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Review

Cultivation, Phytochemistry, Health Claims, and Genetic Diversity of *Sambucus nigra*, a Versatile Plant with Many Beneficial Properties

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Abstract: *Sambucus* is a cosmopolitan plant genus that has been used for centuries for its medicinal properties and nutritional value. *Sambucus nigra*, the most studied species, contains a wide range of bioactive compounds that have been linked to various health benefits. Moreover, the fruit of the elderberry is a rich source of phytochemicals and is used to make a variety of food products. In this review, after an introduction of the species, we outline the main points for its cultivation and production. We then illustrate the major phytochemical components and related beneficial properties, such as antioxidant, antimicrobial, and pharmaceutical activities. We also provide insights into genetic variability, functional diversity, and some evolutionary relationships that were evaluated with DNA-based techniques. We discuss that despite its long history of use and potential benefits, *Sambucus nigra* has received relatively little attention in terms of horticulture, breeding, and molecular genetics, while studies on its biochemical composition and health benefits are well developed. Further research is also needed to better understand the pre-harvest and post-harvest factors that influence plant growth and production, as well as to explore new applications and industrial uses of this underutilized species.

Keywords: elderberry; black elder; European elder; bioactive compounds; DNA markers; horticulture

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1. Introduction

The *Sambucus nigra* L., commonly called elder, black elder, elderberry, or European elder, is a species complex of the Adoxaceae family (previously part of the large Caprifoliaceae family). Numerous subspecies and variants are described in horticulture, which are often used as ornamental plants [1,2].

Elderberries have a lightly tart, sour, and slightly sweet taste, according to the ripeness, with some bitterness and a floral note, and should be eaten after cooking because they are mildly poisonous. A delicate muscat aroma characterizes the flowers, which can be eaten raw or cooked [3]. Different parts of the plants can be used for the preparation of drinks, liqueurs, and food flavorings [4].

The most used part of the *S. nigra* is the fruit, employed to produce jams, jellies, juices, syrups, and other beverages (e.g., wine) [5]. Flowers can be employed to make teas, cordials, and infusions. Leaves, roots, stems, and (fresh) bark, although utilized in some cultures, are considered more toxic because of cyanogenic glycosides and alkaloids, and they should not be regarded as safe to brew beverages and produce alcoholics unless proven otherwise.

Sambucus nigra has been also used for medicinal purposes for centuries [1,2]. Flowers, berries, leaves, and bark have been exploited in different traditional medicines as pain relief and to treat a variety of conditions, such as respiratory infections, digestive discomfort, skin conditions and inflammation [6]. This range of use and ample effects have been attributed to its rich phytochemical makeup, and a number of reviews have been published on the biochemical composition of different parts of the plant (e.g., flowers, berries, and leaves) [5,7,8], the possible health benefits [9,10], and potential use in the treatment of various diseases [3,11,12].

Elderberry has a long history of cultivation and use by various civilizations, dating back to ancient times. In the last century, cultivation has declined due to a shift towards intensive agriculture and low economic value compared to other fruit trees. In contrast to the significant advancements made in fruit trees during the last century, such as the development of dwarfing rootstocks, the introgression of disease and pest-resistance traits, the improvement fruit quality, and the extension of the growing/fruiting seasons, systematic breeding efforts for this species have been very limited. Today, in addition to an increased general interest in functional food, *S. nigra* is being valued within a wildlife and habitat management context.

In this review, we examine the potential of the elderberry in agriculture, its genetic diversity, and breeding efforts. This contribution starts with an overview of the morphological features and cultivation, followed by a brief discussion of the phytochemical composition and bioactivities of the plant and its extract. In addition, the current knowledge regarding genetic diversity and breeding efforts is presented. We conclude with some perspectives, which include suggestions to prioritize research directions.

2. Botany, Ecology, and Cultivation

S. nigra is a fast-growing deciduous shrub (with numerous branches arising from the base) or more rarely, a small tree (especially with old plants where a single trunk becomes dominant), with erect and bushy growth habit and arching branches. The plant has cracked and corky bark, with prominent lenticels, and produces small, white hermaphrodite flowers, gathered in large, flat umbels (umbrella-shaped inflorescences), 10–20 cm wide (Figure 1).

Figure 1. Some distinctive morphological features of *Sambucus nigra*. (**A**) Young trunk with lenticels; (**B**) umbels with white flowers; (**C**) dark-colored fully ripe fruits. Fruits are commercially considered berries.

Flowers can be also colored (from light to dark pink) in the so-called “Black” varieties, which also have darker foliage and are usually employed as garden plants. Botanically, fruits are classified as drupe because they contain a single seed encased in a hard pit (or stone), surrounded by fleshy edible tissue (Figure 1). Nonetheless, they are commercially considered berries because of their size, color, and consistency of the flesh. The infructescence consists of shiny blackish-dark-blue fruits present in a grape-like fruit cluster, whose color originates from the species name (*nigra*). The name of the genus allegedly derives from the Greek word “σαμβύκη” (in Latin: *sambuca*), which indicates a small harp, while the reference to a small flute (typically made from the plant twig) became more widespread during the Middle Ages [5].

S. nigra grows in the whole of Europe, West Asia, North Africa, and the USA [13]. Its adaptability to different environmental conditions and the large distribution range most likely contribute to the difficulties in the taxonomic classification. A continuous native distribution occurs throughout western Europe, whereas an isolated population of this plant species can be found up to northern limits located at latitudes of 61°–65° N (in Norway and Sweden) and to eastern limits at the longitude of 55° E [14]. In addition, the altitudinal limits in Europe are reported between 900 and 1500 m a.s.l. (on Tatra mountains and the Alps, respectively) [14]. These geographical boundaries exclude areas characterized by mean air temperatures in October lower than 7.2 °C, probably because these thermal conditions do not allow a full seed maturation [14], hence limiting the natural diffusion of this species in such environments. Elderberry was also successfully introduced in other continents (Northern Africa, Eastern Asia, and Oceania) [5], and starting from the early 1980s, its commercial cultivation has progressively spread in Europe (Austria, Denmark, Germany, Hungary, and Italy), North America (USA and Canada), Chile, and New Zealand [15]. Even though elderberry can adapt to a large range of soil types (from sandy to clay soils), vegetative growth and yield are best performing in well-drained soils, rich in organic matter and with subacid pH (6.0–6.5).

Plants used for commercial plantations are generally propagated by own-rooted hardwood cuttings using segments of one-year-old shoots collected from mother plants. Seed propagation is limited by the germinability of the small seeds and their orthodox dormancy, besides providing a more variable plant population. Considering that black elder barely tolerates competition with other plants, the soil should be prepared to remove carefully weeds before transplanting (in Fall or Spring). Irrigation and fertilization (mainly nitrogen) should be provided to allow adequate plant growth, which can be very vigorous in favorable conditions [5]. For the same reasons, regular weed control and fertilization should be undertaken throughout the commercial life of the elderberry plantation. The selection of planting distances should consider that (a) elder plants have a spread bushy habit (the canopy of an adult plant occupies a space with a radio of around 0.9–1.2 m depending on the cultivar), and (b) the type of machinery owned by the grower. Suggested planting distances are around 2.1–2.4 m within the row and 3.0–3.6 m between rows (corresponding to planting densities of around 1263–1587 bushes/ha; 4 × 2 m) [5]. Starting from the second year of planting, dormant pruning (applied always before bud break) should be regularly carried out in commercial orchards of elderberry. The main objectives of this canopy management practice should be to remove damaged, diseased, and dead stems/branches and to rejuvenate the canopy by removing three-year or older branches. Productivity significantly declines in four-year-old branches [5]. Even though the adoption of coppicing at the ground level may be interesting for medium- to large-size plantations to significantly reduce the labor costs required for pruning, its regular annual use is not advisable for the European elder because most of the commercial cultivars of this species do not bear fruits on primocanes. Commercial elderberry cultivations achieve full production 3–5 years after planting. Elderberry berries are typically harvested when they are fully ripe and have turned a dark purple or almost black color. The berries are ready to harvest in late summer or early fall, depending on the climate and location. Berries are highly perishable and should be processed or stored (e.g., freezing) promptly after harvest [16]. In professional cultivation, it is recommended to monitor the accumulation of soluble solids in the berries and observe any changes in the color of the berry skin to determine the optimal harvest time. This occurs when berry juice has reached a minimum total soluble solid content of 12 °Brix, and at least 75% of the berries should have dark-violet skins [17]. The target total soluble solid values may change depending on the cultivar and the growing conditions [4,18]. Flowers are usually harvested when they are in full bloom, which typically occurs in late spring or early summer. The flowers are typically picked in the morning when they are still fresh and fragrant. Leaves and bark can be harvested at any time during the growing season but are usually collected in late spring or early summer. It is important to harvest only a small portion of the plant and to avoid damaging the main stems or trunks.

3. Phytochemistry and Biological Activities

Elderberry is long known as a rich source of various phytochemicals with bioactive properties, such as anthocyanins, flavonoids, other phenolic acids, triterpenes, and lectins. The most common type of anthocyanins is cyanidin derivatives, mostly responsible for the reddish-purple to blue color of the fruits. Main anthocyanins are Cyanidin 3-O-glucoside, Cyanidin 3-O-rutinoside, Cyanidin 3-sambubioside-5-glucoside, and Cyanidin 3,5-diglucoside, but non-cyanidin-based compounds (e.g., Pelargonidin-3-O-glucoside and Peonidin 3-O-glucoside) were also reported [4,10,19–21]. A more complete list and information on the range of specific compounds can be found in the literature [10,22]. Elderberry is considered one of the fruits with the highest anthocyanin content, along with blackberries, black currants, and blueberries [23]. Quercetin and kaempferol are the most abundant flavonoids [24]. The most abundant cinnamic acids are related to chlorogenic acids derivatives and flavanol-glycosides, such as quercetin-3-O-rutinoside, quercetin-3-O-glucoside and kaempferol-3-Orutinoside. The ursolic acid and oleanolic acid are the triterpenes present in higher quantities [24–26]. Elderberry is also a source of lectins, carbohydrate-binding proteins that have been shown to have immunomodulatory properties, and Ribosome-Inactivating Proteins (RIP), a class of enzymes that can inactivate ribosomes. These proteins have various biotechnological applications [27]. One of the most studied proteins is the *Sambucus nigra* I agglutinin (SNA), a tetrameric protein belonging to the type 2 RIP, composed of an A-chain with enzymatic activity and a B-chain with carbohydrate-binding activity [28].

3.1. Antioxidant Activity

The high polyphenol content—especially anthocyanins—is a main contributor to the antioxidant activity of elderberry fruits, jams, and syrups [29,30]. Elderberry wine also showed high antioxidant activity, dependent on the quantities of sugar and water used in its production and the degree of fineness of the fruit [31]. For all these reasons, it is believed that a diet comprising products deriving from fruits, or extracts from flowers or leaves, has the potential to protect against oxidative stress and its adverse effects on human physiology. For example, an elderberry extract was able to significantly reduce levels of reactive oxygen species (ROS) and lipid peroxidation in a human colon mucosal cell model in vitro, indicating a protective effect against stress-induced damage [32]. Additionally, the elderberry extract was able to improve cell viability in the presence of hydrogen peroxide. This study also revealed a significant loss of bioactive compounds due to the digestion process, but the remaining part was considered sufficient to elicit their positive effects [32].

Elderberry leaves, flowers, fruits, and their edible products have been confirmed to have significant antioxidant properties using a variety of antiradical activity assays in vitro [10,33]. Specifically, extracts have been found to be effective in scavenging hydroxyl, ABTS, and DPPH radicals, as well as in FRAP, CUPRAC, and TBARS assays, and inhibiting lipid peroxidation in linoleic acid emulsion [10,33–35]. Among different fruits, elderberry and chokeberry had the best antiradical activity based on hydrophilic (H-ORAC) and lipophilic (L-ORAC) compounds in ORAC assay, and the ability to scavenge the peroxyl radical (ROO•) in the ORAC assay was higher in elderberry than in black chokeberry [36].

3.2. Antimicrobial Activity

Although elderberry has a long tradition and history of human consumption, its vegetative tissues, flowers, and unripe fruits contain relevant amounts of cyanogenic glycosides (CG). The main CG is sambunigrin, followed by prunasin, holocain, and zierin [10,37]. CGs are present in thousands of plant species, including cassava, bitter almond, lima beans, and some sorghum varieties. These compounds are generally harmless when consumed in small amounts, but they can be toxic if consumed in large quantities. When the plant tissue is damaged or broken following, for instance, a pest attack, plant enzymes such as beta-glucosidases convert the CGs into hydrogen cyanide (HCN), a potent toxin that causes hypoxia. This compound can be also released on hydrolysis during digestion or maceration. Typically, the release of hydrogen cyanide from CGs happens post-consumption, where the glycosidases of the intestinal microflora facilitate hydrolysis in an alkaline environment and, to a lesser extent, is due to glucosidases in the liver and other tissues [38]. Eating immature fruits or very high quantities of elderberry fruits, therefore, may cause nausea, vomiting, and diarrhea, but food processing through heating strongly reduces the risk also because HCN is volatile [37].

Elderberry contains several compounds that contribute to its antimicrobial activity, including flavonoids (e.g., quercetin, rutin, and kaempferol) as well as phenolic acids (e.g., chlorogenic acid, caffeic acid, and p-coumaric acid) [39,40]. Different scientific articles highlight the effectiveness of elderberry extracts as an antimicrobial product. Rubini® (10%), a standardized liquid extract of *S. nigra*, effectively decreased the growth of *Streptococcus pyogenes*, *Streptococcus* Group C and G, and *Branhamella catarrhalis*, which are all known to cause frequent infections of the upper respiratory tract. A concentration of 20% inhibited bacterial development by 99% [41]. Rubini® has also shown effective antiviral activity against influenza A (KAN-1, H5N1) and B (B/Mass) in a cell culture model [41]. A study based on Direct Binding Assay and DART TOF-MS analysis identified and characterized two anti-influenza flavonoids in an elderberry fruit extract (5,7-dihydroxy-4-oxo-2-(3,4,5-trihydroxyphenyl)chroman-3-yl-3,4,5-trihydroxycyclohexanecarboxylate and 5,7,3′,4′-tetra-O-methylquercetin) as the main determinant of antiviral activities [42]. These compounds directly bind to the Influenza A virus subtype H1N1 particles, preventing them from entering host cells and effectively reducing infection in vitro [30,42]. In addition, the antiviral effects of elderberry extracts include also inhibiting viral proliferation and hemagglutination, as well as stimulating the immune system by increasing the production of cytokines by monocytes [41,43,44]. Another class of biomolecules with known antiviral and antimicrobial properties present in elderberry and other species of the *Sambucus* genus are RIPs. Over 40 RIPs and lectins have been identified and characterized from the *Sambucus* genus, which is considered one of the best sources of these proteins [27,45]. Compared to ricin, probably the most known and well-characterized RIPs, and another toxic type 2 RIPs, RIPs from *Sambucus* have lower toxicity in cell-free systems and animals [27]. Flower extracts also displayed a higher antimicrobial efficacy, but leaf infusion was also found to have inhibitory effects on the growth of bacteria such as *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, and *Staphylococcus aureus* [41]. Conflicting results are reported regarding major nosocomial bacteria, including positive, moderate, and very low inhibitory activities according to the pathogen species [46,47].

Finally, plant extracts have been shown to possess antimicrobial activity due also to the presence of Plant Antimicrobial Peptides (AMPs) [48]. These small cationic peptides are a crucial component of the plant's innate immune system and can kill or inhibit the growth of various microorganisms, including human pathogens [49]. In *S. nigra*, mass spectrometry was utilized to detect cysteine-rich peptides AMPs from flower extracts and the antimicrobial properties of the peptide extracts were assessed against fish pathogens of interest in aquaculture [50].

3.3. Pharmaceutical Activities

Black elder is probably one of the best examples of a plant growing in the wild that has been used as food or medicine across the whole globe for a long time. Elderberry bioactive compounds have a range of possible pharmaceutical activities, which have been proven in several in vitro studies [9]. Nonetheless, there is a limited number of clinical trials that have compared the effects of elderberry extracts with standard treatments on patients suffering from infections or diseases [51]. For instance, while different in vitro and in vivo studies indicated the anti-influenza activities of elderberry extracts [9], clinical studies have shown that elderberry supplements have a positive impact on reducing the duration and severity of colds in patients with viral respiratory diseases, mainly relieving symptoms such as fever, muscle aches, cough, nasal congestion, mucus discharge, and headache [51,52]. On the other hand, evidence that elderberry reduces the duration or severity of influenza was also not found in a double-blind placebo control trial based on a 48 h evaluation [53].

Elderberry extracts, fractions, and various secondary metabolites can be utilized to regulate glucose and lipid metabolism [9]. For instance, studies have identified naringenin as one of the most effective in enhancing glucose utilization and reducing fatty acid accumulation, both of which are characteristic of metabolic syndrome [54]. In a study that involved 34 healthy individuals, the effectiveness of low-dose elderberry juice powder (400 mg spray-dried powder containing 10% anthocyanins) was tested against a placebo for lipid parameters in a randomized, placebo-controlled study [55]. The participants were given capsule powder (equivalent to 5 mL elderberry juice) three times a day for two weeks and were instructed to follow a 35% fat diet. Results showed a slight reduction in all lipid parameters of the low-dose elderberry extract group compared to baseline at two weeks, but the improvement was not statistically significant. At the end of the trial, the total cholesterol level was reduced from 199 mg/dL (baseline) to 190 mg/dL, compared to the placebo (from 192 to 196 mg/dL) [55]. It was discussed that higher doses of elderberry extract may produce more significant benefits and that patients with normal lipid levels may not see significant reductions as their lipids are already within the normal range [55].

Phenolic extracts, anthocyanins, procyanidins, and selected metabolites from elderberry can increase the glucose and oleic acid uptake in human skeletal muscle cells in vitro, with the phloroglucinol aldehyde having the highest activity [56]. Moreover, cyanidin and its glycosides inhibited intestinal α-glucosidase and pancreatic α-amylase, hence potentially playing a role in controlling postprandial hyperglycemia [56]. Inhibition of digestive enzymes, such as α-amylase, α-glucosidase, and pancreatic lipase by elderberry extracts [57,58], has also been associated with a potential reduced intestinal absorption useful to counteract obesity.

Different studies have suggested that elderberry may have potential benefits for cardiovascular health [9,22]. In addition to antioxidant and anti-inflammatory properties, which do have a role in reducing the risk of cardiovascular diseases, elderberry extracts can improve aspects of cardio-metabolic health. In a study with the apoE$-/-$ murine model (a widely used pre-clinical model of atherosclerosis), an anthocyanin-rich elderberry extract caused a reduction in aortic cholesterol levels, despite no significant alterations in plasma lipid levels or a notable change in serum inflammation markers [59]. Treated mice, however, showed an increased expression and serum activity of paraoxonase 1 (an enzyme that is mainly synthesized in the liver and circulates in the bloodstream, bound to High-Density Lipoprotein particles; HDL) and significant alterations in the expression of hepatic genes encoding HDL proteins, indicating a potential impact on aortic cholesterol accumulation [59]. The dietary supplementation with black elderberry extracts improved markers of HDL-C and HDL function (e.g., PON1 activity, endogenous antioxidant defense) in the same murine model, but there was no significant difference in the severity of atherosclerosis between the treatment and control groups based on an *en face* analysis of aortas [60].

The neuroprotective effects of the elderberry extracts arise from their intrinsic antioxidant and mitochondriotropic properties of the anthocyanins, as well as the stimulation of cellular signaling pathways that increase cell survival. Extracts possess some anticonvulsant activity [61], and consumption of elderberry can provide protection against neuronal cell death and microglial activation in a rat model of Huntington's disease induced by 3-NP, a neurotoxin that causes damage to the striatum of the brain [62]. This ameliorating effect was ascribed to a reduction in apoptosis, inflammation, and oxidative stress [62]. A two months dietary supplementation with elderberry mitigated in C57Bl/6J mice the negative effects of BCCA (bilateral common carotid artery) occlusion (a condition in which blood flow to the brain is restricted, leading to behavioral deficits and potential pathology) and significantly alleviated the ischemia-reperfusion (I/R) neuronal damage [63].

3.4. Systematic Reviews of Elderberry in Healthcare

Meta-analyses to identify, evaluate, and synthesize all relevant studies connected with specific health claims have provided evidence of the therapeutic effects of elderberry, although systematic reviews on mono-herbal products are limited in number [64].

A recent meta-analysis of 11 clinical trials focused on the effects of elderberry-based supplements and foods on cognitive performance parameters in elderly individuals with normal cognition or mild cognitive impairment. The study indicated that supplements and foods may have beneficial effects on global cognitive performance, psychomotor function, learning and memory, working memory capacity, executive function, and brain perfusion/activity [65]. Nonetheless, the authors also highlighted the presence of inconsistent findings across the measured outcomes, which was attributed to the heterogeneity of the study design, the intervention type/dose/duration, and the cognitive tasks considered. Moreover, the study discussed that most of the included trials were of poor or modest methodological quality and that the interventions given were heterogeneous and, in some trials, inadequately explained [65]. The latter is also connected to the variability of the berry extracts employed, which ranged from juice (a no-fiber extract) to freeze-dried powder, in which the deterioration and solubility of the active compounds are essentially unknown.

A systematic review of the scientific literature (including pre-prints, conference proceedings, and other scientific sources) was performed to evaluate the effectiveness of elderberry in treating or preventing respiratory illnesses such as colds and influenza [11]. The conclusion was that there is insufficient information to state that elderberry is effective in shortening the duration and reducing the severity of influenza symptoms compared to a placebo, mainly because of the small sample size and methodological differences in the studies analyzed [11]. The systematic review also excluded a boost of the immune response and indicated that elderberry treatment does not seem to be associated with any significant harmful effects (e.g., nausea and vomiting) [11]. Moreover, evidence of the impact of elderberry on clinically relevant outcomes related to inflammation was not found, although there were some statistically significant reductions in cytokines, indicating that elderberry likely has some effect on inflammatory markers [11]. A previous systematic analysis related to the same type of illness provided a different picture. Specifically, a meta-analysis of studies on commercial elderberry supplementation found that it effectively reduces the duration and severity of upper respiratory symptoms (compared to placebo) [66]. The effect size was substantial, and the benefits were seen regardless of whether the patient had received a flu vaccine or not. Elderberry was found to be particularly effective in reducing symptoms caused by the influenza virus [66]. However, the authors discussed that caution should be taken because the analysis was conducted on a small sample size [66]. A literature review based on five studies involving 936 people found that taking preparations of *S. nigra* fruits close to the onset of symptoms and for up to two weeks may help relieve symptoms of the common cold and influenza and duration of illness/rate of recovery [67]. Elderberry products may reduce the duration and severity of fever, headache, nasal congestion, and nasal mucous discharge when associated with an acute viral respiratory infection [67]. Nonetheless, there is some inconsistent evidence that fruit products may relieve cough or help prevent the worsening of cough when attributed to viral infections [67]. A limitation discussed by the authors is that the formulation, dose, and duration of treatment varied between studies, limiting generalizations [67].

While elderberry has been used for centuries as a natural remedy to treat a range of conditions, a “well-established medicinal use” (Directive 2001/83/EC) has not been fully proven through rigorous clinical research in terms of recognized efficacy and acceptable level of safety. Despite its physiological benefits, mostly described in non-clinical trials, to date, there is no strong scientific evidence that elderberry should be used in place of other more proven therapies [64].

3.5. Considerations on the Health Benefits of Elderberry Phytochemicals: A Note of Caution

These findings imply that elderberry extracts from various plant organs could be promoted as a nutraceutical supplement. Mono- or poly-herbal products derived from elderberry can provide a wide range of extra health benefits in addition to the known nutritional value. They may be used as complementary tools to prevent or treat certain health conditions or in conjunction with other therapies to improve overall health and wellbeing. However, the bioavailability of polyphenols, the main contributors to elderberry activities, is complex and the specificity and relevance of these compounds in determining physiological effects after consumption need to be better understood [68,69]. Their clinical effects cannot be simply inferred by in vitro studies. In particular, the therapeutic effects of anthocyanins are reliant on their adequate bioavailability, both at the cellular level and in the whole organism through diet. Due to the complex biochemistry of anthocyanins, much remains to be uncovered about their biochemical activity, bioavailability, metabolism, and impact on (already ongoing) therapeutic interventions [70].

4. Genetic Characterization, Breeding Efforts, and Germplasm Collections

Elderberry is renowned for its broad spectrum of bioactive compounds, which are widely recognized to be associated with numerous health-promoting properties. Genetic diversity in elderberry populations can influence the phytochemistry of the plant, leading to differences in the concentration and types of bioactive compounds present in different varieties or populations of elderberry. Therefore, understanding genetic diversity is important for identifying and selecting varieties with desired phytochemical profiles for various health and industrial applications. Under this perspective, *Sambucus nigra* has been neglected despite the ample number of works on its biochemical characterization. This species has not garnered much interest from the scientific community involved in horticulture, breeding, and molecular genetics.

The genome of *S. nigra* is organized in 18 chromosomes (2n = 36), which is the same as other related (sub-)species of the genus, such as *S. canadensis* (*S. nigra* subsp. *Canadensis* (L.) R. Bolli), *willamisii*, and *ebulus* [71]. Another typical karyotype (2n = 38) is described for other (sub-)species of the genus such as *S. cerulea (S. nigra subsp. Cerulea* (Raf.) R. Bolli). It was hypothesized that the main difference between the two karyotypes derives from a mis-division of chromosome VII in 2n = 36, which resulted in two telocentric chromosomes (XIII and XVI) in 2n = 38 [71]. Despite the limitations of cytotaxonomic approaches for species circumscription of the *Sambucus* genus [71], the close correlation between the major karyotypes and effective chromosome pairing in hybrids supports the hypothesis that several species are interfertile horticultural types. [71,72]. The 2C DNA content of *S. nigra* has been estimated at 28.6 pg [73] and 21.9 pg [74]. The number of publicly available nucleotide sequences at the National Center for Biotechnology Information (NCBI) is 227, archived as 188 genomic sequences (including the plastid genome) and 39 mRNA sequences (https://www.ncbi.nlm.nih.gov; accessed on 1 February 2023). The number of protein sequences is higher (745), with eight 3D structures available, all referring to agglutinin II. Considering NGS experiments, 226 runs/items are publicly available, for an estimated total of 959.90 Gb (including both WGS and RNA-Seq assay types). Among the most frequent studies genes, there are the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL), often employed for taxonomic classification, and gene coding for lectins (AIVl3), thaumatin-like proteins (TLP), RIPs, and allergen-like protein (BRSn20), mainly because of their relevant effect on human physiology and biotechnological value. The plastid genome of *S. nigra* has been estimated at 158,102 bp, a dimension comparable to those of other species of the genus *Viburnum*, with a GC content of 38% and a total of 132 genes [75]. Simple Sequence Repeats (SSRs) with a pentanucleotide core were the most abundant among the detected microsatellites [75].

The phylogenetic inference has been based on conserved nuclear sequences [76] and, more recently, on the full sequence of the plastid genome [77]. Specifically, the analysis of the Internal Transcribed Spacer (ITS) regions ITS4 and ITS5 supported a monophyletic origin of the whole *Sambucus* genus [76]. The full sequencing of the chloroplast genome indicated that this genus is mostly related to the *Viburnum*, within a small clade of the Adoxaceae family [77]. This phylogenetic similarly is consistent with previous evidence based on the rbcL sequence [1] or mitochondrial genes (i.e., cox 1, cox 3, and the nad5/4–5 intron) [78]. The *Sambucus* genus has been split into two major clades, with *S. nigra* L., *S. maderensis* Lowe, *S. peruviana* H.B.K., and *S. canadensis* L. forming a smaller group separated from the other species of the genus [79].

The genetic characterization of *Sambuca nigra* populations has been mainly carried out with anonymous markers such as microsatellites (SSRs) and internal transcribed spacers (ITSs). The first identified SSRs derived from a genomic library [80]. Specifically, 8 nuclear SSRs (selected from 26) were used to fingerprint 21 *S. nigra* and 2 *S. canadensis* accessions using a fluorescent-based capillary electrophoresis (CE) system. The Target Region Amplification Polymorphisms (TRAP) system was also used to discriminate the genetic diversity of 66 elderberry accessions belonging to different *Sambucus* species collected in different regions [81]. A more recent study characterized 30 *S. nigra* samples from five areas of the Varosa Valley in Portugal [82]. The work employed and compared different markers systems such as Inter-Simple Sequence Repeats (ISSRs), SSRs (resolved onto an agarose gel), and PCR-RFLPs based on the amplification of the ITS1–5.8S–ITS2 region. All the marker systems proved to be sufficiently polymorphic to distinguish the germplasm. Specifically, the 11 ISSR primer sets showed the highest percentage of polymorphism (61.86%), followed by the six (out of eight tested) polymorphic SSRs (61.1%), and PCR-RFLPs based on four restriction digestions (out of seven tested) (50%) [82]. The percentage of polymorphism was calculated as the ratio between polymorphic and total bands, with SSRs scored as dominant [82]. Nonetheless, the authors pointed out that also the size of the (undigested) ITS amplicons showed polymorphism among some clones [82]. The use of ITS-based barcoding has allowed the distinction of a native Northern Greek wild population of *S. nigra* from genotypes of other countries, as well as of other *Sambucus* species [83]. Moreover, PCR-RFLP of ITS amplicons allowed for characterizing the genetic nature of interspecific hybrids [73]. On the other hand, *S. nigra* and *S. ebulus*, a species that has been used as a donor in interspecific breeding, have the same matK sequence, one of the most frequently used chloroplast sequences in DNA barcoding [84]. Five fluorescent SSRs resolved in CE have been employed to estimate the diversity of *S. palmensis* Link (*Sambucus nigra* subsp. *Palmensis* (Link) R. Bolli) in the Canary Islands (Spain), where this endemic species is listed as at risk of extinction [85]. The hierarchical partition of the genetic diversity among and within populations and islands indicated little differentiation among islands, while most of the variance was within populations [85]. Cross-species transferability of SSRs among the genus *Sambucus* has been also reported [79,86], and primers specifically developed for *S. palmensis* are available [87].

Germplasm collections of *Sambucus* are limited, probably because the cultivation is scattered in a relatively reduced number of nations and the rapid commercial decline of the cultivation in the last century [88]. Most of the commercial germplasm has been developed decades ago [15], with many cultivars selected from the wild, both in the USA and Europe [89]. Morphological descriptors for germplasm characterization have been proposed also because *S. nigra* is not within the Union for the Protection of New Varieties of Plants (UPOV) species [90]. Efforts were recently performed in the characterization of wild accession, also under an agronomic perspective [83,90]. To our knowledge, the largest publicly available collection of *Sambucus* is available at the USDA ARS-GRIN (Germplasm Resources Information Network) (https://www.ars-grin.gov/; accessed on 16 February 2023) and includes more than one hundred samples taxonomically classified as belonging to the *Sambucus* genus [91]. Accessions are maintained as plants and seeds. The Nordic Baltic Genebanks Information System (https://www.nordic-baltic-genebanks.org, assessed on 16 February 2023) indexed 79 *S. nigra* accession, which are, however, unavailable.

Two breeding approaches have been mainly employed for elderberry, the selection of valuable lines from locally cultivated accessions or wild populations, and intraspecific hybridization. It should be added that *S. nigra* and *S. canadiensis* are often considered distinct species, but a modern revision of the genus *Sambucus* proposed that these two taxa, along with others, should be regarded as subspecies within the expanded *Sambucus nigra* [92]. Besides technical issues (e.g., the presence of small flowers and the need for manual emasculation), interspecific hybridization is limited by the fertility of the crosses. This seems to be higher when employing genetically closer species (e.g., *S. nigra* × *S. canadensis* hybrids are more fertile than *S. nigra* × *S. ebulus*) [93]. *S. nigra* × *S. caerulea* crosses are reported to be sterile, but this trait was analyzed only in the first years of life [73]. For breeding purposes, self-incompatible accessions of *S. javanica* have been identified from the wild and employed in various programs [93], while *S. palmensis* is suspected to be self-incompatible [87]. Although the information is scarce, it is likely that current germplasm releases tend to satisfy the needs of landscape management and gardening, with different leaf shapes and colors of the foliage and flowers [94]. Breeding efforts in the past have been devoted especially towards the improvement of the visual, productive, and quality features of the fruits, such as color (intensity and glossiness), size, weight, firmness, and the pulp-to-seed ratio [72]. There is little doubt that the variability in *Sambucus nigra* and its interspecific hybrids could be sufficient to select superior genotypes at the biochemical level [21,24,88,95], but studies addressing heritability and the G × E interaction are rare and mainly focus on the comparison of varieties in different locations across years. The traits under investigation are agronomic (including phenology) [15,96,97] but also related to the compositional features of the fruits [98]. The content of specific polyphenols in elderberry juice samples from different field replications of the same genotype, location, and year can vary greatly, suggesting that several factors can play a role [99]. Interestingly, the Discriminant Analysis of the fruit composition of three elderberry varieties suggested that each cultivar had a discernible profile across years or locations, implying a relevant genetic base of the polyphenol content of the berries [99].

5. Perspectives and Conclusions

It is probably beyond dispute that few plant species have the remarkable properties of *Sambucus nigra*. This species can be used for a large variety of foods (e.g., cordials, jams, jelly, juice, syrup, and fermented and brewed beverages) and some industrial applications (e.g., cosmetics, supplements, and pharmaceuticals), besides providing a relatively soft wood that can be used for some crafts. Fruits are packed with a wide range of beneficial components such as polyphenols, essential oils, carotenoids, free fatty acids, phenolic acids, vitamins, and minerals. Elderberry extracts can have numerous positive effects on human health. Due to their impressive nutrient profile, they have been rightfully referred to as “superfruits” [100]. Nonetheless, despite its environmental adaptability, fast growth, prunability, and resilience, elderberry is little cultivated in comparison to other fruits and vegetables due to several reasons. Firstly, fruits, like many other true berries, have a relatively short shelf life, are easily perishable, and require appropriate care during transportation and storage, also because they should be picked when fully ripe. Additionally, elderberries are difficult to harvest because they grow on fragile peduncles, which makes mechanical harvesting challenging. Moreover, elderberries are not as well known as other fruits because the species is associated with traditional medicine, herbal products, and landscape management. Besides some efforts in the first decades of the XXcentury, *S. nigra* has not received much attention from plant breeders, which has limited the development of high-yielding and disease-resistant cultivars and, ultimately, made the plant even less competitive compared to more popular fruit trees. Therefore, a revamp of elderberry cultivation should be pursued through the following actions. It is necessary to develop scientific and technical knowledge on the pre-harvest and post-harvest factors that influence plant growth and production, which should be accurately exploited to assist extension services and farmers to optimize and standardize yield, improve the quality of the harvest, and minimize post-harvest loss and spoilage. Information to select cultivars that are well-suited for specific growing conditions and desired end products also needs to be greatly expanded, as varieties differ in cold-hardiness, yield, fruit quality, vigor, and pollination requirements. There is a very large scientific gap to be filled to harness the potential of modern breeding tools such as genetic mapping, genomic selection, marker-assisted selection, genetic transformation, and high-throughput phenotyping. The selection of already existing germplasm and the development of new varieties of elderberry that have a higher yield are more prone to mechanical harvest and are more resistant to pests and diseases is one of the first steps to making cultivation more attractive to professional farmers. It is, however, positive that the literature provides appropriate arguments to increase awareness among consumers about the potential benefits of the plants and to promote the commercialization and development of value-added products (cosmetics, flavorings, supplements, herbal products, wine, etc.). We strongly believe that this knowledge will support the creation of a more profitable market, ultimately contributing to the economic development of the elderberry cultivation sector.

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