

Agro-Morphological Characteristics of Selected Sweet Potato (*Ipomoea batatas* L.) Varieties from Organic Farming and Their Genetic Background

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ABSTRACT.

Sweet potato is one of the most important root crops worldwide. This study aimed to compare agro-morphological characteristics of four sweet potato varieties (Martina, Janja, Lučka, Purple Speclet) from organic farming with additional information about their genetic background. A total of 26 agro-morphological traits were evaluated during vegetation. Pre-grown seedlings were planted in organic fields during the 2021 growing season using the soil ridge cultivation method. The study showed significant differences between varieties in quantitative (except for the extent and intensity of anthocyanin colouration on abaxial veins) and qualitative traits (except for the number and length of primary shoots and internode diameter). The varieties Lučka and Martina proved to be significantly higher yielding compared to the other two varieties. The genetic background of the varieties was evaluated on 8 Simple Sequence Repeat (SSR) loci using allele polymorphisms with a total number of 34 different alleles and an average polymorphic information content of 0.60. The favourable informativeness of the selected markers was confirmed by the global genetic diversity of 0.68. The assignment of each genotype to two genetic groups agreed well with the varietal distribution in the phylogenetic tree and the results of the analysis of the genetic structure (Martina/Janja and Lučka/Purple Speclet). The present study contributes to a better knowledge of the considered sweet potato varieties and their agro-morphological and genetic diversity.

Keywords: Allele polymorphisms, Genetic diversity, Organic farming, Plant descriptor, SSR marker.

INTRODUCTION

Sweet potato (*Ipomoea batatas* L.) is a dicotyledonous angiosperm plant and belongs to the convolvulaceae species (Gobena *et al.*, 2022; Cartabiano-Leite *et al.*, 2020). It is native to South and Central America, where its domestication began about 5,000 years ago. Later, the cultivated types spread throughout the Americas, Asia, and Africa (Escobar-Puentes, 2022; Roullier *et al.*, 2013). Nowadays, it is ranked the seventh most important food crop in the

world and is grown in 117 countries with a global annual production of nearly 90 million tons, with an average yield of 12 t ha⁻¹ (FAOSTAT, 2023; Prakash *et al.*, 2018). Sweet potato is grown mainly by smallholders and serves as a staple food in the poorest regions of the world (Andrade *et al.*, 2017) because it can be planted and harvested flexibly in frost-free areas, has a short growing season, uses non-edible parts for planting, does not form trellises, and requires few soil nutrients (Mukhopadhyay *et al.*, 2011). It is usually grown in areas between sea level and 2,300 m altitude in

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tropical and temperate zones located 40° south and north of the equator (Gobena *et al.*, 2022). Production in Europe, where the largest producers are Spain, Portugal, and Italy, accounts for the smallest share of global production, although the introduction of sweet potato in the central European region has great potential for agriculture and human nutrition due to its variable genetic background potentially suitable for this growing region, including Slovenia (Dinu *et al.*, 2021; Pipan *et al.*, 2017a).

The plant is cultivated for its starchy roots and immature leaves, which are used for human consumption and as animal feed (Gobena *et al.*, 2022; Ferreira *et al.*, 2022). Sweet potato is an admirable crop because it is the only member of its species capable of developing nutritious tuberous roots that feed a large portion of the world's population, especially in the tropics where most of the crop is grown and consumed (Cartabiano-Leite *et al.*, 2020; Ukom *et al.*, 2009). Tubers are a good source of energy with an abundance of proteins, lipids, fiber, sugars, minerals such as potassium, vitamins A, C, D, E, and B complex, and can be used for various purposes. They are rich in starch, which accounts for more than 50% of carbohydrate components (Hayati *et al.*, 2020; Andrade *et al.*, 2017) and are also a valuable source with anti-cancer, anti-diabetic, and anti-inflammatory properties (Mohanraj and Sivasankar, 2014).

Sweet potato is a vine-like perennial herb that spreads on the soil surface. Morphologically, the plant consists of the following main parts: tubers (enlarged root), stem (also called vine) and leaf, flower, fruit and seed, and is grown as an annual plant (Ukom *et al.*, 2009). Sweet potatoes can be propagated by seeds, tubers, or vine cuttings. Vegetative propagation is usually done by planting cuttings from the previous season's crop or by raising selected tubers. Vines/shoots from freshly harvested crop can also be planted in nursery beds to provide sufficient planting material (Mukhopadhyay *et al.*, 2011). Shoot production is increased by the best selection

of storage root sizes to optimize growth and yield. Varieties with different storage root sizes produce a variable number of shoots that vary in length and thickness. Standard characterization of plant genetic resources includes conventional approaches such as the use of descriptor lists for morphological traits or agronomic performance evaluation, complemented by molecular techniques (Maquia *et al.*, 2013). Sweet potato plant diversity can be studied by identifying agromorphological traits. The purpose of identification is to find out the important traits of plant species derived from different clones so that they can be used as a source of genetic diversity to support plant breeding activities. Generally, yields of sweet potato in farmer fields are low due to the use of local genotypes, but could be increased by using improved varieties or new clones (Hayati *et al.*, 2020; Reddy *et al.*, 2018). The success of any genetic conservation and breeding program depends on understanding the distribution of genetic diversity in the gene pool (Zhang *et al.*, 2000). Among the 175 species of *Ipomoea*, *Ipomoea batatas* is the only tuber-forming natural allohexaploid ($2n=6x=90$) species, although many of the species are diploid and tetraploid (Mukhopadhyay *et al.*, 2011; Reddy *et al.*, 2018). There are many varieties of sweet potato known and cultivated worldwide. They have different colours, shapes and sizes of skin and flesh of storage root and differ in taste and texture. The different sweet potato varieties are generally characterized by the skin and flesh color of the storage roots (tubers) and other agronomic traits such as leaf and stem morphology (Amagloh *et al.*, 2021).

The objective of this study was to characterize the agro-morphological variation among four sweet potato varieties grown in organic farming using the soil ridge cultivation method using various agromorphological traits related to overall plant architecture, stem, leaf and tubers, and to determine the main contributors of variation for future selection and breeding programs. In addition, SSR markers were used to

investigate the genetic background and relatedness between the four varieties at the molecular level. Sweet potato is relatively new crop in Slovenia and wider Central European growing conditions. Therefore, agro-morphological and genetic studies on the available varieties creates valuable knowledge for further intensification of cultivation in the region and beyond.

MATERIALS AND METHODS

Plant Material and Experimental Design

A set of four sweet potato varieties (Martina – purple skin and white tuber flesh, Janja – white skin and white tuber flesh, Lučka – orange skin and orange tuber flesh, Purple Speclet – purple skin and purple tuber flesh) were grown in the 2021 growing season, in the organic fields of the Biotechnical Centre in Naklo, Slovenia (46° 16' 18" N, 14° 18' 56" E, 420 m asl). The varieties Martina, Lučka and Janja were registered as protected varieties in the Slovenian National List of Varieties in 2016, while the variety Purple Speclet was commercially available on the European market. A single-factorial field trial was established at the experimental site in a randomized block design with four replicates of 15 seedlings planted 40 cm apart on the soil ridge of each plot. Seedlings were vegetatively propagated in the greenhouse by cuttings from previous growing seasons tubers. The seedlings (about 20 cm high) were planted in the field in early June on the plowed and harrowed soil ridges. Barley straw was used as mulch between plots to prevent roots from growing from each node during vegetation. The soil type is Umbrian planosols with a silty loam texture and a bulk density of 1.61 kg/m³ in the upper 30 cm. Analysis of the upper 30 cm of soil showed a pH of 6.8 and P₂O₅ and K₂O contents of 220 and 500 mg kg⁻¹, respectively. The organic carbon content was 5.3%, while the available Nitrate-

Nitrogen (NO₃-N) was 0.68%. Basic fertilization was carried out before tillage with the organic fertilizer Fertildung Stallatico Umificato Pellettato (3-3-1; Fertigest, Fomet, Italy), while the plants were fertilized twice during the growth period with the organic fertilizer Tiger Dung 3:6:12+2MgO (Fomet, Italy).

Agro-Morphological Characterisation

A total of 26 quantitative and qualitative descriptors related to the architecture of the whole plant, stem, leaves, and tubers developed by the International Union for the Protection of New Varieties of Plants – UPOV (2010) and the International Board for Plant Genetic Resources – IBPGR (1991) for *Ipomoea batatas* were used for the agro-morphological characterization. All qualitative descriptors were assessed visually, while quantitative descriptors were measured using a digital calliper (0.1 mm), a tape measure (1 cm) and a laboratory balance (0.5 g). Data on agro-morphological characteristics were collected according to the descriptor list in Table 1.

Molecular Analysis

Young leaf tissue was frozen and stored at –20°C for further genetic analysis. DNA was extracted from the leaves of four individual plants of each variety. The DNA extraction protocol and genotyping procedure followed the methods described in Pipan *et al.* (2017a, b) using a set of eight Simple Sequence Repeat (SSR) markers (Buteler *et al.*, 1999; Veasey *et al.*, 2008).

Data Analysis

Different univariate and multivariate approaches were applied to the data of the 26 agro-morphological qualitative and quantitative traits using the statistical programming environment version 3.4.4 R

**Table 1.** Agro-morphological characteristics considered in the study.

Type of trait	Organ	Abb	Descriptors		
Qualitative	Whole plant architecture	GH	Growth habit	1 Upright; 3 Semi-upright; 5 Spreading 3 Low (< 50%); 5 Medium (50-74%); 7 High (75-90%); 9 Total (> 90%)	
		GC	Ground cover		
	Stem	ACI	Anthocyanin coloration of internode	1 Absent or weak; 2 Medium; 3 Strong	
		ACT	Anthocyanin coloration of tip	1 Absent or weak; 2 Medium; 3 Strong	
		ACN	Anthocyanin coloration of node	1 Absent or weak; 2 Medium; 3 Strong	
		PT	Pubescence of tip	1 Absent or sparse; 2 Medium; 3 Dense	
	Leaf	LBL	Leaf blade lobes	1 Absent; 2 Three lobes; 3 Five lobes; 4 Seven lobes	
		DOL	Only varieties with leaf blade lobes present: Leaf blade: depth of lobing	1 Very shallow; 3 Shallow; 5 Moderate; 7 Deep; 9 Very deep	
		EACAV	Leaf blade: extent of anthocyanin coloration on abaxial veins	1 Absent or very small; 3 Small; 5 Medium; 7 Large; 9 Very large	
		IACAV	Leaf blade: intensity of anthocyanin coloration on abaxial veins	1 Very weak; 3 Weak; 5 Medium; 7 Strong; 9 Very strong	
PAC		Petiole anthocyanin coloration	1 Absent or very weak; 3 Weak; 5 Medium; 7 Strong		
PSN		Primary shoot number			
Whole plant architecture	NI	Number of internodes			
	LPS	Length of primary shoots (cm)			
	LI	Length of internode (cm)			
	DI	Diameter of internode (mm)			
	NL	Number of leaves/plant			
	PL	Petiole length (cm)			
Quantitative	Leaf	MLS	Mature leaf size (cm)		
		HFW	Haulm fresh weight (g/plant)		
	Tuber	NTP	Number of tubers/plant		
		TW/TP	Total weight of tubers /plant (g)		
		NM/TP	Number of marketable tubers (≥150 g)/plant		
		WMT	Weight of marketable tubers (g)		
		TD	Tuber diameter (cm)		
		TL	Tuber length (cm)		
		Abb			

Core Team (2021) to investigate the patterns of variation within and among the four sweet potato varieties analyzed in this study. First, the “*diverse*” package (Guevara *et al.*, 2016) was used to calculate the frequency of distribution and estimate the Shannon-Weaver diversity index (H') (Shannon and Weaver, 1949) and Pielou’s evenness index (J) to assess the diversity of the 11 qualitative traits. The package “*ggstatsplot*” (Patil, 2021) was used to plot the frequencies of the different classes of the 11 qualitative traits within and between the four varieties, and the differences within and between varieties were tested using Fisher’s exact test implemented in the package “*rstatix*” (Kassambara, 2023), with $P \leq 0.01$ as the significance threshold. For the 15 quantitative traits, a two-way Analysis of Variance (ANOVA) was first performed to determine the effects of variety, block effect, and their interaction on the variation of each trait. Then, a one-way ANOVA was performed, followed by a Tukey HSD at the $P < 0.01$ significance level for the comparison of means among the four varieties. ANOVA and the Tukey HSD test were performed using the “*aov*” and “*TukeyHSD*” functions, respectively, implemented in the “*R-stats*” package of the R programming environment. In addition, the package “*TraitStats*” (Nitesh *et al.*, 2021) was used to calculate the Genotypic Coefficient of Variation (GCV), the Phenotypic Coefficient of Variation (PCV) according to Burton (1951), the broad-sense Heritability (H^2) according to Lush (1940), and the Genetic Advance over Mean (GAM). The latter was calculated as $GAM = (GA/\mu) \times 100$, where GA is the Genetic Advance and was calculated as $GA = k \times (H^2/100) \times \sigma_g$, and μ was the overall mean of the trait, k was the standardized selection differential at a selection intensity of 5% ($k = 2.06$) and σ_g was the genotypic standard deviation (Johnson, 1955). Furthermore, the package “*CorrPlot*” (Taiyun and Viliam, 2017) was used to examine the correlation among the 26 agro-morphological traits using the Spearman

correlation coefficient (ρ). Finally, Multifactorial Analysis (MFA) was performed for all analysed traits using “*FactoMiner*” (Lê *et al.*, 2008) and “*Factoextra*” (Kassambara and Mundt, 2020) to examine the differentiation patterns among the four sweet potato varieties and estimate the contribution of each trait to differentiation.

Genetic diversity parameters and observations of codominant data were evaluated using various population genetics programs. Marker variability and allele patterns of varieties were calculated in MS Toolikit (Park, 2001) and GenAlEx (Peakall and Smousse, 2006). Allelic richness (Ar) was calculated using Fstat (Goudet, 2005). Genetic distances between varieties were calculated using the standard genetic distance of Nei (1972) and the UPGMA (unweighted pair group method with arithmetic mean) clustering method in the software populations (Langella, 2002).

RESULTS AND DISCUSSION

Agro-Morphological Variability and Trait Associations

In this study, sweet potato cultivation of four varieties (Martina, Janja, Lučka, Purple Speclet) was tested in organic fields under subalpine continental climate conditions. Sweet potato varieties are generally distinguished based on agro-morphological characteristics and show great variability in botanical traits related to plant architecture, leaves, stems, and tubers, and productivity among varieties may differ even under the same environmental conditions (Vazhacharickal *et al.*, 2017). Here, analysis of the 11 qualitative traits showed considerable variability among the four varieties studied, with mean values for the Shannon-Weaver diversity index (H') and Pielou’s evenness index (J) of 0.69 and 0.62, respectively (Table 2).



As revealed by Fisher's exact test, the four varieties differed significantly in all qualitative traits ($P < 0.01$), except for the Extent (EACAV) ($P = 0.049$) and Intensity (IACAV) ($P = 0.063$) of anthocyanin coloration on the abaxial veins of the leaf blade (Figure 1). In terms of plant architecture, Ground Cover (GC) was the most variable trait ($H' = 1.14$ and $J = 0.82$) among those studied. Individual plants of the three varieties Janja, Martina, and Purple Speclet had a complete ground cover, with a

few plants having a ground cover that varied from low to high, while the ground cover of Lučka plants was mainly medium and some individuals had low ground cover. Growth Habit (GH) ($H' = 1.01$ and $J = 0.78$) was mostly semi-upright in Lučka and Purple Speclet varieties, spreading in Janja, and upright in Martina.

In stem-related traits, a relatively large variation was observed in Anthocyanin Coloration of the Internode (ACI) ($H' = 1.02$ and $J = 0.93$), Tip (ACT) ($H' = 0.67$ and



Figure 1. Patterns of variability among the four varieties based on 11 qualitative traits.

Table 2. Shannon-Weaver diversity index and Pielou's evenness index of the 11 qualitative traits.^a

Trait	H'	J
GH	1.02	0.93
GC	1.14	0.82
ACI	1.02	0.93
ACT	0.67	0.61
ACN	0.66	0.60
PT	0.81	0.74
LBL	0.56	0.81
DOL	1.08	0.78
EACAV	0.10	0.15
IACAV	0.10	0.15
PAC	0.38	0.27
Average	0.69	0.62

^a H': Shannon diversity index; J: Pielou's evenness index.

J= 0.61), and Node (ACN) ($H^2 = 0.66$ and $J = 0.60$). In the variety Janja, coloration was absent or weak in the internode and the tip and node, in the varieties Lučka and Martina, anthocyanin coloration was mostly medium in the internode and absent or weak in the tip and node, while in Purple Specllet strong coloration was predominant in the internode and medium coloration in the tip and node. In leaf characteristics, the greatest diversity was observed in Leaf Blade Lobe Depth (DOL) ($H^2 = 1.08$ and $J = 0.78$) and Leaf Blade Lobes (LBL) ($H^2 = 0.56$ and $J = 0.81$). Except for Purple Specllet, which had five lobes, the other three varieties had three lobes. The depth of lobing was mostly moderate in Purple Specllet, very shallow in Janja, and shallow in Lučka and Martina. The four varieties did not differ significantly in the extent and intensity of anthocyanin coloration in the abaxial veins (EACAV and IACAV) ($H^2 = 0.10$ and $J = 0.15$) and in Anthocyanin Coloration of the Petiole (PAC) ($H^2 = 0.38$ and $J = 0.27$), and coloration was absent or very weak.

In addition, analysis of variance for the 15 quantitative traits showed significant

differences among sweet potato varieties for all traits, except Number of Primary Shoots (PSN), Length of Primary Shoots (LPS), and Diameter of Internodes (DI) (Table 3), with an average of explained genetic variance (variety component) of 65.38% for all traits. For the significantly different traits, a comparison of means using Tukey's test showed that variety Janja had a significantly higher Number of Internodes (NI) and Leaves (NL) and significantly lower yield (NTP), as it had significantly lower values for all tuber traits compared to the other varieties. However, the varieties Lučka and Martina proved to be significantly higher yielding compared to the other two varieties. Nevertheless, Lučka and Martina differed significantly from each other in some traits, such as Internode Length (LI), Number of Leaves (NL), Mature Leaf Size (MLS), Haulm Fresh Weight (HFW), Number of Tubers per Plant (NTP), and Marketable Tuber Weight (WMT). The variety Purple Specllet was in an intermediate position in terms of tuber production (Figure 2). However, the variance explained by the block and variety×block components was

Table 3. ANOVA showing the effects of variety, block, variety×block and residuals on variation in the 15 quantitative traits.

Trait	Variety		Block		Variety×Block		Residuals
	Explained variance (%)	P	Explained variance (%)	P	Explained variance (%)	P	Explained variance (%)
PSN	17.09	0.375	46.55	0.094	19.94	0.305	16.42
NI	53.61	5.26E-05	0.00	0.989	39.55	7.88E-04	6.84
LPS	0.83	0.593	66.03	0.000	31.83	9.54E-14	1.31
LI	80.42	4.32E-12	10.30	0.102	5.46	0.236	3.82
DI	15.32	0.35012	13.57	0.325	57.18	0.007	13.94
NL	89.43	< 2E-16	3.16	0.275	4.78	0.145	2.64
PL	90.39	7.40E-08	0.33	0.827	2.31	0.803	6.97
MLS	94.26	< 2E-16	0.85	0.527	2.76	0.275	2.13
HFW	77.15	2.53E-07	1.11	0.678	15.30	0.071	6.44
NTP	41.34	0.00559	4.68	0.486	44.38	0.004	9.60
TWTP	79.35	< 2E-16	17.09	0.000	2.74	0.020	0.82
NMTP	82.77	< 2E-16	11.11	0.005	4.70	0.020	1.42
WMT	79.33	< 2E-16	12.45	0.001	7.02	0.001	1.20
TD	88.40	< 2E-16	1.16	0.394	8.85	0.001	1.59
TL	91.05	< 2E-16	4.79	0.050	2.92	0.072	1.24



relatively small, averaging 12.87 and 16.64%, respectively, and was not significant for most traits analyzed. The residual effect was also small, averaging 5.09% for all traits (Table 3).

These results indicate that the agro-morphological variability is mainly due to the genetic differences among varieties, indicating the possibility of improving the agro-morphological traits of the studied sweet potato varieties through genotypic selection. Agro-morphological traits that are generally stable and unaffected by environmental factors include leaf shape, leaf and petiole colour, tuber skin and flesh colour, while traits that changed under environmental influence include petiole length, leaf size, and tuber yield (Hayati *et al.*, 2020). In addition, variability in sweet potato agro-morphology may be related to

factors such as seasonality, crop farming management, climatic conditions, and natural plant characteristics (Leite *et al.*, 2022).

According to Ochieng (2019), sweet potato genotypes grown at two locations differed in terms of internode length and primary shoot (vine) growth rate, petiole length and leaf size, and tuber yield at both locations, with a significant correlation between shoot growth rate and internode length and mature leaf size at one location. At the other side, there was a significant correlation between shoot growth rate and internode length. In our study, there was a correlation between stem length of primary shoots and internode length, between stem length of primary shoots and mature leaf size, the correlation coefficient was low. In the study by Ilodibia *et al.* (2018),

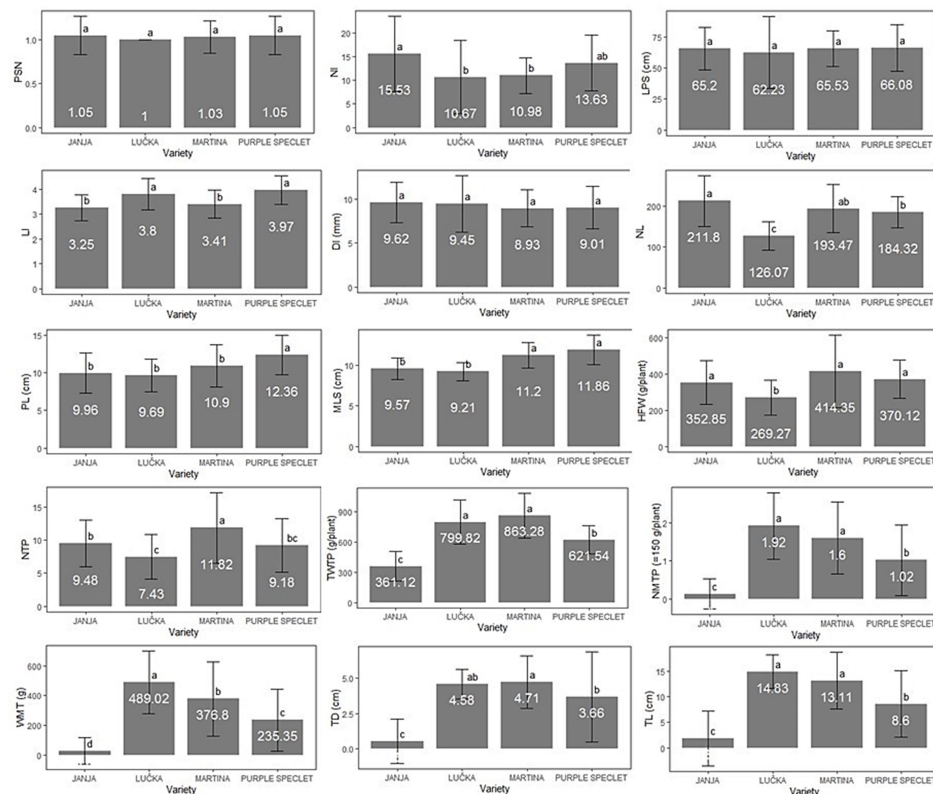


Figure 2. Comparison of mean values for the 15 quantitative traits. Error bars indicate one standard deviation. Different letters indicate significant pairwise differences at $P < 0.01$.

similarities were found in plant shape, leaf type and colour, leaf arrangement shape, leaf margin, veining, tips, shoot shape, texture, and tuber type. Differences were noted in shoot length, colour and diameter, leaf size and colour, and petiole length. Phenotypic characterization of sweet potatoes is done by evaluating variations in plant, shoot, leaf, flower, and storage root traits and is traditionally used to identify sweet potato varieties (Vazhacharickal *et al.*, 2022).

Patterns of agro-morphological variability in quantitative traits were calculated using Phenotypic and Genotypic Coefficients (PCV and GCV). As shown in Table 4, PCV was higher than GCV for all traits analyzed, with average values of 46.16 and 29.08%, respectively. The highest PCV and GCV values were observed for tuber-related traits such as Number (NMTP) (PCV= 91.47% and GCV= 63.85%) and Weight (WMT) (PCV= 93.87% and GCV= 68.35%) of marketable tubers, while the lowest values were recorded for mature leaf size (MLS) (PCV= 16.49% and GCV= 10.86%) and Internode Length (LI) (PCV= 17.47% and GCV= 8.43%). This higher GCV in tuber traits suggests that these traits can be more easily improved by genotypic selection.

Although CVG estimation provides information on genetic variation in quantitative traits, it cannot determine the extent of heritable variability. This was estimated in our study using heritability in a broader sense, which ranged from low ($H^2 < 30\%$) to medium ($30\% \leq H^2 < 60\%$), with an average of 35.57% for the 15 quantitative traits (Table 4). Among the analyzed traits, the Number of Internodes (NI) ($H^2 = 55.75\%$) and tuber-related traits had the highest Heritability ($H^2 > 50\%$), except for Haulm Fresh Weight (HFW) ($H^2 = 17.99\%$). These results indicated that, although PCV performed better than GCV, this substantial amount of heritable variation suggested that environmental factors did not strongly influence phenotypic variation in these traits. Of the contracts, the least heritable variation was found for Number of Primary Shoots (PSN) ($H^2 = 2.54\%$) and Internode Diameter (DI) ($H^2 = 16.93\%$). In addition, Genetic Advance as a percentage of the Mean at a selection intensity of 5% (GAM) showed almost the same trends as PCV, GCV, and H^2 , with values ranging from 0.91% for PSN to 102.51% for WMT (Table 4). This pattern indicates that the mean values for most of the analyzed traits

Table 4. Estimates of phenotypic and genotypic coefficients of variation for 15 quantitative traits.^a

Trait	PCV	GCV	H^2	GAM
PSN	17.52	2.79	2.54	0.92
NI	54.57	40.74	55.75	62.67
LPS	32.04	20.04	39.11	25.81
LI	17.48	8.43	23.26	8.38
DI	27.46	11.20	16.63	9.41
NL	33.53	20.01	35.63	24.61
PL	21.46	9.24	18.53	8.19
MLS	16.49	10.86	43.40	14.74
HFW	41.49	17.60	17.99	15.38
NTP	47.08	22.43	22.71	22.02
TWTP	41.55	31.95	59.15	50.62
NMTP	91.47	63.85	48.72	91.80
WMT	93.87	68.35	53.01	102.51
TD	79.63	53.03	44.35	72.76
TL	76.70	55.73	52.80	83.43

^a PCV: Phenotypic Coefficient of Variation; GCV: Genotypic Coefficient of Variation; H^2 : Broad sense heritability, GAM: Genetic Advance as a percentage of the Mean.

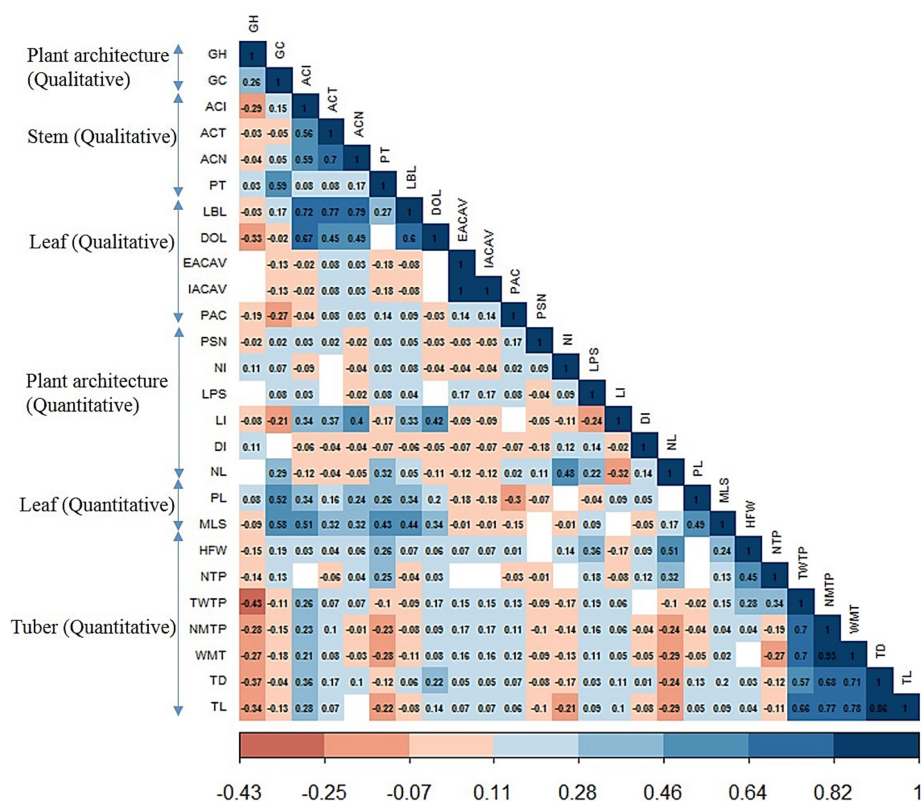
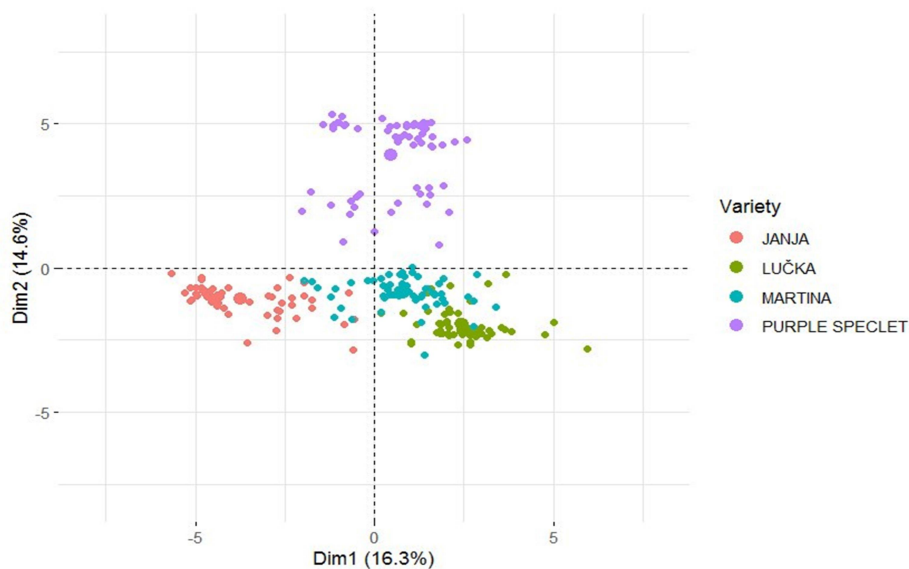


Figure 3. Pattern of correlations among the 26 agro-morphological traits using Spearman's coefficient. Only significant correlations are shown (P < 0.05).



can be strongly modified at a selection intensity of 5%.

Analysis of the association between pairwise traits showed moderate to strong positive correlations were found between leaf- and stem-related traits at the vegetative level (Figure 3). However, relatively low, but significant, correlations were found between traits characterizing plant architecture and all other traits. On the other hand, strong positive correlations were found at the reproductive level between traits characterizing tuber size (TD and TL) and traits related to marketable tubers (TWTP, NMTP, WMT, TD, and TL) ($\rho > 0.65$). However, the Number of Tubers per Plant (NTP) was negatively correlated

with the other tuber traits, except for a moderate positive correlation with Total Tuber Weight per Plant (TWTP) ($\rho = 0.34$), indicating that the plant produced tubers with different sizes. On the other hand, haulm fresh weight (HFW) showed very low but significant positive correlations with the other tuber traits, except for its positive correlation with Number of Tubers per Plant (NTP) ($\rho = 0.45$) and Number of Leaves (NL) ($\rho = 0.51$). It should also be noted that Growth Habit (GH) and Ground Cover (GC), both of which characterize plant architecture, had negative correlations with tuber traits (Figure 3).

To investigate the patterns of agro-morphological differentiation between

Table 5. Contribution of the 26 morpho-agronomic traits to the first two dimensions of MFA.^a

Trait	Dim.1	Dim.2	Dim.1-Dim.2
Explained variance (%)	16.30	14.60	30.90
Qualitative traits	GH	10.74	12.69
	GC	3.46	8.23
	ACI	7.25	16.41
	ACT	1.08	11.51
	ACN	0.80	13.11
	PT	3.15	7.42
	LBL	0.11	16.27
	DOL	7.96	12.75
	EACAV	0.81	1.10
	IACAV	0.81	1.10
	PAC	0.35	0.73
Quantitative traits	PSN	0.22	0.24
	NI	1.62	1.70
	LPS	0.04	0.04
	LI	1.51	4.36
	DI	0.13	0.15
	NL	3.99	4.30
	PL	0.00	4.81
	MLS	0.07	8.39
	HFW	0.08	0.46
	NTP	0.46	0.55
	TWTP	9.27	9.37
	NMTP	11.45	11.74
	WMT	12.15	12.65
	TD	10.48	10.59
	TL	11.99	12.12

^a The traits with the highest contribution to differentiation between varieties are in bold.

**Table 6.** Parameters of genetic diversity among loci.^a

Locus	Range	N	He	PIC	HWE	Ar
Ib242	126-146	4	0.71	0.63	ns	1.71
Ib248	126-190	5	0.79	0.73	ns	1.80
Ib255F1	231-255	5	0.58	0.51	*	1.52
Ib255	172-180	2	0.42	0.32	*	1.31
Ib286	104-120	5	0.74	0.67	ns	1.71
Ib297	104-156	6	0.78	0.71	ns	1.77
Ib316	92-136	3	0.54	0.44	ns	1.53
Ib318	132-138	4	0.83	0.78	*	1.70
Total		34				
Average		4.25	0.68	0.60		1.63

^a Range: Range of allele lengths; N: Number of alleles; He: Expected Heterozygosity; PIC: Polymorphic Information Content; HWE: Hardy-Weinberg Equilibrium, Ar: Allelic richness.

Table 7. Diversity parameters between four studied sweet potato varieties.

	Janja	Martina	Lučka	Purple specklet
No ^a of different alleles	3,000	3,250	2,250	2,250
No of locally common alleles (Freq. ≥ 5%) found in 50% or less varieties	1,250	1,125	0.125	0.500
Unbiased expected heterozygosity	0.638	0.701	0.656	0.488

^a No: Number.

varieties (inter-variety), the data of the 26 traits were analyzed using Multifactorial Analysis (MFA). The results of MFA showed that the first two dimensions explained 16.3 and 14.6% of the total variation, respectively. The plot formed by these two dimensions clearly separated the variety Purple Specklet from the other three varieties on the positive side of the second dimension (Figure 4). The contribution to variation among varieties was much higher for qualitative traits than for quantitative traits (10.77 versus 5.43%) (Table 5).

The most important traits that distinguished this variety from the other varieties were mainly qualitative traits related to Anthocyanin Colouration in the Internode (ACI) (16.41%), Node (ACN) (13.11%) and Tip (ACT) (11.51%), traits related to leaf morphology (LBL and DOL) (16.27 and 12.75%, respectively) and Growth Habit (GH) (10.74%) (Table 5). On the other hand, despite some overlap with the variety Martina, the variety Janja seems

to differ from the varieties Lučka and Martina on the negative side of the first MFA dimension, which in turn tend to differ from each other on the positive side of the first MFA dimension (Figure 4). The major contributors in the differentiation among varieties were the tuber-related traits including WMT (12.5%), NMTP (11.45%), TL (11.99%), and TD (10.48%) (Table 5).

Agro-morphological quality, agricultural management, and productivity of sweet potato plants are important factors directly related to increasing the supply potential of the crop. Thus, the use of selected high-quality plants and good agricultural practices could improve the physical and morphological characteristics of sweet potato plants (Leite *et al.*, 2022). Koussao *et al.* (2014) reported that the greatest differentiation is between the predominant tuber flesh colour and the number of leaf lobes. Flower shape is very important for breeding, as are other visible traits such as plant type, mature leaf colour, immature leaf

colour, general outline of leaves, and predominant shoot colour.

Genetic Background

The SSR markers used in the molecular analysis yielded 34 alleles, with N_e ranging from 2 in Ib255 to 6 in Ib297, with an average of 4.25 alleles per locus (Table 6). The highest level of genetic diversity ($H_e > 0.75$) was found for loci Ib318, Ib248, and Ib297, which were also identified as the most Polymorphic loci ($PIC > 0.7$), while the average PIC value was = 0.60. Deviations from Hardy-Weinberg equilibrium were statistically significant for loci Ib255F1, Ib255, and Ib318. The highest allelic richness was for loci Ib248 and Ib297 ($Ar \geq 1.77$) (Table 6). Diversity parameters among varieties showed that variety Martina had the highest number of alleles (3.250), while the number of locally common alleles with a frequency higher than 5% found in 50% or fewer varieties was calculated for variety Janja. Genetically, the most uniform variety was Purple Speclet, in which the U_{He} was the lowest (0.488) when comparing four varieties (Table 7). The UPGMA clustering showed that varieties Janja and Martina were genetically closer to each other; both were associated with the variety Lučka to Purple Speclet, which is not strongly associated with them, as shown in Figure 5.

As for the genetic background, two genetic clusters (Martina/Janja and Lučka/Purple Speclet) were identified based on the distribution of 16 genotypes within four varieties. The stable genetic structure and uniformity of the already established varieties are reflected in the diversity parameters between loci and among varieties (Tables 6 and 7). The SSR markers were moderately informative as indicated by the PIC value (0.60). Similar results were obtained by Pipan *et al.* (2017a) with the same markers ($PIC = 0.69$).

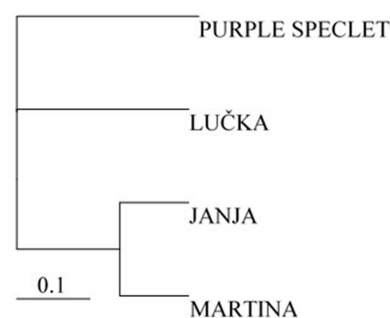


Figure 5. Genetic relationship among assessed sweet potato varieties.

CONCLUSIONS

Sweet potato is a tropical crop, but it can be successfully grown on organic fields in Central Europe using the soil ridge cultivation method. This study is the first in Slovenia to use general morphological traits and genetic evaluation of promising sweet potato varieties of different origins as a basis for discovering relationships between and within genotypes. Clustering analysis showed that varieties Janja and Martina were genetically closer to each other. The varieties Lučka and Martina proved to be significantly higher yielding compared to the other two varieties. From a practical point of view, the results are important for the development and improvement of agromorphological traits, which are becoming increasingly important for breeding.

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REFERENCES

1. Amagloh, F. C., Yada, B., Tumuhimbise, G. A., Amagloh, F. K. and Kaaya, A. N. 2021. The Potential of Sweetpotato as a Functional Food in Sub-Saharan Africa and Its Implications for Health: A Review. *Molecules* **26**: 2971-2992.
2. Andrade, E. K. V. D., Andrade, V. C. D., Laia, M. L. D., Fernandes, J. S. C., Oliveira, A. J. M. and Azevedo, A. M. 2017. Genetic Dissimilarity among Sweet Potato Genotypes Using Morphological and Molecular Descriptors. *ACTA. Sci. Agron.*, **39**: 447-455.
3. Burton, G.W. 1951. Quantitative Inheritance in Pearl Pillet (*P. glaucum*). *Agron. J.*, **43**: 409-417.
4. Buteler, M. I., Jarret, R. L. and LaBonte, D. R. 1999. Sequence Characterization of Microsatellites in Diploid and Polyploid *Ipomea*. *Theor. Appl. Genet.*, **99**: 123-132.
5. Cartabiano-Leite, C. E., Porcu, O. M. and de Casas, A. F. 2020. Sweet Potato (*Ipomoea batatas* L. Lam) Nutritional Potential and Social Relevance. *Int. J. Eng. Res. Appl.*, **10**: 23-40.
6. Dinu, M., Soare, R., Băbeanu, C., Hoza, G. and Sima, R. 2021. Nutraceutical Value and Production of the Sweet Potato (*Ipomoea batatas* L.) Cultivated in South-West of Romania. *J. Cent. Eur. Agric.*, **22**: 285-294.
7. UPOV. 2010. Sweet Potato: *Ipomoea batatas* (L.) Lam. Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability. International Union for the Protection of New Varieties of Plants (UPOV), Geneva, PP. 1-27.
8. Escobar-Puentes, A. A., Palomo, I., Rodríguez, L., Fuentes, E., Villegas-Ochoa, M. A., González-Aguilar, G. A. and Wall-Medrano, A. 2022. Sweet Potato (*Ipomoea batatas* L.) Phenotypes: From Agroindustry to Health Effects. *Foods*, **11**: 1058-1076.
9. 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.
10. FAOSTAT. 2023. *Food and Agricultural Data, Crops and Livestock Products*. Food and Agriculture Organisation of the United States.
11. Ferreira, C. C., Figueira, J. H. M., Soares, D. O. P., Lobato, C. A. N. and Castro, A. P. D. 2022. Morphological Characteristics of Varieties of Sweet Potato, Cenoura, Margarita, Rainha and Roxa, Cultivated in Amazonas, Brazil. *J. Agric. Sci.*, **14**: 170-176.
12. Gobena, T. L., Asemie, M. M. and Firisa, T. B. 2022. Evaluation of Released Sweet Potato [*Ipomoea batatas* (L.) Lam] Varieties for Yield and Yield-Related Attributes in Semen-Bench District of Bench-Sheko-Zone, South-Western Ethiopia. *Heliyon*, **8**: e10950.
13. Goudet, J. 2005. Hierfstat, a Package for R to Compute and Test Hierarchical *F*-statistics. *Mol. Ecol. Notes*, **5**: 184-186.
14. Guevara, M.R., Hartmann, D. and Mendoza, M. 2016. Diverse: An R Package to Analyze Diversity in Complex Systems. *R J.*, **8**: 60-78.
15. Hayati, M. and Anhar, A. 2020. Morphological Characteristics and Yields of Several Sweet Potato (*Ipomoea batatas* L.) Tubers. *IOP Conf. Ser.: Earth Environ. Sci.*, **425**: 012055.
16. Ilodibia, C. V., Arubalueze, C. U., Udearoh, S. N., Okafor, B. I. and Agbanusi, C. 2018. Assessment of Morphological and Nutritional Attributes of Two Varieties of *Ipomoea batatas* (L.) Utilized in Nigeria. *Arch. Agric. Environ. Sci. J.*, **3**: 394-398.
17. IBPGR. 1991. *Descriptors for Sweet Potatoes*. International Board for Plant Genetic Resources, Rome, Italy, PP. 1-52.
18. Johnson, H. W., Robinson, H. F. and Comstock, R. E. 1955. Estimates of Genetic and Environmental Variability in Soybean. *Agron. J.*, **47**: 314-318.
19. Kassambara, A. 2023. *Rstatix: Pipe-Friendly Framework for Basic Statistical Tests [R Package Version 0.7.2]*. Comprehensive R Archive Network

- (CRAN).
<https://rpkgs.datanovia.com/rstatix/>
20. Kassambara, A. and Mundt, F. 2020. Factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R Package Version 1.0.7. <https://CRAN.R-project.org/package=factoextra>
 21. Koussao, S., Gracen, V., Asante, I., Danquah, E. Y., Ouedraogo, J. T., Baptiste, T. J. and Vianney, T. M. 2014. Diversity Analysis of Sweet Potato (*Ipomoea batatas* L. Lam) Germplasm from Burkina Faso Using Morphological and Simple Sequence Repeats Markers. *Afr. J. Biotechnol.*, **13**: 729-742.
 22. Langella, O. 2002. Populations 1.2.31. Population Genetic Software (Individuals or Populations Distances, Phylogenetic Trees). Free Software Foundation Inc., Boston, MA, USA.
 23. Lê, S., Josse, J. and Husson, F. 2008. FactoMineR: An R Package for Multivariate Analysis. *J. Stat. Softw.*, **25**: 1-18.
 24. Leite, C. E. C., Souza, B. D. K. F., Manifio, C. E., Wamser, G. H., Alves, D. P. and de Francisco, A. 2022. Sweet Potato New Varieties Screening Based on Morphology, Pulp Color, Proximal Composition, and Total Dietary Fiber Content via Factor Analysis and Principal Component Analysis. *Front. Plant. Sci.*, **13**: 1-18.
 25. Lush, J.L. 1940. Intra-Sire Correlation and Regression of Offspring on Dams as a Method of Estimating Heritability of Characters. *Proc. Am. Soc. Anim. Prod.*, **33**: 293-301.
 26. Maquia, I., Muocha, I., Naico, A., Martins, N., Gouveia, M., Andrade, I. and Ribeiro, A.I. 2013. Molecular, Morphological and Agronomic Characterization of the Sweet Potato (*Ipomoea batatas* L.) Germplasm Collection from Mozambique: Genotype Selection for Drought Prone Regions. *S. Afr. J. Bot.*, **88**: 142-151.
 27. Mohanraj, R. and Sivasankar, S. 2014. Sweet potato (*Ipomoea batatas* L. Lam) – A Valuable Medicinal Food: A Review. *J. Med. Food*, **17**: 733-741.
 28. Mukhopadhyay, S. K., Chattopadhyay, A., Chakraborty, I. and Bhattacharya, I. 2011. Crops that Feed the World 5. Sweetpotato. Sweetpotatoes for Income and Food Security. *Food Secur.*, **3**: 283-305.
 29. Nei, M. 1972. Genetic Distance between Populations. *Am. Nat.*, **106**: 283-292.
 30. Nitesh, S. D., Parashuram, P. and Shilpa, P. 2021. TraitStats: Statistical Data Analysis for Randomized Block Design Experiments. R Package Version 1.0.1. <https://www.niteshgpb.in/TraitStats.pdf>
 31. Ochieng, L. A. 2019. Agro-Morphological Characterization of Sweet Potato Genotypes Grown in Different Ecological Zones in Kenya. *J. Hort. Plant Res.*, **5**: 1-12.
 32. Park, S. 2001. *Microsatellite Toolkit*. Department of Genetics: Trinity College, Dublin, Ireland.
 33. Patil, I. 2021. Visualizations with Statistical Details: The 'ggstatsplot' Approach. *J. Open Sour. Softw.*, **6**: 3167.
 34. Peakall, R. O. D. and Smouse, P. E. 2006. Genalex 6: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. *Mol. Ecol. Notes*, **6**: 288-295.
 35. Pipan, B., Žnidaršič, D., Kunstelj, N., and Meglič, V. 2017a. Genetic Evaluation of Sweetpotato Accessions Introduced to the Central European Area. *J. Agric. Sci. Technol.*, **19**: 1139-1150.
 36. Pipan, B., Žnidaršič, D. and Meglič, V. 2017b. Evaluation of Genetic Diversity of Sweet Potato [*Ipomoea batatas* (L.) Lam.] on Different Ploidy Levels Applying Two Capillary Platforms. *J. Hortic. Sci. Biotechnol.*, **92**: 192-198.
 37. Prakash, P., Kishore, P., Jaganathan, D., Immanuel, S. and Sivakumar, P. S. 2018. The Status, Performance and Impact of Sweet Potato Cultivation on Farming Communities of Odisha, India. *30th International Conference of Agricultural Economists*, Vancouver, Canada, PP. 1-13.
 38. R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.



39. Reddy, R., Soibam, H., Ayam, V. S., Panja, P. and Mitra, S. 2018. Morphological Characterization of Sweet Potato Cultivars during Growth, Development and Harvesting. *Indian J. Agr. Res.*, **52**: 46-50.
40. Roullier, C., Duputié, A., Wennekes, P., Benoit, L., Fernández Bringas, V. M., Rossel, G., Tay, D., McKey, D. and Lebot, V. 2013. Disentangling the Origins of Cultivated Sweet Potato (*Ipomoea batatas* (L.) Lam.). *PLoS One*, **8**: e62707.
41. Shannon, C. E. and Weaver, W. 1949. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana, IL, **11**: 11-20.
42. Taiyun, W. and Viliam, S. 2017. R Package "corrplot": Visualization of a Correlation Matrix (Version 0.84). *Statistician*, **56**: e24.
43. Ukom, A. N., Ojmelukwe, P. C. and Okpara, D. A. 2009. Nutrient Composition of Selected Sweet Potato [*Ipomea batatas* (L) Lam] Varieties as Influenced by Different Levels of Nitrogen Fertilizer Application. *Pak. J. Nutr.*, **8**: 1791-1795.
44. Vazhacharickal, P. J. and Augustine, A. 2017. Morphological Characterization of Sweet Potato (*Ipomoea batatas* Lam.) Varieties in Kerala: An Overview. *Amazon*: 1-58.
45. Veasey, E.A., Borges, A., Silva Rosa, M., Queiroz-Sila, J. R., de Andrade Bressan, E. and Peroni, N. 2008. Genetic Diversity in Brazilian Sweet Potato (*Ipomoea batatas* (L.) Lam., Solanaes, Convolvulaceae) Landraces Assessed with Microsatellite Markers. *Genet. Mol. Biol.*, **31**: 725-733.
46. Villanueva, R. A. M. and Chen, Z. J. 2019. ggplot2: Elegant Graphics for Data Analysis. *Measurement*, **17**(3): 160-167.
47. Zhang, D., Cervantes, J., Huamán, Z., Carey, E. and Ghislain, M. 2000. Assessing Genetic Diversity of Sweet Potato (*Ipomoea batatas* (L.) Lam.) Cultivars from Tropical America Using AFLP. *Genet. Resour. Crop. Evol.*, **47**: 659-665.

ویژگی‌های زراعی-مورفولوژیکی ارقام منتخب سیب‌زمینی شیرین (*Ipomoea batatas* L.) در کشاورزی ارگانیک و پیشینه ژنتیکی آنها

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چکیده

سیب‌زمینی شیرین یکی از مهم‌ترین محصولات ریشه‌ای در سراسر جهان است. این تحقیق با هدف مقایسه ویژگی‌های زراعی-مورفولوژیکی چهار رقم سیب‌زمینی شیرین (مارتینا Martina، جانجا Janja، لوچکا Lučka، پرپل اسپکلت Purple Speclet) در کشاورزی ارگانیک به همراه اطلاعات تکمیلی در مورد پیشینه ژنتیکی آنها انجام شد. در طول دوره رشد رویشی، در مجموع ۲۶ صفت زراعی-مورفولوژیکی ارزیابی شد. نهال‌های از پیش رشد یافته در طول فصل رشد ۲۰۲۱ با استفاده از روش کشت پشته‌ای (soil ridge cultivation) در مزارع ارگانیک کشت شد. این مطالعه تفاوت‌های معنی‌داری را بین گونه‌ها از نظر صفات کمی (به جز میزان و شدت رنگ آنتوسیانین روی رگبرگ‌های دور محور) و کیفی (به جز تعداد و طول شاخه‌های اولیه و قطر میانگره) نشان داد. گونه‌های لوچکا و مارتینا در مقایسه با دو گونه دیگر، عملکرد قابل

توجهی بالاتری داشتند. پیشینه ژنتیکی گونه‌ها بر روی ۸ جایگاه تکرار توالی ساده (SSR) با استفاده از پلی‌مورفیسم‌های آللی با تعداد کل ۳۴ آلل مختلف و میانگین محتوای اطلاعات چندشکلی ۰.۶۰. / ارزیابی شد. اطلاعات مطلوب نشانگرهای انتخاب شده با تنوع ژنتیکی جهانی ۰.۶۸. / تأیید شد. انتساب هر ژنوتیپ به دو گروه ژنتیکی به خوبی با توزیع گونه‌ها در درخت فیلوژنتیک و نتایج تجزیه و تحلیل ساختار ژنتیکی (Martina/Janja و Lučka/Purple Specklet) مطابقت داشت. این پژوهش به دانش بهتر در مورد گونه‌های سیب‌زمینی شیرین مورد بررسی و تنوع زراعی-مورفولوژیکی و ژنتیکی آنها کمک می‌کند.