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Controlled water stress to improve functional and nutritional quality in quinoa seed

[Estrés hídrico controlado para mejorar la calidad funcional y nutricional en semilla de quinoa]

Susana FISCHER[1], Rosemarie WILCKENS[1], Jorge JARA[2] & Mario ARANDA[3]

[1]Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Concepción, Chillán, Chile.
[2]Departamento de Recursos Hídricos, Facultad de Ingienería Agrícola, Universidad de Concepción, Chillán, Chile
[3]Laboratorio de Estudios Avanzados en Fármacos y Alimentos, Departamento de Bromatología, Facultad de Farmacia, Universidad de Concepción, Concepción, Chile
Contactos | Contacts: Susana FISCHER - E-mail address: sfischer@udec.cl

Abstract
Chenopodium quinoa W. is a pseudocereal with bioactive compounds like polyphenols, carotenoids, dietary fibers and oleic acid, which have acquired importance because of their human health benefits. The present study aimed to determine the effect of controlled water restriction on the potential yield, chemical composition (protein, fat content and crude fiber) and antioxidant capacity in seeds of three genotypes of quinoa. The study was conducted in the south-central zone of Chile under field and controlled conditions in a greenhouse. Main plot treatment was available water level and subplots included three quinoa genotypes. Results indicated an increase of the antioxidant capacity, with an average of 88% in seeds of the three genotypes and 70% in seeds of plants exposed to 95 to 20 % available water. Seed yield potential was reduced, but the extent of reduction varied depending on the genotype. It was possible to produce seeds of higher nutritional value when controlled water stress was applied from 40 to 20% available water, without a considerably reduction on seed yield.

Keywords: Antioxidant activity, seed yield, *Chenopodium quinoa*, pseudocereal, drought stress.

Resumen
Chenopodium quinoa W. es un pseudocereal con sustancias bioactivas como polifenoles, carotenoides, fibras dietarias y ácido oleico, las que han adquirido importancia, principalmente debido a los beneficios que produce en la salud humana. El propósito de este estudio fue en semilla determinar el efecto de la restricción hídrica controlada sobre el potencial de rendimiento, la composición química (proteína, contenido de grasas, fibra cruda) y la capacidad antioxidante, de tres genotipos de quinoa. Este estudio se realizó en la zona centro sur de Chile, en condiciones de campo y en invernadero, en condiciones controladas. El tratamiento principal fue la disponibilidad de agua y las subparcelas los genotipos de quinoa. Se observó en los resultados un incremento en la capacidad antioxidante de un 88% entre genotipos y un 70% en semillas expuestas desde 95 a 20% de la capacidad de campo. Por otra parte el potencial de rendimiento se redujo en diferentes magnitudes entre genotipos. Finalmente, fue posible producir semillas con mayor valor nutritivo cuando se aplicó una restricción hídrica desde un 40 a un 20% de la capacidad de campo sin reducir considerablemente el rendimiento.

Palabras Clave: Capacidad antioxidante, producción de semilla, *Chenopodium quinoa*, pseudocereal, estrés por sequía.

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Abbreviation uses in manuscript:
RCBD: Randomized complete block design; AW: Available water; FC: Field capacity; PWP: Permanent wilting point; AOAC: Official Analytical Chemists; DM: Dry matter; EEa: Lipid content; CP: Crude protein; CF: Crude fiber; NFE: Nitrogen free extract; OM: Organic matter; DPPH: 2,2-diphenyl-2-picryl-hydrazyl

INTRODUCTION

There is currently an increased awareness about healthcare and a greater interest of the role of food in preventing diseases and increasing consumer welfare. Although urbanization seems to bring a number of positive improvements, it has also led to sedentary lifestyles and to a number of unhealthy dietary patterns such as: an increased consumption of fast food and foods with high saturated fat content (Uauy *et al.*, 2001). Therefore, changing dietary habits has become a priority, and a high intake of fruit, vegetables, legumes, whole grain cereals, and pseudocereals is recommended, as these may have a protective effect on cardiovascular diseases (Czerwinski *et al.*, 2004; Gorinstein *et al.*, 2007). Therefore, it is important to promote an increased consumption of functional foods, creating opportunities for the development of crops with good nutritional value, and high content of protein, such as quinoa, focusing on new markets (Wilckens *et al.*, 1996; Hevia *et al.*, 1998; Hevia *et al.*, 2001; Miranda *et al.*, 2010).

Quinoa has been cultivated in the Andean region and Mesoamerica as far back as 5000 to 6000 years ago (Cardozo, 1961; Wahli, 1990; Hernández and León, 1992). It is an annual herbaceous plant with a small shiny seed, presenting a flat and pointed oval shape, with the embryo surrounding the perisperm. Regarding its chemical composition, often determined by proximate analysis (Vega-Gálvez *et al.*, 2010), the protein content of quinoa seed varies from 75 to 221 g kg^{-1} with an average of 138 g kg^{-1}, which is higher than the content of the most commonly consumed grains, such as wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), oat (*Avena sativa* L.), rice (*Oryza sativa* L.), and barley (*Hordeum vulgare* L.) (Bhargava *et al.*, 2006; Comai *et al.*, 2007), but lower than grain legumes (Etchevers and Ávila, 1980; Bhargava *et al.*, 2006).

It is important to note that quinoa's protein is of a high quality, as it has all the essential amino acids for human nutrition. Quinoa's seed has a high content of lysine (5.2 - 8.0%), usually deficient in most plant proteins and absent in wheat, methionine (2.4 - 5.1%), tryptophan (0.7 - 1.0%), and a high content of arginine and histidine. These two amino acids (arginine and histidine) are both essential for infants and children and, therefore, these constitute interesting components to be included in the development of infant food formulas (Ruales and Nair, 1992; Vega-Gálvez *et al.*, 2010). Furthermore, quinoa's seed contains high levels of unsaturated fatty acids such as linoleic acid (50.2 - 56.1%) and oleic acid (22.0 - 24.5%), as well as linolenic acid but in lower levels (5.4 - 7%) (Ruales and Nair, 1992; Abugoch *et al.*, 2009). Quinoa's starch content varies between 510 and 610 g kg^{-1}, and both the leaves and seeds contain carotenoids, α-and β-tocopherol, which act as cell protectors and represent an important source of antioxidants likely to be used for nutraceutical purposes in humans (Bhargava *et al.*, 2006).

The cultivation of quinoa is often restricted to areas where there are different types of abiotic stress, showing a high drought stress tolerance (Jacobsen *et al.*, 2003; Martínez *et al.*, 2009; Geerts *et al.*, 2008).

It has been reported that a number of environmental stresses during plant growth regulate the accumulation of secondary metabolites and other nutraceutical substances that increase synthesis of them, and some also act as cell protectors (Edreva *et al.*, 2008). Moreover, it has been demonstrated that increased antioxidant synthesis in water-stressed plants is explained by an association between oxidative stress and abiotic stress caused by environmental conditions (Cao *et al.*, 1996)

Due to the economic importance that these nutraceutical substances have acquired, the condition described above could be exploited as an opportunity to increase the quality of raw materials for the nutraceutical industry. Therefore, the aim of this study was to evaluate the effect of controlled water stress on the biosynthesis of molecules and antioxidant activities in seeds of three genotypes of quinoa, as well as evaluating its effect on its seed yield potential.

MATERIALS AND METHODS

Experimental condition

The experiments were conducted in Chillán (-36°35'43,2''S, -72°04'39,9'' W and 140 m.a.s.l.), in Ñuble Province, Bío Bío Region, Chile, in the spring and summer of the 2010 - 2011 growing season under controlled and field conditions.

In the controlled-condition experiment, quinoa plants were grown under natural light conditions in a greenhouse at 22 °C ± 3 °C during the day and 18 °C ± 3 °C during the night.

The field experiment was carried out in a soil belonging to the Arrayán series (medial, thermic, Humid Haploxerand), with a leveled topography and good drainage, and an annual average rainfall of 1000 mm (Stolpe, 2006). The climate of this location is classified as temperate Mediterranean (del Pozo and del Canto, 1999).

Measurement of daily maximum air temperature, daily minimum air temperature, and relative humidity were made at a weather station (Datalogger HOBO ® model Pro Series, Boston, MA, USA) for both types of experiments (Table 1).

Table 1
Mean monthly maximum (T° max.) and minimum (T° min.) air temperature, rainfall, and relative humidity (RH) of field condition experiment in Chillán, Chile, in 2010-2011 season.

Month	T° max.	T° min.	Rainfall	RH
	----------°C----------		mm month^{-1}	%
October	19.8	7.4	53.5	60.9
November	23.5	8.6	21.9	56.9
December	25.1	9.8	16.5	53.9
January	27.1	11.9	5.6	51.7

Experimental design

For field and controlled conditions, the experimental design was an RCBD with a split-plot arrangement with 4 replicates. The main plot was the level of available water (AW) once 50% of the grains were in the grain filling stage. Irrigation was applied when the soil water content at 0.6-m depth reached 95%, 70%, 40%, and 20% of AW. The following equation was used to compute AW:

$$AW = (\theta_{fc} - \theta_{pwp})Z$$

where θ_{fc} is the soil water content at field capacity (FC; $m^3\ m^{-3}$) and θ_{pwp} is the soil water content at permanent wilting point (PWP; $m^3\ m^{-3}$) representing the soil water potential at -30 and - 1500 J kg^{-1}, and Z is the root zone depth (0.6 m).

The sub-plot was three quinoa genotypes: Regalona (official variety recorded in a national catalog of the SAG division of the Chilean Ministry of Agriculture), ecotype B080, and the breeding line AG2010 (obtained from Agrogen E-I-R-L, Temuco, Chile). Experimental units under field experiment consisted of 4 rows, 5 m long, and spaced 0.45 m apart. At controlled environment conditions, experimental units consisted of 9 black plastic 5-liter bags (15 cm diameter and 40 cm depth), in which individual quinoa plants were grown spaced at 30 cm within rows and between them. The substrate was a mixture of sandy loam soil with 52.1% total porosity and a bulk density of 1.27 µg m^{-3}. Soil water content varied according the soil depth considering height from surface to down of 0 - 0.3m-; 0.3 - 0.6m-, 0.6-1.0 m in the following values: 0.37, 0.33 , 0.32 $m^3\ m^3$ for the FC and 0.2, 0.22, 0.2 $m^3\ m^3$ for PWP under field conditions, and 0.39 $m^3\ m^3$ for FC and 0.21 $m^3\ m^3$ for PWP under controlled conditions. Seeding dates at each environment was 20 October 2010.

Experiment management

Seeding rate was 10 kg ha^{-1} under field experiment conditions; whereas, three seeds were sown in each bag under controlled environment conditions in order to have one plant per bag with one pair of leaves two weeks later. Fertilizer rates were calculated according to soil test levels for both types of experimental conditions. Phosphorus was applied and incorporated into the soil through tillage at 4-cm depth, in rates of 100 kg P_2O_5 and 50 kg K_20 ha^{-1} at the time of the last tillage operation before seeding. Nitrogen rate was 160 kg N ha^{-1} in both experiments, half of the rate was applied at 2 leaf-stage and half at early reproductive stage.

Broadleaf and grass weeds were controlled during preemergence with glyphosate (N-(phosphonomethyl) glycine) applied at 2 L ha^{-1} (Glifos 480 SL). Postemergence broadleaf weeds were controlled with MCPA dimethylamine salt at 0.46 L ha^{-1} (MCPA 750SL, ANASAC, Chile), while grass weeds were hand weeded.

Irrigation was applied with a drip tape irrigation system (Queen Gi, Bulgary) with drip emitters separated every 10 cm under field conditions and one drip emitter per bag under controlled

conditions, with 4 L h^{-1} flow at 1 MPa of pressure in both types of experiments. Prior to the study, quinoa plants were irrigated maintaining a soil moisture content ranging from field capacity to 90% AW. Water restriction treatments were applied when seeds reached milk stage until dough stage. In order to have uniform soil moisture content, plants were all watered at 100% field capacity, during 2.5 hour, before water restriction treatments started. When the desired level of available water was reached, the different treatments were rewatered at field capacity. Then, a new cycle of water restriction was started until the seeds reached dough stage.

Six bags of each experimental unit under controlled conditions were hand harvested. Also the two-center rows of each plot in the field experiment were swathed and threshed with a stationary plot thresher (Bill's Welding Pullman, WA, USA). Plots were harvested at the end of January and at physiological maturity, when 50% of the panicle was brown in color (Berti *et al.*, 1997).

Evaluations

Volumetric soil water content

In the field experiment, volumetric water content was measured every two days using a neutron probe (CPN, 503-DR Hydroprobe, Campbell Pacific Nuclear International, L.A,CA, USA) calibrated at the site. One month after planting, a neutron meter access tube was installed to a depth of 100 cm between the two-center rows of each experimental unit. Measurements of neutron thermalization were made daily at 0.20-, 0.45-, and 0.75-m depth (Fischer *et al.,* 2013). Simultaneously, soil water content was measured daily in the four center plant bags of each experimental unit in the trial under controlled conditions by using a Theta Probe ML 2x moisture sensor and meter model HH1 (Delta–T Devices, Cambridge, UK) at 15-cm depth, also calibrated at the side.

Seed yield

Under field conditions, plants from 4 m of two center rows of each plot were swathed after discarding 0.5 m from row ends and seven day later threshed with a stationary plot combine. To obtain the seed yield under controlled conditions, six plants of the center row of the experimental unit were harvested according the method described above. Seed samples were cleaned and stored on a shelf at room temperature. Also, the 1000 seed weight was calculated.

Biomass

Biomass samples under controlled conditions were taken from three plants within each experimental unit and at field condition from a 0.6 m^2 area within each plot, where plants were cut at the stem base and dried at 60 °C for 48 h.

Chemical analysis

All methodologies followed the recommendations of the Association of Official Analytical Chemists (AOAC, 1995). Seed dry matter (DM) was measured in duplicate using AOAC method 923.03. The lipid content was determined by cuantification of ether extract in acid medium (EEa), crude protein (CP) using the Kjeldhal method with a conversion factor of 6.25 (AOAC 991.20). HPLC method with diode-array detection was used to identify and quantify amino acid. The crude fiber (CF) was estimated by acid/alkaline hydrolysis of insoluble residues (AOAC 962.09), and total ash was determined by calcination (AOAC 923.03). Organic matter (OM) was calculated by difference between DM and ash. The nitrogen free extract content (NFE) was calculated as the difference between the food OM and the sum of CP, EEa, and CF on a DM basis.

Extracts preparation

Samples of quinoa seeds were ground on a Thomas-Wiley mill (serie 3383 - L10 USA) through a 60 mesh screen. A one-gram-sample for each treatment was extracted with 100 mL of solvent, consisting of methanol 99% (v/v) shaken for 60 min at 18 °C in an orbital shaker. The liquid phase of the extracts was filtered through filter paper Whatman N° 2 and storage at 4 °C until used.

Antioxidant capacity

Free radical scavenging capacity of the samples was determined using the 2,2-diphenyl-2-picryl-hydrazyl (DPPH) method with modifications suggested by Miranda *et al.* (2010). For measurement of scavenging capacity, 0.40 µL of supernatant sample were added to test tubes, and 3,960 µL of DPPH stock solution (absorbance 1.2; Thaipong *et al.*, 2006) was added to each tube. The reaction mixture was vortex-mixed for 20 s and left to stand at dark room for 30 min at 15°C. All measurements were done in triplicate. The absorbance was determined using an UV/VIS spectrophotometer (Optizen Pop) measured at 517 nm. A standard curve was prepared with a gallic acid stock solution (1 mg mL^{-1}).

Statistical analysis

Statistical analysis was conducted using standard procedures for an RCBD with a split-plot arrangement. In order to determine differences among treatments, results of each trial were analyzed by analysis of variance (ANOVA) with the SAS (Statistical Analysis System, 2009) program and the LSD test ($P = 0.05$).

RESULTS

Seed and Biomass yield

Grain yield and grain size, a determinant of its commercial quality, are frequently used as selection criteria for quinoa breeding. As a consequence of the diversity of environmental factors affecting crop yield and quality, quinoa exhibits a strong variability for cultivar-specific responses (Bertero *et al.,* 2004). Seed yield was affected according to genotypes and available water, showing a significant ($P \leq 0.05$) interaction for seed yield between available water and genotypes (Table 2) in both experiments. An average value of 5792 kg ha^{-1} was recorded for genotype Regalona and B080, and 5118 kg ha^{-1} for AG2010 at 95% AW. However, seed yield decreased when water supplementation was restricted at 20% to a range of 3608 and 3754 for Regalona and B080 gentypes, respectively. An average decrease of 36% was observed from 95% to 20% AW. On the contrary, seed yield did not change significantly at any water treatment in AG2010 even though different rewatering cycles were applied. In order to reach the different water contents, rewatering cycles were applied at 7, 10 and 14 days for 70%, 40% and 20% AW, respectively (Fischer *et al.,* 2013).

Table 2
Seed yield of three quinoa genotypes under four levels of available water (AW) and two experimental conditions in Chillán, Chile, in 2010-2011.

	Field condition			Controlled condition		
AW	**Genotypes**					
(%)	**Regalona**	**B080**	**AG 2010**	**Regalona**	**B080**	**AG 2010**
	Seed yield (kg ha^{-1})			**Seed yield (g plant^{-1})**		
95	**5,840aA**	**5,743aA**	**5,118aB**	**24.20aA**	**22.50aA**	**21.40aA**
60	**4,506bA**	**4,486bA**	**4,285aA**	**20.10bA**	**19.60bA**	**19.90abA**
40	**4,179 bA**	**4,258bA**	**4,265aA**	**17.80bA**	**16.30cB**	**18.70bcA**
20	**3,608bB**	**3,754bB**	**4,251aA**	**15.60cB**	**15.70cB**	**18.10cA**
r^2	**0.97**	**0.98**	**0.80**	**0.93**	**0.85**	**0.89**

% AW= Available water (%). Y.curve eq. = Yield curve equation.
Different lowercase letters in the same column indicate significant difference (P ≤ 0.05). Different capital letters in the same row indicate significant difference (P ≤ 0.05).

Under controlled environment and field conditions, strong interaction among genotypes and available water in seed yield occurred because there was a change in the magnitude of the response of genotype AG2010, in which the slope of seed yield response was about half of the slope of the other two genotypes (Table 2).

In biomass of quinoa plants, higher levels of biomass were observed when crop was irrigated at 95% of AW producing 15.9 t ha^{-1} and 57.9 g pl^{-1} under field and controlled conditions, respectively. There was no significant difference between genotypes (Table 3).

Chemical analysis

In this study, only seeds under field conditions were analyzed. The mean values of the chemical analysis of quinoa seeds indicate that water restriction did not affect any of the analyzed variables except for fat content, which increased in quinoa seed irrigated at 20% available water. There were significant differences between genotypes for the crude protein, fat, crude fiber, and ash content ($P \leq 0.05$) (Table 4). The crude protein content observed in this study ranged between 174 and 189 g kg^{-1}.

Protein nutritional quality is determined by the proportion of essential amino acids that cannot be synthesized by animals. Amino acid contents obtained

in the present study (Table 5) are in the range of values described by González *et al.* (1989), Koziol (1992), IESN-Chile (2001) and Wright *et al.*, (2002), except for isoleucine and leucine which were 50% of the values reported by González *et al.*, (1989) and Koziol (1992). In contrast, the values obtained for tryptophan were higher in this study. Significant differences ($P \leq 0.05$) between genotypes were detected for aspartic acid, serine, glutamic acid, glycine, alanine, valine and methionine. However, lysine values did not vary significantly ($P \geq 0.05$) between genotypes or water stress treatments.

Table 3

Biomass yield (B. yield) and 1000-seed weight of three quinoa genotypes under four levels of available water (AW) and two experimental conditions in Chillán, Chile, in 2010-2011.

	Field Condition		Controlled Condition	
AW	Biomass yield	1000-seed weight	Biomass yield	1000-seed weight
%	t ha^{-1}	g	g pl^{-1}	g
95	15.87a	3.01	57.91a	2.98
60	14.65b	3.07	54.48b	2.98
40	13.25c	3.15	51.59c	2.91
20	12.38c	2.92	44.96d	2.90
Means†	14.07	3.04	52.24	2,95
Genotypes				
Regalona	13.97	2.60b	52.34	2.72b
B080	14.40	3.21a	51.69	3.04a
Ag2010	14.10	3.29a	52.68	3.06a
Means‡	14.16	3.03	52.24	2.94

† Mean across available water (AW)

‡ Means across genotypes.

Different lowercase letters in the same column indicate significant difference ($P \leq 0.05$).

Table 4

Chemical characterization of seeds of three genotypes of quinoa under four levels of available water (AW) under field conditions in Chillán, Chile in 2010-2011.

AW	Moisture content	Crude protein	Fat	Crude fiber	Ash	Avail. CHO
%	---------- g kg^{-1} ----------					
95	116± 2	185± 9	59.7± 6b	24± 2	37± 4	694± 9
60	115± 4	184± 6	59.9± 4ab	25± 2	35± 3	696± 7
40	118± 3	180± 6	59.4± 3b	25± 2	36± 2	701± 7
20	117± 3	172± 2	62.8± 6a	25± 4	37±3	704± 3
	---------- g kg^{-1} ----------					
Genotypes						
Regalona	115± 4	174± 2b	64.9± 3a	24± 2	35± 2b	704± 2
B080	117± 3	188± 8a	57.9± 3b	24± 2	37± 3a	693± 8
AG2010	117± 2	179± 5b	58.0± 4b	26± 4	37± 2a	700± 9

Data are means ± standard deviations of 12 measures.

Avail. CHO: Available carbohydrates measured as:

1000 - crude protein (g kg^{-1}) - fat (g kg^{-1}) - crude fiber (g kg^{-1}) ash (g kg^{-1}).

Different lowercase letters in the same column indicate significant difference ($P \leq 0.05$).

Table 5
Amino acid profile in seeds of three genotypes of quinoa under four levels of available water (AW) under field conditions in Chillán, Chile, in 2010-2011.

	AW (%)				Genotype		
Amino acid	**95**	**60**	**40**	**20**	**Regalona**	**B080**	**AG2010**
Asp	**1.5**	**1.7**	**1.5**	**1.5**	**1.7b**	**2.9a**	**1.2b**
Ser	**3.5**	**4.3**	**3.9**	**3.9**	**3.9a**	**4.2ab**	**3.6b**
Glu	**17.6**	**19.9**	**17.2**	**18.5**	**20.1a**	**17.9ab**	**16.8b**
Gly	**6.1**	**6.4**	**6.2**	**6.2**	**6.9a**	**6.4a**	**5.3b**
His	**3.3B**	**4.3A**	**3.9A**	**3.9A**	**3.7**	**3.9**	**3.9**
Arg	**6.9**	**8.0**	**7.6**	**7.5**	**7.3**	**7.9**	**7.3**
Thr	**3.9B**	**5.5A**	**5.0A**	**4.9A**	**4.4**	**5.4**	**4.7**
Ala	**4.3**	**4.7**	**4.5**	**4.5**	**4.5a**	**4.7a**	**4.1b**
Pro	**3.6**	**4.2**	**3.9**	**3.9**	**3.9**	**4.1**	**3.7**
Cys	**0.3**	**0.4**	**0.3**	**0.5**	**0.3**	**0.5**	**0.3**
Tyr	**2.6**	**3.0**	**3.0**	**3.1**	**2.7**	**3.5**	**2.9**
Val	**2.3**	**2.9**	**2.7**	**3.1**	**2.0b**	**3.2a**	**3.1a**
Met	**0.7**	**0.7**	**0.7**	**0.6**	**0.9a**	**0.7b**	**0.4c**
Lys	**5.1**	**5.6**	**5.4**	**5.5**	**5.4**	**5.7**	**5.1**
Ile	**1.7**	**2.0**	**1.9**	**2.0**	**1.7**	**2.1**	**2.0**
Leu	**5.1**	**4.5**	**5.4**	**5.5**	**5.3**	**4.9**	**5.2**
Phe	**3.1**	**3.5**	**3.3**	**3.4**	**3.3**	**3.4**	**3.2**
Trp	**2.3**	**2.1**	**2.1**	**2.1**	**2.5a**	**1.9ab**	**1.9ab**

Different capital letters in the same row indicate significant difference ($P \leq 0.05$) for water restriction.
Different lower case letters in the same row indicate significant difference ($P \leq 0.05$) for genotype.

Antioxidant capacity

Cereals and pseudocereals can be recommended in balanced diets in the same scale as fruits and vegetables (Gorinstein *et al.*, 2007), due to their relatively high antioxidants capacity. In particular, this activity may be partially related to their free radical-scavenging ability, and DPPH is a relatively stable free radical used extensively in evaluating the antioxidant capacity of natural products.

The analysis of variance indicated that the main effects (water restriction and genotype) were significant for DPPH in field and controlled experimental conditions (Table 6). The genotype AG2010 had significantly higher antioxidant capacity ($P \leq 0.05$) than genotype Regalona and B080 in both field and controlled conditions (Table 6). The antioxidant capacity increased between the lowest (95% AW) and highest (20% AW) water restriction treatments in both experimental conditions.

The level of DPPH detected under controlled conditions was greater than in the field experiment, indicating that environmental conditions influenced concentration of antioxidant compound (Table 6).

In this study, quinoa seeds increased the antioxidant capacity when plants were subjected to water restrictions from 95% AW to 20% AW (Table 6). However, these water stress levels produced a reduction in seed yield of an average of 30 and 27% in field and controlled conditions, respectively (Table 2). However, the genotype AG 2010 had a higher level of antioxidant capacity ($P < 0.05$) (Table 6), when irrigation was restricted to 20% AW. Nevertheless, in this genotype, water restriction treatment reduced seed yield in 17% and 15% at field and controlled condition, respectively (Table 2).

Table 6
Antioxidant capacity in seed extract of three genotypes (G) of quinoa under four levels of available water (AW) and two experimental conditions in Chillán, Chile, in 2010-2011.

	Genotype			
Water restriction	**Regalona**	**B080**	**AG 2010**	**Mean‡**
AW (%)	**----------mg GAE g^{-1}----------**			
	Field condition			
95	**1.41**	**1.88**	**3.24**	**2.18c**
60	**1.63**	**2.53**	**3.31**	**2.49c**
40	**2.48**	**3.10**	**3.67**	**3.08b**
20	**2.66**	**3.35**	**5.12**	**3.71a**
†Mean	**2.04C**	**2.71B**	**3.84A**	
% increase	**89**	**78**	**58**	
	Controlled condition			
95	**4.76**	**5.42**	**6.12**	**5.44b**
60	**4.89**	**5.59**	**6.60**	**5.69b**
40	**5.38**	**5.76**	**7.10**	**6.08b**
20	**6.72**	**6.29**	**9.11**	
†Mean	**5.44B**	**5.77B**	**7.23A**	**7.37a**
% increase	**41**	**16**	**49**	
Average	**2.04C**	**2.71B**	**3.84A**	

† Mean across available water (AW) for each genotype.
‡ Means across genotypes for each available water (AW).
Different lower case letters in the same column indicate significant difference (P ≤ 0.05) for available water.
Different capital letters in the same row indicate significant difference (P ≤ 0.05) for genotype.

Discussion

Results indicates that available water has less influence on seed yield reduction in genotype, explained by a phenotypic elasticity of quinoa (Bertero *et al.*, 2004) or indicating that it is more tolerant to water stress (Winkel *et al.*, 2002) (Table 2). Phenotypic elasticity of the genotype included in this study allowed quinoa to maintain its performance even under different irrigation schedules. Seed yields obtained in this study were higher than values reported in a set of 24 cultivars of quinoa tested in 14 international trial environments across three continents during the growing season 1998-1999 (Bertero *et al.*, 2004), but our results fell within the range of values reported for quinoa crop in other studies in Chile (Jacobsen *et al.*, 1994; Berti *et al.*, 1997; Martínez *et al.*, 2009). Variations in the different seed yield response could be attributed to the rusticity of quinoa, that allows genotypes to adapt to the environmental variations generated by the topographical and latitudinal range where there are grown. In fact, this specie exhibits a strong variability for cultivar-specific responses to environmental variation (Bertero *et al.*, 2004; Fuentes and Bhargava, 2011; Burrieza *et al.*, 2012).

Reduction of quinoa biomass could be explained if we consider that when plants are exposed to water stress, different resistant control mechanisms are activated to avoid environmental stress, such as morphological, physiological, and anatomical modifications. In fact, physiological changes start with a decrease in cell elongation and a decrease in shoot and root growth. Jacobsen *et al*. (1994) indicated that some of the resistance showed by quinoa is due to the

ramification of their roots and the development of hygroscopic papillae on leaf cuticle that would reduce transpiration. The plant also avoids the negative effects of drought through the reduction of its leaf area by leaf dropping (Jensen *et al.*, 2000). These might explain why seed weight was similar for all water treatments in spite of a lower biomass development in treatments in which soil was irrigated when there was high soil water depletion (20% AW).

Regardless of the water restriction level and genotype, protein content in this study was higher than the values reported in Chile before for 'Baer, 'Faro', 'Pichaman', and UDEC10 quinoa cultivars, which ranged between 119 and 131 g kg^{-1}, 118 and 135 g kg^{-1}, 141 and 130 g kg^{-1}, and 137 g kg^{-1}, respectively (Etchevers and Ávila, 1980; Hevia *et al.*, 1998; Hevia *et al.*, 2001). Additionally, Koziol (1992) reported protein content ranging from 138 to 165 g kg^{-1} and Wright *et al.* (2002) reported protein content of 148 and 157 g kg^{-1} for sweet and bitter quinoa from Bolivia, respectively. Nevertheless, protein content can range from 80 to 220 g kg^{-1} depending on the genotypes of quinoa, which is higher than the average content of common cereals (Jancurová *et al.*, 2009). According to values observed in this study, it is interesting to note that water restriction did not change the range of protein as previously reported in cereals such as wheat (Campell *et al.*, 1981; Rao *et al.*, 1993) or barley (Hevia *et al.*, 1994). In fact, these authors have reported that protein concentration in grain is higher under drought conditions than favorable water conditions. In addition, variation in protein content between quinoa genotypes have been described by Etchevers and Ávila, (1980) and Hevia *et al.* (1998) and for other cereals by Bassett *et al.* (1988). This indicates that genotype is a key factor in improving quality and that cultivation strategies need to be developed in order to achieve the desirable protein content. Regarding quality of protein, thereby it is suggested that quinoa flour could be a good supplemental ingredient in the preparation of highly nutritious foods (Stikic *et al.*, 2012).

Another important factor is the antioxidant capacity of food. However, there is little information about the variation of antioxidant capacity related to the effect of drought in different genotypes. Steffensen *et al.* (2011) analyzed variations in the content of 11 different seed polyphenols of 18 genotypes of *Amaranthus* sp., observing that *Amaranthus hypochondriacus* displayed the highest content of flavonoids in seeds.

Mpofu *et al.*, (2006) described that growing environmental effect was considerably greater than genotype effects, increasing significantly the level on the total phenolic compounds and DPPH scavenging capacities in hard spring wheat. Similar response was observed when plants were subjected to drought stress, increasing the level of antioxidant enzymes and metabolites that react with active oxygen, minimizing oxidative damage (Yordanov *et al.*, 2000; Reddy *et al.*, 2004). In leaf discs of drought stress maize, resistant strains to water stress, Pastori and Trippi (1992) observed an increased activity of glutathione reductase, stimulated probably as a result of the translation by preexisting mRNA. Basu *et al.* (2010) reported an increment in antioxidant capacity, particularly flavonoids and phenolics production in Pokkali culivars of indica rice (*Oryza sativa* var. *indica*).

The DPPH method is recognized as a simple method to determine the antioxidant activity (Sun and Ho, 2005; Miller *et al.*, 2000; Kedare and Singh, 2011). However, to compare the values of antioxidant activity in extracts of quinoa obtained in this research, using DPPH scavenging, is difficult due to some differences with other research, in the type of solvent (ethanol) used in the extract preparation (Miranda *et al.*, 2010), or methanol (Hirose *et al.*, 2010) or the final results expressed in Trolox µmol equivalent (Hirose *et al.*, 2010).

Reduction of seed yield when water stress was applied is not surprising, since biotic and abiotic conditions are known to influence antioxidant capacity, especially phenolic content in plants (Jaleel *et al.*, 2009). In this particular condition, plants are stressed with a decrease in water availability that, in general, produces an increase in antioxidant capacity with a reduction in yield. Given that seed of AG2010 genotype had a lower reduction of seed yield among water availability, it has been suggested that this genotype had a stable response pattern across environments.

CONCLUSIONS

In the present study, seed yield of quinoa varied among genotypes Regalona, B080 and AG2010 revealing different decreasing pattern in Ag2010 when plants were subjected to water restriction from 95 to 20% available water. Only fat content was affected by water restriction and the crude protein, fat, crude fiber, and ash content were significantly different among

genotypes. Antioxidant content increased as available water for quinoa plant decreased.

Results demonstrated that quinoa has agronomic potential because it could be adapted to produce high seed yields under adverse conditions and at the same time enhance antioxidant capacity in the seeds.

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