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RESEARCH ARTICLE

Combined effect of water and sulfur deficit on the productivity and antioxidant capacity of quinoa in pots

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ABSTRACT

The challenge of feeding the world's population implies producing a greater quantity and better quality of food. Therefore, a deeper knowledge of the adaptation capacity of crops to different edaphoclimatic environments is required to face the challenges of climate change. The objective was to evaluate the effect of water and S availability on agronomic characteristics, yield, total phenol content and antioxidant capacity in quinoa (*Chenopodium quinoa* Willd.) 'Regalona'. The test was out in outdoor pots in the 2019-2020 and 2020-2021 seasons. The substrate was silt loam soil. The design was a two-factor split block, S availability (SA) with 8, 12 and 20 mg kg^{-1} S, and water availability (WA) 25%, 50% and 100% in the grain filling stage. Likewise, there was no interaction ($p > 0.05$) between WA×SA for the agronomic and productive characteristics. However, the lowest WA (25%) decreased ($p \leq 0.05$) yield and height of the plant, while the highest S fertilization increased ($p \leq 0.05$) plant height, panicle length, thousand seed weight and yield. In antioxidants, 100% and 25% WA generated differences ($p \leq 0.05$) in total phenols (TPC) and flavonoids content (TFC). Treatment SA exerted differences ($p \leq 0.05$) between 8 and 20 mg kg^{-1} in the TPC and ferric ion reducing antioxidant power (FRAP) tests. WA×SA generated differences ($p \leq 0.05$) in the TFC and the oxygen radical absorbance capacity (ORAC) in the 2020-2021 season. At 12 mg kg^{-1} SA, the highest TFC was with 25% WA and in ORAC it was at 100% WA. The degree of correlation between TPC and antioxidant activity by the FRAP method, coefficients of 0.76 and 0.66 were found for the 2019-2020 and 2020-2021 seasons, respectively.

Key words: *Chenopodium quinoa*, sulfur-water interaction, phenolic compounds, water availability.

INTRODUCTION

Plants are exposed to environmental factors that affect bioavailability of essential nutrients as well as plant growth, development, and yield (Sanaeifar et al., 2023). Drought, which is a period of below-normal water availability, can trigger physiological, biochemical, and molecular responses by plants (Sharma et al., 2022), with secondary metabolites being particularly important as they can act as osmoprotectants, osmolytes, antioxidants and/or exert protective functions in response to stress conditions. Additionally, water restriction negatively impacts the absorption of macronutrients such as nitrate, phosphate, and sulfate by plants, which produce metabolites to cope with this type of stress (Chan et al., 2013). Sulfur is an essential macronutrient for plants since it is part of a variety of chemical structures such as amino acids (methionine and cysteine), membrane sulfolipids, cell walls, vitamins, pigments, phosphonucleotides, metabolites, and cofactors. The storage of S is limited, being mainly absorbed from the soil, plant or animal residues, or external nutrient supply (Zhang et al., 2020).

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal that belongs to the dicotyledonous class, Amaranthaceae family, Chenopodiaceae subfamily, and *Chenopodium* genus (Contreras-Jiménez et al., 2019). The species can adapt to adverse conditions due to its genetic variability (Pereira et al., 2019), including abiotic stress factors such as drought (Maestro-Gaitán et al., 2022). From a nutritional point of view, quinoa stands out for its high contents of essential amino acids, vitamins, minerals, and phenolic compounds (Ramzani et al., 2017). Phenols are bioactive compounds that act as antioxidants, which have health-promoting effects (Ren et al., 2022) and are associated with the prevention or reduction of the risk of diseases such as cancer, cardiovascular and inflammatory diseases (Gómez-Caravaca et al., 2014), diabetes, obesity, and neurodegenerative diseases (Pereira et al., 2020).

The effect of drought on quinoa has been described in the literature, but the response of quinoa plants to induced water restriction in S-deficient soils has not been studied yet. The objective of this study was to evaluate the effect of S and water availability on the agronomic characteristics, yield, content, and antioxidant capacity of 'Regalona' quinoa (*Chenopodium quinoa* Willd.) seed. The hypothesis was that the restriction in the availability of water and S in the cultivation of quinoa does not negatively affect the agronomic characteristics, plant yield or the antioxidant content and capacity of the seed.

MATERIALS AND METHODS

Experimental conditions and experimental design

The experiment was carried out outdoors in pots during the 2019-2020 and 2020-2021 seasons at the El Nogal Experimental Station (36°35'56.5" S, 72°05'02.8" W), Faculty of Agronomy, University of Concepción, Chile. The site is characterized by a temperate Mediterranean climate, with an average temperature of 13.1 °C (Fischer et al., 2013). The meteorological parameters between sowing and harvest of quinoa (*Chenopodium quinoa* Willd.) were obtained from the Agrometeorological Station of the University of Concepción (36°35.715' S, 72°4.794' W) (Table 1).

Table 1. Average temperature, rainfall, hours of sun, and solar radiation from sowing to harvesting of quinoa in Chillan, Ñuble Region, Chile.

Season	Month	Max. T°	Min. T°	Rainfall	Hours of sun	Solar radiation
		°C	°C	mm mo^{-1}	h	MJ m^{-2} d^{-1}
2019-2020	October	19.5 ± 2.3	6.1 ± 3.1	9.9	8.5 ± 1.4	18.4 ± 4.5
	November	24.3 ± 4.1	9.0 ± 2.5	6.2	9.9 ± 1.6	22.4 ± 4.4
	December	27.1 ± 2.8	11.0 ± 2.0	4.2	10.5 ± 1.2	24.8 ± 4.6
	January	29.2 ± 4.2	12.5 ± 2.3	3.9	10.6 ± 1.4	25.5 ± 3.6
	February	30.0 ± 4.1	11.6 ± 3.2	1.3	10.6 ± 0.4	25.5 ± 1.3
2020-2021	October	21.9 ± 5.4	6.0 ± 3.4	4.7	9.0 ± 2.3	20.5 ± 5.0
	November	24.2 ± 2.3	8.3 ± 1.7	0.0	10.1 ± 1.3	23.1 ± 3.6
	December	26.6 ± 4.1	9.3 ± 2.3	2.2	10.5 ± 1.7	25.4 ± 5.0
	January	28.3 ± 4.3	11.5 ± 2.9	89.1	10.5 ± 1.5	24.9 ± 5.6
	February	26.9 ± 4.3	10.9 ± 2.1	0.0	9.2 ± 1.4	20.2 ± 5.3

The experiment was carried out in a split-block design with two factors (S and water availability) and three levels per factor. Three S availability (SA) were applied in the crop, 8, 12 and 20 mg kg^{-1}, adjusting the content in two different plant development stages. Water availability (WA) was adjusted when 50% of seeds were in the grain filling stage, with levels of 25%, 50% and 100% of available water at field capacity. The experimental unit consisted of four pots of 30 cm in diameter and 50 cm in depth. The substrate used in each pot corresponded a soil with 30.8% sand and 54.8% silt and 15.2% clay, classified as medial, amorphic, Typic Haploxerands (USDA, 1999), with a porosity of 72.4%.

Agronomic conditions of pot experiment

Five 'Regalona' quinoa seeds were sown per pot. Seedlings were thinned when they had four true leaves, leaving one plant per pot. Fertilization applied before and during cultivation was estimated according to the initial soil analysis (Table 2). At sowing, 1.56 g triple superphosphate pot^{-1} (80 kg P_2O_5 ha^{-1}) and 0.78 g urea pot^{-1} (40 kg N ha^{-1}) were added. Subsequently, a second dose of 1.17 g urea pot^{-1} (60 kg N ha^{-1}) was added to all the treatments at the beginning of flowering. When the plants developed two true leaves, two different fertilizers, Ecosul (Ecofos, Santiago, Chile) and Sulpomag (Mosaic Company, Riverview, Florida, USA), were applied to adjust S content and balance K in each treatment. Magnesium content was balanced using a Hoagland nutrient solution. Doses of 1.60 g pot^{-1} Sulpomag and 0.60 g pot^{-1} Ecosul were applied to the 8 and 12 mg kg^{-1} S treatments, whereas respective doses of 2.65 and 0.20 g pot^{-1} were applied for the treatment of 20 mg kg^{-1} S. The dose required for each treatment was corroborated by a subsequent soil analysis.

Table 2. Soil analysis of the silt loam soil used soil classified as medial, amorphic, Typic Haploxerands (USDA, 1999) in the experiments. ECEC: Effective cation exchange capacity.

Determination	Natural grassland	Normal category or level of sufficiency
pH	6.03	6.0-7.0
Organic matter, %	8.41	2.0-8.0
Electrical conductivity, dS m^{-1} (1:2.5)	0.10	< 1.0
Ammonium N-NH_4, mg kg^{-1}	7.50	> 10
Nitrate N-NO_3, mg kg^{-1}	10.30	> 10
Available N, mg kg^{-1}	17.80	20-60
Available P, mg kg^{-1}	3.60	20
Exchangeable K, cmol kg^{-1}	0.35	0.30-0.45
Available K, mg kg^{-1}	135.80	115-175
Exchangeable Ca, cmol kg^{-1}	6.66	4.0-8.0
Exchangeable Mg, cmol kg^{-1}	1.32	0.6-1.5
Exchangeable Na, cmol kg^{-1}	0.17	< 1.0
Sum of bases, cmol kg^{-1}	8.50	5.0-10.0
Available S, mg kg^{-1}	7.80	16-30
Exchangeable Al, cmol kg^{-1}	0.01	< 0.15
ECEC, cmol kg^{-1}	8.50	> 5.0
Al saturation, %	0.17	< 2.0
K saturation, %	9.70	5.0-10.0
Ca saturation, %	78.40	65-75
Mg saturation, %	15.70	5.0-10
Ca/Mg	5.00	4.0-6.0
K/Mg	0.60	0.3-0.6
Fe, mg kg^{-1}	8.60	> 2.5
Mn, mg kg^{-1}	3.00	> 3.0
Zn, mg kg^{-1}	1.00	> 1.0
Cu, mg kg^{-1}	0.60	> 0.5
B, mg kg^{-1}	0.70	0.6-1.5

To prevent the incidence of mildew (*Peronospora variabilis*), a common fungus in quinoa crops, the fungicide mancozeb 80 WP (1.4 g L^{-1}) was applied in two different plant development stages: When the plant had three true leaves and 1 wk after the first application.

Weed control and irrigation were carried out manually. From sowing to grain filling, water availability was maintained at 100% field capacity, with water restriction beginning 85 d from sowing in the 2019-2020 season and 93 d from sowing in the 2020-2021 season, which corresponds to the period when the grain presents a milky-pasty consistency. Water doses to be added were determined using three analog capacitive sensors per block, placed 8 cm deep from the surface of the pot and, vertically, 5 cm from the plant stem.

Evaluations

Agronomic and yield parameters. The following determinations were made in the two evaluated seasons (2019-2020 and 2020-2021). Days to flowering, the period from emergence to 50% flowering of quinoa plants. Days to grain filling, the period from emergence to physiological maturity, in which the grain presents resistance to penetration when pressed by the nails. Days to maturity, period between emergence and physiological maturity, when 50% of the panicles have a brown color. Plant height (cm) and panicle length (cm), plant height was measured from the base of the stem to the apex of the inflorescence on the main stem; and length of the main panicle of each plant was determined at harvest using a tape measure. Seed yield (g), quinoa plants were cut and placed in a paper bag and dried in the open air for 10 d to homogenize humidity. Relative humidity reached 43%, while average maximum and minimum temperatures were 30.3 and 11.5 °C, respectively. Subsequently, the panicles were threshed in a stationary thresher (Baldor 1425 rpm, Baldor Electric Co, USA), and the seeds obtained were dry cleaned at a speed of 21 m s^{-1}. Yield was obtained by determining the mass (g) of seeds per plant in each of the treatments. One thousand seed weight (g), the average mass in a sample of 500 seeds per experimental unit was obtained and multiplied by two.

In vitro antioxidant activity of the extracts. Total phenolic content (TPC) and total flavonoids content (TFC) were determined. Antioxidant activity was assessed by ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays. A Synergy HTX multi-mode microplate reader and Bio Tek Gen5 software (Agilent, Santa Clara, California, USA) were used in all the assays.

Preparation of the extracts. Five grams of ground seeds (Laboratory Mill 3100 Perten, Springfield, Illinois, USA) were sieved through a 1 mm sieve (U.S. standard sieve N°18) and mixed with 50 mL extraction solution (70% v/v acetone acidified to 0.5% v/v glacial acetic acid) in a vortex shaker (Boeco, Hamburg, Germany) at 1600 rpm for 3 min at room temperature. The mixture was placed in a thermoregulated bath (Lauda, Lauda-Königshofen, Germany) at 20 °C for 1 h, and then centrifuged (M-24 A, Boeco) at 3000 rpm for 10 min at 22 °C. The supernatant was collected, and the residue was subjected to extraction again. Both supernatants were combined in an amber bottle and refrigerated until analysis.

Total phenolic compounds were determined according to the methodology of Singleton et al. (1999), with some modifications. The results were expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g DM. Total flavonoid (flavones and flavonols) content (TFC) was calculated using the aluminum chloride colorimetric method according to Lin and Tang (2007), with some modifications. The results were expressed as milligrams of a quercetin equivalent (mg QE) per 100 g DM. The ferric reducing antioxidant power (FRAP) assay was applied according to the method of Ou et al. (2002), with some modifications. The FRAP values were calculated using a $FeSO_4$ calibration curve and the results were expressed as micromoles of Fe (µmol Fe^{2+}) per 100 g DM. The oxygen radical absorbance capacity (ORAC) assay was performed according to the methodology of Wu et al. (2004), with some modifications. The results were expressed as micromoles of Trolox equivalents (µmol TE) per 100 g DM.

Statistical analysis

Data analyzed normality and homoscedasticity using the Shapiro-Wilk and Bartlett tests, respectively. Subsequently, the results of each trial were subjected to an ANOVA and means were compared by the least significant difference test ($\alpha = 0.05$). All statistical analyses were performed using SAS University Edition software (2016; SAS Institute, Cary, North Carolina, USA), including the correlation analysis between the assays of TPC and antioxidant capacity.

RESULTS AND DISCUSSION

Agronomic and yield parameters

The phenology of 'Regalona' quinoa plants varied between the evaluated seasons (Table 3), recording 43 and 48 d from emergence to flowering, 85 and 93 d from emergence to grain filling, and 110 and 121 d from emergence to maturity for the 2019-2020 and 2020-2021 seasons, respectively. These differences could be explained by temperature variation between the seasons, with maximum and minimum temperatures in the

range of 17.2-34.1 and 3.0-14.8 °C in the 2019-2020 season, respectively; and 16.5-31.2 and 2.6-14.4 °C in the 2020-2021 season, respectively. The life cycle span observed in the present study is shorter than that observed by Voronov et al. (2023), who reported 135-140 d when studying 10 commercial accessions of quinoa grown in USA, Peru, Denmark, and Chile. Furthermore, De Santis et al. (2016) reported 61 d from emergence and 50% flowering in a study on 'Regalona' quinoa grown in a clay loamy soil, while Gámez et al. (2019) reported 86 and 111 d from sowing and grain filling for quinoa plants 'Rainbow' (coastal zone of Chile) and 'Illpa' (highlands of Chile), respectively. Difference between the results obtained in the present work and those of the previously mentioned studies could be attributed to edaphoclimatic conditions as well as the effects of nutrient deficiency and water restriction on quinoa plants. In this regard, Maestro-Gaitán et al. (2022) have described that the phenology of quinoa is affected by water stress, resulting in advanced flowering and a shorter grain filling stage.

Table 3. Length of the plant phenological stages of quinoa in the two consecutive seasons.

Number of days	2019-2020	2020-2021
From emergence to flowering	43	48
From emergence to the initial phase of grain filling	85	93
From emergence to seed maturity	110	121

Plant height reached higher values in the 2019-2020 season, ranging from 97.1 to 114.9 cm in the S availability (SA) treatments (Table 4) and from 100.4 and 110.7 cm in the water availability (WA) treatments (Table 5), whereas values in both treatments fluctuated between 70.8 and 82.3 cm and between 70.2 and 80.5 cm in the 2019-2020 and 2020-2021 seasons, respectively. This situation could be explained by differences in rainfall levels between the seasons, reaching respective levels of 25.5 and 6.9 mm. It is important to note that the plants were covered to prevent exposure to the rainfall event (89.1 mm) in January 2021.

Table 4. Mean values of agronomic parameters of quinoa under three levels of S availability in two consecutive seasons. Different letters in a column indicate significant differences according to LSD test ($p \leq 0.05$) for the treatments in the season.

Season	Available S	Plant height	Panicle length	One thousand seed weight	Yield
	mg kg^{-1}	cm	cm	g	g plant^{-1}
2019-2020	20	114.9^{a}	16.0^{a}	2.7^{a}	29.1^{a}
	12	108.3^{a}	13.1^{b}	2.6^{b}	25.9^{b}
	8	97.1^{b}	12.1^{c}	2.5^{b}	21.2^{c}
2020-2021	20	82.3^{a}	18.1^{a}	2.9^{a}	14.6^{a}
	12	75.3^{b}	15.8^{b}	2.6^{b}	13.2^{b}
	8	70.8^{b}	14.4^{c}	2.5^{b}	9.8^{c}

Table 5. Mean values of agronomic parameters of quinoa under three levels of water availability in two consecutive seasons. Different letters in a column indicate significant differences according to LSD test ($p \leq 0.05$) for the treatments in the season.

Season	Available water	Plant height	Panicle length	One thousand seed weight	Yield
	%	cm	cm	g	g plant^{-1}
2019-2020	100	110.7^{a}	14.8^{a}	2.7^{a}	28.9^{a}
	50	105.8^{ab}	13.7^{b}	2.7^{a}	27.3^{a}
	25	100.4^{b}	12.6^{c}	2.5^{a}	19.9^{b}
2020-2021	100	80.5^{a}	16.3^{a}	2.8^{a}	13.6^{a}
	50	78.1^{a}	15.9^{a}	2.7^{a}	12.7^{a}
	25	70.2^{b}	16.1^{a}	2.5^{b}	10.4^{b}

Regarding panicle length, values ranging between 12.1 and 16.0 cm and between 12.6 and 14.8 cm were observed in the 2019-2020 season in the SA and WA experiments, respectively. However, higher values ranging from 14.4 to 18.1 cm and from 15.9 to 16.3 cm were observed in the following season for both factors, respectively (Tables 4 and 5). A study of Reguera et al. (2018) reported a value of 17 cm for 'Regalona', which agrees with the results obtained herein for the treatment of 100% WA and 20 mg kg^{-1} SA.

With respect to one thousand seed weight, values varied between 2.5 and 2.7 g in both the SA and WA trials in the 2019-2020 season. However, differences were observed between the two factors in the 2020-2021 season, with values ranging from 2.5 to 2.9 g and from 2.5 to 2.8 g for the two factors, respectively (Tables 4 and 5). These results are close to those obtained by Voronov et al. (2023), who reported a range of 1.6-3.0 g for this pseudocereal.

Yield per plant varied between 21.2 and 29.1 g and between 19.9 and 28.9 g in the 2019-2020 season for the SA and WA trials, respectively, while values ranged from 9.8 to 14.6 g and from 10.4 to 13.6 g per plant in the 2020-2021 season. These results are lower than those reported by Reguera et al. (2018), who obtained yields between 26.5 and 54.1 g $plant^{-1}$. This can be attributed to the restriction levels established in this study.

In both seasons, 8 mg kg^{-1} S dose showed a negative and significant effect ($p \leq 0.05$) on plant height, panicle length, yield, and one thousand seed weight compared to 20 mg kg^{-1} treatment (Table 4). The negative effect observed on plant growth and yield agrees with the findings of Cao et al. (2023), who reported that a S level of 10 mg kg^{-1} is considered as the critical limit to avoid affecting these parameters, as well as the quality of the crop and the defense mechanisms against the abiotic stress.

Water availability had a significant effect ($p \leq 0.05$) on crop yield and plant height at 25% and 100% WA in both seasons (Table 5). In this sense, Aziz et al. (2018) found that biomass decreased as water deficit increased. In the 2020-2021 season, there was a significant effect ($p \leq 0.05$) of WA on one thousand seed weight at 25% WA compared to the 50% and 100% WA treatments. In this sense, a study conducted by Ruiz et al. (2013) revealed that up to 50% water deficit due to drought has no effect on quinoa yield, which coincides with the findings of the present study. In contrast, Gámez et al. (2019) evaluated 'Rainbow' and 'Illpa' plants with irrigation levels of 100%, 50% and 20% of total field capacity in the grain filling stage and found no differences in yield. The interaction between the factors did not show an effect on the agronomic and productive characteristics.

Total phenolic content, flavonoid content and in vitro antioxidant capacity

When analyzing the S content factor, TPC values per 100 g DM were in the range of 129.0-147.2 mg GAE and 158.3-163.3 mg GAE for the 2019-2020 and 2020-2021 seasons, respectively (Table 6). In addition, significant differences ($p \leq 0.05$) were found between the S levels of 8 and 20 mg kg^{-1} S. Regarding the WA factor, TPC per 100 g DM fluctuated between 135.5 and 145.5 mg GAE and between 153.4 and 170.4 mg GAE for the 2019-2020 and 2020-2021 seasons, respectively. Furthermore, there were significant differences ($p \leq 0.05$) between the 25% and 100% WA treatments in both seasons. These results coincide with Aziz et al. (2018), who reported a significant increase in the TPC of quinoa plants under drought conditions, but differences were not consistent for all the treatments. Similarly, Fischer et al. (2013) conducted a study on quinoa plants subjected to water stress, reporting TPC per 100 g DM values ranging between 330 and 380 mg GAE using a similar methodology for polyphenol quantification and 99% methanol as the extraction solvent. This could explain the lower TPC values obtained herein, using 70% acetone for the extraction. Furthermore, by using a similar extraction solvent and methodology in the determination of TPC, Vega-Gálvez et al. (2018) reported per 100 g DM a range of 97-164 mg GAE for six ecotypes of quinoa, being close to the values determined in this study. In this case, the differences could be related to genotype and cultivation conditions as described by Chaudhary et al. (2023).

In the SA experiment, the total flavonoid content (TFC), specifically flavones and total flavonols, per 100 g DM was in the range of 12.4-12.8 mg QE and 10.9-12.8 mg QE in the 2019-2020 and 2020-2021 seasons, respectively. Meanwhile, in the WA trial, per 100 g DM ranges of 12.3-13.0 mg QE and 11.8-12.4 mg QE were observed in the 2019-2020 and 2020-2021 seasons, respectively (Table 6). The number of flavonoids represents a small portion of the TFC, which agrees with Tang et al. (2015). Additionally, the values obtained in the present study agree with those of Carciochi et al. (2015), who reported values per 100 g DM of 1.65-26.93 mg QE. It should be noted that the 25% and 100% WA treatments showed a significant effect ($p \leq 0.05$) on the flavonoid content in both seasons (Table 7). Likewise, lower water availability tended to result in greater flavonoid content.

Table 6. Mean values of total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) per 100 g DM of quinoa under three S levels in two consecutive seasons. Different letters in a column indicate significant differences according to LSD test ($p \leq 0.05$) for the treatments in the season. n = 12 samples per treatment. GAE: Gallic acid equivalents; QE: Quercetin equivalents; TE: Trolox equivalents.

Season	Available S	TPC	TFC	FRAP	ORAC
	mg kg^{-1}	mg GAE	mg QE	µmol Fe^{2+}	µmol TE
2019-2020	20	129.0^{b}	12.8^{a}	952.4^{b}	$63\,220.0^{a}$
	12	143.2^{a}	12.6^{a}	990.6^{b}	$59\,064.9^{b}$
	8	147.2^{a}	12.4^{a}	1089.4^{a}	$58\,731.6^{b}$
2020-2021	20	158.3^{b}	12.7^{a}	911.5^{b}	$64\,770.9^{b}$
	12	161.3^{ab}	12.8^{a}	969.7^{a}	$68\,956.6^{a}$
	8	163.3^{a}	10.9^{b}	1010.8^{a}	$64\,036.2^{b}$

Table 7. Mean values of total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) per 100 g DM of quinoa under three levels of water availability in two consecutive seasons. Different letters in a column indicate significant differences according to LSD test ($p \leq 0.05$) for the treatments in the season. n = 12 samples per treatment. GAE: Gallic acid equivalents; QE: Quercetin equivalents; TE: Trolox equivalents.

Season	Available water	TPC	TFC	FRAP	ORAC
	%	mg GAE	mg QE	µmol Fe^{2+}	µmol TE
2019-2020	100	135.5^{b}	12.3^{b}	1040.7^{a}	$58\,839.1^{b}$
	50	138.4^{ab}	12.5^{ab}	975.5^{b}	$62\,903.1^{a}$
	25	145.5^{a}	13.0^{a}	1016.2^{ab}	$59\,273.8^{b}$
2020-2021	100	153.4^{c}	11.8^{b}	927.1^{b}	$68\,931.1^{a}$
	50	159.1^{b}	12.2^{a}	949.7^{b}	$62\,689.4^{c}$
	25	170.4^{a}	12.4^{a}	1015.3^{a}	$65\,165.6^{b}$

No differences were found ($p > 0.05$) in terms of TFC when water availability at the three levels interacted with the S level of 20 mg kg^{-1}. The interaction between the factors only generated significant differences between flavonoids content in the 2020-2021 season. The highest flavonoid content was obtained with the treatment of 25% WA and 12 mg kg^{-1} SA (Table 8).

Table 8. Mean values of total flavonoid content (TFC) (mg QE 100 g^{-1} DM) for the interaction between a water and S availability in the 2020-2021 season in Chillán, Chile. Different lowercase letters indicate significant differences according to LSD test ($p \leq 0.05$) within the column. Different capital letters indicate significant differences according to LSD test ($p \leq 0.05$) within the row. QE: Quercetin equivalents.

Available S	Available water		
mg kg^{-1}	25%	50%	100%
8	11.4^{cA}	11.3^{bA}	10.1^{bB}
12	13.4^{aA}	12.6^{aB}	12.4^{aB}
20	12.4^{bA}	12.7^{aA}	12.9^{aA}

The results of the FRAP assay, SA resulted in values per 100 g DM of 952.3-1089.4 µmol Fe^{2+} and 911.5-1010.8 µmol Fe^{2+} in the 2019-2020 and 2020-2021 seasons, respectively. In both seasons, this factor decreased the FRAP values, with differences ($p \leq 0.05$) between the S treatments of 8 and 20 mg kg^{-1}. Regarding the WA factor, per 100 g DM values ranged from 975.5 to 1040.7 µmol Fe^{2+} and from 927.1 to 1015.3 µmol Fe^{2+} for the 2019-2020

and 2020-2021 seasons, respectively (Tables 6 and 7). The FRAP values obtained in the present study are higher than the value per 100 g DM of 750 µmol Fe^{2+} reported by Reguera et al. (2018) for quinoa.

Regarding the ORAC assay, the S content treatments per 100 g recorded values of 58 731.6-63 220.0 µmol TE and 64 036.2-68 956.6 µmol TE for the first and second seasons, respectively. In both seasons, the treatment of 12 mg kg^{-1} SA presented significant differences ($p \leq 0.05$) with respect to the level of 20 mg kg^{-1} SA (Table 6), indicating that S content affects the quality of antioxidants in quinoa seeds. However, the same trend was not observed in both seasons.

Schaich et al. (2015) indicated that the values obtained through the ORAC assay do not allow classifying food nor determining alternatives for food consumption because both the molecules detected through this technique and their physiology need to be considered.

In WA, the ORAC values per 100 g DM were in the range of 58 839.1-62 903.1 µmol TE and 62 689.4-68 931.1 µmol TE for the 2019-2020 and 2020-2021 seasons, respectively (Table 7). These values are higher compared to the results obtained in a study conducted by Rocchetti et al. (2017) using a similar analysis methodology for commercial quinoa, per 100 g DM the value was 34 171 µmol TE, which can be explained by the different edaphoclimatic conditions and extraction solvent used, 70% methanol for the study already mentioned and 70% acetone in this study.

In the 2019-2020 season, the 50% WA treatment allowed obtaining the highest ORAC value, showing a significant effect with respect to the other two WA treatments evaluated (Table 7). However, in the 2020-2021 season, the effect was exerted by 100% WA treatment. Meanwhile, when water availability interacted with the S content factor, the treatment 50% WA and 20 mg kg^{-1} SA presented differences ($p \leq 0.05$) with respect to the other treatments. The treatment 100% WA and 12 mg kg^{-1} SA recorded the highest antioxidant capacity value according to the ORAC assay (Table 9).

Table 9. Mean values of the oxygen radical absorbance capacity (ORAC) (µmol TE 100 g^{-1} DM) for the interaction between water and S availability in the 2020-2021 season in Chillán, Chile. Different lowercase letters indicate significant differences according to LSD test ($p \leq 0.05$) within the column. Different capital letters indicate significant differences according to LSD test ($p \leq 0.05$) within the row. TE: Trolox equivalents.

Available S	Available water		
mg kg^{-1}	25%	50%	100%
8	64 966.8^{aA}	63 408.4^{aA}	63 733.5^{aA}
12	67 330.5^{aA}	66 607.5^{aA}	72 931.8^{aA}
20	63 199.6^{aAB}	60 985.2^{bB}	70 127.9^{aA}

When analyzing the degree of correlation between TPC and antioxidant activity by the FRAP method, coefficients of 0.76 and 0.66 were found for the 2019-2020 and 2020-2021 seasons, respectively. In both cases, the null hypothesis was rejected. However, the correlation coefficient between the variables was greater than 0.75 in the 2019-2020 season, being valid according to Cody (2021). Alvarez-Jubete et al. (2010) studied quinoa of Bolivian origin and reported a high correlation (0.99) between these parameters. The authors explained that discrepancies with other studies were attributed to the interpretation of the results, presence of interfering substances, oxidant solubility, oxidation state, pH of the medium and type of substrate. In contrast, Park et al. (2017) found a negative relationship between TPC and FRAP values when comparing quinoa from Peru, USA, and Korea. The low correlation (-0.744) was attributed to the fact that the main antioxidant compounds in the extracts might be non-phenolic, such as ascorbic acid, phytic acid, α-tocopherol, β-carotene, and saponins, which is consistent with the limitations of the Folin-Ciocalteu reagent-based method described by Filho et al. (2017).

The correlation coefficients between TPC and the ORAC values were 0.39 and 0.42 for the 2019-2020 and 2020-2021 seasons, respectively. Tang et al. (2015) reported a higher correlation coefficient of 0.99 for commercial quinoa extracts, including both free and conjugated phenolic compounds and using a 70% methanol as an extraction solvent.

CONCLUSIONS

In both seasons, the phenological stages of quinoa were shortened, time from sowing to flowering, as well as from sowing to grain filling and from sowing to seed maturity.

The interaction of S and water availability factors did not show an effect on agronomic and productive characteristics. Fertilization with S had a positive effect on plant height, panicle length, yield and thousand seeds in both seasons. Meanwhile, the lower the availability of water, the height of the plants and the yield of the seeds decrease.

In antioxidants, the interaction of the study factors showed that the average dose of S (12 mg kg^{-1}) with the lowest availability of water generates the greatest amount of flavonoids content, while, with the highest availability of water (100%) obtains greater oxygen radical antioxidant capacity (ORAC). Meanwhile, individually, water reduction increases the content of both total phenols and flavonoids. However, the lower the availability of S, the greater the antioxidant capacity in vitro according to the ferric ion reducing antioxidant power (FRAP) test.

In the first season of the study, the content of total phenols and the antioxidant capacity according to the FRAP assay were validly correlated. While no correlation was found between the content of total phenols and the antioxidant capacity by the assay (ORAC).

This study contributes to a better understanding of the impact of drought conditions and soil nutrient deficiency, specifically S content, on agricultural production, which allows for a quantitative approach to the negative effects of those conditions on quinoa.

Author contribution

Investigation: M.O., S.F. Resources: M.O., S.F. Writing-original draft: M.O. Writing-review & editing: M.O. Supervision: S.F. Methodology: M.O., S.F. Software: I.F. Validation: I.F. Formal analysis: S.F. All co-authors reviewed the final version and approved the manuscript before submission.

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