MAGYAR AGRÁR- ÉS
ÉLETTUDOMÁNYI EGYETEM

Hungarian University of Agriculture and Life Sciences

EVALUATION OF ELDERBERRY CULTIVARS TO IMPROVE THE PRODUCTION IN HUNGARY

Virág Bolfné Csorba

Budapest

2021

PhD School:

Name: **Doctoral School of Horticultural Sciences**

Field: Crop Sciences and Horticulture

Head of PhD School: Dr. Éva Zámboriné Németh,
Professor, DSc
MATE, Institute of Horticulture,
Department of Medicinal and Aromatic Plants

Supervisors: Dr. Magdolna Tóth
Professor, DSc

Dr. Levente Kardos
Associate professor, PhD
MATE, Institute of Horticulture,
Department of Agricultural Environmental Studies

…………………………………..   …….…………………………………

Head of PhD School   Supervisors

1. BACKGROUND AND OBJECTIVES

Over the last two decades elderberry (*Sambucus nigra* L.) has become the berry fruit species cultivated in the largest quantities in Hungary. Ever since cultivation was begun, the choice of cultivar has been extremely narrow, and nowadays it is still the Austrian cultivar ‘Haschberg’ that dominates cultivation. Thanks to the work of breeders, however, various other cultivars are now available, allowing the limitations of cultivation based on ‘Haschberg’ (protracted ripening period, uneven maturity within the inflorescences, short processing season) to be eliminated. There is increasing demand from growers for more extensive information on the new cultivars, among which those with early maturity and high yielding ability appear the most promising. The most important parameters for the processing industry are the chemical traits of the cultivars, especially high soluble solids and anthocyanin content.

In Central Europe a large number of wild elderberry biotypes and of genotypes arising from crossing are available for selective breeding, but fast, reliable analytical methods are required to evaluate the fruit quality of breeding materials, which can be collected at different times, and of their progeny, and to compare them objectively with the control cultivar, ‘Haschberg’. Near infrared spectroscopy has been successfully used for the analysis of other fruit species, and has the advantages that it is rapid, cheap, non-invasive and reagent-free.

Apart from the fruit, the flowers of elderberry are also valuable, primarily due to their outstanding mineral content, but few data are available about the cultivars in this respect. Determining the mineral content of the leaves provides a picture about the nutrient supplies in the orchard, but this quantity is influenced not only by the environmental conditions and the technology, but also by the genotype. In addition to mineral nutrients, heavy metals may also be found in the plant organs, particularly in plants growing on roadsides. The majority of orchards have been established for the purpose of fruit production, and inflorescences are mainly collected in the wild for home use. Some of the marketed fruit also originates from wild stocks, so these too may accumulate heavy metals.

Eleven elderberry cultivars (‘Haschberg’, ‘Haidegg 13’, Haidegg 17’, Korsör’, ‘Samocco’, ‘Samdal’, ‘Samidan’, ‘Sampo’, ‘Samyl’, ‘Weihenstephan’ and K3) have been evaluated for adaptability in the elderberry cultivar collection of the Experimental and Research Farm of the Fruit Production Branch of the Hungarian University of Agriculture and Life Sciences (MATE) since 2013. The present work expanded and deepened the research already in progress, with the following main aims:

1. The detailed comparison of the cultivars for phenological traits (observations on flowering and ripening dynamics, determination of flowering and harvesting periods), for growth and fruiting traits, and for the physical (inflorescence weight, inflorescence size, peduncle length) and chemical (titratable acid, soluble solids, polyphenol and anthocyanin contents, antioxidant capacity) parameters of fruit samples.
2. The comparative evaluation of phenological, physical and chemical data over three years (2017, 2018, 2019).
3. Investigations on the suitability of the near infrared technology (FT-NIR) for the analysis of elderberries. Differentiation of genotypes using the NIR technology on the basis of chemical data (titratable acid and soluble solids content) and reference data from the literature (place of origin, breeding method).
4. Determination of the mineral content (K, Ca, Na, Cu, Fe, Zn, B) of inflorescences, fruit and leaves. Evaluation of the results and their comparison for individual minerals and plant organs.
5. Tests on the heavy metal contamination (Pb, Cd, Cu, Zn) of elderberry samples (inflorescences, fruit) collected from roadside trees. Investigation of the effect of distance from roads and traffic volume on the heavy metal quantities.

It is hoped that this work will contribute to the selection of valuable cultivars for the purposes of cultivation and processing, and as basic material for breeding.

2. MATERIALS AND METHODS

2.1. Location of the experiments; origin of the samples

The field experiments were carried out in three consecutive years, 2017, 2018 and 2019, in the gene bank collection of the MATE Experimental Farm of the Fruit Production Branch in Soroksár, where the phenological, growth and yielding characteristics of the cultivars were determined and the plant samples were collected.

2.2. Cultivars included in the experiments

The 11 elderberry cultivars found in the gene bank orchard were included in the experiments. In addition to the best-known, widely cultivated Austrian cultivar ‘Haschberg’, there were two ‘Haidegg’ clones, ‘Haidegg 13’ and ‘Haidegg 17’, also from Austria, and the Danish cultivars ‘Sampo’, ‘Samdal’, ‘Samidan’, ‘Samocco’, ‘Samyl’ and ‘Korsör’. A German genotype, 'Weihenstephan' and the Hungarian experimental genotype K3 were also evaluated.

2.3. Protocol of field experiments

In all three years the first field surveys were carried out in mid-April, at which time five shoots of each cultivar were labelled for observations on flowering and fruit production. At the beginning of flowering a note was made of the number of nodes between the base and tip of the labelled shoots, and the number and location of the inflorescences. Flowering data were recorded at weekly intervals, noting the number of inflorescences still in the budding stage, those in full bloom and those where the petals were falling. The inflorescences were regarded as being in the budding/full bloom/over stage if 95% of the flowers in the clusters were in the given stage.

Fruit ripening was recorded every week from mid-July to the end of August on the labelled shoots. When the fruit were harvested, measurements were made on the weight (g), size [calculated from the width (cm) and length (cm) parameters] and peduncle length (cm) of the harvested inflorescences. The mean inflorescence weight was calculated by dividing the total weight of the harvested clusters by the number of clusters. After ripening the diameter of the nodes and the length of the internodes on the shoots were also recorded.

2.4. Sample collection and preparation

Inflorescence samples from the experimental orchard were collected in full bloom in May or June, depending on the flowering dates of the cultivars. Before measuring element contents, the samples were dried and ground. The fruit were harvested at optimum maturity (when 95% of the berries were dark purple), after which the peduncles were removed and the fruit were homogenised in a food processor and stored frozen at –25°C until required. Leaf samples were taken at fruit maturity, following the sampling guidelines recommended by the National Agricultural Chamber, and like the inflorescences, were dried and ground in preparation for element analysis. Inflorescence and fruit samples were also collected from various wild stands of elderberry for the investigation of heavy metal contamination. These samples were taken at the same time, using the same method as those from the orchard.

2.5. Laboratory measurements

Chemical measurements were performed in the Department of Pomology of MATE. The water-soluble solids content (SSC) of the fruit was analysed using an HI 96801 digital refractometer. The titratable acid (TA) content was determined on the basis of the Hungarian standard (MSZ EN 12147:1998) and was expressed as citric acid equivalent. The antioxidant capacity of elderberries was determined using the FRAP method described by Benzie and Strain, and was defined as ascorbic acid equivalents (mmol AAE/ l00 g) based on the ascorbic acid standard calibration curve. The total polyphenol content (TPC) was measured according to the method of Singleton and Rossi (1965) with slight modifications. The results were expressed as micrograms of gallic acid equivalents (mg GAE/100 g). Total anthocyanin content (TAC) was determined using ethanol and hydrochloric acid, as described by Füleki and Francis (1968) (mg Cy3G/100 g). The results were given on a fresh weight basis in each cases.

For spectral measurements, homogenised fruit samples were analysed using a Bruker MPA FT-NIR instrument (Ettlingen, Germany) in the Department of Applied Chemistry of MATE. From each cultivar sample was measured seven subsamples, so the mean of the results was used for further analysis. The element analysis was performed in the MATE Department of Agricultural Environmental Studies. Before measurements, the plant samples were digested at 150°C with the addition of nitric acid and hydrogen peroxide, after which the quantities of iron, sodium, zinc, copper, boron, cadmium and lead were determined using an Aurora AI1200 atomic absorption spectrometer (AAS), and the calcium and potassium with a PG Instruments FP910 flame photometer.

2.6. FT-NIR spectral messurments

Fruit samples, from the 11 cultivars were collected in 2016 by Szilvia Kovács in the orchard of the Soroksár Experimental Farm of MATE. Four types of reference data were designated for the comparison of the spectra. Four sample groups were formed from both the SSC data and the TA data. The classification of the cultivars into two groups according to geographical origin and breeding method was performed on the basis of data from the literature.

2.7. Evaluation of shoot parameters

Due to differences in the shoot length and node number, shoot parameters can only be compared if the shoots are divided into sections and the data (e.g. number of inflorescences) are summed for each section. The internode lengths and shoot diameters above the nodes recorded for 'Haschberg' were taken as a basis for the determination of section boundaries. The mode of the values recorded in each section of the shoots was calculated and the percentage ratio of the cut-off points was determined. In this way the shoots were divided into four sections and these cut-off points were applied for all the other cultivars.

2.8. Statistical evaluation of the results

The statistical analysis was performed using the IBM SPSS Statistics 25 program package (IBM Corporation, Armonk, NY, USA). The physical parameters of the fruit were evaluated using two-way ANOVA models, and the chemical parameters, element contents and heavy metal data with one-way ANOVA (Dobson, 2002; Hang, 2014). Pearson's correlations and scatter diagrams were employed to evaluate linear correlations. The normality of error terms was checked for all the statistical analyses. Two-tailed tests were applied and in all cases differences at the $p<0.05$ level were considered to be significant.

2.9. FT-NIR results evaluation

OPUS 7.2 (Bruker, Germany) software was employed to analyse the spectral images and identify characteristic light absorption regions. In the course of statistical analysis the same three spectrum transformation operations were applied in all cases: standard normal variation (SNV); multiplicative scatter correction (MSC); first derivative. Statistica 8.0 (StatSoft, USA) and Unscrambler 10.4 (CAMO, Norway) software was used for the statistical evaluation of the data. The analysis and comparison of the samples was performed using the principal component analysis (PCA) and linear discriminant analysis (LDA) techniques. PCA was performed after all three data pre-treatments and no spectral outliers were found at the 95% confidence level. Finally, the groups formed on the basis of reference factors were subjected to LDA analysis for pattern recognition and the discrimination of the genotypes according to various criteria.

3. RESULTS AND DISCUSSION

3.1. Flowering date and flowering dynamics of elderberry cultivars

The whole course of flowering was monitored on the five shoots selected for each cultivar in three consecutive years. After the weekly analysis of flowering dynamics, the numbers of inflorescences that were budding, in full bloom and over were totalled, after which percentages were calculated to determine flowering phenograms. These were used to compare the flowering dates of the cultivars in each of the three years. Flowering began earliest in 2018 (in the last ten days of April), in the first ten days of May in 2019 and, with the exception of one cultivar, in the second ten days of May in 2017. The shortest flowering period, approximately four weeks, was observed in 2019, while in 2017 flowering took five weeks, and in 2018 it was protracted for a period of six weeks. Research conducted in Germany reported that the inflorescences bloomed between the end of May and the middle or end of June (Zeithöfler, 2002; Höhne, 2014), while Hungarian authors (Tóth and Kovács, 2001) found that the cultivars began to flower at the beginning of the second ten days of May.

On the basis of three years of data, the cultivars were divided into five flowering groups (Table 1). The finding that ‘Sampo’ flowered earlier than ‘Haschberg’ was confirmed by the observations of Möhler (2000) and Matejicek et al. (2015). The flowering order observed in the case of the cultivars ‘Haschberg’, ‘Korsör’, ‘Samocco’ and ‘Sampo’ and the experimental cultivar K3 was similar to that reported earlier (Molnár, 2013; Mezősi, 2016).

Table 1: Grouping of elderberry cultivars on the basis of flowering date (Soroksár, 2017–2019)

Flowering groups				
Early	Mid-early	Medium	Mid-late	Late
Samidan	Samocco	Samdal	Haschberg	Korsör
Sampo	K3	Samyl	Haidegg 13	Weihenstephan
			Haidegg 17	

3.2. Distribution of inflorescences over the shoot sections

The number and location of inflorescences at the various nodes were also recorded. These data gave a clear picture of which parts of the shoots were the most fertile. The results recorded for three cultivars (‘Haschberg’, ‘Sampo’, ‘Samyl’) in 2019 will be presented in greater detail. When the data were plotted on a diagram, the curves obtained for all the cultivars exhibited first an increasing and then a decreasing trend. In general the curve reached the maximum for the third section, decreasing at a relatively fast rate in the fourth section. The third section proved to be the most fertile for all three cultivars, with an average of 5–9 inflorescences in this section for ‘Haschberg’, 3–6 for ‘Sampo’ and 7–11 for ‘Samyl’. A considerable number of inflorescences was also formed on the fourth, apical section of the shoots in the case of ‘Haschberg’ (3–8) and ‘Sampo’ (3–8). Unlike the other two cultivars, ‘Samyl’ also produced 4–9 inflorescences on the second section, but only 2-7 on the fourth section. The findings of Mezősi (2016), who reported that for the majority of cultivars the most fertile sections were those nearer the tip, confirmed those recorded in the present work.

3.3. Ripening date of elderberry cultivars

The ripening of inflorescences on the labelled shoots of the eleven cultivars was monitored to determine the ripening order and harvest periods. Differences were found over the three years for both the beginning and duration of ripening. Fruit ripening began earliest in 2018 (July 11th) and 2019 (July 10th), while in 2017 the harvesting period started a week later, on July 17th. In all three years the end of ripening was recorded in the first ten days of September. The total harvesting period was the longest (60 days) in 2018 and the shortest (49 days) in 2017, while in 2019 harvesting continued for 53 days. These observations are in agreement with those of Möhler et al. (2009), who reported that the early cultivars could be harvested from mid-July, while the ripening of the latest cultivars was completed by mid-September.

The cultivars could be divided into five maturity groups on the basis of the harvesting dates (Table 2). Molnár (2013) found that 'Sampo' and 'Samidan' ripened earliest, in the first ten days of August, which was partially reflected in the present work. Höhne (2014), however, reported that in Germany 'Haidegg 17' ripened at the same time or later than 'Haschberg', which was contradicted by the present findings. Möhler et al. (2009), on the other hand, confirmed the present observations that 'Sampo' and 'Samyl' were the earliest ripening and 'Weihenstephan' the latest ripening cultivars. The ripening order of some of the cultivars was fairly constant, e.g. the early ripening of 'Sampo' and 'Samyl' and the late ripening of 'Weihenstephan', but other cultivars ('Haidegg 17', 'Korsör') varied from one year to the next. The ripening order found here for 'Haschberg', 'Korsör' 'Sampo' and 'Weihenstephan' was in accordance with that found in earlier studies (Molnár, 2013).

Table 2: Ripening order and harvesting periods of elderberry cultivars (Soroksár, 2017–2019)

Maturity groups				
Early	Mid-early	Medium	Mid-late	Late
Samyl	Samocco	K3	Haschberg	Weihenstephan
Sampo	Samdal	Haidegg 17	Haidegg 13	
		Samidan		

3.4. Physical parameters of the fruit of elderberry cultivars

Among the physical traits of elderberry inflorescences, the weight and size of the clusters are decisive for the profitability of cultivating the given cultivar (Sipos, 2010). Averaged over the three years, the **cluster size** proved to be the greatest for 'Haidegg 13' (222 cm²), while the smallest clusters were found for 'Samyl' (79 cm²). Those of 'Weihenstephan' and

‘Haschberg’ were also classified in the small size category. Molnár (2013) reported the largest clusters for the experimental cultivar K3, but this was not confirmed in the present work. Statistical analysis revealed that both the year and the cultivar had a significant effect on cluster size: $F(10;1263)>51.46$; $p<0.001$ for the cultivar and $F(2;1263)=16.03$; $p<0.001$ for the year. The year × cultivar interaction, however, was not significant.

‘Haidegg 13’ also proved to be outstanding with respect to **mean cluster weight** (84 g), which was two or in some cases three times as high as for the other cultivars, confirming the results of Möhler et al. (2009). The smallest mean cluster weights were recorded for ‘Samyl’ and ‘Haschberg’ (26–29 g), in agreement with Molnár (2013). Höhne (2014) also reported low cluster weights for ‘Haschberg’. The main effects of cultivar ($F(10;1258=47.83$; $p<0.001$) and year ($F(2;1258)=33.32$; $p<0.001$) proved to be significant for cluster weight, too.

The processing industry requires elderberry clusters to be harvested with as short a stalk as possible, but short stalk length makes harvesting difficult. Experience has shown that a stalk length of approx. 6 cm is the most favourable. The cultivar with the longest stalk was ‘Haidegg 13’ (8.5 cm), while ‘Samyl’ had the shortest (4.9 cm). ‘Haschberg’ and ‘Samidan’ had peduncles of medium length, making them ideal for both harvesting and processing. The sources quoted here from the literature contained no reference to the evaluation of stalk length. The analysis of peduncle length data revealed a significant cultivar × year interaction ($F(20;1263)=1.84$; $p=0.014$).

3.5. Fruit quality traits of elderberry cultivars

The market value of the fruit is determined by the **soluble solids content**, as the processing industry will only purchase fruit with a soluble solids content of at least 12% (Sidor and Gramza-Michalowska, 2015). This criterion was satisfied by all the cultivars except ‘Sampo’, ‘Samyl’ and ‘Weihenstephan’. In the present work, not only ‘Haidegg 17’ (14%) and ‘Korsör’ (14.5%), but also the Hungarian experimental cultivar K3 (13.2%) proved to be promising. The low values recorded for ‘Sampo’ confirm the findings of Safránková (2011). The results obtained for ‘Haschberg’, however, differed from those reported by Kaack (1997), who found a substantially lower soluble solids content for this cultivar than for the other genotypes. The cultivar proved to have a significant effect on the soluble solids content ($F(10;8.74)=9.71$; $p=0.001$).

Variations in the quantity of acids and their ratio to the sugar content influence the taste of the fruit. The combined data for the three years indicated that the **titratable acid content** ranged on average from 0.51 to 0.75%, with the lowest values for ‘Samocco’ and K3 and the highest for 'Samidan' and 'Sampo'. The results obtained for 'Samocco' and 'Sampo' agreed with those reported by Kaack (1997). According to Szalóki-Dorkó (2016), 'Samocco' had a higher acid content than 'Haschberg', but this was not confirmed in the present study. The ANOVA data revealed that the cultivar had a significant effect on the titratable acid content ($F(10;22)=7.91$; $p<0.01$).

The **sugar–acid ratio** of the elderberry cultivars was calculated from the refraction index and the titratable acid content of the fruit. The highest ratio was measured for K3 and 'Samocco' (26–27%), where a moderate refraction index was associated with low acid content. The lowest ratio was obtained for 'Sampo' (14%), which had high acid content and low refraction index. Judging by the sugar–acid ratios calculated by Safránková (2011) from the soluble solids and titratable acid contents, 'Sampo' appears to have had the smallest sugar–acid ratio, as in the present instance. Statistical analysis on the three years of data proved that the cultivar also had a significant effect on the sugar–acid ratio ($F(10;22)=9.361$; $p<0.001$).

Numerous factors influence the **antioxidant capacity** of the fruit, but the genotype also has a considerable effect (Scalzo et al., 2005), as confirmed by the statistical analysis ($F(10;22)=3.61$; $p=0.006$). The FRAP indices of the cultivars ranged from 3.8 mM AS/100 g ('Haschberg") to 8.3 mM AS/100 g ('Haidegg 17'). According to the results obtained by Mlynarczyk et al. (2020) with the ABTS method, the antioxidant capacity of 'Haschberg' was greater than that of 'Sampo' or 'Samyl', but this was not confirmed in the present work. Matejicek et al. (2015) also highlighted the substantial antioxidant capacity of 'Haschberg', while 'Haidegg 13' and 'Korsör' were found to have low values when antioxidant capacity was determined using the DPPH method, based on radical scavenging.

The mean **polyphenol content** of the 11 elderberry cultivars exhibited great variability (853–2541 mg GAE/100 g). One-way ANOVA proved that the genotype had a significant effect on the mean polyphenol content ($F(10;22)=9.77$; $p<0.001$). The best results were obtained for 'Samyl' and 'Weihenstephan', but 'Samidan' all had a substantial polyphenol content. Earlier studies in Hungary (Szalóki-Dorkó, 2016) gave contradictory results, as the Danish cultivars 'Samocco', 'Sampo' and 'Samyl' were found to have polyphenol contents similar to those of 'Haschberg'. Very few reports have been published on this aspect of European cultivars; most research has focussed on cultivars originating from *Sambucus canadensis* L., which is native to America (Özgen et al., 2010; Thomas et al., 2013).

The quantity of **anthocyanins** in the fruit is also an important property from the point of view of processing, as 90% of the harvested fruit is used to produce pigment concentrations for the food industry (Charlebois et al., 2010). The anthocyanin content ranged from 452 mg cy-3-g/100 g ('Haidegg 17') to 1417 mg cy-3-g/100 g ('Samyl'). Möhler et al. (2009) and Kaack (1989) also found the highest values for 'Samyl'. Statistical analysis again found a significant effect of the genotype in the case of anthocyanin content ($F(10;8.52)=36.18$; $p<0.001$).

3.6. Results of spectral measurements; analysis of FT-NIR spectra

The soluble solids content of the cultivars was analysed in 2016 and the results showed that the cultivars could be divided into four groups (A, B, C and D). Even before data pre-processing, spectral pattern recognition showed that the classification was 97.2% reliable at the 95% confidence level. As seen on Figure 1, the four groups could be clearly distinguished, i.e. the grouping on the basis of soluble solids content could be seen to be correlated with the spectra. When data reduction was performed prior to the analysis and the 20 main components obtained were used for statistical evaluation, checks involving random groupings resulted in a mixed picture, clearly proving that the original pattern recognition was not accidental (Figure 1.).

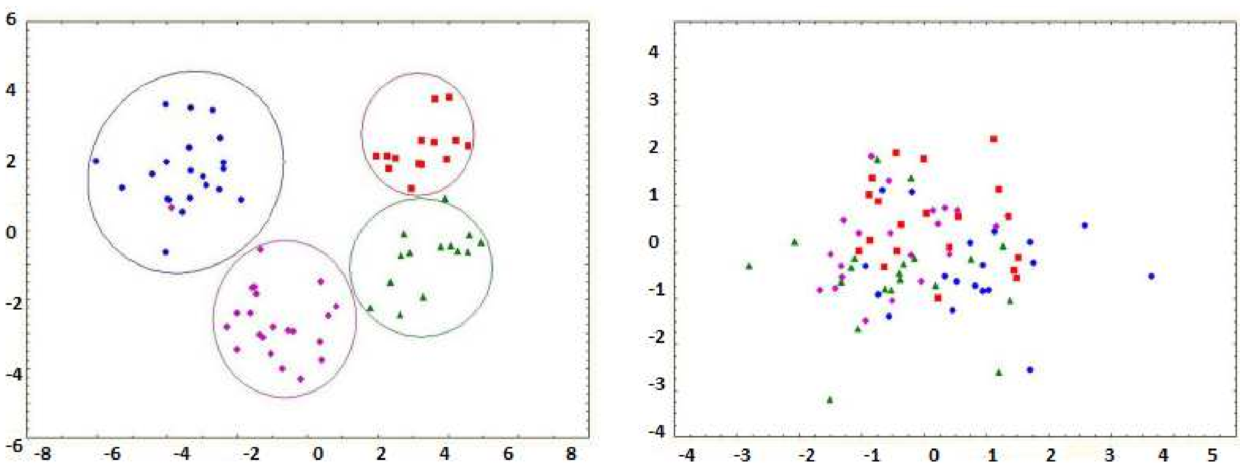

Figure 1.: Pattern recognition and discrimination (left) on the basis of soluble solids content using LDA analysis without preliminary data treatment and (right) random control classification. ● – 10.4–11.37; ■– 11.84–12.23; ♦– 12.67–12.73; ▲– 13.05–13.0 (Brix %)

The cultivars could also be divided into four groups (A, B, C, D) on the basis of titratable acid content, into two on the basis of geographical origin (Northern and Eastern Europe) and into two according to breeding method (selected, hybrid). As in the case of soluble solids content, these analyses were carried out using PCA after data reduction to 20 main components. LDA was then performed first with no data pre-processing and then after SNV and MSC pre-processing. The groups could be clearly distinguished in all three cases; LDA pattern recognition clearly distinguished between the groups, proving the correlation between the analyses. In all cases, checks confirmed that the classification was not random.

3.7. Analysis of element contents

Elderberry inflorescences contain valuable minerals, such as potassium, calcium, sodium, iron, etc. (Mlynarczyk et al., 2020), as confirmed in the present work. Statistical analysis of the element contents led to the conclusion that the genotype had a significant effect for all the elements tested [(K: ($F(10;22)=6.723$ $p<0.001$); Ca: ($F(10;22)=10.84$ $p<0.001$); Na: ($F(10;22)=28.838$ $p<0.001$); Fe: ($F(10;22)=10.301$ $p<0.001$); Zn: ($F(10;22)=16.85$ $p<0.001$); Cu: ($F(10;22)=5.574$ $p<0.001$)]. Based on the element contents of the inflorescences, 'Haidegg 17' was outstanding for its sodium, iron and zinc contents, 'Korsör' for its potassium, calcium and iron contents, and 'Samdal' for its calcium, sodium and copper contents.

Compared with other fruit species, elderberries have very high mineral contents (Souci et al., 2008; Vulic et al., 2008), as confirmed by the present results. ANOVA proved that the genotype had a significant effect on the contents of all the elements studied with the exception of copper [(K: ($F(10;22)=5.075$ $p=0.001$); Ca: ($F(10;8.51)=17.48$ $p<0.001$); Na: ($F(10;8.51)=17.48$ $p<0.001$); Fe: ($F(10,22)=2.741$ $p=0.023$); Zn: ($F(10,22)=15.58$ $p<0.001$)]. On the basis of mineral content, the fruit of 'Korsör' had excellent values for potassium and iron, 'Samocco' for potassium and calcium and 'Weihenstephan' for calcium and zinc.

The nutrient supplies to the leaves are influenced by many factors, among which the cultivar effect is considerable, as seen in the present work (Tagliavini et al., 1992; Holb et al., 2009). A significant effect was detected for all the elements studied with the exception of copper [(K: ($F(10;22)=4.347$ $p=0.002$); Ca: ($F(10;22)=6.189$ $p<0.001$); Fe: ($F(10;22)=14.246$ $p<0.001$); Zn: ($F(10;22)=2.946$ $p=0.017$); B: ($F(10;22)=5.310$ $p=0.001$)]. As regards the leaf element contents, two cultivars gave outstanding values: 'Samocco' for calcium, iron and boron, and 'Sampo' for zinc and boron.

3.8. Analysis of heavy metal contamination

The level of heavy metal (potentially toxic) contamination in the flowers and fruit of elderberry collected by roadsides was examined in two consecutive years, 2018 and 2019. ANOVA revealed that, in the case of inflorescences, the collection site had a significant effect on the cadmium ($F(8;9)=34.912$ $p<0.001$), lead ($F(8;9)=44.945$ $p<0.001$) and copper ($F(8;9)=10.857$ $p=0.001$) contents, while no correlation was detected for the zinc content. In the case of fruit, there was a significant correlation between the collection site and the cadmium ($F(2;3)=130.51$ $p=0.001$) and lead ($F(2;3)=4.82$ $p=0.116$) contents, whereas no correlation was detected for copper or zinc.

As regards the distance between the collected plant samples and the roads, a correlation was found for the lead content of the inflorescences ($p<0.008$) and the fruit ($p<0.034$). The effect of traffic volume was found to be significant for the cadmium ($p<0.013$; $p<0.034$) and lead ($p<0.038$; $p<0.041$) contents of the inflorescences and fruit, respectively. In Poland Kolodziej et al. (2012) carried out observations on elderberry flower and fruit samples and found a positive correlation between the traffic volume and the lead, cadmium, zinc and copper contents of both plant organs. These results were partially confirmed in the present work.

4. CONCLUSIONS AND RECOMMENDATIONS

The results of phenological studies led to the conclusion that elderberry cultivars could be divided into five groups on the basis of flowering date. When these results were compared with data from the literature, the same relative flowering order was obtained in the case of 'Haschberg', 'Korsör', 'Samocco', 'Sampo' and K3 (Molnár, 2013; Mezősi, 2016). Knowledge on the flowering biology traits of elderberry cultivars could be of assistance in planning cultivar combinations to make production more efficient and reliable.

As in the case of flowering, the cultivars could also be divided into five maturity groups on the basis of their ripening dates over the three years. Comparison with studies performed abroad or in Hungary showed that for some cultivars the ripening date was modified by both the location and the age of the plants. The harvest period could be favourably drawn out by cultivating the new cultivars tested here, as, with the exception of 'Weihenstephan' and 'Haidegg 13', all the cultivars matured earlier than 'Haschberg'.

When pruning fruit-bearing trees, it must not be forgotten that for most of the cultivars the third quarter of the shoot from the base is the most fertile, while the fourth quarter, nearest the tip, also bears a considerable number of inflorescences.

The physical and chemical parameters of the fruit depended to the greatest extent on the cultivar. The evaluation of these traits led to the conclusion that several of the cultivars could be promising for cultivation and processing, based on one or other of the fruit parameters.

With regard to the weight and size of the inflorescences, ‘Haidegg 13’ was the most outstanding, followed by ‘Haidegg 17’ and ‘Sampo’, while among the chemical traits the soluble solids content and antioxidant capacity of ‘Haidegg 17’ were exceptional. ‘Samyl’, ‘Samidan’ and ‘Weihenstephan’ had excellent polyphenol and anthocyanin contents. The most suitable cultivar for any given purpose can thus be selected, thus expanding the choice of cultivars grown.

The spectral data obtained using an NIR instrument were correlated in all cases with the cultivar groups identified, as confirmed by validation tests. The results were the first to prove, at the international level, that FT-NIR spectroscopy could be successfully applied for the rapid, non-destructive comparison and analysis of the fruit of elderberry cultivars. Near infrared spectroscopy could thus be a promising approach for the evaluation and comparison of breeding materials for elderberry.

The statistical analysis of the element contents of the plant organs revealed that element quantities were greatly influenced by the genotype. All the elements measured in the inflorescences (K, Ca, Na, Fe, Zn, Cu) exhibited a significant genotype effect, while in the case of fruit and leaves, copper was the only element where no correlation was detected. Among the cultivars, the mineral content of the inflorescences was most favourable in ‘Korsör’ (potassium, calcium and iron content), while in the case of the fruit ‘Samocco’ was found to be valuable for its potassium and calcium content, and ‘Sampo’ for its calcium and sodium content. The analysis of leaf element contents showed that ‘Samocco’ accumulated more iron, calcium and boron, and ‘Sampo’ more zinc and boron than the other cultivars.

In no case did the heavy metal residues in inflorescences and fruit collected in the wild exceed the threshold values. The distance of the growing site from roads and the effect of traffic volume were proved to influence the cadmium and lead values of the samples. However, the research did not cover all the potentially toxic elements; for instance the quantities of arsenic, chromium and nickel were not measured. In addition, roadside plants are also exposed to danger from other health-damaging factors, such as dust deposition. Further tests are therefore needed to form a judgment on their suitability for human consumption.

5. NEW SCIENTIFIC RESULTS

1. The flowering and ripening dates of 11 elderberry cultivars and their relative flowering and ripening orders were determined on the basis of three years of phenological observations, which led to the distinction of five flowering and five maturity groups.
2. Statistical analysis revealed the significant effect of the cultivar and the year on physical fruit parameters (inflorescence size, inflorescence weight, stalk length) and that of the cultivar on chemical traits (titratable acid, soluble solids, polyphenol and anthocyanin contents, antioxidant capacity). The complex evaluation of several fruit quality parameters using the SRD method showed that, among the cultivars tested, 'Haidegg 13', 'Haidegg 17' and 'Samyl' were the most valuable.
3. In the case of elderberry, this work proved for the first time that FT-NIR spectroscopy is a reliable, precise method for the non-invasive measurement of refraction and acid content in the fruit and for the reliable differentiation of the genotypes on the basis of fruit quality, which could lead to the substantial acceleration of breeding activities.
4. The atomic absorption analysis of 11 elderberry cultivars confirmed the genetic determination of mineral contents in leaves, inflorescences and fruit, which was not significantly influenced by the year effect. Among the cultivars investigated, 'Korsör' and 'Samocco' proved to be the best cultivars for all the macroelements tested, and 'Haidegg 17' for the microelements.
5. The analysis of heavy metal contamination in the inflorescences and fruit of elderberry growing wild by the roadside indicated that the accumulation of cadmium and lead could represent health risks.
6. Studies on the location of inflorescences on the shoots confirmed that, in the case of elderberry, flower bud formation is decisively concentrated on the upper half of the shoots.

References

1. BENZIE I. F., STRAIN J. J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of „antioxidant power”: the FRAP assay. *Analytical Biochemistry*, 239: (1) 70–76. p.
2. CHARLEBOIS D., BYERS P. L., FINN C. E., THOMAS A. L. (2010): Elderberry: botany, horticulture, potential. *Horticultural Reviews*, 37: 213–280. p.
3. DOBSON A. J. (2002): An introduction to generalized linear models. (2nd ed). Chapman & Hall/CRC
4. FÜLEKI T., FRANCIS F. J. (1968): Quantitative methods for anthocyanins. II. Determination of total anthocyanin and degradation index for cranberry juice. *Journal of Food Science,* 33: 78–83. p.
5. HANG L. (2014): Foundations of applied statistical methods. Springer International Publishing. Switzerland.
6. HOLB I. J., GONDA I., VÁGÓ I., NAGY P. T. (2009): Seasonal dynamics of nitrogen, phosphorus, and potassium contents of leaf and soil in environmental friendly apple orchards. *Soil Science and Plant Analysis*, 40: 694–705. p.
7. HÖHNE F. (2014): Holunderanbau-was kann wie erreicht werden. Ergebnisse aus Gülzow. Mitteilungen des Obstbauversuchsringes des Alten Landes. Beerenobst, 69:(8) 219-227.
8. KAACK K (1997): ’Sampo’ and ’Samdal’, elderberry cultivars for juice concentrates. *Fruit Varieties Journal*, 51: (1) 28–31. p.
9. KAACK K. (1989): New varieties of elderberry (*Sambucus nigra* L.). *Tidsskrift for Planteavl*, 93:59–65.
10. KOLODZIEJ B., MAKSYMIEC N., DROZDAL K., ANTONKIEWICZ J. (2012): Effect of traffic pollution on chemical composition of raw elderberry (*Sambucus nigra* L.) *Journal of Elementology* 17: (1) 67–78. p.
11. MATEJICEK A., KAPLAN, J., MATEJICKOVA J., VESPALCOVA M. CETKOVSKA J. (2015): Comparsion of substances in elderberry cultivars and wild elderberry. *Acta Horticulturae*, 1074: 105–109. p.
12. MEZŐSI N. (2016): Fekete bodza fajták virágzás és érésdinamikájának, valamint terméshozási sajátosságainak vizsgálata. Budapest, Szent István Egyetem, Kertészettudományi Kar, Gyümölcstermő Növények Tanszék. Szakdolgozat.

13. MLYNARCZYK K., WALKOWIAK-TOMCZAK D., STANIEK H., KIDON M., LYSIAK GP. (2020): The content of selected minerals, bioactive compounds and the antioxidant properties of the flowers and fruit of selected cultivars and wildly growing plants of *Sambucus nigra. Molecules*, 25: (4) 876–887. p
14. MÖHLER M, BLASCHEK W, LOHNWASSER E, WALTHER E (2009): Holunder (*Sambucus nigra* L.). In: HOPPE B. (Szerk.). Handbuch des Arznei- und Gewürzpflanzenbaus. Germany, Bernburg, Grafisches Centrum Cuno. 551–561. p.
15. MÖHLER, M. (2000): 'Haschberg' die beste Sorte für die Farbstoffproduktion – neue Erkenntnisse aus der LVG Erfurt. *Obstbau*, 25: 401–404. p.
16. ÖZGEN M., SCHEERENS JC., REESE RN., MILLER RA. (2010): Total phenolic, anthocyanin contents and antioxidant capacity of selected elderberry (*Sambucus canadensis* L.) accessions. *Pharmacognosy Magazine*, 6: 198–203. p.
17. SAFRÁNKOVÁ P. (2011): Chemical composition of berries juices from some cultivars of European elder. Brno, Brno Univertity of Technology. Diplomamunka.
18. SIDOR A., GRAMZA- MICHALOWSKA A. (2015): Advances research 16etal antioxidant and health benefit of elderberry (*Sambucus nigra*) in food – a review. *Journal of Functional Foods*, 18: 941–958. p.
19. SINGLETON V. L., ROSSI J. A. (1965): Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture,* 16: 144–158. p.
20. SIPOS B. Z. (2010): A fekete bodza termesztése. Budapest, Mezőgazda Kiadó.
21. SOUCI S.W., FACHMANN W., KRAUT H. (2008): Die zussammensetzung der Lebensmittel Nahrwert-Tabellen. Stuttgart, MedPharm Scientific Publishers. 1090–1091. p.
22. SZALÓKI-DORKÓ L. (2016): Fekete bodza színanyagok átfogó analitikai vizsgálata élelmiszertechnológiai eljárások során. Gödöllő, Szent István Egyetem, Élelmiszertudományi Doktori Iskola. Doktori dolgozat
23. TAGLIAVINI M., SCUDELLARI D., MARANGONI B., BASTIANEL A., FRANZIN F., ZAMBORLINI M. (1992): Leaf mineral composition of apple tree: sampling date and effects of cultivar and rootstock. *Journal of Plant Nutrition*, 15: (5) 605–619. p.
24. THOMAS A. L., PERNKINS-VEAZIEB P., BYERSC P. L., FINN C., LEE J. (2013): A comparison of fruit characteristics among diverse elderberry genotypes grown in Missouri and Oregon. *Journal of Berry Research* 3: (3) 159–168. p.

25. TÓTH M., KOVÁCS SZ. (2001): Fekete bodza. In: TÓTH M. (Szerk.). *Gyümölcsészet.* Nyíregyháza, Primom Kiadó. 417–425. p.
26. VULIC J. J., VRACAR L. O., SUMIC Z. M. (2008): Chemical characteristics of cultivated elderberry fruit. *Acta periodica technologica,* 39: 85–90. p.

6. PUBLICATIONS RELATED TO THE TOPIC OF THE THESIS

Journal articles with Impact Factor:

Csorba V., Fodor M., Kovács Sz., Tóth M. (2019): Potential of Fourier transformed near-infrared spectroscopy (FT-NIR) for rapid analysis of elderberry (Sambucus nigra L.) fruits. *Czech Journal of Food Sciences*, 37: (1) 27-28. p. IF: 0,932 (2019), 5-Year IF: 1,322, Q3 (benyújtási évben: Q2)

Csorba V., Tóth M., László A. M., Kardos L., Kovács Sz. (2020): Cultivar and year effects on the chemical composition of elderberry (*Sambucus nigra* L.) fruits. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 48: (2) 770-782. p. IF: 0,33 (2020), Q3

Peer-reviewed journal (MTA list) publications:

Csorba V., Reiczigel Zs., Kovács Sz. (2016): Törzses vázkaros fekete bodza ültetvény generatív és vegetatív paramétereinek vizsgálata különböző mértékű vesszőterhelés mellett. *Kertgazdaság*, 48: (2) 10-19. p.

Csorba V., László A., Tóth M., Mezősi N., Kovács Sz. (2018): Bodzafajták összehasonlító vizsgálata a gyümölcsök fizikai paraméterei alapján. *Kertgazdaság*, 50: (4) 20-28. p.

Csorba V., Tóth M., Kovács Sz. (2020): Bodzafajták virágzási sajátosságai három évjáratban végzett megfigyelések alapján. *Kertgazdaság*, 52: (4) 35-45. p.

Conference full paper:

Csorba V. Kardos L. (2018): Termesztett és vadon termő fekete bodza (*Sambucus nigra* L.) elemtartalom vizsgálata. In: Füleky György (szerk.) XIV. Kárpát-medencei Környezettudományi Konferencia Kiadványa. Gödöllő, 2018. április 5-7. 72-76. p.

Conference proceedings (abstracts):

Csorba V., Tóth M. Kovács Sz. (2018): Bodzafajták gyümölcsvizsgálati eredményei. In: Karsai I., Polgári Zs. (szerk.) XXIV. Növénynemesítési Tudományos Nap. Budapest, 2018. március 6. 76.p. (poszter)

Degree thesis and TDK defence:

Mezősi N. (2016): Fekete bodza fajták virágzás és érésdinamikájának, valamint terméshozási sajátosságainak vizsgálata. Budapest, Szent István Egyetem, Kertészettudományi Kar, Gyümölcstermő Növények Tanszék. Szakdolgozat.

Publications in other journals:

Csorba V. (2019): Hogyan állunk ma a bodzával? *Agrofórum*, 30 (10): 180-184. p.

Csorba V. (2020): Bodzafajta-vizsgálatok új hazai eredményei. *Agrofórum Extra,* 85: 68-70. p.

References in a foreign publication:

Csorba V., Fodor M., Kovács Sz., Tóth M. (2019): Potential of Fourier transformed near-infrared spectroscopy (FT-NIR) for rapid analysis of elderberry (Sambucus nigra L.) fruits. *Czech Journal of Food Sciences*, 37(1): 27-28. p.

Cite:

1. Stefan Stuppner, Sophia Mayr, Anel Beganovic et al. (2020): Near-Infrared Spectroscopy as a Rapid Screening Method for the Determination of Total Anthocyanin Content in Sambucus Fructus. *Sensors, 20*(17), 4983. (open acces)
2. Joanna Szulc, Grażyna Gozdecka, Wojciech Poćwiardowski (2020): The application of NIR spectroscopy in moisture determining of vegetable seeds. *Czech J. Food Sci.*, 38: 131-136. p.

Csorba V., Tóth M., László A. M., Kardos L., Kovács Sz. (2020): Cultivar and year effects on the chemical composition of elderberry (*Sambucus nigra* L.) fruits. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 48(2): 770-782. p.

Cite:

Anete Boroduskea, Kaspars Jekabsonsb, Una Riekstinab, Ruta Mucenieceb, Nils Rostoksa, Ilva Nakurtec (2021): Wild *Sambucus nigra* L. from north-east edge of the species range: A valuable germplasm with inhibitory capacity against SARS-CoV2 S-protein RBD and hACE2 binding in vitro. *Industrial Crops and Products*, 165, July, 113438 (in press)