



# Drought tolerance of Five Quinoa (*Chenopodium quinoa* Willd.) Genotypes and Its Association with Other Traits under Moderate and Severe Drought Stress

A. M. M. Al-Naggar<sup>1\*</sup>, R. M. Abd El-Salam<sup>1</sup>, A. E. E. Badran<sup>2</sup>  
and Mai M. A. El-Moghazi<sup>2</sup>

<sup>1</sup>Department of Agronomy, Faculty of Agriculture, Cairo University, Egypt.

<sup>2</sup>Plant Breeding Unit, Department of Genetic Resources, Desert Research Center, Cairo, Egypt.

## 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

There are genotypic differences in drought tolerance (DT) among quinoa (*Chenopodium quinoa*) cultivars, and most of the known mechanisms of drought tolerance are encountered in this species. The objectives of the present investigation were to identify the most drought tolerant quinoa genotypes, to estimate the superiority of tolerant (T) over sensitive (S) genotypes and to identify the trait(s) of strongest association with DT. Five quinoa genotypes were evaluated in two seasons using a split plot design with five replications under three irrigation regimes, *i.e.* well watering (WW), water stress (WS) and severe water stress (SWS), achieving a field capacity of 95, 65 and 35%, respectively. The highest drought tolerance index (DTI) under WS and SWS was exhibited by

\*Corresponding author: Email: [ahmedmedhatalnaggar@gmail.com](mailto:ahmedmedhatalnaggar@gmail.com), [medhatalnaggar@gmail.com](mailto:medhatalnaggar@gmail.com);

the genotype CICA-17. Seed yield/ha (SYPH) of drought tolerant (T) genotypes was greater than the sensitive (S) ones by 31.8 and 43.6%, respectively under WS and SWS. Superiority of drought tolerant (T) over sensitive (S) quinoa genotypes in SYPH under SWS was due to its superiority in seed yield/plant and its components, water use efficiency, branches/plant, chlorophyll concentration index and leaf area. All studied inflorescence traits (inflorescence weight, diameter and length, 1000-seed weight, inflorescences/plant) were strongly inter-correlated and strongly correlated with DTI. They could be regarded as selection criteria helping plant breeder in selection programs for high drought tolerance if the heritability and genetic advance from selection for these traits are high.

**Keywords:** *Chenopodium quinoa*; drought tolerance index; seed yield components; chlorophyll concentration index; water use efficiency.

## 1. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) has recently gained worldwide attention because of its ability to grow in various stress conditions like soil salinity, acidity, drought, frost, etc. [1,2]. Quinoa has intrinsically low water requirements and therefore displays a strong natural ability to cope with drought. Quinoa is considered to have remarkable drought tolerance. Apart from this, its grain is a rich source of a wide range of minerals, vitamins, high quality oil, protein and natural antioxidants [3].

Drought tolerance may be defined as the mechanism(s) causing minimum loss of yield in a drought environment relative to the maximum yield in constraint-free *i.e.* optimal environment for the crop [4]. Some authors prefer the term 'dehydration' than 'drought' and consequently refer to dehydration tolerance [5]. Plants with better growth under limited water supply were considered to be drought-tolerant [6]. The drought tolerance of quinoa is attributed to morphological characters, such as an extensively ramified root system and presence of vesicles containing calcium oxalate that are hygroscopic in nature and reduce transpiration [7]. Physiological characters indicating drought tolerance is low osmotic potential, low turgid weight/dry weight ratio, low elasticity and an ability to maintain positive turgor even at low leaf water potentials [8].

There are genotypic differences in drought tolerance among quinoa cultivars, and most of the known mechanisms of drought tolerance are encountered in this species [2]. The adaptation capacities of quinoa are huge since we can find varieties developed from sea level up to 4,000 m above and from 40°S to 2°N of latitude [9]. It has been reported to grow with as little as 200 mm in annual precipitation in pure sand [10].

The study of correlation is regarded as an important step in breeding programs of quinoa since the information obtained is useful in estimating the correlated response to directional selection for the formulation of selection indices. Reports on association among drought tolerance and different traits in quinoa are rare; they are based on a few yield components and are based on experiments carried out in America and Europe [11]. Espinola and Gandarillas [12] reported that inflorescence length was the most important component influencing grain yield in quinoa. Risi and Galwey [13] reported strong correlation between plant height, stem diameter, inflorescence length, and inflorescence diameter with each other. Spehar and Santos [14] reported positive association of inflorescence length and diameter with grain yield, which indicated that the selection for these characters may result in more productive genotypes. A strong positive relationship between yield and grain number has also been found in quinoa [15]. Significant correlation among branches/plant, inflorescence length, and inflorescence/plant pointed out that plants with good branching habit tend to develop a large number of long inflorescences [16]. Inflorescence length was also positively associated with plant height, indicating that lines with greater plant height also developed longer panicles, a fact also reported by Rojas [17].

Growing tolerant genotypes of quinoa might be one of the cost-effective strategies for coping with growth constraints, which are significant factors affecting crop production and sustainability in numerous agricultural regions. The objectives of the present investigation were: (i) to assess drought tolerance of five quinoa genotypes in order to identify the best one(s) to be grown in Salhiya and similar newly reclaimed locations in Egypt, where the soil is sandy and suffers from soil moisture deficit, (ii) to estimate the superiority of tolerant (T) over sensitive (S) genotypes and (iii) to elucidate the relationships

between drought tolerance and agronomic and physiological traits of the quinoa germplasm in order to be considered as selection criteria in breeding programs for improving quinoa drought tolerance (DT).

## 2. MATERIALS AND METHODS

This study was carried out in the two successive winter growing seasons 2014 /2015 and 2015/2016 at New Salhiya station, Sharqiya Governorate, Egypt, where the soil is sandy. 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 obtained from Madison University, Wisconsin, USA were used in this study. The pedigree and origin of these genotypes are presented in Table 1.

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

Name	Origin	Seed color
Q-I3	Bolivia	Light yellow
Chipaya	Altiplano Salares, Bolivia	Mixed (white & Paige color)
CICA-17	Peru	Yellow
CO-407	Colrado, USA	Mixed (light yellow & white)
Ollague	Altiplano Salares, Bolivia	Yellow

### 2.2 Field Experiments

On the 19<sup>th</sup> 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/ha (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<sup>2</sup>) 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

The following three different water regimes were used:

#### 2.5.1 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<sup>3</sup> water/ha; thus, the total quantity of water given in the whole season for WW treatment was 8200 m<sup>3</sup> per ha.

#### 2.5.2 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<sup>3</sup> water/ha; thus, the total quantity of water given in the whole season for WS treatment was 5000 m<sup>3</sup> per ha.

#### 2.5.3 Severe water stress (SWS)

Where the field capacity (FC) was about 35%. Irrigation in this treatment (WW) was given each twelve days; with 10 irrigations during the whole season. The water meter recorded at the end of each irrigation about 236.8 m<sup>3</sup> water/ha; thus, the total quantity of water given in the whole season for WW treatment was 2368 m<sup>3</sup> per ha.

### 2.6 Fertilization Regimes

#### 2.6.1 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/ha and was well mixed with the soil two weeks before sowing at a depth of 10-15 cm.

### **2.6.2 Mineral fertilizers**

The following mineral fertilizers were applied: Nitrogen fertilizer at the rate of 70 kg N / ha was applied through irrigation system after 25, 50 and 75 days from sowing (46% P<sub>2</sub>O<sub>5</sub>) at the rate of 30 kg P<sub>2</sub>O<sub>5</sub>/ha was added as soil application in two equal doses, the first (15 kg P<sub>2</sub>O<sub>5</sub>/ha) before sowing during preparing the soil for planting and the second (15 kg P<sub>2</sub>O<sub>5</sub>/ha) after 25 days from sowing. Potassium fertilizer at the rate of 25 kg K<sub>2</sub>O/ha was added as soil application in two doses; before planting (15 kg K<sub>2</sub>O/ha) and after 25 day from sowing (10 kg K<sub>2</sub>O/ha) as Potassium Sulfate (48% K<sub>2</sub>O). Calcium Sulfate or Gypsum (22% Ca, 17% S) at the rate of 20 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%, Magnesium 3%) were added through irrigation system at a rate of half liter/month. Phosphoric acid (52:60% P<sub>2</sub>O<sub>5</sub>) at a rate of two Liters every 15 days was added through irrigation system when needed to open closed drippers.

### **2.7 Parameters Recorded**

1. Days to flowering (DTF) measured as the number of days from the date of emergence to the date at which about 50% of the plants in a plot showed blooming).
2. Days to maturity (DTM) measured as the number of days from the date of emergence to the date when the crop was ready for harvesting, i.e. seeds had become mature and the plant had started drying
3. Plant height (PH) in cm measured on 10 guarded plants plot<sup>-1</sup> as the average height from the ground level to the tip of the inflorescence on the main stem at the time of harvesting.
4. Leaf area (LA) in cm<sup>2</sup> measured on the 3<sup>rd</sup> leaf from the top of the plant using the leaf area meter Model Li-3100 Series No. LAM-1059, USA, when the plant was in full bloom.
5. Chlorophyll concentration index (CCI)% measured on 5 guarded plants/plot by Chlorophyll Concentration Meter, Model CCM-200, USA, as the ratio of transmission at 931 nm to 653 nm through the 3<sup>rd</sup> leaf from the top of the plant.
6. Root length (RL) in cm measured on 10 guarded plants/plot at harvest time by

lifting the plant from the sandy soil with the help of shovel and washing it with running water.

7. Primary branches/plant (BPP) measured as the total number of primary branches growing from the main stem at different node positions, including the basal branches on 5 guarded plants plot<sup>-1</sup>.
8. Inflorescences/plant (IPP) measured as number of inflorescences per plant at the time of harvest on 5 guarded plants plot<sup>-1</sup>.
9. Inflorescence diameter (ID) in cm measured as the diameter of the middle of inflorescence (maximum diameter).
10. Inflorescence length (IL) in cm measured as the mean length of three inflorescences taken randomly from different positions, from the lowest branch to the top of the inflorescence
11. Inflorescence weight (IW) in g measured as the weight of inflorescence from the lowest branch to the top of the inflorescence.
12. Seeds/plant (SPP) measured as number of seeds/plant on 5 guarded plants plot<sup>-1</sup> by multiplying number of inflorescences per plant x number of seeds per inflorescence.
13. Thousand seed weight (TSW) in g: Five samples of 1000 seeds from the bulked seed of each genotype were weighed and averaged.
14. Seed yield/plant (SYPP) in g measured as weight of seeds per plant on 10 guarded plants/plot.
15. Seed yield/ha (SYPH) in kg estimated by converting seed yield per plot to seed yield per hectare (ha).
16. Water use efficiency (WUE) in kg seed/1 m<sup>3</sup> water: This was calculated by the following formula:  $WUE = (\text{Seed yield/ha in kg}) / (\text{quantity of irrigation water/ha in m}^3 \text{ given during the whole season})$ .

### **2.8 Drought Tolerance Index (DTI)**

It is the factor used to differentiate between the genotypes from tolerance point of view and it is calculated by the equation of Fageria [18] as follows:

$$DTI = (Y1/AY1) \times (Y2/AY2)$$

Where, Y1 = trait mean of a genotype at well watering. AY1 = average trait of all genotypes at well watering. Y2 = trait mean of a genotype at water stress. AY2 = average trait of all genotypes at water stress. When DTI is  $\geq 1$ , it indicates that

genotype is tolerant (T) to drought. If DTI is  $<1$ , it indicates that genotype is sensitive (S) to drought.

## 2.9 Biometrical and Genetic Analyses

Analysis of variance of the split-split plot design in RCB arrangement 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, combined analysis for each environment separately across seasons 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. [19]. Simple correlation coefficients were calculated between pairs of studied traits under well watering (WW), water stress (WS), severe water stress (SWS) and combined across all irrigation treatments according to Singh and Narayanan [20].

## 3. RESULTS AND DISCUSSION

### 3.1 Analysis of Variance

Combined analysis of variance across two growing seasons (S) of the split-plot design for the studied morphological, physiological and yield traits of five genotypes (G) of quinoa under three irrigation regimes (T) is presented in Table 2.

Mean squares due to seasons were significant ( $P \leq 0.05$  or  $0.01$ ) for all studied traits, except for days to flowering (DTF), days to maturity (DTM), branches/plant (BPP), inflorescence diameter (ID), inflorescence weight (IW), seed yield/ha (SYPH) and water use efficiency (WUE), indicating significant effect of climatic conditions on nine out of 16 studied traits of quinoa.

Mean squares due to irrigation regimes (T) and quinoa genotypes (G) were significant ( $P \leq 0.05$  or  $0.01$ ) for all studied traits, indicating that irrigation regime and genotype had significant effects on all studied traits. Significant differences among studied quinoa genotypes suggest that improvement of these traits is possible only *via* breeding programs.

Mean squares due to the 1<sup>st</sup> order interaction, *i.e.* T×S, G×S and G×T were significant ( $P \leq 0.05$  or

$0.01$ ) for all studied traits, except for root length (RL), ID and 1000-seed weight (TSW) for T×S and days to maturity (DTM) and branches/plant (BPP) for G×S (Table 2). Significance of G×T indicates that genotype's rank differed from one irrigation regime to another and selection would be efficient for all studied traits under a specific water stress environment, as previously reported by several investigators [21-27].

Mean squares due to the 2<sup>nd</sup> order interaction, *i.e.* G×S×T were significant ( $P \leq 0.05$  or  $0.01$ ) for all studied traits, except for RL, inflorescence length (IL), SYPP and SYPH, indicating that quinoa genotype's performance differed from a combination of treatment and season to another combination for most studied traits.

It is observed from Table 2 that variance due to irrigation treatments was the largest contributor to the total variance in this experiment for all studied traits. Comparing irrigation with season effect, it is clear that irrigation variance showed larger contribution to total variance than season variance for all studied traits, indicating that water stress had more effect than season effect on such traits.

Combined analysis of variance (data not presented) of randomized complete blocks design for studied traits of five quinoa genotypes under three environments (WW, WS and SWS); representing well watering (95% FC), water stress (65% FC) and severe water stress (35% FC) indicated that mean squares due to genotypes, were significant ( $P \leq 0.01$ ) for all studied traits, suggesting the significance of differences among studied quinoa genotypes for all studied traits under all water stress environments and selection would be efficient under all studied environments.

Mean squares due to the interaction genotype × season (G × S) were significant ( $P \leq 0.05$  or  $0.01$ ) for all studied traits under all environments, except RL and WUE under WW, DTF, RL, BPP, IL, SYPP, SYPH and WUE under WS and ID and IL under SWS environment. It is observed that genotypes are the largest contributor to total variance for all studied traits in all environments, except chlorophyll concentration index (CCI) under WW, plant height (PH) under WS and LA, CCI and CCI under SWS, where seasons were the largest contributor and SPP under SWS, where G×S interaction variance was the largest contributor to total variance.

**Table 2. Combined analysis of variance of split plot for studied traits of quinoa genotypes under three irrigation regimes (treatments) across two seasons**

SOV	df	Mean squares							
		Days to 50% flowering	Days to 50% maturity	Plant height	Leaf area	Chlorophyll-concent. index	Root length	Branches /Plant	Inflorescence /plant
Season (S)	1	0.06	0.027	195.4**	68.6**	221.0**	0.5*	0.5	3.53**
R(S)	8	0.76	0.16	5.6	0.1	7.1	0.2	0.4	2.66
Treatment (T)	2	130.21**	777.31**	18739.4**	319.6**	4659.2**	164.4**	619.6**	781.82**
T x S	2	0.78**	0.83*	421.9**	33.1**	305.8**	0.0	1.0*	3.21**
Error (a)	16	0.35	0.45	7.3	0.2	6.2	0.4	0.4	1.08
Genotype (G)	4	31.44**	63.24**	125.6**	39.8**	354.2**	85.4**	174.6*	54.21**
G x S	4	3.24**	0.677	32.8**	13.9**	53.2**	0.6*	0.8	6.31**
G x T	8	8.77**	25.99**	118.4**	13.7**	91.4**	110.2**	42.5**	55.77**
G x S x T	8	1.46**	1.75**	125.6**	7.2**	16.4**	0.5	1.5**	4.24**
Error (b)	96	0.6	0.72	3.3	0.3	4.4	0.3	0.5	1.46
		Inflorescence diameter	Inflorescence length	Inflorescence weight	Seeds /plant	1000-seed weight	Seed yield/plant	Seed yield/ha	Water use efficiency
Season (s)	1	0.3	0.4*	0.0001	137350*	0.54*	0.91*	5.1	98.64
R(S)	8	0.3	0.2	0.019	102274	0.51	0.37	369.3	104.4
Treatment (T)	2	752.5**	381.2**	12.86**	9833577**	18.54**	1200.6**	789450**	1809739*
T x S	2	0.1	0.3*	0.24**	2055197**	0.350	0.66*	411.4*	285.4**
Error a	16	0.4	0.1	0.024	97066	0.353	0.44	179.1	76.55
Genotype (G)	4	202.0**	109.8**	4.3**	1401183**	2.28**	199.8**	194892**	100965**
G x S	4	1.8**	0.6*	0.36**	1774849**	0.60**	1.19**	585.1**	258.5**
G x T	8	12.4**	11.2**	1.57**	1168931**	3.65**	111.96**	75591.5**	65680.8**
G x S x T	8	1.01**	0.2	0.18**	1414597**	0.45**	0.39	145.2	184.8**
Error b	96	0.4	0.3	0.024	109826	0.23	0.33	124.3	53.87

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

### 3.2 Drought Tolerance of Quinoa Genotypes

Drought tolerance index (DTI) values of each studied quinoa genotype estimated using the equation suggested by Fageria [18] and the reductions in seed yield/ha from WW to WS and SWS as another parameter of drought tolerance, are presented in Table 3. According to our scale, when DTI is  $\geq 1.0$ , it indicates that genotype is tolerant (T), If DTI is  $< 1$ , it indicates that genotype is sensitive (S). For relative reduction (Red%) in grain yield due to water stress, the smaller the Red% value the higher the drought tolerance of the genotype and *vice versa*.

**Table 3. Drought tolerance index (DTI) and reduction (Red%) in SYPH from WW to WS and SWS for each genotype under WS (65% FC) and SWS (35% FC) conditions**

Genotype	DTI		Red%	
	WS	SWS	WS	SWS
QL-3	1.12 (T)	0.72 (S)	14.0	55.9
Chipaya	1.02 (T)	1.08 (T)	13.8	27.2
CICA-17	1.14 (T)	1.32 (T)	4.7	12.2
CO-407	1.07 (T)	1.16 (T)	9.9	21.8
Ollague	0.69 (S)	0.73 (S)	9.3	23.3

T=Tolerant, S= Sensitive

Based on DTI estimates, the genotype CICA-17 followed by CO-407 and Chipaya could be considered drought tolerant under both stressed environments (WS and SWS), and QL-3 could be considered drought tolerant under WS only (65% FC). The highest value of DTI was shown by CICA-17 under both environments, *i.e.* it is the best drought tolerant quinoa genotype in this experiment. On the contrary, the genotypes Ollague under WS and SWS and QL-3 under SWS were considered drought sensitive according to DTI estimates.

Based on the reduction (Red%) value in seed yield/ha from WW to WS and SWS, data in Table 3 assured that CICA-17 genotype was the most tolerant one in this experiment, since it showed the lowest reduction (4.7 and 12.2%) under WS and SWS, respectively. In this respect, the genotype CO-407 came in the second place for drought tolerance after CICA-17. On the contrary, based on reduction parameter, the genotype QL-3 exhibited the largest reduction, and therefore was considered sensitive to drought at WS and SWS. According to the

mentioned results, Quinoa CICA-17 variety could be recommended to be grown in Salhiya and similar newly reclaimed locations in Egypt, where the soil is sandy and suffers from soil moisture deficit.

Quinoa is considered to have remarkable drought tolerance. It has been reported to grow with as little as 200 mm in annual precipitation in pure sand [10]. Yields exceeding 1000 kg ha<sup>-1</sup> have been reported with as little as 50 mm irrigation in the Atacama Desert of Northern Chile. However, yields are much improved in arid regions under irrigation [28]. Quinoa also exhibits remarkable agronomic characteristics. It is highly tolerant to drought and soil salinity. Many studies have been conducted to investigate the responses of quinoa to drought or fertilization separately, but little is known about the response of quinoa to nitrogen application under drought conditions.

Quinoa (*Chenopodium quinoa* Willd.) is a traditional Andean seed crop increasingly attracting attention because of its high yield potential under adverse soil and climate conditions such as drought [29]. Quinoa appears to employ a wide variety of drought resistance mechanisms; these include drought escape, tolerance and avoidance. 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 [2,30]. The accumulation of both inorganic (Na<sup>+</sup>,K<sup>+</sup>and Cl) and organic (soluble sugars and proline) osmolytes has been found in quinoa under drought and saline conditions [31,32].

Additionally, quinoa can avoid the negative 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 [30,33,34] and having vesicular glands, small and thick-walled cells. In our previous studies quinoa started to close its stomata when leaf water potential dropped below 1.2 MPa. Such reduced sensitivity in quinoa's stomatal response was found to be one of the special drought tolerant characteristics of this species [30]. However, rather contrasting results were found in follow-up studies which showed that quinoa closed its stomata to maintain leaf water potential and photosynthetic rate and hereby increase photosynthetic water use efficiency when exposed to water deficit [35].

This controversy may be attributed to both genetic variability of quinoa accessions used in these studies, as well as difference in kinetics of drought stress development and experimental conditions. In the latter work, it was found that quinoa was able to increase a photosynthetic water use efficiency by 50% with soil drying to FTSW = 0.7– 0.4 [35]. ABA concentration in the xylem sap was found to increase slightly with mild drought, indicating that ABA regulation on stomatal conductance is one of the mechanisms utilized by quinoa under drought [35], later confirmed by Razzaghi et al. [29]. However, more studies are required on chemical signalling pathway of drought tolerance in quinoa, and the role of ABA in relation to quinoa's drought tolerance. 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.

### 3.3 Superiority of Tolerant (T) Over Sensitive Quinoa Genotype

To describe the differences between tolerant (T) and sensitive (S) quinoa genotype, data of the selected characters were averaged for the two groups of genotypes differing in their drought tolerance (both WS and SWS), as well as in grain yield/plant under WS and SWS (Table 4). Based on DTI, the most tolerant genotype under WS and SWS was CICA-17 and the most sensitive (S) genotype was Ollague (Table 3).

Results averaged for each of the T and S of quinoa genotypes differing in tolerance to WS and SWS indicate that seed yield/ha of tolerant

(T) was greater than that of the sensitive (S) genotype by 25.5, 31.8 and 43.6%, respectively under well watering (WW), water stress (WS) and severe water stress (SWS) conditions, respectively. The superiority of T (CICA-17) to S (Ollague) in seed yield/ha and seed yield/ha was increased by increasing water stress, i.e. by decreasing soil moisture field capacity (FC) from 95% to 65 and 35%.

Superiority of drought tolerant (T) over sensitive (S) quinoa genotypes in SYPH under SWS was due to its superiority in SYPP (46.6%), WUE (43.6%), TWS (44.0%), IL (44.1%), ID (57.1%), IPP (146.2%), BPP (121.4%), CCI (30.6%) and LA (31.4%). Likewise, under moderate water stress, the tolerant genotype showed superiority over S genotype in SYPP (11.0%), TWS (17.7%), IL (17.1%), ID (24.6%), BPP (112.2%), RL (38.1%), CCI (16.9%), LA (14.2%) and WUE (31.8%). Superiority of T over S quinoa genotypes in SYPH under well watering (WW) was due to their superiority in SYPP (90.83%), TWS (52.0%), IL (12.7%), ID (36.7%), IPP (10.4%), BPP (9.9%), RL (11.4%), CCI (12.0%), LA (7.0%) and WUE (25.4%). The superiority of quinoa tolerant genotypes to drought was also attributed to high estimates of inflorescence parameters [12,13], long roots [29,30,33], high chlorophyll concentration [36] and large leaf area [16,29,30,33,34] and high water use efficiency [35].

### 3.4 Trait Associations

Estimates of phenotypic correlation coefficients between each of SYPP and water use efficiency (WUE) and other studied traits across the two

**Table 4. Superiority (%) of the most tolerant (T) (CICA-17) over the most sensitive (S) quinoa genotype (Ollague) for selected traits under well watering (WW), water stress (WS) and severe water stress (SWS)**

Trait	WW (95% FC)			WS (65% FC)			SWS (35% FC)		
	T	S	Super.%	T	S	Super.%	T	S	Super. %
LA	18.4	17.2	7.0**	17.7	15.5	14.2**	15.5	11.8	31.4**
CCI	54.3	48.5	12.0**	54.7	46.8	16.9**	39.3	30.1	30.6**
RL	16.6	14.9	11.4**	22.1	16.0	38.1**	24.0	26.6	-9.8**
BPP	20.0	18.2	9.9**	17.4	8.2	112.2**	15.5	7.0	121.4**
IPP	17.0	15.4	10.4*	14.3	14.6	-2.1	12.8	5.2	146.2**
ID	23.8	19.1	24.6**	23.1	16.9	36.7**	18.7	11.9	57.1**
IL	19.9	17.0	17.1**	17.8	15.8	12.7**	16.0	11.1	44.1**
TWS	4.0	3.4	17.7*	3.8	2.5	52.0**	3.6	2.5	44.0**
SYPP	33.3	30.0	11.0**	31.4	23.9	31.4**	29.9	20.4	46.6**
SYPH	901.7	718.4	25.5**	858.9	651.7	31.8**	791.4	551.1	43.6**
WUE	262.1	209.0	25.4**	409.0	310.4	31.8**	795.3	553.8	43.6**

Superiority =  $100 \times [(T - S)/S]$ , \* and \*\* significant at 0.05 and 0.01 probability levels



seasons under WW, WS and SWS and combined across environments were calculated across all quinoa genotypes and presented in Table 5. Seed yield/plant of quinoa genotypes showed perfect positive phenotypic association with seed yield/ha ( $r= 0.85, 0.98, 0.99$  and  $0.96$ ) under WW, WS and SWS, respectively; that is why the estimates of correlation coefficients between SYPP and other traits would be very close to those between SYPH and the same traits.

**Table 5. Correlation coefficients between seed yield/plant (SYPP) and other studied traits under well watering (WW), water stress (WS), severe water stress (SWS) and combined across environments and across two seasons**

Trait	WW	WS	SWS	Combined
DTF	0.47**	0.31*	0.61**	0.58**
DTM	0.36*	0.58**	-0.18	0.63**
PH	0.26	-0.01	0.59**	0.03
LA	0.39**	0.21	0.71**	0.64**
CCI	0.15	0.38**	0.64**	0.67**
RL	0.82**	0.83**	0.38**	0.60**
BPP	0.28*	0.93**	0.79**	0.91**
IPP	0.13	-0.01	0.86**	0.75**
ID	0.14	0.77**	0.88**	0.91**
IL	0.13	0.36*	0.83**	0.81**
IW	0.81**	0.86**	0.96**	0.94**
TSW	0.81**	0.86**	0.96**	0.94**
SYPH	0.85**	0.98**	0.99**	0.96**

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

Combined across all irrigation treatments, seed yield per plant of quinoa genotypes showed very strong and positive phenotypic association with 1000-seed weight, inflorescence weight, inflorescence diameter, inflorescence length and branches/plant and above average associations with IPP, RL, CCI, LA, DTM and DTF. It is observed that SYPP showed the strongest correlation with TSW and IW ( $r=0.94$ ) followed by ID and BPP ( $r=0.91$ ), IL ( $r= 0.81$ ) and IPP ( $r= 0.75$ ) in combined analysis of correlation across all environments.

Under severe water stress (35% FC), SYPP exhibited significant ( $p\leq 0.05$  or  $0.01$ ) and positive correlation coefficients with 11 traits (TSW, IW, IL, ID, IPP, BPP, RL, CCI, LA, PH and DTF); the strongest ones were with TSW, IW, IL, ID, IPP, BPP, CCI, LA and DTF. Under water stress treatment (65% FC), SYPP showed significant ( $p\leq 0.05$  or  $0.01$ ) and positive

correlation coefficients with 9 traits (TSW, IW, IL, ID, BPP, RL, CCI, DTM and DTF); the strongest of them were TSW, IW, ID, BPP and RL. Under well watering treatment (95% FC), SYPP showed significant ( $p\leq 0.05$  or  $0.01$ ) and positive, but mostly weak correlation coefficients with 7 traits (TSW, IW, BPP, LA, RL, DTM and DTF); the strongest of them were TSW, IW and RL.

From the abovementioned results, it could be concluded that the seven traits TSW, IW, ID, BPP, RL, IL and IPP were strongly correlated with SYPP under each irrigation treatment and across treatments. These traits could help plant breeder for selection of high seed yielding genotypes of quinoa if heritability of the trait(s) is high.

Combined across all irrigation treatments, water use efficiency (WUE) of quinoa genotypes also showed very strong and positive phenotypic association with TSW, IW, ID, IL and BPP and above average associations with IPP, RL, CCI, LA and DTF (Table 6). It is also observed that WUE showed the strongest correlation with TSW and IW followed by ID, BPP, IL and IPP in combined analysis of correlation across all environments.

Under severe water stress (35% FC), WUE exhibited significant ( $p\leq 0.05$  or  $0.01$ ) and positive correlation coefficients with 11 traits (TSW, IW, IL, ID, IPP, BPP, RL, CCI, LA, PH and DTF); the strongest ones were with TSW, IW, IL, ID, IPP, BPP, CCI, LA and DTF. Under water stress treatment (65% FC), WUE showed significant ( $p\leq 0.05$  or  $0.01$ ) and positive correlation coefficients with 9 traits (TSW, IW, IL, ID, BPP, RL, CCI, DTM and DTF); the strongest of them were TSW, IW, ID, BPP and RL. Under well watering treatment (95% FC), WUE showed significant ( $p\leq 0.05$  or  $0.01$ ) and positive, but mostly weak correlation coefficients with 8 traits (TSW, IW, IL, ID, LA, RL, DTM and DTF); the strongest of them were TSW, IW, RL and DTM.

From the abovementioned results, it could be concluded that the seven traits TSW, IW, ID, BPP, RL, IL and IPP were strongly correlated with WUE as well as SYPP under each irrigation treatment and across treatments. These traits could help plant breeder for selection of high seed yielding and water use efficient genotypes of quinoa if the heritability of it is high.

Drought tolerance index (DTI) of quinoa genotypes showed perfect ( $r>0.90$ ), significant

**Table 6. Correlation coefficients between each of water use efficiency (WUE) and drought tolerance index (DTI) and other studied traits under well watering (WW), water stress (WS), severe water stress (SWS) and combined across environments and across two seasons**

Trait	WS	SWS	WW	WS	SWS	Combined
	DTI		WUE			
DTF	0.50**	0.71**	0.53**	0.37**	0.63**	0.53**
DTM	0.56**	0.01	0.74**	0.60**	-0.2	0.48**
PH	-0.12	0.48**	-0.06	-0.04	0.56**	0.02
LA	0.09	0.67**	0.35*	0.15	0.70**	0.68**
CCI	0.31*	0.70**	0.23	0.40**	0.62**	0.68**
RL	0.79**	0.44**	0.72**	0.82**	0.33*	0.38**
BPP	0.88**	0.91**	-0.03	0.92**	0.80**	0.80**
IPP	0.13	0.90**	0.19	0.03	0.88**	0.79**
ID	0.70**	0.93**	0.48**	0.77**	0.87**	0.93**
IL	0.13	0.93**	0.43**	0.29*	0.84**	0.87**
IW	0.75**	0.91**	0.73**	0.83**	0.96**	0.91**
TSW	0.75**	0.91**	0.73**	0.83**	0.96**	0.91**
SYPH	0.93**	0.93**	1.00**	1.00**	1.00**	0.94**
SYPP	0.98**	0.95**	0.85**	0.98**	0.99**	0.96**
WUE	0.98**	0.95**				

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

and positive correlations (Table 6) with WUE, SYPP and SYPH under both stresses (WS and SWS). Under severe water stress (35% FC), DTI showed above average significant and positive correlation ( $r = \geq 0.67$ ) with TSW, IW, IL, ID, IPP and BPP and below average significant and positive correlation ( $r = \geq 0.44$ ) with RL and PH. Under water stress (65% FC), DTI, exhibited above average significant and positive correlation ( $r = \geq 0.56$ ) with TSW, IW, ID, BPP, RL, DTM, DTF and below average significant and positive correlation ( $r = \geq 0.31$ ) with CCI.

Correlation analysis across all irrigation treatments and seasons showed that significant ( $p \leq 0.5$  or  $p \leq 0.01$ ) correlation coefficients existed among all studied traits, except between PH and all other traits (except DTF and CCI),

between LA and each of DTM, CCI and RL (Table 7). The strongest association was found between IW vs TSW (1.00), ID vs IL (0.93), ID vs IW (0.89), ID vs TSW (0.88), IW vs BPP (0.88), TSW vs BPP (0.87), BPP vs ID (0.86), IL vs IPP, IL vs CCI, IL vs IW, RL vs BPP and IL vs TSW (0.79).

Many previous studies reached to similar conclusions of our study on the correlations in quinoa. Espinola and Gandarillas [12] reported that inflorescence length was the most important component influencing grain yield in quinoa. Risi and Galwey [13] reported strong correlation between plant height, stem diameter, inflorescence length, and inflorescence diameter with each other. Spehar and Santos [14] reported positive association of inflorescence length

**Table 7. Correlation coefficients for pairs of selected traits combined across all irrigation treatments and seasons**

	DTM	PH	LA	CCI	RL	BPP	IPP	ID	IL	IW	TSW
DTF	0.52**	-0.33*	0.50**	0.16	0.45**	0.50**	0.51**	0.46**	0.23	0.45**	0.45**
DTM		0.04	0.21	0.51**	0.80**	0.78**	0.52**	0.62**	0.45**	0.63**	0.63**
PH			-0.06	0.36*	0.15	0.22	0.15	0.12	0.26	0.05	0.05
LA				0.27	0.26	0.53**	0.68**	0.59**	0.52**	0.49**	0.49**
CCI					0.39**	0.70**	0.62**	0.77**	0.83**	0.64**	0.64**
RL						0.79**	0.33*	0.46**	0.28*	0.57**	0.57**
BPP							0.72**	0.86**	0.76**	0.88**	0.87**
IPP								0.82**	0.81**	0.71**	0.71**
ID									0.93**	0.89**	0.88**
IL										0.79**	0.79**
IW											1.00**

and diameter with grain yield, which indicated that the selection for these characters may result in more productive genotypes. Bhargava et al. [16] reported significant correlation among branches/plant, inflorescence length, and inflorescence/plant pointed out that plants with good branching habit tend to develop a large number of long inflorescences. Inflorescence length was also positively associated with plant height indicating that lines with greater plant height also developed longer panicles, a fact also reported by Rojas [17] and Ochoa and Peralta [37]. The path analysis revealed that 1000 seed weight had highest positive direct relationship with seed yield, followed by total chlorophyll and branches/plant [16]. Fuentes and Bhargava [36] noticed high correlation between stem diameter and plant weight, stem diameter and plant height, plant weight and plant height, plant weight and inflorescence length, plant height and inflorescence length, and leaf length and leaf width.

The results of the present study indicated that all studied inflorescence traits (IW, ID, IL, TSW, IPP, BPP) were strongly inter-correlated (Table 7) and strongly correlated with SYPP, SYPH, WUE and DTI. They could be regarded as selection criteria helping plant breeder in selection programs for high seed yield, high drought tolerance and high water use efficiency if the heritability and genetic advance from selection are high.

#### 4. CONCLUSIONS

The present investigation identified the highest drought tolerant genotypes under elevated water stress which could be offered to future breeding programs to improve quinoa drought tolerance (DT); they were the cultivar CICA-17 followed by CO-407. It was concluded that tolerant (T) quinoa genotypes produced much higher seed yield/ha than sensitive ones under moderate and severe water stress conditions. The tolerant genotypes of quinoa possessed high values of adaptive traits to drought tolerance, namely seed yield components (inflorescence weight, diameter and length, 1000-seed weight, inflorescences/plant), water use efficiency, number of branches/plant, and chlorophyll concentration index. These traits could be considered as selection criteria helping plant breeder in selection programs for high drought tolerance if the heritability and genetic advance from selection for these traits are high. Further studies should be carried out on more genotypes of quinoa to ascertain the role of

these traits in drought tolerance and on the type of gene action controlling the inheritance of these traits.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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