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Research article

Calcium and magnesium elimination enhances accumulation of cardenolides in callus cultures of endemic *Digitalis* species of Turkey

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ABSTRACT

Elimination of calcium (Ca), magnesium (Mg) or both from the medium of callus cultures of *Digitalis davisiana* Heywood, *Digitalis lamarckii* Ivanina, *Digitalis trojana* Ivanina and *Digitalis cariensis* Boiss. ex Jaub. et Spach increased cardenolides production. Callus was induced from hypocotyl segments from one-month old seedlings were cultured on MS medium containing 0.5 μg ml^{-1} thidiazuron (TDZ) and 0.25 μg ml^{-1} indole acetic acid (IAA). After 30 days of culture, callus was transferred in hormone-free MS medium (MSO) as well as Ca or Mg or both were completely eliminated from same medium. The amount of five cardenolides from *D. davisiana* Heywood, *D. lamarckii* Ivanina, *D. trojana* Ivanina and *D. cariensis* Boiss. ex Jaub. et Spach were compared. Higher amounts of five cardenolides and total cardenolides were obtained when callus of four *Digitalis* species were incubated on MS medium lacking both Ca and Mg. The mean contents of total cardenolides obtained were in the order of *D. lamarckii* (2017.97 μg g^{-1}) > *D. trojana* (1385.75 μg g^{-1}) > *D. cariensis* (1038.65 μg g^{-1}) > *D. davisiana* (899.86 μg g^{-1}) when both Ca and Mg were eliminated from the medium, respectively. This protocol is useful for development of new strategies for the large-scale production of cardenolides.

1. Introduction

Digitalis, commonly known as foxgloves, belongs to the family Plantaginaceae. The genus consists of about 20 species of herbaceous perennials, shrubs and biennials. Members of the genus *Digitalis* L. are medicinally and economically important plants as they contain cardiac glycosides that are used as heart medicines [1] and effective agents in cancer chemotherapy [2–4]. Because of their importance, studies have focused on in vitro culture of several *Digitalis* species including *Digitalis davisiana* [5], *Digitalis lanata* [6], *Digitalis obscura* [7], *Digitalis purpurea* [8,9] and *Digitalis thapsi* [10–13].

Digitalis species is distributed in Europe, Western Asia and the Mediterranean region. *D. davisiana* Heywood, *Digitalis lamarckii* Ivanina, *Digitalis trojana* Ivanina and *Digitalis cariensis* Boiss. ex Jaub. et Spach, which are the most widespread members of the nine *Digitalis* species growing in Turkey and are endemic to Turkey [14,15]. *D. trojana* contains the highest amount of cardiac glycoside in leaves among the endemic *Digitalis* species in Turkey [16]. Large-scale plant cell and tissue culture has been considered as an alternative technique for the production of bioactive secondary metabolites since their production by traditional agriculture is inefficient [17]. The production of cardenolides using tissue culture techniques for *Digitalis* species has been studied for several years, however, the productivity has not yet reached economically desired levels. Most scientists have reported that tissue cultures established from *Digitalis* or other cardenolide containing plants either did not produce cardenolides or contained only at trace amounts. On the other hand, to improve the production of such secondary metabolites, some strategies have been developed [18]. One of them is the employment of various kinds of abiotic stress factors such as changing nutrient conditions, temperature range, UV and chemical application [19].

The aim of this work was to achieve the enhancement of cardenolide accumulation in *D. davisiana* Heywood, *D. lamarckii* Ivanina, *D. trojana* Ivanina and *D. cariensis* Boiss. ex Jaub. et Spach, using calcium and magnesium deprived cultures and to determine the content of digitoxin, digoxin, lanatoside C, gitoxigenin and digoxigenin as secondary metabolites of commercial interest for the pharmaceutical industry.

2. Results

Callus was readily initiated within one week when hypocotyl segments excised from one-month old seedlings were cultured on

Abbreviations: IAA, indole-3-acetic acid; TDZ, thidiazuron; MS, Murashige and Skoog; Lan C, lanatoside C.

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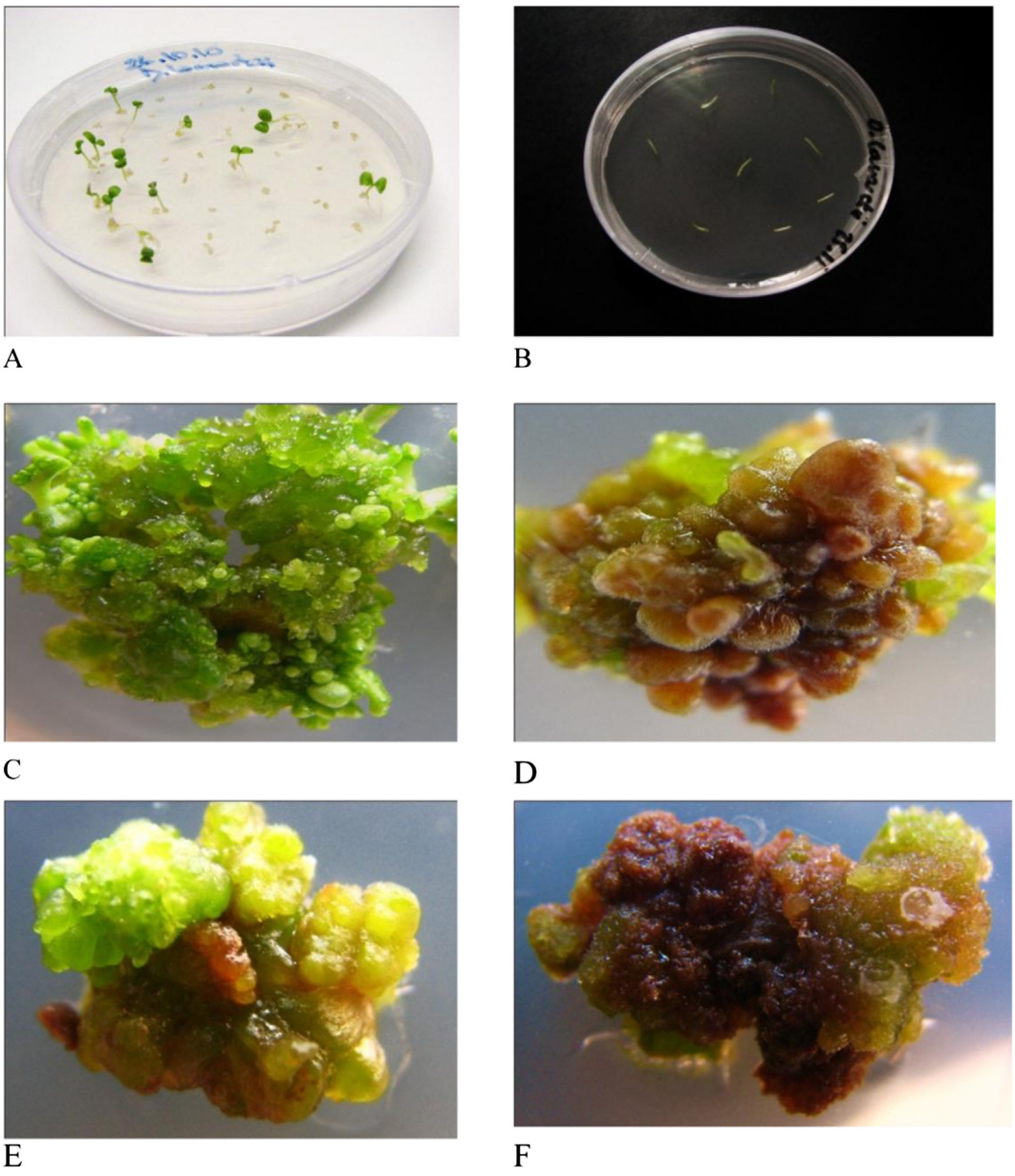

Fig. 1. Experimental design of *D. lamarckii*. Germinated seedlings in vitro (A), hypocotyl segments (5–8 mm) from one-month old seedlings were cultured on MS medium containing 0.5 μg ml^{-1} TDZ and 0.25 μg ml^{-1} IAA (B), growth of callus derived from hypocotyl explants (C), deficiency symptoms of Ca (D), Mg (E), or both Ca & Mg (F) in the callus tissue of *D. lamarckii*.

MS medium supplemented with 0.5 μg ml^{-1} TDZ combined with 0.25 μg ml^{-1} IAA. Following a 30 days culture on this medium, calli were transferred to Ca-, Mg- or both Ca and Mg-eliminated medium for 15 days. Elimination of Ca or Mg or both from the medium slightly reduced the growth of callus without affecting cell viability. The colour of the callus changed from green in the control treatment to yellow–brown on Ca- or Mg-eliminated media, and gradually from yellow–brown to brown on both Ca and Mg-eliminated media after 15 days of culture (Fig. 1).

As seen in the last column of Table 1, the elimination of Ca, Mg or both from callus cultures of four *Digitalis* species clearly promoted the total accumulation of cardenolides. Ca and Mg elimination stimulated the cardenolide accumulation in *D. lamarckii* compared to control group. Deficiency of both Ca and Mg from the medium was followed by a significant increase of Lan C (1962.4 ± 7.84 μg g^{-1}dry weight, dw). The non-treated group produced 251.2 ± 4.62 μg g^{-1}, dw Lan C while those cultured on medium lacking either Ca or Mg producing 883.66 ± 9.71 μg g^{-1}, dw or 561.53 ± 4.55 μg g^{-1}, dw Lan C, respectively. The deficiency of both macronutrients resulted in the highest level of digitoxin (38.55 ± 2.03 μg g^{-1}, dw), digoxigenin (9.74 ± 0.26 μg g^{-1}, dw) and gitoxigenin (7.68 ± 0.40 μg g^{-1}, dw).

For *D. trojana*, Ca and Mg elimination also seemed to enhance the cardenolide accumulation. When both Ca and Mg were eliminated from the medium, the level of Lan C increased significantly (1344 ± 6.97 μg g^{-1}, dw). 225.63 ± 7.58 μg g^{-1}, dw Lan C was produced in the non-treated group compared to those cultured on medium lacking either Ca or Mg, which produced 890.83 ± 9.6 μg g^{-1} or 562.56 ± 7.00 μg g^{-1}, dw Lan C, respectively. Moreover, the highest digitoxin (26.65 ± 4.43 μg g^{-1}, dw) and digoxigenin (8.64 ± 0.38 μg g^{-1}, dw) levels were obtained when both Ca and Mg were eliminated from the medium. There was not a