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The potential use of quinoa as a new non-traditional leafy vegetable crop

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Quinoa (*Chenopodium quinoa* Willd.) is one of the oldest food crops in the world, with evidence of cultivation dating back for more than 7000 years in Andean region. Using of quinoa fresh leaves as a vegetable have not been reported or well-studied yet. Therefore, the present study was conducted during winter growing seasons of 2014 and 2015 at the Experimental Farm of Agricultural Botany Dept., Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt. Intended to examine the potential use of fresh leaves of two quinoa cultivars (Hualhuas and CICA), as a new non-traditional leafy vegetable crop compared to spinach cultivar (Balady). The vegetative growth, yield and leaf chemical compositions for young plants harvested at 45 days after sowing date were evaluated. The experiments were designed in a complete randomized block design with 4 replicates. The obtained results revealed that young quinoa plants of CICA showed the highest performance for all investigated vegetative growth characters and yield compared with Hualhuas and spinach plants in both seasons. Except for leaf area in both seasons and fresh weight of leaves/plant in the second season, whereas the highest values were attained by spinach plant. Concerning leaf chemical compositions, the obtained results showed that Hualhuas cultivar recorded the highest values for leaf pigments, ash, crude fats, crude proteins, total phenolic content (TPC), total flavonoids content (TFC) and antioxidant activity% compared to CICA and spinach plants. However, spinach plants gave the highest values of crude fiber, total soluble carbohydrates (TSC), nitrate (NO3) and nitrite (NO2) contents in both seasons. It is likely that the levels of anti-nutritional factors (NO3, NO2 and oxalate) in the leaves of the three studied cultivars are far below the limits permitted to be found in leafy vegetables. As for leaf mineral contents, the leaves of CICA plants had higher K, Ca and Fe contents, while spinach plants exhibited relatively higher Na, Zn and Cu contents. It is appeared from the obtained results that the contents of evaluated nutritive values were significantly influenced by cultivar, both quinoa cultivars had higher ash, crude fats, crude proteins, TPC, TFC, antioxidant activity%, N, K, Ca and Mn contents compared to spinach cultivar in both seasons. The obtained results confirmed the suitability of potential use of young quinoa plants as a new non-traditional leafy vegetable crop under Egyptian conditions, but further researches are still needed.

Keywords: ***Chenopodium quinoa, Spinacia oleracea,*** **Young plants, Vegetative growth, Yield, Leaf chemical compositions and Anti-nutritional factors.**

INTRODUCTION

Leafy vegetables are essential to human diet and health, since they are being cheap and excellent source for vitamins, minerals;

biologically active substances, pigments and dietary fibers, as well as they have not much fats and calories (Toledo et al., 2003). They are important components of a healthy diet if consumed daily in sufficient amounts, could help in preventing major diseases such as cardiovascular diseases and certain cancers (Patil et al., 2009). Although, they are a short-life cycle crops (Shaheen et al., 2012). However they have a strong tendency to accumulate some of unwanted anti-nutritional factors, nitrate, nitrite (Escobar-Gutierrez and Burns, 2002) and oxalate (Stagnari et al., 2007), because of their detrimental effects on the human health (Hord et al., 2009; Solberg et al., 2015).

Quinoa (*Chenopodium quinoa* Willd., family *Amaranthaceae*) is one of the oldest food crops in the world, with evidence of cultivation dating back for more than 7000 years in Andean region of South America for its nutritious grains (Pearsall, 1992). It has been cultivated as one of the staple food crops for Andes people for hundreds of years, but the crop was marginalized when the Spanish conquest arrived to Latin America (Brinegar et al., 1996). Recently quinoa has been revived as a new food crop, attracted a worldwide attention and increased in popularity; it is cultivated globally in more than 70 countries outside of Andean region (Comai et al., 2007). Owing to the excellent nutritional value (Repo-Carrasco et al., 2003), potential health benefits (Vilcacundo and Hernandez-Ledesma, 2017), wide range of genetic diversity and geographical adaptability (Fuentes et al., 2009) and the exceptional tolerance to various harsh environmental conditions (Choukr-Allah et al., 2016). Quinoa has been selected by FAO as one of the crops destined to offer food security in this century and also declared the *International Year of Quinoa* in 2013 (FAO, 2013). It has a variety of uses in the food, feed, food processing and other non-food/industrial uses (Bhargava et al., 2006). Quinoa is considered as a new non-traditional multipurpose cash crop halophyte newly introduced to Egypt (Eisa et al., 2017).

Quinoa leaves have different colors (green, red or purple), with the reddish color due to the presence of betalain pigment (Gallardo et al., 2000). The fresh leaves of quinoa could be used as a vegetable in human diets (Bhargava et al., 2007). Leaves are typically cooked and served as a side dish similar to amaranth leaves (Mlakar et al., 2010) or spinach (Oelke et al., 1992). A quinoa leaf salad is generally more nutritious than most green salads (Aufhammer, 2000). Fresh leaves and sprouts of quinoa are edible and may be used as a nutritious supplement for vegetarian people, functional or complete foods and fortification (Paśko et al., 2009; Gawlik-Dziki et al., 2015), also they have a higher antioxidant and anticancer activities (Gawlik-Dziki et al., 2013; Świeca et al., 2014). Furthermore, quinoa leaves have been used medicinally as an analgesic, anti-inflammatory, wound healing and disinfectant for urinary tract (Mujica, 1994; Tang and Tsao, 2017).

Quinoa leaves contain an ample amount of ash (3.3%), fibers (1.9%), Na (289 mg/100 g) and nitrates (0.4%) (Koziol, 1992), as well as 82-190 mg/kg of carotenoids and 27-30% of proteins (Prakash et al., 1993). Bhathal et al., (2015) revealed that quinoa fresh leaves contain chlorophyll a (0.48-1.82 mg/g), chlorophyll b (0.25-0.07 mg/g) and much higher amount of carotenoids (230.23-669.57 mg/kg). Quinoa leaves are good source of high quality proteins, it contains an average of 20% proteins with well-balanced amino acids profile (Ostrowski-Meissner et al., 1980) and it has all of the essential amino acids including lysine, histidine, methionine and threonine that are rare in plant origin (Abugoch, 2009; Escuredo et al., 2014). *Chenopodium* spp. is a rich source of minerals like K (6.329), Ca (1.154), Na (8.350) and Fe (83.92 mg/100g) as reported by Bhargava et al., (2010). Furthermore, Akubugwo et al., (2007) revealed that the fresh amaranth leaves contain a significant amount of nutrients (ash 13.80, crude fibers 8.61, crude fats 4.62, crude proteins 17.92 and carbohydrates 52.18%), minerals (P 34.91, K 54.20, Ca 44.15, Mg 231.22, Na 7.43, Fe 13.58 and Zn 3.80 mg/100g DW), phyto chemicals (phenols 0.35 and flavonoids 0.83 mg/100g DW). Moreover, Tang et al., (2014) reported that crude fats in quinoa and amaranth fresh leaves were 2.72-4.18% contained mainly essential fatty acids and had a highly favorable ratio of omega 3/omega 6 (2.28-3.89), respectively. In additions, the average of leaves mineral contents for 30 vegetable amaranth strains studied were, 3.7 for K, 1.7 for Ca and 2.9 g/100 g for Mg, and averages of 1233.8 for Fe, 791.7 for Zn, and 108.1 mg/kg for Mn (Shukla et al., 2006). Also, Mbwambo et al., (2015) declared that both leaves and grains of amaranth contain K (611, 135), P (148, 50), Ca (215, 47), Fe (2.32, 2.1 mg) and protein (2.46, 3.8 g), respectively. Singh *et al.* (2001) pointed out that there were significant differences between leaves of spinach and amaranth concerning their contents of proteins (26.5 and 26.2%), β-carotene (4.0 and 5.4 mg/100 g), Fe (35.8 and 26.8), Zn

(6.0 and 2.4), Mn (10.2 and 3.1) and Cu (1.9 and 0.9 mg/100 g), respectively. Also, spinach leaves contain significant amount of K (3.73), Na (2.98), Mg (1.94), Ca (0.94) and P (0.50%). In addition, appreciable amount of Fe (52.7), Zn (7.78), Mn (16.8) and Cu (1.66 mg/kg) as demonstrated by Bhattacharjee et al., 1998). Accordingly, spinach is considered as an important source of carotenoids, flavonoids, Ca and Mg as well as a good source of Fe (Koh *et al.,* 2012). On the other hand, the contents of nitrate and oxalate could be high, which can adversely affect human health (Solberg et al., 2015). In this context, Santamaria et al., (1999) stated that amongst all studied fresh vegetables, spinach and Swiss chard showed the highest levels of accumulated nitrate and oxalate. Total phenol and flavonoids in leaves of some *Chenopodium album* cultivars ranged between 224.99-304.98 mg GAE/100g and 220.0-406.67 mg/100g DW, respectively. The saponin was also presented in leaves but in lesser amount (0.027-0.867 g/100g). Moreover, oxalate content in the leaves was ranged between 394.19-518.42 mg/100g (Purvika et al., 2012). In this context, quinoa fresh leaves showed amounts of saponins approximately less than 0.015% (Burnouf-Radosevich and Paupardin, 1983), or no detectable amounts of saponins in relative to spinach leaves which contain 0.55% saponins on a fresh weight basis (Fenwick and Oakenfull, 1983). Some of non-traditional vegetables are considered as good sources of bioactive compounds, but leaves of some of them presented high levels of oxalic acid (Morales et al., 2014).

Varietal differences were noticed between quinoa cultivars Altiplano and Salcedo. Salcedo had higher contents of ash and total leaf pigments than Altiplano cultivar. While the total polyphenols and total flavonoids contents are almost similar in both cultivars. However, the antioxidant activity in Altiplano was higher than Salcedo cultivar (Chacaliaza-Rodríguez et al., 2016). Leaf chemical compositions of lettuce were significantly influenced by selected cultivar (Koudela and Petříková, 2008). Also, Pokluda and Kuben (2002) reviewed that cultivar is an important factors that influencing growth, yield and nutritional quality of the plant.

At the best of our knowledge, using fresh leaves of quinoa as a vegetable have not been reported or well-studied yet. Also lack of information on the chemical composition and nutritional value of leaves is existed. Therefore, to fill this knowledge gap, the current study was undertaken to figure out the possibility of potential use of young plants of two quinoa cultivars, as a new non-traditional leafy vegetable in Egypt in comparison with spinach plants. Plant vegetative growth, yield and leaf chemical compositions for young plants harvested after 45 days from sowing date were evaluated.

MATERIALS AND METHODS

The current study was carried out at the Experimental Farm of Agricultural Botany Department (altitude 22 m above sea level, latitude 30° 06' 48'' N and longitude 31° 14' 52'' E), Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt, during the two successive winter growing seasons of 2014 and 2015. In order to evaluate the possibility of potential use of young plants of two cultivars of quinoa (*Chenopodium quinoa* Willd.), Hualhuas and CICA, as a new non-traditional leafy vegetable crop in Egypt, in comparison with spinach (*Spinacia oleracea* L.) plants, Balady cultivar which known as a popular winter leafy vegetable crop. Plant vegetative growth, yield and leaf chemical compositions for young plants harvested at 45 days after sowing date were measured. Physical and chemical properties of the experimental soil are presented in Table 1.

Experimental design

The experiments were set in a complete randomized block design with 4 replicates. Quinoa cultivars (Hualhuas and CICA) and spinach cultivar (Balady) were randomly arranged within the block. Each block consisted of 3 plots, each with 2 m length x 2 m width, with a net area of 12 m^2 for each experimental block (6 m length x 2 m width).

Experimental site preparation and cultivation

Experimental soil was prepared by land plough and then divided into four main experimental blocks. Then the 3 plots were manually constructed within each experimental block. All experimental plots were received compost at rate of 15 ton ha^{-1}, phosphorus at 70 kg P_2O_5 ha^{-1} as calcium super-phosphate (15.5% P_2O_5) and nitrogen at 150 kg N ha^{-1} in the form of ammonium sulphate (20.6% N).

Table 1. Physical and chemical properties of the experimental soil.

Soil physical properties										
Sand (%)	**Clay (%)**	**Silt (%)**	**Texture**	**Saturation (%)**	**Balk density (gcm^{-3})**					
28.1	33.3	38.6	Clay Loam	55	1.50					
Soil chemical analysis										
ECe (dsm^{-1})	**pH**	**OM (%)**	**$CaCO_3$ (%)**	**meq/l**						
				Ca^{++}	**Mg^{++}**	**Na^{+}**	**K^{+}**	**$SO_4^{=}$**	**HCO_3^{-}**	**Cl^{-}**
1.9	7.46	1.51	1.72	7.2	4.1	6.4	1.	6.7	3.8	9.0

Full dose of both compost and phosphorus as well as the half dose of nitrogen was applied during the final preparation of experimental soil and thoroughly mixed with the soil. Whereas, the other half dose of nitrogen fertilizer was added 20 days after sowing date. Also, potassium fertilizer was added at rate of 90 kg K_2O ha^{-1} in the form of potassium sulphate (48% K_2O). The whole amount was added once as a side-dressing among rows of the plants, one month after sowing date.

Seeds of the two quinoa cultivars, Hualhuas (International Potato Center CIP, Lima, Peru), CICA (Centro de Investigación en Cultivos Andinos, Cusco, Peru) and spinach Balady cultivar (Horticultural Research Institute, Agricultural Research Center, Giza, Egypt), were surface sterilized with ethanol 70% for 10 sec., then with sodium hypochlorite solution (5% active chloride) for 10 min. After that, the seeds were thoroughly washed with distilled water several times to ensure complete elimination of chloride traces. Then the seeds were spread on tissue paper and left to dry at room temperature. Washed dried seeds of both quinoa and spinach cultivars were sown on the second week of October in both seasons of 2014 and 2015, in rows with 2 m length and 20 cm inter-row distance inside the experimental plot with a capacity of 10 rows per plot. The normal agricultural practices of regular irrigation, fertilization, controlling of pest, disease and weed were followed. A plant density was maintained in a range of 180-200 plants/m^2, this has been achieved through seeding rate of about 8, 12 and 25 g of seeds per experimental plot of Hualhuas, CICA and spinach, respectively.

Data recorded

Vegetative growth characters and yield

Twenty five young plants of both quinoa and spinach cultivars were randomly harvested from the middle of each experimental plot by cutting the plants at the soil surface, in the morning after evaporation of dew, at 45 days after sowing date. Afterward, harvested plants were transferred to the laboratory for measurements of vegetative growth parameters i.e. plant length, leaf number/plant, leaf area, fresh and dry weights of the leaves and stem/plant, and plant fresh weight. In addition, plant yield as kg/m^2 was calculated by multiplying the plant fresh weight by the number of plant per square meter. Also plant yield as ton/ha was estimated.

Leaf chemical compositions

Another sample of 25 young plants of the two quinoa and spinach cultivars were randomly harvested as previously described. Then leaf samples were separated from their stalks and washed under running distilled water to remove surface adhered dust, and then leaves were spread on tissue paper at room temperature for 1 h. Then the leaves were dried in an electrical oven supplied with fan at 70°C till constant weight. The dried leaf samples were finely ground in a high speed stainless-steel grinder to pass a 1 mm sieve and subjected to different analysis of leaf chemical compositions.

Leaf pigment contents:

Chlorophyll a, b and carotenoids contents were determined according to Moran (1982). Fresh leaf disks (0.5 g) were immersed in 10 ml of N,N-dimethylformamide overnight. The obtained extracts were measured at 663, 647 and 470 nm in a UV/VIS spectrophotometer (T-60, PG instrument, Wibtoft Leicestershire, UK), for chlorophyll a, b and carotenoids, respectively. N,N-dimethylformamide was used as a blank.

Percentage of ash, crude fibers, crude fats and crude proteins:

Ash content of dried leaf samples was determined using a muffle furnace (M110, Heraeus Instruments, Hanau, Germany) at

525±25°C for 12 h, according to the methods described in AOAC (2016). While, crude fibers were measured using the ANKOM Fiber Analyzer A-200 with ANKOM filter bags F-57 (ANKOM Technology Corp., Macedon NY, USA). Furthermore, crude fats were measured using VELP solvent extractors unit SER 148/3 with VELP cellulose thimbles 33x80 mm (VELP Scientific, Inc., Bohemia, NY, USA), for 90 min using petroleum ether with a boiling range of 40-60°C, as an extraction solvent. Moreover, the percentage of total nitrogen content in dried leaf samples of the three studied plants was converted to crude proteins by using the conversion factor of 6.25.

Total soluble carbohydrates (TSC) content: The total soluble carbohydrates in the press sap of the leaves were assayed according to Irigoyen *et al.* (1992). A sample of 1 ml of the diluted press sap (1: 200) was mixed with 1 ml of Anthrone reagent. The formed blue green color was measured at 620 nm against blank in a UV/VIS spectrophotometer. A calibration curve for calculation of the TSC content was prepared using serial concentrations of glucose standard.

Total phenolic (TPC), flavonoids (TFC) contents and antioxidant activity:

A 0.1 g of dried leaf samples was immersed in 5 ml of absolute ethanol. The samples were placed in a shaken water bath at 80°C for 1 h, then samples were cooled and centrifuged at 5000 rpm for 15 min and the supernatants (ethanolic extract) were collected and stored at 4°C for further analyses.

The total phenolic content (TPC) was determined using Folin-Ciocalteau reagent as described by Stratil et al., (2006). Diluted Folin reagent (1:10), 0.75 ml was added to 100 µl of ethanolic extract and mixed well. Then 0.75 ml of 6% (w/v) sodium carbonate was added to the mixture and shaken gently, then allowed to stand at room temperature for 1 h in the dark. Absorbance was read at 725 nm. Gallic acid was used as the standard reference and TPC was expressed as mg gallic acid equivalent (GAE)/g DW.

Total flavonoids content (TFC) was determined using colorimetric method described by Chang et al., (2002). A 0.5 ml of ethanolic extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After 30 min at room temperature, the absorbance of the reaction mixture was measured at 415 nm. The TFC were expressed as µg quercetin equivalent (µg/100 g DW).

The percentage of antioxidant activity (free radical scavenging activity), the ability of ethanolic extract to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was estimated as previously described by Jain *et al.* (2008). An aliquot of 3.9 ml of 0.1 mM DPPH radical in methanol was mixed with 0.1 ml of ethanolic extract of the sample. After incubation for 30 min at room temperature, the absorbance was measured against the blank (pure methanol) at 517 nm. The free radical scavenging activity was expressed as the percentage of antioxidant activity.

Nitrate, nitrite and oxalic acid contents:

Nitrate content was determined according to Cataldo et al., (1975). A sample of 100 mg of dried leaf samples was suspended in l0 ml of deionized water and incubated at 45°C for 1 h. Then samples were centrifuged at 14000 rpm for 15 min and the supernatants were collected. A 0.2 ml of the supernatant was transferred into 50 ml flask and mixed thoroughly with 0.8 ml of 5% (w/v) salicylic acid in concentrated H_2SO_4 (SA-H_2SO_4). After 20 min at room temperature, 19 ml of 2N NaOH were added slowly to raise the pH above 12. Then samples were stand at room temperature for 30 min and the absorbance was determined at 410 nm. A blank of 0.2 ml of deionized water and the normal reagents used was employed.

The nitrite content was determined according to Cemek et al., (2007). A 5 ml of sodium tetra-borate and 100 ml of warm deionized water (80°C) were added to 2.0 g of dried leaf samples and incubated for 15 min in a warm water bath at 45°C. Subsequently, potassium hexa cyanoferrate and zinc acetate dehydrate were added to the solution. Then the solution was transferred to a 200 ml volumetric flask and diluted with deionized water. To determine nitrite, 10 ml of the solution was transferred to a 50 ml volumetric flask. By adding sulfanilamide chloride and 1-naftil ethylene di-amine di-hydrochloride to the solution, a purple colored complex is produced. Then absorbance was measured at 538 nm and finally the nitrite concentrations in samples were determined by the calibration curve.

The oxalic acid content was determined according to Xu and Zhang (2000). A 0.5 g of dried leaf samples was added to 30 ml of 0.25N HCl in a 50 ml volumetric flask, then kept in a boiling water bath for about 15 min, and the volume was made with 0.25N HCl to 50 ml. Oxalic acid content was determined in the reaction mixture of 100-600 µl of standard oxalic acid, 250 µl of 1.0×10^{-3} M bromophenol blue, 400 µl of 0.1 M potassium dichromate and 450 µl of 1 M H_2SO_4. The final volume was made to 10 ml with distilled water. Then kept in a boiling water bath for 10 min, the reaction was stopped by adding 1 ml of 2 M NaOH. The absorbance was read at 600 nm. The absorbance for non-catalytic reaction without oxalic acid (Ab), and for catalytic reaction (As) was recorded. The difference (∆A), between Ab and As was calculated and log (Ab-As) was calculated to determine oxalic acid concentration.

Leaf mineral contents:

A weight of 0.2 g of dried leaf samples was wet digested using a mixture of sulphuric acid (H_2SO_4 98%) and hydrogen peroxide (H_2O_2 30%) as described by Wolf (1982). Acid digested solution was used to determine mineral contents on a dry weight basis. Total nitrogen was determined using the Kjeldahl method and phosphorus was also assayed according the modified colorimetric method using spectrophotometer (SPECTRONIC 20D, Milton Roy Co. Ltd., NY, USA) according to the procedures described by Cottenie et al., (1982). While, K, Ca and Na contents were measured using flame photometer (JENWAY, PFP-7, ELE Instrument Co. Ltd., Staffordshire, UK). In addition Mg, Fe, Zn, Mn and Cu were determined using atomic absorption spectroscopy (AAnalyst-200, Perkin Elmer Inc., MA, USA), as described by Chapman and Pratt (1982).

Statistical analysis

All data sets were tabulated and subjected to statistical analysis of variance procedure using One-way-ANOVA of the Statistical Package for the Social Sciences software (SPSS Inc., 2008, release 17, Chicago, IL, USA). Values are given as an average of four measurements ± standard deviation (SD). Duncan multiple range test was employed to compare the significant differences among means at ($P\leq0.05$) level of probability according to the procedures reported by Gomez and Gomez (1984).

RESULTS

Vegetative growth characters and yield

The obtained results strongly indicated that young quinoa plants of CICA cultivar harvested after 45 days from sowing date (Fig. 1) was superior to Hualhuas and spinach plants in all investigated vegetative growth characters and yield in both seasons of 2014 and 2015. Young quinoa plants of CICA recorded the highest significant values ($P\leq0.05$) of plant length, number of leaves/plant, fresh and dry weights of the leaves and stem/plant, plant fresh weight and yield in both seasons of the study. Whereas, spinach plants (Balady cultivar) gave higher significant values ($P\leq0.05$) of leaf area than both quinoa cultivars (Hualhuas and CICA) in both seasons and higher significant value of fresh weight of leaves/plant than quinoa cultivar of Hualhuas in the second season only (Table 2). Significant differences were observed among the three studied plants on the leaves number/plant, stem fresh and dry weights/plant in both seasons, in addition to leaves fresh weight/plant in the first season only. However, no significant differences were noticed between young quinoa plants of Hualhuas and spinach plants in most cases in both seasons. In the same respect, no significant differences were detected between young quinoa plants of CICA and spinach plants on leaves dry weight/plant and leaves fresh weight/plant in the first and second season, respectively.

Concerning plant yield, it is evident that quinoa cultivar of CICA gave significantly the heaviest plant yield (4.142, 3.656 kg/m^2 and 41.420, 36.560 ton/ha) followed by spinach Balady cultivar (2.203, 2.152 kg/m^2 and 22.030, 21.520 ton/ha) and then by quinoa cultivar of Hualhuas (2.142, 2.056 kg/m^2 and 21.420, 20.560 ton/ha) in the first and second season, respectively, without a significant difference between plants of Hualhuas and spinach in both seasons of the study (Table 2). It is of interest to state that young quinoa plants of CICA cultivar produced a yield of ~ 45% higher compared to spinach and Hualhuas cultivars.

Leaf chemical compositions

Leaf pigment contents

Data presented in Table 3 clearly revealed that leaves of Hualhuas cultivar recorded the highest values of chlorophyll a and carotenoids in the first season, while, in the second season it gave the highest values of all leaf pigment contents (chlorophyll a, b and carotenoids) followed insignificantly by spinach plant. However, leaves of CICA cultivar gave the lowest values of all leaf pigment contents in both seasons of the study. A significant difference was realized between Hualhuas, spinach plants and CICA plants in the contents of chlorophyll a and b in both seasons. Except for chlorophyll a in the second season, a significant difference was observed between plants of Hualhuas and CICA cultivars. Concerning carotenoids content no significant difference was detected among the three studied cultivars in both seasons of the study.

Percentage of ash, crude fibers, crude fats and crude proteins

It is highly apparent that young quinoa plants of Hualhuas and CICA cultivars showed the higher significant values (*P≤0.05*) for the percentages of ash, crude fats and crude proteins than spinach plants in both seasons of the study (Table 3). Young quinoa plants of Hualhuas cultivar recorded the highest values of the percentage of crude fats and the percentage of ash, crude fats and crude proteins in the first and second seasons, respectively, followed insignificantly by CICA cultivar. On the contrary, the lowest values of the percentage of ash, crude fats and crude proteins were attained by spinach plants in both seasons. Regarding the percentage of crude fibers, no significant difference was detected among the three studied plants in both seasons of the study (Table 3).

Figure 1. Cultivation of the two quinoa cultivars, Hualhuas (A) and CICA (B), as a new non-traditional leafy vegetable crop in comparison with spinach cultivar Balady (C) at germination stage and at harvesting stage after 45 days from sowing date.

Table 2. Plant vegetative growth parameters of the two quinoa cultivars as a new non-traditional leafy vegetable crop in comparison with spinach plants harvested at 45 days after sowing date in the winter seasons of 2014 and 2015.

Parameters	Hualhuas		CICA		Spinach	
First season (2014)						
Plant length (cm)	32.72	±2.43 b	36.00	± 0.58 a	33.06	±1.67 b
Leave number/plant	19.89	±1.49 b	40.33	±1.45 a	7.89	±0.10 c
Leaf area (cm^2)	19.34	±2.28 b	15.38	±1.30 b	46.22	±5.99 a
Leave F.W./plant (g)	6.24	±0.28 c	14.31	±1.28 a	12.24	±1.51 b
Leave D.W./plant (g)	0.78	±0.05 b	1.69	±0.10 a	1.16	±0.12 ab
Stem F.W./plant (g)	5.66	±0.54 b	8.71	±0.86 a	0.00	±0.00 c
Stem D.W./plant (g)	0.48	±0.04 b	0.98	±0.09 a	0.00	±0.00 c
Plant fresh weight (g)	11.90	±0.80 b	23.01	±1.90 a	12.24	±1.51 b
Yield (kg/m^2)	2.142	±0.144 b	4.142	±0.342 a	2.203	±0.272 b
Yield (ton/ha)	21.420	±1366 b	41.420	±3258 a	22.030	±2584 b
Second season (2015)						
Plant length (cm)	28.00	±0.88 b	36.33	±0.17 a	26.06	±0.83 b
Leaves number/plant	25.50	±3.75 b	37.67	±3.82 a	8.56	±0.51 c
Leaf area (cm^2)	17.63	±2.80 b	20.57	±1.87 b	38.40	±1.63 a
Leaves F.W./plant (g)	6.26	±0.87 b	11.36	±0.94 a	11.95	±0.54 a
Leaves D.W./plant (g)	0.96	±0.12 b	1.89	±0.21 a	1.14	±0.07 b
Stem F.W./plant (g)	5.14	±1.03 b	9.01	±1.03 a	0.00	±0.00 c
Stem D.W./plant (g)	0.57	±0.09 b	0.87	±0.12 a	0.00	±0.00 c
Plant fresh weight (g)	11.42	±1.66 b	20.31	±1.88 a	11.95	±0.54 b
Yield (kg/m^2)	2.056	±0.299 b	3.656	±0.338 a	2.152	±0.098 b
Yield (ton/ha)	20.560	±2844 b	36.560	±3215 a	21.520	±933 b

Values represent mean of four replicates ± SD.
Different letters within a row indicated significant ($P \leq 0.05$) differences among the three studied cultivars according to Duncan's multiple range test.

Table 3. Leaf chemical compositions of the two quinoa cultivars as a new non-traditional leafy vegetable crop in comparison with spinach plants harvested at 45 days after sowing date in the winter seasons of 2014 and 2015.

Parameters	Hualhuas		CICA		Spinach	
First season (2014)						
Chl a (mg/g FW)	2.43	±0.13 ^{a}	1.63	±0.28 ^{b}	2.32	±0.08 ^{a}
Chl b (mg/g FW)	0.82	±0.06 ^{a}	0.51	±0.08 ^{b}	0.90	±0.05 ^{a}
Carotenoids (mg/g FW)	0.61	±0.01 ^{a}	0.53	±0.07 ^{a}	0.58	±0.04 ^{a}
Ash (%)	2.48	±0.06 ^{a}	2.57	±0.15 ^{a}	2.14	±0.02 ^{b}
Crude fibers (%)	7.76	±0.229 ^{a}	7.47	±0.270 ^{a}	7.97	±0.297 ^{a}
Crude fats (%)	3.01	±0.497 ^{a}	2.84	±0.847 ^{a}	1.71	±0.39 ^{b}
Curde proteins (%)	28.00	±1.49 ^{a}	28.15	±0.48 ^{a}	22.78	±2.08 ^{b}
TSC (mg/ml press sap)	1.05	±0.25 ^{b}	0.93	±0.56 ^{b}	1.57	±0.76 ^{a}
TPC (mg/g DW)	5.44	±0.07 ^{a}	4.36	±0.16 ^{b}	3.32	±0.15 ^{c}
TFC (µg/100g DW)	225.8	±20.6 ^{a}	159.6	±10.9 ^{b}	127.2	±9.95 ^{c}
Antioxidant activity %	49.74	±6.266 ^{a}	30.88	±1.184 ^{b}	22.35	±8.512 ^{c}
Nitrate content (ppm)	10.20	±2.63 ^{b}	11.20	±1.18 ^{ab}	12.43	±0.75 ^{a}
Nitrite content (ppm)	0.94	±0.59 ^{b}	2.02	±0.6 ^{a}	2.46	±1.168 ^{a}
Oxalate content (mg/100g DW)	161.62	±12.58 ^{a}	158.20	±15.59 ^{a}	160.94	±4.78 ^{a}
Second season (2015)						
Chl a (mg/g FW)	3.04	±0.24 ^{a}	2.01	±0.12 ^{b}	2.56	±0.07 ^{ab}
Chl b (mg/g FW)	1.17	±0.03 ^{a}	0.66	±0.03 ^{b}	0.98	±0.04 ^{a}
Carotenoids (mg/g FW)	0.71	±0.10 ^{a}	0.66	±0.03 ^{a}	0.71	±0.06 ^{a}
Ash (%)	2.85	±0.06 ^{a}	2.63	±0.15 ^{a}	2.21	±0.02 ^{b}
Crude fibers (%)	7.66	±0.23 ^{a}	7.37	±0.38 ^{a}	7.79	±0.30 ^{a}
Crude fats (%)	2.89	±0.50 ^{a}	2.42	±0.85 ^{a}	1.53	±0.38 ^{b}
Curde proteins (%)	32.18	±2.88 ^{a}	30.50	±3.25 ^{a}	19.89	±0.38 ^{b}
TSC (mg/ml press sap)	1.62	±1.59 ^{b}	1.19	±0.23 ^{b}	2.19	±1.18 ^{a}
TPC (mg/100g DW)	5.29	±0.06 ^{a}	4.18	±0.16 ^{ab}	3.41	±0.15 ^{b}
TFC (µg/100g DW)	201.79	±20.65 ^{a}	137.51	±10.89 ^{b}	116.44	±9.95 ^{c}
Antioxidant activity (%)	55.43	±3.697 ^{a}	29.89	±1.550 ^{b}	17.83	±2.057 ^{c}
Nitrate content (ppm)	9.70	±2.62 ^{b}	10.69	±0.75 ^{b}	12.60	±1.18 ^{a}
Nitrite content (ppm)	1.00	±0.59 ^{b}	1.86	±0.60 ^{a}	2.12	±1.17 ^{a}
Oxalate content (mg/100g DW)	164.71	±12.57 ^{a}	160.84	±15.59 ^{a}	161.25	±4.78 ^{a}

Values represent mean of four replicates ± SD. Different letters within a row indicated significant (*P≤0.05*) differences among the three studied cultivars according to Duncan's multiple range test.

In spite of no significant difference was detected, it is interest to note that spinach plant gave the highest percentage of crude fibers followed by Hualhuas and then by CICA cultivar in both seasons.

Total soluble carbohydrates (TSC) content

When comparing the total soluble carbohydrates content (TSC) in young plants of the three studied cultivars, it was noticed that spinach plant exhibited the highest significant ($P \leq 0.05$) value, followed significantly by young quinoa plants of Hualhuas and then by CICA cultivars. Moreover, no significant difference was noticed between the two quinoa cultivars, Hualhuas and CICA during both seasons of the study (Table 3).

Total phenolic (TPC), flavonoids contents (TFC) and antioxidant activity

Data presented in Table 3 indicated that young quinoa plants of Hualhuas cultivar gave the best nutritional values of some bioactive (health promoting) compounds i.e. total phenolic content (TPC), total flavonoids content (TPC) and antioxidant activity % when compared with the other studied plants in both seasons. Consequently, young plants of Hualhuas cultivar recorded significantly ($P \leq 0.05$) the highest values of TPC and TFC as well as the percentage of antioxidant activity (DPPH free radical scavenging activity) followed significantly by young plants of CICA cultivar. On the contrary, the lowest significant values were recorded by spinach plants in both season of the study. It was evident that significant differences were noticed among the three studied plants on TPC, TFC and antioxidant activity in the first season and on TFC and antioxidant activity in the second season. However, no significant difference was detected between both quinoa cultivars Hualhuas and CICA on TPC. Similarly, no significant difference was realized between CICA and spinach plants. A significant difference was noticed only between Hualhuas and spinach plants.

Nitrate, nitrite and oxalic acid contents

The obtained results obviously demonstrated that spinach plants recorded the highest accumulated values of nitrate (NO_3) and nitrite (NO_2) contents in the leaves followed by CICA plants, whereas, Hualhuas plants recorded the lowest values in both seasons of the study (Table 3). Significant differences were found between spinach and Hualhuas plants concerning their leaf contents of nitrate and nitrite in both seasons of the study. On the other hand, no significant differences were noticed between spinach and CICA plants in the first season on nitrate and nitrite contents and on nitrite content in the second season. Furthermore, no significant differences were detected on nitrate content between both quinoa cultivars in both seasons of the study.

Regarding, oxalic acid content, the obtained results sharply showed that no significant differences were noticed among the three studied plants in relation to the oxalate content in both seasons (Table 3). In spite of no significant differences were detected among the three studied plants, it could be stated that young plants of Hualhuas showed a higher oxalate content, since it recorded 161.62 and 164.71 mg/100g DW, followed by spinach plants (160.94 and 161.25 mg/100g DW) and finally by CICA plants (158.20 and 160.84 mg/100g DW) in the first and second seasons, respectively.

Leaf mineral contents

Data shown in Table 4 declared that there were significant differences among the three studied plants (Hualhuas, CICA and spinach) in connection with their leaf mineral contents. The superiority of leaf mineral contents of the three studied plants was fluctuated among them. Young quinoa plants of Hualhuas cultivar recorded the highest values of P, Mg and Mn in the first season and N and Mn in the second season. In contrast, it recorded the lowest values of Fe, Zn and Cu in relative to other studied plants in both seasons. In the same regards, young plants of CICA cultivar gave the highest values of N, K, Ca and Fe in the first season and P, K, Ca and Fe in the second season. Moreover, it gave the lowest values of Na in both seasons of the study, and Mg in the second season only. Spinach plants recorded the highest values of Na, Zn and Cu in both seasons of the study, in addition to Mg in the second season. On the contrary, it recorded the lowest values of N, P, K, Ca and Mn in both seasons in addition to Mg in the first season only (Table 4).

It is of interest to mention that significant differences were detected among the three studied plants on Zn content in the first season and on Fe content in the second season. No significant differences were noticed among the three studied plants on the percentage of P and Mg in both seasons of the study. In the same respect,

Table 4. Leaf mineral contents of the two quinoa cultivars as a new non-traditional leafy vegetable crop in comparison with spinach plants harvested at 45 days after sowing date in the winter seasons of 2014 and 2015.

Parameters	Hualhuas		CICA		Spinach	
First season (2014)						
N %	4.48	±0.24 [a]	4.50	±0.08 [a]	3.65	±0.33 [b]
P %	0.53	±0.04 [a]	0.50	±0.03 [a]	0.47	±0.06 [a]
K %	6.42	±0.08 [a]	6.60	±0.09 [a]	5.13	±0.24 [b]
Ca %	1.47	±0.08 [a]	1.65	±0.20 [a]	0.89	±0.14 [b]
Mg %	2.42	±0.21 [a]	2.35	±0.13 [a]	2.05	±0.09 [a]
Na %	0.05	±0.03 [b]	0.03	±0.02 [b]	0.19	±0.05 [a]
Fe (mg/kg)	1308.8	±371 [b]	1480.6	±51.0 [a]	1416.4	±228 [a]
Zn (mg/kg)	49.60	±13.13 [c]	81.33	±20.9 [b]	98.57	±43.25 [a]
Mn (mg/kg)	97.77	±17.56 [a]	93.13	±14.37 [a]	76.93	±2.85 [b]
Cu (mg/kg)	10.80	±1.77 [b]	10.86	±0.52 [b]	11.83	±2.72 [a]
Second season (2015)						
N %	5.18	±0.46 [a]	4.81	±0.52 [a]	3.18	±0.06 [b]
P %	0.39	±0.03 [a]	0.40	±0.12 [a]	0.35	±0.02 [a]
K %	5.65	±0.23 [ab]	5.97	±0.26 [a]	5.27	±0.35 [b]
Ca %	1.84	±0.12 [a]	2.13	±0.20 [a]	1.43	±0.11 [b]
Mg %	1.81	±0.18 [a]	1.58	±0.06 [a]	1.82	±0.05 [a]
Na %	0.20	±0.01 [b]	0.17	±0.003 [b]	0.38	±0.03 [a]
Fe (mg/kg)	894.8	±32.51 [c]	1180.3	±252.5 [a]	1093.0	±92.42 [b]
Zn (mg/kg)	30.32	±2.08 [b]	39.13	±2.89 [a]	44.70	±4.36 [a]
Mn (mg/kg)	59.42	±4.00 [a]	51.33	±4.04 [a]	42.67	±5.69 [b]
Cu (mg/kg)	9.83	±3.21 [b]	11.00	±4.36 [b]	17.33	±2.08 [a]

Values represent mean of four replicates ± SD.
Different letters within a row indicated significant (*P≤0.05*) differences among the three studied cultivars according to Duncan's multiple range test.

significant differences were realized between young plants of quinoa (Hualhuas and CICA) and spinach plants on N, Ca, Na, Mn and Cu in both seasons, in addition to K in the first season only.

DISCUSSION

The obtained results herein strongly showed that amongst the three studied plants, young quinoa plants of CICA gave the vigorous plant vegetative growth and the heaviest plant fresh weight consequently, reflected a higher plant yield as kg/m^2 or ton/ha in both seasons of the study.

The obtained results may be ascribed to varietal differences according to different genetic background and different geographic distribution of the three studied plants. In this concern, Tapia (2015) reported that quinoa ecotypes could be classified into five groups: Valley quinoa, “Altiplano” quinoa, “Sea level” quinoa, “Salar” quinoa and “Subtropical” quinoa according to wide variations and broad genetic diversity (Fuentes et al., 2009) as well as geographical distribution and adaptability to different environments conditions (Choukr-Allah et al., 2016). The two quinoa cultivars used in this study, belong to different groups. Hualhuas is a Peruvian cultivar belonging to a Altiplano ecotype, whereas CICA is a Peruvian cultivar belonging to a Valley ecotype.

The obtained results are in harmony with the results obtained by Ebrahim et al., (2018) who stated that CICA cultivar is highly adapted for cultivation under Egyptian conditions. He also added that Hualhuas plants tended to flower earlier than CICA plants. Moreover, Ahmadi et al., (2010) reported that significant differences were noticed among spinach accessions regarding their vegetative growth and yield. In the same regards, the differences observed in vegetative growth among vegetable amaranth lines might be explained due to genetic variation and/or favorable influence of environmental conditions (Mbwambo et al., 2015). Generally, plant vegetative growth and yield were significantly influenced by cultivar (Pokluda and Kuben, 2002; Koudela and Petříková, 2008).

Concerning leaf chemical compositions, young quinoa plants of Hualhuas cultivar was superior to CICA and spinach plants in both seasons of the study. The differences observed in leaf chemical compositions among the three studied cultivars, may be attributed to varietal difference as described above. In addition, leaf chemical compositions as well as nutritional quality were significantly influenced by cultivar as reported by Pokluda and Kuben (2002) and Koudela and Petříková (2008).

The obtained results are in good accordance with those obtained by Bhathal et al., (2015) and Chacaliaza-Rodríguez et al., (2016) on quinoa fresh leaves, Purvika et al., (2012) on leaves of *Chenopodium album*, Akubugwo et al., (2007) on fresh amaranth leaves, Tang et al., (2014) on quinoa and amaranth leaves and Singh et al., (2001) on leaves of spinach and amaranth. On the contrary, no significant difference on leaf accumulated nitrate was detected among studied Iranian spinach accessions (Ahmadi et al., 2010).

Special attention was given to the levels of nitrate (NO_3), nitrite (NO_2) and oxalic acid ($C_2O_4^{2-}$) contents as anti-nutritional factors, in the leaves of the three studied plants (Hualhuas, CICA and spinach) which could be adversely affected the human health (Hord et al., 2009; Solberg et al., 2015). The obtained results herein showed that young quinoa plants of Hualhuas gave the lowest values of anti-nutritional factors (NO_3 and NO_2 contents). In contrast, spinach plants recorded the highest values of anti-nutritional factors (nitrate, nitrite and oxalic acid contents) in both seasons of the study. It is well known that nitrite content in vegetables is generally very low when compared to nitrate.

A number of factors affecting nitrate accumulation in vegetables, i.e. cultivar, vegetation period, density of plants in the field, soil type, agricultural practices (nitrogen doses and forms), environmental conditions (temperature, photoperiod) and season of harvest (Santamaria, 2006). Vegetable plants belong to *Amaranthaceae* and *Chenopodiaceae* families tended to accumulate large amounts of nitrate and nitrite as well as oxalate in their leaves (Jancurová et al., 2009). Spinach plant is one of the highest nitrate accumulators (Santamaria et al., 1999), likely due to a very efficient uptake and inefficient reductive systems, or unfavorable combination of both (Maynard et al., 1976). Nitrate and oxalate accumulated in New Zealand spinach leaves varied from 449 to 3472 mg/kg FW and from 506 to 1765 mg/100 g FW, respectively, (Jaworska, 2005).

European Commission's Scientific Committee for Food set the maximum acceptable nitrate content as 3000 and 2500 mg/kg on fresh weight basis for crops harvested from November to March and from April to October, respectively. Further, European Commission's regulations set the Acceptable Daily Intake (ADI) of nitrate and nitrite by 0-3.7 and 0-0.06 mg/kg of body weight, respectively, (Anonymous, 2005). According to the EC's regulations, our results indicated that the accumulated nitrate and nitrite in the leaves of young plants of the three studied cultivars are far below the limits permitted to be accepted in leafy vegetables.

Regarding leaf mineral contents the obtained results showed significant differences between young plants of both quinoa cultivars (Hualhuas and CICA) and spinach plants in both seasons of the study. The superiority of the three studied plants was fluctuated regarding their leaf mineral contents. It is worth to mention here that ash content could be used as an index for mineral contents in the plant. The higher ash content in quinoa leaves than spinach leaves might be explained mainly due to the increment of K^+, Ca^{++} contents.

Minerals are important in human diet, they served as cofactors for many physiological and metabolic functions. Significant genotypic differences are existed in the elements uptake by plants (Marschner, 1995). Special attention was given to Ca, Na, Fe and Zn contents in leaves of the three studied plants. Calcium is a major element for bone and teeth and it is necessary for transmission of nerve impulse. The daily requirement of Ca ranged from 500-1200 mg/day. Iron is an essential element for hemoglobin formation, normal functioning in the oxidation of carbohydrates, proteins and fats. Anemia is the most common Fe nutritional deficiency disease. Daily Fe consumption is 20-30 mg/day. Zinc is a trace element essential for protein biosynthesis and it is required for activation of more than 300 enzymes in human body. Zinc deficiency leads to poor wound healing, dermatitis and alopecia. The daily requirement of Zn is 10 mg/day. Sodium is helping in maintaining normal blood pressure and normal function of muscles and nerves. The recommended daily requirement of Na ranged from 1500-2300 mg/day. People suffering from high blood pressure may be instructed to reduce daily Na intake. High blood pressure can increase kidney disease or the risk of heart attack. High Na intake can lead to water retention (Soetan et al., 2010).

Young quinoa plants of CICA showed the higher percentages of Ca (1.65 and 2.13%) and Fe (1480.6 and 1180.3 mg/kg) compared with Hualhuas and spinach plants in the first and second seasons, respectively. In the same respect, spinach plants of Balady cultivar gave higher percentages of Na (0.19 and 0.38%) and Zn (98.57 and 44.70 mg/kg) relative to both quinoa cultivars in the first and second seasons, respectively. It is worth to mention that our results herein revealed that mineral contents in leaves of young quinoa plants could be met a significant portion of daily requirement for human body from minerals especially Ca, Fe and Zn.

As mentioned above the obtained results herein may be ascribed to varietal difference according to the different of genetic background among the three studied cultivars. It is appeared from the obtained results that the contents of evaluated nutritive values were significantly influenced by cultivar. Cultivar is an important factors that influencing nutritional quality and leaf chemical compositions (Pokluda and Kuben, 2002; Koudela and Petříková, 2008).

The obtained results are in agreement with those obtained by Shukla et al., (2006) and Akubugwo et al., (2007) on fresh amaranth leaves, Bhargava et al., (2010) on *Chenopodium* leaves, Bhattacharjee et al., (1998) on spinach leaves and Ahmadi et al., (2010) on leaves of some Iranian spinach accessions. In the same regard, Singh et al., (2001) reported that significant differences were noticed between spinach and amaranth fresh leaves concerning their contents of Fe, Zn, Mn and Cu. Spinach plants considered as a good source of Fe and an important source of Ca and Mg (Koh et al., 2012).

It is of interest to point out that CICA cultivar showed the vigorous plant growth and late flowering than Hualhuas cultivar. This led significantly to the increment of plant biomass, consequently resulted in dilution effect. This might be gave an acceptable explanation for the obtained results herein which revealed that Hualhuas cultivar was superior than CICA cultivar concerning most of leaf chemical and mineral contents.

CONCLUSION

It could be concluded that fresh leaves of both young quinoa plants of Hualhuas and CICA cultivars, harvested after 45 days from sowing date could be supplied a considerable amount of leaves pigments, fats, proteins, health promoting compounds (TPC, TFC and antioxidant activity%) and important amount of mineral contents (N, P, K, Ca, Mg, Fe and Mn). In addition to acceptable amount of anti-nutritional factors (NO_3, NO_2, oxalic acid and Na) compared to spinach plants. Therefore, they could be essential for meeting a favorable portion of daily requirement for human body and to maintain health. These confirmed the possibility of the potential use of young quinoa plants as a new non-traditional leafy vegetable crop. It is of interest to mention that this study is the first step to introduce quinoa fresh leaves as a new leafy vegetable crop for the first time in Egypt, but more researches are still needed.

CONFLICT OF INTEREST

There is no conflict of interest here.

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