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Biotechnological advances in *Vitex* species, and future perspectives

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Abstract *Vitex* is a large genus consisting of 230 species of trees and shrubs with multiple (ornamental, ethnobotanic and pharmacological) uses. Despite this, micropropagation has only been used to effectively propagate and preserve germplasm a limited number (six) of *Vitex* species (*V. agnus-castus*, *V. doniana*, *V. glabrata*, *V. negundo*, *V. rotundifolia*, *V. trifolia*). This review on *Vitex* provides details of published micropropagation protocols and perspectives on their application to germplasm preservation and *in vitro* conservation. Such details serve as a practically useful user manual for *Vitex* researchers. The importance of micropropagation and its application to synthetic seed production, *in vitro* flowering, production of secondary metabolites, and the use of molecular markers to detect somaclonal variation *in vitro*, are also highlighted.

Contents

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1. Introduction

The *Vitex* L. genus (Lamiaceae) [51] contains mainly trees and shrubs and several species have well-established uses in ethnobotany, medicine, pharmacology and landscaping as ornamental plants [27,30,71,109,56,76]. The socio-economic importance of many *Vitex* species is thus irrefutable. However, the over-reliance on natural populations to derive such primary resources can strain the ecological balance of the environment in which they grow naturally, making collection from natural populations, in some cases, unsustainable. Fifteen *Vitex* species (*V. acunae* Borh. & Muniz, *V. ajugaeflora* Dop., *V. amaniensis* W. Piep, *V. cooperi* Standl., *V. evoluta* Däniker, *V. gaumeri* Greenm., *V. heptaphylla* A. Juss., *V. keniensis* Turrill, *V. kuylenii* Standl., *V. lehmbachii* Gürke, *V. longisepala* King & Gamble, *V. parviflora* Juss., *V. urceolata* C.B. Clarke, *V. yaundensis* Gürke, and *V. zanzibarensis* Vatke) are included in the IUCN Red Data list [46] for various reasons, but all related to unsustainable harvesting.

Depulped seeds of *V. doniana* Sweet germinate well after a hot water treatment [1] or after 21 days of hydration-dehydration cycles [31]. The seed germination of other *Vitex* species has not yet been studied. Thus, urgent attention is needed to conserve *Vitex* species. One biotechnological tool, *in vitro* propagation, provides a viable solution for the large-scale propagation of medicinally important plants [53,92,65], including cryoconservation [94] and genetic transformation [96]. The micropropagation of three *Vitex* species (*V. negundo*, *V. agnus-castus* and *V. trifolia*) from 29 published reports of *Vitex* sp. has recently been reviewed [9]. However, that review lacks vital details about disinfection methods, temperature, light source and intensity, photoperiod, basal medium, plant growth regulator type and concentrations, medium pH, carbon source type and concentrations for culture initiation, multiplication and rooting, all of which are essential factors that influence the outcome of the *in vitro* protocol for *Vitex* species. Consequently, our review explores these fine-scale details of the different steps of the plant tissue culture protocols for *Vitex* spp. to allow plant biotechnologists to design new and detailed experiments. This review, which provides a detailed analysis of reports from 1986 to 2016 of the micropropagation of six *Vitex* species (*V. agnus-castus* L., *V. doniana*, *V. glabrata* R. Br., *V. leucoxylon* L., *V. negundo* L., *V. trifolia* L.) (Tables 1 and 2), provides a solid foundation for the sustainable social and economic use of valuable members of this genus. A brief background of the socio-economic importance of *Vitex* species for which micropropagation protocols exist is provided next.

V. agnus-castus (chast berry) is a deciduous shrub native of Mediterranean Europe and Central Asia. The fruit extract of *V. agnus-castus* is used to treat menstrual disorder (amenorrhoea, dysmenorrhoea), premenstrual syndrome, corpus luteum insufficiency, hyperprolactinaemia, infertility, acne, menopause and disrupted lactation [30]. The fruits and leaves of *V. doniana* (black plum) are either consumed raw or after processing while the leaves, fruits, roots, barks and seed of the plant are used in traditional medicinal in Africa to treat a wide range of ailments [28,29,32], and references therein]. *V. glabrata* is a tree commonly known as "Kai Nano" in Thailand whose bark and roots are used as an astringent because the bark accumulates high levels of ecdysteroids, primarily 20-hydroxyecdysone or β-ecdysone [110]. The former compound, 20-hydroxyecdysone, can be synthesized in cell suspension cultures of *V. glabrata* [83,84,23]. *V. leucoxylon* is a large deciduous tree found in India and is commonly known in Marathi as *Songarbhi*. The crude alcoholic extract of its leaves possesses anti-psychotic, anti-depressant, analgesic, anti-parkinsonian, anti-microbial, anti-inflammatory and wound-healing properties [100]. *V. negundo* is a woody, aromatic shrub used in Ayurveda, Unani, Chinese, and folk medicine [109,56], and has mainly anti-inflammatory, analgesic, anti-hyperglycaemic, hepato-protective, anti-microbial and snake venom neutralization activity [71]. *V. trifolia* is a component of a number of commercially available herbal formulations that employ its leaves, and which have antiseptic, aromatic, febrifuge, anodyne, diuretic, and emmenagogue activity, fruits, which have nervine, cephalic, emmenagogue, amenorrhoea-treating and anthelmintic activity, roots, which are used to treat febrifuge, painful inflammation, cough and fever, and flowers, which are used to treat fever [76].

This review highlights the advances made in the micropropagation (including synthetic seed technology), *in vitro* flowering, and production of secondary metabolites of *Vitex* species. Emphasis is also given to the use of molecular markers to detect variation arising from *in vitro* propagation. This review is useful for conservation biologists, plant physiologists and biotechnologists that aim to explore other unexplored *Vitex* species or to expand the repertoire of research existent for the currently investigated species.

2. Selection of suitable starting material and disinfection

The choice of explant often depends on the material that is available, and sometimes even on the season. The selection of explants in *Vitex in vitro* studies tends to be from young and actively growing shoots or branches, with shoot tips and nodal explants with dormant axillary buds being the first choice (Table 2) due to the presence of a predetermined meristem which allows for true-to-type clonal propagation.

2.1. Alternative explant sources

There are several studies available in which other explants were used. For instance, internodes or stem segments were used for callus induction and regeneration studies in *V. negundo* [103,77,26,79], *V. leucoxylon* [25], and *V. trifolia* [15]. Stem-induced callus of *V. glabrata* produced 20-hydroxyecdysone (20-HES) in a liquid culture system [83,84,98]. *V. negundo* leaves were used for callus culture and