REPORT

by Prof. Katya Marinova Georgieva, PhD

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of Doctoral Thesis for awarding the academic degree “Doctor of Sciences” in professional field 4.3. "Biological Sciences", scientific specialty “Plant Physiology”

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Topic: **Physiological role of biogenic isoprene in plants**

General presentation of the procedure and dissertation

The documents presented by Prof. Velikova with respect to the thesis defense procedure are in accordance with the Law for Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation as well as the corresponding Regulations of Bulgarian Academy of Sciences and the requirements of Institute of Plant Physiology and Genetics for acquiring the scientific degree "Doctor of Sciences".

Violeta Velikova graduated from Faculty of Biology, Sofia University “St. Kl. Ohridski” in 1986. Since 1988 he has worked at Institute of Plant Physiology consecutively as a specialist biologist (1988-1998) and a senior assistant (1999-2006). She defended her Ph. D. thesis on “Influence of artificial acid rain on the functional activity of photosynthetic apparatus and the possibilities for overcoming its adverse effects by means of polyamines” in 1998. In 2006 she was elected as an associate professor and in 2012 as a professor. She was a leader of many international and national projects, as well as two successfully defended PhD students. She has specialized in various laboratories in Italy, Germany, the United Kingdom, Portugal, and Greece thanks to fellowships by NATO, ESF, Maria Curie, Alexander von Humboldt and others. The total number of her scientific publications is 102, of which 74 have been published in high-ranking scientific journals (Q1 - 43; Q2 - 10; Q3 - 20; Q4 - 1).

The dissertation presents a summary of 20 scientific publications – 19 in journals from the first quartile in the respective field (Q1) and 1 in Q2, showing the high quality of her scientific work.

The total impact factor of 86.851 and the number of citations of the papers included in the thesis is 1327.

Actuality of the topic

Prof. Velikova's thesis is an in-depth study of the physiological role of biogenic isoprene in protecting plants against abiotic stress. It is known that isoprene can affect the air quality, contributing to the formation of ozone and secondary organic aerosol particles and increasing the amount of methane in the atmosphere. Isoprene emission is highly dependent on environmental factors. The predicted climatic changes associated with rising temperatures and increasing dry periods can lead to an enhanced isoprene emission, which will increase its negative effect on air quality. The study of the impact of environmental factors on isoprene emissions is essential in predicting global climate change as well as in proposing adequate environmental management policies for future. The interest in the study of biogenic isoprene is also associated to its suggested protective role in plants experiencing various stress effects. Thus, clarifying the role of biogenic isoprene in enhancing plant tolerance to abiotic stress is important.

Characterization and evaluation of dissertation work and contributions

The design of the literature review and analysis of the published data, as well as the citation of 379 references demonstrate that Prof. Velikova possesses profound theoretical knowledge and professional skills. The purpose and specific tasks of the thesis are clearly formulated. Various up-to-date methodological approaches are used to solve these tasks. A number of physiological, biophysical, biochemical and structural analyzes are performed. Appropriate statistical methods are applied to analyze the results obtained. In order to clarify the physiological role of biogenic isoprene different approaches are used. Studies are conducted with plant species emitting isoprene as natural metabolite *(Phragmites australis*, *Platanus orientalis* L., *Platanus* x *acerifolia* L., *Populus nigra, Populus* x *canescen*s, *Arundo donax*); non-isoprene emitting species (*Hakonechloa macra*), and plant species with altered ability to emit isoprene by genetic manipulation (*Arabidopsis thaliana*, *Nicotiana tabacum* cv. Samsun; *Populus* x *canescens*); leaves with manipulated isoprene emission; leaves developed under elevated CO_2; leaves in different developmental stages, as well as plants in different ages.

The thesis contains original contributions, which can be characterized as novelty for science and extension of existing knowledge. It has been shown for the first time that endogenous isoprene has an important antioxidant role in plants, limiting the formation of reactive oxygen species. It was experimentally proven that isoprene increased thermotolerance of plants by

preserving the integrity of thylakoid membranes and photochemical activity. It has been found that endogenous isoprene not only protect the leaves against high temperature, but contribute to their better recovery after cessation of stress. The results showed that the isoprene emission depended on plant age. Two-year-old *Platanus orientalis* plants emitted more isoprene, had higher antioxidant and antiradical capacity and were more resistant to heat stress compared to one-year-old plants. However, the combined effect of high temperature and elevated atmospheric CO_2 concentration on biennial *Platanus orientalis* had an adverse effect on isoprene emission and functional activity.

It has been found for the first time that the inhibition of isoprene biosynthesis and emission alter the chloroplast protein profile, which is associated with structural changes in photosynthetic membranes and decreased resistance to oxidative stress. Suppression of isoprene emission has been shown to lead to significant changes in the lipid and fatty acid composition of thylakoid membranes and changes in the ultrastructure of chloroplasts, which proves the positive effect of isoprene on membrane stability.

The experimental data show that isoprene-emitted transgenic tobacco plants have higher drought tolerance. Isoprene-emitting plants show higher drought resistance under moderate drought due to stimulation of isoprene emission under these conditions. Under prolonged drought, the isoprene stimulates the accumulation of non-volatile isoprenoids (abscisic acid and carotenoids), and stimulates phenylpropanoid metabolism. It has been demonstrated that the isoprene-emitting plant *Arundo donax* exhibit better drought resistance than the non-emitting isoprene *Hakonechloa macra*, regardless a greater increase in the phenylpropanoids content of *H. macra*. *A. donax* more effectively regulates water loss through coordinated reduction of mesophyll and stomatal conductance compared to *H. macra*. Furthermore, the comparative study of physiological and metabolic changes in response to drought of two *A. donax* ecotypes adapted to different climatic conditions shows increased biosynthesis of isoprenoids in plants originating from regions with more unfavorable environmental conditions, contributing to better protection of photosynthetic membranes under severe drought and their faster recovery after rehydration.

The thesis abstract fully reflects the main results presented in the dissertation.

CONCLUSION

Prof. Velikov's dissertation is an in-depth study of the physiological role of biogenic isoprene in plants. Interesting approaches and various physiological, biophysical and biochemical methods have been used and significant scientific results have been obtained representing an

original contribution to science. The dissertation shows that she possesses deep theoretical knowledge and professional skills in the field of plant physiology and biochemistry.

The dissertation meets all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, the Regulations of the Bulgarian Academy of Sciences and the rules of Institute of Plant Physiology and Genetics for the acquisition of a scientific degree "Doctor of Sciences".

Based on the above, as well as the overall activity of the candidate as an internationally recognized scientist, I am convinced of my positive assessment of the research, the results achieved and the contributions made, and I suggest that the Honorable Academic Board award the “Doctor of Science” degree to Prof. Dr. Violeta Borisova Velikova in professional field 4.3. Biological Sciences, scientific specialty “Plant Physiology”.

09.03.2020 г. Assessor:

Prof. Dr. Katya Georgieva