J. Agr. Sci. Tech. (2017) Vol. 19: ??-??

Genetic Evaluation of Sweetpotato Accessions Introduced to the Central European Area

B. Pipan[1]*, D. Žnidarčič[2], N. Kunstelj[3], and V. Meglič[1]

ABSTRACT

The objectives of the study were to investigate the genetic relationships of sweetpotato [*Ipomoea batatas* (L.) Lam] genotypes, acquired from different origins and to evaluate the genetic relations among them,using eight SSR markers. A high level of polymorphism was found, with an average of 7.5 alleles per SSR locus. High average values of Shannon's information index (0.864) and expected heterozygosity (0.739) revealed high level of genetic diversity of the Sweetpotato genotypes. Favorable applicability and informativity of selected set of SSR markers was confirmed by high global polymorphic information content (0.690) and low probability of genotype identity (1.4×10^{-8}). The overall fixation index was negative (-0.562), reflecting excess of heterozygosity, due to negative assortative selection as a consequence of vegetative propagation of sweetpotato. Estimation of *Rst* based on AMOVA shows 23% of molecular variance; the first two coordinates of PCoA cumulatively explaining 62.33% of genetic variability. The assignment of individual genotypes into three genetic groups is highly concordant with the PCoA and Bayesian approach in Structure analysis. Our results suggest that selection and breeding can also improve genetic potential and increase genetic uniformity in sweetpotato. Evaluation of genetic background and relationships among and within genotypes provided baseline data for introduction, management, production and conservation of sweetpotato germplasm, regarding its favorable consumer acceptance in Central Europe.

Keywords: Genetic structure, Genotypes, Polymorphism, SSR markers, Sweetpotato.

INTRODUCTION

Sweetpotato [*Ipomoea batatas* (L.) Lam] is a perennial herb, treated as an annual vegetable when cultivated (Moulin *et al*., 2012). It influences food supply chain particularly in developing countries (Escalante-Sanchez *et al*., 2008). Depending upon the variety, virtually all parts of the plant can be used for human consumption, including leaves, young shoots, seeds and storage roots (Takahata*et al*., 2011). Huang *et al*. (2006), estimated that sweetpotato roots and leaves contain phytochemicals with significant medicinal values, such as antioxidant properties that might be beneficial for human health.

Based on the number of related species and the analysis of their morphological variation, the *Ipomoeabatatas* geographical center of origin has been thought to lie between the Yucatan Peninsula in Mexico and the Orinoco River in Venezuela (Manifesto *et al*., 2010). Although sweetpotato has an out-crossing mating system, it is propagated vegetatively (Veasey*et al*., 2008). *I. batatas* is a hexaploid (2n= 6x= 90) with a basic

[1] Department of Crop Science, Agricultural Institute of Slovenia, Hacquetovaulica 17, SI-1000 Ljubljana, Slovenia.
[2] Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia.
[3] Biotechnical Centre Naklo, Strahinj 99, 4202 Naklo, Slovenia.
*Cooresponding author; E-mail: barbara.pipan@kis.si, Phone: +386 1 2805 273

chromosome number of x= 15 (Silva *et al*., 2012).

Sweetpotato is becoming increasingly popular in Slovenia (Kunstelj *et al*., 2013a; b). The introduction of sweetpotato in the Central European area has a great potential for agriculture and human nutrition (Kunstelj *et al*., 2013a; b), due to its variable genetic background which is potentially suitable for our production area. Hence, the collection of Sweetpotato germplasm of commercial value and breeding lines from Africa, Asia and USA. These accessions have the potential adaptation to local environment for higher yields and diseases and pests resistance. However, little is known about the genetic diversity and characteristic of these accessions.

Differences among accessions are revealed by the application of molecular markers or agro-morphological markers. According to Shanjani *et al*. (2012), it is generally much easier to evaluate differences between accessions for molecular markers than for morphological characters. This is because most morphological characters are multigenic, not available at all growth stages and are also influenced by environmental factors, making it difficult to assess them quickly and objectively, requiring repeated observations (Nováková *et al*., 2009). Different marker systems such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), simple sequence repeats (SSR) and Inter-Simple Sequence Repeat (ISSR) have been used to assess genetic diversity in different agronomical and horticultural important species (Mansour *et al*., 2015; Naghavi *et al*., 2009; Salahlou *et al*., 2016; Beigi *et al*., 2013). SSRs are highly applicable in genetic studies of different plant species (Pipan *et al*., 2013; Rusjan *et al*., 2012; 2015). This is because; SSR markers are more polymorphic and render more consistent results (Erayman *et al*., 2014). Application of SSR markers obtains high level of polymorphism due its codominant nature. SSR markers for sweetpotato have been developed (Jarret and Bowen, 1994; Buteler *et al*., 1999; Hu *et al*., 2004). Some of those were successfully used for genetic differentiation of Brazilian sweetpotato landraces (Veasey *et al*., 2008). Additionally, nuclear and chloroplast SSRs were applied for assessing genetic origin and diversity of sweetpotato in New Guinea, as a secondary center of diversity (Roullier *et al*., 2013). Determination of genetic relationship between cultivars, derived from polycross breeding programs was assessed by Hwang *etal*. (2002) using SSR markers. Some recently published studies also described diversity of sweetpotato accessions using 30 SSR markers (Koussao *et al.,* 2014; Yang *et al.,* 2015). Baffi *et al.* (2015), also studied 76 sweetpotato accessions for 40 agro-morphological and physico-chemical traits using 25 SSR markers.

The objectives of the present study were to investigate the genetic relationships of Sweetpotato genotypes to be cultivated in the Central European area, and to determine their population structure using SSR markers.

MATERIALS AND METHODS

Plant Material

Fifteen Sweetpotato genotypes including two reference cultivars were used in the study (Table 1).The work was done at the Biotechnical Faculty Ljubljana, Slovenia. Ten clones per genotype were cultivated under field conditions in a Randomized Complete Block Design (RCBD) with four replications. Thirty to fifty young leaves of each accession were sampled from 8 to 10 plants that were free of pests and diseases.

DNA Extraction

Genomic DNA was extracted from frozen leaves of four different plants individually from each of 13 genotypes and from one different plant from each of two reference cultivars. BioSprint 15 DNA Plant Kit

Table 1. Origin and morphological characteristics of the sweetpotato accessions.

Accession	Origin	Growth habit	Root shape	Skin colour	Flesh colour
SVNBe-01	Porto Rico	Semi-upright	Elliptic	Cream	White-cream
SVNRo-02	USA	Spread	Elliptic	Pale-orange	Orange
SVNVi-03	USA	Semi-upright	Elliptic	Purple-read	Cream
SVNK1-04	USA	Spread	Round	Light-copper	White-cream
SVNK2-05	USA	Half spread	Ovate	Cream	White-cream
SVNK3-06	Porto Rico	Semi-upright	Long-eliptic	Light-copper	Snow-white
Jewel	USA	Spread	Obovate	Copper	Pale-purple
SVNOk-08	Japan	Spread	Round-eliptic	Light-brown	Purple
SVNLe-09	USA	Semi-upright	Long-eliptic	Dark-orange	Orange
SVNNo-10	USA	Spread	Elliptic	Light-copper	Orange
SVNPs-11	USA	Half spread	Long-eliptic	Deep purple	Red-purple
SVNNv-12	USA	Half spread	Elliptic	Light-purple	White-cream
SVNMc-13	Porto Rico	Spread	Round-eliptic	Red-purple	Cream-white
Purple	USA	Half spread	Oblong	Purple	Deep purple
SVNMo-15	USA	Spread	Ovate	Pale-orange	Orange

(Qiagen) and MagMax (Applied Biosystems) nucleic acid isolation robot was used, following the modified method from manufacturer's instructions, primarily optimized for application using King Fisher mL (Thermo).

Genotyping Procedure

Eight SSR primer pairs were used (Table 2). Applied primer sequences were described by Buteler *et al*. (1999) and Veasey *et al*. (2008). PCR reactions were performed in a final volume of 11 µl, containing 1ng of genomic DNA and following reagents with starting concentrations of: 10X PCR buffer (Biotools), 10 mM of each dNTP's, 50 mM $MgCl_2$ (Biotools), 10 µM of each primer, 10 µM 5' fluorescently labelled universal primer (6-FAM, NED, HEX) and 0. 5 U of *Taq* DNA polymerase (Biotools). The forward primer of each SSR marker was appended with 18 bp tail sequence 5'-*TGTAAAACGACGGCCAGT*-3' (M13(-21) as described by Schuelke (2000). PCR analyses were performed on ABI 9700

Table 2. Parameters of genetic diversity among loci.[a]

Locus	Ra[a]	N[b]	He[c]	Ho[d]	No[e]	PI[f]	PIC[g]	Fst[h]	Nm[i]
Ib242	126-148	6	0.75	0.97	-0.131	0.102	0.71	0.16	1.28
Ib248	126-194	8	0.83	0.90	-0.044	0.055	0.79	0.30	0.58
Ib255F1	231-255	7	0.72	0.90	-0.109	0.119	0.67	0.27	0.65
Ib255	108-180	8	0.62	0.60	0.012	0.217	0.54	0.44	0.30
Ib286	98-120	7	0.78	1.00	-0.127	0.083	0.74	0.24	0.78
Ib297	104-182	8	0.81	0.91	-0.061	0.064	0.77	0.27	0.66
Ib316	92-316	7	0.56	0.47	0.051	0.263	0.49	0.21	0.90
Ib318	128-146	9	0.80	0.89	-0.054	0.068	0.77	0.26	0.70
Average		**7.5**	**0.73**	**0.83**	**-0.058**		**0.69**	**0.27**	**0.73**
Total		**60**				**1.4×10^{-8}**			
sd		0.9	0.09	0,19	0.065	0.077	0.11	0.08	0.28

[a] Range of allele lenghts, [b] number of alleles; [c] expected Heterozygosity; [d] observed Heterozygosity; [e] estimated frequency of Null alleles; [f] Probability of Identity; [g] Polymorphic Information Content ; [h] Inbreeding coefficient, and [i] Number of migrants. Standard deviation is defined as *sd*.

(Applied Biosystems) under the following ‘touch-down’ conditions: 94°C for 4 minutes, thirty cycles at 94°C for 1 minute, auto increment temperature from 49.5°C for 0.5°C per cycle for 30 seconds, 72°C for 1 minute, followed by thirty cycles at 94°C for 30 seconds, auto increment temperature from 49.5°C for 0.5°C per cycle for 30 seconds, 72°C for 1 minute and final extension for 5 minutes at 72°C. Fragment analysis was performed on 3130XL Genetic Analyzer (Applied Biosystems), the allele lengths were determined by comparison with size standard GeneScan-350 ROX (Applied Biosystems) using GeneMapper 4.0 (Applied Biosystems).

Data Analysis

Parameters of genetic diversity among loci including Range of allele lengths (Ra), numbers of alleles (n), frequencies of Null alleles (N_0) and Probability of Identity (PI) were calculated using Identity v.1.0 software (Wagner and Sefc, 1999). The degree locus-specific genetic differentiation, considering inbreeding coefficient (Fst) and Number of migrants (Nm) were done using GenAlEx v.6.4 (Peakall and Smousse, 2006). The MsToolkit (Park, 2001) was used to evaluate locus-specific expected Heterozygosities (He), observed Heterozygosities (Ho) and Polymorphic Information Content (PIC). The calculations of accession-specific statistics parameters including proportion of polymorphic loci, Numbers of effective alleles (Ne), expected Heterozygosities (He), Shannon’s Information index (I) and pairwise Nei’s genetic correlations were obtained using GenAlEx v.6.4 (Peakall and Smousse, 2006). Two commonly used estimators of volunteer and feral population differentiation are: F_{ST}, based on allele identity, and R_{ST}, which incorporates SSR-specific stepwise mutation model. Calculations of both estimations was performed using GenAlEx v.6.4 (Peakall and Smousse, 2006), where the estimation of R_{ST} was using Analysis of Molecular Variance (AMOVA) with 999 permutations. Bayesian method was used to assess genetic structure of Sweetpotato genotypes. This analysis was performed using the model-based software Structure v.2.3.3 (Pritchard *et al*., 2009) that infers the number of genetic groups *K*, present in a sample by comparing the posterior probability for different numbers of putative populations specified by the user and assigns individuals giving a percentage of membership (Q value), for these clusters. The admixture model with 100,000 MCMC (Markov Chain Monte Carlo) repetitions and 10,000 burn-in periods was employed. Eleven independent runs were performed without prior information on groups and assuming correlated allele frequencies. The real number of clusters was estimated by computing *ad hoc* statistic ΔK, based on the rate of change in the log probability of the data between successive *K* values described by Evanno *et al*. (2005) (Pascher*et al*., 2010). Genetic relatedness across genotypes from different accessions was estimated in PCoA *via* covariance matrix with data standardization using GenAlEx v.6.4 (Peakall and Smousse, 2006).

RESULTS AND DISCUSION

Knowledge of the genetic structure of this collection is essential for rational germplasm conservation and utilization (Zhang *et al*., 2000). There are also recent reports on the use of SSR markers successful for the study of genetic structure in common bean (*Phaseoulus vulgaris* L.) (Maras *et al*., 2015) and purity testing of commercial hybrid soybean (*Glycine max* L.) (Zhang *et al*., 2014). In this study, genetic evaluation within and between perspective accessions of sweetpotato, was performed. A high level of polymorphism was found, with an average of 7.5 alleles per SSR locus (Table 2). Veasey *et al*. (2008) evaluated 78 Brazilian accessions using the same set of eight SSR markers. Their results were obtained on the basis of binary scoring,

showing an average of 6 polymorphic bands per SSR locus, reflecting lower genetic diversity in their study. Therefore, high level of genetic diversity has been maintained and conserved for Sweetpotato genotypes, introduced to the Central European area.

The SSR markers, were highly informative and polymorphic, due to the high *PIC* value of 0.69 and low probability of identity of the different genotypes (1.4×10^{-8}), obtained in our study (Table 2). Additionally, codominant diploid determination of alleles is highly suitable in genetic diversity studies among sweetpotato genome, considering the fact, that *I. batatas* is a hexaploid species. High capability of genetic differentiation was found for Ib248, Ib297, Ib318, Ib297 and Ib242 loci. Moreover, for highly informative locus, Ib242 had the greatest Number of migrants (Nm= 1.2811), but the lowest inbreeding coefficient (Fst= 0.163). These findings indicate that locus Ib242 was very informative due to the number of migrants found, but *Fst* value represents the lowest level of genetic differentiation, compared to other loci. The positive values of frequencies for null alleles were found for Ib255 and Ib316, reflecting in its lowest values for Polymorphic Information Content (PIC< 0.550)(Table 2).

Accession-specific allelic patterns revealed a high average polymorphic loci (92.5%) reflecting in relatively high average number of effective alleles (Ne= 2.402) across accessions. Veasey *et al*. (2008) reported a similar level of polymorphism (95.8%). The PCoA showed an actual intra- and inter-accession genetic relatedness, based on covariance distance matrix. According to the common accession-specific patterns and PCoA, the greatest genetic uniformity was found in SVNPs-11 due to thelowest Shannon's information index and expected heterozygosity, confirming the minimal genetic distances between all genotypes from observed accessions. The eight SSR markers revealed a total of 60 different alleles across the 15 genotypes. The number of alleles ranged from 6 (Ib242) to 9 (Ib318), with an average of 7.5. Calculated mean frequency of null alleles (No) was -0.058 (Table 2). The higher number of migrants was found on the locus Ib242 where the detected No was the lowest (Table 2). Therefore, locus Ib242 could be used to attainthe changes in genetic structure of *I. batatas* genome due to temporal and spatial conditions. The average expected Heterozygosity (He) was 0.739 (Table3). Favorable applicability and informativity of selected set of SSR markers on co-dominant diploid level was confirmed by high global polymorphic information content (PIC= 0.690) and low probability of genotype identity (PI= 1.4×10^{-8}) (Table 2) considering the fact that such determination of alleles within genotypes across the same species occurred. The degree of genetic differentiation across genotypes was evaluated by global inbreeding coefficient (Fst= 0.273) and the average Number of migrants (Nm= 0.735). The inbreeding values at the Ib255 and Ib248 loci were the highest (Table 2), due to their homozygote frequency and hence allele fixation. Three alleles with a frequency > 0.25 (138, 148 and 249) were found as private alleles for SVNRo-02 on three different loci (Ib242, Ib248, Ib255F1). The accession SVNOk-08 has its three private alleles with a frequency > 0.167 on the same locus, Ib255. At the locus Ib316, three private alleles were found; allele 136 with a frequency of 0.50 for SVNNo-10, allele 104 with a frequency of 0.33 for SVNLe-09 and allele 316 with a frequency of 0.125 for SVNMo-15 (data not shown).

Global Shannon's information index of 0.864 suggests a high level of genetic diversity across the dataset (Table 3). The overall Fixation index (F) is negative (-0.562; sd= 0.036) reflecting excess of heterozygosity, due to the negative assortative selection as a consequence of vegetative propagation of sweetpotato (data not shown).Related to that, observed heterozygosity is higher than the expected heterozygosity(Table 2) and negative values for null allele frequency have been conducted. Considering Shannon's

Table 3. Parameters of genetic differentiation across accessions (both reference genotypes are excluded).

Accessions	Number of genotypes	Percent polymorphic loci	Mean No. of effective alleles	Shannon's Information index (mean) (I)	Expected Heterozygosity (mean) (He)
SVNK2-05	4	87.50	2.241	0.786	0.493
SVNK1-04	4	100	2.841	1.057	0.608
SVNMc-13	4	100	2.195	0.817	0.526
SVNNv-12	4	100	2.541	0.942	0.582
SVNBe-01	4	87.50	2.692	0.956	0.559
SVNVi-03	4	100	2.758	1.040	0.610
SVNNo-10	4	87.50	2.159	0.729	0.481
SVNK3-06	4	62.50	1.500	0.433	0.313
SVNRo-02	4	100	2.567	0.927	0.547
SVNLe-09	4	87.50	2.313	0.812	0.510
SVNMo-15	4	100	2.880	1.060	0.586
SVNPs-11	4	87.50	1.906	0.664	0.425
SVNOk-08	4	100	2.631	1.008	0.599
Average	4	92.5	2.402	0.864	0.526

Information index (I) and expected Heterozygosity (He), SVNK3-06, is the most genetical uniform (Table 3), which is not relevant due to the expanded frequency of null alleles, 62.5% of polymorphic loci and the lowest Ne across three genotypes. Therefore, the SVNPs-11, SVNNo-10 and SVNK2-05 exhibited the greatest genetic uniformity (He< 0.5 and I< 0.80) (Table 3). Conversely, the greatest variability was detected across SVNK1-04 SVNMo-15 and SVNok-08 accessions, expressing the highest values of effective alleles, *I* and *He* (Table 3). These results demonstrate that selection and breeding increase genetic uniformity, and thus might be used to improve obtained genetic potential.

The estimation of *Rst* (by stepwise mutation model using AMOVA) shows 23% of molecularvariation among accessions, 74% of variationamong screened genotypes and 4% within sweetpotato genotypes (Table 4). The first two coordinates in the PCoA cumulatively explain 62.33% of genetic variability considering covariance distance matrix. The sweetpotato accessions were distributed in PCoA into three genetic groups, as shown in Figure 1. Additionally, cluster analysis using Bayesian approach also generated three genetic clusters, colored as green, red and blue. The average genetic distances between genotypes in the first cluster (red) is 0.674 (Fst= 0.161), followed by 0.737 (Fst= 0.065) in the second cluster (green) and 0.663 (Fst= 0.236) for the third genetic cluster (blue) (Figure 2). Genotypes in the first (red) cluster were SVNK2-05, SVNNo-10 and SVNMo-15. Those in the second (green) were SVNK3-06, SVNRo-02, SVNLe-09, SVNPs-11, SVNOk-08 and the reference genotypes Jewel and Purple, while those in the third (blue)cluster were SVNK1-04, SVNNc-13, SVNNv-12, SVNBe-01 and SVNVi-03, respectively (Figure 2). Thus, we note that the assignment of individual genotypes into the three genetic groups is highly concordant with the PCoA. Pairwise genetic

Table 4.Analysis Of Molecular Variance (AMOVA) for the sweetpotato accessions.

Source of variation	*df*	*SS*	*EV*	*% TV*	*P*
Among accessions	12	1346311	7680.070	23	0.010
Among genotypes	39	1979329	24784.128	74	0.010
Within genotypes	52	61556	1183.769	4	0.010
Total	103	3387196	33647.967	100	

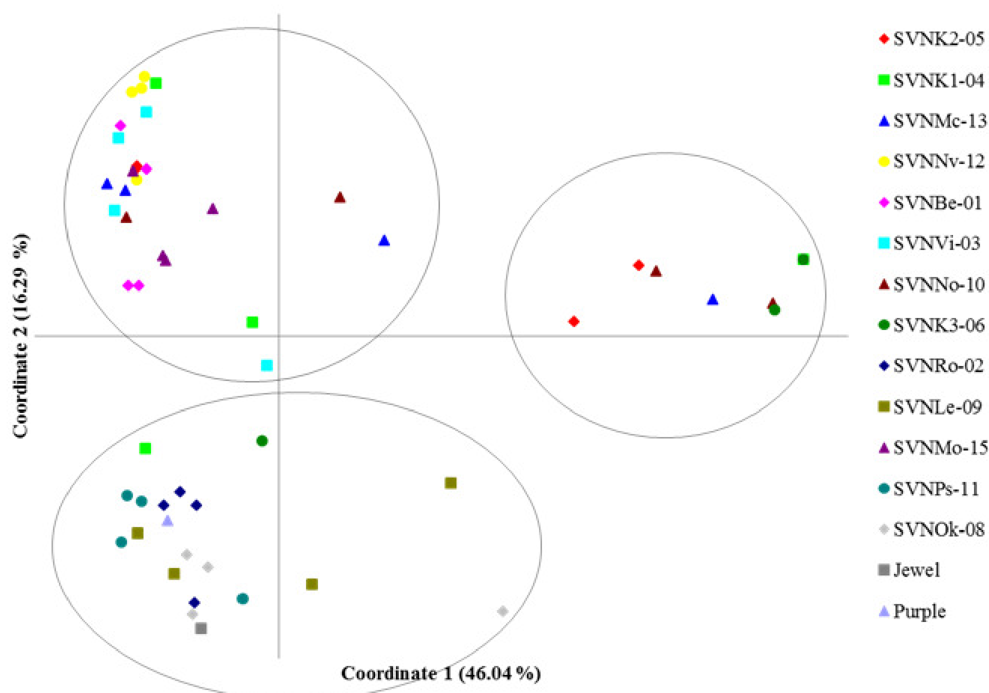

Figure 1. PCoA distribution of sweetpotato accessions.

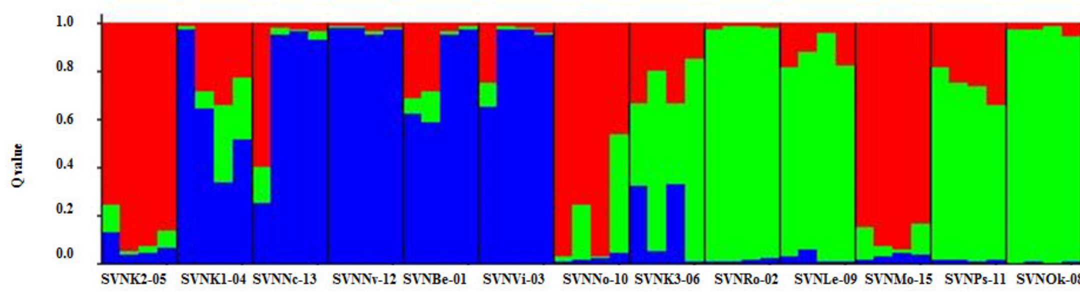

Figure 2. Genetic structure of individual genotypes from sweetpotato accessions.

comparisons across the sweetpotato accessions shows the highest genetic identity of 90.4% between SVNK2-05 and SVNNo-10 (Table 5), both originating from the USA (Table 5). The lowest genetic linkages were obtained between SVNNv-12 and Jewel (16.1%).Jewel was weakly related to SVNK2-05 (19.0%). Global pairwise genetic identity between genotypes in our study, based on Nei's genetic distance (Nei, 1972), shows the greatest genetic similarity for SVNVi-03 and SVNMc-13 compared to the others genotypes. Both accessions have purple skin color and creamy-flesh color, originating from the USA and Porto Rico, and belonging to the third genetic cluster (blue), according to the Bayesian approach. The lowest level of genetic association with other genotypes was observed for Jewel and SVNRo-02. These two accessions are representatives of the second (green) genetic cluster, originating from the USA, demonstrating the spread of growth habit.

Sweetpotato is asexually propagated *via* vine cuttings and adventitious buds arising from storage roots, which may result in the

Table 5. Pairwise matrix of Nei genetic identity (under diagonal) and ranking levels of genetic identity among accessions (above diagonal); 1: High level (0.690 – 0.999), 2: Moderate level (0.4 - 0.699), 3: Low level (< 0.399).

	SVNK2-05	SVNK1-04	SVNMc-13	SVNNv-12	SVNBe-01	SVNVi-03	SVNNo-10	SVNK3-06	SVNRo-02	SVNLe-09	SVNMo-15	SVNPs-11	SVNOk-08	Jewel	Purple
SVNK2-05	1.000	2	2	2	1	1	1	2	3	2	1	2	3	3	2
SVNK1-04	0.570	1.000	1	1	1	1	2	2	2	2	2	2	2	3	2
SVNMc-13	0.598	0.819	1.000	1	1	1	2	2	2	2	1	2	2	3	2
SVNNv-12	0.673	0.733	0.787	1.000	1	1	2	3	2	2	2	2	3	3	2
SVNBe-01	0.699	0.776	0.785	0.691	1.000	1	2	3	3	2	1	2	2	3	2
SVNVi-03	0.706	0.887	0.850	0.900	0.829	1.000	2	2	2	2	2	2	2	3	2
SVNNo-10	0.904	0.539	0.579	0.548	0.626	0.608	1.000	2	3	2	1	2	3	3	2
SVNK3-06	0.439	0.565	0.479	0.354	0.329	0.495	0.463	1.000	2	2	2	2	2	3	2
SVNRo-02	0.327	0.451	0.511	0.489	0.380	0.513	0.252	0.433	1.000	2	3	2	2	3	2
SVNLe-09	0.494	0.594	0.546	0.443	0.420	0.569	0.517	0.655	0.614	1.000	2	1	2	2	2
SVNMo-15	0.795	0.661	0.707	0.585	0.723	0.653	0.784	0.566	0.332	0.474	1.000	2	3	3	2
SVNPs-11	0.610	0.605	0.627	0.446	0.609	0.574	0.617	0.527	0.466	0.741	0.614	1.000	2	2	2
SVNOk-08	0.276	0.532	0.474	0.239	0.523	0.444	0.237	0.455	0.466	0.465	0.398	0.597	1.000	2	2
Jewel	0.190	0.333	0.363	0.161	0.298	0.289	0.222	0.389	0.371	0.427	0.345	0.559	0.625	1.000	2
Purple	0.450	0.494	0.529	0.410	0.499	0.531	0.440	0.412	0.591	0.600	0.486	0.641	0.594	0.530	1.000

accumulation of random mutation (Veasey *et al*., 2008) reflecting in *Rst* statistic from our AMOVA results.

In this study, analysis was performed according to general morphological traits and genetic evaluation of perspective genotypes, acquired from different origins. Revealing their genetic background, linkages and relationships among and within genotypes, baseline data for introduction, sustainable management, production and conservation of Sweetpotato germplasm in Central Europe were provided.

ACKNOWLEDGEMENTS

This research was supported financially by grant P4-0072 from the Slovenian Research Agency.

REFERENCES

1. Baffi, E., Gracen, V. E., Blay, E. T., Ofori, K., Manu-Aduening, J. and Carey, E. E. 2015. Evaluation of Sweetpotato Accessions for End-User Preferred Traits Improvement. *Afr. J. Agric. Res*., **10(50):** 4632-4645.
2. Beigi, S., Zamanizadeh, H., Razavi, M. and Zare, R. 2013. Genetic Diversity of Iranian Isolates of Barley Scald Pathogen (*Rhynchosporiumsecalis*) Making Use of Molecular Markers. *J. Agr. Sci. Tech*., **15**: 843-854.
3. Buteler, M. I., Jarret, R. L. and LaBonte, D. R. 1999. Sequence Characterization of Microsatellites in Diploid and Polyploid Ipomoea. *Theor. Appl. Genet.*, **99**: 123–132.
4. Eivazi, A.R., Naghavi, M.R., Hajheidari, M., Pirseyedi, S.M., Ghaffari, M.R., Mohammadi, S. A., Majidi, I., Salekdeh, G. H. and Mardi, M. 2008. Assessing Wheat (*Triticumaestivum* L.) Genetic Diversity Using Quality Traits, Amplified Fragment Length Polymorphisms, Simple Sequence Repeats and Proteome Analysis. *Ann. Appl. Biol.*, **152:** 81–91.
5. Erayman, M., İlhan, E., Güzel, Y. and Eren, A. H. 2014. Transferability of SSR Markers from Distantly Related Legumes to *Glycyrrhiza* Species. *Turk. J. Agric. For.*, **38**: 32–38.
6. Escalante-Sánchez, E., Rosas-Ramírez, D., Linares, E., Bye, R. and Pereda-Miranda, R. 2008. Batatinosides II–VI, Aacylated Lipooligosaccharides from the Resin Glycosides of Sweetpotato. *J. Agric. Food Chem.,* **56:** 9423–9428.
7. Evanno, G., Regnaut, S. and Goudet, J. 2005. Detecting the Number of Clusters of Individuals Using the Software STRUCTURE: a Simulation Study. *Mol. Ecol.*, **14**: 2611–2620.
8. Hernandez, T. P., Hernandez, T. and Miller, J. C. 1964. Frequency of Somatic Mutations in Several Sweetpotato Varieties. *P. Am. Soc. Hortic. Sci.*, **85**: 430–434.
9. Hu, J, Nakatani, M. and Mizuno, K. and Fujimura, T. 2004. Development and Characterization of Microsatellite Markers in Sweetpotato. *Breed. Sci.*, **54:**177–188.
10. Hu, J., Nakatani, M., Lalusin, A. G., Kuranouchi, T. and Fujimura, T. 2003. Genetic Analysis of Sweetpotato and Wild Relatives Using Inter-Simple Sequence Repeats (ISSRs). *Breed. Sci.*, **53**: 297–304.
11. Huang, Y., Chang, Y. and Shao, Y. 2006. Effects of Genotype and Treatment on the Antioxidant Activity of Sweetpotato in Taiwan. *Food Chem.*, **98:** 529–538.
12. Hwang, S. Y., Tseng, Y. T. and Lo, H. F. 2002. Application of Simple Sequence Repeats in Determining the Genetics Relationships of Cultivars Used in Sweetpotato Polycross Breeding in Taiwan. *Sci. Hort.*, **93**: 215–224.
13. Jarret, R. L. and Bowen, N. 1994. Simple Sequence Repeats (SSRs) for Sweetpotato Germplasm Characterization. *Plant. Genet. Resour. Newsl*., **100**: 9–11.
14. Koussao, S., Gracen, V., Asante, I., Danquah, E. Y., Ouedraogo, J. T., Baptiste, T. J., Jerome, B. and Vianney, T. M. 2014. Diversity Analysis of Sweetpotato (*Ipomoea batatas* [L.] Lam) Germplasm from Burkina Faso Using Morphological and Simple Sequence Repeats Markers. *Afr. J. Biotechnol*., **13(6)**: 729-742
15. Kunstelj, N., Žnidarčič, D. and Šter, B. 2013. Employing Artificial Neural Networks and Regression in Analysis on Knowledge about Sweetpotato (*Ipomoea batatas* L.) in Slovenia. *Ital. J. Food Sci.,* **25**: 263–274.
16. Kunstelj, N., Žnidarčič, D. and Šter, B. 2013. Using Association Rules Mining for Sweetpotato (*Ipomoea batatas* L.) in

Slovenia: a Case Study. *Int. J. Food, Agric. and Environ.,* **11:** 253–258.
17. Manifesto, M. M., Costa Tártara, S. M., Arizio, C. M., Alvarez, M. A. and Hompanera, N. R. 2010. Analysis of the Morphological Attributes of a Sweetpotato Collection. *Ann. Appl. Biol.,* **157:** 273–281.
18. Mansour, E., Ben Khaled, A., Triki, T., Abid, M., Bachar, K. and Ferchichi, A. 2015. Evaluation of Genetic Diversity among South Tunisian Pomegranate (*Punicagranatum* L.) Accessions Using Fruit Traits and RAPD Markers. *J. Agr. Sci. Tech*., **17**: 109-119.
19. Maras, M., Pipan, B., Šuštar-Vozlič, J., Todorović, V., Đurić, G., Vasić, M., Kratovalieva, S., Ibusoska, A., Agić, R., Matotan, Z., Čupić, T. and Meglič V. 2015. Examination of Genetic Diversity of Common Bean from the Western Balkans. *J. Amer. Soc. Hort. Sci.*, **140(4)**: 208-316.
20. Matos, E. L. S., Oliveira, E. J., Jesus, O. N. and Dantas, J. L. L. 2013. Microsatellite Markers of Genetic Diversity and Population Structure of *Carica papaya*. *Ann. Appl. Biol.*, **163**: 298–310.
21. Moulin, M. M., Rodrigues, R., Gonçalves, L. S. A., Sudré, C. P., dos Santos, M. H. and da Silva, H. R. 2012. Collection and Morphological Characterization of Sweetpotato Landraces in North of Rio de Janeiro State. *Hortic. Bras.*, **30**: 286–292.
22. Naghavi, M. R., Malaki, M., Alizadeh, H., Pirseiedi, M. and Mardi, M. 2009. An Assessment of Genetic Diversity in Wild Diploid Wheat (*Triticum boeoticum)* from West of Iran Using RAPD, AFLP and SSR Markers. *J. Agr. Sci. Tech*., **11**: 585-598
23. Nei, M. 1972. Genetic Distance between Populations. *Amer. Nat.*, **106:** 283–392.
24. Nováková, A., Šimáčková, K., Bárta, J. and Čurn, V. 2009. Potato Variety Identification by Molecular Markers Based on Retrotransposon Analyses. *Czech J. Genet. Plant Breed.,* **45:** 1–10.
25. Park, S. 2001. *Microsatellite Toolkit*. Department of Genetics, Trinity College, Dublin, Ireland.
26. Pascher, K., Macalka, S., Rau, D., Gollman, G., Reiner, H., Glössl, J. and Grabherr, G. 2010. Molecular Differentiation of Commercial Varieties and Feral Populations of Oilseed Rape (*Brassica napus* L.). *BMC Evol. Biol.*, **10**: 63. doi: 10.1186/1471-2148-10-63.
27. Peakall, R. and Smouse, P. E. 2006.GenAlEx 6: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. *Mol. Ecol. Note*., **6**: 288–295.
28. Pipan, B., Šuštar-Vozlič, J. and Meglič, V. 2013. Genetic Differentiation among Sexually Compatible Relatives of *Brassica napus* L. *Genetika*, **45(2)**: 309–327.
29. Pritchard, J. K., Wen, X. and Falush, D. 2009. *Documentation for STRUCTURE Software*: Version 2.3. Department of Human Genetics, University of Chicago and Department of Statistics, University of Oxford, USA.
30. Roullier, C., Kambouo, R., Paofa, J., McKey, D. and Lebot, V. 2013. On the Origin of Sweetpotato [*Ipomoea batatas* (L.) Lam.] Genetic Diversity in New Guinea, a Secondary Centre of Diversity. *Heredity (Edinb.)*, **110(6)**: 594–604.
31. Rusjan, D., Pipan, B., Pelengić, R. and Meglič, V. 2012 Genotypic and Phenotypic Discrimination of Grapevine (*Vitis vinifera* L.) Varieties of the 'Vitovska' and 'Garganja' Denominations. *Eur. J. Hortic. Sci.*, 77**(2)**: 84–94.
32. Rusjan, D., Pelengić, R., Pipan, B., Or, E., Javornik, B. and Štajner N. 2015. Israeli germplasm: phenotyping and genotyping of native grapevines (*Vitisvinifera* L.). *Vitis*, **54**: 87-89.
33. Salahlou, R., Safaie, N. and Schams-Bakhsh, M. 2016. Genetic Diversity of *Macrophomina phaseolina* Populations, the Causal Agent of Sesame Charcoal Rot Using Inter-Simple Sequence Repeat Markers. *J. Agr. Sci. Tech*., **18**: 277-287.
34. Schuelke, M. 2000. An Economic Method for the Fluorescent Labelling of PCR Fragments. *Nature Biotechnol.*, **18**: 233–234.
35. Shanjani, P. S., Jafari, A. A. and Kohi, L. 2012. Genetic Variation and Relationships between Local and Exotic Germplasm of *Dactylis glomerata*, Based on Morphological and Total Protein Markers. *Rom. Agric. Res.*, **29**: 103–113.
36. Silva, G. O., Ponijaleki, R. and Suinaga, F. A. 2012. Genetic Divergence among Sweetpotato Accessions Based on Root Traits. *Hortic. Bras.*, **30**: 595–599.
37. Takahata, Y., Kai, Y., Tanaka, M., Nakayama, H. and Yoshinaga, M. 2011. Enlargement of the Variances in Amount and Composition of Anthocyanin Pigments

in Sweetpotato Storage Roots and their Effect on the Differences in DPPH Radical-Scavenging Activity. *Sci. Hortic.*, **127**: 469–474.
38. Veasey, E. A., Borges, A., Silva Rosa, M., Queiroz-Sila, J. R., de Andrade Bressan, E. and Peroni, N. 2008. Genetic Diversity in Brazilian Sweetpotato [*Ipomoea batats* (L.) Lam., Solanaes, Convolvulaceae] Landraces Assessed with Microsatellite Markers. *Genet. Mol. Biol.*, **31(3)**: 725–733.
39. Wagner, H. W. and Sefc, K. M. 1999. *Identity 1.0*. Centre for Applied Genetics, University of Agricultural Sciences, Vienna, Austria.
40. Yang, X. S., Su, W. J., Wang, L. J., Lei, J., Chai, S. S. and Liu, Q. C. 2015. Molecular Diversity and Genetic Structure of 380 Sweetpotato Accessions as Revealed by SSR Markers. *J. Integr. Agric.*, **14(4)**: 633-641.
41. Zhang, D., Cervantes, J., Huamán, Z., Carey, E. and Ghislain, M. 2000. Assessing Genetic Diversity of Sweetpotato [*Ipomoea batatas* (L.) Lam.] Cultivars from Tropical America Using AFLP. *Genet. Resour. Crop. Ev.*, **47**: 659–665.
42. Zhang, D. P., Rossel, G., Kriegner, A. and Hijmans, R. 2004. AFLP Assessment of Diversity in Sweetpotato from Latin America and the Pacific Region: Its Implications on the Dispersal of the Crop. *Genet. Resour. Crop. Ev.*, **51**: 115–120.
43. Zhang, C. B., Peng, B., Zhang, W. L., Wang, S. M., Sun, H., Dong, Y. S. and Zhao, L. M. 2014. Application of SSR Markers for Purity Testing of Commercial Hybrid Soybean (*Glycine max* L.). *J. Agr. Sci. Tech.*, **16**: 1389-1396.

ارزیابی ژنتیکی الحاقیه سیب زمینی شیرین معرفی شده به منطقه مرکزی اروپا

ب. پیپان، د. زنیدارکیک، ن. کنستلی، و و. مگلیک

چکیده

هدف این مطالعه بررسی روابط ژنتیکی ژنوتیپ های سیب زمینی شیرین [*Ipomoea batatas* (L.) Lam] بدست آمده از مناطق مختلف با استفاده از ۸ نشانگر SSR می باشد. سطح بالایی از پلی مورفیسم، با میانگین ۷.۵ الل در هر لوکوس SSR پیدا شد. مقادیر متوسط بالا شاخص شانون اطلاعات (۰.۸۶۴) و هتروزیگوسیتی (۰.۷۳۹) ، سطح بالایی از تنوع ژنتیکی ژنوتیپ های سیب زمینی شیرین را نشان داد. کاربرد مطلوب و مقدار اطلاعات نشانگرهای SSR انتخاب شده توسط محتوای اطلاعات چندشکلی جهانی (۰.۶۹۰) و احتمال کم هویت ژنوتیپ ($1.4x10^{-8}$) تایید شد. شاخص تثبیت کلی به دلیل انتخاب منفی assortative به عنوان نتیجه تکثیر رویشی سیب زمینی شیرین منفی بود (-۰.۵۶۲)که منعکس کننده هتروزیگوسیتی زیاد می باشد. برآورد RST بر اساس AMOVA، ۲۳ درصد از واریانس مولکولی را نشان داد که؛ دو مختصات اول PCoA توضیح ۶۲.۳۳٪ از تنوع ژنتیکی می باشد. انتساب ژنوتیپ فردی به سه گروه ژنتیکی بسیار هماهنگ و همسو با PCoA و رویکرد Bayesian، در تجزیه و تحلیل ساختار می باشد. نتایج ما نشان می دهد که انتخاب و پرورش نیز می تواند پتانسیل ژنتیکی را بهبود بخشد و یکنواختی ژنتیکی در سیب زمینی شیرین را افزایش دهد. بررسی زمینه ژنتیکی و روابط بین و درون ژنوتیپ ، با توجه به علاقه مصرف کننده در اروپای مرکزی، یک

اطلاعات پایه ای برای معرفی، مدیریت، تولید و حفاظت از ژرم پلاسم سیب زمینی شیرین ارائه کرده است.