salinity and drought (Jacobsen *et al.*, 2012).

39 Quinoa appears to employ a wide variety of drought resistance mechanisms; these
40 include drought escape, tolerance and avoidance Gonzalez *et al.* 2009 . The escape appears as a
41 faster development of the vegetative growth and early maturing. Drought tolerance is mainly
42 achieved through quinoa's tissue elasticity and putative low osmotic potential (Jensen *et al.* 2000
43 and Jacobsen *et al.* 2003). The accumulation of both inorganic and organic osmolytes has been
44 found in quinoa under drought and saline conditions (Gonzalez *et al.* 2009, Hariadi *et al.* 2011,
45 Shabala *et al.* 2012 and Adolf *et al.* 2013).

46 Additionally, quinoa can avoid the negative effects of drought by growing a deep and
47 dense root system along with the reduction of leaf area, leaf dropping, developing special
48 epidermal cell bladders which may serve as external water reservoirs and having vesicular
49 glands, small and thick-walled cells (Jensen *et al.* 2000, Tester and Davenport 2003 and Shabala
50 and Mackay 2011). The knowledge gained by exploring those differences could be used in
51 breeding program aimed at developing more suitable quinoa **linesvarieties** for specific
52 **conditionsenvironments., as well as potentially extrapolated to breeding other crops for**
53 **drought tolerance.**

Comment [ie1]:

54 The increasing population in Egypt **require demands** an increase in food production
55 along with **a shift towards environmentally sound** sustainable agriculture. Expansion of
56 agriculture is only available in the newly reclaimed lands in desert areas of Egypt. There is a
57 need for cultivation of crops **or varieties** that require **minmal water requirements,minimum**
58 **inputs including soil moisture availability in these areas. Drought-tolerant Quinoa varieties**
59 **Quinoa crop** is qualified to be cultivated in such **regions areas, especially the drought tolerant**
60 **varieties of this crop.** Information on leaf anatomy of tolerant and susceptible quinoa genotypes
61 in response to **imposition of** water stress are **generally** limited. **The delay of plant breeders to**
62 **incorporate drought stress tolerance into breeding programs is related to the lack of**
63 **understanding the genetic behavior of such a complicated character and especially biochemical**
64 **constituents and anatomical attributes responsible for drought tolerance (Al-Naggar *et al* 2002a,**
65 **b). Reports on heritability and genetic advance from selection for leaf anatomical traits in quinoa**

Comment [ie2]: This sentence is very long. To improve readability, consider breaking this into multiple sentences.

Comment [ie3]: Is that something special for this crop?

66 leaves are scarce. The present investigation aimed at: (i) the main objective of this study was to
 67 studying the effect of different soil moisture levels (95, 65 and 35% of field capacity; FC) of
 68 imposed drought stress on quinoa leaf anatomical traits and its heritability., genetic advance
 69 from selection and (ii) describing differences among drought tolerant and susceptible
 70 genotypes in such anatomical traits following the imposition of water deficit.

Comment [ie4]: Again how about the other crops

Formatted: No underline, Underline color: Auto

71 2. MATERIALS AND METHODS

72 This study was carried out in the growing winter season 2015/2016 at New Salhiya
 73 station, Sharqiya Governorate, Egypt. The station is located at 30° 18' 24" N latitude and 31° 6'
 74 47" E longitude with an altitude of 20 meters above sea level.

75 Plant materials

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

79 **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

81 Field experiment

82 On the 19th of November, the seeds were planted along the irrigation pipes of drip
 83 irrigation system. Each pipe (row) length was 90 meter and keeping row to row distance of 60
 84 cm and hill to hill of 60 cm. Seeds (7-10) were sown in each hill, thereafter (after 35 days) were
 85 thinned to three plants/hill to achieve a plant density of 35,000 plants/fed (83,300 plants/ha).
 86 Each experimental plot included three rows of 0.6 meter width and 12.0 meters long (plot size =
 87 21.6 m²) with a 1.0 meter ally between irrigation treatments.

88 Experimental design

89 A split-plot design arrangement in randomized complete block (RCB) arrangement
 90 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.

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.

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 m³ water/ha; thus, the total quantity of water given in the whole season for WW treatment was 8200 m³ 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 m³ water/ha; thus, the total quantity of water given in the whole season for WS treatment was 5000 m³ per ha.

3. Severe water stress (SWS), where the field capacity (FC) was about 35%. Irrigation in this treatment (WW) was given each nine days; with ten10 irrigations during the whole season. The water meter recorded at the end of each irrigation about 236.8 m³ water/ha; thus, the total quantity of water given in the whole season for WW treatment was 2368 m³ per ha.

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 atwith 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 equal doses as ammonium nitrate (33.5 % N). Triple Superphosphate

Comment [ie5]: Convert Area units

Comment [ie6]: Convert area units

Comment [ie7]: Add the chemical sign for ammonium nitrate

Fertilizer (46% P₂O₅) at the rate of 30 kg P₂O₅/fed was added as soil application in two equal doses, the first (15 kg P₂O₅/fed) before sowing during preparing the soil for planting and the second (15 kg P₂O₅/fed) after 25 days from sowing. Potassium fertilizer at the rate of 25 kg K₂O/fed was added as soil application in two doses; before planting (15 kg K₂O/fed) and after 25 day from sowing (10 kg K₂O/fed) as Potassium Sulfate (48% K₂O). 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%, Magnesium 3%) were added through irrigation system at a rate of half liter/month. Phosphoric acid (52:60% P₂O₅) 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 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).

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 age of 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. Leaves were transferred through different levels of Ethyl Alcohol to get the leaves dried, i.e. Ethyl alcohol 70% 2h, Ethyl alcohol 85% 2h, Ethyl alcohol 95% 2h, Ethyl alcohol absolute 24h, Ethyl alcohol 3:1 chloroform 2h, Ethyl alcohol 2:2 chloroform 2h, 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 24h, 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 (1g one gram gelatin in 100 ml warm water) to prevent specimen from falling of the surface of the slide, then

Formatted: No underline, Underline color: Auto

Comment [ie8]: Under the room temp??

Formatted: No underline, Underline color: Auto

left it to dry. After the slide got dried it was ready to go to dying stage, consisting of 16 dye solution (Xylene 24h, Xylene +Ethyl absolute (0.5:0.5) 2min, Ethyl absolute 2 min, Ethyl alcohol 95% 2min Ethyl alcohol 85% 2min, Ethyl alcohol 70% 2min, Safranin (overnight), Ethyl alcohol 70% 2min, Ethyl alcohol 85% 2min, Ethyl alcohol 95% 2min, Ethyl absolute 2 min, Fast green, light green “sec”, Ethyl absolute, Xylene + Ethyl absolute (0.5:0.5) 2min and Xylene 1min). The slides were covered by thin fine glass cover using Canada Balsam as an 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.

Biometrical and genetic analyses

Analysis of variance of for the split-split plot design arrangement in randomized complete block (RCB) arrangement design was performed on the basis of individual plot observation using the MIXED procedure of MSTAT ®. Combined analysis of variance across the two growing seasons was also performed if the homogeneity test was non-significant. Moreover, a combined analysis for each environment separately across seasons was conducted performed as RCB design. Least significant difference (LSD) values were calculated to test the significance of differences between means according to Steel *et al* (1997). Expected mean squares at separate treatments were estimated from ANOVA table (Table 2) according to Hallauer *et al* (2010).

Table 2. Analysis of variance 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.

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:

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

Comment [ie9]:

Comment [ie10]: Please consider rewording. This sentence is very long. To improve readability, consider breaking this into multiple sentences.

Comment [ie11]: This sentence is very long. To improve readability, consider breaking this into multiple sentences.

Comment [ie12]: Please consider providing the linear model

Formatted: Do not check spelling or grammar

Formatted: Pattern: Clear

Comment [ie13]: Please rewrite this part

Formatted: No underline, Underline color: Auto

Comment [ie14]: You have to provide the layout for splitplot with and across seasons as well.

179 Where: σ_g^2 = genetic variance, and δ_{ph}^2 = phenotypic variance.

180 Expected genetic advance from selection

181 Expected genetic advance from selection for all studied traits as a percent of the mean
182 was calculated according to Singh and Narayanan (2000) as follows: $GA (\%) = 100 K h_b^2 \sigma_{ph} / \bar{x}$,
183 Where: $\bar{x}$ = General mean, σ_{ph} = Square root of the denominator of the appropriate heritability,
184 h_b^2 = The applied heritability, K = Selection differential (K = 1.76, for 10% selection intensity
185 used in this study).

186 3. RESULTS AND DISCUSSION

187 3.a. Analysis of variance

188 Analysis of variance (Table 3) of leaf anatomical traits for five quinoa genotypes
189 evaluated in 2015/2016 season under three soil moisture regimes (WW, WS and SWS), revealed
190 significant ($p \leq 0.01$) differences among genotypes and among irrigation regimes for the five
191 anatomical traits, except irrigation treatments for lower epidermis, which were not significant.
192 Moreover, mean squares due to genotype x irrigation regimes interaction were significant
193 ($p \leq 0.01$ or $p \leq 0.05$) for all studied anatomical traits, suggesting that thickness of leaf and different
194 leaf layers of quinoa varies with water supply. A similar conclusion was reported by several
195 investigators (Chartzoulakisa *et al* 2012, Dawood *et al* 2014 and Faycal *et al* 2014).

196 | **Table (3). Analysis of variance of split-plot for leaf anatomical traits of five quinoa genotypes (G)**
197 **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

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

199 Analysis of variance of randomized complete blocks design for studied leaf anatomical
200 traits of five quinoa genotypes under three environments (WW, WS and SWS) is presented in
201 Table (4). Mean squares due to genotypes were significant ($P \leq 0.01$ or $p \leq 0.05$) for all leaf
202 anatomical traits, indicating the significance of differences among studied quinoa genotypes for
203 all leaf anatomical traits under all irrigation treatments and selection would be efficient under a
204 specific water stress environments.

Comment [ie15]: Rewrite this part

Comment [ie16]: This is not split plot ANOVA table

Comment [ie17]: I think you switched the tables

205
206
207
208

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

SOV	df	Leaf thickness	Upper epidermis	Mean squares 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

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

213 3.b. Effect of water stress on leaf anatomical traits

214 The effects of soil moisture stress levels on the means of leaf anatomical traits across all
215 quinoa genotypes are presented in Table (5). Thickness of leaf was significantly decreased due to
216 water stress by 3.42 and 6.16% under WS and SWS, respectively.

217 **Table (5). Summary of means $\pm$ SE (standard error), reduction (Red%) from well watering (WW)**
218 **to water stress (WS) and severe water stress (SWS), minimum (Min) and maximum**
219 **(Max) values for thickness of leaf and studied layers across all quinoa genotypes.**

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

SWS 0.38±0.01 5.00 0.48 0.26

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 (Naz *et al* 2014, Fernando *et al.*, 2017), but others (Dolatabadian *et al* 2011, Chartzoulakisa *et al.* 2012, Dawood *et al.* 2014 and Faycal *et al.* 2014) 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 (Jensen *et al.* 2000, Jacobsen *et al.* 2003). Additionally, quinoa can avoid the negative effects of drought by developing special epidermal cell bladders which may serve as external water reservoirs (Jensen *et al.* 2000, Tester and Davenport 2003 and Shabala and Mackay 2011) and having vesicular glands, small and thick-walled cells.

3.c. 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.

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
LSD₀₅	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
LSD₀₅	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				
LSD₀₅	0.04	0.04	0.03	0.03				

246

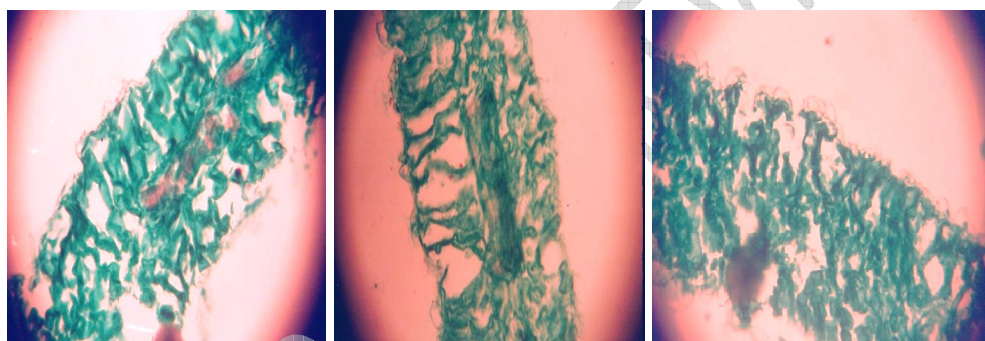
247 It is observed from Table (5) that the thickest upper epidermis was shown by CICA-17
248 followed by QL-3 under all and across environments. On the contrary, the genotype Ollague
249 (sensitive) had the thinnest upper epidermis under all and across environments. Regarding lower
250 epidermis, the thickest genotype was QL-3 followed by CICA-17 (the most drought genotype)
251 under WW, SWS and combined across all environments. The thinnest lower epidermis was
252 shown by CO-407 followed by Ollague (sensitive) under WW, WS and combined across
253 environments. For palisade, the thickest layer was exhibited by CICA-17 and CO-407 (drought
254 tolerant genotypes) under most studied irrigation regimes. On the contrary, the thinnest palisade
255 layer was shown by the genotype QL-3 (sensitive). The genotypes Chipaya and CICA-17 (both
256 are drought tolerant) had the thickest spongy layer under most environments, but the genotype
257 CO-407 followed by QL-3 had the thinnest spongy layer under WW and WS, respectively.

258 From the abovementioned results presented in Table (5), it could be concluded that the
259 genotype CICA-17 (the most tolerant genotype) was the first in thickness for upper epidermis,
260 and leaf and second in lower epidermis, palisade and spongy layers. On the other hand, the
261 genotypes Ollague (sensitive), CO-407, Chipaya and QL-3 (sensitive) had the thinnest layers in 2
262 (upper and lower epidermis), 1 (spongy layer), 1 (leaf) and 1 (palisade layer) cases, respectively.

263 3.d. Description of leaf transverse sections of quinoa genotypes

3.d.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.



95% FC

65% FC

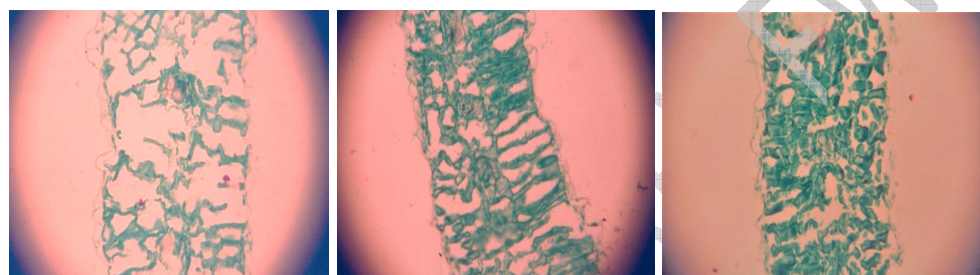
35% FC

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).

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.d.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 rapture 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.



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 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.d.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 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.

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).

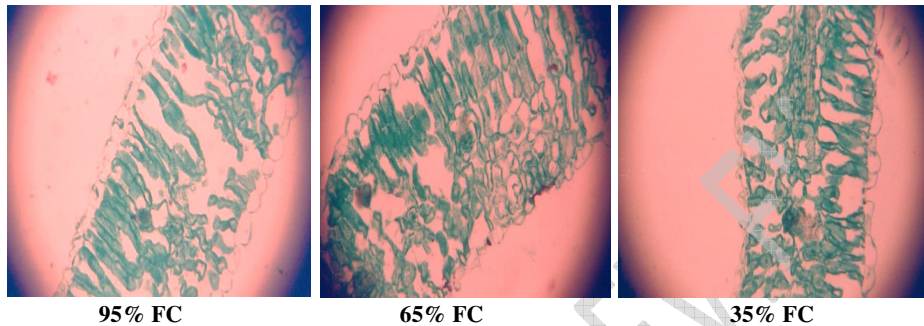


Fig. 3. Leaf transverse section for quinoa genotype CICA-17 at the soil moisture of 95% FC showing that the air spaces are large, upper and lower epidermis are normal, moderate soil moisture stress (65% FC) showing the three layers of palisade cells are well organized in the upper epidermis; upper and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

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 1.70μ (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.d.4. CO-407 genotype

Under the optimum moisture conditions, the air spaces of genotype CO-407 were found in the lower epidermis and the thickness of the layer was 1.39μ (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.

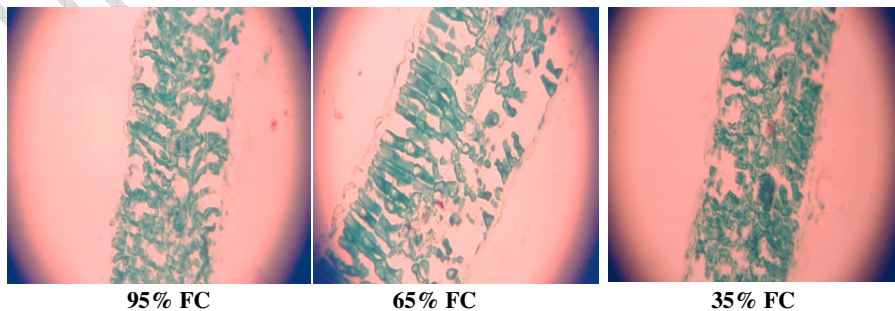


Fig. 4. Leaf transverse section for quinoa genotype CO-407 at the soil moisture of 95% FC showing that the air spaces are small size; upper and lower epidermis are not normal, soil moisture of 65% FC showing upper and lower epidermis are normal and soil moisture of 35% FC showing upper and lower epidermis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

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.d.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.

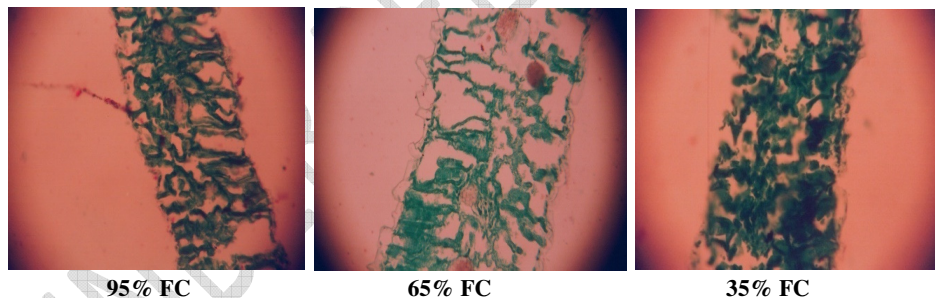


Fig. 5. Leaf transverse section for quinoa genotype Ollague at the moisture 95% F.C showing that the air spaces are small size, upper and lower epidermis are normal, moisture 65% F.C showing that the air spaces are small size, upper and lower epidermis are not exist and moisture 35% F.C showing that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

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.

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 Nour- El-Din et al. (1999), Asish et al (2004), Brokar et al (2009), Dolatabadian et al (2011), Chartzoulakisa et al. (2012), Dawood et al (2014) and Faycal et al. (2014), 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 (Jensen et al. 2000, Jacobsen et al. 2003). 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 (Jensen et al. 2000, Tester and Davenport 2003, Shabala and Mackay 2011). 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 (Naz *et al* 2014, Fernando *et al.*, 2017). On the other hand, thick palisade helps in more mesophyll conductance and hence enhances the CO₂ diffusion that may increase the photosynthesis rate (Nandy et al, 2005). 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 (Xie and Luo 2003). 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 (Kano and Fukuoka 2006).

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 (6). 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.

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

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).

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 (Blum, 1988, Hefny, 2007, Al-Naggar *et al*, 2009 and 2011, 2016 a, b, Al-Naggar and Shehab El-Deen, 2012 and Al-Naggar and Atta 2017). The second group of researchers found that heritability and GA from selection for grain yield is higher under non-stress than those under stress (Shabana *et al*, 1980; Atlin and Frey, 1990; Banziger and Laffite, 1997; and Worku, 2005). 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.

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.

REFERENCES

1. **Jacobsen, S.E. 1997.** Adaptation of quinoa (*Chenopodium quinoa*) to Northern European agriculture: studies on developmental pattern. *Euphytica*. 96: 41–48
2. **Jacobsen, S.E. and Stolen, O. (1993).** Quinoa-morphology, phenology and prospects for its production as a new crop in Europe. *Eur. J. Agron.* 2, 19–29.
3. **Jacobsen, S.E., Hill, J., Stolen, O. (1996).** Stability of quantitative traits in quinoa (*Chenopodium quinoa* Willd.). *Theo. Appl. Gen.* 93 (1–2), 110–116.
4. **Jacobsen SE, Jensen CR, Liu F (2012)** Improving crop production in the arid Mediterranean climate. *Field Crop Res.* 128:34–47.
5. **Gonzalez, J.A., M. Gallardo, M. Mirna Hilal, M. Rosa, and F.E. Prado (2009).** Physiological responses of quinoa (*Chenopodium quinoa* Willd.) to drought and waterlogging stresses: dry matter partitioning. *Bot. Stud.* 50: 35–42.
6. **Jensen, C.R., Jacobsen, S.E., Andersen, M.N., Nunez, N., Andersen, S.D., Rasmussen, L. and Mogensen, V.O. (2000).** Leaf gas exchange and water relation characteristics of field quinoa (*Chenopodium quinoa* Willd.) during soil drying. *Eur. J. Agron.* 13, 11–25.
7. **Jacobsen S.-E., Mujica A., Jensen C. R. (2003).** The resistance of quinoa (*Chenopodium quinoa* Willd.) to adverse abiotic factors. *Food Rev. Int.* 19 99–109. 10.1081/FRI-120018872
8. **Hariadi, Y., K. Marandon, Y. Tian, S. E. Jacobsen, and S. Shabala (2011).** Ionic and osmotic relations in quinoa (*Chenopodium quinoa* Willd.) plants grown at various salinity levels. *J. Exp. Bot.* 62, 185–193.
9. **Shabala, L, A. Mackay, YuTian, S.E. Jacobsen, D. Zhou and S. Shabala (2012).** Oxidative stress protection and stomatal patterning as components of salinity tolerance mechanism in quinoa (*Chenopodium quinoa* Willd.). *Physiol. Plant* 146(1):26–38
10. **Adolf, V.I., S.E. Jacobsen and S. Shabala (2013).** Salt tolerance mechanisms in quinoa (*Chenopodium quinoa* Willd.). *Environ Exp Bot* 92:43–54.
11. **Tester, M., and R. Davenport, 2003:** Na⁺ Tolerance and Na⁺Transport in Higher Plants. *Ann. Bot.* 91, 503–527.
12. **Shabala S. and Mackay A (2011)** Ion transport in halophytes. *Adv Bot Res* 57:151.
13. **Al- Naggari, A. M. M., O. O. El-Nagouly and Zeinab S. H. Abo-Zaid (2002a).** Genotypic differences in leaf free amino acids as osmoprotectants against drought stress in grain sorghum. *Egypt. J. Plant Breed.* 6 (1): 85 – 98.

14. **Al- Naggar, A. M. M., O. O. El-Nagouly and Zeinab S. H. Abo-Zaid (2002b).** Genetic behaviour of the compatible osmolytes free amino acids that contribute to drought tolerance in grain sorghum. Egypt. J. Plant Breed. 6 (1): 99 – 109.
15. **Steel, R.G.D., G.H. Torrie and D.A. Dickey (1997).** Principles and Procedures of Statistics: A Biometrical Approach. 3rded. McGraw-Hill, New York, USA, 450 p.
16. **Hallauer, A. R., Carena, M.J. and J. B. Miranda Filho (2010).** Quantitative Genetics in Maize Breeding. Springer Science + Business Media, LLC 2010, New York, NY 10013, USA. pp 663
17. **Singh, P., and S. S. Narayanan (2000).** Biometrical Techniques in Plant Breeding. Kalayani Publishers, New Delhi, India, 249 p.
18. **Chartzoulakisa, K.; A. Patakasb; G. Kofidisc; A. Bosabalidisc and A. Nastoub. (2012),** Water stress affects leaf anatomy, gas exchange, water relation and growth of two avocado cultivars. Scientia Horticulture 95 (1-2): 39-50.
19. **Dawood, M.G.; H. A. A. Taie, R. M. A. Nassar, M. T. Abdelhamid and U. Schmidhalter (2014).** The changes induced in the physiological, chemical and anatomical characteristics of *Vicia faba* by the exogenous application of proline under sea water level stress. South africans. of Botany, 93: 54-63.
20. **Faycal B., Abdellaouil R., Ben-Brahim N. and Neffati M. (2014).** Anatomical adaptation of *Astragalus gom biformis* Pomel. Under drought stress. Cent. Eur. J. Biol. 9 (12):1215-1225.
21. **Naz N, Rafique T, Hameed M, Ashraf M, Batool R, Fatima S. (2014).** Morpho-anatomical and physiological attributes for salt tolerance in sewan grass (*Lasiurus scindicus* Henr.) from Cholistan Desert, Pakistan. Acta Physiologiae Plantarum. 2014; 36(11):2959–74. doi:10.1007/s11738-014-1668-8.
22. **Fernando E. Prado, Mirna B. Hilal, Patricia L. Albornoz, , Miriam G. Gallardo and Verónica E. Ruiz 2017.** Anatomical and physiological responses of four quinoa cultivars to salinity at seedling stage. Indian Journal of Science and Technology, Vol 10(8), DOI: 10.17485/ijst/2017/v10i8/93709
23. **Dolatabadian, A.; S.A.M.M Sanavy and F. Ghanati. (2011).** Effects of salinity on growth, Xylem structure and anatomical characteristics of soybean. Not Sci Bio, 3(1):41-45.
24. **Nour-El-Din, Nahed M.; A.M. Ahmed and M.A. Hammouda (1999).** Ecological responses and anatomical adaptation of *Acacia* spp. Form Gebel Elba Egypt. Desert Inst. Bull., Egypt 49(2):299-319.
25. **Asish, K.P.; A. B. Das and B. Mirttra (2004).** Effect of salt on growth ion accumulation photosynthesis and leaf anatomy of the mangrove, *Bruguiera Parviflora*. J. Trees. 18(2)167-174.
26. **Borkar, M.V.; R.P. Athalya and Q. Goldin (2009)** Salinity induced changes in the leaf anatomy of the mangrove (*Avicennia marina*) along the anthropogenically stressed tropical creek. J. of coastal Devel. 14(3):191-201.
27. **Nandy P., Das S., Ghose M. (2005).** Relation of leaf micro-morphology with photosynthesis and water efflux in some Indian mangroves. Acta Botanica Croatica. 2005; 64(2):331–40.
28. **Xie S. and Luo X. (2003).** Effect of leaf position and age on anatomical structure, photosynthesis, stomatal conductance and transpiration of Asian pear. Botanical Bulletin of Academia Sinica. 2003; 44(4):297–303.
29. **Kano Y. and Fukuoka N. (2006).** Comparison of cell size and sugar accumulation in melons (*Cucumis melo* L.) grown early or late in summer. Environmental Control in Biology. 2006; 44(2):93–102. doi:10.2525/ecb.44.93.
30. **Blum, A. (1988).** Plant breeding for stress environment. CRC pres Inc Boca Raton. Florida.
31. **Hefny, M. M. (2007).** Estimation of quantitative genetic parameters for nitrogen use efficiency in maize under two nitrogen rates. Int. J. Pl. Breed. Genet., 1:54-66.
32. **Al- Naggar, A. M. M.; Shabana, R.; Mahmoud, A. A.; Abdel El-Azeem, M. E. M. and Shaboon, S. A. M. (2009).** Recurrent selection for drought tolerance improves maize productivity under low-N condition. Egypt. J. Plant Breed., 13:53-70.
33. **Al-Naggar, A. M. M., S. M. Soliman and M. N. Hashimi (2011).** Tolerance to drought at flowering stage of 28 maize hybrids and populations. Egypt. J. Plant Breed., 15(1): 69-87.

- 547 34. **Al-Naggar, A. M. M; A.M.A. Abdalla, A.M.A. Gohar and E. H. M. Hafez (2016a)**. Heritability, genetic
548 advance and correlations in 254 maize doubled haploid lines $\times$ tester crosses under drought conditions. Archives
549 of Current Research International 6(1): 1-15.
- 550 35. **Al-Naggar, A. M. M; M. M. M. Atta, M. A. Ahmed and A. S. M. Younis (2016b)**. Influence of deficit
551 irrigation at silking stage and genotype on maize (*Zea mays* L.) agronomic and yield characters. Journal of
552 Agriculture and Ecology Research International, 7(4):1-16.
- 553 36. **Al-Naggar, A. M. M. and M.T. Shehab- El- Deen (2012)**. Predicted and actual gain from selection for early
554 maturing and high yielding wheat genotypes under water stress conditions. Egypt. J. Plant Breed., 16 (3) : 73 -
555 92.
- 556 37. **Al-Naggar, A.M.M. and Atta M.M.M. (2017)**. Combining Ability and Heritability of Maize (*Zea mays* L.)
557 Morphologic Traits under Water Stress and Non-stress at Flowering Stage. Archives of Current Research
558 International 7(1): 1-16.
- 559 38. **Shabana, R.; Bailey, T. and Fery, K. J. (1980)**. Production traits of oats selected under low; medium and high
560 productivity. Crop Sci. 20:739-744.
- 561 39. **Atlin, G. N. and Frey, K.J. (1990)**. Selection of oat lines for yield low productivity environments. Crop Sci.,
562 30:556-561.
- 563 40. **Banziger, M. and H.R. Lafitte (1997)**. Efficiency of secondary traits for improving maize for low-nitrogen
564 target environments. Crop Sci., 37:1110-1117.
- 565 41. **Worku, M. (2005)**. Genetic and Crop-Physiological Basis of Nitrogen Efficiency in Tropical Maize. Ph.D.
566 Thesis. Fac. Agric. Hannover Univ. Germany, 122 p.

567