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Method

An approach for the mass propagation of *Cupressus sempervirens* L. (Cupressaceae), for quality propagule production

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Abstract

The scenarios of climate change in Mediterranean regions bring back the need for tree species tolerant to drought and disease, for agro- and urban-forestry purposes, landscape rehabilitation, as well as for post-fire and quarry restoration plans. Therefore, forest industries focus on selecting, propagating and growing superior trees. The *Cupressus sempervirens* L. is such a coniferous tree species, with a fundamental ecological, financial and ornamental role in the Mediterranean region. The aim of this study was to develop an efficient macropropagation protocol, which would lead to mass-selected genotypes or phenotypes of *C. sempervirens* f. *sempervirens*. For this purpose the axillary shoot multiplication method was used, by adopting both cutting propagation and intermittent mist methods. These methods were used for the first time in the macropropagation of *C. sempervirens* f. *sempervirens*. For shoot proliferation, 24 different modified macropropagation treatments at different concentrations and combinations were tested: shoot cuttings, concentrations of K-IBA and rooting systems. The elongation and root induction was carried out in an intermittent mist system for 50 days, then the new plantlets were moved for acclimatization for 15 days in a greenhouse. The current workflow presents an effective preliminary protocol with clear steps and treatments for macropropagation of *C. sempervirens* f. *sempervirens*. The developed protocol for

macropropagation of tree species ensures cost- and time-efficient propagation. Produced plantlets were developed efficiently under *in vivo* conditions, allowing to propagate and store genetic material for conservation and domestication. This protocol can be generated for other tree species of the *Cupressus* genus and the Cupressaceae family.

Keywords

Cupressaceae, K-IBA, Mediterranean cypress, macropropagation, mist system, plant propagation, woody trees

Introduction

Propagation techniques for woody trees are used for producing plantlets and imply the culture of aseptic small sections of tissues and organs in vessels with defined culture and under controlled environmental conditions. These techniques have become an increasingly important tool for both science and commercial application in recent years (Kumar and Reddy 2011). The research on tissue culture of forest trees resulted in optimisation and increased efficiency of most of the culture techniques for trees, especially in the form of axillary shoots (or buds) regeneration and somatic embryogenesis (Wilhelm 2005). Therefore, propagation methods under specific conditions, gain ground over conventional vegetative propagation since they could be linked to the propagation of a great number of pathogen-free plants in a short time with high uniformity (e.g. Yasodha et al. 2004, Wilhelm 2005, Kumar and Reddy 2011).

The success of propagation involves several factors, such as the composition of the culture medium, culture environment, and genotype (Kumar and Reddy 2011). The propagation by leafy stem cuttings (axillary and adventitious shoot multiplication) has been mentioned as one of the most technically effective propagating techniques (Hartmann et al. 2002, Mateja et al. 2007). However, a key point of plant propagation from stem cutting is minimising the water stress caused from the cutting, until roots are formed (Mesén et al. 2001, Mateja et al. 2007). For overcoming this stress, propagation systems need to maintain an atmosphere with low evaporative demand, thus reducing water loss from transpiration and avoiding extreme tissue-water loss. For this purpose, the systems that were used ensured that the fog or mist on leaves allowed evaporative cooling, resulting in 90-100% humidity (e.g. intermittent mist and fogging systems of high-humidity, or non-mist polypropagators) (Harrison-Murray and Thompson 1988, Spethmann 1997, Hartmann et al. 2002).

The scenarios of climate change in Mediterranean regions bring back the need for the preference of tree species tolerant to drought and disease, for both timber production and for urban forestry purposes. The *Cupressus sempervirens* L. (Mediterranean cypress) is a coniferous tree species, which has a fundamental ecological, financial and ornamental role in the Mediterranean region (Giovanelli and de-Carlo 2007). *Cupressus sempervirens* is a medium-sized evergreen coniferous tree and is a monoecious wind-pollinated plant (Caudullo and de-Rigo 2016). *Cupressus sempervirens* is a pioneer species, growing

quickly when young on most substrates but not on clay or waterlogged ones, and up to 35-40 m with trunks 1 m in diameter, rarely over 2 m (Eckenwalder 2009, Farjon 2010). *Cupressus sempervirens* has a long horticultural history in the Mediterranean region, and its natural distribution nowadays is the remnant of an extensive cypress forest of the Pliocene (López-Sáez et al. 2019). The present natural distribution of *C. sempervirens* occurs in the southwest Mediterranean basin over several geographically non-adjacent areas reaching, eastwards, Caucasus and southwestern Iran, on elevations from sea level (Crete) up to 2000 m (Turkey) (Eckenwalder 2009, Caudullo and de-Rigo 2016). The *C. sempervirens* is a light-demanding, drought- and heat-tolerant species, surviving in habitats with as little as 200 mm of annual rainfall (Caudullo and de-Rigo 2016). In winter it is quiescent, while in summer it remains dormant, quickly resuming after rainfall, owing to its extensive and shallow root system (Santini and Donardo 2000). The species favours rich, deep, moist and well-aerated soil with neutral pH, however also grows on rocky, dry and compact soils (Caudullo and de-Rigo 2016). It can live for longer than 100 years, while older individuals (of 200-500 year of age) are not uncommon in natural stands. The *C. sempervirens* was initially characterised by two varieties: (i) the wild type var. *horizontalis* (Miller) Aiton, widely represented in native populations, with a broad conical crown and the main branches forming a wide angle with the trunk and (ii) the fastigiated var. *pyramidalis* Nymann (= var. *stricta* Aiton), widely planted and cultivated, with a dense columnar crown and main branches growing upwards close to the trunk (Zacharis 1977, Tutin et al. 1993). However, according to the recent taxonomy rank, the variety relating to the growth form of fastigiated *var. pyramidalis* does not correspond to a taxon (Farjon 2010). Therefore, the columnar cultivated form is referred to as *C. sempervirens* f. *sempervirens* and the spreading form as *C. sempervirens* f. *horizontalis* (Farjon 2010, Korakis 2015).

As regards to the *C. sempervirens* timber, it has interesting characteristics of high natural durability and straightness. From ancient times its wood has been particularly appreciated for its resistance to fungi and insects, especially if immersed in water (Hadjikyriakos 2007). Despite the fact that *C. sempervirens* wood is suitable for small carpentry, exterior woodworks (doors, windows, garden furniture, etc.) and ship-building, the ecological importance of the species is more favourable for the climatic conditions nowadays. The species was used since Greek and Roman times as an ornamental tree for shading gardens, cemeteries, as a windbreak along roads, as well as for vegetable and fruit tree crops, and it has become a characteristic feature of the Mediterranean coastal and urban landscapes (Farjon and Filer 2013, Bagnoli et al. 2009). More recently, and owing to its ecological qualities, *C. sempervirens* was used in hot areas for forest protection against desertification and soil conservation, where soil is shallow and degraded and other forest tree species cannot grow (Caudullo and de-Rigo 2016). It is also considered as a valuable species for landscape rehabilitation, soil reclamation and quarry restoration (Korakis 2015).

Since the 1970s, and after the introduction of the serious disease of the “Cypress canker” in Europe (most likely from the USA), which is caused by the *Seiridium cardinal* fungus, recorded over large Mediterranean areas and leading to damage in forests, nurseries and ornamental plantations, the disease has become a limiting factor to cypress planting. Fungus management requires the removal of infested trees and the use of genetically

resistant forms, and hence, clones have been recently patented (clonal selection in natural stands where the disease was present or by cross-breeding between selected trees in experimental fields) (Giovanelli and de-Carlo 2007). In addition, another fungus, the *Diplodia pinea* f. spp. *cupressi* causes cankers on the stem and branches of water-stressed cypresses.

Although a number of reports have indicated that propagation can be successfully applied to *Cupressus* species (Fossi et al. 1981, Hřib and Dobrý 1984, Franco and Schwarz 1985, Spanos et al. 1997, Giovanelli and de-Carlo 2007) in the past decades, the use of a more efficient propagation (and rooting) method for mass-selected genotypes or phenotypes of the targeted species can lead to increase of the conservation effort, as well as to commercial use for agroforestry (and urban forestry purposes) of *C. sempervirens* f. *sempervirens*. Hence, the current study aims to develop and present an effective protocol for the macropropagation of *C. sempervirens* f. *sempervirens* using axillary shoot multiplication by adopting both cutting propagation and intermittent mist methods. This protocol could be adopted to address the need for rapid propagation and cultivation of selected genotypes with commercial and ecological/silvicultural interest.

Materials and Method

Sampling of shoots

For sampling of plant materials, the general guidelines for plant propagation need to be adopted (e.g. Moe and Andersen 1988, Hartmann et al. 2002, Lambardi et al. 2013). Due to the fact that *C. sempervirens*' growth is potentially a continuous and undetermined process, affected by limiting environmental conditions, such as low temperature and/or drought (Mateja et al. 2007) and that bud burst cannot be taken into account for determining the onset of growth, repeated collections of plant material can be advised during mid-winter (February) and early spring (March). For the current study, a cohort of young trees of *C. sempervirens* f. *sempervirens* (>15 years old) was chosen. This cohort is located southwest of Klirou village (Cyprus),in an altitude of 450 m. From each tree lateral healthy side-veneer shoots (on 1-year old rootstocks) were collected from the shady side of each tree, located into the lower third of the crown. Each shoot had a length of 30-45 cm. Shoots were excised in the morning and brought to the nursery in iceboxes with temperature 10-12ºC and covered by a wet cotton cloth. The shoots collection was performed just before the onset of vegetative growth, around the end of winter growth cessation (February).

Experiment design

Methodology

The current experiment addressed the need of simplifying the processes, as well as the minimisation of the propagation time and steps for *C. sempervirens* f. *sempervirens*. The experiment was based on the general stages of propagation process, including the following steps where different treatments in each step were tested:

- **Culture initiation (tissues preparation)**: This stage aimed to initiate axenic cultures. It was carried out in the nursery after the field-sampling of tissues. Sampled shoots were divided into three cuttings (basal, middle and terminal part of sampled shoot), where each cutting had a length of approximately 10-12 cm. Remarkable is the fact that in each cut axillary shoot, a bud in its base (footing) was kept up, as well as 4-6 fully formed leaves at the top side of each of the cut shoot part. All shoot cuttings were carried out using a sterilised cutter (with ethanol).
- **Plant growth substances**: In this stage the masses of shoot tissues were repeatedly subcultured. The "quick dip" method was adopted for this study, due to the type of tissues material. Thus, the talcum base (where bud exists) of cuttings, approximately 4-5 cm, was immersed in a rooting hormones solution for 10 seconds. The *indole-3-butyric acid* (K-IBA), was used as the rooting hormones solution (K-IBA by Merck KGaA, Darmstadt, Germany). In this experiment four different concentrations of K-IBA were tested (2500 ppm, 4000 ppm, 5500 ppm, 7000 ppm), as well as the neutral concentration without K-IBA (0 ppm; only deionised water) (Hortus USA – see http://www.rooting-hormones.com/IBAmethd.htm).
- **Elongation and root induction or development (rooting phase)**: This stage was designed to induce the establishment of both fully developed roots and plantlets. This stage was carried out in continuation to the previous stage, and, hence, after immersing the basal end of the cut shoot in K-IBA, the shoot was immediately planted for 4-5 cm in root trainers. For this experiment perlite (3-5 mm) with pitmus substrate in a 4:1 ratio was used as the rooting medium, into two different root systems: bench and root trainer. The rooting phase was carried out in a greenhouse with a high-pressure intermittent mist system, at the Athalassa Forest Nursery (Department of Forests; Ministry of Agriculture, Rural Development and Environment, Cyprus). The intermittent mist system assured constant high humidity (95%) in the house, and house temperature of 19ºC on a 24-hour basis (standby). The rooting phase took place for 50 days, and after this period the plantlets were moved to the next stage (see *Acclimatization*). Notable is the fact that masses of tissues must be repeatedly subcultured under aseptic conditions; therefore shoots were irrigated with a fungicide (i.e. Topam).
- **Acclimatization**: The last stage of the experiment was the environmental adaptation of plantlets in growing conditions in the field (outside the green house). The shoots that developed root were planted in small plastic flowerpots and kept for

15 days in a greenhouse before being moved to natural conditions in Athalassa Forest Nursery, Department of Forests (Nicosia). Under this framework the young plantlets were cultivated and their viability was monitored for two years (2017 & 2018) in a nursery (Athalassa Forest Nursery).

In overview, the current experiment tested the effectiveness of quality propagule production for numerous parameters, namely: (i) germination ability of different parts of the shoot (basal, middle and terminal cuttings), (ii) four different concentrations of K-IBA (2500 ppm, 4000 ppm, 5500 ppm, 7000 ppm; including neutral concentration of K-IBA = 0ppm), (iii) two different rooting systems (bench and root trainer). In total 3000 axillary shoot multiplications were used for this experiment (2400 axillary shoots for test propagation and 600 axillary shoots as neutral markers – Suppl. material 1) in order to develop a protocol for the *in vivo* germination of *C. sempervirens* f. *sempervirens* with cost- and time-efficient propagation.

Statistical analyses

For the statistical analyses the rooting shoot development (number of shoots/explant) was recorded after the elongation stage for each tested parameter (treatments) and by first removing from the analysis the samples that were dipped into the solution without K-IBA (neutral samples: 600 axillary shoots). The *T-test* and analysis of variance (ANOVA) were used for statistical analysis of the results, and in order to check the statistical signal among the different parameters. The statistical analyses were carried out using the IBM SPSS Statistics 20 software.

Data outcomes

The *T-test* was used for comparing the mean value of successful rooting of the different cuttings of sampled shoots, between the two different rooting systems (bench and root trainer). This test detected statistically significant differentiation between rooting bench (M= 0.20; SD= 0.401) and root trainer (M= 0.17; SD= 0.37), with the mean difference being equal to 0.32 (t= 2.7, df= 2398, F= 18.7, p<0.01). Thus, the tested cuttings had slightly higher (but significant) amount of rooting in bench than in root trainer (Suppl. material 2). ANOVA was used to detect the differences among treatments (culture initiation and concentration of K-IBA) in successful rooting of shoot cuttings (Suppl. material 3). Statistically significant differences among the treatments were tested with the Tukey test at 0.95 confidence level, and the homogeneity of variance test was tested (showing non-significant level statistic). The ANOVA distinguished that the rooting percentage differed significantly among the three different parts of the shoot cuttings (basal, middle and terminal) (F= 15.348; p<0.05) with the basal cutting recording the highest rooting ability and the middle cutting recording the lowest percentage of rooting (Fig. 1). The concentration of K-IBA was also a critical point for the success of *C. sempervirens* f. *sempervirens* propagation. In addition, the ANOVA detected significant differentiation among the different K-IBA concentrations (F= 33.810; p<0.05) regarding the mean value of cuttings that rooted. More specifically, the concentration of K-IBA of 5500 ppm was the one

with the highest presentence of rooting success compared to the other concentrations (Fig. 2).

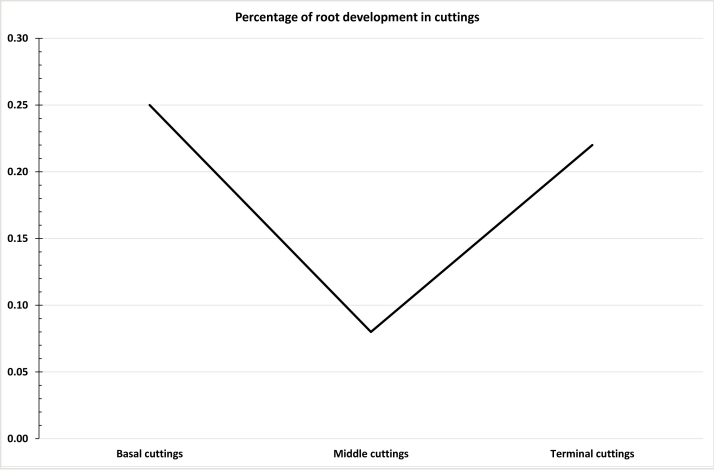

Figure 1. doi

Effectiveness of shoots rooting according to the sampled part from each shoot (basal, middle and terminal cutting).

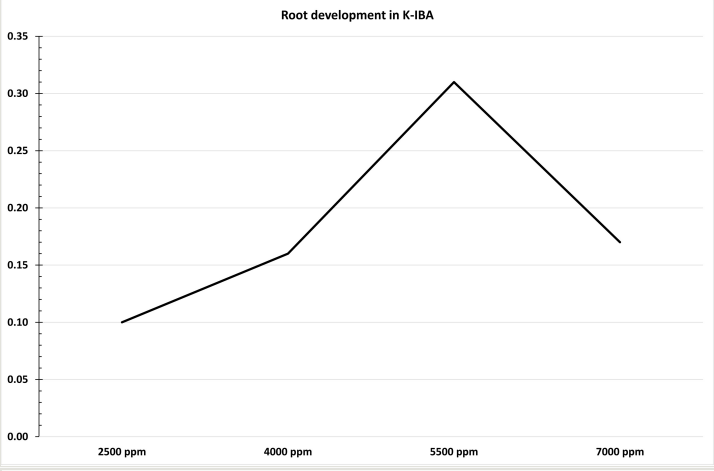

Figure 2. doi

Effectiveness of shoots rooting after dipping in different concentrations of K-IBA.

Remarkable is the fact that the survival percentage of new plantlets under the environmental adaptation (acclimatization) in growing conditions outside the green house was: (i) 99% for the first year and (ii) for the second year all (100%) of the survived plantlets of the first year. The acclimatization was carried out in Athalassa Forest Nursery, Cyprus Department of Forests, and followed all necessary maintenance steps for plants in a nursery (watering, manuring, etc.).

Discussion

Today, the expanding of knowledge in the area of developmental tree physiology, in order to improve propagation systems, is important for several purposes in the context of propagation population, clonal forestry, and for conservation purposes, as well as for use of tree species with specific shapes and growth characteristics in urban forestry (and in agriculture for windbreaks). In all these cases, the purpose of vegetative propagation is to produce clones of elite trees that have been selected from populations of genetically variable siblings (Wilhelm 2005). Thus, the vegetative propagation is essentially the reproduction of plant material in a way in which the offspring contains the genetically determined characteristics of the parent plant. For conservation efforts, propagation could support the maintenance and preservation of selected genotypes and/or phenotypes with proved resistance to disease, or with desirable crown shape. These arguments, as mentioned above, become more important in the case where serious constraints lead to the delay or failure of seeds to germinate in the nursery and/or where there is a need for mass production of plantlets for a specific plant species.

In the case of *C. sempervirens* previous studies reported protocols based on micropropagation methods of tissues (especially shoots) (e.g. Spanos et al. 1997, Giovanelli and de-Carlo 2007) for the mass-propagation of this target species. The current protocol adopted the most frequently used method for vegetative multiplication of the cutting propagation (macropropagation). Thus, the outcomes from this study come to support previous general guides about the propagation of plant species, and at the same time to argue that the macropropagation of *C. sempervirens* stem cuttings can be successfully implemented. The present sampling method allowed a flexibility with the handling of sampled shoots, because sampling was carried out during late winter to early spring, on an evergreen hardwood, and since cuttings were taken from stock plants from well-ripened, vigorous one-year-old shoots. In such case the shoot is dormant and thus there is considerably more flexibility with its handling (Wilhelm 2005). The semi-ripe wood and evergreen hardwood propagation is an important method for many conifers and broadleaved evergreen species for their cutting propagation (Wilhelm 2005).

A critical point of propagation is root formation, where for this formation, auxins have a central role. Despite the general assumption that different species exhibit varying rooting success for cuttings taken from basal, middle and terminal cuttings (parent stem) (Wilson 1993), more recent studies support more specific features of auxins. In practice, when a shoot is cut, auxins, which are produced in the shoot apex, accumulate at the shoot base to induce rooting (Wilhelm 2005). In this study the impact of this physiology mechanism is obvious, since cutting from the basal and the terminal of shoot showed higher ability of rooting than cutting from the middle. Hence, cutting from the basal and the terminal of a single shoot of *C. sempervirens* could hasten root initiation, increase the number and quality of roots and encourage uniformity of rooting. Besides, the observation from this study supports the argument that rooting percentage of cuttings was increased by specific auxin treatment (e.g. Assareh and Sardabi 2005, Kipkemoi et al. 2013), in this case the optimum concentration of K-IBA. On the other hand, the support of bench as rooting

system (perlite with pitmus substrate in a 4:1 ratio), ensures the low cost for rooting, the high percentage and the quality of root cuttings, from this protocol. This observation is in accordance with the assumption that optimum propagation medium should provide sufficient porosity to allow good aeration and this ensures adequate oxygen availability for the developing roots system (Hartmann et al. 2002).

The protocol and workflow (Fig. 3) presented in this study, are directly applicable for nurseries interested in the quantitative reconstruction of mass-propagation of *C. sempervirens* f. *sempervirens*. It is particularly useful at smaller nurseries with limited funding and personnel, or those without laboratories or facilities for applying micropropagation (*in vitro*). Streamlining tasks and workspaces improved the speed at which specimens were being processed and increased the flow of data, while minimizing the perdition of cutting materials. In addition, simplifying the workflow improved outcomes, as this is supported by the survival of produced clones (plants) in the acclimatization stage (100% survival after the second year). The proposed propagation protocol could be adopted or used as the baseline knowledge for the massive propagation of other species of the Cupressaceae family.

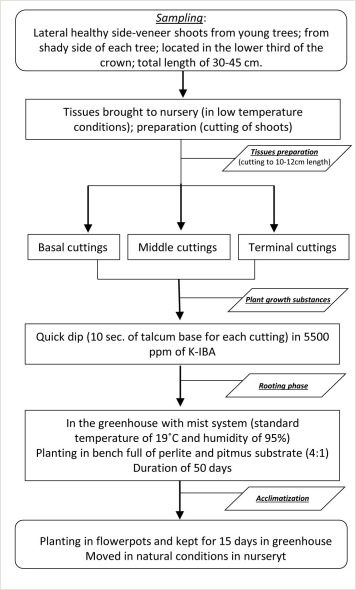

Figure 3. doi

Generalized workflow for the new propagation protocol of *C. sempervirens* f. *sempervirens*.

Conclusions

As mentioned above, *C. sempervirens* is resilent to extreme climate and soil conditions in the Mediterranean region. Thus, the species is highly preferred for several restoration purposes. In all cases, plants macropropagation provides a mass production of plantlets,

whilst maximising efficient plantlets multiplication on site, operating in a non-aseptic environment, reducing labour requirements and overcoming a lack of plant stock material at the industrial site (Hartmann et al. 2002, Woods and Woods 2006). However, a key point for plantlets mass propagation by using the macropropagation method is the development of specific protocols with clear statements, as this is suggested in the present study for *C. sempervirens*.

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Author contributions

C. Pericleous contributed to the fieldwork and the protocol development. N.-G. H. Eliades carried out the data analyses. Both authors contributed to the manuscript writing.

References

- Assareh MH, Sardabi H (2005) Macropropagation and micropropagation of *Ziziphus spina-christi* . Pesquisa Agropecuária Brasileira 40 (5): 459-465. https://doi.org/10.1590/s0100-204x2005000500006
- Bagnoli F, Vendramin GG, Buonamici A, Doulis AG, González-Martínez SC, La Porta N, Magri D, Raddi P, Sebastiani F, Fineschi S (2009) Is *Cupressus sempervirens* native in Italy? An answer from genetic and palaeobotanical data. Molecular Ecology 18 (10): 2276-86. https://doi.org/10.1111/j.1365-294X.2009.04182.x
- Caudullo G, de-Rigo D (2016) *Cupressus sempervirens* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz J, de-Rigo D, Caudullo G, Durrant T, Mauri A (Eds) European atlas of forest tree species. Publication Office of the European Union, Luxembourg, 88-89 pp.
- Eckenwalder J (2009) Conifers of the World: The complete reference. Timber Press, London.
- Farjon A (2010) A handbook of the world's conifers. Brill, Leiden, Boston. https://doi.org/10.1163/9789047430629

- Farjon A, Filer D (2013) An Atlas of the world's conifers: An analysis of their distribution, biogeography, diversity and conservation status. Brill, Leiden, Boston. https://doi.org/10.1163/9789004211810
- Fossi D, Lipucci D, Tognoni F (1981) Induzione in vitro di gemme ascellari della specie *Cupressus sempervirens* L. Rivista di Ortoflorofrutticoltura Italiana 65: 293-299.
- Franco E, Schwarz J (1985) Micropropagation of two tropical conifers: *Pinus oocarpa* Schiede and *Cupressus lusitanica* Miller. In: Henke R, Hugies K, Constantin M, Hollaender M (Eds) Tissue culture in forestry and agriculture. Plenum Press, New York, 195-213 pp. https://doi.org/10.1007/978-1-4899-0378-5_15
- Giovanelli A, de-Carlo A (2007) Micropropagation of Mediterranean cypress (*Cupressus sempervirens* L.). In: Jain S, Häggman H (Eds) Protocols for Micropropagation of woody trees and fruits. Springer, Dordrecht, The Netherlands, 93-105 pp. https://doi.org/10.1007/978-1-4020-6352-7_9
- Hadjikyriakos G (2007) Aromatic and spicy plants in Cyprus - From antiquity to the present day. Bank of Cyprus Cultural Foundation, Nicosia, Cyprus. [ISBN 978-9963-42-852-6]
- Harrison-Murray RS, Thompson R (1988) In pursuit of minimum stress environment for rooting leafy cuttings: comparison of mist and fog. Acta Horticulturae 227: 211-216. https://doi.org/10.17660/actahortic.1988.227.34
- Hartmann H, Kester D, Davies F, Geneve R (2002) Plant propagation, principles and practices. Prentice Hall, New Jersey.
- Hřib J, Dobrý J (1984) An explant culture of Tassilian cypress *Cupressus dupreziana* (A. Camus). Forest Ecology and Management 8: 235-242. https://doi.org/10.1016/0378-1127(84)90056-2
- Kipkemoi N, Kariuki N, Wambui N, Justus O, Jane K (2013) Macropropagation of an endangered medicinal plant, *Strychnos henningsii* (Gilg), (Loganiaceae) for sustainable conservation. Journal of Medicinal Plant Research 2: 247-253.
- Korakis G (2015) Forest botany. Hellenic Academic Ebook, Athens, 619 pp. [In Greek]. [ISBN 978-960-603-282-0]
- Kumar N, Reddy M (2011) In vitro plant propagation: A Review. Journal of Forest Science 27: 61-72.
- Lambardi M, Ozudogru E, Jain S (2013) Protocols for micropropagation of selected economicallyiImportant horticultural plants. Humana Press, Springer Science+Business Media, New York. https://doi.org/10.1007/978-1-62703-074-8
- López-Sáez JA, Glais A, Tsiripidis I, Tsiftsis S, Sánchez-Mata D, Lespez L (2019) Phytosociological and ecological discrimination of Mediterranean cypress (*Cupressus sempervirens*) communities in Crete (Greece) by means of pollen analysis. Mediterranean Botany 40 (2): 145-163. https://doi.org/10.5209/mbot.59789
- Mateja Š, Dominik V, Franci Š, Gregor O (2007) The effects of a fogging system on the physiological status and rooting capacity of leafy cuttings of woody species. Trees 21 (4): 491-496. https://doi.org/10.1007/s00468-006-0121-z
- Mesén F, Leakey R, Newton A (2001) The influence of stockplant environment on morphology, physiology and rooting of leafy stem cuttings of *Albizia guachapele* . New Forests 22: 213-227. https://doi.org/10.1023/A:1015668011884
- Moe R, Andersen A (1988) Stock plant environment and subsequent adventitious rooting. In: Davis T, Haissig B, Sankhla N (Eds) Adventitious root formation in cutting. Dioscorides Press, Oregon, 214-234 pp.

- Santini A, Donardo V (2000) Genetic variability of the 'bark canker resistance' character in several natural provenances of *Cupressus sempervirens* . Forest Pathology 30 (2): 87-96. https://doi.org/10.1046/j.1439-0329.2000.00188.x
- Spanos K, Pirrie A, Woodward S (1997) Micropropagation of *Cupressus sempervirens* L. and *Chamaecyparis lawsoniana* (A. MURR.) PAR. Silvae Genetica 46: 87-96.
- Spethmann W (1997) Autovegetative Gehölzvermehrung. In: Krüssmann G (Ed.) Die Baumschule. Parey Verlag, Berlin, 382-449 pp.
- Tutin T, Heywood V, Burges N, Moore D, Valentine D, Walters S, Webb D (1993) Flora Europaea. I. Cambridge University Press, Cambridge.
- Wilhelm E (2005) Micro- and macropropagation of forest trees. In: Geburek T, Turok J (Eds) Conservation and Management of Forest Genetic Resources in Europe. Arbora Publishers, Zvolen, 623-650 pp.
- Wilson PJ (1993) Propagation characteristics of *Eucalyptus globulus* Labill. ssp. *globulusstem* cuttings in relation to their original position in the parent shoot. Journal of Horticultural Science 68 (5): 715-724. https://doi.org/10.1080/00221589.1993.11516404
- Woods S, Woods J (2006) Methods and apparatus for the micro-and macropropagation of reed grasses. US Patent 7,052,912 B1, US Patent, US.
- Yasodha R, Sumathi R, Gurumurthi K (2004) Micropropagation of quality propagule production in plantation forestry. Indian Journal Biotechnology 3: 159-170.
- Zacharis A (1977) Forests of Crete from Antiquity to today. Ministry of Agriculture, Athens.

Supplementary materials

Suppl. material 1: Number of shoots per tested parameters on axillary shoots proliferation in *C. sempervirens* f. *sempervirens*. doi

Authors: Constantinos Pericleous & Nicolas-George Homer Eliades
Data type: Table of data
Download file (606.90 kb)

Suppl. material 2: Results of T-test between independent samples of rooting shoots in bench and root trainers. doi

Authors: Constantinos Pericleous & Nicolas-George Homer Eliades
Data type: Table of Data
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Suppl. material 3: Full package of ANOVA based on the different treatments applied for propagation of *C. sempervirens* f. *sempervirens*. doi

Authors: Constantinos Pericleous & Nicolas-George Homer Eliades
Data type: Table of Data
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