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In vitro culture of *Digitalis* L. (Foxglove) and the production of cardenolides: An up-to-date review

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ABSTRACT

Digitalis L. (Foxglove, Plantaginaceae) genus is a representative of several medicinal ornamental plants that are widely used in the production of herbal medicines. Since the eighteenth century, human civilizations have been using the extracts from several *Digitalis* species for treating heart-related ailments. The active ingredient in the medicine is cardiac glycosides. Cardenolides, which are constituents of cardiac glycosides, have an important role in tumor therapy. Certain pharmacologically active compounds including cardenolides are isolated from plants, as the structural complexity of cardenolides impede an easy chemical synthesis. In modern plant biotechnology research, production of cardenolides in large-scale using *in vitro* techniques has become the need of the hour. The reasons are twofold: first, to reduce the excessive use of natural *Digitalis* population and second, to improve the plant quality *vis-à-vis* genetic preservation of the superior seeds for future use. The production of useful secondary metabolites depends on the overall wellness of the plant from which extraction is to be made. The subject matter of this review includes concurrent development and propagation of several *Digitalis* species based on direct and indirect regeneration methods. Herein, a compilation of up-to-date published research reports on *in vitro* culture of *Digitalis* L. has been presented, including the authors' latest and yet-to-be-published work on *Digitalis davisiana* Heywood. The important steps to be followed for the implementation of any plant improvement/preservation program must include topic-wise requirements at various stages of micropropagation (viz., culture establishment, shoot multiplication, root induction and acclimatization) and also the requirements for plant regeneration (viz., somatic embryogenesis and organogenesis). These have been reviewed thoroughly and different methods for the *in vitro* production of cardenolides have been discussed. Critical comments on the prospects of highly scalable cultures and their importance to meet the ever-growing demand for *Digitalis*-derived products in pharmaceutical industries, have also been included.

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1. Introduction

Digitalis L. constitutes flowering plants of the Plantaginaceae family; some of the species are endemic to the Mediterranean region, Turkey (*D. davisiana* Heywood, *D. cariensis, D. lamarckii* Ivan, and *D. trojana* Ivan), Ikaria island in Greece (*D. leocophaea* Sm. Subsp. *leucophaea* and *D. leocophaea* Sm. Subsp. *ikarica*) and Samtskhe-Javakheti in Armenia-Georgia region (*D. ferruginea* L.) (*i.e.*, *D. ferruginea* subsp. *ferruginea* L., and *D. ferruginea* subsp. *schischkini*). *Digitalis* species are native to the Balkans, Hungary, Italy, Germany, Spain, Lebanon, Romania, Transcaucasia, Turkey, Japan and India. This genus was traditionally placed in Scrophulariaceae family, but recent phylogenetic research has placed it in the much-enlarged family of Plantaginaceae (Olmstead et al., 2001). There are 20 species of herbaceous perennials, shrubs and biennials that are commonly called foxglove. *Digitalis* species include *D. ciliata*; *D. dubia*; *D.* × *fulva*; *D. grandiflora*; *D. isabelliana*; *D. laevigata*; *D. lanata*; *D. mariana*; *D. lutea*; *D. micrantha*; *D. obscura*; *D. parviflora*; *D. purpurea*; *D. thapsi*; *D. sceptrum*; *D. viridiflora*; and *D. canariensis*. Many of these have medicinal uses that are highly valued in the pharmaceutical industries (Fig. 1A–J). *Digitalis* is also popular in many households as ornamental and decorative plants.

The first known use of the *D. purpurea* extract was in 1785 (Goldthorp, 2009; Withering, 2014) for treating heart ailments in humans. The extract contains cardiac glycosides which helps to increase cardiac contractility in an affected patient making it easy to breath. Cardiac glycosides are also known for their antiarrhythmic action to control the heart rate. Extract of several *Digitalis* species is often prescribed to the patients in atrial fibrillation, especially if they have been diagnosed with congestive heart failure. Due to their usefulness in producing life-saving medicines, *Digitalis* species have high economic value. Owing to large-scale and uncontrolled exploitation to meet the ever-increasing demand of *Digitalis* by pharmaceutical industries, coupled with limited cultivation and insufficient attempts to replenishment it in the wild, the population of such an important plant species has been depleted markedly. Natural propagation of *Digitalis* through seeds is feasible; but this method is not as effective in producing a sufficient number of planting stocks as the germination frequency of the *Digitalis* seeds is rather poor. Besides these, uncontrolled collection of various *Digitalis* species from the natural population makes the vegetation disappear quickly, when the plants are harvested even before the seed setting. The limitations listed above can be partially overcome using *in vitro* propagation methods under controlled environmental conditions, facilitating the rapid multiplication of superior clones and the extraction of cardenolides throughout the year without any seasonal constraints. This necessitates establishment of superior tissue culture protocols. As the *Digitalis* species is biennial, plants in the open fields often fail to germinate after the second vegetation period. Therefore, an effective protocol for the propagation of *Digitalis* species is needed to produce large quantities of 'quality plants' for the extraction of valuable metabolites as well as for the ornamental plants market, while avoiding the exploitation of the wild populations. Even in the twenty-first century, the cardenolides are still extracted from the plants, since the structural complexity of the cardenolides makes it difficult to effect a facile synthesis in the laboratory. Furthermore, genetic engineering practices are most effective in conjunction with the *in vitro* culture techniques (Kothari et al., 2010), which could provide cardenolides producers with opportunities to improve the quality and quantity of cardenolides derivatives raising the economic potential of the *Digitalis* species. In 1988, Rücker published an account on the *in vitro* culture, regeneration and production of cardenolides and other secondary products of the *Digitalis* species (Rücker, 1988). Since then many advanced techniques in biotechnology research have been developed. Similarly, the search for economically viable cardenolides production by more and more members of the *Digitalis* family (other than those described by Rücker) has been expanding. This has motivated the authors to make a thorough search in the current literature dealing solely with the *in vitro* cultures of *Digitalis* species and cardenolides production. The exercise resulted with the preparation of this up-to-date review.

The present article includes a balanced compilation of up-to-date published research reports on *in vitro* culture of *Digitalis* L. The latest, yet-to-be-published work on *Digitalis davisiana* Heywood, by the present authors, is also included here. Topic-wise requirements at various stages of micropropagation (viz., culture establishment, shoot multiplication, root induction and acclimatization) have been summarized. The requirements for plant regeneration (viz., somatic embryogenesis and organogenesis) have been discussed in detail. This review article is expected to serve as a guide for the use of *in vitro* tissue culture of *Digitalis* L. for the production of cardenolides and aims to provide for a hands-on knowledge of the *Digitalis* family, focussing on the generation, propagation and preservation of the species.

2. Importance of *Digitalis* and the applications of *Digitalis* extracts

Amongst the *Digitalis* species, *Digitalis purpurea* is the best-known and most widely used as an ornamental plant in households. The plant blossoms into vivid flowers with color range from purple tints, several shades of light gray and pure white. Besides multiple colors, the *Digitalis purpurea* flowers also possess eye-catching spottings and marks on them which make them more attractive. *Digitalis lanata*, also called as wooly foxglove or Grecian foxglove, has its distinctive appearance due to the wooly texture of the leaves. Like other *Digitalis* species, *D. lanata* is toxic and its extract is rich with powerful cardiac glycosides. The *Digitalis* species, namely, *D. purpurea* and *D. lanata* have been extensively studied by horticulturists as well as plantation agronomists for their medicinal, ornamental and economical values.

The use of *Digitalis* extracts in the treatment of heart ailments was first reported by William Withering in 1785 (Withering, 2014), who made use of the active ingredient in the extract, later identified to be a cardiac glycoside (also known as a cardiotonic steroid). The molecule has a steroid-like structure including an unsaturated lactone ring. The cardiac glycosides contain sugar