REVIEW

of the dissertation on "Physiological role of biogenic isoprene in plants"
for the award of the scientific degree “Doctor of Science”

to **Prof. Dr. Bioleta Borisova Velikova**

in the higher education field 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty Plant Physiology

by Prof. Stefka Germanova Taneva, DSc

Materials Received for Review

Documents for review: Dissertation; Summary of dissertation; List of scientific publications indexed in Web of Science and Scopus; The publications on which the dissertation is based in full text; List of citations that do not overlap with those provided for the scientific and educational degree “Doctor”; List of participation in scientific forums.

The following administrative documents are presented: CV of the candidate; Copy of the diploma for the educational and scientific degree “Doctor”; Protocols for Extension of the Scientific Seminar (No 791 / 11.11.2019) and for the Extended Scientific Seminar (No 47 / 05.12.2019) of the Laboratory of Photosynthesis - Activity and Regulation at the Institute of Plant Physiology and Genetics, BAS, held for discussion of a project of the dissertation work; Rules for the Regulations for Scientific Development of the Academic Staff of the IPPG-BAS.

All documents presented by Prof. Violeta Borisova Velikova for the award of the degree of “Doctor of Science” are carefully prepared and fully in compliance with the requirements of the ZRASRB, the Regulations for its implementation and the specific requirements of the IPPG-BAS for the degree of “Doctor of Science" at the Institute of Plant Physiology and Genetics, BAS.

General Presentation of the Author of the Dissertation

Prof. Velikova graduated in Ecology and Environmental Protection at the Faculty of Biology at Sofia University "St. Kliment Ohridski". She defended her PhD Thesis in 1998. Her scientific career began as a biologist at the Institute of Plant Physiology “M. Popov”, Bulgarian Academy of Sciences; from 1998 to 2006 she worked as a research associate I - II degree. In 2006 she was habilitated as an Associate Professor and in 2012 she was elected for the academic position of Professor. Prof. Velikova is a head of the Laboratory of Photosynthesis - Activity and Regulation, at the Institute of Plant Physiology and Genetics, BAS.

Prof. Velikova has specialized in prestigious research centers in Italy, Germany, UK, Portugal, Greece: postdoc in Italy (2010 - 12 months); Specialization in ESF – VOCBAS, Italy (2009 - 4 months); Specialization in line ESF – VOCBAS, UK (2006 - 4 months); ACCENT specialization, Italy (2006 - 3 months). She had been awarded a NATO Fellowship (Portugal - 2003-2004 - 12 months; Italy - 2003; 2000-2001 - 12 months in total; Greece - 2001 - 3 months); Alexander von Humbold (18 months, 2012 - 2014); Marie Curie Fellowships (Industry - Academy (PTR-TOF) (Italy 2011 - 12 months) and Training Program (ISONET) (Italy 2007 - 3 months)). Currently she has active collaborations with leading scientists from abroad.

Prof. Velikova has published 102 scientific publications, 74 of which in renowned international journals (43 publications fall in journals with rank Q1, 9 with Q2, 20 with Q3 and 1 with Q4); with a total IF of 190.489. Her works have been cited over 4 000 times (Web of Sci / Scopus), h-index 25 (Scopus, Web of Sci), which shows the high quality of her scientific production.

She has coordinated or participated in national and international funded research projects.

Prof. Velikova supervised 2 successfully defended PhD students.

She is a member of the Union of Scientists in Bulgaria, the Federation of European Societies of Plant Biology (FESPB), and the Society for Experimental Biology (SEB).

Since 2014 Prof. Velikova is the chairman of the Scientific Board of IPPG - BAS.

Relevance of the dissertation topic

Prof. Velikova's dissertation is devoted to an up-to-date research topic about the physiological role of biogenic isoprene, the most widespread volatile hydrocarbon, which emissions (44% of the total emissions of biogenic volatile organic compounds) are associated with significant energy metabolite and carbon consumption, and that plays a significant role in atmospheric chemistry and air quality. Elucidation of the factors that control the emission of isoprene from plants is particularly important both for predicting changes in atmospheric chemistry and for selecting plant species suitable for industrial pollution areas as well as for establishing the presumed protective role of isoprene in plants under various stresses.

Knowledge of the Scientific Problem

The author of the dissertation has used 379 literature sources to justify the relevance of the problem under investigation. The survey of the scientific literature and the analysis of the published data and scientific achievements in the field show excellent knowledge of the problem, solid understanding and competence in the research area. The literature review is written concisely and competently in 5 sections focused on: 1) plants as sources of isoprene; 2) the role of isoprene in atmospheric chemistry; 3) biosynthesis of isoprene in plants; 4) factors controlling isoprene emission; 5) the role of biogenic isoprene in protecting plants against abiotic stressors.

The author reviewed the scientific achievements in the field; pointed out the isoprene emissions impact on air quality, global troposphere chemistry and climate change; highlights the important questions on biogenic isoprene emission the current studies are seeking answers, namely "what is the biosynthetic pathway of isoprene?" and "why the plants produce isoprene?" to which the dissertation work is devoted.

Research Approaches and Methods

Modern experimental techniques and various methods are applied: biophysical; biochemical; spectroscopic (for measuring chlorophyll fluorescence); proteomics; chromatographic; antioxidant assays; statistical etc., to conduct studies at physiological and structural level, which determines the high quality of the results obtained.

Various plant species were selected for the studies: (1) isoprene-emitting (Phragmites australis, Platanus orientalis L., Platanus x acerifolia L., Populus nigra, Populus x canescens, Arundo donax) and non-emitting isoprene (Hakonechloa macra) plant species; plants subjected to gene manipulation (Arabidopsis thaliana, Nicotiana tabacum cv. Samsun; Populus x canescens) resulting in altered ability to secrete isoprene as a natural metabolite; (2) isoprene emission manipulated leaves: chemically with fosmidomycin, which specifically blocks the MEP biosynthetic pathway in chloroplasts; leaves developed in an atmosphere with high concentration of CO2; leaves at different stages of ontogenetic development; plants of different age.

Characteristics and Evaluation of the Dissertation

The dissertation is presented on 422 pages. The main text is structured in 6 thematic chapters (Literature Review, Aims and Objectives, Materials and Methods, Results and Discussion, Conclusion, Contributions) on 148 pages; illustrated with 67 figures and diagrams; 379 references are cited. A bibliography of the published works related to the dissertation and copies of the publications are attached.
The literature review shows an extremely thorough understanding of the subject; summarizes the knowledge on plant sources of isoprene; isoprene biosynthesis in plants; the role of biogenic isoprene in protecting plants against abiotic stress and in atmospheric chemistry.

Elucidation of the physiological significance of biogenic isoprene as a tool for plant protection against abiotic stress is a major goal of the dissertation that summarizes investigations performed to test the following hypotheses:

(1) the biogenic isoprene is essential for plant tolerance at functional, proteome, metabolic and structural levels; environmental factors have a strong effect on isoprene emission and at the same time it represents a non-trivial carbon loss to the plants;

(2) the endogenous isoprene has a regulatory role on the accumulation of reactive nitrogen and oxygen products in cells and hence for the response of plants to stress;
(3) the synchronized action of biogenic isoprene as part of the plant's antioxidant system, along with other protective metabolites, provides better protection against stress.

To achieve the goal of the dissertation, specific research tasks are formulated to examine:
- the possible protective effect of isoprene against various abiotic factors (ozone, singlet oxygen, high temperature, high concentration of CO_2 in the ambient air, drought, anthropogenic pollution);
- the interaction between isoprene and nitric oxide (NO) in planta and the consequence of this interaction under oxidative stress;
- the effects of inhibition of the isoprene emission on the proteome, lipid and fatty acid composition of photosynthetic membranes, as well as on the chloroplast ultrastructure;
- the relationship between isoprenoids and phenylpropanoids under optimal and stressful conditions.

The main part of the dissertation - Results and Discussion contains 7 thematic sections.

The first section 4.1 presents results for the antioxidant capacity of biogenic isoprene, and includes studies on the role of endogenous isoprene in protecting the photosynthetic apparatus against oxidative stress (induced by ozone and Rose Bengal treatment) and against singlet oxygen as well as transgenic tobacco plants against oxidative stress.

The next section 4.2 is dedicated to the thermo-protective role of biogenic isoprene studied in leaves in which isoprene emission is chemically manipulated by fosmidomycin and leaves from plants at different age that emit different amounts of isoprene. In addition, the combined effect of increased atmospheric concentration of CO_2 and high temperature on isoprene emission, and the functional and structural characteristics of plants were investigated. Disturbances in photosynthetic function due to the inhibition of isoprene emission after treatment with high temperature and stabilizing effect of biogenic isoprene on photosynthetic membranes have been observed. Endogenous isoprene has been found to exert a protective role against high temperature and also to contribute to leaf recovery after drought stress. The better thermal tolerance of isoprene-emitting leaves is explained by the ability of isoprene to stabilize photosynthetic membranes and reduce reactive oxygen species. The results also show that isoprene emission depends on the age of the plants and that the antioxidant and antiradical ability of the plants is significantly increased after exposure to high temperature.

In section 4.3 the effects of isoprenoids on transgenic tobacco plants have been discussed. Data suggest that isoprene-emitting tobacco plants are better protected against drought than non-emitting plants, which is corroborated by other studies. New data have been obtained for plants recovery from applied drought stress and for stimulation of the production of non-volatile isoprenoids and phenylpropanoids, leading to additional plant protection under stress. During the drought process the isoprene emission increases almost twice in the initial stages of drought, and decreases significantly only at the highest degree of drought.

Section 4.4 refers to the interaction between endogenous isoprene and nitric oxide in plants, the effect of the inhibition of isoprene emission on the nitric oxide formation and the ability of isoprene and nitric oxide to increase the resistance to oxidative stress. Besides confirming that isoprene-emitting leaves are better protected against oxidative stress, it has been found that isoprene-inhibited leaves experiencing oxidative stress produce greater amounts of nitric oxide. The interaction between isoprene and nitric oxide upon protein nitrosylation is discussed. Exogenous nitric oxide, fed as gas or through the donor SNP, has a beneficial effect on leaves exposed to oxidative stress, reducing the inhibition of photosynthesis, the accumulation of reactive oxygen species and membrane damage products.

It has been demonstrated for the first time that nitric oxide accumulates only in isoprene-inhibited leaves of P. australis treated with ozone, whereas nitric oxide is not localized in isoprene-emitting and isoprene-inhibited leaves that do not experience ozone stress or in isoprene-emitting ozone treated leaves.

Special attention is paid to the proteins, lipids and ultrastructure of chloroplasts in plants with isoprene-inhibited emission in section 4.5, and also to the changes in the chloroplast proteome after genetic manipulation of isoprene emission in poplar; the changes in the lipid matrix of the thylakoid membranes and poplar chloroplast ultrastructure, and in S-nitrosylation of proteins as a result of inhibition of the isoprene emission. It is worth noting that a new method for proteomic studies (isotope-encoded protein tagging technique) was applied for the first time, that allowed determination of the inhibition of the biosynthesis and emission of isoprene resulting in alteration of the protein profile of chloroplasts, structural changes in photosynthetic membranes and reduction of plant resistance to oxidative stress. The levels of chloroplast proteins involved in photosynthesis have been shown to decrease in non-isoprene poplar genotypes, while the levels of histones and ribosomal proteins are increased.

Section 4.6 summarizes data on the correlation between the effects of isoprenoids and phenylpropanoids in stress; for the synchronization of the individual components of the plant antioxidant system for the protection and integration between isoprenoids, volatiles and non-volatiles, and flavonoids in plants in response to environmental stressors (high temperature and light intensity, and drought in plane trees (Platanus x acerifolia)) and for their physiological role in species of the subfamily Arundinoideae with different metabolism in drought conditions. Isoprene has been found to reduce photooxidative stress, reducing reactive oxygen and nitrogen forms, and enhancing heat resistance thylakoid membranes.

New convincing evidence is presented for the synchronization of the individual components of the plant antioxidant system for the protection and combined action of isoprenoids, volatiles and non-volatiles, and flavonoids in plants in response to environmental stress (high temperature and light intensity, and drought).

Analysis of two A. Donax ecotypes shows stimulation of isoprene biosynthesis, which has a protective function and prevents metabolic damage during severe drought in the ecotype adapted to harsh compared to that adapted to moderate climate conditions.

The last section 4.7 focuses on the effects of anthropogenic environmental pollution on isoprene emission, the effect of heavy metal nickel on photosynthesis, mesophilic conductivity and isoprenoid emissions in Populus nigra, and changes in the primary metabolism and isoprene emission at different phosphorus levels. Ni has been shown to adversely affect plant metabolism, an effect that depends on the amount of Ni accumulated in the leaves and the stage of their development, and for the first time the photosynthetic efficiency has been shown to decrease when leaves were treated with Ni due to reduced mesophilic conductance, electron transport and maximum carboxylating Rubisco activity, which depends on the stage of ontogenetic development of the leaves. These results are important for the development of models predicting the emission of constitutive and induced isoprenoids under stress.

High levels of plant phosphorus stimulate respiratory metabolic processes without increasing the release of biogenic isoprene into the atmosphere and, on the other hand, stimulate primary carbon metabolism by enhancing photosynthesis, which can stimulate biomass accumulation and increase phosphorus removal.

The dissertation ends with a Conclusion and Scientific contributions that present the most important scientific achievements of the dissertation.

Scientific Contributions of the Dissertation

The formulated major scientific contributions contain new and original information, some are affirmative, and correctly reflect the direct experimental evidences of the three hypotheses for the role of isoprene.

The evidences on (i) the endogenous isoprene-induced increase in the thermostability of thylakoid membranes, related to its effect on the lipid composition of the membranes, the amount of basic lipid classes and their fatty acid composition; (ii) the dependence of chloroplast ultrastructure and the chloroplast proteome on the inhibition of isoprene biosynthesis; (iii) the correlation of the isoprene emission with the level and fatty acid composition of the major lipid classes in thylakoids, confirm the hypothesis of the essential role of isoprene in the increased plant tolerance, inducing changes at structural, proteome, metabolic and functional levels.

By controlling the levels of S-nitrosylation of enzymes metabolizing reactive forms, endogenous isoprene regulates the levels of reactive oxygen and nitrogen forms, which supports the second hypothesis that isoprene indirectly modulates plant response to stress.

The results for synchronized action of biogenic isoprene with other protective metabolites (volatile and non-volatile isoprenoids and phenylpropanoids) that provides additional "antioxidant" protection of plants under stress, support the third hypothesis.

Evaluation of the Publications Related to the Dissertation and Personal Contribution of the Author

The dissertation summarizes the scientific contributions of 20 publications, all published in ISI Web of Science refereed journals with high IF (9 of them published in journals with impact factor over 5 (from 5.08 to 7.21), total impact factor 86.851; 19 of publications fall in journals with rank Q1 and 1 in Q2. The results have been reported in 72 scientific forums.

The dissertation related publications are of a high scientific level and with high quotability (1326). There are publications cited: 561 times (Plant Physiology 127: 1781-1787, 2001); 125 (Plant Cell and Environment 32: 520-531, 2009); 119 (Plant Cell and Environment 28, 318-327, 2005); 4 publications are cited more than 47 times, etc.

Prof. Velikova is the first author of 13 of the publications. This shows her leading role, despite being co-authored with bulgarian and foreign researchers.

Dissertation Summary

The dissertation summary is presented in Bulgarian and English, and reflects the content of the dissertation, the scientific contributions and includes list of publications related to the dissertation.

Personal impressions

I have known Prof. Velikova for many years as a highly qualified, dedicated to research investigations scientist as evidenced by her solid scientific output. I believe that Prof. Velikova is a researcher with a great potential and capacity to realize original scientific projects in the future.

CONCLUSION

The dissertation presented by Prof. Violeta Borisova Velikova summarizes a large amount of experimental results, original publications in peer-reviewed scientific journals that received a wide international recognition. The scientific data represent a valuable contribution to the science and meet all the recommended requirements for the degree of “Doctor of Science” of the Act for the Development of the Academic

Staff in the Republic of Bulgaria (ADASRB), the Regulations for the Application of ADASRB in BAS and the specific requirements of the IPPG-BAS.

This gives me reason to express my positive opinion about the dissertation, the in-depth research, the results achieved and the contributions to science and to recommend to the Honorable Scientific Jury to award the “Doctor of Science” degree to Prof. Violeta Velikova in the higher education field 4. Natural sciences, mathematics and informatics, professional field: 4.3. Biological Sciences, in Plant Physiology.

Sofia
12th of March 2020 /Prof. Stefka Germanova Taneva, DSc/