Research Article

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Interrelationships among seed yield, total protein and amino acid composition of ten quinoa (*Chenopodium quinoa*) cultivars from two different agroecological regions

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Abstract

BACKGROUND: Quinoa is a good source of protein and can be used as a nutritional ingredient in food products. This study analyses how much growing region and/or seasonal climate might affect grain yield and nutritional quality of quinoa seeds.

RESULTS: Seeds of ten quinoa cultivars from the Andean highlands (Bolivia/Argentina site) and Argentinean Northwest (Encalilla site) were analysed for seed yield, protein content and amino acid composition. Grain yields of five cultivars growing at Encalilla were higher, and four were lower, compared with data from the Bolivia/Argentina site. Protein contents ranged from 91.5 to 155.3 and from 96.2 to 154.6 g kg^{-1} dry mass for Encalilla and Bolivia/Argentina seeds respectively, while essential amino acid concentrations ranged from 179.9 to 357.2 and from 233.7 to 374.5 g kg^{-1} protein respectively. Significant positive correlations were found between the content of essential amino acids and protein percentage.

CONCLUSION: It appears that there are clear variations in seed yield, total protein content and amino acid composition among cultivars from the two sites. Essential amino acid composition was more affected than grain yield and protein level. The study revealed that both environmental and climatic factors influence the nutritional composition of quinoa cultivars growing in different agroecological regions.

Keywords: quinoa; protein; amino acids; seed yield

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), the 'mother grain' of the Andean peoples, contains gluten-free high-quality protein. The protein of quinoa seed is rich in essential amino acids, particularly methionine, threonine and lysine, which are the limiting amino acids in most cereal grains.[1] For human and animal nutrition the quality of protein is determined by its biological value (BV), which serves as an indicator of protein intake by relating nitrogen uptake to nitrogen excretion. The highest values of BV correspond to proteins of whole egg (93.7%) and cow milk (84.5%).[2] The protein of quinoa has a BV of 83%, which is higher than that of fish (76%), beef (74.3%), soybean (72.8%), wheat (64%), rice (64%) and corn (60%) proteins.[3] According to the FAO/WHO nutritional requirements for 10–12-year-old children, the protein of quinoa possesses adequate levels of phenylalanine, tyrosine, histidine, isoleucine, threonine and valine.[4] Consequently, there is no need to combine quinoa seeds with other protein sources to cover the human requirements of essential amino acids.

Quinoa is an annual species able to tolerate extreme environmental conditions such as salinity, cold, high solar radiation and drought; it is also able to grow over a wide latitudinal range (nearly 50°) and from sea level to over 4000 m above sea level (a.s.l.) in the Andean highlands (Altiplano region).[3] The Altiplano is a wide area lying in the highlands of the central Andes, mainly occurring in Bolivia but also occupying parts of Chile, Argentina (Northwest region) and Peru. The Bolivian Altiplano is an extremely complex population of environments that affect both crop yield and protein quality through a latitudinal range.[5] This fact has led to the creation of many specific response genotypes with different characteristics and high variability regarding prevailing environmental conditions in the Andean highlands.[6] At present, quinoa is grown

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Table 1. List of quinoa cultivars evaluated in this study and their origin, source, ecotype, altitude and grain colour

Cultivar	Origin	Source (location/country)	Ecotype	Altitude (m a.s.l.)[a]	Grain colour
Amilda	Bolivia	Patacamaya (Bolivia)	Altiplano	3960	White
Chucapaca	Bolivia	Patacamaya (Bolivia)	Altiplano	3960	White
CICA	Peru	Iruya (Argentina)	Valley	2780	Yellow
Kamiri	Bolivia	Patacamaya (Bolivia)	Altiplano	3960	White
Kancolla	Peru	Patacamaya (Bolivia)	Altiplano	3960	White
Ratuqui	Bolivia	Patacamaya (Bolivia)	Altiplano	3960	White
Robura	Bolivia	Patacamaya (Bolivia)	Altiplano	3960	White
Sajama	Bolivia	Patacamaya (Bolivia)	Altiplano	3960	White
Samaranti	Bolivia	Patacamaya (Bolivia)	Altiplano	3960	White
Sayaña	Bolivia	Patacamaya (Bolivia)	Altiplano	3960	Pale yellow

[a] Corresponds to location altitude.

as a minor crop over extensive areas in the Andean highlands as well as in India, Mexico, Egypt, USA, Canada, Chile, Argentina, Peru, Colombia, Ecuador, Italy, Greece, Spain and other countries.[7] Moreover, quinoa has been described as an 'alternative crop' or a 'new crop' thanks to possibilities of using its seeds and leaves in the food industry and pharmacy.[3] Nevertheless, no study evaluating relationships among nutritional quality, environmental variables and potential to inherit grain traits has yet been reported. Thus the aim of the present study was to evaluate during two consecutive periods the seed yield, total protein content and amino acid composition of ten quinoa cultivars growing under drought conditions in a defined agroecological site located at 1995 m a.s.l., compared with similar seeds from two agroecological sites located at 3960 m a.s.l. (Bolivian Altiplano) and 2780 m a.s.l. (Andean Northwest region of Argentina). As a general hypothesis to sustain the objective of this work, we take into account the finding that the protein composition of wheat grains depends primarily on genotype but is also dependent on environmental factors and their interactions.[8]

MATERIALS AND METHODS

Plant material

The material for this study comprised nine cultivars of quinoa (*C. quinoa* Willd.) from the Bolivian Altiplano (Patacamaya Experimental Station, 17° 15′ S, 67° 55′ W, altitude 3960 m a.s.l.) and one from the Andean Northwest region of Argentina (Iruya, 22° 46′56″ S, 65° 14′ W, altitude 2780 m a.s.l.). The nine cultivars from the Bolivian Altiplano were Amilda, Chucapaca, Kamiri, Kancolla, Ratuqui, Robura, Sajama, Samaranti and Sayaña, while the cultivar from the Andean Northwest region of Argentina was CICA (Table 1).

Experimental sites and climatic conditions

Field experiments were carried out in two consecutive periods (2007–2008 and 2008–2009) at the Encalilla site, an arid mountain region of the Argentinean Northwest (Amaicha del Valle, 22° 31′ S, 65° 59′ W, altitude 1995 m a.s.l.). The climate of Encalilla is classified as desert type (BWkaw) according to the Köppen classification system.[9] The annual rainfall is 200 mm, with over 70% (∼150 mm) falling during the growing season (September–March). Maximum and minimum air temperatures recorded during the growing season were 30.4 and 11.1 °C respectively, while maximum and minimum values of relative humidity (RH) were 44.2 and 54.2% respectively. The velocity of wind ranged from 10 to 25 km h^{-1}, while the photosynthetic active radiation (PAR) values recorded at midday under cloudy and sunny conditions were 1403 and 1993 µmol m^{-2} s^{-1} respectively. Day length was 9.8 h during the early spring and 11.3 h during the summer. Climatic parameters were recorded using an automatic weather station (Pegasus EP1000, Buenos Aires, Argentina). Air temperature and RH were measured using a thermocouple with a temperature range between −20 and 70 ± 1 °C and an RH range between 25 and 95 ± 1% (Hobo H8 RH/Temp data logger, Onset Computer Corp., Bourne, USA). PAR was measured using a quantum sensor (LI-190SA) coupled to a data logger (LI-1000) (Li-Cor, Lincoln, NE, USA). The soil of Encalilla is classified as Xeric Torriorthent type[10] with sandy clay loam texture (0–50 cm depth) and the following physicochemical parameters: pH 8.4, electrical conductivity (EC) 2.0 dS m^{-1}, exchangeable sodium (ES) 38.6% and cation exchange capacity (CEC) 12.3 cmol kg^{-1} (Table 2). The soil evapotranspiration calculated by a modification of the Penman method[11] was 350 mm, i.e. 2.3-fold higher than the rainfall value. In comparison with data of the Encalilla site, we also performed a screening of available climatic and soil data corresponding to the Patacamaya region (Bolivian Altiplano). This site has an annual rainfall of 370 mm with marked seasonality (over 80% of the total precipitation falls in summer). Maximum and minimum air temperatures during the growing season (October–April) were 24 and −0.8 °C respectively, while corresponding RH values were 35 and 40% respectively. The velocity of wind ranged between 7.2 and 10.5 km h^{-1}. The daily total solar radiation recorded in summer was 3634 µmol m^{-2} s^{-1}, reaching a midday maximum value of 5980 µmol m^{-2} s^{-1} on a very sunny day.[12] The soil of Patacamaya has a sandy loam texture (0–30 cm depth) with low clay content (6.0%), pH 6.6, organic matter 0.5% and exchangeable aluminium (EA) negligible.[13] It is shallow, sandy (79% sand content) and stony with granular structure, being classified as Haplic Xerosol type according to the FAO–UNESCO soil taxonomy.[14] The average soil evapotranspiration is 1022 mm (3.4-fold higher than rainfall)[15] (Table 2).

Experimental design

The experimental design was a randomised block with three replications for each cultivar. Plot sizes were 18 m^2 with five rows each, 30 cm plant-to-plant and 50 cm row-to-row spacing in an E–W row direction. One day prior to seed sowing, the plots were surface irrigated to provide about 10 mm of water. Seeds were hand sown in 10 cm-spaced holes at 2–3 cm depth (ten

Table 2. Soil classification, chemical and physical properties of Encalilla and Patacamaya topsoils

Parameter	Encalilla	Patacamaya
Soil order	Entisol	–
Type	Xeric Torriorthent	Haplic Xerosol[13]
Sand (%)	48	79[13]
Silt (%)	22	15[13]
Clay (%)	30	6[13]
Soil texture	Sandy clay loam	Sandy stony[13]
pH of suspension in H_2O (1 : 1)	8.4	6.6[13]
Organic matter (%)	0.60	0.50[13]
Total nitrogen (%)	0.055	0.066[13]
C/N ratio	10.9	7.6[13]
P-Olsen (mg kg^{-1})	23.5	20.5[34]
$CaCO_3$ (%)	0.68	0.45[34]
ES (%)	38.6	Negligible[13b]
EC (dS m^{-1})	2.0	7.0[34]
Exchangeable cations		
K^+ (mg kg^{-1})	390.2	424.4[34]
Na^+ (mg kg^{-1})	615.2	–
Mg^{2+} (mg kg^{-1})	342.7	279.2[34]
CEC (cmol kg^{-1})[a]	12.3	8.9[34]

ES, exchangeable sodium; EC, electrical conductivity; CEC, cation exchange capacity.
[a] Centimoles of positive charge per kilogram of dry soil.
[b] Corresponds to exchangeable aluminium at pH 6.6.

seeds per hole). Sowing dates were 17 September 2007 and 23 September 2008. When the first two leaves emerged, seedlings were hand thinned to a final stand density of 80 000 plants ha^{-1}. During the growing season, plots were irrigated weekly in the morning to get a water soil profile of 250 mm per cropping cycle at the end of the experiment. Weeds were eliminated by hand at 15 day intervals. No additional fertilisation and no control of fungal diseases and pests were performed during the cropping cycle. At physiological maturity, defined as the date when seeds from the main panicle become resistant when pressed[6] (~140 days after sowing), plants were hand harvested on a 4 m row segment from three central rows for each cultivar. Prior to harvesting, plant height was measured on the main stem from soil level to inflorescence tip (panicle) using a plastic ruler (0.5 mm accuracy). Harvested plants were kept at 4 °C before transport to the laboratory for analytical determinations.

Laboratory methods

Plants were divided into roots, aerial parts (leaves plus stems) and panicles and dried at 70 °C until constant weight (~48 h). To determine the grain yield, seeds from hand-threshed panicles were exposed to ventilation to separate residues and dust. After cleaning, the seeds should be dried to a moisture content of 130 g kg^{-1} to prevent fungal growth. The grain yield was expressed as kg ha^{-1}. Thereafter the seeds were ground to a fine meal in a grinding mill (Wiley Intermediate, Arthur H Thomas Co., Philadelphia, PA, USA) with a 150 µm mesh setting. The seed grinding was conducted carefully to avoid overheating and to prevent both oxidation and loss of amino acids. Meal samples were used to determine nitrogen, total protein, amino acid and tryptophan contents. All chemical determinations were carried out at the Graduate School of Human Life Science, Osaka City University (Osaka, Japan).

Nitrogen and total protein content

The nitrogen content in each sample was quantified by the micro-Kjeldahl method with colorimetric ammonium (NH_4^+) determination[16] and expressed as % seed dry mass. Total protein content was calculated from the nitrogen content using a conversion factor of 6.25.[17] Results were given as g kg^{-1} dry mass. The quality index (QI), a measure of protein quality, was calculated as the tryptophan/protein ratio in the sample and expressed as %.[18]

Amino acid composition and tryptophan content

Amino acid analysis was performed after hydrolysis of seed samples with 6 mol L^{-1} HCl and 0.5 g L^{-1} β-mercaptoethanol in sealed vacuum-evacuated tubes at 110 °C for 21 h. After cooling, the hydrolysates were centrifuged at 6000 × *g* for 30 min and the precipitates were discarded. HCl and β-mercaptoethanol were removed by evaporation under vacuum and the amino acid composition was determined after chromatography on an automatic amino acid analyser (Hitachi L8500, Tokyo, Japan). For tryptophan determination, samples were decolourised with half-saturated *n*-butanol solution and digested in 75 mmol L^{-1} KOH containing 0.5 g L^{-1} β-mercaptoethanol at 110 °C for 24 h in screw-capped test tubes. After centrifugation at 6000 × *g* for 30 min the resulting supernatants were used for colorimetric tryptophan determination.[19] The concentration of amino acids was expressed as g kg^{-1} protein.

Soil analysis

Physicochemical parameters and mineral composition of the soil from the Encalilla site were determined by standard analysis methods (Laboratorio de Suelos, Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina). The soil water content was determined by gravimetry.[20]

Statistics

To verify the statistical significance of measured parameters, a triplicate analysis for each measurement was conducted for each cultivar. Data were analysed using SAS/STAT Version 8 (SAS Institute Inc., Cary, NC, USA). Analysis of variance was performed on data of each year separately. Multiple comparisons of means were made using the Student–Newman–Keuls test ($P < 0.05$). Differences between years were not tested because there were no significant differences between them. Correlation coefficients (r and r') were calculated from linear regression analyses to detect significant relationships between the analysed parameters. The linear equation was in the form $y = a + bx$, where y is the dependent variable (mg amino acid g^{-1} dry mass or g amino acid kg^{-1} protein), a is the intercept, b is the slope of the line and x is the independent variable (% protein).

RESULTS AND DISCUSSION

Because no significant differences in climatic conditions, grain yield and evaluated chemical parameters between 2008 and 2009 trials were observed, the reported results correspond to averages of the two field experiments.

Metabolic and physiological traits of crops are affected by the environment, with soil and weather being the most important factors.[21] In this context it is expected that changes in both grain yield and seed nutritional quality might occur in quinoa cultivars growing at the Encalilla site when compared with cultivar data from different agroecological sites. Table 3 shows growth and seed yield data of the ten quinoa cultivars grown at the Encalilla site as well as seed yield data corresponding to cultivars grown at the Patacamaya site. (Data on the seed yield of the CICA cultivar from the Andean Northwest region of Argentina were not available.) According to the available literature, quinoa cultivars exhibit great genetic diversity, showing variability in the colouring of plant, inflorescence and seeds, inflorescence types, protein, saponin and betacyanin contents, and calcium oxalate crystals in leaves. Thus a wide adaptation to different environmental conditions (soil, rainfall, temperature, altitude, frost, drought, salinity or acidity) may be seen.[6] Agreeing with their genetic adaptation capacity, in this study, except for four cultivars, no great changes in grain yield among quinoa cultivars from the Encalilla and Patacamaya sites were observed. The success of crops in different agroecological regions depends mainly on their metabolic adaptation.[22] Because the metabolism of nitrogen-containing compounds, i.e. proteins and amino acids, may be strongly affected by environmental conditions,[8] we also analysed the nitrogen and total protein contents and amino acid composition of quinoa seeds from the Encalilla site as well as from the Bolivian Altiplano (Patacamaya site) and the Andean Northwest region of Argentina (Iruya site). Hereinafter the two latter locations are named the Bolivia/Argentina site. The seed nitrogen content based on seed dry mass ranged between 1.46% (minimum) and 2.48% (maximum) among cultivars from the Encalilla and Bolivia/Argentina sites. Although maximum and minimum values practically did not differ between seed types, significant inter- and intracultivar differences were observed (data not shown). The total protein content based on dry mass ranged from 91.5 to 155.3 g kg^{-1} with a mean of 126.9 g kg^{-1} in Encalilla seeds and between 96.2 and 154.6 g kg^{-1} with a mean of 122.6 g kg^{-1} in seeds from the Bolivia/Argentina site. Similar to nitrogen, individual protein contents showed significant inter- and intracultivar variations at both sites. Highest and lowest protein contents respectively were found in Ratuqui and Sajama seeds from the Encalilla site, while for Bolivia/Argentina seeds they corresponded to CICA and Robura cultivars (Table 4). Seeds of Ratuqui, Chucapaca and Sayaña cultivars from the Encalilla site showed protein increases of 49.6, 22.9 and 21.9% compared with seeds from the Bolivia/Argentina site. By contrast, in Sajama, Samaranti and CICA seeds the protein content was decreased by 31.2, 31.3 and 14.9% respectively. Amilda, Kamiri, Kancolla and Robura seeds did not show significant changes between sites. Furthermore, Sajama and Samaranti cultivars growing at the Encalilla site had much lower seed yields than other cultivars (Table 3). According to the soil data given in Table 2, the soil nitrogen content was slightly higher in the Patacamaya region than in the Encalilla region. The natural water supply (rainfall percentage) during the growing season was also higher in Patacamaya than in Encalilla; however, the higher soil evapotranspiration observed in the former determines that both sites exhibit a similar strong drought condition requiring an additional water supply. Despite the fact that both sites can be characterised as very arid environments, some climatic parameters differ between them, so it can be assumed that they correspond to different agroecological regions. Therefore the data reported here show clearly that significant changes in protein content take place in quinoa seeds from different agroecological regions. Interestingly, almost all seeds from the Encalilla site had higher protein content than cereals cultivated worldwide,[3] but in seeds of Sajama and Samaranti cultivars it was significantly lower (Table 3). Although we cannot explain this trend, it possibly occurs through unknown environmental and/or genotype × environment interactions.

Table 3. Two-year mean values of root biomass, aerial biomass, seed biomass, plant height and grain yield of ten quinoa cultivars grown under drought conditions at Encalilla site. For comparison, literature data on grain yield obtained in the Patacamaya region are also shown

Cultivar	Root (%)	Aerial biomass (%)	Seed (%)	Plant height (cm)	Encalilla grain yield (kg ha^{-1})	Bol/Arg grain yield (kg ha^{-1})[a]
Amilda	8.9a	41.1a	25.4b	84.3b	2110c	2140[32]
Chucapaca	6.7b	34.0b	33.0a	102.9a	2755b	2500[33]
CICA	9.1a	46.9a	18.7c	92.0ab	2344bc	2050[26]
Kamiri	5.9b	27.2c	31.8a	104.2a	2192c	2500[33]
Kancolla	4.8c	41.3a	24.5b	83.8b	2846b	2200[33]
Ratuqui	7.0b	29.4c	35.1a	98.6ab	2110c	1800[33]
Robura	8.0a	32.4b	19.5c	89.7ab	1441d	2150[32]
Sajama	7.2b	31.1bc	11.1d	71.0c	1069e	2100[33]
Samaranti	7.3ab	39.5a	6.7e	61.4c	376f	∼1900[26]
Sayaña	8.0a	33.2b	34.1a	115.6a	3855a	1950[33]

Values followed by the same letter within a column are not significantly different at $P < 0.05$ ($n = 10$ per year).
[a] Corresponds to average value.

Feeding of both humans and animals requires certain quantities of essential amino acids (high-quality protein) from biologically available sources as part of a larger protein/nitrogen intake. In contrast to animal-derived proteins, most plant proteins contain very low levels of essential amino acids, being particularly scarce in tryptophan, lysine and methionine.[2] Thus it is important to determine the relative efficiency (protein quality) with which plant proteins meet these requirements. The quality of plant proteins can be evaluated by different methods and expressed in terms of various parameters such as protein efficiency ratio (PER), net protein utilisation (NPU), biological value (BV), protein digestibility (PD) and quality index (QI), the last two being extensively used to assess the quality of cereal proteins.[23] The QI, defined as the tryptophan/total protein ratio, of quinoa seeds from the two sites was significantly higher than that reported for common cereals.[23] Seeds from the Bolivia/Argentina site generally had higher QI than seeds from the Encalilla site. QI values ranged from 4.74 to 10.12% in Bolivia/Argentina seeds and from 4.04 to 9.87% in Encalilla seeds (Table 4). Significantly higher QI values of Bolivia/Argentina seeds were observed in Sayaña (+137.6%), Chucapaca (+37.9%), Kamiri (+31.9%), Sajama (+29.1%), Amilda (+26.3%) and CICA (+21.9%) cultivars. Samaranti was the only cultivar from the Encalilla site that had a higher QI value (+17.1%). The changes in QI observed could be in agreement with previous studies showing that in field-grown maize the content of tryptophan in a determined protein is more stable than the protein content itself,[24] and also that the quality and quantity of seed proteins are dependent on the soil fertility (nitrogen availability) and drought condition.[25] Moreover, it was reported that the protein composition of field-grown wheat depends primarily on the

Table 4. Two-year mean values of total protein content and protein quality index of quinoa seeds from both growing sites

	Total protein (g kg^{-1} dry mass)		Protein quality index (%)	
Cultivar	Encalilla	Bolivia/ Argentina	Encalilla	Bolivia/ Argentina
Amilda	125.0a	114.1a	5.20b	6.57a
Chucapaca	143.4a	116.7b	4.04b	5.57a
CICA	134.6b	154.6a	5.94b	7.24a
Camiri	131.2a	139.8a	5.64b	7.44a
Kancolla	151.7a	144.4a	4.28a	4.78a
Ratuqui	155.3a	103.8b	6.05a	6.94a
Robura	104.3a	96.2a	9.87a	9.36a
Sajama	91.5b	120.0a	6.78b	8.75a
Samaranti	93.4b	122.6a	8.78a	7.50b
Sayaña	138.5a	113.6b	4.26b	10.12a
Mean	126.9a	122.6a	6.08b	7.43a

Values followed by the same letter for each pair of data within a row are not significantly different at $P < 0.05$ ($n = 3$ per year).

genotype but is also affected by environmental factors and their interactions.[8] Therefore the differences in QI between Encalilla and Bolivia/Argentina seeds may be due to a complex interaction of environmental factors and soil fertility rather than heritable genetic characters.

In spite of the importance of QI, amino acid composition is a more important trait for the assessment of protein quality.[25] Quinoa seeds from both sites contained large amounts of glutamic acid, arginine and aspartic acid along with lesser amounts of glycine, leucine and lysine, constituting about 60% of the total free amino acids (Table 5). The profile of amino acids showed significant differences among cultivars and growth sites. Seeds harvested in Encalilla from Amilda, CICA, Kamiri, Sajama, Samaranti and Sayaña cultivars showed significantly lower contents of total amino acids than corresponding Bolivia/Argentina seeds, the highest decrease being observed in Sajama seeds (−95.9%). By contrast, Ratuqui and Robura seeds from the Encalilla site had significantly higher amino acid contents (+61.5 and +20.7% respectively) than corresponding seeds from the Bolivia/Argentina site. Chucapaca and Kancolla seeds did not show significant differences in amino acid content between sites. Essential amino acids, i.e. methionine, leucine, lysine, phenylalanine, tyrosine, isoleucine, threonine, tryptophan and valine, were present in both seed types, but their distribution patterns were different among cultivars. Total essential amino acids ranged from 179.9 (Sajama) to 357.2 (Ratuqui) g kg^{-1} protein in Encalilla seeds and between 233.7 (Ratuqui) and 374.5 (CICA) g kg^{-1} protein in Bolivia/Argentina seeds. In general, essential amino acid contents were higher in Bolivia/Argentina seeds. Only seeds of Ratuqui and Robura cultivars from the Encalilla site showed higher contents of essential amino acids than corresponding Bolivia/Argentina seeds (Table 6). The parenteral lines of all cultivars tested in this study are from the Andean highlands.[26] Considering that quinoa breeding programmes were conducted mainly on an Andean local basis and exploiting local adaptations, it could be expected that cultivars growing in the Andean highlands would exhibit better gene expression related to essential amino acid synthesis. Therefore it could be assumed that the content of essential amino acids in seeds from the Bolivia/Argentina site must be higher than that in seeds from the Encalilla site. Seeds from both sites contained lysine, threonine and methionine in relatively adequate amounts to give nutrient equilibrium for both human and animal feeding[1] (Table 6). Concentrations of aromatic essential amino acids (phenylalanine and tyrosine) and similarly isoleucine, threonine and valine were also sufficient according to the FAO/WHO[4] suggested requirements for 10–12-year-old children. In comparison, lysine, tyrosine and tryptophan seem to be limiting amino acids for 2–5-year-old children. Moreover, the essential amino acids present in quinoa seeds have high chemical scores,[27] which determine that these seeds contain protein with a high BV (data not shown).

In order to analyse differences in the content and profile of essential amino acids in seeds from the two sites, linear regression analysis was carried out. Correlation coefficients between protein and essential amino acid contents computed for quinoa cultivars are given in Table 7. Correlation coefficients (r) between the concentration of each essential amino acid, based on seed dry weight, and % protein ranged from 0.78 to 0.92 for Bolivia/Argentina seeds and from 0.60 to 0.80 for Encalilla seeds, with tryptophan giving the lowest values (0.78 and 0.60). Excepting tryptophan, the r values obtained indicate that linear increases in essential amino acids correlate strongly and positively with increases in protein content for both seed types. The lower r values found for tryptophan may be related to the lack of reliable quantification methods.[28] Correlation coefficients (r') between the concentration of each essential amino acid, expressed as g kg^{-1} protein, and % protein ranged from 0.41 to 0.61 and from −0.16 to 0.57 for Bolivia/Argentina and Encalilla seeds respectively. Excepting methionine, lysine and threonine, the r' values obtained vary significantly between the two sites (Table 7). The lower r' values of Encalilla seeds could indicate that the content of essential amino acids in the protein does not change markedly when the protein content increases; that is, the essential amino acid composition is independent of the amount of protein in the seed.[29] In contrast, the r' values of Bolivia/Argentina seeds were relatively higher than those of Encalilla seeds, ranging between 0.41 and 0.61. This fact could indicate that quinoa cultivars are genotypically better adapted to the Andean highland environment. According to Triboï *et al.*,[8] if different genotypes of a crop produce the same protein in different amounts, the main factor responsible for this trend will be the content of an essential amino acid composing the protein. However, seeds from the Encalilla site do not agree with this assumption, because the content of each essential amino varies differently in relation to the protein content. A similar lack of correlation between essential amino acid concentration and protein content has been reported previously for maize and wheat varieties.[30] Therefore we considered that the use of regression equations to estimate genotypic traits between different quinoa cultivars must be tempered, because they do not discriminate between different components of the seed protein pool. This is composed of several fractions, i.e. albumin-1 (Alb-1), albumin-2 (Alb-2), globulin (Glo) and glutelin (Glut), which constitute active proteins located within cells. Moreover, each protein fraction has a unique amino acid composition, so changes in the balance between fractions differing in their amino acid composition may result in significant changes in determined essential amino acids in the whole seed protein.[31] A new study using isolated protein fractions is being planned and will be reported elsewhere. However, despite the differences in seed yield, protein and amino acid composition between Encalilla and

Table 5. Two-year mean values of amino acid (AA) composition of quinoa seeds from Encalilla and Bolivia/Argentina

AA (g kg^{-1} protein)	Amilda	Chucapaca	CICA	Kamiri	Kancolla	Ratuqui	Robura	Sajama	Samaranti	Sayaña
Encalilla										
Aspartic acid	78.3	67.4	71.4	79.9	72.8	93.6	102.8	51.5	61.0	69.1
Threonine	30.1	25.5	28.9	31.1	30.2	43.1	38.6	20.9	23.8	25.8
Serine	41.6	36.0	38.3	42.1	38.4	59.4	53.1	27.2	31.4	25.8
Glutamic acid	122.1	106.0	119.4	123.7	117.1	179.7	150.6	73.7	90.6	110.3
Glycine	50.8	43.1	44.7	49.9	47.7	71.0	64.3	33.6	40.4	41.7
Alanine	33.4	29.4	33.3	34.0	32.1	49.0	47.0	25.8	32.6	29.1
Valine	29.6	21.9	27.2	29.9	31.9	39.1	37.4	23.3	27.7	24.6
Methionine	13.1	11.0	11.6	12.4	11.8	17.9	15.7	7.3	9.1	10.8
Isoleucine	22.9	16.5	21.9	24.0	25.9	31.0	28.5	18.9	22.1	19.9
Leucine	52.3	43.6	49.3	54.7	52.1	74.6	67.1	37.5	43.0	44.7
Tyrosine	24.9	21.0	22.3	25.1	24.5	34.6	33.3	18.8	21.8	21.1
Phenylalanine	32.4	26.2	29.2	33.1	30.5	45.2	41.8	22.6	26.1	27.2
Lysine	43.0	36.2	39.4	43.3	44.4	62.3	52.2	24.4	29.8	37.3
Histidine	24.7	20.9	23.8	24.7	24.3	36.3	29.2	13.6	17.1	21.5
Arginine	78.2	66.4	68.5	75.4	70.1	98.4	84.2	36.8	45.9	66.5
Proline	31.2	26.0	29.0	35.7	31.4	43.5	40.8	22.1	25.7	26.1
Tryptophan	6.5	5.8	8.0	7.4	6.5	9.4	10.3	6.2	8.2	5.9
Total	715.1a	602.9a	666.2a	726.4a	691.7a	988.1b	896.9b	464.2a	556.3a	616.9a
Bolivia/Argentina										
Aspartic acid	112.7	78.1	105.6	106.1	87.1	43.1	85.8	103.1	99.5	84.9
Threonine	44.3	32.1	45.9	41.5	34.9	28.7	35.4	42.1	41.2	33.8
Serine	59.0	41.7	58.7	54.3	46.0	34.8	44.7	53.7	51.1	42.6
Glutamic acid	165.5	107.9	165.9	161.5	130.8	96.0	114.1	149.7	148.9	126.1
Glycine	66.3	48.6	59.8	61.8	54.3	42.0	53.0	62.5	60.4	49.4
Alanine	50.9	36.3	49.9	44.6	39.0	31.7	39.5	45.5	43.1	36.5
Valine	41.1	28.7	43.9	40.1	32.3	27.3	31.1	38.7	40.4	30.7
Methionine	18.6	13.4	18.7	16.6	15.9	14.8	15.0	16.7	16.1	14.1
Isoleucine	29.5	19.8	34.0	31.0	23.9	21.2	23.2	31.6	31.6	23.7
Leucine	73.7	50.9	72.0	69.1	57.0	47.3	58.2	65.1	67.9	55.3
Tyrosine	34.0	23.2	36.1	32.1	27.0	21.2	25.8	29.8	31.0	24.1
Phenylalanine	43.4	30.0	45.5	40.7	34.2	27.2	33.5	39.6	38.2	32.8
Lysine	64.1	46.5	67.2	59.9	49.9	38.8	50.9	60.1	59.8	48.4
Histidine	34.8	23.6	37.9	33.1	26.8	19.7	24.4	31.5	32.7	26.0
Arginine	108.8	65.4	99.0	102.8	82.3	59.6	66.1	90.1	97.6	78.0
Proline	40.1	28.3	44.1	38.9	31.8	26.6	33.1	39.3	38.0	31.3
Tryptophan	7.5	6.5	11.2	10.4	6.9	7.2	9.0	10.5	9.2	8.3
Total	994.3b	680.1a	995.4b	944.5b	780.1a	611.9a	742.8a	909.6b	906.7b	746.0b

Total amino acid values followed by the same letter within a column, i.e. for each cultivar at both sites, are not significantly different at $P < 0.05$ ($n = 3$ per year). For clarity, significant differences in individual amino acids between sites are not indicated.

Bolivia/Argentina seeds, our data showed that different cultivars of quinoa could constitute an alternative crop for people living in arid mountain regions.

CONCLUSION

The results presented in this paper shed light on the influence of both environmental and climatic variations on the grain yield and seed protein quality of ten quinoa cultivars. Clear variations in investigated traits between two sites were observed. The essential amino acid profile was found to be more affected than the grain yield and total protein content. A positive correlation exists between the essential amino acid content and % protein. We demonstrated that environmental effects influenced the observed variations for most traits, allowing selection based on environmental changes. In fact, we considered that in quinoa cultivars growing in different agroecological sites, apart from genetic variability, effects of environmental factors on the relative performance of plants have a decisive potential to influence the success of a determined cultivar in a particular agroecological region. Therefore quinoa cultivars should be carefully investigated in relation to different environments before being considered for release as a valuable food for widespread human consumption. However, despite the differences in protein and amino acid composition among cultivars from both sites, we believe that quinoa seeds contain an adequate amount of high-quality protein that could be used as a nutritional substitute for scarce animal protein by people in arid mountain regions of developing countries.

Table 6. Two-year mean values of essential amino acid (EAA) composition of quinoa seeds from Encalilla and Bolivia/Argentina

EAA (g kg^{-1} protein)	Amilda	Chucapaca	CICA	Kamiri	Kancolla	Ratuqui	Robura	Sajama	Samaranti	Sayaña
Encalilla										
Leucine	52.3	43.6	49.3	54.7	52.1	74.6	67.1	37.5	43.0	44.7
Lysine	43.0	36.2	39.4	43.3	44.4	62.3	52.2	24.4	29.8	37.3
Methionine	13.1	11.0	11.6	12.4	11.8	17.9	15.7	7.3	9.1	10.8
Phenylalanine	32.4	26.2	29.2	33.1	30.5	45.2	41.8	22.6	26.1	27.2
Threonine	30.1	25.5	28.9	31.1	30.2	43.1	38.6	20.9	23.8	25.8
Isoleucine	22.9	16.6	21.9	24.0	25.9	31.0	28.5	18.9	22.1	19.9
Tyrosine	24.9	21.0	22.3	25.1	24.5	34.6	33.3	18.8	21.8	21.1
Valine	29.6	21.9	27.2	29.9	31.9	39.1	37.4	23.3	27.7	24.6
Tryptophan	6.5	5.8	8.0	7.4	6.5	9.4	10.3	6.2	8.2	5.9
Cysteine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total	254.8a	207.8a	237.8a	261.0a	257.8a	357.2b	324.9b	179.9a	211.6a	217.3a
Bolivia/Argentina										
Leucine	73.7	50.9	72.0	69.1	57.0	47.3	58.2	65.1	67.9	55.3
Lysine	64.1	46.5	67.2	59.9	49.9	38.8	50.9	60.1	59.8	48.4
Methionine	18.6	13.4	18.7	16.6	15.9	14.8	15.0	16.7	16.1	14.1
Phenylalanine	43.4	30.0	45.5	40.7	34.2	27.2	33.5	39.6	38.2	32.8
Threonine	44.3	32.1	45.9	41.5	34.9	28.7	35.4	42.1	41.2	33.8
Isoleucine	29.5	19.8	34.0	31.0	23.9	21.2	23.2	31.6	31.6	23.7
Tyrosine	34.0	23.2	36.1	32.1	27.0	21.2	25.8	29.8	31.0	24.1
Valine	41.1	28.7	43.9	40.1	32.3	27.3	31.1	38.7	40.4	30.7
Tryptophan	7.5	6.5	11.2	10.4	6.9	7.2	9.0	10.5	9.2	8.3
Cysteine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total	356.2b	251.1b	374.5b	341.4b	282.0a	233.7a	282.1a	334.2b	335.4b	271.2b

Total essential amino acid values followed by the same letter within a column, i.e. for each cultivar at both sites, are not significantly different at $P < 0.05$ ($n = 3$ per year). For clarity, significant differences in individual amino acids between sites are not indicated. ND, not detected.

Table 7. Two-year means of correlation coefficients between mg essential amino acid g^{-1} dry mass *vs* % protein (r) and between g essential amino acid kg^{-1} protein *vs* % protein (r') in seeds of ten cultivars from Encalilla and Bolivia/Argentina sites

Essential amino acid	Encalilla		Bolivia/Argentina	
	r	r'	r	r'
Leucine	0.77a	0.37A	0.87a	0.54B
Lysine	0.76a	0.57A	0.87a	0.54A
Methionine	0.80a	0.49A	0.92a	0.52A
Phenylalanine	0.75a	0.32A	0.88a	0.58B
Threonine	0.79a	0.42A	0.88a	0.52A
Isoleucine	0.75a	0.22A	0.85a	0.56B
Tyrosine	0.74a	0.25A	0.89a	0.61B
Valine	0.74a	0.20A	0.87a	0.58B
Tryptophan	0.60a	−0.16A	0.78b	0.41B

Values followed by the same letter within a row are not significantly different at $P < 0.05$ ($n = 3$ per year). Lowercase letters are used to denote significance between r values. Uppercase letters are used to denote significance between r' values.

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