**Hydroponic technologies as a means of protection and cultivation of medicinal and conservation significant plant species**

**Boryanka Dimitrova Traykova**

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## Summary

Hydroponic technologies encompass various methods of growing plants without soil using aqueous solutions of nutrients, with or without an artificial substrate (perlite, gravel, expanded clay pebbles, coconut husks or other inert material) acting as a mechanical support. One of the main advantages of hydroponic gardening is the significant increase in yields, as plants are cultivated under controlled environmental conditions (optimal temperature and light regime) and receive balanced nutrients according to their specific needs. Production is independent of seasons, pest infestations, soil type and pH, and soil-related weeds, diseases and pests. Hydroponic technologies lend themselves to mechanization and automation, many species are grown year-round, space is saved by introducing multi-storey vertical farming, water is used much more efficiently by recovering and recycling excess solution. In the context of climate change, hydroponic technologies are very promising and are considered to be decisive for the future of agriculture.

For the successful application of hydroponic technologies to new species, it is necessary to experimentally determine the optimal amount and ratio of the components in the nutrient solution, to choose an appropriate type of hydroponic system and substrate, as well as to know the biology of the respective species and to comply with its specific environmental requirements. Developments always begin at the laboratory level and, with favourable circumstances, reach scale-up and industrial production.

The aim of the present dissertation was the establishment of protocols for propagation and growth acceleration of selected medicinal and conservation important plant species, characterized by propagation difficulties (low germination and/or slow growth), by means of various hydroponic technologies. In addition, it was intended to verify whether the hydroponically propagated individuals retain the species-specific biosynthetic ability with respect to secondary metabolites. The following 11 species were the subject of research: *Haberlea rhodopensis, Thymus longedentatus, T. pannonicus, T. zygioides, Vaccinium vitis-idaea, Arctostaphylos uva-ursi, Lilium rhodopaeum, Hippeastrum papilio, Alkanna tinctoria, Salvia officinalis* and *Echinacea purpurea*, some of which are endemic or endangered, most with real or potential economic significance. The species were selected so that the possibilities of both seed and vegetative propagation from leaves, cuttings and bulbs could be explored. Soilless cultivation can be considered as a part of plant biotechnologies, and in some species slow growing *in vitro* propagated plantlets were used as starting material in order to accelerate their growth.

For the first time, plants of *Haberlea rhodopensis* were propagated from leaves using two aero-hydroponic systems: the vertical Green Diamond and the horizontal Aeroflo-20. Perlite is the most suitable substrate, allowing rooting of 47% of the fresh leaves and formation of 2.9 well-formed rosettes per leaf; or survival of 85% of pre-rooted leaves forming 2.2 rosettes per leaf. A total of 47 rosettes were planted in soil and acclimatized in a greenhouse, where they flowered for the past 3 years. For the first time, plants of the three target *Thymus* species were obtained hydroponically by vegetative propagation from cuttings, thus overcoming the

difficult and slow rooting of cuttings in a soil substrate. For *V. vitis-idaea*, the age of the cuttings was crucial: the best results were obtained with semi-hardened cuttings treated with IBA (100% rooted and surviving) on a Flood & Drain system with perlite substrate.

The protocol for *in vitro* propagation of the slow-growing bulb species *L. rhodopaeum* and *H. papilio* was improved by upgrading with a new step: hydroponic cultivation of the *in vitro* bulblets to accelerate their growth. This resulted in overcoming the dormancy in which *in vitro* propagated *L. rhodopaeum* bulblets fall upon repeated sub-cultivation. In 8 months, lily bulblets with well-developed roots grew 22 times, regardless of their initial weight. The presence of *Trichoderma harzianum* in the nutrient solution stimulated bulblets' rooting, while the higher values of the solution electrical conductivity led to formation of new bulblets. Many of the soil-adapted plants under greenhouse conditions formed flower-bearing stems already in the first season. From placing the *in vitro* bulbs on a hydroponic system to acclimating the plants in the *ex situ* collection and reaching flowering, it took about 2.5 years. Regarding *H. papilio*, in 16 weeks the weight of plants on the Cutting Board system increased on average 59.1 ± 24.0 times, while for the soil control it was only 11.1 ± 4.7 times.

In *S. officinalis*, seed germination and stem branching were significantly accelerated on a vertical aero-hydroponic system, with 3-month hydroponically grown plants reaching the size of 7-month soil-grown plants. The differences persisted after the acclimatization of the plants in the *ex situ* collection: the biomass yield increased by 18% and twice as many flowering stems were formed, which doubled the amount of essential oil. The time from seed germination to harvest was reduced by 5 months. In *E. purpurea* and *A. tinctoria*, it was necessary to stimulate the germination of the seeds before their hydroponic cultivation. In *E. purpurea*, the use of an aero-hydroponic system stimulated the formation of a well-developed root system, which shortened twice the time to maturity, compared to plants germinated in terrine with soil. In *A. tinctoria*, transferring the plant sprouts to the Cutting Board system led to their rapid growth and shortened the period from seed germination to the flowering phase from 12 to 6 months.

The hydroponically cultivated plants of the investigated phytochemical species retained their ability to synthesize their typical biologically active substances. Galanthamine was the major alkaloid in all *H. papilio* leaf samples taken from hydroponically cultivated plants, soil-grown control plants, and wild-type plants (about 90% of the total alkaloid content). In all samples, the related 7 alkaloids were the same. The slight decrease in galanthamine content in the dry matter of the hydroponically cultivated plants was compensated by their intensive growth: 5 times more biomass than in the soil-cultivated plants. Hydroponic cultivation of *S. officinalis* did not affect the concentration of the essential oil, but it did affect its composition and the amount of its components to some extent; however, the amount of essential oil remained twice as high during the second year of cultivation in the *ex situ* collection, which was due to the significantly higher number of generative stems formed by the hydroponically grown plants. The metabolic profiles of the hydroponically grown plants of the investigated thyme species corresponded to those of the parental plants. The methanolic extract of *T. zygioides* differed from that of *T. longedentatus* by the high content of thymol and carvacrol and the presence of some specific compounds such as geranic acid and hydroquinone

In most studied species, protocols have been established for the successful application of this innovative method at the laboratory level. The *ex situ* collection of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences has been enriched with 10 new species propagated by hydroponic technologies.