ESTABLISHMENT OF IN VITRO CELL CULTURES OF ARTEMISIA ANNUA L. FOR THE PRODUCTION OF THE ANTIMALARIAL PHYTOCHEMICAL ARTEMISININ


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Artemisia annua L. is an aromatic annual herb which has been used in Chinese medicine for centuries in the treatment of fevers. However, the plant now grows wild in many other countries in Europe and America. There is a great interest in this plant due to its ability to synthesize and accumulate a variety of secondary metabolites which are biologically active compounds. Among them, artemisinin, an endoperoxide sesquiterpene lactone, is an antimalarial drug effective against multidrug resistant strains of Plasmodium, the malarial parasite. At present, the major production of artemisinin for pharmacological use is obtained by extraction and purification from field grown plants, although with quite variable yields. The highest artemisinin content has been found in leaves and flowering buds of the plant, and it is influenced by several environmental factors. As a result, the supply of artemisinin is far from enough in the international market. On the other hand, the chemical synthesis has not proved to be commercially feasible. Efforts are needed in order to enhance the production of artemisinin in plants or, alternatively, to exploit cell and tissue culture technology, which has not been fully explored in this species.

The aim of this work is the establishment of cell and tissue cultures of Artemisia annua L. for the in vitro production of artemisinin.

Axenic leaf discs were incubated in the dark on various media in order to induce callus cultures. A broad spectrum experimental design using MS basal medium supplemented with 49 combinations of NAA or 2,4D with 6-BAP at different concentrations was carried out. Quantitative and qualitative evaluations of callus proliferation were performed in several subcultures allowing to identify the most suitable conditions for the establishment of cell suspensions. The best results were obtained when 2,4D and 6-BAP were added in the ratio of 5:1 to MS basal medium.

Starting from axenic meristem tips it was also possible to obtain shoot cultures which were micropropagated on MS basal medium supplemented with 6-BAP (0.5 mg/L) and NAA (0.01 mg/L). This result was obtained when the optimal medium composition able to reduce the occurrence of vitrification was identified, by lowering auxin and increasing agar concentration.