REVIEW

on a Dissertation submitted for the degree of "Doctor of Science"
professional field 4.3. Biological Sciences, scientific speciality Plant Physiology

Author of the Dissertation: Violeta Borisova Velikova, PhD, Professor in the Laboratory "Photosynthesis - activity and regulation" of the Institute of Plant Physiology and Genetics at the Bulgarian Academy of Sciences

Thesis topic: **Physiological role of biogenic isoprene in plants**

Reviewer: Professor Liliana Georgieva Gigova, PhD, Institute of Plant Physiology and Genetics at the Bulgarian Academy of Sciences

This review has been prepared on the basis of Order of the Director of IPPG-BAS № 971/17.12.2019 and decision of the Academic board approved on 15.01.2020.

In the procedure of defense, Prof. Violeta Velikova presented all documents in accordance with the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (LDASRB or ZRASRB), the Rules for its implementation, as well as the Regulations for the conditions and the procedure for acquiring scientific degrees and occupation of academic positions at BAS and IPPG-BAS.

Career profile of V. Velikova

Violeta Borisova Velikova acquired the qualification of Biologist at the Faculty of Biology at Sofia University "St. Cl. Ohridski "in 1986. In 1988 she was appointed Specialist Biologist at the IPP „Acad. M. Popov"- BAS. In 1998 she successfully defended her dissertation and obtained the educational and scientific degree "Doctor". In 2006, Violeta Velikova was elected as a Senior Research Associate (Associate Professor) at the IPP, and in 2012 - as a Professor at the IPPG. In 2006 she specialized in Italy (3 months) and in the UK (4 months). In 2009 and 2010 she specialized in Italy 4 and 12 months, respectively. In the period 2001-2014 she won 6 scholarships abroad (Italy, Germany, Great Britain, Portugal, Greece) with a total duration of 60 months. Specializations in leading scientific institutions and cooperation with leading scientists enrich V. Velikova's knowledge in the field of plant physiology, expand her

skills and competences for the application of various techniques and methods for scientific research (gas-chromatography, proteomics, biophysics), computer programs for data processing and analysis. This helps her establishing as one of the recognized researchers in the field of biogenic organic compounds. Professor Velikova is a co-author of 102 scientific articles, 74 of which have been published in prestigious international journals (**Q1 – 43**; Q2 – 9; Q3 – 20; Q4 – 2, total IF=**190.489**), with noted citations over **4000** (WoS/Scopus). Her h-index is **25** (Scopus). Currently, Prof. Velikova is Leader of the Laboratory "Photosynthesis - activity and regulation" and since 2014 has been the Chairman of the IPPG Scientific Council. She is a member of national (Union of Scientists in Bulgaria) and international (Federation of European Societies of Plant Biology, FESPB) organizations.

Characteristics and evaluation of the dissertation

Prof. Violeta Velikova's dissertation is devoted to clarifying the role of biogenic isoprene in reducing the negative effects of abiotic stress on plants and the possible mechanisms of its protective action. As a part of current research on biogenic isoprene emission, the dissertation contributes to answer to the question: "why do plants produce isoprene". Since isoprene biosynthesis is costly, a large investment of carbon and energy in the production of this molecule must have relevant functional reasons. However, the functional causes of isoprene emissions are still a debatable topic. The topic is up-to-date and its importance is emphasized by the fact that isoprene emission has a tangible impact on atmospheric chemistry, air quality and climate change. This effect is associated with the high reactivity of isoprene, which leads to an increase in tropospheric ozone concentration, methane life span, and affects the nucleation, condensation, or coagulation of secondary organic aerosols.

The dissertation is written on 148 pages. The main text is presented in 7 parts, illustrated with 67 figures (incl. 4 photographs, 3 electron microscopic photographs, 4 schemes and 2 diagrams) and 1 table. 359 references are cited. The achievements of 20 scientific publications (19 in Q1 and 1 in Q2, with a total impact factor of 86,851) are summarized.

The concise (17 pages) and informative Literature Review clearly shows the author's good awareness in the context of the experimental tasks to be performed. The focus is on the factors controlling isoprene emission (light, temperature, circadian rhythm, CO_2 concentration, soil moisture, nutrient supply). Available literature data are presented and analyzed for the role

of biogenic isoprene in protecting plants against abiotic stressors (high temperature, ozone), as well as the putative mechanisms of its protective function. The 2 figures, adapted from correctly cited sources, illustrate (1): the sources of isoprene and its role in atmospheric chemistry and (2): the biosynthetic pathways of plant isoprenoids and their cellular localization.

Based on the data in the literature and the accumulated research experience on the problem under study, Prof. Velikov clearly formulates the purpose of the dissertation, 3 hypotheses and 4 specific tasks to achieve the goal. Various experimental models have been used and they are well illustrated in a figure. A multidisciplinary approach (physiological, biophysical, biochemical and structural studies) has been applied and a rich set of relevant classical and modern methods and techniques have been used for each study level. For example, I will mention the methods used for quantitative and qualitative proteomics - Isotope-coded protein labeling (ICPL) of chloroplast proteins, in combination with polyacrylamide gel electrophoresis (PAGE) and tandem mass spectrometry (LC-MS/MS) as well as Lable-Free LC-MS/MS, Blue Native PAGE of native proteins and protein complexes, Acid-Urea-PAGE of histone proteins, Biotin-Switch and LC-MS/MS for analysis of *S*-nitrosylated proteins. **For the first time** the biophysical techniques (circular dichroism, electrochromic shift at 515 nm and thermoluminescence) were used to clarify the physiological role of isoprene. The experimental results were processed with adequate statistical methods using ANOVA followed by Tukey's test and Student's *t*-test. The results of proteomic analyzes are subjected to multivariate analysis with statistical methods of Principal Component Analysis and Orthogonal Partial Least Squares.

Results and Discussion: A large volume of well-documented and illustrated (67 figures and 1 table) research data was obtained and analyzed. Each of the 7 parts of the results contains an introduction (including a rationale for the research objective and a hypothesis), a statement, a discussion, and a conclusion that correctly reflects the relevant results. The studies are logically planned in accordance with the formulated three hypotheses for the physiological role of endogenous isoprene. The experimental results clearly show that in isoprene-emitting plants, photosynthetic activity is less impaired under different stress conditions compared to non-isoprene-producing plants. **For the first time** endogenous isoprene is shown to play an important antioxidant role, protecting plants from ozone and singlet oxygen induced oxidative stress. It is established that isoprene-emitting plants are not only better protected at high temperature and drought than non-emitting plants, supporting available studies, but additionally also

demonstrated that isoprene-emitting plants recover faster after cessation of stress. The protective role of isoprene has been associated with its ability to reduce the formation of reactive oxygen and nitrogen forms and to limit lipid peroxidation of cell membranes, to directly interact with singlet oxygen, or to promote electron flow through photosynthetic/photorespiratory pathways. I would like to acknowledge and appreciate the studies related to the effect of biogenic isoprene on photosynthetic membranes. Three appropriately selected biophysical techniques have been applied, exploring different aspects of the photosynthetic apparatus. **For the first time** it is experimentally proven by the obtained results that isoprene improves the integrity and functionality of photosynthetic membranes under high temperature conditions, increasing the thermal tolerance of the plants. Isoprene has been suggested to improve the thermal stability of thylakoid membranes by affecting the lipid composition of the membranes, which has been proven in subsequent experiments. The inhibition of isoprene emission leads not only to significant changes in the lipid and fatty acid composition of the thylakoid membranes, but also to changes in the chloroplast ultrastructure. **Impressive and of particular interest** are the results of proteomic analysis. By applying for the first time the method of stable isotope-encoded protein labeling, it has been found that inhibition of isoprene biosynthesis alters the chloroplast protein profile in the direction of structural changes in photosynthetic membranes and reduced protection against oxidative stress. An interesting fact is that changes in the chloroplast protein profile determine the development of alternative protective mechanisms, such as photorespiration and non-photochemical quenching, to compensate for the lack of isoprene. **New data are presented** for the accumulation of nitric oxide only in isoprene-inhibited ozone-treated leaves, which is associated with more pronounced changes in protein *S*-nitrosylation. The comparative profile of the *in vivo S*-nitrosoproteome of isoprene- emitting and non-emitting plants shows increased nitrosylation of a number of antioxidant enzymes, leading to the conclusion that isoprene indirectly regulates the formation of reactive oxygen species (ROS) via the control of the *S*-nitrosylation level of ROS-metabolizing enzymes. **Original and significant results** were obtained by examining the action of individual components of the antioxidant plant protection system under conditions of single and combined stress. **New evidence** for a daily orchestration of antioxidant protective agents is provided, and it reveals an unforeseen joint action of volatile, nonvolatile isoprenoids and flavonoids in plants (*Platanus* x *acerifolia*), experiencing the combined effect of high temperature, high light and drought. Under severe

drought, the presence of isoprene stimulates the synthesis of non-volatile isoprenoids (abscisic acid and carotenoids) and phenylpropanoids, providing additional protection of transgenic tobacco plants. The results of this study highlight the central role of isoprene in stress-induced metabolic tuning. By using *Populus nigra* as a model system, it is shown that not only isoprene, but other, higher molecular weight isoprenoids, such as cis-β-ocimene and linalool, may play an important role in plant resistance mechanisms against heavy metal stress.

Particularly noteworthy are the **scientific-applied aspects** of these fundamental researches. In the present dissertation, it is shown **for the first time** that constitutive isoprene emission and induced isoprenoid emissions in *Populus nigra* (black poplar) increase under Ni stress. The combined effect of high temperature and elevated atmospheric concentration of CO_2 ($[CO_2]$) has been found to have an adverse effect on the isoprene emission, functional and structural characteristics of *Platanus orientalis* (oriental plane). In addition, elevated $[CO_2]$ not only inhibits isoprene emission but also stimulates methanol emission. It has also been found that *Phragmites australis* (common reed) can accumulate high levels of phosphorus without increasing the supply of biogenic isoprene into the atmosphere. On the other hand, high levels of phosphorus stimulate photosynthesis, which can accelerate the accumulation of biomass and increase the removal of phosphorus from polluted waters. These results would be a useful basis for developing adequate models for the quantitative and qualitative prediction of constitutive and induced isoprenoid emissions from plants under stress (anthropogenic pollution, climate change), models for the impact of these emissions on air quality on a regional and global scale, as well as for the selection of suitable plant species for reforestation, inc. remediation of areas with different anthropogenic pollution.

In general, **the scientific achievements** can be summarized as **providing new experimental evidences** to support the formulated three hypotheses for the physiological role of endogenous isoprene, namely:

- isoprene is essential for increased plant tolerance, exerting effects at functional, proteome, metabolic and structural levels;

- isoprene modulates plant response to stress indirectly through the regulated generation of active oxygen and nitrogen forms;

- isoprene is a part of the plant's antioxidant system and acts in sync with other protective metabolites, providing better protection against stress.

Dissertation publications

The scientific findings of Prof. Velikova are original and extremely significant. A strong argument in support of my appreciation for them is the fact that the results presented in 20 scientific articles are published in journals with impact factor (total **IF 86.851**) and **Q1** rank (**19** articles) and **Q2** rank (**1** article). All publications are in reputable international journals: New Phytologist (IF 7.210); Plant, Cell and Environment (IF 6.960); Plant Physiology (IF 6.456); Journal of Proteome Research (IF 4.245); etc. In recognition by the international scientific community, **1327** citations of the publications included in the dissertation are noted, of which 1159 are in scientific publications referred and indexed in Web of Science and Scopus. In 13 of the publications Prof. Velikova is the first author, in two - the second, and in one - the last corresponding author, which makes her personal contribution undoubted. Seven of the scientific publications included in the dissertation are outside the habilitation work for the occupation of the academic position of "Professor". In 2 of these publications Prof. Velikova is the first author, in one she is the first and corresponding author and in one she is the corresponding author, which is in accordance with this specific requirement of the IPPG. In addition, the scientific results have been reported at 26 international and 3 national scientific forums. In 14 of the international reports, Prof. Velikova is the first author (including 2 invited oral presentations and 3 oral presentations). The dissertation of Prof. Velikova includes the results of a total of 9 projects (6 international and 3 national), in 6 of which she is the project coordinator.

Obviously, the scientometric indicators of Prof. Velikova are extremely high, and their values and quality far exceed the requirements for acquiring the scientific degree "Doctor of Science".

I accept the author's reference for the **achievements** presented in the dissertation. The clearly outlined 15 scientific contributions objectively reflect the real achievements of the work, including what was achieved for the first time in the science.

The **SUMMARY** (78 pages) contains all the basic data and messages of the dissertation and is well illustrated.

Notes and Recommendations:

- Fig. I.2. C-F and Fig. I.6. from the abstract are missing in the dissertation;
- Fig. II.2. C - photosynthesis has not been shown to be slightly inhibited in leaves undergoing a reconstruction experiment in which exogenous isoprene was fed, as indicated in the text on page 31 of the dissertation;
- Part of the achievements could be exempt from details and combined in meaning. For example, achievements 1-3 and achievements 8 and 10;
- In my opinion, the results of the dissertation publications should not be presented and discussed in the Literature Review, nor should these publications be included in the References list.

I would like to point out that the comments and recommendations made are not substantive and do not affect the merits of the dissertation.

CONCLUSION

The analysis of the dissertation submitted for review shows that it is a logically designed, precisely performed multidisciplinary research, with a number of original scientific contributions that fully meets the requirements of the ZRASRB, the Rules for its implementation, as well as the Regulations for the conditions and the procedure for acquiring scientific degrees and occupation of academic positions at BAS and IPPG-BAS. Many years of focused researches, extensive theoretical knowledge and experimental experience, high and valuable publication activity and citation counts characterize Prof. Velikova as an internationally recognized, leading scientist in a specific field of research related to the physiological role of biogenic isoprene and the mechanisms of its protective action under conditions of abiotic stress. All this gives me a reason **to positively evaluate** the reviewed scientific work, the results achieved and the original contributions, and **to unconditionally support** the acquisition of the scientific degree "Doctor of Science" by the author of this dissertation. I dare to strongly recommend to the Honorable Academic board to award to **Prof. Violeta Borisova Velikova** the scientific degree "**Doctor of Science**" in the professional field Biological Sciences, scientific specialty "Plant Physiology".

06.03.2020 Reviewer:

/Prof. Liliana Gigova, PhD/