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Heritability and Interrelationships for Agronomic, Physiological and Yield Traits of Quinoa (*Chinopodium quinoa* Willd.) under Elevated Water Stress

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Authors' contributions

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

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ABSTRACT

Strong associations between yield and given traits along with high heritability for such traits would allow plant breeder to use these traits as selection criteria for selecting high yielding genotype. The present investigation aimed at elucidating the relationships among agronomic, physiological and yield traits of five quinoa genotypes and estimating heritability in broad sense (h^2_b) and genetic advance (GA) from selection for these traits. A two-year experiment was carried out in a split plot experiment with five replications. The main plots were devoted to 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, and sub plots to five quinoa genotypes. The results indicated that correlations (r) between each of seed yield/plant (SYPP), water use efficiency (WUE), drought

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tolerance index (DTI) and other studied traits became stronger as water stress became severer; the strongest correlations were with thousand seed weight (TSW), inflorescence weight (IW), inflorescence diameter (ID), branches/plant (BPP), root length (RL), inflorescence length (IL) and inflorescences/plant (IPP). The highest h^2_b (>0.99%) under all irrigation treatments was shown by RL, WUE and seed yield/ha (SYPH), while the lowest estimate (0.0%) was shown by IPP under WW and WS and SPP under WW and SWS. Estimates of GA increased as water stress increased; the highest GA was shown by IPP (101.87%) followed by BPP (68.04%) under SWS, while the lowest one (1.32%) was shown by days to maturity under WS. Results indicated that the best secondary traits for high drought tolerance and high WUE of quinoa were TSW, IW, ID, IL, IPP, BPP, RL, SYPP and SYPH traits, since they showed high (r), high (h^2_b) and high (GA) estimates. Further studies should be carried out on other quinoa germplasm to ascertain this conclusion.

Keywords: Chinopodium quinoa; genetic advance; correlation coefficients; water use efficiency; drought tolerance index.

1. INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal and is one of the 250 species included in the genus *Chenopodium*, commonly known as ‘goosefoot’ genus [1]. It is a dicotyledonous annual species belonging to the family Amaranthaceae (formerly Chenopodiaceae). 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%) [2,3]. Due to its high starch content (51–61%) it can be used in the same way as cereals for flour production [2]. In addition, quinoa is a good source of vitamins, oil with high linoleate and linolenate content (55–66% of the lipid fraction), natural antioxidants such as a- and c-tocopherol, and a wide range of minerals [3,4]. In addition, quinoa is an undemanding crop that has remarkable productive advantages of cultivation under adverse environmental conditions [4,5] 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 [4,6]. 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 [7].

The study of correlation is regarded as an important step in breeding programs of quinoa since the information obtained is useful estimating the correlated response to directional selection for the formulation of selection indices. Reports on variability and association among different traits in quinoa are rare, based on few yield components and are based on experiments carried out in America and Europe [8,9]. Inflorescence length was the most important component influencing grain yield in quinoa [10]. A strong correlation was found between plant height, stem diameter, inflorescence length, and inflorescence diameter with each other [8]. However, the associations between the durations of the developmental phases were weak, suggesting that there is a great scope for manipulation of the pattern of development through breeding. Bhargava et al. [11] calculated the correlation coefficients among various traits and their direct and indirect effects on grain yield in quinoa grown on normal and sodic soils. Stem diameter and dry weight/plant were positively correlated with grain yield on both soil types. It was concluded that selection of thick-stemmed plants with more number of inflorescences and high dry weight would be beneficial in breeding for high grain yield in quinoa on sodic soils. Positive association was reported of inflorescence length and diameter with grain yield, which indicated that the selection for these characters may result in more productive genotypes [12]. Positive correlation was also observed between plant height and inflorescence length which suggested that high grain yield can be attained by selecting for stem/inflorescence ratio. A strong positive relationship between yield and grain number has also been found in quinoa, whereas grain weight has shown a conservative behavior [13]. In a more detailed study, the interrelationships among yield and yield components were elucidated in 27 germplasm lines of quinoa by Bhargava et al. [14]. 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 [15]. Grain yield integrates two main components, grain number per m^2 and average grain weight, where yield variations in grain crop species are usually associated with grain number [16]. De Santis et al. [17] stated that all of the morphological traits except days to flowering and days to maturity showed significant positive associations with seed yield, while the quality traits were negatively correlated with seed yield.

The estimation of the 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 [18]. 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 [18,19]. 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 [20]. The estimations of high coefficients of heritability are associated with a greater genetic variability, greater selective accuracy [21] and greater possibilities for success in selecting genotypes with higher productivity of grain. De Santis et al. [17] stated that heritability estimates in quinoa were relatively high for almost all of the traits considered.

The objectives of the present investigation were: (i) to elucidate the relationships between the seed yield and agronomic and physiological traits of the quinoa germplasm and (ii) to estimate the heritability of these traits, in order to determine the selection criteria for high seed yield under elevated drought stress conditions.

2. MATERIALS AND METHODS

This study was carried out in the two successive growing winter 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 meters above sea level.

2.1 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-l3	Bolivia	Light yellow
Chipaya	Altiplano Salares, Bolivia	Mixed (white & Paige color)
CICA-17	Peru	Yellow
CO-407	Colorado, USA	Mixed (light yellow & white)
Ollague	Altiplano Salares, Bolivia	Yellow

2.2 Experimental Procedures

On the 19th of November the seeds were planted along the irrigation pipes of drip irrigation system. Each pipe (row) length was 90 meter and keeping row to row distance of 60 cm and hill to hill of 60 cm. Seeds (7-10) were sown in each hill, thereafter (after 35 days) were thinned to three plants/hill to achieve a plant density of 35,000 plants/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^2) with a 1.0 meter ally between irrigation treatments.

2.3 Experimental Design

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

2.4 Irrigation System

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

2.5 Water Regimes

The following three different water regimes were used:

1. **Well watering (WW)**, where the field capacity (FC) was about 95%. Irrigation in this treatment (WW) was given each three days; with 40 irrigations during the whole season. The water meter recorded at the end of each irrigation about 205 m^3 water/ha; thus, the total quantity of water given in the whole season for WW treatment was 8200 m^3 per ha.
2. **Water stress (WS)**, where the field capacity (FC) was about 65%. Irrigation in this treatment (WS) was given each six days; with 20 irrigations during the whole season. The water meter recorded at the end of each irrigation about 250 m^3 water/ha; thus, the total quantity of water given in the whole season for WS treatment was 5000 m^3 per ha.
3. **Severe water stress (SWS)**, where the field capacity (FC) was about 35%. Irrigation in this treatment (WW) was given each twelve days; with 10 irrigations during the whole season. The water meter recorded at the end of each irrigation about 236.8 m^3 water/ha; thus, the total quantity of water given in the whole season for WW treatment was 2368 m^3 per ha.

2.6 Fertilization Regimes

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 28 tons/ha and was well mixed with the soil two weeks before sowing at a depth of 10-15 cm.
2. **Mineral fertilizers:** The following mineral fertilizers were applied: 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% P_2O_5) at the rate of 70 kg P_2O_5/ha was added as soil application in two equals doses, the first (35 kg P_2O_5/ha) before sowing during preparing the soil for planting and the second (35 kg P_2O_5/ha) after 25 days from sowing. Potassium fertilizer at the rate of 60 kg K_2O/ha was added as soil application in two doses; before planting (35 kg K_2O/ha) and after 25 day from sowing (25 kg K_2O/ha) as Potassium Sulfate (48% K_2O). Calcium Sulfate or Gypsum (22% Ca, 17% S) at the rate of 50 kg /ha was added as soil application in two equal doses, the first time during preparing the soil for planting and the second time 75 days after sowing. Trace elements (Chelated Iron 3%, Chelated Zinc 2%, Boron 0.5%, 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.

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^{-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 cm^2** 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.
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^3 water:** This was calculated by the following formula:

    WUE = (Seed yield/ha in kg)/(quantity of irrigation water/ha in m^3 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 [22] 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 ≥ 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. [23]. Expected mean squares at separate and across the three treatments were estimated from ANOVA table according to Hallauer et al. [24].

Genotypic (σ^2_g), phenotypic (σ^2_{ph}), genotype x treatment, genotype x treatment x season, genotype x season and error variances were computed as follows: $\sigma^2_g = (M_3 - M_2)$ /sr, $\sigma^2_{ph} = \sigma^2_g + \sigma^2_{gs}/ rs + (\sigma^2_e / rs)$. Where r = number of replications, g= number of genotypes and s= number of seasons.

2.10 Heritability in the Broad Sense

Heritability in the broad sense (h^2_b) for a trait in a separate environment and combined across environments was estimated according to Singh and Narayanan [25] using the following formula:

$$h^2_b\ \% = 100 \times (\delta^2_g / \delta^2_p)$$

Where: σ^2_g = genetic variance, and δ^2_p = phenotypic variance.

2.11 Expected Genetic Advance from Selection

Expected genetic advance from selection for all studied traits as a percent of the mean was calculated [25] as follows: GA (%) = 100 K $h^2_b\ \sigma_{ph} / \overline{x}$, Where: $\overline{x}$ = General mean, σ_{ph} = Square root of the denominator of the appropriate heritability, h^2_b = The applied heritability, K = Selection differential (K = 1.76, for 10% selection intensity, used in this study).

2.12 Trait Interrelationships

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 [25].

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Combined analysis of variance 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) is presented in Table 2. Mean squares due to genotypes, were significant ($P \leq 0.01$) for all studied traits, indicating 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 from Table 2 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.

3.2 Mean Performance

Means of studied traits of each of the five quinoa genotypes under each environment (WW, WS and SWS) across the two seasons are presented in Table 3. The high means of all studied traits were considered favorable, except earliness traits (DTF and DTM), where high means were considered unfavorable.

Under severe water stress, quinoa genotypes varied greatly in SYPH (from 1883.5 kg for CICA-17 to 1311.6 kg for Ollague), SYPP (from 29.9 g for CICA-17 to 14.8 g for QL-3), WUE (from 795.3 g/m^3 for CICA-17 to 416.2 g/m^3 for QL-3), TSW (from 3.6 g for CICA-17 to 1.8 g for QL-3), SPP (from 8412 for CICA-17 to 7872 for CO-407), IW (from 3.92 g for Ollague to 2.03 for CO-407), IL (from 16.0 cm for CICA-17 to 11.1 cm for Ollague), ID (from 18.7 cm for CICA-17 to 11.9 cm for Ollague), IPP (from 12.8 for CICA-17 to 4.1 for QL-3), BPP (from 15.5 for CICA-17 to 7.0 for Ollague), RL (from 24.0 cm for CICA-17 to 15.1 cm for Chipaya), CCI (from 39.3% for CICA-17 to 30.1% for Ollague), LA (from 15.5 cm^2 for CICA-17 to 10.1 cm^2 for QL-3), PH (from 57.0 cm for CICA-17 to 48.8 cm for QL-3), DTF (from 60.8 for Chipaya to 56.6 for Ollague) and DTM (from 134.1 for QL-3 to 127.7 for Ollague).

In general, the quinoa genotype CICA-17 had the highest (favorable) means for 12 out of 16 traits, namely WUE, SYPH, SYPP, IW, IL, ID, IPP, BPP, RL, CCI, LA and PH. In the second highest place, came the quinoa genotype CO-407, for SYPH, SYPP, SPP, ID, I/P, BPP, CCI and PH, the genotype QL-3 for IW, RL and CICA-17 for IL and Chipaya for LA.

On the contrary, the lowest means across all environments were shown by the quinoa genotype Ollague for nine traits, namely SYPH, SYPP, IW, ID, IPP, BPP, CCI, DTF and DTM, QL-3 for LA, IL, WUE and CO-407 for SPP. For earlines traits (DTF and DTM), the genotype Ollague was the earliest.

The variability among quinoa genotypes in all studied traits in the present investigation were in agreement with several investigations [2,8,9]. Quinoa's traditional range of cultivation stretches as far north as Columbia and as far south as southern Chile. As a result of its wide distribution, the crop is adapted to a wide range of environments and forms a diverse range of ecotypes [8].

3.3 Trait Interrelationships

Estimates of phenotypic correlation coefficients between each of SYPP and water use efficiency (WUE) and other studied traits across the two seasons under WW, WS and SWS and combined across environments were calculated across all quinoa genotypes and presented in Table 4. 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.

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.

Table 2. Combined analysis of variance across seasons of randomized complete blocks design for studied traits of five quinoa genotypes under well watering (95% FC), water stress (65% FC) and severe water stress (35% FC)

SOV	**df**	**Mean squares**							
		Well watering (95% FC)							
		Days to 50% flowering	**Days to 50% maturity**	**Plant height**	**Leaf area**	**Chlorophyll concentration index**	**Root length**	**Branches /Plant**	**Inflorescence /plant**
Season (S)	1	0.02	0.32*	10.0**	2.2**	367.2**	0.13	0.5	2
Error	8	0.21	0.31	3.8	0.2	17.3	0.1	0.4	2.65
Genotype (G)	4	10.95**	21.55**	86.2**	6.0**	218.8**	34.61**	32.2**	5.75**
G x S	4	3.27**	0.27*	33.9**	1.8**	34.7**	0.21	1.9**	8.75**
Error	32	0.44	0.25	3.1	0.2	10.2	0.21	0.3	2.34
		Inflorescence diameter	**Inflorescence length**	**Inflorescence weight**	**Seeds/plant**	**1000-seed weight**	**Seed yield /plant**	**Seed yield /ha**	**Water use efficiency**
Season (S)	1	0.26*	0.03	20.5**	59030.5	0.10*	0	113.1	0.61
Error	8	0.17	0.2	0.6	106053.9	0.08	0.3	78.5	0.21
Genotype (G)	4	64.84**	42.5**	22.4**	135550.9**	0.13*	31.1**	75261.36**	6156.9**
G x S	4	1.06*	0.23*	2.9**	894667**	0.28**	0.7**	100.8**	0.198
Error	32	0.5	0.18	0.7	57358.8	0.08	0.3	71.95	0.222
		Water stress (65% FC)							
		Days to 50% flowering	**Days to 50% maturity**	**Plant height**	**Leaf area**	**Chlorophyll concentration index**	**Root length**	**Branches /Plant**	**Inflorescences /plant**
Season (S)	1	1.28*	1.28*	937.5**	35.5**	330.8**	0.2	0.5*	0.72**
Error	8	0.4	0.4	15.2	0.2	1	0.8	0.3	0.07
Genotype (G)	4	9.17**	8.97**	167.5**	16.9**	190.5**	55.6**	120.2**	1.97**
G x S	4	0.53	1.43*	199.7**	13.0**	18.5**	0	0.9	2.37**
Error	32	0.54	0.71	6.2	0.3	2.2	0.3	0.6	0.27

		Inflorescence diameter	Inflorescence length	Inflorescence weight	Seeds /plant	1000-seed weight	Seed yield /plant	Seed yield /ha	Water use efficiency
Seasons (S)	1	0	0.9**	0.28**	1522512**	0.01**	0.07	103.7	48.06
Error	8	0.2	0.1	0.01	67758	0.002	0.6	470.6	76.41
Genotype (G)	4	85.8**	46.4**	2.37**	3187435**	0.39**	75.7**	60581**	13820.9**
G x S	4	2.6**	0.3	0.44**	2637323**	0.04**	0.6	177.2	27.10
Error	32	0.3	0.3	0.02	102753	0.01	0.4	178.5	37.95
				Severe water stress (35% FC)					
		Days to flowering	**Days to maturity**	**Plant height**	**Leaf area**	**Chlorophyll concentration index**	**Root length**	**Branch /Plant**	**Inflorescence /plant**
Seasons (S)	1	0.32	0.08	91.66**	97.2**	134.6**	0.2	1.6**	7.22*
Error	8	0.62	0.35	11.24	0.16	1.1	0.2	0.5	2.1
Genotype (G)	4	28.87**	74.72**	108.1**	44.3**	127.7**	215.5**	107.9**	158.0**
S x G	4	2.37**	2.48*	50.40**	13.36**	32.8**	1.3**	1.1*	3.67*
Error	32	0.83	1.21	0.73	0.34	0.75	0.4	0.5	1.76
		Inflorescen. diameter	**Inflorescen. length**	**Inflorescen. weight**	**Seeds /plant**	**1000-seed weight**	**Seed yield /plant**	**Seed yield /ha**	**Water use efficiency**
Seasons	1	0.3	0	0.19**	2666202**	1.122	2.1**	611.1*	620.789
Error	8	0.6	0.2	0.03	122594	1.14	0.4	178.5	180.861
Genotype (G)	4	76.1**	43.3**	**4.49	425060**	**9.05	316.9*	**210233****	212349.1**
S x G	4	0.2	0.5	0.122**	1072052**	*1.19	0.7*	597.4**	600.77*
Error	32	0.4	0.4	0.04	169367	0.618	0.3	122.5	123.43

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

Table 3. Mean performance of studied traits of each quinoa genotype under well watering (WW), water stress (WS) and severe water stress (SWS) across two seasons

Genotype	WW	WS	SWS	WW	WS	SWS
	Days to 50% flowering			Days to 50% maturity		
QL-3	62.1	61.8	57.4	137.4	136.3	134.1
Chipaya	62.4	60.9	60.8	138.4	136.5	128.7
CICA-17	63.1	60.2	59.7	138.3	137.4	132.6
CO-407	61.6	60.3	58.9	139.4	137.8	129.3
Ollague	60.3	59.2	56.6	135.5	135.4	127.7
L.S.D. 0.05	0.5	0.5	0.6	0.3	0.6	0.8
	Plant height (cm)			Leaf area (cm^2)		
QL-3	94.0	77.3	48.8	18.1	14.2	10.1
Chipaya	87.4	73.8	49.1	18.6	16.6	12.4
CICA-17	86.6	82.6	57.0	18.4	17.7	15.5
CO-407	87.7	82.8	51.8	16.8	15.5	14.3
Ollague	88.8	82.7	52.2	17.2	15.5	11.8
L.S.D. 0.05	1.6	2.3	0.8	0.4	0.5	0.5
	Chlorophyll concentration index (%)			Root length (cm)		
QL-3	51.9	46.4	31.9	19.8	20.3	19.5
Chipaya	46.2	45.9	35.5	17.7	18.1	15.1
CICA-17	54.3	54.7	39.3	16.6	22.1	24.0
CO-407	58.1	55.9	33.1	15.9	17.9	17.9
Ollague	48.5	46.8	30.1	14.9	16.0	26.6
L.S.D. 0.05	2.9	1.3	0.8	0.4	0.5	0.5
	Branches/plant			Inflorescences/plant		
QL-3	18.9	13.4	8.9	16.6	15.4	4.1
Chipaya	15.7	12.6	10.4	15.3	14.9	8.9
CICA-17	20.0	17.4	15.5	17.0	14.3	12.8
CO-407	13.6	15.5	12.5	15.7	15.2	12.3
Ollague	18.2	8.2	7.0	15.4	14.6	5.2
L.S.D. 0.05	0.5	0.7	0.6	1.0	0.4	0.9
	Inflorescence diameter (cm)			Inflorescence length (cm)		
QL-3	20.0	19.3	12.2	17.8	13.0	11.8
Chipaya	21.3	19.2	14.9	17.9	14.0	13.5
CICA-17	23.8	23.1	18.7	19.9	17.8	16.0
CO-407	25.1	23.9	15.2	22.1	17.7	15.0
Ollague	19.1	16.9	11.9	17.0	15.8	11.1
L.S.D. 0.05	0.7	0.5	0.5	0.4	0.5	0.6
	Inflorescence weight (g)			Seeds/plant		
QL-3	2.10	1.88	3.61	8589	9152	8208
Chipaya	2.12	1.85	2.66	8517	9640	8071
CICA-17	1.96	2.20	2.34	8409	8312	8412
CO-407	2.05	1.90	2.03	8553	8653	7872
Ollague	1.95	1.64	3.92	8729	9509	8277
L.S.D. 0.05	0.1	0.0	0.2	163	218	279
	1000-seed weight (g)			Seed yield/plant (g)		
QL-3	4.1	3.2	1.8	34.9	28.9	14.8
Chipaya	3.8	2.9	2.9	32.5	27.6	23.7
CICA-17	4.0	3.8	3.6	33.3	31.4	29.9
CO-407	3.8	3.3	3.2	32.2	28.9	25.0
Ollague	3.4	2.5	2.5	30.0	23.9	20.4
L.S.D. 0.05	0.3	0.1	0.7	0.5	0.6	1.2

	Seed yield/ha (kg)			Water use efficiency (g/m^3)		
QL-3	2234.6	1921.9	985.6	271.2	384.5	416.2
Chipaya	2133.0	1838.3	1553.4	260.5	367.8	656.1
CICA-17	2146.0	2044.2	1883.5	262.1	409.0	795.3
CO-407	2133.7	1921.4	1668.1	260.6	385.9	704.4
Ollague	1709.8	1551.0	1311.6	209.0	310.4	553.8
L.S.D. 0.05	18.3	29.0	24.0	0.4	5.6	10.1

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.

Table 4. 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	Comb
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*

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 5). 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 is high.

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

Table 5. 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 seasons

Trait	WS	SWS	WW	WS	SWS	Comb
	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*

correlation (r= ≥ 0.67) with TSW, IW, IL, ID, IPP and BPP and below average significant and positive correlation (r= ≥ 0.44) with RL and PH. Under water stress (65% FC), DTI, exhibited above average significant and positive correlation (r= ≥ 0.56) with TSW, IW, ID, BPP, RL, DTM, DTF and below average significant and positive correlation (r= ≥ 0.31) with CCI.

Correlation analysis across all irrigation treatments and seasons showed that significant (p≤ 0.5 or p≤ 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 6). 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).

The study of correlation is regarded as an important step in breeding programs since the information obtained is useful estimating the correlated response to directional selection for the formulation of selection indices. Espinola and Gandarillas [10] reported that inflorescence length was the most important component influencing grain yield in *C. quinoa*. Risi and Galwey [8] reported strong correlation between plant height, stem diameter, inflorescence length, and inflorescence diameter with each other. However, the associations between the durations of the developmental phases were weak suggesting that there is a great scope for manipulation of the pattern of development through breeding. Ortiz et al. [26] noted high correlation between stem and inflorescence color which confirmed the presence of partial common genetic control for pigmentation in the crop. Bhargava et al. [11] calculated the correlation coefficients among various traits and their direct and indirect effects on grain yield in quinoa grown on normal and sodic soils. Stem diameter and dry weight/plant were positively correlated with grain yield on both soil types. It was concluded that selection of thick-stemmed plants with more number of inflorescences and high dry weight would be beneficial in breeding for high grain yield in quinoa on sodic soils. Spehar and Santos [12] 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. Positive correlation was also observed between plant height and in florescence length which suggested that high grain yield can be attained by selecting for stem/inflorescence ratio. In a more detailed study, the interrelationships among yield and yield components were elucidated in 27 germplasm lines of C. quinoa and two lines of *C. berlandieri subsp. nuttalliae* by Bhargava et al. [14]. Significant correlation among branches/plant, inflorescence length, and inflorescence/plant pointed out that plants with good branching habit tend to develop a large

Table 6. 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 ** indicate significant at 0.05 and 0.01 probability levels, respectively*

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 [15] and Ochoa and Peralta [27]. The path analysis revealed that 1000 seed weight had highest positive direct relationship with seed yield, followed by total chlorophyll and branches/plant. It was concluded that seed yield and seed protein were the only traits exhibiting high positive direct path and significant positive association with harvest index, indicating a true relationship among these traits [14]. Fuentes and Bhargava [4] 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 6) 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 were high.

3.4 Heritability and Expected Genetic Advance from Selection

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 watering (WW), water stress (WS) and severe water stress (SWS) conditions are presented in Tables 7.

Heritability estimates in the broad sense were, on average, higher under WW than other environments for five traits (DTM, PH, LA, IL and SYPH); under WS for six traits (DTF, CCI, RL, BPP, SPP and TSW) and under SWS for four traits (IPP, ID, IW and SPP). On average, the highest h^2_b estimate (99.96%) was shown by root length under WS followed by SYPH (99.90%) under WW. On the contrary, the lowest h^2_b (0.0%) was shown in five cases (IPP, TSW and SPP under WW, PH and IPP under WS).

The average expected genetic advance (GA%) from selection of the best 10% was generally higher under severe water stress than under well watering for all studied traits, except for CCI and SPP, where the opposite was true (Table 7).

Under severe water stress, the maximum predicted GA% from selection was achieved from IPP (101.87%), followed by BPP (68.04%), TSW (62.10%), SYPP (56.02%) and SYPH (52.80%). These five traits (SYPP, SYPH, IPP, BPP and TSW) are therefore 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. On the contrary, the lowest GA% (0.0%) was shown in five cases (IPP, TSW and SPP under WW, PH and IPP under WS).

Since the efficiency of selection would depend upon the magnitude of heritable variability, higher heritability accompanied with high expected genetic advance for the characters studied should be quite valuable. It is obvious from the results of this study, that the traits RL, BPP, IPP, ID, IW, TSW, SYPP and SYPH were characterized by having high heritability accompanied by high values of expected genetic advance under SWS conditions.

Table 7. Heritability (h^2_b) and genetic advance (GA%) from selection for studied traits of quinoa under WW, WS and SWS

Parameter	WW	WS	SWS	WW	WS	SWS
	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
	Inflorescences diameter			**Inflorescences 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
	Inflorescences 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
	Seed yield/ha			**WUE**		
h^2_b	99.9	99.70	99.70	99.99	99.45	99.43
GA%	22.60	22.60	52.80	17.28	17.55	40.85

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 [28-36]. The second group of researchers found that heritability and GA from selection for grain yield is higher under non-stress than those under stress [37-40]. Our results are in agreement with the first group for most studied traits.

Based on the correlation analyses between all studied traits and each of SYPP, WUE and DTI under all water treatments and their corresponding estimates of broad-sense heritability and expected genetic advance from selection, it is evident that the best secondary traits for high seed yield, drought tolerance and high water use efficiency of quinoa in our study are TSW, IW, ID, IL, IPP, BPP, RL, SYPP and SYPH traits, since they show high r, high h^2_b and high GA estimates. These traits could be helpful criteria for plant breeding programs aiming at improving drought tolerance in quinoa crop.

4. CONCLUSIONS

The significant differences among quinoa genotypes in this investigation indicated that selection would be efficient under a specific water stress environment. The quinoa genotype CICA-17 had the highest (favorable) means for most studied traits and could therefore be considered the most drought tolerant in our study. The results indicated that correlations (r) between each of seed yield/plant (SYPP), water use efficiency (WUE), drought tolerance index (DTI) and other studied traits became stronger as water stress became severer. Results indicated that the traits TSW, IW, ID, IL, IPP, BPP, RL, SYPP and SYPH could be considered selection criteria in breeding programs for improving drought tolerance (DT) and water use efficiency (WUE) in quinoa, since they showed strong correlation with DT and WUE, high heritability and high genetic advance estimates. Further studies should be carried out on other quinoa germplasm to ascertain this conclusion.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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