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Agro-morphological characteristics of selected sweet potato (Ipomoea batatas L.) varieties from organic farming and their genetic background

Nataša Kunsteli¹, Lovro Sinkovič²*, Barbara Pipan², Mohamed Neji², and Vladimir Meglič² ٤

ABSTRACT ٥

Sweet potato is one of the most important root crop worldwide. This study aimed to compare ٦ ٧ agro-morphological characteristics of four sweet potato varieties (Martina, Janja, Lučka, Purple Speclet) from organic farming with additional information about their genetic background. A ٨ total of 26 agro-morphological traits were evaluated during vegetation. Pre-grown seedlings ٩ ۱. 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 ۱١ ۱۲ the extent and intensity of anthocyanin colouration on abaxial veins) and qualitative traits ۱۳ (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 ١٤ 10 varieties. The genetic background of the varieties was evaluated on 8 SSR loci using allele ١٦ polymorphisms with a total number of 34 different alleles and an average polymorphic ۱۷ 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 ۱۸ ۱٩ genetic groups agrees 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 ۲. present study contributes to a better knowledge of the sweet potato varieties considered and ۲١ their agro-morphological and genetic diversity. ۲۲

۲۳ Keywords: Diversity, Organic farming, Plant descriptor, SSR marker, Sweet potato.

INTRODUCTION

Sweet potato (Ipomoea batatas L.) is a dicotyledonous angiosperm plant and belongs to the ۲۷ convolvulaceae species (Gobena *et al.*, 2022; Cartabiano-Leite *et al.*, 2020). It is native to South ۲۸ and Central America, where its domestication began about > 5,000 years ago. Later, the ۲۹ 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

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¹ Naklo Biotechnical Center, Strahinj 99, Naklo, Slovenia.

² Department of Crop Science, Agricultural Institute of Slovenia, Hacquetova Ulica 17, Ljubljana, Slovenia. *Corresponding author, e-mail: lovro.sinkovic@kis.si

average yield of 12 t ha⁻¹ (FAOSTAT, 2023; Prakash *et al.*, 2018). Sweet potato is grown mainly ٣٢ ٣٣ 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 ٣٤ season, uses non-edible parts for planting, does not form trellises, and requires few soil nutrients ۳0 ٣٦ (Mukhopadhyay et al., 2011). It is usually grown between sea level and 2,300 m altitude in ۳۷ tropical and temperate zones located 40° south and north of the equator (Gobena *et al.*, 2022). ۳۸ Production in Europe, where the largest producers are Spain, Portugal, and Italy, accounts for ۳٩ the smallest share of global production, although the introduction of sweet potato in the central ٤٠ 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 ٤١ ٤٢ al., 2021; Pipan et al., 2017a).

٤٣ The plant is cultivated for its starchy roots and immature leaves, which are used for human ٤٤ consumption and as animal feed (Gobena et al. 2022; Ferreira et al. 2022). Sweet potato is an 20 admirable crop because it is the only member of its species capable of developing nutritious ٤٦ tuberous roots that feed a large portion of the world's population, especially in the tropics where ٤٧ most of the crop is grown and consumed (Cartabiano-Leite et al., 2020; Ukom et al., 2009). ٤٨ 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. ٤٩ ٥. 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, 01 ٥٢ and anti-inflammatory properties (Mohanraj and Sivasankar, 2014).

٥٣ 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) 0 2 00 and leaf, flower, fruit and seed and is grown as an annual plant (Ukom *et al.*, 2009). Sweet ٥٦ potatoes can be propagated by seeds, tubers, or vine cuttings. Vegetative propagation is usually ٥٧ 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 ٥٨ 09 sufficient planting material (Mukhopadhyay et al., 2011). Shoot production is increased by the ٦. best selection of storage root sizes to optimize growth and yield. Varieties with different storage ٦1 root sizes produce a variable number of shoots that vary in length and thickness. Standard ٦٢ characterization of plant genetic resources includes conventional approaches such as the use of ٦٣ descriptor lists for morphological traits or agronomic performance evaluation, complemented ٦٤ by molecular techniques (Maquia et al., 2013). Sweet potato plant diversity can be studied by ٦٥ identifying agro-morphological 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 ٦٦ ٦٧ 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 ٦٨ ٦٩ varieties or new clones (Hayati et al., 2020; Reddy et al., 2018). The success of any genetic ٧. conservation and breeding program depends on understanding the distribution of genetic ۷١ diversity in the gene pool (Zhang et al., 2000). Among the 175 species of Ipomoea, Ipomoea ۲۷ *batatas* is the only tuber-forming natural allohexaploid (2n = 6x = 90) species, although many ٧٣ of the species are diploid and tetraploid (Mukhopadhyay et al., 2011, Reddy et al., 2018). There ٧٤ are many varieties of sweet potato known and cultivated worldwide. They have different ۷٥ 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 ٧٦ ٧٧ storage roots (tubers) and other agronomic traits such as leaf and stem morphology (Amagloh *et al.*, 2021). ٧٨

٧٩ The objective of this study was to characterize the agro-morphological variation among four ٨٠ sweet potato varieties grown in organic farming using the soil ridge cultivation method using various agro-morphological traits related to overall plant architecture, stem, leaf and tubers, and ۸١ ۸۲ to determine the main contributors of variation for future selection and breeding programs. In ۸۳ addition, SSR markers were used to investigate the genetic background and relatedness between ٨٤ 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 ٨0 ٨٦ on the available varieties creates valuable knowledge for further intensification of cultivation ۸٧ in the region and beyond.

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MATERIALS AND METHODS

9. Plant Material and Experimental Design

۹١ A set of four sweet potato varieties (Martina – purple skin and white tuber flesh, Janja – white ٩٢ 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 ٩٣ Biotechnical Centre in Naklo, Slovenia (46° 16' 18" N, 14° 18' 56" E, 420 m asl). The varieties ٩٤ 90 Martina, Lučka and Janja were registered as protected varieties in the Slovenian National List ٩٦ of Varieties in 2016, while the variety Purple Speclet is commercially available on the European ٩٧ market. A single-factorial field trial was established at the experimental site in a randomised ٩٨ block design with four replicates of 15 seedlings planted 40 cm apart on the soil ridge of each 99 plot. Seedlings were vegetatively propagated in the greenhouse by cuttings from tubers from

previous growing seasons. The seedlings (about 20 cm high) were planted in the field in early ۱.. 1.1 June on the ploughed 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 1.1 planosols with a silty loam texture and a bulk density of 1.61 in the upper 30 cm. Analysis of 1.7 1.5 the upper 30 cm of soil showed a pH of 6.8 and P₂O₅ and K₂O contents of 220 and 500 mg kg⁻ 1.0 ¹, respectively. The organic carbon content was 5.3%, while the available Nitrate-Nitrogen ۱.٦ (NO₃-N) was 0.68%. Basic fertilisation was carried out before tillage with the organic fertiliser ۱۰۷ Fertildung Stallatico Umificato Pellettato (3-3-1; Fertilgest, Fomet, Italy), while the plants were ۱۰۸ fertilised twice during the growth period with the organic fertiliser Tiger Dung 3:6:12+2MgO (Fomet, Italy). The application rate/dosage was in accordance with the manufacturer's 1.9 11. recommendations. 111 ۱۱۲ **Agro-morphological Characterisation** A total of 26 quantitative and qualitative descriptors related to the architecture of the whole ۱۱۳ 112 plant, stem, leaves, and tubers were developed by the International Union for the Protection of 110 New Varieties of Plants - UPOV (2010) and the International Board for Plant Genetic 117 Resources - IBPGR (1991) for Ipomoea batatas were used for the agro-morphological 117 characterisation. All qualitative descriptors were assessed visually, while quantitative ۱۱۸ descriptors were measured using a digital calliper (to 0.1 mm), a tape measure (to 1 cm) and a

laboratory balance (to 0.5 g). Data on agro-morphological characteristics were collected
 according to the descriptor list summarised in Table 1.

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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 agromorphological qualitative and quantitative traits using the statistical programming environment version 3.4.4 R Core Team (2021) to investigate the patterns of variation within and among the four sweet potato varieties analysed 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 180 ١٣٦ the frequencies of the different classes of the 11 qualitative traits within and between the four ۱۳۷ varieties, and the differences within and between varieties were tested using Fisher's exact test ۱۳۸ implemented in the package "*rstatix*" (Kassambara, 2023), with $P \le 0.01$ as the significance threshold. For the 15 quantitative traits, a two-way Analysis Of Variance (ANOVA) was first 139 ١٤. performed to determine the effects of variety, block effect, and their interaction on the variation 151 of each trait. Then, a one-way ANOVA was performed, followed by a Tukey HSD at the 158 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, 153 122 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 120 127 Variation (GCV), the Phenotypic Coefficient of Variation (PCV) according to Burton (1951), ١٤٧ the broad-sense Heritability (H²) according to Lush (1940), and the Genetic Advance over Mean ١٤٨ (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 129 10. standardized selection differential at a selection intensity of 5% (k= 2.06) and σ_g was the 101 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 101 107 using the Spearman correlation coefficient (ρ). Finally, Multifactorial Analysis (MFA) was performed for all analysed traits using "FactoMiner" (Lê et al., 2008) and "Factoextra" 105 100 (Kassambara and Mundt, 2020) to examine the differentiation patterns among the four sweet 107 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 (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

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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. 2022). 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).

140 As revealed by Fisher's exact test, the four varieties differed significantly in all qualitative 177 traits (P<0.01), Except for the extent (EACAV) (P = 0.049) and Intensity (IACAV) (P=0.063) 177 of anthocyanin coloration on the abaxial veins of the leaf blade (Figure 1). In terms of plant ۱۷۸ architecture, Ground Cover (GC) was the most variable trait (H'= 1.14 and J= 0.82) among ۱۷۹ 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, ۱۸. while the ground cover of Lučka plants was mainly medium and some individuals had low 141 ۱۸۲ ground cover. Growth Habit (GH) (H'= 1.01 and J= 0.78) was mostly semi-upright in Lučka ۱۸۳ and Purple Speclet varieties, spreading in Jania, and upright in Martina. In stem-related traits, ۱۸٤ a relatively large variation was observed in Anthocyanin Coloration of the Internode (ACI) 110 (H'= 1.02 and J= 0.93), Tip (ACT) (H'= 0.67 and J= 0.61), and Node (ACN) (H'= 0.66 and J = 0.60). In the variety Janja, coloration was absent or weak in the internode and the tip and ۱۸٦ ۱۸۷ 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 Speclet, strong coloration was ۱۸۸ ۱۸۹ predominant in the internode and medium coloration in the tip and node. In leaf characteristics, 19. the greatest diversity was observed in Leaf Blade Lobe Depth (DOL) (H'= 1.08 and J= 0.78) 191 and Leaf Blade Lobes (LBL) (H'= 0.56 and J= 0.81). Except for Purple Speclet, which had five 198 lobes, the other three varieties had three lobes. The depth of lobing was mostly moderate in ۱۹۳ Purple Speclet, very shallow in Jania, and shallow in Lučka and Martina. The four varieties did 192 not differ significantly in the extent and intensity of anthocyanin coloration in the abaxial veins 190 (EACAV and IACAV) (H'= 0.10 and J= 0.15) and in Anthocyanin Coloration of the Petiole 197 (PAC) (H'= 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), Lngth 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

۲.۳ had significantly lower values for all tuber traits compared to the other varieties. However, the ۲.٤ varieties Lučka and Martina proved to be significantly higher yielding compared to the other ۲.0 two varieties. Nevertheless, Lučka and Martina differed significantly from each other in some ۲.٦ 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 ۲.۷ ۲۰۸ (WMT). The variety Purple Speclet was in an intermediate position in terms of tuber production ۲.۹ (Figure 2). However, the variance explained by the block and variety×block components was relatively small, averaging 12.87 and 16.64%, respectively, and was not significant for most ۲١. 117 traits analysed. The residual effect was also small, averaging 5.09% for all traits (Table 3). 212 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 ۲۱۳ 212 traits of the studied sweet potato varieties through genotypic selection. Agro-morphological 210 traits that are generally stable and unaffected by environmental factors include leaf shape, leaf ۲۱٦ and petiole colour, tuber skin and flesh colour, while traits that changed under environmental ۲۱۷ influence include petiole length, leaf size, and tuber yield (Hayati et al., 2020). In addition, ۲۱۸ variability in sweet potato agro-morphology may be related to factors such as seasonality, crop 219 farming management, climatic conditions, and natural plant characteristics (Leite et al., 2022). ۲۲. According to Ochieng (2019), sweet potato genotypes grown at two locations differed in terms 221 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 222 ۲۲۳ length and mature leaf size at one location. At the other side, there was a significant correlation ٢٢٤ between shoot growth rate and internode length. In our study, there was a correlation between 220 stem length of primary shoots and internode length, between stem length of primary shoots and 222 mature leaf size, the correlation coefficient was low. In the study by Ilodibia et al. (2018), ۲۲۷ similarities were found in plant shape, leaf type and colour, leaf arrangement shape, leaf margin, ۲۲۸ veining, tips, shoot shape, texture, and tuber type. Differences were noted in shoot length, 229 colour and diameter, leaf size and colour, and petiole length. Phenotypic characterization of ۲۳۰ sweet potatoes is done by evaluating variations in plant, shoot, leaf, flower, and storage root ۲۳۱ traits and is traditionally used to identify sweet potato varieties (Vazhacharickal et al., 2022).

YTTPatterns of agro-morphological variability in quantitative traits were calculated usingYTTPhenotypic and Genotypic Coefficients (PCV and GCV). As shown in Table 4, PCV was higherYTTthan GCV for all traits analysed, with average values of 46.16 and 29.08%, respectively. TheYTThighest PCV and GCV values were observed for tuber-related traits such as Number (NMTP)YTT(PCV = 91.47% and GCV = 63.85%) and Weight (WMT) (PCV= 93.87% and GCV= 68.35%)

۲۳۷ of marketable tubers, while the lowest values were recorded for mature leaf size (MLS) ۲۳۸ (PCV= 16.49% and GCV= 10.86%) and Internode Length (LI) (PCV= 17.47% and ۲۳۹ GCV= 8.43%). This higher GCV in tuber traits suggests that these traits can be more easily ۲٤. improved by genotypic selection. Although CVG estimation provides information on genetic 251 variation in quantitative traits, it cannot determine the extent of heritable variability. This was 252 estimated in our study using heritability in a broader sense, which ranged from low ($H^2 < 30\%$) 252 to medium $(30\% \le H^2 < 60\%)$, with an average of 35.57% for the 15 quantitative traits (Table 4). Among the analysed traits, the Number of Internodes (NI) ($H^2 = 55.75\%$) and tuber-related traits 755 250 had the highest Heritability ($H^2 > 50\%$), except for Haulm Fresh Weight (HFW) ($H^2 = 17.99\%$). 252 These results indicated that, although PCV performed better than GCV, this substantial amount ۲٤٧ of heritable variation suggested that environmental factors did not strongly influence ۲٤٨ 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 7 2 9 addition, Genetic Advance as a percentage of the Mean at a selection intensity of 5% (GAM) 10. showed almost the same trends as PCV, GCV, and H^2 , with values ranging from 0.91% for PSN 101 101 to 102.51% for WMT (Table 4). This pattern indicates that the mean values for most of the 207 analysed traits can be strongly modified at a selection intensity of 5%.

Analysis of the association between pairwise traits showed moderate to strong positive 70 E 200 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 202 201 plant architecture and all other traits. On the other hand, strong positive correlations were found ۲٥٨ at the reproductive level between traits characterizing tuber size (TD and TL) and traits related 209 to marketable tubers (TWTP, NMTP, WMT, TD, and TL) ($\rho > 0.65$). However, the Number of ۲٦. Tubers per Plant (NTP) was negatively correlated with the other tuber traits, except for a 221 moderate positive correlation with Total Tuber Weight per Plant (TWTP) ($\rho = 0.34$), indicating 222 that the plant produced tubers with different sizes. On the other hand, haulm fresh weight ۲٦٣ (HFW) showed very low but significant positive correlations with the other tuber traits, except 225 for its positive correlation with Number of Tubers per Plant (NTP) ($\rho = 0.45$) and Number of 220 Leaves (NL) (ρ = 0.51). It should also be noted that Growth Habit (GH) and Ground Cover 222 (GC), both of which characterize plant architecture, had negative correlations with tuber traits ۲٦۷ (Figure 3).

To investigate the patterns of agro-morphological differentiation between varieties (intervariety), the data of the 26 traits were analysed using Multifactorial Analysis (MFA). The results of MFA showed that the first two dimensions explained 16.3 and 14.6% of the total

171 variation, respectively. The plot formed by these two dimensions clearly separated the variety 277 Purple Speclet from the other three varieties on the positive side of the second dimension ۲۷۳ (Figure 4). The contribution to variation among varieties was much higher for qualitative traits ۲۷٤ than for quantitative traits (10.77% versus 5.43%) (Table 5). The most important traits that 200 distinguished this variety from the other varieties were mainly qualitative traits related to 272 Anthocyanin Colouration in the Internode (ACI) (16.41%), Node (ACN) (13.11%) and Tip ۲۷۷ (ACT) (11.51%), traits related to leaf morphology (LBL and DOL) (16.27 and 12.75%, ۲۷۸ respectively) and Growth Habit (GH) (10.74%) (Table 5). On the other hand, despite some ۲۷۹ overlap with the variety Martina, the variety Janja seems to differ from the varieties Lučka and ۲٨٠ 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 ۲۸۱ differentiation among varieties were the tuber-related traits including WMT (12.5%), NMTP ۲۸۲ (11.45%, TL (11.99%), and TD (10.48%) (Table 5). ۲۸۳

۲۸٤ Agro-morphological quality, agricultural management, and productivity of sweet potato ۲۸٥ plants are important factors directly related to increasing the supply potential of the crop. Thus, ۲۸٦ the use of selected high-quality plants and good agricultural practices could improve the ۲۸۷ physical and morphological characteristics of sweet potato plants (Leite et al., 2022). Koussao ۲۸۸ et al. (2014) reported that the greatest differentiation is between the predominant tuber flesh ۲۸۹ colour and the number of leaf lobes. Flower shape is very important for breeding, as are other ۲٩. visible traits such as plant type, mature leaf colour, immature leaf colour, general outline of 291 leaves, and predominant shoot colour.

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Genetic Background

295 The SSR markers used in the molecular analysis yielded 34 alleles, with Ne ranging from 2 290 in Ib255 to 6 in Ib297, with an average of 4.25 alleles per locus (Table 6). The highest level of 297 genetic diversity (He> 0.75) was found for loci Ib318, Ib248, and Ib297, which were also ۲۹۷ identified as the most Polymorphic loci (PIC> 0.7), while the average PIC value was = 0.60. ۲۹۸ Deviations from Hardy-Weinberg equilibrium were statistically significant for loci Ib255F1, 299 Ib255, and Ib318. The highest allelic richness was for loci Ib248 and Ib297 (Ar≥ 1.77) ۳.. (Table 6). Diversity parameters among varieties showed that variety Martina had the highest 3.1 number of alleles (3.250), while the number of locally common alleles with a frequency higher ۳.۲ than 5% found in 50% or fewer varieties was calculated for variety Janja. Genetically, the most ۳.۳ uniform variety was Purple Speclet, in which the UHe was the lowest (0.488) when comparing ۳.٤ four varieties (Table 7). The UPGMA clustering showed that varieties Janja and Martina are

- genetically closer to each other; both are 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).
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۳۱٤ CONCLUSIONS

310 Sweet potato is a tropical crop, but it can be successfully grown on organic fields in Central 312 Europe using the soil ridge cultivation method. This study is the first in Slovenia to use general 311 morphological traits and genetic evaluation of promising sweet potato varieties of different origins as a basis for discovering relationships between and within genotypes. Clustering 311 319 analysis showed that varieties Janja and Martina are genetically closer to each other. The ۳۲. varieties Lučka and Martina proved to be significantly higher vielding compared to the 371 other two varieties. From a practical point of view, the results are important for the ۳۲۲ development and improvement of agro-morphological traits, which are becoming increasingly ۳۲۳ important for breeding.

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۲۲۰ ACKNOWLEDGEMENTS

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REFERENCES

 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.

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.

347	3. Burton, G.W. 1951. Quantitative inheritance in Pearl millet (<i>P. glaucum</i>). Agron. J. 43 :
۳۳۹	409-417.
٣٤.	4. Buteler, M.I., Jarret, R.L., and LaBonte, D.R. 1999. Sequence characterization of
351	microsatellites in diploid and polyploid Ipomea. Theor. Appl. Genet. 99: 123–132.
322	5. Cartabiano-Leite, C.E., Porcu, O.M., and de Casas, A.F. 2020. Sweet potato (Ipomoea
322	batatas L. Lam) nutritional potential and social relevance. Int. J. Eng. Res. Appl. 10: 23-40.
٣٤٤	6. Dinu, M., Soare, R., Băbeanu, C., Hoza, G., and Sima, R. 2021. Nutraceutical value and
320	production of the sweet potato (Ipomoea batatas L.) cultivated in South-West of Romania. J.
321	Cent. Eur. Agr. 22: 285-294.
٣٤٧	7. UPOV. 2010. Sweet potato: <i>Ipomoea batatas</i> (L.) Lam. Guidelines for the conduct of
٣٤٨	tests for distinctness, uniformity and stability. International Union for the Protection of New
329	Varieties of Plants (UPOV), Geneva: 1-27.
۳0.	8. Escobar-Puentes, A.A., Palomo, I., Rodrígues, L., Fuentes, E., Villegas-Ochoa, M.A.,
301	González-Aguilar, G.A., and Wall-Medrano, A. 2022. Sweet potato (Ipomoea batatas L.)
302	phenotypes: from agroindustry to health effects. Foods 11: 1058-1076.
303	9. Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of
305	individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14: 2611–2620.
800	10. FAOSTAT. 2023. Food and agricultural data, Crops and livestock products, Food and
307	Agriculture Organisation of the United States.
Tov	11. Ferreira, C.C., Figueira, J.H.M., Soares, D.O.P., Lobato, C.A.N., and Castro, A.P.D.
Тол	2022. Morphological characteristics of varieties of sweet potato, Cenoura, Margarita, Rainha
809	and Roxa, cultivated in Amazonas, Brazil. J. Agric. Sci. 14: 170-176.
31.	12. Gobena, T.L., Asemie, M.M., and Firisa, T.B. 2022. Evaluation of released sweet potato
311	[Ipomoea batatas (L.) Lam] varieties for yield and yield-related attributes in Semen-Bench
322	district of Bench-Sheko-Zone, South-Western Ethiopia. Heliyon 8: e10950.
222	13. Goudet, J. 2005. Hierfstat, a package for R to compute and test hierarchical <i>F</i> -statistics.
325	Mol. Ecol. Notes 5: 184-186.
820	14. Guevara, M.R., Hartmann, D., and Mendoza, M. 2016. Diverse: an R package to analyze
٣٦٦	diversity in complex systems. The R Journal 8: 60-78.
37V	15. Hayati, M., and Anhar, A. 2020. Morphological characteristics and yields of several
37 J	sweet potato (Ipomoea batatas L.) tubers. IOP Conference Series: Earth and Env. Sci. 425:
٣٦٩	012055.

- 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. Agri. Env. Sci. J. 3: 394-398.
- TVT 17. IBPGR. 1991. Descriptors for sweet potatoes. International Board for Plant GeneticTV£ Resources, Rome, Italy: 1-52.
- TVo
 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.
- TVV 19. Kassambara, A. 2023. Pipe-friendly framework for basic statistical tests [R package
 TVA rstatix version 0.7. 2]. Comprehensive R Archive Network (CRAN).
- ^{rva} 20. Kassambara, A., and Mundt, F. 2020. Factoextra: extract and visualize the results of
 ^{rva} multivariate data analyses. R package version 1(5).
- 7A) 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.

- ^{*}^{Ao} 22. Langella, O. 2002. Populations 1.2.31. Population genetic software (individuals or
 ^{*}^{A7} populations distances, phylogenetic trees). Free Softwarw Fundation Inc.: Boston, MA, USA.
- TAV 23. Lê, S., Josse, J., and Husson, F. 2008. FactoMineR: an R package for multivariate
 TAA analysis. J. Stat. Softw. 25: 1-18.
- ^{۳۸۹} 24. Leite, C.E.C., Souza, B.D.K.F., Manifio, C.E., Wamser, G.H., Alves, D.P., and de
 ^{۳۹۰} Francisco, A. 2022. Sweet potato new varieties screening based on morphology, pulp color,
 ^{۳۹۱} proximal composition, and total dietary fiber content via factor analysis and principal
 ^{۳۹۲} component analysis. Front. Plant. Sci. 13: 1-18.
- Clush, 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.

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.

^{rqq} 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.

Nei, M. 1972. Genetic distance between populations. The Am. Nat. 106: 283-292. ٤ • ٤ 29. ٤.0 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. ٤.٦ ٤٠٧ 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. ٤٠٨ ٤٠٩ 32. Park, S. 2001. Microsatellite Toolkit. Department of Genetics: Trinity College, Dublin, ٤١٠ Ireland. ٤١١ Patil, I. 2021. Visualizations with statistical details: The'ggstatsplot' approach. J. Open 33. ٤١٢ Sour. Softw. 6: p3167. ٤١٣ 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. ٤١٤ Pipan, B., Žnidaršič, D., Kunstelj, N., and Meglič, V. 2017a. Genetic evaluation of 210 35. sweetpotato accessions introduced to the central European area. J. Agric. Sci. Technol. 19: ٤١٦ 1139-1150. ٤١٧ Pipan, B., Žnidarčič, D., and Meglič, V. 2017b. Evaluation of genetic diversity of sweet ٤١٨ 36. ٤١٩ potato [Ipomoea batatas (L.) Lam.] on different ploidy levels applying two capillary platforms. ٤٢٠ J. Hortic. Sci. Biotechnol. 92: 192-198. Prakash, P., Kishore, P., Jaganathan, D., Immanual, S., and Sivakumar, P.S. 2018. The ٤٢١ 37. ٤٢٢ status, performance and impact of sweet potato cultivation on farming communities of Odisha, India. 30th International Conference of Agricultural Economists, Vancuver, Canada: 1-13. ٤٢٣ ٤٢٤ R Core Team. 2021. R: A language and environment for statistical computing. R 38. 270 Foundation for Statistical Computing, Vienna, Austria. 577 Reddy, R., Soibam, H., Ayam, V.S., Panja, P., and Mitra, S. 2018. Morphological 39. ٤٢٧ characterization of sweet potato cultivars during growth, development and harvesting. Indian J. ٤٢٨ Agr. Res. 52: 46-50. ٤٢٩ 40. Roullier, C., Duputié, A., Wennekes, P., Benoit, L., Fernández Bringas, V.M., Rossel, ٤٣٠ G., Tay, D., McKey, D., and Lebot, V. 2013. Disentangling the origins of cultivated sweet ٤٣١ potato (Ipomoea batatas (L.) Lam.). PLoS One 8: p.e62707. ٤٣٢ Shannon, C.E., and Weaver, W. 1949. The mathematical theory of communication. 41. ٤٣٣ Urbana, IL: University of Illinois Press, 11: 11-20. ٤٣٤ 42. Taiyun, W., and Viliam, S. 2017. R package "corrplot": Visualization of a correlation 270 matrix (Version 0.84). Statistician 56: e24.

- 43. Ukom, A.N., Ojimelukwe, 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. Pakistan 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 batats (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
 - ٤٤٦ (2nd ed.). Measurement **173**: 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.
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Table 1. Agro-morphological characteristics considered in the study.

Type of trait	Organ	Abb	Descriptor	Scores			
6980-	Whole plant	GH	Growth habit	2 Upright; 3 Semi-upright; 5 Spreading			
8	architecture	GC	Ground cover	3 Low (< 50%); 5 Medium (50-74%); 7 High (75-90%); 9 Total (> 90%)			
		ACI	Anthocyanin coloration of internode	1 Absent or weak; 2 Medium; 3 Strong			
	C.	ACT	Anthocyanin coloration of tip	1 Absent or weak; 2 Medium; 3 Strong			
	Stem	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			
Qualitative		LBL	Leaf blade lobes	1 Absent; 2 Three lobes; 3 Five lobes; 4 Sever 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			
	Leaf	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				
		NI	Number of internodes				
	Whole plant	LPS	Lenght of primary shoots (cm)				
	architecture	LI	Lenght of internode (cm)				
		DI	Diameter of internode (mm)				
		NL	Number of leaves/plant				
	Leaf	PL	Petiole length (cm)				
Quantitative		MLS	Mature leaf size (cm)				
	Tuber	HFW	Haulm fresh weight (g/plant)				
		NTP	Number of tubers/plant				
		TWTP	Total weight of tubers /plant (g)				
		NMTP	Number of marketable tubers (≥150 g)/plant				
		WMT	Weight of marketable tubers (g)				
		TD	Tuber diameter (cm)				
		TL	Tuber length (cm)				

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٤٥٤ Abb, abbreviation.

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 \mathfrak{sor} **Table 2.** Shannon-Weaver diversity index and Pielou's evenness index of the 11 qualitative traits.

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

H', Shannon-Weaver diveristy index; J, Pielou's evenness index.

Trait	Variety		Block		Variety×Block		Residuals	
	Explained variance (%)	Р	Explained variance (%)	Р	Explained variance (%)	Р	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	0.350	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	0.006	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	

 $\mathcal{L}_{\mathcal{V}}$ **Table 3.** ANOVA showing the effects of variety, block, variety×block and residuals on variation in the 15 quantitative traits.

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

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

 $\xi \vee \circ$ TL76.7055.7352.8083.43 $\xi \vee \vee$ PCV, phenotypic coefficient of variation; GCV, genotypic coefficient of
variation; H², broad sense heritability; GAM, genetic advance as a
percentage of the mean.

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

Trait		Dim 1	Dim.2	Dim.1-Dim.2
Explained variance (%	6)	16.30	14.60	30.90
	GH	10.74	1.95	12.69
	GC	3.46	4.77	8.23
	ACI	7.25	16.41	23.67
	ACT	1.08	11.51	12.59
	ACN	0.80	13.11	13.91
Qualitative traits	PT	3.15	4.27	7.42
	LBL	0.11	16.27	16.39
	DOL	7.96	12.75	20.71
	EACAV	0.81	0.29	1.10
	IACAV	0.81	0.29	1.10
	PAC	0.35	0.38	0.73
	PSN	0.22	0.02	0.24
	NI	1.62	0.07	1.70
	LPS	0.04	0.00	0.04
	LI	1.51	2.84	4.36
	DI	0.13	0.02	0.15
	NL	3.99	0.31	4.30
	PL	0.00	4.81	4.81
Quantitative traits	MLS	0.07	8.32	8.39
	HFW	0.08	0.38	0.46
	NTP	0.46	0.08	0.55
	TWTP	9.27	0.10	9.37
	NMTP	11.45	0.29	11.74
	WMT	12.15	0.50	12.65
	TD	10.48	0.10	10.59
	TL	11.99	0.14	12.12

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

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Locus He PIC HWE Range Ν Ar Ib242 126-146 0.71 1.71 0.63 4 5 5 ns Ib248 126-190 0.73 0.79 1.80 ns Ib255F1 231-255 0.58 0.51 1.52 2 0.32 * Ib255 172-180 0.42 1.31 5 0.74 104-120 Tb286 0.67 ns 1.71 104-156 6 Ib297 0.78 0.71 ns 1.77 Ib316 92-136 0 54 1 53 3 0.44 ns 1.70 Ib318 132-138 4 0.83 0.78 Total 34 4.25 0.68 0.60 1.63 Average

Table 6. Parameters of genetic variability among loci.

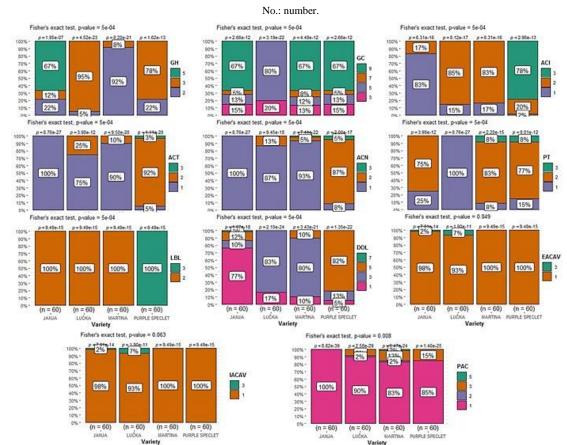
Range, range of allele lengths; N, number of alleles; He, expected heterozygosity; PIC, polymorphic information content; HWE, Hardy-Weinberg equilibrium; Ar, allelic richness.

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

	Janja	Martina	Lučka	Purple Speclet
No of different alleles	3.000	3.250	2.250	2.250
No of locally common alleles (freq. \geq 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

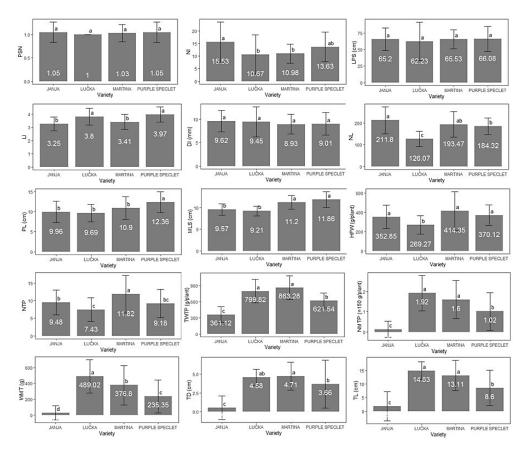


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Figure 1. Patterns of variability among the four varieties based on 11 qualitative traits.

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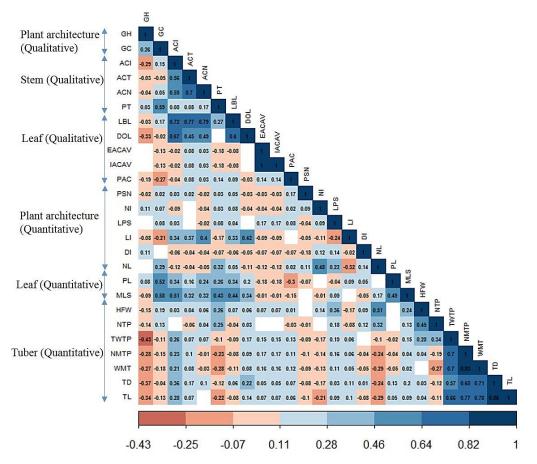
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Figure 2. Comparison of mean values for the 15 quantitative traits. Error bars indicate one

standard deviation. Different letters indicate significant pairwise differences at P < 0.01.

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••• Figure 3. Pattern of correlations among the 26 agro-morphological traits using Spearman's

 $\circ \cdot \uparrow$ coefficient. Only significant correlations are shown (P< 0.05).

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