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Phenolic Compounds and Saponins in Quinoa Samples (*Chenopodium quinoa* Willd.) Grown under Different Saline and Nonsaline Irrigation Regimens

Ana María Gómez-Caravaca,*[,†,§] Giovanna Iafelice,[#] Antonella Lavini,[⊗] Cataldo Pulvento,[⊗] Maria Fiorenza Caboni,[†] and Emanuele Marconi[#]

[†]Dipartimento di Scienze degli Alimenti, Università di Bologna, Piazza Goidanich 60, 47521 Cesena (FC), Italy

[§]Department of Analytical Chemistry, University of Granada, c/Fuentenueva s/n, 18071 Granada (GR), Spain

[#]DISTAAM, Università degli Studi del Molise, Via De Sanctis, 86100, Campobasso, Italy

[⊗]CNR-ISAFOM Institute for Agricultural and Forest Systems in the Mediterranean, National Research Council (CNR) of Italy, Via Patacca 85, 80056 Ercolano Naples, Italy

ABSTRACT: Quinoa is a pseudocereal from South America that has received increased interest around the world because it is a good source of different nutrients and rich in antioxidant compounds. Thus, this study has focused on the effects of different agronomic variables, such as irrigation and salinity, on the phenolic and saponin profiles of quinoa. It was observed that irrigation with 25% of full water restitution, with and without the addition of salt, was associated with increases in free phenolic compounds of 23.16 and 26.27%, respectively. In contrast, bound phenolic compounds were not affected by environmental stresses. Saponins decreased if samples were exposed to drought and saline regimens. In situations of severe water deficit, the saponins content decreased 45%, and 50% when a salt stress was added. The results suggest that irrigation and salinity may regulate the production of bioactive compounds in quinoa, influencing its nutritional and industrial values.

KEYWORDS: *quinoa, phenolic compounds, saponins, irrigation level, salt water*

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a grain crop that has been cultivated in South America for centuries. It belongs to the family Amaranthaceae, and it is a pseudocereal because it can be used in the same manner as wheat and rice. Until recently, its cultivation was restricted to subsistence farming in some regions of South America.[1] However, there has been an increasing interest in quinoa due to its perceived superior nutritional quality compared to other grains. Quinoa has a high protein content and is a good source of essential amino acids such as lysine and methionine.[2] Furthermore, it is rich in vitamins, minerals, fiber, and a large variety of antioxidant compounds.[3] For these reasons, it has aroused a level of high interest in the United States, Europe, and Asia, where demand for quinoa has recently increased.[4]

Quinoa is able to grow in very different climate conditions. It is a drought-tolerant crop with low water requirements. It is able to grow in regions where the annual rainfall is in the range of 200−400 mm, but it also can be grown in southern Chile, with an annual precipitation as high as 3000 mm.[5]

Strong tolerance has also been demonstrated for other stressful conditions such as salty soils and a cold climate.[6,7] Quinoa can be grown on various types of soils, including marginal soils, under a wide range of soil salinity (from pH 6.0 to 8.5). In addition, it tolerates a wide range of temperatures from approximately −1 to 35 °C.

The deleterious effects of salinity on plant growth are associated with a low water potential of soil solution (water stress), nutritional imbalance, specific ion effects (salt stress), or a combination of these different factors.[8] During the onset and development of salt stress within a plant, all of the major processes are affected. Abiotic stresses may also cause significant changes in the yield and composition of secondary metabolites, such as polyphenols.[9,10]

As mentioned above, quinoa possesses a large number of minor compounds. Of these, phenolic compounds are of great interest because of their activities as antioxidants and their antiallergic, anti-inflammatory, antiviral, anticarcinogenic, and cardiovascular protective properties.[11,12] Another important family of compounds present in quinoa is the family of saponins. These compounds, mainly concentrated in the pericarp layer, are responsible for an unpleasant bitter taste, making the removal of saponins during food processing necessary. However, saponins have antimicrobial reactivity,[13] are toxic to brine shrimps, act against viral diseases, have cholesterol-lowering effects, and have enhanced drug absorption through mucosal membranes. They act as immunological and absorption adjuvants to enhance antigen-specific antibody and mucosal responses.[14]

Traditionally, the phenolic compounds in quinoa have been determined by spectrophotometric analysis,[3,15,16] and only a few studies have been performed using HPLC[3,17] or NMR.[18] Saponins have been analyzed by biological tests as well as by

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gravimetric, hemolytic, and foam-based assays. These methods are nonspecific and liable to produce considerable errors. Thus, a good approach for the analysis of saponins was achieved by HPLC, GC, and GC-MS. With these methods, all saponins can be detected.[19−22]

The aim of this work was to evaluate the impact of different irrigation levels and the use of salt water for irrigation on bioactive compounds, such as polyphenols and saponins, in quinoa. Quinoa is of interest because of its high nutritional value and because its consumption in Europe is increasing. Furthermore, quinoa is a gluten-free food that can represent a healthy alternative to frequently used ingredients in gluten-free products.[23] It is also important to highlight that, to our knowledge, this is the first time that abiotic stresses have been studied in terms of their effect on the polyphenol and saponin content in quinoa.

MATERIALS AND METHODS

Chemicals and Reagents. HPLC grade acetonitrile, water, methanol, acetone, acetic acid, ethyl acetate, *n*-hexane, and diethyl ether were purchased from Merck KGaA (Darmstadt, Germany). Ferulic acid, rutin, and oleanolic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Experimental Field and Samples. The study was conducted on the Danish cultivar of quinoa (Q52) provided by Dr. Sven Erik Jacobsen of the University of Copenhagen. The field trials were carried out during the 2009−2010 season at the experimental station of the National Research Council (CNR), Institute for Agricultural and Forest Mediterranean Systems (ISAFoM) at Vitulazio (CE) in the lower part of the Volturno river plain (southern Italy, at 25 m asl; 14° 12′ E 4° 07′ N). The climate characteristics of the trial site are typical of a subhumid Mediterranean area.

The quinoa samples were harvested as a completely randomized block with three replicates of three irrigation levels: a control with 100% of the water necessary to replenish the soil to field capacity and two treatments with 50 and 25% of the water volume used for the control treatment. Each irrigation level had a plain water irrigation treatment (Q100, Q50, and Q25) and a corresponding saline water irrigation treatment (Q100S, Q50S, and Q25S). The chemical characteristics of plain water and saline water are the same as described by Pulvento et al.;[24] the saline water was prepared by adding sodium chloride (NaCl), calcium chloride ($CaCl_2$), potassium chloride (KCl), magnesium chloride ($MgCl_2$), and magnesium sulfate ($MgSO_4$) to well water, obtaining water with an electrical conductivity of approximately 22 dS m^{-1} and a ionic content similar to that obtained by mixing water and saline water in the ratio 1:1. The irrigation water was supplied weekly using a surface drip irrigation system.

During the two trials four and five irrigations occurred in the first and second years, respectively. The control treatment (Q100) received a seasonal water volume of approximately 3600 m^3 ha^{-1} and approximately 3000 m^3 ha^{-1} in the first and in the second years of the trial, respectively.

Extraction of the Free Polar Fraction of Quinoa. The protocol used to isolate the free phenolic fraction was that of Gómez-Caravaca et al.[21] In brief, 2 g of quinoa flour was extracted in an ultrasonic bath (20 min) in 30 mL of a solution of methanol/water (4:1 v/v) with 0.1% of acetic acid. After centrifugation at 1000*g* for 10 min, the supernatant was removed and the extraction was repeated. The supernatants were collected, evaporated, and reconstituted with 2 mL of methanol/water (1:1 v/v). The final extracts were filtered through 0.22 μm PTFE syringe filters and stored at −18 °C until use.

Extraction of the Bound Phenolic Fraction of Quinoa. After the extraction of free phenolic compounds, bound phenols were collected by alkaline hydrolysis following the method of Verardo et al.[25] with some modifications. Then, 2 g of whole flour was digested with 100 mL of 2 M NaOH at room temperature for 20 h while shaking under nitrogen gas. The mixture was then brought to pH 2−3 by adding 10 M hydrochloric acid in a cooling ice bath and extracted with 500 mL of hexane to remove the lipids. The final solution was extracted three times with 100 mL of 1:1 diethyl ether/ethyl acetate (v/v). The organic fractions were pooled and evaporated to dryness. The phenolic compounds were reconstituted with 2 mL of methanol/water (1:1 v/v). The final extracts were filtered through 0.22 μm PTFE syringe filters and stored at −18 °C until use.

HPLC-DAD-ESI-MS Analysis of Phenolic Compounds. The LC data were gathered using an Agilent 1100 series LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a degasser, a binary pump, an autosampler, a column heater, a diode array detector (DAD), and a quadrupole mass spectrometer detector (MSD, model G1946A). Separation was carried out on a fused core type column Kinetex C18 (100 mm × 4.6 mm, 2.6 μm) (Phenomenex, Torrance, CA, USA). The gradient elution was programmed using a mobile phase A consisting of acidified water (1% acetic acid) and a mobile phase B consisting of 60% phase A and 40% acetonitrile. The elution program was as follows: an isocratic step, 2% phase B from 0 to 3.5 min; from 2% B to 6% B, 3.5−4.5 min; from 6 to 10% B, 4.5−6 min; from 10 to 17% B, 6−7.5 min; from 17 to 36% B, 7.5−13 min; from 36 to 38.5% B, 13−14 min; from 38.5 to 60% B, 14−19 min; from 60 to 100% B, 19−24 min; 100% B, 24−30 min; and from 100 to 2% B, 2 min. The flow rate was constant at 0.8 mL/min, and the column temperature was maintained at 25 °C. The injection volume was 10 μL, and UV spectra were recorded from 200 to 600 nm, whereas the chromatograms were registered at 240, 280, and 330 nm.

The MS analyses were carried out in full-scan mode (range m/z 50−1000) using an electrospray (ESI) interface and the following conditions: drying gas flow, 9.0 L/min; nebulizer pressure, 35 psig; gas drying temperature, 350 °C; capillary voltage, 3000 V; and fragmentor voltage, 80 V.

Saponin Evaluation. To assess the saponin content, quinoa seeds were ground using a laboratory mill (model IKA A10-IKA Werke GmbH & Co., Staufen, Germany). As reported by Ridout et al.,[22] quinoa flour was defatted by a Soxhlet extraction. After air-drying, the defatted sample was hydrolyzed in reflux for 3 h with a methanolic solution of hydrochloric acid (2 N). The product of the hydrolysis was cooled and neutralized with ammonium hydroxide and dissolved in 5 mL of distilled water. Sapogenins (the hydrolyzed product of saponins) were then extracted with ethyl acetate (3 × 20 mL). The combined ethyl acetate extracts were dried, dissolved in bis-(trimethylsilyl)trifluoroacetamide (BSTFA) (100 μL) and dry pyridine (100 μL), and derivatized for 20 min at 50 °C. The total sapogenins were estimated by gas chromatography as described by Ridout et al.[22] with some modifications. In particular, 1 μL of derivatized sample was injected into a Perkin-Elmer GC Clarus 500 (Waltham, MA, USA) fitted with a ZB-5HT capillary column (Phenomenex). Injector and detector (FID) temperatures were set at 370 °C, whereas the oven temperature was programmed in increments of 8 °C min^{-1} from 180 to 230 °C and in increments of 6 °C min^{-1} from 230 to 350 °C.

The analyses were performed in duplicate for each cultivar. The sapogenin contents were calculated using their peak area relative to that of the internal standard. The identification of sapogenins was based on relative retention times compared with the commercial standards and confirmed with the mass spectral data obtained by GC-MS (GCMS-QP2010 Plus, Shimadzu, Tokyo, Japan) using the same chromatographic conditions for GC-FID. The quadrupole was used in the electronic impact mode (70 eV), and a mass range of m/z 50−800 was monitored in full scan.

Statistical Analysis. One-way analysis of variance, ANOVA (Tukey's honest significant difference multiple comparison), was evaluated using Statistica 8.0 software (StatSoft, Tulsa, OK, USA), and p values < 0.05 were considered to be statistically significant.

Factorial ANOVA univariate analysis was also used to evaluate the effects of irrigation and salinity on phenolic compounds and saponins in quinoa samples (Statistica 8.0 software, StatSoft).

All chemical analyses were carried out in triplicate, and the analytical data were used for statistical comparisons.

Table 1. Free Phenolic Compounds (Expressed as Milligrams Analyte per 100 g of Quinoa)[a]

peak[b]	compound	Q25	Q50	Q100	Q25S	Q50S	Q100S
1	1-*O*-galloyl-*β*-D-glucose[c]	2.93 a	2.04 c	2.51 ab	2.33 bc	2.30 bc	2.20 bc
2	acacetin/questin/apigenin-7-methyl ether[d]	49.05 a	34.86 bcd	39.99 b	37.18 bc	30.49 cd	27.37 d
3	protocatechuic acid 4-*O*-glucoside[c]	1.53 a	1.34 b	1.34 b	1.31 b	1.17 c	1.29 bc
4	vanillic glucoside[c]	1.76 a	1.63 ab	1.78 a	1.56 b	1.56 b	1.36 c
5	penstebioside[d]	1.38 a	0.75 c	0.87 b	0.72 cd	0.61 de	0.55 e
6	canthoside A/2-hydroxybenzoate 2-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside[c]	1.28 a	1.19 abc	1.25 ab	1.13 c	1.17 bc	1.14 bc
7	ferulic acid 4-*O*-glucoside[c]	3.27 a	2.41 b	2.30 bc	2.38 b	2.50 b	1.88 c
8	ethyl-*m*-digallate[c]	2.30 a	1.88 b	2.03 b	1.96 b	1.88 b	1.76 b
9	(epi)gallocatechin[d]	2.51 a	1.04 cd	1.31 bc	1.53 b	0.79 d	1.05 cd
10	quercetin 3-*O*-(2,6-di-*α*-L-rhamnopyranosyl)-*β*-D-galactopyranoside[d]	23.65 a	18.86 b	20.79 ab	24.61 a	18.29 b	16.77 b
11	kaempferol 3-*O*-*β*-D-apiofuranosyl(1‴→2″-O-[*α*-L-rhamnopyranosyl(1‴→6″]-*β*-D-galactopyranoside[d]	17.67 a	10.44 d	13.23 bc	15.52 ab	6.98 e	12.06 cd
12	kaempferol 3-*O*-*β*-D-apiofuranosyl(1‴→2″-*O*-[*α*-L-rhamnopyranosyl(1‴→6″]-*β*-D-galactopyranoside isomer[d]	2.97 a	1.78 b	1.93 b	2.68 a	1.69 b	1.89 b
13	kaempferol 3-*O*-(2,6-di-*α*-L-rhamnopyranosyl)-*β*-D-galactopyranoside (mauritianin)[d]	18.32 a	11.68 bc	14.18 b	14.27 b	10.97 c	11.64 bc
14	quercetin 3-*O*-[*β*-D-apiofuranosyl(1‴→2″)]-*β*-D-galactopyranoside[d]	11.78 a	6.86 bc	6.18 c	10.48 a	6.85 bc	7.97 b
15	rutin[d]	6.09 a	4.26 bc	4.27 bc	4.81 b	3.27 d	3.53 cd
16	quercetin glucuronide[d]	14.92 a	5.74 bcd	5.29 cd	7.59 b	4.72 d	7.07 bc
17	quercetin 3-*O*-glucoside[d]	3.03 a	1.97 cd	2.01 cd	2.61 ab	1.57 d	2.45 bc
	total	164.46 a	108.74 cd	121.25 c	132.70 b	96.82 d	101.97 d

[a]Q100, Q50, and Q25 (quinoa samples irrigated with 100% of the water necessary to replenish soil to field capacity and two treatments with restitution of 50 and 25% of the water volume used for the control treatment). Q100S, Q50S, and Q25S (a corresponding treatment irrigated with saline water). Different letters in the same row indicate significant differences ($p < 0.05$). [b]Peak numbers from Figure 1a. [c]Expressed as mg/100 g of ferulic acid. [d]Expressed as mg/100 g of rutin.

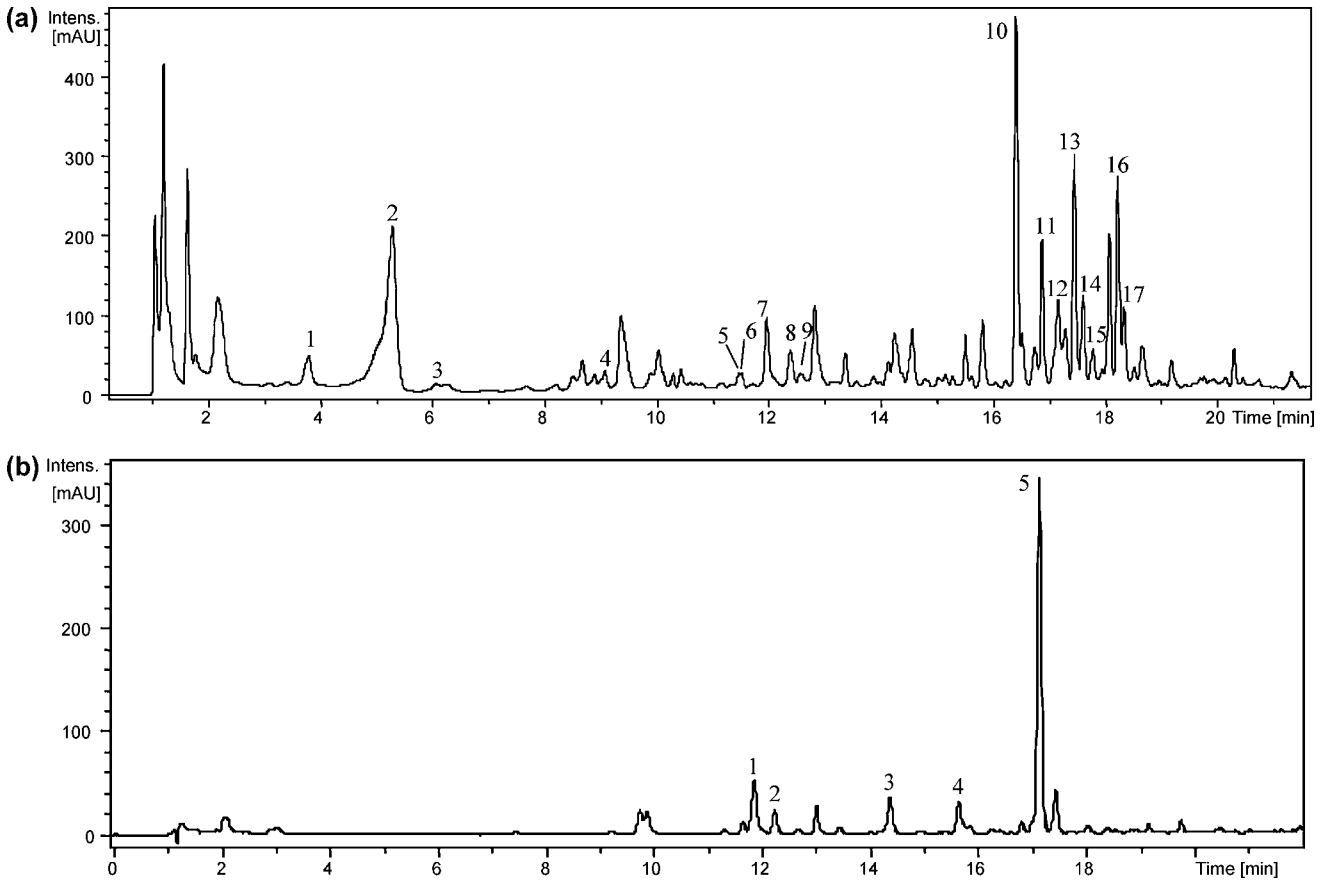

Figure 1. (a) Chromatogram of the free phenolic compounds of quinoa at λ = 280 nm; (b) chromatogram of the bound phenolic compounds of quinoa at λ = 280 nm obtained by HPLC. See Tables 1 and 2 for identification of phenolic compounds.

Table 2. Bound Phenolic Compounds (Expressed as Milligrams Analyte per 100 g of Quinoa)[a]

peak[b]	compound	Q25	Q50	Q100	Q25S	Q50S	Q100S
1	benzoic acid	2.34 ab	2.49 a	1.90 b	2.26 ab	1.38 c	1.98 b
2	vanillic acid	1.46 bc	1.90 a	1.17 c	1.53 b	1.52 b	1.91 a
3	vanillin	2.28 a	2.12 ab	1.53 b	2.19 ab	1.55 b	2.12 ab
4	coumaric acid	1.64 ab	2.21 a	1.46 b	1.69 ab	1.73 ab	1.67 ab
5	ferulic acid	7.87 a	8.64 a	5.66 a	8.58 a	8.36 a	7.51 a
	total	15.60 a	17.37 a	11.72 a	16.25 a	14.54 a	15.19 a

[a]Q100, Q50, and Q25 (quinoa samples irrigated with 100% of the water necessary to replenish soil to field capacity and two treatments with restitution of 50 and 25% of the water volume used for the control treatment). Q100S, Q50S, and Q25S (a corresponding treatment irrigated with saline water). Different letters in the same row indicate significant differences ($p < 0.05$). [b]Peak numbers as in Figure 1b.

RESULTS AND DISCUSSION

Determination of Free Phenolic Compounds in Quinoa by HPLC-DAD-ESI-MS. Seventeen phenolic compounds have been identified and quantified by HPLC in the free phenolic fraction of different quinoa samples. The concentrations of the total and individual free phenolic compounds determined in these samples are reported in Table 1, and the chromatographic profile obtained is shown in Figure 1a.

It is known that plant adaptability to different types of stresses is associated with an increase in antioxidant capacity and antioxidants, [26] such as polyphenols, that can scavenge harmful radicals and stabilize lipid oxidation.[27,28]

In fact, the results showed that both drought and salt stresses affect the amount of phenolic compounds. The decrease in irrigation volumes is associated with an increase in phenolic compounds in samples that were treated only with water and in samples irrigated with salt water. If the water volume was 50% of the fully watered samples, no significant differences in phenolic compounds were observed, whereas if the water volume was 25% of the fully watered samples, the concentration of phenolics was significantly higher than in the Q100 and Q100S samples. The increase in phenolic compounds was 26.3% in Q25 samples and 23.2% in Q25S samples compared to Q100 and Q100S, respectively.

Despite the samples irrigated with plain water and samples irrigated with saline water showing the same behavior in terms of free phenolic compounds, total free phenolic compounds in the saline water irrigated samples were lower than in the samples exposed only to water if the same percentages of water volume are compared. The decrease in phenolic compounds was in the range of 10.9−19.3%. Reports on the effect of salinity on phenolic contents are limited and contradictory depending on the matrices studied. Several studies have found that an enhancement of phenolics metabolism is a response to abiotic stress.[29,30] However, other studies are in agreement with the presented results, where salinity negatively influences the content of phenolic compounds in quinoa samples.[31]

It is important to highlight that flavonol derivatives were the most abundant free phenolic compound family in the samples and that these compounds showed a large increase after the stress suffered by the quinoa plant.

This result is in agreement with the literature because it has been demonstrated that flavonols are the phenolic compounds with the highest detoxification activity, reducing oxidative damage in cases of stress.[32]

In the samples irrigated with plain water, the compound illustrated as peak 16, which is quercetin glucuronide, increased to the highest percentage after drought stress (64.6%), followed by (epi)gallocatechin and quercetin 3-*O*-[*β*-D-apiofuranosyl-(1‴-2″)]-*β*-D-galactopyranoside, which increased 47.8 and 47.6%, respectively. In contrast, there were compounds, such as vanillic glucoside and canthoside A, that presented no significant differences in concentration after being exposed to water deficits.

The samples treated with salt water had some differences. Compounds found with the highest in saline water treatments differed from those that changed after the exposure to only water deficit, and the increase in the concentrations of these phenolic compounds was slightly lower than in cases when only a water deficit was suffered. (Epi)gallocatechin, quercetin 3-*O*-(2,6-di-*α*-L-rhamnopyranosyl)-*β*-D-galactopyranoside, and kaempferol 3-*O*-*β*-D-apiofuranosyl(1‴→2″-*O*-[*α*-L-rhamnopyranosyl(1‴→6″]-*β*-D-galactopyranoside showed higher increases after saline water stress (31.44, 31.83, and 29.65%, respectively). Protocatechuic acid 4-*O*-glucoside and canthoside A were not significantly different in concentration after saline water stress.

Similar progress in terms of water stress has also been found in different plants, such as *Salvia officinalis* L.,[28] cherry tomato,[33] and lettuce.[34]

Determination of Bound Phenolic Compounds in Quinoa by HPLC-DAD-ESI-MS. Bound phenolic compounds under irrigation deficit and salinity were also evaluated by HPLC (Table 2). The chromatographic profile of bound phenolic compounds can be observed in Figure 1b.

Total bound phenolic compounds were not affected by salinity and irrigation deficit conditions. No significant differences were found among samples, neither those exposed to water deficit only nor those irrigated with salt water.

The bound phenolic compound present at the highest concentration was ferulic acid, but the concentration was not significantly different among the samples. However, small increases in benzoic acid, vanillic acid, and coumaric acid concentrations were noted in the Q50 samples. This finding indicates that the concentration of most bound phenolic compounds in quinoa increases after abiotic stresses.[30] However, unlike total free phenolic compounds, total bound phenolic compounds in quinoa were not affected by environmental abiotic stresses.

The response of the quinoa plant against oxidative damage produced by drought and salinity conditions is a clear increase in phenolic compounds after water deficit (in the presence and the absence of salinity); nevertheless, the increase in phenolic compounds is lower in the presence of salt water than in the samples for which plain water was used for irrigation.

Saponins Evaluation. Saponins were evaluated in terms of sapogenins, and their contents in quinoa seeds are presented in

Table 3. Sapogenin Content in Quinoa (Milligrams per 100 g Dry Weight)[a]

compound	Q25	Q50	Q100	Q25S	Q50S	Q100S
oleanolic acid	248.8 e	359.6 cd	384.5 bc	409.5 b	339.3 d	660.8 a
hederagenin	191.6 e	161.6 f	351.1 b	273.3 c	245.1 d	497.3 a
phytolaccagenic acid	189.3 f	251.7 e	404.5 b	307.8 d	357.8 c	475.2 a
total	629.7 e	772.9 d	1140.1 b	990.6 c	942.1 c	1633.3 a

[a]Q100, Q50, and Q25 (quinoa samples irrigated with 100% of the water necessary to replenish soil to field capacity and two treatments with restitution of 50 and 25% of the water volume used for the control treatment). Q100S, Q50S, and Q25S (a corresponding treatment irrigated with saline water). Different letters in the same row indicate significant differences ($p < 0.05$).

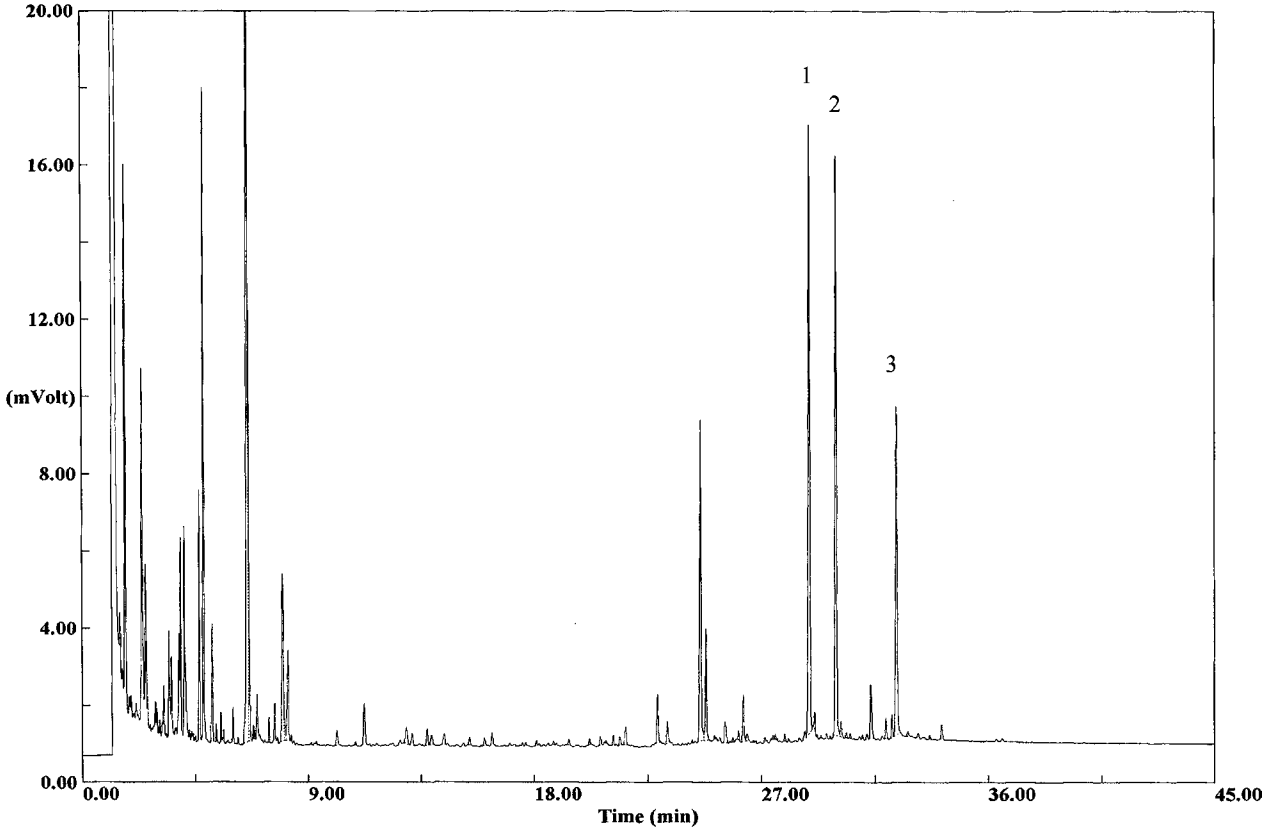

Figure 2. GC-FID profile of the sapogenins of quinoa samples. Peaks: 1, oleanolic acid; 2, hederagenin; 3, phytolaccagenic acid.

Table 3. The GC procedure was applied for the evaluation of saponin aglycones (sapogenins) derived from the acid hydrolysis of samples, as reported previously.[22,35,36] A chromatographic profile of quinoa saponins is shown in Figure 2. Three major quinoa saponin aglycones were identified: oleanolic acid (36−50% total), hederagenin (27−28%), and phytolaccagenic acid (21−36%). These results are in agreement with the data reported by other authors.[35,37,38] These sapogenins were identified by their retention times and their mass spectra data obtained by GC-MS in comparison with the commercial standards. Oleanolic acid was identified by its most important fragment signals at m/z 600 (3.5%), m/z 585 (7.6%), m/z 482 (21.4%), m/z 320 (33.9%), and m/z 203 (100%). These data were in agreement with previous data found in the literature.[39,40] Hederagenin reported the principal fragments at m/z 688 (3.2%), m/z 598 (3.3%), m/z 570 (14.5%), m/z 320 (42.2%), and m/z 203 (100%) as previously described by Burnouf-Radosevich et al.[40] Fragment signals found at m/z 732 (1.2%), m/z 642 (1.9%), m/z 614 (11%), m/z 364 (21.5%), m/z 247 (85%), and m/z 207 (100%) were in agreement with the fragmentation pattern of phytolaccagenic acid.

Although the literature provides much information about the influence of irrigation on agronomic performance in quinoa, very little knowledge is available as to how the irrigation level affects saponin concentration in quinoa seeds. Abiotic and biotic stresses cause fluxes between plant primary and secondary metabolism, resulting in a diversion of available resources from growth to defense, resulting in the production of different secondary metabolites, including saponins.[41,42]

Statistical analysis showed significant differences between saline and nonsaline treatments and among the three irrigation volumes used. The highest saponins values were observed in samples obtained without deficit irrigation treatments (1633.3 mg/100 g for Q100S and 1140.1 mg/100 g dw for Q100, respectively).

When considering the total amount of saponins, it was observed that the genotype Q52 is a bitter-type quinoa variety. In fact, quinoa seeds that contain a saponin concentration >0.11% are usually considered to be a bitter genotype.[1,5,38]

Table 4. Factorial ANOVA (Univariate Results)[a]

compound	W	S	W×S
free phenolic compounds			
1-*O*-galloyl-*β*-D-glucose	0.04*	0.08	0.16
acacetin/questin/apigenin-7-methyl ether	0.00***	0.00***	0.00**
protocatechuic acid 4-*O*-glucoside	0.00**	0.00**	0.29
vanillic glucoside	0.27	0.00**	0.02*
penstebioside	0.00***	0.00***	0.00**
canthoside A/2-hydroxybenzoate 2-*O*-*β*-D-apiofuranosyl(1→6)-*O*-*β*-D-glucopyranoside	0.13	0.03*	0.16
ferulic acid 4-*O*-glucoside	0.02*	0.02*	0.17
ethyl *m*-digallate	0.05	0.08	0.25
(epi)gallocatechin	0.00***	0.00**	0.04*
quercetin 3-*O*-(2,6-di-*α*-L-rhamnopyranosyl)-*β*-D-galactopyranoside	0.00**	0.61	0.01*
kaempferol 3-*O*-*β*-D-apiofuranosyl(1‴→2″-*O*-[*α*-L-rhamnopyranosyl (1‴→6″]-*β*-D-galactopyranoside	0.00***	0.00***	0.00***
kaempferol 3-*O*-*β*-D-apiofuranosyl(1‴→2″-*O*-[*α*-L-rhamnopyranosyl(1‴→6″]-*β*-D-galactopyranoside Isomer	0.00**	0.46	0.64
kaempferol 3-*O*-(2,6-di-*α*-L-rhamnopyranosyl)-*β*-D-galactopyranoside (mauritianin)	0.00***	0.00**	0.02*
quercetin 3-*O*-[*β* -D-apiofuranosyl(1‴→2″)]-*β*-D-galactopyranoside	0.00***	0.69	0.16
rutin	0.00***	0.00***	0.15
quercetin glucuronide	0.00***	0.00***	0.00***
quercetin 3-*O*-glucoside	0.00**	0.58	0.20
total free phenolics	0.00***	0.00***	0.01*
bound phenolic compounds			
benzoic acid	0.00**	0.00**	0.00***
vanillic acid	0.02*	0.03*	0.00***
vanillin	0.03*	0.83	0.01*
coumaric acid	0.03*	0.45	0.05*
ferulic acid	0.12	0.31	0.49
total bound phenolics	0.13	0.70	0.09
saponins			
oleanolic acid	0.00***	0.00***	0.00***
hederagenin	0.00***	0.00***	0.00***
phytolaccagenin	0.00***	0.00**	0.00***
total saponins	0.00***	0.00***	0.00***

[a]Column W, significant effect of % water irrigation; column S, significant effect of salinity; column W×S, significant effect of irrigation and salinity. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

In contrast, the irrigation deficit was associated with a minor accumulation of saponins in quinoa seeds. The samples that were treated with only a water deficit (Q25 and Q50) showed a decrease in the saponin content when compared to Q100. The Q50 samples, compared with the Q100 ones, showed a decrease in saponins of 32%, whereas the samples grown with a higher irrigation deficit (Q25) showed a 45% decrease in saponins.

These results are in agreement with the study reported by Soliz-Guerrero et al.,[43] who reported that the content of saponins is affected by a soil−water deficit, such that high water deficits promote low saponin contents.

Samples treated with saline water also showed significant differences at different irrigation levels (Q100S, Q50S, and Q25S); the decrease in saponins in the Q50S and Q25S samples was very high compared to Q100S (40 and 42% for Q25S and Q50S, respectively).

By comparison of the results obtained in samples treated with plain water and saline water, it could be observed that the saponins content is higher in samples irrigated with saline water in all cases. Saponins in Q100S are 30% higher than in Q100, in Q50S are 18% higher than in Q50, and in Q25S are 36% higher than in Q25. These results indicate that the saline water treatment increase the saponin content in quinoa samples.

When the effects of irrigation treatments on individual saponins, such as oleanolic acid, hederagenin, and phytolaccagenic acid are examined, a different trend is evident between plain water and saline water irrigation. In samples grown using plain water, hederagenin acid and phytolaccagenic acid are more sensitive to water stress, with the highest reduction in Q25, whereas in samples grown with saline water, oleanolic acid and hederagenin showed the highest reduction, in particular in the Q50S sample.

Factorial ANOVA Univariate Analysis. A univariate analysis of variance was used to indicate the effects of the variables and between the variables (Table 4).

The p level shows substantial differences among free phenolic compounds, bound phenolic compounds, and saponins if the samples are affected by salinity and irrigation deficits.

In most cases, changes in free and bound phenolics and saponin contents were associated with the combined effects of salinity and drought conditions.

Single and total saponin contents were influenced by salinity and irrigation drought conditions and by the interaction of both experimental factors.

In general, free phenolic compounds were affected by the irrigation level and by salinity. In most cases, the interaction of both factors had a great influence on them as well.

Finally, as for the exception of ferulic acid, single bound phenolic compounds were also influenced by the different experimental conditions. However, the ferulic acid content was

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much higher than that of the other phenolic acids and, because of that, total bound phenolics were not significantly affected by irrigation level, salinity, or irrigation−salinity effects.

Our results showed that drought and salinity have a marked influence on the content of bioactive secondary metabolites in quinoa (*C. quinoa* Willd.). However, there is variation depending on the degree of water deficit and the analyzed compounds (free and bound phenolic compounds and saponins). The increase in the phenolic content after water deficit agrees with data in the literature,[26−28] indicating that this effect is most likely a response to the generation of reactive oxidative species. Severe water deficits produced the highest increment in the phenolic content; however, the total phenolic content was slightly lower in the samples from the saline treatments. These results also show that drought can be successfully used to enhance the content of health-promoting phytochemicals in quinoa and that it is possible to increase the content of bioactive desirable compounds by changing the agronomic conditions. Irrigation with salt water produced a small decrease in the free phenolic compounds but, despite that fact, quinoa is tolerant to saline conditions.

Saponins decrease under saline and drought conditions; therefore, a deficit of irrigation is an interesting sustainable practice to reduce the saponin levels in quinoa seeds. This effect can be seen as a good way to control the saponin levels in quinoa and, thus, to avoid the elimination of the outer layers of the seeds where vitamins and minerals are concentrated.

Finally, it is also important to highlight that, to our knowledge, this is the first time that the behavior of phenolic compounds and saponins in quinoa has been studied under abiotic stress conditions.

AUTHOR INFORMATION

Corresponding Author

*Phone: +39-(0)547-338117. Fax: +39-(0)547-382348. E-mail: anagomez@ugr.es.

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