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Full Length Research Paper

Molecular characterization of the Indian Andigena potato core collection using microsatellite markers

Jagesh K Tiwari[1]*, B. P Singh[1], Jai Gopal[1,2], Poonam[1] and V. U Patil[1]

[1]Central Potato Research Institute, Shimla-171 001, Himachal Pradesh, India.
[2]Directorate of Onion and Garlic Research, Rajgurunagar, Pune - 410 505, Maharashtra, India.

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Twenty-four (24) microsatellite (SSR) markers of a new PGI kit were used to validate the genetic diversity of the 77 Indian Andigena potato core collections. In SSR analysis, polymorphic information content (PIC), allelic richness per locus of microsatellite loci and cluster analysis showed the high diversity of core collection. In total, 214 SSR alleles were detected in the core collection, out of which 208 alleles were polymorphic with absolute frequencies between 2 to 58. The PIC values of SSR loci ranged from 0.61 to 0.90. SSR-based dendrogram revealed eight main groups (Clusters I to VIII) including 26 single accessions at Dice similarity coefficient value of 0.37. None of the accession showed full similarity with any other accession, except that the maximum similarity (0.83) was observed between the accessions JEX/A-316 and JEX/A-317. PCA revealed 47.31% variation in the first three components. Analysis of molecular variance (AMOVA) analysis which resulted into maximum variation was due to within country origins and yield types. The genetic diversity of the core collection based on the microsatellite data appeared to have quite distinct genotypes that were formed by the morph-agronomic traits. These findings not only demonstrate the diverse core collection but are also useful for selecting genetically distinct potato materials to widen the genetic background of the potato gene pool.

Keywords: Core collection, genetic diversity, potato, *Solanum tuberosum* subsp. *andigena*, SSR.

INTRODUCTION

Genetic diversity is a key to progress in crop improvement programmes. The best way to increase the diversity of crops is to exploit the germplasm stored in the gene banks by introduction into the crop breeding programs. There are more than 3900 accessions of the cultivated Tuberosum (*Solanum tuberosum* subsp. *tuberosum*) and wild species of potato including more than 1200 accessions of the Andigena (*S. tuberosum* subsp. *andigena*) at National Active Germplasm Repository, Shimla. These accessions were imported during 1960 to 1980s from South America and are maintained for their utilization in potato breeding. The Andigena potato is cultivated at elevations of 2500 to 4300 m in the Andean highlands of South America this is adapted to tuberization under short-days. Since, more than 90% of potato crop is grown under short-days of winter season in the Indo-Gangetic plains of India, Andigena potato is a good source for potato improvement (Gopal, 2006).

A large number of Andigena accessions make it comprehensive and impractical to maintain accurate descriptions. Since these accessions are conserved in the clonal form, the maintenance costs of field gene banks are expensive and also exposed to damage by poor management, inappropriate environments and pathogens. An alternative to the efficient exploitation of genetic resources is the construction of a core collection, which retains most of the genetic diversity of the original collection in a smaller number of accessions (Huamán et al., 2000a). Therefore, Central Potato Research Institute (CPRI) has recently constructed an Andigena core collec-

*Corresponding author. E-mail: jageshtiwari@gmail.com. Tel: +91-177-26225073. Fax: +91-177-2624460.

tion consisting of 77 (~10% of whole) accessions representing entire 740 *S. tuberosum* subsp. *andigena* accessions. The core collection was constructed based on morphological, agronomic, disease and pest descriptors (Unpublished data).

There are several criteria to construct a core collection, such as morphological descriptors (Huamán et al., 2000a), biochemical data (Huamán et al., 2000b) and DNA markers (Spooner et al., 2005). Studies were conducted in the past to construct a core collection to facilitate the utilization of diverse genetic resources. Huamán et al. (2000a) developed an Andigena core collection of 306 accessions based on 25 morphological descriptors and validated by 38 isozyme markers to provide a more reliable estimate of genetic diversity (Chandra et al., 2002). Later, Ghislain et al. (2006) examined the superior polymorphism detection power of nuclear SSR markers compared to random amplified polymorphic DNA (RAPD) markers for similar number of markers for construction of a core collection in potato. Today, molecular markers are used for germplasm management to address genetic identification, redundancy, and genetic variation. Measure of variations at molecular level is very suited to assess relationship among individuals and constructing core collection. Among the DNA markers, simple sequence repeats (SSR) or microsatellite provides excellent marker system for discriminating closely related genotypes. Due to its locus specific, co-dominant inheritance, robustness, amenability to high throughput and capable to detect allelic variation in the genome, SSR marker has become a tool of choice for researchers in germplasm management. SSR markers have been used widely in potato for genetic diversity (Spooner et al., 2007; Ispizúa et al., 2007; Gavrilenko et al., 2010; Lung'aho et al., 2011), fingerprinting (Galarreta et al., 2011), construction of core collection (Ghislain et al., 2006) and identification duplicate collections (Del Rio et al., 2006). Recently, a new 24 microsatellite locus-specific markers namely 'potato genetic identity (PGI) Kit' was developed for molecular characterization of potato (Ghislain et al., 2009). Moreover, SSR markers have been used in constructing core collection of several crop species such as peanut (Wang et al., 2011), pea (Zong et al., 2009), rice (Li et al., 2010), soybean (Kuroda et al., 2009), common bean (Blair et al., 2009) and pearl millet (Upadhyaya et al., 2011). The core collection has been proposed as useful tools for the study, utilization, and management of genetic diversity maintained in large germplasm collection. Genetic diversity in a core gene pool is an important tool to capitalize high heterosis (Brown, 1995) and gene isolation (Van Hintum et al., 2000) in crop breeding.

In this study, we report on validation of the genetic diversity of the core collection of 77 Indian Andigena accessions using new 'PGI kit'. To our knowledge, this is the first ever report on microsatellite characterization of the Indian Andigena core collection.

MATERIALS AND METHODS

Plant materials

The core collection used in this study was consisted of 77 Andigena accessions which was developed from 740 accessions of *S. tuberosum* subsp. *andigena* at CPRI, Shimla (Table 1). The tuber material of each accession was obtained from Central Potato Research Station, Jalandhar (Punjab) and grown in the earthen pots at Shimla for molecular analysis.

DNA isolation and SSR analysis

Genomic DNA was isolated from leaf samples of the core collection obtained from the tuber grown plants (~100 mg) using the GenElute Plant Genomic DNA Kit (Sigma-Aldrich, St. Louis, USA). DNA quality and quantity were assessed with NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA), and quality was also assessed on 0.8% (w/v) agarose gel followed by the dilution to 10 ng μL^{-1} for SSR analysis.

A PGI kit of 24 SSR markers was used to analyze the genetic diversity of the core collection (Ghislain et al., 2009). The polymerase chain reaction (PCR) was performed in a Mastercycler Gradient (Eppendorf, Hamburg, Germany) in a total volume of 25 µl and consisted of 50 ng DNA templates in 1× PCR buffer, 2.5 mM $MgCl_2$, 200 µM dNTP, 0.2 µM each primer, 1 Unit Taq Polymerase (Qiagen). PCR procedure included: 4 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at annealing temperature (Ta), and 1 min at 72°C, with a final extension of 7 min at 72°C. Ta was used as described in the PGI kit. The amplified DNA products were separated by an automated chip based electrophoresis system using DNA 1000 kit in Agilent 2100 Bioanalyzer (Agilent Technologies, Germany). Results of the fragment sizes were automatically scored in base pairs based on the relative migration of the internal size standard (15 to 1500 bp DNA ladder) with 2100 Expert software (Agilent Technologies).

Statistical analysis

A data matrix of SSR allele counts of the 77 core collection was constructed on the basis of presence (1) or absence (0) of bands of the amplified DNA fragments. Missing data were scored as "9". An accession was assigned a null allele where an amplification product could not be detected and so not considered in the analysis. Number of alleles, allele size, frequencies and polymorphic information content (PIC) of each SSR were calculated for the 77 core collection. The PIC of each SSR marker was calculated according to the formula: $PIC = 1 - \sum(Pi^2)$, where Pi is the frequency of the ith allele of a marker detected in all accessions (Nei, 1973). Genetic diversity analysis was performed with the program NTSYS-PC 2.21 (Rohlf, 2006). A similarity matrix was calculated by Dice coefficient and the dendrogram was generated using unweighted pair-group method (UPGMA) clustering method.

To assess the genetic association of the core collection, a principal component analysis (PCA) of the 77 core collection was conducted using NTSYS-PC 2.21 based on the similarity matrix of 214 SSR alleles. PCA plots of the first three resulting principal components were made to assess the accession associations and to identify genetically distinct accessions.

An analysis of molecular variance (AMOVA) that was based on the dissimilarity matrix of pairwise accessions was also performed using Arlequin version 3.5.1.2 (Excoffier et al., 2005) to assess the genetic structure of the core collection. Two sources of genetic structuring were examined as accessions of various country origins and yield types. Significance of resulting variance components was tested with 10 000 random permutations.

Table 1. A core collection of 77 Indian Andigena with origin, yield performance and SSR polymorphism.

S/N	Accession	Origin	Yield[a]	Cluster/subgroup/single	SSR alleles
Cluster I					
1	JEX/A-10	Unknown	Low	I (Single)	32
2	JEX/A-93	Peru	Medium	I (Single)	49
3	JEX/A-595	Columbia	Medium	I1	41
4	JEX/A-801	Argentine	Medium	I1	49
Cluster II					
1	JEX/A-1046	Peru	Medium	II1	41
2	JEX/A-1081	Peru	Medium	II1	42
3	JEX/A-1061	Peru	Medium	II (Single)	37
Cluster III					
1	JEX/A-14	Peru	Medium	III1	48
2	JEX/A-15	Peru	Medium	III1	50
3	JEX/A-21	Peru	Low	III1	60
4	JEX/A-457	Columbia	High	III2	72
5	JEX/A-459	Columbia	High	III2	77
6	JEX/A-379	Columbia	Low	III (Single)	64
7	JEX/A-763	Bolivia	Low	III (Single)	57
8	JEX/A-612	Columbia	Medium	III3	52
9	JEX/A-622	Columbia	Medium	III3	52
10	JEX/A-107	Bolivia	Medium	III (Single)	53
11	JEX/A-122	Bolivia	High	III (Single)	57
12	JEX/A-132	Bolivia	Very low	III (Single)	37
13	JEX/A-804	Argentine	High	III4	50
14	JEX/A-827	Argentine	Very high	III4	46
Cluster IV					
1	JEX/A-164	Columbia	Medium	IV1	44
2	JEX/A-616	Columbia	Low	IV1	45
3	JEX/A-274	Columbia	High	IV2	45
4	JEX/A-275	Columbia	Medium	IV2	37
5	JEX/A-288	Columbia	Very low	IV2	52
6	JEX/A-638	Columbia	Medium	IV3	59
7	JEX/A-668	Columbia	Medium	IV3	59
8	JEX/A-683	Columbia	Medium	IV3	38
9	JEX/A-912	Peru	Medium	IV (Single)	31
10	JEX/A-1092	Peru	Low	IV4	54
11	JEX/A-1152	Peru	High	IV4	68
12	JEX/A-267	Columbia	Medium	IV (Single)	48
13	JEX/A-215	Columbia	Medium	IV5	32
14	JEX/A-865	Unknown	Medium	IV5	27
15	JEX/A-468	Columbia	Medium	IV (Single)	35
16	JEX/A-296	Columbia	Medium	IV (Single)	29
17	JEX/A-390	Columbia	Low	IV (Single)	42
18	JEX/A-232	Columbia	Low	IV (Single)	39
19	JEX/A-413	Columbia	Low	IV6	48
20	JEX/A-617	Columbia	Low	IV6	44
21	JEX/A-380	Columbia	Medium	IV (Single)	50
22	JEX/A-368	Columbia	Medium	IV (Single)	54
Cluster V					
1	JEX/A-30	Columbia	Very high	V1	54
2	JEX/A-907	Unknown	Medium	V1	54

Table 1: Continued.

S/N	Accession	Origin	Yield[a]	Cluster/subgroup/single	SSR alleles
Cluster V					
3	JEX/A-539	Columbia	Medium	V (Single)	62
4	JEX/A-705	Chile	Very high	V2	52
5	JEX/A-707	Chile	Very high	V2	45
6	JEX/A-708	Chile	Medium	V3	42
7	JEX/A-1038	Peru	Medium	V3	40
8	JEX/A-76	Columbia	Medium	V (Single)	36
9	JEX/A-79	Columbia	Medium	V4	45
10	JEX/A-298	Columbia	Medium	V4	58
11	JEX/A-299	Columbia	Medium	V4	50
12	JEX/A-202	Columbia	Medium	V5	46
13	JEX/A-316	Columbia	Medium	V5	58
14	JEX/A-317	Columbia	Medium	V5	55
15	JEX/A-329	Columbia	Low	V5	46
16	JEX/A-361	Columbia	Medium	V6	44
17	JEX/A-506	Columbia	Medium	V6	43
18	JEX/A-513	Columbia	Medium	V6	54
19	JEX/A-216	Columbia	Medium	V (Single)	46
20	JEX/A-498	Columbia	Very low	V (Single)	22
21	JEX/A-947	Peru	High	V (Single)	57
22	JEX/A-745	Peru	Low	V (Single)	32
23	JEX/A-918	Peru	Medium	V7	39
24	JEX/A-920	Peru	Medium	V7	39
Cluster VI					
1	JEX/A-42	Columbia	Medium	VI1	53
2	JEX/A-58	Columbia	Medium	VI1	45
3	JEX/A-197	Columbia	Medium	VI2	58
4	JEX/A-198	Columbia	Medium	VI2	43
5	JEX/A-199	Columbia	Medium	VI (Single)	42
Cluster VII					
1	JEX/A-19	Peru	Medium	VII1	46
2	JEX/A-26	Peru	Low	VII1	51
3	JEX/A-45	Columbia	Medium	VII (Single)	42
Cluster VIII					
1	JEX/A-189	Columbia	Medium	VIII (Single)	36
2	JEX/A-877	Unknown	High	VIII (Single)	16

[a]Average yield (g/plant) under short-days [Very low, <50; Low, 51 to 100; Medium, 101 to 200; High, 201 to 300; Very high, >300] are adopted from Kumar et al (2008).

RESULTS

SSR polymorphism

A total of 24 SSR markers were used to characterize the 77 Andigena potato core collections. Table 1 shows each accession, origin, cluster and SSR polymorphism in the core collection. Table 2 shows the detected polymorphism, number of alleles, allelic absolute frequencies and PIC values of 24 SSR loci in the core collection. SSR analysis detected a total of 214 SSR alleles with an average of 6 (STI0001 and STM0037) to 14 (STI0012) alleles per SSR locus. The 208 polymorphic alleles showed a varying degree of polymorphisms in terms of absolute frequencies from 2 (STG0010-188, STG0016-120, STI0001-206, STI0004-117, STI0030-142, STM0031-185 and STM0037-154) to 58 (STM0037-95). Six alleles namely STG0001-129, STI0004-83, STI0030-96, STI0033-111, STM0019-81 and STM5121-271 were monomorphic in the core collection. The highest PIC value of SSR locus was observed in STM1052 (0.903) followed by STI0012 (0.896) and

Table 2. Polymorphism of 24 microsatellite markers detected in the 77 Andigena core collection.

SSR Marker[a]	Map location	No. of SSR fragments	No. of polymorphic SSR fragments	Detected SSR alleles (in Bp) and their absolute frequencies[b] (in brackets)	PIC[c]
STG0001	XI	10	9	129 (68), 139 (14), 145 (11), 149 (21), 155 (7), 168 (3), 175 (7), 180 (5), 190 (5), 206 (10)	0.708
STG0010	III	7	7	121 (4), 128 (6), 145 (5), 150 (4), 157 (14), 163 (8), 188 (2)	0.798
STG0016	I	12	12	120 (2), 127 (24), 134 (7), 137 (34), 142 (9), 154 (9), 162 (9), 177 (13), 184 (7), 192 (9), 211 (13), 217 (10)	0.877
STG0025	X	11	11	187 (4), 196 (51), 202 (16), 208 (22), 215 (12), 219 (3), 223 (4), 239 (22), 248 (5), 264 (18), 277 (7)	0.832
STI0001	IV	6	6	175 (26), 184 (47), 194 (21), 206 (2), 212 (4), 237 (23)	0.740
STI0003	VIII	8	8	137 (19), 149 (56), 158 (48), 170 (35), 179 (48), 188 (27), 232 (36), 247 (39)	0.862
STI0004	VI	10	9	83 (69), 95 (24), 101 (19), 107 (24), 117 (2), 129 (24), 136 (10), 147 (8), 154 (8), 166 (11)	0.798
STI0012	IV	14	14	158 (38), 168 (33), 175 (23), 183 (30), 187 (14), 191 (8), 201 (12), 204 (7), 207 (6), 212 (6), 220 (7), 225 (8), 234 (16), 266 (24)	0.896
STI0014	IX	11	11	117 (8), 121 (12), 125 (27), 129 (19), 133 (10), 155 (5), 159 (9), 163 (5), 171 (6), 190 (5), 196 (6)	0.866
STI0030	XII	12	11	96 (65), 104 (16), 109 (8), 114 (10), 119 (23), 125 (7), 136 (8), 142 (2), 145 (13), 150 (4), 156 (9), 164 (10), 171 (4)	0.797
STI0032	V	8	8	106 (11), 118 (40), 122 (28), 130 (37), 142 (8), 151 (10), 157 (30), 187 (38)	0.840
STI0033	VII	6	5	111 (71), 121 (11), 131 (23), 137 (6), 159 (7), 199 (22)	0.671
STM0019	VI	7	6	81 (71), 215 (5), 221 (8), 229 (20), 237 (22), 243 (10), 252 (35)	0.766
STM0031	VII	7	7	185 (2), 197 (17), 203 (11), 211 (14), 218 (16), 223 (12), 230 (15)	0.833
STM0037	XI	6	6	95 (58), 101 (11), 109 (24), 116 (5), 134 (4), 154 (2)	0.613
STM1052	IX	13	13	207 (41), 212 (16), 220 (16), 226 (12), 230 (17), 235 (14), 243 (15), 250 (14), 256 (11), 263 (11), 268 (11), 279 (9), 296 (20)	0.903
STM1053	III	8	8	167 (41), 174 (38), 180 (16), 186 (11), 191 (5), 196 (7), 205 (6), 211 (24)	0.806
STM1064	II	8	8	186 (24), 191 (30), 195 (20), 201 (11), 206 (9), 213 (7), 219 (10), 225 (16)	0.846
STM1104	VIII	9	9	161 (27), 168 (49), 177 (10), 204 (7), 211 (14), 221 (4), 226 (6), 232 (12), 241 (12)	0.805
STM1106	X	10	10	133 (22), 145 (15), 152 (44), 159 (41), 166 (12), 178 (5), 184 (4), 200 (17), 211 (10), 240 (25)	0.853
STM5114	II	7	7	278 (15), 286 (31), 291 (33), 297 (17), 302 (11), 312 (3), 330 (7)	0.800
STM5121	XII	8	7	271 (67), 280 (13), 286 (10), 292 (10), 297 (17), 303 (12), 309 (3), 316 (15)	0.741
STM5127	I	8	8	237 (8), 241 (8), 245 (6), 248 (4), 258 (14), 263 (16), 267 (5), 304 (7)	0.847
STPA58	V	8	8	226 (24), 230 (36), 235 (19), 244 (10), 249 (5), 256 (25), 263 (15), 269 (23)	0.848
Total		214	208		

[a]SSR repeat motifs, primer sequences, Annealing temperature (T°a), allele size, number of alleles and other details are described in Ghislain et al. (2009). [b]Absolute frequencies may not reach 77 due to missing values, [c]Polymorphic information content.

STG0016 (0.877) and the lowest in STM0037 (0.613). The number of detected SSR alleles in the core collection varied from 16 (JEX/A-877) to 77 (JEX/A-459) with an average of 46.3 alleles per accession. The most informative loci that were reported in more than 50% core collection accessions were STM1053-167, STI0001-184, STI0032-118, STI0003-149, STM1104-168; STM1052-207, STG0025-196 and STM1106-152. This analysis also detected some of the null alleles (or missing values) because it was difficult to separate non-amplification due to experimental errors from null alleles.

Cluster analysis

Figure 1 shows a dendrogram of the core collection based on the total SSR polymorphism at the Dice similarity coefficient value that ranged between 0.22 to 0.83. Accordingly, the core collection was characterized into different clusters (including single accession) as shown in Table 1. The cophenetic matrix derived from the cluster analysis is in good agreement with the original similarity matrix (r^2 = 0.66). Setting the cut-off point of similarity coefficient at 0.37, eight main groups (Clusters I to VIII) were formed and each cluster is further distinguished into different subgroups including 26 single accessions, which show certain relationship with the cluster. The highest similarity of 0.83 was found between Columbian accessions JEX/A-316 and JEX/A-317, while the lowest similarity of 0.038 was detected between JEX/A-877 (unknown origin) and JEX/A-76 from Colombia: Moreover, at the 0.53 similarity coefficient, 48 main groups including 26 single accessions could be formed. The dendrogram represents wide genetic diversity present in the core collection. Average similarities were always higher in members of intra cluster

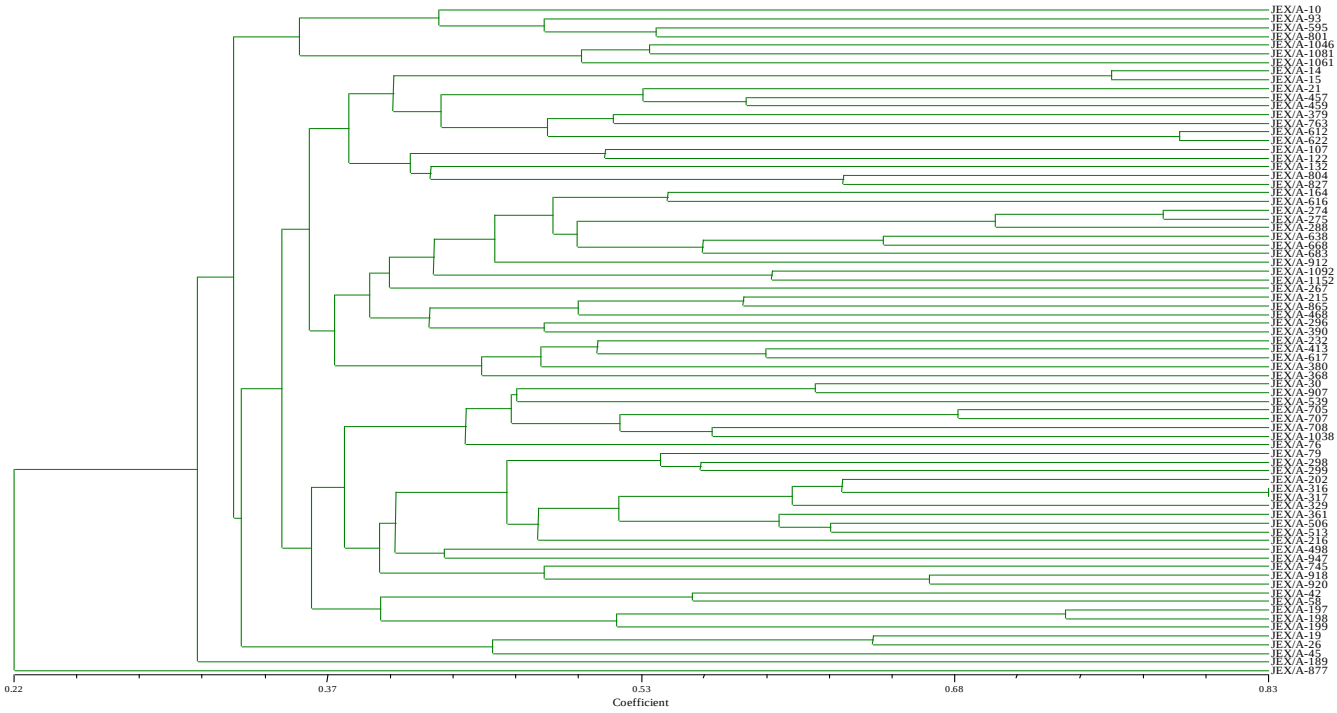

Figure 1. Dendrogram based on the Dice similarity coefficient showing the 77 Andigena core collection.

than inter cluster or subgroups. Details of the morpho-agronomic attributes of the core collection are shown in Supplementary Tables 1 and 2.

The first main group (Cluster I) contained 4 accessions from Peru, Columbia and Argentine which are low and medium yielding types and possessed SSR alleles counts between 32 to 49. Second main group (Cluster II) was formed by 3 accessions from Peru which were medium yielding type and hold high SSR allele counts between 37 to 41. Third main group (Cluster III) was formed by 14 accessions from Peru, Columbia, Bolivia and Argentine which varied between very low to very high yielding types and showed wide genetic diversity at the allelic counts by SSR polymorphisms and possessed SSR alleles counts between 37 to 77. The second largest, fourth main group (Cluster IV) was composed of 22 accessions from Columbia and Peru which varied between very low to high yielding types and showed wide genetic diversity at the allelic counts between 27 to 68 by SSR polymorphisms.

The largest fifth main group (Cluster V) was composed of 24 accessions from Columbia, Chile and Peru which varied between very low to very high yielding types and showed wide genetic diversity at the allelic counts between 22 to 62 by SSR polymorphisms. The sixth main group (Cluster VI) was composed of five accessions from Columbia which were medium yielding types and showed wide genetic diversity at the allelic counts between 42 to 58 and SSR polymorphism levels. The seventh main group (Cluster VII) was composed of three accessions from Peru and Columbia, which were low to medium yielding types and showed allele counts between 42 to 51 by SSR polymorphisms.

The eighth main group (Cluster VIII) was composed of only two single accessions JEX/A-189 from Columbia and JEX/A-877 (unknown origin). Accessions were medium and high yielding types, and SSR allele counts were 16 and 36.

Principal component analysis (PCA)

Variation among the first three principal components accounted for 25.64, 12.11 and 9.57% variation, respectively of the total variance summing up to 47.31%. A matrix plot of the first two components is presented in Figure 2 showing distribution of 77 core collections obtained from the first two principal components (Dim-1 and Dim-2). Cluster established by the cluster analysis were not clearly separated by the PCA and smaller differences in the position of accessions were observed. Clusters I, II, III and VII were located on the top of the plot, while clusters IV, V, VI and VIII were located in the middle to bottom of the plot. In addition, accessions were analyzed for PCA according to their origin that showed variable degree of cumulative percentage as shown in Supplementary Table 3. PCA analysis of accessions from Argenitna, Bolivia, Chile and unknown origin showed 100% variation. However, accessions from Columbia and Peru showed only 52.43 and 51.85% variation,

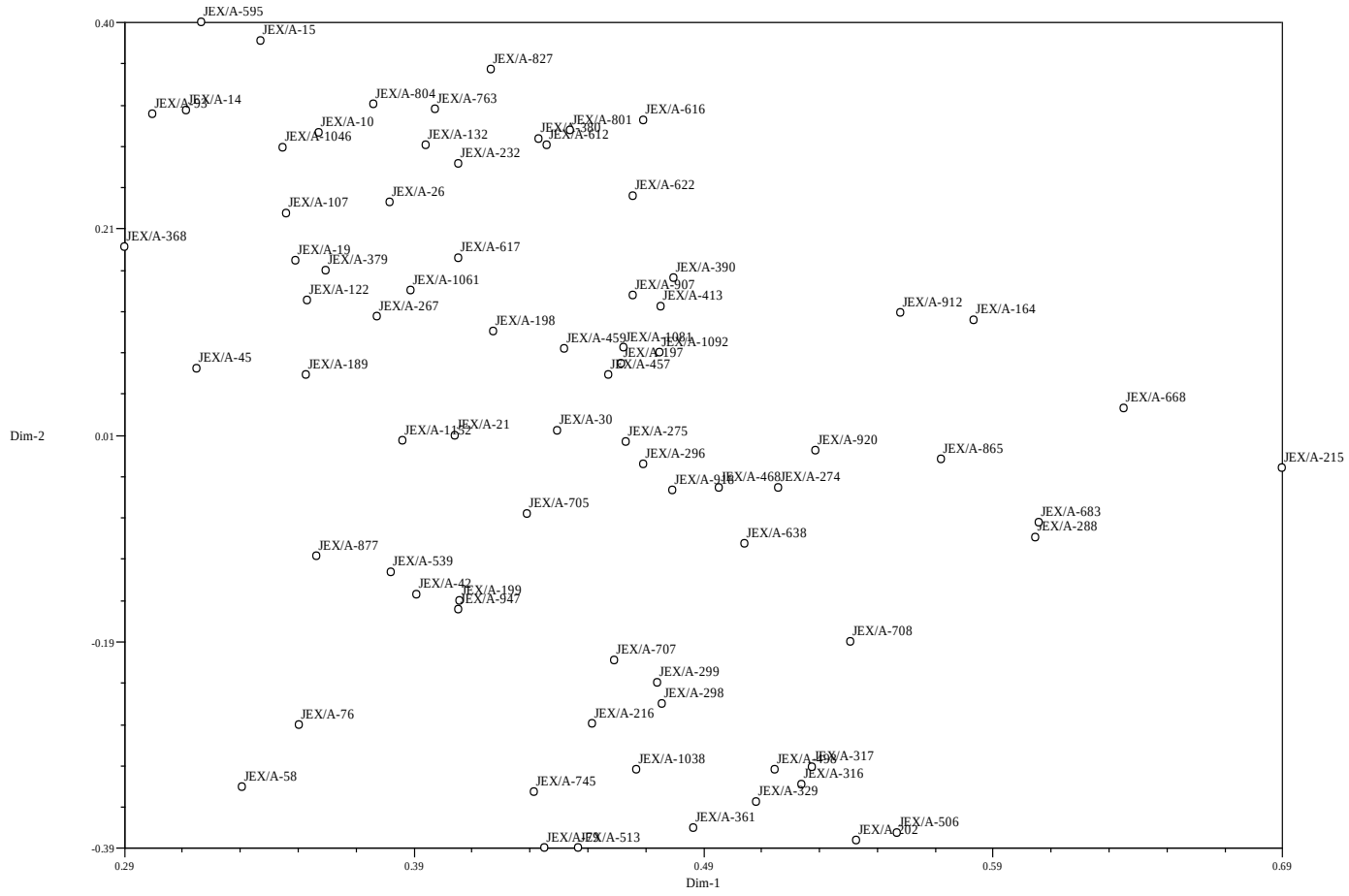

Figure 2. Principal component analysis showing distribution of the 77 Andigena core collection.

Table 3. Results of analyses of molecular variance (AMOVA) of 77 core collection.

Source	*df*	Sum of square	Variance component	Percentage of variation	*P*-value
Germplasm of various origins					
Between countries	5	29777	395	733	<0.001
Within countries	71	354779	4997	9267	
Various yield types[a]					
Between yield types	4	34969	842	1460	<0.001
Within yield types	72	354779	4927	8540	

[a] Yield type includes ‘very low, low, medium, high, and very high accessions.

respectively.

AMOVA

Analysis of molecular variance of the total genetic variations among the 77 accessions indicated that variance components were significant ($P < 0.001$) (Table 3). 7 % of the total SSR variation was distributed among countries while 92.67% was partitioned within counties. Of the total genetic variation, 14.6% was distributed among yield types, whereas 85.4% occurred within yield types.

DISCUSSION

A set of 24 SSR markers were employed to analyze the genetic diversity within Andigena core collection. The common use of a fixed set of reference markers that

reported earlier in potato (Galarreta et al., 2011), while it was similar to the results of Ghislain et al. (2009). Both the average allele number and PIC values are indicative of genetic diversity richness in the Andigena core collection. High level of polymorphism and heterozygosity in the microsatellite regions of potato suggested that microsatellite may be a useful tool to detect genetic differences between closely related taxa (Raker and Spooner, 2002). The higher the genetic diversity in the core collection indicates the larger content of genes, and the greater opportunity for a gene of interest to be mined from the collection. In other crop species such as in soybean, Kuroda et al. (2009) found high levels of allelic diversity (5 to 28 per locus) using 20 SSR marker loci in soybeans.

Zong et al. (2009) used 21 SSR loci to construct a core collection of Chinese landraces of *Pisum* genotypes. Using mini-core collections, resistance to grain mold and downy mildew was described in sorghum (Sharma et al., 2010), and also resistance to *Sclerotinia minor* Jagger (Chenault et al., 2010). In the cluster analysis, all the accessions originated from South American countries (Peru Argentine, Columbia, Bolivia and Chile); cluster analysis was not able to distinguish them clearly into different clusters. Although there was over-representation from Columbia, there was under-representation from Chile and Argentina in the core collection. However, there was a consistency of selection of genotypes with diverse representation in all the clusters. Thus, the dendrogram based on 24 SSR markers reflects an obvious distance and distinctiveness between accessions from different countries or with different morpho-agronomic traits in the core collection.

Fu et al. (2009) revealed ten groups by clustering of 169 accessions, but the groups were not distantly separated and found narrow genetic base of the Canadian potato germplasm. PCA analysis of the core collection indicated that only 47% variations in the first three components may be due to diverse genetic background of the core collection. Fu et al. (2009) showed that the 1.8% SSR variation was explained by country origin in Canadian germplasm and exotic potato accessions. Cluster analysis and PCA were generally in good agreement, but minor differences were observed in the location of accession between PCA plot and dendrogram.

Similarly, Galarreta et al. (2011) also showed that some smaller differences were detected in association of groups and genotypes in the cluster analysis and Principal Coordinate Analysis methods. AMOVA analysis of the core collection indicated that 7.33% variation was explained by between countries whereas 92.67% variation was observed within countries. Further, 14.60% variation was explained by between yield types whereas 85.40% variation were explained by the within yield type of accessions. A small fraction of the variation was partitioned between countries and this was demonstrated in the accessions from different counties of the one continent South America whereas accessions from within countries also showed major distinct genotypes. These results are in agreement with other studies of genetic diversity in landraces. For example, Ispizúa et al. (2007) found by means of SSR markers that most of the genetic variation of 155 potato accessions occurred among sites within localities and among varieties in Argentina.

The Andigena core collection was validated using SSR markers that harbored considerable genetic variation with important morph-agronomic traits. Microsatellite data of core collection generated was not only to enable efficient management of the core collection, but also provided useful information for selecting specific germplasm with distinct genetic background for diversifying potato breeding program. Moreover, using this basic information, it may be possible to explore the Andigena gene pools more effectively with population genetics-based approaches, such as association mapping and SNP markers.

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REFERENCES

Blair MW, Díaz LM, Buendía HF, Duque MC (2009). Genetic diversity, seed size associations and population structure of a core collection of common beans (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 119:955-972.

Brown AHD (1995). The core collection at the crossroads. In: Hodgkin T, Brown AHD Van Hintum ThJL, Morales EAV (Eds.) Core Collections of Plant Genetic Resources. John Wiley and Sons, West Sussex, UK, pp. 3-20.

Chandra S, Huaman Z, Krishna HS, Ortiz R (2002). Optimal sampling strategy and core collection size of Andean tetraploid potato based on isozyme data - a simulation study. Theor. Appl. Genet. 104:1325-1334.

Chenault KD, Melouk HA, Payton ME (2010). Evaluation of the U.S. peanut mini core collection using a molecular marker for resistance to *Sclerotinia minor* Jagger. Euphytica 172:109-115.

Del Rio A, Bamberg J, Huamán Z (2006). Genetic equivalence of putative duplicate germplasm collections held at CIP and US potato genebanks. Am. J. Potato Res. 83:279-285.

Excoffier L, Laval G, Schneider S (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinf. Online 1:47-50.

Fu Y-B, Peterson GW, Richards KW, Tarn TR, Percy JE (2009). Genetic diversity of canadian and exotic potato germplasm revealed by simple sequence repeat markers. Am. J. Potato Res. 86:38-48.

Galarreta JIR, Barandalla L, Rios DJ, Lopez R, Ritter E (2011). Genetic relationships among local potato cultivars from Spain using SSR markers. Genet. Resour. Crop Evol. 58:383-395.

Gavrilenko T, Antonova O, Ovchinnikova A, Novikova L, Krylova E, Mironenko N, Pendinen G, Islamshina A, Shvachko N, Kiru S, Kostina L, Afanasenko O, Spooner D (2010). A microsatellite and morphological assessment of the Russian National cultivated potato collection. Genet. Resour. Crop Evol. 57:1151-1164.

Ghislain M, Andrade D, Rodríguez F, Hijmans RJ, Spooner DM (2006). Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. Theor. Appl. Genet. 113:1515-1527.

Ghislain M, Nuñez J, Herrera MR, Pignataro J, Guzmán N, Bonierbale M, Spooner DM (2009). Robust and highly informative microsatellite-based genetic identity kit for potato. Mol. Breeding 23:377-388.

Gopal J (2006). Consideration for successful breeding. In: Gopal J, Khurana, SMP (Eds.), Handbook of Potato Production, Improvement and Post-Harvest Management. Haworth's Press, Inc., New York, pp. 77-108.

Huamán Z, Ortiz R, Gómez R (2000a). Selecting a *Solanum tuberosum* ssp. *andigena* core collection using morphological, geographical, disease and pest descriptors. Am. J. Potato Res. 77:183-190.

Huamán Z, Ortiz R, Zhang D, Rodríguez F (2000b). Isozyme analysis of entire and core collections of *Solanum tuberosum* subsp. *andigena* potato cultivars. Crop Sci. 40:273-276.

Ispizúa VN, Guma IR, Feingold S, Clausen AM (2007). Genetic diversity of potato landraces from northwestern Argentina assessed with simple sequence repeats (SSRs). Genet. Resour. Crop Evol. 54:1833-1848.

Kumar R, Kumar V, Gopal J, Luthra SK, Pandey SK (2008). Inventory of Potato Germpalsm (Group Andigena) Collection. Technical Bulletin No. 86, CPRI, Shimla. pp. 1-100.

Kuroda Y, Tomooka N, Kaga A, Wanigadeva SMSW, Vaughan DA (2009). Genetic diversity of wild soybean (*Glycine soja* Sieb. et Zucc.) and Japanese cultivated soybeans [*G. max* (L.) Merr.] based on microsatellite (SSR) analysis and the selection of a core collection. Genet. Resour. Crop Evol. 56:1045-1055.

Li X, Yan W, Agrama H, Hu B, Jia L, Jia M, Jackson A, Moldenhauer K, McClung A, Wu D (2010). Genotypic and phenotypic characterization of genetic differentiation and diversity in the USDA rice mini-core collection. Genetica 138:1221-1230.

Lung'aho C, Chemining'wa GN, Fu Y-B, Shibairo SI, Hutchinson MJ, Paniagua HG (2011). Genetic diversity of Kenyan potato germplasm revealed by simple sequence repeat markers. Am. J. Potato Res. 88:424-434.

Nei M (1973). Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321-3323.

Raker CM, Spooner DM (2002). Chilean tetraploid cultivated potato, *Solanum tuberosum*, is distinct from the Andean populations: microsatellite data. Crop Sci. 42:1451-1458.

Rohlf F (2006). NTSYSpc: Numerical taxonomy system (ver. 2.2). Exeter Publishing, Ltd., Setauket, NY, USA.

Sharma R, Rao VP, Upadhyaya HD, Reddy VG, Thakur RP (2010). Resistance to grain mold and downy mildew in a mini-core collection of sorghum germplasm. Plant Dis. 94:439-444.

Spooner DM, Nuñez J, Trujillo G, Herrera MR, Guzmán F, Ghislain M (2007). Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. Proc. Natl. Acad. Sci. USA 104:1939819403.

Spooner DM, Van Treuren RR, de Vicente MC (2005). Molecular markers for germplasm and genebank management. IPGRI Tech Bull 10. International Plant Genetic Resources Institute, Rome.

Upadhyaya HD, Yadav D, Reddy KN, Gowda CLL, Singh S (2011). Development of pearl millet minicore collection for enhanced utilization of germplasm. Crop Sci. 51:217-223.

Van Hintum TJL, Brown AHD, Spillane C, Hodgkin T (2000). Core collections of plant genetic resources. IPGRI Tech Bull 3. International Plant Genetic Resources Institute, Rome.

Wang ML, Sukumaran S, Barkley NA, Chen Z, Chen CY, Guo B, Pittman RN, Stalker HT, Holbrook CC, Pederson GA, Yu J (2011). Population structure and marker-trait association analysis of the US peanut (*Arachis hypogaea* L.) mini-core collection. Theor. Appl. Genet. 123:1307-1317.

Zong X, Redden RJ, Liu Q, Wang S, Guan J, Liu J, Xu Y, Liu X, Gu J, Yan L, Ades P, Ford R (2009). Analysis of a diverse global *Pisum* sp. collection and comparison to a Chinese local *P. sativum* collection with microsatellite markers. Theor. Appl. Genet. 118:193-204.