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REVIEW

Of the member of the scientific jury: **Prof. Lilyana Rumenova Nacheva**, **Ph.D.**, Fruitgrowing Institute - Plovdiv, Agricultural Academy, professional field 6.1. Horticulture, scientific specialty "Fruitgrowing" (Professor) and 4.3. Biological Sciences, scientific specialty Plant Physiology (Associate professor), appointed as a member of the Scientific Jury according to Order № 62/27.09.2024 of the Director of The Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences

regarding the PhD Thesis for awarding the educational and scientific degree "**Doctor**" (PhD) in field of higher education: 4. Natural sciences, mathematics and informatics; professional direction: 4.3. Biological Sciences, scientific specialty: Botany

PhD Student: Boryanka Dimitrova Traykova

Title: „Hydroponic technologies as a means of protection and cultivation of medicinal and conservation significant plant species“

I. General characteristics of the dissertation - volume and structure

In recent years, hydroponic technologies have successfully entered agriculture for the cultivation of vegetables, spices and herbs. They provide a number of advantages such as year-round production regardless of climatic conditions, mechanization of activities and limiting the use of pesticides. In addition, there is a growing demand for biologically active substances isolated from rare and endangered species. In this sense, the dissertation submitted to me for review examines many current problems related not only to the protection of biodiversity, but also to the modern possibilities of obtaining valuable secondary metabolites with applications in the cosmetic, food and pharmaceutical industries.

The dissertation presents an interdisciplinary scientific study of the possibilities of applying hydroponic technologies as a means of conservation and cultivation of medicinal and endangered plant species, some of which are Balkan endemics, included in the Red Book of the Republic of Bulgaria, and their transformation into cultivated species. To the best of my knowledge, this is the first dissertation in our country to apply the hydroponic culture method to medicinal plants.

The dissertation is sufficient in volume, contains all the necessary sections, which are well structured and balanced. It is written on 178 pages, with the literature review covering 18% of its volume. The majority is devoted to the results and their discussion. The work is well illustrated with 17 tables and 42 figures. In essence, the dissertation covers 8 publications related to the hydroponic cultivation of rare, endangered medicinal and/or conservation important plant species. Five of the publications are in publications referenced in the global databases Web of Science and/or Scopus. Their publication in prestigious journals is another proof of the high quality of the research conducted and the presentation of the results obtained from them.

II. Literary awareness and theoretical preparation of the candidate

The literature review covers the main literature sources related to the hydroponic cultivation of plants. The advantages and disadvantages of the technology are highlighted, as well as the main achievements in the cultivation of medicinal plants.

The cited literature includes 138 sources, 129 in Latin and 9 in Cyrillic, and most of the cited sources are from the last 10 years.

III. Methodical approach

Eleven plant species were used in the development of the dissertation: *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*. All these species are medicinal and/or conservation important plants. The possibilities for vegetative and seed propagation and accelerated growth in different types of hydroponic systems have been investigated in detail, with the main goal being to create reliable protocols for their propagation. In some of the medicinal plant species, the biosynthetic ability of the hydroponically propagated individuals was also investigated in relation to their characteristic secondary metabolites. Six research tasks were set and successfully completed.

A wide range of methods and techniques was used, focusing on the main subject of the study. All experiments were carried out in a sufficient number of replicates so that the obtained results were subject to statistical processing. A number of important indicators related to the possibilities of seed and vegetative reproduction from leaves and cuttings of the target species were investigated by testing different hydroponic systems, the acceleration of bulb growth, adaptation of the hydroponically cultivated plants to soil substrate and subsequent

acclimatization to greenhouse conditions and found in the ex situ collections, as well as a comparative analysis of the results of chromatographic determination of the biologically active substances of some of the hydroponically grown plants compared to the control ones.

IV. Significance and persuasiveness of the obtained results, interpretations and conclusions

The classical methods of seed and vegetative propagation in medicinal species of conservation importance (*Haberlea rhodopensis*, *Thymus pannonicus*, *T. zygioides, T. longedentatus*, *Vaccinium vitis-idaea*, *Lilium rhodopaeum*, *Hippeastrum papilio*, *Alkanna tinctoria*) are slow and ineffective, and cannot to meet the growing demand for sources of secondary metabolites for the pharmaceutical and food industries. Innovative approaches are needed on the one hand for the protection of biodiversity, and on the other - for the industrialization of future productions. A huge amount of work has been done.

Species-specific features of hydroponic vegetative reproduction have been established.

The conclusions drawn as a result of the study are logical, the most important of which are:

- ✓ Due to the better developed root system, in the target species tested (*Haberlea rhodopensis*, *Thymus pannonicus*, *T. zygioides* and *T. longedentatus*, *Vaccinium vitis-idaea*) the rooting and survival of leaves or cuttings was much more successful in hydroponic systems compared to with the control in soil substrate;
- ✓ Of essential importance for the success of the propagation of *Haberlea rhodopensis*, when using aero-hydroponic systems, are the type of substrate and the preliminary rooting of the leaves in moist perlite;
- ✓ For *Vaccinium vitis-idaea*, the best results were achieved on the F&D system with perlite substrate with semi-woody cuttings treated with IBA;
- ✓ IBA treatment improved rooting only in *Vaccinium vitis-idaea*, while in *Haberlea rhodopensis* it stimulated the formation of numerous miniature rosettes at the base of the newly obtained plant;
- ✓ Hydroponic technologies are suitable for accelerating the growth of slow-growing bulb species using bulbs obtained from seeds or propagated in vitro. This method helps to overcome the dormancy that in vitro propagated bulbs of *Lilium rhodopaeum* fall into when they are subcultivated on nutrient medium, the F&D system with perlite substrate being more suitable;
- ✓ The growth of seed- or *in vitro*-propagated bulbs of *Hippeastrum papilio* was more efficient on CB hydroponic system with expanded clay substrate compared to F&D system with perlite substrate;

- ✓ A significant acceleration of the growth and development of some of the studied species was found when cultivating seeds or ponies of the GD aerohydroponic system. The use of this system in GA3-treated *Echinacea purpurea* seeds stimulated the formation of a well-developed root system. In *Salvia officinalis*, cultivation on a GD system led to a significant acceleration of growth and development, with differences persisting after acclimation of the plants in the ex situ collection;
- ✓ Extremely low seed germination in *Alkanna tinctoria* was overcome by combined seed treatment with GA_3 and monochromatic light. This approach shortens the period from seed germination to flowering phase from 12 to 6 months;
- ✓ Application of the CB system with a substrate of peat or coconut cubes is unsuitable for *Haberlea rhodopensis* due to the excessively slow growth of panicles and the development of algae, moss and pest larvae on the substrate;
- ✓ It was established that the hydroponically cultivated plants of the phytochemically studied species retain their ability to synthesize their typical biologically active substances;
- ✓ Galantamine and the accompanying 7 alkaloids were the same in all examined *Hippeastrum papilio* leaf samples: hydroponically grown plants of F&D and CB systems, soil-grown control plants, and wild-type plants;
- ✓ An inverse proportionality was found between the biomass growth of *H. papilio* and its galantamine content, being the highest in the leaves of plants grown in a soil substrate. The slight reduction in galantamine content of CB system is compensated by intensive growth and biomass (5 times more than in soil cultivated plants);
- ✓ The concentration of essential oils in conventionally and hydroponically obtained *Salvia officinalis* plants did not differ in both years of their cultivation in the ex situ collection;
- ✓ Hydroponic cultivation of *S. officinalis* affects the composition of the essential oil and the amount of its components. Differences in the amount of essential oil persisted in the second year as well, which was due to the significantly greater number of generative stems formed in the hydroponically obtained 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* differs from that of *T. longedentatus* with a higher content of thymol and carvacrol and the presence of some specific compounds such as geranic acid and hydroquinone.

V. Critical notes to the dissertation work

I have no significant remarks on the dissertation work. The commercial brands of the nutrient solutions are indicated. It would be good to give their mineral composition as well.

I have a few ***questions:***

1. Does the PhD student have observations on the application of hydroponic techniques in the acclimatization of other *in vitro* propagated species, beyond those investigated in the dissertation?
2. Are there additional observations of the target plant species after the results are published?
3. Do you think it would be promising to scale up the hydroponic cultivation of the studied species from the river *Thymus* and *Haberlea rhodopensis* in order to apply the method in production?

VI. Nature of scientific contributions

I accept the author reference for the contributions. They are very well summarized. I would classify them into the following two groups:

A. Contributions of an original nature:

1. *Haberlea rhodopensis* plants were obtained for the first time by hydroponic propagation, using leaves, on two different aero hydroponic systems: the vertical Green Diamond (best results) and the horizontal Aeroflo 20. Several years of observations show a slow but steady growth of the rosettes in the greenhouse and flowering in three consecutive years, proving sustained acclimatization and reliability of the method.

2. For the first time, plants of 3 types of thyme (*Thymus pannonicus*, *T. zygioides* and the Balkan endemic *T. longedentatus*) were obtained through vegetative propagation from cuttings, using hydroponic technologies. The method is very suitable for the tested species, given the difficult and slow rooting of the cuttings in soil substrate;

3. The metabolic profiles of the hydroponically grown plants of the investigated thyme species corresponded to those of the parental plants;

4. Reliable protocols have been created for the propagation of medicinal and conservation important plant species with the application of hydroponic technologies at the laboratory level through: vegetative propagation (*Haberlea rhodopensis*, *Thymus longedentatus*, *T. pannonicus*, *T. zygioides* и *Vaccinium vitis-idaea*) and seed propagation (*Echinacea purpurea*, *Salvia officinalis*, *Alkanna tinctoria*);

5. Hydroponic cultivation of *in vitro* obtained bulbs of the slow-growing species *Lilium rhodopaeum* and *Hippeastrum papilio* was successfully applied in the process of their acclimatization to *ex vitro* conditions to accelerate their growth.

B. *Contributions of a confirmatory nature:*

1. The hydroponically cultivated plants of the phytochemically studied species retain their ability to synthesize their typical biologically active substances.

VII. Evaluation of the quality of the scientific works reflecting the dissertation research

In connection with the procedure, 8 publications in English are presented, which contain results of the development of the dissertation work. They have undergone a peer review process and have been published in reputable scientific journals. Five of them were published in scientific publications referenced in the globally recognized databases Web of Science and/or Scopus with quartiles Q2 – Q4. This confirms their significance and scientific value.

Two of the publications were cited seven times, which is another confirmation of the high scientific level of the conducted research.

The PhD student has presented the results of her research at 10 international scientific forums - 3 abroad and 7 in Bulgaria.

The presented abstract fully and accurately reflects the research included in the dissertation work.

VIII. A motivated answer to the question to what extent the dissertation research is primarily the personal work of the doctoral student

Doctoral student Boryanka Traikova has acquired the necessary knowledge in the relevant scientific field - research into the possibilities of cultivating rare and endangered medicinal or conservation-important plant species through hydroponic systems. She has conducted a large-scale interdisciplinary scientific study of the possibilities for the conservation and cultivation of eleven species of medicinal and conservation significant plants. She mastered many and varied methods of research, with which she fulfilled one of the main goals of the educational and scientific degree "Doctor". I had the opportunity to see her presentation at an international scientific forum, where she showed an excellent command of the materials presented.

Based on her educational program, the doctoral student has covered study material from specialized courses that correspond to the required 130 credits. The doctoral student meets the requirements set by the Training Center at BAS for the approval of the implementation of the scientific program, presentation of the results on the topic of the dissertation in publications and at scientific forums, and has collected a total of 470 credits, with a mandatory minimum of 250.

CONCLUSION

On the basis of the various research methods learned and applied by the doctoral student, the correctly performed experiments, the generalizations and conclusions made, I believe that the presented dissertation represents an original contribution to science, meets the requirements of the ZRASRB and the Regulations for the conditions and procedures for the acquisition of scientific degrees and for occupying academic positions at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences, which gives me reason to evaluate him POSITIVELY.

I allow myself to propose to the honorable Scientific Jury to also vote positively and award **Boryanka Dimitrova Traikova** the educational and scientific degree **"Doctor" (PhD)** in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction: 4.3. Biological Sciences, scientific specialty "Botany".

14.11.2024 г. Reviewer: .....

Plovdiv (Prof. Lilyana Nacheva, PhD)