Variation of Seed Mineral Concentrations in Seven Quinoa Cultivars Grown in Two Agroecological Sites

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ABSTRACT

Variations in concentration and tissue distribution of mineral elements in seeds of seven quinoa cultivars from both a "natural" habitat (Patacamaya, Bolivia, 3,960 m above sea level) and a "nonnatural" habitat (Encalilla, Argentina, 1,980 m above sea level) were analyzed. Data clearly showed inter- and intravarietal differences in seed mineral concentrations between the two sites. Correlation analysis revealed that concentrations of major and minor dietary minerals as well as essential ultratrace elements of Encalilla seeds showed, in general, higher correlations with both seed protein and seed yield than did element concentrations of Patacamaya seeds. Results of scanning electron microscopy combined with energy dispersive X-ray spectroscopy showed clearly differences in the contents of major mineral elements (calcium, magnesium, potassium, phosphorus, and sulfur) in the pericarp and embryonic axis (cotyledon + radicle). Obtained data could indicate that genotype × environment interactions are responsible for mineral variations occurring in quinoa cultivars. Results can also be useful for developing mineral biofortification strategies for the world's poorest regions.

Quinoa (*Chenopodium quinoa* Willd.) is a native South American seed crop. Quinoa has been cultivated in the Andean region for thousands of years as food, being the major source of high-quality protein that often is used as a meat substitute in the diet (Ruiz et al 2014). The high nutritional value of quinoa seed is mainly because of its exceptional essential amino acid balance and its nutritionally favorable lipid content (Repo-Carrasco et al 2003; González et al 2012). Furthermore, quinoa contains higher levels of carotene, riboflavin, tocopherol, folic acid, and minerals than major cereals. Quinoa seed contains high amounts of calcium, magnesium, potassium, iron, copper, manganese, and zinc (Table I). In fact, quinoa can supply the daily requirement of certain vitamins and several minerals for children aged 1–3 years (Ruales and Nair 1993). Minerals are important in the human diet, filling physiological functions as metabolic cofactors. Further, they are important components of bones, teeth, soft tissues, hemoglobin, muscle, blood, and nervous cells, being also vital to mental and physical well-being (Rawat et al 2013). In this regard, increasing iron, zinc, and calcium in plant foods through plant breeding was proposed as a strategy for combating widespread mineral deficiencies in human populations (White and Broadley 2009). Despite increasing interest in quinoa as an alternative food crop in many regions outside South America (Jacobsen 2003), no significant efforts have been made for qualitative improvement of its mineral content (Bhargava et al 2008). Although the mineral composition of quinoa seed has been reported (Ruales and Nair 1993; Konishi et al 2004; Stikic et al 2012), effects of environmental and genetic factors on its composition and variability have been poorly studied. Therefore, genotype stability of quinoa seeds for mineral content and composition under different environmental conditions still remain unclear. One can ask whether the mineral composition of quinoa seeds will change significantly when plants are cultivated in different agroecological sites. Thus, the assessment of environmental effects on concentrations of major and minor dietary minerals in quinoa seeds becomes important for reliable and useful confirmation of its value as a food for humans and animals. The main aim of this study was to analyze variations of mineral concentrations in seeds of seven quinoa cultivars from two different agroecological sites. Correlations between mineral concentration and seed protein and grain yield were analyzed. Approximate tissular contents of major mineral elements were also estimated. The hypothesis was that mineral composition and nutritional quality of quinoa seeds are affected by both genetic and environmental factors. We began with the assumption that quinoa genotypes that grow in the Andean highlands ("native" habitat) are better adapted and have better nutritional mineral composition than those growing outside the Andean region ("nonnative" habitat).

MATERIALS AND METHODS

Plant Material. Seeds of seven quinoa cultivars (Amilda, Chucapaca, CICA, Ratuqui, Sajama, Samaranti, and Sayaña) were selected for this study. These cultivars were from the city of Patacamaya (Bolivian Altiplano, 17°14′S, 67°55′W, 3,960 m above sea level).

Site Description and Experimental Design. The experiment was conducted during the summer months (September to February) of 2009–2010 (hereafter 2010) and 2010–2011 (hereafter 2011) in the Encalilla site, an arid mountainous area in the Northwest of Argentina (22°31′S, 65°59′ W, 1,980 m above sea level). Climate of the site has been classified as an arid environment with cold winters and hot summers. Annual rainfall is 200 mm, with more than 70% occurring between September and February (González et al 2008). Climatic parameters were recorded daily during the cropping period. Minimum and maximum air temperature and relative humidity were measured with a thermocouple (Hobo H8 RH/Temp

TABLE I
Mineral Composition of Quinoa Seed and Common Edible Cereals (mg/kg dw)[z]

Minerals	Quinoa	Wheat	Rice	Corn	Barley	Oats
Calcium	874	430	151	300	822	940
Potassium	9,520	5,020	1,180	3,770	5,800	4,600
Magnesium	2,621	1,520	1,122	1,401	1,450	1,380
Iron	81	38	7	21	32	41
Phosphorus	4,120	4,280	2,601	3,100	4,120	4,700
Zinc	48	35	18	31	31	30
Copper	25	8	3	2	9	5
Manganese	33	26	25	24	15	22
Sodium	115	40	300	251	490	280

[z] Sources: Ruales and Nair (1993), Konishi et al (2004), and Stikic et al (2012).

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family data logger, Onset Computer, U.S.A.). Photosynthetic active radiation was measured between 12:00 and 14:00 (local time) with a quantum sensor (LI-190S coupled to LI-1400 data logger, LI-COR, U.S.A.). Rainfall and wind velocity were recorded with an automatic weather station (EP1000, Pegasus, Argentina). Climatic data for the Patacamaya site were from available data of the Patacamaya Meteorological Station (17°23′S, 67°92′W, 3,907 m above sea level). Comparative climatic data between the Encalilla and Patacamaya sites are given in Table II. The texture of Encalilla soil (50 cm depth) is sandy loam clay, and it is classified as Xeric Torriorthent type according to the FAO/UNESCO soil taxonomy (Batjes 1997), with pH 8.4 (1:2 soil/water); electrical conductivity, 2.0 dS/m (2:1 extracted soil paste); exchangeable sodium percentage, 38.6%, cation exchange capacity (CEC), 12.3 cmol/kg; organic matter, 0.6%; nitrogen, 0.055%; $CaCO_3$, 0.68%; potassium, 39.0 mg/100 g; sodium, 61.5 mg/100 g; and magnesium, 34.3 mg/100 g (González et al 2012). Soil evapotranspiration calculated with the Penman–Monteith equation was 350 mm, that is, 2.3-fold higher than total rainfall during the cropping cycle. Patacamaya soil is shallow with a sandy loam texture and stony granular structure (30 cm depth) and is classified as Haplic Xerosol type according to the FAO/UNESCO soil taxonomy (Batjes 1997). It has a low clay content (6.0%); pH 6.6 (1:2 soil/water); exchangeable aluminum percentage, negligible; electrical conductivity, 7.0 dS/m; CEC, 8.9 cmol/kg; organic matter, 0.5%; nitrogen, 0.066%; $CaCO_3$, 0.45%; potassium, 42.4 mg/100 g; and magnesium, 27.9 mg/100 g. Soil evapotranspiration is 1,022 mm (3.4-fold higher than total rainfall) (García et al 2003).

A randomized complete block design experiment was used with two replicates in 2010 and two replicates in 2011. For each cultivar, the plot size was five rows of 5 m long (east to west row direction) with 30 cm interplant spacing and 50 cm inter-row spacing. Seeds were sowed in 2–3 cm depth holes (10 seeds per hole) with 10 cm interhole spacing. Sowing dates were September 19, 2009, and September 16, 2010. Plots were watered up to 10 mm rainfall equivalent one day before sowing seeds. When the first two leaves emerged, seedling thinning was performed manually to achieve a seedling population of 100,000 plants/ha. Hand-weeding was performed every two weeks to remove weeds. No additional fertilization and no control of fungal diseases and pests were performed during cropping cycles. During the two cropping cycles, plots were slightly watered (equivalent to 5 mm rainfall) once a week in the morning to achieve at the end of the cultivation period a soil water profile equivalent to 350 mm annual rainfall, to approximate rainfall volume of the Patacamaya growing site. The growing period lasted 130 days. Harvest of plants was done manually at physiological maturity of seeds, which was defined as the date when seeds from the main panicle became resistant when pressed (Bertero et al 2004). Plants from the three central rows only were harvested. After drying (48 h at 65°C), panicles were hand-threshed and seeds collected. For mineral composition analysis and the estimation of approximate tissular content of major mineral elements, seeds from two replicate plots were combined separately for each evaluated cultivar. For intersite comparison, seeds of the Patacamaya region were obtained during the 2011 cropping cycle.

TABLE II
Weather Data Recorded at the Two Experimental Sites[y]

Parameter	Encalilla	Patacamaya
Maximum air temperature (°C)	27.3 ± 1.4	21.0 ± 1.0
Minimum air temperature (°C)	14.1 ± 1.2	–0.8 ± 0.2
Relative humidity (%)	44.2 ± 5.1	35–40
Midday PAR (μmol/m^{2}s)	1,893 ± 46	3,634 ± 125
Wind velocity (km/h)	12.5 ± 1.5	7.2–10.5
Day length (h)	10.3 ± 0.5	…
Rainfall (mm/year)	200 ± 20[z]	370 ± 30

[y] Data for the Encalilla site, excepting rainfall, correspond to mean values of 2010 and 2011 cropping cycles. Data for Patacamaya correspond to the 2010 cropping period and were obtained from Patacamaya Meteorological Station. PAR = photosynthetic active radiation.

[z] Mean of data recorded during the last 50 years.

Mineral Content. Dry seeds were ground to a fine powder in a mortar with a pestle, passed through a 60 mesh sieve, and ashed in an oven at 575°C for 16 h. After cooling, ash subsamples (0.1 g) were digested with 10 mL of $HF/HClO_4/HNO_3$ (5.0/2.5/2.5, v/v) mixture in a closed Teflon vessel (EvapoClean, Analab, Spain) at 90°C for at least 12 h. Once digestion finished, the remaining acid was evaporated on a sand bath to incipient dryness. Next, 1 mL of HNO_3 was added twice and was evaporated again to incipient dryness. Residual sediment was dissolved in HNO_3 (1 mL) and transferred into a 100 mL volumetric flask. The digestion vessel was rinsed with deionized water several times, and washing water was also transferred to the volumetric flask. Flask volume was made up with deionized water and then used for chemical element analysis (Ruggieri 2011). A total of 56 elements including major (e.g., calcium, potassium, magnesium, and phosphorus), minor (e.g., zinc, iron, copper, manganese, cobalt, and sodium), and ultratrace mineral elements (e.g., chromium, lithium, arsenic, nickel, molybdenum, selenium, tin, and vanadium) were determined by inductively coupled plasma mass spectroscopy (Element XR HR-ICP-MS, Thermo Scientific, U.S.A.) at the Institute of Earth Sciences Jaume Almera (Spanish Research Council [CSIC], Barcelona, Spain). Quality control of element determinations was carried out by using internal standards and control samples of known composition for each analyzed element (BDH Chemical, England). The quantification of elements was performed by using appropriate calibration curves obtained with standard element solutions in the same acid matrix. Element concentration was expressed on a dry weight (dw) basis. For each replicate plot, element determination was repeated twice, and the average was taken. To analyze variations in seed mineral concentrations between sites, we used the relative mean concentration (RMC), expressed as a percent, which corresponds to an increased or decreased mineral concentration average of all cultivars from Encalilla when compared with a 100% value that corresponds to the average mineral concentration of Patacamaya seeds.

Localization and Distribution of Mineral Elements. Scanning electron microscopy combined with energy dispersive X-ray spectroscopy (SEM/EDX) and dot map analysis were used to assess the distribution and approximate content of major mineral elements (e.g., magnesium, phosphorus, sulfur, potassium, and calcium) in the pericarp and embryonic axis of seeds from the Encalilla and Patacamaya sites. SEM/EDX micrographs and dot maps were obtained from transversely hand-sectioned seed samples (five seeds per cultivar and for each site). Seed sections were examined with a JEOL 6300 SEM outfitted with an Oxford/Link Pentafet model 6699 energy-dispersive X-ray spectrometer equipped with a SiLi detector and a thin window (beryllium window) for elements from boron to uranium. In all cases, the accelerating voltage was 20 kV, probe current 10 nA, and collection time 50 s (Psaras and Manetas 2001). Element distribution was done from dot maps with Oxford/Link ISIS software (series 300), which enabled the system to give directly the percentage of elemental composition for each selected element. Micrographs and X-ray dot maps were taken in digital form. SEM/EDX microscopy and dot map analysis were carried out at the Laboratory of Electron Microscopy and Microanalysis (School of Sciences, University of Patras, Greece). To estimate approximate contents of major elements in the seeds we used an empirical symbol-based scale.

Mineral composition was also analyzed in relation to seed protein and seed yield. Analytical methods and data of seed protein content and seed yield have been previously reported (González et al 2012).

Statistical Analyses. SAS/STAT version 8.0 statistical package (SAS Institute, U.S.A.) was used for statistical analyses. Student's *t* test was used to compare means of mineral nutrient concentrations between Encalilla and Patacamaya seeds. Linear regression analysis was done with OriginLab software (version 6.1, OriginLab, U.S.A.).

RESULTS

Mineral Composition. Because no large differences in climatic parameters and seed mineral composition were observed between the 2010 and 2011 cropping cycles, data reported here correspond to the average of the two years. Concentrations of 56 mineral elements were determined in seeds from both the Encalilla and Patacamaya sites; for brevity, data of 18 elements that are particularly important to human nutrition only are presented here. When comparing mineral composition between sites, significant inter- and intravarietal differences in the concentrations of major (potassium, phosphorus, calcium, magnesium, and sodium) and minor or trace (iron, cobalt, zinc, copper, and manganese) dietary minerals as well as in ultratrace elements (molybdenum, selenium, chromium, lithium, nickel, tin, vanadium, and arsenic) were observed among all tested cultivars. Among major mineral elements, potassium, phosphorus, calcium, and sodium showed significantly higher values of RMC (142.7, 125.7, 202.3, and 131.2%), whereas the RMC value corresponding to magnesium was lower (83.1%). Higher RMC values were also found for important minor dietary minerals such as zinc, copper, and manganese (122.6, 119.8, and 121.9%). In contrast, RMC values of minor minerals such as iron and cobalt were significantly lower (69.6 and 54.8%) compared with the reference value. Essential ultratrace elements of Encalilla seeds such as chromium, molybdenum, lithium, and tin showed significantly higher RMC values (Table III). Interestingly, RMC corresponding to molybdenum was approximately sixfold higher than the reference.

Localization and Distribution of Mineral Elements. By SEM/EDX microscopy and dot map analysis, we assessed the tissular localization and distribution of potassium, phosphorus, magnesium, calcium, and sulfur in the pericarp and embryonic axis (cotyledon + radicle) of quinoa seeds. Figure 1 shows the tissular distribution of minerals in quinoa seed. The highest contents of calcium, magnesium, and potassium were localized in pericarp tissue (Fig. 1B–D), whereas phosphorus was higher in the embryonic axis (Fig. 1E). Sulfur occurred in both the pericarp and embryonic axis to a similar extent (Fig. 1F). Table IV shows approximate tissular contents of major elements in seeds of different quinoa cultivars from Encalilla and Patacamaya. Intravarietal and intersite differences in the pericarp and embryonic axis were clearly observed.

TABLE III
Seed Mineral Concentrations (mg/kg dw) and Relative Mean Concentrations (RMCs) of Seven Quinoa Cultivars from the Patacamaya and Encalilla Sites[z]

Mineral	Amilda	Chucapaca	CICA	Ratuqui	Sajama	Samaranti	Sayaña	RMC (%)
Encalilla								
Potassium	9,605.28bA	9,952.35bA	12,595.39aA	9,321.46bA	8,932.68bcA	9,021.26bA	8,870.41bcA	142.7
Phosphorus	2,623.21abA	3,133.04aA	3,309.55aA	3,270.56aA	1,237.20cB	1,341.52cB	2,772.61abA	125.7
Calcium	570.24bA	499.96bA	907.54aA	526.67bA	823.34abA	1,166.03aA	531.88bA	202.3
Magnesium	1,229.85bA	1,461.32aA	1,514.39aA	1,549.22aA	855.36cB	968.42cB	1,394.93aA	83.1
Iron	89.72aA	95.34aA	98.44aA	74.16bB	47.56dB	61.45cA	74.72bB	69.6
Zinc	38.53aA	37.23aA	42.24aA	31.03bA	21.23cA	20.62cA	26.79bA	122.6
Copper	11.19aA	10.17aA	9.40abA	7.90bA	4.81dA	6.12cA	7.61bA	119.8
Manganese	16.95cA	24.16bA	38.53aA	20.04bcA	24.63bA	31.32aA	20.45bcA	121.9
Sodium	37.76bA	35.37bA	73.28aA	18.76dA	19.06dA	26.45cA	20.50dA	131.2
Cobalt	0.05aB	0.06aA	0.04bA	0.05aB	0.05aB	0.05aB	0.04bB	54.8
Molybdenum	0.78aA	0.69bA	0.78aA	0.58cA	0.50cA	0.51cA	0.52cA	566.4
Lithium	0.18bA	0.18bA	0.26aA	0.13cA	0.14cA	0.20bA	0.12cA	242.3
Chromium	0.23bA	0.16bA	0.15cB	0.13cB	0.23bA	0.48aA	0.20bA	120.9
Selenium	0.11aA	0.06bA	<0.001cB	<0.001cB	<0.001cB	<0.001cB	<0.001cB	39.8
Tin	0.24aA	0.04cA	0.05cA	0.21aA	0.10bA	0.10bA	0.10bA	216.1
Vanadium	0.09aA	0.10aA	0.06bA	<0.001dB	0.03cB	0.03cB	<0.001dB	50.3
Nickel	0.39aB	0.14bB	0.16bA	0.14bB	0.13bB	0.14bB	0.14bB	30.9
Arsenic	0.07aA	0.06aA	0.06aA	0.04bB	0.03bcA	0.03bcA	0.02cA	75.6
Ash (%)	2.29aA	2.98aA	3.66aA	2.72aA	2.50abA	2.78aA	2.59abA	104.1
Patacamaya								
Potassium	6,933.95bB	4,984.71bcB	8,080.83aB	6,804.71bB	7,376.13bB	5,237.50bcB	8,444.71aA	100
Phosphorus	2,502.35aA	1,899.58abB	1,989.55abB	2,111.77aB	1,682.81bA	1,991.80abA	1,891.85abB	100
Calcium	328.89bB	251.52cB	607.69aB	290.48bcB	318.03bB	295.97bcB	391.03bA	100
Magnesium	1,718.19aA	1,336.55abA	1,606.57aA	1,605.81aA	1,406.85abA	1,511.44aA	1,608.59aA	100
Iron	113.37bA	53.43dB	90.60cA	240.40aA	106.31bA	68.09dA	105.86bA	100
Zinc	33.08aA	16.51bB	31.25aA	23.96abA	20.31abA	29.19aA	23.21abA	100
Copper	8.77aA	3.48cB	5.86bA	6.49bA	6.89bA	8.47aA	7.81aA	100
Manganese	24.35aA	17.23bA	19.53bB	23.41aA	18.74bA	15.46bB	25.66aA	100
Sodium	24.87bB	20.05bB	50.19aB	22.19bA	18.12bcA	17.42bcA	23.33bA	100
Cobalt	0.15aA	0.03bB	0.02bB	0.12aA	0.10aA	0.10aA	0.10aA	100
Molybdenum	0.09bB	0.15aB	0.14aB	0.09bB	0.09bB	0.10bB	0.11bB	100
Lithium	0.05bB	0.03cB	0.15aB	0.11aA	0.06bB	0.04cB	0.06bB	100
Chromium	0.19aA	0.17aA	0.21aA	0.21aA	0.21aA	0.14bB	0.18aA	100
Selenium	0.03bB	0.05bA	0.09aA	0.05bA	0.06bA	0.05bA	0.10aA	100
Tin	0.05bB	0.05bA	0.05bA	0.05bB	0.04bB	0.09aA	0.06bB	100
Vanadium	0.08bA	0.04cB	0.06bA	0.06bA	0.15aA	0.08bA	0.15aA	100
Nickel	0.81aA	0.24cA	0.16dA	0.66bA	0.62bA	0.71abA	0.81aA	100
Arsenic	0.02bB	0.02bB	0.02bA	0.27aA	0.02bA	0.03bA	0.03bA	100
Ash (%)	2.95aA	2.17abA	3.04aA	2.66aA	2.66aA	2.53aA	2.75aA	100

[z] Concentration of a particular element represents the average of two year means. Values followed by the same lowercase letter for each element and for each site within a row are not significantly different. Values followed by the same uppercase letter for each element and for each cultivar in both sites are not significantly different at $P < 0.05$ ($n = 4$ per year).

Correlation Analysis. Correlation analysis between mineral concentrations and both seed yield and seed protein content are shown in Table V. For Encalilla seeds, phosphorus, iron, magnesium, copper, and zinc showed highly significant positive correlations with seed yield. Correlation coefficients ranged from r = 0.724 (phosphorus) to r = 0.55 (zinc). Positive lower correlation coefficients ranging between 0.127 and 0.312 were observed with manganese, molybdenum, sodium, potassium, and arsenic. Negative correlations were found for calcium, chromium, and lithium, being significantly higher only for calcium (r = –0.698). Further, vanadium, tin, nickel, and cobalt did not correlate significantly with seed yield. Regarding the seed protein content, iron, magnesium, copper, and phosphorus were significantly positively correlated. Correlation coefficients ranged between r' = 0.804 (magnesium) and r' = 0.595 (phosphorus). Significant positive correlations were also observed for potassium, zinc, manganese, sodium, and cobalt, but their correlation coefficients were lower, ranging between r' = 0.167 (cobalt) and r' = 0.292 (zinc). Strong negative correlations were found for molybdenum (r' = –0.738), calcium (r' = –0.635), and selenium (r' = –0.515). Lower negative correlations occurred with lithium, chromium, tin, vanadium, nickel, and arsenic. Patacamaya seeds showed lower correlations between minerals and seed yield than those of Encalilla seeds. Except for phosphorus, molybdenum, manganese, and magnesium, minerals of Patacamaya seeds showed significant negative correlations with seed yield. Correlation coefficients ranged from r = –0.111 (cobalt) to r = –0.510 (vanadium). Correlation analysis between mineral concentration and seed protein content showed significant positive correlations for calcium, zinc, molybdenum, sodium, lithium, and selenium. Correlation coefficients ranged from r' = 0.383 (zinc) to r' = 0.826 (calcium). By contrast, phosphorus, magnesium, iron, manganese, cobalt, vanadium, nickel, and arsenic were significantly negatively correlated with seed protein content. Correlation coefficients ranged between r' = –0.162 (manganese) and r' = –0.409 (cobalt).

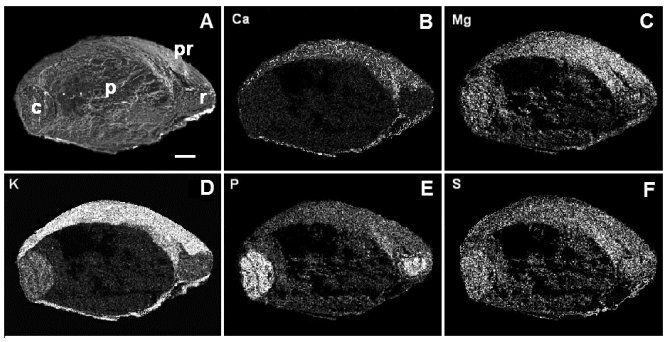

Fig. 1. Transverse sectioned seed (Samaranti cultivar from the Patacamaya site) showing internal structure and localization of mineral elements. **A,** Internal structure; **B,** calcium; **C,** magnesium; **D,** potassium; **E,** phosphorus; and **F,** sulfur. White dots indicate the presence of the corresponding element. c = cotyledon; p = perisperm; pr = pericarp; and r = radicle. Bar = 200 µm.

DISCUSSION

Quinoa seed is one of the most complete foods in nature because it contains a well-balanced essential amino acid composi-

TABLE V
Correlation Coefficients of Mineral Concentrations with Seed Yield (r) and Seed Protein Content (r')[z]

	Encalilla		Patacamaya	
Mineral	r	r'	r	r'
Potassium	0.243*	0.279*	−0.135*	0.077
Phosphorus	0.724**	0.595**	0.133*	−0.313*
Calcium	−0.698**	−0.635**	−0.081	0.826***
Magnesium	0.609**	0.804***	0.062	−0.246*
Iron	0.556**	0.770***	−0.484*	−0.247*
Zinc	0.550**	0.292*	−0.139*	0.383*
Copper	0.576**	0.718**	−0.289*	−0.051
Manganese	0.178*	0.286*	0.026	−0.162*
Molybdenum	0.312*	−0.738***	0.169*	0.424*
Cobalt	0.060	0.167*	−0.111*	−0.409*
Sodium	0.282*	0.219*	−0.117*	0.548**
Lithium	−0.184*	−0.126*	−0.151*	0.601**
Chromium	−0.464*	−0.306*	−0.348*	−0.079
Selenium	0.127*	−0.515**	−0.271*	0.612**
Tin	−0.102*	−0.492*	−0.166*	0.103
Vanadium	0.064	−0.330*	−0.510**	−0.299*
Nickel	0.047	−0.192*	−0.192*	−0.381*
Arsenic	0.257*	−0.164*	−0.503**	−0.352*

[z] *, **, and *** = significant at $P < 0.05$, 0.01, and 0.001, respectively.

TABLE IV
Contents of Magnesium, Calcium, Phosphorus, Sulfur, and Potassium in Pericarp and Embryonic Axis (EA) of Quinoa Seeds Obtained from SEM/EDX Micrographs[z]

	Magnesium		Calcium		Phosphorus		Sulfur		Potassium	
Location, Cultivar	**Pericarp**	**EA**	**Pericarp**	**EA**	**Pericarp**	**EA**	**Pericarp**	**EA**	**Pericarp**	**EA**
Encalilla										
Amilda	+	++	16.9%	…	…	++	+	…	+++	+
Chucapaca	+	+	+	+	…	+	+	…	++	+
CICA	++	+	++	+	…	++	+	+	++	+
Ratuqui	+	+	+	…	…	++	…	…	++	+
Sajama	+	…	+	+	…	++	+	+	+++	+
Samaranti	++	+	++	…	…	++	+	++	+++	+
Sayaña	…	…	+	+	…	++	+	+	+++	+
Patacamaya										
Amilda	++	+	+	…	…	++	9.0%	…	++	+
Chucapaca	+	+	12.7%	…	…	+	++	…	++	+
CICA	…	…	+	+	…	+++	+	+	++	+
Ratuqui	+	++	++	+	…	14.7%	+	+	++	+
Sajama	…	…	+	…	…	+	+	+	+++	+
Samaranti	++	++	++	+	+	+++	++	++	+++	+
Sayaña	+	…	+	…	…	+	…	…	++	+

[z] Empirical symbol-based scale: + = only a few dots can be visualized on the image; ++ = isolated dots are clearly visible on the image; and +++ = many separated dots can be visualized on the image and some even appear as dot-fused areas. When the accumulated element exceeds the symbol-based scale estimation, the numerical value of element concentration is indicated in the table. SEM/EDX = scanning electron microscopy combined with energy dispersive X-ray spectroscopy.

tion, enzymes, vitamins, minerals, fiber, antioxidants, and other phytonutrients (Ruales and Nair 1993; Repo-Carrasco et al 2003). Quinoa minerals represent a good source of major and minor dietary minerals compared with common cereals (Table I). Data of the present study showed that quinoa seed can also be an important source of ultratrace essential minerals for people, especially those with scarce provision of animal-derived foods such as meat, milk, and eggs (Table III). Mineral concentration of seeds showed a high variability depending upon both genetic and environmental factors (Gomez-Becerra et al 2010). In the absence of fertilization, seed mineral concentration can be affected by both soil composition and soil physicochemical characteristics. Electrical charges on soil colloids determine the ability of a soil to retain minerals against the force of water moving through the soil, which, in turn, affects the soil capacity to supply mineral nutrients over time (Bonfil and Kafkafi 2000). Soil capacity to retain positively charged ions is enhanced with increasing negative charges onto its particles. Negative charges on soil particles constitute the CEC. Soils with high CEC can retain higher amounts of positively charged minerals and are better able to buffer or to avoid the rapid exchange of retained minerals with others from the surrounding solution, particularly when their concentrations become depleted (Evans 1989). In this way, an excessive amount of a replaceable cation on soil particles (primarily clay) can affect the mobility and availability of other cations for plants.

Effects of soil type on seed mineral composition were analyzed in different pseudocereals (Konishi et al 2004; Kibria et al 2009). However, many of these studies were performed in a greenhouse under controlled climatic conditions; those carried out under field conditions are scarce (Karyotis et al 2003; Huang et al 2014). Indeed, comparative studies showing the effects of soil type and environmental conditions on mineral composition of quinoa seed are still rare (Karyotis et al 2003). In this study, we analyzed the seed mineral composition of seven quinoa cultivars grown in two different agroecological sites, Encalilla and Patacamaya. Because the soil of Encalilla had a higher clay content and CEC than Patacamaya soil, one might expect that the mineral composition of seeds was different between both sites. Data showed that all analyzed mineral elements were present in seeds from both sites, but significant inter- and intravarietal differences in element concentrations were observed. Seeds from the Encalilla site had higher RMC for important major and minor dietary minerals than seeds from the Patacamaya site. Interestingly, of eight analyzed ultratrace elements, four (chromium, lithium, tin, and molybdenum) showed significantly higher RMC in Encalilla seeds (Table III). In agreement with our results, lithium has also been found in seeds of quinoa and amaranth (*Amaranthus caudatus*) consumed in the north of Argentina (Coelho et al 2011). An additional nutritional feature related to high concentrations of chromium, lithium, and molybdenum found in Encalilla seeds can be linked to human health, because they have been recognized as essential ultratrace elements in human metabolism (Nielsen 2000). By contrast, the essentiality of lithium and chromium for plants has not been recognized, and in many plants, even those growing on chromium- or lithium-enriched soils, these elements are absent or are present in rather low concentrations. Despite lithium and chromium having little to no effect on plant metabolism, their presence in quinoa seeds deserves additional study.

Besides soil characteristics, environmental factors such climatic conditions also affect the mineral composition of plants (Porder and Chadwick 2009). In this regard, adaptation of wild cereals to arid habitats depends upon their abilities to concentrate inside the seed high amounts of limited elements occurring in the soil, without affecting the plant productivity (Chatzav et al 2010). In these conditions the seed size tends to decrease, giving a mineral concentration effect (Nevo 2011). In contrast, in natural habitats plants produce larger seeds and higher seed yields, which cause a mineral dilution effect (Karaköy et al 2012). According to Murphy et al (2008), if plants grow under conditions of mineral dilution, the seed mineral concentration and grain yield frequently show significant negative correlations. Agreeing with this assumption, many minerals of seeds harvested at the Patacamaya site were negatively correlated with seed yield, whereas most minerals occurring in Encalilla seeds showed positive correlations (Table V). Therefore, it could be assumed that a mineral concentration effect occurs when the quinoa is cultivated in arid regions outside its natural habitat (Andean highlands). Supporting our assumption, the size and weight of Encalilla seeds were lower than those of the Patacamaya seeds (J. A. González, *unpublished data*). Occurrence of the mineral concentration effect in cultivars grown in Encalilla should be taken with caution because seed yield data were obtained from different cropping trials, one on a small scale with well-controlled plant development (Encalilla) and the other on a large commercial scale without plant development control (Patacamaya).

Although mineral variability in species grown at different sites depends upon changes occurring in species-specific genes (Alonso-Blanco et al 2009), it also depends on environmental factors and even on agricultural practices (Gomez-Becerra et al 2010; Farahani et al 2011). Thus, comprehensive understanding of genetic networks governing the mineral composition of seeds also requires a careful attention to environmental factors impacting on the growth site. In this sense, significant intervarietal and intersite differences observed in the mineral concentration of quinoa seeds suggest that genetic factors are not the sole seed traits involved in mineral variations. In agreement with previous results obtained by Bhargava et al (2008), our results also indicate that variations in quinoa mineral composition can be explained better by genotype (cultivar) × environment (soil and climate conditions) interactions.

In many cereals and legumes the mineral composition of seeds is closely related to their protein concentration. Moreover, most correlation analyses between seed protein and minerals show highly positive correlations (Peleg et al 2008; Zhao et al 2009; Chatzav et al 2010). Negative correlations were rarely observed, and their occurrence has been explained by assuming that lower-yielding genotypes have smaller seeds and lower mineral dilutions than larger seeds occurring in higher-yielding genotypes (Feil et al 2005). In the present study, both positive and negative correlations were observed in seeds from the two sites (Table V). Based on previous results showing that there are no significant variations in seed protein concentration and seed yield between the Encalilla and Patacamaya sites (González et al 2012), we assume that negative correlations do not constitute an evolutive strategy of quinoa to get a higher mineral concentration. In contrast, we believe that in quinoa there is no one-way selection for both higher protein content and increased mineral concentration. Nonetheless, phenotypic plasticity of seed protein composition and mineral accumulation are triggered by genetic and environmental factors (Bourgeois et al 2009; Fenner 2010); therefore, to explain correlations reported here, multilocation testing is needed to get a more realistic insight into the relative contributions of genes and environment to relationships between minerals and protein occurring in quinoa cultivars grown in natural and non-natural habitats.

To better understand how genetic and environmental factors can affect mineral distribution in quinoa seeds, we analyzed by SEM/EDX microscopy and dot map analysis the tissular content of major minerals in the pericarp and embryonic axis. In agreement with previous studies carried out in amaranth (*A. hypochondriacus*) (Konishi et al 1998), tartary buckwheat (*Fagopyrum tataricum*) (Pongrac et al 2013), and quinoa (Konishi et al 2004), our SEM/EDX micrographs also showed a heterogeneous tissular distribution of mineral elements in quinoa seed (Fig. 1). Estimated contents of major dietary minerals in the pericarp and embryonic axis differed according to analyzed element. Interestingly,

element contents obtained empirically by using a symbol-based scale were similar to element concentrations determined quantitatively by Ando et al (2002). However, when analyzing the distribution pattern among cultivars and also between sites, significant differences were found (Table IV). These results contribute to confirm that genetic and environmental factors were responsible for observed variations in accumulation and distribution of minerals in quinoa seeds obtained from plants cultivated under different agroecological conditions.

CONCLUSIONS

We analyzed seed mineral concentrations of seven quinoa cultivars grown at two different agroecological sites. Patterns of mineral accumulation were different among analyzed cultivars for each site and between both sites. Minerals were positively or negatively correlated with both seed yield and seed protein content, but correlation patterns were different between sites and also among cultivars within each site. These results suggest that the accumulation of minerals and its relationships with yield traits exhibit a highly plasticity, being probably controlled by genetic × environment interactions. The dataset of this work provides a valuable resource for genetic improvement trials and the creation of new and better varieties to solve crop-adaptation problems for places where climatic and soil conditions are limiting for other crops, particularly in mountainous regions of the world where hunger is still an unsolved issue. Undoubtedly, finding sustainable solutions to mineral malnutrition will not be achieved in the foreseeable future if we do not start to adopt agriculturally based tools to attack this important global crisis in human health and well-being.

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