

***In vitro* Propagation and the Production of Secondary Metabolites from *Arbutus andrachne* L.**

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Introduction

Arbutus andrachne L, Ericacea, the “Grecian Strawberry tree”, is an evergreen small tree, widely spread from the East Mediterranean to northern Black Sea (Davis, 1978). In the Palestinian Territories the plant occurs on rocky hills with high clay content and low aeration (Danin, 2005). The reddish stems and evergreen foliage make the tree very attractive with high ornamental value (Hedrick, 1972; Facciola, 1990). Grecian strawberry tree is being used in folk medicine as astringent and urinary antiseptic and for the treatment of urinary system, in addition to blood tonic and cancer (Said *et al.* 2002; Sakar *et al.* 1991). (Sakar *et al.* 1999) reported that there are triterpenoides, sterols, and lipids isolated from the bark, leaves, and fruits of *A.andrachne*. Also shown the active medicinal compounds in *A.andrachne* as arbutin. In the past few decades, populations of Grecian strawberry tree and many other wild plants in Palestine are facing severe habitat fragmentation, over-exploitation, urbanization, and damages resulted from extensive agricultural and human activities (ROTEM, 2002). Very limited recovery of plants in the Palestine has been observed possibly due to difficult germination under natural conditions and slow plant growth. Micropropagation techniques offer a good alternative to propagate endangered plant species. Extracting secondary metabolites using plant tissue culture has some advantages include: (1) the sources of these metabolites, i.e., most of higher plants have specific acclimation requirements. Hence, specific metabolites can be produced in cultures and available all in any place of the world even in places where these crops are not grown. (2) The already limited supply of these raw materials cannot be exhausted considering the future needs (Narula *et al.* 2004). Additional advantage of culturing medicinal plants *in vitro* is the possibility of producing beneficial bioactive secondary metabolites and novel chemical compounds (Srivastava *et al.* 2005).

The present study is aimed at studying some chemical and physical factors influencing the *in vitro* propagation of Grecian strawberry tree. Additionally, callus tissue culture will be initiated and maintained for examining further secondary metabolite production. It is expected that this project will allow us to better understand the composition of bioactive material in Grecian strawberry tree, and allow us to efficiently propagate this endangered wild tree.

Material and methods

a- Establishment of plant material: *in vitro* motherstock plants will be initiated from seeds. Following surface sterilization, seeds will be implanted on basal growth medium for germination. Media will be supplemented with different levels and combinations of auxins, cytokinins and gibberellin to test the best shoot proliferation and rooting rates. Rooted plantlets will gradually be acclimatized and transfer to *ex vitro* conditions for further growth.

b- Callus culture: callus tissue will be initiated by placing seeds, and leaf discs on media supplemented with different levels of 2,4-D and cytokinins (BA, kinetin, and 2i-P). Callus will be subcultured on media with different PGR levels to test growth performance and estimate the growth curve.

Results

We are successfully breaking seeds dormancy by stratification, and using gibberellins treatment. After examined different types of basal media, seeds successfully germinated at (woody plant medium) called WPM, which developed by Lloyd and McCown (1980). A protocols of shooting and rooting were established for *in vitro* *A.andrachne* plants, rooted

plants now transfer to acclimatize. And also callus induced and maintained from different explants.

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