

## **REVIEW**

**of the official reviewer Liudmyla Bondarieva**

for the dissertation of

**He Songtao**

**«Complex ecological, physiological and genetic analysis of the response of *Cucurbita* genus representatives to salt stress»,**

submitted for a Doctor Philosophy Degree (PhD)

Field of study: 10 – Natural Sciences

Specialty: 101 – Ecology

**Relevance of the dissertation topic.** Global environmental issues related to climate changes affect the functioning of natural systems as well as agro-ecosystems. An increase in the frequency and intensity of droughts is becoming one of the determining factors of crop yields, especially in arid and semi-arid regions. This factors is becoming a decisive factor in crop productivity, especially in arid and semi-arid regions. Drought, in turn, enhances the mobilization and accumulation of salts in the upper soil layers and activates soil salinization processes in space and time. High salt concentrations in the soil not only complicate water infiltration but can also directly harm plants and even disrupt soil structure by reducing its porosity and impairing water absorption capacity.

Due to the decrease in crop yield and product quality resulting from soil salinity, the annual losses of world agriculture currently amount to about 12 billion dollars. As a result, salinization has become a significant factor limiting agricultural productivity in the world.

Therefore, research on the mechanisms of plant adaptation to salt stress is becoming increasingly important each year. Studies on the mechanisms of salt tolerance in major agricultural crops, including representatives of the *Cucurbitaceae*

family, are particularly necessary and crucial. Most of these crops are quite sensitive to excess salts in the soil solution.

Considering the above, a relevant and practically significant aspect of the dissertation under review is the establishment of the ecological, physiological, and genetic foundations of salt tolerance in representatives of the genus *Cucurbita*. Additionally, it involves studying the potential adaptation of this group of agricultural crops to excessive salt content in the soil using classical experimental-field methods (morphometric, vitality analysis) and a range of modern laboratory ecophysiological methods (chemical, spectrometric, fluorescent analysis).

**Connection of the research with scientific programmes, plans, and topics.**

The work was done according to the plans of the research institute work of the Department of Ecology and Botany at Sumy National Agrarian University and according to the plans of the research institute work of Henan University of Science and Technology. In particular, research was supported by grants from the Key Research and Promotion Projects of Henan Province (No.212102110410 & No. 202102110202).

**The scientific novelty of the obtained results.** For the first time, based on the results of observations during the entire growing season, the influence of salt stress on a complex of quantitative and qualitative morphological characteristics of pumpkin plants was studied. The features and regularities of the accumulation of ions of mineral substances in the vegetative and generative organs of pumpkins under conditions of salt stress have been established. Physiological and biochemical transformations that appear in pumpkins against the background of salt stress were comprehensively investigated and clarified.

At the outset, the influence of salt stress on a complex of quantitative and qualitative morphological characteristics of pumpkin plants was studied based on the results of observations during the entire growing season. The features and regularities of ion accumulation of mineral substances in the vegetative and generative organs of pumpkins under conditions of salt stress were established.

Physiological and biochemical transformations that manifest in pumpkins against the background of salt stress were comprehensively investigated and clarified.

Twelve transcription factors belonging to the WRKY family in pumpkins were examined in response to salt stress. Their phylogenetic relationships, spatio-temporal expression patterns, tissue-specific expressions, and transcriptional activities under salt stress were analyzed comprehensively.

In a groundbreaking effort, the ecological and physiological interactions during the cultivation of *Cucurbitaceae* using grafting technology and arbuscular mycorrhizal fungi were meticulously studied and elucidated.

Methodological improvements were made in assessing salt stress impact during pumpkin ontogenesis, incorporating vitality analysis to evaluate the plant's condition.

A highly effective transient transformation system was developed to facilitate the study of gene function and protein subcellular localization in pumpkins.

Progress was made in advancing both theoretical and practical principles concerning the utilization of salt-tolerant pumpkin rootstock for watermelon cultivation.

**The practical significance of the obtained results.** The dissertation materials are found application in the academic curriculum of the Department of Ecology and Botany at Sumy National Agrarian University. These resources are utilized in the instruction of courses such as "Ecological Physiology of Plants," "Agroecology," and "Biology."

**The main results obtained personally by the author.** The dissertation under review conducts research on the mechanisms of response to salt stress and adaptation to it in plants of the genus *Cucurbita* at various levels of their organization. Specifically:

- At the molecular level:

Identification and analysis of the expression of the WRKY gene family in pumpkin under salt stress conditions was carried out

- At the cellular metabolism level:

It has been demonstrated that salt stress affects indicators and characteristics related to the the process of photosynthesis, water exchange, amino acid metabolism, as well as influences the accumulation of mineral ions and their distribution among various plant organs. By the way, at this level, pumpkin plants have exhibited a high level of cultivar individuality.

- At the individual and agro-population levels:

The influence of salt stress on morphometric parameters and vitality of plants has been established. It has been shown that an increase in salt concentration leads to a reduction in plant size parameters and vitality. Negative symptoms such as yellowing of leaves and curling manifest against the backdrop of increased salt concentration, affecting photosynthetic capacity and plant productivity. Vitality analysis also confirmed the higher sensitivity of plants of the variety Miben to the influence of increased salt concentrations.

- At the interspecies level:

It is proved expediency of use of plant grafting in order to adapt plants to salt stress, increase crop yields, and obtain high-quality products.

- At the biocenotic level

It has been demonstrated that the use of arbuscular mycorrhizal fungi in the cultivation of Cucurbitaceae plants leads to a change in plant metabolism and an increase in their productivity.

An analysis of the plagiarism check report for the presence of textual borrowings (Strike Plagiarisma Program) was carried out. The reviewer concluded that He Songtao's dissertation of on the topic «Complex ecological, physiological and genetic analysis of the response of *Cucurbita* genus representatives to salt stress» is the result of independent research of the applicant and does not contain elements of plagiarism and borrowing in accordance with the resolution of the Cabinet of Ministres of Ukraine dated 12.01.2022 No. 44, paragraph 9. The used ideas, results and texts of other authors have a link to the corresponding information sources.

**Number of scientific publications.** According to the materials these 12 scientific papers were published: three articles were published in a scientific journal, included in the database of Scopus, Web of Science, three articles were published in professional scientific journals of Ukraine (B), six publications were published in materials and abstracts of reports at international and national conferences.

**Remarks for the thesis content.** In a positive evaluation of He Songtao's dissertation work, it is evident that the research holds substantial scientific-theoretical and practical significance. The novel insights and relevance of the research are commendable. Here are some appropriate comments and wishes:

*Regarding statistical processing and presentation of data.* In the reviewer's opinion, in certain cases, for a more representative presentation of the data, it would be advisable to apply not only analysis of variance but also to present the results not only in the form of histograms but also as regression equations. This would allow for a better understanding of the overall response trends of each variety to the influence of factors (Fig. 3.2 (p. 68-69), Fig. 3.8. (p.83-84). Table 3.3. (p. 70).

*Regarding formatting.* In the dissertation, some figures (3.8, 3.9, 3.10, etc.) consist of several components (A, B, C). In the reviewer's opinion, it would be more appropriate to split each of them into three separate figures, representing the content of ions in the root, stem, and leaves (at the author's discretion).

*Technical errors.* Table 3.2. (p.70): «Nacl»; Fig. 3.4 (p. 75): the name of the Fig 3.4 is not written completely; p. 96: «Na<sup>+</sup> and Mg<sup>2+</sup>...».

These remarks do not reduce the scientific level and practical significance of the research, but primarily concern the design of the work and the presentation of the research results.

**Correspondence of the dissertation to the specialty and the profile of the specialized academic council.** Dissertation by He Songtao «Complex ecological, physiological and genetic analysis of the response of *Cucurbita* genus

representatives to salt stress», which was submitted for defense to the specialized academic council for the degree of Doctor of Philosophy in the field of knowledge Field of study: 10 – Natural Sciences, Specialty: 101 – Ecology in terms of relevance, scientific and theoretical level, main results of validity, provisions and results published in professional publications, novelty of the formulation and practical significance meets the requirements of the order Ministry of Education and Science of Ukraine No. 40, January 12, 2017 "On approval of requirements for the preparation of a dissertation" and Resolution of the Cabinet of Ministers of Ukraine of 12-th January, 2022, No. 44 "On approval of the Procedure for awarding the degree of Doctor of Philosophy and cancellation of the decision of the one-time specialized academic council of the institution of higher education, scientific institution on awarding the degree of Doctor of Philosophy" with changes introduced in accordance with Resolution of the Cabinet of Ministers, No. 341 dated 03/21/2022. He Songtao deserves to be awarded the scientific degree of Doctor of Philosophy , in the Specialty of 101 – Ecology

Official Reviewer:

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ПІДПИСАНО  
ЗАСВІДЧУЮ  
ПРОВІДНИЙ ФАХІВЕЦЬ  
*Людмила Бондарієва*

