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Research Article

Asexual propagation for sustainable utilization of Greek indigenous germplasm of common dogwood (*Cornus sanguinea* L.), a neglected but noteworthy ornamental and nutraceutical species

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Abstract

The common dogwood (*Cornus sanguinea* L., Cornaceae) across the European continent represents a species with validated ornamental and nutraceutical value that has been utilized by humans for thousands of years but has been abandoned during modern times. It still represents however, a valuable but neglected native germplasm resource. The development of an asexual propagation protocol as a first step towards utilization of Greek *C. sanguinea* germplasm is presented herein for the first time. Plant material was collected from *C. sanguinea*'s natural habitat during winter dormancy (hardwood cuttings) as well as during early vegetative growth (softwood cuttings). The propagation protocol was developed through preliminary trials for the successful propagation of the material sourced directly from the wild to establish enough starting material (*ex situ* adapted mother plants); consecutively, cutting propagation experiments over a two-year period coupled with early plant growth and survival assessment were conducted. The results showed that the use of external indole-3-butyric acid (IBA) rooting hormone application between 2,000 and 4,000 ppm delivered very high rooting rates (80-100%) via the use of primary softwood, leafy cuttings with minimum amount of lignification under mist. The proposed protocol is considered fast, reliable, easy to implement and economically viable. The study provides first time data on domestication and propagation of Greek *C. sanguinea* germplasm and at the same time paves the way for further research on the sustainable utilization of this species.

Keywords: biodiversity; cuttings; domestication; *ex situ* conservation; indole-3-butyric acid; neglected and underutilized germplasm; nutraceutical plants

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Introduction

Native germplasm resources which often are neglected or underutilized represent a valuable source of genetic material for sustainable agronomic exploitation (Libiad *et al*., 2021). Several countries across the Mediterranean region including Greece undertake efforts to exploit such resources under strategic planning which can deliver diverse long-term results of national and international importance (Maloupa *et al*., 2008a; Krigas *et al*., 2021). Such germplasm resources involve a wide variety of plant species, including small trees of potentially high agronomical value on which particular attention is given in several countries over recent years (Ercisli, 2004; Verma *et al*., 2010; Botu *et al*., 2017; Manco *et al*., 2019). Local endemic tree species of given regions can have ecological and ornamental value and can be a prolific source of natural antioxidants (Cosmulescu *et al*., 2017; Che and Zhang, 2019; Bourgou *et al*. 2021), thus conservation and exploitation of such species is considered significant. The rich biodiversity of Greece is an important source for locating, selecting, and utilising relevant valuable plant germplasm of potentially high ecological, ornamental, nutritional and medicinal value through integrated research efforts (Krigas *et al*., 2021).

The common dogwood (*Cornus sanguinea* L., Cornaceae) is a small deciduous tree indigenous to Greece which naturally occurs in most of the central and north part of the country, at altitudes up to 1400 m along creeks, shrubland areas and forests (Boratynski *et al*., 1992). It reaches up to 4 meters in height with resilient shoots that develop into hardy trunks. It bears deep green, ovate leaves during spring and it flowers at summer producing spherical corymbs of small creamy-white flowers that develop into small purple/black drupes in late summer through early fall (Ball, 1968; Popescu *et al*., 2016). This species has been known and used by humans for thousands of years, and recent palaeontological and palaeobotanical data indicate the use of *C. sanguinea* fruits and its hardy wood during the Late-Neolithic period in Europe (Out, 2008; Tolar *et al*., 2021). Nowadays *C. sanguinea* is considered a valuable ornamental species with significant seasonal phenotypic transitions upon summer flowering with its bright white flowers contrasting against its deep green, ovate leaves bringing about a vivid appearance, but also during autumn with young shoots changing colour from green to red/crimson as they enter dormancy making it a successful species in winter botanical gardens (Upson and Kerley, 2007). In addition, native germplasm of *C. sanguinea* that is evolutionary adapted to its native climate coupled with its natural resistance to pests and diseases (Popescu *et al*., 2016) can be used in botanical garden restoration work across its original habitat zones as well as for biodiversity conservation at national parks (Di Martino *et al*., 2020).

Although *C. sanguinea* fruits are not toxic, their unpleasant taste has turned people away from using these fruits for commercial alimentary purposes. However, earlier studies have shown that *C. sanguinea* fruit and leaf extracts have significant antioxidant capacity in terms of total phenolic and total flavonoid content (Stanković and Topuzović, 2012). In more recent studies, members of the genus *Cornus* including *C. sanguinea*, *Cornus alba* and *Cornus officinalis* have shown pharmaceutical value as a source of valuable secondary metabolites with free radical scavenging potential exhibited through *in vitro* assays (Wang *et al*., 2018; Barut and Şöhretoğlu, 2020; Truba *et al*., 2020), being rich in iridoid glucosides that are synthesised through the mevalonic acid pathway and have recognized anti-inflammatory properties among others (Viljoen *et al*., 2012). In addition, evidence have been found recently on the antioxidant activity of *C. sanguinea* fruit extracts *in vivo* acting synergistically with metallic nanoparticles (David *et al*., 2020). Relevant research on *C. sanguinea* antioxidant properties is on-going with new secondary metabolites being isolated and new, diverse properties being discovered such as anti-aging effects on human cells (Iannuzzi *et al*., 2021).

Although the agronomical traits of *C. sanguinea* have only been sporadically studied in the past (Krüsi and Debussche, 1988; Guitián *et al*., 1996) along with its ecology (Lindelof *et al*., 2020), its propagation has not been given significant attention in contrast with *Cornus mas* (Klimenko, 2004; Marković *et al*., 2014;

Karapatzak *et al.*, 2022b). The relative profusion of information on asexual propagation of *C. mas* as opposed to *C. sanguinea* may stem from the fact that *C. mas* fruits have received significant nutritional attention over recent years (Szczepaniak *et al.*, 2019).

The basic steps towards the utilization of native germplasm resources employ firstly the evaluation of the studied species in terms of utilization potential, and secondly, the development of a solid asexual propagation protocol aiming at the production of genetically uniform stock material with stable transfer of agronomical traits for further utilization and *ex situ* conservation work (Krigas *et al.*, 2021; Karapatzak *et al.*, 2022a). Furthermore, a successful and economically sustainable asexual propagation protocol may pave the way for the agronomical exploitation of the germplasm on a commercial scale (Maloupa *et al.*, 2021). Asexual propagation protocols via cuttings of wild-type germplasm of the genus *Cornus* have been developed across several countries with *C. mas* being the most studied species (Pirlak, 2000; Kosina and Baudyšová, 2011), including Greece (Karapatzak *et al.*, 2022b). Relevant literature on vegetative propagation of *C. sanguinea* native germplasm across its south European distribution range, however, is highly scarce.

A popular hormonal substance that has been used in the past for the propagation of *Cornus* spp. is indole-3-butyric acid (IBA) (Pirlak, 2000; Marković *et al.*, 2014), frequently achieving high rooting rates. However, successful adaptation of *ex situ* raised plants should also be secured for long-term sustainable exploitation of the germplasm, especially medicinal and aromatic plants (MAPs) in man-made settings (Grigoriadou *et al.*, 2020). Consecutively, a solid propagation protocol should be paired with trials monitoring *ex situ* early plant growth and acclimatization for the effectiveness and repeatability of the protocol to be secured (Maloupa *et al.*, 2008b).

In the above framework, the development of an asexual propagation protocol of native *C. sanguinea* germplasm occurring in Greece was scoped for the first time. Consequently, the aims of the current study were: (1) The collection of Greek *C. sanguinea* plant material (hardwood cuttings and fresh softwood plant parts with leaves) directly form the wild; (2) The development of a fast, reliable, easy to implement and economically viable asexual propagation protocol for *C. sanguinea* originating from the collected and taxonomically identified germplasm samples. The second part of the work, commenced with preliminary trials for the successful propagation of the material that was directly collected from the wild aiming at providing enough acclimatized starting material (*ex situ* adapted mother plants) for further experimentation. Consecutively, experiments were conducted over a two-year period to study the rooting capacity of the *ex situ* adapted material in more detail. The results were complemented with early plant growth and survival data aiming at providing information on the physiological basis of rooting of cuttings and its effects on early plant establishment in man-made settings. The overall work provided enough documentation that can pave the way for the sustainable utilization of *C. sanguinea* as an ornamental and pharmaceutical species with significant agronomic potential.

Materials and Methods

Collection of plant material

The collection of *C. sanguinea* plant material was conducted under a special permit to the Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization Demeter (Permit 82336/879 of 18/5/2019 & 26895/1527 of 21/4/2021) issued by the Greek Ministry of Environment and Energy which is issued yearly after detailed reporting of collections made. The samples were collected during the winter 2018 – 2019 and spring 2019 from the wild-growing populations at the region of Epirus (Arta, Mountain range Tzoumerka, N 39.371394, 21.13897 E; 817 m of altitude). The material was taxonomically identified (Strid, 2016, Figure 1) and was consisted of dormant stem cuttings (twigs) during the winter collection, and fresh soft-wood, leafy stem cuttings and runner shoots during spring collection that were used as starting propagation material. After collection and taxonomic identification, the material was promptly transferred to the laboratory where it was allocated the unique IPEN (International Plant Exchange Network) accession number GR-1-BBGK-19,199 given by the Balkan Botanic Garden of Kroussia of the Institute of Plant Breeding and Genetic Resources (IPB&GR) of the Hellenic Agricultural Organization-Demeter (ELGO-Demeter), and subsequent analyses and preliminary propagation trials were commenced

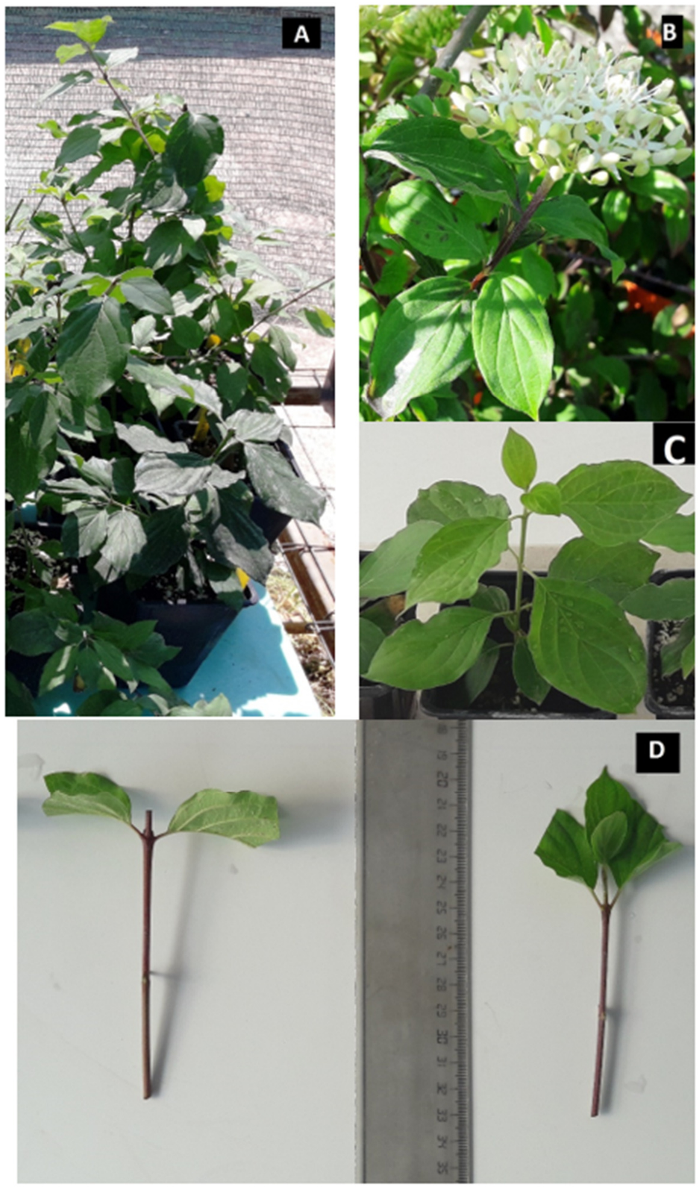

Figure 1. Morphological characteristics of *Cornus sanguinea* Greek native germplasm adapted *ex situ* from the wild-growing population sample GR-1-BBGK-19,199. (A) Typical appearance of the *exsitu* acclimatized mother plants used in trials, (B) Inflorescence, (C) Opposite, elliptical or ovate leaves, (D) Apical (right) and sub-apical (left) cuttings prepared from the mother plants for propagation trials

Preliminary trials and establishment of mother plant stock material

The collected material was used in preliminary asexual propagation trials (Figure 1). Different external hormone application treatments of indole-3-butyric acid (IBA) were applied coupled with different cutting types taken at different seasons from varying stages of mother plant growth. Cuttings were set for rooting in propagation trays with peat (Klasmann, KTS 1): perlite at 1:3 v/v on mist bench in a plastic greenhouse at ambient temperature with relative humidity (RH) maintained >85% where they were attended weekly to assess their rooting capacity. The produced mother plants were kept *ex-situ* at the laboratory's nursery at the grounds of IPB&GR under ambient conditions for plant adaptation. The produced plants were transplanted in 3 L pots using a mixture of peat (Klasmann, KTS 2) and perlite (3:1, v/v) and were watered regularly. This allowed the establishment of mother plants directly from the wild for conservation purposes (Figure 1), but it also enabled the supply of mother plant stock material for further experimentation.

Propagation experiments

Based on the results of the preliminary trials, two experiments were conducted during the summers of 2019 and 2020. For the 2019 experiment, cuttings were taken from 1st year growth (soft wood) in July from stems that just started to lignify and were characterized as 'semi-lignified' cuttings (Bryant and Trueman, 2015; Pacholczak *et al*., 2017). Two types of cuttings were made; 10-12 cm stem sections from the upper part which included the apical meristem and were characterized as 'semi-lignified apical cuttings' as well as internode sections from lower parts of the stem which included at least two axillary buds and were characterized as 'semi-lignified sub-apical cuttings' (Bryant and Trueman, 2015). In all cuttings one or two fully developed leaves were kept. The 2020 experiment exploited the results of preliminary trials and the 2019 propagation experiment; thus cuttings (apical and sub-apical cuttings) were taken in early June (a month earlier compared to 2019) prior to the onset of shoot lignification.

Cuttings in each experiment using Indole-3-butyric acid (IBA) as rooting enhancer were allocated to each treatment immediately after excision from the mother plant to avoid turgor loss (Pradeep Kumar *et al*., 2020). The hormone application employed 0 ppm IBA (control) in apical or in sub-apical cuttings; 2,000 ppm, 4,000 ppm and 6,000 ppm IBA (each dissolved in 50% ethanol) in apical or sub-apical cuttings. The application method used was a quick dip of the base of each cutting into the hormone solution for 5 - 7 sec (Blythe *et al*., 2007). Cuttings were put in propagation trays with a high perlite ratio substrate for improved physical properties (Silber *et al*., 2010), namely 3 perlite : 1 peat (v/v) (Klasmann, KTS 1) substrate and were placed under mist (70-85% RH) located within ambient greenhouse conditions.

Cutting performance and growth measurements

Observations on the progress of cuttings were taken weekly and when a treatment reached 100% or >80% rooting, the trays were taken out of mist and measurements were taken on rooting frequency per treatment coupled with root number and average root length per cutting. At the same time, rooted cuttings were transplanted in 1 L pots with 3 peat (Klasmann, KTS 1) : 1 perlite substrate and were kept for the first two weeks within a greenhouse with automated irrigation for plant establishment. After this period, plant survival rates and growth measurements were taken at fortnightly intervals up to 45 days after rooting (DAR). More particularly, total plant or main stem height and total number of emerged leaves per survived plant were recorded. At the end of the above period, plant biomass production was assessed by a destructive harvest and measurement of total plant, shoot and root dry weight with the use of an indoor dryer.

Experimental design and statistical analysis

The two experiments conducted in 2019 and in 2020 abided by an identical design. Each experiment followed a complete randomised block design with two blocks. Each block included the above four hormone treatments (including control) and each treatment included the two cutting types described above. The set up resulted in eight treatments within each block that consisted of 10 replicate cuttings per treatment for each block in 2019 and six replicate cuttings per treatment for each block in 2020. The rooting and plant biomass data were subjected to analysis of variance (GLM-ANOVA) to establish overall treatment effects. Part of plant biomass data, namely root/total dry weight ratio expressed as the proportion of mass taken by root that were recorded in the form of percentages were arcsine transformed prior to the ANOVA to improve normality. Consecutively, following the results of the GLM-ANOVA, to dissect specific treatment effects for each variable (hormone level, cutting type) data were split into each cutting type or hormone level and separate analyses of variance were conducted to assess the effects of hormone level within each cutting type and the effects of cutting type within each hormone level accordingly. Means were compared using Tukey's HSD post hoc test at the a = 0.05 significance level. For the early plant growth data of plant height and leaf number that were measured over time, treatment effects were evaluated through repeated measures ANOVA. All analyses were conducted using the IBM-SPSS 23.0 software (IBM Corp., Armonk, NY, USA) software and graphs were drawn using Microsoft Excel.

Results

A clear superiority of the actively growing, softwood material against dormant, hardwood material in terms of rooting capacity has been observed in preliminary propagation trials of *C. sanguinea* GR-1-BBGK-19,199 (Table 1). Differences in rooting capacity were observed among trials at different seasons. Rooting of hardwood cuttings in winter treated with 10,000 ppm IBA reached 40%, whereas rooting of softwood cuttings the following spring treated with 2,500 ppm IBA reached 90% at a faster rate. Consecutively, in the final trial on softwood material that started to lignify (harden) in the summer and treated with 4,000 ppm IBA, rooting capacity reached 50.6% (Table 1).

Table 1. Results of preliminary propagation trials at consecutive growth stages of Greek native *Cornus sanguinea* GR-1-BBGK-19,199 utilizing the initial material collected directly from wild-growing populations. The table summarizes the most successful treatments used in terms of rooting frequencies

Month/Year – Season	Hormone treatment (ppm IBA)	Mother plant development stage**	Cutting type	Rooting (%)
12/2018 – Winter	10,000	Dormancy	Hardwood	40.0
05/2019 – Late Spring	2,500*	Early growth (bud break)	Softwood	90.0
07/2019 – Summer	4,000	Advanced growth	Softwood	50.6

* Hormone treatment applied through powdering; ** Early and advanced growth refer to the annual vegetative growth cycle

In the propagation experiments, the rooting capacity of *C. sanguinea* GR-1-BBGK-19,199 softwood cuttings reached 85% within 30 days in 2019 and 100% within 25 days in 2020 via use of 4,000 ppm IBA treatment in both cutting types (Figure 2). Comparatively, the 2,000 ppm IBA hormone treatment presented similarly high rooting rates ≥ 80% in both cutting types in both years (Figure 2).

According to the statistical analysis of root number and length data, external hormone application significantly affected root length but not root number in 2019 ($p < 0.05$). More specifically, 6,000 ppm IBA showed the highest root length in semi-lignified apical cuttings, whereas in semi-lignified sub-apical cuttings, 2,000 ppm IBA showed the highest length ($p < 0.05$, Figure 2, A1 and A2). The above high rates of root length that were observed were also significantly different between the two cutting types of the same hormone treatment ($p < 0.05$). In 2020, on the other hand, a significant effect of external hormone application was only observed in root number in apical cuttings with the control treatment being the lowest ($p < 0.05$, Figure 2, B1) showing no effect between cutting types. However, higher root number rates were observed in 2020 softwood cuttings compared to respective root length rates in 2019 semi-lignified cuttings across the three hormone levels applied (Figure 2, Figure 5).

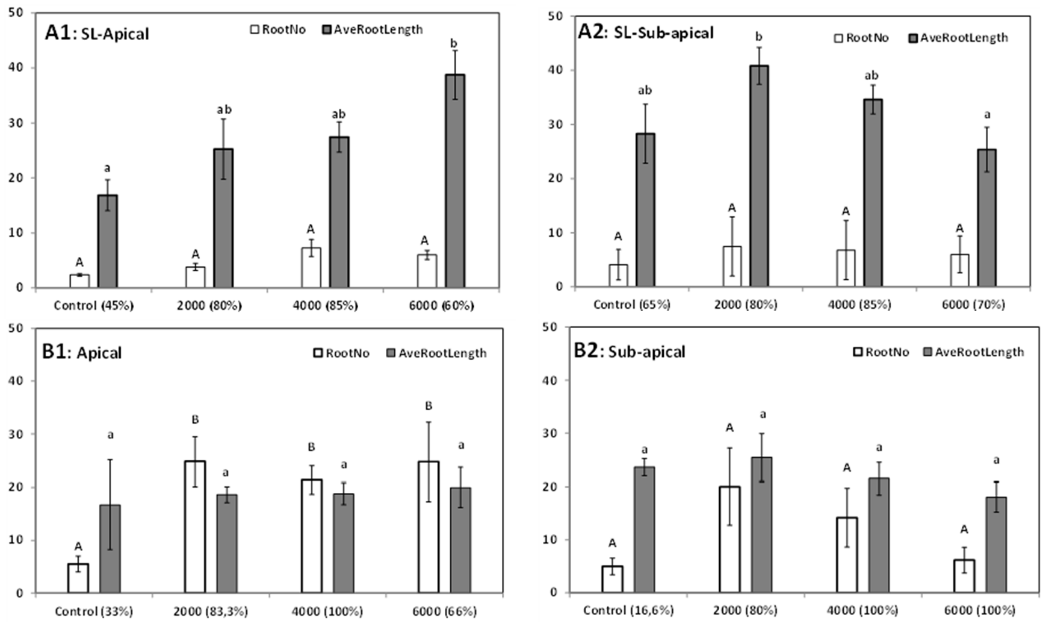

Figure 2. Mean root number (white bars) and average root length (mm) (grey bars) of rooted cuttings for each hormone treatment (ppm IBA) and cutting type from the rooting trials of the Greek native *Cornus sanguinea* genotype GR-1-BBGK-19,199 across the two years of experimentation. A1: 2019 semi-lignified apical cuttings (SL-Apical); A2: 2019 semi-lignified sub-apical cuttings (SL-Sub-apical); B1: 2020 Apical cuttings; B2: 2020 Sub-apical cuttings. All cuttings were leafy sections of 1st year soft wood. Apical cuttings included the stem's apical meristem and sub-apical cuttings were internode sections. The substrate used in all trials was 3 perlite : 1 peat (v/v) under mist conditions. Standard errors of the means are shown on the bars ($p < 0.05$). Bars that do not share the same letter are significantly different (Tukey HSD, $p < 0.05$, capital letters for root number and lowercase letters for root length). Across the horizontal axis of each graph, the total rooting percentage (%) is given for each treatment next to treatment name

The observed rooting rates were reflected in the respective early plant vegetative growth in terms of plant height and leaf number in 2019. Particularly, 2,000 ppm and 4,000 ppm IBA presented higher rates of early plant growth and survival, without however being significantly different from each other, excepting leaf number of semi-lignified sub-apical cuttings where 4,000 ppm IBA treatment showed higher leaf number at 40 DAR ($p < 0.05$, Figure 3, B2). In addition, the control treatment showed significantly lower plant height and leaf number rates in semi-lignified apical cuttings ($p < 0.05$, Figure 3, A1 and A2). Based on the 2019 results, early plant height and leaf number after rooting in 2020 were measured more frequently, thus revealing faster growth rates of apical cuttings compared to sub-apical ones which however resulted at the same pace, despite the fact that the effect of cutting type on early growth over time was significant ($p < 0.05$, Figure 4, Figure 6). The faster-growing apical cuttings similarly to 2019 showed higher rates of growth under the rooting treatments of 2,000 ppm and 4,000 ppm IBA, without however being significantly different between them ($p < 0.05$). In sub-apical cuttings in 2020 on the other hand, a more obscure picture was emerged in terms of comparisons between hormone treatments especially in leaf number, but treatment differences (not over time) were not found to be significant ($p < 0.05$).

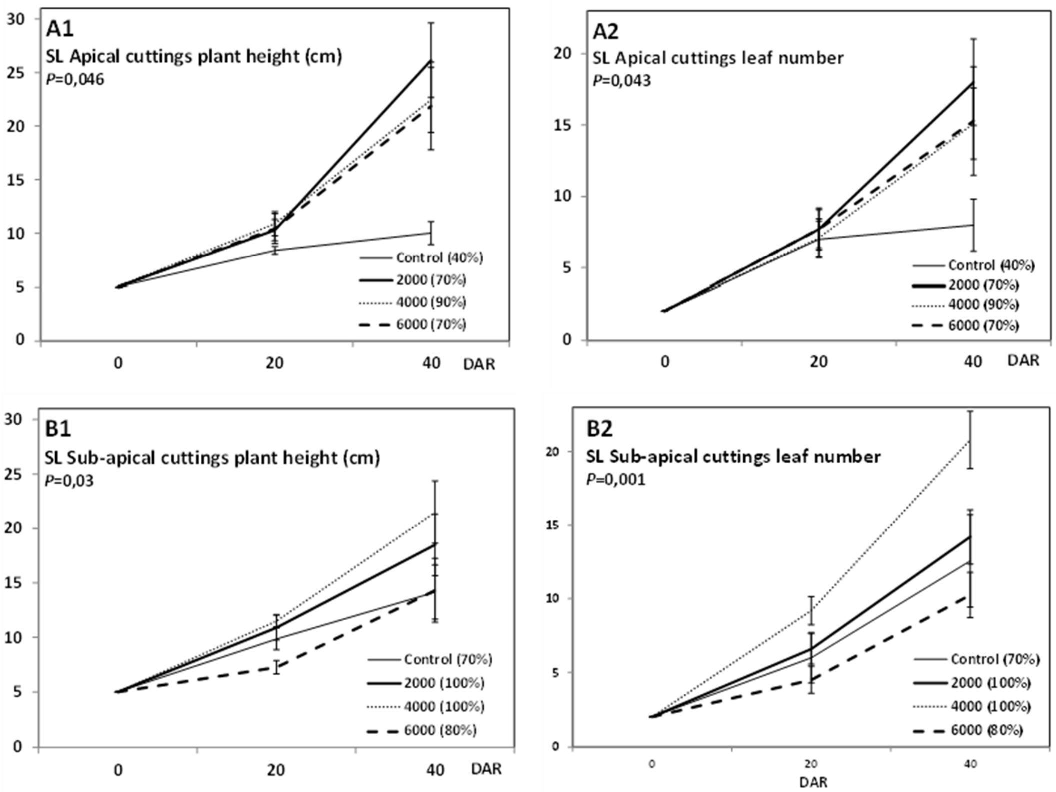

Figure 3. Plant growth patterns after rooting (DAR: days after rooting) of rooted cuttings in the 2019 experiment of Greek native *Cornus sanguinea* genotype GR-1-BBGK-19,199. A1 and B1: Mean plant height (cm) for each hormone treatment (ppm IBA) presented for the two cutting types, i.e., semi-lignified (SL) apical cuttings that included the stem's apical meristem and semi-lignified (SL) sub-apical cuttings that were internode sections. A2 and B2: Mean number of leaves for each hormone treatment (ppm IBA) presented for the same two cutting types. Standard errors of the means are shown on the graphs ($p < 0.05$) as well as the respective p-values of a Repeated Measures ANOVA conducted on hormone treatment effects (between-subjects effects) on each measured variable for each cutting type presented in each graph ($p < 0.05$). Treatment effects on growth over time (within-subjects effects) were not found to be significant in 2019. On each graph's legend, the total plant survival percentage (%) for each treatment is given next to treatment name

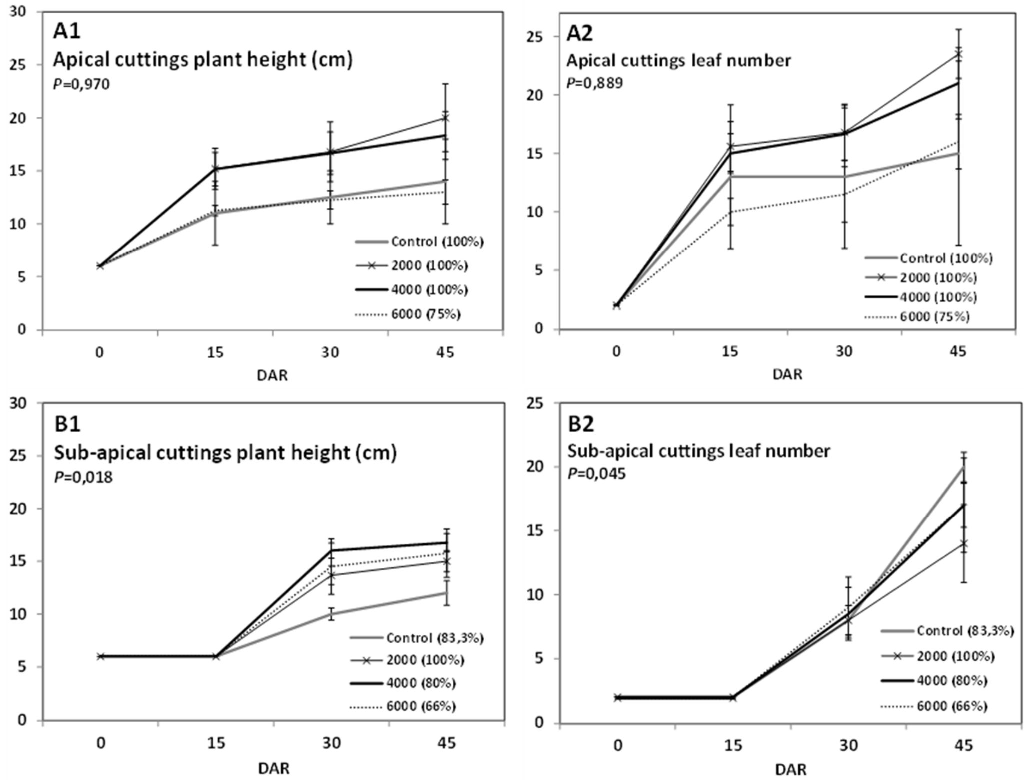

Figure 4. Plant growth patterns after rooting (DAR: days after rooting) of rooted cuttings in the 2020 experiment of Greek native *Cornus sanguinea* genotype GR-1-BBGK-19,199. A1 and B1: Mean plant height (cm) for each hormone treatment (ppm IBA) presented for the two cutting types, i.e., apical cuttings that included the stem's apical meristem and sub-apical cuttings that were internode sections. A2 and B2: Mean number of leaves for each hormone treatment (ppm IBA) presented for the same two cutting types. Standard errors of the means are shown on the graphs ($p < 0.05$) as well as the respective p-values of a Repeated Measures ANOVA conducted on treatment effects on growth over time (within-subjects effects) for each measured variable for each cutting type presented in each graph across the measured dates ($p < 0.05$). Hormone treatment effects between treatments (not over time, between-subjects effects) were not found to be significant in 2020. On each graph's legend, the total plant survival percentage (%) for each treatment is given next to treatment name

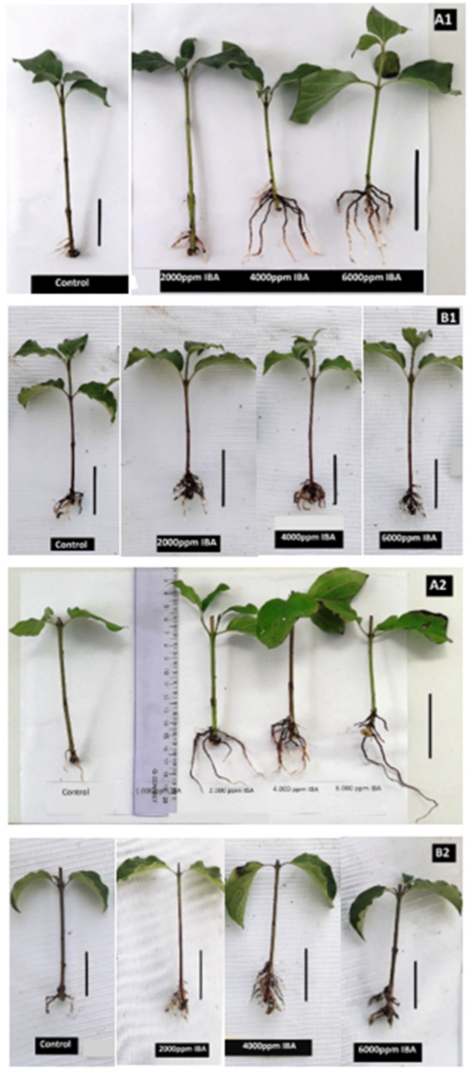

Figure 5. Comparative illustrations of cutting propagation results of the Greek native *Cornus sanguinea* genotype GR-1-BBGK-19,199 using four hormone level treatments (indole-3-butyric acid, IBA) in two years of experimentation. (A1) Apical softwood cuttings in peat–perlite (1:3, v/v) during 2019; (B1) Apical softwood cuttings in peat–perlite (1:3, v/v) during 2020; (A2) Sub-apical softwood cuttings in peat–perlite (1:3, v/v) during 2019; (B2) Sub-apical softwood cuttings in peat–perlite (1:3, v/v) during 2020. The differences in number of emerged roots per hormone treatment after two years of experimentation is outlined. Bars in photos represent 5 cm

Finally, plant biomass accumulation which was measured in the 2020 experiment presented higher values of total plant and root dry weight in 2,000 ppm and 4,000 ppm IBA treatments which were significantly affected by hormone treatment with root dry weight in particular, being affected by hormone in interaction with cutting type ($p < 0.05$, Table 2). The effect of treatments on root dry weight and root/total dry weight ratio expressed as the proportion of mass taken by root was evident in both cutting types but had a more pronounced effect in apical cuttings ($p < 0.05$, Table 2, Figure 6).

Table 2. Plant biomass accumulation expressed as mean dry weight values (g ± SEM) for the 2020 experiment of Greek native *Cornus sanguinea* genotype GR-1-BBGK-19,199. TDW: Total plant dry weight, RDW: Root dry weight, SDW: Shoot dry weight, RDW (%): Proportion of mass taken by the root out of the total dry weight. Values that do not share the same letter (lowercase letters for apical cuttings and capital letters for sub-apical cuttings) within each column are significantly different (Tukey HSD, $p < 0.05$)

	Treatment	TDW	RDW	SDW	% RDW
Apical cuttings	Control	1.249 ± 0.499 a	0.400 ± 0.173 a	0.900 ± 0.346 a	27.80 ± 1.789 a
	2,000	2.154 ± 0.138 a	0.800 ± 0.057 b	1.350 ± 0.104 a	37.35 ± 0.492 ab
	4,000	1.947 ± 0.111 a	0.775 ± 0.048 b	1.200 ± 0.091 a	38.87 ± 1.187 b
	6,000	1.485 ± 0.153 a	0.575 ± 0.047 ab	0.950 ± 0.150 a	39.90 ± 4.747 b
Sub-apical cuttings	Control	1.247 ± 0.245 A	0.325 ± 0.094 A	0.975 ± 0.184 A	24.35 ± 6.677 A
	2,000	1.470 ± 0.061 A	0.350 ± 0.028 AB	1.050 ± 0.028 A	25.50 ± 1.212 A
	4,000	1.620 ± 0.126 A	0.625 ± 0.047 AB	0.975 ± 0.170 A	39.77 ± 4.989 A
	6,000	1.900 ± 0.302 A	0.700 ± 0.100 B	1.225 ± 0.209 A	35.37 ± 2.089 A

Figure 6. Representative illustrations of the successfully established plants at 45 days after rooting across hormone level treatments (indole-3-butyric acid, IBA) from the 2020 propagation experiment of the Greek native genotype of *Cornus sanguinea* GR-1-BBGK-19,199. The photos were taken prior to destructive harvest for biomass accumulation assessment via dry weight. The photos are grouped according to cutting type treatments: (A) Apical softwood cuttings in peat–perlite (1:3, v/v); (B) Sub-apical softwood cuttings in peat–perlite (1:3, v/v). The differences in plant height, leaf number and root volume between the two cutting types as well as across hormone treatments within each cutting type are discernible. Bars in photos represent 10 cm

Discussion

The current study provided interesting data for the first time on asexual propagation via cuttings for Greek native germplasm of *C. sanguinea* originating from material collected directly from the wild. The preliminary trials indicated a clear superiority of softwood leafy, summer material in terms of rooting capacity under hormone treatment. Even though high rooting rates under hormonal treatment was achieved during the winter with hardwood cuttings on other *Cornus* species (Pirlak, 2000), winter cuttings tend to take much longer to root than summer cuttings which is believed not to be economically efficient. The current results of the subsequent experimentation on rooting capacity of summer softwood leafy cuttings of Greek *C. sanguinea* germplasm GR-1-BBGK-19,199 exhibited high rooting levels in a prompt manner in both years of experimentation. The rates reached 100% under the use of 4,000 ppm of indole-3-butyric acid (IBA) followed by 80% rooting under 2,000 ppm IBA within 25-30 days in both cutting types. At the same time, control treatment with no IBA showed lower rooting capacity with 45% and 65% in apical and sub-apical cuttings in 2019, respectively and similarly, in 2020 the control treatment reached 33% and 16,6% rooting in apical and sub-apical cuttings, respectively. The above observation indicates that the application of external hormone is essential for achieving high rooting rates (Hartmann *et al*., 2000), at least for the types of cuttings that were used herein. This, commensurate with similar work on other *Cornus* species where the application of external hormone greatly enhanced rooting of softwood cuttings during the summer (Bounous *et al*., 1992; Kosina and Baudyšová, 2011; Marković *et al*., 2014). With hormone application levels above 4,000 ppm, on the other hand, our cuttings did not perform equally well, with the application of 6,000 ppm IBA delivering lower rooting rates in both years, excepting sub-apical cuttings in 2020. Generally, excessive hormone application can have adverse effects on rooting and cutting quality, which is also related with species identity (Blythe *et al*., 2007; Karapatzak *et al*., 2022a).

A difference in rooting patterns in terms of root number and length was observed between the two years of experimentation. In particular, cuttings in 2020 presented higher number of roots that were shorter in length throughout (Figure 5), which is considered a valuable trait in terms of rooting quality (Scoggins, 2006). External hormone application and cutting type has also significantly affected the rooting quality in terms of root number and length in softwood cuttings of *Dianthus* spp. (Kumar *et al*., 2014), as well as in tropical woody species (Noor Camellia *et al*., 2009). The current observation of increased root numbers in 2020 may be linked to the higher level of lignification of the cuttings in 2019 which was just starting to take place when the cuttings were obtained. Semi-lignified tissue can act as a physical barrier for emerging roots at the base of cuttings. Pacholczak *et al*. (2017) induced anatomical changes on the stem tissue of mother plants of *C. alba* through shading which included reduced diameters of stem and pith and thinner layers of cortex, epidermis and collenchyma; this resulted in cuttings coming from shaded mother plants presenting higher number of roots and better rooting. Even though, mother plants of this study in 2020 were under *ex situ* acclimatization for a year more than mother plants of 2019, the collected cuttings in 2020 were of a softer tissue. The developmental stage and age of mother plants have been shown to affect rooting of softwood stem cuttings in other species such as the Chinese peony (Xian Feng *et al*., 2009). Another support related to the higher rooting quality of 2020 cuttings is that these cuttings reached 100% rooting under 4,000 ppm IBA. Thus, it is argued that a less lignified and softer material can absorb and utilize external hormone better, thus enhancing the ease of rooting (Costa *et al*., 2017). Following the above observations, higher plant survival rates were also observed in 2020 under 2,000 ppm and 4,000 ppm IBA in apical cuttings. A higher number of roots can result in higher root surface area and, as such, higher root volume and capacity of the newly formed plant to absorb nutrients and exploit the substrate, enabling higher assimilate partitioning and faster growth, survival, and acclimatization *ex situ*. To this end, high root volume in cuttings has been correlated with root surface area and quality of rooted cuttings in herbaceous perennials (Twardoski *et al*., 2012).

The faster growth rates that were observed in 2020 apical cuttings compared to sub-apical cuttings (Figure 6), could be linked to the effect of apical dominance and faster development of the already active apical bud (Srivastava, 2002); however, it is unclear whether the axillary/lateral buds on sub-apical cuttings had completed their differentiation by the time the cuttings were taken. Nonetheless, the axillary buds of the 2020 sub-apical cuttings did emerge due to absence of apical dominance but they did not emerge as fast as the apical buds developed on apical cuttings, still showing, however, a steadfast total plant growth (Bredmose, 2003; Bredmose and Costes, 2017). In addition, external hormone application significantly enhanced the production of more roots in apical cuttings which is also reflected in the biomass production data providing a clearer picture of the hormonal effect on the course of early plant development than in sub-apical cuttings. External IBA application is shown to significantly enhance rooting and plant survival in apical, leafy softwood cuttings during the summer in *Prunus yedoensis* (Kim and Kim, 2012). Similar results on the combination of IBA and apical cuttings have been found on *Prunus* rootstock germplasm using hardwood cuttings (Exadaktylou *et al*., 2009).

Concerning genotype effects, it has been shown in *C. mas* that different genotypes exhibit differences in rooting capacity and quality in interaction with external hormone application (Balta *et al*., 2019). Similar genotype effects on rooting of cuttings have also been found on Greek native *C. mas* genotypes (Karapatzak *et al*., 2022b) but also in members of other genera such as Greek native *Sambucus nigra* genotypes (Karapatzak *et al*., 2022a). Nevertheless, further experimentation on more Greek *C. sanguinea* genotypes is required to affirm whether the observed responses are genetically based or are a result of the combination of factors involved in rooting.

Another asexual propagation method for *Cornus* spp. that has been developed by other investigators employs the use of grafting for the production of high-quality stock material. Grafting a desirable genotype based on fruit characteristics onto another genotype based on ease of root development has given positive results in the past on European populations of *Cornus mas* (Bijelić *et al*., 2016). However, it is believed that the propagation and successful adaptation of self-rooted material can provide a more uniform population which can be a quicker and more economic method. Nonetheless, further research is required for the *C. sanguinea* germplasm studied herein regarding the production of new plants by grafting.

Although IBA which is readily available and low-cost hormone have shown very positive results on various *Cornus* species including the herein studied *C. sanguinea*, other substances and biostimulators have also been proposed as good rooting enhancers for *Cornus spp*. cuttings (Pacholczak *et al*., 2017). This direction should also be further investigated for *C. sanguinea* as well.

Conclusions

In this study, the use of external hormone application between 2,000 and 4,000 ppm IBA is proposed for successful propagation of *C. sanguinea* by softwood, leafy cuttings with minimum amount of lignification under mist. Cuttings should be taken during the summer from healthy, actively growing donor plants that have been acclimatized. The suggested protocol is considered fast, reliable, easy to implement and economically viable and is considered the first step towards conservation and utilisation of Greek *C. sanguinea* germplasm. Bulk production of plant material can facilitate prompt transfer to cultivation which, in turn, will lead to commercial production of this noteworthy ornamental and pharmaceutical species.

Authors' Contributions

Conceptualization NK and EK; Data curation EK, KP, DK, PY, GP, NN and NK; Formal analysis EK, KP and NK; Funding acquisition EM, NN and GP; Investigation EK, KP, DK, PY, GP, NN and NK; Methodology EM, EK, KP, DK, PY, GP, NN and NK; Project administration EM and GP; Resources EM and GP; Software EK and NK; Supervision NK, EM and GP; Validation EK, KP, DK, PY, NN, EM, GP and NK; Visualization EK and NK; Writing - original draft EK and NK; Writing - review and editing EK, KP, DK, PY, NN, EM, GP and NK. Please note: All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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