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ORIGINAL ARTICLE

In vitro conservation of centennial Austrian Cornelian cherry accessions

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Abstract

Cornelian cherry (*Cornus mas*) appears in a list of fruit and nut species growing in Europe considered neglected and underused economically. Although *C. mas* has a long-standing traditional medicinal use, only in recent years interest in products and food made from Cornelian cherries, said to have health-promoting effects, increased. This in turn raises the demand for improved planting material. In the Pielach Valley Region, Lower Austria, hundreds of centenary specimens of *Cornus mas*, but even a few millennial plants can still be encountered. The occurrence of these plants requested an active intervention to genetically characterize and preserve this valuable biodiversity, particularly in the light of changing environmental conditions. Efforts for the establishment of an in vitro collection of this valuable germplasm of centenary cornelian cherries yielded 193 mericlones initiated from single node explants from 41 selected plants. The selected donor plants were grouped by estimated age ranging from 10 years, > 50 years, > 100 years, > 200 years, > 400 years and 1000 years. The final goal of our efforts is to preserve these genetic resources, also checked for genetic and phytosanitary quality, for future generations and to use the superior clones for further breeding programs.

Keywords *Cornus mas* · Underutilized crop plant · Centenary individuals · Mericlones · Genetic characterization · In vitro collection

Abbreviations

BA	6-Benzylaminopurine
FeEDTA	Ethylenediaminetetraacetate ferric sodium
FeEDDHA	Ethylenediamine di 2-hydroxyphenylacetate ferric
8-HQS	8-Hydroxy-quinolinol-sulfate
IBA	Indole-3-butyric acid
MS	Murashige and Skoog
NAA	1-Naphthalenacetic acid
WPM	Woody plant medium

Introduction

Cornus mas appears in a list of 27 fruit and nut species growing in Europe considered more or less neglected by researchers or which have been underused economically. Although cultivars and ecotypes are mentioned, there exist no orchestrated efforts for germplasm conservation (Maggioni 2000). For the purpose of ex situ conservation of valuable plant germplasm both in vivo and in vitro genebanks are generally recognized measures (IBPGR 1988).

Cornelian cherry (*Cornus mas)* of the dogwood family *Cornaceae*, is a shrub or small tree distributed over Southern and Central Europe, the Black Sea basin and the Caucasus (Da Ronch et al. 2016). It bears large elliptical or roundish fruits each containing a single stone enclosed by some sweet pulp. The ripe fruits are collected from the wild still today and used for the preparation of sherberts and jellies, and for the fermentation of alcoholic beverages. Cornels were also held to have medicinal virtues in antiquity and the Middle Ages (Gismondi et al. 2012). Even today, products and food made from Cornelian cherry fruit are said to have health-promoting effects. As a consequence of the traditional uses as medicinal plant, researchers got attentive and the identification and quantification of possibly health-related substances became an important research topic (Borroto Fernandez et al. 2021a).

While Turkey is the leader in production and processing of Cornelian cherry (Ercisli 2004), the highest genetic variability

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has been reported from the Caucasus region, Georgia and Ukraine (Pirc 2015). In Lower Austria, in the Pielach Valley Region, 200–300-year-old specimen of *C. mas* from the time of Empress Maria Theresia (1717–1780), but even millennial plants can still be encountered (Pirc 2015). The occurrence of these centenary *Cornus mas* plants challenged us to establish a protocol for in vitro conservation of these valuable genetic resources.

The most common way of propagation of wild Cornelian cherry is done via seedlings, where seeds need to be stratified after harvest and germinate in a 3-year period. First inflorescences will appear only after 6–8 years (Pirc 2015). Alternatively, vegetative propagation of selected cultivars by budding onto 2-year old seedling rootstock bears the advantage of the offsprings already bearing fruits in the fourth year (Pirc 2015). However, this method encounters several limitations such as seasonal dependence, low coefficient of propagation, seed stratification requirements, etc. The propagation by layering is even more difficult, with uncertain results and non-applicable for a commercial scale (Ďurkovič 2008).

First efforts for tissue culture of *Cornus* spp. were reported from North American and Asian species (Lattier et al. 2014), mainly the highly valued ornamental species *C. nuttalii* Audubon (Edson et al. 1994), *C. florida* (De Klerk and Korban 1994; Kaveriappa et al. 1997; Trigiano et al. 1992), *C. canadensis* L. (Feng et al. 2009) and *Cornus alba* (Ilczuk and Jacygrad 2016). On the other side, also the medicinal and edible plants *C. officinalis* Torr. ex Dur. (Lu 1985; Park et al. 1993; Xue et al. 2003), *C. kousa* (Ishimaru et al. 1998; Hadziabdic 2005) and *C. mas* Schönbrunner Gourmet (Swanson 2018) and selected high-yielding cultivars of the biofuel plant *C. wilsoniana* Wangerin in China (Li et al. 2015) were cultured in vitro.

Starting material in all cases involved either juvenile (seeds or seedlings) from breeding programs or 20–30-year-old specimen from plant collections. Ďurkovič (2008) describes the establishment of tissue culture from a mature 27-year-old tree of *Cornus mas* 'Macrocarpa' and the challenges encountered in establishing a stable in vitro culture.

Here we describe the efforts for the establishment of an in vitro collection of valuable germplasm of centenary cornelian cherries. The final goal of our efforts is to preserve these genetic resources for future generations and to use the superior clones for further breeding programs in the light of changing environmental conditions.

Materials and methods

Plant material

The in vitro conservation of valuable genetic resources of many centenary *Cornus mas* plants requested a protocol for in vitro establishment and maintenance. To achieve this goal, we addressed our questions subsequently to two populations.

The control population of 4 to 8 years old grafted individuals of 5 cultivars of Cornus mas, very popular in Austria, 'Bulgarico', 'Flava', 'Kanzanlak, 'Schönbrunner Gourmet' and 'Schumener' was maintained in the greenhouse.

The main population consisted of centenary plants from different locations in Lower Austria (Table 1). The plants were grouped by estimated age ranging from 10 years (1 plant), > 50 (9 plants), > 100 (9 plants), > 200 (20 plants), > 400 (1 plant) and 1000 years (1 plant, Fig. 2a). The age of the plants was estimated from the information provided by the respective owners. The youngest plant and the plants in the category > 50 years have been planted by the owners. The plants > 100 were planted by the grand parents of the current owners. The plants in the categories > 200 and > 400 have achieved an impressive trunk size, and moreover have a historic link with the first mention of the farms in a given site. The oldest plant is even described as a national monument.

Genetic comparisons by microsatellite analyses revealed, that the 41 trees from 27 selected sites (at different elevations) represented unique genotypes, which could be explained by the pollination biology of the species (Borroto Fernandez et al. 2021b, Table 1). In brief, total genomic DNA was extracted from flower buds collected during ripening time. Seven different primer combinations (Wadl et al. 2014) were used for amplification of DNA from the different accessions.

To identify the population genetic structure of the selected genotypes of *Cornus mas* a model-based cluster analysis using STRUCTURE (Pritchard, Stephens and Donnelly, 2000) was implemented with a burn-in period and Markov chain Monte Carlo (MCMC) repetitions set to 5000 and 10,000, respectively. The admixture model with correlated allele frequencies was selected; K was set at 1–10 ($K = 1–10$) and K values were average across three iterations. The results from STRUCTURE were graphed on Microsoft Excel (2019) to obtain the plot (Fig. 1). A comparison with the entire population analysed (447 individuals) corroborated these results (Borroto Fernandez et al. 2021b).

A set of the following criteria were adopted for the selection of the individual genotpyes: fruit size, shape, and colour, yield and taste properties, and the fact, that the individuals belonged to the main different clusters as categorized by Borroto Fernandez et al. (2021b). Biochemical analyses were carried out on fruits from 11 plants (Borroto Fernandez et al. 2021a) and some plants exhibited particular traits, e.g. growing in a very humid or shady location, having an extremely small leaf size, or showing a different phyllotaxy (Table 1).

Table 1 Mericlones were successfully established from 24 explants from 41 plants selected from 27 sites in 4 regions in Austria (G, P, T, V: Gölsen Valley, Pielach Valley, Traisen Valley, Vienna region) and maintained on WPM medium with a pH adjusted to 7.0 and supplemented with 0.36 mg/L BA and 0.02 mg/L NAA

Plant Code	Site nr., origin (P, G, T, V)	Elevation above sea level (m)	Age (yrs)	Special notes	Fruit size, shape	Fruit color	Nr. of mericlones established successfully	Year of establishment
1	20, P	280	10		Large, round	Medium red	4	2019
2	11, P	600	> 50	Early bearing	Large, elliptical	Medium red	1	2016
3	11, P	600			Large, elliptical	Yellow	6	2019
4	11, P	600			Medium, elliptical	Medium red	8	2017
5	2, P	320			Medium, elliptical	Light red	5	2016
6	3, V	220			Medium, elliptical	Medium red	8	2016
7	4, V	151		Closed vegetation	n.d	n.d	7	2016
8	5, P	320			Medium, elliptical	Medium red	7	2017
9	17, V	180			large, elliptical	Medium red	3	2019
10	11, P	600			Medium, elliptical	Medium red	4	2019
11	1, V	180	> 100	Botanical Garden Vienna	Medium, elliptical	Medium red	5	2016
12	22, P	500			Medium, round	Orange	3	2016
13	24, P	400			large, elliptical	Light red	2	2016
14	26, G	450			LARGE, elliptical	Light red	1	2016
15	12, P	600			Medium, elliptical	Medium red	7	2019
16	13, T	570		Small leaves	Medium	Dark	1	2019
17	15, P	600		Water logged site	Large, elliptical	medium red	2	2017
18	16, V	160			Very large, pyriform	dark red	14	2017
19	18, P	372			Large, elliptical	dark red	2	2017
20	6, P	344	> 200		Medium, round	dark red	1	2017
21	7, G	369			Large, round	light red	2	2019
22	7, G	369			Large elliptical	yellow	6	2017
23	7, G	369			Large, elliptical	yellow	3	2018
24	7, G	369			Medium, elliptical	Orange	14	2017
25	8, G	600			Large, elliptical	Medium red	4	2017
26	8, G	600	> 200		Large, elliptical	Medium red	4	2019
27	8, G	600			Large, elliptical	Dark red	4	2019
28	8, G	600			Large, elliptical	Medium red	1	2019
29	9, P	572			Medium, round	Light red	1	2019
30	10, P	580			Medium, elliptical	Orange	4	2017
31	14, T	356			Large	Dark red	2	2019
32	14, T	356			Large	Dark red	1	2019
33	19, P	400			Large, elliptical	Light red	15	2019
34	21, G	600			Large, elliptical	Dark red	11	2019
35	22, P	600			Large, elliptical	Medium red	3	2019
36	22, P	600			Large, elliptical	Medium red	6	2017
37	24, P	460			Small, elliptical	Medium red	4	2019
38	27, G	600			Large, elliptical	Medium red	4	2018
39	10, P	580			Large, elliptical	Medium red	3	2017
40	25, P	460	400		Medium, elliptical	Dark red	4	2018
41	26, T	450	1000	Sub-opposite leaf position, triple nodes	Medium, elliptical	Medium red	6	2019

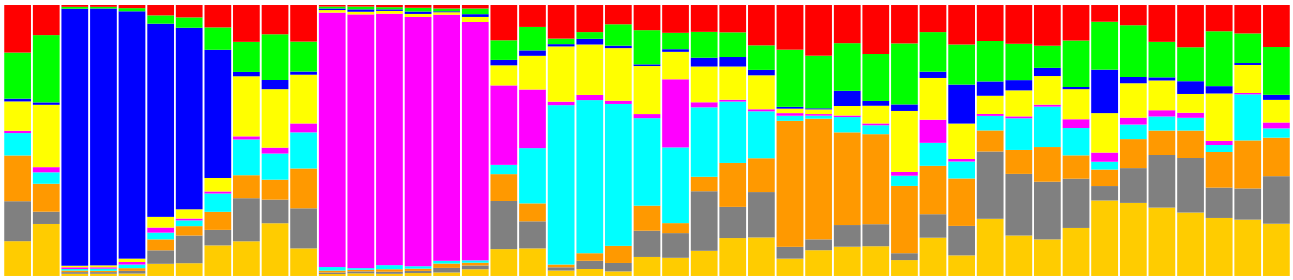

Fig. 1 Clustering of 47 *Cornus mas* individuals (6 cultivars and 41 wildtypes) analysed with a $K=9$ value by structure

In vitro establishment

Culture initiation of selected cultivars

During vegetative shoot growth in springtime 2016 buds located at different positions on a twig were taken as explants from five greenhouse grown cultivars 'Bulgarico', 'Flava', 'Kanzanlak, 'Schönbrunner Gourmet' and 'Schumener'. According to Debergh and Maene (1981) the state of the donor plant essentially enhances tissue culture establishment.

Freshly collected twigs were cut into sections, rinsed under tap water and a few drops of liquid detergent for 20 min and incubated in 70% ethanol for 2–3 min. A fractionated treatment (5 min + 15 min) with 20% and 15% (v/v) commercial bleach (2.8% NaOCl) solutions with 2–3 droplets of Tween® 20 (Sigma-Aldrich Corporation, St. Louis, MO) was followed by 5 steps of washing with sterile water. Individual mericlones were initiated from single node explants from precisely marked sections to understand the impact of topophysis: **A** for apical buds, **B** for the first thin section of the twig, **C** for the central and **D** for the basal sections of the twigs. Special care was given to the nomenclature allowing to trace back the individual buds initiated from the same nodium on the branch.

Alternatively, a method for the in vitro establishment of *Malus domestica* from adult material (Laimer da Câmara Machado et al. 1991) was modified for *Cornus mas*, including important findings from Ďurkovič (2008). Twigs were immersed in an 0.1% 8-hydroxy-quinolinol-sulfate (8-HQS, Chinosol, Riedel de Haen) solution at 10 °C overnight, before buds were excised and processed as described before. Single buds were excised under a stereo microscope and placed in multiwell-plates (24 wells), covered with 0,1% 8-HQS overnight and subculture to fresh culture medium after 24 h, as originally described (Laimer da Câmara Machado et al. 1991). Initial culture media were based on MS medium (1962), supplemented with only 0.5 mg/l BA or in combination with 0.05 mg/l GA_3 at pH 5.8.

Culture initiation of wildtypes from open field

Results obtained with the greenhouse grown material led us to elaborate a modified establishment procedure for the material from open field in spring 2016 (8 accessions), 2017 (12 accessions), 2018 (3 accessions) and 2019 (18 accessions). One of the techniques Durzan (1990) recommended for in vitro establishment of valuable individuals having reached the mature state, is the use of stump sprouts at the trunk of the tree. In fact, the first decision was already taken in the field, when harvesting the twigs with 40 vegetative buds, when available. Thin twigs were excluded, and only strong vital shoots were considered. Twigs were transported back to the lab, immersed and shaken in a 0.1% 8-HQS bath for 30 min, wrapped in tissue towels wetted in 0.1% 8-HQS solution and stored in the cold-room until further use. The next day twigs were rinsed under tap water and a few drops of liquid detergent for 20 min, incubated in 70% ethanol for 2–3 min and cut into 2–3 node sections to avoid tissue damage during surface sterilization. The fractionated surface sterilization involved a treatment (5 min + 15 min) with 20% and 15% (v/v) commercial bleach (2.8% NaOCl) with 2–3 droplets of Tween® 20.

Explants were cultured on a propagation medium consisting of WPM basal nutrients and vitamins, replacing the chelating agent for iron with EDDHA supplemented with 0.7 mg/l BA alone or in combination with 0.05 mg/l IBA or NAA, 100 mg l-1 myo-inositol, and 20 g l-1 sucrose. Medium was solidified with 7 g l-1 agar (Type A, Sigma-Aldrich) and a pH adjusted to 6.8–7.0.

Maintenance of the collection

Explants were cultured on propagation medium consisting of WPM basal nutrients and vitamins, replacing the chelating agent for iron with EDDHA, supplemented with 0.7 mg/l BA in combination with 0.05 mg/l IBA or NAA, 100 mg l-1 myo-inositol, and 20 g l-1 sucrose. The medium was solidified with 7 g/l-1 agar (Type A, Sigma-Aldrich) and the pH adjusted to 6.8–7.0. Regenerated axillary shoots

were maintained by transferring to fresh regeneration medium every 4 to 6 weeks and incubated under standard culture conditions [24 ± 2 °C and a 16-h photoperiod of 100 µmol m-2 s-1 provided by cool-white fluorescent lamps].

Results and discussion

Culture initiation and long-term conservation of selected cultivars

On the single node explants after 28 days either one or both buds developed. To reduce the impact of apical dominance of one over the other, at the first subculture care was taken to split the original explant in two and to subculture the individual buds separately as *x* and *y*.

After 3 months from 124 established explants (Table 2), a total of 89 stably growing mericlones could be regenerated. Depending on the genotype, the number was variable from 29 of Schumener, 27 of Schönbrunner Gourmet, 16 of Kazanlak, 9 of Flava to 8 mericlones of Bulgarico. In total the most responsive buds were located at section **D** (31 mericlones), followed by the apical explants with 24 mericlones, and 17 mericlones for section **B** and **C** respectively, which corresponds to only 54% of section **D**. This could be most prominently observed in the cultivar Schumener, while it was not as pronounced, but still visible in the other cultivars. Regarding the cultivars Kazanlak, Flava and Bulgarico we observed less vitality and growth. Several buds turned brown and stopped growth, especially in the explants from the youngest section of the twigs. Interestingly from the apical explants the main meristem frequently developed into a small shoot and then died off, while the lateral buds formed on the first internode developed further and were therefore treated as individual mericlones *x* and *y*, which allowed to determine that 10/24 initially established nodes produced two vital shoots in the case of the cultivar Schumener, and 9/24 in the cultivar Schönbrunner Gourmet (data not shown).

Ďurkovič (2008) attempted to obtain axenic cultures by the application of commercial plant preservative mixtures and 0.1% mercuric chloride ($HgCl_2$) solution; however, without mentioning the sterilisation rate. Given the toxicity of mercuric chloride, we searched for alternative solutions. The exposure of students and workers to $HgCl_2$ solutions and the fact, that the washing water needs to be treated as contaminated waste, represent unnecessary occupational risks (Havermans et al. 2015). 8-HQ on the contrary has been known for its antimycotic and bactericidal activity, and apart from its use in human healthcare (Auterhoff et al. 1999) has been used for the treatment of budwood of fruit trees (Duhan, pers. comm). Read and Yang (1989) also showed that 8-hydroxy-quinoline citrate produced positive effects during the establishment of tissue cultures of woody species.

Therefore, even when starting from 24 original explants for every cultivar it was possible, after 28 days, to have enough material established to begin experiments for the optimization of the multiplication medium (Laimer da Câmara Machado et al. 1991). Both establishment methods yielded high numbers of vital shoots, which in dependence on the genotype varied from 33.3 to 100% (Table 2). A similar observation of the impact of the genotype, and even of the individual donor tree has been impressively documented already by Edson et al. (1994). Comparisons with data obtained with other *Cornus* species also indicated this high variability. Kaveriappa et al. (1997) reported that nodal sections from seedlings of *C. florida* grown in the greenhouse were easily established in culture after a treatment with 20% commercial bleach and a few drops of Triton X-100. Only 2–5% of the cultures were lost to contaminations, which agrees well with other studies that utilized seedling material grown under similar conditions (Declerck and Korban 1994). Edson et al. (1994) treated *C. nuttallii* bud material with NaOCl concentrations of 1.5%. Ilczuk and Jacygrad (2016) incubated *C. alba* buds in 70% EtOH for 2 min, followed by a treatment with 3% NaOCl for 15 min. In comparison, the NaOCl concentrations in all these experiments were substantially higher, opening further optimization

Table 2 Mericlones successfully established from 24 explants of 5 cultivars of *Cornus mas* popular in Austria after 28 days on WPM medium with a pH adjusted to 7.0 and supplemented with 0.7 mg/L BA and 0.05 mg/L NAA in 2016

Cultivar	Nr. of explants	Nrs. of mericlones successfully established				% axenic explants
Position on the branch		A	B	C	D	
Bulgarico	24	3	2	n.d	3	33.3
Flava	24	3	2	2	**2**	37.5
Kazanlak	24	n.d	n.d	7	9	66.6
Schumener	24	8	8	3	10	100
Schönbrunner Gourmet	24	10	5	5	7	100
Total	124	24	17	17	31	71.7

perspectives by increasing the concentration without risking higher explant losses.

The cytokinin BA alone was reported to be sufficient for shoot culture establishment and multiplication of *C. nuttallii* (Edson et al. 1994), *C. florida* (De Klerk and Korban 1994; Kaveriappa et al. 1997), and *C. kousa* (Hadziabdic 2005). However, low concentrations of auxin were necessary for efficient in vitro growth in other dogwood species (Lattier et al. 2014). Since we did not observe any beneficial impact when applying GA_3 (data not shown), we concluded to omit this variant from future trials.

Culture initiation and long-term conservation of wildtype accessions

Among several parameters considered of importance for a successful establishment, both genotype and environment were recognized as highly influential. Here we discuss the approach under two perspectives: (a) the choice of the explant and (b) the role of the culture medium to allow vigorous growth.

Mericlones developing from actively growing buds from different donor plants varied from a single bud (4,1%) up to 15 buds (62.5%) from the originally established 24 explants (Table 1). From the youngest individual (planted 8 years ago) 4 buds (16.6%) gave rise to actively growing mericlones. Similar ratios were obtained from plant material from the oldest plants, the 400 and the 1000-year-old specimen, namely 4 and 6 (16.6 and 25%), respectively (Table 1). Among the plants in the age class of > 50, only one particularly early ripening individual developed only from a single explant, while the other plants yielded 3–8 (12.5–33.3%) actively growing mericlones. Interestingly, among the 9 plants in the age class of > 100 2 plants developed a single mericlone, while 1 plant yielded 14 (58.3%) mericlones. Out of the 20 plants in the age class of > 200 4 plants yielded only a single viable mericlone, while others yielded up to 11, 14 or even 15 actively growing mericlones (45.8, 58.3 and 62.5%), respectively. In conclusion, the success rate does not correlate with the age of the donor plants, but must be clearly attributed to the physiological stage of the explant material at the time of excision.

The major problem encountered were fungal contaminations, with infection rates ranging from 68.6 to 72.4%, depending on the sterilization method. However, these results are comparable to those reported by Edson et al. (1994). The methods applied were quite efficient in controlling bacterial contaminations, since they only accounted for the loss of 2.9% and 5.5% of explants. Contrary to the observation of Edson et al. (1994), that most of the bacterial contaminations disappeared after a few subcultures in *C. nuttallii*, here infected cultures of *C. mas* were discarded.

Sterility of explants is probably the most difficult challenge with field grown explants due to their high contamination levels (Lazo-Javalera et al. 2016, Laimer da Câmara Machado et al. 1991). The establishment of in vitro cultures from woody plant material collected from *Cornus* species growing in the open field since centuries is reported to be a difficult task, owing to explant losses by fungal contaminations (Edson et al. 1994) and to detrimental conditions such as vitrification or chlorosis (Ilczuk and Jacygrad 2016). In fact, Edson (1994) stated that more explants from the forest trees of *Cornus nuttallii* Audubon were contaminated with fungi than from the greenhouse seedlings (75% versus 18%). Trigiano et al. (1992) reported high contamination rates with explants from forest trees of *C. florida* L. Most of these cultures became necrotic after 3 months and only 14/400 original explants survived.

Orthotropic shoots originating from the base of the trunk or shoots that arise from epicormic buds in several hardwoods are more juvenile than branches from other parts of the plant (Bonga 1987). These shoots arise from arrested of from adventitious buds. Epicormic buds are stimulated to grow and produce shoots as a result of stress i.e. sudden environmental change, thinning, crown dieback, heavy pruning, root death, cold, change in the water table. In temperate regions this phenomenon has been reported in oak (*Quercus*), elm (*Ulmus*), linden (*Tilia*), poplar (*Populus*), rowan (*Sorbus*) and willow (*Salix*). Examples of angiosperm trees micropropagated from stemp sprouts are *Quercus robur, Tilia cordata* (Chalupa 1984; Youn et al. 1988) and from buds of almost 100-year-old *Morus nigra*, *Sorbus domestica* and *Liquidambar orientalis* (Durkovic et al. 2012; Durkovic and Misalova 2009; Bayraktar et al. 2015).

Although most known tissue culture media were tested for *Cornus* species (Lattier et al. 2014), basal nutrients of the woody plant medium (WPM, Lloyd and McCown 1980) have preferentially been used for the micropropagation of a diverse range of dogwoods, including *C. nuttallii* (Edson et al. 1994), *C. officinalis* (Xue et al. 2003), *C. mas* (Ďurkovič 2008) and *C. florida* (Kaveriappa et al. 1997). Ishimaru et al. (1998) established cultures of *C. kousa* on Broadleaf Tree (BW) medium, Hadziabdic (2005) on WPM or half-strength BW medium (Chalupa 1984).

A strong dependency of explant vitality, lack of it and rate of proliferation on the cultivation conditions has reported by multiple authors (Ilczuk and Jacygrad 2016; Edson et al. 1994), whereas no mention in the literature was found on direct influences of the establishment method on the later vitality of the explants. As described by Ďurkovič (2008) cultures of *Cornus mas* Macrocarpa are prone to decline either by re-appearing infections and culture media conditions. Growth of cultures, initially established on WPM medium with a pH adjusted to 5.8 ceased, shoot tip necrosis appeared, and cultures gradually died. For this reason, the

living shoots were transferred to a modified WPM with the pH adjusted to 6.8–7.0. Similarly, explants of *C. mas* Schönbrunner Gourmet after the initial 6 weeks on the medium described by Ďurkovič (2008) showed leaves that were slender and exhibited vitrification. These phenotypes disappeared after a transfer of the cultures to Long and Preece medium (Long et al. 1995; Swanson 2018). Shoots of *C. florida* exhibiting fasciated growth patterns, hyperhydricity and inhibited elongation where reported exposed to TDZ as plant growth regulator (Kaveriappa et al. 1997).

In our cultures of *Cornus mas* signs of vitrification disappeared after the transfer from MS (Murashige and Skoog 1962) to WPM medium. The most successful medium with a pH adjusted to 7.0 was supplemented with 0.7 mg/L BA and 0.05 mg/L NAA. After several subcultures on this medium all explants were growing actively, with long shoots and large vital green leaves (Fig. 2). Mericlones were regularly subcultured and maintained with 12–18 plantlets each on a medium supplemented with 0.36 mg/L BA and 0.02 mg/L NAA. This population size allows the safe maintenance of valuable genotypes and a rapid multiplication for experimental purposes or release. The documentation in an in house developed database allowed continuous monitoring of the status of cultures.

Similar media compositions including WPM as a basis as well as supplementation by BA and NAA at moderate pH values have been reported as optimal for various *Cornus* species (Ilczuk and Jacygrad 2016). In most in vitro shoot regeneration studies on dogwoods BA has been used as an effective cytokinin, and alone was sufficient for shoot proliferation of *C. nuttallii* (Edson et al. 1994), *C. florida* (De Klerk and Korban 1994; Kaveriappa et al. 1997), and *C. kousa* (Hadziabdic 2005) (see Lattier et al. 2014). However low concentrations of auxin were necessary for efficient in vitro growth in other dogwood species. Ďurkovič (2008) reported shoot regeneration using BA supplemented with NAA for *C. mas* 'Macrocarpa'. Xue et al. (2003) reported proliferation of *C. officinalis* shoots on media containing BA, zeatin and NAA.

Chlorosis appeared in later subcultures and in some cases even led to explant death. Since we attributed it to the non-optimal cultivation conditions rather than to the establishment methods, we searched for alternative chelating agents. The beneficial influence of FeEDDHA on in vitro culture was noticed for several horticultural crops, e.g. increased shoot length and reduced chlorosis, enhanced regeneration from somatic embryos in rose, reduced chlorosis in raspberry and higher adventitious regeneration in blackberry (Van der Salm et al. 1994; Zawadzka and Orlikowska 2006; Tsao and Reed 2002). Development of chlorosis in shoot cultures of raspberry was caused by a shortage of iron when supplied as FeEDTA (Zawadzka and Orlikowska 2006), known to be less stable than FeEDDHA due to its susceptibility to light (Van der Salm et al. 1994), or its precipitation with other nutrient compounds (Dalton et al. 1983; Schenk et al. 1991). An obvious increase in the quality of the raspberry leaves (chlorophylls and iron content)—and thus reduction of chlorosis—was attributed by Zawadzka and Orlikowska (2006) to the higher availability of iron when supplied as FeEDDHA over FeEDTA, known

Fig. 2 Millennial *Cornus mas* **a** in vivo with an impressive trunk perimeter and **b** in vitro developed mericlones

to be less stable due to its susceptibility to light (Van der Salm et al. 1994). However, these authors also stressed, that the extent of this influence was cultivar dependent.

In vitro propagation offers also major contributions for any conservation programme and for the preservation of the genepool (Laimer and Maghuly 2008). In fact, Gepts (2006) correctly states that Plant Genetic Resources Conservation and Utilization are two tightly linked aspects important for the future generations. As far as efforts for germplasm conservation are concerned, in vivo and in vitro genebanks are generally recognized measures for ex situ conservation of valuable plant germplasm (IBPGR 1988; Yilmaz et al. 2009; Rop 2010). Thorough evaluation of the genetic resources of the native genotypes is essential for selecting most useful genotypes for future breeding programs designed to introduce traits such as hardiness and disease resistance from wild genotypes into cultivated varieties (Klimenko 2004; Ercisli et al. 2008; Borroto Fernandez et al. 2021b).

As climate is a key agro-ecosystem driving force, climate change could have a severe impact on cultivated as well as on wild growing plants. Many assessments have been carried out to date on the possible effects of climate change on plant physiology (Salinari 2006). In particular, plant pathogens may be specifically responsive to global change, given both short generation times and effective dispersal mechanisms (Coakley et al. 1999). Because of altered temperature and precipitation regimes, climate change may alter growth stages and/or rates of development in the life cycle and pathogenicity of pathogens, as well as modify the physiology and resistance of host plants (Chakraborty 2005). EU directives define the demands to the genetic and phytosanitary quality of intensively grown crop plants, but the perception of what this means for the native flora or extensively grown plants is largely missing, except when it acts as possible reservoir of inoculum. Changing climate conditions and reduced space availability will increasingly replace them with selected plant material. Healthy planting material can be made available, which provides the basis for a successful production of healthy food. With data presented in this paper, we are able to apply our long-lasting experience in the conservation of temperate fruit cultivars and landraces, as well as of small fruits, e.g. *Rubus* and *Vaccinium* to the conservation of Cornelian cherries.

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