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Genetic Diversity in Argentine Andean Potatoes by Means of Functional Markers

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Abstract

Andean Potato is a major crop for farmers in the Andes and represents an important gene pool for potato improvement. We present the population structure and genetic diversity of 88 Andigena accessions collected in Northwestern Argentina based on functional markers (25 SSR) distributed along 12 chromosomes. Polymorphic information content ranged from 0.40 to 0.87. A Bayesian approach, a Principal Coordinate Analysis and a Cluster Analysis revealed the presence of: I) a major group containing most of the Andean accessions and II) a smaller group including the out-group cv. Spunta and the sequenced genotype DM. Several group specific alleles were detected. AMOVA showed that 81% of the variability was within each group. Eleven of the SSRs analyzed are linked or within genes reported to regulate traits of nutritional and industrial interest. Additionally, the allelic variant of a photoperiod dependent tuberization regulator gene, StCDF1.2, was exclusively detected in accessions clustered in group II.

Resumen

La papa andina es un cultivo importante para los agricultores en los Andes y representa un importante reservorio de genes para el mejoramiento de la papa. Aquí presentamos la estructura poblacional y la diversidad genética de 88 introducciones Andígenas, colectadas en el noroeste de Argentina, en base a marcadores funcionales (25 SSRs) distribuidos a lo largo de los 12 cromosomas. El contenido de la información polimórfica fluctuó entre 0.40 y 0.87. Una aproximación Bayesiana, un análisis de coordenadas principales y un análisis de agrupamientos, revelaron la presencia de: I) Un grupo principal constituido por la mayoría de las introducciones Andinas y II) un grupo más pequeño que también incluía a la variedad Spunta y al genotipo secuenciado DM. Se detectaron algunos alelos específicos de grupo. El análisis AMOVA mostró que el 81 % de la variabilidad estaba dentro de cada grupo. Once de los SSRs analizados están ligados o dentro de genes reportados asociados a caracteres de interés nutricional e industrial. Además, la variante alélica del gen regulador de la tuberización dependiente del fotoperíodo, StDF1.2, se detectó exclusivamente en introducciones del grupo II.

Keywords Andean potato · SSR · Population structure · Genetic diversity · Nutritional & industrial quality · CDF

Introduction

Potato (*Solanum tuberosum* L.) is the third food crop of global importance in human diet and the first non-cereal food crop (FAOSTAT 2015). It belongs to the Solanaceae family which also includes other economically important crops such as tomato, pepper, eggplant, petunia and tobacco. Potato tubers are the storage organs of this species, also employed as the vegetative propagation system. The interest in potato has been reinforced with the complete sequencing of its genome in 2011 (PGSC 2011) and its subsequent physical and genetic map (Sharma et al. 2013).

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There is a controversy regarding the classification of cultivated potato (Spooner and van den Berg 1992; Spooner et al. 2014). According to Dodds (1962), *Solanum tuberosum* is a unique species comprised by five cultivar groups: Stenotonum, Phureja, Chaucha, Andigena and Tuberosum, the latter including lowland tetraploid chilean potatoes that were introduced and cultivated in Europe about 400 years ago and spread worldwide as 'modern potatoes' (Ames and Spooner 2008; Spooner et al. 2014). On the other hand, group Andigena comprises native Andean potatoes domesticated 7000–10,000 years ago in the South American Andes uplands (Milbourne et al. 2007). For simplicity, we refer to *S. tuberosum* group Andigena as 'Andigena' and *S. tuberosum* group Tuberosum as 'Tuberosum'. It has been reported that 'modern potatoes' have a narrower genetic base than Andean potatoes, as demonstrated by morphological descriptors and molecular markers (Sukhotu et al. 2005; Ispizúa et al. 2007; Ghislain et al. 2009; Gavrilenko et al. 2013). The presence of high contents of vitamins, proteins, antioxidant compounds and mineral micronutrients confers special nutritional value to this group (Hawkes and Hjerting 1969; Andre et al. 2007) constituting a basic food source for rural populations. Moreover, these nutritional features have led to consideration of Andean potatoes as a functional food source (Campos et al. 2006).

There are several agronomic, industrial and nutritional traits that define potato quality. Some of them include tuber yield, tuber starch yield, tuber starch, reducing sugars, chlorogenic acid and steroidal glycoalkaloid content as well as chip quality, pathogen resistance and plant maturity (Krits et al. 2007; Öztürk et al. 2010; Hirsch et al. 2013; Slater et al. 2016). Recent results show that Andean potatoes of NW Argentina present wide variation of phenotypes for reducing sugar content and chip quality traits (Colman et al. 2017).

Since potato origin is in the Andes region near the equator, plants are adapted to short photoperiod conditions. Andigena is more dependent on short days for tuberization than Tuberosum and photoperiod dependence is linked to the regulatory gene Cycling DOF Factor 1 (*St*CDF1) (Kloosterman et al. 2013). *St*CDF1 belongs to the family of DOF transcription factors (DNA-binding with one finger) and it has been postulated to be involved in the regulation of timing of tuber formation and plant maturity in response to photoperiod. Three different *St*CDF1 alleles have been reported to date: one 'late' *St*CDF1.1 allele (present in Tuberosum, Andigena and Phureja), and two 'early' *St*CDF1.2 and *St*CDF1.3 alleles, both reported to be present only in Tuberosum (Morris et al. 2013). *St*CDF1.2 has a 7 bp insertion in 3′-terminal end and *St*CDF1.3 shows a ~800 bp insertion in 3′-terminal end that have been shown to cause loss of photoperiod regulatory domain (Kloosterman et al. 2013).

In NW Argentina, in the provinces of Jujuy, Salta and Catamarca, several Andigena landraces are cultivated. The germplasm bank of the EEA INTA-Balcarce (BAL) has been collecting and preserving this material for more than 40 years. Andean potatoes present differences in development, flower color, tuber shape, skin and flesh color, nutrient and sugar content as well as different behavior under diseases and abiotic factors such as droughts and cold (Ross 1986; Ochoa 1990). The existing variability among landraces is valuable because it is a potential source of alleles for the improvement of commercial varieties (Brush et al. 1992). In addition, characterization of genetic diversity is crucial to ensure germplasm protection (Esquinas-Alcázar 2005) and the establishment of core collections. Germplasm conservation has gained relevance in the past few decades since gene erosion can lead to a decrease of genetic variability. Detailed studies of genetic diversity revealing allelic variants of relevant genes and addressing of population structure of the Andigena germplasm may contribute to enlarge the cultivated potato gene pool employed in breeding programs.

Intergenic molecular markers are useful for assessment of genetic variability in biodiversity studies, phylogenetic analysis and cultivar identification (Das et al. 2013), since they are not subjected to selection pressure. Functional markers -on the other hand- are advantageous over intergenic DNA markers given their linkage to characterized genes (Andersen and Lübberstedt 2003). Microsatellite markers or simple sequence repeats (SSRs) are short tandem repeats of di-, three or tetranucleotides, amplified with specific primers and resolved by gel electrophoresis. SSRs present codominance, multiallelism, high quality reproducible bands and high degree of polymorphism (Luque Cabrera and Herráez 2001). They have been employed in potato for genetic linkage maps, QTL localization, varietal identification, phylogeny, germplasm characterization and population structure (Milbourne et al. 1998; Ghislain et al. 2001; Norero et al. 2004; Raimondi et al. 2005; Yıldırım et al. 2009; Favoretto et al. 2011). Expressed sequences tags (ESTs)-derived SSRs are functional markers particularly useful for quality trait evaluation (Oliveira et al. 2009). All these features make microsatellites a reliable and low cost method for genetic analysis (Spooner et al. 2005).

Ispizúa et al. (2007) and Atencio (2011) have reported the genetic variability of the Andigena potato germplasm from NW Argentina employing a few SSRs. However, the population structure of this germplasm has not been deeply explored yet. The goal of this study was to link genetic diversity to allele variability of genes of agronomic importance, as a source for breeding and to determine population structure in a collection of 88 Andigena potatoes from NW Argentina employing a set of functional SSR markers and *St*CDF1 alleles.

Materials and Methods

Plant Material

A total of 88 *Solanum tuberosum* Andean Potato accessions (*Solanum tuberosum*, group Andigena) (4X) from the NW

region of Argentina were analyzed (Fig. 1). The doubled monoploid line used as the reference genome DM1–3516 R44 (hereafter referred to as DM) (*Solanum tuberosum* Phureja Group) (2×), the commercial cultivar Spunta (hereafter referred to as SP) (*Solanum tuberosum* Tuberosum Group) (4×) and one accession of *Solanum curtilobum* (hereafter referred to as '83') were included as out-groups. Seventy-nine accessions were provided by the BAL, three by the Jujuy National University and seven accessions of 'Imilla Negra' were provided by Cauqueva Cooperative (Table 1). 'Imilla Negra' accessions were selected from the same landrace seed pool for showing molecular variability in preliminary analysis (data not shown). The BAL material has been collected from farmers in the provinces of Salta and Jujuy from 1976 to 2001 (Okada 1976, 1979; Okada and Clausen 1984; Clausen 1989; Clausen et al. 2005) and stored in vitro under 16/8 photoperiod, with controlled temperature and relative humidity conditions. The BAL accessions were selected to represent variability in tuber morphological traits such as shape, skin and flesh color, and bud distribution (Atencio 2011).

DNA Isolation

Genomic DNA was extracted from leaves of two-week-old in vitro plants from each accession with the methodology described by Haymes (1996). DNA quality and concentration were examined in 1% agarose gels run in 1X TBE (89 mM Tris-Borate, 20 mM ethylenediaminetetraacetic acid) and stained with GelRed (GenBiotech, Argentina). Quantification of DNA was performed in a SmartSpecTM 3000 Spectrophotometer (BIORAD, USA). DNA final concentrations were adjusted to 10 ng/μl.

Genotyping

A total of twenty-five simple sequence repeats (SSRs) markers were employed in the present analysis (Table 2). Twenty EST-derived SSR markers were selected according to reported genome coverage, locus-specific information content and reproducibility, covering all twelve chromosomes (Milbourne et al. 1998; Feingold et al. 2005; Sørensen et al. 2008 and Ghislain et al. 2009). Five markers were newly identified on the sequenced genome browser (www.potatogenome.net) as repetitive motifs in the proximity or within the selected invertases and chlorogenic acid synthesis pathway enzymes genes that may regulate quality traits. Primers were designed for invertases genes SSRs: InVac_2.1_III (PGSC0003DMG400013856) (F:TGAGAGCTTTTCCTTTTGTCTC, R:CTAATCCTACACCAGATGGCAG) and STM1106.2 (PGSC0003DMG401028252) (F:GGTTGACGAAAATGTTGGGC, R:GGCTGGCACCTAAGTTTGAC) (Colman 2014), and for chlorogenic acid synthesis enzymes genes SSRs: HQT_VII (PGSC0003DMG400011189) (F:ATTTTCCTCGACATTGGTGG, R:GCAAAGCCTCGTGTTAGTATG), HCT_III (PGSC0003DMG400014152) (F:AGGAGAAGCATAAT

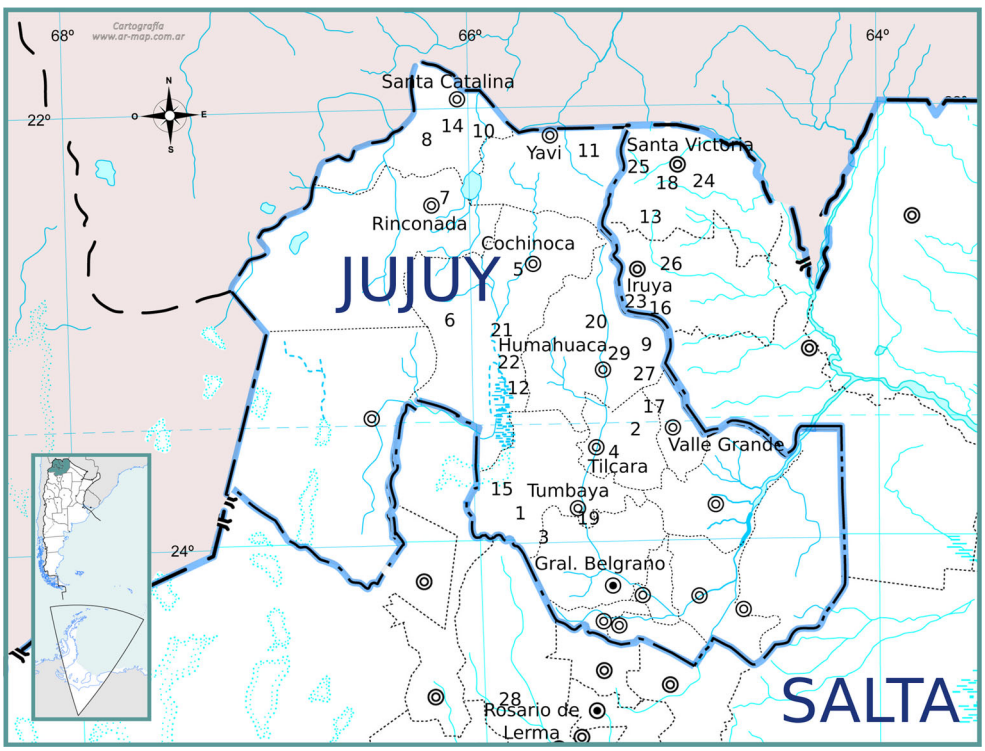

Fig. 1 Sampling area in Northwestern Argentina. Samples were collected in several departments in the provinces of Jujuy and Salta. Numbers indicate collection sites (see Table 1)

Table 1 Accessions analyzed and geographical sampling locations

Number	Germplasm Bank accession	Common name	Location/Source	Geographical position in Fig.1
1	CCS 1350	BLANCA ALARGADA	Jujuy Tumbaya El Angosto	1
2	CS 1418	CHAQUEÑA	Jujuy Tilcara Papachacra	2
3	CS 1432	COLLAREJA REDONDA	Jujuy Gral. Belgrano Cuevas	3
4	CCS 1172	MORADITA REDONDA	Jujuy Tilcara Casa Colorada	4
5	CCS 1251	CHACARERA	Jujuy Cochinoca Cochinoca	5
6	CCS 1330	MORADITA	Jujuy Cochinoca Rachaite	6
7	CCS 1255	DESIREE	Jujuy Rinconada Rinconada	7
8	CCS 1307	MORADITA	Jujuy Santa Catalina Cabrería	8
9	CCS 1166	CUARENTONA COLORADA	Jujuy Tilcara Casa Colorada	4
11	CCS 1199	TUNI BLANCA	Jujuy Humahuaca Palca de Aparzo	9
12	CCS 1349	COLORADITA	Jujuy Tumbaya El Angosto	1
13	CCS 1185	TUNI MORADA	Jujuy Humahuaca Aparzo	9
14	CCS 1201	AZUL	Jujuy Humahuaca Varas	9
15	CCS 1288	DESIREE	Jujuy Santa Catalina Cieneguillas	10
16	CS 1430	CUARENTONA	Jujuy General Belgrano Cuevas	3
17	CL 658	SANTA MARÍA	Jujuy Yavi Yavi	11
20	CCS 1371	CHACARERA	Jujuy Cochinoca Quebraleña	12
21	CCS 1205	CHURQUEÑA	Jujuy Humahuaca Varas	9
23	CL 621	CHORCOYEÑA	Salta Santa Victoria Nazareno	13
25	CCS 1271	BLANCA	Jujuy Santa Catalina Morco Esquina	14
26	CCS 1284	SANI	Jujuy Yavi	11
27	CCS 1366	OVERA	Jujuy Tumbaya El Moreno	15
28	CCS 1327	BAYISTA	Jujuy Cochinoca Rachaite	6
31	CL 728	CUARENTONA	Salta Iruya Colanzulí	16
32	CL 769	SALLAMA GRANDE	Jujuy Valle Grande Santa Ana	17
33	CL 482	ROSADA	Salta Santa Victoria Rodeopampa	18
34	CCS 1383	PERA O SEÑORITA	Jujuy Tumbaya Patacal	19
35	CL 748	OVERA	Jujuy Humahuaca Chaupi Rodero	20
36	CCS 1323	COLORADA	Jujuy Cochinoca Agua Caliente	21
37	CCS 1303	YURUMA	Jujuy Santa Catalina Casira	10
39	CCS 1374	MORADITA	Jujuy Cochinoca Agua Castilla	22
40	CL 631	ALLO	Salta Iruya Campo Carreras	23
41	CCS 1384	CORBATILLA	Jujuy Tumbaya Patacal	19
42	CCS 1385	MORADITA	Jujuy Tumbaya Patacal	19
44	CL 516	CHORCOYEÑA	Salta Santa Victoria Chorro	24
45	CL 650	COLORADA	Salta Santa Victoria Poscaya	25
46	CL 576	RUNA	Salta Santa Victoria Lizoite	25
48	CL 548	PAPA PALTA	Salta Santa Victoria Trigohuaico	25
49	CL 641	RUNA	Salta Santa Victoria Poscaya	25
51	CL 835	AIRAMPÍA	Jujuy Valle Grande Santa Ana	17
52	CL 790	OVERA	Jujuy Valle Grande Santa Ana	17
53	CCS 1170	OJOS COLORADOS	Jujuy Tilcara Casa Colorada	4
54	CL 836	AIRAMPÍA	Jujuy Valle Grande Santa Ana	17
56	CL 708	RUNA	Salta Iruya Colanzulí	16
57	CL 820	NEGRA REDONDA	Jujuy Valle Grande Santa Ana	17
58	CCS 1309	BLANCA REDONDA	Jujuy Santa Catalina Cabreria	8
62	LC 348	IMILLA NEGRA	Jujuy Humahuaca Huachichocana	9
64	CL 528	COLORADA	Salta Santa Victoria Chorro	24

Table 1 (continued)

Number	Germplasm Bank accession	Common name	Location/Source	Geographical position in Fig.1
67	CL 634	COLLAREJA	Salta Santa Victoria Arpero	26
68	CL 636	COLLAREJA	Salta Santa Victoria Abra Colorada	25
69	CL 712	HUAREÑA	Salta Iruya Colanzulí	16
70	CL 739	RUNA	Jujuy Humahuaca Chaupi Rodero	20
71	CL 750	RUNA	Jujuy Humahuaca Chaupi Rodero	20
72	CL 752	BLANCA	Jujuy Valle Grande Santa Ana	17
73	CL 782	TUNI ROSILLA	Jujuy Valle Grande Santa Ana	17
74	CL 793	SALLAMA	Jujuy Valle Grande Santa Ana	17
75	CL 814	HOLANDESA COLORADA	Jujuy Valle Grande Santa Ana	17
77	CL 815	BOLIVIANA	Jujuy Valle Grande Santa Ana	17
78	CL 821	CUARENTILLA TOSCRA	Jujuy Valle Grande Santa Ana	17
79	CL 832	ABAJEÑA OVERA	Jujuy Valle Grande Santa Ana	17
80	CCS 1196	AZUL OVERA	Jujuy Humahuaca Palca de Aparzo	9
81	CCS 1200	PAPA OCA	Jujuy Humahuaca Varas	9
82	CCS 1206	PAPA OCA	Jujuy Humahuaca Varas	9
84	CCS 1218	RUNA	Jujuy Humahuaca Ocumazo	27
86	CCS 1257	OJOSA	Jujuy Rinconada Rinconada	7
88	CCS 1300	HOLANDESA	Jujuy Santa Catalina Casira	10
89	CCS 1321	ROSADA	Jujuy Cochinoca Agua Caliente	21
90	CCS 1378	CHURQUEÑA NEGRA	Jujuy Tumbaya Patacal	19
91	CS 1402	OJOS COLORADOS	Jujuy Tumbaya Carce	19
92	CS 1408	CHAQUEÑA OVERA	Jujuy General Belgrano Papachacra	2
93	CS 1413	PAPA VALLISTA	Jujuy General Belgrano Papachacra	2
94	CS 1414	CUARENTONA MORADA	Jujuy General Belgrano Papachacra	2
95	CS 1417	CUARENTONA ROSADA	Jujuy General Belgrano Papachacra	2
97	LC 335	TONCA	Jujuy Humahuaca Patacal	20
98	CL 783	NAVECILLA	Jujuy Valle Grande Santa Ana	17
99	CL 849	BALCACHA	Salta Rosario de Lerma El Gólgota	28
101	CCS 1375	TUNI BLANCA	Jujuy Tumbaya Tumbaya	19
102	CCS 1221	COLORADA	Jujuy Humahuaca Coctaca	29
104		AZUL	UNJu	
105		SANTA MARÍA PULPA BLANCA	UNJu	
106		SANTA MARÍA PULPA ROJA	UNJu	
IN 1		IMILLA NEGRA	CAUQUEVA	
IN 2		IMILLA NEGRA	CAUQUEVA	
IN 3		IMILLA NEGRA	CAUQUEVA	
IN 4		IMILLA NEGRA	CAUQUEVA	
IN 5		IMILLA NEGRA	CAUQUEVA	
IN 6		IMILLA NEGRA	CAUQUEVA	
IN 7		IMILLA NEGRA	CAUQUEVA	
83	CCS 1209	*S. curtilobum* genotype	outgroup	
SP		Spunta (*S.t.* Group Tuberosum)	outgroup	
DM		DM1–3516-R44	Sequenced Potato Genome	

GGGTTTC, R:CTGGCAATCCAAGAAGCAC) and CQ-S3H_X (PGSC0003DMG400018934) (F:GGCTCAAA TCCAAAACATTCAG, R:GCCCATTACCATTTATGTCC) (Carboni et al. 2013). StI002 (PGSC0003DMG200008942) (Feingold et al. 2005) primers for apoplastic invertase gene on chromosome IX were redesigned to avoid stuttering and

renamed as StI002.2 (F: GTTACAGGAATCACACCTGC, R: CACTTCAACATCTGCCTGTC). STM1106.2 primers were designed to amplify a repetitive motif in an intronic region of an invertase gene located in chromosome X, which coincidentally matched the same motif as STM1106 (Milbourne et al. 1998), and was renamed accordingly.

Polymerase chain reactions were performed in a 20 μl final volume containing 1X PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTP mix, 0.2 μM of forward and reverse primers, 0.3 U of Taq One polymerase (NEB, USA) and 30 ng of template DNA. Reactions were performed in 96-well plates in an MJ thermocycler (PTC-100 MJ Research, Watertown, Mass., USA) under the following profile: 3 min initial denaturing step at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at annealing temperature according to authors' description, and 1 min at 72 °C, with a final extension step of 5 min at 72 °C. For StI002.2, InVac_10 and STM1106.2 markers a 63–58 °C annealing temperature touchdown profile (Don et al. 1991) was used and for HQT_VII, HCT_III and CQ-S3H_X markers a 60–54 °C touchdown profile was employed. PCR products were resolved on 6% denaturing polyacrylamide gels and silver-stained as detailed by Benbouza et al. (2006). Band size was determined by comparison with 25 bp DNA ladder (Promega, USA) employing Gel Pro Analyser V3.1 (Media Cybernetics, USA).

In order to complement a marker set for important traits, specific primers were designed for the 3′ exonic region of *St*CDF1 gene (F: CAAATGAAGCGGCTAAAAGC, R: ATTGTACCACGAGCCGAATC). Amplification was performed employing 60–54 °C touchdown profile and detection of *St*CDF alleles was performed by urea denaturing 6% polyacrylamide gel electrophoresis as described above.

Allele Scoring and Genetic Diversity Analysis

Since potato is a tetraploid species, each amplified SSR band was considered as an allele and treated as dominant. Allele presence (band) was scored as '1' and absence as '0'. Alleles found in less than 2% of the accessions under study, were considered as 'rare'. A 1/0 matrix was constructed for each marker and was used to calculate genetic distance among the accessions according to Nei's distance (Nei 1972). A dendrogram was constructed using the Neighbour Joining clustering method (NJ) employing aboot from *poppr* (v.2.2.0) package for R software (Ihaka and Gentleman 1996). Support for clusters was given by a bootstrap analysis with 1000 bootstrap replicates.

Polymorphic information content (PIC) was calculated according to Nei (1973) as: PIC = 1 - ∑ pi2, where pi is the allele frequency of the íth′ allele of a certain SSR locus.

Population structure was evaluated with a Bayesian-based model procedure implemented by STRUCTURE software version 2.3.4 (Pritchard et al. 2000). The program was run assuming admixture with independent allele frequencies. A burn-in period of 5000 and 10,000 Markov Chain Montecarlo (MCMC) cycles were employed. Ten independent iterations were performed for each simulated value of K ranging from 1 to 10. The optimal value of K was determined using the DeltaK method (Evanno et al. 2005) with Structure Harvester software (Earl and Vonholdt 2011). Permutations of the output of the STRUCTURE analysis were done with CLUMPP software (Jakobsson and Rosenberg 2007) using independent runs to obtain a consensus matrix. Accessions were assigned to a group when their membership coefficient was >0.50.

A Principal Coordinate Analysis (PCoA) was carried out using *ape* (v.3.5) package for R (Paradis et al. 2004). The amount of variation among clusters was assessed by partitioning genetic diversity using analysis of molecular variance (AMOVA) with the software GenAlex v.6501 (Peakall and Smouse 2006). The number of exclusive alleles for each group was also determined.

Results

Genetic Diversity Analysis

SSR primer pairs generated well-defined bands of the expected fragment sizes according to genome sequence information (PGSC 2011) and showed polymorphism and reproducibility. The 25 SSRs analyzed rendered a total of 157 different alleles. All of the SSRs were polymorphic in the 91 accessions. Among Andigena accessions the highest allele number (64) was exhibited by 'Imilla Negra 2' (IN 2) accession, while the lowest (36) was shown by accession '32'. As expected, due to its homozygous (double monoploid) nature, genome reference genotype DM had the lowest allele number (25). Among markers, StI023 and StI030 detected the highest number of alleles with 11 each while StI014, STM1053, STM1064, HCT_III and CQ-S3H_X detected the lowest with three each. The mean number of alleles per locus was 6.3. StI002.2 presented the largest number of patterns or allele combinations (43) and unique patterns (23). PIC values ranged from 0.39 for STM1105 to 0.87 for StI023 with an average value of 0.69 for all SSRs. A total of 13 rare alleles were identified from the analyzed loci. StI030 generated the highest number of rare alleles (four alleles) followed by StI004 and STM1106.2 with two alleles each. StI001, StI002.2, STM1024, STM0037, and STM5127 produced one rare allele each. Accessions '32' and '83' (*S. curtilobum*) presented three rare alleles each, the maximum number for this collection. Accession '58' had two rare alleles while '2', '6', '86', '99' and DM showed one each.

Out of the 25 SSRs analyzed, 11 are linked to genes implicated in agronomically important traits (Table 2). StI002.2, InVac_2.1 and STM1106.2 markers are located in invertases

Table 2 Polymorphism in Andean potato collection from NW Argentina detected by SSR markers

SSR Marker	Source	Linkage Group	No. SSR Frag.	Detected SSR allele size (in bp)	No.of patterns	No. of unique patterns	PIC
StI001	a	IV	7	172,178,184,188,193,198,202	20	7	0.76
StI002.2*	e[i]	IX	10	108,111,117,123,125,126,129,132,134,135	43	23	0.76
StI004	a	VI	10	70 80 83 86 92 97,105,112,117,121	21	10	0.78
StI012	a	IV	7	139,150,155,158,165,176,180	23	8	0.80
StI014	a	IX	3	137,144,149	7	1	0.65
StI021	a	VI	7	92 99,106,111,115,118,122	34	18	0.82
StI023	a	X	11	123,134,142,153,167,173,193,198,201,228,245	41	21	0.87
StI024	a	II	7	89,104,118,127,135,146,153	31	8	0.81
StI030	a	XII	11	92 94 95 97 98,102,107,111,119,125,143	28	12	0.79
StI032	a	V	8	114,116,121,124,128,132,141,145	33	14	0.81
StI033	a	VII	8	115,123,126,133,141,148,154,157	22	10	0.70
StI060	a	III	5	113,121,133,142,157	18	8	0.70
STM0037*	b	XI	7	79 82 95 99,104,117,135	14	5	0.70
STM1024	b	VII	7	166,170,172,176,180,190	11	4	0.61
STM1053*	b	III	3	141,150,156	7	1	0.59
STM1064*	b	II	3	184,189,195	8	1	0.48
STM1105*	b	VIII	4	181,187,218,222	5	0	0.40
STM2005*	b	XI	5	120,127,136,145,184	15	7	0.68
STM5127	c	I	6	260,275,281,285,291,327	23	3	0.77
stPoAc58	d	V	5	232,239,244,250,254	19	9	0.74
InVac_2.1_III*	e	III	4	204,209,214,217	12	5	0.62
STM1106.2*	e[ii]	X	8	242,245,251,254,257,263,266,269	18	8	0.57
HQT_VII*	f	VII	3	340,364,409	5	2	0.52
HCT_III*	f	III	5	141,143,145,147,149	13	5	0.48
CQ-S3H_X*	f	X	3	340,344,352	7	2	0.47

Source of SSR markers is indicated as follows, **a**: Feingold et al. (2005); **b**: Milbourne et al. (1998); **c**: Sorensen et al. (2008); **d**: Gishlain et al. (2004); **e**: Colman (2014) and **f**: Carboni et al. (2013)

* SSRs linked to agronomically relevant genes

[i] Redesigned primers on StI002 (Feingold et al. 2005)

[ii] Redesigned from same repetitive motive than STM1106 (Milbourne et al. 1998)

genes on chromosomes IX, III and X respectively, that have been associated to 'cold sweetening' (Draffehn et al. 2010). StI002.2 presented a total of ten alleles constituting the most polymorphic invertase marker. STM1106.2 showed a total of 8 alleles and 18 patterns and InVac_2.1, presented four alleles and 12 different patterns.

HQT_VI, HCT_III and CQ-S3H_X markers map in Hydroxycinnamoyl-Coenzyme A Quinate Transferase (HQT), hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (HCT) and p-coumaroyl quinate/shikimate 3′-hydroxylase (C3'HCQ/S3'H) genes in chromosomes VI, III and X respectively, which have been reported to be involved in antioxidant chlorogenic acid (CGA) biosynthesis (Sonnante et al. 2010). HCT_III presented five fragments with 13 patterns within the studied collection, while HQT_VI and CQ-S3H_X markers exhibited only three alleles each with five and seven patterns, respectively.

STM1053 is located in the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) gene. Steroidal glycoalkaloids (SGA) are toxic secondary metabolites and high levels are associated with high expression of HMGR (Valkonen et al. 1996). This marker showed three fragments with seven different patterns in the collection.

STM1064 maps within a putative Snakin gene. Snakin/GASA proteins have a large range of functions including defense against potato pathogens such as *Rhizoctonia solani* and *Erwinia carotovora* (Almasia et al. 2008). STM1064 displayed three bands with a total of eight patterns.

STM1105 and STM2005 are ESTs derived markers coding for putative plant glycogen synthase and Nucleotide-diphospho-sugar transferase/glycosyltransferase respectively, enzymes involved in carbohydrates metabolism (Campbell et al. 1997; Szydlowski et al. 2009). STM1105 presented four alleles with five patterns while STM2005 resulted more polymorphic with five alleles and fifteen patterns.

STM0037 is a SSR located in an intergenic region near the sucrose transporter Sut1 and a putative invertase or pectinase inhibitor (Schönhals et al. 2016). This marker exhibited seven alleles with 14 different patterns.

From the previously reported *St*CDF1 allelic variants, only two were detected in this collection. Expected *St*CDF1.1 allele size was found in all accessions studied while *St*CDF1.3 allele was not detected. In addition to *St*CDF1.1, accessions '2', '25', '45' and '92' displayed *St*CDF1.2 allele showing the same banding pattern as Spunta.

Population Structure Analysis

The assignment into groups performed with STRUCTURE v. 2.3.4 and assessed with Structure Harvester software suggests that this collection is most likely to be described by two groups (Fig. 2). One major group (I) contained 74 accessions, with different tuber shapes, skin and flesh color. The second group (II) is morphologically less diverse and included 17 accessions along with cv. Spunta. Thirty-one group exclusive alleles were detected among accessions from cluster 'I', 11 of which were rare. Cluster 'II' presented four exclusive alleles, one of them was rare.

Clustering of Potato Accessions

Neighbor-joining relationships among accessions (Fig. 3) were in good agreement with results obtained from STRUCTURE with two main clusters distinguished. Cluster 'II' contained 16 accessions, including '2', '25', '45', '92', which as cv. SP, showed the *St*CDF1.2 allele. The remaining 75 accessions grouped in cluster 'I' where more SSR diversity was found. Unlike the Bayesian analysis, the NJ assigned DM to the major group and '4' to group 'II'. Additionally, cluster 'I' presented a subgroup containing a few landraces with purple-violet colored skin and (or) flesh ('14', '12', '105', '104', '89', '39', '106' and '17'). Surprisingly, the 'IN' accessions were split in two distant sub-clusters within cluster I; 'IN2' and 'IN4' grouped together separated from the remaining 'IN' accessions.

Principal Coordinates Analysis (PCoA) detected two major clusters in agreement with STRUCTURE and NJ analysis: (A) represented by most of the Andigena accessions and (B) which included accessions that showed the presumptive early maturity allele *St*CDF1.2 (Fig. 4). However, there were a few landraces ('4', '45', '15', '25', '6' and DM) in intermediate positions in the center of the PCoA plot (C) that were difficult to associate with any of the previous groups. Additionally, the previously mentioned set of 'purple-violet colored' accessions was located on the top of the plot (D) and might be considered as a subgroup within cluster 'A'. The overall variation explained by the PCoA was 21.46% with coordinates 1, 2 and 3 explaining, 9.51%, 6.90% and 5.05%, respectively.

AMOVA results showed that 81% of the allelic diversity is within each group while only 19% is distributed between the two groups.

Discussion and Conclusions

Molecular markers have proved to be a useful tool for several analyses as described previously. Particularly in potato, AFLP and RFLP have been employed for high-resolution mapping (Meksem et al. 1995), DNA fingerprinting (McGregor et al. 2000) and phylogenetic studies (Spooner et al. 2005). RAPD and ISSR markers have been used for marker-assisted sampling (Ghislain et al. 1999) and genetic diversity studies (Hoque et al. 2014; Siddappa et al. 2014; Onamu et al. 2016). Among these markers, SSRs of functional nature were chosen for this study due to their high polymorphism level, reproducibility, and ease of use. Functional SSRs have been used in several crop genetic diversity studies (Thiel et al. 2003; Choudhary et al. 2008; Marconi et al. 2011).

Genetic diversity of potato germplasm has been described by means of SSRs (Ispizúa et al. 2007; Fu et al. 2008; Kandemir et al. 2010; Ruíz de Galarreta et al. 2010; Juyó et al. 2015). Many of the SSRs employed in this work are routinely applied in potato genetic diversity analyses. Ghislain et al. (2008), described a 24 SSR 'Potato Genetic Identity Kit' (PGI) based on the fingerprinting of 742 potato landraces belonging to different taxonomic groups. In this work, 20 SSRs described in above mentioned analyses (12 from the PGI kit) and five newly developed SSRs were employed.

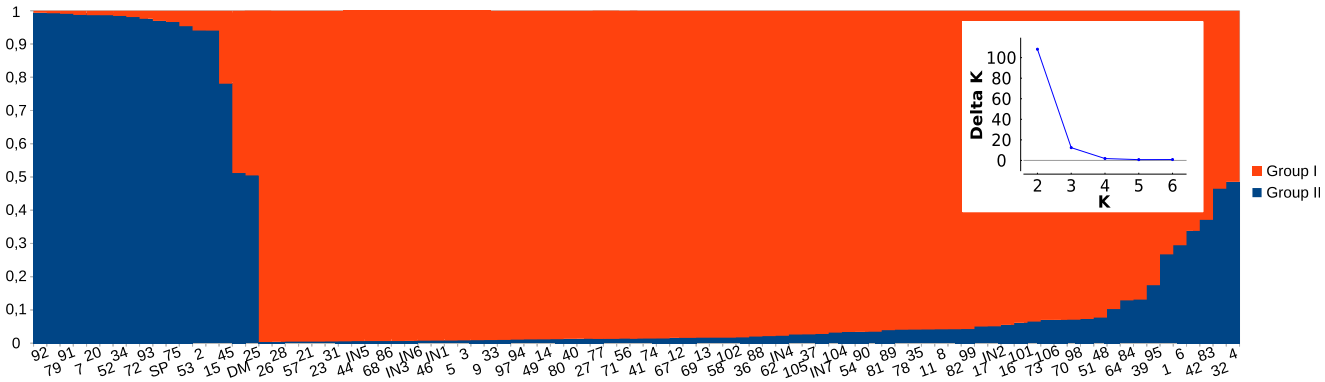

Fig. 2 STRUCTURE analysis. Population structure of the 88 Andean potato accessions according to microsatellite markers: each vertical line represents one accession partitioned into two colored segments according to the estimated membership to the two inferred clusters. The ΔK plot derived from genotypic data shows that the accessions form two distinct clusters

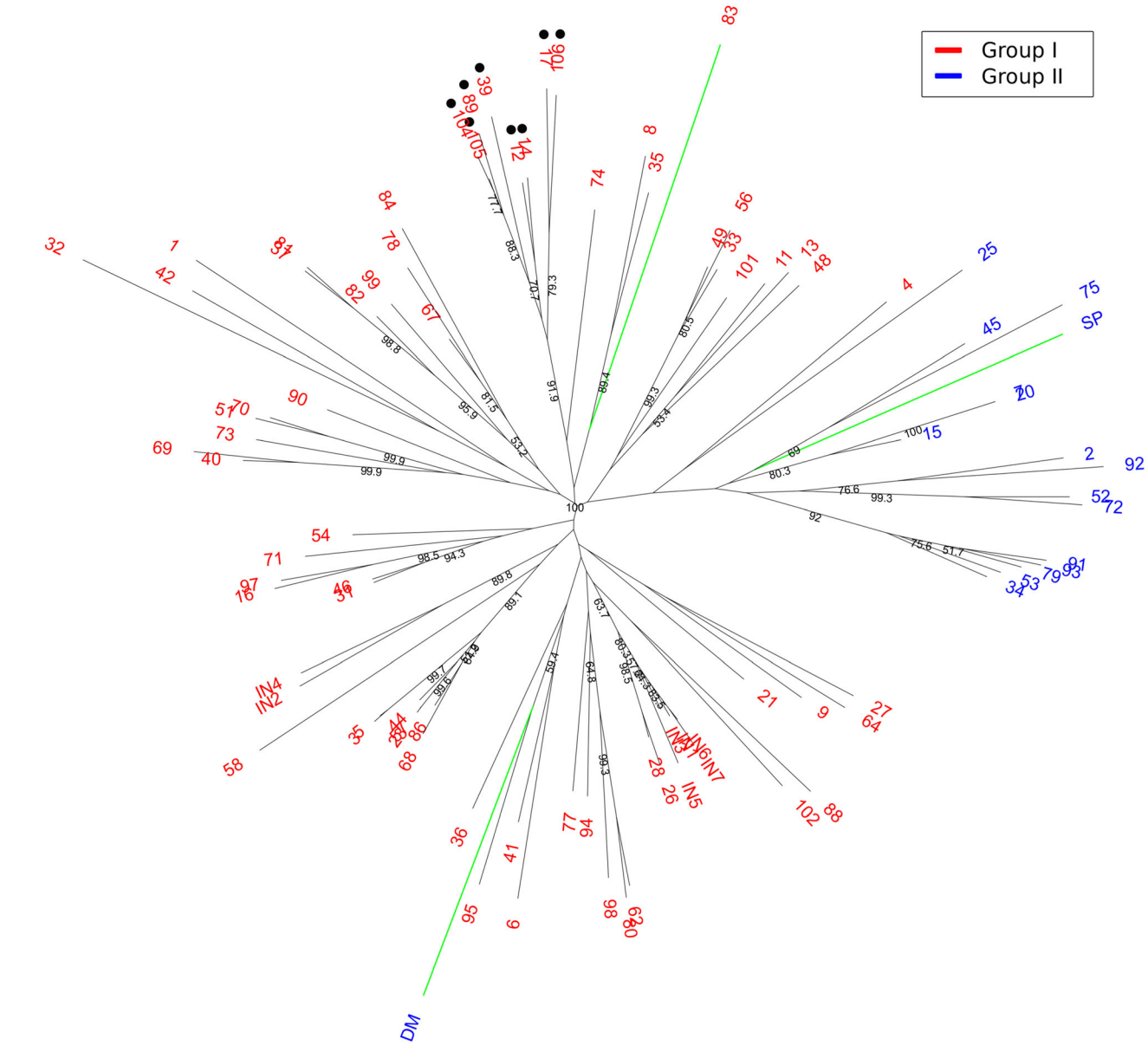

Fig. 3 Neighbor-Joining unrooted dendrogram of the 91 potatoes accessions using SSR markers. Accession numbers are colored in blue and red according to group membership from Structure results. Green-shaded lines indicate outgroups DM (*S. tuberosum* group Phureja), "83" (*S. curtilobum*) and cv. Spunta (*S. tuberosum* group Tuberosum). Black dots indicate accessions from "purple-violet colored" subcluster. Numbers on nodes are bootstrap probabilities estimated by testing with 1000 permutations (shown only for values greater than 50)

Our results are in agreement with previous analyses where morphological diversity in Andean potato is reflected by the large number of genetic patterns. Ispizúa et al. (2007) analyzed 155 accessions with four highly polymorphic SSRs and found 72 genotypes indicating the possible presence of duplicates. Atencio (2011) has described 205 genotypes in a collection of 335 Andigenum accessions, employing three SSRs (StI001, StI004 and StI012). In the present study, 86 different patterns were distinguished from a total of 88 Andean accessions (98%). Regarding the presence of possible duplicated clones our results showed the same electrophoretic pattern for '7' and '20' and for '23' and '57'. Considering the number of SSR markers employed (25), both pairs of accessions showing the same pattern may be considered as duplicates.

Eighty-six accessions exhibited a membership coefficient > 0.6 in the Bayesian analysis. Only '4', '32', '25' and DM did not reach that proportion and consequently showed some clustering inconsistencies. Indeed, '4' and '25' grouped together in a sub branch in the smaller cluster in the neighbour-joining analysis and in an intermediate position in the PCoA (Figs. 3 and 4). Both accessions had the lowest membership coefficient (≈0.5). DM genotype also showed a coefficient ≈0.5

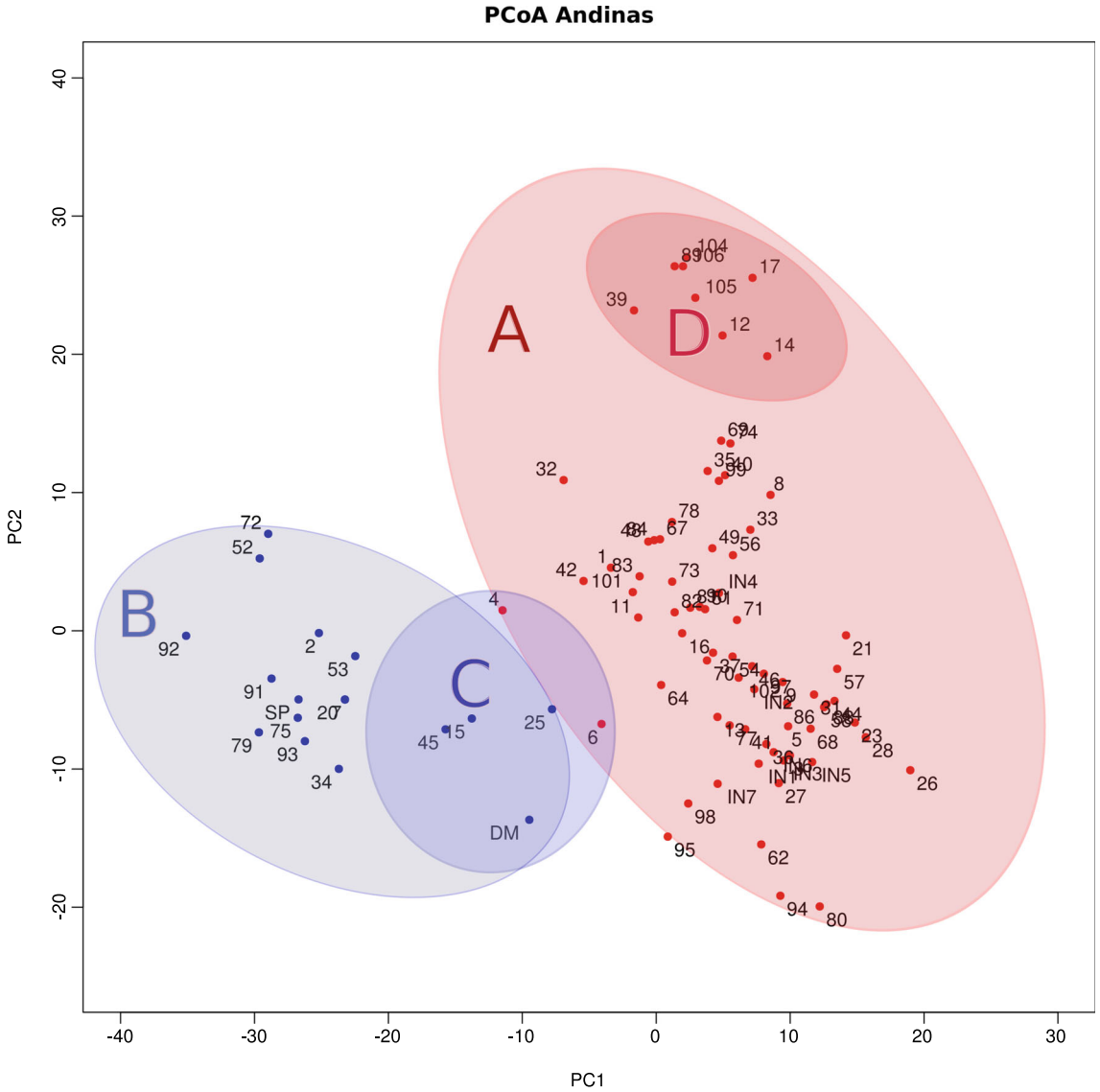

Fig. 4 Principal coordinate plot showing associations among accessions. Accessions are colored by group identity according to results from Structure. A: Cluster containing most of the Andean accessions; B: Cluster with few accessions with CDF1.2 allele (2, 25, 45, 92 and cv. Spunta); C: subcluster including accessions with unclear grouping; D: subcluster with "purple-violet colored" accessions

and was poorly associated to any of the two main groups. This is consistent with the origin of DM from a Phureja Group genotype (PGSC 2011). Although located in the center of PCoA (Fig. 4), '15' and '45' had a strong membership coefficient (0.94 and 0.78 respectively) and this is noted for their location in the cluster II in the neighbour-joining analysis. The same occurred for accession '6' with a coefficient of 0.7 located in cluster I. Most of the accessions registered at the BAL under the same common name grouped exclusively in one of the two main groups indicating related genetic identity. Nevertheless, accessions belonging to 'Chacarera', 'Overa', 'Colorada' and 'Blanca' varieties were represented in both main clusters. This may arise from the fact that folk naming typically reflects only tuber morphology (mainly shape, color and depth of buds) while other plant traits genetically determined are not considered (Hawkes 1947; Brush et al. 1980; Quiros et al. 1990; Raimondi et al. 2005; Haan et al. 2007; Atencio 2011). Therefore, naming information does not exactly illustrate genetic relatedness. Additionally, there is an absence of a consensual system of naming Andean potato cultivars which difficults its identification and classification (Quiros et al. 1990; Haan et al. 2007).

Accessions from the same collection site were often in different clusters and likewise accessions from different collection sites were clustered together indicating the possibility of gene flow between sites and regions. Sexual polyploidization and hybridization with other Andigena and Tuberosum reintroduced landraces as well as crop wild

relatives also contribute to enhance the variability (Ugent 1970; Astley and Hawkes 1979; Johns and Keen 1986; Sukhotu et al. 2005; Camadro 2012).

The most distributed local variety in NW Argentina, 'Collareja' (Huarte et al. 1991), represented in this collection by accessions '3', '67' and '68', clustered in the major group I (Fig. 3) and cluster A (Fig. 4). Surprisingly, allelic profile of '3' (Collareja Redonda) and '5' (Chacarera) only differed in StI024 and STM1064 patterns. This tendency in the 'Collareja Redonda' landraces to differentiate from the rest of 'Collareja' has been described previously (Atencio 2011).

Another commonly cultivated landrace is Runa, represented by seven different accessions (Table 1) which grouped all together in the largest cluster A (Fig. 4). Despite the reduced sampling area for this variety (Fig. 1) the genetic diversity is relatively wide as shown in the analysis (Figs. 3 and 4). Moradita is also an example of a local variety that showed broad morphological and molecular variability. Indeed, accession '39' grouped in the purple-violet subgroup D while remaining accessions from this landrace ('6', '8' and '42'), were located in distant positions within the main cluster A (Fig. 4).

'Imilla Negra' (IN) is a main landrace in Bolivia and south Peru and exhibits large diversity presumably caused by hybridization (Ochoa 1990). Ispizúa et al. (2007) proposed to consider this group as a complex landrace. Five accessions ('IN 1', 'IN 3', 'IN 5', 'IN 6' and 'IN 7') clustered together in a sub branch in neighbour-joining analysis closer to '26' ('Sani') and '28' ('Bayista') (Fig. 3). The two remaining accessions provided by Cauqueva grouped together but distant from the rest of IN accessions (Fig. 3). This separation into two remote subgroups was an unexpected outcome considering the single origin of the germplasm. The unique accession for this landrace provided by the BAL ('62') grouped next to '80' ('Azul Overa') and '98' ('Navecilla') in a closer position to the biggest IN subgroup although in a different sub branch.

As expected, out-group '83' (*S. curtilobum*) was distantly positioned from the rest of the accessions (Fig. 3), due to the presence of three rare alleles. A special situation was observed for '32' which also displayed three rare alleles: it was the only accession that showed a maximum of two alleles for each of the SSRs indicating that it might be a diploid cultivar or a different species. Allelic dosage determination or cytogenetic analysis will clarify this issue.

An assessment of variability in genes related to agronomic and quality important traits is crucial for establishing breeding strategies. In this work allelic variants of some of these genes have been assessed. StI002.2 has been associated to chip quality and tuber starch content (Li et al. 2008) and to reducing sugar content (Colman 2014). StI002.2 123 bp allele has been associated to lower reducing sugar content as well as good chip quality at harvest and after storage. It was present in nearly half of the accessions from each cluster. StI002.2 132 bp allele has also been associated to lower reducing sugar content after storage and good chip quality only at harvest (Colman 2014). This allele was present in 50% of the accessions from group I and 75% from group II. StI002.2 126 bp allele has been associated to higher reducing sugar content and poor chip quality. It was present only in six accessions from group I and Spunta. STM1106.2 266 bp allele has been reported to be associated to lower tuber starch content (Schönhals et al. 2016) and poor quality in chips (Colman 2014). It was a highly frequent allele that was present in 80% of the accessions within group I and all accessions from group II. InVac_2.1 214 bp allele, also associated to sugar content reduction after cold storage (Colman 2014), was present in 30% of the accessions from group I and 12% from group II.

Regarding markers linked to CGA biosynthesis enzymes, preliminary results from our group have shown an allele for the HCT_III marker to be associated with a higher CGA content (Carboni et al. 2013).

STM0037 presented a good degree of polymorphism within the collection. The presence of STM0037-a (STM0037 135 bp) allele has been reported to be associated to good chip quality at harvest and after storage and higher tuber starch content (Li et al. 2008). It was present in 14% of accessions from group I and was absent in group II. The allele STM0037-g (STM0037 79 bp) has been associated to lower tuber starch content and tuber starch yield (Li et al. 2008; Schönhals et al. 2016). It was present in 95% of accessions from group I and all group II accessions with the exception of DM.

In order to complement SSR analysis, additional information has been obtained through StCDF1. It co-localizes with a major QTL for plant maturity on chromosome V and 3′ insertion allelic variants are postulated to cause loss of photoperiod regulated domain and had allowed the adaptation at latitudes distant from the tropics (Kloosterman et al. 2013). In light regulated tuberization model postulated by Kloosterman et al. (2013), as a result of the interaction with StGI1 and StFKF1, StCDF1.1 alleles are tagged for proteasome degradation under long day conditions, leading to repression of StSP6A tuberization activator through StCO1/2 and StSP5G accumulation. In contrast, StCDF1.2 (7 bp insertion) and StCDF1.3 (~800 bp insertions) alleles lacking StFKF1 interaction domain, due to open reading frame shifts caused by the insertions, evade long day degradation and inhibit StCO1/2-StSP5G mediated repression of StSP6A triggering tuberization under either long or short day conditions. Early maturity alleles *St*CDF1.2 and *St*CDF1.3, were both reported in a Tuberosum diploid population (Kloosterman et al. 2013) and *St*CDF1.2 was also present in Neo-Tuberosum (Morris et al. 2013). Additionally, 16 SNPs were recently reported within *St*CDF1 gene in an upstream region from 1.2 and 1.3 insertions (Schönhals et al. 2016). One of the novel SNP alleles was associated to tuber and starch yield but none of them was associated to plant maturity phenotype (Schönhals et al. 2016). In agreement with previous results we have shown the

presence of *St*CDF1.1 in all the accessions studied. Interestingly, there were four accessions ('2', '25', '45' and '92') that exhibited *St*CDF1.2 allele type along with Spunta and grouped in the same cluster (II). Previous efforts to detect *St*CDF1 'early' alleles in tetraploid populations have failed (Schönhals et al. 2016). The presence of alleles associated to early maturity phenotypes and the genetic similarity to Spunta suggests that those accessions might be derived from 'modern potatoes' that have been reintroduced in NW Argentina. Misidentification of Tuberosum landraces as Andigena at collection sites has been reported previously (Ghislain et al. 2009). Preliminary results obtained by our group on in vitro tuberization assays have evidenced that accessions carrying *St*CDF1.2 allele might present a photoperiod independent tuberization (data not shown). In addition to clustering and genetic information, these findings would support the possible re-introduction of 'modern' accessions with early maturity alleles. The absence of a commercial seed production for Andean potatoes encourages an informal exchange among farmers in which seeds from unknown origin are incorporated into the fields, sometimes across country boundaries. This situation establishes a context where 'modern potato' germplasm may be reintroduced. The presence of accessions exhibiting names with European roots such as 'Desiree' and 'Holandesa' in cluster II reinforces this idea.

As urbanization and tourism have increased in NW Argentina in the last 30 years, cultivation of different native species is threatened. People leave valleys to immigrate to bigger cities looking for better living opportunities. As a result the communities in the valleys are becoming smaller and this impacts crop production since there are less people committed to traditional agriculture. The culture of culinary-important and pathogen resistant 'modern' or native landraces in detriment of others, has also contributed to erode the native gene pool. In June of 2016 the Agrobiotechnology Laboratory from EEA Balcarce-INTA in collaboration with IPAF-NOA and INTA-EEA-Abra Pampa have restored several andean potato accessions from this collection to native communities in Jujuy, NW Argentina, in an effort to promote in situ biodiversity conservation and cultivation of ancestral varieties (http://intainforma.inta.gov.ar/?p=33834).

This work provides an overview of the genetic diversity of native potatoes in Argentina assessed by functional markers highlighting those that are suitable to explore variability and allelic valuation in genes related to industrial, nutritional and agronomic traits. The reintroduction of modern potato germplasm has been also evidenced, a situation that should be considered in sampling campaigns and germplasm preservation. Results from this research may be of interest for designing and implementing strategies aimed to conserve local biodiversity as well as to develop programs addressed to the improvement of commercial and Andean potato varieties through conventional and/or marker assisted selection.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interests.

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