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Research article

Genotypic differences in agro-physiological, biochemical and isotopic responses to salinity stress in quinoa (*Chenopodium quinoa* Willd.) plants: Prospects for salinity tolerance and yield stability

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ABSTRACT

Quinoa is an important nutritive crop that can play a strategic role in the development of marginal and degraded lands. Genotypic variations in carbon isotope composition ($\delta^{13}C$), carbon isotope discrimination ($\Delta^{13}C$), ratio of intercellular to atmospheric CO_2 concentration (*Ci/Ca*), intrinsic water use efficiency (iWUE), seed yield and grain protein contents were analyzed in 6 quinoa cultivars grown in the field under saline conditions (0, 10, 20 dS m^{-1}). Significant variations occurred in dry biomass, seed yield, plant height, number of branches, number of panicles, panicle weight, harvest index, N and C content. Some genotypes produced yields with values significantly higher than 2.04 t ha^{-1} (Q12), with an average increased to 2.58 t ha^{-1} (AMES22157). The present study indicates a large variation in $\Delta^{13}C$ for salinity treatments (3.43‰) and small magnitude of variations among genotypes (0.95‰). Results showed that Δ might be used as an important index for screening, and selection of the salt tolerant quinoa genotypes with high iWUE. Quinoa genotypes differs in foliar ^{13}C and ^{15}N isotope composition, which reflected complex interactions of salinity and plant carbon and nitrogen metabolisms. Grain protein contents were found higher in Q19 and Q31 and lowest in Q26. The study demonstrates that AMES22157 and Q12, were salt tolerant and high yielder while the AMES22157 was more productive. This study provides a reliable measure of morpho-physiological, biochemical and isotopic responses of quinoa cultivars to salinity in hyper arid UAE climate and it may be valuable in the future breeding programs. The development of genotypes having both higher water use efficiency and yield potential would be a very useful contribution for producers in the dry region of Arabian Peninsula.

1. Introduction

Arable lands are significantly affected by serious problem of salinization and is increasing globally, especially in the drylands and marginal environment (Munns, 2011; Hussain et al., 2016). According to an estimate, 1 billion hectares of world soil is seriously affected due to water and soil salinity. Moreover, it is increasing at a rate of about 10% annually and increased salinization of arable land will result to 50% land loss by the middle of the 21st century (Jamil et al., 2011; Al-Dakheel and Hussain, 2016). High levels of salinity in soils is mainly due to the presence of soluble salts in the irrigation water, low precipitation, high temperature and over-exploitation of available ground water resources (Munns and Tester, 2008; Munns, 2011). New solutions are necessary to mitigate and counteract the detrimental effects of salinity on agricultural production. In this case, particular emphasis will be given to screening, selection and evaluation of suitable crop genotypes that show potential for adaptation to abiotic stresses. The use of halophytic plants that can tolerate high salt concentrations in the soil and allow irrigation with saline water are one of the possible ways to proceed, especially in arid and semi-arid regions (Koyro and Eisa, 2008).

Quinoa (*Chenopodium quinoa* Willd.) is an annual seed plant that has been cultivated in Andean region for thousand of years (Jacobsen et al., 2003). Quinoa is a highly nutritious food crop and its seeds contains essential amino acids (lysine, methionine, threonine), minerals (Ca, Fe, K, Mg, Mn, P, Zn) and fatty acids (Vega-Gálvez et al., 2010). The quinoa has shown a good tolerance potential to different resource constraint because of its great ecological plasticity and hardiness and has adapted

Abbreviations: FW, fresh weight; DW, dry weight; *Ci/Ca*, ratio of intercellular to atmospheric CO_2 concentration; $\delta^{13}C$, carbon isotope composition; iWUE, intrinsic water use efficiency; Δ, ^{13}C-carbon isotope discrimination; $\delta^{15}N$, Nitrogen isotope composition; C, Carbon concentration; N, Nitrogen concentration

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in marginal environment (Bazile and Baudron, 2015). It has the ability to tolerate low temperatures (−8 °C) (Jacobsen et al., 2007), drought (Jacobsen et al., 2009) and salinity (Jacobsen et al., 2003; Rosa et al., 2009). Therefore, the cultivation of salt-tolerant quinoa genotypes has been proposed as an alternate option for the successful production in semi-arid areas. Evaluation of different plant ecophysiological traits (CO_2 rate, leaf stomatal conductance, leaf water relation, carbon and nitrogen isotope composition) can help in integrating the growth and physiological characteristics and their interaction with external environment (Farquhar et al., 1989; Centritto et al., 2003; Condon et al., 2004; Hussain and Reigosa, 2015, 2017). Photosynthetic carbon isotope discrimination ($\Delta^{13}C$), provides a time-integrated measurement of the plant's transpiration efficiency (i.e. the ratio of carbon gain to water transpired) over the period during which dry matter is assimilated (Condon et al., 2002). Furthermore, carbon isotope discrimination also very helpful in evaluating the relation to long-term water and nutrient use efficiency (Araus et al., 1997, 2003; 2013; Adiredjo et al., 2014; Yousfi et al., 2012; Cernusak et al., 2009). Soil salinity results in the reduction of CO_2 rate, transpiration rate, and CO_2 uptake through decreased stomatal conductance (Farquhar et al., 1989). The decrease in stomatal conductance result in a low internal leaf CO_2 concentration and consequently, a decrease in CO_2 concentration at the carboxylation site of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), thereby decreasing net photosynthetic rate (Isla et al., 1998; Yousfi et al., 2009). By limiting transpiration, stomatal closure can also improve plant water use efficiency (WUE) and therefore indirectly influence productivity under salt and water stress (Yousfi et al., 2009). However, under field conditions, application of WUE has been largely limited by the time-consuming and expensive screening process in breeding programs due to large populations. Carbon isotope discrimination ($\Delta^{13}C$), through its negative relationship with transpiration efficiency has been demonstrated to be a simple but reliable measure of WUE (Farquhar et al., 1989; Farquhar and Richards, 1984; and their negative correlation has been used for indirect selection of WUE under selected environments (Adiredjo et al., 2014).

Differences in plant N isotope composition ($\delta^{15}N$) has been proposed as a useful attribute for screening, as it is linked to plant N metabolism, even though there is no precise knowledge of the underlying mechanisms or function (Handley et al., 1997; Robinson et al., 2000; Coque et al., 2006). Nitrogen isotopes have potential to provide integrated information for nitrogen fluxes, assimilation pathways and allocation (Evans, 2001). Different reports indicate that abiotic stresses such as salinity and drought can either decrease (Handley et al., 1999; Robinson et al., 2000) or increase $\delta^{15}N$ (Ellis et al., 2002; Lopes and Araus, 2006) relative to control. Osmotic stress caused by salinity, led to increase the $\delta^{15}N$ in the roots, but decrease in the leaves (Yousfi et al., 2012). In broccoli, it was found that osmotic and ionic stress caused by high salinity decreased the nitrogen isotope discrimination but increased carbon isotope discrimination in leaf dry matter (Del Amor and Cuadra-Crespo, 2011). In another study, Robinson et al. (2000), proposed that measuring the natural abundance of both $\delta^{13}C$, $\delta^{15}N$ may give an indication of responses to environmental stresses such as drought and nitrogen starvation. In this context, the natural abundance of carbon and nitrogen stable isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$) is widely used to study the physiology of salt tolerance in cereals such as in barley (Ellis et al., 2002). However, to our knowledge, few studies used C and N isotope ratios to assess salinity stress responses in quinoa.

Adaptation and tolerance of crop plants to salinity is generally associated with the induction of defense mechanisms for the protection of several physiological features. However, the underlying genotypic variability of quinoa with respect to physiological responses has not been well-documented. Understanding the physiological adaptive responses will assist breeders to identify key physiological process for salt tolerance breeding in this crop. Studying the genotypic variability with respect to agro-physiological and biochemical traits (plant height, number of branches, leaf C, N ionic concentration, C:N ratios, plant biomass, grain protein contents), yield stability traits (number of panicles, average panicle length, harvest index) and stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ can provide useful stress indicators that may explain the physiological basis for salt adaptation in quinoa. We hypothesis that signatures of $\delta^{13}C$ and $\delta^{15}N$ in the leaf dry matter might indicate genotypic tolerance to salinity better than other more conventional parameters, such as ion concentration, do. This is because the former directly reflect the effect of salinity on carbon and nitrogen metabolisms and thus on plant growth and yield. Meanwhile, the $\delta^{15}N$ and $\delta^{13}C$ and their correlation with biomass, seed yield and across growing conditions may provide some clues as how the salinity affects these physiological attributes. Quinoa's tolerance to salinity offers an alternate pathway, not only in terms of recovery of these lands, but also to produce food of high nutritional value.

2. Materials and methods

This study was conducted between November 2014 and May 2015 in the experimental field facilities located at the International Center for Biosaline Agriculture (ICBA, Dubai, U.A.E) and at CACATI (Centro de Apoio Cientifico Tecnologico a la Investigacion), University of Vigo, Spain. Two experimental studies were carried out to (i) assess the salinity tolerance potential of 6 quinoa genotypes (ii) identify the role that saline water plays in interfering the plant growth, development, yield and biochemical attributes (grain protein contents) (iii) signatures of stable isotope composition of $\delta^{13}C$, $\delta^{15}N$ was measured in leaf dry matter to evaluate the genotypic responses to salinity and pattern of relationships between $\delta^{15}N$ and $\delta^{13}C$ with seed yield and biomass among genotypes and across growth conditions. The 6 quinoa genotypes used in the present study was acquired from US Department of Agriculture (Table 1). The genotypes were selected based on their availability, experiment site, agriculture inputs and handling capacity. The climatic data during the experiment has been presented in Fig. 1. The research station has latitude of 25°13 N longitude of 55°17 E and experimental field soil is classified as the carbonatic, hyperthermic typic torripsamment with a negligible level of inherent soil salinity (0.2 dS m^{-1}). The soil has fine sand and moderately alkaline (pH 8.2) throughout the soil profile, and has a pH range 7.0–7.93, with low organic matter (< 0.5%), and electrical conductivity of the saturation extract (ECe) 1.37–3.44 dS m^{-1}.

2.1. Saline water treatment, irrigation scheduling and experimental design

The experiment was organized in a two-factor (quinoa accessions × salinity) factorial randomized complete block design with three replications. Each plot had 5 rows (3-m-long) spaced 50 cm apart and each row had 25 plants separated at 25 cm from each other and 1 m distance was maintained between two genotypes. Drip laterals of 16 mm in diameter had in-line emitters spaced 0.25 m apart, each

Table 1
List of quinoa (Chenopodium quinoa Willo.) genotypes used in this study.

S.No.	Code	Germination line	Source	Origin	Status	Seed color
1	Q 12	Chenopodium quinoa (Accession)	USDA	Colorado, USA	Cultivar	Light
3	Q 19	C.quinoa (Accession)	USDA	Bio-Bio, Chile	–	Light
6	Q 26	C. quinoa (Accession)	USDA	Chile	–	Light
7	Q 27	C. quinoa (Accession)	USDA	Chile	–	Light
9	Q 31	*Regalona Baer*	Imported	Chile		
10	AMES 22157	C. quinoa (Accession)	USDA	Chile		

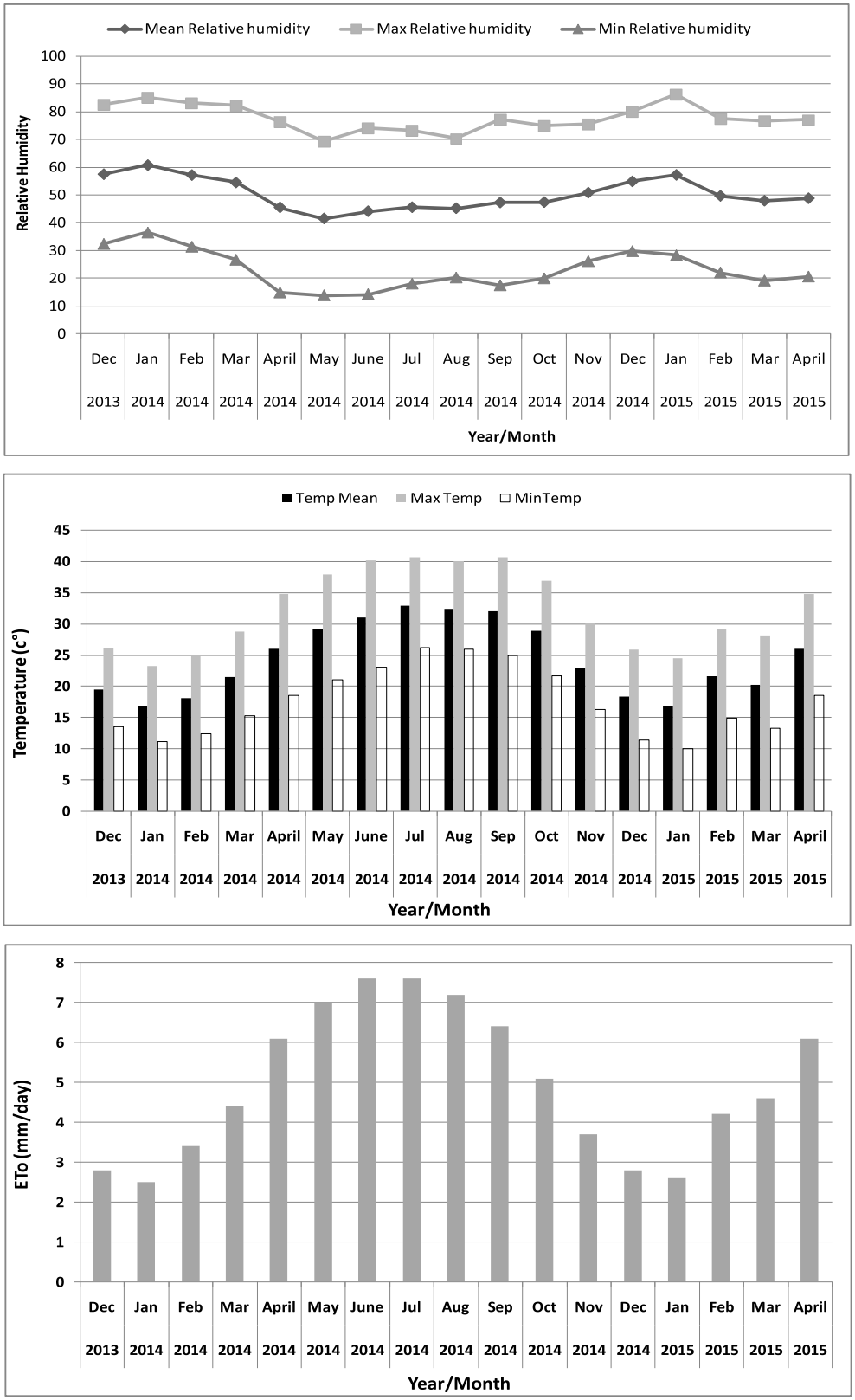

Fig. 1. Monthly average values of (a) mean (T mean), maximum (T maxi), and minimum (T mim) air temperature and reference evapotranspiratin (ETo) in the ICBA weather station, Dubai, UAE from December 2013 to April 2015.

delivering 4.0 L h^{-1} at an operating pressure of 100 kPa. The experimental plots were equipped with three irrigation valves from RainBird Company. One valve was handling fresh, other saline and third valve (Solinoid valve of 2" size) controlling both saline and fresh water after the main control valve and operate irrigation according to main controller instructions. The experimental field plots were supplied with saline water collected at a large reservoir; passing through the experimental area with main irrigation line. Salt treatment (EC_w = 0, 10,

20 dS m^{-1}) was initiated 30 days after seed sowing and continued till the end of the study using drip irrigation system.

2.2. Seedling establishment, plant growth and agronomic practices

Six quinoa (*C. quinoa* Willd.) genotypes are reportedly either salt tolerant or salt sensitive based on previous screening work for salinity tolerance in the field or under control conditions (own unpublished data) were used. Seeds were surface sterilized using 1% sodium hypochlorite, then repeatedly washed with distilled water, and soaked for 12 h in distilled water.

Seeds from quinoa genotypes were sown by hand in the rows at 50-cm row spacing on 26 November 2014. The seed bed was prepared by cultivating the field with disc plough and followed by harrowed to ensure an even seedbed. The organic fertilizer (pH 7.7; C:N 16.5; moisture 1.64%; organic matter 41%; N 1.5%; K 1.65%; Na 1.22%) from Al Bayadir®, Jabel Ali, Dubai, UAE) was applied (@ 40 t ha^{-1}) and incorporated into soil to improve the soil fertility. The chemical fertilizer {NPK (20:20:20)} from Growfert Solub™) was applied at the rate of 50 Kg ha^{-1} in two split doses by banding alongside the rows and is the recommended rate for the region. During the whole crop season, hand weeding was carried out when needed, without applying any herbicide. The research measurements were conducted from middle 1 m of the two central rows. Five plants were selected from each subplot to record the growth, physiological and biochemical measurements. The average plant height (cm) from ground surface to the tip of inflorescence on the main stem was measured at physiological maturity. The total number of branches from the main stem at different node positions, including the basal branches were recorded. At the time of harvest, from each plot, number of inflorescence per plant was counted from five plants and averaged. The mean length (cm) of three inflorescence was taken randomly and averaged. The fresh biomass was recorded at physiological maturity and dry matter yield was determined by drying the samples initially under the sun for two days and then in a forced-air oven at 80 °C for 48 h.

2.3. Grain yield

A sample line of 1 m length was harvested and seeds were removed from the panicle of plants/plot, threshed, and weighed (g m^{-2}) and then converted into t ha^{-1}.

2.4. Harvest index

Harvest index was calculated by using the following formula.

Harvest index (%) = Grain yield / dry biomass × 100

2.5. Stable carbon and nitrogen isotope analysis

Stable carbon and nitrogen isotope analysis was conducted at Isotopes and Mass Spectrometry Facility at CACATI (Centro de Apoio Cientifico Tecnologico a la Investigacion), University of Vigo, Spain. The leaf samples from each treatment/plot and control were collected, oven dried and ground into a fine powder. Total N and C contents (% dry matter) were measured by elemental analysis (Flash EA-1112, Swerte Germany). Dry ground plant material was weighed (1700–2100 μg) using high precision analytical balance (Metler Toledo GmbH, Greifensee, Switzerland), and filled in tin capsules (5 × 3.5 mm, Elemental Microanalysis Limited, U.K.). Tin capsules (pressed are in the shape of a microball) and combusted (1600–1800 °C) using an automated elemental analyser coupled to an Isotope Ratio Mass-Spectrometer (Finnegan: Thermo Fisher Scientific, model MAT-253, Swerte Germany). The Isotopic Ratio Mass Spectrometer has an analytical precision better than 0.3‰ for ^{15}N and 0.05‰ for ^{13}C.

Carbon and nitrogen isotope compositions were calculated as;

$$\delta\,(‰) = [(R_{sample}/R_{standard}) - 1)]\ \text{x}\ 1000 \quad (1)$$

Where R_{sample} is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and $R_{standard}$ were the standards used. Atmospheric N_2 was the standard for nitrogen while Vienna PeeDee Belemnite (VPDB) was the standard for carbon. The accuracy and reproducibility of the measurements of $\delta^{13}C$ and $\delta^{15}N$ were checked with an internal reference material (NBS 18 and IAEA-C6 for C), and (IAEA-310A and IAEA-N1 for N), and acetanilide for C/N % ratios, respectively.

Carbon isotope discrimination is a measure of the carbon isotopic composition in plant material relative to the value of the same ratio in the air on which plants feed:

$$\Delta\,(‰) = [(\delta a - \delta p)/\,(1 + \delta p)]\ \text{x}\ 1000 \quad (2)$$

Where Δ represents carbon isotope discrimination, δa and δp refer to $\delta^{13}C$ of air CO_2 and plant material, respectively.

Farquhar et al. (1989), Farquhar and Richards (1984) indicates that carbon isotope discrimination in leaves of plants can be expressed in relationship to CO_2 concentrations inside and outside of leaves in its simplest form as:

$$\Delta = a + (b - a)\,Ci/Ca$$

$$\Delta = 4.4 + (27 - 4.4)\,Ci/Ca \quad (3)$$

Where, *a* is discrimination that occurs during diffusion of CO_2 through the stomata (4.4‰), *b* is discrimination by Rubisco (27‰), and *Ci/Ca* is the ratio of the leaf intercellular CO_2 concentration to that in the atmosphere *Ci/Ca*-ratio of intercellular to atmospheric CO_2 concentration. Equation (3) establishes a direct and linear relationship between Δ and *Ci/Ca*. Therefore, measurement of Δ gives an estimation of the rate–weighed value of *Ci/Ca*.

2.5.1. Intrinsic water use efficiency (iWUE)

The term “intrinsic water-use efficiency” can be defined as the ratio of the instantaneous rates of CO_2 and transpiration at the stomata. Intrinsic water use efficiency (iWUE) was calculated according to the following equation:

$$\text{iWUE} = A/g = ca\,[1 - (Ci/Ca)]\ \text{x}\ (0.625) \quad (4)$$

Where, A is the rate of CO_2 and “*g*” is the stomatal conductance.

Carbon isotope discrimination ($\Delta^{13}C$), ratio of the leaf intercellular CO_2 concentration to that in the atmosphere (*Ci/Ca*) and intrinsic water use efficiency (iWUE) was determined according to the theory documented by Farquhar et al. (1989), Farquhar and Richards (1984). The close relationship between $\Delta^{13}C$ and *Ci/Ca* has been explained on the basis that the observed differences reflect the variation of *Ci/Ca* in the carboxylation step of photosynthesis, in response to environmental constraints that affect stomatal regulation. Both *Ci/Ca* and iWUE were derived from $\delta^{13}C$ basic data using equations (3) and (4) as reported previously (Hussain and Reigosa, 2012, 2017).

2.6. Grain protein contents measurements

From each quinoa genotypes, 200 mg FW (three replicates/treatment) were employed for quantification of grain protein contents using commercial bovine seroalbumin (BSA) through Bradford assays (Bradford, 1976) as reported previously (Hussain and Reigosa, 2011).

2.7. Statistical analysis

The quinoa genotypes agro-hysiological and biochemical responses to salinity and genotype (G) and environment interaction (G X S) on the studied parameters were analyzed through General Linear Modeling (GLM) procedure and analysis of variance (ANOVA) using the SPSS for Windows version 23.0 (SPSS Inc., Chicago, IL, USA). Difference

between treatments means were compared using Tukey's HSD test.

(1) The physiological parameters was divided into two categories; physiological traits ($\delta^{13}C$, $\Delta^{13}C$, *Ci/Ca*, iWUE, $\delta^{15}N$, N%, SY, HI, grain protein contents); agro-morphological traits (PDM, BN, PN). For each trait category, the genotype–treatment combinations (i.e. six genotypes crossed with three treatments) were subjected to GLM analysis to summarize the relative merit of genotypic effect and growing conditions as causes of changes in the plant attributes. A Pearson's correlation matrix was conducted to assess the relative contribution of ecophysiological trait associations towards the seed yield at overall salinity.
(2) A static yield stability index was calculated according to environmental variance (S^2) as documented by Roemer (1917). Meanwhile, a dynamic yield stability index was presented following Wricke's ecovalence (W^2) (Wricke, 1962).

3. Results

3.1. Effect of salt stress on agro-morphological characteristics

Plant dry biomass (PDM) was significantly affected following saline water treatment that results in 23.7% and 36% reduction in biomass at 10 and 20 dS m^{-1} respectively. The highest dry biomass was produced by Q19 (8.20 t ha^{-1}) and lowest by Q26 (5.59 t ha^{-1}) (Table 3). The plant height was decreased by 23% and 24% following salt treatment at 10 and 20 dS m^{-1}, respectively, compared to control (Table 2). AMES 22157 and Q12 exhibited higher plant height than other genotypes (Table 3). Quinoa genotypes (AMES 22157, Q27, Q26, Q12,) produced highest number of panicles/plant that was 16.22, 16.11, 15.9 and, 14.8, respectively and Q31 produced the lowest number of panicles (13.3). Salinity stress (10, 20 dS m^{-1}) significantly decreased panicle numbers and panicle length as compared to control. Similarly, Q12 and AMES 12157 produced highest panicle length (20.1 cm, 19.7 cm), followed by Q26 and Q27 that exhibit 17.9 and 17.3 cm, panicle length, respectively. The genotype Q31 exhibit the short panicle length (14.3 cm) than rest of the genotypes (Table 3).

3.2. Effect of salt stress on carbon (C) and nitrogen (N) and C/N ratios

The leaf C % was not much affected among the quinoa genotypes. The Q19 exhibited higher (30.2%) while Q26 showed lowest (28.8%) C concentration. The genotype effect was significant for N concentration, with values being higher in Q19, followed by AMES22157 genotype while Q26 showed minimum values for this traits (Table 3). Salinity decreased the C concentration at both levels (10 and 20 dS m^{-1}) as compared to control. The C/N ratios was significantly higher in control plants as compared to treated quinoa genotypes at 10 and 20 dS m^{-1}. Quinoa genotype Q26 has highest C/N ratio (13.30) that was significantly higher than all other genotypes. The present results indicate that N% increased after salinity treatments, compared to control, showing the highest values under medium and high salinity, respectively (Table 3).

3.3. Salinity impact on seed yield, water use efficiency, carbon and nitrogen isotope signature

Averaged across all genotypes, increased salinity generally decreased seed yield (Table 4). Seed yield was decreased by 62.6%, and 48.9% under 20, and 10 dS m^{-1} NaCl treatments, respectively, compared to the control (Table 4). Genotypes, AMES 22157, demonstrated higher seed yield (2.57 t ha^{-1}) followed by Q12 (2.04 t ha^{-1}) than all other genotypes. The lowest yield was produced by Q19 (1.08 t ha^{-1}) that was 58% less than the salt tolerant genotype AMES 22157 (Table 5). A continuous decrease in the values of harvest index (HI) was observed with increasing salinity level. Our results revealed that HI (%) was decreased to 56.83%, and 30.04% at 20, and 10 dS m^{-1} salinity, respectively, as compared to control (Table 4). The maximum values of HI was observed in genotype AMES 22157 (32.81%), followed by Q12 (30.22%). The mínimum HI values was documented in Q19 (14.91%) (Table 5).

The ratio of intercellular to ambient CO_2 concentration (*Ci/Ca*), were significantly less (0.588 and 0.642) after treatment with 20 and 10 dS m^{-1} as compared to control (0.739), indicating closing of stomata and inhibition of CO_2 (Table 4). The maximum value of *Ci/Ca* was observed in genotype AMES 22157 and Q26 (0.67), while a lowest value was found in Q31 (0.63) (Table 5). The intrinsic water use efficiency (iWUE) significantly increased following salinity treatment. Our results revealed that iWUE was increased to 58.45%, and 37.85% at 20, and 10 dS m^{-1} NaCl treatments, respectively, as compared to non-saline condition (Table 4). The maximum values of iWUE was observed in genotype Q31 (8.02), followed by Q27 (7.84). The minimum iWUE values was documented in Q26 (7.09) (Table 5). The $\delta^{13}C$ values were less negative (−25.25) and (−26.41) after treatment with saline water (20 and 10 dS m^{-1}) as compared to control (−28.52), respectively. Genotypic variability with respect to $\delta^{13}C$ was observed under both conditions. Genotypes AMES 22157 and Q26 showed higher values of $\delta^{13}C$ under salt stress condition. Quinoa, Q31, showed less negative values of $\delta^{13}C$ (−26.22).

The carbon isotope discrimination ($\Delta^{13}C$) values were higher in Q26 and AMES22157, 19.66 and 19.60, while lowest $\Delta^{13}C$ values was observed in Q31, respectively. A significant difference ($p > 0.05$) was observed after salinity treatment in carbon isotope discrimination ($\Delta^{13}C$), that was in the range of 19.66–18.71. Genotypic differences between pairs of tolerant and susceptible genotypes for $\delta^{15}N$ traits was also examined for salinity treatment. The leaf N concentration was higher in treated plants as compared to control plants. Quinoa genotype Q27 has significantly higher nitrogen isotope (12.58) values followed by Q31 (12.46) and Q19 (12.41) while the lowest $\delta^{15}N$ values was obtained in Q26 (10.55). The leaf N concentration and the $\delta^{15}N$ of

Table 2
Effect of salt stress on biomass and agro-physiological traits, and yield components across all quinoa genotypes.

	Plant Dry Biomass (t/ha)	Plant Height (cm)	Number of branches/plant	No. of Panicle/plant	Average panicle length (cm)	Leaf N%	Leaf C%	C:N ratio
Control	7.55a	96.88a	18.38a	16.16a	18.17a	2.21c	31.12a	14.85a
10 dS m-1 Nacl	5.76b	73.61b	14.66c	13.44c	17.44b	2.89ab	28.73b	10.09b
20 dS m-1 Nacl	4.83c	73.72b	17.11b	15.44b	17.37b	2.91a	28.36b	9.93c
Salinity Treatment (T)	**	**	ns	ns	ns	**	**	**
Genotype (G)	**	**	ns	ns	ns	**	ns	ns
T × G interaction	**	ns	ns	**	**	**	ns	ns

Values in a single column sharing the same letter are not significantly different ($p < 0.05$) according to Tukey's honestly significant difference (HSD) test. NS, (*), (**) are non-significant or significant at $p < 0.05$ or 0.001, respectively. Treatment values are the means of the 54 measurements (six genotypes and three replications per genotype).

Table 3
Quinoa genotype difference in biomass and agro-physiological traits, and yield components across all salinity treatments.

	Plant Dry Biomass (t/ha)	Plant Height (cm)	Number of branches/plant	No. of Panicle/plant	Average panicle length (cm)	Leaf N%	Leaf C%	C:N ratio
Accession Q12	7.37b	84.9b	16.33c	14.77bc	20.13a	2.6d	29.6b	11.74c
AMES 22157	7.94b	107a	18.11a	16.11a	19.7b	2.71b	29.12b	11.05c
Q26	7.02b	81b	17.88b	15.9b	17.89c	2.28c	28.83bc	13.3a
Q27	5.6c	68d	17.66b	16.22a	17.33c	2.67b	29.14b	11.75c
Q31	5.59c	75.5c	14.11d	13.33d	14.3e	2.63b	29.54b	12.04b
Q19	8.20a	72.11c	16.22c	13.7d	16.66d	3.14a	30.2a	9.87d

Genotype values are the means of 9 measurements (three treatments and three replications per treatment). Means with different letters within a column for a given trait are significantly different at $p < 0.05$) according to Tukey's honestly significant difference (HSD) test.

Table 4
Genotype and treatment effects on seed yield, harvest index, carbon and nitrogen isotope attributes of six quinoa genotypes grown under different water salinity levels.

	SY	HI	*Ci/Ca*	iWUE	$\delta^{13}C$	$\Delta^{13}C$	$\delta^{15}N$
Control	2.78a	36.65a	0.739a	5.68c	−28.52a	21.12a	10.75c
10 dS m^{-1}	1.42a	25.64a	0.642b	7.83b	−26.41b	18.90b	12.12b
20 dS m^{-1}	10.4c	15.82c	0.588c	9.00a	−25.52c	17.69c	12.66a
Salinity Treatment (T)	*	*	*	*	*	*	*
Genotype(G)	*	*	ns	ns	ns	ns	**
Tx G interaction	*	**	ns	ns	ns	ns	*

SY, Seed yield (t ha^{-1}); HO, harvest index (%); *Ci/Ca*, ratio of intercellar to ambient CO_2 concentration; iWUE, intrinsic water-use efficiency; $\delta^{13}C$, stable carbon isotope composition (‰).$\Delta^{13}C$ carbon isotope discrimination (‰). SY, seed yield (t ha^{-1}); $\delta^{15}N$, stable nitrogen isotope composition. Values in a single column sharing the same letter are not significantly different ($p^{<}0.05$) according to Tukey's honestly significant difference (HSD) test. NS, (*), (**) are non-significant or significant at $p^{<}0.05$ or o.001, respectively. Treatment values are the means of the 54 measurements (six genotypes and three replications per genotype).

Table 5
Genotype and treatment effects on seed yield, harvest index, carbon and nitrogen isotope attributes of six quinoa genotypes grown under different water salinity levels.

	SY	HI	*Ci/Ca*	iWUE	$\delta^{13}C$	$\Delta^{13}C$	$\delta^{15}N$
Genotypes Q12	2.04b	30.22b	0.65b	7.58b	−26.65b	19.16a	12.30a
AMES 22157	2.58a	32.81a	0.67a	7.15b	−27.07a	19.60a	10.75b
Q26	1.91c	27.25c	0.67a	7.09b	−27.12a	19.66a	10.55b
Q27	1.49d	24.42e	0.64b	7.84b	−26.39b	18.89b	12.58a
Q31	1.39d	26.61d	0.63b	8.02a	−26.22b	18.71b	12.46a
Q19	1.08e	14.91f	0.66b	7.34b	−26.88b	19.40b	12.41a

SY, Seed yield (t ha^{-1}); HI, harvest index (%); *Ci/Ca*, ratio of intercellular to ambient CO_2 concentration; $\delta^{13}C$, stable carbon isotope composition (‰). $\Delta^{13}C$, carbon isotope discrimination. SY, Seed yield (t ha^{-1}); $\delta^{15}N$, stable nitrogen isotope composition. Genotype values are the means of 9 measurements (three treatments and three replications per treatment). Means with different letters within a column for a given trait are significantly different at $p < 0.05$) according to Tukey's honestly significant difference (HSD) test.

tolerant genotypes was reduced to a greater extent than sensitive ones at all salinity stress, thus causing a significant G × T interaction (Table 5).

3.4. Grain protein contents

The grain protein contents (GP) changed significantly at all salt treatments. Significant increases ($P < 0.05$) in GP contents were observed in all genotypes following salt stress treatments relative to the controls (Fig. 2). GP contents were different according to genotypes over all NaCl concentrations. The highest GP contents were found in "Q19" and "Q31" (21.0 and 19.0 mg/g DW), respectively) and the lowest in "Q26" and "Q27" (16.2 and 16.5 mg/g DW protein) (Fig. 2), following salt stress treatment at 20 dS m^{-1}. GP contents remarkably increased 16.5 and 16.2 at 20 dS m^{-1} salinity, respectively, relative to control in genotypes Q27 and Q26. Although salt stress generally enhanced the activity of GP contents at 10 and 20 dS m^{-1} salinity compared to the control; while there was significant difference between 10 and 20 dS m^{-1} salinity treatments, except in genotype Q19 and AMES22157.

3.5. Trends in grain yield stability

The quinoa genotypes, demonstrated the highest mean grain yield across the treatments (mi), (Table 6). The quinoa genotypes exhibited very different scores for both static environmental variance (S^2) and dynamic Wricke's ecovalence (W^2). The static environment variance for grain yield among the 6 quinoa genotypes ranged from 0.127 to 3.265 while Wricke's ecovalence varied from 0.090 to 2.329. In these stability analysis, the lowest values demonstrate the stability in yield over saline environments. The variety 'Q31' was static stable and high yielder, ranking 1st for S^2i grain yield index across all saline environments. The quinoa 'Q12' and 'AMES22157' were found to produce the 2nd and 3rd highest static yield index across all treatments. The genotype 'Q12′ showed stable mean yield (W^2i) and ranked 1st among all the genotypes across all environments. Moreover, variety 'AMES22157' was static stable (S^2i) and high yielder, ranking the 2nd for W^2i grain yield index (Table 6).

3.6. Correlations between seed yield, agro-physiological and yield attributes

Pearson's correlations analysis showed significantly positive relationships between PH, TB, HI, C% and C:N ratio with seed yield (SY). However, significant and negative correlations were observed between N%, nitrogen and carbon isotopes, protein contents and seed yield (Table 7). The NOB and NOI exhibited significant + ve correlation with AIL, TB, C:N ratio while same showed –ve relation with N%, nitrogen isotope and protein contents. Biological yield displayed positive correlations with the N% and protein content. TB showed highly significant negative relation with HI, nitrogen and carbon isotopes attributes and C:N ratio. A significant negative correlation was exhibited between the HI and nitrogen and carbon isotopes and protein contents while same trait showed positive relation with C%. Both nitrogen and carbon isotopes showed positive correlation with protein content (Table 7). The carbon isotope discrimination ($\Delta^{13}C$) presented highly significant and positive correlation with seed yield (0.544), and HI (0.469) (Table 7). The relations between $\Delta^{13}C$ and C% and $\delta^{15}N$ was also highly significant and positive.

4. Discussion

Salt tolerance is a complex trait and attributed to a plethora of interconnected morphological, physiological and biochemical mechanisms. These mechanisms are linked to the major constraints of salinity on plant growth (osmotic effects, restriction of CO_2 gas exchange, ion

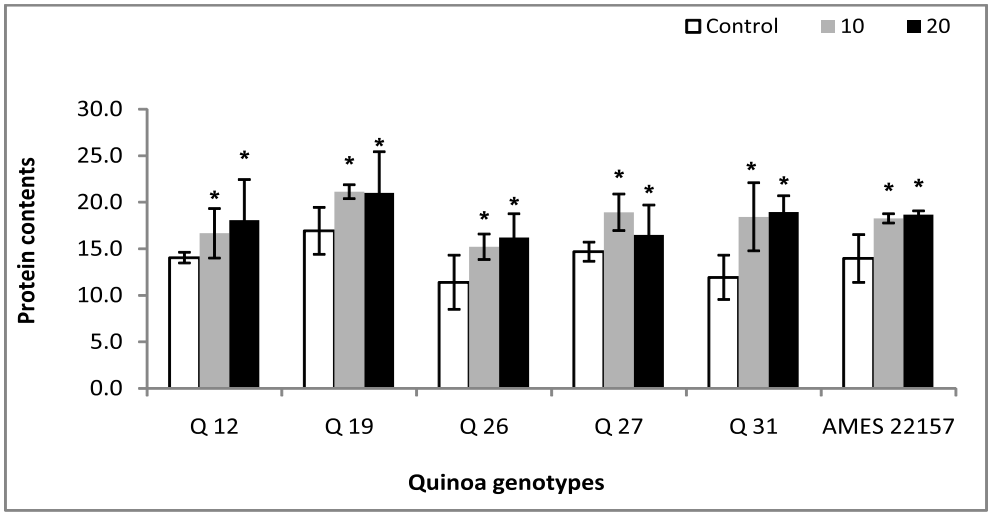

Fig. 2. Changes in grain protein contents (mg g^{-1}) in 6 quinoa genotypes following exposure to three different salinity levels (0, 10, 20 dS m^{-1}). Each bar represents the mean (± S.E.) of three replicates. *Asterisks indicate significant differences as compared to control for $p < 0.05$ according to Tukey's HSD test.

Table 6
Environmental variance (S2i) and Wricke's ecovalence (W2i) over the ambient treatment and three climate treatments for the 6 quinoa genotypes with highest averaged mean yield across treatments (mi).

S.No.	Genotypes name	mi	S2i	W2i
1	Q 12	2.043	0.517	0.090
2	Q 19	1.082	1.361	1.242
3	Q 26	1.919	1.052	0.169
4	Q 27	1.494	3.265	2.329
5	Q 31	1.398	0.127	1.533
6	AMES 22157	2.577	0.734	0.435

toxicity, and nutritional imbalance) and operate in coordination to alleviate both the cellular hyperosmolarity and ion disequilibrium (Flowers and Colmer, 2008; Geissler et al., 2010; Hussain et al., 2015). In this context, the screening and selection of salt tolerant quinoa genotypes is an important step to persue their adaptation under marginal and nutrient poor UAE sandy soils. Our results indicate significantly difference exist among tested traits of quinoa genotypes. The plant dry biomass was decreased in all the genotypes and highest reduction occurred in Q26. However, other researchers documented that salinity induced growth stimulation in Peruvian and Bolivian quinoa cultivars (Hariadi et al., 2011). The Peruvian quinoa cultivar "Hualhuas" was slightly increased after 20% seawater salinity (Eisa et al., 2012). Quinoa has the ability to adjust its leaf water potential by accumulating salt ions in tissues, enabling the plant to maintain cell turgor and limit the transpiration under saline conditions (Jacobsen et al., 2003, 2009). Several halophytes posses different physiological and biochemical mechanisms to tolerate salinity through sequestration of Na^+ and Cl^- (free osmolytes) in the cell vacuoles and use the compatible solutes fot osmotic adjustment in the cytosol (Shabala et al., 2012). About 85% osmotic adjustment occurs in quinoa young leaves through accumulation of inorganic ions in plants treated with saline water (Hariadi et al., 2011). Other workers also reported that quinoa has excellent tolerance to high salinity (Rosa et al., 2009; Hariadi et al., 2011). According to recent study, it was found that salinity significantly affects carbon metabolism, plant growth due to ionic toxicity, induced nutritional deficiency, water stress and oxidative damage (Munns and Tester, 2008; Araus et al., 2013; Hussain et al., 2016). In the present research, salinity stress caused significant reduction in the plant height and biomass. Similar results were reported by other colleagues (Munns and James, 2003; Yousfi et al., 2012). The reduction in plant growth

Table 7
Pearson's correlations among physiological and seed yield traits of Quinoa as a result of genotypic collective response for all three salinity levels. Grey color cells indicate no correlation between the different variables.

	SY	PH	NOB	NOI	AIL	TB	HI	N%	C%	CN Ratio	$\delta^{15}N$	$\Delta^{13}C$	Protein
SY	1												
PH	0.66**	1											
NOB	0.15	0.201	1										
NOI	0.11	0.172	.948**	1									
AIL	0.2	.320*	.383**	.429**	1								
TB	0.25*	.319**	.275**	0.213	.276**	1							
HI	0.841**	.496**	-0.024	-0.035	0.062	-.179**	1						
N%	-.475**	-.283**	-.304**	-.349*	-.261**	.180**	-.492**	1					
C%	.374**	.396**	0.066	-0.033	-0.094	0.346	.218**	0.004	1				
CN Ratio	.540**	.337**	.313**	.339**	0.173	-.028**	.515**	-.924**	.236**	1			
$\delta^{15}N$	-.338**	-.379**	-.359**	-.410**	-0.178	-.107**	-.282**	.576**	-0.203	-.590**	1		
$\Delta^{13}C$	.544**	.514**	0.108	0.077	0.068	.024**	.469**	.475**	.592**	.559**	.508**	1	
Protein	-.475**	-0.283	-0.304	-0.349	-0.261	.180**	-.492**	.	.004**	-.924**	.576**	.475**	1

**Correlation significant at p > 0.05 according to Tukey's HSD test; SY: Seed yield, PH: Plant Height, NOB, Branch number; NOI: Influorescence number, AIL: Average Influorescence Length, TB: Total Biomass, HI: Harvest Index, N%, Nitrogen concentration; C%, Carbon concentration; δ15N, Nitrogen isotope composition; δ13C, Carbon isotope composition.

attributes (height, biomass) were mainly consequences of inhibition of cell growth and cell division due to Na^+ accumulation (Munns and Tester, 2008). Restriction of leaf growth is the first visible toxicity of sainity, due in part to reduce hydraulic conductance in plants (Steudle, 2000; Taleisnik et al., 2009).

Crop yield is directly correlated to the leaf growth and leaf area development, photosynthesis, and nitrogen utilisation (Hay and Porter, 2006). Salinity reduces leaf growth and limits the grain yield and yield attributes (Taleisnik et al., 2009). The presents results demonstrate that quinoa seed yield was decreased and varied from 48 to 62% when salinity increased from 10 to 20 dS m^{-1}. Meanwhile, ratio of intercellular to ambient CO_2 concentration (*Ci*/*Ca*), were significantly less after salinity treatment indicate closing of the stomata (Table 4). The stomatal closure can restrict the CO_2 supply to carboxylation sites, and thus reduced the activity of Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), carbon synthesis and translocation (Farquhar and Richards, 1984; Isla et al., 1998; Yousfi et al., 2009), storage (Rivelli et al., 2002; Farquhar et al., 2007) and ultimately grain yield. Other workers also demonstrated that stomatal closure (e.g. water stress, either directly or through salinity) affect plant growth via reduced carbon assimilation and its transfer to the sink (i.e. grain) which ultimately lower the SY (Araus et al., 2013). There was significant variation in the seed yield and harvest index between AMES22157 and Q12 that might be due to variation in their genetic makeup. Such differences are even more evident in genotypes Q6 and Q27 which showed 3% HI variation. Different quinoa genotypes were evaluated for their salinity tolerance potential showed higher *Ci*/*Ca*. and yield potential that demonstrate their adaptation to Dubai climatic condition. This reflects the greater adaptability of some of the quinoa genotypes to the agro-climatic conditions of UAE. This is in agreement with Jacobsen (2003), who confirmed that the Chilean lines were more suited to new areas (because of less sensitivity to the photoperiod), which gives them good yield stability.

The carbon isotope discrimination can provides an integrated measure of stomatal control of internal CO_2 concentration and elaborate the long–term photosynthetic carbon of C_3 plant species (Farquhar and Richards, 1984; Merah et al., 1999). The $\Delta^{13}C$ was higher in Q26 and AMES22157 and lowest Δ in Q31. According to present results, genotypes AMES 22157 and Q12 showed highest photosynthetic CO_2 rate (*Ci*/*Ca*), yield and productivity and were most suitable and well -adapted genotypes under Dubai marginal soil environment. By contrast Q19 and Q31 exhibited lowest rates (5.8 fold lower *Ci*/*Ca* than AMES 22157 and Q12) being the less adapted ones. Remaining genotypes (Q26 and Q27) did not show significant differences in *Ci*/*Ca*-ratio of intercellular to atmospheric CO_2 concentration. In general, under water limited and saline conditions; stomatal conductance becomes the controlling point at which the net flux of CO_2 into the leaf can be regulated (Lawlor, 2002). These results were agreement with those reported by Adolf et al. (2012), whom found the significant differences in both photosynthetic CO_2 and stomatal conductance among tested genotypes under salinity stress. Genotype, “Utusaya,” demonstrated only 25% reduction in net CO_2 due to high stomatal conductance while “Titicaca” showed a significant reduction in stomatal conductance (67%) and CO_2. Utusaya variety has a genetically improved osmoregulator mechanism to counteract the deleterious osmotic effects of salt stress and has less need to reduce water loss by transpiration (Adolf et al., 2013). Other researchers demonstrated that salt stress caused osmotic stress coupled with decrease in the leaf water content ultimately lead to decrease in the quinoa grain yield and carbon isotope discrimination (González et al., 2011). The ratio of intercellular to ambient CO_2 concentration (*Ci*/*Ca*), was less than control plants, that is a typical indication of stomata closing and inhibition of CO_2. Therefore, we consider that genotypic differences in CO_2 observed in quinoa genotypes can well be explained by salinity-induced changes in stomatal conductance. Consistent with this observation, it has been postulated that quinoa stomatal conductance is a heritable trait associated with both abiotic-stress avoidance and yield increase (Jensen et al., 2000).

The C and N balance between sink and source strengths will be an essential objective for maximising the response of crops to growth under different C and N availability conditions (Aranjuelo et al., 2013). With this aim in mind, through the use of stable isotopes, the allocation and partitioning of C and N throughout the plant and between organs can be traced. The C:N ratios are considered an important characteristics that might play a significant role in the carbon sequestration potential of that particular crop and variety. In the present study, quinoa genotype Q19 showed higher C% and N% as compared to all other genotypes. It might be due to higher nitrogen uptake and C accumulation by Q19 and low uptake in Q26. Several authors have reported that different crops and genotypes showed different C:N, and it was varied in sorghum, rice, finger millet (Kushwah et al., 2014). Carbon, nitrogen ratios in plants are also affected by concentration of N in labile pool, root proliferation for N (Dotaniya et al., 2013), crop growth pattern and plant species (Lemaire et al., 1985). Based on the C:N ratio and yield of the crop biomass, the carbon sequestration potential of a particular crop can be calculated (Lakaria et al., 2012).

In comparison to $\delta^{13}C$, relatively few studies have addressed genotypic variation in plant $\delta^{15}N$ in response to stress conditions, and mostly to drought (Robinson et al., 2000; Evans, 2001; Peuke et al., 2006). Difference in the N isotope composition ($\delta^{15}N$) has been proposed as a useful trait for screening, as it is linked to plant N metabolism, even though there is no precise knowledge of the underlying mechanisms or function (Pritchard and Guy, 2005; Coque et al., 2006). In this study, salinity stress significantly increased the $\delta^{15}N$ and highest nitrogen isotope values was observed in Q27 while lowest values in Q26. Previously, it was documented that abiotic stresses (salinity, drought, allelochemical) can either decrease (Handley et al., 1997; Robinson et al., 2000) or increase $\delta^{15}N$ relative to controls (Hussain and Reigosa, 2014). Different nitrogen uptake mechanisms and pathways, can participate in discriminate against ^{15}N (Evans, 2001). Moreover, plants depend not only on the $\delta^{15}N$ of N sources, but also on the balance between enzyme activity and external concentration. Such patterns contrast with the well established decrease in $\Delta^{13}C$ (or increase in $\delta^{13}C$) associated with these stresses in the same studies, and illustrate the relative complexity of the mechanisms determining $\delta^{15}N$ signatures in plants. Except for leaf N concentration, no significant G × T interaction was detected among other traits. Moreover, both $\delta^{13}C$ and $\delta^{15}N$ have been used to phenotype the response of quinoa mapping populations to salinity, as the natural abundances of these isotopes are strongly affected by salinity and there is genotypic variability in both stable isotopes. However, further research is necessary in order to understand the mechanisms controlling the quinoa plant C isotope discrimination that will further enhance our knowledge of the acquisition and allocation of N and C in plants under different climate scenarios.

The correlations between various agro-morphological attributes and the relative yield of quinoa genotypes at overall salinity were shown in Table 7. There was positive correlation existed between various traits (NOB, NOI and other attributes like AIL, TB, C:N ratio), while same traits (NOB, NOI) showed –ve correlation with N%, nitrogen isotope composition and protein contents. The $\Delta^{13}C$ exhibited significant and positive correlation ($p > 0.05$) with seed yield. Several researchers have documented that simultaneously increasing the WUE of crops will lead to increase the yield and productivity (Richards et al., 2002). The association between yield potential, **Δ**, and WUE is often misunderstood, which in turn was leading to conceptual oversight and wrong decisions in implementing breeding programs for drought prone environments (Blum, 2005). Confusion was largely provoked by the fact that the relations between Δ (or WUE) and yield were positive or negative, and sometimes there was no correlation (Ngugi et al., 1996), depending on the crop and growing conditions.

5. Conclusion

Stable C and N isotope ratios of terrestrial plants have the potential to provide unique insights into physiological processes and their interactions with surrounding environment. Continued research into environmental and physiological determinants of Δ will further increase this potential. For C_3 plants (like quinoa), a priority should have to understand the mechanisms that lead to coordination between stomatal conductance, *Ci/Ca*, thereby muting the response of crop plants to salt stress. This is also important for engineering plants that have both high water use efficiency and high photosynthetic capacity. These results are encouraging regarding the possibility of using Δ as an effective selection index in quinoa to obtain genotypes with high WUE. The development of genotypes having both high yield performance and a higher WUE would be a very useful contribution for producers in the dry regions like Arabian Desert environment. AMES22157 and Q12 were proved to be superior genotypes that demonstrated the high seed yield that might be used as genetic material for future use in breeding programs and for scaling up in the Arabian Peninsula. Although, our study is only a first approach to test the relationship between Δ and seed yield in quinoa, it highlighted the complexity of determining a definitive relationship between these two parameters under salinity. The combined measurement of $\Delta^{13}C$ and $\delta^{15}N$ in plant tissues is of particular interest in crop management and breeding due to their relationship to photosynthetic, nitrogen uptake, translocation and yield performance. This may eventually help agronomists and plant breeders to identify crop management practices and to select genotypes that are better suited to many different combinations of growing conditions.

Author contributions

M.I.H., designed and perform research, collected data; A.J.D, analyzed data, critical review of the article, obtaining of funding; M.J.R., Critical review of the article.

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References

Adiredjo, A.L., Navaud, O., Lamaze, T., Grieu, P., 2014. Leaf carbon isotope discrimination as an accurate indicator of water-use efficiency in sunflower genotypes subjected to five stable soil water contents. J. Agron. Crop Sci. 200, 416–424.

Adolf, V.I., Jacobsen, S.E., Shabala, S., 2013. Salt tolerance mechanisms in quinoa (*Chenopodium quinoa* Willd.). Environ. Exp. Bot. 92, 43–54.

Adolf, V.I., Shabala, S., Andersen, M.N., Razzaghi, F., Jacobsen, S.E., 2012. Varietal differences of quinoa's tolerance to saline conditions. Plant Soil 357, 117–129.

Al-Dakheel, A.J., Hussain, M.I., 2016. Genotypic variation for salinity tolerance in *Cenchrus ciliaris* L. Front. Plant Sci. 7, 1090.

Araus, J.L., Cabrera-Bosquet, L., Serret, M.D., Bort, J., Nieto-Taladriz, M.T., 2013. Comparative performance of $\delta^{13}C$, $\delta^{18}O$, and $\delta^{15}N$ for phenotyping durum wheat adaptation to a dry land environment. Funct. Plant Biol. 40, 595–608.

Araus, J.L., Villegas, D., Aparicio, N., García del Moral, L.F., El Hani, S., Rharrabti, Y., Ferrio, J.P., Royo, C., 2003. Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. Crop Sci. 43, 170–180.

Araus, J.L., Bort, J., Ceccarelli, S., Grando, S., 1997. Relationship between leaf structure and carbon isotope discrimination in field grown barley. Plant Physiol. Biochem. 35, 533–541.

Aranjuelo, I., Tcherkez, G., Molero, G., Gilard, F., Avice, J.C., Nogués, S., 2013. Concerted changes in N and C primary metabolism in alfalfa (*Medicago sativa*) under water restriction. J. Exp. Bot. 64, 1–17.

Bradford, M.M., 1976. A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Ann. Biochem 72, 248–254.

Bazile, D., Baudron, F., 2015. The dynamics of the global expansion of quinoa growing in view of its high biodiversity. In: Bazile, D., Bertero, H.D., Nieto, C. (Eds.), State of the Art Report on Quinoa Around the World in 2013. FAO and CIRAD, Rome, pp. 42–55.

Blum, A., 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Aust. J. Agric. Res. 56, 1159–1168.

Centritto, M., Loreto, F., Chartzoulakis, K., 2003. The use of low CO_2 to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt stressed olive saplings. Plant Cell Environ. 26, 585–594.

Cernusak, L.A., Winter, K., Turner, B.L., 2009. Physiological and isotopic ($\delta^{13}C$ and $\delta^{18}O$) responses of three tropical tree species to water and nutrient availability. Plant Cell Environ. 32, 1441–1455.

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D., 2004. Breeding for high water-use efficiency. J. Exp. Bot. 55, 2447–2460.

Condon, A.G., Richards, R.A., Rebetzke, G.R., Farquhar, G.D., 2002. Improving intrinsic water-use efficiency and crop yield. Crop Sci. 42, 122–131.

Coque, M., Bertin, P., Hirel, B., Gallais, A., 2006. Genetic variation and QTLs for ^{15}N natural abundance in a set of maize recombinant inbred lines. Field Crop. Res. 97, 310–321.

Del Amor, F., Cuadra-Crespo, P., 2011. Alleviation of salinity stress in broccoli using foliar urea or methyl-jasmonate: analysis of growth, gas exchange, and isotope composition. Plant Growth Regul. 63, 55–62.

Dotaniya, M.L., Datta, S.C., Biswas, D.R., Meena, B.P., 2013. Effect of solution phosphorus concentration on the exudation of oxalate ions by wheat (*Triticum aestivum* L.). Proc. Natl. Acad. Sci., India, Sect. B Biol. 83, 305–309.

Eisa, S., Hussin, S., Geissler, N., Koyro, H.W., 2012. Effect of NaCl salinity on water relations, photosynthesis and chemical compositionof quinoa (*Chenopodium quinoa* Willd.) as a potentialcash crop halophyte. Aust. J. Crop Sci. 6, 357–368.

Ellis, R.P., Forster, B.P., Gordon, D.C., Handley, L.L., Keith, R.P., Lawrence, P., Meyer, R., Powell, W., Robinson, D., Scrimgeour, C.M., Young, G., Thomas, W.T.B., 2002. Phenotype/genotype associations for yield and salt tolerance in a barley mapping population segregating for two dwarfing genes. J. Exp. Bot. 53, 1163–1176.

Evans, R.D., 2001. Physiological mechanism influencing plant nitrogen isotope composition. Trends Plant Sci. 6, 121–126.

Farquhar, G.D., Cernusak, L.A., Barnes, B., 2007. Heavy water fractionation during transpiration. Plant Physiol. 143, 11–18.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. 40, 503–537.

Farquhar, G.D., Richards, R.A., 1984. Isotope compositionof plant carbon correlates with water use efficiency of wheat genotypes. Aust. J. Plant Physiol. 11, 539–552.

Flowers, T.J., Colmer, T.D., 2008. Salinity tolerance in halophytes. New Phytol. 179, 945–963.

Geissler, N., Hussin, S., Koyro, H.W., 2010. Elevated atmospheric CO2 concentration enhances salinity tolerance in Aster tripolium L. Planta 231, 583–594.

González, J.A., Bruno, M., Valoy, M., Prado, F.E., 2011. Genotypic variation of gas exchange parameters and leaf stable carbon and nitrogen isotopes in ten quinoa cultivars grown under drought. J. Agron. Crop Sci. 197, 81–93.

Handley, L.L., Austin, A.T., Robinson, D., Scrimgeour, C.M., Raven, J.A., Heaton, T.H.E., Schmidt, S., Stewart, G.R., 1999. The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects measures of water availability. Aust. J. Plant Physiol. 26, 185–199.

Handley, L.L., Robinson, D., Forster, B.P., Ellis, R.P., Scrimgeour, C.M., Gordon, D.C., Nero, E., Raven, J.A., 1997. Shoot $\delta^{15}N$ correlates with genotype and salt stress in barley. Planta 201, 100–102.

Hariadi, Y., Marandon, K., Tian, Y., Jacobsen, S.E., Shabala, S., 2011. Ionic and osmotic relations in 10 quinoa (*Chenopodium quinoa* Willd.) plants grown at various salinity levels. J. Exp. Bot. 62, 185–193.

Hay, R.K., Porter, J.R., 2006. The Physiology of Crop Yield. Blackwell Publishing.

Hussain, M.I., Lyra, D.A., Farooq, M., Nikoloudakis, N., Ahmad, N., 2016. Salt and drought stresses in safflower: a Review. Agron. Sustain. Dev. 36, 4.

Hussain, M.I., Reigosa, M.J., 2011. Allelochemical stress inhibits growth, leaf water relations, PSII photochemistry, non-photochemical fluorescence quenching and heat energy dissipation in three C_3 perennial species. J. Exp. Bot. 62, 4533–4545.

Hussain, M.I., Reigosa, M.J., 2012. Seedling growth, leaf water status and signature of stable carbon isotopes in C_3 perennials exposed to natural phytochemicals. Aust. J. Bot. 60, 676–684.

Hussain, M.I., Reigosa, M.J., 2014. Higher peroxidase activity, leaf nutrient contents and carbon isotope composition changes in *Arabidopsis thaliana* are related to rutin stress. J. Plant Physiol. 171, 1325–1333.

Hussain, M.I., Reigosa, M.J., 2015. Characterization of xanthophyll pigments, photosystem II photochemistry, heat energy dissipation, reactive oxygen species generation and carbon isotope discrimination during artemisinin-induced stress in Arabidopsis thaliana. PLoS One 10, e0114826.

Hussain, M.I., Reigosa, M.J., 2017. Evaluation of photosynthetic performance and carbon isotope discrimination in perennial ryegrass *(Lolium perenne* L.) under allelochemicals stress. Ecotoxicology 26, 613–624.

Hussain, M.I., Reigosa, M.J., Al-Dakheel, A.J., 2015. Biochemical, physiological and isotopic responses to natural product *p*-hydroxybenzoic acid in Cocksfoot (*Dactylis glomerata* L.). Plant Growth Regul. 75, 783–792.

Isla, R., Aragüés, R., Royo, A., 1998. Validity of various physiological traits as screening criteria for salt tolerance in barley. Field Crop. Res. 58, 97–107.

Jacobsen, S.E., Liu, F., Jensen, C.R., 2009. Does root-sourced ABA play a role for regulation of stomata under drought in quinoa (*Chenopodium quinoa* Willd.). Sci. Hortic. 122, 281–287.

Jacobsen, S.E., Mujica, A., Jensen, C.R., 2003. The resistance of quinoa (*Chenopodium*

quinoa Willd.) to adverse abiotic factors. Food Rev. Int. 19, 99–109.
Jacobsen, S.E., 2003. The worldwide potential of quinoa (*Chenopodium quinoa* Willd.). Food Rev. Int. 19, 167–177.
Jacobsen, S.E., Monteros, C., Corcuera, L.J., Bravo, L.A., Christiansen, J.L., Mujica, A., 2007. Frost resistance mechanisms in quinoa (*Chenopodium quinoa* Willd.). Eur. J. Agron. 26, 471–475.
Jamil, A., Riaz, S., Ashraf, M., Foolad, M.R., 2011. Gene expression profiling of plants under salt stress. Crit. Rev. Plant Sci. 30, 435–458.
Jensen, C.R., Jacobsen, S.E., Andersen, M.N., Núñez, N., Andersen, S.D., Rasmussen, L., Mogensen, V.O., 2000. Leaf gas exchange and water relation characteristics of field quinoa (*Chenopodium quinoa* Willd.) during soil drying. Eur. J. Agron. 13, 11–25.
Koyro, H.W., Eisa, S.S., 2008. Effect of salinity on composition, viability and germination of seeds of *Chenopodium quinoa* Willd. Plant Soil 302, 79–90.
Kushwah, S.K., Dotaniya, M.L., Upadhyay, A.K., Rajendiran, S., Coumar, M.V., Kundu, S., Subba Rao, A., 2014. Assessing carbon and nitrogen partition in kharif crops for their carbon sequestration potential. Natl. Acad. Sci. Lett. 37, 213–217.
Lakaria, B.L., Singh, M., Reddy, K.S., Biswas, A.K., Jha, P., Choudhary, R.S., Singh, A.B., Rao, A.S., 2012. Carbon addition and storage under integrated nutrient management in soybean-wheat cropping sequence in a Vertisol of central India. Natl. Acad. Sci. Lett. 35, 131–138.
Lawlor, D.W., 2002. Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. Ann. Bot. 89, 871–885.
Lemaire, G., Cruz, P., Gosse, G., Chartier, M., 1985. Study of the relationship between the dynamics of levy nitrogen dynamics and dry matter growth of a stand of alfalfa (*Medicago sativa* L.). Agron. J. 5, 685–692.
Lopes, M., Araus, J.L., 2006. Nitrogen source and water regime effects on durum wheat photosynthesis, and stable carbon and nitrogen isotope composition. Physiol. Plantarum 126, 435–445.
Merah, O., Deleens, E., Monneveux, P., 1999. Grain yield, carbon isotope discrimination, mineral and silicon content in durum wheat under different precipitation regimes. Physiol. Plantarum 107, 387–394.
Munns, R., 2011. Plant adaptations to salt and water stress: differences and commonalities. Adv. Bot. Res. 57, 1–32.
Munns, R., James, R.A., 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. Plant Soil 253, 201–218.
Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651–681.
Ngugi, E., Austin, R., Galwey, N., Hall, M., 1996. Associations between grain yield and carbon isotope discrimination in cowpea. Eur. J. Agron. 5, 9–17.
Peuke, A.D., Gessler, A., Rennenberg, H., 2006. The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes. Plant Cell Environ. 29, 823–835.
Pritchard, E.S., Guy, R.D., 2005. Nitrogen isotope discrimination in white spruce fed with low concentrations of ammonium and nitrate. Trees Struct. Funct. 19, 89–98.
Richards, R., Rebetzke, G., Condon, A., Van Herwaarden, A., 2002. Breeding opportunities for increasing the efficiency of water use and crop yield in temperate cereals. Crop Sci. 42, 111–121.
Rivelli, A.R., James, R.A., Munns, R., Condon, A.G., 2002. Effects of salinity on water relations and growth of wheat genotypes with contrasting sodium uptake. Funct. Plant Biol. 29, 1065–1074.
Robinson, D., Handley, L.L., Scrimgeour, C.M., Gordon, D.C., Forster, B.P., Ellis, R.P., 2000. Using stable isotope natural abundances ($\delta^{15}N$ and $\delta^{13}C$) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. J. Exp. Bot. 51, 41–50.
Roemer, T., 1917. Sind die ertragsreichen Sorten ertragssicherer? Mitteilung Deutsche Landwirtschafts-Gesellschaft 32, 87–89.
Rosa, M., Hilal, M., González, J.A., Prado, F.E., 2009. Low-temperature effect on enzyme activities involved in sucrose-starch partitioning in salt-stressed and salt-acclimated cotyledons of quinoa (*Chenopodium quinoa* Willd.) seedlings. Plant Physiol. Biochem. 47, 300–307.
Shabala, L., Mackay, A., Tian, Y., Jacobsen, S.E., Zhou, D., Shabala, S., 2012. Oxidative stress protection and stomatal patterning as components of salinity tolerance mechanism in quinoa (*Chenopodium quinoa*). Physiol. Plantarum 146, 26–38.
Steudle, E., 2000. Water uptake by plant roots: an integration of views. Plant Soil 226, 46–56.
Taleisnik, E., Rodriguez, A., Bustos, D., Erdei, L., Ortega, L., Senn, M.E., 2009. Leaf expansion in grasses under salt stress. J. Plant Physiol. 166, 1123–1140.
Vega-Gálvez, A., Miranda, M., Vergara, J., Uribe, E., Puente, L., Martínez, E.A., 2010. Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* willd.), an ancient Andean grain: a review. J. Sci. Food Agric. 90, 2541–2547.
Wricke, G., 1962. Uber eine Methode zur Erfassung der okologischen Streubreite in Feldversuchen. Z. Pflanzenzuchtg 47, 92–96.
Yousfi, S., Serret, M.D., Araus, J.L., 2009. Shoot $\delta^{15}N$ gives a better indication than ion concentration or $\Delta^{13}C$ of genotypic differences in the response of durum wheat to salinity. Funct. Plant Biol. 36, 144–155.
Yousfi, S., Serret, M.D., Márquez, A.J., Voltas, J., Araus, J.L., 2012. Combined use of $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ tracks nitrogen metabolism and genotypic adaptation of durum wheat to salinity and water deficit. New Phytol. 194, 230–244.