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Research article

Genetic diversity of quinoa (*Chenopodium quinoa* Willd.) germplasm as revealed by sequence-related amplified polymorphism markers

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is an excellent example of a functional food being rich in antioxidants and well balanced regarding carbohydrates, lipids and protein, providing an ideal source of essential amino acids for human nutrition. Its nutritional characteristic has been emphasized both in developed and undeveloped regions resulting in many countries growing quinoa as a healthy food. Breeding programs for quinoa in new regions need diverse genetic variety. Therefore, the genetic diversity of 135 quinoa accessions was determined using 21 sequence-related amplified polymorphism (SRAP) markers generated from eight primer pairs. The polymorphic information content (PIC) was in the range 0.10–0.25 with an average of 0.19. Overall genetic diversity was relatively low in the range 0.17–0.40, with a mean of 0.32. Population structure, neighbor-joining tree and principal coordinate analyses revealed that the quinoa accessions were clustered into two groups. One was composed of 85 accessions mainly from the USA and Peru and some accessions from Chile and Bolivia. The other one comprised 50 accessions largely from Bolivia and Chile and some accessions from Peru. The results from this study suggested that the genetic diversity of the quinoa accessions from the USA was relatively low compared with other reports. Therefore, plant breeders need to develop new cultivars by crossing quinoa from different genetic groups or to access additional *ex situ* germplasm collections.

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Introduction

Quinoa (*Chenopodium quinoa* Willd.), an Andean grain crop (with regional importance in Bolivia and Peru) also known as pseudocereal, has been cultivated for 5 millennia in the Andes and is distributed in different agro-ecological zones within South America (Food and Agriculture Organization of United Nations, 2012). Currently, quinoa is cultivated as a food crop in many countries and has received worldwide attention because of its ability to thrive in harsh climates involving drought, frost, saline and acid soils and with minimum inputs (Jacobsen et al., 2003). Quinoa grains show great nutritional value, being high in protein content (7.5–22.1%) and free of gluten, while having a low glycemic index and plenty of essential amino acids (especially lysine, threonine and methionine), fiber, vitamins and minerals (Vega-Galvez et al., 2010). Due to the great adaptability of quinoa to various environments as well as its dietary benefits, this crop has been introduced into new growing areas outside its native region. More than 70 countries have trialed growing quinoa as a healthy food by applying modern breeding techniques to improve the yield and agronomic performance (Food and Agriculture Organization of United Nations, 2012).

Genetic diversity is crucial in plant breeding programs to improve agronomic traits and facilitate the global expansion of quinoa (Friedt et al., 2007). Crop molecular diversity analysis using DNA markers provides not only better understanding of the biological function of eco-geographic distribution of crop plants, but also helps in managing genetic resources and developing breeding strategies for crop plants. There have been some reports of molecular genetic diversity analyses of quinoa. Mason et al. (2005) developed 208 simple sequence repeat (SSR) markers of which only 67 were highly polymorphic and separated quinoa germplasm into two distinct groups (Chilean coastal ecotypes and Altiplano ecotypes). Castillo et al. (2007) studied the genetic relationships of quinoa from four distinct regions (the North, Center and South of the altiplano and inter-Andean valleys of Bolivia) using random amplified polymorphic DNA (RAPD) markers and showed that quinoa has a strong population structure and high intra-population variation due to geographical structure. Christensen et al. (2007) evaluated the genetic diversity of quinoa accessions from the USA Department of Agriculture (USDA) and the International Potato Center-Food and the Food and Agriculture Organization international nursery collections using SSR markers and classified quinoa germplasm into two distinct gene pools: Andean highland and lowland. Andean highland quinoa consisted of accessions from Peru, Bolivia, Ecuador, Argentina and northeastern Chile, while the Andean lowland consisted of accessions from Chile, which is a second center of quinoa diversity. In addition, Fuentes et al. (2009) used SSR markers to classify Chilean quinoa accessions and showed that they separated the accessions into two discrete groups: the southern lowlands and the northern highlands.

Sequence-related amplified polymorphism (SRAP) markers are polymerase chain reaction (PCR)-based-markers that are generated using primer pairs each with 17 or 18 nucleotides in length (Li and Quiros, 2001). The primers preferentially amplify open reading frames (ORFs) in the genome (Li and Quiros, 2001). SRAP is a relatively simple and highly reproducible DNA-based method and shows higher discriminating power than other molecular markers (Budak et al., 2004) especially, RAPD and amplified fragment length polymorphism (AFLP; Ferriol et al., 2003), which are dominant markers like SRAP. SRAP is a powerful marker for revealing genetic relationships among related cultivars (Ferriol et al., 2003; Lin et al., 2004) and can be used for linkage map construction (Li and Quiros, 2001; Yeboah et al., 2007) and comparative genetics of different species. For example, Xu et al. (2014) used 13 SRAP primers to evaluate genetic relationships among the genus *Malus* Mill. They reported that SRAP primers showed a high degree of polymorphism and some of the grouped genotypes were highly congruous for geographical distribution. Furthermore, genetic diversity analysis using SRAP markers is more congruent to the morphological variation than AFLP markers (Ferriol et al., 2003) which could result from the fact that SRAP markers have been designed from the ORF region which could be linked to genes, thus making them related to phenotype.

Even though Christensen et al. (2007) evaluated the genetic diversity of quinoa from the USDA and other resources with SSR markers, in the current study used SRAP markers to investigate the diversity and population genetic structure of 135 quinoa accessions from the USDA which were 30% different from Christensen et al. (2007). The objective of the current study was to determine the degree of similarity or dissimilarity among germplasms and to determine the extent of genetic diversity for proper utilization in breeding programs.

Materials and Methods

Plant materials

Samples (135) quinoa accessions from various origins were obtained from the USDA. These accessions were grown

in an experimental field of the Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom, Thailand and were used in this study (*Supplementary Table 1*).

DNA extraction

Total genomic DNA was extracted from the young leaves of each plant using a modified cetyl trimethyl ammonium bromide method described by Lodhi et al. (1994). The quality and quantity of the DNA were determined using agarose gel electrophoresis and a spectrophotometer (NanoDrop 8000; Nanodrop Technologies; USA). The total DNA was diluted in a tris-ethylene-diamine-tetraacetic acid buffer to a concentration of 10 ng/μL for SRAP marker analysis.

Sequence-related amplified polymorphism marker analysis

In total, 154 SRAP primer combinations were used to screen for amplification and polymorphism in the quinoa accessions from five different origins (the USA, Peru, Bolivia, Mexico and Chile). PCR amplification was carried out in a total volume of 10 μL comprising 20 ng of genomic DNA, 5 mM each of forward and reverse primers, 1× *Taq* buffer, 200 mM dNTP, 2mM $MgCI_2$, and 1 U *Taq* DNA polymerase (Thermo Scientific; USA. Amplification was performed on a PCT-100™ Thermal Controller (Applied Biosystems; USA) with the following profile: 94°C pre-denaturation for 4 min, followed by 5 cycles of 94°C denaturation for 1 min, annealing at 35°C for 1 min, 72°C extension for 1 min, followed by 35 cycles of 94°C denaturation for 1 min, annealing at 50°C for 1 min, 72°C extension for 1 min and the final extension at 72°C for 10 min. The PCR products were run on 3% agarose gel using a 1 kb ladder (Thermo Scientific; USA as the standard size, stained with ethidium bromide and visualized under ultraviolet light. The primer combinations showing polymorphic DNA bands were selected and used to analyze the DNA of all quinoa accessions.

Data analysis

The polymorphic markers (band) were each scored as binary data ("1" for presence and "0" for absence of each marker locus). The polymorphism information content (PIC) of each locus was calculated according to Anderson et al. (1993). The gene diversity of each locus was calculated using the POPGENE 1.31 software (Yeh et al., 1999).

Population structure analysis was performed using the STRUCTURE 2.3.4 software (Pritchard et al., 2007). The software applies a Bayesian algorithm for genetic clustering. The optimum number of clusters (subpopulation; *K*) was determined based on the Δ*K* method (Evanno et al., 2005) by running 20 independent simulations of the STRUCTURE using K from 1 to 10 with the "admixture" and "allele-frequency independent" model, a burn-in period of 10,000 and 50,000 replicates of Markov Chain Monte Carlo (MCMC). Subsequently, the software was run with the optimum *K*, burn-in period of 100,000 and 500,000 replicates of MCMC to assign individuals to each subpopulation.

Jaccard's coefficient (Jaccard, 1901) among individual accessions was determined using the R software program (R Development Core Team, 2013) and then subjected to principal coordinate analysis (PCoA) using the NTSYSpc 2.2 software (Rohlf, 1993) to reveal relationships among them. The coefficient was also used to construct a phylogenetic tree based on neighbor joining (NJ) analysis using the MEGA6 software (Tamura et al., 2013). A cophenetic correlation coefficient (*r*) was estimated to specify the quality level of dataset clustering (Sokal and Rohlf, 1994) using the same software for the PCoA analysis.

Results

Sequence-related amplified polymorphism

Among the 154 SRAP primer combinations screened for amplification and polymorphism in the five quinoa accessions, 145 (94.16%) were able to amplify the DNA of quinoa. Among the amplifiable primer combinations, 95 (65.51%) were polymorphic, while the remaining 50 (34.49%) were monomorphic. However, only eight primer combinations showed clearly scorable polymorphic DNA bands and were selected for diversity analysis in all the DNA accessions (Table 1). The polymorphic primer combinations produced a total of 21 polymorphic loci, ranging from 1 locus (Em7-Me1) to 4 loci (Em5-Me2). Among these primers, Em7-Me1 had the lowest percentage of polymorphic bands (50%) while Em6-Me5, Em9-Me1, Em11-Me2 and Em14-Me2 had 100% polymorphic bands. The mean PIC value for all primers (0.19) was low. The highest (0.25) and the lowest (0.10) PIC values were obtained from the Em7-Me1 and Em8-Me3 primer combinations, respectively. The genetic diversity was in the range 0.17–0.40 with a relatively low average of 0.32.

Table 1 Number of polymorphic bands, genetic diversity and polymorphic information content (PIC) value of eight polymorphic sequence-related amplified polymorphism primer combinations amplified in 135 quinoa accessions

Primer combination	Total number of bands	Number of polymorphic bands	Polymorphic bands (%)	Genetic diversity	PIC
Em5-Me2	5	4	80	0.35	0.22
Em6-Me2	4	3	75	0.30	0.23
Em6-Me5	3	3	100	0.34	0.14
Em7-Me1	2	1	50	0.17	0.25
Em8-Me3	3	2	67	0.21	0.10
Em9-Me1	2	2	100	0.39	0.18
Em11-Me2	2	2	100	0.40	0.15
Em14-Me2	4	4	100	0.39	0.24
Total	25	21	-	-	-
Average	3.13	2.63	83.96	0.32	0.19

Population structure analysis

The STRUCTURE software was used to perform population structure analysis with the marker data generated from all 21 polymorphic marker loci. Based on the *ad-hoc* Δ*K* analysis (Evanno et al., 2005), there were two subpopulations for the 135 quinoa accessions (Fig. 1). Subpopulation I consisted of 85 accessions (Fig. 2 and *Supplementary Table 2*), consisting of 42 accessions from the USA, 25 from Peru, 5 from Bolivia, 7 from Chile, 2 from Mexico and 4 from Argentina. Subpopulation II consisted of 50 accessions (Fig. 2 and *Supplementary Table 2*), with 28 from Bolivia, 10 from Chile, 7 from Peru, 4 from the USA and 1 from Ecuador.

Neighbor-joining analysis

A neighbor-joining tree based on the genetic distances of the 135 quinoa accessions (Fig. 3) showed a similar clustering pattern to that revealed by STRUCTURE. All quinoa accessions were grouped into two major clusters (I and II). The neighbor-joining tree was well supported by a high cophenetic correlation coefficient of 0.86. Cluster I consisted of 91 accessions, with 44 from the USA, 12 from Bolivia, 6 from Chile, 2 from Mexico, 24 from Peru, and 3 from Argentina. Cluster II consisted of 44 accessions with 2 from the USA, 21 from Bolivia, 11 from Chile, 7 from Peru, and 1 each from Argentina and Ecuador.

Principal coordinate analysis

The PCoA based on Jaccard's similarity revealed that the first, second and third axes explained 15.95%, 9.27%, and 8.08%, respectively, or 33.30% of the total variation. The PCoA plot revealed a similar grouping of accessions to the neighbor-joining tree and STRUCTURE analyses. A two-dimensional principal component (PC) plot (PC1 versus PC2) of the 135 quinoa accessions is illustrated in Figure 4. PC1 separated the accessions into two major groups (populations), namely I and II, which were mainly distributed in the upper half of the plot. Group I was in the upper right of the plot and comprised all accessions except two from the USA, almost all accessions from Peru, some accessions from Bolivia, about one-half of the accessions from Chile, all three accessions from Argentina and both the accessions from Mexico. Group II was in the upper left of the plot and was composed of some accessions from Bolivia and one-half of the accessions from Chile.

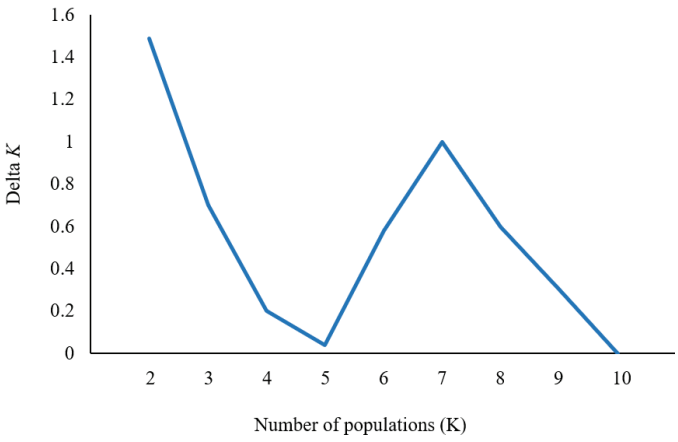

Fig. 1 Determination of optimum number of clusters based on ΔK (Delta K) method (Evanno et al., 2005) for K = 1 to 10

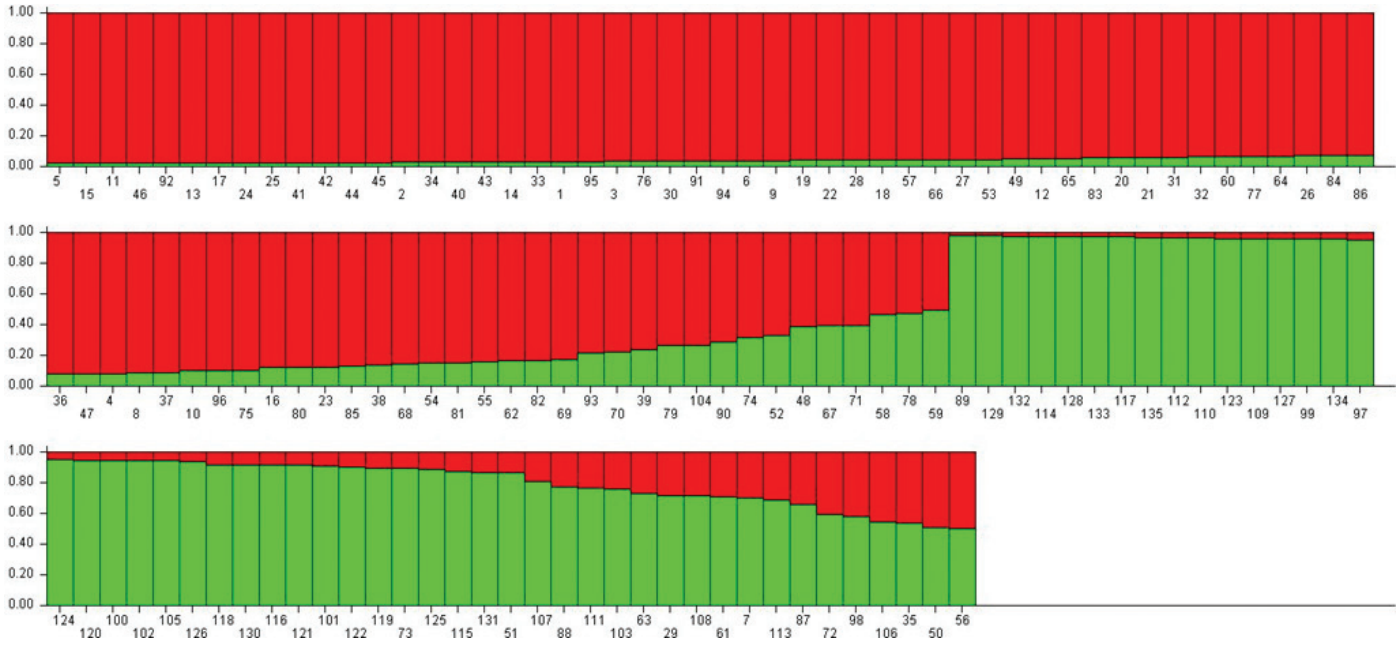

Fig. 2 Population structure of 135 quinoa accessions as determined using STRUCTURE analysis based on sequence-related amplified polymorphism information, where each bar is divided into segments indicating its genetic composition (red indicates subpopulation I membership and green indicates subpopulation II membership) and numbers below bar plots represent sample numbers in *Supplementary Table 2*

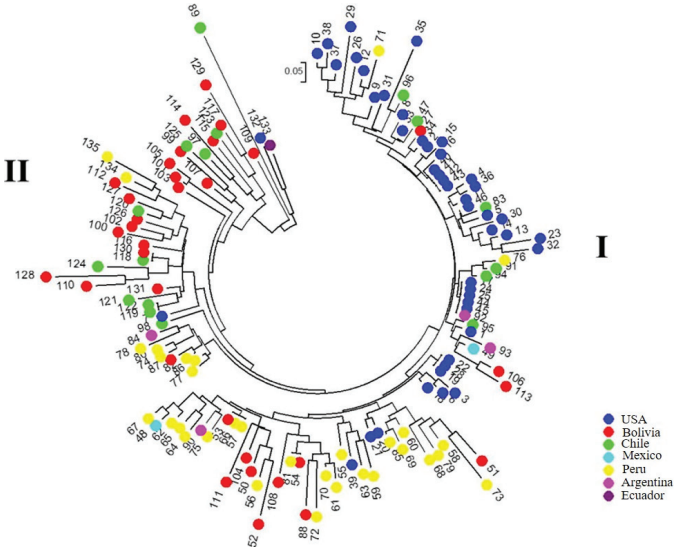

Fig. 3 Neighbor-joining trees of 135 quinoa accessions based on genetic distance using sequence-related amplified polymorphism markers, where color of each sample corresponds to origin of sample as shown in *Supplementary Table 2*

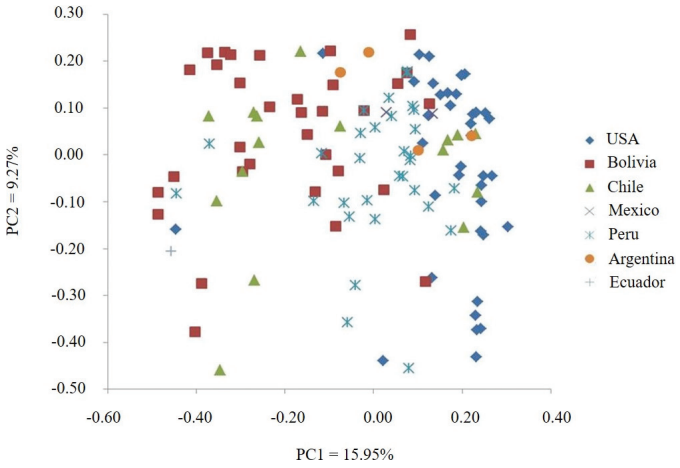

Fig. 4 Two-dimensional plot of first two principal components (PC1 and PC2) from principal coordinate analysis based on genetic distance among 135 quinoa accessions

Discussion

The current study was the first to use SRAP as a basic molecular marker of genetic variability among quinoa germplasm. The SRAP amplification showed that the number of polymorphic bands per primer pair ranged from only 1 up to 4 with an average of 2.63 bands. This was quite low compared with other studies. For example, Gulsen et al. (2009) reported 5.4 bands per primer in 182 bermudagrass (*Cynodon dactylon* (L.) Pers.) accessions. Soleimani et al. (2012) reported 10.2 bands per primer in 63 pomegranate (*Punica granatum* L.) accessions, while Zhao et al. (2020) found 13.5 bands per primer in 33 *Paris polyphylla* accessions. The low number of polymorphic loci per primer pair in the current study was very likely due to the fact that the SRAP markers preferentially amplify open-reading frames of genes, which differ in each species (Li and Quiros 2001; Ferriol et al., 2003). The PIC value was used to measure the efficiency of polymorphic loci in revealing genetic diversity among the accessions. In the current study, the PIC value for the SRAP markers was in the range 0.1–0.25 with an average of 0.19, indicating that the quinoa accessions had a low range of genetic diversity. However, the highest PIC value was related to Em7-Me1, which suggested it as the most informative for genetic diversity studies among these quinoa genotypes, despite producing only one polymorphic band. The average genetic diversity (0.32) in the quinoa germplasm from the USDA detected using the SRAP markers was relatively low, indicating low diversity in this germplasm. However, this finding contrasted with that of Christensen et al. (2007) who reported high genetic diversity (0.75) for 153 quinoa germplasm accessions. This dissimilarity could have been due to Christensen et al. (2007) bulking the DNA of each accession, resulting in greater diversity although low phenotypic variation was observed. In addition, different types of markers were used in the current study and by Christensen et al. (2007). The SRAP used in the current study revealed low polymorphic information content due to this type of marker amplifying the ORF region which is conserved in the genome, while the SSR markers used by Christensen et al. (2007) amplify the repetitive sequence 2–6 bp long which is abundant in the genome. This could have influenced finding low genetic diversity in the current study.

The STRUCTURE, Δ*K* values, PCoA and neighbor-joining analyses based on SRAP data consistently demonstrated that the 135 quinoa accessions from diverse origins were clustered into two groups (populations) as shown in Figs. 1, 2 and 3. The results agreed with previous findings reported by Zhang et al. (2017) and Christensen et al. (2007), who reported two centers of diversity in quinoa: 1) the Andean highlands of Peru and Bolivia; and 2) the lowlands of the USA and Chile. However, in contrast to their results, in the current study there was one major group of quinoa (subpopulation I, cluster I and group I based on the STRUCTURE, NJ and PCoA analyses, respectively) mostly comprised of germplasm from the USA and Peru, while the other major group of quinoa (subpopulation II, cluster II and group II based on the STRUCTURE, NJ and PCoA analyses, respectively) comprised germplasm from Bolivia and Chile (Figs. 1, 2 and 3). Due to the close genetic relationship between the germplasm from the USA and Peru in the current study, it was assumed that the quinoa accession from the USA might have originated from Peru because there was no original information on quinoa from the USA collected by Emigdio Ballón as reported by Christensen et al. (2007). The clustering of the two groups (Bolivian or Chilean quinoa germplasm) in the current study was in agreement with results showing that one major group consisted of Bolivian quinoa germplasm (Castillo et al., 2007) and the second group consisted of Chilean quinoa germplasm from the north (Andean highlands) and the south (lowland or coastal) according to Mason et al. (2005) and Fuentes et al. (2009).

In the current study, the genetic diversity was greatest in the quinoa germplasm from Bolivia; however, the genetic diversity of the germplasm from Chile was comparable with that from Bolivia. The germplasm from these two countries constituted subpopulation II. The genetic diversity in subpopulation II was much higher than in subpopulation I that was consisted of the germplasm from several countries (some from Bolivia and Chile were included in subpopulation I). The clustering of germplasm from Bolivia and Chile in subpopulation II supported the hypothesis proposed by Wilson (1988a) that quinoa germplasm from Chile was more similar to quinoa germplasm from Bolivia than elsewhere. Based on the genetic diversity together with genetic cluster analyses, the current results suggested that a center of diversity of quinoa is the Bolivian-Chilean region. Based on the high morphological diversity of germplasm from the Peruvian-Bolivian region, Gandarillas (1979) proposed the Peruvian and Bolivian southern highlands as the center of domestication of quinoa. Based on morphological and isozyme variations in 98 diverse quinoa accessions, Wilson (1988b) proposed that the origin of diversity of quinoa was around Lake Titicaca, a border region between Bolivia and Peru. This was supported by the high molecular diversity of the quinoa germplasm found in the Bolivian-Peruvian region (Christensen et al., 2007). The high diversity in Chilean quinoa found in the current study may

have resulted from extensive natural hybridization between cultivated quinoa and wild quinoa *Chenopodium hircinum* (Shrad.) (Fuentes et al., 2009). Nonetheless, the results from the current and these other studies illustrated that germplasm from Bolivia and Chile had high genetic diversity, thus indicating that the region of Bolivia and Chile is a center of diversity of quinoa.

Ethical Statements

Informed consent was obtained from all individual participants included in the study. This article does not contain any studies involving human participants performed by any of the authors.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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