



## Minireview

Smoke tree (*Cotinus coggygria* Scop.) propagation and biotechnology: A mini-reviewJaime A. Teixeira da Silva<sup>a,\*</sup>, Andrzej Pacholczak<sup>b,\*</sup>, Agnieszka Ilczuk<sup>b,\*</sup><sup>a</sup> P. O. Box 7, Miki-cho post office, Ikenobe 3011-2, Kagawa-ken 761-0799, Japan<sup>b</sup> Warsaw University of Life Sciences, Faculty of Horticulture, Biotechnology and Landscape Architecture, Department of Ornamental Plants, Nowoursynowska 159, 02-787 Warsaw, Poland

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## ABSTRACT

*Cotinus coggygria* Scop. (Anacardiaceae), commonly referred to as 'smoke tree' or 'smoke bush', is an attractive ornamental tree or large bush that has medicinal properties and multiple bioactivities. Seed germination can be high after treating seeds with H<sub>2</sub>SO<sub>4</sub>, but to avoid growing male trees that will not form attractive inflorescences, the rooting of cuttings from female trees is a preferred method of vegetative propagation. One method to intensify the rooting of stem cuttings is by shading mother plants and simultaneously applying an exogenous auxin or biostimulator. This method can increase the quality of rooted cuttings. In addition, shading causes anatomical changes in the stem structure and leads to the formation of adventitious root primordia. New EU regulations have forced member states to change or withdraw existing authorization for products used in plant protection that contain active substances like auxin (indole-3-butyric acid, IBA). Consequently, there is an active search for alternative measures to support the process of rooting woody plants. Examples of such preparations are biostimulators, including AlgaminPlant, HumiPlant and Route®, which can increase the rooting of cuttings, affect processes associated with oxidative stress, and increase the intensity of gas exchange and the content of organic compounds. The most effective *in vitro* micropropagation protocol for *C. coggygria* involves the use of Murashige and Skoog basal solid or liquid media enriched with one of two cytokinins, <sup>6</sup>N-benzyladenine or *meta*-metoxytopolins. The use of IBA has shown most success, both *ex vitro* and *in vitro*. All other aspects of *Cotinus* species biotechnology still need to be developed.

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## 1. Introduction

*Cotinus coggygria* Scop. (Anacardiaceae; [The Plant List, 2017a](#)) has nine synonymous varieties or subspecies and even though it has 21 species (accepted, synonymous, or contested; [The Plant List, 2017b](#)), published literature exists on the propagation biology and biotechnology of only one, *C. coggygria*. The common names 'smoke tree' or 'smoke bush' have been assigned to *C. coggygria*, which is a small tree or bush ([Gilman and Watson, 1993](#)) that is distributed primarily from southern Europe to western China, and is a hardy (e.g. 'Nordine Red') or purple-leaved (e.g. 'Royal Purple') ornamental ([Fig. 1](#)) ([Pijut, 2000](#)). Purple coloring of leaves results from the accumulation of anthocyanins in response to 300–400 nm of UV light and cold temperature (4–9 °C) ([Oren-Shamir and Levi-Nissim, 1997](#)). In the Northern hemisphere,

**Abbreviations:** 2,4-D, 2,4-dichlorophenoxyacetic acid; ABA, abscisic acid; BA, <sup>6</sup>N-benzyladenine; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; IAA, indole-3-acetic acid; IAAO, oxidase indole-3-acetic acid; IBA, indole-3-butyric acid; LS, Linsmaier and Skoog medium; MemT, *meta*-metoxytopolin; MemTR, *meta*-metoxytopolin riboside; MS, Murashige and Skoog medium; NAA, 1-naphthaleneacetic acid; PPF, photosynthetic photon flux density; POX, peroxidase; PPO, polyphenol oxidase; QL, Quoirin and Lepoivre medium.

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smoke tree flowers in the summer and sets seed in autumn. This makes several *C. coggygria* cultivars an attractive option for landscaping, including in rooftop gardening where it has been shown to exhibit mild cold (–23 °C) tolerance ([Fan and Wang, 2011](#)). Plants grow well when potted in 70 peat:30 bark (v/v) ([Cameron et al., 2005](#)). There is a risk of fungal infection, especially by *Verticillium dahliae*, which causes plant stunting and early leaf senescence, thus negatively affecting its ornamental properties ([Gilman and Watson, 1993](#); [Xiong et al., 2014](#)).

A comprehensive review highlighted the pharmacological and phytochemical constituents of *C. coggygria* with a wide range of biological activities ("antioxidative, antibacterial, antifungal, antiviral, anticancer, antigenotoxic, hepatoprotective and anti-inflammatory") derived from the plant's essential oils and extracts found in shoots, leaves, flowers and heartwood ([Matić et al., 2016](#)). Thus, in addition to its ornamental value, *C. coggygria* has important medicinal properties. This review explores the propagation of this ornamental, both *via* conventional seed or cutting propagation and by *in vitro* tissue culture.

## 2. Seed germination and seedling establishment

Sexual or seed propagation of *C. coggygria* is a common method to produce many progenies but these are genetically heterogeneous.



Fig. 1. Five-year-old plants of *Cotinus coggygria* 'Royal Purple' grown in autumn.

Even though as many as 100,000–119,000 seeds/kg can be collected, some studies have indicated that *C. coggygria* seeds exhibit some dormancy and reduced (75%) germination ability, limiting the ability to propagate this plant (Hartmann et al., 1997; Olmez et al., 2008; Deng et al., 2010). Seeds have a hard testa and display internal dormancy (physical and physiological) (Stilinovic and Grbic, 1988; Olmez et al., 2009).

Several methods are available to break seed dormancy, including scarification (mechanical, physical and chemical), cold stratification, soaking in gibberellic acid ( $GA_3$ ) or a combination of all of these (Stilinovic and Grbic, 1988; Pijut, 2000; Olmez et al., 2008; Guner and Tilki, 2009; Olmez et al., 2009; Deng et al., 2010; Pipinis et al., 2014). Seeds of *C. coggygria* can be harvested and sown in autumn without any treatment or in spring with a pre-treatment that can involve 30 min scarification in  $H_2SO_4$  and subsequent stratification in moist sand for 45–60 days at 3 °C (Pijut, 2000). Olmez et al. (2008) soaked seeds in hot water (88–100 °C) for 24 h and cooled them immediately or soaked them for 24 h in tap water. To scarify seeds, they used 98%  $H_2SO_4$  for 20, 50, or 80 min then placed seeds in the cold for 20, 40, or 60 days. Seeds were sown in pots in a greenhouse or in a field. Seeds immersed for 20–80 min in  $H_2SO_4$  then cold stratified for 60 days and left for 22–25 days under greenhouse conditions resulted in 77.1–82.8% germination. In the control treatment, only 19.3% of seeds germinated after 52 days while under field conditions germination was even lower. Olmez et al. (2009), in ensuing experiments, immersed seeds in  $H_2SO_4$  for 50 min and cold stratified (5 °C) them for 15 days, resulting in 88.1% germination in the laboratory, but in greenhouse conditions, 70.2% of seeds germinated after 10 min treatment with  $H_2SO_4$  and 30-day stratification. Guner and Tilki (2009) noted that the germination of *C. coggygria* seeds increased significantly when stratification period was extended (16.8% after 30 days, 72.3% after 120 days and 1.7% in the control). In addition, 10–40 min scarification in  $H_2SO_4$  together with cold stratification for 30 or 60 days significantly improved germination percentage (95.8–99.0%) compared to other treatments. Pipinis

et al. (2014) evaluated the effect of  $H_2SO_4$ -based scarification, cold stratification and  $GA_3$  to break seed dormancy. Seeds that were only cold stratified for up to 120 days resulted in very low germination (0–15.8%). The best results were obtained when seeds were acid-scarified for 60 min, treated for 24 h with 0.5–2.0 mg L<sup>-1</sup>  $GA_3$  and cold stratified for 1–3 months. Germination was 65–73.3%, independent of  $GA_3$  concentration and the duration of chilling. The application of  $GA_3$  could not replace the stratification period but could stimulate seed germination (30.8–35.8% versus 5.0% in the control). Similar results (40.7%) were obtained by Deng et al. (2010) when seeds of *C. coggygria* var. *cinerea* were scarified and then immersed in 346.4 mg L<sup>-1</sup>  $GA_3$  (Table 1). The differences between the results of those research groups can be attributed to the provenance of seeds. Seed used by Olmez et al. (2008, 2009) and by Guner and Tilki (2009) were from Turkey, those by Stilinovic and Grbic (1988) from Serbia and those by Pipinis et al. (2014) from Greece. It can be inferred that seeds from Turkey had lower dormancy than those from Serbia. It is also possible that seeds from Greece and Serbia had a similar hard testa that was impenetrable to water.

### 3. Vegetative propagation

Seed germination tends to result in the production of male plants that are then not able to produce the attractive inflorescences typical of this ornamental. For this reason, vegetative propagation has received the greatest attention in terms of research output to date. Thus, it is common to propagate vegetative stem cuttings of female plants with indole-3-butyric acid (IBA) at 1.0–3.0 g L<sup>-1</sup> and under misting in moist soil. Roots develop between 4 and 8 weeks, as was already shown by earlier studies, mainly with *C. coggygria* 'Royal Purple' (Kelley and Foret, 1977; Siftar, 1981; Blakesley et al., 1991, 1992; Pijut, 2000). Kelley and Foret (1977) claimed 33% rooting success of stems sampled in early June in the absence of any special treatments, increasing up to 95% when IBA was used. Blakesley et al. (1991) used young shoots 8–10 cm long denuded of leaves in the basal 3–4 cm section. Even though these shoots were dipped in rooting medium, no roots formed during the summer growth period (July and August). In their study, high levels of rooting were associated with high levels of free indole-3-acetic acid (IAA) in plants. A subsequent study by the same authors (Blakesley et al., 1992) showed that etiolated shoots collected in May could induce higher levels of rooting using the same treatment. Cameron et al. (2001a) showed how removal of the shoot tip reduced rooting percentage from 95% to 40%, while fogging resulted in about 20% higher rooting than misting. In contrast to most of those studies where leaves were normally removed from shoots prior to rooting, Cameron et al. (2001b) dipped shoots in 1.26 g L<sup>-1</sup> IBA for 5 s, and suggested leaving all leaves intact to allow for maximum leaf area. They obtained >80% rooting in contrast to 44% rooting when leaf area was reduced and <22% rooting when lateral branches were trimmed, perhaps explaining the 0% rooting observed by Blakesley et al. (1991). This may be

Table 1  
Methods of breaking seeds dormancy of *Cotinus coggygria* Scop.

Method	References
24 h soak in hot (88–100 °C) water and immediately cooling	Olmez et al. (2008)
24 h soak in tap water	Olmez et al. (2008)
98% $H_2SO_4$ scarification during 20–80 min	Stilinovic and Grbic (1988), Pijut (2000), Olmez et al. (2008), Guner and Tilki (2009), Olmez et al. (2009), Pipinis et al. (2014)
20–120 days stratification at 3–5 °C	Stilinovic and Grbic (1988), Pijut (2000), Olmez et al. (2008), Guner and Tilki (2009), Olmez et al. (2009), Pipinis et al. (2014)
24 h soak in $GA_3$	Deng et al. (2010), Pipinis et al. (2014)

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