A Comparative Study of some Local Genotypes with Commercial Cultivar of Black Elder (*Sambucus nigra* L.) Regarding Vegetative and Reproductive Traits

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### Summary

The main goal of this study was to evaluate three black elder (Sambucus nigra L.) genotypes ("Prigorje", "Korčula", "Zagreb") and compare them with cv. Haschberg as standard cultivar. Diameter of inflorescences, cluster mass, berry/stem mass ratio, mass of 10 berries and SSC were higher in season 2014 than in season 2015, while length of one-year-old shoot and flowering density showed no significant differences. Interaction between season and genotype (S  $\times$  G) was not significant for any of studied parameters. Genotype "Korčula" had significantly smaller diameter of inflorescence (11.26 ± 1.5 cm) than genotypes "Prigorje", "Zagreb" and cv. Haschberg (16.81  $\pm$  2.38, 18.05  $\pm$  2.39, 17.5  $\pm$  2.23 cm, respectively). Genotype "Korčula" (0.09 ± 0.03 flowers⋅cm<sup>-1</sup>) had significantly smaller flowering density than genotypes "Prigorje", "Zagreb" and cv. Haschberg (0.15  $\pm$  0.07, 0.15  $\pm$  0.04, 0.16  $\pm$ 0.04 flowers·cm<sup>-1</sup>, respectively). Genotype "Korčula" had significantly smaller cluster mass (23.35 ± 10.71 g) than genotypes "Prigorje", "Zagreb" and cv. Haschberg (54.07  $\pm$  22.09, 56.24  $\pm$  22.76, 67.29  $\pm$  24.56 g, respectively). Cv. Haschberg had significantly higher mass of 10 berries (1.35  $\pm$  0.2 g) than genotypes "Zagreb" (1.15  $\pm$  0.17 g) and "Korčula" (1.14 ± 0.19 g). Genotype "Zagreb" had significantly higher value of total soluble solids (10.23  $\pm$  1.74 %Brix) than genotype "Korčula" (8.99  $\pm$  1.89 %Brix). Among studied genotypes and cv. Haschberg no significant differences were found for length of one-year-old shoot. Since no significant differences were recorded between cv. Haschberg and genotypes "Zagreb" and "Prigorje" in majority of parameters, these genotypes should be further evaluated for longer period in order to bring final conclusions about their commercial and breeding potential.

## Key words

black elder, elderberry, fruit quality, genotypes, Sambucus nigra L.

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## Introduction

Sambucus is a genus in the family Adoxaceae (Applequist, 2015). Black elder (Sambucus nigra L.) is found widely throughout the sunny locations or sites in all of the European countries, as well as some African (Algeria, Tunisia), Asian (Iran, Iraq, Turkey, Armenia, Azerbaijan, Georgia, Russia) and North American (USA) countries (Christensen et al., 2007; Jemrić, 2007). It is a deciduous shrub or a small tree tall up to 8 m or more (Jemrić, 2007). Black elder usually grows in the wilderness. Some parts of black elder plant, particularly stems and immature fruits, can be toxic due to a variety of toxic alkaloids and cyanogenic glycosides (Lewis and Elvin-Lewis, 2003).

According to Valles et al. (2004) black elder is one of the most versatile plants because it can be used in many ways. According to the same authors its health promoting properties are known from ancient times and nowadays numerous studies confirm it. Barak et al. (2002), Roschek et al. (2009), Zakay-Rones et al. (1995) reported that European elder (*Sambucus nigra* L.) and American elder (*Sambucus canadensis* L.) have numerous health benefits such as anti-inflammatory, antibacterial and antiviral properties.

In addition, black elder can be used as an ornamental plant (Christensen et al., 2007) or its flowers and fruits as a material for the production of food and beverages (Dawidowicz et al., 2006; Jemrić, 2007; Simonovik et al., 2007). Lately, cultivation of black elder has received increased attention due to its high anthocyanin levels in berries (Mustafa et al., 2009), which are used as food colorants primarily in the beverage industry (He and Giusti, 2010). Possibility of usage of black elder berries as a source of natural colorants is very interesting because it is suspected that synthetic dyes that are commonly used in the food industry cause adverse behavioral and neurological effects (McCann et al., 2007).

Due to the several benefits mentioned above there is an increasing demand of black elder fruit in food industries and it has become an important horticultural crop nowadays (Simonovik et al., 2007). Due to a limited number of cultivars of black elder there is a need of evaluating other genotypes in order to expand existing breeding programs and to overcome current cultivation problems of black elder in Croatia. Particular attention should be devoted to the selection of new cultivars from the local populations of black elder as they can be easily adapted under the local soil and climatic conditions. The current study is a step towards introducing new cultivars by exploring three local genotypes of black elder from different parts of Croatia by comparing the vegetative and reproductive traits with the standard cultivar (cv. Haschberg).

# Materials and methods

### Plant material

The samples were collected from the black elder genotypes ("Korčula", "Prigorje", "Zagreb") and cv. Haschberg grown at Experimental Field Research Station 'Jazbina' of Dept. of Pomology, Faculty of Agriculture, University of Zagreb, Croatia. The studied genotypes of black elder at the research station originate from different regions. Genotype "Korčula" originate from island of Korčula (Mediterranean Croatia), while genotypes

"Zagreb" and "Prigorje" belong to Zagreb country (continental Croatia). The black elder orchard at 'Jazbina' research station is in shape of slope vegetation. Fruit samples were randomly harvested at optimal maturity stage and analyzed at the end of August and September during two seasons (2014 and 2015) at research laboratory of Dept. of Pomology. During the seasons only pruning and weed removal was carried out.

# Morphological and Chemical analysis or measurements

The measurements regarding vegetative and reproductive characteristics were collected from every genotypes and cv. Haschberg in three replicates in spring and summer seasons of 2014 and 2015. Length and width of 5 inflorescences (cm), length of three one-year-old shoots (cm) and the number of inflorescences on the one-year-old shoots were measured in month of May. Whereas, the flower density was obtained by dividing the number of inflorescences with length of one-year-old shoot. Measurements were done on four clusters from each tree. Mass of cluster with the stem and the mass of separated stem were measured on analytical balance (OHAUS Adventurer AX2202, Ohaus Corporation Parsippani, NJ, USA). Berry/stem mass ratio was calculated according to formula: 100-(mass of the separated stem × 100/mass of the cluster with the stem). For each genotype, mass of 10 berries and soluble solids content (SSC) were randomly measured from four clusters per tree using ATAGO 3810 PAL-1 digital refractometer (ATAGO, Tokyo, Japan) and expressed as %Brix.

## Statistical analysis

Data was analyzed using analysis of variance (ANOVA) and the significance of differences among means were obtained using LSD test at p<0.05 and the standard deviation calculated by using SAS statistical software ver. 9.4 (SAS Institute, NC).

# Results and discussion

The ANOVA revealed that the factor or variable season caused no significant difference regarding length of one-year-old shoot and flowering density of shoot; as well as factor or variable genotype regarding length of one-year-old shoot and SSC in fruits (Table 1). In season 2014 the diameter of inflorescences, mass of cluster, berry/stem mass ratio, mass of 10 berries and SSC were significantly higher than in season 2015. The interaction between season and genotype (S  $\times$  G) was not significant for any of studied parameters. Therefore, differences among genotypes are expressed as an average of two seasons (2014 and 2015) in results.

Genotype "Zagreb" had highest diameter of inflorescences (18.05  $\pm$  2.39 cm) followed by cv. Haschberg (17.5  $\pm$  2.23 cm), genotype "Prigorje" (16.81  $\pm$  2.38 cm) and lowest in genotype "Korčula" (11.26  $\pm$  1.5 cm). Genotype "Korčula" had significantly smaller diameter of inflorescences than genotypes "Prigorje", "Zagreb" and cv. Haschberg. There were no significant differences between cv. Haschberg and genotypes "Prigorje" and "Zagreb" (Fig. 1).

Cv. Haschberg had highest length of one-year-old shoot (106.13  $\pm$  38.84 cm) followed by genotypes "Zagreb" (95.5  $\pm$  16.94 cm), "Prigorje" (94.63  $\pm$  34.22 cm) and lowest in genotype

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Source of variability	Diameter of inflorescences (cm)	Length of one- year-old shoot (cm)	Flowering density (number of flowers·cm <sup>-1</sup> )	Cluster mass (g)	Berry/stem mass ratio (%)	Mass of 10 berries (g)	SSC (%Brix)
Season (S)	0.0018**	0.0591 n.s.	0.8132 n.s.	0.0046**	<0.001***	0.0223*	<0.001***
Genotype (G)	<0.001***	0.4576 n.s.	0.0040**	<0.001***	0.0410*	0.0025**	0.0892 n.s.
$S \times G$	0.3291 n.s.	0.8311 n.s.	0.9631n.s.	0.4700 n.s.	0.3269 n.s.	0.3200 n.s.	0.8448 n.s.
			Season (S) (	Mean ± SD)			
2014	16.29±3.71	84.05±26.05	0.13±0.07	57.56±26.65	92.82±1.71	1.29±0.16	10.66±1.38
2015	14.91±3.13	106.38±35.48	$0.14\pm0.04$	41.72±24.08	90.04±2.5	1.19±0.21	8.68±1.34

Table 1. ANOVA table for vegetative and fruit quality trait of four genotypes of black elder studied over two seasons

Note: n.s. \*, \*\*, \*\*\* - nonsignificant or significant at  $P \le 0.05$ , 0.01 or 0.001 level, respectively

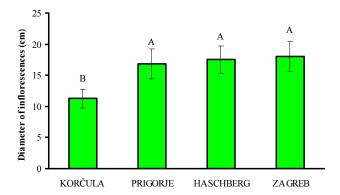


Figure 1. Diameter of inflorescences (cm) of three black elder genotypes and cv Haschberg (average values from seasons 2014 and 2015); vertical bars represent SD

"Korčula"  $(84.75 \pm 30.64 \text{ cm})$ , but there were no significant differences found among them and hence data not shown here.

Highest flowering density was recorded in cv. Haschberg (0.16  $\pm$  0.04 flowers·cm $^{-1}$ ) and was closely followed by genotypes "Prigorje" (0.15  $\pm$  0.07 flowers·cm $^{-1}$ ) and "Zagreb" (0.15  $\pm$  0.04 flowers·cm $^{-1}$ ), while genotype "Korčula" had lowest flowering density (0.09  $\pm$  0.03 flowers·cm $^{-1}$ ). Genotype "Korčula" had significantly lower flowering density in comparison with genotypes "Zagreb" and "Prigorje" and cv. Haschberg. Regarding this parameter there were no significant differences between cv. Haschberg and genotypes "Zagreb" and "Prigorje" (Fig. 2).

Highest cluster mass had cv. Haschberg (67.29  $\pm$  24.56 g) followed by genotypes "Zagreb" (56.24  $\pm$  22.76 g), "Prigorje" (54.07  $\pm$  22.09 g) and lowest in genotype "Korčula" (23.35  $\pm$  10.71 g). Genotype "Korčula" had significantly lower cluster mass than cv. Haschberg and genotypes "Zagreb" and "Prigorje". Between genotypes "Zagreb" and "Prigorje" and cv. Haschberg no significant differences were recorded (Fig. 3). The results can be compared with the findings of Bošnjaković et al. (2012). These authors have reported that average mass of cluster of 8 evaluated genotypes ranged from 64.95 to 241.52 g (per each genotype) and cv. Haschberg had an average mass of 121.32 g. The possible explanation for the results obtained in our study can be justify due to the unfavorable growing conditions (slope vegetation) which prevented trees to show their full productive potential. Additional support to this explanation can be found in the study conducted

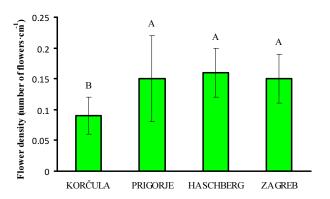


Figure 2. Density of flowering of shoot (number of flowers·cm<sup>-1</sup>) of three black elder genotypes and cv Haschberg (average values from seasons 2014 and 2015); vertical bars represent SD

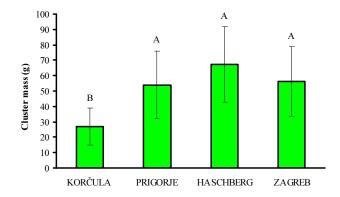


Figure 3. Cluster mass (g) of three black elder genotypes and cv Haschberg (average values from seasons 2014 and 2015); vertical bars represent SD

by Finn et al. (2008) who found significant effect of area of cultivation (site) to the productive parameters of American elderberry (*Sambucus canadensis* L.). In above mentioned study in year 2004 cv. Adams II grown in Mountain Grove had average cluster mass of 58.6 g, while in Mt. Vernon of 30.1 g.

Cv. Haschberg (92.24  $\pm$  2.48 %) had the highest berry/stem mass ratio followed by genotype "Prigorje" (91.7  $\pm$  2.09 %), genotype "Korčula" (91.34  $\pm$  2.07 %) and lowest in genotype "Zagreb" (89.51  $\pm$  3.49 %). Genotype "Zagreb" had significantly

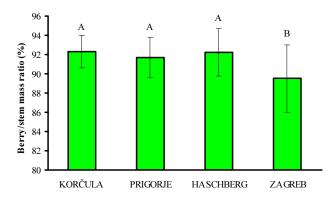


Figure 4. Berry/stem mass ratio (%) of three black elder genotypes and cv Haschberg (average values from seasons 2014 and 2015); vertical bars represent SD

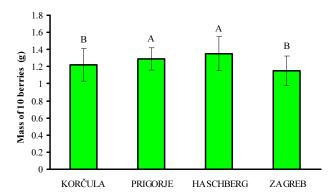


Figure 5. Mass of 10 berries (g) of three black elder genotypes and cv Haschberg (average values from seasons 2014 and 2015); vertical bars represent SD

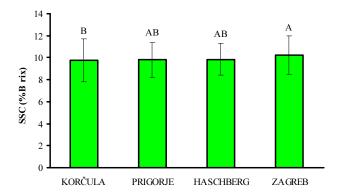


Figure 6. Fruit SSC (%Brix) of three black elder genotypes and cv Haschberg (average values from seasons 2014 and 2015); vertical bars represent SD

lower berry/stem mass ratio than cv. Haschberg and genotypes "Prigorje" and "Korčula". Regarding this parameter, between cv. Haschberg and genotypes "Prigorje" and "Korčula" no significant differences were recorded (Fig. 4).

Cv. Haschberg had the highest mass of 10 berries (1.35  $\pm$  0.2 g) followed by genotypes "Prigorje" (1.29  $\pm$  0.13 g), "Zagreb" (1.15  $\pm$  0.17 g) and lowest in genotype "Korčula" (1.14  $\pm$  0.19 g).

Cv. Haschberg and genotype "Prigorje" had significantly higher mass of 10 berries than genotypes "Zagreb" and "Korčula". Between cv Haschberg and genotype "Prigorje" no significant differences were recorded, as well as between genotypes "Zagreb" and "Korčula" (Fig. 5). Bošnjaković et al. (2012) reported that mass of 10 berries from seven potentially perspective genotypes of black elder from natural populations of Serbia varied from 1.11 to 1.9 g, with average value of 1.47 g while average value of 10 berries of cv. Haschberg was 1.77 g. Similarly, Mratinić and Fotirić (2007) reported an average mass of single berry from 4 genotypes from Serbia ranged from 0.13 to 0.21 g (equals 1.3 to 2.1 g for 10 berries). In addition, Romero Rodriguez et al. (1992) found average mass of single berry of black elder from Galicia (Spain) ranged from 100 to 130 mg (from 1 to 1.3 g for 10 berries). Mustafa et al. (2009) reported that average mass of one berry of black elder of different genotypes was between 0.14 and 0.20 g (from 1.4 to 2 g for 10 berries). According to Akbulut et al. (2009) an average mass of single berry from four genotypes form Artvin (Turkey) ranged from 0.14 to 0.2 g (from 1.4 to 2 g for 10 berries). Our results regarding this parameter are around the lower limit in comparison to the results reported by Akbulut et al. (2009), Bošnjaković et al. (2012), Mratinić and Fotirić (2007) and Mustafa et al. (2009) while similar with Romero Rodriguez et al. (1992). As discussed previously, possible explanation for such results can be found in unfavorable growing conditions (slope vegetation), that prevented studied genotypes to show full productive nature or behavior.

Genotype "Zagreb" had the highest SSC (10.23 ± 1.74 %Brix) followed by cv. Haschberg (9.84 ± 1.46 %Brix), genotype "Prigorje"  $(9.81 \pm 1.58 \text{ %Brix})$  and lowest in genotype "Korčula"  $(8.99 \pm$ 1.89 %Brix). Genotype "Zagreb" had significantly higher SSC than genotype "Korčula". Between genotype "Zagreb" and genotype "Prigorje" and cv. Haschberg no significant difference was recorded, as well as between genotype "Korčula" and genotype "Prigorje" and cv Haschberg (Fig. 6). Galić et al. (2009) reported quite higher average SSC values from black elder fruits located near Gospić (Croatia) of 17.00 ± 0.50 %Brix. However, Lee and Finn (2007) reported that SSC within 10 genotypes of black elder varied from 11.2 to 15.4 %Brix. Mustafa et al. (2009) reported that the SSC values of four genotypes of black elder varied from 11.74 to 12.62 %Brix. The SSC from four genotypes of black elder from Serbia varied from 14.8 to 17.19 %Brix (Mratinić and Fotirić, 2007). According to Bošnjaković et al. (2012) the SSC for 8 different genotypes from Serbia varied from 12.34 to 17 %Brix, while cv. Haschberg had 12.84 %Brix. According to Akbulut et al. (2009) SSC from four genotypes ranged from 11.74 to 12.62 %Brix. The results for this parameter are lower than above findings possibly due to the unfavorable growing conditions (slope vegetation).

# Conclusion

In most of the studied parameters, genotype "Korčula" showed significantly lower values than other genotypes and hence not recommended for selection and cultivation purposes under agro-climatic conditions of continental Croatia. However, due to its Mediterranean origin, this genotype might show some different and better results if tested in more appropriate agro-climatic conditions.

In majority of studied parameters, no significant differences were recorded between cv. Haschberg and genotypes "Zagreb" and "Prigorje". Therefore, these genotypes should be further evaluated for longer period on multiple locations in different agro climatic conditions in order to bring final conclusions.

#### References

- Akbulut M., Ercisli S., Tosun M. (2009). Physico-chemical characteristics of some wild grown European elderberry (*Sambucus nigra* L.) genotypes. Phcog Mag 5(20): 320-323.
- Applequist W.L. (2015). A brief review of recent controversies in the taxonomy and nomenclature of *Sambucus nigra* sensu lato. Acta Hortic 1061: 25-33.
- Barak V., Birkenfeld S., Halperin T., Kalickman I. (2002). The effect of herbal remedies on the production of human inflammatory and anti-inflammatory cytokines. Isr Med Assoc J 4: 919-922.
- Bošnjaković D., Ognjanov V., Ljubojevic M., Barac G., Predojevic M., Mladenovic E., Cukanovic J. (2012). Biodiversity of wild fruit species of Serbia. Genetika 44: 81-90.
- Christensen L. P., Kaack K., Fretté X. C. (2007). Selection of elderberry (*Sambucus nigra* L.) genotypes best suited for the preparation of elderflower extracts rich in flavonoids and phenolic acids. Eur Food Res Technol 227: 293-305.
- Dawidowicz A. L., Wianowska D., Baraniak B. (2006). The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. (antioxidant properties of extracts). Lwt-Food Sci Technol 39: 308-315.
- Finn C. E., Thomas A. L., Byers P. L., Serce S. (2008). Evaluation of American (*Sambucus canadensis*) and European (*S. nigra*) Elderberry Genotypes Grown in Diverse Environments and Implications for Cultivar Development. HortScience 43(5): 1385–1391.
- Galić A., Dragović-Uzelac V., Levaj B., Bursać Kovačević D., Pliestić S., Arnautović S. (2009). The Polyphenols Stability, Enzyme Activity and Physico-Chemical Parameters During Producing Wild Elderberry Concentrated Juice. Agric Conspec Sci 74: 181-186
- He J. and Giusti M. M. (2010). Anthocyanins: Natural Colorants with Health-Promoting Properties. Annu Rev Food Sci Technol 1:163-187.

- Jemrić T. (2007). Bazga Važnost, uporaba i uzgoj. Hrvatska sveučilišna naklada, Zagreb, 81 pp.
- Lee J., Finn C. E. (2007). Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. J Sci Food Agr 87: 2665-2675.
- Lewis W. H., Elvin-Lewis M. P. F. (2003). Medical Botany: Plants Affecting Human Health. 2nd Ed., John Wiley and Sons Inc, Hoboken, New Jersey, 832 pp.
- McCann D., Barrett A., Cooper A., Crumpler D., Dalen L., Grimshaw K., Kitchin E., Lok K., Porteous L., Prince E., Sonuga-Barke E., Warner J. O., Stevenson J. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebocontrolled trial. Lancet 370: 1560–1567.
- Mratinić E., Fotirić M. (2007). Selection of black elderberry (*Sambucus nigra* L.) and evaluation of its fruits usability as biologically valuable food. Genetika 39: 305-314.
- Mustafa A., Sezai E., Murat T. (2009). Physico-chemical characteristics of some wild grown European elderberry ( *Sambucus nigra* L.) genotypes. Pharmacogn Mag 5: 320-323.
- Romero Rodriguez M. A., Vazquez Oderiz M. L., Lopez Hernandez J., Simal Lozano J. (1992). Studio della composizione chimica, caratteristiche fisiche ed indici di maturazione del lauroceraso (*Prunus laurocerasus* L.) e delle bacche di sambuco (*Sambucus nigra* L.). Industrie Alimentari 31: 911-917.
- Roschek B. Jr., Fink R. C., Mcmichael M. D., Li D., Alberte R. S. (2009). Elderberry flavonoids bind to and prevent H1N1 infection in vitro. Phytochemistry 70: 1255-1261.
- Simonovik B., Ivancic A., Jakse J., Bohanec B. (2007). Production and genetic evaluation of interspecific hybrids within the genus *Sambucus*. Plant Breeding 126: 628-633.
- Valles J., Bonet M. A., Agelet A. (2004). Ethnobotany of Sambucus nigra L. in Catalonia (Iberian peninsula): The integral exploitation of a natural resource in mountain regions. Econ Bot 58: 456-469
- Zakay-Rones Z., Varsano N., Zlotnik M., Manor O., Regev L., Schlesinger M., Mumcuoglu M. (1995). Inhibition of several strains of influenza virus in vitro and reduction of symptoms by an elderberry extract (*Sambucus nigra* L.) during an outbreak of influenza B Panama. J Altern Complem Med 1: 361-369.

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