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QUINOA AS A NEW LEAFY VEGETABLE CROP IN EGYPT

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ABSTRACT

The present work was aimed to evaluate *Chenopodium quinoa* cultivar CICA (*Chenopodium quinoa* Willd. cv. CICA), in field experiments, as a new and non-traditional leafy crop in Egypt under saline (EC_e 17.9 dSm^{-1}) and non-saline (EC_e 1.9 dSm^{-1}) soil conditions. Production of biomass, some morphological, physiochemical and yield components traits were estimated at 40 days from sowing date. Biomass production of young quinoa shoot under saline soil was significantly higher by 25% than non-saline soil. Quinoa plants cultivated under saline soil also showed significant high performances for most of morphological traits. Although salinity led to accumulate Na^+ concentrations in the leaves by six folds higher than that found in the leaves produced under non-saline soil conditions, but no significant reduction has been observed for K^+ concentrations. Moreover, salinity was significantly increased magnesium concentrations in quinoa leaves. On the other hand, no significant increase has been detected of proline or total soluble carbohydrates concentrations in leaves of quinoa grown under saline soil as compared to non-saline soil. This clearly indicated that quinoa plants, during early growth stage, tended to utilize inorganic ions rather than organic solutes to regulate its osmotic potential under saline conditions. Chlorophyll a, chlorophyll b and carotenoid concentrations were significantly decreased under saline soil. Also, concentrations of crude fiber, crude fat and iron in the leaves of quinoa plants grown under saline soil conditions were significantly decreased. Meanwhile, salinity has no significant influence on crude protein concentrations. These results revealed that the quinoa has the ability to grow and produce considerable high leafy vegetable yield with good quality, in terms of high protein, in land unsuitable for conventional vegetable crops.

INTRODUCTION

Salinity is one of the most deleterious abiotic stresses that limits the development and productivity of crop especially in arid and semi-arid regions. Using salt tolerant species that can tolerate high salinity in soil and allow irrigation with saline water is one of the options proposed recently to mitigate and counteract the adversely effects of salinity in agricultural production **(Munns & Tester, 2008 and Koyro et al 2008)**. *Chenopodium quinoa* Willd. is one of the promising candidates for sustainable agriculture in salt affected regions **(Eisa et al 2017)**. *Chenopodium quinoa* is a facultative halophyte and could be used as an alternative cash crop for land and water unsuitable for conventional crops in arid and semi-arid regions (**Eisa et al 2005**). Quinoa attracted worldwide attention, during the recent time, because of its exceptional tolerance to various unfavorable environmental conditions (**Choukr-Allah et al 2016**). Quinoa has the ability to grow and complete its life cycle under high salinity levels equal to those found in seawater (**Koyro & Eisa, 2008; Shabala et al 2013 and Panuccio et al 2014**). However, there have been extensive studies carried out on the potential of quinoa as a grain crop either under non-saline or saline conditions, but, there have been very few field trials set up to evaluate the potential of quinoa as a leafy vegetable crop. In this concern, **Bhargava et al (2010**) reported that the foliage of many

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species of *Chenopodium* (*C. album*, *C. murale, C. bushiamum, C.giganteum, C. murale, C. quinoa and C. ugandea*) is a rich source of minerals like calcium, potassium, iron and sodium. **Tang et al (2014)** added that quinoa leaves had high concentrations of carotenoids and xanthophyll's. For example, Carotenoids play an important role for human nutrition as precursors to vitamin A, beside its role as antioxidants **(Bhargava et al 2007)**. On the other hand, it is important to take into consideration that successful use of quinoa, as a cash halophyte crop, for leafy vegetable potential depends on its biomass production, nutritive value under saline condition. Therefore, this work is may be the first report for evaluating biomass production and its nutritive value of quinoa cultivated for vegetable purpose under high salinity condition.

MATERIALS AND METHODS

Field trail experiments were conducted to evaluate the growth performance of quinoa plants in two different locations. The first location was characterized by high saline soil (ECe 17.9 dSm^{-1}) and located in northwestern part of Sinai (32° 28' 44" N, 31° 56' 35" E). The second location was characterized by non-saline soil (ECe 1.9 dSm^{-1}) and located in north Cairo at the experimental station of the Faculty of Agriculture, Ain Shams University (30° 06' 48" N, 31° 14' 52" E)**.**

Soil and water analyses of the selected locations

Soil samples from non-saline and saline locations were collected before sowing to a depth of 0-30 cm. The collected soil samples were air dried ground and sieved through 2 mm stainless steel sieve and stored in plastic vials for different analyses. Soil pH value was determined in the soil paste by using a glass electrode pH meter. Electrical conductivity (ECe) was determined for saturated extract by using standard conductivity bridge at 25^{o}C. Soil physical and chemical prosperities **(Table 1)**, and water chemical proprieties **(Table 2)**, were assessed according to the standard methods published by **Page et al (1982).**

Soil preparation and seed sowing

Soil in both locations was prepared for cultivation by land plough and ridges construction. Phosphorus at rate of 32 kg P_2O_5 fed^{-1} were added during the final preparation of land and thoroughly mixed with the soil. While, nitrogen as calcium nitrate was added in two equal portions (during soil preparation and after 25 days seeding) at the rate of 60 kg N fed^{-1} .

Seeds of quinoa cultivar CICA [origin: International Potato Center (CIP), Lima, Peru] were sown under field experiments during two successive winter seasons of years 2013 and 2014. Seeds surface were sterilized before sowing with ethanol 70% for 10 sec, then with sodium hydrochloride solution (5% active chloride) for 10 minutes. Seeds were then washed with distilled water several times.

About 10-12 seeds per hill were sown at a density of 240,000 plants per feddan.

Seeds of quinoa were sown in non-saline location on October 8 and 22, while in saline soil location on October 26 and 23 for the first and second season respectively. The young plants at both sites were harvested at 40 days after sowing.

The experimental design was complete randomized design with 6 replicates; each experimental plot area was 6.0 m^2.

Morphological, yield, chemical composition and mineral concentrations

Twenty five plants from each experimental plot were randomly taken at 40 days after sowing date and data were recorded on these plants for morphological traits as the following:

Plant height (cm): the average height from ground level to the maximum extension of the tip leaves on the stem.

Leaf fresh weight per plant (g): all green and fully expanded leaves were immediately cut and weighted.

Leaf dry weight per plant (g): samples of leaves were oven dried at 70 C till constant weight.

Stem fresh weight per plant (g): weight of stem after excluding the leaves.

Stem dry weight per plant (g): samples of stem were oven dried at 70 C till constant weight.

Leaf mass area (LMA): Leaf mass to area ratio or leaf succulence is a definition for water content per unit leaf area, was determined by taking 4 leaves from each plants. Samples were dried for 48 h at 70 C to determine dry mass (DM) after measuring leaf area (LA) and fresh mass (FM) for each sample. Water content, LMA= (FM-DM), for each sample was expressed on a leaf area basis (g cm^2).

Table 1. Physical and chemical proprieties of soil samples collected from non-saline and saline locations

Parameters	Non-saline soil	Saline soil
Sand (%)	28.1	61.6
Silty (%)	38.6	29.9
Clay (%)	33.3	8.3
Soil texture	Clay Loam	Sandy Loam
pH (soil paste)	7.46	8.24
ECe (dSm^{-1})	1.9	17.9
Soluble Na^{+} meq l^{-1}	6.4	131
Soluble K^{+} meq l^{-1}	1.4	4.3
Soluble Ca^{++} meq l^{-1}	7.2	29.0
Soluble Mg^{++} meq l^{-1}	4.1	43.5
Soluble Cl^{-} meq l^{-1}	9.0	116
Soluble $SO_4^{=}$ meq l^{-1}	6.7	81.0
Soluble $CO_3^{=}$ meq l^{-1}	0.0	0.0
Available Fe mg kg^{-1}	4.6	6.1
Available Mn mg kg^{-1}	6.9	2.4
CaCO3 (%)	1.72	1.1

Table 2. Some chemical proprieties of irrigation water collected from non-saline and saline locations

Parameters Soil	Non-saline	Saline
EC dSm^{-1}	0.43	1.3
pH	7.1	7.7
K^{+} meq l^{-1}	0.2	0.5
Na^{+} meq l^{-1}	1.4	5.8
Ca^{++} meq l^{-1}	1.0	3.6
Mg^{++} meq l^{-1}	1.1	1.8
Cl^{-} meq l^{-1}	1.2	8.2
HCO_3^{-} meq l^{-1}	0.6	4.0
$CO_3^{=}$ meq l^{-1}	0.0	0.0
$SO_4^{=}$ meq l^{-1}	2.0	3.0

Vegetable yield (kg $feddan^{-1}$): whole plants, excluding the roots, from one square meter of the central rows were cut and immediately weighted.
As for, some chemical constituents and some mineral concentrations in leaves were estimated as the following:

Chlorophyll-a, -b and total carotenoids concenration were determined in representative fresh leaves samples according to **Moran** (**1982**), using dimethylformamide as a solvent.

Total soluble carbohydrate concentrations in press sap of leaves were measured according to the method of **(Irigoyen et al 1992).**

The concentration of proline was determined according to the method of **(Bates et al 1973).**

According to the methods described in **AOAC (1995)**, the crude fiber (method 962.09) was measured using Ankom Fibre Analyzer A-200 (ANKOM Technology, Macedon NY, USA). Crude fat (method 920.39) was determined using VELP solvent extractors unit SER 148/3 (VELP Scientific, Inc., Bohemia NY, USA) using petroleum ether as a solvent.

For determination of Mineral concentrations, Leaves were oven dried at 70 C until constant weight; then the dry samples of leaves finely ground and assayed for mineral-ions concentration. Dried ground material (0.5 g) of leaves was wet digested in hydrogen peroxide and sulphuric acid for the analysis of N, P, K, Mg, Na, Fe and Mn.

Total nitrogen were determined using micro-kjeldahl method and its concentration is used to calculate the crude protein percentoge (protein % = total N% x 6.25).

Phosphorus was determined by modified colorimetric method using spectrophotometer (SPECTRONIC 20D, Milton Roy Co. Ltd., USA).

Sodium and potassium percentages were measured using flame photometer (JENWAY, PFP-7, ELE Instrument Co. Ltd., UK).

The concentrations of Mg, Fe and Mn were determined using Atomic-absorption spectroscopy (Analyst 200, Perkin Elmer, Inc., MA, USA).

Statistical analysis

Combined analysis for the raw data of both experimental years was statistically analyzed according to statistical analysis system **(SAS, 1999)**. Separation between means was carried out by using Duncan Multiple Range test **(Duncan, 1955**).

RESULTS AND DISCUSSION

1- Morphological and yield traits

The mean values for different morphological and yield traits are present in **Table (3)**. No significant difference was observed in plant height be-

tween plants grown under saline and non-saline soil conditions. similar finding was obtained by **Gomez-Pando, et al (2010)** who reported no significant reduction in plant height of quinoa plants due to the effect of salinity, even increases was observed when electrical conductivity not exceeding 25 dSm^{-1}. however, we have to take in account that the quinoa plants in our present study exposed to salinity for short period (40 days from sowing date to harvest date), and this may be a possible explanation for weakly effects of salinity on the halophytic plants such as quinoa. In this concern, **Nguyen (2016)** found that the plant height of quinoa was decreased when salt stress period takes place for time period range from 35 - 45 days after sowing date. On the other hand, salinity significantly stimulated leaf fresh weight, stem fresh weight and consequently led to high biomass yield **(Table 3)**. In the same context, **Bosque-Sanchez et al (2003)** stated that the total plant biomass of quinoa was not negatively influenced by salinity up to 20 dSm^{-1}. Also, in field experiment conducted in southern Italy, **Pulvento et al (2012)** found that the quinoa plants had no significant differences in the yield when irrigated with saline water of 22 dSm^{-1}. An interesting observation, in the present study, was detected with regard to succulence or high leaf mass to area ratio (LMA) under saline soil conditions. High LMA is demonstrated in many dicotyledonous halophytes that inhabit saline stress by deposing ions of salt in large vacuoles to maintain low Na concentrations in cytosol and in the active metabolic tissues **(Munns and Tester, 2008)**. This suggested that quinoa as a halophytic species might utilize salt inclusion mechanism and consequently could enhance or keep plant growth under saline condition. Similar observation has been reported by **Nguyen et al (2013)** who mentioned that the halophytes could keep growing up to 25-35 dSm^{-1}.

2- Pigment concentrations, K^+/Na^+ ratio, Total soluble carbohydrate and Proline concentrations

Data illustrated in **Fig. (1)** Shows an inverse relationship between salinity and the concentrations of photosynthetic pigment. Whereas, the reduction of Chl (a), Chl (b) and carotenoid concentrations were 53%, 33% and 65.5% under saline soil as compared to non-saline one, respectively. Reduction in chlorophyll concentrations is widely reported under salt stress **(Khan et al 2000; Kaya et al 2001; Geissler et al 2009 and Eisa et al 2012)**. Enhancement of chlorophyll and carotenoid levels under saline conditions may be considered as a desirable trait for salt resistance because it indicates a low degree of photoinhibition **(Adolf et al 2012)**. However, **Koyro et al (2013); Geissler et al (2015)** reported that the reduction in the chlorophyll concentration seems to be an adaptive mechanism to cope with saline stress, since it may be lead to lower the over reduction of the photosynthetic electron transport chain and hence the generation of ROS on one hand. On the other hand, it would lead to suppress the net photosynthetic rate (**Ignatova et al 2005)**.

Table 3. Mean performance of quinoa cultivar CICA for morphological and yield traits under non-saline and saline soil conditions

Traits	Non-saline soil	Saline soil
Plant height (cm)	21.9 a	22.0 a
Leaf fresh weight (g $plant^{-1}$)	4.5 b	6.6 a
Leaf dry weight (g $plant^{-1}$)	0.64 a	0.61 b
Stem fresh weight (g $plant^{-1}$)	3.2 b	3.8 a
Stem dry weight (g $plant^{-1}$)	0.35 a	0.36 a
Leaf mass area (g cm^2)	0.32 b	0.52 a
Vegetable yield (kg $feddan^{-1}$)	1864 b	2496 a

As for K^+/Na^+ ration, the results clearly revealed that the quinoa grown in saline soil accumulated higher Na^+ concentrations, but no significant reduction has been observed for K^+ concentrations in compared with non-saline **(Table 4)**. This led consequently to induce reduction in K^+/Na^+ ration **(Fig. 1-d)**. Salinity resistance in quinoa is attributed to its highly efficient of K retention in leaves **(Shabala et al 2010 and Hariadi et al 2011).** This is counterintuitive in the light of the traditional view about salt-induced K^+ deficiency **(Marschner, 1995**), but consistent with other studies of quinoa **(Orsini et al 2011 and Adolf et al 2012)**. Also, the results of the present work indicated higher accumulation of Mg^{++} in leaves of

quinoa grown under saline soil than those in non-saline **(Table 4)**. This might be due to the high Mg concentration in the saline soil location **(Table 1)**. However, increasing $Na^{+,}$ K^{+} and Mg^{++} concentrations in leaves of quinoa grown in saline soil may be clearly evident that quinoa plants utilizing of inorganic ions to regulate its osmotic potential under saline stress. Osmotic adjustment by accumulation of inorganic ions has been reported in many halophytic species **(Debez et al 2006; Koyro et al 2008 and Hussin et al 2013)**, including *C. quinoa* **(Hariadi et al 2011 and Eisa et al 2012).** This strategy is considered to be less carbon energy demanding (metabolically cheap osmoticum) compared to the De novo synthesis of active organic substances such as proline and total soluble carbohydrates (TSC). These observations were in agreement with our results for proline and total soluble carbohydrates **(Fig. 1-e&f)**.

Table 4. Some chemical composition and nutrient concentrations in leaves of quinoa under non- saline and saline soil conditions

Parameters	Non- saline	Saline
Protein (%)	26.4a	25.0a
Fiber (%)	7.4a	6.3b
Fat (%)	2.3a	1.9b
Phosphorus (%)	0.45a	0.50a
Potassium (%)	6.0a	5.8a
Na (%)	0.18b	1.1a
Magnesium (%)	1.2b	2.7a
Fe (mg kg^{-1})	1355a	1019b
Mn (mg kg^{-1})	46b	70a

3- Yield components

Data in **Table (4)** showed that salinity had no significant influence on protein, phosphorus and potassium concentrations in quinoa leaves. Meanwhile, crude fiber, crude fat and iron concentrations significantly decreased in leaves of quinoa due to salt stress. Therefore, the present results gave some interesting observation with reference to quality traits for the leafy vegetable potential of quinoa particularly in marginal regions, where the low incomes and malnutrition is common. Utilizing quinoa as a promising cash halophyte crop for land and water unsuitable for conventional crops in arid and semi-arid regions is an urgent need for obtaining low cost sources of protein and essential nutrients such as potassium and iron to combat malnutrition which dominantly in the marginal areas of developing countries. Also, carotenoids play an important role in human nutrition as precursors for vitamin A, besides its function as antioxidant **(Pavia and Russel, 1999)**. Moreover, **(Gupta and Wagle, 1988; Prakash and Pal, 1991; Shukla et al 2003)** mentioned that the leaf carotenoid content in quinoa was higher than that reported in spinach. Finally, further investiration shoud be made to evaluate palatability of quinoa as fresh leafy vegetable crop in Egypt.

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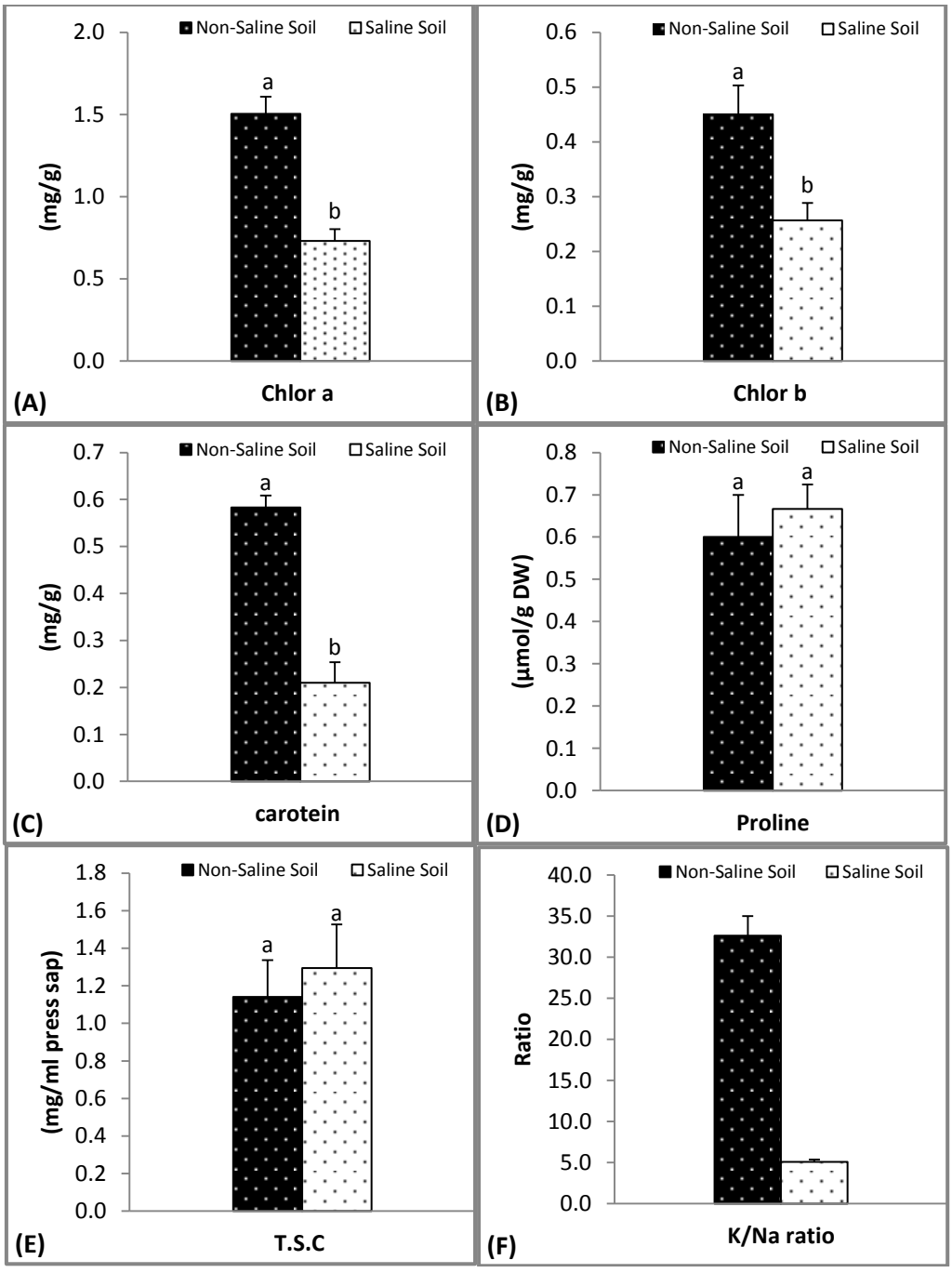

Fig. 1. Chl.a , Chl.b ,Carotenoids, proline, T.S.C concentrations and K^+/Na^+ ratio in leaves of quinoa (40 days after sowing) grown under non-saline and saline soil conditions.

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