GENETIC DIVERSITY OF *PLUKENETIA VOLUBILIS* L. ASSESSED BY ISSR MARKERS*

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The diversity and genetic relationships in 173 sacha inchi samples were analyzed using ISSR markers. Thirty ISSR primers were used, only 8 showed variability in tested samples. ISSR fragments ranged from 200 to 2500 bp. The mean number of bands per primer was 12 and the average number of polymorphic bands per primer was 11. The lowest percentages of polymorphic bands (27%), gene diversity (0.103), and Shannon's information index (0.15) were exhibited by the Santa Lucia population, which was also geographically most distant. This fact may be attributed to a very small size of this group. In contrast, the Dos de Mayo population exhibited the highest percentage of polymorphic bands (78%), and the Santa Cruz population the highest Nei's gene diversity index (0.238) and Shannon's information index (0.357). The obtained level of genetic variability was 36% among tested populations and 64% within populations. Although the diversity indices were low, a cluster analysis revealed 8 clusters containing mainly samples belonging to individual populations. Principal coordinate analysis clearly distinguished Chumbaquihui, Pucallpa, Dos de Mayo, and Aguas de Oro populations, the others were intermixed. The obtained results indicated the level of genetic diversity present in this location of Peru, although it is influenced by anthropological aspects and independent on the geographical distances.

genetic variability; molecular markers; populations; Sacha inchi

doi: 10.1515/sab-2015-0029
Received for publication on May 27, 2015
Accepted for publication on August 15, 2015

INTRODUCTION

The Amazon is considered one of the most important centres of biodiversity. Several important crops, now used in agriculture worldwide (e.g. cassava, pineapple, cocoa, and rubber), have their origin and were domesticated in this region. However, there still have been several valuable species of Amazonian plants less investigated. A typical example of these species is sacha inchi (*Plukenetia volubilis*), a perennial semi-ligneous plant from the Euphorbiaceae family (Alvarado, 2008). Sacha inchi was probably cultivated by the pre-Incas and the Incas as a highly nutritious traditional food crop of the Peruvian Amazon and in early 1980's it was rediscovered as a very promising plant (Flores, 2010). Seeds of sacha inchi are rich mainly in high quality oil (Hamaker et al., 1992; Guillen et al., 2003; Bondiolli et al., 2006) and contain considerable amounts of proteins (Sathe et al., 2002). Sacha inchi is not only valued for its importance in alimentation, culture, and history, but also for its high economic value. It is a potential economically efficient crop with great possibilities for industrialization (Arévalo, 1995).

There is a significant research gap in understanding its genetic diversity. The estimation of the existing genetic diversity in such an interesting plant as *Plukenetia volubilis*, is essential for conservation, effective selection of plant materiál, and future breeding (Gupta et al. 2008). Concerning the *Plukenetia* genus, some studies have so far been devoted to the description of morphological variability; e.g. in *Plukenetia volubilis* (Alvarado, 2008), *Plukenetia huayllabambana* (Bussmann et al., 2009), and *Plukenetia carolis-vegae* (Bussmann et al., 2013). A few studies on the genetic variability of the *Plukenetia* genus are

* Supported by the Internal Grant Agency of the Czech University of Life Science Prague (CIGA), Project No. 20135004, by the Internal Grant Agency of the Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague (IGA), Project No. 20145020, and by the Foundation Nadání Josefa, Marie a Zdeňky Hlávkových.

Table 1. Collection sites and their description

No. of population	Name of population	n	Coordinates	Description of the site	Weight of hundred seeds (WHS) (g)
1	Mishquiyacu (MIS)	20	latitude 6°21,673‘ S longitude 76°34,998‘ W altitude 470 m a.s.l., southern orientation	ca. 400 m2 plot close to a small village, about 200 plants in the plot, cultivated by local villagers	139.74
2	Chumbaquihui (CHU)	20	latitude 6°21,991‘ S longitude 76°34,504‘ W altitude 364 m a.s.l.	close to Mishquiyacu, ca. 200 plants on 400 m2, cultivated together with banana plants by locals	177.78
3	Dos de Mayo (2DM)	22	latitude 6°47,573‘ S longitude 76°32,108‘ W altitude 335 m a.s.l.	a population distant from the others, recognizable for its small seeds. Ca. 350 plants cultivated at a slope in the edge of the forest on the area of 1000 m2	91.29
4	Pucallpa (PUC)	21	latitude 6°25,676‘ S longitude 76°34,689‘ W altitude 455 m a.s.l.	cultivation on the top of the ridge, area of 1.5 ha with irregular spacing of plants (ca. 1200) of a significantly shrubby growth	117.3
5	Aucaloma (AUC)	18	latitude 6°24,816‘ S longitude 76°26,143‘ W altitude 740 m a.s.l.	a small plot (300 m2) close to Pacchila plantation and intercropped together with banana and maize plants. 100 plants in total	120.14
6	Pacchilla (PAC)	20	latitude 6°25,694‘ S longitude 76°27,729‘ W altitude 703 m a.s.l.	commercial plantation, area of 15 ha, spacing 3 × 3 m, ca. 16 500 plants in total	131.08
7	Ramón Castillo (RAC)	7	latitude 6°35,244‘ S longitude 76°07,884‘ W altitude 210 m a,s,l.	several (7) plants scattered in the area of an abandoned sacha inchi field near the Huallaga River	102.11
8	Santa Cruz (SCR)	23	latitude 6°36,803‘ S longitude 76°44,452‘ W altitude 425 m a.s.l.	a garden on the edge of the village with robust plants cultivated in rows on wires; 150 plants per 0.1 ha	131.8
9	Aguas de Oro (ADO)	20	latitude 6°17,570‘ S longitude 76°39,200‘ W altitude 385 m a.s.l.	quite isolated cultivated population in the valley of the Huallaga River, the seeds were transported from Churuzapa village. Ca. 300 plants on 0.4 ha	126.56
10	Santa Lucía (SLU)	2	latitude 9°07,622‘ S longitude 76°01,040‘ W altitude 562 m a.s.l.	only two plants were encountered in this location several km from Tingo María. Both were grown binding a tree and seemed as an old cultivation	131.91
Total number of individuals		173			

SLU = Santa Lucia, SCR = Santa Cruz, AUC = Aucaloma, PUC = Pucallpa, PAC = Pacchilla, ADO = Aguas de Oro, MIS = Mishquiyacu, RAC = Ramón, CHU = Chumbaquihui, 2DM = Dos de Mayo

Table 2. List of ISSR markers with their scored bands, polymorphism and level of gene diversity

Primer	Sequence 5´- 3´	Scored bands			Diversity		
		band size (bp)	total bands	NPB	PPB (%)	h + SD	I + SD
UBC809	(AG)8 G	220–2000	13	9	69.2	0.081 ± 0.106	0.147 ± 0.168
UBC824	(AG)8 YT	200–2000	18	18	100	0.329 ± 0.127	0.499 ± 0.159
UBC826	(AC)8 C	700–2000	7	5	71.4	0.102 ± 0.120	0.181 ± 0.183
UBC836	(AG)8 YA	300–1500	9	9	100	0.250 ± 0.148	0.400 ± 0.190
UBC844	(CT)8 RC	300–2500	11	11	100	0.340 ± 0.129	0.513 ± 0.153
UBC845	(CT)8 RG	300–1500	13	13	100	0.234 ± 0.080	0.391 ± 0.107
UBC847	(CA)8 RC	400–2000	15	15	100	0.335 ± 0.134	0.506 ± 0.161
UBC859	(TG)8 RC	500–2500	11	10	90.9	0.312 ± 0.155	0.471 ± 0.204
Total		200–2500	97	90			
Mean			12.125	11.3	91.4	0.260 ± 0.158	0.405 ± 0.313

h = Nei's gene diversity, I = Shannon's information index, NPB = number of polymorphic bands, PPB = percentage of polymorphic bands, SD = standard deviation

available, too (Corazón-Guivin et al., 2008; Rodríguez et al., 2010; Rodrigues et al., 2013). The ISSR markers are considered to be a powerful tool for plant diversity determination and have been successfully applied in genetic studies for the species of Euphorbiaceae family (Gupta et al., 2008).

The objective of the present study was to assess the genetic variability within the species using the ISSR markers. The study has tried to capture the current level of genetic variability at the place of origin of this promising crop, sacha inchi, which is essential for future research, crop improvement, and suitable conservation management.

MATERIAL AND METHODS

Plant sample collection

Samples of *Plukenetia volubilis* were collected in June–August 2012 in the San Martín region of Peru. The plants from each location were randomly selected and seeds and leaves were collected. Leafy material of 173 individual plants from 10 localities was immediately stored in silica gel (Carl Roth GmbH, Karlsruhe, Germany). The collection sites were selected according to the Peruvian Amazon Research Institute's preferences (Table 1).

Extraction of DNA and ISSR analysis

The DNA (deoxyribonucleic acid) material of the leaf samples of *Plukenetia volubilis* L. was extracted with the cetyltrimethylammonium bromide (CTAB) method (Doyle, Doyle, 1987). The DNA quality was determined by 0.8% agarose gel electrophoresis and using a Micro-spectrophotometer UVS-99 (ACT Gene, Piscataway, NJ, USA). The final concentration of all the DNA samples was adjusted to 50 ng.μl^{-1} for Polymerase Chain Reaction (PCR), and stored at –20°C. Within optimization 30 available ISSR primers (Integrated DNA Technologies, Leuven, Belgium) were used, out of which only 8 were polymorphic (Table 2). The PCR amplification reactions were carried out in a total volume of 20 μl containing 0.5 μl of each primer, 10 μl PPP Master Mix (Top-Bio, Prague, Czech Republic), 0.2 μl Bovine Serum Albumin (BSA) (Thermo Fisher Scientific, Vilnius, Lithuania), 7.3 μl PCR Water (Top-Bio). The PCR amplification was performed in T100TM Thermal Cycler (Bio-Rad Laboratories, Berkeley, USA). The annealing temperatures in PCR were optimized for each primer (Table 2). The cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 95°C for 1 min, 1 min at specific annealing temperature in the range of 48–55°C, 2 min at 72°C (extension). These 40 cycles were afterwards followed by a final extension step at 72°C for 10 min. The PCR products were resolved in 2% agarose gels in 1X TBE buffer (P-Lab) using the following programme: 180 min at 55 V and 120 mA. The gels were stained with ethidium bromide staining (Carl Roth GmbH, Karlsruhe, Germany) and the bands were visualized and acquired under UV light (Cleaver Scientific, Rugby, UK). The size of the amplified products was estimated using a 100 bp ladder (Thermo Fisher Scientific, Lithuania).

Data analysis

All experiments were performed in duplicates, and the consistent and well-resolved fragments were manually scored. The scoring of the amplified DNA fragments was done on the basis of presence (1) or absence (0) in the gel. The genetic association was

Table 3. Measures of genetic diversity in the 10 populations of P. volubilis

Population	NPB	PPB	h + SD	I + SD	G_{st}	Nm*
Dos de Mayo (2DM)	70	77.78	0.173 ± 0.157	0.280 ± 0.228	–	–
Aucaloma (AUC)	65	72.22	0.174 ± 0.162	0.278 ± 0.237	–	–
Santa Lucia (SLU)	24	26.67	0.103 ± 0.180	0.150 ± 0.262	–	–
Pucallpa (PUC)	59	65.56	0.181 ± 0.190	0.277 ± 0.271	–	–
Ramón Castillo (RAC)	45	50.00	0.131 ± 0.167	0.207 ± 0.247	–	–
Mishquiyacu (MIS)	65	72.22	0.213 ± 0.196	0.322 ± 0.276	–	–
Santa Cruz (SCR)	69	76.67	0.238 ± 0.201	0.357 ± 0.279	–	–
Pacchilla (PAC)	69	76.67	0.184 ± 0.174	0.292 ± 0.246	–	–
Chumbaquihui (CHU)	69	76.67	0.212 ± 0.178	0.329 ± 0.253	–	–
Aguas de Oro (ADO)	67	74.44	0.188 ± 0.170	0.297 ± 0.245	–	–
Mean	60.2	66.89	0.180 ± 0.178	0.280 ± 0.254	0.290	1.227

NPB = number of polymorphic bands, PPB = percentage of polymorphic bands, h = Nei's gene diversity, I = Shannon's information index, SD = standard deviation, N_m = gene flow value, G_{st} = Nei's gene differentiation

*estimate of gene flow N_m from G_{st}: $N_m = 0.5(1 – G_{st})/G_{st}$

Table 4. Genetic distances among investigated populations (Coefficient: Standard Jaccard. Distance Transformation: d=1-s)

	2DM	AUC	SLU	PUC	RAC	MIS	SCR	PAC	CHU	ADO
2DM	0									
AUC	0.242	0								
SLU	0.231	0.197	0							
PUC	0.405	0.268	0.366	0						
RAC	0.426	0.438	0.258	0.524	0					
MIS	0.287	0.224	0.245	0.39	0.352	0				
SCR	0.273	0.229	0.273	0.374	0.379	0.183	0			
PAC	0.465	0.387	0.434	0.394	0.474	0.417	0.335	0		
CHU	0.332	0.348	0.229	0.356	0.419	0.327	0.303	0.472	0	
ADO	0.422	0.334	0.264	0.442	0.362	0.349	0.342	0.397	0.375	0

2DM = Dos de Mayo, AUC = Aucaloma, SLU = Santa Lucia, PUC = Pucallpa, RAC = Ramón, MIS = Mishquiyacu, SCR = Santa Cruz, PAC = Pacchilla, CHU = Chumbaquihui, ADO = Aguas de Oro

evaluated by calculating Jaccard similarity coefficient for pairwise comparison based on the proportion of shared amplified DNA fragments produced by the primers using Darwin software (Version 5.0.158). The percentage of polymorphic bands (PPB), Nei's (1973) gene diversity (h), and Shannon's information diversity index (I) were estimated using POPGENE software (Version 1.32) under the assumption of Hardy–Weinberg equilibrium. The two comparable estimators, Nei's gene diversity (h) and Shannon's information indices (I), were used to calculate the genetic diversity for each population.

RESULTS

ISSR band variation and level of polymorphism

In total, 97 clear and scorable bands were amplified by 8 out of 30 ISSR markers tested with an average of 12 bands per primer having an ISSR fragment size ranging from 200 to 2500 bp. The number of polymorphic bands ranged from 5 to 18 attributing to 90 total polymorphic bands and 11 polymorphic bands per primer. The PPB was 69% for primer 809, 71% for primer 826, 91% for primer 859, and 100% for primers 824, 836, 844, 845, and 847 (Table 3).

The highest values for Nei's gene diversity index (0.34) and Shannon's information index (0.513) were exhibited by primer 844. In contrast, primer 809 showed the lowest Nei's diversity and Shannon's information index with values of 0.081 and 0.147, respectively. The mean Nei's gene diversity and Shannon's information index for all primers were 0.260 and 0.405, respectively (Table 3). Gene diversity ranged from 0.103 for Santa Lucia (SLU) to 0.238 for Santa Cruz (SCR) with a mean of 0.18, and the same pattern was observed for the Shannon's information index which ranged from 0.15 for SLU to 0.357 for SCR with the mean of 0.280. The populations of Aucaloma (AUC), Pucallpa (PUC), Pacchilla (PAC), and Aguas de Oro (ADO) exhibited gene diversity close to the mean of 0.18.

Genetic variability

Values for Nei's genetic diversity (h) ranged from 0.103 to 0.231 with the mean of 0.180, and for the Shannon's information index (I) the values ranged from 0.150 to 0.357 with the mean of 0.280. The analysis of molecular variance (AMOVA) indicated a total of 64% within population and 36% among populations variation. The calculated Nei's gene differentiation (G_{st}) was 0.290 (Table 3) with gene flow value (Nm) estimated at 1.227 (Table 3).

Overall, the value of Jaccard similarity coefficient ranging from 0.183 to 0.524 was obtained. The pairwise comparison of Jaccard value showed that Mishquiyacu (MIS) and SCR as well as AUC and SLU were the closest populations with Jaccard distance coefficients of 0.183 and 0.197, respectively. PUC and Ramón (RAC) were the most distant populations with the distance coefficient of 0.524 (Table 4).

Principal coordinate analysis

The results of three-dimensional principal coordinate analysis (PCoA) (Fig. 1) indicated a relatively clear differentiation of sacha inchi individuals from four localities – Chumbaquihui (CHU), Dos de Mayo (2DM), PUC, and ADO. On the other hand, PCoA revealed the tendency of samples from other localities (MIS, PAC, RAC, AUC, SLU) and some admixed individuals from remaining localities to cluster together.

Cluster analysis

The Neighbor Joining (NJ) dendrogram of genetic distance between 10 populations (Fig. 2) clearly shows eight main clusters segregated according to the localities where the samples were collected. Cluster 4 branched into two visible sub-clusters 4-A and 4-B, while Cluster 2 and Cluster 8 contained only four and eight individuals, respectively. In the analysis each cluster was dominated by samples belonging to a specific population with some intermixing with samples from other populations. Cluster 1 contained nearly the entire CHU population, with samples 15 and 19 located in different clusters. Along with the CHU population, Cluster 1 contained three samples (DM04, DM06, and DM08) from the 2DM population. Cluster 2 was formed by only four individuals (CHU19, AUC14, AUC15, and AUC18). Cluster 3 contained samples mainly from the 2DM and AUC populations but also two samples from SLU, and MIS01, PUC09, PUC12, CHU15, and SCR22 samples. Sub-cluster 4A contained the sample PAC19 and all PUC samples except PUC09 and PUC12, which were included in Cluster 3. Sub-cluster 4B contained the whole PAC population, except PAC19, along with sample PAC19

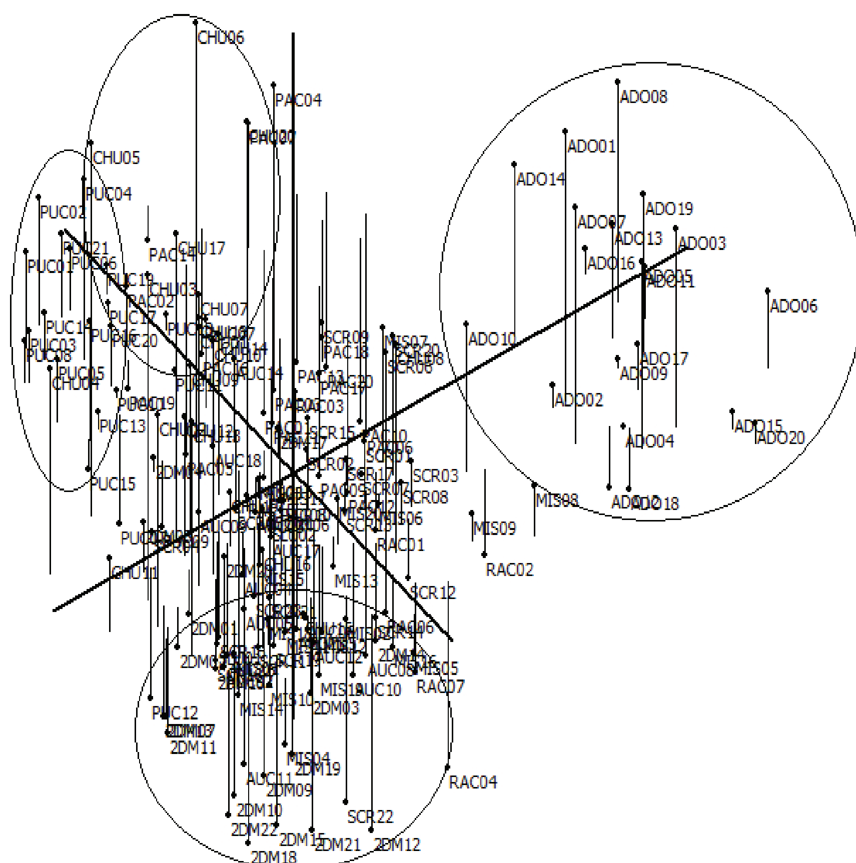

Fig. 1. Three-dimensional principal coordinate analysis (PCoA) of the genetic data based on Nei's genetic coefficients for tested 173 individuals of Plukenetia volubilis marking four distiguished compact populations

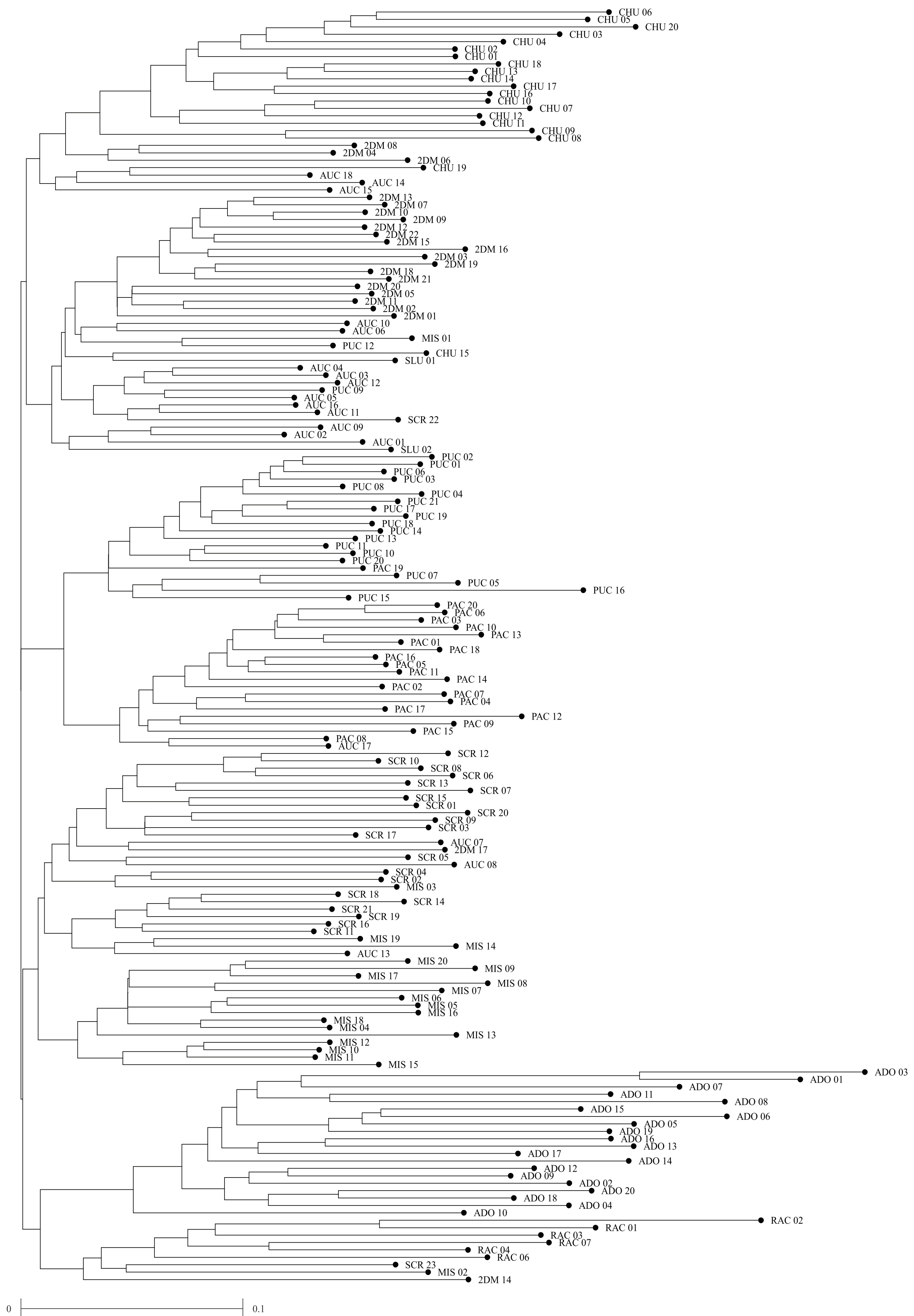

Fig. 2. Neighbor-joining (NJ) based analysis of 173 individuals of *P. volubilis* using Jaccard similarity coefficient

from the AUC population. Cluster 5 was formed of 21 out of 23 samples from the SCR population along with MIS03, AUC07, AUC08, and 2DM17 samples. Cluster 6 was formed exclusively by MIS samples, with the missing samples scattered among other clusters. Cluster 7 consisted of all twenty ADO samples. Finally, Cluster 8 was composed of all six RAC samples and three samples from different locations (MIS02, 2DM14, and SCR23). The dendrogram showed a clear differentiation of five populations (CHU, SRC, MIS, ADO, and RAC). The population collected in PAC, which was the only location included to the study where sacha inchi was cultivated on a commercial plantation, was closely related to the population from the PUC location. On the other hand, samples from the most distant populations of 2DM and SLU were merged together with samples from the AUC locality. Neither the cluster analysis, nor the principal coordinate analysis revealed any relation between the level of genetic diversity and geographical distance in the studied populations. The RAC population from the abandoned field created a separate cluster and exhibited a great divergence from the populations of PUC, 2DM, and CHU.

DISCUSSION

This study uncovered the level of genetic diversity within 10 populations of *Plukenetia volubilis* collected at different locations in Peruvian Amazon. The estimation of genetic diversity is essential for choosing an adequate breeding programme and strategy for diversity conservation. Because the genetic diversity based on morphological and biochemical parameters has limitations due to the influence of different environmental conditions, the application of molecular biology methods is desirable. According to the obtained results the application of ISSR DNA finger printing method was efficient and successful for disclosing the diversity between 173 samples of sacha inchi, scoring a range from 5 to 18 polymorphic bands. In total 8 ISSR primers generated 90 polymorphic bands.

The obtained level of genetic variability, 36% among the tested locations, can be considered as a lower diversity in the samples. In addition, in *Plukenetia volubilis* the estimated level of genetic diversity among populations was lower than that in populations. This result corresponds with the fact that long-living, outcrossing plants with wide and continuous flowering retain most of their diversity within populations. The lower genetic variabilities among populations than in populations were dicovered also in the study on *P. volubilis* (Corazón-Guivin et al., 2008) and in other allogamous plants like *Thymus* spp. (Hadian et al., 2014).

Corazón-Guivin et al. (2008) studied the intravarietal and intervarietal level of genetic diversity in four populations from the region of San Martín in the Peruvian Amazon using DALP primers and noticed a strong differentiation in four natural populations attributable to gene flow restriction as a result of factors like the presence of natural barriers, geographical distance, and mixed system of pollination. Also the effect of deforestation could cause isolation and fragmentation of the tested populations with the consequence of their strong genetic differentiation. In this study exploiting dominant ISSR markers, the G_{st} value was used for measuring the genetic differentiation of populations (Nybon, 2004). The estimated G_{st} value was 0.29 and the result for gene flow value Nm was 1.227. Hartl, Clark (2007) considered the values of Nm smaller than 2 present considerable opportunity for genetic population divergence. The obtained statistical data pointed to genetic diversity with adequate gene flow, supporting the belief that the populations are genetically different but not to such extent as described by Corazón-Guivin et al. (2008). The obtained G_{st} value (0.29) for the sacha inchi populations from the San Martín region indicates that an exchange of alleles (migration) has been taking place there, which is in line with the G_{st} values published by Fisher et al. (2000). The G_{st} value calculated with the employment of RAPD markers was 22% for perennial plants and 19% for outcrossing species. The above discussed results of the study by Corazón-Guivin et al. (2008) were probably influenced by the choice of investigation methods. The use of DALP technique with only three polymorphic primers may be responsible for the inaccuracy of the obtained results. Another reason for the discrepancy could be the type of sampling. In this study the samples of sacha inchi were collected on a substantially larger area of the San Martín region and the obtained results should reflect the situation in the genetic diversity of this species more realistically. Rodrigues et al. (2013) studied the genetic diversity in 37 *Plukenetia volubilis* samples from four localities, provided by the Embrapa Amazônia Ocidental gene bank, using the AFLP technique. The value of Jaccard dissimilarity coefficient for the tested samples was in the range of 0.338–0.900. The results of our study revealed lower genetic variability among all the 173 samples tested, with Jaccard dissimilarity coefficients ranging 0.183–0.524. The studied samples collected from the forests, home gardens, and one plantation seem to be genetically more related that those studied by Rodrigues et al. (2013) stored in the gene bank and collected at two different localities of Brazil.

The cluster analysis successfully distinguished 10 populations and PCoA distinguished 4 populations. The most distant population, SLU, was found in deep forest and showed the lowest genetic diversity due to the small size of population (Dostálek et al., 2014). On the other hand, in the dendrogram SLU individuals were placed to the same cluster with individuals of

2DM and AUC. All three populations were cultivated in great distance to each other and were cultivated in very extensive way (slopes and intercropped), moreover the size of seeds collected in the 2DM location was significantly smaller. On the other hand, the cluster analysis revealed that samples from the localities PUC and PAC are closer to each other. PAC plantations are new and the seeds are bigger, probably due to their selection by the farmers. Also ADO samples were placed together in one cluster with RAC, both localities were small and isolated. This proves the genetic diversity in the region of Peruvian Amazon predominates especially among extensively and intensively cultivated plants. PCoA and NJ showed a strong clustering in most of individuals, however in some clusters the admixed individuals were revealed as a possible consequence of gene flow through pollen or seed, migration of people from place to place, short and long distance marketing of seeds. Rodrigues et al. (2013) found out a certain degree of genetic similarity in the tested sacha inchi samples which may be attributed to the long cultivation history of this crop in the region. Aliyu, Awopetu (2007) described the situation in cashew nut and explained that during the pre-research era, exchange of planting materials must have taken place among farmers and this could be the reason why samples from different geographical regions have often been more closely related than the samples from neighbouring locations. Rao et al. (2012) attributed the loss of genetic diversity in *Solanum pimpinellifolium* to the migration of the species from Peru to Ecuador resulting in the selection towards autogamy. Heywood, Iriondo (2003) considered the anthropological influence crucial for the biodiversity conservation. In our study, both the cluster analysis and the principal coordinate analysis did not reflect any relationship between the level of genetic diversity and geographical distances in the studied populations. Also Beebee, Rowe (2008), Rodrigues et al. (2013), Shilpha et al. (2013), and Bekele et al. (2014) did not find any clear pattern of clustering according their geographical locations.

The result of both the cluster analysis and the PCoA successfully identified diversity among samples from different locations. The study revealed difference among samples from abandoned or older cultivation places and new plantations independently on the geographical distance. Presumably the genetic diversity is strongly influenced by the anthropological effect and the ways of distribution are connected with human activities such a migration of people from place to place, short and long distance marketing of seeds (Bekele et al., 2014).

CONCLUSION

So far, a few genetic information studies using different molecular markers have been published. The present research intended to extend the available information on *Plukenetia volubilis* focusing on the genetic analysis in order to describe the current genetic diversity and obtain information useful for conservation strategies and future breeding programmes with the aim to explore heterosis. The detection of the current genetic diversity could also help cultivating this important plant for human consumption in other areas. The used method of ISSR markers provided segregation of the tested individuals to appropriate clusters with the attribution of the place of their distribution and independently of their geographical distance. The genetic diversity in sacha inchi and the detected genetic variability were influenced by the isolation of populations and limited gene flow. The present level of a certain similarity is due to its historical long lasting use and seed transmission by people. Any future research of this species should be focused at the comparison of samples from other South American regions and the employment of other molecular biology methods with the aim of deeper genetic diversity analysis.

ACKNOWLEDGEMENT

We wanted to thank The Academy of Science of The Czech Republic (AVCR) and the National Council for Science, Technology and Technological Innovation (CONCYTEC), Peru, through the binational Project entitled ‘Morphological and genetic diversity of indigenous tropical trees in the Amazon’; and Bilateral Agreement between the CULS Prague and UNU in Pucallpa for the additional financial support during samples collection.

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