

## CROPS AND SOILS RESEARCH PAPER

# Implications of farmers' seed exchanges for on-farm conservation of quinoa, as revealed by its genetic diversity in Chile

F. F. FUENTES<sup>1\*</sup>, D. BAZILE<sup>2,3</sup>, A. BHARGAVA<sup>4</sup> AND E. A. MARTÍNEZ<sup>5</sup>

<sup>1</sup>Departamento de Agricultura del Desierto y Biotecnología, Universidad Arturo Prat, Casilla 121, Iquique, Chile

<sup>2</sup>CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), UPR47, GREEN, Montpellier, France

<sup>3</sup>PUCV, Instituto de Geografía, Av. Brasil 2241, Valparaíso, Chile

<sup>4</sup>Amity Institute of Biotechnology, Amity University Lucknow Campus, Lucknow-226010, India

<sup>5</sup>Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile and PhD Program on Applied Biology and Ecology (Dr-BEA), Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile

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## SUMMARY

Quinoa cultivation in Chile presents an ancient and active complex of geographic, climatic, social and cultural interactions that has determined its current biodiversity in the three main growing zones (north, central and south). Importantly, these interactions involve the participation of farmers, whose activities are at the base of seed exchange networks due to their knowledge and *in situ* conservation of genetic diversity. The present study reports how a better understanding of farmers' seed exchanges and local production practices could impact the genetic structure and diversity of quinoa at national scale in Chile. Using field interviews and characterization of 20 microsatellite genetic markers in a multi-origin set of 34 quinoa accessions representative of Chile and the South American region, the phenetic analysis of germplasm was consistent with the current classification of quinoa ecotypes present in Chile and Andean zone. This allowed the identification of five populations, which were represented by quinoa of Salares (northern Chile), Coastal/Lowlands (central and southern Chile), Highlands (Peru, Bolivia and Argentina) and Inter-Andean Valleys (Ecuador and Colombia). The highly informative quality of the markers used revealed a wide genetic diversity among main growing areas in Chile, which correlated well with natural geographical–edaphic–climatic and social–linguistic context to the expansion of quinoa biodiversity. Additionally, in addition to ancient seed exchanges, this process is still governed by the diverse agricultural practices of Andean farmers. Genetic erosion is considered an imminent risk due to small-scale farming, where the influence of increased migration of people to urban systems and export-driven changes to the agro-ecosystems may further reduce the diversity of quinoa plants in cultivation.

## INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), an Andean grain crop, has recently gained worldwide attention because of its nutritional value and functional features (Bhargava *et al.* 2006; Hirose *et al.* 2010; Vega-Gálvez *et al.* 2010), as well as its ability to grow under conditions of soil salinity, drought, frost and a wide pH range (Jacobsen *et al.* 2003; Fuentes & Bhargava 2011). Because of these characteristics, quinoa has been introduced to new areas outside its native region, especially

in Europe and the subtropical regions of the world, where it has provided good yields and so demonstrated the potential of quinoa as a grain and also as a fodder crop (Mujica *et al.* 2001; Casini 2002; Jacobsen 2003; Bhargava *et al.* 2007a; Pulvento *et al.* 2010).

Quinoa has been cultivated for c. 7000 years in the Andes of South America (Jacobsen 2003; Mujica 2004). Its current distribution is from Nariño (1°39'N; Colombia) to the Salares of southern Bolivia (21°15' S), including countries such as Peru, Ecuador, northern Argentina (Jujuy and Salta provinces), northern Chile (Tarapaca and Antofagasta regions) and the southern extreme of the Andes in the Chilean lowlands (39°48'S)

\* To whom all correspondence should be addressed. Email: francfue@unap.cl

(Wilson 1990; Bhargava *et al.* 2006; Fuentes *et al.* 2009c), with an altitudinal range from sea level to 4300 m asl (Cancosa, Chile). Its diversity, at a continental scale, is associated with five main ecotypes: Highlands, Inter-Andean valley, Salares (salt flats in the Andean High plateau), Yungas (a warm, rainy and humid stretch of forest along the eastern slope of the Andes Mountains, transitional between the Andean highlands and the eastern forests; stretching from central Bolivia to south-eastern Peru) and Coastal/Lowlands; each of these are associated with sub-centres of diversity that originated around Lake Titicaca (Risi & Galwey 1984).

Thus, the genetic diversity of quinoa probably evolved as ancient societies along the Andean range tested new soils and climates through cycles of seed exchange and domestication processes (Wood & Lenné 1997; Tagle & Planella 2002; Badstue 2006; de Haan 2009). It has been proposed that at least three genetic events could have created 'bottlenecks' and affected this biodiversity (Jellen *et al.* 2011). The first, and potentially most severe, event could have occurred at the very beginning when the two diploid ancestry of quinoa hybridized. The second event occurred when quinoa was domesticated from its wild tetraploid relatives. The third can be considered a political bottleneck, starting more than 400 years ago during the Spanish conquest period and has continued until the present time, reflecting the different national histories of current South American countries. During this 400-year period, quinoa was excluded from the production process due to its importance in the indigenous social and religious beliefs (Cusack 1984; National Research Council 1989; Fuentes *et al.* 2009a). A new, fourth bottleneck event can be added viz. the migration from rural zones of the Andes to modern urban centres, resulting in abandoned villages and farms (Bazile *et al.* 2011). In Chile, the last national census (INE 2004) revealed that 13.5 million people of 15.6 million, i.e. 0.87 of the population, live in urban areas. This social change has exposed quinoa to a risk of genetic erosion, particularly considering that germplasm diversity is conserved mostly on farm or *in situ* within zones where there are no massive intervention programmes of agricultural modernization (Wale 2008; Martínez *et al.* 2010). This agrobiodiversity is held by indigenous farmers – often women – at very small spatial scales, especially in Chile. This genetic diversity reflects the wide range of quinoa ecotypes adapted to different agroecological zones in the Andean area (Fuentes *et al.* 2009b), where farmers have used the

grain as a staple food source because of its nutritional significance (Rana *et al.* 2010; Fuentes & Bhargava 2011). In this context, the Andean farmers might be considered as *resource persons* (Bazile & Abrami 2008) due to the fact that: (i) they conserve the genetic diversity of quinoa in their fields as *in situ* collections (Cleveland *et al.* 1994); (ii) they know the agronomic behaviour of each quinoa archetype; and (iii) they know the complex networks that explain the seed fluxes in their territories (Bazile & Weltzien 2008; Aleman *et al.* 2010). However, although farmers have a broad knowledge of quinoa archetypes, they do not appreciate the diversity at much larger spatial scales, ignoring most of these diverse varieties. Hence, it is necessary to share the traditional knowledge more widely, to recognize and thus to avoid the risks of genetic erosion (Brookfield *et al.* 2002; Bazile & Negrete 2009). The lack of systematic assessment of seed systems on-farm, as a key gap in seed security, is a longstanding issue (Sperling *et al.* 2008). Nevertheless, it has been possible in recent years to relate the major factors (e.g. natural disasters and farmer's seed exchanges/selection) shaping population dynamics at micro-regional levels with the genetic diversity pattern by using a molecular marker-based approach (Pressoir & Berthaud 2004; Ferguson *et al.* 2011).

Polymorphic DNA markers have been used in diverse applications such as population genetics, conservation biology, studies of evolutionary history and for developing conservation strategies as well as core collections in a important number of crop plants (Matus & Hayes 2002; Woodhead *et al.* 2005; Orabi *et al.* 2007; Angioi *et al.* 2010; Vargas *et al.* 2010; Bellucci *et al.* 2011). Among them, the microsatellites or simple sequence repeats (SSRs) have become a powerful tool for diversity analysis since they are highly polymorphic, multi-allelic, frequently co-dominant, highly reproducible, and randomly and widely distributed in the genome (Bhargava & Fuentes 2010). In quinoa, the study of genetic diversity has been carried out using morphological data (Rojas 2003; Bhargava *et al.* 2007b; Fuentes & Bhargava 2011) as well as by using molecular tools (Christensen *et al.* 2007; Fuentes *et al.* 2009c; Rana *et al.* 2010), resulting in important advances in describing the relationships among quinoa ecotypes at regional spatial scales. This information has also been useful in planning actions for conservation and use of germplasm applied at a wider scale (regional and national). However, these approaches to the diversity analysis of quinoa do not consider, for example, the

dynamics of seed distribution through on-farm exchanges, as an anti-risk strategy for food security using the diversity of the agro-ecological sites (Subedi *et al.* 2003; Chevassus-au-Louis & Bazile 2008).

The goals of the present study were to relate the agro-ecosystem factors, the farmers' seed exchanges and their local production practices on-farm with the emerging genetic pattern of quinoa at national county scale in Chile using microsatellites molecular markers.

## MATERIALS AND METHODS

### Study area

The study was based in the three main quinoa growing areas of Chile, which exemplify a wide range of geographic areas, ecosystems and cultural groupings from the north to the south of the country between 18 and 39°S. In the northern zone (Tarapaca region, between 18 and 22°S), quinoa is cultivated as a main crop by indigenous communities (Aymara culture). The crop is grown on small farms under highland conditions, with a mean altitude of 3500 m asl, on saline soils and an annual precipitation of 100–200 mm from December to February (Fuentes 2008). The characteristic dry climate leads farmers to cultivate the same field only once every 2 years, permitting water accumulation in the upland soils (endorheic geographical component) for the fallow period. The central zone (O'Higgins and Maule regions) is characterized by a temperate climate with dry summers and winters, having precipitation c. 500–800 mm per year (DMCH 2011). The agriculture is based mainly on vineyard, tree fruits and cereals: quinoa is cultivated without irrigation in marginal areas as an alternative crop at an altitude of c. 100–200 m between 34 and 36°S. The southern zone (Araucania region) has a rainy oceanic climate with annual average precipitation reaching up to 2000 mm per year (DMCH 2011); the principal agricultural activities are cultivation of forages, cereals and vegetables. Here quinoa is cultivated by indigenous communities (Mapuche culture) and other smallholders at various altitudes from 50 to 600 m between 37 and 39°S.

### Fieldwork

In 2008, a field survey was carried out to characterize the diversity of agroecosystems of quinoa production in the three areas. Initially, a participatory rapid appraisal (PRA) with 10–15 people in each site was conducted, to present the objectives of the work, to

understand the general agriculture dynamics in the zone and to obtain precise information about quinoa diversity production and management in this sector. Then, semi-directed interviews of farmers were conducted to establish the importance of quinoa cultivation in the farms and the management of local varieties. The sample size was 21 farmers in the north, 13 in the centre and 5 in the south, reflecting the relative importance of quinoa crop revealed in the latest available national agricultural census (INE 2007). A qualitative treatment of data allowed descriptions of production systems, management of local varieties and external variables affecting the farming system. In 2009, a second round of field surveys was carried out in the same areas. In order to identify the details of practices of local varieties management undertaken by farmers and to assess the key elements of quinoa cultivation and the roles of different actors (farmers and institutions), semi-constructed and qualitative interviews with farmers and institutions (three or four villages from each zone) were conducted. In total, 92 interviews were conducted: 31, 26 and 35 in the northern, central and southern zones, respectively. The database constructed following these interviews allowed determination of who provided seeds to whom through local networks, and analysis of the level of diversity of local varieties and the diffusion of associated knowledge regarding crop adaptation and seed management. The analysis of information was performed through multiple factor analysis using Statistica 6.0<sup>®</sup> Software (StatSoft 2001).

### Genetic materials

Genetic analysis was performed on 34 quinoa accessions, which were provided by diverse institutional seed banks as seed pools collected from farmers (Table 1). Twenty-six accessions were geographically representative of the three main quinoa growing areas in Chile (northern, central and southern zones) (Fuentes *et al.* 2009c) and the remaining eight were included as controls from the Andean zone outside of Chile (Christensen *et al.* 2007).

### DNA extraction and microsatellite analysis

All quinoa accessions were sown and grown in pots at 25 °C and 12 h photoperiod in a growth chamber at Arturo Prat University (Iquique, Chile) until four true leaves formed. DNA was extracted from bulked leaf material from three individual plants per accession

Table 1. *Passport data of quinoa accessions utilized in the present study*

No.	ID accession	Origin	Source*	No.	ID accession	Origin	Source*
1	QPC001	Chile, Tarapaca	UNAP	18	Regalona	Chile, Araucania	Baer
2	QNC003	Chile, Tarapaca	UNAP	19	BO01	Chile, Araucania	Baer
3	QNCH006	Chile, Tarapaca	UNAP	20	BO07	Chile, Araucania	Baer
4	QRP010	Chile, Tarapaca	UNAP	21	BO13	Chile, Araucania	Baer
5	QRC012	Chile, Tarapaca	UNAP	22	BO14	Chile, Araucania	Baer
6	QRE013	Chile, Tarapaca	UNAP	23	BO17	Chile, Araucania	Baer
7	QACC018	Chile, Tarapaca	UNAP	24	BO25	Chile, Araucania	Baer
8	QRCOL025	Chile, Tarapaca	UNAP	25	Puc	Chile, Araucania	INIA
9	QAS2	Chile, Antofagasta	UNAP	26	RU-2	England (Chilean origin)	CIP-FAO
10	Palmilla	Chile, O'Higgins	UNAP	27	Jujuy	Argentina, Jujuy	CIP-FAO
11	Mau	Chile, O'Higgins	INIA	28	Kamiri	Bolivia	CIP-FAO
12	Javi	Chile, O'Higgins	INIA	20	Chucapaca	Bolivia	CIP-FAO
13	UdeC9	Chile, Maule	INIA	30	L-P	Bolivia	CIP-FAO
14	BO38	Chile, Bio-Bio	Baer	31	Ingapirca	Ecuador	CIP-FAO
15	B042	Chile, Bio-Bio	Baer	32	Nariño	Colombia	CIP-FAO
16	BO46	Chile, Bio-Bio	Baer	33	Illpa-INIA	Peru, Puno	CIP-FAO
17	Hueque	Chile, Araucania	UNAP	34	Salcedo	Peru, Puno	CIP-FAO

\* UNAP (Arturo Prat University, Chile), Baer (Seeds Baer Company, Chile), INIA (Institute of Agricultural Research, Chile), CIP-FAO (International Potato Center and Food and Agriculture Organization).

(c. 0.1 g from each), according to protocol described by Lodhi *et al.* (1994).

Twenty di-/tri-nucleotide loci microsatellites utilized by Fuentes *et al.* (2009c) were reviewed and chosen in accordance with their reproducibility and clear amplification of bands (Table 2). The polymerase chain reaction (PCR) mix was performed with 60 ng of genomic quinoa DNA (3 µl), 1 mM of Cresol Red (1.5 µl), 2 mM of deoxynucleotide triphosphates (dNTPs) (1.5 µl), 25 mM of magnesium chloride (MgCl<sub>2</sub>) (1.5 µl), 10 times of PCR buffer (1.5 µl), 10 µM of each primer (1.5 µl), and 1 unit of Taq polymerase (Bioline USA Inc.) (0.5 µl), for a total volume per reaction of 15 µl.

The amplification parameters of DNA were as follows: 18 cycles at 94 °C for 60 s, 67 °C for 30 s and 72 °C for 60 s, followed by 20 cycles at 58 °C as annealing temperature with a final extension cycle of 72 °C for 10 min on a thermocycler Eppendorf Mastercycler Gradient® (Hamburg, Germany).

The amplicons were visualized and photo-documented under a UV transilluminator Digi Doc-It System (UVP, Biolmaging system, Upland, CA, USA) using 0.03 (w/v) Metaphor® agarose gel (CambrexBio Science, East Rutherford, NJ, USA) stained with ethidium bromide. The size fragments were measured using the Gel-Pro Analyser 6.0 software (Media Cybernetics, Bethesda, MD, USA). A standard ladder of 100 bp (Bioline USA Inc., Boston, MA, USA) and a set of fragments of known size reported by Fuentes

*et al.* (2009c) were utilized as reference for size fragment measuring.

#### Molecular data analysis

Molecular data were converted into a binary matrix, which registered the presence (1) or absence (0) of alleles at each microsatellite *locus* per accession. A pairwise analysis of molecular data was performed using Jaccard's similarity coefficient using the FreeTree® program (Pavlicek *et al.* 1999). An unweighted pair group method with arithmetic mean (UPGMA) cladogram was computed after 500 replicates for a bootstrap test and constructed with help of Tree View (Win32) ver. 1.6.6. software (Page 2001). Levels of heterozygosity (*H*) per *locus* and Nei's (1972, 1978) identities/distances among populations, as well as Wright's *F*<sub>ST</sub>-statistics value (Wright 1951) were calculated using TFPGA software, version 1.3 (Miller 1997). Comparison of polymorphic nucleotide motifs were made using unpaired Student's *t* test; *P* ≤ 0.05 with INFOSTAT (Infostat 2008) statistical software.

## RESULTS

#### Microsatellite analysis

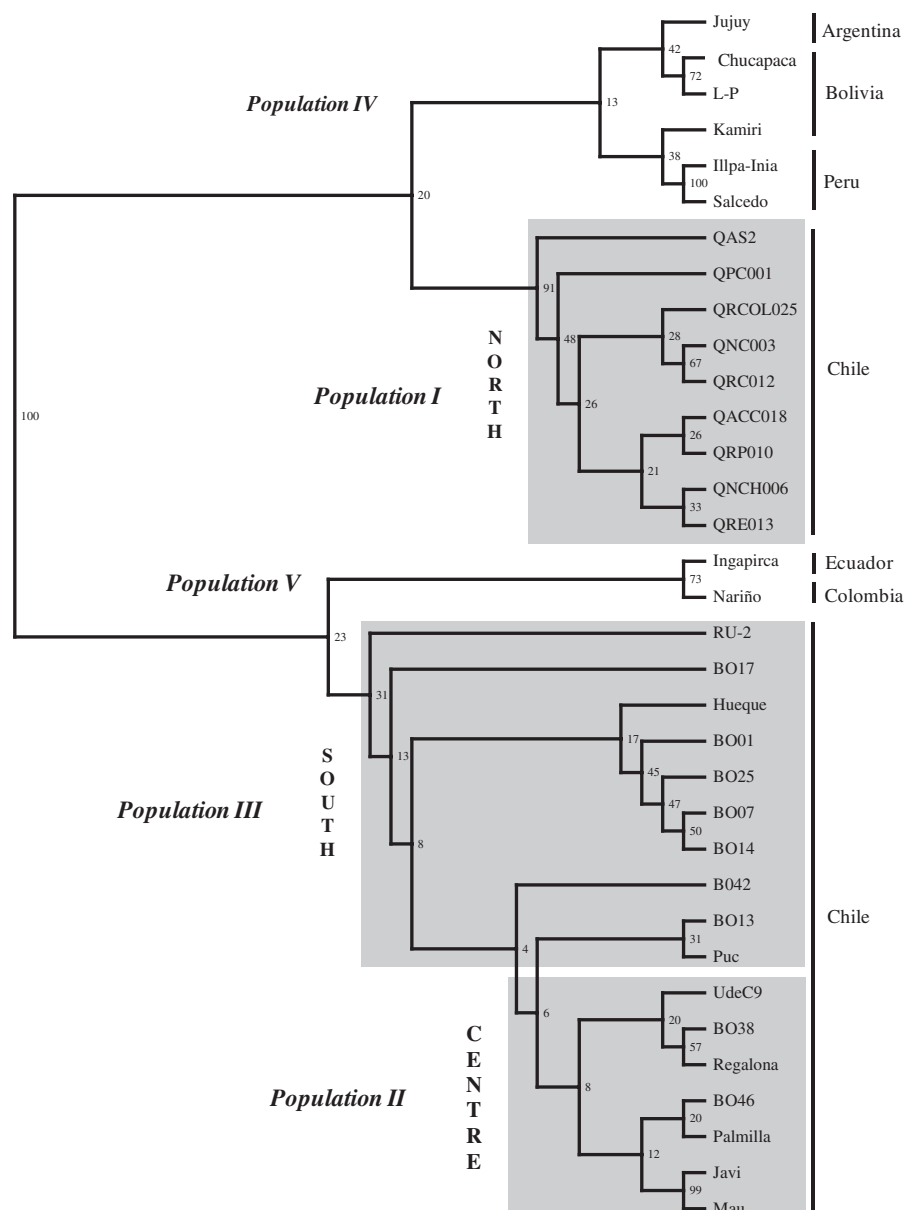
The molecular analysis yielded 118 polymorphic markers for all quinoa accessions assessed, with a mean value of alleles per locus of 5.9. The QAAT76 locus had the highest record for alleles number (*n* = 11)

Table 2. Name, motif, primer sequences, GenBank accession number, number of alleles, *H* value modified and Wright's  $F_{ST}$ -statistics value for each locus microsatellites used in the present study

	Locus microsatellite	Principal motif	Primer forward (5' → 3')	Primer reverse (5' → 3')	GenBank accession no	No of alleles	<i>H</i> Value*	$F_{ST}$ †
1	KGA16	(GA)22	ccctgcttaatctccgtgaa	gcttctcgaaccaagactacgaaaca	DQ462130	4	0.64	0.61
2	QAAT24	(ATT)10	gcttctaccataacagcaccacctt	agggatcaatctgttcattca	DQ462136	8	0.84	0.24
3	QCA48	(CA)13	gcttctacaatacatacaaccaatattcaa	tggaaatgtcactatgattgga	AY458240	4	0.61	0.52
4	QAAT74	(ATT)14	gcttctatggaacacccatccgataa	atgcctatcctcatcctcca	DQ462141	8	0.79	0.39
5	QCA24	(CT)13TT(CT)12CA(CT)7(CA)17	agatgagcttgaatcattacatc	gcttcttacatactgtaaatcatgccaaa	DQ462151	6	0.63	0.56
6	KGA20	(CT)22	gcttctcacctacctcggtaaaggaaa	ggagcagatgatgaacatgg	DQ462131	5	0.70	0.37
7	QAAT70	(ATT)15	tgaacaggatcgtcatagtcaa	gcttctcgttcatcatctgacccaat	DQ462139	7	0.74	0.23
8	QCA120	(TG)4CG(TG)8(AG)6	gcttctgacgcacataacgtgtagaattg	tcctcattcccttctccatc	DQ462156	4	0.30	0.39
9	QCA88	(TG)10(CA)5CGCG(CA)7	gcttctctggctgctccacctaata	cagtcccgaatcgtactc	DQ462154	2	0.40	0.02
10	QGA17	(TG)8AAG(GA)16	ttacggttctcccggctctc	gcttctgcaacaagaagaagcatgaag	DQ462158	4	0.12	0.17
11	QCA57	(CA)22(TA)5	gcttctgcaaggaaaccatcttgg	tgctcagcagtcacacctaca	AY458243	6	0.80	0.43
12	QGA02	(AG)25	gcttctgaaccttaataggctgtaccaaatac	aagaaatgtcacaagcaagca	DQ462157	7	0.76	0.33
13	QAAT50	(ATT)17	ggcacgtgctgctactcata	gcttctatggcgaatggtaatttgc	DQ462137	8	0.74	0.37
14	QAAT78	(ATT)22	agcgaaggaaattggaact	gcttcttaacgatacgtccaaggaa	DQ462143	7	0.80	0.27
15	QCA38	(CT)10(CA)13	gcttctcatttcccaactgcatgaat	atgtgtgtgcgtgtgagtg	DQ462152	8	0.79	0.30
16	KGA003	(GA)16	attgccgacaatgaacgaat	gcttctatgtaaatggcatgtcccaac	DQ462129	5	0.70	0.33
17	QATG86	(CAT)9(TGA)2TGTTGA	aatcgcagcctaaactgagc	gcttctagtccatttcgaccatatgataa	DQ462147	4	0.51	0.35
18	QAAT76	(ATT)30	gcttcatgtgtataaaaatgccaat	gcttcttctcggctcccactaatttt	DQ462142	11	0.87	0.04
19	QCA14	(GT)21(GA)12	gcttctcctgagctgattatcaaaggac	cctcttgcgagatttctgct	DQ462150	4	0.42	0.33
20	QCA37	(CA)13CT(CA)16	gcttctcgttctccagaccaattc	tcatgagccacttcatacacg	AY458227	6	0.81	0.23
					Mean	5.9	0.649	0.324

\* Nei (1978).

† Wright's  $F_{ST}$  statistics value with 0.95 confidence interval for groups identified (Wright 1951).



**Fig. 1.** UPGMA cladogram based on Jaccard's similarity coefficient of 34 quinoa accessions performed after 500 replicates for bootstrap test (percentage number between each node). Grey rectangles represent the main growing areas of quinoa in Chile. (Population I: Salares/Northern Chile; Population II: Lowland/Central Chile; Population III: Lowland/Southern Chile; Population IV: Highland/Peru–Bolivia–Argentina; Population V: Inter-Andean Valley/Ecuador–Colombia).

and QCA88 locus the lowest record ( $n = 2$ ) (Table 2). In general, it was observed that loci with tri-nucleotide motifs were significantly more polymorphic than those loci with two-nucleotide motifs ( $t$ -test;  $P \leq 0.05$ ). The  $H$  for all quinoa accessions ranged between 0.12 (QGA17) and 0.87 (QAAT76) and a mean value of 0.65 (Table 2).

The UPGMA analysis using the Jaccard coefficient identified two major groups, which were subdivided into five populations. Population I contained nine

accessions representative of the northern zone of Chile, population II seven accessions of the central zone, population III included nine accessions from the southern zone and only one from the central zone (B042), population IV contained six accessions from the highlands of Peru, Bolivia and Argentina, and finally population V contained two accession from Ecuador and Colombia (Fig. 1).

The identification of populations allowed the comparison of allelic diversity patterns (Table 3); the

Table 3. Pattern of total alleles, mean alleles per locus, unique alleles, average heterozygosity (Nei 1978), proportion of polymorphic loci (PPL) and Nei's (1972, 1978) identities/distances of populations identified in the study\*

	Population I (n=9)	Population II (n=7)	Population III (n=10)	Population IV (n=6)	Population V (n=2)
Allelic diversity					
Total alleles	58	48	70	53	31
Mean alleles per locus	2.9	2.4	3.5	2.7	1.6
Unique alleles	14	4	8	5	1
Average heterozygosity	0.45	0.38	0.52	0.52	0.26
PPL	0.90	0.75	0.95	0.95	0.55
Nei's identities/distances (1972, 1978)					
Population I	–	1.51	1.25	0.69	1.44
Population II	0.22	–	0.32	0.97	1.02
Population III	0.29	0.73	–	0.76	0.87
Population IV	0.50	0.38	0.47	–	1.09
Population V	0.24	0.36	0.42	0.34	–

\* Population I: northern zone of Chile; population II: central zone of Chile; population III: southern zone of Chile; population IV: Peru, Bolivia and Argentina highlands; population V: Inter Andean valleys of Ecuador and Colombia.

highest values found for average  $H$  and proportion of polymorphic loci were observed in populations III (lowland) and IV (highlands outside Chile). Additionally, population III had the highest values for mean alleles per locus and total alleles, in spite of having less geographic dispersion than population IV (Peru, Bolivia and Argentina) (Fig. 2). Population I (northern zone of Chile) exhibited the maximum value for unique alleles, followed by populations III (southern zone of Chile) and IV (highlands outside Chile). Finally, populations II and V yielded lower values for all variables that described the allelic diversity pattern (Table 3).

Nei's identities/distances values among populations (Nei 1972, 1978) were consistent with their geographical origin. Thus, quinoa populations from highlands (I and IV) and lowlands (II and III) had the lowest genetic distances. Interestingly, quinoa from the Inter-Andean Valley was shown to be genetically closer to quinoa from lowlands (Table 3).

Through the analysis of Wright's  $F_{ST}$  values, it was possible to describe a new sub-set of informative loci for population discrimination (Table 2). Thus, within all SSR markers the loci with higher  $F_{ST}$  values, which were the most biologically significant, stood out: KGA16, QCA24, QCA48 and QCA57. In contrast, the loci QCA88 and QAAT76 were not significant, having  $F_{ST}$  value <0.1.

#### Assessment of fieldwork surveys

In the northern zone, it was possible to detect a high level of variability in phenotypically selected cultivated quinoa, which gives farmers a degree of security against climate change (drought and frost). The size of these farms ranged from 1 to 4 ha per farmer, with quinoa grown mostly for self-consumption. The terms *variety* or *landrace* are used by farmers to describe a quinoa line that is adapted to a specific locality and recognized by phenotypic characteristics, principally by panicle colour. Thus, farmers had on average 2.4 varieties each; however 0.32 of farmers have only one variety and 0.15 of them have four or more varieties (Table 4). Among the communities assessed, 0.90 of farmers use the same landrace, called 'white' because of its grain colour and its suitability for cooking. Those farmers who manage two or more other varieties as well as the dominant white one always use this variety in association with the others, i.e. biodiverse farmers tend to adopt this strategy of maintaining more than one variety through different cultivation periods.

In the central zone, the diversity of soils is limited to sandy type with or without salt. Cultivation area ranges from 1 to 5 ha per farmer. Farmers throughout the area make reference to a single landrace (Table 4), called *white*, independently of conditions and properties affecting the farm system (soil texture,



**Fig. 2.** Model of biodiversity dynamics of quinoa associated with populations identified in the study (Population I: Salares/Northern Chile; Population II: Lowland/Central Chile; Population III: Lowland/Southern Chile; Population IV: Highland/Peru-Bolivia-Argentina; Population V: Inter-Andean Valley/Ecuador-Colombia). Arrows show most likely seed migration routes, as inferred from genetic similitude and from ancestral people's interactions and cultural exchanges.

salinity, etc.). However, although they only talk about one landrace, there was evidence of synonyms for the same kind of seed (e.g. white, golden and yellow).

The interviews in the southern zone revealed a total of eight landraces, with 0.32 of the farmers having a single landrace, 0.49 two landraces and 0.18 can distinguish three landraces using various criteria such as grain colour before and after saponin extraction, or the colour of leaves and panicles. These farms were the smallest in the present study, with an area of c. 0.1 ha per farmer (Table 4).

In all of the regions, farmers classified varieties of quinoa based on colour, whether of the flower or grain, which implies also different type of uses (cultural or economic). Each farmer manages diversity via different management practices. This contributes to the diversity of the production system within each area through the growing of different crop or horticultural species and also generates more diversity within the quinoa

species, through growing different ecotypes or landraces.

Clearly, even though several scientific or social projects have been carried out in Chile, the farmers themselves are the main actors in biodiversity conservation (Table 4). Also, social and professional networks are important: two types of exchange take place in the communities and represent the main access to seeds. The first is an *individual* exchange (between individuals or families) inside the community or between close communities. The second is a *collective* exchange that is organized during events such as local markets or traditional ceremonies. Each type of exchange uses the same main participants. These are largely women in the south (0.85), and have been termed *seed curators* (Pérez 2005), in contrast with 0.95 of men in the north and centre. These farmers typically maintain high levels of crop diversity and have knowledge regarding crop adaptations and



Table 4 *Principal agronomic and social variables of quinoa in the three ancestral production zones of Chile*

Variable	North	Centre	South
i Number of producers	> 170	> 70*	> 50*
ii Hectares	1374	130*	10*
iii Production (tonnes)	802	100*	2,9
iv Grain yield (kg/ha)	580	978	1074
v Mean number of landraces per field crop	3–5	1	1–3
vi Photoperiod sensibility of landraces	Absence or lesser sensitivity to photoperiod during fill grain, with not good yields south of 25°S	Sensitivity to photoperiod, with not good yields north of 25°S	Sensitivity to photoperiod, with not good yields north of 25°S
vii Growth period	August–May	September–April	October–March
viii Seeds origin	Family inheritance during many generations within communities and Aymara fairs with Bolivia or Peru	Family inheritance during many generations, barter with neighbour and new diffusion with <i>Agricola Las Nieves Ltda</i> (Paredones)	Family inheritance during many generations, <i>Traikintu</i> (seeds exchange; barter) and programmes of agricultural modernization
ix Breeding	Red and yellow populations selected under a broad genetic basis (UNAP)	Search of improved varieties for the firm <i>Agricola Las Nieves</i> (Paredones)	The only improved variety registered in Chile: ' <i>Regalona</i> ' (Baer Seed Company)
x Link among producers	Strong communities but in competition around power, territory conflicts	Isolated	Strong communities with good links among several sectors of region. Importance of the Mapuche identity with permanent fights for ethnic issues (forest and water rights)
xi Public institutions supporting rural extension	Subsidies supporting livestock	Technical support and subsidies for fertilizer acquisition	Local varieties diffusion (NGO) and also diffusion of <i>Regalona</i> variety (INDAP/State decentralized institution)
xii Producers organizations	Two cooperatives that could spend efforts to sell quinoa under specific conditions according to market demand. Difficulty of organizations to offer attractive prices to producers. Looking after Bolivian organizations associated with international market (Fair Trade)	The only cooperative in the zone presents conflict of interest with some producers because a few of them are members and prices are variable between those who are or are not members. Cooperative is member of the <i>Agricola Las Nieves</i> firm, which focused only on export activities with better expectative in a short term for organic certification	DAWE project led by CET-Sur (NGO) have developed a participatory rural auto-certification for diversity, seed conservation and to promote local market and valuation of seed diversity in local markets and within direct contacts for restaurant chains

i, ii, iii and iv: information from 2007 National Agricultural Census (INE 2007) completed and corrected by field works.

\* Minimum estimation.

seed management, and can be categorized as nodal farmers because they play an essential role in seed exchanges. These nodal farmers participate in all the formal exchanges organized in their regions, thus giving a dynamic to the exchange and confidence to the various farmers they collaborate with.

## DISCUSSION

### Microsatellite analysis

The significant difference (*t* test;  $P \leq 0.05$ ) of polymorphism between two- and three-nucleotide motifs confirms the observation in other studies of

microsatellites, also carried out in quinoa, by Mason *et al.* (2005), and recently in *Amaranthus* by Mallory *et al.* (2008), where it was concluded that the development of highly polymorphic microsatellites markers should be focused to use tri-nucleotide motifs with repeat of >20 bp.

Previous assessments of microsatellites markers in multi-origin quinoa germplasm have reported  $H$  values of 0.57 (Mason *et al.* 2005), 0.75 (Christensen *et al.* 2007), 0.57 (Jarvis *et al.* 2008) and 0.65 (Fuentes *et al.* 2009c). Comparison with the  $H$ -value of 0.65 (ranging from 0.12 to 0.87) found in the present study suggests that there was a wide genetic diversity in the quinoa samples of the present study, and also confirmed the highly informative quality of the markers used. 0.60 ( $n=12$ ) of them were classified as highly polymorphic ( $H \geq 0.7$ ) according to the classification established by Ott (1992).

The geographic origin of accessions and the populations identified by UPGMA analysis were consistent with the ecotype classifications described by Risi & Galwey (1984) and the production zones focused in the present study. In this sense, population I was representative of Salares (North of Chile), population II and III of Coastal/Lowlands quinoa (South of Chile), population IV of Highlands (Peru, Bolivia and Argentina) and population V of the northernmost Inter-Andean Valleys (Ecuador and Colombia) (Fig. 2).

The pattern of high genetic diversity within Coastal/Lowlands ecotypes has been described in previous studies of southern Chile ecotypes using dominant AFLP markers (Anabalón Rodríguez & Thomet Isla 2009) and co-dominant SSR markers (Fuentes *et al.* 2009c). It has been suggested that Coastal/Lowlands ecotypes are representative of active crop/weed complexes having a monophyletic coevolving behaviour (Rana *et al.* 2010). This might explain why lowland breeders find it difficult to obtain pure quinoa cultivars, because of its close affinity to weed populations of *C. album* and/or *C. hircinum* (Fuentes *et al.* 2009c). This is also suggested by the large number of alleles recorded within population III, in spite of its smaller geographic dispersion than population IV (representative of a broad geographic area surrounding the centre of origin of quinoa). Finally, the close genetic relationship among lowland and Inter-Andean Valley representations found in the present study supports the hypothesis proposed by Fuentes *et al.* (2009c), which is based on the artificial introduction of alien germplasm to lowland areas. An example of this is the case of the *Regalona* cultivar

from the Baer Seed Company, obtained by artificial hybridization between parents from Araucania region (Baer II) and Ecuador (I. von Baer, personal communication).

Values for average  $H$  and proportion of polymorphic loci in population II (central zone) were lower than those recorded for population III (southern zone). Thus, the diversity for the central zone revealed a sub-pattern of a geographic bottleneck within lowland/coastal quinoa. This pattern matches well with the isolation among farmers (Table 4), resulting in a fragmented pattern of diversity among these two geographic areas (Fig. 2). Meanwhile, the lowest  $H$  value, observed for population V, was limited by the small size of the sample considered. This situation has also been reported in previous quinoa diversity analyses, because of its poor representation in seed banks (Christensen *et al.* 2007).

The Wright's  $F_{ST}$  values of each microsatellite locus reported interesting features related to the detected polymorphism at population level (Table 2). This information may make the partial use of some microsatellite loci in faster diversity assessing or marker-assisted selection purposes possible in the future (Forapani *et al.* 2001; Skøt *et al.* 2005).

#### Dynamics of seed exchanges

Even the most cautious interpretation of the observed molecular diversity with respect to geographic, edaphic, climatic, social and cultural groupings, correlates well with the reported information. Thus, Salares and Highlands ecotypes, even if they are geographically separated, do remain close in the dendrogram because they are in similar edaphic and climatic contexts (populations I and IV, respectively) (Fig. 2). They share the high Andes climatic conditions and also similar photoperiods and soils (Rojas 2003; Martínez *et al.* 2009a). The Aymara–Quechua people currently occupy areas in Peru, Bolivia and Chile and lowland accessions form a clear distinct grouping (Fig. 1), which correlates well with differences in the geographic/edaphic/climatic and cultural contexts.

In Chile, quinoa is mainly used for the farmers' own consumption and it has remained a marginal crop, different from others such as maize, potatoes or wheat, which are sold in the domestic markets. Quinoa is recognized for its culinary diversity and its properties as a high-quality food. It is also grown as an old habit acquired through the knowledge of their parents, who cultivated it since their childhood, associating it with

family memories in different ancient cultures. The generation changes are already affecting rural areas; indeed, one consequence of the mass migration from rural areas into cities in Chile is that agro-industries hire immigrants from the neighbouring countries of Peru and Bolivia for harvesting and other rural activities, due to the lack of local people. This is a global phenomenon (Taran & Geronimi 2004) from which Latin America, and particularly women, is not exempt (Staab 2003). However, quinoa is still maintained in the three major areas of its cultivation in Chile as well as in the rest of the Andes (Bhargava *et al.* 2006; Fuentes *et al.* 2009c), being an essential part of the intangible cultural rural heritage and identity (Nuñez *et al.* 2010).

In the southern Mapuche communities, it is still possible to identify the key elements of the quinoa history associated with the role of the different modern actors (farmers and institutions) involved in the biodiversity dynamics of this crop. The crop almost disappeared following the Spanish conquest, replaced by rice and wheat in the Mapuche diet first and then throughout the Chilean people. Nevertheless, some families never stopped growing quinoa and conserved their family *population varieties* as local collections of landraces. According to Anabalón Rodríguez & Thomet Isla (2009), they even maintained low levels of seed exchange between Coastal/Lowlands and Andean piedmont regions, separated by not more than 150 km (this is also supported by genetic analysis). However, the important role played by *curators* at intra- and extra-regional scales (Pérez 2005; Bazile *et al.* 2010a) could change the future dynamic of seed exchanges, and it will be necessary to determine the impact of these public exchanges on biodiversity dynamics.

This particular situation at the Coastal/Lowlands also exists in other regions of quinoa production. In the highlands, the altitude and climatic constraints determine a natural limit to wider seed exchanges. However, a better understanding of the Aymara crop system and the extent of trans-border trading could reveal the real diversity between Chile, Bolivia and Peru zones in the highlands (Fuentes *et al.* 2009c; Bazile *et al.* 2010c). The diversity of local varieties of quinoa has the same ranking among the people of the northern highlands of Chile (Villages of Colchane and Cancosa, near Bolivia) and south (Village of Socaire, near Argentina). The present results show that farmers define different varieties by agro-morphological characteristics such as the colour of the panicle of

quinoa. There is also a sub-classification of plants by size and panicles for each colour. In the present surveys, farmers identified more than 18 landraces, conserved at the village scale, including four to five colours, as was also observed in Bolivian diversity of the Quinoa Real type. Thus, for example in the village of Colchane (northernmost region of quinoa production for Chile), 0.90 of the farmers sow the *white quinoa*, then comes a second landrace in the crop system: the yellow, red and pink, used by 0.30–0.40 of farmers. Besides these four landraces there is another one which is used by less than 0.10 of the farmers. A future study on the dynamics of what farmers sow every year (number of farmers sowing a particular landrace and sown areas), and under what criteria they have saved certain seed types (and within what conditions of conservation) would be interesting.

The information provided by farmers in the central zone revealed the existence of a single landrace. However, the analysis of practices showed that sowing and harvest periods can vary within this landrace, with harvest being February to April. However, the existence of many differences in sowing to flowering and harvesting dates means that it is not possible to validate that all the farmers have the same landrace. The practices give evidence of two groups of landraces, one harvested early and one later (4 and 7 months growth duration, respectively), and each group has different coefficients of photoperiodism to justify the heterogeneity of practices, as shown in Table 4. Thus, the diverse handling of the production system by farmers in the central zone has generated a high biodiversity of quinoa types as revealed by the relationships within population II (Fig. 1). It is also true that some farmers do select for higher tolerance to salt stress by sowing on lands naturally invaded by brackish coastal waters close to river outlets (Ruiz-Carrasco *et al.* 2011).

However, the strengthening of international markets and export incentives for other products in the area could cause a loss of seed diversity in the future (Marti & Pimbert 2007). This could be caused by changes in land use or by homogenizing the seed throughout the zone, to respond to increasing market demands for quinoa (Martínez *et al.* 2010). This highlights the importance of linking sociological and agronomic studies to further a deeper analysis with molecular markers in order to validate different hypotheses of diversity levels such as, for instance, whether there is a hidden higher diversity within a single landrace, *white quinoa*, in the area of central Chile. In that case, even if

a great diversity of practices is observed along with high genetic diversity, the genetic erosion risk farms is very high, particularly due to small scale (Martínez *et al.* 2009b; Bazile *et al.* 2010b). Thus, this agricultural diversity needs to be measured and protected before the risk of it disappearing becomes too great.

Even today, farmers who inherited the traditions of quinoa consumption and associated culture, but belong to different highland and lowland regions, do not share quinoa germplasm. This is also due to different sowing and harvesting seasons, derived from very different photoperiods (Bertero *et al.* 1999; Bertero 2001), a limit that could be established around 25°S (Fig. 2). In previous experiments where quinoa ecotypes from highland and lowland seed sources were sown at 30°S (Coquimbo region), greater production was achieved by southern ecotypes than by high Andes ones (Martínez *et al.* 2007, 2009a, b). It is considered that 3–5 years are needed to adapt a landrace transferred between zones from a particular local region and 10 years for landraces from other regions further away (Aleman 2009).

Farming practices have a significant role in the distribution and conservation of genetic variation, thus increasing the resilience of the systems to production constraints and environmental changes (Sperling *et al.* 2008; Ferguson *et al.* 2011). Many plant genetic resources such as quinoa are increasingly being lost from traditional agricultural landscapes through external factors (e.g. population migration and commercial pressures). The utility of *ex situ* gene banks to conserve germplasm is not enough for two reasons: firstly, it is very difficult to capture or collect all the cultivated diversity with so many different landraces and variants, and secondly even if that were possible, the *ex situ* conservation cannot integrate other key elements associated with the genetic resources when conserved *in situ*, e.g. the agricultural knowledge and practices, and the associated culture, which are specific to the diverse array of agrobiodiversity conservation systems. To maintain this co-evolutionary adaptation and selection between the genetic resources and their *in situ* users, the central point is to conserve the characteristics of the agrobiodiversity dynamics, considering the whole associated social context.

The main conclusion from the present work is that the highly polymorphic microsatellite alleles present in the three main growing areas of quinoa in Chile (as well as of the rest of the Andean region) suggests the

existence of a large amount of genetic diversity and confirms the status of previously recognized ecotypes. The genetic information allows the detection of variation among and within the populations identified, which matches well with natural geographical–edaphic–climatic constraints to the expansion of quinoa biodiversity. This grouping also correlates well with the social–linguistic context of ancient people inhabiting the Andes region, where agronomic and cultural traditions that have survived until the present time are very different. Risks to biodiversity in this species are postulated due to export incentives for agricultural products that might favour none or fewer quinoa genotypes. Better incomes from export systems are normally obtained in a short time scale, although the highest profit is generally achieved by bigger land owners, whose greater numbers of hectares are cultivated with much higher investment. In such circumstances, small-scale farmers migrate or become employees of the agro-industry, and risk losing the culture and the agro-biodiversity of their landscapes.

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