ASSESSMENT OF GENETIC DIVERSITY OF *Smallanthus sonchifolius* (Poepp. & Endl.) H. ROBINSON LANDRACES BY USING AFLP MARKERS

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Ovesná J., D. Russo, D. Frescura, E. F. Cusimamani, E. Svobodová, L. Milella (2018): *Assessment of genetic diversity of Smallanthus sonchifolius (Poepp. & Endl.) h. Robinson landraces by using AFLP markers.-* Genetika, Vol 50, No.3,803-816.
AFLP (Amplified Fragment Length Polymorphism) analysis was carried out on *Smallanthus sonchifolius* to increase the knowledge on its genetic diversity. It is an ethnomedical and edible plant native of Peru and cultivated in many other countries. Thirteen landraces were analyzed by selected AFLP primer combinations generating a number of 185 fragments, of which 180 were polymorphic (97.00% of polymorphism). The mean value of fragments per primer combination was 37, but *Mse*I (*M*)-CAG/*Eco*RI (*E*)-ACT primer combination reported the highest number with 63 amplicons, instead only 27 were revealed by *M*-CAG/*E*-ACC. The marker attributes such as resolving power (RP), marker index (MI) and polymorphism information content (PIC) were determined. RP values varied from 11.54 (*M*-CAG/*E*-ACC) to 27.54 (*M*-CAG/*E*-ACT), PIC ranged from 0.25 (*M*-CAG/*E*-AGC) to 0.28 (*M*-CAG/*E*-ACA), whereas MI values were found to

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be in the range from 6.18 (*M*-CAG/*E*-ACC) to 15.95 (*M*-CAG/*E*-ACT). Cluster analysis and PCA were evaluated for determining relationships among yacon landraces. We concluded that AFLP markers showed a highest efficiency in estimating genetic diversity in yacon despite to previous paper in which 3 times lower samples have been analyzed.

            Keywords: Amplified Fragment Length Polymorphism; DNA; genetic variation; molecular markers; Yacon.

INTRODUCTION

            The genetic variability describes genetic differences among individuals of the same species and it is an important tool for plant breeding and conservation programs (MINN *et al.,* 2015). Physiological (phenotypic characterization) and morphological characters were the only parameters for evaluation of genetic variation, but with this approach a reduced set of loci is estimated and the bias derived from environmental variability was not considered (KHANLOU *et al.*, 2011). Genetic molecular markers, used to determine the molecular variability, are not affected from environment and they can be applied to DNA derived from each growth stage. Molecular markers are DNA sequences or gene localized on chromosomes that encode a specific trait and they are applied to define and interpret the genetic variability in several organisms (SAKER *et al.,* 2005; SOUZA *et al.*, 2008; HASANOVA *et al.*, 2017). A number of DNA markers such as Inter-Simple Sequence Repeat (ISSR), Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Single Nucleotide Polymorphisms (SNPs) are used to quantify genetic variation in various plant species (MILELLA *et al.*, 2011; FERNANDEZ *et al.*, 2006; YONEMOTO *et al.*, 2007; SHARIFOVA *et al.,* 2017). The high discriminating ability of these molecular practices can be applied for identification of plant species, for evolutionary and taxonomic studies and breeding programs via marker-assisted selection, to detect plant species closely related and interspecific hybrids (PADULA *et al.,* 2013). Among all molecular markers, AFLPs, first described by VOS *et al.* (1995), represent dominant markers and provide multilocus profiles screening at the same time many different DNA regions of a genome. Unlike of other molecular markers, no sequence knowledge is required for AFLPs to develop species specific primers (PEAKALL *et al.*, 1998) and their cost is related to the number of samples to analyze. AFLP has been used to measure phylogenic and genetic variability in several plant species as pistachio, cashew, almond, apricot, chickpea, jatropha, echinacea, and hevea (GEUNA *et al.,* 2003; KAFKAS, 2006; NGUYEN, *et al.,* 2004; SINHA *et al.,* 2016; TATIKONDA *et al.,* 2009). Furthermore, some indigenous medicinal plants widely exploited from the wild population need immediate attention and information on their genetic structure and the diversity of populations are essential for their conservation programs (KUMAR *et al.,* 2014).

            Smallanthus sonchifolius (Poepp. & Hendl.) H. Robinson, named also yacon (Asteraceae family) is a perennial plant, but regarding the system of cultivation it is an annual plant (FERNANDEZ *et al.*, 2007; TATIKONDA *et al.,* 2009). Nowadays, *Smallanthus sonchifolius* can be found in several parts of the world (New Zealand, United States, Japan), but in Europe is mainly cultivated in Czech Republic. Morphologically, it is 1.5 to 3.0 m in height, with roots that can weigh until 20 kg (4 - 20 edible tubers), and its aerial parts show large green leaves and yellow flowers (FERNANDEZ *et al.* 2006). Yacon is an important food crop for Andean population; they used tuberous roots as food, but other plant parts are used as a vegetables (aerial parts) and for medicinal purposes (roots and leaves). Scientific studies reported that roots of *S.*

sonchifolius can be considered an ideal food for people with obesity problems because they have a high amount of fructooligosaccharides (40-70 % dry weight), natural compounds not metabolized in the digestive tract of humans (OJANSIVU *et al.*, 2011; FERNANDEZ *et al.*, 2007; RUSSO *et al.,* 2015a). In addition, studies on diabetic rats reported that leaf aqueous extract of this plant decreases glycemia and increases the concentration of insulin (AYBAR *et al.*, 2001). The specie is also a rich source of antioxidants of a phenolic character contained, mainly in the leaves (LACHMAN *et al.*, 2003; RUSSO *et al.*, 2015b). Several studies showed that leaves possess several biological effects, for example immunomodulation (VALENTOVÁ and ULRICHOVÁ, 2003), antioxidant and cytoprotector effects (RUSSO *et al.,* 2015b; VALENTOVÁ *et al.,* 2005; SIMONOVSKA *et al.*, 2003; VALENTOVA *et al.*, 2003). So, the agronomic conditions, the productivity and the identified bioactive phytochemicals (in particular flavonoids, caffeic acid and its derivatives) made yacon a good natural source for breeding and marketing programs, because the consumer demand of healthy stuff is growing. Thus, molecular and metabolomic approaches provide data useful for the management and the use of plant genetic resources. To date, few studies of the genus Smallanthus and its relatives were performed. Analysis of ITS regions demonstrated to be able to detect and differentiate among species and also between our germplasm and other germplasm (ŽIAROVSKÁ *et al.,* 2014). Thirty accessions of yacon were investigated by RAPD markers (MANSILLA *et al.,* 2006). A collection of yacon landraces and its relatives was retained at the Czech University of Life Sciences Prague; five were investigated by RADP and AFLP markers (MILELLA *et al.* 2011) and, successively, a study of several clone–type landraces were carried by ISSR (SVOBODOVÁ *et al.,* 2013; VIEHMANNOVA *et al.,* 2014; LORENZONI *et al.,* 2017). The only report (MILELLA *et al.*, 2011) that used the AFLP technique to assess genetic difference on yacon, demonstrated a higher variability, about 3 times greater than what is present in literature. This study aimed to investigate the molecular characterization of *Smallathus sonchifolius* landraces by using AFLP markers due to their ability to generate a very large number of bands and thus enable the identification of many polymorphic markers. In this way the knowledge and structuring of the genetic variability in wild and polyploid yacon landraces is increasing for potential breeding programs.

MATERIALS AND METHODS

Plant material

Leaf tissue of thirteen landraces of *Smallanthus sonchifolius,* selected for their different morphological traits, named PER01-PER11, ECU43 and ECU44, were harvested in October, 2013. The plant material, acquired since 1993, has been vegetatively propagated as previously described (SVOBODOVÁ *et al.,* 2013) and now maintained at the Faculty of Tropical AgriSciences (FTA), Czech University of Life Sciences Prague (CULS). A voucher specimen for all landraces (CZU-ECU43, ECU44 and PER01-11) is deposited at the Faculty of Tropical Agriculture herbarium, Czech University of Agriculture, Prague. PER01-PER11 are natural landraces, whereas ECU43 and ECU44 are *in vitro* artificial polyploids and their number of chromosomes and ploidy level were listed in a previous report (SVOBODOVÁ *et al.,* 2013).

DNA extraction

The extraction of DNA from plant samples is the first step for all molecular marker analysis. Yield and high quality of isolated genomic DNA is suitable for any DNA based detection systems. Genomic DNA was extracted from fresh young leaf tissue (about 0.1 g) using

Invisorb Spin Plant Mini Kit (STRATEC Molecular, Germany). The quality and concentration of total DNA was checked by 1% agarose gel and Lambda DNA (Thermo Scientific, USA) at different concentrations (5.0 and 7.5 ng/μL).

AFLP assay and profile analysis

AFLP markers were obtained with the Applied Biosystems kit for plant genome (Applied Biosystems). Eleven primer combinations were applied, but only five were used for further analysis. The choice of these primer pair combinations is due to the high number of produced fragments ranging from 100 to 500 bp. The step of the selective amplification, by using *Mse*I primers and fluorescently marked *Eco*RI primers, was carried out as multiplex PCR in a reaction mix of 10 μl of 10×Taq polymerase buffer (Qiagen, Germany) with 15 mM MgCl$_2$, 5 mMol dNTP (Invitrogen, USA), 5 pmol *Mse*I selective primer, 1 pmol *Eco*RI 6-FAM labelled primer, 1 pmol *Eco*RI JOE labelled primer, 1 pmol *Eco*RI NED labelled primer, 0.5 U Taq polymerase, and 1 μl diluted (1:20) preselective amplification reaction. Nuclease-free sterile water was added to the final volume 10 μl. The fragments were analyzed by capillary electrophoresis (ABI PRISM 310, Perkin Elmer, Covina, CA, USA) and ROX-500 (Applied Biosystems) was used as an internal size standard as reported by OVESNÁ *et al.,* (2011). The results were analyzed using GeneScan and Geno-typer software and the profiles obtained by each primer combination were registered. Experiments were done in triplicate.

Statistical analysis

All AFLP fragments were scored for presence (1) or absence (0) of homologous bands and a binary matrix was produced. Only reproducible and clearly distinct bands were scored for the analysis. The neighbour-joining cluster analysis of AFLP data focused on the Jaccard dissimilarity coefficient was calculated using the XLSTAT software 2016 to visualize the genetic relationships among landraces. Principal Component Analysis (PCA) by XLSTAT software 2016 was also applied, because the advantage of PCA is to develop a new and easier model with a smaller number of artificial analyses that accounts for most of the variance in the data set.

Marker attributes such as marker index (MI), polymorphism information content (PIC) and resolving power (RP) were used to evaluate the discriminatory ability of AFLP primer combinations. PIC for each AFLP fragment refers to a marker for measuring polymorphism within a population, depending on the total measurable alleles and the distribution of their frequency. The PIC value of the *l* allele was calculated as proposed by (NAGY *et al.,* 2012).

$$PIC = 1 - \sum_{i=1}^{l} P_i^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^{l} 2P_i^2 P_j^2$$

where Pi and Pj are the population frequency of the ith and j[th] allele. PIC was calculated considering the fragments for each primer combination.
The marker index was calculated as reported by (VARSHNEY *et al.,* 2007).

$$MI = PIC \; x \; EMR$$

where EMR (Effective Multiplex Ratio) is the number of polymorphic fragments per primer combination.

For each AFLP primer combination, the resolving power (RP) was measured according previous report (PREVOST and WILKINSON, 1999).

$$RP = \sum Ib$$

where *Ib* represents the fragment informativeness and it can be found into a 0-1 scale by the following formula:

$$Ib = 1 - [2 \, x \, |0.5 - p|]$$

where *p* is the proportion of the 13 landraces having the fragment.

RESULTS

In the present investigation, five primer combinations were applied to create the AFLP profiles of 13 yacon landraces considering only the fragments well resolved. A number of 185 amplicons were generated, of which 180 were polymorphic (97.00% of polymorphism) and only 5 were monomorphic. The bands obtained from AFLP markers were scored for presence (1) or absence (0), and then converted into 0/1 binary matrix.

All obtained fragments by AFLP were in a range from 27 (*M*-CAG/*E*-ACC) to 63 (*M*-CAG/*E*-ACT), showed 37 fragments per primer combination as mean value. The percentage of polymorphic fragment ranged from 92.59% to 100.00% (average value of 97.00% per primer combination). Considering the frequency distribution, the highest number of polymorphic bands were observed in 0.60–0.70 and 0.80–0.90 frequency classes (Fig. 1).
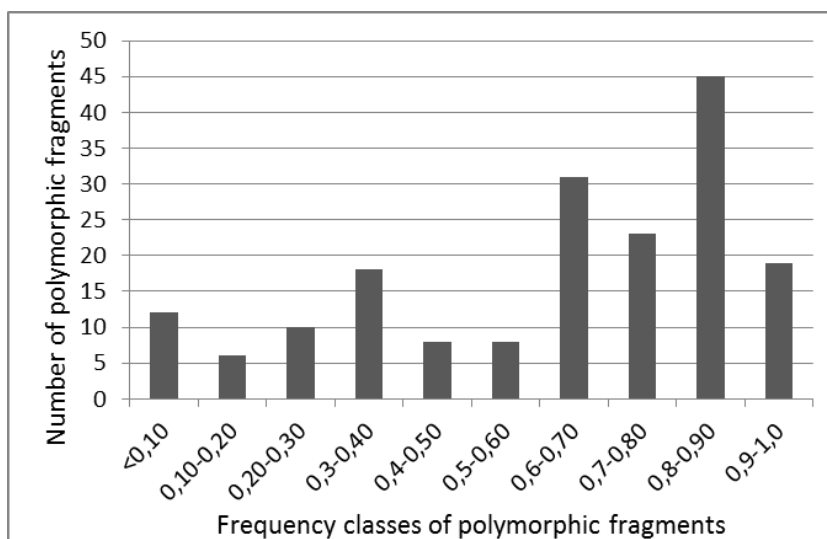

Fig. 1 Frequency distribution of polymorphic AFLP fragments in *Smallanthus sonchifolius* landraces

Taking into account only the polymorphic fragments, they can be organized in unique (UF), shared (SF) and similar fragments. The unique fragments, are considered as present in one accession for a primer combination and they varied from 1 (*M*-CAG/*E*-ACA) to 6 (*M*-CAG/*E*-AGC and *M*-CAG/*E*-ACT) with a total of 11. As reported by previous study (TATIKONDA *et al.,* 2009) AFLP fragments present in less than 10% of the yacon landraces were measured as rare fragments (RF), but no rare fragments were observed. Shared fragments were those that were scored in 70.00% of landraces. A total of 81 SF between polymorphic ones were detected ranging from 9 (*M*-CAG/*E*-ACC) to 26 (*M*-CAG/*E*-ACT). Similar fragments (SiF) were the fragments present in more than 70% of landraces and with the five used AFLP primer combinations a total of 87 were generated as similar (average value of 17.40 per primer combination, Table 1).

Table 1. Degree of polymorphism information and information content obtained from for 5 AFLP primer combination binary matrix

	TF[a]	MF[b]	PF[c]	% P[d]	UF[e]	SF[f]	SiF[g]
M-CAG/*E*-ACA-FAM	35	1	34	97.14	1	17	16
M-CAG/*E*-AGC-NED	28	0	28	100.00	6	11	11
M-CAG/*E*-AGG-JOE	32	1	31	96.88	2	18	11
M-CAG/*E*-ACC-NED	27	2	25	92.59	1	9	15
M-CAG/*E*-ACT-FAM	63	1	62	98.41	2	26	34
TOTAL	185	5	180		12	81	87
MIN	27	0	25	92.59	1	9	11
MAX	63	2	62	100.00	6	26	34
AVERAGE	37	1	36	97.00	2.4	16.2	17.4

a) Total fragments (TF). b) Monomorphic Fragments (MF). c) Polymorphic Fragments (PF)
d) Percentage of polymorphism (%).   e) Unique Fragments (UF).   f) Shared Fragments (SF).
g) Similar Fragments (SiF).

Marker attributes as PIC, MI and RP were used. The PIC value for 180 polymorphic fragments were found in the range from 0.05 to 0.50 with a mean value of 0.26. The maximum value of PIC for dominant markers is 0.50 since two alleles per locus are considered and both are affected by number and frequency of the alleles (Table 2). The highest value of PIC (0.28) was reported for the primer combination *M*-CAG/*E*-ACA whereas the lowest (0.25) for *M*-CAG/*E*-AGC primer combination. In our analysis 49 fragments showed a PIC value between 0.20 and 0.25, whereas only one fragment reported a PIC value of 0.05. The remaining fragments ranged mainly between 0.10 and 0.15 and between 0.25 and 0.35 (Fig. 2). The PIC values of polymorphic bands derived from a single primer combination were calculated to have its average PIC value. The distinctive ability of MI for AFLP data is associated to the EMR component and related to the large number of detected polymorphic bands generated for each profile. MI ranged from 6.18 (*M*-CAG/*E*-ACC) to 15.95 (*M*-CAG/*E*-ACT) with an average of 9.39 per primer combination. The resolving power (RP) allows the evaluation of the most informative primers to

be able for investigated cultivars differentiation and results of these 5 AFLP primer combinations fluctuated from 11.54 (*M*-CAG/*E*-ACC) to 27.54 (*M*-CAG/*E*-ACT) with an average of 18.09.

Table 2 Polymorphism information of the five AFLP primer combinations

Primer combinations	PIC	EMR	MI	RP
M-CAG/*E*-ACA-FAM	0.28	34	9.59	23.69
M-CAG/*E*-AGC-NED	0.25	28	6.89	11.85
M-CAG/*E*-AGG-JOE	0.27	31	8.34	15.85
M-CAG/*E*-ACC-NED	0.25	25	6.18	11.54
M-CAG/*E*-ACT-FAM	0.26	62	15.95	27.54
MIN	0.25	25	6.18	11.54
MAX	0.28	62	15.95	27.54
AVERAGE	0.26	36	9.39	18.09

Polymorphic information content (PIC); Effective multiple ratio (EMR); Marker index (MI); Resolving power (RP).
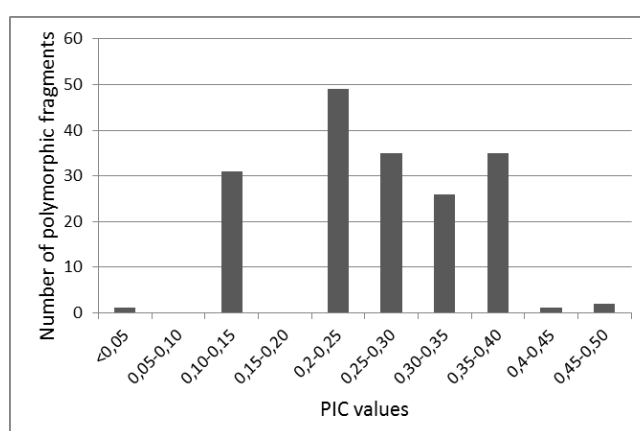

Fig. 2 Polymorphism information content (PIC) distribution for polymorphic fragments generated by AFLP primer combinations

Results of all 180 polymorphic fragments were used to generate a genetic dissimilarity matrix by using Jaccard's dissimilarity coefficient by XLSTAT software.

Based on Jaccard dissimilarity matrices (Table 3), a dendrogram was created by using UPGMA (Unweighted Pair Group Method of Arithmetic Mean). The dissimilarity coefficient values of the dendrogram ranged from 0.09 (PER04-PER10) to 0.89 (PER11-ECU43).

In the neighbor-joining tree, all 13 landraces were grouped in 6 clusters I-VI (Fig. 3). The clusters I, II, III and IV included each only one landrace, PER11, ECU43, PER08 and PER01, respectively. The remaining landraces were divided in cluster V (PER06, PER05, PER03, PER4, PER10) and VI (PER02, PER07, PER9 and ECU44).

Table 3 Jaccard dissimilarity matrix obtained by AFLP markers

|	PER 01	PER 02	PER 03	PER 04	PER 05	PER 06	PER 07	PER 08	PER 09	PER 10	PER 11	ECU 43	EC U44
PER01	0												
PER02	0.31	0											
PER03	0.39	0.30	0										
PER04	0.33	0.22	0.20	0									
PER05	0.37	0.27	0.22	0.21	0								
PER06	0.43	0.33	0.23	0.23	0.29	0							
PER07	0.31	0.21	0.28	0.25	0.24	0.37	0						
PER08	0.47	0.46	0.36	0.37	0.41	0.39	0.46	0					
PER09	0.33	0.20	0.31	0.23	0.24	0.35	0.20	0.44	0				
PER10	0.35	0.25	0.19	0.09	0.23	0.25	0.24	0.40	0.25	0			
PER11	0.83	0.83	0.83	0.83	0.83	0.86	0.82	0.81	0.84	0.84	0		
ECU43	0.76	0.71	0.73	0.72	0.74	0.74	0.73	0.79	0.73	0.72	0.89	0	
ECU44	0.39	0.28	0.31	0.21	0.27	0.33	0.28	0.44	0.26	0.25	0.84	0.70	0
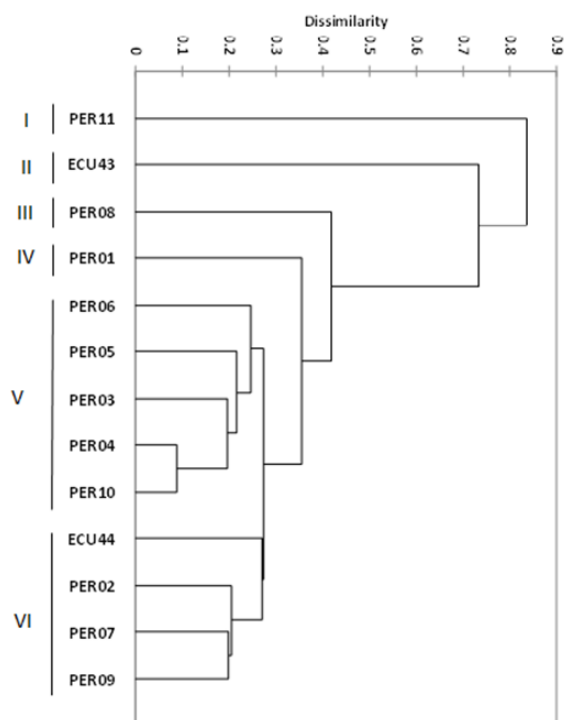
Fig. 3 AFLP dendrogram based on Jaccard dissimilarity coefficient of yacon landraces
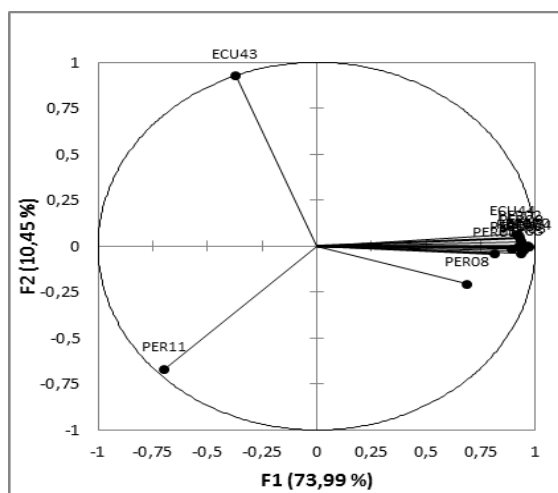
Fig. 4 Principal component Analysis based on Jaccard dissimilarity matrix obtained by AFLP primer combination

Genetic dissimilarity matrix obtained based on Jaccard's dissimilarity coefficient was also subjected to the PCA. Two dimensional graph separated the yacon landraces in 4 groups explaining 84.44% of dissimilarity with the first 2 component F1 and F2 (Fig. 4). PCA analysis formed 4 groups of which 3 comprised just one landraces, (PER08, PER11 and ECU43). The remaining yacon landraces are grouped together in the last group.

DISCUSSIONS

The evaluation of genetic variability can usually be observed through morphological, biochemical, and molecular characteristics (GONÇALVES *et al.,* 2009).
Smallanthus sonchifolius is a vegetative propagation species. Vegetative propagation results into genetically identical clone and preserves a stock of the desired variety, thus the use of molecular markers, in this case, has the advantage to underline genetic variability on a more detailed level among investigated landraces of yacon.

AFLP is a multilocus marker technique, highly informative and reproducible, that employs the use of restriction enzymes (RFLP tecnhique) and RAPD (LIU *et al.,* 2010). Several researchers evaluated the efficiency of the AFLP technique to analyze the genome of different herbal species comparing the results obtained with other molecular markers (RFLPs, RAPDs, ISSRs and SSRs) and finally they reported that the efficiency of AFLP is higher than the other techniques (ADAWY *et al.,* 2005; KOUNDAL *et al.,* 2006).

Previous study on molecular markers such as ISSR, RAPD and AFLP have been applied to measure the genetic diversity among *S. sonchifolius* individuals. RAPD markers tested on thirty cultivated yacon accessions from Peru (MANSILLA *et al.,* 2006) showed 30.70% of polymorphism. Five yacon landraces instead were analyzed by RADP and AFLP markers reporting only 28.70% and 23.40% of polymorphism, respectively (MILELLA *et al.,* 2011). ISSR markers applied to 27 clone–type landraces (SVOBODOVÁ *et al.,* 2013), showed 97.40% of

polymorphism, but considering only *S. sonchifolius* clones, the percentage of polymorphism was 80.30%.

Studies on the genetic diversity among yacon landraces were reported by scientific community, but the genetic variability of the plant still needs to be investigated.
In this study, 11 primer combinations from *Eco*RI and *Mse*I were investigated, and 5 of them gave a clear polymorphic marker profile for the investigated yacon landraces. The effectiveness of a molecular marker is related to the percentage of polymorphism it can detect. In this study the five primer combinations exhibited high percentage of polymorphism with an average of 97.00%. To the best of our knowledge, no study reported the distinguishing of unique, shared and similar fragments obtained by AFLP molecular markers on yacon. In the present study, the detection of 12 unique fragments would be a useful data for the selection of yacon landraces for plant improvement. The highest number of unique fragments were found in PER06 (6 UF), followed by ECU43 (4 UF), PER02 (1 UF) and PER08 (1 UF). One primer combination (*M*-CAG/*E*-AGC) was found to be the most effective. The feature of the unique bands to have a single occurrence in the genome, allows to convert them into sequence tagged site (STS) markers and then they can be applied to detect combinations between landraces (LAURENTIN and KARLOVSKY, 2007).

PIC, EMR, MI values of a primer help in determining its effectiveness in genetic diversity analysis. In our study the evaluation of genetic diversity was performed using PIC, RP and MI as marker parameters allowing the selection of informative primers (AVENDAÑO *et al.,* 2015). The PIC attribute has been applied to diversity/marker studies (TATIKONDA *et al.*, 2009), PREVOST and WILKINSON (1999) presented the meaning of resolving power (RP) in order to verify the discriminatory capacity of the 5 used primers showing an average PIC of 0.26. The highest PIC value was 0.28 and it was found with *M*-CAG/*E*-ACA, whereas the highest values of MI and RP were found with *M*-CAG/*E*-ACT. Considering these results, *M*-CAG/*E*-ACA and *M*-CAG/*E*-ACT primer combinations should be the most informative and usable for distinguishing among yacon landraces.

The applicability of integrative analysis are important to better understand the genetic diversity and the comparison of data from different molecular markers may reveal more relationship information. Results from UPGMA dendrogram and PCA were compared and discussed. The analysis of the genetic diversity in the yacon landraces was calculated through Jaccard's similarity index. The Jaccard coefficient measures the similarity between finite sample sets and it is complementary to the Jaccard distance. The UPGMA is the simplest agglomerative hierarchical clustering method, that uses the matrix of pairwise distances to create the phylogenetic tree in a stepwise manner. The dissimilarity coefficient values of the dendrogram ranged from 0.088 (PER04-PER10) to 0.892 (PER11-ECU43). PERR11 reported high Jaccard dissimilarity values with all other landraces; the highest value were observed with ECU43. Also ECU43 showed high dissimilarity values, suggesting a high genetic variation among evaluated yacon landraces. Analysis of dendrogram reported no clear geographical separation were observed, as well as by PCA.

In fact, both UPGMA-dendrogram as well as PCA display similar grouping of landraces showing small differences. Interestingly PER11, ECU43 and then PER8 yacon landraces showed the highest genetic dissimilarity in comparison to the other landraces in both dendrogram and PCA (**Fig. 3**-**4**). Results from UPGMA-dendrogram were more informative, in fact the remaining landraces are grouped together in a single group by PCA, whereas the same landraces were

grouped in three clusters by UPGMA-dendrogram, one of them included only PER01. Similar results have been showed by using ISSR markers and, considering the same yacon landraces, no strong separation of the investigated samples was observed in relation to their geographical origins (SVOBODOVÁ *et al.,* 2013). The same consideration was observed in other plant species as garlic, in fact, as reported by previous study (OVESNÁ *et al.,* 2011), the geographical origin of clones was not strictly correlated to the cluster analysis. Then, the two *in vitro* polyploids (ECU43 and ECU44), with 116 chromosomes, were not clustered together by AFLP markers. ISSR analysis (SVOBODOVÁ *et al.,* 2013) has not clearly separated, between plants with different number of chromosomes (58, 87 and 116 chromosomes); PER11 and PER5 with 87 chromosomes were grouped in two different clusters, one of them included only PER11.

This fact could be explained as previously suggested in other species, in which the plant tissue cultures may react differentially to the *in vitro* conditions and lead to the abnormal expression of a set of genes related to the normal cellular control for genetic and epigenetic fidelity (WANG *et al.,* 2013).

CONCLUSIONS

The medicinal properties of *Smallanthus sonchifolius* and its high consumer demand, attracted more attention from the researchers for conservative programs. The conservation of plant genetic resource requires an accurate identification of its accessions, thus this study aimed to assess the genetic diversity of 13 *Smallanthus sonchifolius* landraces using the powerful AFLP technique. AFLP are dominant markers that allow to detect a high level of polymorphism and gave reliable and reproducible results. A total of 185 AFLP fragments were observed and out of them 180 were polymorphic showing a percentage of polymorphism of 97.3, much higher than previous reports. The high number of polymorphic bands and the values of the marker attributes suggested that AFLPs are powerful and extremely discriminatory markers for fingerprinting analysis and genomic diversity in yacon compared with previous results. The evaluation of the variability level among landraces or cultivars through molecular techniques can be a useful tool for breeding and conservation programs, in particular for the selection of segregating populations. Therefore, combining the data obtained from the different types of markers may reveal more informative genetic relationships.

ACKNOWLEDGEMENTS

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OCENA GENETIČKOG DIVERZITETA *Smallanthus sonchifolius* (Poepp. & Endl.) H. ROBINSON POPULACIJA POMOĆU AFLP MARKERA

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Izvod

AFLP (Polimorfizam amplifikovanih dužina fragmenata) analiza je urađena na *Smallanthus sonchifolius* u cilju proučavanja genetičkog diverziteta. To je etnomedicinska jestiva biljka autohtona u Peruu i kultivisana u mnogim drugim zemljama. Trinaest populacija je analizirano odabranim AFLP kombinacijama prajmera, koji su dali ukupno 185 fragmenata, od kojih je 180 bili polimorfno (97.00% polimorfizma). Prosečan broj fragmenata po kombinaciji prajmera bio je 37, ali kombinacija *Mse*I (*M*)-CAG/*Eco*RI (*E*)-ACT je imala najveći broj od 63 amplikona, dok je kombinacija *M*-CAG/*E*-ACC imala samo 27. Određene su osobine markera kao što su snaga (RP), marker indeks (MI) i sadržaj informacija o polimorfizmu (PIC). RP je varirao od 11.54 (*M*-CAG/*E*-ACC) do 27.54 (*M*-CAG/*E*-ACT), PIC od 0.25 (*M*-CAG/*E*-AGC) do 0.28 (*M*-CAG/*E*-ACA), dok je MI bio u rasponu od 6.18 (*M*-CAG/*E*-ACC) do 15.95 (*M*-CAG/*E*-ACT). Klaster i PCA analiza primenjene su za utvrđivanje međuzavisnosti jakon populacija. Zaključili smo da su AFLP markeri pokazali najveću efikasnost u proceni genetičke raznovrsnosti kod jakona.