RESEARCH ARTICLE

Ecogeographic structure of phenotypic diversity in cultivated populations of quinoa from Northwest Argentina

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Abstract

A set of 34 quinoa populations from the Northwest Argentina region was characterised using quantitative and qualitative phenotypic traits in an experiment conducted in the province of Jujuy, Argentina. A selection of quinoa descriptors from the Bioversity International (former IBPGR) list was applied, and data were analyzed using descriptive and multivariate techniques. Morphological and phenological traits variation was observed among accessions collected in contrasted ecogeographic zones of this Andean region. On the basis of quantitative traits, both the principal component analysis and the Cluster Analysis differentiated between accessions from the highlands, transition zone, central dry valleys and eastern valleys. On the other hand, the principal coordinates analysis based on qualitative traits only discriminated accessions from transition zone and eastern valleys. The correlation between both characterisations was fairly low suggesting that individual characterisations offer information that can be complementary. The accessions from the highlands and dry valleys presented the more advanced domesticated traits, while accessions from transition zone and eastern valleys showed traits more similar to wild-type related Chenopods from the Andean region. These differences are discussed on the basis of previous hypotheses about the domestication and crop diffusion processes from the southern Andes suggested for this species.

Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an Andean grain crop of exceptionally high nutritive quality adapted to grow in the harsh conditions that characterise much of the Andean highlands (Altiplano) (Wilson, 1988; Maughan *et al.*, 2007). Christensen *et al.* (2007) estimated that there are ≈ 5000 diverse accessions and commercial cultivars of quinoa maintained in collections worldwide, although an undefined number of accessions exist in Colombia, Ecuador, Chile and Argentina. There are few published efforts to characterise this genetic diversity (Jacobsen & Mujica, 2002). An essential aspect for the breeding and use of those quinoa germplasm collections is their characterisation.

Early biosystematic studies, which included the full range of species distribution and were based on morphometric leaf and fruit traits as well as allozyme variation, showed that cultivated populations of quinoa presented a bimodal pattern of morphometric and allozyme differentiation: Andean (quinua) and coastal (quingua) populations (Wilson, 1981, 1988, 1990). In the more variable Andean group, subtle distinctions were detected between northern and southern populations. According to these early studies, populations from the highlands of Peru, Bolivia, Northwest Argentina (NWA) and Chile exhibited a relatively high level of variation but lacked a consistent structure as far as origin or elevation are concerned. Moreover, these types showed

a tenuous phenetic linkage to both northern Andean and coastal populations. On the basis of this, Wilson (1988) hypothesised that the southern highlands of the Peruvian/Bolivian region are quinoa's centre of diversity and putative centre of origin. Recent studies performed by Rojas (2003) and del Castillo *et al.* (2007) using sampling strategies more detailed than those of the initial works of Wilson or Ruas *et al.* (1999) revealed distinct ecogeographic patterns within highlands populations but did not question Wilson's early hypothesis regarding quinoa's centre of origin.

Northwest Argentina represents the southern end of what is known as the *C. quinoa* Andean complex, and quinoa is considered marginal in terms of its cultivation in this region (Bertero, 2001). Native quinoa crops are found in three ecoregions, from the western arid highlands of the Puna to the eastern slopes of the Cordillera Oriental, passing through the dry valleys of the Quebrada de Humahuaca and the Valles Calchaquíes (Curti *et al.*, 2010). No descriptive studies of phenotypic or genetic diversity of NWA quinoa accessions have been carried out before, and only a few accessions were included in previous works (e.g. Wilson, 1981, 1988; Rojas, 2003; Christensen *et al.*, 2007) not being representative of the variability observed in the region (Costa Tártara *et al.*, 2008; Curti *et al.*, 2010). Because previous quinoa characterisations found a strong association between environmental variation and morphological characteristics of the cultivated populations of the species (Risi & Galwey, 1989a,1989b; Ortiz *et al.*, 1998; Rojas, 2003), environmental heterogeneity of quinoa collection sites in NWA leads us to hypothesise that phenotypic differences would be found among materials of different geographic origins. In addition, expanding Wilson's hypothesis (1988) that the centre of origin and diversity of *C. quinoa* is located in the central Andean region of Peru and Bolivia, it is our hypothesis that diffusion of the crop into the NWA region has been mediated by founding events associated with early dispersal from that centre of origin. The objectives of this study were to: (a) evaluate the phenotypic variation in quantitative and qualitative traits among a set of cultivated quinoa populations collected in the NWA region; (b) compare the patterns of phenotypic variation obtained according to different multivariate techniques and (c) compare the phenotypic grouping arising from these characterisations with the results of previous studies on quinoa ecogeography.

Materials and methods

Study area

Northwest Argentina is located between 22 and 28°S and between 62 and 69°W, including the provinces of Jujuy, Salta, Tucumán, Santiago del Estero, Catamarca and La Rioja (Bianchi *et al.*, 2005). Within this region, quinoa is grown in the provinces of Salta and Jujuy (Fig. 1) (Bertero, 2001) and rarely in Catamarca (Babot, pers. comm.). In NWA, the complex Andean topography is a major factor affecting climate and rainfall spatial distribution and defining contrasting environments and landscapes (Bianchi *et al.*, 2005). The Puna, located north of latitude 27°S, is the most arid sub-region within the quinoa distribution area in Argentina. It is a highland with large mountain ranges that separate relatively flat lands (between 3400 and 4500 m.a.s.l) with a large latitudinal gradient in annual average rainfall and mean temperature, from 37 mm and 3.9°C to the SW up to 322 mm and 9.4°C to the NE (Bianchi *et al.*, 2005). The Puna is bounded by the mountain ranges of Santa Victoria and Zenta to the east, a continuation to the south of the Eastern Bolivian Cordillera (Fig. 1). It presents a wide altitudinal range in the north–south direction, from 4700 to 3000 m.a.s.l, and annual average rainfall and mean temperature from 300 to 400 mm and 6°C to 8°C, respectively (Bianchi *et al.*, 2005). Towards the east, in the ecological layer known as *cerro* (high mountains foggy grasslands, between 2500 and 3500 m.a.s.l), the eastern valleys of Santa Victoria Oeste (Salta province) appear with contrasting climatic conditions (annual precipitation and mean temperature are between 350 and 700 mm and 13°C and 17°C, respectively) (Grau & Brown, 2000). Between the Puna and the Zenta mountain range, a narrow dry valley called Quebrada de Humahuaca descends from the Puna between 3500 and 2000 m.a.s.l in the north-south direction (Fig. 1), with an annual average rainfall range and mean temperature of between 200 and 400 mm and 12.3°C and 13°C, respectively (Bianchi *et al.*, 2005). Towards the south and located at the same altitude range, a second dry valley called Valles Calchaquíes (Fig. 1) presents a climate similar to that of the Quebrada de Humahuaca. The geographic range of origin of the accessions covered the distribution of the crop in the NWA region, showing contrasting latitudes (between 22°10′ and 25°14′S), longitudes (between 64°58′ and 67°31′W), altitudes (between 2334 and 4012 m.a.s.l), precipitation and temperature (decreasing in the east–west direction) (Bianchi *et al.*, 2005).

Experimental site

The germplasm characterisation was conducted in Calete, Department of Humahuaca (23°12′S, 65°20′W; 2939 m.a.s.l), province of Jujuy, Argentina, during the 2008–2009 growing season (austral summer). Growing season rainfall, average daily mean, maximum and minimum temperatures were 168 mm, 12°C, 27°C and
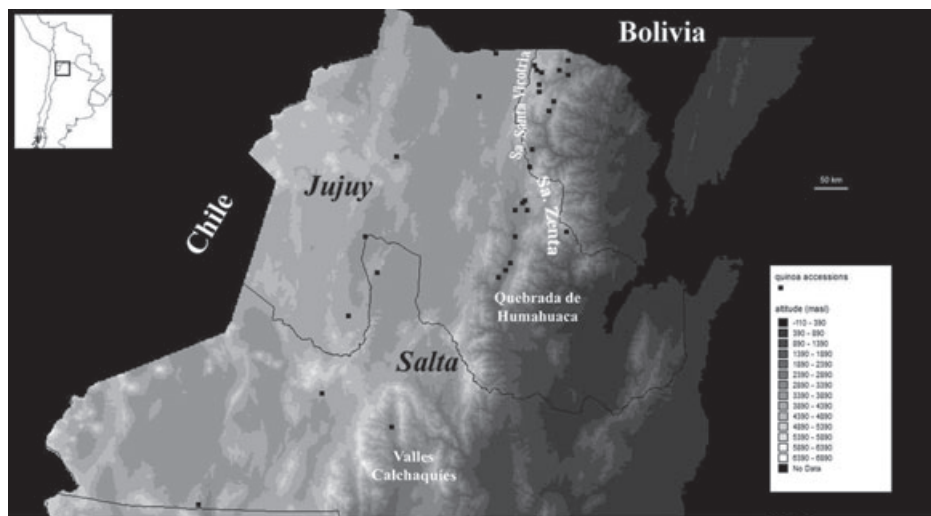
Figure 1 Geographical location of the 34 populations of *C. quinoa* assessed in the present study. Digital Elevation Model of the NWA region with data from the SRTM (The Shuttle Radar Topography Mission; Farr *et al.*, 2007). Neighbouring countries and provinces and mountain ranges of Santa Victoria and Zenta are shown. (Sa. corresponds to the local name Serranía). Inset: the location of Northwest Argentina region in Central Andes of South America.

$3°C$, respectively. Quinoa accessions were sown in two replicate plots per accession in a fully randomised design. Each plot consisted of three 5-m-length rows spaced 0.5 m apart. Sowing density was 14 seeds m^{-1}, equivalent to 280 000 seeds ha^{-1}. The experiment was kept free of weeds and pests and fertilised at a rate of 43 kg N ha^{-1} 35 days after crop emergence. The harvest took place based on accessions earliness; as the accessions from the western highlands showed more earliness, they were harvested in early March (120 days after sowing) followed by accessions from the dry valleys in late April (170 days after sowing), whereas the eastern humid and transition zone accessions were harvested in early May (180 day after sowing). The experiment received deep irrigation before sowing and then every 10 or 15 days following local practices (furrow irrigation) to avoid water deficits during the crop cycle. Because of the experimental design, all accessions were exposed to the same irrigation regime.

Descriptors

We used the species descriptors list (IBPRG 1981, currently Bioversity International) to measure (quantitative) and score (qualitative) phenotypic descriptors in 10 plants (5 in each replicate plot) randomly chosen from the middle plot row (Tables 1 and 2). Development stages (recorded when at least 50% of the plants in the plots had reached the stage) were determined as emergence, visible floral bud (VFB), first anthesis (at least one flower opened) and physiological maturity (visually determined

Table 1 Qualitative descriptors (adapted from IBPRG 1981) used for the characterisation of the *C. quinoa* germplasm

Qualitative Descriptors
Axil pigmented (0, absent; 1, present)
Striation pigmented (0, absent; 1, present)
Striation colour (1, yellow; 2, red; 3, other)
Main stem colour (1, yellow; 2, green; 3, grey; 4, red; 5, purple)
Stem colour intensity (3, low; 5, medium; 7, high)
Branching (0, absent; 1, present)
Leaf margin (0, untoothed; 2, toothed)
Panicle colour at maturity (1, white; 2, red; 3, purple; 4, yellow; 5, orange; 6, brown; 7, grey; 8, black; 9, red and green)
Panicle colour intensity at maturity (3, low; 5, medium; 7, high)
Panicle colour at harvest (1, white; 2, red; 3, purple; 4, yellow; 5, orange; 6, brown; 7, grey; 8, black; 9, red and green)
Panicle colour intensity at harvest (3, low; 5, medium; 7, high)
Type of panicle (1, terminal; 2, non-differentiated)
Shape of panicle (1, glomerulate; 2, amaranthiform)
Compactness of panicle (3, lax; 5, medium; 7, compact)
Cotyledon pigmented (0, absent; 1, present)
Intensity cotyledon pigmented (3, low; 5, medium; 7, high)
Intensity hypocotyl pigmented (3, low; 5, medium; 7, high)

in the middle third of the inflorescence) (Bertero & Ruiz, 2008). Branch number was determined on the main stem between the basal and the last node below the panicle. The shape of the top fully expanded leaf on the main stem was defined as the length-to-width ratio. Most of the phenotypic traits were taken at the anthesis stage, save for seedling traits and physiological maturity.

The inflorescence of quinoa is a panicle with a principal axis, from which secondary or tertiary axes originate.

Table 2 Descriptive statistics of central trend and dispersion for each quantitative trait ($n = 34$)

Quantitative Trait	Mean	Range	SD	CV
Number of branches on the main stem	8.7	0–24	8.1	93.7
Teeth number	8	3.8–16.4	3.6	45.9
Shape of the top leaf	1.5	1–1.9	0.2	13.6
Stem diameter (mm)	6.2	2.8–9.2	1.7	27.8
Petiole length (mm)	40.1	16.4–59.2	12.1	30.1
Leaf length (mm)	65.8	33.3–94.4	16	24.2
Leaf width (mm)	54.2	26.3–75.8	13.7	25.2
Glomeruli length (mm)	9.2	5.1–11.8	2	22.1
Length of cotyledon (mm)	15.9	9.3–19.7	2	15
Length of hypocotyl (mm)	18.9	13.9–22.1	2	10.8
Grain diameter (mm)	1.8	1.4–2.5	0.2	13.8
Panicle length (cm)	32	5–61.4	13.2	41.2
Plant height (cm)	114.6	23.2–181	50.7	44.2
Days from sowing to emergence	7.8	5–10	1.8	23.4
Days from sowing to visible floral bud	41.8	18–71	15.5	37
Days from sowing to first anthesis	65.5	33–95	16.7	25.6
Days from sowing to physiological maturity	158.2	88–217	26.1	16.5

CV, coefficient of variation, expressed in percentage (%); SD, standard deviation.

Two inflorescence types have been described for quinoa: amaranthiform and glomerulate. In the amaranthiform type, the glomeruli (short branches bearing a group of flowers or grains) are inserted directly in second-order axes while, in the glomerulate type, the glomeruli are inserted in third order axes. In the amaranthiform type, glomeruli length was measured in the middle third of the panicle, whereas in the glomerulate type, it was measured in the lower third (Bertero *et al.*, 1996).

Statistical analyses

Descriptive and multivariate analyses were carried out to characterise the phenotypic diversity of quinoa accessions collected in the NWA region. The mean, range, standard deviation and coefficient of variation (CV) were used to estimate and describe the position of the accessions in relation to each quantitative descriptor. Pearson's correlation coefficients were used to calculate the magnitude and type of association between each pair of descriptors. Both statistical analyses were conducted using the statistical program Infostat (2009).

A set of multivariate analyses [principal component analysis (PCA), principal coordinates analysis (PCoA) and Cluster Analysis (CA)] was used to simultaneously examine several variables for each accession and to describe genotypic variation patterns in the germplasm characterised (Franco & Hidalgo, 2003). Principal component analysis was performed with Euclidean standardised quantitative variables and depicted in a two-dimensional scatter plot. Eigenvalues >1 were considered as they describe significant variation in a data set (Cuadras, 2010). For classification (CA), the hierarchical agglomerative method with the incremental sum of squares as fusion criteria (Ward, 1963) was chosen using standardised Euclidean distance. Statistical analyses were conducted using the statistical program PCORD 4 (McCune & Mefford, 1999).

A simple matching coefficient of similarity was used for qualitative descriptors and the relationships among the 34 accessions were investigated via PCoA and depicted in a two-dimensional scatter plot (Bramardi *et al.*, 2005). A minimum spanning tree (MST) from the corresponding distance-similarity matrix was added, contributing to the interpretation of similarity between accessions. The NTSYS (Numerical Taxonomic System version 2.11) statistical software was used for this analysis (Rohlf, 2002). A Mantel test (Mantel, 1967) was employed to establish the relationships between the quantitative and qualitative distance matrices.

Results

Quantitative descriptors

Descriptive statistics for each quantitative descriptor are summarised in Table 2. A large dispersion in relation to mean and CV was observed for main stem branch and leaf teeth numbers, petiole and panicle lengths, plant height and time to VFB. Of the 136 correlation coefficients evaluated, 30 showed highly significant associations ($P < 0.01$) with an $r \geq 0.4$ and 21 exhibited significant associations ($P < 0.05$) with a $0.35 < r < 0.49$ (Table 3).

The PCA results show that the first five components concentrate 79.3% of total variation. The first component (PC1) explained most of this variation (39.9%) and ordered accessions according to a gradient of plant size and time to first anthesis. As indicated by Fig. 2, accessions with taller plants, thicker stem diameter, longer and wider leaves, longer panicles and glomeruli and longer time to first anthesis were placed to the left of the graph, as these accessions presented larger negative scores for eigenvector coefficients of these traits. Most of these accessions are from the eastern (CHEN 212, 451, 452, 456, 458, 461 and 463) and dry valleys (CHEN 58, 182, 185, 214, 215, 216, 231, 232, 252, 275 from the Quebrada de Humahuaca and CHEN 414 from the Valles Calchaquíes); accessions CHEN 183 and 435 come from an area that is usually regarded as part of the highlands because of its altitude (Table 4) but is within the most humid and

Table 3 Pearson's coefficient of correlation among quantitative descriptors of quinoa accessions

	SD	NB	STL	TN	PLE	LLE	LW	PALE	GLE	LEC	LEH	SEM	SVFB	SFLO	SPM	HEI	GD
SD	1																
NB	0.2	1															
STL	0.3	0.2	1														
TN	−0.02	0.2	−0.4[a]	1													
PLE	0.8[b]	0.2	0.3	0.07	1												
LLE	0.7[b]	0.4[a]	0.1	0.3	0.7[b]	1											
LW	0.7[b]	0.3	−0.1	0.5[b]	0.7[b]	0.9[b]	1										
PALE	0.7[b]	−0.08	0.2	−0.1	0.6[b]	0.5[b]	0.5[b]	1									
GLE	0.5[a]	0.3	0.05	0.01	0.4[a]	0.5[a]	0.5[b]	0.6[b]	1								
LEC	0.3	0.1	0.07	0.2	0.3	0.4[a]	0.5[b]	0.3	0.2	1							
LEH	0.3	−0.3	0.4[a]	−0.4[a]	0.2	0	−0.1	0.07	−0.1	0.03	1						
SEM	−0.3	0.01	−0.07	0.06	−0.2	−0.2	−0.1	−0.2	−0.06	−0.01	−0.3	1					
SVFB	0.4[a]	0.4[a]	0.2	0.4[a]	0.3	0.3	0.5[b]	0.2	0.4[a]	0.2	−0.3	−0.1	1				
SFLO	0.5[b]	0.4[a]	0.3	0.3	0.4[a]	0.4[a]	0.6[b]	0.3	0.5[b]	0.08	−0.2	−0.1	0.9[b]	1			
SPM	0.6[b]	0.3	0.2	0.2	0.5[a]	0.3	0.5[a]	0.3	0.4[a]	0.04	−0.01	−0.08	0.7[b]	0.7[b]	1		
HEI	0.9[b]	0.3	0.2	0.2	0.8[b]	0.7[b]	0.8[b]	0.6[b]	0.5[b]	0.3	0.06	−0.3	0.7[b]	0.7[b]	0.7[b]	1	
GD	0.03	−0.2	−0.3	0.02	−0.06	0.2	0.06	0.2	0.1	0.05	0.05	−0.2	−0.4[a]	−0.4[a]	−0.5[b]	−0.1	1

GD, grain diameter; GLE, glomeruli length; HEI, plant height; LLE, leaf length; LW, leaf width; LEC, length of cotyledons; LEH, length of hypocotyls; NB, number of branch on the main stem; PALE, panicle length; PLE, petiole length; SD, stem diameter; SEM, days from sowing to emergence; SFLO, days from sowing to flowering; SPM, days from sowing to physiological maturity; STL, shape of the top leaf; SVFB, days from sowing to visible floral bud.

[a] Significant at 0.05 level.
[b] Significant at 0.01 level.

warmer part of this region. Accessions at the right side of axis 1 presented higher positive scores and showed shorter cycle, shorter plants, thinner stems, narrower leaves, shorter panicles and glomeruli. They originated from the western highlands (CHEN 420, 426, 427, 431, 432 and 438). Accessions CHEN 60, 256, 261, 465, 466, 468, 481 and 482 are located in an intermediate place on axis 1 and are characterised by morphological traits similar to those from the highlands and by phenological traits similar to those from the dry valleys (Table 5). They all come from the transition zone and cover a wide range of altitudes of origin, from 3000 to 3960 m.a.s.l.

PC2 explained 14.4% of variation and distinguished between accessions from dry and eastern valleys according to contrasting eigenvector coefficients for morphology and phenology. The eigenvector coefficients for teeth and branch number and for all phenological traits (time to VFB, first anthesis and physiological maturity) were negative, whereas the rest of the morphological traits were positive. The accessions from eastern valleys showed higher negative scores for teeth and branch number as well as for all phenological traits and were positioned to the lower left part of the graph on PC2 (Fig. 2). These accessions showed higher teeth and branch number and longer time to VFB, first anthesis and physiological maturity but lower stem diameter, panicle and glomeruli length and grain diameter than the dry valley accessions (Table 5). On the other hand, dry valleys accessions (average 3100 m.a.s.l, Quebrada de Humahuaca and Valles Calchaquíes; Fig. 1 and Table 4) have positive higher morphological and lower phenological scores (lower time to VFB, first anthesis and physiological maturity) than accessions from eastern valleys and were positioned to the top left part of the graph on PC2 (Fig. 2). All other components [PC3 (12.4%), PC4 (6.5%) and PC5 (6.1%)] explained less variation and did not differentiate between accessions according to origin or elevation.

The dendrogram resulting from CA shows two main groups formed at a cut-off of 16.08 Euclidean distance (Fig. 3). This clustering pattern is consistent with accessions distribution on axes 1 and 2 in the PCA (see symbols in Fig. 2). The first cluster was formed by accessions which were clearly differentiated into two subgroups: the transition zone (G1) and western highlands (G2). An exception was found with CHEN 256, which comes from the transition zone but was clustered together with accessions from the western highlands (Fig. 3). It is noteworthy that this accession was positioned closer to accessions from the same origin on PC1 (Fig. 2).

The second cluster also distinguished two subgroups: one formed by accessions from the eastern valleys (G3) and another by a mixture of accessions from different origins (G4) but dominated by dry valley
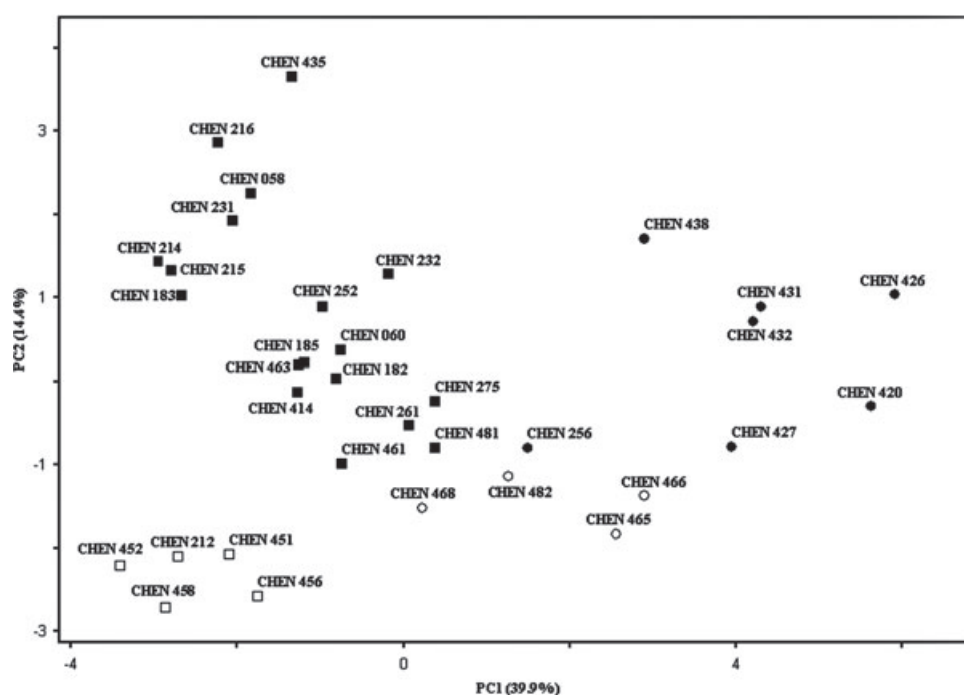
Figure 2 Ordination of quinoa accessions on the first two principal components of the principal component analysis. ●, accessions from the western highlands; ○, accessions from the transition zone; ■, accessions from the dry valleys; and □, accessions from the eastern valleys according to hierarchical agglomerative clustering.

accessions (Fig. 3). A comparison of quantitative data between the four groups revealed that morphological traits distinguished accessions according to altitude (G1 + G2 versus G3 + G4) and longitude (western versus eastern), while phenological traits differentiated between early maturing accessions from the western highlands (G2), and the rest of which are late or intermediate to mature (G2 + G3 + G4) (Table 5). On the other hand, quinoa accessions from the highlands and dry valleys showed more variability for most traits than accessions from the transition zone and eastern valleys (Table 5).

Qualitative descriptors

The five main components of the PCoA on the simple matching similarity matrix explained 31.7%, 17.2%, 10.7%, 7.9% and 6.7% of total variation, respectively. Fig. 4 shows cultivars configuration on the plane defined by the first two coordinates and a MST. One group is formed to the left and two to the right sides of the plane, respectively. Almost all accessions from the western highlands (CHEN 183, 420, 426, 431, 435 and 438) and seven from the dry valleys (CHEN 58, 215, 216, 231, 232, 252 and 275) were positioned to the top right side of the graph in one group (G A) (Fig. 4). These accessions lack pigmented axils and branched

growth habit; present red pigmented striation colour, intermediate green stem colour, dentate leaves; terminal, compact and amaranthiform or glomerulate panicle shape with white or yellow intermediate colour at maturity or harvest, respectively (Table 6).

A second group (G B) of accessions was localised to the left side of the graph and includes almost all accessions from the transition zone and eastern valleys (CHEN 60, 212, 256, 261, 451, 456, 461, 463, 468 and 482) and three accessions from the dry valleys (CHEN 182, 185 and 214). These accessions lack pigmented axils, present yellow pigmented striation colour, low green stem colour, dentate leaves and branched growth habit; panicles were less terminal (or differentiated) and compact than for first group accessions and glomerulate shape prevailed with white or yellow light colour at maturity or harvest, respectively (Table 6). The last group (G C) includes accessions from several origins such as eastern valleys (CHEN 452 and 458), transition zone (CHEN 465, 466 and 481), dry valleys (CHEN 414) and highlands (CHEN 427 and 432) and was positioned to the bottom right side of the graph (Fig. 4). These accessions present pigmented axils, purple pigmented striation colour and intermediate green stem colour, branched growth habit and less dentate leaves. The panicle was even less terminal and compact than for second group accessions and

Table 4 Passport data of the 34 quinoa accessions from Northwest Argentina (NWA)

Accession No.	Origin (Location, Department)	Province	Longitude	Latitude	Altitude (m.a.s.l)
CHEN 58	Coctaca, Humahuaca	Jujuy	65°17′	23°09′	3215
CHEN 060	Abralaite de Colanzuli, Iruya	Salta	65°14′	22°54′	3711
CHEN 182	QQ 95-NSL 106394, Humahuaca	Jujuy	65°20′	23°12′	2939
CHEN 183	QQ 101-NSL 106396, Yavi	Jujuy	65°28′	22°07′	3457
CHEN 185	LP 128-PI 587173, Tilcara	Jujuy	65°22′	23°34′	2461
CHEN 212	San Felipe, Santa Victoria Oeste	Salta	64°58′	22°16′	2507
CHEN 214	Yacoraite, Tilcara	Jujuy	65°20′	23°23′	2700
CHEN 215	Cienaguillas, Tilcara	Jujuy	65°27′	23°40′	2400
CHEN 216	Coctaca, Humahuaca	Jujuy	65°17′	23°09′	3200
CHEN 231	Ocumaso, Humahuaca	Jujuy	65°15′	23°12′	3000
CHEN 232	Pucará, Humahuaca	Jujuy	65°16′	23°08′	3000
CHEN 252	Maimará	Jujuy	65°24′	23°37′	2334
CHEN 256	Campo Luján, Iruya	Salta	65°13′	22°47′	3000
CHEN 261	Río Grande de Colanzuli, Iruya	Salta	65°12′	22°52′	3600
CHEN 275	1485 Coctaca, Humahuaca	Jujuy	65°17′	23°09′	3215
CHEN 414	La Poma	Jujuy	66°11′	24°42′	3016
CHEN 420	Antofallita, Los Andes	Salta	67°31′	25°14′	3498
CHEN 426	Santa Rosa Pastos Grandes, Los Andes	Salta	66°40′	24°28′	3939
CHEN 427	Puesto Sey, Susques	Jujuy	66°29′	23°56′	4012
CHEN 431	Susques	Jujuy	66°22′	23°23′	3619
CHEN 432	Cobres, La Poma	Salta	66°17′	23°38′	3591
CHEN 435	Cangrejillos, Yavi	Jujuy	65°35′	22°25′	3583
CHEN 438	Rachaite, Cochinoca	Jujuy	66°09′	22°50′	3640
CHEN 451	Rodeo Pampa, Santa Victoria Oeste	Salta	65°02′	22°14′	2898
CHEN 452	Rodeo Pampa, Santa Victoria Oeste	Salta	65°02′	22°14′	2898
CHEN 456	Trigo Huaico, Santa Victoria Oeste	Salta	65°02′	22°21′	3230
CHEN 458	Morro de Pucará, Santa Victoria Oeste	Salta	64°58′	22°10′	2645
CHEN 461	Poscaya, Santa Victoria Oeste	Salta	65°04′	22°27′	3208
CHEN 463	Aguadas, Santa Victoria Oeste	Salta	65°06′	22°31′	3073
CHEN 465	Santa Cruz del Aguilar, Santa Victoria Oeste	Salta	65°10′	22°23′	3955
CHEN 466	San José del Aguilar, Santa Victoria Oeste	Salta	65°10′	22°20′	3960
CHEN 468	Santa Ana, Valle Grande	Jujuy	64°59′	23°21′	3379
CHEN 481	Pie de la cuesta de Lizoite, Santa Victoria Oeste	Salta	65°11′	22°14′	3711
CHEN 482	Lizoite, Santa Victoria Oeste	Salta	65°09′	22°15′	3310

glomerulate shape prevailed with purple or yellow dark colour at maturity or harvest, respectively (Table 6).

As results of the individual characterisations produced different configurations, a Mantel test was used to establish the relationships between the distance matrices of quantitative and qualitative traits. This association was weak ($r = 0.22$) but highly significant at a 0.01 level. This low but significant relationship between configurations means that both provided information that was independent and complementary.

Discussion

Argentinean quinoa germplasm is highly diverse at the phenotypic level reflecting variation in the environment of origin. In this study, quinoa accessions from the NWA region showed a wide range of variability in phenological and morphological attributes. The variability

in phenological traits is promising from the viewpoint of genetic improvement as it will be possible to cope with abiotic limitations such as frost and drought, two factors which highly affect local crop production (Geerts *et al.*, 2006; Pouteau *et al.*, 2011; Winkel *et al.*, 2011). On the other hand, variation in morphological traits is relevant for future comparative studies of new quinoa accessions from NWA or other countries, as it is associated with the site of origin or elevation of accessions and is consistent with previous characterisations of collections from Peru, Bolivia and Chile (Gandarillas, 1968; Risi & Galwey, 1989a; Ortiz *et al.*, 1998; Rojas, 2003; Anabalón Rodriguez & Thomet Isla, 2009).

Trait combinations observed in accessions from the eastern valleys and western highlands were congruent with the accessions from similar origin in Peruvian and Bolivian collections, respectively (Ortiz *et al.*, 1998; Rojas, 2003). Dry valleys accessions showed similar cycle

Table 5 Variation in quantitative traits for the groups recognised with principal component analysis and cluster analysis[a]

Descriptors	Traits	(G1)[b] Transition zone (n = 4)[c]	(G2)[b] Highlands (n = 7)	(G3)[b] Eastern Valleys (n = 5)	(G4)[b] Dry Valleys (n = 18)
Quantitative	Stem diameter (mm)	5 ± 0.4	3.7 ± 0.4	6.4 ± 0.1	7.4 ± 0.2
	Number of branches on the main stem	9.2 ± 1.0	4.1 ± 2.0	18.4 ± 1.9	7.6 ± 2.1
	Shape of the top leaf	1.7 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.04
	Teeth number	4.7 ± 0.6	8.1 ± 1.2	12.3 ± 1.5	7.4 ± 0.8
	Petiole length (mm)	27.7 ± 4.1	28 ± 3.7	44.7 ± 3.6	46.3 ± 2.1
	Leaf length (mm)	44.3 ± 4.8	52.7 ± 4.6	74.3 ± 4.7	73.4 ± 2.9
	Leaf width (mm)	33.6 ± 3.3	41 ± 3.0	68.1 ± 2.4	60.1 ± 1.9
	Panicle length (cm)	25 ± 3.2	20 ± 7.2	30.2 ± 9.8	38.7 ± 2.2
	Glomeruli length (mm)	8.6 ± 0.8	6.8 ± 0.8	10.1 ± 0.7	10.1 ± 0.4
	Length of cotyledon (mm)	12.6 ± 1.3	15.3 ± 1.1	17.2 ± 0.9	16.5 ± 0.4
	Length of hypocotyl (mm)	19.5 ± 0.3	18.6 ± 0.8	16.2 ± 1.0	19.7 ± 0.4
	Plant height (cm)	81.6 ± 13.2	39.5 ± 8.3	156.8 ± 6.7	139.4 ± 7.0
	Grain diameter (mm)	1.5 ± 0.1	2 ± 0.1	1.7 ± 0.03	1.9 ± 0.1
	Days to emergence	7.7 ± 0.8	8.4 ± 0.9	8.4 ± 1.0	7.5 ± 0.4
	Days from sowing to visible floral bud	45.5 ± 3.8	21 ± 1.4	67.8 ± 1.2	42 ± 1.9
	Days from sowing to first anthesis	70 ± 4.5	39.2 ± 1.9	89.4 ± 3.2	68.1 ± 1.6
	Days from sowing to physiological maturity	173.2 ± 14.6	118 ± 9.3	179 ± 1.9	164.7 ± 1.9

[a]For each quantitative trait mean ± 1 standard error is expressed.
[b]Genotype groups identified by hierarchical agglomerative clustering on the basis of quantitative traits.
[c]In parenthesis, the number of accessions in each group.

Table 6 Variation in qualitative traits for the groups recognised in principal coordinates analysis[a]

Descriptor	Traits	G A: Highlands + Dry Valleys (n = 13)[b]	G B: Eastern Valleys + Transition Zone (n = 13)	G C: Highlands + Dry Valleys + Transition Zone + Eastern Valleys (n = 8)
Qualitative	Axil pigmented	0.16 (present)	0 (present)	1.00 (present)
	Striation pigmented	0.91 (present)	0.66 (present)	1.00 (present)
	Striation colour	0.75 (red)	0.50 (yellow)	0.62 (purple)
	Main stem colour	1.00 (green)	1.00 (green)	0.66 (green)
	Stem colour intensity	0.54 (medium)	0 (medium)	0.50 (medium)
	Branching	0 (present)	0.92 (present)	1.00 (present)
	Leaf margin	0.91 (toothed)	1.00 (toothed)	0.62 (toothed)
	Panicle colour at maturity	0.83 (white)	1.00 (white)	0.75 (purple)
	Panicle colour intensity at maturity	0.53 (high)	0.15 (high)	0.88 (high)
	Panicle colour at harvest	0.66 (yellow)	0.92 (yellow)	0.62 (yellow)
	Panicle colour intensity at harvest	0.46 (low)	1.00 (low)	0.38 (low)
	Type of panicle	1.00 (terminal)	0.71 (terminal)	0.25 (terminal)
	Shape of panicle	0.58 (glomerulate)	0.71 (glomerulate)	0.87 (glomerulate)
	Compactness of panicle	0.92 (compact)	0.23 (compact)	0 (compact)
	Cotyledon pigmented	0.25 (present)	0 (present)	0.50 (present)
	Intensity cotyledon pigmented	0.16 (medium)	0 (medium)	0.25 (medium)
	Intensity hypocotyl pigmented	0.08 (low)	0.92 (low)	0.25 (low)

[a]The relative frequency of each trait (parenthesis) is expressed.
[b]In parenthesis, the number of accessions in each group.

length than accessions from the transition zone but were morphologically more similar to accessions from eastern valleys. Furthermore, they do not seem to be easily compared with any accessions group of collection mentioned above for neighbouring countries. On the other hand, the transition zone accessions were morphologically similar to those from the highlands except in growth habit

and grain diameter but phenologically similar to dry valleys accessions. As shown by their intermediate values in plant size and cycle duration in Table 3 and their intermediate position in Fig. 2, they occupy a transitional zone (in geographical and morphophenological terms) between highlands and eastern valleys accessions, a pattern similar to that observed by Rojas (2003). The PCA
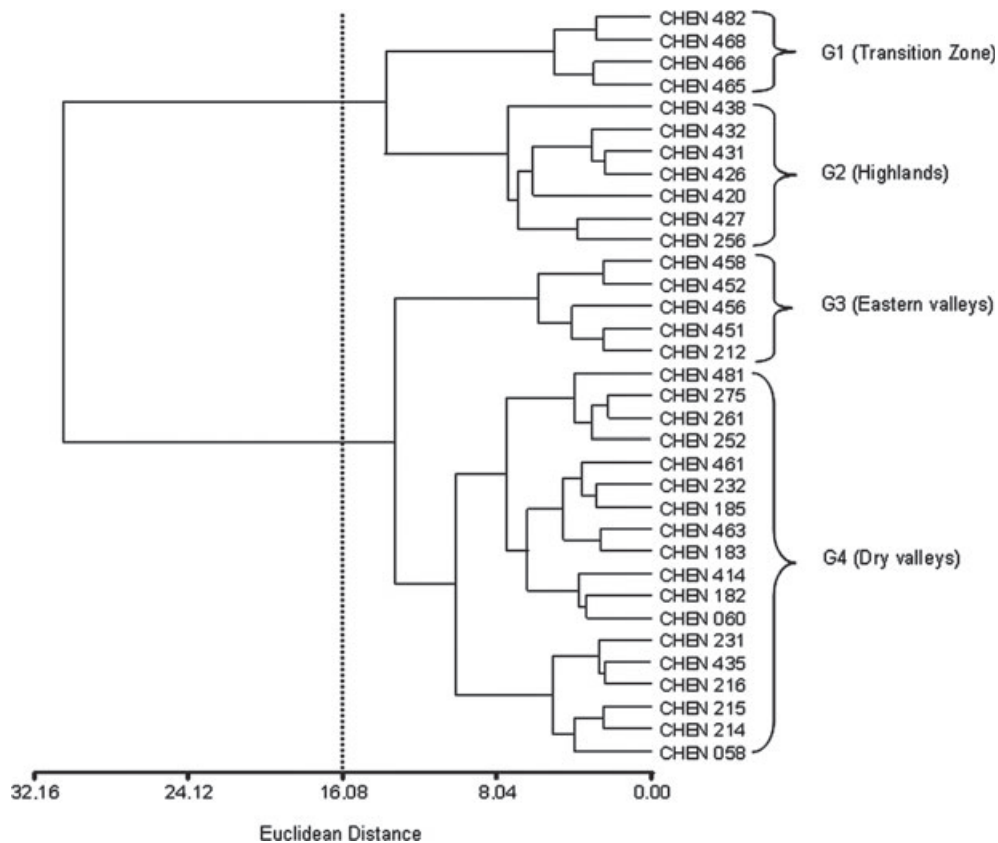
Figure 3 Dendrogram showing 34 quinoa accessions in the Ward cluster analysis according to quantitative traits.

and CA reveal that the phenotypic diversity for quantitative traits was structured according to site of origin or elevation, whereas the PCoA shows a grouping related to the 'domestication level' of accessions. In quinoa, the combinations of traits seen in the transition zone and eastern valleys accessions are generally more similar to those found in the ancestral wild type, while accessions from the highlands and dry valleys are morphologically closer to the domesticated type (Wilson, 1980, 1990). According to Harlan (1975), the evolutionary changes observed in cereals were from open and lax panicles or spikes and branched growth habit seen in the wild type to a large, heavy, compact and terminal inflorescence without branching habit such as in the domesticated type. In Chenopods and specially quinoa, these changes were similar (Wilson, 1980, 1990).

Wilson (1988) suggested that quinoa accessions from the Southern Andes showed the widest variation range in morphological and molecular traits and that a consistent pattern regarding elevation or site of origin within this group was not found. Thus, valleys and highlands populations were not well differentiated in that study. On the basis of quantitative traits, quinoa accessions from

NWA tend to group according to elevation or site of origin. The four groups recognised in PCA present similarities with some of those proposed by Rojas (2003) in the Bolivian germplasm accessions, which also discriminate quinoa ecotypes from the highlands, transition zone and eastern valleys. These ecotypic differentiations based on morphometry were later confirmed by del Castillo *et al.* (2007) using random amplification of polymorphic DNA (RAPD). This correspondence suggests that similar processes of genetic differentiation might have affected quinoa all over the southern Andes and that gradients of frost and aridity could be of major importance in quinoa ecotypes differentiation.

Christensen *et al.* (2007) recently analysed the genetic diversity of quinoa in the United States Department of Agriculture (USDA) and Centro Internacional de la Papa-Food and Agriculture Organization of the United Nations (CIP-FAO) collections. They suggested that the Argentinean accessions represent introductions from both the southern Bolivian highlands and the Chilean lowlands. According to our results, the Argentinean accessions likely represent introductions from southern Bolivia because the accessions from highlands (G2), transition
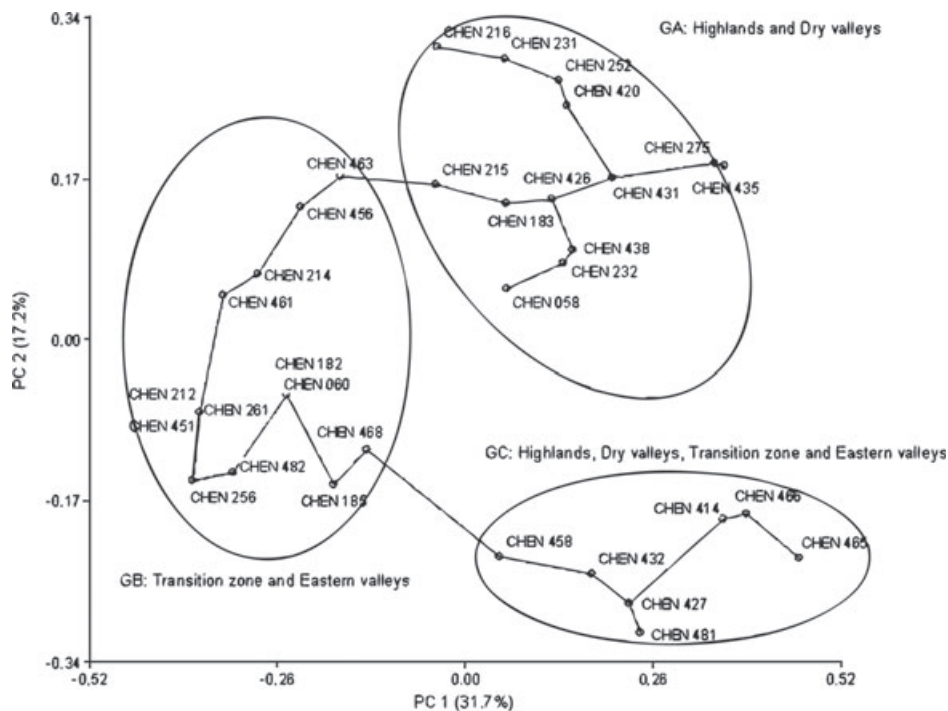
Figure 4 Ordination of quinoa accessions on the first two principal coordinates of the principal coordinates analysis and minimum spanning tree superimposed. The three main groups recognised are named and enclosed by circles. The accession CHEN 452 is located next to the 458 and is not shown.

zone (G1) or eastern valleys (G3) exhibit characteristics that seem to relate them to some introductions from the Bolivian southern (i.e. G1 in Rojas, 2003) or the northern highlands (i.e. G4, *ibid*) and eastern valleys (i.e. G7, *ibid*). However, this does not exclude the possibility that the Chilean highlands could have acted as an alternative road for quinoa introduction into Argentina, as accessions of the western highlands exhibit characteristics that relate them to some populations from the Chilean highlands, notably the lack of branched growth habit, leaf traits such as teeth number, blade length and width and panicle colour (Fuentes & Bhargava, 2010).

The results of this study hold several implications for quinoa conservation and improvement programs in the NWA region. Generally speaking, phenotypic diversity observed for NWA quinoa accessions is structured according to the site of origin. Thus, future conservation and breeding programs should consider the genetic structure of this crop species throughout the region. Because breeding programs established in the Andean countries are guided by grain attributes (size and colour), the western highlands and dry valleys accessions would be the most promising to include in those breeding programs, while other accessions (eastern valleys) could experience less use and even abandonment posing a serious hazard

of genetic erosion of this germplasm unless an alternative use, like forage, is promoted for these tall, branched, leafy ecotypes. Another issue to consider is the different amount of diversity within the groups recognised in this study. According to our results, quinoa accessions from the highlands and dry valleys showed more variability than accessions from the eastern valleys. These differences could be the result of founder events associated with early dispersal from the centre of origin as these cultivars showed traits more similar to the wild type, while both the highlands and dry valleys accessions showed traits more advanced in terms of domestication (Harlan, 1975) and could represent more recent introductions of materials from the southern Bolivian highlands. Future breeding programs should focus on not reducing too much this genetic diversity while at the same time achieving the required homogeneity for commercial production, as they grow in a stressful and unpredictable environment and reduction in genetic variability could hamper their adaptability to these environments.

Acknowledgements

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