

HERITABILITY AND GENETIC ADVANCE FROM SELECTION FOR MORPHOLOGICAL, BIOCHEMICAL AND ANATOMICAL TRAITS OF *Chenopodium quinoa* UNDER WATER STRESS

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ABSTRACT

The present investigation aimed at estimating heritability in broad sense (h^2_b) and genetic advance (GA) from selection for agronomic, physiological, leaf amino acids and anatomical traits of quinoa (*Chenopodium quinoa* Willd.) under elevated water stress. A two-year experiment was conducted in a split plot experiment with five replications. The main plots were devoted to three irrigation regimes, *i.e.* well irrigation (WI), moderate irrigation stress (MIS) and severe irrigation stress (SIS), achieving a field capacity of 95, 65 and 35%, respectively, and sub plots to five quinoa genotypes. For agronomic and physiological traits, h^2_b ranged from 0.0% for inflorescences/plant (IPP), 1000-seed/plant (TSW) and seeds/plant (SPP) under WI, plant height (PH) and IPP under MIS to > 97.0% for root length, inflorescence length and seed yield/plant under all irrigation treatments. For amino acids, h^2_b ranged from 78.01% (Serine) to 99.95% (Proline) under WI, from 73.66% (Tyrosine) to 100.0% (Valine) under MIS and from 93.69% (Asparagine) to 99.98% (Proline) under SIS. For anatomical traits, h^2_b ranged from 33.33% (lower epidermis) to 100% (upper epidermis) under SIS. GA for agronomic and physiological traits generally increased as water stress increased and ranged from 0% (IPP, TSW, SPP) to 26.04% (inflorescence diameter) under WI, from 0% (PH, IPP) to 58.27% (branches/plant; BPP) under MIS and from 0% (SPP) to 101.87% (IPP, BPP) under SIS. For amino acids, GA ranged from 12.26% (Glutathione) to 26.00% (Leucine) under WI, from 16.94% (Lysine) to 25.56% (Threonine) under MIS and from 16.03% (Alanine) to 87.79% (Methionine) under SIS. For anatomical traits, GA ranged from 30.40% (leaf thickness) to 87.12% (spongy layer) under WI, from 52.66% (leaf thickness) to 82.72% (palisade layer) under MIS and from 15.40% (upper epidermis) to 72.97% (palisade layer) under SIS. Results indicated that the best selection environment was SIS for all studied traits, except for upper epidermis and spongy layer, which was WI.

Keywords: Quinoa, heritability, selection gain, amino acids, leaf anatomy, inflorescence.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) can be termed 'underutilized', particularly in Egypt, since in spite of its

wide adaptability, rusticity and nutritional superiority, its commercial potential has remained untapped. Quinoa is a dicotyledonous annual species belonging to the family

Amaranthaceae (formerly *Chenopodiaceae*). Quinoa is a highly nutritious Andean pseudo cereal crop that has been a staple food for over 5000 years for the Inca Empire and among pre-Columbian Andean farming communities in South America (Planella *et al.*, 2015). Quinoa has an exceptional balance between oil (4–9%), protein (averaging 16%, with high nutritional relevance due to the ideal balance of its essential amino acid content) and carbohydrates (64%) (Vega-Galvez *et al.*, 2010). In addition, quinoa is a good source of vitamins, oil with high linoleate and linolenate content (55–66% of the lipid fraction), natural antioxidants and a wide range of minerals (Vega-Galvez *et al.*, 2010; Fuentes and Bhargava, 2011). Moreover, quinoa has remarkable productive advantages of cultivation under adverse environmental conditions (Ward 2000; Jacobsen *et al.*, 2003; Fuentes and Bhargava, 2011), resulting in a very good alternative for marginal environments and low-input agriculture. Efforts to introduce quinoa as an alternative crop have been made in numerous countries, and successful adaptation of this species has been reported in Europe, North America, Africa and India (Jacobsen, 2003 and Fuentes *et al.*, 2009b). Quinoa was formally put in field trials in the Sinai Peninsula with 13 varieties and strains being tested in deserts of South Sinai governorate (near Nuwaiba city) which proved to be a success (Shams, 2011).

International interest in quinoa began to rise in the late 1970s and 1980s when the first breeding programs outside of South America were begun. In Europe, programs were established in the UK, Denmark, and the

Netherlands (Jacobsen, 2003). In North America, efforts were begun in 1983 to grow quinoa in high altitude locations of Colorado through a partnership between Colorado State University and Sierra Blanca Associates (Johnson, 1990).

Quinoa can grow under extremely dry conditions (Sun *et al.*, 2014; Walters *et al.*, 2016), including drought prone areas of Africa (Jacobsen *et al.*, 2003). In arid and semiarid agroecosystems, drought and salinity are the main abiotic stresses damaging the potential yield and causing yield instability in quinoa (Pulvento *et al.*, 2010, Fuentes and Bhargava, 2011, Razzaghi *et al.*, 2011 a, b). 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 and 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 greater production of carbohydrates (Xie and Luo, 2003).

Many plants including halophytes, accumulate compatible osmolytes, such as amino acids, sugar alcohols (e.g. pinitol), other sugars (e.g. fructans) and quaternary ammonium compounds (e.g. glycine betaine) when they are exposed to drought or salinity stress (Delauney and Verma, 1993). It has been suggested that compatible osmolytes do not interfere with normal biochemical reactions and act as osmoprotectants during osmotic process. Cytoplasmic osmolyte accumulation is believed to reduce cellular water potential below the external water potential, while avoiding deleteriously high ionic strength (Hare and Cress, 1997), which enables water to move into the cell and can be maintained there. Maintenance of turgor pressure is essential for continued growth. While several amino acids are known to accumulate in response to osmotic stress, proline apparently has a specific protective role in the adoption of plant cells to water deprivation (Handa *et al.*, 1986) and appears to be preferred organic osmoticum in many plants.

The estimation of heritability, genetic and gains from selection is important for genetic improvement programs because these estimates facilitate the choice of methods and characters used in the initial and advanced phases of improvement programs, thereby allowing the study of mechanisms, genetic values and variability for one character (Cruz *et al.*, 2012 and Vasconcelos *et al.*, 2012). Heritability is a very useful parameter for breeders because it allows one to predict the possibility of

success with the selection, as it reflects the proportion of phenotypic variation that can be inherited; in other words, the heritability coefficient measures the reliability of the phenotypic value as an indicator of genotypic value (Vasconcelos *et al.*, 2012). Heritability estimates facilitate the choice of methods and characters used in the initial and advanced phases of improvement programs, thereby allowing the study of mechanisms, genetic values and variability for one character (Cruz *et al.*, 2012 and Vasconcelos *et al.*, 2012). The variability among cultivars reflects the heterogeneity of the genetic material improves food security threatened by climate change and offers the possibility of identifying promising material for use in a plant breeding program (Ruiz *et al.*, 2014). It is also emphasized in different plant species (Yazici and Bilir, 2017 and Dutkuner *et al.*, 2008). The estimations of high coefficients of heritability are associated with a greater genetic variability, greater selective accuracy (Cargnelutti Filho *et al.*, 2009) and greater possibilities for success in selecting genotypes with higher productivity of grain. Al-Naggar *et al.* (2002 a, b, c, d) estimated heritability and expected selection gain for free amino acids that contribute to drought tolerance in grain sorghum. They found that maximum heritability estimate in the narrow sense was obtained for methionine (24%) under water stress and valine (36.9%) under control. Maximum predicted genetic advance from selection was obtained under water stress conditions for lysine (41.0%), followed by proline (40.3%) and methionine (39.7%). De Santis *et al.* (2016) stated that heritability estimates in quinoa were relatively high for almost all of the traits considered.

The knowledge gained by exploring estimates of heritability and selection gain could be used in breeding program aimed at developing more suitable quinoa varieties

for adverse conditions, as well as potentially extrapolated to breeding other crops for drought tolerance. Information on heritability and genetic advance from selection leaf anatomy and amino acids of quinoa in response to imposition of water stress are generally limited. The objectives of the present investigation were (i) to estimate the heritability and genetic advance from selection for 14 agronomic and physiological traits, 16 leaf free amino acids and 5 leaf anatomical traits of quinoa under elevated water stress levels and (ii) to identify the selection environment for selecting drought tolerant genotypes.

MATERIALS AND METHODS

Plant Materials

Seeds of five quinoa (*Chenopodium quinoa* Willd.) genotypes were obtained from Madison University, Wisconsin, USA. 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-13 | Bolivia | Light yellow |
| Chipaya | AltiplanoSalares, Bolivia | Mixed (white & Paige color) |
| CICA-17 | Peru | Yellow |
| CO-407 | Colorado, USA | Mixed (light yellow & white) |
| Ollague | AltiplanoSalares, Bolivia | Yellow |

Field experiments

The field experiment was carried out in the two successive growing seasons 2014 /2015 and 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 m above sea level. On November 19, 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 m²) with a 1.0 m ally between irrigation treatments.

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 irrigation (WI), moderate irrigation stress (MIS) and severe irrigation stress (SIS). 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 irrigation (WI)**, where the field capacity (FC) was about 95%. Irrigation in this treatment (WI) 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 WI treatment was 8200 m³ ha⁻¹.

2. **Moderate irrigation stress (MIS)**, where the field capacity (FC) was about 65%. Irrigation in this treatment (MIS) 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 MIS treatment was 5000 m³ ha⁻¹.
3. **Severe irrigation stress (SIS)**, where the field capacity (FC) was about 35%. Irrigation in this treatment (SIS) 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 m³ water/ha; thus, the total quantity of water given in the whole season for SIS treatment was 2368 m³ ha⁻¹.

Fertilization Regimes

First: Organic fertilizer

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

Second: Mineral fertilizers

Nitrogen fertilizer at the rate of 165 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% P₂O₅) at the rate of 70 kg P₂O₅/ha was added as soil application in two equals doses, the first (35 kg P₂O₅/ha) before sowing during preparing the soil for planting and the second (35 kg P₂O₅/ha) after 25 days from sowing. Potassium fertilizer at the rate of 60 kg K₂O/ha was added as soil

application in two doses; before planting (35 kg K₂O/ha) and after 25 day from sowing (25 kg K₂O/ha) as Potassium Sulfate (48% K₂O). 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%, 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 mEq/l were Ca (2.45), Mg (5.8), Na (8.5), K (6.8). Soluble anions of soil in mEq/l were Cl (5.3), CO₃ (0.0), SO₄ (2.39). Irrigation water EC was 0.67 dSm⁻¹. Soluble cations of water in mEq/l were Ca (1.4), Mg (0.4), Na (4.9), K (0.3). Soluble anions of water in mEq/l were Cl (3.0), CO₃ (0.0), SO₄ (0.0).

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⁻¹ 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 cm²** measured on the 3rd 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 3rd 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⁻¹.
8. **Inflorescences/ plant (IPP)** measured as number of inflorescences per plant at the time of harvest on 5 guarded plants plot⁻¹.
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⁻¹ 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.

Leaf free amino acids determination

In 2015/2016 growing season, samples were taken from three replication of each irrigation treatment from the mature leaves of five quinoa genotypes at age of 50 days after emergence (leaf on the third node from the top of the main stem). The 16 leaf free amino acids Asparagine, Threonine, Serine, Glutathione, Glycine, Alanine, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Lysine, Arginine and Proline were determined in the laboratory as follows:

Principle

The acid hydrolyzed amino acids by amide bond breakage were determined according to Pellet and Young (1980). Ninhydrin is used for the detection of amino acids at λ 440 for proline and 570 nm for the other amino acids through an oxidative decarboxylation reaction of the amino acids with ninhydrin, to give Ruhemann's purple compound, which could be detected by the spectrophotometer. Aliquot of 515.46 ml of 36% HCl (6N) was completed to 1000 ml distilled water. Sodium acetate buffer (0.1 N) of pH 2.2 was used as sample dilution buffer.

Procedure

Acid hydrolysis

From each fresh sample of quinoa (leaves collected from plants of age 50 days after emergence from the 3rd node from the top of main stem), 1 g was hydrolyzed in sealed evacuated Pyrex test tube using 5 ml of 6 N HCl at 110°C for 24 h. At the end of this period, hydrolysate was transferred quantitatively to other containers and the hydrochloric acid was then evaporated to dryness at 50 – 60°C on water bath.

Distilled water (5 ml) was added to the hydrolysate and then evaporated to dryness to remove the excess HCl. Further addition of distilled water was carried out till complete removal of excess HCl and samples were dried till the dry film was obtained. The obtained dry film was dissolved in a known volume of sample dilution buffer (0.1N sodium acetate buffer, pH 2.2) and the solution was filtered through a 0.45 mm membrane filter, and then stored frozen in sealed vials until fractionation of the amino acids by amino acid analyzer.

Separation of amino acids

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

| | |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Column | Hydrolysate column SYKAM (S4300) – (150x4.6 mm) of a temperature of 57°C |
| Sample | 100 µl |
| Buffer system | Sodium acetate, buffer A (pH 3.45), buffer B (pH 10.85) and buffer C (regeneration solution) |
| Flow rate | 0.25 ml/min for ninhydrin pump 0.45 ml/min for quaternary pump |
| Detection | Ninhydrin is used for the detection of amino acids spectrophotometrically at λ 440 for proline and 570 nm for the other amino acids through an oxidative decarboxylation reaction to give ruhemann's purple color |

Calculation of amino acids

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

Leaf anatomical traits determination:

In 2015/2016 season, the leaf samples from control (95% FC) and drought at 65 and 35% FC treatments were taken from the field of quinoa genotypes at age of 70 days from emergence at the 3rd node from the top of main stem. Leaves were preserved in a solution of 1-5 ml formaldehyde acetic acid (FAA), 2-5 ml glacial acetic acid (GAA) and 90 ml Ethyl

alcohol 70% and kept in vials. Leaves were transferred through different levels of Ethyl Alcohol to get the leaves dried, *i.e.* Ethyl alcohol 70% 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 gelatin in 100 ml worm water) to prevent specimen from falling of

the surface of the slide, then left it to dry. After the slide got dried it was ready to go to dying stage, consisting of 16 dye solution (Xylene 24h, Xylene + Ethyl absolute (0.5:0.5) 2 min, 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% 2 min, 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 fine glass cover using Canada Balsam as adhesive before we examined it under the microscope (Lica, Germany) at 40x and 80x eye length. Finally, photographs were taken with a digital camera (Canon) attached to a microscope. Measurements were taken on the thickness of five layers, namely leaf, upper epidermis, lower epidermis, palisade and spongy layers.

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.* (1997). Expected mean squares at separate and across years were estimated from ANOVA table according to Hallauer *et al.* (2010). Across seasons, genotypic (σ_g^2), phenotypic (σ_{ph}^2), genotype x season (σ_{gs}^2) and error (σ_e^2) variances were computed as follows:

$$\bar{\sigma}_g^2 = (M_3 - M_2) / rs, \quad \bar{\sigma}_{gs}^2 = (M_2 - M_1) / r$$

$$\text{and } \sigma_{ph}^2 = \sigma_g^2 + \sigma_{gs}^2 / r + \sigma_e^2 / rs.$$

Where r=number of replications, g=number of genotypes and s=number of seasons.

Heritability in the broad sense

Heritability in the broad sense (h_b^2 %) for a trait in a separate environment and combined across environments was estimated according to Singh and Narayanan (2000) using the following formula:

$$h_b^2 \% = 100 \times (\bar{\sigma}_g^2 / \bar{\sigma}_{ph}^2)$$

Expected genetic advance from selection

Expected genetic advance from selection for all studied traits as a percent of the mean was calculated as follows:

$$GA (\%) = 100 K h_b^2 \sigma_{ph} / \bar{x} \quad (\text{Singh and Narayanan, 2000})$$

Where: \bar{x} =General mean, K= Selection differential=1.76 for 10% selection intensity, used in this study).

RESULTS

Analysis of Variance for Agronomic, Physiological and Yield Traits

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) and inflorescence weight (IW), indicating significant effect of climatic conditions on nine out of 14 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 via breeding procedures.

Mean squares due to the 1st 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 3). Mean squares due to the 2nd 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 of randomized complete blocks design for studied traits of five quinoa genotypes under three environments (WI, MIS and SIS); representing well irrigation (95% FC), moderate irrigation stress (65% FC) and severe irrigation stress (35% FC) indicated

that mean squares due to genotypes, were significant ($P \leq 0.01$) for all studied traits (Table 3), suggesting the significance of differences among studied quinoa genotypes for all studied traits under all water stress 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 under WI, DTF, RL, BPP, IL and SYPP under MIS and ID and IL under SIS 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 WI, plant height (PH) under MIS and LA, CCI and CCI under SIS, where seasons were the largest contributor and SPP under SIS, where G×S interaction variance was the largest contributor to total variance.

Heritability and genetic advance for agronomic, physiological and yield traits

Estimates of heritability in the broad sense (h^2_b) and expected genetic advance from selection as a percentage of the mean (GA%) for studied quinoa traits under well irrigation (WI), moderate irrigation stress (MIS) and severe irrigation stress (SIS) conditions are presented in Table (4). On average, the highest h^2_b estimate (99.96%) was shown by root length under MIS. On the contrary, the lowest h^2_b (0.0%) was shown in five cases (IPP, TSW and SPP under WI, PH and IPP under MIS). h^2_b for agronomic and physiologic traits ranged from 0.0% for inflorescences/plant (IPP), 1000-seed/plant (TSW) and seeds/plant (SPP) under WI, plant height (PH) and IPP under MIS to > 97.0% for root length, inflorescence length and seed yield/plant under all irrigation treatments.

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 |
| Season (S) | 1 | 0.06 | 0.027 | 195.4** | 68.6** | 221.0** | 0.5* | 0.5 |
| R(S) | 8 | 0.76 | 0.16 | 5.6 | 0.1 | 7.1 | 0.2 | 0.4 |
| Treatment (T) | 2 | 130.21** | 777.31** | 18739.4** | 319.6** | 4659.2** | 164.4** | 619.6** |
| T x S | 2 | 0.78** | 0.83* | 421.9** | 33.1** | 305.8** | 0 | 1.0* |
| Error (a) | 16 | 0.35 | 0.45 | 7.3 | 0.2 | 6.2 | 0.4 | 0.4 |
| Genotype (G) | 4 | 31.44** | 63.24** | 125.6** | 39.8** | 354.2** | 85.4** | 174.6* |
| G x S | 4 | 3.24** | 0.677 | 32.8** | 13.9** | 53.2** | 0.6* | 0.8 |
| G x T | 8 | 8.77** | 25.99** | 118.4** | 13.7** | 91.4** | 110.2** | 42.5** |
| G x S x T | 8 | 1.46** | 1.75** | 125.6** | 7.2** | 16.4** | 0.5 | 1.5** |
| Error (b) | 96 | 0.6 | 0.72 | 3.3 | 0.3 | 4.4 | 0.3 | 0.5 |
| | | Inflorescence /Plant | Inflorescence diameter | Inflorescence length | Inflorescence weight | Seeds /Plant | 1000-seed weight | Seed yield/plant |
| Season (s) | 1 | 3.53** | 0.3 | 0.4* | 0.0001 | 137350* | 0.54* | 0.91* |
| R(S) | 8 | 2.66 | 0.3 | 0.2 | 0.019 | 102274 | 0.51 | 0.37 |
| Treatment (T) | 2 | 781.82** | 752.5** | 381.2** | 12.86** | 9833577** | 18.54** | 1200.6** |
| T x S | 2 | 3.21** | 0.1 | 0.3* | 0.24** | 2055197** | 0.35 | 0.66* |
| Error a | 16 | 1.08 | 0.4 | 0.1 | 0.024 | 97066 | 0.353 | 0.44 |
| Genotype (G) | 4 | 54.21** | 202.0** | 109.8** | 4.3** | 1401183** | 2.28** | 199.8** |
| G x S | 4 | 6.31** | 1.8** | 0.6* | 0.36** | 1774849** | 0.60** | 1.19** |
| G x T | 8 | 55.77** | 12.4** | 11.2** | 1.57** | 1168931** | 3.65** | 111.96** |
| G x S x T | 8 | 4.24** | 1.01** | 0.2 | 0.18** | 1414597** | 0.45** | 0.39 |
| Error b | 96 | 1.46 | 0.4 | 0.3 | 0.024 | 109826 | 0.23 | 0.33 |

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

Table 3. Combined analysis of variance across seasons of randomized complete blocks design for studied traits of five quinoa genotypes under well irrigation (95% FC), moderate irrigation stress (65% FC) and severe irrigation stress (35% FC)

| SOV | | Mean squares | | | | | | |
|--------------------------------------------|----|--------------------------|---------------------------|-------------------------|-------------------------|-------------------------------|---------------------|----------------------|
| Well irrigation (95% FC) | | | | | | | | |
| | df | Days to 50% flowering | Days to 50% maturity | Plant height | Leaf area | Chlorophyll concent. index | Root length | Branches /plant |
| Season (S) | 1 | 0.02 | 0.32* | 10.0** | 2.2** | 367.2** | 0.13 | 0.5 |
| Error | 8 | 0.21 | 0.31 | 3.8 | 0.2 | 17.3 | 0.1 | 0.4 |
| Genotype (G) | 4 | 10.95** | 21.55** | 86.2** | 6.0** | 218.8** | 34.61** | 32.2** |
| G x S | 4 | 3.27** | 0.27* | 33.9** | 1.8** | 34.7** | 0.21 | 1.9** |
| Error | 32 | 0.44 | 0.25 | 3.1 | 0.2 | 10.2 | 0.21 | 0.3 |
| | | Inflorescences /plant | Inflorescence diameter | Inflorescence length | Inflorescence weight | Seeds /plant | 1000-seed weight | Seed yield /plant |
| Season (S) | 1 | 2 | 0.26* | 0.03 | 20.5** | 59030.5 | 0.10* | 0 |
| Error | 8 | 2.65 | 0.17 | 0.2 | 0.6 | 106053.9 | 0.08 | 0.3 |
| Genotype (G) | 4 | 5.75** | 64.84** | 42.5** | 22.4** | 135550.9** | 0.13* | 31.1** |
| G x S | 4 | 8.75** | 1.06* | 0.23* | 2.9** | 894667** | 0.28** | 0.7** |
| Error | 32 | 2.34 | 0.5 | 0.18 | 0.7 | 57358.8 | 0.08 | 0.3 |
| Moderate irrigation stress (65% FC) | | | | | | | | |
| | | Days to 50% flowering | Days to 50% maturity | Plant height | Leaf area | Chlorophyll concent. index | Root length | Branches /plant |
| Season (S) | 1 | 1.28* | 1.28* | 937.5** | 35.5** | 330.8** | 0.2 | 0.5* |
| Error | 8 | 0.4 | 0.4 | 15.2 | 0.2 | 1 | 0.8 | 0.3 |
| Genotype (G) | 4 | 9.17** | 8.97** | 167.5** | 16.9** | 190.5** | 55.6** | 120.2** |
| G x S | 4 | 0.53 | 1.43* | 199.7** | 13.0** | 18.5** | 0 | 0.9 |
| Error | 32 | 0.54 | 0.71 | 6.2 | 0.3 | 2.2 | 0.3 | 0.6 |

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| | | Inflorescences /plant | Inflorescence diameter | Inflorescence length | Inflorescence weight | Seeds /plant | 1000-seed weight | Seed yield /plant |
|------------------------------------------|----|----------------------------------|-----------------------------------|---------------------------------|---------------------------------|---------------------------------------|-----------------------------|------------------------------|
| Seasons (S) | 1 | 0.72** | 0 | 0.9** | 0.28** | 1522512** | 0.01** | 0.07 |
| Error | 8 | 0.07 | 0.2 | 0.1 | 0.01 | 67758 | 0.002 | 0.6 |
| Genotype (G) | 4 | 1.97** | 85.8** | 46.4** | 2.37** | 3187435** | 0.39** | 75.7** |
| G x S | 4 | 2.37** | 2.6** | 0.3 | 0.44** | 2637323** | 0.04** | 0.6 |
| Error | 32 | 0.27 | 0.3 | 0.3 | 0.02 | 102753 | 0.01 | 0.4 |
| Severe irrigation stress (35% FC) | | | | | | | | |
| | | Days to 50% flowering | Days to 50% maturity | Plant height | Leaf area | Chlorophyll concent. index | Root length | Branches /plant |
| Seasons (S) | 1 | 0.32 | 0.08 | 91.66** | 97.2** | 134.6** | 0.2 | 1.6** |
| Error | 8 | 0.62 | 0.35 | 11.24 | 0.16 | 1.1 | 0.2 | 0.5 |
| Genotype (G) | 4 | 28.87** | 74.72** | 108.1** | 44.3** | 127.7** | 215.5** | 107.9** |
| G x S | 4 | 2.37** | 2.48* | 50.40** | 13.36** | 32.8** | 1.3** | 1.1* |
| Error | 32 | 0.83 | 1.21 | 0.73 | 0.34 | 0.75 | 0.4 | 0.5 |
| | | Inflorescences /plant | Inflorescence diameter | Inflorescence length | Inflorescence weight | Seeds /plant | 1000-seed weight | Seed yield /plant |
| Seasons | 1 | 7.22* | 0.3 | 0 | 0.19** | 2666202** | 1.122 | 2.1** |
| Error | 8 | 2.1 | 0.6 | 0.2 | 0.03 | 122594 | 1.14 | 0.4 |
| Genotype (G) | 4 | 158.0** | 76.1** | 43.3** | **4.49 | 425060** | **9.05 | 316.9* |
| G x S | 4 | 3.67* | 0.2 | 0.5 | 0.122** | 1072052** | *1.19 | 0.7* |
| Error | 32 | 1.76 | 0.4 | 0.4 | 0.04 | 169367 | 0.618 | 0.3 |

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

Table 4. Heritability (h^2_b) and genetic advance (GA%) from selection for studied traits of quinoa under WI, MIS and SIS

| Parameter | WI | MIS | SIS | WI | MIS | SIS |
|-----------|----------------------------------------|-------|-------|-----------------------------|-------|--------|
| | Days to 50% flowering | | | Days to 50% maturity | | |
| h^2_b | 70.14 | 94.22 | 91.79 | 98.75 | 84.06 | 96.68 |
| GA% | 2.69 | 3.39 | 6.04 | 2.39 | 1.32 | 4.60 |
| | Plant height | | | Leaf area | | |
| h^2_b | 60.71 | 0.00 | 53.38 | 69.55 | 23.01 | 69.83 |
| GA% | 4.56 | 0.00 | 7.70 | 6.87 | 4.27 | 26.05 |
| | Chlorophyll concentration index | | | Root length | | |
| h^2_b | 84.14 | 90.31 | 74.37 | 99.40 | 99.96 | 99.40 |
| GA% | 17.27 | 18.15 | 17.77 | 24.74 | 28.42 | 50.88 |
| | Branches/plant | | | Inflorescences/plant | | |
| h^2_b | 94.25 | 99.25 | 99.01 | 0.00 | 0.00 | 97.68 |
| GA% | 21.55 | 58.27 | 68.04 | 0.00 | 0.00 | 101.87 |
| | Inflorescence diameter | | | Inflorescence length | | |
| h^2_b | 98.37 | 96.99 | 99.68 | 99.46 | 99.46 | 98.91 |
| GA% | 26.04 | 31.57 | 42.97 | 24.58 | 31.09 | 34.74 |
| | Inflorescence weight | | | Seeds/plant | | |
| h^2_b | 67.92 | 81.51 | 97.29 | 0.0 | 17.0 | 0.0 |
| GA% | 9.30 | 28.70 | 52.65 | 0.0 | 2.41 | 0.0 |
| | 1000-seed weight | | | Seed yield/plant | | |
| h^2_b | 0.00 | 89.74 | 86.87 | 97.76 | 99.23 | 99.78 |
| GA% | 0.00 | 21.26 | 62.10 | 12.04 | 22.06 | 56.02 |

The average expected genetic advance (GA%) from selection of the best 10% was generally higher under severe irrigation stress than under well irrigation for all studied traits, except for CCI and SPP, where the opposite was true (Table 4). GA ranged from 0% for IPP, TSW and SPP to 26,04% for inflorescence diameter under WI, from 0% for PH and IPP to 58.27% for branches/plant under MIS and from 0% for SPP to 101.87% for IPP and BPP under SIS.

Analysis of variance for leaf amino acids

Analysis of variance (Table 5) of 16 leaf free amino acids and their total content of five quinoa genotypes evaluated in 2015/2016 season under three soil moisture

regimes (WI, MIS and SIS), revealed significant ($p \leq 0.01$) differences among genotypes and among soil moisture regimes for the 16 amino acids and their total. Moreover, mean squares due to genotypes x irrigation regimes interaction were significant ($p \leq 0.01$ or $p \leq 0.05$) for all free amino acids and their total content.

Combined analysis of variance of randomized complete blocks design for 16 different free amino acids and their total of five quinoa genotypes under three environments (WI, MIS and SIS) (data not presented) revealed that mean squares due to genotypes, were significant ($P \leq 0.01$ or $p \leq 0.05$) for all amino acids and their total content.

Table 5. Analysis of variance of split plot for leaf free amino acids of five quinoa genotypes (G) under three irrigation treatments (T) in 2014/2015 season

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

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

Table 6. Heritability in broad sense (h^2_b) and genetic advance from selection (GA) for free amino acids of quinoa under WI, MIS and SIS environments in 2015/2016 season

| Amino acids | h^2_b % | | | GA% | | |
|----------------|-----------|--------|-------|-------|-------|-------|
| | WI | MIS | SIS | WI | MIS | SIS |
| Asparagine | 99.53 | 99.98 | 93.69 | 16.67 | 19.42 | 16.42 |
| Therionine | 99.54 | 97.79 | 99.57 | 20.85 | 25.56 | 22.17 |
| Serine | 78.01 | 79.68 | 99.78 | 19.83 | 19.34 | 21.89 |
| Glutathione | 86.50 | 99.97 | 99.98 | 12.26 | 20.51 | 24.13 |
| Glycine | 99.67 | 99.67 | 99.15 | 20.71 | 24.22 | 20.59 |
| Alanine | 99.69 | 99.75 | 99.87 | 19.93 | 19.53 | 16.03 |
| Valine | 99.53 | 100.00 | 99.92 | 18.70 | 18.31 | 23.55 |
| Methionine | 92.86 | 87.50 | 99.66 | 22.28 | 14.32 | 87.79 |
| Isoleucine | 98.60 | 99.77 | 99.96 | 17.24 | 20.28 | 23.16 |
| Leucine | 99.33 | 99.84 | 99.97 | 26.00 | 20.26 | 23.67 |
| Tyrosine | 98.77 | 73.66 | 98.26 | 19.61 | 24.97 | 16.17 |
| Phenyl alanine | 99.67 | 98.83 | 99.95 | 20.01 | 21.72 | 22.07 |
| Histidine | 98.60 | 100.00 | 96.77 | 18.72 | 18.40 | 22.56 |
| Lysine | 99.56 | 99.57 | 93.79 | 20.34 | 16.94 | 18.44 |
| Arginine | 99.22 | 99.30 | 99.36 | 20.35 | 19.94 | 20.62 |
| Proline | 99.95 | 98.86 | 99.98 | 22.12 | 24.85 | 21.83 |
| Total | 99.87 | 98.93 | 99.93 | 18.55 | 19.45 | 20.78 |

Heritability and genetic advance for leaf amino acids

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 free amino acid contents under well irrigation (WI), moderate irrigation stress (MIS) and severe irrigation stress

(SIS) conditions are presented in Table (6). In general, heritability estimates in the broad sense for all amino acids were very high in magnitude (>92%), except for Serine (79.68%), Methionine (87.5%) and Tyrosine (73.66%) under moderate irrigation stress (MIS) and Serine (78.01%) under well irrigation (WI).

Heritability ranged from 78.01% for Serine to 99.95% for Proline under well irrigation (WI), from 73.66% for Tyrosine to 100.0% for Valine under moderate moderate irrigation stress (MIS) and from 93.69% for Asparagine to 99.98% for Proline under severe irrigation stress (SIS).

The genetic advance (GA%) from selection (Table 8) ranged from 12.26% for Glutathione to 26.00% for Leucine under WI, from 16.94% for Lysine to 25.56% for Threonine under MIS and from 16.03% for Alanine to 87.79% for Methionine under SIS conditions.

Analysis of variance for leaf anatomical traits

Analysis of variance (Table 7) of leaf anatomical traits for five quinoa genotypes evaluated in 2015/2016 season under three soil moisture regimes (WI, MIS and SIS), 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 regimes

interaction were significant ($p \leq 0.01$ or $p \leq 0.05$) for all studied anatomical traits.

Analysis of variance of randomized complete blocks design for studied leaf anatomical traits of five quinoa genotypes under three environments (WI, MIS and SIS) (data not presented) indicated that mean squares due to genotypes were significant ($P \leq 0.01$ or $p \leq 0.05$) for all leaf anatomical traits.

Heritability and genetic advance for leaf anatomical traits

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 irrigation (WI), moderate irrigation stress (MIS) and severe irrigation stress (SIS) conditions are presented in Table (8). 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 WI, MIS and SIS, respectively. The highest h^2_b estimate (100%) was shown by upper epidermis under severe irrigation stress.

Table 7. 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 8. Heritability in broad sense (h^2_b) and genetic advance from selection (GA) for leaf anatomical traits of quinoa under WI, MIS and SIS environments in 2015/2016 season

| Anatomical traits | h^2_b % | | | GA% | | |
|-------------------|-----------|-------|--------|-------|-------|-------|
| | WI | MIS | SIS | WI | MIS | SIS |
| 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 |

For anatomical traits, GA ranged from 30.40% (leaf thickness) to 87.12% (spongy layer) under WI, from 52.66% (leaf thickness) to 82.72% (palisade layer) under MIS and from 15.40% (upper epidermis) to 72.97% (palisade layer) under SIS.

DISCUSSION

The significance of mean squares due to seasons indicates significant effect of climatic conditions on nine out of 16 studied agronomic, physiological and yield traits of quinoa. Significance of mean squares due to irrigation regimes (T) and quinoa genotypes (G) for all studied traits, indicates that irrigation regime and genotype had significant effects on all studied agronomic, physiologic and yield traits. Significant differences among studied quinoa genotypes suggest that improvement of these agronomic, physiologic and yield traits are possible *via* breeding procedures. Significance of G×T indicates that genotype's rank differed from one irrigation regime to another and selection would be efficient for all studied agronomic, physiologic and yield traits under a specific water stress environment, as previously reported by several investigators (Al-Naggar *et al.*, 2009, 2011 a, b, c and 2016 a,b). Significance of mean squares due to G×S×T for all studied agronomic, physiologic and yield traits, except for root length (RL), inflorescence length (IL) and SYPP, indicates that quinoa genotype's performance differed from a combination of treatment and season to another combination for most studied agronomic, physiologic and yield traits. It is observed from Table (6) 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 all studied agronomic, physiologic and yield traits. Significance of mean squares due to genotypes under separate environments for all studied traits, indicates the significance of differences among studied quinoa genotypes for all studied agronomic, physiological and yield traits under all water stress environments and selection would be efficient under all studied environments. It is observed that genotype is the largest contributor to total variance for all studied traits in all environments, except chlorophyll concentration index (CCI) under WI, plant height (PH) under MIS and LA, CCI and CCI under SIS, where season was the largest contributor and SPP under SIS, where G×S interaction variance was the largest contributor to total variance.

Analysis of variance for the 16 amino acids indicated significant differences among genotypes and among soil moisture regimes for the studied 16 amino acids and their total. Results indicated the significance of differences among studied quinoa genotypes for all studied amino acids and their total content under each of studied environments and selection would be efficient under a specific water stress environment. Moreover, significance of mean squares due to genotypes x irrigation regimes interaction for all free amino acids and their total content, suggests that content of each free amino acid and their total in quinoa leaves varies with water supply. Al-Naggar *et al.* (2002 a, b, c, d) reported a similar conclusion in sorghum.

Analysis of variance for the five anatomical traits, indicated significant differences among genotypes and among irrigation regimes, except irrigation treatments for lower epidermis, which were

not significant. Moreover, significance of mean squares due to genotype x irrigation regimes interaction for all studied anatomical traits, suggested that thickness of leaf and different leaf layers of quinoa varies with water supply. A similar conclusion was reported by Chartzoulakisa *et al.* (2012), Dawood *et al.* (2014) and Faycal *et al.* (2014). Results indicated the significance of differences among studied quinoa genotypes for all leaf anatomical traits under all irrigation treatments and selection would be efficient under a specific water stress environments.

Heritability estimates in the broad sense of for agronomic, physiologic and yield traits, were, on average, higher under WI than water stressed environments for five traits (DTM, PH, LA and IL; under MIS for six traits (DTF, CCI, RL, BPP, SPP and TSW) and under SIS for four traits (IPP, ID, IW and SPP). The five traits SYPP, IPP, BPP and TSW (inflorescence traits) were the most responsive to selection in quinoa genotypes. Few cycles of selection for these traits would lead to improve these traits either under water stress conditions. These five traits could be considered the best secondary traits for selecting drought tolerant genotypes of quinoa. The average expected genetic advance (GA%) from selection of the best 10% was generally higher under severe irrigation stress than under well irrigation for all studied traits, except for CCI and SPP, where the opposite was true. Under severe irrigation stress, the maximum predicted GA% from selection was achieved from IPP (101.87%), followed by BPP (68.04%), TSW (62.10%), and SYPP (56.02%).

For amino acids, results indicated that environment had very small effect on the phenotype of free amino acids in leaves of quinoa. The severe irrigation stress showed

the highest h^2_b estimates for amino acids as compared to MIS and WI environments. Highest heritability in the broad sense was shown by Proline (99.95%) under well irrigation (WI), by Valine (100.0%) under moderate irrigation stress (MIS) and by Proline (99.98%) under severe irrigation stress (SIS). The genetic advance (GA%) from selection was generally higher under severe irrigation stress (for 9 amino acids) than under moderate water stress (6 amino acids) and well irrigation (3 amino acids).

For leaf anatomical traits, results suggested that environment had very small effect on the phenotype for most studied anatomical traits in leaves of quinoa. The highest h^2_b estimate (100%) was shown by upper epidermis under severe irrigation stress. The genetic advance (GA%) from selection was generally higher under moderate irrigation stress (MIS) for 3 anatomical traits, namely leaf thickness, lower epidermis and palisade layer and under well irrigation for two traits, namely upper epidermis and spongy layer.

Since the efficiency of selection would depend upon the magnitude of heritable variability, higher heritability accompanied with high-expected genetic advance for the amino acids studied should be quite valuable. It is obvious from the results of this study, that the traits RL, BPP, IPP, ID, IW, TSW and SYPP, all quinoa amino acids, its total and palisade and spongy layers under all environments were characterized by having high heritability accompanied by high values of expected genetic advance, especially under severe irrigation stress conditions. 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, El-Ganayni *et al.*, 2000, Hefny, 2007; Al-Naggar *et al.*, 2009, 2011, 2016 a, b, Al-Naggar and Shehab El-Deen 2012, 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 first group for most studied agronomic, physiological, yield traits and most amino acids, but are in agreement with the second group for upper epidermis and spongy layer and with the first group for palisade layer and leaf thickness.

To the best of our knowledge these results on heritability and genetic advance on leaf free amino acids and leaf anatomical traits in quinoa under WI, MIS and SIS environments are believed to be the first record in the literature. Further investigations on the type of gene action controlling the inheritance of these traits are needed to help plant breeders in improving drought tolerance trait.

CONCLUSION

Significance of variances due to the two factors (irrigation regimes and quinoa genotypes) and their interaction for studied agronomic, physiological and yield traits, amino acids and anatomical traits, suggested that all studied traits in quinoa varies with water supply and selection would be efficient under a specific water stressed environment. Out of the studied traits, the five traits SYPP, IPP, BPP and TSW (inflorescence traits) were the most responsive to selection in quinoa genotypes particularly under SIS. It is also obvious that

all quinoa leaf free amino acids, leaf thickness, palisade and spongy layers were characterized by having high heritability accompanied by high values of expected genetic advance, especially under severe irrigation stressed environments. Few cycles of selection for these traits would lead to improve such traits either under water stress or non-stress conditions. Results indicated that the best selection environment was SIS for all studied traits, except for upper epidermis and spongy layer, which was WI.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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