Bertsouklis K *et al.* (2023)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 51, Issue 1, Article number 13062
DOI:10.15835/nbha51113062
Research Article

In vitro regeneration from adult node explants of *Juniperus oxycedrus*

Konstantinos BERTSOUKLIS*, Angeliki T. PARASKEVOPOULOU, Evdoxia PETRAKI

*Agricultural University of Athens, School of Plant Sciences, Department of Crop Science, Laboratory of Floriculture and Landscape Architecture, Iera Odos 75, 11855 Athens, Greece; kber@aua.gr (*corresponding author); aparas@aua.gr; evdopetraki@gmail.com*

Abstract

In the present study the *in vitro* propagation from adult native plants of *Juniperus oxycedrus* was investigated. The effect of seasonal explant collection (spring, summer, winter), in varying media and cytokinin type were examined. The decontamination process of explants taken in the summer was more efficient than that of the other seasons (57.3% of the explants survived). In the spring a low performance in shoot formation took place both on hormone free Murashige and Skoog (MS) media and Rugini Olive Medium (OM) or each containing 6-benzyladenine (BA) at 1.0 mg L^{-1} by which shoots did not elongate more than 0.5 cm. In the summer OM was used, and a higher *in vitro* performance was observed on medium containing 0.1 mg L^{-1} 6-(γ,γ-Dimethylallylamino)purine (2iP); the shoot formation percentage was 72.0%, and respectively 3.1 shoots, 1.4 cm in length, were formed. In the winter, the shoot formation percentage was 92.0-100.0%, and respectively 3.9 shoots of 0.4 cm length were formed on medium containing 1.0 mg L^{-1} 2iP. The multiplication phase on cytokinin-containing media led to a satisfactory proliferation rate in terms of shoot formation percentage and shoot number. The higher shoot number (3.3) was recorded on media containing 2iP at 1.0 mg L^{-1}, while the shoot length was longer (2.0) cm, on hormone-free media. Initiation of roots was not observed during the rooting phase on hormone-free half-strength OM, or supplemented with 1.0 and 2.0 mg L^{-1} IBA. Spontaneous rooting at a low percentage (10.0%) was observed on OM media supplemented with 0.1 mg L^{-1} 2iP and successful acclimatisation took place on a 1:1 (v/v) ratio of peat to perlite substrate.

Keywords: Cupressaceae; cytokinins; explant decontamination; olive medium; 2iP

Introduction

The genus *Juniperus* (f. Cupressaceae) family includes 67 species constituting it the second-largest genus of conifers; its members are native mainly to the northern hemisphere (Adams, 2004). There are eight species in Greece: *Juniperus drupacea*, *J. communis, J. oxycedrus, J. macrocarpa, J. phoenicea, J. foetidissima, J. excelsa,* and *J. sabina*. *J. oxycedrus*, commonly known as 'prickly juniper', is also known by the name 'cade' or 'sharp cedar'. It is a composing species of the Mediterranean sclerophyll scrublands, spreading around the Mediterranean Sea over to Caucasus and Iran at altitudes ranging from sea level to 2,200 m asl. It can be found on dry, rocky slopes, sand dunes, and at higher altitudes in pastures, that are usually overgrazed (Farjon, 2010). *J. oxycedrus* has been assessed for The IUCN Red List of Threatened Species in 2011 and is listed as Least

Received: 06 Jan 2023. Received in revised form: 20 Jan 2023. Accepted: 07 Feb 2023. Published online: 22 Feb 2023.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Concern (Farjon, 2013). The form of the plant may vary from a shrub to a small conical-shaped tree up to 12.0 m in height, having branches that hang down to the ground. The colour of its bark is gray-brown and its leaves are needle-shaped, hard, without articulation, and arranged in whorls of threes. It is a dioecious plant with monogenic flowers that grow in the axils of the leaves. The seed cones are berry-like, with a diameter of up to 12.0 mm, and usually contain two or three seeds (Korakis, 2015).

Essential oils are extracted from the *Juniper* berries and are used by the pharmaceutical industry for their beneficial properties (Unlu *et al.*, 2008; Ahani *et al*., 2013; Al-Ramamneh *et al*., 2017), and by the distillery and perfume industry as flavor and aroma compounds respectively (Ilashi, 1986), while the *Juniper* forests are exploited for timber (Berhe and Negash, 1998).

Regarding the use of conifers in the urban and suburban landscape, it is mentioned that they possess particular traits and ornamental forms suitable for landscaping (Torchik, 2010). Additionally, coniferous, forest, woody species used for ornamental purposes can provide significant ecosystem services enhancing biodiversity conservation and facilitating pest control (de Foresta *et al.*, 2013). The hardy nature and drought tolerance of *Juniperus* species constitute it of high ornamental value for xeriscaping, coastal planting, and regenerating degraded soil areas, as it is suitable for areas threatened by soil erosion (Ceballos and Ruis, 1971). Many *Juniperus* species have a limited reproductive capacity, with low seed production as well as decreased seed viability (Garcia, 2001; Ferradous *et al*., 2013); this may be due to reduced pollination, low viability of pollen, as well as local prevailing harsh environmental conditions (Ortiz *et al*., 1998; Arista *et al*., 2001). Furthermore, often dispersed seeds (by gravity, surface runoff, and animals) are deposited in areas with unfavorable seed germination conditions (Ortiz *et al*., 1998; Chambers *et al*., 1999). All of the above-mentioned factors as well as overgrazing, fires, and human activity, have led to the reduction of *Juniperus* natural habitats, of which some species are already listed as rare or endangered (Hazubska-Przybył, 2019). A variety of nutrient media, explant types, auxin and cytokinins have been used for the *in vitro* propagation of different *Juniperus* species (Gómez and Segura, 1995; Loureiro *et al*., 2007; Kocer *et al*., 2011; Khater and Benbouza, 2019; Bertsouklis *et al*., 2020) indicating the difficulty and need to meet certain particularities for their micropropagation. *In vitro* propagation could enhance the mass production and spread of *J. oxycedrus.* To our knowledge, there is one study on axillary shoot proliferation of *J. oxycedrus* on modified Schenk and Hildebrandt medium (SH), (Gómez and Segura, 1995), showing a low *in vitro* performance.

Therefore, this study aims to examine the effect of seasonal explant collection (spring, summer, winter) in varying media (Murashige and Skoog and Rugini Olive Medium) and cytokinin types (BA, 2iP and KIN) with the potential to enhance *in vitro* performance of *J. oxycedrus* contributing to its preservation and protection as well as its use as an ornamental plant in arid and semi-arid or degraded Mediterranean landscapes.

Materials and Methods

Plant material

Nodal explants were excised from the apical part of actively growing stems of an adult tree growing in the wild (Marathon, Attica, Greece) in May (spring), June (summer), and January (winter) 2020. Following excision, explants well washed under running tap water, were surface-sterilized with absolute alcohol solution (90.0%, v/v) for 10 sec and then with 30.0% (v/v) commercial bleach (4.6% w/v sodium hypochlorite), supplemented with 0.1% Tween-20 for 10 min. Afterwards they were rinsed with sterile distilled water for 3 min, three times.

Establishment and multiplication

The establishment of the initial cultures was examined in three seasons (spring, summer and winter) and took place on either Murashige and Skoog (MS) (Murashige and Skoog, 1962) or Rugini Olive Medium (OM) (Rugini, 1984) media. MS was used for the spring cultures, while OM was used for the spring, summer and winter cultures. In more detail, the spring establishment experiment on MS and OM was either hormone free (Hf) or supplemented with 1 mg L^{-1} 6-benzyladenine (BA); the summer establishment experiment, on OM media was either Hf or supplemented with 0.1 mg L^{-1} 6-(γ,γ-Dimethylallylamino) purine (2iP); and the winter establishment experiment, on OM media was either Hf or supplemented with either 1.0 mg L^{-1} 2iP, or with 1.0 mg L^{-1} kinetin (KIN). The initial cultures contained 48-96 explants / replicates per treatment.

On the other hand, the multiplication phase examined a total of three subcultures on OM media: hormone-free, or supplemented with BA (1.0 mg L^{-1}) or 2iP (0.1 and 1.0 mg L^{-1}). The multiplication phase subcultures each contained 20-68 explants per treatment.

In-vitro culture conditions and data collection

Initial cultures took place in 50.0 ml cylindrical test tubes (diameter 25.0 mm and height 10.0 cm), filled with 10.0 ml media and contained one explant per tube. On the other hand, sub-cultures and rooting experiments took place in 100.0 mL Sigma vessels, sealed with Magenta B-caps, that were filled with 25.0 ml media, and contained four explants or micro-shoots per vessel. The cultures were incubated at 25.0±2.0 °C, with a 16-h photoperiod at 37.5 μmol m^{-2} s^{-1} provided by cool-white, fluorescent lamps. All the media contained 30.0 g L^{-1} sucrose, were solidified with 8.0 g L^{-1} agar, and their pH was adjusted at 5.7-5.8 before agar addition and autoclaving at 121.0 °C for 20 min. Data on the survival, infection, and damage of the cultures as well as the effectiveness of the decontamination process were collected after 7 days of culture. Data on shoot proliferation percentages, shoot numbers per explant and shoot lengths were collected 60 days after the culture, according to Gómez and Segura (1995). The "multiplication index" (MI) was calculated by multiplying the percentage of explants that produced shoots by the mean number of shoots per responding explant, then dividing by 0.6 (the length of each explant used for sub-culture) to obtain the proliferation potential of the cultures.

Rooting and ex vitro acclimatisation

Half-strength OM (OM/2), supplemented with indole-3-butyric acid (IBA) at 1.0 and 2.0 mg L^{-1} was used in the rooting stage. Twenty-four micro-shoots were cultured into each of the three different rooting OM/2 media. 1.5-2.5 cm long, thoroughly rinsed under running tap water to remove the medium were transferred to 500.0 mL plastic containers (8 plantlets/container, 2 containers) filled with a soilless substrate composed of peat (pH 5.5-6.5, Klasmann-Delimann GmbH, Geeste, Germany) and perlite (particles diameter 1.0-5.0 mm, Perloflor, Isocon S.A., Athens, Greece) at a 1:1 (v/v) ratio. The containers were covered with transparent plastic wrap to maintain humidity, and placed for one week in a growth chamber at 25±2.0 °C and photoperiodic lighting {16 h cool white fluorescent light (37.5 μmol.m^{-2} s^{-1}):8 h dark}. Next, containers were uncovered and transferred to a bench in a heated glasshouse of the Laboratory of Floriculture & Landscape Architecture of the Agricultural University of Athens (lat. 37°58′58.0″ N, and long. 23°42′19.2″ E) and 14 days later, data on the acclimatisation of the plantlets were recorded.

Statistical analysis

The experiment followed the completely randomized design method, and the significance between treatments was by one-way analysis of variance (ANOVA) (JMP 14.0 software, SAS Institute Inc., Cary, NC, USA, 2013). Data on percentages were arcsine-transformed before statistical analysis was performed. The treatment means were compared using Student's t-test at $P < 0.05$ (JMP 14.0 software, SAS Institute Inc., Cary, NC, USA, 2013). Concerning, the multiplication phases the collected data from the three subcultures were pooled together. Note that the number of replicates per treatment ('*n*') differed between experiments and are stated in the tables presented.

Results and Discussion

Culture establishment

The season of explant collection can play a pivotal role in the *in vitro* establishment of initial cultures starting from adult mother plants. In the present study, summer explant collection showed to have the best response to the decontamination process followed, and the least percentage of infection compared to spring and winter explant collections. More specific, the survival explants percentage was 57.3% for summer establishment, followed in descending order by 29.9% for corresponding winter and 16.7% for corresponding spring establishment (Figure 1). The seasonal effect of explant collection has been reported for many other woody species having an important role in aseptic culture establishment (Kumar *et al*. 2005; Kartsonas and Papafotiou 2007; Wang and Yao, 2017) as shown similarly in the current study. In future studies, the use of a fungicide solution prior to applying the sodium-hypochlorite solution could possibly enhance the efficiency of the decontamination process as has been reported for other *Juniperus* species (Loureiro *et al.* 2007; Baravardi *et al*. 2014).

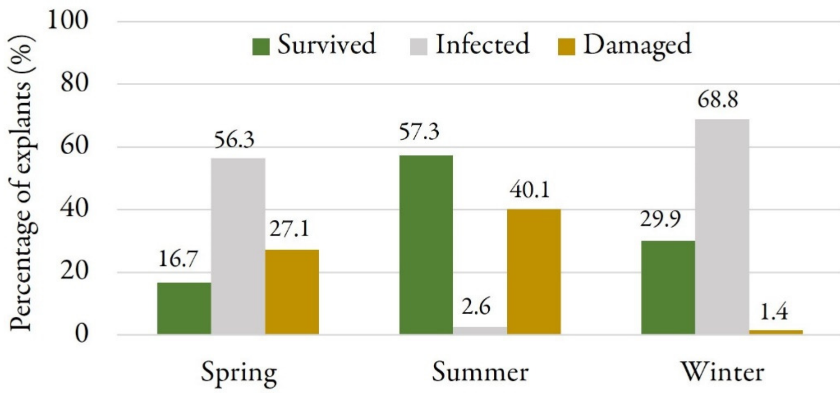

Figure 1. Percentage (%) of survival, infection, and damage of *Juniperus oxycedrus* explants recorded 7 days after the decontamination process, using 90.0% (v/v) ethanol for 10 sec, followed by 30.0% sodium hypoclorite supplemented with 0.1% Tween-20 for 10 min, and finally rinsed with sterile distilled water for 3 min, three times (*n*=48-96).

In the spring the initiated shoots showed stunted growth in line with Gómez and Segura (1995). MS medium was rated as insufficient in terms of shoot formation; none shoot formation was observed on hormone free MS medium and relatively small percentage of shoot formation (40.0%) was observed on MS media containing 1.0 mg L^{-1} BA. Despite a relatively large number of shoots/explant (5.8 shoots/explant) were formed on MS containing BA, these shoots were too short (0.2 cm) (Table 1). On the other hand, OM media demonstrated a slightly higher performance in terms of shoot formation and shoot length (0.5 cm) which was used for the summer and winter *in vitro* establishment. The inefficiency of MS media has also been demonstrated for the *in vitro* propagation of *Juniperus phoeniceae* (Loureiro *et al*., 2007; Bertsouklis *et al*., 2020).

Higher shoot formation percentages (92.0%-100.0%) were observed in the winter, on OM media without hormone or supplemented with either 1.0 mg L^{-1} 2iP or KIN in line with Gómez and Segura (1994) (Table 1). The highest multiplication index (5.2) was recorded in the summer, on OM supplemented with 0.1

(mg L^{-1}) 2iP (Table 1); although the corresponding shoot formation percentage in the summer (72.0%) was less compared to winter, both the number of shoots formed (3.1) and shoot length (1.4 cm) were satisfactory (Figure 2A, Table 1). Gómez and Segura (1995) found higher shoot formation percentages (100.0%) on modified Schenk en Hildebrandt (SH) media (Schenk and Hildebrandt, 1972), and with non-significant influence of the explant collection season on both the shoot formation percentages and the number of shoots formed per explant (1.6 to 2.0).

Table 1. The combined effect of seasonal explant collection and media on the *in vitro* initial culture shoot proliferation of *Juniperus oxycedrus* explants

Season	Media[1]	Shoot formation (%)	Shoot number	Shoot length (cm)	MI[2]
Spring	OM	50.0 b	2.3 c	0.5 b	0.9 b
	OM +1 mg L^{-1} BA	44.0 b	4.3 b	0.3 b	0.9 b
	MS	-	-	-	-
	MS +1 mg L^{-1} BA	40.0 b	5.8 a	0.2 b	0.8 b
Summer	OM	46.0 b	2.1 c	1.2 a	1.9 b
	OM +0.1 mg L^{-1} 2iP	72.0 ab	3.1 c	1.4 a	5.2 a
Winter	OM	92.0 a	2.6 c	0.6 ab	2.4 ab
	OM +1 mg L^{-1} 2iP	100.0 a	3.9 b	0.4 b	2.6 ab
	OM +1 mg L^{-1} KIN	95.0 a	3.0 c	0.5 ab	2.4 ab
$F_{\text{one-way ANOVA}}$		*	***	**	**

[1]MS: Murashige and Skoog; OM: Olive Medium; BA, 2iP and KIN: cytokinins BA, 2iP and KIN respectively.
[2]MI: multiplication index = shoot formation (%) × mean shoot number per explant × mean shoot length/0.6. *, **, ***: denote significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. Differences between means within columns are shown with different letters (Student' s t test, P <0,05), *n*= 24-48.

Multiplication phase

Based on the reduced performance of MS media compared to OM in the *in vitro* initial culture of *J. oxycedrus* explants, only OM media were examined during the multiplication phase. These findings are in accordance with Khater and Benbouza (2019) who reported that after two weeks on MS, during the second subculture of *Juniperus thurifera* L. an initial basal browning gradually progressing to the necrosis of the entire stem length was observed. Therefore, the effect of OM without hormone or with BA and 2iP were examined. The proliferation rate in terms of shoot formation percentage was greater on OM supplemented with either 2iP or BA (81.0%-100.0%), while in terms of shoot number was greater on OM supplemented with 2iP at 1.0 mg L^{-1} (Table 2, Figure 2B). On the other hand, the longest shoots (2.0 cm) were observed on OM hormone-free media. During the multiplication phase, an increase in shoot length compared to the establishment phase was observed (Table 1, Table 2). Micro-shoots produced during the multiplication phase on OM media containing 2iP were vigorous and well-formed compared to the micro-shoots on BA media that showed, progressive loss of morphogenetic potential (data not shown), in line with Gómez and Segura (1995) who also reported the development of hyperhydration of *J. oxycedrus* micropropagated shoots.

Table 2. The effect of Olive Medium media, hormone free or supplemented with cytokinins (BA at 1.0 mg L^{-1}or 2iP at 0.1 or 1.0 mg L^{-1}) on the shoot proliferation of *Juniperus oxycedrus* explants

Cytocinin[1]	Concentration (mg L^{-1})	Shoot formation (%)	Shoot number	Shoot length (cm)	MI[2]
Hormone free	0	50.0 b	1.8 b	2.0 a	3.0 a
BA	1.0	100.0 a	1.3 b	1.2 b	2.6 a
2iP	0.1	98.0 a	1.9 b	1.1 bc	4.2 a
2iP	1.0	81.0 a	3.3 a	0.8 c	3.0 a
$F_{\text{one-way ANOVA}}$		**	***	***	ns

[1]BA, 2iP and KIN: cytokinins BA, 2iP and KIN respectively. [2]MI: multiplication index = shoot formation (%) × mean shoot number per explant × mean shoot length/0.6. ns, *, **, ***: denote non signicance at $P < 0.05$ or significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. Differences between means within columns are shown with different letters (Student' s t test, P <0,05), n=20-68.

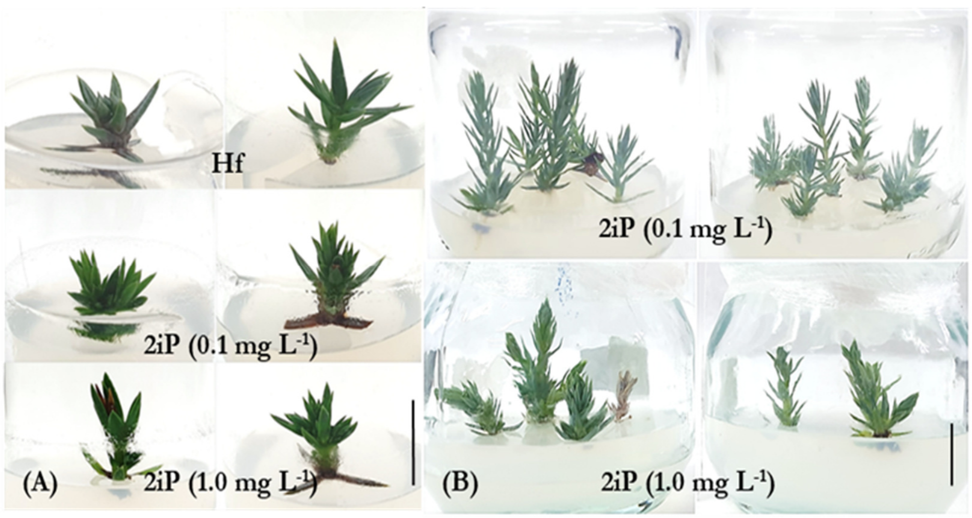

Figure 2. The establishment phase of initial cultures of *Juniperus oxycedrus* explants collected from adult plants on Olive Medium (OM) (A) and the corresponding multiplication phase on OM medium (B). The vertical bar represents the length of 1.0 cm.

Rooting phase and ex vitro acclimatisation

Initiation of roots was not shown on OM/2 that was hormone free or contained IBA at 1.0 and 2.0 mg L^{-1}. These findings are in accordance with Brito (2000) and Bertsouklis *et al.* (2020) who found that several *Juniperus phoenicea* genotypes had been recalcitrant to rooting. *In vitro* rooting is a challenge concerning the members of the Cupressaceae f. (Negussie, 1997; Oliveira *et al.*, 2003) making it difficult to develop commercially efficient *in vitro* protocols. Spontaneous rooting took place in subsequent subcultures on OM media supplemented with 0.1 mg L^{-1} 2iP by which micro-shoots rooted at 10.0% (data non shown, Figure 3).

Figure 3. Spontaneous rooted micro-shoots of *Juniperus oxycedrus* 60-days old (A) and plantlets established on peat:perlite (1:1 v/v) substrate at the end of the acclimatization process (B).
Bars represent the length of 1.0 cm

The later results agree with Gómez and Segura, (1994) who also reported spontaneous rooting of the species *J. oxycedrus*. After three weeks in the acclimatisation phase, the survival rate was 100.0%, while two to three well-formed, relatively thick, and approximately 2.0-3.0 cm in length roots, were formed. The high acclimatisation rate of *J. oxycedrus* in the present study, is promising for establishing an efficient *in vitro* propagation method compared to other *Juniperus* species, such as *Juniperus thurifera* L. which demonstrated a relatively low survival rate (≤ 50.0%), of *in vitro* rooted micro-shoots transplanted into peat:perlite mixture (1:1, v/v ratio) (Khater and Benbouza, 2019). Low rooting percentages of *J. oxycedrus* (7.0-10.0 %) have been reported also by Gómez and Segura (1995) on media supplemented with IBA with non-significant differences from other auxins or auxin combinations tested. Gómez and Segura (1994) established high rooting percentages with adventitious shoots regenerated from leaves of mature *J. oxycedrus* suggesting that the micropropagation method affected the rooting ability of the regenerated shoots based on a rejuvenation process observed in other woody species.

Conclusions

J. oxycedrus is currently under threat, with several desirable characteristics such as drought tolerance and possible application as an ornamental plant species. *In vitro* propagation constitutes an effective method to promote the protection, reintroduction, and restoration of threatened species, and studies on *in vitro* propagation of *J. oxycedrus* is limited. The study herein examined the potential to advance information on *in vitro* performance of *J. oxycedrus* contributing to its preservation and protection. The study showed that summer explant collection generated in the establishment phase of the *in vitro* propagation of *J. oxycedrus*, a satisfactory multiplication rate on OM containing 0.1 mg L^{-1} 2iP, and concomitantly the most effective disinfection process. The use of OM containing 0.1 mg L^{-1} 2iP also proved to be sufficient for the multiplication stage. Further research is needed to examine the possibility to facilitate and increase the percentage of rooting induction of *J. oxycedrus* micro-shoots. Despite the shown low percentage of rooted *J. oxycedrus* micro-shoots in this study, the survival of plantlets in the acclimatisation phase was successful and is promising for contributing to the preservation and protection of *J. oxycedrus* as well as its use as an ornamental plant in arid and semi-arid, or degraded Mediterranean landscapes.

Authors' Contributions

Conceptualization: K.B. and A.T.P.; Data curation: K.B., A.T.P. and E.P.; Formal analysis: K.B., A.T.P. and E.P.; Investigation: K.B. and E.P.; Methodology: K.B. and A.T.P.; Supervision; K.B., and A.T.P.; Visualization: K.B., A.T.P. and E.P.; Writing - original draft: K.B. and A.T.P.; Writing - review and editing: K.B. and A.T.P. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

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

References

Adams RP (2004). *Junipers* of the World: the genus *Juniperus*. Trafford Publishing Co., Bloomington, Indiana.

Ahani H, Jalilvand H, Hosseini Nasr SM, Soltani Kouhbanani H, Ghazi MR, Mohammadzadeh, H (2013). Reproduction of juniper (*Juniperus polycarpos*) in Khorasan Razavi, Iran. Forest Science and Practice 15:231-237. *https://doi.org/10.1007/s11632-013-0307-6*

Al-Ramamneh EAD, Daradkeh N, Rababah T, Pacurar D., Al-Qudah M (2017). Effects of explant, media and growth regulators on *'in vitro'* regeneration and antioxidant activity of *Juniperus phoenicea*. Australian Journal of Crop Science 11(7):828-837. *https://doi.org/10.21475/ajcs.17.11.07.pne479*

Arista M, Ortiz PL, Talavera S (2001). Reproductive cycles of two allopatric subspecies of *Juniperus oxycedrus* (Cupressaceae). Flora 196(2):114-120. *https://doi.org/10.1016/S0367-2530(17)30026-9*

Baravardi H, Ranjbar GA, Kamali S, Abadi F (2014). Investigation of the effects of growth regulators on callus induction in *Juniperus excelsa* L. Bulletin of Environment, Pharmacology and Life Sciences 4(1):80-84.

Berhe D, Negash L (1998). Asexual propagation of *Juniperus procera* from Ethiopia: a contribution to the conservation of African pencil cedar. Forest Ecology and Management 112(1-2):179-190. *https://doi.org/10.1016/S0378-1127(98)00327-2*

Bertsouklis K, Paraskevopoulou AT, Zarkadoula N (2020). *In vitro* propagation of *Juniperus phoenicea* L. Acta Horticulturae 1298:331-334. *https://doi.org/10.17660/ActaHortic.2020.1298.45*

Brito, G (2000). Micropropagação de duas espécies autóctones da Ilha de Porto Santo (*Olea europaea* L. ssp. *maderensis* Lowe e *Juniperus phoenicea* L.) e estudo da resposta de rebentos *in vitro* a stress osmótico. M.Sc. thesis (Aveiro: University of Aveiro)

Ceballos L, Ruiz J (1971). Arboles y arbustos de la España peninsular. Instituto Forestal de Investigaciones Agrarias y Escuela Técnica Superior de Ingenieros de Montes, Madrid, Spain, pp 512.

Chambers RM, Meyerson LA, Saltonstall K (1999). Expansion of *Phragmites australis* into tidal wetlands of North America. Aquatic Botany 64(3-4):261-273. *https://doi.org/10.1016/S0304-3770(99)00055-8* de Foresta H, Somarriba E, Temu A, Boulanger D, Feuilly H, Gauthier M (2013). Towards the assessment of trees outside forests. Forest Resources Assessment Working Paper 183. FAO, pp 335

Farjon A (2010). A Handbook of the world's conifers. Koninklijke Brill, Leiden

Farjon A (2013). *Juniperus oxycedrus*. The IUCN red list of threatened species 2013: e.T42243A2965838. Retrieved 2022 December 24 from *https://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T42243A2965838.en*

Ferradous A, Alifriqui M, Hafidi M, Duponnois R (2013). Essais de régénération artificielle du Genévrier thurifere (*Juniperus thurifera* L.). Ecologia Mediterranea 39(1):115-120

Garcia D (2001). Effects of seed dispersal on *Juniperus communis* recruitment on a Mediterranean mountain. Journal of Vegetation Science 12:839-848. *https://doi.org/10.2307/3236872*

Gomez MP, Segura J (1994). Factors controlling adventitious bud induction and plant regeneration in mature *Juniperus oxycedrus* leaves cultured *in vitro*. *In vitro* Cellular and Developmental Biology-Plant 30:210-218. *https://doi.org/10.1007/BF02823034*

Gómez MP, Segura J (1995). Axillary shoot proliferation in cultures of explants from mature *Juniperus oxycedrus* trees. Tree physiology 15(9):625-628. *https://doi.org/10.1093/treephys/15.9.625*

Hazubska-Przybył T (2019). Propagation of *Juniper* Species by Plant Tissue Culture: A Mini-Review. Forests 10(11):1028. *https://doi.org/10.3390/f10111028*

Ilashi I (1986). *Juniper* (*Juniperus polycarpos* C. Koch) In: Bajaj YPS (Ed). Biotechnology in Agriculture and Forestry. Springer, Berlin, Heidelberg, Germany pp 321-325.

Kartsonas E, Papafotiou M (2007). Mother plant age and seasonal influence on *in vitro* propagation of *Quercus euboica* Pap., an endemic, rare and endangered oak species of Greece. Plant Cell Tissue Organ Culture 90:111-11. *https://doi.org/10.1007/s11240-007-9232-5*

Khater N, Benbouza H (2019). Preservation of *Juniperus thurifera* L.: a rare endangered species in Algeria through *in vitro* regeneration. Journal of Forestry Research 30:77-86. *https://doi.org/10.1007/s11676-018-0628-3*

Kocer ZA, Gozen AG, Onde S, Kaya Z (2011). Indirect organogenesis from bud explants of *Juniperus communis* L: effects of genotype, gender, sampling time and growth regulator. Dendrobiology 66:33-40

Korakis G (2015). Forest Botany [Undergraduate textbook]. Kallipos, Open Academic Editions. *https://hdl.handle.net/11419/742*

Kumar R, Sharma K, Agrawal V (2005). *In vitro* clonal propagation of *Holarrhena antidysenterica* (L.) Wall. through nodal explants from mature trees. *In vitro* Cellular and Developmental Biology-Plant 41:137-144. *https://doi.org/10.1079/IVP2004624*

Loureiro J, Capelo A, Brito G, Rodriguez, E, Silva S, Pinto G, Santos C (2007). Micropropagation of *Juniperus phoenicea* from adult plant explants and analysis of ploidy stability using flow cytometry. Biologia Plantarum 51(1):7-14 *https://doi.org/10.1007/s10535-007-0003-2*

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15(3):473-497. *https://doi.org/10.1111/j.1399-3054.1962.tb08052.x*

Negussie A (1997). *In vitro* induction of multiple buds in tissue culture of *Juniperus excelsa*. Forest Ecology and Management 98:115-123. *https://doi.org/10.1016/S0378-1127(97)00034-0*

Oliveira P, Barriga J, Cavaleiro C, Peixe A, Potes AZ (2003). Sustained *in vitro* root development obtained in *Pinuspinea* L. inoculated with ectomycorrhizal fungi. Forestry 76:579-587. *https://doi.org/10.1093/forestry/76.5.579*

Ortiz PL, Arista M, Talavera S (1998). Low reproductive success in two subspecies of *Juniperus oxycedrus* L. International Journal of Plant Sciences 159(5):843-847. *https://doi.org/10.1086/297605*

Rugini E (1984). *In vitro* propagation of some olive (*Olea europaea* L. var. *sativa*) cultivars with different root-ability, and medium development using analytical data from developing shoots and embryos. Scientia Horticulturae (Amsterdam) 24 (2):123-134 *https://doi.org/10.1016/0304-4238(84)90143-2*

Schenk RV and Hildebrandt AC (1972). Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Canadian Journal of Botany 50:199-204. *https://doi.org/10.1139/b72-026*

Torchik V (2010). Evaluation of an assortment of ornamental forms of conifers in Central Botanical Garden of Belarus National Academy of Science. Acta Horticulturae 885:375-381. *https://doi.org/10.17660/ActaHortic.2010.885.53*

Unlu M, Vardar-Unlu G, Vural N, Dönmez E, Cakmak O (2008). Composition and antimicrobial activity of *Juniperus excelsa* essential oil. Chemistry of Natural Compounds, 44: 129-131. *https://doi.org/10.1007/s10600-008-0040-x*

Wang Y, Yao R (2017). Plantlet regeneration of adult *Pinus massoniana* Lamb. trees using explants collected in March and thidiazuron in culture medium. Journal of Forestry Research 28:1169-1175. *https://doi.org/10.1007/s11676-017-0412-9*