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Development and Use of Microsatellite Markers for Genetic Diversity Analysis of Canahua (*Chenopodium pallidicaule* Aellen)

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Development and Use of Microsatellite Markers for Genetic Diversity Analysis of Cañahua (*Chenopodium pallidicaule* Aellen)

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ABSTRACT

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Cañahua (*Chenopodium pallidicaule* Aellen) is a poorly studied, annual subsistence crop of the high Andes of South America. Its nutritionally value (high in protein and mineral content) and ability to thrive in harsh climates (drought, extreme elevations, etc.) make it an important regional food crop throughout the Andean region. The objectives of this study were to develop genetic markers and to quantify genetic diversity within cañahua. A set of 43 wild and cultivated cañahua genotypes and two related species (*C. quinoa* and *C. petiolare*) were evaluated for polymorphism using 192 microsatellite markers derived from random genomic sequences produced by 454 pyro-sequencing of cañahua genomic DNA. In addition, another and 424 *C. quinoa* based microsatellite markers were evaluate as potential cross-species marker loci. A total of 48 polymorphic microsatellite marker loci were identified which detected a total of 168 alleles with an average of 3.5 alleles per marker locus and an average heterozygosity value of 0.47. A cluster analysis, based on Nei genetic distance, grouped the cultivated cañahua into a single dominant branch clearly separated from wild cañahua genotypes and the outgroup species. Within the cultivated genotypes, two dominant subclades were present that were further partitioned by AMOVA analysis into five model-based clusters. Significant correlations were found between genetic distance and morphological traits. The isolation by distance test

displayed no significant correlation between geographic collection origin and genotypic data, suggesting that cañahua populations have moved extensively, presumably via ancient food exchange strategies among native peoples of the Andean region. The molecular markers reported here are a significant resource for ongoing efforts to characterize the extensive Bolivian and Peruvian cañahua germplasm banks, including the development of core germplasm collections needed to support emerging breeding programs.

Keywords: Genetic diversity, cañahua, microsatellite markers, population structure.

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Chapter 1. Development and Use of Microsatellite Markers for Genetic Diversity Analysis of Cañahua (*Chenopodium pallidicaule* Aellen)

INTRODUCTION

Cañahua (*Chenopodium pallidicaule* Aellen) is a nutritionally important annual crop with regional importance in Bolivia and Peru. It is an annual diploid (2*n*=2x=18) species of the large, but poorly studied, Amaranthaceae family (subfamily Chenopodioideae, Muller and Borsch, 2005; Bisby et al., 2002). Cañahua's growth habits can be classified as erect, semiprostrate or prostrate. Height (ranging from 25 to 60 cm tall, Galwey, 1989; Gade, 1995) and maturity (95 to 173 days, Flores, 2006; Rodriguez, 2007) range dramatically among ecotypes. Current cultivation of cañahua occurs in two major centers, namely the in the departments of La Paz, Bolivia and Puno, Perú, although minor cultivation extends into the Bolivian departments of Oruro, Cochabamba, and Potosi. Cañahua yields in these departments range from 375 to 2968 kg /ha (Marin, 2002; Flores, 2006; Rodriguez, 2007). Cañahua is uniquely adapted to the harsh climatic conditions that characterize much of the Andean Altiplano, including extreme elevations (~4000 m.a.s.l), frequent frosts and hail, and arid-saline soils (rain fall between 500 to 600mm, pH 4.8) (Risi and Galwey, 1984; NRC, 1989; Tapia and Fries, 2007). Indeed few other cultivated species can be cultivated on the Altiplano, a region inhabited by nearly three million subsistence farmers, reinforcing cañahua's importance as a regional food security crop.

The percent protein content of the cañahua grain ranges from 14 to 19 % (IPGRI et al., 2005), with a superior balance of essential amino acids – similar to that of soybean (Repo-Carrasco et al., 2003). Moreover, cañahua flour is high in dietary fiber, phytates and antioxidants, as well as iron and calcium (Repo-Carrasco et al., 2003; Wood and Eyzaguirre, 2004; Repo-Carrasco et al., 2009). Traditional uses of cañahua include the use of flour made from toasted grain to treating altitude sickness and fatigue (Bonifacio, 2003). Anciently cañahua was an integral part of the subsistence farmers' reciprocal food exchange/acquisition strategy – a

seasonal food-trading network practiced between farming communities located at different altitudes (Rist, 2000; Marti and Pimbert, 2007). Such strategies were important for insuring food security and nutrition. Similar food network were also practiced in Central America (Gumerman, 1997).

Genetic diversity is an important aspect of any improvement program (Friedt et al., 2007). A primary concern regarding the future improvement of cañahua is the loss of *in-situ* genetic diversity. Poverty, political unrest and climate destabilization have accelerated a massive human exodus from the Andean Altiplano. Efforts to conserve the genetic diversity of cañahua have led to the collection and development of two main germplasm banks (IPGRI et al., 2005; Flores, 2006). The largest collection, consisting of 801 accessions, is maintained at the National Germplasm Bank of High Andean Grains at the Foundation for the Promotion and Investigation of Andean Products (PROINPA) in Quipaquipani, Bolivia, while another 460 accessions are maintained by the Universidad del Altiplano in Illpa-Puno, Perú (UNA). Initial attempts are being made to develop core-breeding collections based on eco-geographical descriptors, morphology and molecular data. Here we report the development of the first molecular markers for cañahua. Several of these markers are based on microsatellite sequences first identified in *C. quinoa* (Mason et al., 2005), a related Andean crop species, while others are cañahua specific. The specific aims of this project were to i) assess the potential use of *C. quinoa* microsatellite markers in cañahua; ii) develop and characterize new microsatellite markers specific to cañahua developed via random 454-pyrosequencing of the cañahua genome; iii) evaluate the correlation between morphologic characteristics and genetic distance using microsatellite genotypic data; and iv) determine the patterns of population structure among cañahua genotypes.

MATERIALS AND METHODS

Plant material

A total of 43 diverse cañahua genotypes were used in this study, including thirty genotypes from Bolivia and 13 genotypes from the USDA-ARS germplasm collection (Table 1). Additionally, a single accession of *C. quinoa* (cv. 'Ollague') provided by A. Mujica; UNA, Puno, Peru and a single genotype of *C. petiolare* Kunth, a sympatric diploid weed species collected by F. Fuentes; UNAP, Iquique, Chile, were also included in our analysis for comparative purposes. We note that we use the terminology "genotypes" to describe the cañahua *C. petiolare* samples as they represent subsamples from potentially heterogeneous germplasm collections. Passport (origin) data, domestication status and morphological data are reported in Table 1. Morphological characteristics, collected from greenhouse grown plants (Provo, Utah, 2008) and field plots (Letanias, Bolivia, 2005) included: i) growth habit (prostrate, shrubby, erect); ii) plant color (yellow, pink, red); iii) seed coat color (white, brown, black); iv) pericarp color (beige, grey, khaki, light orange, dark orange, light brown, dark brown, firebrick, saddle brown); and v) downy mildew symptoms (present, absent).

Cañahua plants grown in the greenhouses at Brigham Young University (Provo, Utah, USA) were grown in 12 cm pots using Sunshine Mix II (Sun Grow, Bellevue, WA), supplemented with 2 g per pot of Osmocote Plus® fertilizer (Scotts, Marysville, OH). Plants were maintained at 18˚C under broad-spectrum halogen lamps with a 16-h light.

Cañahua specific microsatellite discovery, primer design and PCR

Genomic DNA used for sequencing and PCR was extracted from 30 mg of freeze-dried apical leaf tissue from greenhouse grown plants following procedures described by Todd and Vodkin

(1996). To identify cañahua specific microsatellites, randomly sheared genomic DNA of the genotype "Ames 13223" was sequenced using 454 FLX pyrosequencing technology (BYU-DNA Sequencing Center, Provo, Utah). Raw DNA sequences were cleaned of vector sequences scanned for perfect microsatellite motifs using the computer program MISA (Thiel et al., 2003) with the following parameters: di-, tri- and tetra-nucleotide motif repeat units of 8, 6 and 4, respectively. Forward and reverse primers were designed using the computer program Primer3 v2.0 (Rozen and Skaletsky, 2000) with the following parameters: product size = 150–225, max Tm difference = 1˚C and max polyX = 3. All primers were screened on a preliminary panel of eight cañahua genotypes (Table 1). The screening panel was used to eliminate primer pairs that failed to amplify or amplified complex banding pattern. Primer pairs that produced simple banding patterns (presumable single loci) were then screened on the full panel of 43 diverse cañahua genotypes. Cross species *C. quinoa* microsatellites primer pairs were also screened for polymorphism using a smaller panel of three cañahua genotypes and one *C. quinoa* genotype (Table 1). Polymorphic markers derived specifically from cañahua genomic sequence were sequentially named with the suffix "CP" (*C. pallidicaule)* followed by microsatellite motif type (AT, AAT, and AAAT). *C. quinoa* derived microsatellites are labeled with the suffix "Q" or "K" as described by Mason et al. (2005) and Jarvis et al. (2008; Table 2).

PCR amplification of the microsatellite markers was performed in 10-µl PCR reactions containing 30 ng genomic DNA, 0.5 units of HotStarTaq DNA polymerase (Qiagen-Aldrich, Inc., Saint Louis, MO), 0.5 μM forward primer, 0.5 μM reverse primer, and 0.1 *m*M cresol red. Thermal cycling profiles were as follows: 94˚C for 30 s, followed by 19 cycles of 94˚C for 60 s, 55˚C for 30 s (decreasing 0.5˚C every cycle), 72˚C for 60 s; 34 cycles of 94˚C for 30 s, 55˚C for 60 s, 72˚C for 60 s, followed by a final extension at 72˚C for 10 min. PCR products and

molecular ladder were separated on 3% Metaphor agarose gels (Cambrex Bio Science, East Rutherford, NJ) at 120 V for 5 hours. All gels were run in 0.5X TBE buffer and visualized using ethidium bromide staining with UV transillumination.

Statistical analysis

Alleles for each microsatellite marker were scored as present, absent, or missing and converted into a binary matrix and analyzed using various statistical analysis programs. Evaluation of microsatellite marker polymorphism was measured by the heterozygosity (*H*) value for each marker as described by Nei (1978) using PowerMarker v3.0 (Liu and Muse, 2005). The degree of inbreeding in the cañahua was estimated by fixation index (*F*), while the apparent outcrossing rates (t_a) were obtained from the inbreeding coefficient *F* [$t_a = (1- F) / (1+ F)$] on the basis of the average expected and observed heterozygosities (Campos Vaz et al., 2009).

The genetic distance among genotypes was calculated based on the matrices of allele frequencies using PowerMarker v3.0 (Liu and Muse, 2005) and Nei distance to infer intrapopulation variation (Nei and Li, 1979). The clustering criterion used was neighbor joining and the resulting dendrogram was rooted using *C. quinoa* and *C. petiolare* genotypes as the out-groups.

The population structure analysis was based on a Bayesian approach using the computer programs Structurama (Huelsenbeck et al., 2007) and Structure (Pritchard et al., 2000; Evanno et al., 2005). Structurama was used to assign individuals to each subpopulation. The subpopulation numbers (*k*) were summarized using a Bayesian Markov Chain Monte Carlo (MCMC) analysis of population structure using the mean partition (Huelsenbeck and Andolfatto, 2007). The sitting parameters for Structurama were: MCMC ngen=2,000, samplefreq=1,000,

printfreq=1,000 and nchains=2. Later the predefined population number (k = 6) was placed into Structure (Pritchard et al., 2000) to infer the population structure using a length period of 100,000, number of MCMC reps after burning period of 1,000,000, and a model allowing for admixture and correlated alleles frequencies (Achigan-Dako, 2008). An admixture proportion was given by the average value of Q, which gave the probability that each genotype corresponded to each cluster.

An analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was carried out using Arlequin 3.5.1.2 (Excoffier et al., 2005; Schneider et al., 2000) to estimate genetic variance components and to partition the total variance within and among clusters generated from the Structure program. The significance of variance components was tested using non-parametric permutations of the data set with 1,000 permutations (Weir and Cockerham, 1984: Excoffier et al., 1992; Weir, 1996). The partitioned genetic effects were attributable to differences among hierarchical clusters (F_{ST}), among clusters within hierarchical cluster (F_{SC}) and among genotypes across the entire collection (F_{CT}). A Pearson correlation analysis was used to identify pattern of association between genetic distances, morphological characteristic, and clusters found by the Structure program.

The isolation by distance (IBD) (Wright, 1943) analysis was tested by ade4 package in R program (Bisby et al., 2002; Dray and Dufour, 2007). First, the matrix pairwise Nei genetic distances (Nei and Tajima, 1983) were calculated using PowerMarker v3.0 (Liu and Muse, 2005). Second, geographic locations of the genotypes were converted into negative values and then arranged in a matrix. Third, the IBD test was performed by assessing the correlation between these two matrices in order to understand the correlation between spatial location of the genotypes where were collected and the genetic distance. Then, the correlation matrix was

calculated and plotted. This correlation tested the null hypothesis that the Bolivian and Peruvian genotypes' correlation is exactly zero.

RESULTS

Marker Characterization in Cañahua. 454-pyrosequencing produced 116,912 sequences (~28.986 Mb). With a genome size of 1044 Mb (estimated by flow cytometry, data not reported), approximately 0.0277X of the total genome was sequenced. Automated searches for microsatellite motifs within the preassembled sequence using the computer program MISA analysis identified a total of 1000 potential microsatellite markers, including 389 di-, 538 tri- and 73 tetra-nucleotide repeat motifs or an approximate microsatellite density of 1 per 29 kb. The most abundant di-, tri-, and tetra-nucleotide motifs identified were AT (71%), AAT (68%), and AAAT (70%), respectively (Fig. 1). When 192 of these putative microsatellite loci were tested for polymorphism, 161 (85%) produced simple amplification products; however only 36 (26%) were polymorphic in the full screening panel (Table 2; Fig. 2). Of these 36 polymorphic microsatellite loci, the AT-rich motifs were clearly the most polymorphic, representing greater than 75% of all polymorphic motifs. To accumulate additional microsatellite loci, we also tested the transferability of microsatellite markers, originally identified in *C. quinoa,* to cañahua. In total, 424 *C. quinoa* microsatellite markers (Mason et al., 2005; Jarvis et al., 2008) were similarly evaluated for polymorphism using the cañahua preliminary and full screening panels. Of these markers, 170 (40%) produced simple banding patterns including 12 (6%) that were clearly polymorphic (Table 3). Of these 12 microsatellites loci, the most common polymorphism motifs were the dinucleotide CA (42%) and the tri-nucleotide CAA (33%). We note that the change in the most common polymorphic motif is likely due to the use of enriched motif libraries

during the development of the *C. quinoa* markers (Mason et al., 2005). Table 2 describes each polymorphic marker according to marker name, primary motif, complexity (simple/compound), type (perfect/imperfect), amplification primer sequences, and expected PCR product size.

Genetic diversity. The information content of a microsatellite marker is a measure the observed number of alleles (ONA) and their corresponding relative frequencies within the test population and is often expressed in terms of heterozygosity (*H*) (Höglund, 2009). Screening the full panel of 43 cañahua genotypes identified 168 alleles spread across the 48 microsatellite loci, ranging from a low of 2 to a high of 8 alleles per locus, or an average of 3.5 alleles per marker locus (Table 4). Heterozygosity values for the microsatellite loci ranged from 0.101 to 0.767, with a mean value of 0.467. According to the criteria of Ott (1992), a marker locus is considered highly polymorphic if $H \geq 0.70$, and polymorphic if $H \geq 0.10$. Thus, all but three of the markers were considered polymorphic and three markers were considered highly polymorphic (Table 3; Fig. 2). We note that these *H* values may be underestimated since 3% Metaphor agarose was used to resolve the microsatellite alleles. In our experiments, 4-bp resolution was achieved, however, others report 2-bp resolution with 3.5% Metaphor agarose (Groben and Wricke, 1998). The use of Metaphor agarose parallels electrophoresis technology available in many developing countries and represents a cost-effective method to analyze microsatellite markers in terms of technical expertise and the reagent cost.

Relatedness of the cañahua genotypes. The unrooted tree shows two dominant branches, one of which contains a sub-branch consisting of the wild cañahua genotypes (cp 43, cp17 and cp44) and the outgroup species (Fig. 3A). Similarly, the rooted Neighbor Joining (NJ) analysis revealed two dominant clades (Fig. 3B). The first clade was characterized by a single wild cañahua genotype (cp44) and the out-group species. The second clade consisted of all the

cultivated cañahua genotypes and a highly dissimilar subclade consisting of the remaining two wild cañahua genotypes (cp43 and cp17). We note that the wild genotypes (cp44, cp43, and cp17) were collected at the periphery of cultivated fields and were categorized as wild cañahua based sole on morphological similarities. Unfortunately, almost no phylogenetic research has been conducted in this genus, thus the question of the species relationship among the wild and cultivated genotypes is unclear. Indeed, our analysis suggest that at least one of the wild genotypes (c44) is genetically more closely associated with the outgroup species *C. petiolare* than it is with the cultivated or other wild genotypes, indicating that it may have been misclassified as *C. pallidicaule* and may represent a different (although related) species or an interspecific hybrid. The positioning of the other wild genotypes, cp43 and cp17 is also of interest as they are grouped within the cultivated cañahua clade (Fig. 3B) but are clearly genetically dissimilar, suggesting their potential to serve as novel sources of genetic variation within emerging cañahua breeding programs.

The cultivated genotypes were clearly distributed into two main clades, each with numerous subclusters. Analysis of the data with the computer program Structure produced the highest log likelihood scores when the number of subclusters (*k*) was set at six, including five cultivated subclusters (Fig. 3B). Of the five cultivated clusters, little differentiation based on morphological characteristics was observed, except for cluster 3, which consisted of six of the eight erect growth habit genotypes. The remaining two erect growth habit genotypes were found in clusters 4 and 5. In addition to the 26 genotypes that were clearly identified as belonging to a single model-based cluster, 17 genotypes were identified as having admixed ancestry (Fig. 3B). The genetic differentiation among the clusters was analyzed by AMOVA and significant ($P < 0.001$) genetic variance occurred within and among clusters. The variance within clusters

accounted for the largest portion (70.6%) of the total variance, whereas 29.4% of the variance was conserved among clusters.

An isolation by distance test, used to determine the relative influences of geographic distance (origin of collection) on genetic similarity among genotypes, was not significant (R^2 = 0.0037; P > 0 .05). Correlation analysis of the genetic and phenotypic data for all morphological traits revealed a significant positive, but low correlation (R^2 = 0.0629; P = 0 .0214). Similarly, the determination coefficient of individual traits with genetic distance was also low (growth habit, R^2 = 0.053; plant color, R^2 = 0.010; seed coat color, R^2 = 0.022; and pericarp color, R^2 = 0.027) and suggests that individually these morphological traits are not predictive of genetic diversity, perhaps due to the limited variation within the traits. Lastly, the average fixation index, which refers to the degree of inbreeding in the population, was high (F = 0.98), and was reflected as low level of predicted outcrossing (t_a = 0.011; Table 3).

DISCUSSION

The transferability of microsatellite loci across plant genera and species is based on the successful amplification of simple and polymorphic amplification products in the target species. In this study we showed the potential transferability of microsatellite loci originally identified in *C. quinoa* to its sister taxon cañahua. Indeed, forty percent of all *C. quinoa* microsatellite markers successfully amplified a single band of similar length in cañahua – a result that confirms the close ancestry of the two species. While the number of polymorphic cross-species microsatellites was low (12%) these markers will undoubtedly be useful for establishing syntenic relationships between the *C. quinoa* and cañahua genomes and suggest the possibility to exploit genetic information across the two species. Similar levels of cross-species transferability have

been reported among *Vitis* species (Di Gaspero et al., 2000), *Phaseolus* species (Gaitán-Solís et al., 2002) and *Amaranthus* species (Mallory et al., 2008).

The most common microsatellite motifs in the cañahua-specific microsatellites were AAT and AT. High frequencies of AT-rich microsatellites have also been observed in many other species of the Chenopodioideae, including quinoa and sugar beet (Mörchen et al., 1996; Jarvis et al., 2008). Mason et al. (2005) correlated the degree of polymorphism with the motif length in *C. quinoa* and suggested that the rate of polymorphism increases dramatically when the tandem repeat length is greater than 20 bp. Similar results were found by Mallory et al. (2008) in the related Amaranthoideae subfamily species amaranth (*A. hypochondriacus*), suggesting that the future development of microsatellites in cañahua should focus on the identification of markers with repeat lengths of greater than 20 bp in AT-rich motifs in order to ensure high discovery rates of polymorphic markers. Such parameters are invaluable, especially in light of the ease of microsatellite discovery from genomic DNA using next-generation sequencing technology. For example, a single pico-titer plate of 454-pyrosequencing Titanium® technology is relatively inexpensive (~$5,000 USD) and can produce more than 500 Mb of DNA sequence. Given the high density of microsatellite loci in plant genomes and automated microsatellite discovery algorithms (e.g., MISA), a single next-generation sequencing run can identify thousands of putative microsatellite loci. The use of motif type and repeat length can be important parameters for minimizing screening cost while maximizing the polymorphic loci discovery rate.

The five cultivate cañahua clusters found in Structure did not show any geographical differentiation. Indeed, Bolivian and Peruvian genotypes are mixed throughout the clusters (Fig. 3B). The lack of geographic differentiation among the cañahua genotypes is suggestive of

extensive mixing of the cañahua genotypes across its endemic cultivation area, presumably as a result of trade or bartering among native peoples. The inconsistency of geographic distance data was corroborated by the isolation by distance test. Interestingly, the marker data does identify several admixed genotypes, suggesting that genotypes, once traded, experienced gene flow – presumably through a low level of outcrossing, estimated here at about 1.1% (Table 3). This low-level of outcrossing is also reflected in the high degree of homozygosity ($F = 0.98$) identified at the microsatellite loci and is likely a function of cañahua's cleistogamous breeding strategy (Risi and Galwey, 1984). From a practical germplasm perspective, inbreeding simplifies the maintenance of long-term germplasm collections, as curators do not have to be concerned about gene flow during the propagation of the germplasm bank. Similarly, inbreeding facilitates the development of pure (fixed) lines that are important to core-breeding collections and the development of commercial varieties.

Anciently, cañahua was an integral part of the Andean farmers reciprocal food exchange/acquisition strategy – a seasonal food trading network practiced between farming communities located at different altitudes (Altiplano, yunga, valley and low lands regions; Rist, 2000). Besides cañahua, crops traditionally incorporated in the food strategy (reciprocity and barter market) included potato (*Solanum andigenum, S. tuberosum, S. juzepczukii*), oca (*Oxalis tuberosa*), isaño or mashua (*Tropaeolum tuberosum*), quinoa (*Chenopodium quinoa*), coca (*Erythroxylum coca*), yuca (*Manihot esculenta*), dehydrated maize (*Zea mayz*), legumes, and several medicinal plants (Marti and Pimbert, 2007). Trading foods items from one Andean region to another continues to be an important strategy for ensuring food security and nutrition. Even the development of motor transportation seems to have had little impact on this practice.

Consequently, the effects of these trading systems on cañahua germplasm diversity and distribution should be an important consideration in any future germplasm conservation efforts.

CONCLUSIONS

We report the first set of polymorphic molecular markers for cañahua (*C. pallidicaule*), a food crop of regional importance for subsistence farmers across the Andean region of South America. The markers reported are of particular value in ongoing efforts to extend and characterize the cañahua germplasm collections in Bolivia and Peru, including the development of core breeding collections needed for emerging cañahua improvement programs across the region. Initial phylogeny analyses using these markers indicate that geographic origin and morphology are not fully predictive of genetic distance, suggesting that future germplasm collection efforts should account for ancient food exchange strategies and incorporate molecular marker data to assure comprehensive germplasm collections. Current efforts are aimed at developing new molecular markers, including single nucleotide polymorphisms, and diverse segregating populations to facilitate the development of linkage maps for cañahua – an important first step towards the development of marker-assisted breeding programs.

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Tables and Figures

Table 1. Passport data and morphological characteristics for the *Chenopodium* genotypes used in microsatellite assays. The microsatellite preliminary screening panels consisted of samples marked with * (*C. quinoa* based microsatellites) and ** (*C. pallidicaule* specific microsatellites). Morphological traits include domestication status (DS), growth habit (GH), seed coat color (SC), pericarp color (PC), and disease presence (DI).

ID	Name	Source	Country of origin	Latitude	Longitude	Altitude	DS[b]	GH[c]	PT[d]	SC[e]	PC[f]	DI[g]
cp1	0012-1	PROINPA	N/A	N/A	N/A	N/A	2	2	1	2	1	2
cp2*	0102-1	PROINPA	N/A	N/A	N/A	N/A	2	3	2	2	1	2
cp3*	0112-1	PROINPA	N/A	N/A	N/A	N/A	2	3	2	2	1	2
cp4	0113-1	PROINPA	N/A	N/A	N/A	N/A	2	3	1	2	1	2
cp5	0120-1	PROINPA	N/A	N/A	N/A	N/A	2	2	2	2	1	2
cp6	0181-1	PROINPA	N/A	N/A	N/A	N/A	2	2	2	2	1	2
cp7*	0271-1	PROINPA	Bolivia, LP[a], Murillo	-16.5	-68.3	4100.8	2	2	2	3	6	2
cp8	0351-1	PROINPA	Bolivia, LP[a], Murillo	-16.6	-68.2	3602.4	2	2	1	2	1	2
cp9	LRN-2	PROINPA	Bolivia, LP[a], Desaguadero	-16.6	-69.0	3886.8	2	2	2	3	2	2
cp10	LRB-1	PROINPA	Bolivia, LP[a],Desaguadero	-16.6	-69.0	3886.8	2	2	2	2	1	2
cp11**	Line-1(05)	PROINPA	Bolivia, LP[a], Caquiaviri	-16.9	-68.5	4022.4	2	2	2	2	8	2
cp12*	Line-2(05)	PROINPA	Bolivia, LP[a], Comanche	-16.8	-68.4	3960.6	2	2	2	2	1	2
cp13	Line-3(05)	PROINPA	Bolivia, LP[a], Curahuara	-17.5	-68.5	3832.9	2	3	3	2	4	2
cp14	Line-4(05)	PROINPA	Bolivia, LP[a], Curahuara	-17.5	-68.5	3832.9	2	2	3	2	8	2
cp15	0019-1	PROINPA	N/A	N/A	N/A	N/A	2	3	2	2	1	2
cp16	0102-2	PROINPA	N/A	N/A	N/A	N/A	2	2	2	3	2	2
cp17*	0441-1	PROINPA	Bolivia, LP[a], Aroma	-17.3	-68.0	3813.7	1	2	2	3	6	2
cp18	0472-1	PROINPA	Bolivia, LP[a], Aroma	-17.3	-68.0	3813.7	2	2	1	2	1	2
cp19	0475-1	PROINPA	Peru, Puno	-15.7	-70.1	3828.6	2	2	3	3	9	2
cp20	0517-1	PROINPA	Bolivia, LP[a], Ingavi	-16.8	-69.0	3828.0	2	2	2	2	1	2
cp21	0017-1	PROINPA	N/A	N/A	N/A	N/A	2	2	2	2	3	2
cp22	0225-2	PROINPA	N/A	N/A	N/A	N/A	2	2	2	2	1	2
cp23	0468-1	PROINPA	Bolivia, LP[a], Aroma	-17.3	-68.0	3813.7	2	2	2	2	1	2

LP[a] = La Paz; **DS[b]**: Wild = 1, Cultivated = 2; **GH[c]:** Prostrate=1, Lasta=2, Saihua=3; **PT[d]:** Yellow=1, Pink=2, Red=3; **SC[e]:** White=1, Brown=2, Black=3; **PC[f]:** Beige=1, Grey=2, Khaki=3, Orange=4, Dark orange=5, Brown=6, Dark brown=7, Firebrick=8, Saddle brown=9; **DI[g]**: presence=1 absent=2, and N/A = Not available.

Table 1. Continued

ID	Name	Source	Country of origin	Latitude	Longitude	Altitude	DS[b]	GH[c]	PT[d]	SC[e]	PC[f]	DI[g]
cp24	0468-2	PROINPA	Bolivia, LP[a], Aroma	-17.3	-68.0	3813.7	2	2	2	2	2	1
cp25	0568-1	PROINPA	Bolivia, LP[a], Omasuyus	-16.0	-68.7	3842.6	2	3	3	2	5	2
cp26	Line-9(05)	PROINPA	Bolivia, LP[a], Omasuyus	-17.6	-68.4	3864.9	2	3	2	2	1	2
cp27	0568-2	PROINPA	Bolivia, LP[a], Omasuyus	-16.0	-68.7	3842.6	2	2	3	2	7	2
cp28	0636-1	PROINPA	Bolivia, LP[a], Coromata	-16.2	-68.5	3983.7	2	2	2	3	2	2
cp29**	PI 478406	USDA	Bolivia, La Paz	-16.3	-68.6	3891.7	2	2	2	3	2	2
cp30	PI 478407	USDA	Bolivia, La Paz	-16.3	-68.5	3912.1	2	3	3	2	4	2
cp31	PI 510525	USDA	Peru, Puno	-15.7	-70.1	3839.0	2	2	2	2	1	2
cp32	PI 510526	USDA	Peru, Puno	-16.4	-69.2	3883.5	2	2	2	2	1	2
cp33*	Ames 13221	USDA	Bolivia, La Paz	-16.2	-68.8	4259.0	2	2	2	2	1	2
cp34	Ames 13222	USDA	Bolivia, La Paz	-16.2	-68.8	4258.1	2	2	2	2	1	2
cp35	PI 510531	USDA	Peru, Puno	-16.0	-69.8	3834.1	2	2	2	2	6	2
cp36	PI 510529	USDA	Peru, Puno	-15.9	-70.0	3833.5	2	2	2	2	1	2
cp37	PI 510528	USDA	Peru, Puno	-16.1	-69.7	3853.9	2	2	2	2	1	2
cp38	Ames 13223	USDA	Bolivia, La Paz	-16.3	-68.6	3880.4	2	2	3	2	4	2
cp39**	Ames 13224	USDA	Bolivia, La Paz	-16.3	-68.5	3912.7	2	2	2	3	2	2
cp40	PI 510527	USDA	Peru, Puno	-16.2	-69.4	4007.5	2	2	2	2	1	2
cp42	0351Mut-45	PROINPA	Bolivia, LP, Ingavi	-16.6	-68.2	3603.0	2	2	1	2	1	2
cp43	Wild-2	PROINPA	Bolivia, LP, Ingavi	-16.6	-68.2	3603.7	2	2	1	3	7	1
cp44*	Wild-4	PROINPA	Bolivia, LP, Ingavi	-16.6	-68.2	3604.3	1	1	2	3	7	2
Cpetiolare	564	UAP	Chile, Chijo	-19.4	-68.6	3918.0	1	N/A	1	3	6	N/A
*Cquinoa***	cv. Ollague	CIP-FAO	Chile, Ollague	-21.2	-68.3	4122.4	2	N/A	3	1	N/A	1

LP[a] = La Paz; **DS[b]**: Wild = 1, Cultivated = 2; **GH[c]:** Prostrate=1, Lasta=2, Saihua=3; **PT[d]:** Yellow=1, Pink=2, Red=3; **SC[e]:** White=1, Brown=2, Black=3; **PC[f]:** Beige=1, Grey=2, Khaki=3, Orange=4, Dark orange=5, Brown=6, Dark brown=7, Firebrick=8, Saddle brown=9; **DI[g]**: presence=1 absent=2, and N/A = Not available.

Table 2. Cañahua and *C. quinoa* microsatellite characteristics, including primary motif, complexity, expected PCR type, flanking regions, flanking regions (forward primer 5'-3' and reverse primer 5'-3'), amplification primer product size (PRO), observed number of alleles (ONA), expected heterozygosity or gene diversity (*H*), fixation index (*F*), and outcrossing rates (t_a).

	Marker name[a]	Primary motif	Complexity	Type	Forward primer (5'-3')	Reverse primer (5'-3')	PRO	ONA	*H*	*F*	t_a
1	CPAAT003	(AAT)14	Simple	perfect	TTTGACTTTCTTGCCCTATTTACA	CGCTATGTATTCCAATGCGA	228	2	0.3084	1.0000	0.000
2	CPAAT015	(AAT)13	Simple	perfect	CGTACAAAGTATAATTAGTGGCGA	CAGAGCACGACAGAGCAGAA	152	4	0.5680	1.0000	0.000
3	CPAC001	(AC)8	Simple	perfect	GTTAAGCTCCACAATCCTTTGA	ATTCCACCGGTTGTCACTTT	225	2	0.2832	1.0000	0.000
4	CPAT002	(AT)8	Simple	perfect	AAGTGTATTAACCGCCCGTG	AGCTCCTGGCTTAGTTTCAAGA	214	2	0.3261	1.0000	0.000
5	CPAT005	(AT)9	Simple	perfect	GGAATCCAACACTTCAGTGGA	AGTTAGTCCGGGTAAAGCCC	202	2	0.4955	1.0000	0.000
6	CPAT032	(AT)9	Simple	perfect	GGAATCCAACACTTCAGTGGA	AGTTAGTCCGGGTAAAGCCC	198	2	0.4875	1.0000	0.000
7	CPAT034	(AT)9	Simple	perfect	CAAACAGAAAGTAAAATAGCAAAAGAA	ATATGCAGGGTCTTGGTTGG	141	4	0.6675	1.0000	0.000
8	CPAT045	(AT)9	Simple	perfect	ATGGTATCGGAGCCAACAAG	GCTGCGAGACCAGCTTTATT	138	8	0.6936	1.0000	0.000
9	CPAT046	(AT)9	Simple	perfect	TCATTTAGTTCAGTTTTAATTTCGG	TCCGGCTCTAGGCCTTTTAT	187	2	0.4950	1.0000	0.000
10	CPAT050	(AT)9	Simple	perfect	GGAATCCAACACTTCAGTGGA	CCCCTAGACGGCGTATTTTT	184	2	0.4898	1.0000	0.000
11	CPAT055	(AT)10	Simple	perfect	AGTCAATGTCGTGTCTCAATATACA	GGTTAATGAAAGGTCCAGGC	172	4	0.5944	0.9609	0.020
12	CPATA010	(ATA)9	Simple	perfect	AAGAGGAGGAGGAGTAGTAGTTGTAG	AGAAAACCAGAAGACTGGTCC	182	7	0.7670	1.0000	0.000
13	CPATT005	(ATT)13	Simple	perfect	GTTTGGGTGAAGAGCGAATC	CCGCGTTAGTATCATCAATCG	126	5	0.5635	1.0000	0.000
14	CPATT006	(ATT)30	Simple	perfect	TTGTTAATTTGGGCAAGCAA	TCTCGTGACCTGACTGTGAGA	210	4	0.4551	1.0000	0.000
15	CPATT013	(ATT)12	Simple	perfect	TCCGATTTAATTCAAATTCTCTTACA	AAGTGTGACACATATAATCGGAGC	162	5	0.1363	0.8292	0.093
16	CPATT017	(ATT)12	Simple	perfect	TCCGATTTAATTCAAATTCTCTTACA	AAGTGTGACACATATAATCGGAGC	162	4	0.1015	0.7466	0.145
17	CPATTT009	(ATTT)6	Simple	perfect	TTTCTGTTGAAAACTCATTGCC	CAACTTGGCCAAAATGATGA	149	3	0.2506	1.0000	0.000
18	CPCA003	(CA)12	Simple	perfect	CCACATCACTTCTGCCGATA	AAGTTAAATCAGTTTGGCTCCTTT	136	2	0.4124	0.9449	0.028
19	CPCAG001	(CAG)14	Simple	perfect	GGGTTATTGTCAATGCTAGTGAA	TTGAACACTCTTGACCTTCTTCT	107	4	0.4082	1.0000	0.000
20	CPGTA001	(GTA)9	Simple	perfect	AGTTAGGGCGGTAGGGAGAG	GGCTATGGAGGTCGACAAAT	103	2	0.4497	1.0000	0.000
21	CPGTA002	(GTA)9	Simple	perfect	AGTTAGGGCGGTAGGGAGAG	GGCTATGGAGGTCGACAAAT	103	2	0.4444	1.0000	0.000
22	CPTA001	(TA)8	Simple	perfect	TCAAACTCAAGTAATATTGCAGATCA	TTCCATGGCATTTCTTCACA	179	4	0.4536	1.0000	0.000
23	CPTA012	(TA)9	Simple	perfect	TCGGTAACGCGACATAAAGA	GGAATCCAACACTTCAGTGGA	150	2	0.4932	1.0000	0.000
24	CPTA015	(TA)9	Simple	perfect	AGTTAGTCCGGGTAAAGCCC	GGAATCCAACACTTCAGTGGA	201	3	0.6247	1.0000	0.000

[a]Suffix “CP” primers derived from *C. pallidicaule*; suffix “Q” and “K” primers derived from *C. quinoa.*

Table 2. Continued.

	Marker name	Primary motif	Complexity	Type	Forward primer (5'-3')	Reverse primer (5'-3')	PRO	ONA	*H*	*F*	t_a
25	CPTA017	(TA)9	Simple	perfect	CGGAACGTGATTGGTTTCTA	TGGATTTCATTTCATTTATCATTTAG	233	2	0.4997	1.0000	0.000
26	CPTA046	(TA)9	Simple	perfect	TCCGGCTCTAGGCCTTTTAT	TCATTTAGTTCAGTTTTAATTTCGG	186	2	0.4997	1.0000	0.000
27	CPTA056	(TA)9	Simple	perfect	CCCCTAGACGGCGTATTTTT	GGAATCCAACACTTCAGTGGA	183	2	0.4997	1.0000	0.000
28	CPTA058	(TA)9	Simple	perfect	CCCCTAGACGGCGTATTTTT	GTTTCAGTTTTAATTTCGGTTTTT	146	2	0.4986	1.0000	0.000
29	CPTA062	(TA)9	Simple	perfect	TCCGGCTCTAGGCCTTTTAT	TCATTTAGTTCAGTTTTAATTTCGG	186	2	0.5000	1.0000	0.000
30	CPTAA001	(TAA)12	Simple	perfect	GGTGGAGGAGAACAACCTCA	GACATTATAATTGGAAGTCCACCC	187	5	0.3602	0.8737	0.067
31	CPTAA003	(TAA)12	Simple	perfect	GGTGGAGGAGAACAACCTCA	GACATTATAATTGGAAGTCCACCC	188	4	0.3226	0.9295	0.037
32	CPTAT011	(TAT)13	Simple	perfect	TTGCTGACCCCTTCTTCTTC	TCACAACAATTAAAATAAAACCCC	197	3	0.4259	1.0000	0.000
33	CPTAT012	(TAT)13	Simple	perfect	TTGCTGACCCCTTCTTCTTC	TCACAACAATTAAAATAAAACCCC	198	5	0.4033	1.0000	0.000
34	CPTTA016	(TTA)9	Simple	perfect	AGTTTGACTCCTTCACCCCC	CCTACCAGCTTCACGTCACA	172	4	0.5152	1.0000	0.000
35	CPTTA017	(TTA)10	Simple	perfect	CGTTGACTTGCAGAGCTGAG	CGAATTGAGCTGAATCGAACT	201	2	0.4800	0.8842	0.061
36	CPTTA022	(TTA)14	Simple	perfect	AGTTGGTGTGAAACCCGTACT	TGCGATTCAATATGCTTTATCA	136	7	0.6851	0.9661	0.017
37	KCAA010	(GGT)4	Simple	perfect	TGGGTCGTAGTTCTGGGTTC	CTTATCACCAGCAGCAGCAC	191	4	0.5809	1.0000	0.000
38	KCAA041	(GTT)8	Simple	perfect	TGGTCGTAGACCACCATTT	CGGATCACTCCACCCTTGTA	197	6	0.7290	1.0000	0.000
39	KCAA132	(CAA)5	Simple	Imperfect	CAAACTGCAGGCACCACA	CAACTTCACCATAGGCCAAAATGATGA	220	2	0.4444	1.0000	0.000
40	KCAA139	$(CAA)_3$	Simple	Imperfect	GAACACCCAACCTGCAAACT	CAACTTCACCATACGCATTCA	180	4	0.2101	0.8918	0.057
41	QAAT076	$(ATT)_{30}$	Simple	perfect	GCTTCATGTGTTATAAAATGCCAAT	TCTCGGCTTCCCACTAATTTT	197	6	0.7215	1.0000	0.000
42	QAAT100	$(ACTACC)_8$	Compound	perfect	GGCATCCAGAGGTCAGTCTT	GCAATTCTTCCTAATAACAACAACAA	349	6	0.3611	0.8680	0.071
43	QATG001	$(CAT)_8$	Simple	perfect	CATAACATCCTATAGAACCGTTGC	TGCTCCTACTTTACTCATTGACTCAT	200	4	0.3682	0.9368	0.033
44	QCA005	$(CA)_{16}$	Simple	perfect	GTGGTTCATGGCTGATCCTT	CTTGCCATCAGGGCATATCT	185	3	0.3534	0.9357	0.033
45	QCA024	$(CA)_{17}$	Compound	perfect	AGATGAGCTTGAATCATTACATC	TACATACTGTAAATCAGCCAAA	250	3	0.5714	0.9583	0.021
46	QCA038	$(CT)_{10}(CA)_{13}$	Compound	perfect	CATTTCCCAAACTGCATGAAT	ATGTGTGTTGCGTGTGAGTG	197	3	0.5559	0.8649	0.072
47	QCA 055	$(TG)_{14}$	Simple	perfect	GGGCATATCTGAAGGAAAGA	ACGCAGGTAGCACTTCCAGT	198	3	0.3534	0.9357	0.033
48	QCA076	$(CA)_{12}$	Simple	perfect	AGGGAGGGTTGAAGGAAAGA	TCTTGTGGACTTGCATGCTT	150	3	0.5214	1.0000	0.000
	Mean						182	3.5	0.4673	0.9786	0.011

[a]Suffix "CP" primers are derived from *C. pallidicaule*; while suffix "Q" and "K" primers are derived from *C. quinoa.*

Table 3. Summary of the statistical evaluation of the polymorphic microsatellite using 43 cañahua (*C. pallidicaule*) genotypes.

	Microsatellite derived from *C. quinoa*	Microsatellite derived from *C. pallidicaule*
Total tested microsatellites	424	192
Amplified microsatellites	170	161
Polymorphic microsatellites	12	39
Total observed alleles (ONA)	168	
ONA range	2 - 8	
Average of ONA	3.5	
Expected heterozygosity value range (*H)*	0.1015 - 0.7670	
Average of expected heterozygosity (*H*)	0.4673	
Fixation index (*F)*	0.7466 - 1	
Average of *F*	0.9786	
Outcrossing rates ($t_{a)}$	0.017 - 0.145	
Average of t_a	0.011	
No. of samples tested	43	

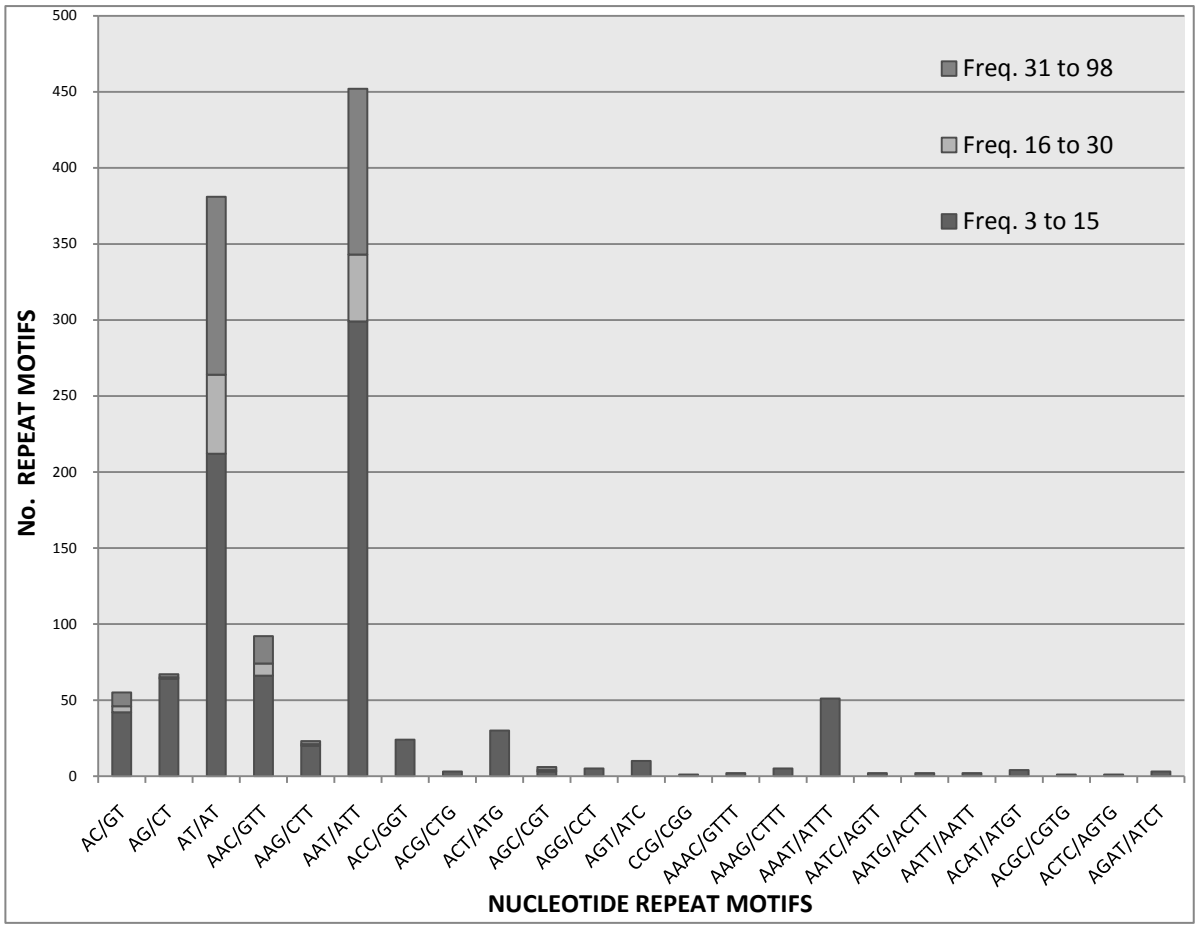

Figure 1. Frequency of microsatellite class repeat types identified in cañahua using 454 pyro-sequencing technology. A total of 1000 microsatellite motifs were identified from the 454-pyrosequencing data using the computer program MISA.

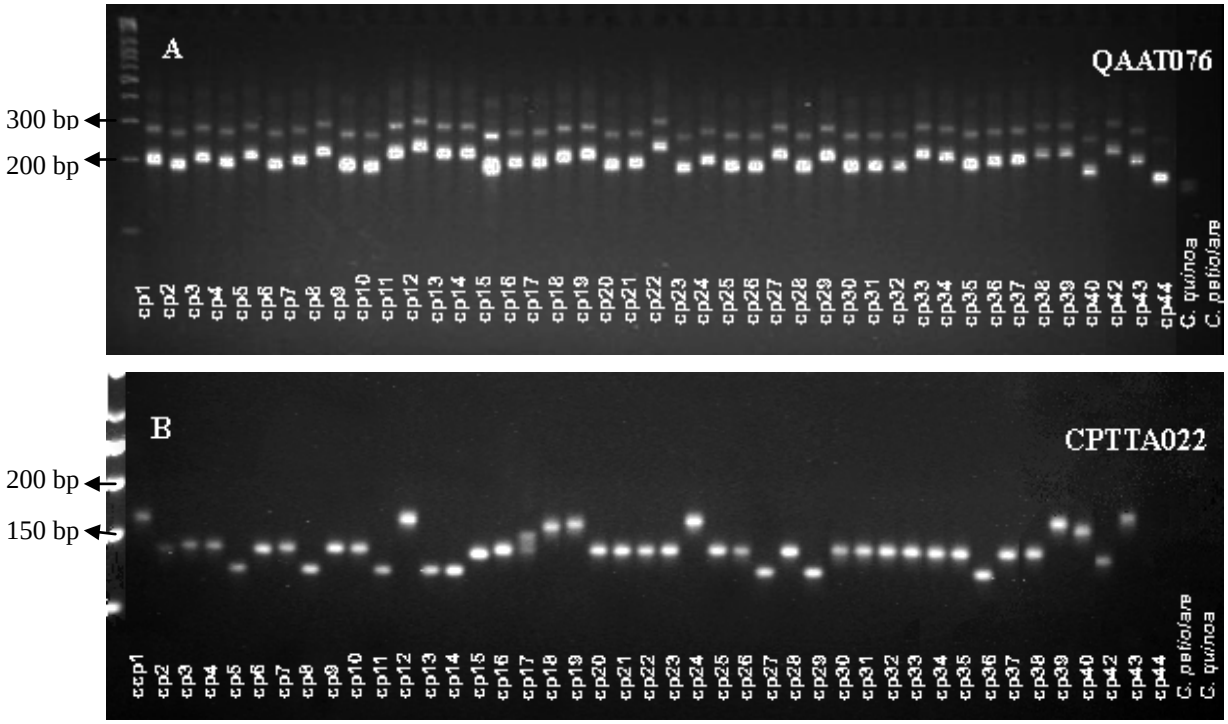

Figure 2. An example of polymorphic microsatellite markers in cañahua across 43 diverse genotypes of *C. pallidicaule* (lanes 2-44), one *C. petiolare* and one *C. quinoa genotype*. Panel (A) shows marker QAAT076 and panel (B) shows marker CPTTA022. Standards are loaded in the outside lanes (lane 1).

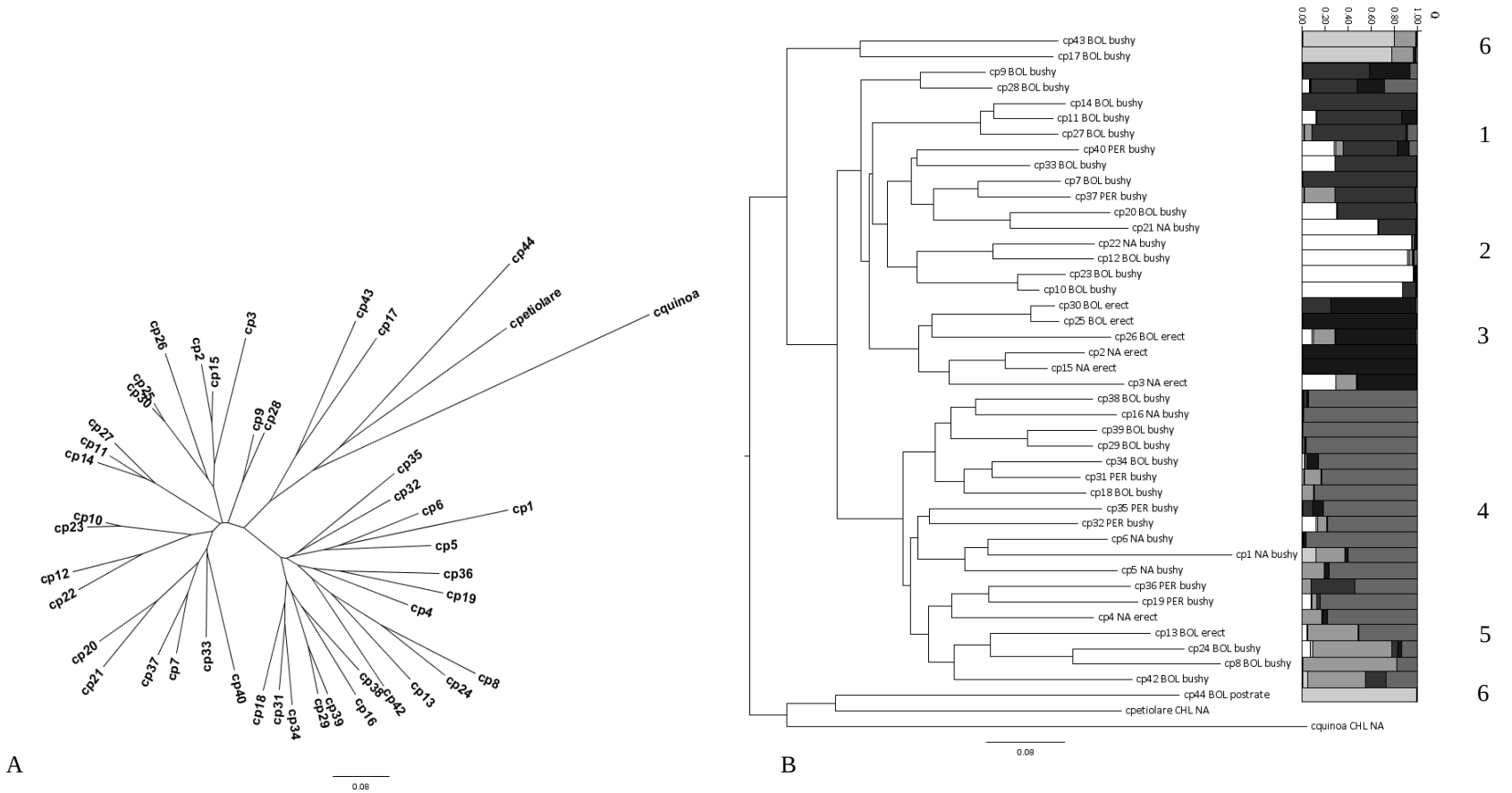

Figure 3. A) Unrooted neighbor joining tree showing the genetic relationship among of individuals of the cañahua (*C. pallidicaule*) genotypes based on the combined set of microsatellite markers. B) Rooted neighbor joining dendrogram and population structure based on 43 genotypes and 48 microsatellite markers using structure (Pritchard et al. 2000). Identification codes of plants correspond to those given in Table 1. Branch lengths are proportional to genetic distance. Each genotype is represented by a line partitioned in six gray colored segments that represent the individual's estimated membership fractions (Q) to each one of the six clusters. Outgroup species are *C. petiolare* and *C. quinoa* (cv. Ollague).

Chapter 2. Literature Review

INTRODUCTION

Cañahua (*Chenopodium pallidicaule* Aellen) is a major component to a balanced diet for millions of people in the Andean region of South America where it was cultivated anciently and continues to be grown for sale and food. It is important nutritionally because seeds are high in protein, vitamins, minerals and all essential amino acids (Bonifacio, 2003; IPGRI et al., 2005). Cañahua is considered nutritionally balanced and is better than wheat, rice, sorghum or millet (Risi and Galwey, 1984; Repo-Carrasco et al., 2003; Repo-Carrasco et al., 2009), and similar to the composition of the protein of milk (casein, Repo-Carrasco et al., 2003). In addition to its high nutritional value, cañahua can thrive at high altitudes and under extreme environmental conditions such as frost, hail, drought, and salty soils. Interest worldwide is growing because of its nutritional qualities and could be considered an important crop for the diversification of future agricultural systems.

Research on cañahua has tried to resolve the problem of uniform grain maturation, small grain kernels, shattering seeds, and high rates of segregation tendencies (Senda et al., 2006). All of these problems are associated with non-domestication and evolution of weediness and must be eliminated to make cañahua a suitable plant for food production.

IMPORTANCE, DOMESTICATION, AND BOTANIC CHARACTERISTIC

Cañahua (*Chenopodium pallidicaule* Aellen) is an important annual crop with regional significance in Bolivia and Perú because of its nutritional value, and its center of diversity near Lake Titicaca (Risi and Galwey, 1984; Ibish and Merida, 2003; IPGRI et al., 2005). Cañahua, an annual self-pollinated diploid (2*n*=2x=18) species, is a member of Amaranthaceae family, subfamily Chenopodioideae (Muller and Borsch, 2005) and was classified by Hunziker (1943)

into the intensely pigmented black-seeded form *Melanospermum*, similar to *C. melanospermum* specie. Paredes (1966) distinguished a wild variety of cañahua, prostrate and highly ramified with black seeds, named macho cañihua that probably corresponded to Hunziker's *Melanospermum*. Paredes also suggested classifying cultivated types as erect with limited branching and black or brown seeds, and semierect, branching with black and brown seeds. Current classifications follow Paredes (1966) suggestions and identify cañahua as erect, prostrate or semiprostrate. Moreover, semiprostrate is known as "bushy" or "shrubby" or "Lasta" and erect type is known as "saihua", while the prostrate type are associated with wild forms (Fig.1, IPGRI et al., 2005). The erect types are characterized by presenting 3-5 basal branches and grow faster during 70 days after that the dry-matter production ceases. However, semierect or bushy "lasta" types present more than six basal branches and it grows throughout the growing season producing more stems. Consequently, it produces high dry matter and yield grain. While, wild type produces low yield grain and dry matter because of its early maturity which is earlier than the others erect and bushy types (NRC, 1989).

Cañahua likely had a great significance among early Andean cultures (Tiahuanacotan and Incan) because of its unique adaptation to harsh climate conditions such as high altitude environments (~4000 m above sea level), extreme frost tolerance, and early maturity (Galwey, 1989). According to Tapia (1979) it was first mentioned by Diego Cabeza in 1586 in his "Description of the city of La Paz" and Hunziker (1943) suggested it was cultivated in the highlands of the northeast of Argentina. Its name reflects the historical importance as a food crop in the Tiahuanacotan and Incan culture. In Aymara and Quechua cultures it is known as cañahua kañawa, isualla hupa, ajara jupa (Aymara); kañiwa, cuchiquinua, kañagua (Quechua).

In Spanish, it is known as cañahua, cañihua, and cañagua (Risi and Galwey, 1984; Wood and Eyzaguirre, 2004).

PRODUCTION AREA

Domestic cultivation of cañahua occurs in two major centers, La Paz, Bolivia and in Puno, Peru. Minor cultivation extends into the Bolivian departments of Oruro, Cochabamba, and Potosi. Cañahua yields range from 375 to 2968 kg /ha (Marin, 2002; Flores, 2006; Rodriguez, 2007). Its height ranges from 25 to 60 cm tall (Galwey, 1989; Gade, 1995; Wood-Paéz and Eyzaguiree, 2004), and matures from 95 to 173 days (Flores, 2006; Rodriguez, 2007). It is able to with stand the harsh environment of the Altiplano and grows at high elevations (~4000 m.a.s.l), survives frequent frosts and hail, and arid-saline soils with little water (rain fall between 500 to 600mm, pH 4.8, Risi and Galwey, 1984; NRC, 1989; Tapia and Fries, 2007). Few other cultivated species can be cultivated on the Altiplano because of the harsh environmental conditions.

NUTRITIVE VALUE AND USES

Cañahua was used and continues to be used an important nutritional component of the Andean farmers' diet. The percent protein content of the grain ranges from 13 to 19 % (Rastrelli et al., 1996; IPGRI et al., 2005; Repo-Carrasco et al., 2009) and contains a good balance of essential amino acids (Repo-Carrasco et al., 2003) that are similar in quality to soybean meal and is far superior to corn (Table 1, Johnston et al., 2007). High levels of dietary fiber, phytates and phenolic compounds were also observed by Repo-Carrasco et al. (2009), while Wood and Eyzaguirre (2004) and reported high levels of iron and calcium (Table 2). According to the National Research Council (1989), cañahua is used as flour made from toasted grain known as

pito. Traditionally, pito was used among llama traders for altitude sickness and alleviate fatigue as they traveled the Altiplano (Bonifacio, 2003). It can also be consumed directly or as a drink by adding sugar. Cañahua flour can also be consumed with milk, added to soups, or mixed with wheat flour to make bread, cookies, pastries, noodles, juice, and snacks mixed with chocolate (Wood and Eyzaguirre, 2004; IPGRI et al., 2005). Non-food uses of cañahua include supplementary forage for sheep, llamas, guinea pig and cows (unpublished data). The chaff from seed threshing is burned and the ash is collected to make llujta or llipta, which is chewed with coca leaves and works as a natural stimulant (Bonifacio, 2003).

Anciently cañahua was an integral part of the subsistence farmers reciprocal food exchange/acquisition strategy – a seasonal food trading network practiced between farming communities located at different altitudes (Altiplano, yunga, valley and low lands regions, Rist, 2000; Marti and Pimbert, 2007). Besides cañahua, crops traditionally incorporated in the food strategy (reciprocity and barter market) included potatoes (*Solanum andigenum, S. tuberosum, S. juzepczukii*), oca (*Oxalis tuberosa*), isaño or mashua (*Tropaeolum tuberosum*), quinoa (*Chenopodium quinoa*), coca (*Erythroxylum coca*), yuca (*Manihot esculenta*), dehydrated maize (*Zea mayz*), legumes, and several medicinal plants (Rist, 2000; Marti and Pimbert, 2007). Similar food trading networks were also practiced in Central America among the Maya society (Gumerman, 1994; Gumerman, 1997), demonstrating the importance of trade in insuring food security and nutrition.

A new awareness of the importance of cañahua's nutritional, economic, and cultural value is contributing to an increased scientific interest in cañahua. Studies involving cañahua have focused on morphologic and agronomic research including the response to nitrogen fertilization, plant density, spacing between and within rows, planting date, grain shattering,

harvest maturity, and post-harvest methods (Marin, 2002; Flores, 2006; Rodriguez, 2007). This and further information is essential for establishing cañahua as a cash crop and extending its cultivation to new areas.

Recently published efforts to characterize agronomic and morphological traits (IPGRI et al., 2005) among cañahua germplasm collections have determined that there are approximately 1000 diverse accessions of cañahua maintained in collections worldwide. The largest collection of 801 accessions (in The National Germplasm Bank of High Andean Grains at The Foundation for the Promotion and Investigation of Andean Products (PROINPA) in Bolivia is an extensive germplasm resource for research, conservation, and improvement. Approximately 460 other accessions are located on Universidad del Altiplano Puno, Peru (UNA), and 13 accessions are in the USDA National Plant Germplasm System (NPGS). Core collections have been developed for cañahua based on passport descriptor, eco-geographical and morphological variability by using qualitative and quantitative data. However, there is no molecular characterization, which is essential for identifying the genetic diversity among cañahua accessions in order to formulate strategies for its conservation, including development of core collections.

Development of specific genetic markers for cañahua is necessary in order to describe the diversity among cañahua accessions and develop core collections. Genetic studies based on transferability and potential utility of *C. quinoa* microsatellite markers (SSRs) as genetic markers for cañahua were assessed by cross-species amplification. This study reported that 67% of microsatellites amplified successfully in cañahua but little research has been applied to describing cañahua core collections using the cross-species amplification procedure (Mason et al., 2005; Maughan et al., 2007; Orihuela, 2008).

GENETIC DIVERSITY

Genetic diversity is an important aspect of any improvement program. A primary concern for the future improvement of cañahua is the loss of *in-situ* genetic diversity. Poverty, political unrest and climate destabilization has accelerated a massive exodus from the Altiplano. Efforts to conserve the genetic diversity of cañahua have led to the collection and development of two main germplasm banks for cañahua (IPGRI et al., 2005). However, the ex situ conservation of cañahua is even much more limited in comparison with quinoa due to the lower priority assigned to this crop (Bonifacio, 2003). Undoubtedly these germplasm resources will be critical for the future improvement of the species by plant breeders. The largest collection of cañahua is maintained in Bolivia and Peru. Eight hundred one accessions are maintained at the National Germplasm Bank of High Andean Grains at the Foundation for the Promotion and Investigation of Andean Products (PROINPA) in Quipaquipani, Bolivia, while another 460 accessions are maintained by the Universidad del Altiplano in Illpa-Puno, Perú (UNA). Within cañahua accessions there a great variation in plant color, stem pigmentation, canopy, seed size, seed coat and pericarp color, leaf serrations and shape, and plant shape which is used in germplasm characterization (IPGRI et al., 2005).

The increasing interest in the development of new cultivars with high quality and processing characteristics, as required by export markets, has heightened the need for molecular tools suitable to predict the presence of desirable genes that can be applied in early generations. Consequently, shortening the time for releasing new cultivars of particular interest and complexity are the quantitative traits such as yield, grain size, popping quality, non-shattering plants, salt resistance, drought, flooding, and frost tolerance. Recently, studies of molecular markers have become an important topic in order to effectively apply the marker-assisted

selection (MAS) in breeding programs (William et al., 2007). The findings of MAS applied in other crops (rice, corn, wheat, and beans) provide strong evidence that MAS can be applied effectively in quinoa and cañahua. Therefore, further investigations are needed in order to determine and find the reliability to improve desirable traits.

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Tables and Figures

Table 1. Comparing protein quality of soybeans, cañahua, quinoa, and corn grain.

	Amino Acid % of Protein			Amino acid Comparisons	
	Soybean	Cañahua	Corn	Cañahua	Corn
	%	%	%	% of Soy	% of Cañahua
Tryptophan	1.52	1.34	0.9	87.7	68.1
Cystine	1.2	1.5	1.2	123.1	79.9
Methionine	1.1	1.9	1.8	171.4	96.6
Threonine	3	4.2	3.6	139.4	86.8
Serine	4	4.2	4	104	96.4
Glycine	3.2	6.1	3.7	191	61.6
Valine	3.6	5.2	4.8	145.7	92.9
Isoleucine	3.7	4.3	3.5	117.7	82
Leucine	6.4	7.1	12	110.7	169.2
Tyrosine	2.8	3	13.1	109.4	103.1
phenylalanine	3.9	4.7	4.6	121.3	99.4
Lysine	8.6	5.9	3	68.2	51.5
Histidine	2.4	3.2	2.7	132.5	84.8
Arginine	8.2	10.6	4.3	128.4	41.1

Data provided by [1]Johnston, N. P., [1]Dunn, M., and [2]Bonifacio, A. 2007of Department of Nutrition, Dietetics and Food Science at Brigham Young University, data not published.

Table 2. Comparing protein and mineral content in rolled cañahua and quinoa (brown and white) flakes.

mg/100g	White quinoa	Brown quinoa	Cañahua
% Protein	7.85	12.58	15.01
% S	0.1	0.17	0.19
% N	1.26	2.01	2.4
% P	15	0.51	0.38
% Ca	0.12	0.08	0.16
% Mg	0.13	0.27	0.33
% K	0.58	1.11	0.76
ppm Zn	32.25	49.37	38.47
ppm Fe	76.25	101.4	176.3
ppm Mn	15.24	21.24	47.52
ppm Cu	8.21	12.1	12.06
ppm Na	86.61	36.25	160.3

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Figure 1. Growing habits observed in cañahua: erect type “saihua”, shrubby or bushy type “lasta”, and prostrate type “pampalasta” (IPGRI et al., 2005).